PHARMACOGNOSY OF *SKIMMIA LAUREOLA* (DC.) SIEBOLD. & ZUCC. EX WALP. AND *ZANTHOXYLUM ARMATUM* DC., FAMILY RUTACEAE

Ph. D. THESIS

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PHARMACOGNOSY OF *SKIMMIA LAUREOLA* (DC.) SIEBOLD. & ZUCC. EX WALP. AND *ZANTHOXYLUM ARMATUM* DC., FAMILY RUTACEAE

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DECLARATION

The materials contained within this thesis are my original work and have not been previously submitted to this or any other university.

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CERTIFICATE OF APPROVAL

This Dissertation, entitled "**PHARMACOGNOSY OF *SKIMMIA LAUREOLA* (DC.) SIEBOLD. & ZUCC. EX WALP. AND *ZANTHOXYLUM ARMATUM* DC., FAMILY RUTACEAE**." submitted by Barkatullah is hereby approved and recommended as partial fulfillment for the award of Degree of Doctor of Philosophy in Botany.

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اقرأ
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DEDICATION

This Dissertation is dedicated to my father, late KHANIMULLAH, who not only raise and nurtured me but also taxed himself dearly over the years for my education and intellectual development.

And

To my mother, whose prayers are the source of motivation and strength during moments of despair and discouragement

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PHARMACOGNOSY OF *SKIMMIA LAUREOLA* (DC.) SIEBOLD. & ZUCC. EX WALP. AND *ZANTHOXYLUM ARMATUM* DC., FAMILY RUTACEAE

ABSTRACT

Present study is about *Skimmia laureola* and *Zanthoxylum armatum* belonging to Family Rutaceae, comprises phytosociology, ethnobotany, pharmacognostic study, physicochemical and pharmacological activities of these plants.

Phytosociological attributes of *S. laureola* were studied in six different localities of khyber Pukhtunkhwa, Pakistan showing that this plant grows gregariously in laomy and clay loamy soil at high altitude ranging from 2400- 3400 meters toward North facing slopes. *S. laureola* was found dominent in five out of six localities in association with a total of 44 plants with a density of 312 to 4437.5 hectare^{-1}. Various other ecological, ethnobotanical and commercial aspects of the plants are also worked out. Similar studies of *Z. armatum* showed that the plants grows in association with 51 species on the North and North West slopes in the foothills of khyber Pukhtunkhwa at an elevation of 850-1600 meters with a density ranging from 560 to 1020 hectare^{-1}.

Pharmacognostic study included leaf and bark of *S. laureola* and leaf, bark and fruit of *Z. armatum.* Leaf of *S. laureola* is punctate with glabrous surfaces. Transverse section of the leaf through the midrib region showed usual bifacial structure with prominent oil cavities in the midrib regions. Other leaf features such as palisade ratio (7.8±0.21), vein islets number (15.4±0.63 per mm^2), vein termination number (19.1±0.43 per mm^2), stomatal number (196.1±3.07 per mm^2) and stomatal index (12.96±0.14) were worked out. Eight different stomatal types were detected, in which actinostephanocytic was the most frequently occurring one . Stomatal cluster was also observed. Morphology and anatomy of *Z. armatum* was also carried out. Leaf of this plant lacks any type of trichome, where as in the midrib region, prominent oil cavities were observed. Palisade ratio (8.2±0.32), vein islets number (16.8±0.64 per mm^2), vein termination number (11.3±0.47 per mm^2), stomatal number (122.1±4.32 per mm^2) and stomatal index (12.32±

0.26) were cworked out. Anatomy of *Z. armatum* fruit showed two portion i.e. fruit wall and seed, the later being non endospermic and contained small elongated embryo. Powder drug microscopy of the parts was carried out. Ash analysis for both plants were carried out. Results of qualitative and quantitative preliminary phytochemical screenings of selected parts of *S. laureola* and *Z. armatum* are given, showing the presence of carbohydrates, proteins, alkaloids, phytosterols, triterpenoids, phenols, flavonoids, tannins, anthocyanins, saponins, glycosides, fixed and volatile oils. Similarly results of extractive values and fluorescence analysis are also given. Elemental analysis showed presence of Zn, Mn and Cr in fairly good amounts and may contribute to hypoglycemic effect of these plants. Correlations among various elements were also determined. Proximate analysis of both plants showed carbohydrate, proteins, fibers, fats and moisture contents in fairly large amounts.

Results of essential oils obtained from the leaves of *S. laureola* (SVO) and *Z. armatum* (ZVO) and fixed oils (ZHO and ZEO) from the fruit of *Z. armatum,* extracted with different solvents were evaluated for physicochemical characteristics including color, odor, % yield, density, optical activity, refractive index, specific gravity, carbon residue, absolute viscosity, kinematic viscosity, total acid number, iodine number and saponification value are presented. A total 31 different components in SVO and 34 in ZVO, 14 in ZHO and 14 in ZEO were identified through GC-MC analysis and their percent concentration is given.

Toxicological studies showed that both plants are safe for human use. Antipyretic effect was found dose dependant. SLE showed maximum antipyretic action of 72.31%, ZLE showed 85.42 percent pyrexia inhibition of whereas ZFE showed a maximum antipyretic action of 83.84%. Hypoglycemic effect of SLE was found to be dose dependent and like the standard allopathic drug and it induced reduction of blood glucose level after 2 hour of dose administration. All the doses showed significant reduction ($p < 0.05$) in glucose level at 6th hour post administration, but 300 mg/Kg body weight dose showed maximum reduction of blood glucose level at 6 hour (80.54±0.04).

Antispasmodic effects of ethanolic and *n*-hexane extracts of leaf (SLE, SLH), bark (SBE, SBH) and leaf essential oil (SVO) of *S. laureola* and ethanolic and *n*-hexane extracts of leaf (ZLE, ZLH), bark (ZBE, ZBH), fruit (ZBE, ZBH) and leaf essential oil of *Z. armatum* on the isolated rabbit jejunum for both spontaneous and KCl induced contractions showed that SVO and ZVO were the most efficient one, causing 100 % relaxation of the smooth muscles at very low concentrations, thus providing a scientific proof for its ethnopharmacological use as an antispasmodic drug.

The two plants were also evaluated for cytotoxic, phytotoxic, antibacterial and anti fungal activities. SLE, SLH and SVO of *S. laurola* and ZBE, ZBH and ZVO of *Z. armatum* showed outstanding cytotoxic results with LD_{50} values of 5.34, 7.44, 11.01, 16.79 17.06 and 15.90 μg/ml. respectively. *Lemna minor* phytotoxicity asssay of SBE showed 100% inhibition at 1000 μg/ml followed by SBH, SLE and SLH with FI_{50} of 25, 1.38, 4.54 and 8.67 μg/ml respectively. ZBE, ZFE and ZLH also showed excellent inhibitions with FI_{50} values of 7.98, 9.24 and 19.13 μg/ml respectively.

Antibacterial bioassays showed that all the samples were effective against various bacterial strains (*Micrococcus leutus, Escherichia coli, Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Bacillus subtilis,* and *Streptococcus viridanes*). SVO and ZVO being the highly effective against all the test strains. Results of various extracts of both plants against various bacterial strains are presented in this dissertation. Dose dependent antifungal activities against test species (*Trichophyton longifusis, Candida albicans, Fusarium solani, Microsporum canis, Aspergillus flavus* and *Candida glabrata*) were found for all the samples but SVO and ZVO inhabited all the test strains and *C. albicans*, *A. flauus*, *T. longifusis* and *F. solani* being the most susceptible species.

The present study has revealed the immense and diverse medicinal properties of *S. laureola* and *Z. armatum*, both can be exploited for therapeutic preparations on commercial scale.

PUBLICATIONS

1. Barkatullah , Muhammad Ibrar , Naveed Muhammad and Lubna Tahir. 2012. Antimicrobial evaluation, determination of total phenolic and flavoniod contents in *Zanthoxylum armatum* DC. *Journal of Medicinal Plants Research*, 6 (11): 2105-2110. **Impact factor; 0.87.**
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LIST OF CONTENTS

S. No.	Contents	Page No.
	Acknowledgment	i
	Abstract	iii
	Publications	vi
	CHAPTER-1 INTRODUCTION	**1-19**
1.1	Pharmacognosy	1
1.2	Medicinal plants	3
1.3	Phytosociology	4
1.4	Ethnobotany	5
1.5	Pharmacognostic study	6
1.5.1	Standardization of herbal drugs	6
1.5.2	Identification and authentication of plant materials	7
1.5.3	Anatomy	7
1.5.4	Phytochemical analysis	8
1.5.5	Physicochemical analysis	8
1.6	Essential oil	10
1.7	Fixed oil	11
1.8	Physicochemical characteristics of oil	11
1.9	Pharmacology	12
1.9.1	Acute toxicity study	13
1.9.2	Antispasmodic activities	13
1.9.3	Hypoglycemic activities	13
1.9.4	Antipyretic activities	14
1.9.5	Cytotoxic activities	14
1.9.6	Phytotoxic activities	15
1.9.7	Antimicrobial activities	15
1.10	Family description	15
1.11	*Skimmia laureola* (DC.) Sieb. & Zucc. ex Walp.	16
1.11.1	Taxanomic position of *Skimmia laureola*	16
1.11.2	Distribution in Pakistan	17
1.11.3	Ethnobotanical uses	17
1.12	*Zanthoxylum armatum* DC.	17
1.12.1	Taxonomic position of *Zanthoxylum armatum* DC	18
1.12.2	Distribution in Pakistan	18
1.12.3	Ethnobotanical uses	18
	CHAPTER-2 REVIEW OF LITERATURE	**20-65**
2.1	Review of literature for *Skimmia laureola*	20
2.2	Review of literature for other species of *Skimmia*	21

2.3	Review of literature for *Zanthoxylum armatum*	22
2.4	Review of literature for other species of *Zanthoxylum*	23
2.5	Review of literature for some other members of family Rutaceae	25
2.6	Phytosociology	27
2.7	Ethnobotany	30
2.8	Pharmacognostic studies	34
2.8.1	Ashing	36
2.8.2	Extractive values	37
2.8.3	Fluorescence study	37
2.8.4	Preliminary phytochemical analysis	38
2.8.5	Elemental analysis	40
2.8.6	Proximate analysis	42
2.9	Physicochemical analysis of oil	44
2.10	GC-MS Analysis	46
2.11	Pharmacological activities	49
2.11.1	Acute toxicity study	49
2.11.2	Antipyretic activity	50
2.11.3	Hypoglycemic activity	52
2.11.4	Antispasmodic activity	55
2.11.5	Cytotoxicity	58
2.11.6	Phytotoxicity	59
2.11.7	Antibacterial activity	61
2.11.8	Antifungal activity	63
	CHAPTER-3 MATERIALS AND METHODS	**66-115**
3.1	Morphology of the Plants	66
3.2	Phytosociology of the research plants	66
3.3	Soil analysis	68
3.3.1	Soil texture	68
3.3.2	Organic matter	68
3.3..3	Nitrogen (N)	69
3.3.4	Phosphorus (P)	69
3.3.5	Pottassium (K)	69
3.3.6	Iron, Zinc and Cupper	69
3.3.7	pH	69
3.4	Ethnobotany	69
3.5	Market survey	70
3.6	Plant collection	70
3.7	Pharmacognosy	72
3.7.1	Macroscopic studies.	72

3.7.2	Anatomy	73
3.7.3	Leaf surface study.	73
3.8	Physicochemical characteristics of powder drugs	76
3.8.1	Powder microscopy	76
3.8.2	Ash analysis	76
3.9	Florescence study	79
3.10	Extractive values determination	79
3.11	Elemental analysis	80
3.12	Nutritional analysis	82
3.12.1	Determination of ash	82
3.12.2	Determination of the moisture	82
3.12.3	Determination of proteins by “Macrojeldahl distillation method	83
3.12.4	Determination of fat (ether extract)	84
3.12.5	Determination of crude fiber	85
3.12.6	Carbohydrates contents	86
3.13	Organic solvent extractions	86
3.14	Qualitative chemical identification tests	86
3.14.1	Carbohydrates detection tests	86
3.14.2	Proteins & amino acids detection tests	87
3.14.3	Alkaloid detection tests	87
3.14.4	Phytosterols and triterpenoids detection test	88
3.14.5	Phenols detection test	88
3.14.6	Flavonoids detection tests	88
3.14.7	Tannins detection tests	89
3.14.8	Anthocyanins detection test	89
3.14.9	Saponin detection test	90
3.14.10	Steroidal glycosides detection test	90
3.14.11	Fxed oils detection tests	90
3.14.12	Volatile oil detection test	90
3.15	Quantitative chemical analysis	90
3.15.1	Alkaloids determination	91
3.15.2	Saponin determination	91
3.15.3	Tannins determination	92
3.15.4	Sterols determination	93
3.15.5	Total phenols determination	94
3.15.6	Total flavoniods determination.	94
3.16	Essential oils extraction	94
3.17	Physicochemical characteristics of oil	95
3.17.1	Color determination	95

3.17.2	Odor determination	95
3.17.3	Determination of percentage oil yield	95
3.17.4	Determination of optical rotation	95
3.17.5	Determination of refractive index	96
3.17.6	Determination of specific gravity	96
3.17.7	Carbon residue	97
3.17.8	Determination of viscosity	97
3.17.9	Kinematic Viscosity	98
3.17.10	Total acid number (TAN)	98
3.17.11	Iodine value determination	99
3.17.12	Determination of the saponification value	100
3.18	Statistical Analysis of physicochemical analysis	100
3.19	Gas Chromatography-Mass Spectrometery (GC-MS) of essential oil.	101
3.20	GC-MS analysis of fixed oil	102
3.21	Pharmacology	104
3.21.1	Acute toxicity test	104
3.21.2	Anti-pyretic activity	105
3.21.3	Hypoglycemic activity	106
3.21.4	Antispasmodic activity	108
3.21.5	Cytotoxicity	109
3.21.6	Phytotoxicity Activity	110
3.21.7	Anti bacterial activities	112
3.21.8	Anti-fungal activities	113
	Objectives of the study	**115**
	CHAPTER- 4 RESULTS AND DISCUSSION	**116-267**
4.1	Morphology of the research plants	116
4.1.1	Morphology of *Skimmia laureola*	116
	Morphology of *Zanthoxylum armatum*	116
4.2	Phytosociology	117
4.2.1	Phytosociology of *Skimmia laureola*	117
4.2.2	Phytosociology of *Zanthoxylum armatum*	120
4.3	Ethnobotany	123
4.3.1	Ethnobotany of *Skimmia laureola*	124
4.3.2	Ethnobotany of *Zanthoxylum armatum*	125
4.4.	Market servey	126
4.4.1	Market survey of *Skimmia laureola*	126
4.4.2	Market survey of *Zanthoxylum armatum*	127
4.5	Pharmacognosy	139
4.5.1	Pharmacognostic studies of *Skimmia laureola*	139

4.5.1.1	Macroscopy	139
4.5.1.2	Microscopy	140
4.5.1.3	Physicochemical characteristics	146
4.5.2	Pharmacognostic studies of *Zanthoxylum armatum*	158
4.5.2.1	Macroscopy	158
4.5.2.2	Microscopy	159
4.5.2.3	Physicochemical characteristics	167
4.6	Physicochemical analysis of oil	220
4.7	GC-MS Analysis	222
4.7.1	GC-MS Analysis of essential oil of *Skimmia laurola* leaf.	222
4.7.2	GC-MS Analysis of essential oil of *Zanthoxylum armatum* leaf	224
4.7.3	GC-MS analysis of Fixed oils of *Zanthoxylum armatum* fruit	226
4.8	Pharmacology	234
4.8.1	Biological activities of *Skimmia laureola*	234
4.8.1.1	Acute toxicity test	234
4.8.1.2	Antipyretic activity	235
4.8.1.3	Antidiabetic activity	236
4.8.1.4	Antispasmodic activity	237
4.8.1.5	Cytotoxicity	239
4.8.1.6	Phytotoxicity	240
4.8.1.7	Antibacterial activity	240
4.8.1.8	Antifungal avtivity	243
4.8.2.	Biological activities of *Zanthoxylum armatum*	244
4.8.2.1	Acute toxicity study	244
4.8.2.2	Antipyretic activity	244
4.8.2.3	Antispasmodic activity	245
4.8.2.4	Cytotoxicity	248
4.8.2.5	Phytotoxicity	248
4.8.2.6	Antibacterial activity	249
4.8.2.7	Antifungal	251
	Conclusions	**268**
	Recommendations	**272**
	References	**273-325**

LIST OF TABLES

Table. No.	Title	Page No.
Tab. 3.1	Conditions applied for detection of various elements	81
Tab. 3.2	Column oven programming for GC-MS analysis of essential oils	101
Tab. 3.3	Column oven programming for GC-MS analysis of fixed oils	103
Tab. 3.4	Composition of E-Medium	111
Tab. 3.5	Bacterial cultures used for antibacterial screening.	112
Tab. 4.1	Localities selected for phytosociological studies of *Skimmia laureola*.	128
Tab. 4.2	Summary of the phytosociological attributes of *Skimmia laureola* and associated flora in sampling areas. Species are listed in alphabetical order.	128
Tab. 4.3	Dominant shrubby species on the bases of importance value (IV) in the selected localities of *S. laureola*.	130
Tab. 4.4	Physicochemical analysis of the soil in different localities of *Skimmia laureola*	130
Tab. 4.5	Localities selected for phytosociological studies of *Zanthoxylum armatum.*	131
Tab. 4.6.	Summary of the phytosociological attributes of *Zanthxylum armatum* and associated flora in sampling areas. Species are listed in alphabetical order.	131
Tab. 4.7	Dominant shrubby species on the bases of importance value (IV) in the selected localities of *Zanthoxylum armatum*.	133
Tab. 4.8	Physicochemical analysis of the soil in different localities of *Zanthoxylum armatum*	133
Tab. 4.9	Market value chain of minimum, maximum and average prices in Pakistani Rupees (PRs.) per kilogram of *Skimmia laureola* at different market points on the basis of data collected from local dealers, hakims and Pansaries.	134
Tab. 4.10	Market value chain of minimum, maximum and average prices in Pakistani Rupees (PRs.) per kilogram of *Zanthoxylum armatum* fruit at different market points on the basis of data collected from local dealers, hakims and Pansaries.	134
Tab. 4.11	Macroscopic features of *Skimmia laureola* leaf.	180
Tab. 4.12	Macroscopic features of *Skimmia laureola* stem bark.	180
Tab. 4.13	Leaf constant values of *Skimmia laureola* leaf.	181
Tab. 4.14	Stomatal diversity with frequency and quantitative features in the lower epidermis of *Skimmia laureola* leaf.	181
Tab. 4.15	Ash analysis of leaf and stem bark of *Skimmia laureola.*	182
Tab. 4.16	Preliminary phytochemical screening of *Skimmia laureola* leaf.	182
Tab. 4.17	Preliminary phytochemical screening of *Skimmia laureola* stem bark.	183
Tab. 4.18	Quantitative chemical analysis of *Skimmia laureola*. All values are mean± SEM of three determinations. All values are expressed in mg/g.	184

Tab. 4.19	Fluorescence analysis of *Skimmia laureola* leaf and stem bark powder with different reagents.	184
Tab. 4.20	Fluorescence analysis of *Skimmia laureola* leaf and stem bark extracts.	185
Tab. 4.21	Percent extractive values of leaf and stem bark and fruit of *Skimmia laureola* with different solvents.	185
Tab. 4.22	Concentration of various elements in leaf and stem bark of *Skimmia laureola*. All values are mean±SEM of three values.	186
Tab. 4.23	Proximate analysis of *Skimmia laureola* leaf and stem bark. All values are mean±SEM of three values.	186
Tab. 4.24	Macroscopic features of *Zanthoxylum armatum* leaf.	187
Tab. 4.25	Macroscopic features of *Zanthxylum armatum* stem bark	187
Tab. 4.26	Macroscopic features of *Zanthxylum armatum* fruit.	188
Tab. 4.27	Leaf constant values of *Zanthoxylum armatum* leaf.	188
Tab. 4.28	Stomatal diversity with frequency and quantitative features in the lower epidermis of *Zanthoxylum armatum* leaf.	189
Tab. 4.29	Ash analysis of *Zanthoxylum armatum* leaf and stem bark.	189
Tab. 4.30	Preliminary phytochemical screening of *Zanthoxylum armatum* leaf.	190
Tab. 4.31	Preliminary phytochemical screening of *Zanthoxylum armatum* stem bark.	191
Tab. 4.32	Preliminary phytochemical screening of *Zanthoxylum armatum* fruit.	192
Tab. 4.33	Quantitative chemical analysis of *Zanthoxylum armatum*. All values are mean ± SEM of three determinations. All values are expressed in mg/g.	193
Tab. 4.34	Fluorescence analysis of leaf, stem bark and fruit powder of *Zanthoxylum armtum* with different reagents	193
Tab. 4.35	Fluorescence analysis of leaf, bark and fruit extracts of *Zanthoxylum armatum*.	194
Tab. 4.36	Percent extractive values of leaf, bark and fruit of *Zanthoxylum armatum* with different solvents.	195
Tab. 4.37	Concentration of various elements in different parts of *Zanthoxylum armatum*. All values are mean±SEM of three values.	196
Tab. 4.38	Proximate analysis of different parts of *Zanthoxylum armatum*. All values are mean±SEM of three values.	196
Tab. 4.39	Physicochemical characteristics of S VO, ZVO, ZHO and ZEO. All values are mean±SEM of three values.	228
Tab. 4.40	GC-MS profile of the *Skimmia laureola* leaf essential oil (SVO).	228
Tab. 4.41	GC-MS profile of *Zanthoxylum armatum* leaf essential oil (ZVO).	229
Tab. 4.42	GC- MS profile of n-hexane extracted fixed oil of Zanthoxylum *armatum* fruit (ZHO).	231
Tab. 4.43	GC- MS profile of Pet. Ether extracted fixed oil of *Z. armatum*	231

	fruit (ZEO).	
Tab. 4.44	Acute toxicity test of *Skimmia laureola* leaf in mice, monitored for 24 h.	253
Tab. 4.45	Antipyretic effect of ethanolic extract of *Skimmia laureola* leaf (100, 200 and 300 mg/kg i.p.) and pracetamol (150 mg/kg).	253
Tab. 4.46	Hypoglycemic effect of ethanol extract of *Skimmia laureola* leaf on blood glucose level of alloxan-induced diabetic rabbits.	254
Tab. 4.47	EC $_{50}$ (half maximal effective concentration values) of Ethanolic and n-hexane extracts of leaf, bark and leaf essential oil of *Skimmia laureola*	255
Tab. 4.48	Cytotoxicity of ethanolic and n-hexane extracts of leaf, bark and leaf essential oil of *Skimmia laureola*.	255
Tab. 4.49	Phytotoxicity of ethanolic and n-hexane extracts of leaf, bark and of leaf essential oil of S*kimmia laureola*.	255
Tab. 4.50	Antibacterial activities of the ethanolic and n-hexane extracts of leaf, bark and of leaf essential oil of *Skimmia laureola*. All values are mean ± SEM of three determinations.	256
Tab. 4.51	MIC (Minimum inhibitory concentration) values of ethanolic and n-hexane extracts of *Skimmia laureola* leaf, bark and leaf essential oil.	256
Tab. 4.52	Antifungal activities of ethanolic and n-hexane extracts of *Skimmia laureola* leaf, bark and leaf essential oil.	257
Tab. 4.53	24 hours acute toxicity test of *Zanthoxylum armatum* leaf and fruit in mice.	257
Tab. 4.54	Anti pyretic ffect of ethanolic crude extract of *Zanthoxylum armatum* fruits and leaf (100, 200 and 300 mg/kg i.p.) and paracetamol (150mg/kg).	258
Tab. 4.55	EC_{50} ((half maximal effective concentration values) of ethanolic and n- hexane extract of leaf, bark, fruit and of leaf essential oil of *Zanthoxylum armatum*.	259
Tab. 4.56	Cytotoxicity of ethanolic and n-hexane extracts of leaf, fruit, bark and of leaf essential oil of *Zanthxylum armatum*. All values are expressed as mean±SEM of three determinations.	259
Tab. 4.57	Phytotoxicity of ethanolic and n-hexane extracts of *Zanthxylum armatum* leaf, fruit, bark and leaf essential oil. Data is expressed as mean ±SEM of three determinations.	259
Tab. 4.58	Antibacterial activity of ethanolic and n-hexane extracts of leaf, fruit, bark and leaf essential oil of *Zanthoxylum armatum* DC. All values are mean ± SEM of three determinations.	260
Tab. 4.59	MIC value of ethanolic and n-hexane extracts of *Zanthoxylum armatum* leaf, fruit, stem bark and leaf essential oil.	260
Tab. 4.60	Antifngal activities of ethanolic and n-hexane extracts of leaves, fruit, bark and leaf essential oil of *Zanthoxylum armatum*. All values are mean± SEM of three determinations.	261

LIST OF FIGURES

Fig. No.	Title	Page No.
Fig. 1.1	*Skimmia laureola* growing in natural habitat.	19
Fig. 1.2	*Zanthoxylum armatum* growing in natural habitat.	19
Fig. 3.1.	Map showing natural habitats of *Skimmia laureola* and *Zanthoxylum armatum*	71
Fig. 4.1	*Skimmia laureola* a. leaves b. fruits c. stem	135
Fig. 4.2	*Zanthoxylum armatum*. a. flowers b. fruits c. leaves d. stem	136
Fig. 4.3	Bars representing the density per hactare of *Skimmia laureola* in Different localities.	137
Fig. 4.4	Regression among some variables for phytosociological study *of Skimmia laureola*	137
Fig. 4.5	Bars reperesenting density hectare^{-1} values of *Zanthoxylum armatum* in different localities.	138
Fig. 4.6	Regression among some variables for phytosocialogical study of *Zanthoxylum armatum.*	138
Fig. 4.7	Macroscopic features and powder of *Skimmia laureola* leaf. a. Adaxial surface b. Abaxial surface	197
Fig. 4.8	Macroscopic features and powder of *Skimmia laureola* bark. a. Adaxial surface b. Abaxial surface	197
Fig. 4.9	T. S. of *Skimmia laureola* leaf lamina.	198
Fig. 4.10	T.S. of *Skimmia laureola* leaf midrib	198
Fig. 4.11	*Skimmia laureola* leaf. a. Palisade cells arrangement under epidermal cells b.veins arrangement in lamina	199
Fig. 4.12	*Skimmia laureola* leaf. a. Upper epidermis b. lower epidermis epidermises.	199
Fig. 4.13	Various types of stomata in the lower epidermis of *Skimmia laureola* leaf	200
Fig. 4.14	T. S. of *Skimmia laureola* stem bark	200
Fig. 4.15	*Skimmia laureola* leaf powder	201
Fig. 4.16	*Skimmia laureola* stem bark powder.	201
Fig. 4.17a	Comparison of total ash (TA), acid insoluble ash (AIA) and water soluble ash (WSA) percent values of *Skimmia laureola* leaf powder non exhausted (SL) and exhausted with ethanol (SLE) and n-hexane (SLH).	202
Fig. 4.17b	Comparison of total ash (TA), acid insoluble ash (AIA) and water soluble ash (WSA) percent values of *Skimmia laureola* bark powder non exhausted (SB) and exhausted with ethanol (SBE) and n-hexane (SBH).	202
Fig. 4.18	Bars representing percent values of flavonoids, phenols, alkaloids, tannins, saponins and sterols in the ethanolic extracts of *Skimmia laureola* leaf (SLE) and bark (SBE).	203
Fig. 4.19	Correlations of various trace elements in *Skimmia laureola* leaf.	204
Fig. 4.20	Correlations of various trace elements in *Skimmia laureola* stem bark.	205

Fig. 4.21	Bars representing ratios among various elements in *Skimmia laureola* leaf (SL) and stem bark (SB).	206
Fig. 4.22	*Zanthoxylum armatum* leaf; a. Adaxial surface, b. Abaxial surface.	207
Fig. 4.23	*Zanthoxylum armatum* stem bark; a. Adaxial surface, b. Abaxial surface.	207
Fig. 4.24	*Zanthoxylum armatum* fruit; a. Whole, b. powder.	207
Fig. 4.25	T. S. of *Zanthoxylum armatum* leaf lamina.	208
Fig. 4.26	T. S. of *Zanthoxylum armatum* leaf midrib	208
Fig. 4.27	*Zanthoxylum armatum* leaf; a. Palisade cells arrangement under epidermal cells, b. veins arrangement in lamina.	209
Fig. 4.28	*Zanthoxylum armatum* leaf a. upper epidermis b. lower epidermises.	209
Fig. 4.29	Various types of stomata in the lower epidermis of *Zanthoxylum armatum* leaf .	210
Fig. 4.30	T. S. of *Zanthoxylum armatum* bark.	211
Fig. 4.31	*Zanthoxylum armatum* fruit. a. T. S of fruit; b. T. S. of fruit wall.	211
Fig. 4.32	*Zanthoxylum armatum* leaf powder.	212
Fig. 4.33	*Zanthoxylum armatum* bark powder	212
Fig. 4.34	*Zanthoxylum armatum* fruit powder	213
Fig. 4.35a	Comparison of total ash (TA), acid insoluble ash (AIA) and water soluble ash (WSA) percent values of *Zanthoxylum armatum* leaf powder; non exhausted (ZL) and exhausted with ethanol (ZLEE) and n-hexane (ZLEH).	213
Fig. 4.35b	Comparison of total ash (TA), acid insoluble ash (AIA) and water soluble ash (WSA) percent values of *Zanthoxylum armatum* bark powder; non exhausted (ZB) and exhausted with ethanol(ZBEE) and n-hexane (ZBEH).	214
Fig. 4.35c	Comparison of total ash (TA), acid insoluble ash (AIA) and water soluble ash (WSA) percent values of *Zanthoxylum armatum* fruit powder non exhausted (ZF) and exhausted with ethanol(ZFEE) and n-hexane (ZFEH).	214
Fig. 4.36	Bars represent percents values of flavonoids, phenols, alkaloids, tannins, saponins and sterols in ethanolic extracts *Zanthoxylum armatum* leaf (ZLE), bark (ZBE) and fruit (ZFE).	215
Fig. 4.37	Correlations of various trace elements in *Zanthoxylum armatum* leaf.	216
Fig. 4.38	Correlations of various trace elements in *Zanthoxylum armatum* stem bark.	217
Fig. 4.39	Correlations of various trace elements in *Zanthoxylum armatum* fruit	218
Fig. 4.40	Bars representing ratios among various elements in leaf (ZL), bark (ZB) and fruit (ZF) of *Zanthoxylum armatum*.	219
Fig. 4.41	Typical GC-MS chromatogram of *Skimmia laureola* leaf essential oil (SVO) showing the separation of chemical components.	232

Fig. 4.42	Typical GC-MS chromatogram of *Zanthoxylum armatum* leaf essential oil (ZVO) showing the separation of chemical components.	232
Fig. 4.43a	Typical GC-MS chromatogram of *Zanthoxylum armatum* fruit fixed oil, extracted with n-hexane (ZHO) showing the separation of chemical components.	233
Fig. 4.43b.	Typical GC-MS chromatogram of *Zanthoxylum armatum* fruit fixed oil, extracted with petroleum ether (ZEO) showing the separation of chemical components.	233
Fig. 4.44	Antipyretic effect of SLE (100, 200 and 300 mg/kg) and (PSM) paracetamol (150mg/kg) on brewer yeast induced pyrexia in mice after 1, 2, 3, 4 and 5h.	262
Fig. 4.45	Hypoglycemic effect of SLE on alloxan-induced diabetic rabbits treated with extracts of SLE (100, 200 and 300 mg/kg) and glibinclamide (GLCL) (100 mg/kg) after 2, 4, 6, 8 and 12h. Bar represent percent ihibition of blood glucose level	262
Fig. 4.46	Dose response curve of the SLE, SLH, SBE, SBH and SVO on isolated rabbit's jejunum preparations. All values are Mean ±SEM, n = 5).	263
Fig. 4.47	Antispasmodic effect of *Skimmia laureola*.	264
Fig. 4.48	Effect of ZLE on brewer yeast pyrexia in mice treated with extract of 100, 200 and 300 mg/kg and paracetamol (PSM) (150 mg/kg) after 1, 2, 3, 4 and 5h. bar represent the percent ihibition of pyrexia.	265
Fig. 4.49	Effect of ZFE on brewer yeast induced pyrexia in mice treated with extract of 100, 200 and 300 mg/kg and paracetamol (PSM) (150 mg/kg) after 1, 2, 3, 4 and 5h. Bar represent the percent ihibition of pyrexia.	265
Fig. 4.50	Dose response curve of the ZLE, ZLH, ZBE, ZBH, ZFE, ZFH, and ZVO on isolated rabbit's jejunum preparations. All values are Mean ±SEM, n = 5).	266
Fig. 4.51	Antispasmodic effect of *Zanthoxylum armatum*.	267

ABBREVIATIONS

SL *Skimmia laureola* leaf

SLE *Skimmia laureola* Leaf ethanolic extract

SLH *Skimmia laureola* Leaf n-hexane extract

SLEE *Skimmia laureola* Leaf powder exhausted with ethanol

SLEH *Skimmia laureola* Leaf powder exhausted with n-hexane

SB *Skimmia laureola* stem bark

SBE *Skimmia laureola* stem bark ethanolic extract

SBH *Skimmia laureola* stem bark n-hexane extract

SBEE *Skimmia laureola* stem bark powder exhausted with ethanol

SBEH *Skimmia laureola* Leaf powder exhausted with n-hexane

SVO *Skimmia laureola* leaf volatile oil

ZL *Zanthoxylum armatum* leaf

ZLE *Zanthoxylum armatum* Leaf ethanolic extract

ZLH *Zanthoxylum armatum* Leaf n-hexane extract

ZLEE *Zanthoxylum armatum* Leaf powder exhausted with ethanol.

ZLEH *Zanthoxylum armatum* Leaf powder exhausted with n-hexane

ZB *Zanthoxylum armatum* stem bark

ZBE *Zanthoxylum armatum* stem bark ethanolic extract

ZBH *Zanthoxylum armatum* stem bark n-hexane extract

ZBEE *Zanthoxylum armatum* stem bark powder exhausted with ethanol

ZBEH *Zanthoxylum armatum* Leaf powder exhausted with n-hexane

ZF *Zanthoxylum armatum* Fruit

ZFE *Zanthoxylum armatum* fruit ethanolic extract

ZFH *Zanthoxylum armatum* fruit n-hexane extract

ZFEE *Zanthoxylum armatum* fruit powder exhausted with ethanol

ZFEH *Zanthoxylum armatum* fruit powder exhausted with n-hexane

ZVO *Zanthoxylum armatum* leaf volatile oil

ZHO *Zanthoxylum armatum* fruit fixed oil extracted with n- hexane

ZEO *Zanthoxylum armatum* fruit fixed oil extracted with petroleum ether

CHAPTER-1

INTRODUCTION

1.1. Pharmacognosy

The detail study of medicines originated from natural sources is described as Pharmacognosy. According to American Society of Pharmacognosy "Pharmacognosy is the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources" (Tyler, 1999). As practised today, pharmacognosy includes the extensive study of natural products from plants, bacteria, fungi and marine organisms, botanical dietary supplements as well as herbal remedies (Cardellina, 2002). Pharmacognosy can also be defined as "the scientific and systematized study of physical, chemical, structural and biological features of crude drugs as well as their history, method of cultivation, collection and preparation for the commercial purposes"(Gokhele *et al.,* 2008). It is the science which provides infrastructure for the evolution of novel medicines. It is a long-established pharmaceutical science which has played an alternative role in finding, characterization, standardization and manufacturing of plant material as well as phytomedicines regarding their macroscopic, microscopic and biochemical characteristics (Kaplan, 2001; Kinghorn, 2002; Gokhele *et al.,* 2008).

Pharmacognosy is the scientific study of crude drugs originated from four different natural sources namely plants, animals, minerals and metals. It is estimated that 90% of the crude drugs are originated from plant sources while the remaining are from other three sources (Joy *et al.,* 1998). Pharmacognostic study of crude drugs involves five customary parameters i.e. the botanical, organoleptic, physical, chemical, and pharmacological parameters. These parameters used to disseminate the unique features of crude drugs in three different stages namely identification, isolation of compounds/ active principles and screening for biological activities.

Pharmacognosists often come across two acquainted practices, adulteration and substitution' which are widespread in trade presently. Adulteration, in general, is the

degradation of any article, which involves conditions such as inferiority, admixture, deterioration, spoilage, sophistication and substitution. Adulterating the crude drugs by any of the said conditions is considered detrimental in the crude drug industry. The word 'substitution' means 'when an article is put in place of another article which is no longer available or put in exchange for', where as pharmacognostically, it is defined as "an entirely different article that is used or sold in place of the required or requested article as cottonseed oil sold as olive oil and American saffron sold as Spanish saffron are examples of substitution (Selvam, 2010).

Pharmacognosy, although closely related to Botany and Phytochemistry, has a natural link with other scientific fields such as Pharmacology, Analytical Chemistry, Microbiology, Plant Tissue Culture, Biotechnology and Genetic Engineering etc and encapsulates all of these fields into a distinct interdisciplinary science (Rangari, 2002; Balunasa & Kinghornb, 2005).

Pharmacognosy has also a very vital link with pharmaceutics and various traditional systems of medicines which help the pharmacognosists to dispense formulate and manufacture drugs of natural origin in the best accepted allopathic form. The knowledge of chemotoxy, extraction, purification, plant tissue culture etc helps in the complete understanding of pharmacognosy along with coming up of better technologies for collection and preparation of crude drugs (Jarald & Jarald, 2007).

Pharmacognosy is also considered as a good example of a modern multidisciplinary discipline that could serve to arouse the interesting medicinal sciences. Increased interest in the study of natural products in drug development, as well as rapidly altering investigation strategies are the driving forces, modernizing the pharmacognosy. Pharmacognosy, now a day focuses on finding novel and unique molecules and revealing unknown targets by studying such molecules in nature. It is now well understood that pharmacognosy is one of several scientific disciplines that have an inimitable strategic position in connecting biology with chemistry and even medicine. New and improved strategies regarding the selection of organism selection, bioassays techniques, isolation procedures, and structure elucidation are constantly devoloped based on the latest advancements in pharmacognosy (Bruhn & Bohlin, 1997; Claeson & Bohlin, 1997).

Pharmacognosy provide basis for the study of secondary metabolites (natural product molecules) which are beneficial for their ecological, medicinal, gustatory or other functional properties. The natural species which are the basis for medicinally important compounds are of the origin of biological kingdoms, particularly marine invertebrates, plants, fungi, and bacteria. The field of Pharmacognosy is not limited to special area and is constantly being reinvigorated by input from time to time by new developments in scientific fields and technologies. This is the reason that now a days Pharmacognosy is a good option for those who like to work at the interface of many diverse but harmonizing branches of science that relate to the natural world (Kinghorn, 2002; Samuelsson, 2004).

Pharmacognosy is largely related to medicinal plants, which have inherited active substances for treating various ailments (Okigbo *et al.,* 2008). Plants have been considered as potential source of medicines for curing various ailments and disorders since the dawn of civilization and led to the establishment of the conventional knowledge of plants all around the sphere. Initially these medicines were utilized in the form of crude drugs, poultices, teas, tinctures, powders, and other herbal formulations. The particular plants to be used and the methods of application for a specific ailment were passed down through verbal communication (Ahmad *et al.,* 2006a; Balick & Cox, 1997; Samuelsson, 2004). Owing to poverty, unawareness and unavailability of contemporary health facilities, most people, especially rural people are still compelled to practise traditional medications for their day to day illnesses (Khan, 2002).

1.2. Medicinal plants

Plants containing inborn potentially active ingredients used to cure disease or relieve pain are called medicinal plants (Okigbo *et al.,* 2008). Plants play a therapeutic and restorative role in protecting human beings from the adverse effects of diseases and other complications, thus considered to have a beneficial role in healthcare system. That is the reason that large proportion of population of the developing countries still rely on herbal medicines. Despite their importance, medicinal plants are seldom handled within an organized manner and most are of them are exploited with little or no respect for the future (Srivastava *et al.,* 1996; Nair *et al.,* 2005). Significant increase in medicinal plants usage has been recorded continuously both for traditional users and pharmaceutical industry.

Medicinal plants provide opportunities for biological screening, methods useful for the industry and trends in the pharmacological investigations of natural products (Ozturk & Ozturk, 2008). Plants are the natural and most easy accessible source of theurapeutically active biological principles, thus there is a dire need to screen out plant for development of new drugs. For this purpose plants have been assayed widely but still large number of them has not arrived to the conventional health care system (Esimone *et al*, 2003; Bhattarai *et al*., 2006). Therefore, search for new drugs from microorganisms, fungi, plants and animals must be persistent and these can be the sources of innovative and prevailing restorative agents for newer, safer and accessible drugs (Lindequist *et al*., 2005). Now a day, due to advancement of modern and new sophisticated methods, plant scientists are taking more intrust in exploring new drugs from natural and biologically active compounds of the plants, which could be serve as inexhaustible resources for pharmaceutical industries (Yakuba *et al*., 2007).

Pakistan has a unique position among developing countries, having about 6000 taxa of angiospermic plants including a variety of medicinal plants due to variation in topographic conditions (Rahim & Hasnain, 2010). Moreover it is interesting to say that about 50% of the population in Pakistan is being treated with local herbal preparations by almost 50,000 hakims (traditional herbal practitioners) (Zaidi, 2006). More than 350 herbal items (as whole herbs or with specific parts) have been reported, which are used in Unani herbal preparations by various Dawakhanas (herbal drugs manufacturing laboratories) in Pakistan (Ahmad *et al*., 2008). Pakistani flora offers great opportunity for the discovery of new bioactive compounds for various ailments (Haq, 1983).

1.3. Phytosociology

Phytosociology is a sub discipline of plant ecology that describes the co-occurrence of plant species in communities (Ewald, 2003). Vegetation and soil characteristics are so interacted and inter-dependent that they become indicative of each other. A habitat under certain existing ecological conditions would permit plants being adjusted to these conditions, thus soil-plant relationship becomes so close that plants reveal the ecological situation of the inhabited locality (Anonymous, 1991; Boggs, 2000). Vegetation diversity is primarily determined by a combination of interacting physical and chemical factors like

water temperature, solar radiation, current flow velocity, which play a major role in determining floristic diversity in a given area (Hinterlang, 1992). The physicochemical analysis of the soil and its interpretation is a significant symptomatic tool to explore the interrelationship of elemental concentration of plants and soils (Itoh *et al.,* 2007). Many plants grow equally in many localities having similar ecological conditions, and as these conditions alter in an area, the cultivation and collection of a medicinal plant may change accordingly (Evans, 2002). The level of essential elements in plants is dependent on geochemical characteristics of the soil and on the form of their bond with the components of the soil. Plants obtain these elements through roots (Bin *et al.,* 2001).

1.4. Ethnobotany

The traditional uses of plants in native cultures are manifold and very diverse. Many people still depend on plants for their economy, medicine, food, construction material, fire wood, dyes, ornamentals purposes etc. The aim of the ethnobotanical study is to create a better understanding of the local uses, to make improved use of resources, to find new ways for transferring this knowledge to future generations and to search for new pharmaceuticals to be used in biomedicine (Kufer *et al.,* 2005). Similarly ethnobotanical knowledge establishes priorities in the local communities and assists taxonomist, ecologists, pharmacologists, watershed and wild life managers in their efforts for improving the economic status of the area (Ibrar *et al.,* 2007). "Ethnobotany is the knowledge of plants usage by the native people and their usefulness as understood to the people of a particular ethnic group, since information concerning a particular plant varies from one ethnic group to another" (Tor-Anyiin *et al*, 2003; Igoli *et al*, 2005). An immense knowledge can be accumulated about the usage of plants against different illnesses, in areas where plants are still of immense significance (Diallo *et al*, 1999). Ethnobotanical survey encourages the persistent search of natural products from plant for pharmaceutical preparations and is one of the major significant approaches to select plants for pharmacological screening (Igoli *et al.,* 2005).

1.5. Pharmacognostic study

In the last few decades there has been an enormous development in the field of herbal medicine. It gets commercializing in developing and developed countries due to its natural derivation and less significant side effects. Herbal drugs play a significant role in health care programs, especially in developing countries (Mulla & Swamy, 2010). Because of the popularity, herbal praparations for various ailments are now being prepared on a large scale in mechanical units, where availability of good quality and authentic raw materials, availability of standards, appropriate standardization procedure of drugs and formulations, quality control parameter etc are some of the problems facing by the manufacturer. Due to these discrepancies, it is now necessary to make efforts for the plants materials standardization, to be used as medicine. This standardization procedure can be achieved by stepwise pharmacognostic assessment (Ali *et al.,* 2005; Agarwal, 2005).

Despite the modern techniques, standerdization and authentication of plant drugs by pharmacognostic procedures is more trustworthy. The morphological and anotomical description of a medicinal plant is the first step towards standardization of plant materials and should be carried out before the commencement of any experimental procedure for the detection of adulterations and impurities (WHO, 1995).

1.5.1. Standardization of herbal drugs

Recently there is an increased trend to the manufacturing and utilization of herbal products but the key issue in these drug and medicines is standardization. "Standardization is the process of producing herbal medicines or extracts in which product potency is guaranteed through consistency of active compound at a satisfied level. This process requires high skill of phytochemical analysis and technology to assess quality control" (Fernandez-Bolanos *et al*., 2006). Standardization of medicinal plants due to their potential therapeutic significance is an indispensable necessity for the whole plant, plant parts or their extracts in order to authenticate quality control (Venkatesh *et al.,* 2004).

Plants are referred to as God's own pharmacy (Treben, 1986), and serves as raw material for important drugs in modern medicine system (Singh *et al.,* 2002). Plant origin drugs are used as whole plant or part of it or in the form of plant extract. Therefore, there is dire need to standardize and utilize medicinal plants, which show appropriate biological effect (Sofowora, 1982). Knowledge about medicinal plants has exploded due to long and

dangerous self-experiences of the people. Progress towards better understanding of plants derived medicines depends on two factors i.e. the development of progressively strict criteria for the proof that a medicine surely does what it is claimed for and identification of the active compound in the plant by chemical analysis (Holiman, 1989).

1.5.2. Identification and authentication of plant materials

The most important step with respect to standardization of herbal drugs is the correct taxanomic identification of the concerned species, whether in fresh, dried or powdered state (Springfield *et al*., 2005). Accurate identification and quality assurance of the starting materials is a necessary prerequisite step for reproducible quality of herbal medicine. Pharmacognostic techniques used for standardization of plant material include macroscopic, microscopic and biochemical description of the plant materials. Pharmacognostic evaluation helps in identification and confirmation of the plant material. (Anonymous, 1998).

1.5.3. Anatomy

Anatomy helps in study of the internal structure of plants and is considered to be a source of fascination for correct identification of plant taxa. Anatomical study centres on the spatial arrangement of the dermal, ground, and vascular tissue systems, the patterning of tissue and cell types and nature of individual specialized cell types (Nancy & Dengler, 2002). Foliar epidermal microscopic features of leaf i.e. shape of epidermal cell, type of stomata, presence or absence of pubescence and cell wall thickness are considered as useful tools for correct taxa identification and its affinity in a family with other taxa. These features are significant not only in making taxonomic conclusions but also in developmental and evolutionary studies (Stace, 1984; Babalola & Victoria, 2009).

Since leaf epidermal studies are considered important in phylogeny and taxonomy, therefore, plant taxonomists have given much attention towards leaf epidermal anatomical studies to resolve the taxonomic problems (Taia, 2005).

Microscopic evaluation is crucial step in the preliminary identification of plants as well as for detection of small fragments of crude or powder drugs and detection of adulterants like insects, animal's feces, molds, fungi etc by identifying characteristic tissue features. Other techniques like linear measurements, determination of leaf constants and

quantitative microscopy are also used for drug evaluation. Linear measurments includes size of starch grain, length and width of fibers, trichome etc. Stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio are the leaf constants, widely employed in the microscopic evaluation of crude leaf drugs (Jarald & Jarald, 2007).

1.5.4. Phytochemical analysis

Phytochemicals are plant derived chemicals, beneficial to human health and having the capability of disease prevention (Chung *et al*., 1998). Secondary metabolites from plants are an important source of drugs since ancient times and now almost 50% of the practical drugs used are derived from natural sources (Wang *et al*., 2008a). Secondary metabolites of plants like alkaloids, tannins, flavonoids, saponins, anthraquinones, cardiac glycosides and cyanogenic glycosides etc are of pivotal importance. Chemical evaluation of the plants for secondary metabolites includes qualitative, quantitative and biochemical tests. Qualitative chemical tests are carried out for identification of various phytoconstituents. Similarly quantitative and biochemical tests are also of the prime importance in drugs evaluation (Rangari, 2002) for the detection of inferior or exhausted materials or substitution by of a worthless article (Jarald & Jarald, 2007).

1.5.5. Physicochemical analysis

Physicochemical characteristics of powder drug assess the estimation of amount of impurities like earthy and other particles present in the drug. Some of the characteristic physicochemical analyses in this category are,

a. Ash values

Ashing is an important tool for detecting of adulteration in crude drugs. Different types of ash values are used for detection of crude drugs like total ash, acid insoluble ash and water soluble ash. Total ash value is useful for detection of any siliceous contamination, chalk powder, lime or other earthy matter. Acid insoluble ash is used to detect excessive earthy materials, which has varying amount of calcium oxalate crystals in the cells while water soluble ash is used to detect the presence of water exhausted material (Jarald & Jarald, 2007).

b. Extractive values

Extractive values play an imperative role in the evaluation of the crude drugs. Extraction with different solvents assures various types of adulteration and exhausted materials e.g. Alcohol and water soluble extractive values are indicative of the presence of the adulterants, defective processing and poor quality of the drug. Petroleum ether soluble extractive value indicates lipid contents present in crude drug (Madhavan *et al*., 2009; Kokate, 1994).

c. Fluorescence study

Fluorescence phenomenon exhibited by plant powder or extract is primarily due to its chemical composition. The same material may appear dissimilar in different wavelength of light. Some constituents of the extract show fluorescence in the visible range in daylight while some florescence only in ultra violet light. If substances do not show fluorescence phenomena, then they may be made fluorescent by applying various reagents to their decomposition products or their derivatives. Through this technique some crude drugs are often assessed qualitatively for standardization. Fluorescence study therefore can be used as a finger print for crude drug identification (Ansari, 2006; Reddy & Chaturvedi. 2010).

d. Elemental analysis

Trace elements have both therapeutic and restorative role in combating against various health problems. There is a great opportunity to make use of the curative and preventive role of various trace elements like Cu, Zn, Cr etc (Kaneez *et al.,* 1998). Mineral elements though make a small proportion of total chemical composition and body weight of the plant materials, but their physiological importance especially metabolic process and pharmacological activities cannot be ignored (Bamiro *et al*., 1995).

e. Nutritional analysis.

Plants are considered as basic nutritional source as they contain protein, carbohydrates, fats and oils, minerals, vitamins, and water, obligatory for growth and development in man and animals. These phytochemicals have been considered of crucial nutritional importance in the prevention of chronic disease such as cardiovascular disease, cancer, and diabetics (Aruoma, 2003). Some plants chemicals have been regarded as anti nutritional or antioxidants but have potentials to reduce the risk of several deadly diseases in

humans (Agte *et al*., 2000). Proteins, fat, carbohydrates and minerals including trace elements, vitamins and water are the essential nutrients, which contribute to caloric and metabolic requirements of human (Underwood, 1977). Proteins in seeds both qualitatively and quantitatively are of utmost significance in the selection of plants for their nutritional values, taxonomic classification and nutrition promotion programs (Siddique, 1998).

Most countries in the world have been facing malnutrition problems especially proteins deficiency in human food and animal feed. Now a days, the need for good quality of proteins has been increasing due to high population growth rate. Similar situation also has been prevailing in Pakistan where the protein gap might be increased unless well-planned programs are launched to cope the situation (Nisar *et al*., 2009). It is therefore very essential to raise protein production by utilizing all the available resources. Efforts have been made conventionally to increase production; new chemical and biological techniques have been employed in recent years to enhance protein yields in foods and feeds (Shah & Khalil, 1988). Similarly high carbohydrate and crude fiber contents suggest the suitability of plant as animal feed (Abighor *et al*., 1997).

1.6. Essential oil

The volatile oils also known as essential oils can be defined as "the oils entirely or almost entirely volatile without decomposition". The essential oils can be produced in flowers, buds, stems, leaves, fruits, seeds and roots etc. These oils are stored in cavities, channels, secretary cells and epidermal cells (Jarald & Jarald, 2007; Hussain *et al*., 2008a). Almost all odoriferous plants yield essential oils. Essential oils can be extracted from fresh, partially dehydrated or dried plant materials (Asekun *et al*., 2007; Hussain *et al*., 2008b). Essential oils have very complex and highly variable chemical compositions, being a mixture of organic volatile substances with different concentrations (Burt, 2004; Bakkali *et al*., 2008). Some of the essential oils or their bioactive components like limonene, carvone, geranyl acetate are useful in toothpaste and other hygienic products preparations. These components are also useful as additives and preservatives, also exhibit biological activities especially antimicrobial, since ancient times. Recently essential oils are also used in aromatherapy in various body complications (Amvam *et al*., 1998; Silva *et al*.,, 2000; Hajhashemi *et al*., 2003). Chemically essential oil contains palmitic acid, myristic acids,

sesquiterpene alcohol, dimethyl ether, cineole, levorotatory borneol, levorotatory camphor, limonene, pyrocatechic tannin and glycoside. GC-MS analysis of volatile oil is used to determine the presence of various types of compounds in oil, in order to know its application in various industrial products (Morallo-Rejesus *et al.,* 1990). Gas chromatography analysis assess in identification of saturated and unsaturated aliphatic hydrocarbons and a few unknown ones in oil (Solanki *et al.,* 2011).

1.7. Fixed oil

Fats and oils are the highest source of energy per unit weight whether it is of animal, vegetable or marine origin represent. Although these are the prime source of reserved energy, fats deposit insulates the body against heat loss and protects vital organs from mechanical injury. Fixed oils are important source of food for man and are also extensively used in nutritional, cosmetic and other industries (Ranken & Kill, 1993). Seed oils are significant sources of dietary oils, industrial and pharmaceutical products. The characteristics features of oils from various sources depend mainly on their composition and other physicochemical characteristics (Mohammed & Jorf-Thomas, 2003).

1.8. Physicochemical characteristics of oil

Study of various physicochemical characteristics explores the practical importance of herbal oils in daily life. Physicochemical properties of oil like colour, odour, density, specific gravity, refractive index, optical rotation, acid value, iodine value, saponification value etc indirectly influence the quality of both essential and fixed oils. The commercial significance of oils mostly depends on these physicochemical properties, which provide baseline data to establish its appropriateness for human consumption (Bamgboye & Adejumo, 2010; Parthiban *et al*., 2011). “Viscosity is a measure of resistance of a fluid to deform under shear stress. It is commonly perceived as thickness, or resistance to pouring”. Viscosity describes a fluid's internal resistance to flow and may be thought of as a measure of fluid friction. It determines the rheological proprieties of these oils. (Kimbonguila *et al*., 2010).

The refractive index is the degree of the deflection of light beam that occurs when it passes from one transparent medium to the other. It increases with increase in number of

carbon atoms and length of the carbon chains. Therefore, the refractive index determines evidences that the sample might be unsaturated long carbon chain (Pearson, 1976).

The iodine value is useful tool, through which drying properties of oils can be detected (Akinhanmi *et al*., 2008). The high iodine value of oils indicates the high content of unsaturation, suggesting the usefulness of oils as drying agent for the manufacturing of oil paints, varnishes, cosmetics and for cocking oil manufacturing index (Adelaja, 2006). The iodine value is also an index of assessing the ability of oil to go rancid. It is also used for determining the level of oxidative deterioration of the oil by enzymatic or chemical oxidation (Dawodu & Omole, 2009).

Acid value is an important physicochemical property index of oil which is used to determine the quality, age, edibility and suitability of oil for industrial use such as paint (Akubugwo *et al*., 2008). This value is used to measure the extent of glycerides in the oil, which have been decomposed by lipase and other physical factors such as light and heat (Demian, 1990).

Saponification value is an index of average molecular mass of various fatty acids in oil samples. The lower value of saponification means molecular weight of fatty acids is lower and has lower limit of use in industry (Denniston *et al*., 2004). The saponification value suggests the usefulness of oil in production of lather shaving cream, liquid soap, and shampoos (Oderinde *et al*., 2009).

1.9. Pharmacology

Plants are among the most common and accessible sources of potentially active drugs for various combating various ailments. Therefore, it is imperative to search biological properties of medicinal plants for the development of new drugs. A lot of work has been done on plants but still there is need to work more in this respect (Alade & Irobi, 1991; Esimone *et al*., 2003). Pharmacology offers various scientific strategies like screening of extracts, fractions and compounds obtained from plants in the form of bioassays in the field of phytochemical research. (Nelms, 1997). Different bioassays are suggested for screening out various medicinal plants extracts for different purposes (Srirama *et al*., 2007). The following pharmacological activities are included in the present study.

1.9.1. Acute toxicity study

Acute toxicity study is a technique for toxicity detection by raising dose till the appearance of toxicity signs. Several different new techniques have been developed for more modern approach to toxicology (Combe *et al*., 2004), the use of animals in safety and toxicological evaluations in acute systemic toxicity testing is still in practice (Anonymous, 2000).

1.9.2. Antispasmodic activities

Diarrheal diseases are one of the major cause children mortality and morbidity in deeloping countries. Each year more than 1000 million casualties of diarrhea and 5 millions deaths occur in children (Carlos & Saniel, 1990). Despite enormous technological expansion in the world of medicine, herbal drugs are still in practice for controlling and curing diarrhea in developing countries (Ojewole, 2004; Agunu *et al*., 2005). The WHO highly appreciates the treatments and protective measures of diarrhea through conventional medical practices (Atta & Mouneir, 2004). To evaluate phytomedicines, In vitro techniques are preferred, as these are easy to handle and conducted in very short span of time (Baker *et al*., 1995). Now day's isolated organ(s) are considered a valuable tool for assessment of such pharmacological potential of drugs of plants origin (Enna *et al*., 2002).

1.9.3. Hypoglycemic activities

Diabetes mellitus type-2 is one of the most severe metabolic disorders characterized by chronic hyperglycemic condition and disturbance in the metabolism of carbohydrates, proteins and fats due to complete or partial lack of insulin secretion (Jia *et al*., 2004; Grover & Yadav, 2004). It is one of the major disorders responsible for mortality in most countries of the world. According to an estimate, approximately 376 million people will be affected worldwide by diabetes by the end of year 2030, presently Pakistan is ranked 6th among mostly affected countries by diabetes; it will replace 5th position of Japan at the end of 2030 (Wild *et al*., 2004). Plants are used to cure and control diabetes in many countries. Many plant extracts are conformed as anti hyperglycemic agents (Kar *et al*., 2003; Virdi *et al*., 2003). Indeed, synthetic drug therapy is obligatory and in practice in clinical treatments, but it does have severe side effect like hematological effects and coma etc. It also disturbs

kidney and liver functions with the passage of time (Lapshina *et al.,* 2006; Zakir *et al.,* 2008). In addition they have also been proved unfit during pregnancy (Larmer, 1985). In Comparison to synthetic drugs, drugs of plant origin are considered to be non toxic with no or very few side reactions (Momoin, 1987).

1.9.4. Antipyretic activities

Pyrexia or fever is not disease itself but is a secondary impact of infections, malignancy or other unhealthy situation. It is the body's natural defence system to create unsuitable environment for the survival of infectious agents or damaged tissues (Chattopadhyay *et al.,* 2005). Normally the infected or injured tissue starts the formation of cytokinens (pro-inflammatory mediators) which stimulates the synthesis of prostaglandin E2 (PGE2) near the hypothalamus area which triggers the hypothalamus, thus raise the body temperature (Spacer & Breder, 1994). Antipyretic activity of large number of plants or their natural products assess to guide the isolation and purification of easily available biologically active principles (Amole & Onbanjo, 1999).

1.9.5. Cytotoxic activities

Screening of plants, active compounds leads to the innovation of new drugs which are efficient in protecting and curing various damaging diseases including cancer (Amara *et al.,* 2008). *Artemia salina,* the brine shrimp, is an invertebrate inhabiting saline aquatic and marine environment and is an important component of energy flow of marine ecosystem. It is considered to be an important laboratory tool for bioassay to determine the toxicity by calculating the medium lethality concentration LC_{50}, which has been reported for large number of toxins and extracts from plants (Meyer *et al.,* 1982; Lagadic & Caquet, 1998). Brine shrimp *(A. salina* nauplii*)* lethality bioassay is used as a convenient source for screening bioactive natural products to ascertain the cytotoxic potential. Brine shrimp lethality assay was described in literature (McLaghlin *et al.,* 1988) and is considered to be a useful tool for preliminary assessment for the detection of cytotoxicity by plant extract, heavy metals, cyanobacteria toxins etc (Moshafi *et al.,* 2009).

1.9.6. Phytotoxic activities

In Pakistan the major problem is the huge wastage of cereal crops due to poor weed control. The extent of weed damage is usually more pronounced than that of pests and diseases but its effects are unseen. Competition for available resources also affects crop yields due to growth of weeds with cereal crops. So, a strategy for weeds control is very essential for increasing production of various crops. In this regards, *lemna* bioassay technique is applied for exploring natural inhibitors of weeds. *L. minor* is a simple aquatic monocot, having a central oval frond, to which are attached two daughter fronds and a filamentous root. This plant reproduces vegetatively by producing buds from preexisting fronds and from pouches on the sides of the main frond (Atta-ur-Rehman, 2001).

1.9.7. Antimicrobial activities

Microbial infections are observed to be a significant cause of mortality and morbidity in spite of advancement in synthetic medicine and new antifungal agents (McNeil *et al.,* 2001). Since microbial strains with multiple antibiotic resistances are increasing worldwide, and have created such a situation that common and less expensive antimicrobial agents are losing efficacy against microorganisms (Montefore *et al.,* 1989). Herbal drugs are now considered as an alternative in such situations (Sofowora, 1993). Now it is of great importance to explore effective treatments of microbes. Researchers are therefore are taking much attention in folk medicine in search of better drugs against microbial infections (Srinivasan *et al.,* 2001).

1.10. Family description

Rutaceae is a large family including both cultivated and wild plants comprising 150 genera and 1200 species, mostly distributed in the subtropical tropical and tropical regions of Mediterranean countries, North America, Australia, South East Asia, and South Africa (Hassan-Ud-Din & Ghazanfar, 1980). These are strongly aromatic having essential oils. In Pakistan this family is represented by 11 genera and 27 species, most of which have been naturalized and hybridized for ornamental, medicinal and edible purposes (Hassan-Ud-Din & Ghazanfar, 1980). In present study *Skimmia* (represented in Pakistan by a single species

Skimmia laureola) and *Zanthoxylum* (represented in Pakistan by a single species *Zanthoxylum armatum*) are selected for pharmacognostic and other studies studies.

Plants description

1.11. ***Skimmia laureola*** (DC.) Sieb. & Zucc. ex Walp.

Skimmia laureola (Fig. 1.1) is evergreen strong-scented shrub, up to 1 m tall, bearing grayish green dichotomous branches., leaves whorled in terminal clusters, with a citrus-leaf odour, glabrous, glossy, oblanceolate to lanceolate in appearance with entire margins and attenuate base. Adaxial surface is shiny with translucent oil glands. Midrib vein is slender, secondary veins hardly distinguishable. Flowers are sub sessile having greenish-white color. A calyx whorl is five lobed, obtuse and persistent. Petals oblong and white in color. Stamen 5, about as long as the petals, glabrous, absent in female flower. Ovary ovoid, 2-5-locular with oil glands, style short with small stigma. Fruit is ovoid berry, bright red (Hassan-Ud-Din & Ghazanfar, 1980).

Flowering period: April to late June.

Synonym: *Limonia laureola* DC.

Common name: Ner (English)

Local names: In India: Shimshar, patti, dhoop, kasturi, Pathra, Chumlani (Chauhan, 2006).

In Pakistan: Namer, Nazar Panra (Pashto) Patar, Barru (Kashmiri), Ner (Gujri), Nera (Hindko), Sheshar (Punjabi) (Shah & Khan, 2006).

1.11.1. Taxonomic position

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Sapindales

Family: Rutaceae

Genus: *Skimmia*

Species: *Skimmia laureola* (DC.) Siebold. & Zucc. ex Walp

1.11.2. Distribution in Pakistan

Skimmia laureola grows at an altitude of 1800-3000 meters, under shady conditions in forest. It is common in the Hazara region, Murree Hills and Kashmir, in Upper Swat and Shangla, (Hamayun *et al*., 2006; Hassan-Ud-Din & Ghazanfar, 1980). Upper and Lower Dir. In Nathia gally the plant is growing gregariously around the tract leading to Mukshpuri top.

1.11.3. Ethnobotanical uses

The leaves are used medicinally. When crushed, the leaves give a musky odour due to the presence of a poisonous compound skimnianine (Hassan-Ud-Din & Ghazanfar, 1980). Dried leaves smoke is used to ward off evils. Leaves are also used as coughs remedy (Joan *et al*, 2004). Leaves are commercially harvested and are used in food as flavouring agent, in traditional healing and cultural practices, being made into garlands and considered sacred (Bhattarai & Karki, 2006). The leaves of *S. laureola* are dried, pulverized to powder form, and given to livestock with wheat flour for treating anthelmintic diseases. Smoke of leaves and twig is considered demon repellent (Hamayun *et al*., 2006). The smoke of the dry leaves is used for nasal tract clearness. It is also used for cold, fever and headache treatment. The leaves are used as insecticides and pesticides (Qureshi *et al*., 2009).

1.12. ***Zanthoxylum armatum*** DC.

Zanthoxylum armatum (Fig. 1.2) is a small xerophytic tree or shrub. Leaflet blades usually with prickles. Leaves are compound, imparipinnate with 3-7 foliolate and pellucid-punctate. Petiole and rachis are winged. Leaflets are sessile, elliptic to ovate-lanceolate with crenate or entire margins. Flowers born axiliary, minute and polygamous. Calyx 6-8-acute lobed. Petals absent. Male flowers with 6-8 stamens with rudimentary ovary. Female flowers with 1-3 carpels. Ovary 1-3 locular. Fruit small drupes with red color, splitting into two when ripe. Seed are rounded and shining black (Hassan-Ud-Din & Ghazanfar, 1980).

Synonym: *Z. alatum* Roxb.

1.12.1. Taxonomic position of *Zanthoxylum armatum* DC

Kingdom: Plantae

Division: Tracheophyta

Class: Magnoliopsida

Order: Rutales

Family: Rutaceae

Genus: *Zanthoxylum*

Species: *armatum*- DC.

Botanical name: - *Zanthoxylum armatum* DC.

English names: Bamboo-Leaved Prickly Ash, Prickly Ash, Toothache Tree, Winged Prickly Ash, Wing leaf Prickly Ash.

Other names: Dambara (Pashtu) (Bakatullah *et al*., 2009), Dambrary, Tamur (Urdu) (Dar, 2003) Darman, Darmar, (Hindi) (Kalaivani *et al*., 2009), Ci Zhu Ye Hua Jiao, Qin Jiao, Zhu Ye Jiao (Chinies) (Kwon *et al*., 2011).

1.12.2. Distribution in Pakistan

Z. armatum prefers sunny or semi shady places for its growth. It grows wild in foothills starting from about 800 meter to 1800 meter in Malakand, Dir, Swat, Buner, Hazara, Muree hills and Rawalpindi (Shinwari *et al.,* 2006).

1.12.3. Ethnobotanical uses

Z. armatum is used locally as medicinal plants and fuel wood species. Fruits and seeds are edible and used as potherb species (Haq *et al*, 2010). The plant is used for Pneumonia and tick infestation (Sindhu *et al.*, 2010). Young shoots are used as toothbrush and useful for curing gum diseases. Fruit is used for toothache, dyspepsia, as a carminative and stomach ache. Seeds are used as condiment and flavoring agent. Wood is used to make walking sticks (Arshad & Ahmad, 2004; Abbasi *et al.*, 2010). Powdered fruit is mixed with *Mentha spp* and table salt, eaten with boiled egg for chest infection and digestive problems (Islam *et al*, 2006, Sher *et al*., 2011).



Fig. 1.1. *Skimmia laureola* growing in natural habitat.



Fig. 1.2. *Zanthoxylum armatum* growing in natural habitat.

CHAPTER-2

REVIEW OF LITERATURE

Skimmia laureola and *Zanthoxylum armatum* belonging to family Rutaceae were studied for their Pharmacognosy. The present review focused on various studies related to these plants.

2.1. Review of literature for *Skimmia laureola*

Review of research work done on *Skimmia laureola* is as follows

Atta-Ur-Rahman *et al*. (2002) isolated a new triterpene O-methyl cyclolaudenol and a new coumarin, (+)-7-methoxy-6-(2'R-methoxy-3'-hydroxy-3'-methyl butyl) and five previously known coumarins i.e. isogospherol, heraclenol, 5,8-dimethoxy coumarin-2H-1-benzopyran-2-one, 7-methoxy-6[2'-oxo-3'-methyl butyl] coumarin,, and (+)-ulopterol for the first time from *S. laureola*.

Shah *et al*. (2003) analyzed essential oil of *S. laureola* from Gulmarg region of Jammu & Kashmir in different seasons and reported variations in the content of linalyl acetate and linalool ranging from 37% to 64% and 4% to 28%, respectively.

Sultana *et al*. (2005) reported a new tyrosinase inhibitor fatty ester, (+)-skimmidiol and a new alkaloid ribaliprenyline from the aerial parts of *S. laureola*. The compound exhibited inhibitory activity against the enzyme tyrosinase.

Yousaf (2006) studied the chemical constituents of *S. laureola* and found triterpendiod (+)-taraxerone, three coumarins, two esters, and two quinoline alkaloids. These compounds, which were reported for the first time from this plant, have also shown antimicrobial activities against a number of human pathogenic bacteria and fungi. Atta-ur-Rahman, *et al*. (2006) isolated one new coumarin and three quinoline alkaloids from the aerial parts of *S. laureola*. Quinoline alkaloids were found to be inhibitors of acetylcholinesterase, which were found to be most effective for impaired cholinergic functions in Alzheimer's disease and related dementias. They also reported a dose-dependent spasmolytic activity on the isolated rabbit jejunum preparation, which were found dose dependent with EC_{50} = 0.1 mg/mL in spontaneous and EC_{50} = 0.4 mg/mL in K (+)-induced

contractions. Bhattarai & Karki (2006) reported that the percentage of essential oil contents in the fresh leaves of *S. laureola* in Nepal ranges from 0.93 to 1.12 percent.

Sultana & Khalid (2008) reported (+)-skimmilaureol and a new triterpene from the aerial parts of *S. laureola*. They also conducted enzymatic bioassays for the previously isolated compounds from this plant for the first time and found to be prolyl endopeptidase inhibitors. Sultana *et al*. (2008) isolated new fatty ester and a new triterpene from the aerial parts of *S. laureola*. Five known compounds previously isolated from this plant were also subjected to enzyme inhibition bioassays for the first time and were found to be acetyl-cholinesterase, prolyl endopeptidase and butyryl-cholinesterase inhibitors.

Donnell *et al*. (2009) isolated skimmone from *Skimmia laureola* and some other species. Hussain *et al*. (2009a) studied the chemistry of triterpenoid isolated from *S. laureola*. Kumari *et al*. (2009) reported the presence of Linalyl acetate and linalool from *S. laureola*.

2.2. Review of literature for other species of *Skimmia*

Reported research work on some other species of *Skimmia* is presented below

Kostova *et al*. (1996) isolated and established structure of skimmiwallichin, skimmi-wallin and a new cycloartanol type compound skimmiwallinin from *S. wallichii*.

Ponchia & Zanin (2000) studied in *vitro* propagation of *Skimmia japonica* and reported that low concentration of IBA was effective for root promotion, but acclimatization of the explants were difficult.

Fukuda *et al*. (2007) reported pollen dimorphism from *Skimmia japonica*, which is a morphologically very variable species and was extensively surveyed from its whole distribution area. The pollen grains were striate to striate-reticulate with sculpture exine, found not different in different varieties. However, the aperture number showed a geographical variation, ranging from 4 to 6 in *S. japonica*.

Sharma *et al*. (2008) isolated a new coumarin glucoside from the leaves of *Skimmia anquetelia* together with five other known coumarins. All these compounds showed strong antibacterial potential against *Pseudomonas syringae, Agrobacterium tumifaciens* and *Pactobacterium carotovorum*.

2.3. Review of literature for *Zanthoxylum armatum*

Previous work done on various aspects of *Z. armatum* is as follows

Yoshihito *et al*. (2000) reported 91 compounds from the essential oil constituents of *Z. armatum* fruit by GC and GC/MS analysis, cultivated in Nepal. They include linalool (62.2%), limonene (12.6%) and methyl cinnamate (8.8%). The minor compounds such as citronellal and citronellol were also detected and found responsible for characteristic odour of the oil.

Mahmood *et al*. (2005) carried out pharmacognostic study of the powdered miswak of *Z. armatum* and showed vessels, fibres and pith with few cells.

Singh *et al*. (2007) tested the allelopathic influence of aqueous extracts of leaf, bark, and fruit pulp of *Z. armatum* on some important winter field crops (*Triticum aestivum*, *Hordeum vulgare*, *Brassica campestris*, and *Lactuca culminaris*). Dose dependant significant effects of these bioassays were noticed on germination and growth of all the test crops.

Ramanujam *et al*. (2008) evaluated the effect of ethyl alcohol extract of *Zanthoxylum armatum* fruits in different tissues of *Heteropneustes fossilis* (a carnivorous air-breathing catfish) and found that Mg^{2+}- and Na^{+}, K^{+}-ATPase activity was both dose and time dependent. Mg^{2+}-ATPase activity was found more inhibitory in tissue extract of brain than muscles and gill in *in- vitro* studies. Mohini *et al*. (2008) analysed and reported 28 compounds, consisting mainly of oxygenated monoterpenes (75%) and monoterpenes (22%) from the essential oils of *Z. armatum* fruit. He also explored essential oil from the seeds of *Z. armatum* for larvicidal potential against three important species of mosquito vectors i.e. *Anopheles stephensi*, *Aedes aegypti,* and *Culex quinquefasciatus*. Among the three mosquito species tested, *C. quinquefasciatus* was found the most sensitive with LC_{50} = 49 ppm followed by *A. aegypti* (LC_{50} = 54 ppm) and *A. stephensi (*LC_{50} = 58 ppm). Linalool, a

major constituent (57%) of the oil, was failed to show any significant activity when tested alone.

Gilani *et al*. (2010a) conducted spasmolytic activity for crude methanolic extract of *Z. armatum* and found to be concentration-dependent relaxation of both spontaneous and high K^+ induced contractions in isolated rabbit jejunum preparations. The plant was confirmed when pre-treatment of the tissues with crude extract showed right shifted Ca^{++} concentration-response curves as caused by verapamil. It was suggested that this effect might be mediated through Ca^{++} antagonist mechanism. Verma & Khosa., (2010) investigated hepatoprotective activity of the ethanolic extract of *Z. armatum* leaf. The extract significantly reduced the level of serum glutamyl pyruvate transaminase, glutamyl oxalacetic acid transaminase, serum bilurubin and inflammation of liver. This study was also supported by histopathological studies of the liver. Batool *et al*. (2010) evaluated antioxidant activities of ethanolic extract of *Z. armatum* fruit. There was found significant inhibition against pro-oxidants induced Thiobarbituric acid reactive substances in the homogenates of rat liver, brain and kidney. The extracts also caused the scavenging of 2, 2-diphenlyl-1-picrylhydrazyl DPPH with IC_{50} = 4.56 ± 1.3 mg/ mL, and hydroxyl radicals, and exhibited Fe^{2+} chelating activity. Upadhyaya & Ashok (2010) reported four major chemical constituents i.e. 1, 8 – cineole, linalool, a- terpeniol and ß- cubebene from fruit oil of *Z. armatum* and foud that the essential oil of *Z. armatum* fruit has strong antioxidant and concentration dependent DPPH scavenging activity.

2.4. Review of literature for other species of *Zanthoxylum*

Some of the reported research work on other species of *Zanthoxylum* is presented below

Ngaine *et al*. (2000) examined 90% aqueous-ethanol extracts of leaves, roots and stem barks of *Z. leprieurii* and *Z. xanthoxyloides* for their antimycotic activities against nine fungal species. Results indicated inhibitory effect of these extracts to the *in-vitro* growth of test species of fungi.

Olila *et al*. (2001) investigated *Z. chalybeum* (seed) and isolated a pure crystalline alkaloid (27–135D) which was characterized as skimianine. Bio assay showed that *Z. chalybeum* extract had neither antifungal nor antibacterial activities.

Jullian *et al*. (2006) demonstrated the antiplasmodial activity of alkaloid fraction of *Z. rhoifolium* bark. Further fractionation of extract has yielded Seven benzophenanthridine alkaloids were obtained on further fractionation, of which five compounds i.e., oxyavicine, oxynitidine, fagaridine, avicine and nitidine were evaluated for antimalarial activity. Of these nitidine was found to be the most active against *Plasmodium falciparum*. Kusuda (2006) reported that polymeric compound (proanthocyanidin) isolated from *Z. piperitum* fruit (Rutaceae) suppressed beta-lactamase activity and chiefly decreased the bacterial cell membrane stability of *Staphylococcus aureus*, which was found methicillin-resistant.

Penali *et al*. (2007) isolated three alkaloids and two amides from the stem bark of *Z. rubescens* (Rutaceae). The anti-Plasmodium activities of the tested alkaloids of *Z. rubescen*s were low, hence discouraging the use of this plant as antimalarial drug. Jun *et al*. (2007) isolated "auraptene" (apoptogenic ingredient) from methylene chloride extract of *Z. schinifolium* and found that this compound caused stress-mediated activation of caspase-12 and -8 of endoplasmic reticulum and subsequent apoptotic events including FLICE inhibitory protein cleavage, c-Jun N-terminal Kinase (JNK) activation, release of mitochondrial cytochrome c, degradation of poly (ADP-ribose) polymerase, caspase-9 and -3 activation, and apoptotic DNA fragmentation in a dose-dependent manner. Pachon *et al*. (2007) isolated a new benzo- phenanthridine-type alkaloid, Rutaceline, from the stem bark powder of *Z. madagascariense* and evaluated it for antiproliferative capacity on the human colorectal adenocarcinoma (Caco-2) and the African green monkey kidney (Vero) cell lines. The 50% inhibition of cell growth (IC_{50}) obtained after 24 h incubation was similar for both cells lines. Rutaceline as revealed by agarose gel electrophoresis also induced DNA fragmentation, and a dose-dependent clastogenic effect in both cell lines. Silva *et al*. (2007) tested the essential oil of *Z. rhoifolium* for cytotoxic effects against human cervical carcinoma, human lung carcinoma, human colon adenocarcinoma, Vero (monkey kidney) cell lines and mice macrophages. Some of the terpenes of its essential oil (caryophyllene, α-

humulene, α-pinene, myrcene and linalool) were also tested to verify their possible influence in cytotoxic activity against tumoral cells.

Donnell *et al*. (2009) reported xanthoxylone obtained from *Z. rhetsa* has ketone in the seven-position and it is possible that 3-ketone form of this compound might be present in this plant but has not yet been explored. Bhattacharya & Zaman (2009) extracted essential oils from *Z. nitidum* fruits and leaves which were analyzed by GC-MS. A total of 17 components including 75% monoterpenes, 12.5% sesquiterpenes and 12.5% straight chain hydrocarbons were reported from fruit essential oils, whereas 16 components including 60 % monoterpenes, 13.3% sesquiterpenes, and 26.7% straight chain hydrocarbons were detected in leaf essential oils.

Ngoumfo (2010) isolated three new compounds from the fruits and root of *Z. leprieuriii*. They also evaluated the chloroform extract of the fruits for the brine-shrimp lethality test and found modest cytotoxicity with LD_{50} of 13.1µg/mL. Isolated compounds were also found to be moderately active against lung carcinoma cells, colorectal adenocarcinoma cells and normal cells with IC_{50} values ranging from 27-77mM.

Review of literature on *Skimmia loureola* and *Zanthoxylum armatum* showed that no work has been done on these plants as proposed in the present study.

2.5. Review of literature for some other members of Family Rutaceae.

Venkatachalam & Jabanesan (2001) studied repellent activity of *Ferronia elephantum* leaves methanolic extract against *Aedes aegypti*. There was found 100% repellent activity at dose of 1.0 and 2.5 mg/cm^2 for the period of 2.14±0.16 h and 4.00±0.24 h, respectively. Lis-Balchin *et al*. (2001) evaluated the action of essential oils from two *Agathosma betulina* and *A. crenulata* from the Cape region of South Africa. At high concentration, the oils had an initial spasmogenic activity followed by spasmolysis. Kim *et al*. (2001) reported that hesperetin isolated from *Citrus junos* inhibited the influenza A virus.

Sivagnaname & Kalyanasundaram (2004) evaluated the leaf methanolic extracts of *Atlantia monophylla* for mosquitocidal activity at immature stages of three mosquito species, *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* under lab conditions. Larvae of *A. stephensi* and *C. quinquefasciatus* were found more susceptible, with LC_{50} values of

0.14 mg/l and 0.05 mg/l, respectively. Cvetni & Vladimir-Kne (2004) tested ethanolic extract of seed and pulp of grapefruit (*Citrus paradisi*) seed and pulp against 20 bacterial and 10 yeast strains. The extract exhibited antimicrobial effect against tested bacteria and yeasts. They also determined 3.92% of total polyphenols and 0.11% of flavonoids spectrometrically in crude ethanolic extract.

Wansi *et al*. (2006) isolated two alkaloid derivative, oriciacridone A and B from the stem bark of *Oriciopsis glaberrima*. The extract exhibited in vitro significant antimicrobial activity against a range of micro-organisms.

Moolla et *al*. (2007) studied antimicrobial and anti-oxidant activities of the extracts of 17 indigenous *Agathosma* species (19 samples) in order to confirm the traditional usage of *Agathosma* species in healing of various diseases. *Agathosma ovata* showed the best antimicrobial activity against *S. aureus* and *B. cereus* with MIC values of 0.16 mg/ml and 0.13 mg/ml, respectively. Most of the extracts also exhibited moderate to poor antioxidant activity using DPPH assay.

Kuete *et al*. (2008) studied stem bark methanolic extract together with three alkaloids of *Teclea afzelii* for their antimicrobial potential against Gram-positive and negative bacterial strains, fungi and *Mycobacterium smegmatis.* The lowest MIC value (19.53µg/ml) of the extract was recorded for *Escherichia coli*, *Bacillus subtilis* and *Microsporum audorium*. Kamkaen *et al*. (2008) distilled out volatile oils from the fresh leaves of *Aegle marmelos*, *Toddalia asiatica* and *Zanthoxylum budrunga* using a Clevenger apparatus. The resulting essential oils were analysed by GC/MS and tested for antimicrobial activity using *Bacillus subtilis*, *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The main constituents of *A. marmelos* were sylvestrene (82.49%), sabinene (8.93%) and germacrene D (3.54%), whilst those of *T. asiatica* were tricyclene (12.75%), 9-*epi*-(*E*)-caryophyllene (10.95%) and (*E*)-nerolidol (8.88%). The main constituents of *Z. budrunga* were limonene (31.09%), terpinen-4-ol (13.94%) and sabinene (9.13%). The essential oils exhibited antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* with *Z. budrunga* showing the strongest activity against *S. aureus* while A. *marmelos* showed the strongest activity against *B. subtilis*

Ilongo *et al*. (2009) tested the extract of fruit pulp of *Limonia acidissima* for glycaemia and level of antioxidant enzymes in Alloxan induced diabetic rats. The extract significantly (P<0.01) reduced the blood glucose level in Alloxan induced hyperglycemia. There was also found significant (P<0.01) decrease in per oxidation products of blood serum. The extract was also found to enhance antioxidant enzymes activities such as SOD, CAT r in the blood serum of diabetic animals. Kalaivani *et al*. (2009) conducted hepatoprotective activity of *Aegle marmelos*. The aqueous and methanolic leaf extracts showed moderate activity as compared to control. The results confirmed the traditional uses of this plant as a potential hepatoprotective agent. Muthumani *et al*. (2009) reported that column extracts of the plant *Murraya koenigi* significantly reduced the number of cancer cells and tumor weight both in *in-vitro* and *in-vivo* condition in male BALB/c mice. In vitro studies showed that all the column extracts exhibited moderate activity. Zanatta *et al*. (2009) studied the gastroprotective properties of alkaloidal extract and 2-phenylquinoline obtained from *Galipea longiflora* bark at a dose of 50 mg/kg, The extract significantly inhibited ulcerative lesions. The possible mechanisms concerned in anti-ulcer activity might be related to a decrease in gastric secretion and increase in gastric mucus content and of involvement of nitric oxide in the gastroprotector mechanisms.

Campelo *et al*. (2011) studied the effect of essential oil of *Citrus limon* on lipid peroxidation level, nitrite content, glutathione reduced (GSH) concentration, and activities of antioxidant enzymes like superoxide dismutase, catalase, and glutathione peroxidase in mice hippocampus. The lipid peroxidation level and nitrite content were found to be significantly reduced but increase was recorded in the GSH levels and activities of the SOD, catalase, and GPx in the tested animals treated with oils.

2.6. Phytosociology

Phytosociology simply explains the association of plant species in communities (Ewald, 2003). Plants grow well in various localities having similar environmental conditions. The cultivation and collection of medicinal plants may change with change in economic situation in an area (Evans, 2002). Similarly physicochemical analyses are used as important management tool to establish the relationship of elemental composition between

plants and soils (Itoh *et al.,* 2007). Following are some of the studies in Pakistan related to plant association among themselves and with environmental factors.

Sher & Khan (2007) reported vegetation of Chagharzai Valley, (District Buner), Pakistan. The study comprised of 222 plant species of 88 families, in which dicot were represented by 78 families, monocot by 7 families and gymnosperms by three families. The biological spectrum showed 38.56% therophytes and 18.38% nanophanerophytes. Leaf spectra of plants consisted of 54.70% microphylls, 19.28% mesophylls 13.00% nanophylls 8.96%leptophylls and 4.03% megaphylls.

Ahmad *et al*. (2008) carried out phytosociological study and reported *Acacia modesta* as the most abundant species in Soone Valley, Punjab, Pakistan. They selected Kufri site of the valley on the basis of some ecological features like soil type, topography and the nature of existing disturbances. *Propsopis juliflora* formed a mono-species stands with complete absence of *Dalbergia sissoo* in this area. *Olea ferruginea* was found in a good association with *Acacia modesta* at higher altitude. Throughout the examined site, *Dodonaea viscosa* and *Justicia adhatoda* were found resistant grazing and fuel needs, therefore occurred very abundantly. Perveen *et al*. (2008) documented the floristic and ecological data for the threatened habitats of Dureji Game reserve. Density, relative density, cover, relative cover, frequency and relative frequency were investigated along with the structure and composition of vegetation. Qureshi (2008) recognized five plant communities in protected forest in Sawan valley of Nara Desert. These were 1) *Calligonum-Dipterygium-Salvadora* in desert; 2) *Phragmites-Typha-Saccharum* in wetland; 3) *Desmostachya-Brachiaria-Cynodon* in agriculture habitat; 4) *Saccharum- Pluchea-Typha* in marshland and 5) *Salvadora-Desmostachya-Posopis* in protected forest. Density, frequency and Cover were also recorded. *Euphorbia prostrata* was found the most frequent species present in all the habitats, followed by *Alhagi maurorum, Desmostachya. Saccharum spontaneum* was reported in 4 habitats. Qureshi and Bhatti (2008) showed differences in species richness in five habitats of Nara Desert, (Pakistan). Flat habitat of the studied area was found with highest species richness of 77.24%. The vegetation over major area was characterized by xerophytes. Wahab *et al*. (2008) carried out phytosociological sampling, age, structure and growth rates in 5 different habitats of District Dangam, (Afghanistan). On the basis of

Importance Value Index of tree species and floristic composition, two monospecific and one bispecific communities were recognized in the study area. It was observed that age and growth rates in *Picea smithiana* were not significantly correlated. Lack of tree seedlings indicates poor regeneration status of the forests.

Abbas *et al*. (2009) conducted a phytosociological analysis of a habitat, spread over some 5,000 km^2 in Himalayan grey goral (*Naemorhedus goral*), inhibited by almost half of the endangered species. Study revealed high overall species diversity in different stands. *Pinus roxburghii* was indicator species of habitat. Ahmed *et al*. (2009a) recognized 10 plant communities in forests dominated by *Olea ferruginea* in lower Dir District of Pakistan. Most of these communities showed same floristic composition with varied numerical values, though no significant relation was found between elevation/density, density/basal area and elevation/basal area. Ahmed *et al*. (2009b) analyzed the floristic data of Abbottabad roadsides vegetation and soil, using multivariate analysis techniques. Five major communities were recognised in the study area. They also investigated the vegetation structure and its relationships to selected ecological factors. Siddiqui *et al*. (2009) carried out phytosociological attributes of *Pinus roxburghii* in Hindu Kush and Lesser Himalayan range of Pakistan. Quantitative attributes viz. relative density, relative frequency and relative basal area and absolute values were calculated. Pine seedlings were recorded in nine stands showing regeneration. The common angiospermic species were found in association with Chir pine where *Dodonaea viscosa*, *Punica granatum*, *Erodium cicutarium*, *Medicago denticulata* and *Vicia sativa*.

Ahmad *et al*. (2010) identified four communities which differ mainly on the basis of their ecological amplitudes along the road verges of Islamabad- Lahore Motorway (M-2). The data will be used as initial source which can be used to study the successional changes in future with reference to different environmental conditions. Khan *et al*. (2010) conducted Phytosociological attributes of *Quercus* forests in Hidukush range Chitral, Pakistan. A total of eight stands were sampled at elevation ranging from 1770 −2370 m. Five stands were found with pure vegetation of *Quercus baloot*, while at 3 locations at high altitude, *Q. dilatata* was found co-dominant with maximum water holding capacity and high soil moisture contents. It was also observed that *Quercus baloot* and *Q. dilatata* were at the risk

of elimination due to anthropogenic activities. Sher & Al-Yemeni (2010) reported five herbs-shrubs-trees communities and one meadow community from various parts of Malam Jabba Valley, (Swat). These communities comprises of economically important plant (and to quantify the availability of species and to suggest suitable method for their production and conservation).

The review of literature reveals that no such autecological studies so far have been done for *Skimmia laureola* and *Zanthoxylum armatum*; consequently it is highly advisable to study their association with other species and related ecological conditions especially soil composition, which will be helpful for their cultivation and commercialization in future.

2.7. Ethnobotany

Ethnobotany is the knowledge of plants usage by the native people, which provide opportunities for better understanding of the traditional uses, make improved use of the available resources, find new ways of transfering this knowledge to future generations and explore new pharmaceuticals for biomedicine (Tor-Anyiin *et al*., 2003; Kufer *et al*., 2005). Some of the ethnobotanical studies reported from Pakistan are,

Dar (2003) explored ethnobotanical information of Lawat and its allied areas (District Muzzaffar abad and reported 52 species consisting of 2 Gymnospermic families (3 species) and 35 angiospermic families (49 species). The plants were used medicinally and for other purposes. The medicinal plants were used singly or in mixtures by the local inhabitants. Due to unplanned exploitation, most of the medicinal plants have become endangered.

Wazir *et al*. (2004) carried out ethnobotanical study of Chapursan Valley (Gilgit) comprises of 41 species of wild herbs, shrubs and tree, belonging to 29 families. These plant species were found to be used as medicinal by the inhabitants in the valley.

Iqbal & Hamayun (2005) carried out ethnobotanicof Malam Jabba (Swat) and reported a total of 187 species belonging to 75 families. These plants were categorised as agricultural tool making, agro forestry based plants, honey bee attracting, ornamental, medicinal plants, vegetable and pot herb, plants yielding edible fruits, fencing and hedge

plants, thatching and sheltering, poisonous and timber yielding plants. Due to high biotic pressure, many important medicinal plants like *Acorus calamus*, *Paeonia emodi*, *Podophylum hexandrum* and *Valeriana jatamansi* were at the edge of extinction. Hamayun *et al.* (2005) conducted ethnomedicinal study in Hindukush-Himalayan valleys of Utror and Gabral (District Swat) comprises of 36 commonly used folk medicinal recipes of the area. Local medicinal plants collection methods and their processing for market were also explored. It was found that due to improper collection, garbling, storage and packing techniques, each year huge amount of medicinal plants were lost. Shah & Khan (2006) reported 80 plant species belonging to 49 families during ethnobotanical studies in Siran Valley (District Mansehra, Pakistan). They arrange all the plants alphabetically by botanical names, followed by local name, family, part used and ethnomedicinal uses. Ahmad *et al*. (2006a) conducted ethnobotanical study of Booni Valley, (Chatral, Pakistan). 75 species from 43 families were reported as medicinal plants. Out of the 75 recorded species, 70 were dicot (40 families), 2 each of monocots and gymnosperms and 1 of fungi. Local names of these collected medicinal plants were also recorded.

Hazrat *et al*. (2007) reported fifty-one local uses of 14 genera of 39 species belonging to family Ranunculaceae of District Dir. The local medicinal uses include anthelmintic, anticancer, anti-inflammatory, aphrodisiac, asthma, carminative, cardiotonic, cough, diuretic, febrifuges, painkiller, tonic, stomachache, dyspepsia, jaundice, leprosy, ulcers and vomiting etc. Ahmad (2007) reported 81 plants belonging to 44 families from in and around Lahore- Islamabad motor way, used locally for curing various ailments like dysentery, fever, snakebite, skin diseases and jaundice etc. Hussain *et al*. (2007) recorded 111 species of 46 families as plant resources used traditionally in Mastuj, (District Chitral, Pakistan). The study revealed 90 fodders, 52 medicinal, 40 firewood, 19 vegetable, 15 thatching/fencing, 13 timber and 9 fruit species. Two species, *Haloxylon griffithii* and *Vaccaria pyramidata* were found soap making species while 4 species were used in basketry, 4 species were preferred as furniture wood species and 8 species were agricultural tool making species.

Khan & Khatoon (2008) conducted ethnobotanical servey of Haramosh and Bugrote Valleys Gilgit, Northern Areas of Pakistan, comprises of 48 species of trees and shrubs.

These species were used in locally as agricultural tools, medicine, shelter and fuel. The population of the region was primarily dependent upon plant resources for their domestic needs. Hussain et *al*. (2008c) reported 45 plants species from Hattar, District Haripur, (KPK, Pakistan), locally used by the people. The plants were categorised as 17 perennials/biannual species, 20 were spring species, while 8 were autumn species. Plant specimens were collected, identified and mounted on herbarium sheets, deposited in the Herbarium, Qarshi Herb Centre, Hattar, (Haripur). Ilahi (2008) explored the ethnobotanical importance and regeneration problems of herbs in Kohat Region of Pakistan. Selected herbs were grown in the Medicinal plants farm of the Kohat University for preservation.

Jan *et al*. (2009) collected ethnobotanical data of four gymnosperms families i.e. Cupressaceae, Ephedraceae, Pinaceae and Taxaceae. A total of eleven species were reported from Dir Kohistan Valleys, among which *Abies pindrow, Cedrus deodara, Pinus roxburgii, P. wallichiana,* and *Taxus wallichiana* were the famous gymnosperms of Dir Kohistan Valley, not used as a source of timber only but also used as fuel and for medicinal purposes. Qureshi *et al*. (2009) conducted plant Survey of Gilgit District and surrounding areas of the Northern Pakistan and reported 27 range land species. The researchers concluded that there is need for sustainable development and conservation strategies in the area taking into consideration of local needs, perspectives and economic development opportunities. Ahmad *et al*. (2009a) recorded traditionally uses of indigenous plants for controlling and curing diabetic in District Attock. Fabaceae with five species were the largest family used for this purpose, followed by Poaceae (4 *Spp*.) and Liliaceae (3 *Spp*.). As investigated from the local inhabitants, these plants were grouped into 29 phytotherapies clases. These traditional recipes include extracts, leaves, fruits, powders, seeds, vegetables and herbal mixtures. Ali & Qaiser (2009) reported a total of 83 wild taxa, being used locally in Chitral Valley for various purposes. Most traditional recipes were prepared form the root in the form of decoction from freshly collected plant parts. Barkatullah *et al*. (2009) reported 100 plants in an ethnobotanical study of Charkotli Hills, Batkhela, District Malakand, Pakistan. The reported species were classified as medicinal, fruit and edible, furniture, fodder or forage, fuel, thatching, hedge or fencing timber, poisonous, ketchup, fixed oil yieldings, miswak yielding species etc. Sher & Hussain (2009) conducted an ethnobotanical study on the

medicinal and economic plants of Malam Jabba, District Swat, comprising of 50 species of plants belonging to 33 families. They documented an ethnobotanical inventory composed of medicinal plants, conservation status of the medicinal plants, marketing and market chain starting from collectors to consumers.

Abassi *et al*. (2010) conducted survey in 30 remote sites of Khyber Pukhtunkhwa and reported seventy-five medications related to skin diseases and cosmetics. These recipes were applied topically as well as ingested orally with water, milk, oil, eggs, ghee and butter. Badshah & Farrukh (2010) reported 41 plant species commonly used by local people in District Tank for curing various diseases. Thirteen of them were frequently mentioned, of which. *Citrullus colocynthis, Withania coagulans* and *Fagonia cretica* widely used for treating fever, rheumatism, diarrhoea, asthma and piles. Ajaib *et al*. (2010) reported the ethnobotanical profile of shrubs from District Kotli, (Azad Jammu & Kashmir, Pakistan). Thirty eight species of 36 genera belonging to 25 families were used traditionally by local inhabitants as medicinal, fuel, fodder/forage, shelter and agricultural tools species. Hazrat *et al*. (2010) enlisted the medicinal plants wealth in Usherai Valley, (District Dir). In total 50 species, belonging to 32 families of wild herbs, shrubs and trees were found to be used as medicinal plants by the inhabitants of the valley. Qasim *et al*. (2010) reported 48 wild coastal plant species belonging 26 families from Hub, Lasbela Districts, (Balochistan). These include 56% fodder species, 22% medicinal species, 5% food species, 5% household utensils species, 3% species for increasing milk production in cattle and 8 % miscellaneous uses species.

Sher & Al-Yemeni (2011) documented economically important plant communities in different parts of Malam Jabba (Swat). A total of 90 species were recorded having medicinal, food, fruit, fodder, timber, fuel and many other uses.

Review of literature reveals a lot of ethnobotanical references about traditional usage of plants. Therefore detailed ethnobotanical studies about the research plants were therefore carried out in various localities.

2.8. Pharmacognostic studies

Pharmacognostic evaluation helps in identification and authentication of the plant material. Correct identification and quality assurance of the starting material is a necessary prerequisite step for quality herbal drugs preparation. The pharmacognostic study of the herbal drugs assists in standardization for quality, purity and sample identification (Anonymous, 1998; Singh *et al*., 2010). Standardization of herbal drugs is done by studying the plants microscopic and macroscopic features, ash values, extractive values, fluorescence studies and secondary metabolites both qualitatively and quantitatively.

Following are the some of the work done regarding macroscopic and microscopic features of herbal drugs.

Maiti *et al*. (2002) studied ten species of the family Solanaceae applying techniques of pharmacognosy and histochemistry for the differentiation and identification of each of the species. Various types of stomata, trichomes, glands, crystals and palisade parenchyma were the anatomical features used for differentiating these species.

Bo *et al*. (2007) studied shape of cells, pattern of anticlinal walls, cuticular membrane and wax ornamentation of nineteen leaf epidermis samples from six species of *Apios* and two of *Cochlianthus* using both light microscopy and scanning electron microscopy. These leaf epidermal characters of these two closely related genera were usually stable within species and thus of the immense significance in distinguishing different genera. Geng *et al*. (2007) investigated macroscopic and powder characteristics of *Ginkgo* leaf in different areas of Gansu province (China) and provide scientific base for its identification.

Thomas *et al*. (2008) pharmacognostically evaluated of ripe fruit of *Averrhoa carambola* and stated that the presence of trichomes and large oval lysigenous oil cavities were the distinguishing anatomical markers; can be used for its proper identification. Bhagwat (2008) subjected *Euphorbia hirta* (Family: Euphorbiaceae) for pharmacognostic study. Venkatesh *et al*. (2008) evaluated *Dodonaea viscosa* pharmacognostically in view of its medicinal importance, taxonomic misunderstanding, morphological and microscopic characters as referential information for identification of this crude drug. Ismaeel & Sultana

(2008) subjected samples of *Morinda umbellata* (Family *Rubiaceae*) leaf, stem and root to anatomical investigations for correct identification.

Sharma *et al*. (2009) demonstrated macroscopic, microscopic evaluation in terms of organoleptic, microscopic and physical parameters of various parts of *Holoptelea integrifolia*. Kalidass *et al*. (2009) conducted microscopic evaluation including anatomy and powder drug study of root and stem of *Ichnocarpus frutescens*. Nirmal *et al*. (2009) carried out macroscopical and microscpical features of *Sesbania sesban* wood to pharmacognostically standardize this important drug. Nikam *et al*. (2009) carried out pharmacognostic study of the leaves of *Sesbania sesban* for various parameters like morphology, microscopy and other salient diagnostic features in order to establish pharmacognostic standards.

Radhika & Begum (2010) carried out organoleptic, microscopic and physical evaluation of the leaves of *Bixa orellana*. Gupta *et al*. (2010) conducted pharmacognostical investigation on the leaf of *Acacia leucophloea*. The study included macroscopical and microscopical characters, determination of leaf constants. Pande & Pathak (2010) carried out pharmacognostic evaluation of the roots of *Mimosa pudica* Linn. (Mimosaceae) including examinations of morphological and microscopic characters and powder analysis. Shah *et al*. (2010) carried out the detailed pharmacognostic study including macroscopy and microscopy of *Lagenaria siceraria* leaf in order to standardize some features, could be useful for future experimental studies. Singh *et al*. (2010) carried out pharmacognostic evaluation of *Trichosanthes dioica* laef in fresh and powder form to determine its macro and microscopical features in order to standardization for quality, purity and sample identification. Singhal *et al*. (2010) studied the microscopic, macroscopic and anatomical characters of *Geniosporum prostratum* that enable the identification for future investigation and to form an important aspect of drug studies. Najafi & Deokule (2010) reported detailed pharmacognostic account of *Tylophora dalzellii* which include macroscopic and microscopic characters for the correct botanical identification of the drug. Chirikova, *et al*. (2010) carried out pharmacognostic study of the aerial parts of *Scutellaria baicalensis* (Lamiaceae). Nayak *et al*. (2010) carried out detailed pharmacognostic study including

macroscopy, microscopy, anatomy and powder drug study of *Jatropha curcas* leaf, to lay down standards for future reference.

Balakrishnan *et al*. (2011) reported macroscopic, microscopic and powder drug study of *Amaranthus spinosus* (Amaranthaceae) in order to develop the diagnostic parameters for quality control of leaf and stem to distinguish the drug from its other species. Bisht *et al*. (2011) reported a comparative botanical analysis as biomarker for *Swertia chirayita* along with two allied species of *Swertia*. In a pharmacognostic study Subha *et al*. (2011) worked out organoleptic, anatomical and powder microscopic analysis of *Acorus calamus*. Chayarop *et al*. (2011), pharmacognostically studied the fresh leaves of *Pseuderanthemum palatiferum*. The microscopic characteristics showed the arrangement of palisade cells into two layers, and the presence of multicellular trichomes with a warty wall in the upper epidermis. Kaneria & Chanda (2011) provided pharmacognostical, physicochemical and phytochemical details of the leaves of the *Psidium guajava* which are useful in laying down standardization and pharmacopoeial parameters of this plant. Zunjar *et al*. (2011) studied the microscopic evaluation of leaves of *Carica papaya* to establish the salient diagnostic features for the leaf. The leaf shows abundant sphaeraphides and rhomboidal calcium oxalate crystals. These results could be beneficial for setting some diagnostic indices for identification and preparation of the monograph of this plant.

2.8.1. Ashing

Ashing is also an important means for detection of adulteration in herbal drugs. Different types of ash values are used for detection of crude drugs like total ash, acid insoluble ash and water soluble ash (Jarald & Jarald, 2007). A lot of references available for ash values determination to authenticate the crude drugs, some of which are Sugumaran & Vetrichelvan, 2008 (Leaves of *Bauhinia purpurea* Linn.), Hussain *et al*. 2009b (different parts of *Piper sarmentosum*), Kalidass *et al*., 2009 (root and stem of *Ichnocarpus frutescens* Linn.), Nikam *et al*., 2009 (leaves of *Sesbania sesban*), Gupta *et al*., 2010 (leaf of *Acacia leucophloea*), Shah *et al*., 2010 (*Lagenaria siceraria*), Mathur *et al*., 2010 (leaves of *Amaranthus spinosus*), Jain *et al*., 2010 (leaves of *Lawsonia inermis*) Singhal *et al*., 2010 (leaf and stem of *Geniosporum prostratum*), Najafi & Deokule, 2010 (*Tylophora dalzellii*), Nayak *et al*., 2010 (leaf of *Jatropha curcas*), Balakrishnan *et al*., 2011 (*Amaranthus*

spinosus), Bisht *et al*., 2011 (*Swertia chirayita*), Subha *et al*., 2011 (*Acorus calamus*), Hussain *et al*., 2011a (*Hygrophila auriculata*), Kumar *et al*., 2011 (*Crocus sativus*) and Zunjar *et al*., 2011 (leaves of *Carica papaya*)

2.8.2. Extractive values

Extractive values play an imperative role in the assessment of the crude drug. Extraction with different solvent assures various types of adulteration and exhausted materials (Jarald & Jarald, 2007). Here is a brief account of the recent work done in this regard. Thomas *et al*., 2008 (fruit of *Averrhoa carambola*), Bhagwat *et al*., 2008 (leaves of *Euphorbia hirta*), Sugumaran & Vetrichelvan, 2008 (Leaves of *Bauhinia purpurea*), Kalidass *et al*., 2009 (root and stem of *Ichnocarpus frutescens*), Nikam *et al*., 2009 (leaves of *Sesbana sesban*), Pande & Pathak, 2010 (roots of *Mimosa pudica*), Gupta *et al*., 2010 (leaf of *Acacia leucophloea*), Shah *et al*., 2010 (*Lagenaria siceraria*), Mathur *et al*., 2010 (leaves of *Amaranthus spinosus*), Jain *et al*., 2010 (leaves of *Lawsonia inermis*), Singhal *et al*., 2010 (leaf and stem of *Geniosporum prostratum*), Nayak *et al*., 2010 (leaf of *Jatropha curcas*), Balakrishnan *et al*., 2011 (*Amaranthus spinosus*), Subha *et al*., 2011 (*Acorus calamus*), Hussain *et al*., 2011a (*Hygrophila auriculata*), Kumar *et al*., 2011 (*Crocus sativus*) and Zunjar *et al*., 2011 (leaves of *Carica papaya*).

2.8.3. Fluorescence study

Fluorescence phenomenon exhibited by plant extract is because of different chemical composition. Some constituents of the extract show fluorescence in the visible range in daylight. The ultra violet radiation produces characteristic fluorescence in many natural substances which are not visible in daylight (Ansari, 2006; Reddy & Chaturvedi, 2010). *Morinda umbellatai* (Ismaeel & Sultana, 2008), root and stem of *Ichnocarpus frutescens* (Kalidass *et al*., 2009), *Holoptelea integrifolia* (Kumar & Kiladi, 2009), leaves of *Amaranthus spinosus* (Mathur *et al*., 2010), leaves of *Catunaregum spinosa* (Shrivastava & Leelavathi, 2010), *Tylophora dalzellii* (Najafi & Deokule, 2010), *Lagenaria siceraria* (Shah *et al*., 2010), *Acorus calamus* (Subha *et al*., 2011), *Hygrophila auriculata* (Hussain *et al*., 2011a) and *Crocus sativus* (Kum ar *et al*., 2011) are some of the crude drugs which have been tried to standardize through fluorescence studies.

2.8.4. Preliminary phytochemical analysis

Plant secondary metabolites are vital, as they are considered to be an imperative source of drugs since ancient times and now a day about 50% of the practical drugs are derived from natural sources (Wang *et al*., 2008a). Chemical evaluation of drug for secondary metabolites includes both qualitative and quantitative tests and chemical assays etc. of the plant material (Rangari, 2002). Plant secondary metabolites, including free carbohydrates, polysaccharides, alkaloids, flavonoids, amino acids, phenolic acids, tannins, anthocyanins, photosynthetic pigments, lipids, and triterpenoids compounds can be detected through preliminary phytochemical analysis, which are of common practice in the field of pharmacognosy. Some of the studies in this respect are given below.

Maiti *et al*. (2002) conducted histochemical tests for proteins, tannin, and alkaloids in ten species of the family Solanaceae.

Okwu, & Josiah (2006) analyzed chemical composition, vitamins and minerals in *Aspilia africana* and *Bryophyllum pinnatum*.

Geng *et al*. (2007) determined flavonoids content in Genko leaf in different areas of Gansu province, in order to rationalize the utilization of Ginkgo in Gansu province. Magaji *et al*. (2007) chemically investigated the leaves, stem bark and root bark of *Securinega virosa* for preliminary phytochemical analysis and found that the three extracts contained almost similar phytochemical composition. Audu *et al*. (2007) carriedout phytochemical screening of the ethanolic extract of *Lophira lanceolata* leaf and detected the presence of flavonoids, anthraquinones, carbohydrate, glycoside, phenols, saponin steroid, tannin and free reducing sugar.

Thomas *et al*. (2008) carried out preliminary phytochemical analysis of the fruit of *Averrhoa carambola* and detected alkaloids, saponins, tannins and flavonoids. Bhagwat *et al*. (2008) screened out different extracts of *Euphorbia hirta* for its preliminary phytochemical analysis and quantitative estimation of total phenolic and flavonoid content. Ismaeel & Sultana (2008) subjected extract samples of *Morinda umbellata* for preliminary phytochemical screening including TLC, paper chromatography and the Rf values determination.

Kumar & Kiladi (2009) carried out preliminary phytochemical screenings of the leaf and stem extracts of *Holoptelea integrifolia* (Ulmaceae). Patra *et al*. (2009a) detected alkaloids, proteins, steroids, flavonoids, tannins, fats & oils, mucilage and organic acids in the leaf of *Hygrophila spinosa* (Acanthaceae). Janapati *et al*. (2009) carried out phytochemical studies and showed the presence of alkaloids, flavonoids, flavanones, tannins, terpenoids, amino acids and carbohydrates.

Chirikova *et al*. (2010) reported free carbohydrates, polysaccharides, amino acids, alkaloids, organic acids, phenolic acids, anthocyanins, flavonoids, tannins, lipids, photosynthetic pigments, and triterpene from the aerial parts of *Scutellaria baicalensis* (Lamiaceae). Koche *et al*. (2010) performed the preliminary phytochemical analysis from eight ethnomedicinal plants, *Ocimum sanctum, Hyptis suaveolens, Croton tiglium, Physalis minima, Tephrosia villosa, Malachra capitata, Cleome viscosa,* and *Galphimia glauca* from Akola District, India. Shrivastava & Leelavathi (2010) carried out preliminary phytochemical investigation of *Catunaregum spinosa* and detected the presence of carbohydrates, phytosterols, glycosides, saponins, triterpenoids, fixed oils & fats and phenolic acids/tannins.

Chayarop *et al*. (2011) carried out phytochemical screening of *Pseuderanthemum palatiferum* leaves and detected flavonoids, phenolic compounds, unsaturated lactone rings and steroid nuclei. Flavonoids by TLC fingerprint using various solvent systems. Hussain *et al*. (2011a) carried out preliminary phytochemical screening and showed the presence of alkaloids, tannins, flavonoids, steroids, triterpenoids and saponins in *Hygrophila auriculata*. Kalyan *et al*. (2011) subjected the ethanolic extract of *Clitoria ternatea* seeds for preliminary phytochemical investigations which revealed the presence of various phytoconstituents like carbohydrates, proteins, alkaloids, sterols, glycosides, tannins, saponins, phenolic compounds and flavonoids. Kumar *et al*. (2011) detected alkaloids, flavonoids, carbohydrate, glycosides, tannins, terpeniods, phenol, steroids and saponins in different petal extracts of *Crocus sativus* using various histochemical tests.

These studies revealed that no such pharmacognostic work has been done on *Zanthoxylum armatum* and *Skimmia laureola*. Detailed pharmacognostic evalvation of both plants was therefore carried out in the present study.

2.8.5. Elemental Analysis

Trace and other elements are important because they play both restorative and preventive role in treating various diseases (Kaneez *et al*., 1998). It is therefore of utmost importance to detect levels of various elements in medicinal plants. Various studies regarding to this aspect of herbals were carried out, some of which are given below

Islam & Adams (2000) investigated seasonal variations of elemental composition in *Atriplex amnicola* and *A*. *nummularia and* found high concentrations of nitrogen (N) in winter as compared with summer while sodium and phosphorus were found almost uniformly distributed in all seasons in both species.

Ferrara *et al*. (2001) analysed ten commercial teas from various countries and determined variable results for their comparative elemental composition for different samples analysed.

Yusuf *et al*. (2003) evaluated the levels of Cd, Cu and Ni in five different edible vegetables including *Celosia trigyna, Corchorus olitorus, Talinum triangulare, Venomia amygydalina* and *Telfaria accidentalis* and also in the soils in which these vegetables were grown. The results showed high accumulation of Cu and Ni by *Corchorus olitorus* than the other vegetable studied.

Narendhirakannan *et al*. (2005) determined mineral composition in the leaves of four medicinal plants i.e. *Murraya koenigii, Mentha piperitae, Ocimum sanctum,* and *Aegle marmelos* using atomic absorption spectroscopy. The levels of Na, K, Zn, Cu and Ni were present in trace amounts, where as very minute level of Fe, Cr, and V were detected. Ozcan (2005) determined macro and micro mineral contents in the flower buds, young shoots, caper berries (fruit) and seeds of *Capparis ovate.* All materials contained high amounts of Na, K, Ca, Mg, P, Pb, and Zn. Very low amount of Li, Ba, Cr, Cd, Cu, Ni and Se were found in caper plant organs.

Aremu *et al*. (2006) demonstrated mineral composition (mg / 100g) of cashew nut (*Anarcaduim occidentale*) showing Na (22.8±0.2), Ca (21.9±0.3), K (38.2±0.1), Mg (36.4±0.2), P (18.6±0.2), Cu (0.4±0.1), Mn (1.6±0.2), Zn (0.8±0.1), Fe (0.8±0.1) while Pb, Cd and Hg were however not detected. Okwu & Josiah (2006) analyzed the minerals in *Aspilia africana* and *Bryophyllum pinnatum* and found to be important sources of minerals like Na, Ca, K, P, Mg, Fe and Zn. The importance of these minerals was also discussed with respect to their role in ethnomedicine in Nigeria. Demirezen & Aksoy (2006) evaluated Ni, Cd, Cu, Pb and Zn contents of various vegetables (bean, tomato, cucumber, lettuce, parsley, onion, green pepper, peppermint, eggplant, pumpkin and okra) and the soil in which these were grown. The concentrations of Ni, Cu and Zn in vegetables were found in the recommended international standards range. The results also showed that peppermint (76.5µg/g) and onion (0.97µg/g) have much ability to accumulate Cd and Cu as compared to other studied vegetables.

Bukhsh *et al*. (2007) determined the trace elements contents i.e. Na, K, Ca, Mg, Fe, Mn, Cu, Cr, Zn, Mo and P in *Carthamus oxyacantha* (Asteraceae) *Eruca sativa* (Brasicaceae)and *Plantago ovata* (Plantaginaceae).

Rehman & Iqbal (2008) evaluated comparative accumulation of heavy metals Fe, Pb, Fe, Cu, Zn and Cr in the foliage of some plants including *Abutilon indicum*, *Prosopis juliflora*, and *Senna holosericea*, naturally grow in non industrial (Control) area and industrial area in Karachi. The level of Cu, Cr and Zn was found in highest concentration in industrial area as compared to control area. Hameed *et al*. (2008) determined elements like Na, K, C, O, Al, Mg, S, Si, Ti, P, Cl, Ca, Fe and Br in some medicinal plants viz., *Rumex dentatus, R. hastatus, R. nepalensis, Rheum australe, Polygonum plebejum* and *Persicaria maculosa* of the family Polygonaceae.

Naser *et al*. (2009) determined the levels of Ni, Pb and Cd in spinach, tomato and cauliflower and in the rizosphere soils of industrially polluted and non-polluted areas. Pb concentration was found higher in tomato, followed by spinach and cauliflower irrespective of the inhabiting area. Cd and Ni concentration were found in spinach followed by tomato and cauliflower, especially in the areas polluted with industrial effluents. Correlation was

found between the concentration of metals in vegetable samples and in the soil in which they were grown. Metal transfer factors from soil to vegetables were found to be significant for Ni, Cd and Pb. Ahmad *et al*. (2009b) determined the concentration of heavy metals like Ni, Pb and Cr in the forage plants with respect to the nutrient necessity of the grazing ruminants in the salt range Soon Valley (Punjab, Pakistan). From the available data, it was found that the concentration of these three minerals were different in different pastures and even in different parts of a plant. Ni, Pb and Cr concentration was found higher than the recommended level in the salt range forage plants. It might be causing toxicities problems in animals grazing the area.

Saeed *et al*. (2010) evaluated the concentration of various micronutrients and macronutrients of *Polygonatum verticillatum* and reported the presence Fe, Zn, Mn, Cu, Cr and Ni. It was noticeable that Ni (1.80 to 2.40 ppm) and Zn (60 ppm) concentrations were found higher than the permissible limits for plants i.e. 1.5 ppm and 50 ppm respectively. Sultan *et al*., (2010) determined the mineral composition i.e. K (0.47-1.29 %), Ca (1.01-2.7%), Mg (0.012-0.032 %), P (0.016-0.064 %), Zn (12.4-41.3 ppm), Cu (14-25 ppm), Mn (9-12 ppm) and Co (0.012-0.061 ppm) in *Adhatoda vesica, Myrsine affricana, Indigoferra gerardiana* and *Impatiens bicolor*.

Review of literature reveals that no such reference is, however lined with the proposed study.

2.8.6. Proximate analysis

Plants are considered basic nutritional source as they contain protein, carbohydrates, fats, oils, minerals, vitamins, and water, which are obligatory for growth and development in human and animals (Aruoma, 2003). References available to study plants, as they are the source of nutrients or not. Following are some of proximate analysis studies to evaluate plants for this purpose.

Ferrara *et al*. (2001) analysed ten commercial teas from various countries and obtained variable results. Polyphenols and flavonoids were found linked to different origins of the plant.

Aremu *et al*. (2006) carried out proximate composition of *Anarcaduim occidentale* using standard analytical techniques, and reporting mean values of various nutrients i.e. carbohydrate (29.4%), crude protein (25.3±0.2%), crude fibre (1.2±0.3%), ether extract (36.7±0.1%), ash (4.4±0.1%), moisture (5.7±0.2%) and energy (2242.8KJ/100g). Adebowale & Adedire (2006) determined physico-chemical properties of the seeds of *Jatropha curcas* and indicated high acid value, peroxide value, fee fatty acids and iodine value.

Anwar & Rashid (2007) examined the physico-chemical characteristics of seed from *Moringa oleifera* and found Protein (31.65%), fiber (7.54%), moisture (8.90%) and ash contents (6.53%). Bukhsh *et al*. (2007) worked on the nutritional value of some medicinal plants i.e. *Carthamus oxyacantha* (Asteraceae), *Eruca sativa* (Brassicaceae) and *Plantago ovata* (Plantaginaceae). Results showed that total proteins, crude proteins and fats in seeds and total carbohydrates in leaves were significantly higher in *Eruca sativa* as compared to *Carthamus oxyacantha* and *Plantago ovata*.

Anwar *et al*. (2008) carried out proximate analysis of the seeds of four citrus plants i.e. *Citrus limetta*, *Citrus paradisi*, *Citrus sinensis*, and *Citrus reticulata* and found protein (3.9–9.6%), fiber (5.0–8.5%) and ash contents (4.6–5.6%). Hameed *et al*. (2008) carried out proximate analysis of *Rumex dentatus, R. hastatus, R. nepalensis, Polygonum plebejum, Rheum australe* and *Persicaria maculosa* of family polygonaceae for carbohydrates, protein, crude fibers, fats, moistures and ash contents.

Bano *et al*. (2009) determined carbohydrates, protein, proline and abscisic acid (ABA) contents in the leaves of four herbaceous alpine plant species. Endogenous ABA were found higher in *Galium aparine*; Carbohydrates and protein contents were found maximum in *Onobrychis dealbata* stocks, whereas maximum proline was found in *Polygonum alpinum*. There was a general trend of increased accumulation carbohydrates, Protins, proline and ABA in the leaves at high altitude. Chitravadivu *et al*. (2009) reported qualitative analysis of the leaves and roots of four medicinally important plants i.e. *Acalypha indica*, *Cassia auriculata*, *Eclipta alba* and *Phyllanthus niruri*. Sultan *et al*. (2009) determined dry matter, organic matter, crude protein, ash, neutral detergent fiber, acid

detergent fiber, lignin contents and hemi-cellulose and for *Athyrium acrotiochoides, Atrimisia maritima, Chenopodium album, Cynoglossum lanceolatum, Hackalia macrophyla*, *Lespedeza spp*, *Onosma hispida*, *Polygonum amplexicaule, Plantago ovata* and *Urtica dioca.*

Hussain *et al*. (2010a) worked on eight vegetable pecies viz., *Abelmoschus esculentus, Cucumis sativus, Cucurbita moschata, Luffa acutangula, Solanum melongena, Spinacia oleracea, Praecitrullus fistulosus* and *Trianthema portulacastrum* for their nutritional values. Highest carbohydrate contents were found in *Cucurbita moschata* followed by *Luffa acutangula*, *Cucumis sativus,* and *Solanum melongena* while other vegetable species had insignificant carbohydrate composition. Protein contents of *Spinacia oleracea* and *Trianthema portulacastrum* had higher amount compared to other plants. Sultan *et al*. (2010) determined and compared nutritive value of *Adhatoda vesica, Impatiens bicolor, Indigoferra gerardiana* and *Myrsine affricana.* Higher amount of dry matter was found in *Indigoferra gerardiana* and *Impatiens bicolor* (38.1%). *Myrsine africana* showed maximum amount of ash contents and crude proteins contents. Highest hemicellulose (42%) and lignin (7.9%) contents, and lowest acid detergent fiber (22%) were detected in *Impatiens bicolor*.

Standardization of herbal drugs is not just an analytical process of detection of few constituents. It, rather, embodies a set of standards for authentication. Keeping this in view, crude powders from the proposed plants were analyzed for proximate analysis to consider it as a source of nutrients.

2.9. Physicochemical analysis of oil

The physico-chemical features of oil are significant as they play a major role in its effectiveness. The physicochemical analysis of both essential and fixed oils is also helpful, determining in order to see if they are of relevance in pharmaceutical, dietary and perfumery industries or not. Lot of work has been done on the physicochemical features of oils, some of which is

Ahmad *et al*. (2006b) studied some physical parameters of essential oils of *Citrus reticulata*, var. mandarin, *C. reticulata*, var. tangerine, *C. sinensis* var. malta, *C.sinensis* var.

mousami, *C. paradisi* and *C. limon*. *C. limon* had the highest peel portion i.e. 45.0%. Similarly, *C. sinensis* var. Malta had the highest oil yield i.e. 1.21%. Physical evaluation of oils delineated that *C. reticulata*, var. tangerine early peel oil had the lowest specific gravity i.e. 0.841, whereas, highest refractive index and optical rotation were found in *C. paradisi*.

Anwar & Rashid (2007) examined the physico-chemical characteristics of seed oil from *Moringa oleifera* with an iodine value (68.63), refractive index (0.4571), density ($0.9032 gcm^{-3}$), acidity as oleic acid (0.81%), saponification value (181.4) and unsaponifiable matter (0.74%).

Ali *et al*. (2008) analysed some fats samples from the local slaughterhouses in Chackdara, (KPK, Pakistan) for the determination of acid value, iodine value, peroxide value, saponification value, anisidine value, ash content and cholesterol content. These results were compared with Codex standard for the edible animal fats. Anwar *et al*. (2008) described the physico-chemical features of the seed oils from *Citrus limetta*, *C. paradisi*, *C. sinensis* and C. reticulata. The extracted oils showed acid value (0.5–2.2 mg KOH/g of oil), iodine value (99.9–110.0), density (0.920–0.941 mg/mL), refractive index (1.4639–1.4670), saponification value (180.9–198.9) and unsaponifiable matter (0.3–0.5%). Aremu *et al*. (2008) reported the physicochemical characteristics of the oil from cashew nut (*Anarcaduim occidentale*) which included colour (yellow), acid value (0.82 ± 0.4 mg KOH/g), refractive index (1.465), iodine value (44.4 ± 0.1 mg Iodine/g), peroxide value (3.1±0.2), specific gravity (0.964), saponification value (168.3 ± 0.3mg KOH/g) and free fatly acids (28.4±0.1 mg/g).

Shabbir *et al*. (2009) extracted oil from freshly collected flower petals of *Rosa centifolia* and determined various physicochemical characteristics like colour, specific gravity, refractive index, optical rotation, acid number, congealing point and ester number.

Bamgboye & Adejumo (2010) concluded values of physicochemical features for Roselle seed oil and compared with standard values, indicating that the oil is edible. Ibrahim *et al*. (2010) examined essential oils from the seeds of nutmeg and ginger roots for their comparative physicochemical properties. Othman & Ngassapa (2010) reported physicochemical properties (refractive index, saponification value, free fatty Acid content,

iodine value, tocopherol content, acid value, peroxide value) of seven imported edible vegetable oils and fat sold in shops in DaresSalaam, (Tanzania) using standard procedures. These results were compared with recommended physicochemical properties. Saeed & Bashir (2010) extracted oils from the seeds of *Ximenia americana* and reported its percentage yield, physical and chemical properties. 51% w/v oil was obtained having reactive index (1.477), density (0.9376 g/ml), boiling point (157°C) and viscosity 42 at 70°C and 227.58 at 25°C, iodine value (47.59), acid value (0.2805), peroxide value (30), saponification value (11.43), ester value (9.82), and the ratio value (35.009).

No physicochemical data of oils extracted from *Skimmia laureola* and *Zanthoxylum armatum* has yet been reported. Therefore, the present study has been done to analyze the physico-chemical characteristics.

2.10. GC-MS Analysis

The oils, their derivatives and extracts obtained from plants have recently gained much popularity and scientific interest because of their pharmacological, nutritional and perfumery significance (Zahed *et al.,* 2011). Essential oils and their derivatives also play a vital role in the innovation of novel useful components from higher plants to combat serious diseases (Moorthy, 2007). Following are some of the work done on chemical analysis of the oil from different plants.

Tzakou *et al*. (2001) distilled volatile oil from *Micromeria graeca* collected from different localities and identified sixty-two constituents through GC and GC–MS.

Hamid *et al*. (2002) extracted fixed oils from the seeds of canola and rape plants and studied comparatively their fatty acid composition by GLC. Rape seed oil samples contained erocic acid, palmitic acid, stearic acid, oleic acid, linoeleic acid, linoelenic acid, arachidic acid and lignoceric acid. Crucic acid, myristic acid and behenic acid were the components found in canola seed oil other than found in rape seed oil. Krauze-Baranowska (2002) analyzed chemically the essential oils through GC/MS from the needles of various pine speciesa i.e. *Pinus ponderosa*, *P. resinosa*, and *P. strobe*. α-pinne was found in all the three essential oils with variable amounts (42.4%, 45.7% and 7.9% respectively). It was concluded that higher content of α-pinne was found in *P. resinosa* followed by *P. ponderosa*

and *P. strobes.* Moreover the essential oil of *P. resinosa* showed maximum concentration of myrcene (15.9%). Estragole and alpha-3-carene (8% each) were identified only in *P. ponderosa*.

Goren *et al*. (2003) analyzed leaf essential oil of *Coridothymus capitatus* through GC/MS, comprises of 98.9% monoterpenes in which 55.6 % were oxygenated hydrocarbons and 43.6% were non-oxygenated hydrocarbons. The major components identified were *p*-cymene (21.0%), carvacrol (35.6%), *γ*-terpinene (12.3%), myrcene (3.0%), thymol (18.6%), *α*-terpinene (3.2%) and *α*-thujene (1.3%).

Baydara *et al*. (2004) analysed essential oils from four lamiaceae members i.e. *Origanum minutiflorum*, *Origanum onites*, *Thymbra spicata* and *Satureja cuneifolia*. Cavracrol was the major constituent detected through GC/MS in all oil samples i.e *O. onites* (86.9%), *O. minutiflorum* (84.6%) 75.5% in *T. spicata* (75.5%) and in *S. cuneifolia* (53.3%). Morteza-Semnani *et al*. (2004) analysed essential oils extracted from the dried leaves and flowers of *Phlomis herbaventi* (Labiatae) by GC and GC–MS. Germacrene D (33.9%) was the largest components followed by hexadecanoic acid (12.9%) and α-pinene (9.4%), where as hexadecanoic acid (33.1%) was the major constituents in the flower essential oil followed by 6,10,14-trimethylpentadecan-2-one (16.2%), 3-methyltetradecane (6.7%) and germacrene D (6.7%).

Nurbas & Yeliz (2005) studied fixed oils from the fruit and essential oil from the dried leaves of *Laurus nobilis* and their chemical composition were determined using GC/MS method.

Adebowale & Adedire (2006) evaluated chemical composition of *Jatropha curcas* seed and found that triacylglycenol was the leading lipids while 1, 2-Dioleoyl-3-linoleoyl-rac-glycerol was the major triacyglycerol. Linolenic acid was the dominant fatty acid in the oil.

Anwar & Rashid (2007) determined the GC/MS analysis of essential oils from *Moringa oleifera* seeds and concluded that β-sitosterol (46.16%) was the component followed by campesterol (17.59%), stigmasterol (18.80%) and avenasterol (9.26%).

Adinee *et al*. (2008) carried out GC and GC/MS analysis of the essential oils from lemon balm flower and identified a total of 12 compounds. Trans-carveol (28.89%), was the dominant compound followed by citronellol (25.24%), carene (5.26%), citronellal (4.9%), geraniol (2.2%), spathulenol (2.06%) and 1-octene-3-ol (2.03%). Anwar *et al*., (2008) studied the chemical composition of seeds oils from *Citrus limetta*, *Citrus paradisi*, *Citrus sinensis* and *Citrus reticulata*. These oils mainly consisted of 36.1–39.8% linoleic acid, 25.8–32.2% palmitic acid, 21.9–24.1% oleic acid, 3.4–4.4% linolenic acid and 2.8–4.4% stearic acid. Verdian-Rivi (2008) studied the chemical composition essential oil obtained from the aerial parts of *Ziziphora clinopodioides*. Twenty-six components were identified including pulegone (36.45%), piperitenone (19.12%), Menth-2-en-1-ol (5.31%), carvacrol (5.10%) neomenthol (4.78) and menthone (4.46%).

Chitravadivu *et al*. (2009) carried out qualitative analysis of essential oil from leaves and roots of four medicinally important plants including *Acalypha indica*, *Cassia auriculata*, *Eclipta alba* and *Phyllanthus niruri*. Bhuiyan *et al*. (2009) carried out GC-MS analysis of essential oil, extracted from the leaves of *Blumea balsamifera* and identified 50 components leaded by borneol (33.22%), caryophyllene (8.24%), ledol (7.12%), tetracyclo [6,3,2,0, (2.5).0 (1,8) tridecan-9-ol, 4, 4-dimethyl (5.18%), phytol (4.63%), caryophyllene oxide (4.07%), guaiol (3.44%s), thujopsene-13 (4.42%), dimethoxydurene (3.59%) and γ-eudesmol (3.18%). Sarac *et al*. (2009) extracted essential oil from *Thymbra spicata* var. *intricata* and determined 24 components through GC/MS analysis, comprising of 75.74% carvacrol, 9.28% γ-terpinene, 7.17% *p* -cymene, 1.39% myrcene, 1.13% β-caryophyllene and 0.15% thymol.

Formisano *et al*. (2010) analysed essential oils from *Teucrium divaricatum* ssp. *villosum* through GC, GC/MS and identified 60 components including sesquiterpenes (64.6%), E-caryophyllene (30.1%) and caryophyllene oxide (6.1%). Saeed & Bashir (2010) analysed oils from the seeds of *Ximenia americana*, in which methyl-14, 14- dimethyl–18-hydroxy heptatriacont-27, 35-dienoate were identified as major components.

Li *et al*. (2011) analyzed volatile oil of three cultivars of Resina ferulae i.e. *Ferula sinkiangensis, F. fukangensis*, and *F. ovina* by GC-MS. Twenty-six compounds

were identified in *F. sinkiangensis*, 21 compounds in *F. fukangensis* and 25 compounds in *F. ovina*.

In the present study essential and fixed oil were analyzed by GC/MC for determination of their chemical composition.

2.11. Pharmacological activities

Pharmacology offers various scientific techniques like screening of extracts, fractions and compounds obtained from plants in the form of bioassays in the field of phytochemical research (Nelms, 1997). Different extracts of medicinal plants are screened out for different purposes through bioassays, which are easy to handle and carried out in short span of time (Srirama *et al.,* 2007). In the present study *Skimmia laureola* and *Zanthoxylum armatum* were screened out for the following bioassays.

2.11.1. Acute toxicity study

It is of utmost importance to study all aspects of medicinal plant research including its safety and potential adverse effects. For this purpose animal toxicity studies are carried out to establish its efficacy against various ailments. Several studies are available in this regard, some of which are

Narayanan et *al*. (2000) tested the alcoholic extract of *Premna herbacea* root for acute toxicity test and found safe up to a dose of 8.0 g/kg, when administered orally to mice.

Ali & Blunden (2003) reported pharmacological actions of the crude extracts of *Nigella sativa* seeds. Administration of either the seed extract or seed oil produced no pronounced adverse effects on the metabolism of liver or kidney.

Abdel-Zaher *et al*. (2005) reported that *Zizyphus spina-christi* leaves appear to be a safe alternative of glibenclamide to lower blood glucose.

Magaji *et al*. (2007) carried out toxicity study of different extracts of the leaf, stem bark and root bark of *Securinega virosa* with LD_{50} values of 1265, 288.5 and 774.6mgkg-1 respectively showing that stem bark is more toxic as compared to other parts.

Selvamani *et al*. (2008) determined LD_{50} value of 894.43 mg/kg body weight for *Capparis sepiaria* during acute toxicity study, indicating that this plant is safe for medicinal usage.

Coker *et al*. (2009) evaluated toxicity test of *Ficus thonningii* leaves. The extracts were given in doses of 0.2, 0.4, and 1.0g/ kg body weight. As compared to control, there were no significant haematological and visible tissue pathological changes in the blood samples of treated groups suggesting *F. thonningii as* non toxic. Danmalam *et al*. (2009) studied the methanolic extract of the leaves of *Hyptis suaveolens* (Lamiaceae) for acute toxicity and showed LD_{50} of 2154.1 mg/Kg body weight in rats, suggesting the plant non toxic. Janapati *et al*. (2009) evaluated ethanolic extract of *Holostemma ada kodien* Schults non toxic up to 5 g/kg body weight in mice. Mowla *et al*. (2009) reported that no acute toxicity was observed for ethanol extract of *Trigonella foenum-graecum* seed, even at a high dose up to 3 g/kg body weight. Murugan & Reddy (2009) found that different fractions of *Mucuna pruriens* leaf showed no adverse effects even at a higher dose up to 2000mg/kg body weight in Wister rats.

Udem & Asogwa (2010) reported that no lethality was observed for leaf aqueous extract of *Ipomoea batatas* even at the highest dose of 1,600 mg/kg.

To test toxicity, the present study also included acute toxicity study on various extract of the *Skimmia laureola* and *Zanthoxylum armatum*.

2.11.2. Antipyretic activity

Pyrexia or fever is a secondary impact of malignancy, infection or other diseased conditions (Chattopadhyay *et al*. 2005). Antipyretic activity of large number of plants or their natural products assess to guide the isolation and purification of easy accessible biologically active principles (Amole & Onbanjo, 1999). Following are some of the previous literature related to antipyretic activities.

Hajare *et al*. (2000) studied antipyretic effect of *Dalbergia sissoo* leaf in Brewer's yeast-induced pyrexia in rats. The result showed significant decrease in pyrexia in the test animals throughout the observation period of 6 h.

Ahmadiani *et al*. (2001) examined antipyretic potential of the *Trigonella foenum-graecum* (TFG) leaf extract and found a significant decrease in hyperthermia of experimental animals induced by brewer's yeast. They suggested that alkaloids present in plant, may be an antipyretic agent.

Chitme *et al*. (2004) evaluated antipyretic activity of *Calotropis gigantea* roots by using Brewer's yeast and TAB (Typhoid) vaccine induced pyrexia in rats and rabbits. In both the cases, the pyrexia was significantly reduced to normal body temperature by injecting 200 and 400 mg/kg doses intraperitoneally. Arul *et al*. (2005) noticed significant decrease in hyperpyrexia in laboratory rates by applying a series of extracts from *Aegle marmelos* leaf.

Sundararajan *et al*. (2006) reported Screening of different extracts and fractions of *Bidens pilosa* (Asteraceae) for antipyretic potential in *in-vivo* models. The promising materials were the methanolic and its ethyl acetate fraction, but little correlation was observed in the degree of antipyretic activity between tested drugs and standard drug.

Panthong *et al*. (2007) reported that the yellow gum-resin secreted from *Garcinia hanburyi* were found effective in lowering the body temperature in yeast-induced hyperthermic rats. Khan *et al*. (2007) carried out antipyretic activity of chloroform and petroleum ether fractions of ethanol extract from the roots of *Laportea crenulata* and showed that both fractions significantly reduced the elevated body temperature at a dose of 80 mg/kg body weight in rats, comparable with standard antipyretic drugs.

Bhargava *et al*. (2009) evaluated antipyretic activities of aqueous extract of *Swertia chirata* root (ASC) in yeast induced pyrexia in albino rats and Paratyphoid A, B vaccine induced pyrexia in rabbits. Significant decrease ($p<0.001$) in hyperpyrexia was observed in both models at a dose of 200 and 400 mg kg^{-1} body weight. Patra *et al*. (2009) investigated the anti-pyretic effect of alcoholic, aqueous, chloroform and petroleum ether extracts of *Hygrophila spinosa* leaf against brewer's yeast-induced pyrexia in rats and found significant results.

Padhan *et al*. (2010) evaluated antipyretic activity of methanolic extract from *Capparis zeylanica* and showed a significant ($P < 0.01$) dose dependent antipyretic effect in yeast induced hyperthermia t in experimental Wister strain albino rats. Chomchuen *et al*. (2010) investigated antipyretic activity of the ethanolic extract of *Ficus racemosa* root (EFR) compared to acetylsalicylic acid (ASA), using lipopolysaccaride (LPS) and brewer's yeast-induced fever in rats. All doses of EFR significantly ($p<0.05$) reduced pyrexia just as ASA did. Hallal *et al*. (2010) evaluated antipyretic effects of fresh leaves aqueous extract from *Chenopodium ambrosioides*, which produced a significant inhibition ($P < 0.01$) in yeast induced pyrexia in rats.

In the present study *Skimmia laureola* and *Zanthoxylum armatum* were evaluated for anti-pyretic activity, as no such work has been reported so far.

2.11.3. Hypoglycemic activity

Diabetes mellitus type-2 is a chronic metabolic disorder regarded as a disorder of glucose, proteins and lipids metabolism, causing deaths to millions of people worldwide. A large number of plants have been screened for hypoglycemic potential, some of which are

Babu *et al*. (2003) reported hypoglycemic effect of *Cassia kleinii* leaf extract in streptozotocin induced diabetic rats. There was found significant decrease in hyperglycemia by alcohol extract at a dose of 200 mg/kg as observed from body weight, serum glucose level and liver glycogen levels. Coumarin, saponins and terpenoids detected in the chloroform fraction, may be responsible for hypoglycemic effect.

Luo *et al*. (2004) conducted hypoglycemic effects of water decoction, crude polysaccharide extracts and purified polysaccharide fractions from *Lycium barbarum* fruit in alloxan-induced diabetic rabbits and found significant decrease in the blood glucose level, serum level, triglyceride level and total cholesterol level and at same time markedly increase high density lipoprotein cholesterol levels after 10 days treatment.

Chakrabarti *et al*. (2005) evaluated different extracts of *Caesalpinia bonducella* seed kernel and showed that aqueous and ethanolic extracts have potent hypoglycemic activity in chronic type 2 diabetic model. Andrade-Cetto *et al*. (2005) studied the

antidiabetic effects of aqueous, ethanolic and butanolic extracts from *Malmea depressa* root in streptozotocin induced diabetic rats. Results indicated a significant decrease in plasma glucose level by oral administration of water, ethanolic and butanolic extracts in diabetic rats within three hours at different doses. Njike *et al*. (2005) studied hypoglycaemic potential of aqueous and methanol extracts of *Bersama engleriana* leaf in hyperglycaemic rats in order to rationalize its traditional therapeutic use. Both the extracts reduced blood glucose level by 37.7% and 49.11% respectively after 8 hour of administration.

Djumeni *et al*. (2006) investigated the hypoglycemic effect of *Ceiba pentandra* root bark extract in both normal and streptozotocin-induced diabetic rats. Blood glucose levels were found to be significantly reduced at doses of 40 and 75 mg/kg of the extract in fasted normal and diabetic groups 8 hour after administration. Okokon *et al*. (2006) conducted hpoglycemic activity of the leaf ethanolic extract of *Croton zambesicus* using alloxan-induced hyperglycaemic rats at a dose of 150mg/kg body weight. Single dose of the extract significantly reduced ($p < 0.01$) the blood glucose level, which is comparable to the reference standard drugs Chlorpropamide in prolonged treatment of 7 days.

Shirwaikar *et al*. (2007) explored hypoglycemic effect of *Holostemma annulare* root alcoholic extract and found significant ($p < 0.05$) reduction of blood glucose levels in fasting and normal as well as in diabetic rats. It was also observed that serum insulin level was stimulated in the diabetic animals, when treated with this extract.

Barik *et al*. (2008) evaluated hypoglycemic activity of aqueous root extract of *Ichnocarpus frutescens* in streptozotocin induced type-II diabetes in rats. Significant reduction ($P<0.05$) was observed at doses of 250 and 500 mg/kg of blood glucose levels in fasted animals on the 10th and 15th day respectively. Rajagopal & Sasikala (2008) carried out antidiabetic evaluation of hydro-ethanolic extract of *Nymphaea stellata* flower in normal and alloxan-induced diabetic rats. As compared to control diabetic group, this extract significantly reduced the high blood glucose level and also exhibited a significant increase in liver glycogen, insulin and HDL level. Gayathri & Kannabira (2008) reported significant decrease in the blood glucose level of fed, fasted and glucose loaded diabetic rates by oral administration of root aqueous extract of *Hemidesmus indicus*. Arumugam *et al*. (2008)

evaluated the methanolic extract of leaf and of the callus obtained from leaf explant of *Aegle marmelos* significantly reduced blood glucose level in streptozotocin induced diabetic rabbits. Bhagwat *et al*. (2008) studied the hypoglycaemic activity of different extracts of *Tridax procumbens* leaf in Wistar rats and reported significant decrease in blood glucose level at a dose of 200 mg kg^{-1} body weight. Selvamani *et al*. (2008) reported maximum fall of plasma glucose level of 9.40%, 13.57% and 15.25%, observed after 12th hour's administration of ethanolic extract of *Capparis sepiaria* at doses of 100, 200 and 300 mg/kg respectively as compared to 18.80% reduction with glibenclamide (10 mg/kg) dose. Kumar *et al*. (2008) reported that ethyl acetate and methanol extracted as well as an isolated compounds (Mycaminose) from *Syzygium cumini* produced a significant ($p<0.05$) reduction in blood glucose level in streptozotocin induced diabetic rates.

Janapati *et al*. (2009) explored that the ethanolic extract of *Holostemma ada* significantly decrease the blood glucose level in normal, glucose fed and alloxan-induced diabetic rats. Sivaraj *et al*., (2009) demonstrated anti-diabetic activity of the combined aqueous extracts of two medicinal plants i.e. *Cassia auriculata* and *Aegle marmelos* on streptozotocin induced diabetic rats. This extract significantly ($P<0.001$) reduced hyperglycemia and hyperlipidemia as compared to diabetic control rats. Adeneye & Olagunju (2009) investigated the hypoglycemic effects of aqueous extract of *Carica papaya* seeds through oral route administratin in normal male Wistar rats for 30 days. There was significant and progressive lowering of the fasting blood sugar level. Abubakar *et al*. (2009) reported significant hypoglycemic effect of ethanolic extract of *Nauclea latifol*ia leaf in streptozotocin-induced diabetic rats. Patra *et al*. (2009) studied the antidiabetic activity of aqueous extract of *Eucalyptus citriodora* leaf in alloxan-induced diabetic and glucose loaded rats. In both the tests, the extract has shown significant and considerable antidiabetic effect in a dose dependent manner. Mowla *et al*. (2009) studied the effects of ethanol extract of *Trigonella foenum-graecum* (Fenugreek) seeds on the blood sugar levels in alloxan-induced diabetic rats at different doses (0.1g/kg, 0.5g/kg, 1g/kg and 2g/kg). Significant decrease in blood glucose level was observed, varying from dose to dose.

Gunjan *et al*. (2010) explored that daily dose 200 mg/kg body weight of *Coccinia indica* fruit extracts for 14 days significantly reduced the blood glucose level of

diabetes induced animals as compared to diabetic control group in the 7th and 14th days of the diabetes induction. Rajesh *et al*. (2010) reported that the alcohlic extract of *smilax zeylanica* leaves significantly reduced the blood glucose level of hyperglycemic rats. Vishnu *et al*. (2010) investigated hypoglycemic activity of *Costus igneus* leaf extract in induced diabetic albino rats in comparison with a known antidiabetic drug i.e. glibenclamide (600 µg/kg body wt.). The extract significantly lowered the blood glucose level and also prevented body weight loss in experimental animals.

Kalyan *et al*. (2011) evaluated antidiabetic potential of seed ethanolic extract of *Clitoria ternatea*. The extracts at two dose levels (200mg and 400mg/kg body weight) in Streptozotocin induced diabetic rats (60mg/kg, i.p.). The 400mg/kg dose showed significant decrease in blood glucose ($p < 0.001$), cholesterol ($p < 0.05$), alkaline phosphatase ($p < 0.001$), aspartate amino transferase ($p < 0.001$) and alanine amino transferase ($p < 0.001$), as compared to diabetic control group. Sah *et al*. (2011) explored the antidiabetic activity of petroleum ether extract of *Citrus medica* seeds in streptozotocin induced diabetic model in rats. The extracts (200 and 400 mg/kg) induced significant reduction ($p < 0.05$) of fasting blood glucose, serum cholesterol, serum triglycerides, LDL and VLDL in dose dependent manner after 15 days of drug administration.

In Malakand Division *Skimmia laureola* is used as anti- diabetic agent, therefore this plant is screened for this purpose.

2.11.4. Antispasmodic activity

Gastrointestinal disorders have affected millions of people, especially children in the developing countries. The in vitro techniques are considered helpful in evaluation of phytomedicine, as these techniques are easily accessible (Baker *et al*., 1995). For this purpose on responces of isolated organ(s) are studied to evaluate the effectiveness of natural drugs (Enna *et al*., 2002). Following are some of the studied antispasmodic activities of different plants.

Shaphiullah *et al*. (2003) reported that methanol extract of *Ludwigia hyssopifolia* showed significant antidiarrheal potential by reducing diarrhoeal episodes in serotonin and

castor oil induced diarrhoea in laboratory mice at a dose higher than 100 mg/kg body weight as compared to standard drug.

Chitme *et al*. (2004) evaluated scientifically the anti-diarrhoeal effect of *Calotropis gigantea (Asclepiadaceae*) and reported significant reductions in fecal output and rate of recurrence of droppings when the plant extracts were administered intra peritoneally at a doses of 200 and 400 mg/kg doses as compared to control. All doses of the plant extracts also significantly retarded the castor-oil induced enter pooling and intestinal transit. Mahomed *et al*. (2004) examined pharmacological potential of aqueous extract of the secondary root of *Harpagophytum procumbens* on isolated gastro-intestinal smooth muscle preparations of rabbit, guinea-pig and chick. The results indicated that the treated extract significantly induced dose dependant contractions of the isolated preparations.

Mathad *et al*. (2005) investigated antidiarrhoeal potential of the alcohlic extract of *Benincasa hispida* fruit against various diarrhoea induced models in laboratory rats and showed significant reduction in recurrence of castor oil induced diarrhoea and repressed PGE_2 induced enter pooling in rats.

Oben *et al*. (2006) investigated antidiarrhoeal potential of the aqueous extract of *Eremomastax speciosa* leaf (Acanthaceae). The administration of oral doses, 400 and 800 mg/kg body weight reduced the number of stools by 42.50 and 48.35% respectively. This ant diarrhoeal effect might be due to the presence of the flavonoids and tannins, detected in *Eremomastax speciosa*.

Magaji *et al*. (2007) investigated the leaf, stem and root barks methanolic extract of *Securinega virosa* for antidiarrhoeal activity. It was found that root bark extract produced a dose-dependent inhibition of castor oil- induced diarrhoea while the leaf extract showed antidiarrhoeal effect but was not dose-dependent. The stem bark extract did not show any effect against diarrhoea.

Naseri *et al*. (2008) reported alcoholic extract of onion (*Allium cepa*) bulb induced spasmolytic activities and suggested that quercetin in onion peel extract induce spasmolytic effect via calcium channels.

Ahmad *et al*. (2009c) confirmed possible calcium channel blocking and cholinomimetic activities of different doses of crude methanolic extract of *Tylaphora hirsuta* in isolated rabbit's jejunum preparations. The extract also inhibited K^+ induced contractions, suggesting spasmolytic action which was further confirmed by right shift in the dose response curves of the isolated tissues in calcium free tyrode's solution. Mohammed *et al*. (2009) investigated the hydromethanolic extract of aerial part of *Indigofera pulchra* for antidiarrhoeal activity, using castor oil-induced diarrhoea in mice and on isolated rabbit ileum. The results showed dose dependent relaxation of rabbit ileum. Akter *et al*. (2009) investigated antidiarrhoeal potential of *Xanthium indicum* leaves using charcoal induced gastrointestinal motility test and castor oil and $MgSO_4$-induced diarrhoeal in mice. There was found significant reduction in the frequency and severity of diarrhoea in test animals throughout the study. The extract also significantly delayed charcoal meal transit in the intestine of test animals as compared to control. Gandhimathi *et al*. (2009) investigated ethanolic (90%) extract of inner bark of *Guettarda speciosa* for its efficacy as antidiarrhoeal agent at 200 and 400 mg/kg body weight doses and showed significant reduction in the number, weight and volume of diarrhoeal stools in intestinal contents. The results obtained substantiated the folkloric claim of the plant as anti- diarrheal agent.

Saralaya *et al*. (2010) evaluated hydroalcoholic (50:50) extract of root of *Moringa oleifera* against castor oil induced diarrhoea models in rats. The extract produced a significant decrease in the severity and rate of recurrence of diarrhoea, volume of intestinal content, intestinal fluid accumulation and intestinal transit compared to normal saline control group. Teke *et al*. (2010) evaluated methanolic extract, fractions and isolated compound of *madagascariense* stem bark for its preventive and restorative antidiarrhoeal effects in rats. Significant decrease ($P \leq 0.05$) was observed in purging indices, fecal recurrence rate, blood cell counts, sera creatinine, fecal *Shigella* load and intestinal enter pooling in a dose dependant manner.

Azam *et al*. (2011) evaluated antispasmodic action of the crude alcohlic extract of the aerial parts of *Myrsine africana* on spontaneous rabbit's jejunum preparations at different doses and showed that the tissue completely abolished at a concentration of 5.0 mg/ml. Ali & Shah (2011) studied the relaxant effect of crude methanolic extract of aerial

parts of *Teucrium stocksianum* on spontaneous rabbits' jejunum preparations at different concentrations. The positive results confirm the folkloric use of *Teucrium stocksianum* as an antispasmodic drug.

2.11.5. Cytotoxicity

Plants are explored for innovation of new natural substances for protection and treatment of various ailments including cancer (Amara *et al*., 2008). Brine shrimp toxicity bioassay is preliminary study to detect toxicity and development of anti cancer drugs. Some of the studies in this regard are

Khan & Khan (2007) evaluated *Rhazya stricta* for brine shrimp lethality test using various solvent fractions. Methanol fraction showed significant cytotoxicity with LC50 17.809µg/ml, having mortality rate 73.33 % at highest dose. Tolulop (2007) studied Aqueous-methanolic extract of *Hibiscus sabdariffa* for cytotoxicity using brine shrimps lethality assay and showed significant cytotoxicity (LC_{50} value = 55.1 ppm).

Amara *et al*. (2008) carried out brine shrimp toxicity bioassay and determined LC_{50} values for seeds of *Gossypium barbadense*, *Nigella sativa*, *Ricinus communis*, *Sesamum indicum*, *Vinca rosea* and *Melia azedarah*; *Xanthium occidental* fruit; *Atriplex nummularia* flower; barks of *Cinnamomum zeylanicum* bark; *Ficus carica* latex and rhizomes of *Curcuma longa* and *Zingiber officinale*.

In an *in-vitro* test, Attard & Cuschieri (2009) studied various extracts of ten plants (Asteraceae) for their effects on T-lymphocytes after positive identification for brine shrimp lethality test. The results obtained indicated that the petroleum ether extract of *Calendula arvensis* is relatively non toxic to peripheral lymphocytes suggesting its potential use as an immune booster. Islam *et al*., (2009) reported significant tumor inhibition at 100 ppm and 1000 ppm of leaf methanol extract *Oldenlandia diffusa*. Manilal *et al*. (2009) reported that red alga, *Laurencia brandenii,* may be cytotoxic as it showed a LD value of 93µg/ml. Ateeq-ur-Rehman *et al*. (2009) evaluated methanol extract of *Thymus serpyllum* (Labiateae) using brine shrimp cytotoxic bioassay and showed highly significant impact on percentage death of brine shrimp with LD_{50} of 466 ppm. Nisar *et al*. (2009)

investigated *Indigofera gerardiana* for cytotoxicity using brine shrimp lethality assay and found no assessable cytotoxicity.

Ayatollahi *et al*. (2010) investigated that chloroform fraction from methanolic extract of *Euphorbia aellenii* showed favorable cytotoxicity while the ethyl acetate fraction was found to be less effective. Hussain *et al*. (2010b) determined the cytotoxicity of the crude methanolic extracts of *Rumex dentatus, R. hastatus, R. nepalensis, Polygonum persicaria*, *Polygonum plebejum* and *Rheum australe* of family Polygonaceae using brine shrimp lethality test at a doses of 10, 100 and 1000 µg/ml. *R. hastatus, R. dentatus* and *R. nepalensis* showed significant cytotoxicity at higher doses. Saeed *et al*. (2010) evaluated the cytotoxicity of *Polygonatum verticillatum* using different doses of different solvent extract. There was no sign of brine shrimp cytotoxicity except in the chloroform fraction (LD_{50} was 1205.07µg/mL). Patel *et al*. (2010) explored that *Rubia cordifolia* could be source of potent pharmacophore for treatment various diseases like cancer.

Bulbul *et al*. (2011) evaluated cytotoxic activities of the n-hexane, chloroform and ethyl acetate extracts of leaves of *Luffa cylindrica* L. and *Luffa acutangula*. All extracts/fractions showed considerable toxicity towards brine shrimps. Ramachandran *et al*. (2011) carried out Brine shrimp lethality test for *Agave cantula* to detect its cytotoxic potential in term of lethality concentration (LC_{50}) and showed that aqueous and alcoholic extract exhibited potent LC_{50} values 15 and 12.5 mg respectively.

Similar studies have been carried out on proposed research plants.

2.11.6. Phytotoxicity

Pakistan is an agriculture country and one of the major producers of some crops, but due to poor weed control management, major portion of the crop has been damaged. So weeds controlling is of utmost significance for enhancement of crop production. Various attempts were made to explore natural remedy for this purpose, some of which are summarized bellow.

Khan *et al*. (2008) evaluated some medicinal plants like *Trichodesma indicum*, *Aconitum spp* and *Sauromatum guttatum* for phytotoxic potential and showed that plants extracts exhibited excellent phytotoxicity tested against *Lemna minor*. Zaidi *et al*., (2008)

studied the methanolic extract of *Arceuthobium oxycedri* and showed that it was extremely toxic for *Lemna spp,* thus exhibiting strong phytotoxicity.

Aliferis *et al*. (2009) studied different herbicides, like mesotrione, norflurazon, paraquat and the phytotoxin pyrenophorol against *Lemna minor* to detect toxicological studies and reported no toxicitymacroscopic symptoms were observed, while metabolic changes were detectable by ^{1}H NMR spectra analysis. Ateeq-ur-Rehman *et al*. (2009) tested phytotoxicity of methanolic extract of *Thymus serpyllum* (Labiateae) against *Lemna minor* showing significant results ($P < 0.05$). Nisar *et al*. (2009) tested crude extract and various fractions of *Indigofera gerardiana* for phytotoxic potential and found that all the fractions except crude extract were highly significant herbicidal potency against *Lemna minor* at the concentration of 1000µg/mL.

Islam *et al*. (2009) carried out phytotoxic assay for leaf methanol extract *Oldenlandia diffusa* and showed significant inhibition at concentrations of 100ppm and 1000ppm. Onocha & Ali (2010) reported that methanolic extracts of the leaves of *Phyllanthus muellerianus* were significantly phytotoxic at a concentration of 1000 µg/m against *Lemna minor*. They also concluded that antitumor compounds can inhibit the growth of *Lemna minor*. Hussain *et al*. (2010b) determined phytotoxicity of the crude extracts of *Rumex dentatus, R. hastatus, R. nepalensis, Polygonum persicaria*, *Polygonum plebejum* and *Rheum australe* of family Polygonaceae using *Lemna minor* as test species. All the plants except *R. hastatus* showed significantactivity at a concentration of 1000 µg/ml. Moderate activity was shown by *R. australe*, *R. nepalensis* and *P. persicaria* at the concentration of 100µg/ml. All the plants showed low phytotoxic activity at concentration of 10µg/ml. Ayatollahi *et al*. (2010) investigated that chloroform fraction of *Euphorbia Aellenii* and showed significant phytotoxicity. Gilani *et al*. (2010b) screened out 81 medicinal plants of KPK, Pakistan, in which *Seriphidium kurramense, Andrachne cordifolia* and *Rhazya stricta* were found strongly phytotoxic as compared to the other species.

Ahmad *et al*. (2011) reported that the ethyl acetate fraction of *Zizyphus jujuba* was moderately phytotoxic against *Lemna minor* at 1000 µg/ml as compare to other extract which showed low phytotoxic activity. Dzoyem *et al*. (2011) carried out *in-vitro* phytotoxic

activity of the of the methanol extract, fractions and isolated compounds from the stem bark of *Diospyros canaliculata* and showed phytotoxicity similar to standard phytotoxic inhibitor, paraquat. Nisar *et al*. (2011) proved that crude methanolic extract and various fractions of *Zizyphus oxyphylla* stem were strongly phytotoxic at higher doses, as tested against *Lemna ninor*.

Both the proposed plants were screened out for phytotoxic potential in the present study.

2.11.7. Antibacterial activity

Undesirable side effects of antibiotics have been reported some uncommon serious infections, which forced scientists to explore new sources of potent antimicrobial drugs. For this purpose plants has been explored and found to be a potential source of effective antibiotic drugs (Maurer-Grimes *et al.,* 1996; Rabe & Van Staden, 1997; Marchese & Shito, 2001). Some of the work carried out in this regard is

Nanasombat & Lohasupthawee (2005) studied the anti bacterial effect of methanolic extracts and essential oils of 14 aromatic plant spices using disk diffusion method. The result showed that clove extract and oils from clove, Cardamom, Coriander, and Cumin inhibited the growth of all the tested bacterial strains.

Aliero & Afolayan (2006) investigated antimicrobial activity of methanol, acetone and aqueous extracts of *Solanum tomentosum* leaf against ten bacterial strains. Results indicated that Acetone and methanol extracts inhibited the growth of some Gram positive and Gram negative bacteria. None of the extracts inhibited the growth of *Escherichia coli*, *Klebsiella pneumonae, Penicillium notatum* and *Staphylococcus aureus,*. Prabuseenivasan *et al*. (2006) evaluated the antibacterial activity of 21 essential oils of plants origin against four gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*) and two gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, in which oils form Cinnamon, Clove, Geranium, Lemon, Lime, Orange and Rosemary exhibited significant inhibitory effect.

Eftekhar *et al*. (2007) studied antibacterial activity of the aerial parts of *Xanthium brasilicum*. Using different extracts and their fractions, significant antibacterial

activity was shown. Bbosa (2007) investigated different extracts of *Mangifera indica* leaf for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The ethanolic extract was most active with MIC ranging from 5481.0 to 43750.0 μg ml^{-1}. Hoque *et al*. (2007) determined antibacterial activity of guava (*Psidium guajava*) and neem (*Azadirachta indica*) extracts against 21 strains of foodborne pathogens. Both the plant showed higher antimicrobial activity against Gram-positive bacteria as compared to Gram-negative bacteria. Mandal *et al*. (2007) tested Steam distillate, petroleum ether and ethanol extracts of *Hyptis suaveolens* leaves for their antimicrobial activity *in vitro* and reported that broad-spectrum antibacterial against the tested organisms. Tolulope (2007) studied aqueous-methanolic extract of *Hibiscus sabdariffa* for antimicrobial activity against *Bacillus cereus*, *Bacillus stearothermophilus*, *Clostridium sporogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Staphylococcus aureus*, *Serratia mascences*, and *Pseudomonas fluorescence* exhibited MIC value ranging from 0.30 ± 0.2- 1.30 ± 0.2 mg/ml.

Farrukh *et al*. (2008) investigated *In-vitro* antibacterial activity of of *Coccinia grandis* leaf and stem extracts against various bacterial strains indicated that aqueous extract of leaves significantly inhibited *Shigella boydii* while ethanolic extract of stem showed significant activity against *Pseudomonas aeruginosa* respectively. Vyas *et al.,* (2008) studied antibacterial activity of Arodent™ (herbal dentifrice) against *Lactobacillus acidophilus* and *Streptococcus mutans* using Colgate as standard drug using cup well method. Both bacterial strains were isolated and identified by standard methods. Arodent inhibited *L. acidophilus* and *S. mutans* producing 5.5 and 10 mm zones of inhibition, respectively as compared to standard, showing its efficacy as antibacterial agent. Akhtar (2008) studied antibacterial activities of methanol, aqueous, acetone and petroleum ether extracts of *Pimpinella anisum* and reported that aqueous and methanol extracts were effective against all the test bacterial strains.

Nwinyi *et al*. (2009) screened out aqueous and ethanol extracts of leaves of *Ocimum gratissimum* and *Piper guineense* for antibacterial activity using *Escherichia coli* and *Staphylococcus aureus* as test species. Both extracts to exhibited selective inhibition against the isolates but the ethanol extracts showed more inhibiton as compared to the

aqueous extracts. Kirbaşlar *et al*. (2009) tested peel oils of various samples of the *Citrus* fruits for antimicrobial activities. C*itrus* peel oils exhibited strong antimicrobial potential against the test organisms. Lemon and bergamot peel oils were found much inhibitory as compared to other *Citrus* peel oils.

Ahameethunisa & Hopper (2010) tested six organic solvent extracts of *Artemisia nilagirica* for antimicrobial potential against phytopathogens and clinically important reference bacterial strains and reported that all the extracts were inhibitory to most of gram-positive and gram-negative bacteria. Hoskeri *et al*. (2010) studied bactericidal activity of crude extracts from lichen *Ramalina pacifica* against 20 clinical pathogenic strains which belong to *Escherichia coli, Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi, Salmonella paratyphi,* and *Staphylococcus aureus*. The extracts exhibited predominant antibacterial activity against all the multi-resistant strain.

Arirudran *et al*. (2011) carried out the antimicrobial activity of successive extracts using different solvents of *Ruellia tuberosa* against different bacterial and fungal organisms. The result showed that all the extracts were active against all the tested bacteria. Debnath *et al*. (2011) reported that the ethanolic extracts of Banana, pineapple and musambi, Jackfruit and papaya showed no significant activity against bacterial.

Anti bacterial potential of the proposed plant has been tried to explore in the present study.

2.11.8. Antifungal activity

Various types of fungal pathogens have been reported to cause severe type of diseases in human beings. Plants have been found to be a potential source of anti fungal drugs (Khan *et al*. 2004). Keeping this, scientists are trying to explore more and more new antifungal drugs from plants, some examples of which are

Krauze-Baranowska (2002) studied antifungal activities of analyzed essential oils from needles of *Pinus ponderosa*, *P. resinosa* and *P. strobus* and noted strongest antifungal activity of the essential oil from *P. ponderosa*.

Sridhar *et al*. (2003) studied thirteen essential oils from Indian herbs for *in-vitro* antifungal activity against plant and Food mold rot and reported that only oil from *Cymbopogon* exhibited inhibition to all tested plants and food molds.

Khan *et al*. (2004) reported that *Tamarix dioca* extract has been used topically as an antifungal agent, found resistant to tested six strains of fungi. Gaspar *et al*., (2004) reported that sesquiterpenoids (+)-curcuphenol and (+)-curcudiol isolated from the Caribbean sponge *Didiscus oxeata* showed inhibitory effects against several filamentous fungi. Phongpaichit *et al*. (2004) reported antifungal activities of Crude methanol extracts of *Cassia alata, C. fistula* and *C. tora* leaves for their antifungal activities against three pathogenic fungi (*Microsporum gypseum, Trichophyton rubrum* and *Penicillium marneffei*). Among 3 species, *C. alata* was most effective against *T. rubrum* and *M. gypseum* at 0.5 and 0.8 mg/ml, respectively, whereas the extract of *C. fistula* was the most potent inhibitor of *P. marneffei*.

Oliveira *et al*. (2006) carried out antifungal activity of *Propolis* extract against 67 yeasts in *in-vitro* tset and showed excellent performance regarding its antifungal activity. Bajwa *et al*. (2006) investigated antifungal potential of aerial parts aqueous extracts of *Cicer arietinum*, *Drechslera tetramera* and *Drechslera hawaiiensis* and reported that the extract showed most significant antifungal activity even at lower concentration (5%).

Khan & Khan (2007) reported antifungal activity of *Rhazya stricta* against *Aspergillus flavus, Candida albicans, Fusarium solani, Microsporum canis* and *Trichophyton longifusis*.

Parekh & Chanda (2008) studied the methanol extract of 9 Indian medicinal plants for in *vitro* antifungal activity against some yeasts and reported that *Saussurea lappa* showed the best antifungal activity. Essien *et al*. (2008) reported that essential oil of *Citrus medica* inhibited 14 tested storage fungi of *Arachis hypogea*.

Hadizadeh *et al*. (2009) evaluated essential oils extracted from five plant species against *Alternaria* rot of stored tomatoes. Ruttoh *et al*. (2009) carried out antifungal activity of leaves, fruits, root and stem barks of *Tabernaemontana stapfiana* against *Candida*

albicans, Cryptococcus neoformans, Microsporum gypseum and *Trichophyton mentagrophytes* and reported significant inhibition of these species.

Ali *et al*. (2010) investigated antifungal activity of Hydroxychavicol, isolated from *Piper betle* leaf (Piperaceae) and reported that the compound has inhibitory effect on fungal species of clinical significance. Segismundo *et al*. (2010) reported significant anti fungal activity of *Gouania javanica* leaves against *Candida albicans*, *Trichophyton mentagrophytes* and *Aspergillus niger*.

Similar study has been carried out on proposed plants of the present study.

CHAPTER-3

MATERIALS AND METHODS

3.1. Morphology of the Plants

Morphology of the research plants were studied in their natural habitat following Hassan-Ud-Din & Ghazanfar, 1980.

3.2. Phytosociology of the research plants

Many trips to different localities were undertaken for phytosociological study of the proposed plants (*Skimmia laureola and Zanthoxylum armatum*) during 2008-2010. For this purpose six localities i.e Malamjabba (Swat), Bahrain (Swat) Matiltan (Swat) and Barawal (Upper Dir), Jagam (Upper Dir) and Patrak (Upper Dir) for *Skimmia laureola* and six localities i.e Batkhela (Malakad), Laram (Lower Dir), Maidan (Lower Dir), Peto Dara (Lower Dir), Kabal (DistrictSwat) and Warsak (Buner) for *Zanthoxylum armatum* were selected (Fig. 3.1). Vegetation sampling was carried out in places where there were no sign of recent disturbance. For each stands altitude, latitude and exposure were recorded (Siddique *et al*., 2009). Quantitative data was recorded using 10 quadrates of 10x10 m, 4x 4m, and 1x 1m for trees, shrubs and herbs respectively. The herb cover was determined by the Daubenmire's cover scale (Daubenmire, 1959). Tree diameter at breast height (1.5m) (dbh method) was measured to obtain basal area (Hussain, 1989). Density, cover and frequency were measured and then these values were converted to relative density, relative cover and relative frequency for each species (Phillips, 1959). The Importance Value (IV) for the species was determined as the sum of the relative frequency, relative density and relative coverage. Dividing this value by 3, Importance value index (IVI) was obtained (Curtis & Cottam, 1956). IV is used for determining dominant species in each stand. Density per hectare was calculated for the two research plants following Mueller-Dombois & Ellenberg, 1974. Various formulae used for analyzing the data are given below.

i. Density

Density is the average number of individuals of a species in unit / area

$$\text{Density} = \frac{\text{No.of individuals of a species}}{Total\ No.\ of\ Quadrates}$$

Density was then converted into relative density (RD) as follows

$$\text{RD} = \frac{\text{Density of a species}}{\text{Total densities of all species}} \times 100$$

ii. Cover

Cover is the vertical projection of foliage shoots crown of a species to the ground surface expressed as fraction or percent of a surface area.

$$\text{Cover} \quad \frac{\text{Cover of a species}}{\text{Total cover of all species}}$$

Cover was then converted into relative cover (RC) as follows

$$\text{RC} = \frac{\text{Cover of a species}}{\text{Total Cover of all species}} \times 100$$

iii. Frequency

Frequency is the percentage occurrence of species in an area.

Frequency was determined as follows.

$$\text{Frequency} = \frac{\text{No.of quadrats in which a species was present}}{\text{Total number of quadrates applied}}$$

Frequency was then converted into relative frequency (RF) as follows

$$\text{RF} = \frac{\text{Frequency of a species}}{\text{Total frequencies of all species}} \times 100$$

iv. Importance Value index(IV)

The importance values Index of each species was calculated by adding relative values of density, cover and frequency. A community was named after the three leading

species having the highest importance values as follows (Mueller-Dombois & Ellenberg, 1974)

Importance value (IV) = RD+RC+RF

Importance value index (IVI) $= \frac{RD+RC+RF}{3}$

v. Determination of Constancy

Plant species were classified into following constancy classes following Raunkiaer (1934).

I. Present in up to 1-20% of the stand Rare

II. Present in up to 21-40% Seldom

II. Present in up to 41-60% Often

IV. Present in up to 61-80% Mostly present

V. Present in up to 81-100% Constantly present

3.3. Soil analysis

Soil samples from the habitat of the proposed plants were collected at a depth of 0-15 cm. The samples were air dried and analyzed for physicochemical characteristic following standard procedure.

3.3.1. Soil texture

Soil texture textural classes were determined by using International Pipette method (Piper, 1966).

3.3.2. Organic matter

Soil organic matter in the soil samples was calculated by oxidizing Sulphuric Acid medium with Potassium Dichromate under standard wet combustion method of Rayan *et al*. (1997).

3.3.3. Nitrogen (N)

Soil Nitrogen contents were determined by Alkaline Permanganate Method of Subbiah & Asija (1956) metohd.

3.3.4. Phosphorus (P)

Available P in the given soil samples was determined by Ascorbic Acid reduced molybdophosphoric blue colour method using Genesys 2PC Spectrophotometer. Standard Nitrogen solution was prepared and run through spectrophotometer and a standard curve was obtained with different concentrations. A diluted solution with lithium sulphate solution and sample solution were run to determine the ppm phosphorous contents in the sample by comparing with standard curve of phosphorous (Watanabe & Olsen, 1965).

3.3.5. Pottassium (K)

Pottassium contents in different soil samples were determined by neutral normal NH_4 OAC Extract Method using EEL flame photometer (Jackson, 1958).

3.3.6. Iron, Zinc and Copper

Available Fe, Zn, Cu were determined by 0.1NHCl Extract Method using Perkin Elmer Analyst 300 atomic absorption spectrophotometer (Jackson, 1958)

3.3.7. pH

pH of the soil samples were measured with pH meter by making 1: 5 soil water suspension (Hussain, 1989).

3.4. Ethnobotany

To know the local traditional uses of the two research plants, an open questionnaire was prepared to interview local inhabitants, hakims and pansaries (local medicine man) during the field surveys of different localities. Questionnaire was filled on the spot as majority of the respondents were illiterate. Vernacular names, harvesting, collection, processing methods and manner of recipe used to treat human ailments along with other ethno botanical uses were recorded for the proposed plants. Eighty four respondents, including pansaries, elder knowledgeable people (both men and women), having information about these plants, were interviewed. Only that information was considered

authentic and reported, when at least 10 interviewees attested to the name and usage of the plants (Barkatullah *et al*., 2009).

3. 5. Market survey

Market surveys were carried out in nearby area of their natural habitats in order to get data from local collectors, purchasers and dealers of the proposed research plants for their collection, storage, packing and marketing status (Hamayun *et al*., 2003).

3.6. Plant collection

Fresh collection of *Skimmia laureola* and *Zantthoxylum armatum* was undertaken from Patrak (Upper Dir) and Batkhela (District Malakand) respectively. Plant materials were cleaned, washed and garbled. Some of the fresh specimen of *S. laureola* and *Z. armatum* were pressed, dried, mounted on herbarium sheets, given voucher number (Bot. 8815 & Bot. 8820 respectively) and kept in the herbarium of Department of Botany, University of Peshawar, Pakistan for ready references. The present pharmacognostic, phytochemical and biological research work is carried out on the leaf and stem bark of *S. laureola* and leaf, stem bark and fruit of *Z. armatum*. Fresh samples were used to study macroscopic and microscopic characteristics, while rest of the collection was dried in shade. The dried plant parts were then pulverized into powder with electric grinder. These powders were stored in air tight bottles to protect them from moisture, molds, insects attack and other adverse climatic conditions. A portion of each of these powders was used for physicochemical analysis while rests were used for various biochemical and biological research studies.

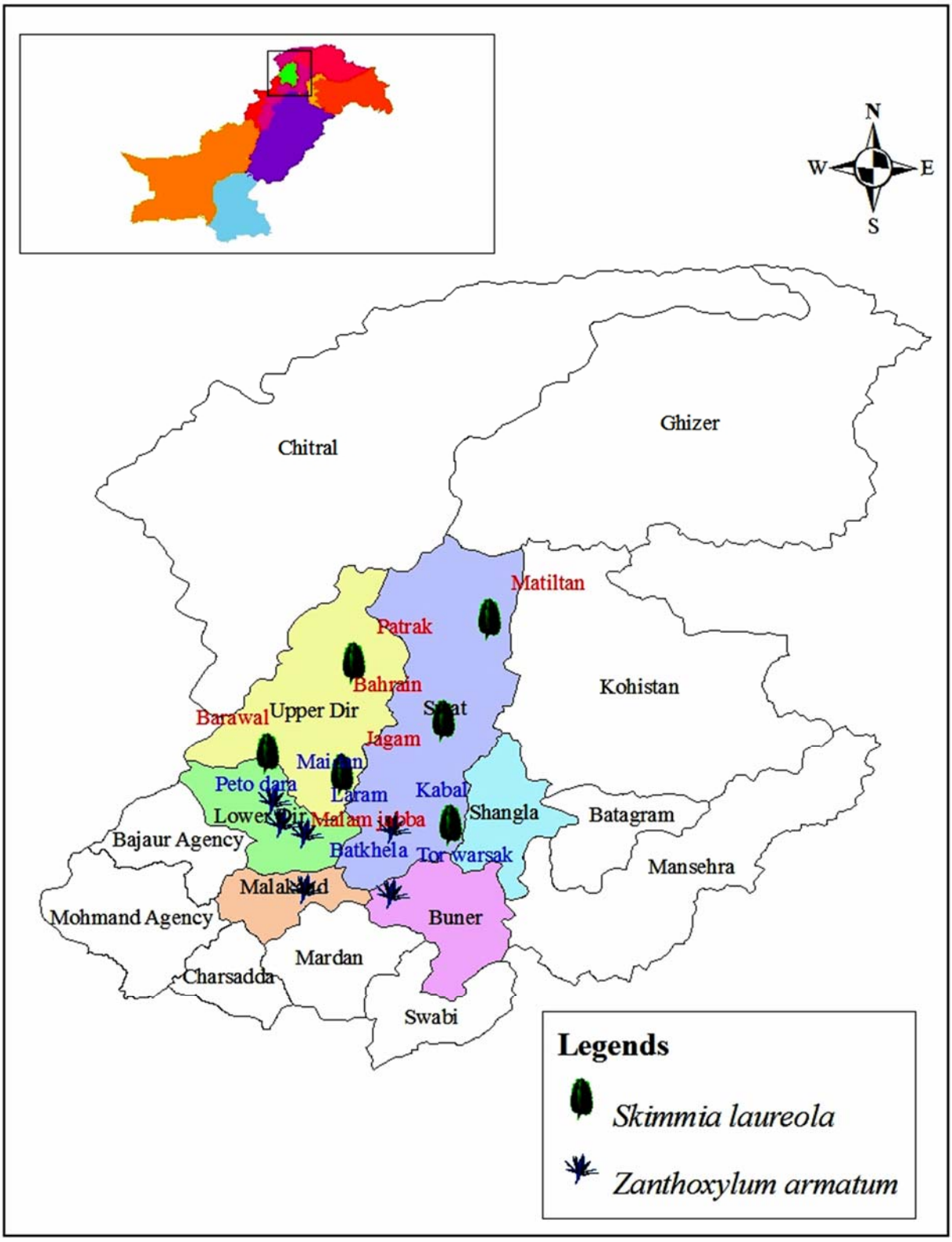


Fig. 3.1. Map showing natural habitats of *Skimmia laureola* and *Zanthoxylum armatum*

3.7. Pharmacognosy

3.7.1. Macroscopic studies.

Morphological observation of the leaves, bark and fruit of *Skimmia laureola* and *Zanthoxylum armatum* were studied by organoleptic methods following Wallis (1985) and Evans (2002).

Leaf was studied for

i. Size
ii. Color
iii. Odor
iv. Taste
v. Phyllotaxis
vi. Insertion
vii. Leaf base
viii. Petiole
ix. Lamina
    a. composition
    b. incision
    c. venation
    d. margin
    e. apex
    f. base
    g. general outline
    h. surface
    i. texture (Wallis, 1985; Evans, 2002)

Characteristic studied for bark were

i. Shape
ii. Dimension
iii. Colour
iv. Outer surface
v. Inner surface

vi. Fracture

vii. Thickness

viii. Color

ix. Odour

x. Taste (Wallis, 1985; Evans, 2002)

Fruit was characterized by the following features

i. Kind

ii. Dimensions

iii. Shape

iv. Insertion

v. Dehiscence

vi. Pericarp

a. colour

b. taste

c. odour

d. texture

e. marking

f. sutures

g. differentiation

h. seeds (Wallis, 1985)

3.7.2. Anatomy

Thin transverse sections of the selected plants parts were made with the help of sharp razor. Large numbers of transverse sections were made, from this, thin sections were selected and stained with phloroglucinol and hydrochloric acid, mounted in glycerin on glass slide and studied under Nikon microscope fitted with camera (Chaffey, 2001).

3.7.3. Leaf surface study.

As in powder drugs most of the cells and their arrangement are destroyed, therefore a cytomicroscopical features of the leaves were carried out for evaluation of drug, which include

a. Stomatal Number and Stomatal Index,

b. Vein islets and vein termination number,

c. Palisade cell ratio.

a. Stomatal Number and Stomatal index

The Stomatal number (SN) and Stomatal Index (SI) are most specific and distinctive feature for identification, standardization and characterization of leaf crude drugs. Two guard cells along with pore constituted a single unit. Stomatal Number or Stomatal Density is the number of stomata per square mm of epidermis on each surface of a leaf. Stomatal Index is the percentage of stomata to the total number of epidermal cells in the leaf (Evans, 2002; Bozoglu & Karayel, 2006).

Procedure:

Epidermis from both sides of fresh leaf was peeled off. With the help of a pair of forceps, the peel was removed and mounted in dilute glycerin and observed under microscope. Numerical data i.e. number of epidermal cells and number of stomata per squre mm was recorded with help of 100x magnification. Other informations like size of epidermal cell, presence an absence of stomata, type of stomata, size of guard cells and ratio of open and closed stomata were also recorded. The size of epidermal cells and stomata was determined using ocular micrometer and stage micrometer. Stomatal indices of the leaves of the two plants were calculated using the above data and following formula (Chaudhary and Imran, 1997; Evans, 2002).

$$I = \frac{S}{S+E} \times 100$$

Where as

I= Stomatal Index

S= Number of stomata per unit area

E= No. of epidermal cells per unit area

Statistical analysis

The statistics analysis of the stomata and epidermis including mean, variance, coefficient of variation and standard error were carried out (Choudhary & Kamal, 2004).

b. Vein islets and vein termination number

The tinny area of the photosynthetic tissue surrounded by ultimate divisions of veins in leaf is known as vein islet and their number per square mm of the leaf is called vein-islet number. A vein termination is ultimate free termination of vein-let and their number per square mm is known as vein termination number (Evans, 2002). These values are constant in range for a species and can be calculated at the same time and used as important tool for identification of a leaf drug.

Procedures

Many pieces from the leaves of proposed plants were taken midway from margin to midrib and cleared by boiling in 200% Choral hydrate solution in a test tube placed on a boiling water bath (Choudhary & Kamal, 2004). Using 4mm objective lens, and stage micrometer, an area of 1 mm was fixed. Next, the stage micrometer was removed and a slide mounted with cleared leaf specimen was focused. The iris diaphragm was closed to a degree to adjust the illumination on the 1 mm focused area. Starting from one side, all the vein islets were counted inside the square and also included those, which were on the boundary of the square. Along with veins-islets, veinlet terminations were also counted, which were inside the square only. To get exact and standard values, 10 readings were taken from continuous squares and vein-islets within it were counted (Evans, 2002).

c. Palisade Cell-Ratio

The average number of Palisade cells present beneath each upper epidermal cell is called Palisade cell ratio (Evans, 2002). Like other leaf features, this value is also constant in range for a species and is not affected by geographical variation; therefore it is a diagnostic feature for characterization, standardisation and identification of particular species (Shruthi *et al*., 2010).

Procedure

Small pieces of the leaves were taken midway between margin and midrib, which were cleared by boiling in 200% Chloral Hydrate solution (Shruthi *et al*., 2010). The cleared pieces were mounted and examined under microscope. The microscope was focused in such, so as the epidermal cells and the palisade cells lying below them could be seen

simultaneously with minor adjustment. A number of groups of each of four epidermal cells were focused, and then by minor rotation of the fine adjustment under lying the palisade cells were focused within the Epidermal Cells. Palisade Cells including those which were more than half covered by the epidermal cells were counted. Palisade ratio was then obtained by dividing the resulted figure by 4. Then readings were taken from different pieces in order to obtain accurate values (Evans, 2002).

3.8. Physicochemical characteristics of powder drugs

3.8.1. Powder microscopy

Powder drug of *Skimmia laureola* (leaves and bark) and *Zanthoxylum armatum* (leaves bark and fruits) were studied in detail for their physical and microscopical characteristics. Physical characteristics included color, odour and taste, while microscopy was carried out to know the characteristic structure present in the respective powder. The following microscopic procedures were adopted for the proposed study.

A pinch of fine powder was taken on a glass slide, treated separately with water, Chloral Hydrate and iodine solutions and observed under microscope using 45X and 10X objective lenses for observation of different structures, which were then sketched. Some of the fragments of powder were also observed under Nikon microscope fitted with camera (Wallis, 1985; Youngkin, 1948).

3.8.2. Ash analysis

Ash analysis of powder drugs included total ash, acid insoluble ash and water soluble ash tests for crude powder as such and exhausted with ethanol and hexane were carried out for leaves and bark of *S. laureola* and leaves, bark and fruits of *Z. armatum*.

Principle

The ash is an analytical term used for the inorganic residue which remains after ignition of organic matter. The resulting ash is not of the same composition as that present in original plant materials because some of it may be lost due to volatilization or chemical interaction among the constituents (Jarald & Jarald, 2007). Ash tests are used for the detection of earthy or sandy adulteration, present or added in the drug and also for the detection of exhausted drugs (Wallis, 1985).

a. Total ash determination

Equipment and glassware

Muffle furnace, silica crucible electric balance, desiccators, oven and Benson burner.

Procedure

Flat bottomed silica crucible was thoroughly washed and was kept for 30 minutes in oven at 70^0C for drying. Crucible was then ignited and tarred, cooled in desiccators and weighted (W_1) and 4 gram of the plants parts (samples) were transferred to it, spread evenly. The loaded crucible was heated gently on Bunsen burner and then transferred to muffle furnace which was ignited and temperature was gradually increased to 550 ^{o} C. This temperature was maintained for many hours until all the carbon in the drug had been burnt away, turning it to white appearance. After turning off the furnace, the crucible containing ash was transferred to desiccators, cooled and weighted (W_2). Percent ash and total ash values were calculated as follows (Wallis, 1985; A. O. A. C., 2000).

Weight of the empty crucible = W_1

Weight of the empty crucible + ash = W_2

Total ash (mg/g) = $W_2 - W_1$ of the sample= mg/gram

$$\% \text{ Ash} = \frac{W2 - W1}{\text{Wt of the sample}} \times 100$$

b. Acid Insoluble Ash

This test is carried out for those drug samples, which may have variable amount of ca-oxalate crystals and which can be adulterated with lime, sand or clay (Wallis, 1985; Rangari, 2002)

Procedure

Total ash (mg/g) was obtained as per 3.7.2a above. 25 ml HCl was added to the crucible containing the specific amount of ash of the respective sample, covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot distilled water and added this liquid to the crucible. The insoluble matter was collected on an ashless

filter-paper, which was drained with hot water until the filtrate became neutral. The filter-paper containing the insoluble matter was transferred to its respective crucible, dried on a hot-plate and ignited at 500ºC in furnace till constant weight. The crucible was then allowed to cool in desiccators for 30 minutes and weighted without delay. The acid-insoluble ash was then calculated in mg per g of air-dried material as follows. (Wallis, 1985; A. O. A. C., 2000).

Weight of the empty crucible = W_1

Weight of the empty crucible + sample = W_2

Acid insoluble ash = $W2 - W_1 = X$ mg/gram

c. Water-soluble ash

This test is carried out to detect the presence of water exhausted material (Jarald & Jarald, 2007).

Procedure

Total ash (mg/g) was obtained as per 3.7.2a above. 25 ml water was added to the pre weighted crucible (W_1) containing the specific amount of ash (W_2) of the respective sample, covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was drained with 5 ml of hot distilled water and added this liquid to the crucible. The insoluble matter was collected on an ash less filter. The filter-paper containing the insoluble matter was transferred to respective crucible, dried on a hot-plate and ignited in furnace at 500ºC for 15 minutes. The crucible was then allowed to cool in desiccators for 30 minutes and weighted without delay (W_3), from which insoluble sample amount was calculated (X mg/g). The content of water-soluble ash (Y mg/ g) of air-dried material was then calculated by subtracting this amount from total ash (Wallis, 1985; AOAC., 2000).

Weight of the empty crucible = W_1

Weight of the empty crucible + sample = W_3

Weight of the sample = $W_3 - W_1 = X$ mg/gram

Water soluble ash = $W_2 - X = Y$ mg/gram

3.9. Florescence study

Certain drugs fluorescence differently when studied in day light and under ultraviolet radiation, therefore this technique is used as identification tool for evaluation of whole and powder drugs (Jarald & Jarald, 2007).

Equipment and glassware:

UV tube (254 nm, 366 nm), glass slides etc.

Reagents:

50% HNO3	50% H2SO4
50% HCl	NH3 solution
NaOH in water	NaOH in ethanol
Picric acid	Iodine solution
10% FeCl3 solution	

Procedure

The fluorescence analysis of the whole dry parts i.e. leaves and bark of *Skimmia laureola*; leaves, bark and fruits of *Zanthoxylum armatum* and of their powder (I gm) as such and those treated with different reagents (50% HNO3, 50% H2SO4, 50% HCl, NH3 solution, NaOH in water, NaOH in ethanol, Picric acid, Iodine solution and 10% FeCl3 solution) was carried out by observing the samples under day and UV light of both short and long wave lengths (Chase & Pratt, 1949; Brain & Turner, 1975; Evans, 2002; Nikam *et al*., 2009).

3.10. Extractive values determination

The extract obtained by various solvent is approximate measure of their chemical constituents. Water extracts glycosides, mucilage etc; alcohol extracts tannins and resins; ether extracts fatty and oily substances. These values are helpful in detection of adulterants in the crude drugs (Jarald & Jarald, 2007; Kokate, 1994)

Solvents: Distilled water Ethanol n- Hexane Methanol
Butane Chloroform Acetone

Equipments:

Balance, beakers, rotary evaporator, filter paper, funnel etc.

Procedure:

Extractive values of the drugs were carried out following Ansari *et al*., (2006). 10 gram of the crude powder drug was dissolved in 200ml of different solvents in airtight bottles for seven days with occasional shaking. Each extract was filtered and the filtrate was dried in rotary evaporator. Each of the process was repeated thrice, combined the three extract of each solvent and their percent extractive valves were calculated as under

$$\text{Percent (\%) extractive value (W/W)} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times 100$$

3.11. Elemental analysis

Elemental analysis of powder from the selected parts was carried out with atomic absorption spectrophotometery for the following trace and heavy metals.

Lead (Pb)	Manganese (Mn)	Zinc (Zn).	Cobalt (Co)
Chromium (Cr)	Copper (Cu)	Iron (Fe)	Potassium (K)
Sodium (Na)	Nickel (Ni)		

Reagents and Equipments

Double distilled water, Nitric acid (HNO_3), Sulphuric acid (H_2SO_4), Hydrogen per oxide (H_2O_2), Hydrogen fluoride (HF), Perchloric acid ($HClO_4$) and Hydrochloric acid (HCl). All the reagents used were from Merck (Darmstadt, Germany). Pb, Cd, Co and Mn sigma made and Cu, Zn and Fe Aldrich made. Glasswares and plastic equipment were thoroughly washed with water, followed by rinsing with distilled water prior to use.

Sample preparation

Samples were prepared by wet digestion method (Hseu, 2004). For this purpose 1 g of the respective powder drug was taken in a conical flask and then added 10 ml of concentrated HNO_3 (67%) and kept overnight (24 h) at room temperature followed by the addition of 4 ml of $HClO_4$ (67%). After 30 minutes, the contents of each flask were heated on hot plate to evaporate, until a clear solution of approximately 1 ml was left. After cooling, the solution was made to a final volume of 100 ml by addition of double distilled

water and filtered through Whatman # 42 filter paper. The filtrate served as stock solution for each sample. The samples were stored in airtight containers for elemental analysis through atomic absorption spectrophotometer (Eslami *et al.,* 2007). Each sample was then analyzed by flame atomic absorption spectrophotometer (Polarized Zeeman Hitachi 2000) and flame photometer (Jenway PFP7, UK) in triplicate. Calibration standard of each metal was prepared by appropriate dilution of the stock solutions (Saeed *et al*. 2010).

Procedure:

The respective cathode lamp for each element was turned on and allowed to warm up for 10 minutes after adjusting the instrument according to the conditions given in the table below. After warming of cathode lamp the air acetylene flame was ignited. The instrument was calibrated and standardized with working standards of 2.5, 5, and 10 ppm for respective element (Table 3.1). The element standard solutions used for calibration were prepared by diluting a stock solution of Pb, Cr, Co, Mn, K (Sigma) , Cu, Fe, Na (Aldrich) Zn and Ni (Parkin Elmer) Working standards were run as unknown to verify the standardization. The sample stock solution was aspired into the flame and the concentration in ppm of each element was calculated by comparing with the standard curve of respective metal (Tuzen *et al*., 2003).

Table 3.1. Conditions applied for detection of various elements.

Elements	Flame type	Wavelength (nm)	Slit width (nm)	Acetylene flow (L/min)	Air oxide flow (L/min)	Cathode lamp current (mA)
Pb	Air/ acetylene	283.3	0.7H	2.0	17	10
Zn	Air/ acetylene	213.9	0.7H	2.0	17	15
Mn	Air/ acetylene	279.5	0.2H	2.0	17	20
Co	Air/ acetylene	240.7	0.2H	2.0	17	30
Cr	Air/ acetylene	357.9	0.7H	2.5	17	25
Cu	Air/ acetylene	324.8	0.7H	2.0	17	15
Fe	Air/ acetylene	248.3	0.2H	2.3	17	30
K	Air/ acetylene	766.5	0.7H	2.0	17	12
Na	Air/ acetylene	589	0.2H	2.0	17	0.8
Ni	Air/ acetylene	232	0.2H	2.0	17	25

Statistical analysis

The elemental data was analyzed statistically using arithmetic mean and standard deviation (Saeed *et al*., 2010).

3.12. Nutritional analysis

Plants provide nutritional requirements as they contain protein, carbohydrates, fats and other nutrients, obligatory for growth and development of humans (Aruoma, 2003). The following parameters have been estimated in the proposed plants.

3.12.1. Determination of ash (Methodology for determination of ash given in section 3.7.2).

3.12.2. Determination of the moisture

Equipment and glassware

Electric oven, Petri dish, desiccators and electric balance

Procedure

About 2 gram of respective plant sample was taken in a clean weighted Petri-dish (W_1). The Petri-dish was partially covered with lid, placed in oven at temperature of 105°C for 4-6 hours, till constant weight was obtained and was then transferred to desiccators for 30 minutes to cool down; after that, the Petri dish was weighted again (W2). Percent moisture contents were calculated using the following formula (AOAC, 2000).

$$\% \text{ Moisture} = \frac{X}{\text{Wt of the sample}} \times 100$$

Where

X = Weight of the sample (after heating) = W_2 - W_1

W_2 = Weight of the empty Petri dish + sample (after heating

W_1 =Weight of the empty Petri dish

3.12.3. Determination of proteins by "Macrojeldahl distillation method"

Reagents

Conc. H_2SO_4, 4% Boric Acid, 32% NaOH, K_2SO_4, $CuSO_4$ and 0.1 N standard HCl solution.

Mixed indicator

Prepared by dissolving 0.016g of methyl red and 0.03g of bromocresol green in 100 ml of alcohol.

Apparatus

Kjeldahl flask, digestion and distillation apparatus, burette etc.

Procedure

Protein (% Nx6.25) was determined by Macro kjeldahl method. The procedure is given below.

0.5 gram of dry ground sample was taken in digestion flask. Digestion mixture (Copper sulphate, Potassium sulphate and ferrous sulphate in the ratio of 5, 94 and 1 respectively) and 25 ml of concentrated Sulphuric acid were added to the flask and digested in digestion flask (kjeldatherm) for 6 hours. The flask was then removed, cooled and the contents were then transferred to 250 ml flask. Small amount of distilled water was added to make the volume to the level 50 ml of the above solution. 10 ml of strong alkali was added to make it alkaline.

About 50ml of 4% Boric Acid solution was transferred to the distillation flask along with 3-5 drops of mixed indicator. 50 ml of water and 60 ml of 32% NaOH solution were then added to it. After distillation, it was then collected in flask for titration.

Titration was done by taking 0.1 N HCl in burette with and the content of the flask. The reading was noted and the percentage of protein was determined using the following formulae (AOAC., 2000).

$$(\text{N }\%) = \frac{(\text{V1}-\text{V2}) \times 14.01 \times 0.5 \times 100}{(\text{Sample in mg})}$$

V1= Titration reading of sample

V2= Titration reading of blank

14.01= Atomic weight of Nitrogen (N)

Crude percent protein contents were calculated for all the samples by multiplying the nitrogen content of the sample by 6.25.

Protein (%) = % Nitrogen × 6.25.

3.12.4. Determination of fat (ether extract)

Equipment, chemicals and glassware

Petroleum ether B.P (40-60°C) H.T (Tecator).

Extraction thimbles, Soxhlet extraction apparatus, heating mantle, water bath.

Procedure:

Soxhlet apparatus was used for the extraction of crude Fat (Zarnowski & Suzuki. 2004). 2 gram of the sample was packed in cellulose extraction thimble made of filter paper which was placed in extraction chamber of the apparatus. A clean and dried pre weighted 250 ml round bottom flask was filled with Petroleum ether and connected to the extraction tube containing thimble. The soxhlet apparatus was run for 5-6 hours. The solvent from the extract in the round bottom flask was evaporated using water bath and reweighted (W_2). Fats percentage was then calculated by the following equation (AOAC., 2000).

$$\% \text{ fats (Ether extract)} = \frac{X}{\text{Wt of the sample}} \times 100$$

Where

X = Weight of the fats = W2 - W_1

W_1 =Weight of the empty flask

W_2 = Weight of the empty flask + sample after evaporation of solvent

3.12.5. Determination of crude fiber

Equipment and Glassware

Crude fiber extraction apparatus (Fiber Tec System M. Tecator), Suction pump, Muffle furnace, oven.

Reagents

Sulphuric Acid – 0.255N

Sodium Hydroxide - 0.313 N

Asbestos, Petroleum Ether, Ethyl Alcohol

Procedure

3 gram of the respective sample was dried in the oven to constant weight. 2 gram of this material was extracted with Petroleum Ether to remove crude fats. The residue materials were transferred to digestion flask along with 0.5g asbestos. To this, about 200 ml boiling 0.255 N, H_2SO_4 was added. The flask was connected to the condenser and boiled for 30 minutes. The contents were then filtered through linen cloth in fluted funnel. The residue was washed to remove the acids and transferred again to the digestion flask with boiling 0.313 N NaOH. Addition of NaOH was continued till the volume to exactly 200 ml. The flask was then connected to the reflux condenser and boiled for 30 minutes. This hot material was then filtered through Gooch crucible prepared with asbestos mat. It was thoroughly washed with boiling water followed by 15 ml of Ethyl Alcohol. The contents were taken to a crucible and dried at 110°C in hot air oven till constant (W_1). The crucible was then transferred to the muffle furnace, ignited till white and weighted (W_2). Crude fibers were then calculated (AOAC, 2000).

$$\% \text{ Crude fibers} = \frac{W2 - W1}{\text{Weight of sample}} \times 100$$

Where

$W_2 - W_1$ = Crude fiber

3.12.6. Carbohydrates contents

Carbohydrates contents were calculated by subtracting the sum of the weights of proteins, fat, crude fibers, ash, and moisture contents from 100 (Merrill & Watt, 1973).

100 – (Protein+ fats +crude fiber +ash + moisture contents) = % Carbohydrates

PHYTOCHEMISTRY

3.13. Organic solvent extractions

1 kg of respective powder of *Skimmia laureola* (leaves and bark) and *Zanthoxylum armatum* (leaves bark and fruit) were soaked separately in 5-6 liters of Ethanol and n-Hexane for 12-15 days with occasional shaking. The extracts were then filtered and concentrated with the help of rotary evaporator. Each of these processes were repeated thrice and at the end all the three extracts of each drug were combined and preserved in refrigerator for further research work including qualitative screening, quantitative studies and pharmacological activities. (Miliauskas *et al*., 2004).

3.14. Qualitative chemical identification tests

Various qualitative chemical tests were conducted for detection of carbohydrates, proteins, alkaloids, phytosterols, triterpenoids, phenols, flavinoids, tannins, anthocyanins, saponins, glycosides, fixed oil and volatile oil in the different extracts of *Skimmia laureola* and *Zanthoxylum armatum*.

3.14.1. Carbohydrates detection tests.

a. Molisch's test (Alcoholic α-napthol solution**)**

Few drops of alcoholic α-napthol were added to the respective extract solution. Then 0.2 ml of concentrated H_2SO_4 was added through the sides of the test tube. Presence or absence of carbohydrates was detected by appearance of purple to violet colour ring at the junction (Evans, 2002).

b. Benedict's test (alkaline solution containing cupric citrate complex)

Extract solution was treated with few drops of Benedict's reagent and then boiled on water bath. Presence of reducing sugars was detected by reddish brown precipitate (Evans, 2002).

c. Fehling's test

Equal volume of Fehling's A (Copper Sulphate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) were treated with few drops of sample solution and boiled. Presence of reducing sugar was confirmed by a brick red precipitate of cuprous oxide formation (Evans, 2002).

3.14.2. Proteins & amino acids detection test

a. Millon's test (Mercuric nitrate in nitric acid containing traces of nitrous acid)

2 ml of Millon's reagent were added to the respective extract. Presence or absence of proteins or amino acids was detected whether i.e. white precipitate appeared, which turned red upon gentle heating or not (Evans, 2002).

b. Ninhydrin Test (Indane 1, 2, 3 trione hydrate)

Extract solution were boiled with 0.2% solution of Ninhydrin in test tube; violet colour appearance was the indication of proteins if present otherwise not (Kumar & Kiladi, 2009).

3.14.3. Alkaloid detection

a. Mayer's test (Potassium mercuric iodide solution).

To the sample solution, few drops of Mayer's reagent were added, creamy white precipitate will be produced if alkaloids were present (Khandelwal, 2004).

b. Wagner's test (Solution of Iodine in Potassium Iodide).

To the sample, few drops of Wagner's reagent were added, Presence of alkaloids were detected by production of reddish brown precipitate (Khandelwal, 2004).

c. Hager's test (Saturated solution of Picric acid)

Few drops of Hager's reagent were added to the sample solution. Alkaloids were detected by appearance of yellow precipitate formation (Khandelwal, 2004).

3.13.4. Phytosterols and triterpenoids detection test

a. Libermann - Burchard test

Different extracts were treated with few drops of acetic anhydride; boiled, cooled and concentrated sulphuric acid was added from the side of the test tube. A brown ring if formed at the junction of two layers and the upper layer turned green would indicated the presence of sterols while a deep red color would indicate the presence of triterpenoids (Harborne, 1998).

b. Salkowski's test

Different solutions in chloroform were treated with few drops of concentrated Sulfuric acid and shaked well. Red colour appearance in the lower layer would indicate the presence of sterol while yellow color would show the presence of triterpenoids. Absence of colours in the upper layer indicates absence of phytosterol and triterpenoids (Harborne, 1998).

3.14.5. Phenol detection test

Ferric chloride test.

2 ml of extract solution was added to 2ml of ferric chloride solution ($FeCl_3$) in a test tube. Formation of deep bluish green solution would indicate the presence of phenols otherwise no phenols would be present (Dahiru *et al.*, 2006).

3.14.6. Flavonoids detection

a. Alkali reagent test:

Extract solution was treated with sodium hydroxide solution. Presence of flavonoids is detected by appearance of yellow to red precipitation (Kokate, 1994).

b. Zn-HCl acid reduction test

Extract solution was treated with a mixture of Zinc dust and conc. Hydrochloric acid. Appearance of yellowish, yellow-orange, occasionally orange colour after few minutes indicates the presence of flavonoids (Kokate, 1994).

c. Shinoda test (Magnesium Hydrochloride reduction test)

To the extract solution, a few fragments of magnesium ribbon and concentrated Hydrochloric acid were added drop wise. After few minutes, pink scarlet, crimson red or occasionally green to blue color will appear if flavonoids were present. (Chitravadivu *et al.*, 2009).

3.14.7. Tannins

a. Gelatin test

To an extract 1% gelatin solution was added containing NaCl. White precipitate will be formed if tannins were present (Kokate, 1994).

b. Ferric chloride test

FeCl3 was mixed with an extract solution. Presence of tannins were detected by blue green colouration. (Kokate, 1994)

c. Alkali reagent test:

Sodium hydroxide was added to extract solution. Presence of tannins were detected by yellow to red precipitate formation in short time (Kokate, 1994)

3.14.8. Anthocyanins detection tests

Hydrochloric acid test

2 ml of 2N HCl were added to sample. The appearance of a pink-red color, which turns blue violet by the addition of ammonia would indicate presence of anthocyanins (Harborne, 1998).

3.14.9. Saponin detection test

Frothing test.

5 ml of an extract was shaken vigorously in a test tube. Formation of froth and its persistence for some time indicated the presence of saponins (Chaouche *et al.*, 2011).

3.14.10. Steroidal glycosides detection test

Killaer kilani test

2 ml of extract was treated with glacial acetic acid, one drop of $FeCl_3$ (5%) and concentrated H_2SO_4. Appearance of reddish brown colour at the junction of two liquid layers and bluish green in the upper layer is the indication of the presence of steroidal glycosoids (Harborne, 1998).

3.14.11. Fixed oils detection tests

a. Spot test

Various extracts were separately pressed between two filter papers. Existence of oil stain on the paper indicates presence of fixed oil (Gomathi, 2010).

b. Alkali test

0.5N alcoholic potassium hydroxide was added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or fractional neutralization of alkali indicates the presence of fixed oils and fats (Gomathi, 2010).

3.14.12. Volatile oil detection test

Various extracts were pressed separately between two filter papers. Absence of permanent stain indicated the presence of volatile oil (Kumar & Kiladi, 2009).

3.15. Quantitative chemical analysis

Quantitative analysis of the selected parts of the research plants were carried out to determine alkaloids, sterols, tannins, saponins, phenols and flavonoids quantitatively.

3.15.1. Alkaloids determination

Materials required

Ethanolic extract, acetic acid, filter paper, beaker, balance, water bath

Procedure

Alkaloids in all selected parts were quantitatively determined following the method of Harborne (1998). 2 gram of the ethanolic extract was treated with 100 ml of 10% acetic acid in a beaker, covered and kept for 4 hours. The mixture was then concentrated on a water bath to 1/4th of its original volume, to which concentrated ammonium hydroxide was added drop wise for precipitation, This precipitate was collected on pre weighted (W_1) Whitman filter paper, which was then washed with dilute ammonium hydroxide. The residue along with filter paper was dried, weighed (W_2) and amount of alkaloid in mg/g as well as percent was calculated as.

$$\text{Amount of alkaloid (mg/g)} = \frac{X}{\text{Weight of sample}}$$

$$\%\ \text{alkaloids} = \frac{X}{\text{Weight of sample}} \times 100$$

Where

X= Weight of alkaloids = W_2- W_1

W_1= Weight of filter paper

W_2 = Weight of filter paper + ppt

3.15.2. Saponin determination

Material required

Ethanolic extract, n-butanol, Dimetyle ether, 5% sodium chloride solution, separatory funnel, filter paper, balance, water bath

Procedure

Saponin quantity was determined by method of (Obadoni & Ochuko, 2001). 2 gram of an ethanolic extract was dissolved in 20 ml of distilled water and transferred to 250 ml

separating funnel. 20 ml of diethyle ether was added to the funnel and shaken vigorously. Ether layer was drained out and the aqueous layer was mixed with 60 ml n-butanol in which saponine precipitated. The saponin was colleceted on pre weighted (W_1) Whitman filter paper, which was then washed twice with about 10 ml of 5% aqueous sodium chloride solution. The precipetate was then dried in oven (40°C) to constant weight (W_2) and the saponin content in mg/g as well as percentage of the initial weight of sample taken was calculated as.

$$\text{Amount of saponin (mg/g)} = \frac{X}{\text{Weight of sample}}$$

$$\text{Saponin (\%)} = \frac{X}{\text{Weight of sample}} \times 100$$

X =Weight of saponin = W_2- W_1

W_1 =Weight of filter paper

W_2=Weight of filter paper + residue

3.15.3. Tannins determination

Material required

Ethanolic extracts, lead acetate solution, sulphuric acid, filter paper, balance, water bath, hot air oven

Procedure

Percent tannins in the respective samples were determined following Van-Buren & Robinson (1969). 2 gram of ethanolic extracts was dissolved in 75 ml distilled water to form a suspension, which was filtered, treated with saturated solution of lead acetate to get tannins as lead- tinnate precipitate. The residue was then dissolved in 20 ml of distilled water and then axcess of dilute sulfuric acid was added to this mixture, which removes the lead sulphate on acidification. The mixture was next filtered and the tannins were collected on the pre-weighted Whitman filter paper (W1). Filter paper was then dried in hot air oven at 60°C. The amount of tannin was estimated by taking the weight of the filter paper again

and tannins content in mg/g as well as percentage of the initial weight of sample taken was calculated as.

$$\text{Amount of tannins (mg/g)} = \frac{X}{\text{Weight of sample}}$$

$$\text{Tannins (\%)} = \frac{X}{\text{Weight of sample}} \times 100$$

Where X =Weight of tannins = W_2- W_1

W_1 =Weight of filter paper

W_2=Weight of filter paper+ residue

3.15.4. Sterols determination

Materials:

Ethanolic 10% potassium hydroxide solution, petroleum ether, separatory funnel, flasks, filter paper, Balance, water bath

Procedure:

Percentage of sterol was determined by dissolving 2 gram of respective sample in 75 ml of distilled water, to which 25 ml of 10% KOH was added. This treatment converted the chlorophylls into water soluble salt called chlorophyllins. This mixture was then extracted thrice with 75ml petroleum ether in separatory funnel. Ether fraction was transferred into a pre weighted (W_1) flask and concentrated on hot water bath, dried and cooled in desiccator. The flask was weighted (W_2) again and sterol contents in mg/g as well as percentage was calculated (Huang *et al*., 2010)

$$\text{Amount of sterols (mg/g)} = \frac{X}{\text{Weight of sample}}$$

$$\text{Sterols (\%)} = \frac{X}{\text{Weight of sample}} \times 100$$

Where

X = Weight of sterols= W_2- W_1

W_1 = Weight of flask

W_2= Weight of flask+ residue

3.15.5. Total phenols determination

Material

Folin-Denis reagent, tannic acid, 20% Na_2CO_3, distilled water, tannic acid, spectronic 20D.

Procedure

The percent phenol concentration of ethanolic extracts was determined following Mc Donald *et al*. (2001). About 2 gram of ethanolic extract was treated with about 20 ml of Folin-Denis reagent, 30 ml of 20 % Na_2CO_3 and diluted by a factor 100 with distilled water. The resulting mixture was kept as such at room temperature for 30 minutes and then filtered. With Spectronic 20D (Milton Roy), the absorbance was measured at 770 nm against the blank. The total phenol content of each sample was calculated by comparing with a standard curve of tannic acid using as blank.

3.15.6. Total flavoniods determination

Total flavonoid contents were determined by following aluminum chloride colorimetric method of Chang *et al*. (2002). 1 gram of extract was dissolved in 10 ml of ethanol, from which 0.5 ml were mixed with 1.5 ml of methanol, 0.1ml of 10% Aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8ml of distilled water. After 30 minutes, the absorbance of the reaction mixture was measured at 415nm.The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to100 µg/ml in methanol.

3.16. Essential oils extraction

A Modified Clevenger type apparatus were used for the extraction of essential oil from the leaves of *Skimmia laureola* and *Zanthoxylum armatum* through hydro-steam

distillation. The leaves were thoroughly washed, cut into small pieces, placed in distillation flask and subjected to hydro- steam distillation for about 4 hours. The steam and vaporized oil were condensed into liquid by a vertical condenser and collected in measuring tube. Being immiscible and lighter than water, the volatile oil separated out as an upper layer. The oil was then separated from water and collected in small bottles, dried with anhydrous sodium sulphate, sealed, labeled and stored in light resistant vials at 4–6°C for further use (Essien *et al*., 2008).

3.17. Physicochemical characteristics of oil

Physicochemical characteristics provide a base line for suitability of oils (Parthiban *et al*., 2011). The physicochemical properties of the oil determined were color, odor, % yield, density, optical activity, refractive index, specific gravity, carbon residue, absolute viscosity, viscosity index, kinematic viscosity, total acid number, iodine number and saponification value.

3.17.1. Color determination

Color of the respective oils was determined by physical observation in day light and under ultraviolet radiation of 254 and 366 nm using ultra violet chamber (Bamgboye & Adejumo, 2010).

3.17.2. Odor determination

Odor of the respective oils was determined by organoleptic evaluation following Evans (2002).

3.17.3. Determination of percentage oil yield

The percentage oil yield was calculated by using following relation (AOAC, 2000).

$$\text{Percentage oily yield (W/W)} = \frac{\text{Weight of oil}}{\text{total weight of materials used for oil extraction}} \times 100$$

3.17.4. Determination of optical rotation

Apparatus required

Polari meter, oils samples,

Procedure

In case of optical rotation determination, 10ml Polari meter tube containing oil was placed in the trough of the instrument between polarizer and analyzer. Care was taken in filling the tube to avoid the of air bubble which could disturb the rotation of light. Analyzer was slowly turned until both the halves of the field were viewed through the telescope. The direction of rotation was determined, if the analyzer was turned counter clock wise from the zero position to obtain the final reading, the rotation is levo (-) if clock wise and dextro (+) if anti clockwise (Juliani *et al*., 2004).

3.17.5. Determination of refractive index

Apparatus required

Abe refractometer, oils samples

Procedure

The refractive index of the oil samples was determined with the help of Abbe refractometer model A 80251 (BS). Two drops of respective oil were placed on the prism with the help of syringe and the prism was firmly closed by tightening the screw head. The apparatus was allowed to stand for 5 min, after that reading was recorded from the display screen (Juliani *et al*., 2004).

3.17.6. Determination of Specific gravity

Specific gravity is the ratio of the density of a respective substance to the density of water at 4^0C (Bamgboye & Adejumo, 2010). This value is also calculated for the oils.

Materials

Specific gravity bottle, Balance, oil samples

Procedure

For the determination of specific gravity of oils, a clean 50 ml specific gravity bottle was weighted (W_0). Then the bottle was filled to the brim with water and stopper was inserted. Extra water spilled out. The water on the stopper and bottle were carefully wiped off and reweighed (W_1). Same process was repeated, but using oil samples instead of water and weighted again (W_2). The specific gravity of the all oil samples were calculated using the following formula (AOAC., 2000; Bamgboye & Adejumo, 2010)

$$\text{Specific gravity of test sample} = \frac{(W2 - W0)}{(W1 - W0)}$$

Where

W_0 = Weight of empty specific gravity bottle

W_1 = Weight of water + specific gravity bottle

W_2 = Weight of test sample + specific gravity bottle.

3.17.7. Carbon residue

A sample of known amount was taken in a silica crucible, heated strongly till the vapoures and smoke disappeared, in a sheath iron hood. The sample was then cooled down in desiccators and cooled down. Carbon residue was then calculated by the following formula (AOAC., 2000).

$$\text{Carbon residue (\%)} = \frac{W_1}{W_2} \times 100$$

Whereas W_1= Carbon residue in crucible

W_2= Weight of sample

3.17.8. Determination of viscosity

Viscosity is the resistance to the flow and it was determined by using viscometer. Viscosity plays an important role in determining the structure of liquids (Mehrotra *et al*., 1996).

Materials

Viscometer, Oil samples

Procedure

The viscosity of the respective oil was determined by using viscometer with a selection of spindle number four which was properly fixed to the holder. The container having the oil was carefully placed below the rotor holding the spindle. The spindle was allowed to immerse into the oil inside the container. The meter was turned on and adjusted to a speed of 6

m/s. then the spindle was allowed to rotate in the oil for a period of 30 min until stable reading displayed on the meter's display screen. The viscosity value of the oil was measured in centipoises (AOAC., 2000).

3.17.9. Kinematic Viscosity

Kinematic viscosity is the ratio of viscosity to density without any force involvement. It can be obtained by dividing the viscosity of a fluid with its density (Yadav *et al*., 2005)

$$\nu = \mu / \rho$$

Where

ν = kinematic viscosity

μ = absolute or dynamic viscosity

ρ = density

3.17.10. Total acid number (TAN)

The number of milligram of KOH, which are required to neutralize the free fatty acids present in one gram of oil. The acid number measures the amount of acids present in oil (Akber *et al*., 2009).

Material

Oil samples, conical flask, Methylated spirit, Potassium hydroxide, Phenolphthalein indicator, Burette.

Procedure

2.5g of oil was taken in a flask. 50 ml of methylated spirit was added to the flask, shake well and titrated against 0.1N KOH solution using phenolphthalein as indicator. Alkali was added till a pink color was established for a few seconds. The TAN was then calculated using the following formula (AOAC, 2000).

$$\text{Acid number} = \frac{(V \times N \times 56.1)}{W}$$

Where as

V = volume of potassium hydroxide used

N = normality of Potassium hydroxide

W = weight in g of the sample

3.17.11. Iodine value determination

Iodine value is a Number of grams of iodine absorbed per 100 gram of fat or oil is known as Iodine value. It is the measure of unsaturation in oil (AOCS, 1998).

Materials required.

Iodine, Carbon tetrachloride, Glacial acetic acid, Potassium iodide, Distilled water, Sodium thiosulphate, Starch, titration flask, burette etc.

Preparation of wij, s solution

Iodine values for the oil samples were determined using wij,s solution. Wij,s solution was prepared by the combination of two solutions; 0.9 gram of Iodine was dissolved in 300 ml of carbon tetrachloride (Solution A) and 8 g of iodine trichloride was dissolved in 200 ml glacial acetic acid (Solution B). Solution A and B were mixed and diluted 1000 ml with glacial acetic acid (AOAC, 1998).

Procedure

0.2 g of respective oil was weighed into a conical flask. 10 ml of carbon tetrachloride and 20 ml of the Wij's solution were added to the flask and the solution was kept in dark for 30 min at room temperature. 15 ml of 10 per cent potassium iodide solution with 100 ml of distilled water were added to the flask. The resulting solution was titrated against 0.1 M sodium thiosulphate ($Na_2S_2O_3$), using starch as indicator till the end point where the blue black coloration becomes colorless. A blank titration was carried out at the same time starting with 10 ml carbon tetrachloride. Iodine value was then calculated by the following formula (AOCS, 1998).

$$\text{Iodine number} = \frac{\{(B - S) \times N \times 12.69\}}{\text{Weight of the sample}}$$

Where

B = 0.1 N sodium thiosulfate required (ml) by blank

S = 0.1 N sodium thiosulfate required (ml) by sample

N = Normality of sodium thiosulfate solution.

3.17.12. Determination of the Saponification value

The number of milligram of KOH, which are required for the complete saponification of one gram of oil is called saponification value (Parthiban *et al.,* 2011).

Materials

Oil sample, alcoholic potassium hydroxide, phenolphthalein indicator, HCl, Titration flask, bureett etc

Procedure

2 g of each oil sample was weighted into a clean dried conical flask and 25 ml of alcoholic potassium hydroxide $(K(OH)_4$ was added. A reflux condenser was attached to the flask and heated for an hour with periodic shaking. The appearance of clear solution indicated the completion of saponification. Then 1 ml of 1 % phenolphthalein indicator was added and the hot excess alkali was titrated with 0.5 M hydrochloric acid (HCl) until it reached the end point where it turned colourless. A blank titration was carried out at the same time and under the same condition. The Saponification value was calculated as (AOAC, 2000).

$$\text{Saponification value} = \frac{b-a}{m} \times 8.05$$

Where

b = 0.5 N HCl required (ml) by the blank

a = 0.5 N HCl required (ml) by the sample.

3.18. Statistical Analysis of physicochemical analysis

During all the physicochemical analysis of respective oil, chemicals of analytical grade and calibrated glassware were used. All the procedures were carried out in triplicate and the recorded data were reported as mean ± SEM (Steel *et al*., 1997).

3.19. Gas Chromatography- Mass Spectrometery (GC-MS) of essential oil.

The essential oil from the leaf of *Skimmia loureola* and *Zanthoxylum armatum* and fixed oil (extracted with n-Hexane and petroleum ether) from fruit of *Zanthoxylum armatum* were analyzed by GC-MS Model QP 2010 plus ((Shimadzu) operating in EI mode at 70 ev equipped with a split-splitless injector (Split ratio, 1:50 was used). Helium was used as carrier gas with flow rate of 1ml/min. A capillary column (Length: 30 m, id: 0.25 mm, thickness: 0.25µm, DB-5MS Agilent technologies, USA) treated with 95 % dimethyle and 5 % diphenyle poly silphenylene. Dichloromethane HPLC grade was used as reagent. The following conditions were maintained during the operation of GC-MS analysis.

Inject temperature: 240 ^{0}C

Ion source temperature (EI): 240 ^{0}C

Interface temperature: 240 ^{0}C

Pressure: 80 KPa

GC program time: 46.67 minutes total

Solvent cut time: 2.5 minutes

MS start time: 3 minutes

MS end time: 46 minutes

Acquisition time: scan

M/Z: 40-500

The process was programmed as per temperature condition given in the table 3.2.

Table 3.2. Column oven programming for GC-MS analysis of essential oils

Rate (^{0}C/min)	Temprature(^{0}C)	Hold (minutes)
-	40	0
3	90	0
10	240	15

Sample preparation:

About 40 mg of diluted essential oil sample was accurately weighed, mixed with 2 ml of dichloromethane and filtered through 0.45µm-membrane filter. One µl of the filtered materials was injected into GC- MS using auto injection system (AOAC, 2000)

Identification of the components

The identification of the constituents was based on comparison of their retention times (RT) and mass spectra of the samples with those obtained from standards used. Relative percentage of compounds was calculated from the total chromatogram by computer (AOAC, 2000)

3.20. GC-MS analysis of fixed oil

Chemical analysis of the fixed oil from the fruit of *Zanthoxylum armatum* was carried through GC-MS. Gas chromatograph–mass spectrometry system, operating in EI mode at 70 ev equipped with a split-splitless injector (Split ratio, 1:50was used). A capillary column (Length: 30 m, id: 0.35 mm, thickness: 0.25µm, TRB- FFAP, Technochroma) treated with poly ethylene glycol. The following conditions were maintained during the operation of GC-MS analysis.

Reagents

Boron trifloride solution: 10 % in methanol

Sodium hydroxide solution: methanolic; 0.5 N

Hexane: HPLC grade

Methanol: HPLC grade

Tridecanoic acid methyle ester (Internal standard)

Sodium chloride solution in water: saturated

Fattyacids methyle esters (FAMES) standard: 37 components(External standards)

GC- MS conditions

Inject temperature: 240 ^{0}C

Ion source temperature (EI): 250 ^{0}C

Interface temperature: 240 ^{0}C

Pressure: 70 KPa

Acquisition time: scan

M/Z: 85-380

The process was programmed as per following temperature conditions (Table 3.3).

Table 3.3. Column oven programming for GC-MS analysis of fixed oils

Rate (^{0}C/min)	Temperature (^{0}C)	Hold (minutes)
-	50	1
15	150	0
2.5	175	5
2.5	220	5

Standards preparation

a. Internal standard (Tridecanoic acids methyle ester)13.7 mg/ml in hexane

b. External standards: 10 mg of ampoule of 37- components FAMES mix standard, (Sigma, USA) diluted to 10 ml with dichloromethane.

Sample

About 25 mg of oil accurately weighed up to 0.1 ml of internal standard (equivalent to 1.37 mg of tridecanoic acid methyle ester) and 1.5 methanolic sodium hydroxide solution(0.5N) was added to a 20 ml test tube. The test tube was sealed with ateflon-lined cap and heated on boiling water bath for 5 minutes to hydrolyze and after cooled. 2.5 ml of boron trifloride solution (10% in methanol) was added. The test tube was again sealed with cap and heated in boiling water bath for 30 minutes with occasional shaking. The esterified solution was cooled and 5 ml of saturated sodium chloride solution was added to decrease the solubility of FAMES and to facilitate extraction. The solution was extracted twice with I ml hexane (1ml each) and filtered through 0.45µm membrane filter. Then one µl of this filterate was injected to GC-MS using auto injection system (AOAC, 2000)

3.21. Pharmacology

The following pharmacological studies were carried out, in order to know the therapeutic potential of *Skimmia laureola* and *Zanthxylum armatum*.

3.21.1. Acute toxicity test

Animals

BALB/c mice (20 to 25 g) of either sex were used in the experiments for evaluating acute toxicity of *Skimmia laureola* (leaves) and *Zanthxylum armatum* (leaf and fruit) following Danmalam *et al*. (2009). The animals were kept in cages, housed under standard condition of 12: 12 h light/dark cycle, fed with NIPRD formulated food and had water *ad libitum* and were acclimatized for 14 days to the laboratory conditions prior to experiments. Before start of experiments, all the animals were deprived from food overnight with free excess to tap water and were distributed randomly into the following ten groups, each with six animals.

Group-I Treated with saline water

Group-II Treated with ZLE 500 mg/kg

Group-III Treated with ZLE 1000 mg/kg

Group-IV Treated with ZLE 2000 mg/kg

Group-V Treated with ZFE 500 mg/kg

Group-VI Treated with ZFE 1000 mg/kg

Group-VII Treated with ZFE 2000 mg/kg

Group-VIII Treated with SLE 500 mg/kg

Group-IX Treated with SLE 1000 mg/kg

Group- X Treated with SLE 2000 mg/kg

Animals were observed continuously for the first 4 hours and then for the next 24 hours for any toxic symptom.

3.21.2. Antipyretic activity

The antipyretic activity of *Zanthoxylum armatum* leaves and fruits was evaluated using BALB/c mice (25–30 g) of either sex. The animals were acclimatized for 14 days with laboratory condition prior to the experiment. All the animals were divided into seven groups, with each group comprising six animals. All groups were fasted overnight while allowing them free accesses to drinking water (Taesotikul *et al*., 2003).

Yeast-induced pyrexia

Normal temperature was recorded for all experimental animals using digital thermometer. The animals were then made hyperthermic by subcutaneous injection of 20% aqueous suspension of Brewer's yeast in 0.9% saline solution at the dose of 10 mL/kg (Patra *et al*., 2009). After 18 hours yeast injection, the rectal temperature was recorded and only those animals were selected for the experiment which showed a rise in rectal temperature of more than 1.2 °C. The corresponding groups were injected subcutaneously with respective treatment as given below.

Group-I	Treated with saline water
Group- II	Treated with paracetamol 150 mg/kg
Group-III	Treated with ZLE 100 mg/kg
Group-IV	Treated with ZLE 200 mg/kg
Group-V	Treated with ZLE 300 mg/kg
Group-VI	Treated with ZFE 100 mg/kg
Group-VII	Treated with ZFE 200 mg/kg
Group-VIII	Treated with ZFE 300 mg/kg

Rectal temperature was recorded periodically at 1, 2, 3, 4 and 5 hours of drugs administration.

The percent reduction in pyrexia was calculated by the following formula

$$\text{Percent reduction} = \frac{B - Cn}{B - A} \times 100$$

Whereas

A = normal rectal temperature

B = Temperature after yeast injection

Cn = body temperature after 1, 2, 3, 4 and 5 hours (Taesotikul *et al*. 2003).

Statistical analysis

The experimental results were represented as mean ±SE (standard error of the mean). One way ANOVA followed by Tukey-Kramer posthoc test was used for evaluation of data and $p<0.05$ accepted as significant (Barkatullah *et al*., 2011).

3.21.3. Hypoglycemic activity

Hypoglycemic activity of the crude ethanolic extract of leaf of *S. laureola* was carried out following Atta-Ur-Rahman *et al*. (2001) and Danmalam *et al*. (2009).

Materials:

Alloxan monohydrate (Sigma Chemical Co., USA)

Glibenclamide (Sanofi-aventis Pakistan limited)

Carboxy methyle cellulose (CMC)

Xylene

Adult healthy local breed rabbits (1200-1500g)

Wooden rabbit holder

Stainless steel feeding needles

Distilled water

Disposable syringe 1cc

Cotton

Standard

Glucometer (Glucotrend, Boehringer, Roche, Germany)

Glucose Strips (Accutrend glucose, Roche, Germany)

Induction of diabetes in Rabbits

About forty rabbits were made diabetic by intra venous injection of 100mg/kg body weight alloxane monohydrate through marginal ear vein, which were made swollen for injection by applying xylose solution (Wang *et al.,* 2003). After a week, blood glucose level of all rabbits was determined using glucometer and rabbits with fasting blood glucose level higher than 120 mg/dl were selected for the study.

Rabbits grouping

Each of the normal and diabetic rabbits were randomly divided into five groups of five animals each. To each group, drug was administered orally, slow and steadily using stainless steal needle attached to plastic syringe and inserted into the stomach through esophagus. All groups were fasted overnight while allowed free accesses to drinking water prior to experiments.

Group I: non diabetic and feed orally with 10 ml of 1% CMC in water (Negative control).

Group II: diabetic and feed with glibenclamide (100mg/kg) dissolve in 1% CMC in water (Positive control).

Group III: diabetic and feed with SLE(100mg/kg) dissolve in 1% CMC in water.

Group IV: diabetic and feed with SLE (200mg/kg) dissolve in 1% CMC in water.

Group V: diabetic and feed with SLE (400mg/kg) dissolve in 1% CMC in water

Blood sampling and determination of blood glucose level

Blood glucose level of all groups was determined at 0, 2, 4, 6, 8 and 12 hours after administration of dose. Rabbit was held in wooden rabbit holder and pricking ear marginal

vein with blood lancet. The oozed blood drop was applied on the strip and glucose level was determined with glucometer.

Statistical analysis

The data were also subjected to two way analysis of variance (ANOVA) and differences between samples were determined by t-test using the Graphpad prism5, Germany, Germany. P Values < 0.05 were regarded as significant. P Values >0.05 were regarded as non-significant (Khan *et al.,* 2009).

3.21.4. Antispasmodic activity

Ethanolic and n- hexane extracts of *Skimmia laureola* (Leaves and bark) and *Zanthxylum armatum* (leaves, bark and fruit) and essential oil of leaves of both the plants were evalvated for antispasmodic activity.

Drugs and other Chemicals

Acetylcholine (BDH Chemicals, Poole, England), Potassium chloride (E. Merck Germany), Tyrode's solution (Prepared from its Constituents with their respective concentrations (mM) NaCl 136.9, KCl 2.68, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.90, $CaCl_2$ 1.8, and glucose 5.55 dissolved in 1 letter distilled water).

Animals

Local breed rabbits of either sex with weights in the range of 1.0-1.4 kg were used. The animals were kept for 14 days before starting the experiments at the "Animal House of Department of pharmacy, University of Malakand" under standard conditions mentioned in the "Animals Bye-Laws 2008 of the University of Malakand (Scientific Procedures Issue-1)." and fed on standard diet and tap water. The animals were kept in fasting condition 24 h prior to the start of experiments with free excess to water.

Rabbit's jejunum preparations

Experiments on rabbit's jejunum preparations were carried out following Gilani *et al*. (2005). Slaughtered animals were dissected to open abdomen and jejunum portion(s) were extracted and kept in freshly prepared Tyrode's solution, aerated with carbogen gas (5% Carbon dioxide and Oxygen mixture) to keep them alive and ready for use. Quiescent

sub-maximal doses of acetylcholine (0.3 µ M) to the tissues were used when needed for keeping the tissue viable and alive. (Qayum, 2004). About 1.5 cm length tissue was mounted in 10 ml tissue bath containing Tyrode's solution and stabilized for 25-30 minutes. All the processes were carried out at 37+ 1^0C with constant aeration and kept under 1 gram pressure. On attaining reproducible response, test samples at the doses of 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 5.0, and 10.0 mg/ml were applied to the bath solution (Gilani *et al*., 2005; Ali *et al*., 2009). The processes were repeated thrice (n=3) and fall in spontaneous activity was observed to be change of the sample tested. For the determination of possible mode of action, the tissue was pretreated with high concentration of KCl (80 mM in final bath solution). KCl cause depolarization and keep the tissue in a position of sustained contraction (Farre *et al.,* 1991). The extract was then applied in cumulative manner to obtain a dose dependent curve and relaxation. Intestinal responses data were recorded using Force Transducer (Model No: MLT 0210/A Pan Lab S.I.) attached with Power lab (Model No: 4/25 T) AD Instruments, Australia. Data was recorded at range of 20 mv, low pass 5 Hz X 10 gain using input 1, rate 40/S (Ali *et al*., 2009). Results were expressed as % of KCl induced contraction (Van Rossum, 1963).

Statistics and interpretation

Chart 5 (AD Instruments) was used to interpret the graph tracings. Student "t" test was used at 95 % confidence interval (CI). 'P' values less or equal to 0.05 was considered as statistically significant (Ali *et al*., 2009).

3.21.5. Cytotoxicity

The preliminary cytotoxic potential of the ethanolic and n-hexane extracts and essential oil from the leaves of *Skimmia laureola* and *Zanthoxylum armatum* were carried out using brine shrimp assay by following the method of Attaurrahman *et al*. 2001.

Requirement

Test sample *Artemia salina* (shrimps eggs), sea salt solution (38 g/L of distilled water, pH 7.4), hatching tray with perforated partition, illuminating source (lamp) to attract brine-shrimp larvae, magnifying lense, Pasture's peppete, micro pipette (5, 50,500µl), vials tray, vials, ethanol and n- hexane.

Storage of *Artemia salina* eggs

Brine shrimp eggs are viable for years if placed under low temperature. For the present study these eggs were stored at low temperatures (4°C).

Hatching techniques

The hatching tray (a rectangular dish (22x32 cm) was filled half with filtered brine solution. The tray was partitioned into two parts with perforated partition. One part was sprinkled with 25 mg of brine shrimp eggs powder and covered with black paper. The other half was left open. The hatching tray was kept at room temperature for hatching of eggs. A lamp was suspended above the tray so as to illuminate the open part of the tray. After hatching the napualli were observed to move through the perforated partition towards the enlightend part.

Sample preparation

20 mg of an extract was dissolved in 2ml of respective solvent and from the solution 5, 50 and 500 µl were transferred to vials (3 vials /concentration) which was equivalent to 10, 100 and 1000 µg/ml respectively. The solvent was allowed to evaporate overnight and 5 ml seawater water solution (38 gram/L) was added to each vial. After 60 hours of hatching and maturation as nauplii, 10 larvae were transferred to each vial using a Pasteur pipette. The vials were then placed at room temperature (25-27 °C) under illumination. For negative and positive controls, vials filled with brine solution; reference cytotoxic drug respectively were used.

Statistical analysis.

After 24 hours the number dead and live napualli was counted and the data was analyzed with Finney computer program to determine LD_{50} values with 95% confidence intervals (Saeed *et al*., 2010; Barkatullah *et al*., 2011).

3.21.6. Phytotoxicity Activity

The phytotoxic activity of *Skimmia laureola* (leaves and bark) and *Zanthoxylum armatum* (leaves, bark and fruits) and essential oil from the leaves of both plants was evaluated using *Lamna minor* as test species following (Atta-Ur-Rahman *et al*, 2001).

Requirements

Lemna minor (test species), distilled water, flask 250 ml, micropipette (10-100 µl, 100-1000 µl), test samples (extracts), filter paper, oven, flasks, E- medium, luminar flow hood, glass vials, brush etc.

Preparation of media

E-medium was prepared from different mineral nutrients with specific amount (Table 3.4) for *Lemna minor* assay. The salts were first weighed in the required amount dissolved in distillated water and the volume was mad up to 1000 ml. The pH was adjusted between 5.5-6.00 by addition of KOH.

Table 3.4. Composition of E-medium.

S. No	Chemical Name	g/L
1.	Potassium dihydrogen phosphate (KH_2PO_4)	0.68
2.	Potassium nitrate(KNO_3)	1.515
3.	Calcium nitrate ($Ca(NO_2)_2.4H_2O$)	1.180
4.	Magnesium sulfate ($MgSO_4.7H_2O$)	0.492
5.	Boric acid (H_3BO_3)	0.00286
6.	Manganous chloride ($MnCl_2.4H_2O$)	0.00362
7.	Ferric chloride ($FeCl_2$.4H2O)	0.00540
8.	Zinc sulfate ($ZnSO_4$.5H2O)	0.00022
9.	Copper sulfate ($CuSO_4.5H_2O$)	0.00022
10.	Sodium molybdate ($Na_2MO_4.2H_2O$)	0.00012
11.	Ethylene diamino tetra acetic acid (EDTA)	0.01120

Procedure

15 mg of respective extract was dissolved in 1.5 ml of the respective solvent and from this solution 5, 50 and 500 µl were transferred to the sterilized flask (3 flask for each concentration), which was equivalent to 10, 100 and 1000 µg/ml respectively. The solvent were allowed to evaporate overnight under sterilized conditions in laminar flow. 20 ml of E. medium was added to each flask.Other flasks (3 for each) which were supplemented with E. medium and standard drug (Atrazine) served as negative and positive controls. To each flask ten plants with 2-3 fronds were transferred and were kept under about 12 hours day length conditions. Plants were observed daily and on the seventh day the numbers of fronds were counted. Percent growth inhibition was recorded with reference to the negative control using the following formula

$$\text{Inhibition \%} = 100 - \frac{\text{Number of fronds in test sample}}{\text{Number of fronds in negative control}} \times 100$$

Statistical analysis.

The phytotoxic results were analyzed with Finney computer program to determine FI_{50} values with 95% confidence intervals (Saeed *et al*., 2010; Barkatullah *et al*., 2011).

3.21.7. Anti bacterial activities

Anti bacterial screening was conducted for ethanolic and n- Hexane extract of *Skimmia laureola* (leaves and bark) and *Zanthoxylum armatum* (leaves, bark and fruits) and essential oil from the leaves of both the plants, following Carron *et al*., (1987) .

Requirments

Test organism (Table 3.5) obtained from PCSIR labs, Lahore Pakistan), test samples, Nutrient broth, nutrient agar, petri dishes, micropipettes, sterile cotton swab, sterile cork borer, Standard antibiotics (imipenem), test tubes, incubator, Laminar flow hood

Table 3.5. Bacterial cultures used for antibacterial screening.

S. No	Bacterial strain	Nature
1	*Micrococcus leutus*	Gram positive
2	*Escherichia coli*	Gram negative
3	*Staphylococcus aureus*	Gram positive
4	*Pasturella multocidia*	Gram negative
5	*Pseudomonas aeruginosa*	Gram negative
6	*Bacillus subtilis*	Gram positive
7	*Streptococcus viridines*	Gram positive

Procedure

Antibacterial activities of the plant were carried out by agar well diffusion method as described by Carron *et al*. (1987). Bacterial strains were first cultured on nutrient broth and incubated for 24 hours prior to experiments. Nutrient agar was melted, cooled to 40°C and poured into sterilized petridishes. Wells were then bored in media using 6mm diameter with

the help of sterile metal cork borer and keeping a distance of 24mm between two adjacent wells. 4-8 hour old bacterial culture was spread on the surface of nutrient agar with the help of sterilized cotton swab. These processes were repeated thrice turning the plate 60° between each streaking. About l00 µl of 3mg/ ml of respective extract, dissolved in DMSO was then added to the wells. Other wells were supplemented with DMSO and 10µg Imipenem served as positive and negative controls. The plates were then incubated for 24 hours at 37°C. The plates were then observed for zones of inhibition. All the experiments were conducted in triplicate.

Minimum Inhibitory Concentration (MIC)

MIC was estimated for those extract, which showed significant antibacterial activity. The MIC estimation of an extract was determined following Tolulope (2007). 1 ml of the respective extract solution with 20 mg/ml concentration was added to 1 ml of pre sterilized nutrient broth. Subsequently, 1 ml from the first test tube was transferred to the second test tube containing 1 ml of nutrient broth and this process was continued up to the seventh test tube. Thereafter, 1 ml of 24 hours culture of each test bacterium (1.0 x 10^{6}cells/ml) was inoculated into each of the seven test tubes and mixed thoroughly. The test tubes were incubated at 37°C for 24 hours. The MIC was taken as the lowest concentration that prevented the growth of the test bacterium, as indicated by absence of cloudiness of the content.

3.21.8. Anti-fungal activities

Anti-fungal screening of ethanolic and n- Hexane extract of *Skimmia laureola* (leaves and bark) and *Zanthoxylum armatum* (leaves, bark and fruits) and essential oil from the leaves of both plants were conducted using agar dilution method following Sridhar *et al*. (2003) and Parekh & Chanda (2008).

Material

Seven days old fungal cultures (PCSIR labs Lahore Pakistan), test samples, sabouraud dextrose agar, Dimethyle sulphoxide (DMSO), screw cap test tubes, micropepites, autoclave, incubator, standard antibiotic (Miconazole).

Test fungi

Trichophyton longifusis	*Candida albicans*	*Fusarium solani*
Microsporum canis	*Aspergillus flavus*	*Candida glabrata*

Methodology

Twenty four mg of crude extract was dissolved in 1ml sterile dimethyl sulfoxide (DMSO) serving as a stock solution. 4 ml sabouraud dextrose agar (SDA) growth media was transffered to each screw capped tube under sterile conditions and autoclaved at 121^0C for 15 minutes. These tubes were then allowed to cool to 50° C and 400µg/ml test sample was added to nonsolidify SDA tubes, which were then allowed to solidify at room temperature. Next each glass tube was inoculated with 4 mm diameter piece of inoculum removed from 7 days old fungal culture, where as agar streak was employed in case of non-mycelial growth. Other media supplemented with DMSO and miconazole antibiotic were used as a negative and positive control respectively. The tubes were incubated at 28±1°C for 7 days. Cultures were observed twice weekly during incubation. Growth in the media was estimated by measuring linear growth (mm) in the in media loaded with sample, DMSO and miconozole respectively and then percentage inhibition of fungal growth was calculated as follows (Umadevi *et al*., 2003; Ali-Shtayeh & Suheil, 1999).

$$\% \text{ Mycelia inhibition} = \frac{Gn - Gt}{Gn} \times 100$$

Where

Gn= Mycelial growth in normal

Gt= Mycelial growth in test

OBJECTIVES OF THE STUDY

Following objectives are achieved through the present research studies on *Skimmia laureola* and *Zanthoxylum armatum*.

- Habit, habitat, physical and chemical characteristics of the soil in which these two plants grow are determined, which will provide guide line for commercial cultivation of these medicinal plants.
- Medicinal and economic significance of these plants are brought to lime light.
- Morphology of the parts used as drugs were carried out for correct identification.
- Leaf features including occurrence, type, size of stomata, stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio are detemined. Anatomy of other parts i.e. bark of both plants and fruit of *Z. armatum* is also explored which will be helpful in correct identification of the drugs.
- Organoleptic and microscopic features of the powder drugs have been worked out, which are necessary for determination of quality and purity of powder drugs and to detect adulteration in powder drugs.
- Physicochemical analysis, including ash analysis, fluorescence characteristics, bio elemental and nutritional composition of powder drugs are carried out.
- Microchemical screening tests for detection of various secondary metabolites like carbohydrates, alkaloids, proteins, fatty materials, saponins, anthraquinones derivatives and tannins etc is carried out. Flavonoids, phenol, alkaloids, tannins, saponin and sterol were also quantified in the studies of these plants.
- Physicochemical and GC-MS analysis of essential oils and fixed oils of these two medicinal plants were carried out.
- Pharmacological potentials like antipyretic, hypoglycemic, antispasmodic, cytotoxic, phytotoxic, antibacterial and antifungal activities of these plants are determined in the present work.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Morphology of the research plants

4.1.1. Morphology of *Skimmia laureola*

Skmmia laureola grows gregariously in shaddy forest at altitude ranged from 7000 to 8800 feet. It is woody shrub up to 1 meter tall with grayish green stem which is dichotomously branched. Leaves are simple lanceolate or sublanceolate with short petiole and entire margins and arranged in whorls with terminal cluster. Leaf ranges from 25 to 80mm in length and 16 to 30mm in width. Adaxial surface shinny dark green with translucent oil glands. Lower surface is light green. Midrib prominent with reticulate venation. Flower small, sessile or subsessile, greenish white in color. Flowers polygamous i.e. both unisexual and bisexual, calyx; 5 sepals, corolla; 5 petals, androcium; 5 stamens, gyoecium; 2-5 carpels. Fruit bright red ovoid berry (Fig. 4.1).

These characteristic are diagnostic taxonomic features for identification of *Skmmia laureola*. More or less similar description was also given by Hassan-Ud-Din & Ghazanfar (1980) and Polunin & Stainton (1984), which confirmed the present taxanomic observations.

4.1.2. Morphology of *Zanthoxylum armatum*

Zanthoxylum armatum grows in sunny or semi shady places at altitude of 2800 to 4700 feet. It is spiny shrub or small tree. Stem is greyish brown and provided with spines. Leaves imperpinnate compound, winged petiolate with three to eleven leaflets. Leaflets sessile to subsessile with acute apex and slightly incised margins having central prominent vein and reticulate venation. Leaflets are from 20 to 80 mm in length and 12 to 25mm in width. Adaxial surface slightly shinny, dark green with translucent oil glands. Lower surface is light green. Flower small, sessile, yellowish white in color. Flowers polygamous i.e. both unisexual and bisexual, calyx; 6-8 sepals, corolla; absent, androcium; 6-8 stamens, gyoecium; 2-3 carpels. Fruit is red colored drupe, splitting into two when ripe. Seeds are rounded and black shining (Fig 4.2).

These observed features are of taxonomic characterization taxonomically and could be used for proper identification of *Z. armatum*. This plant was described with more or less similar features by Hassan-Ud-Din & Ghazanfar (1980) and Polunin & Stainton (1984), which confirmed the present observation.

4.2. Phytosociology

The structural property of a community is the quantitative relationship between the species growing around. The quantitative study of vegetation is called phytosociology and its principal aim is to describe vegetation, explain or predict its pattern and classify it in a meaningful way (Ilorkar & Khatri, 2003). There is a gradual decrease in flora richness which might be due to overexploitation of resource base and degradation of vegetation (Qureshi, 2008). Forests are unstable and degraded and would be vanished if not maintained properly. The original vegetation structure has been altered due to deforestation and overgrazing and there is a need for restoration of habitat (Siddiqui *et al.,* 2009).

A lot of work has been done on the phytosociological studies of communities in various parts of Pakistan but very little information is available on the phytosociological attributes of a single species (Khan *et al*., 2010). Some of the previous work reported in this respect is on *Olea ferruginea* (Ahmad, 2007)*, Picea smithiana* (Wahab *et al*., 2008), and *Quercus baloot* (Khan *et al*., 2010). In the present study phytosociology of two ethno botanically important plants i.e. *Skimmia laureola* and *Zanthoxylum armatum* was carried out.

4.2.1. Phytosociology of *Skimmia laureola*

Skimmia laureola is a shrub growing gregariously in shaddy forests floor at altitude ranging from 5500 to 10000 feet (Hassan-Ud-Din & Ghazanfar, 1980). It is an important medicinal plant which is locally considered sacred and is used for various ailments (Hamayun *et al*., 2006). The smoke produced from burning of dry leaves is used in curing nasal tract infections, cold, fever and headache. The dried leaves are also used as disinfectants, insecticide and pesticide (Qureshi *et al*., 2009).

Six different localities in the natural habitate of Khyber Pukhtunkhwah, Pakistan were selected for phytosociological study of *S. laureola* (Table 4.1). This plant is

distributed at the north facing slope at the elevation ranging from 7000 to 8800 feet. Hamayun *et al*. (2006), Hussain *et al*., (2008c) and Qureshi *et al*. (2009) also reported it from the same altitudes, strengthening the present findings.

A total of 44 species were present in association with *Skimmia laureola* in different localities. Seven species including *Adiantum venustum, Fragaria vesica, Indigofera heterantha, Isodon rugosus, Podophyllum hexandrum, Pteridium aquilinum* and *Taxus baccata* were found to be the constant species in all stands. Four species, *Abies pindrow, Barberis lyceum, Rubus fruticosus* and *Voila odorata* were the mostly present species, while six species including *Bergenia himalaica*, *Pinus wallichiana, Ranunculus laetus, Rumex dentatus, Trifolium repens, ,* and *Valeriana jatamansi* were the often present species in association with *S. laureola*. The associated flora also includes 14 seldom and 12 rare species (Table 4.2).

Dominant plants in all sites were determined on the basis of importance value. *S. laureola* was found dominant in all sites except one where it was third dominant species (Table 4.3). The average importance value for *S. laureola* was 26.88, with average importance value index of 8.96. Maximum IV was 48.89 at Bahrain, District Swat while minimum IV was 4.86 at Tajaka, Barawal Upper Dir. Associated shrubs found in co-dominance with *S. Laureola* were *Indigofera heterantha, Isodon rugosus,* and *Rubus fruiticosus* with variable importance values (Table 4.3). The number of herbaceous species in each stand ranged from 12-19, showing that the ground flora exhibited somewhat variation. Among the herbaceous flora *Adiantum venustum, Fragaria vesica, Podophyllum hexandrum* and *Pteridium aquilinum* were the constant species with IV of 12.33, 38.36, 3.74 and 29.67 respectively. *Voila odorata* was the only species that fell in "mostly present" class of Raunkiaer's constancy classes. Most of the herbs were categorized as seldom and rare species having narrow range of distribution with *S. laureola*. Five trees were found in all localities in association with *S. laureola,* in which *Taxus baccata* was the only constant tree species. *Abies pindrow* was found to be mostly present while *Pinus wallichiana* was oftenly present spp. *Picea smithiana* and *Cedrus deodara* were the seldom and rarely present species in all the localities. *Cedrus deodara,* although present in only one stand had the highest importance values of 13.48 among the tree species. Similar

phytosociological studies have also been conducted for *Chenopodium album* (Malik, 2004), *Olea ferruginea* (Ahmed *et al*., 2009a), *Pinus roxburghii* (Siddique *et al*., 2009) and *Quercus baloot* (Khan *et al*., 2010) with more or less similar results.

Ahmad *et al*. (1990) has reported density hectare^{-1} values (54-154 plant/ hectare) for *Juniper* forest of Rodhmallazi, Baluchistan. Similarly Siddique *et al*. (2009) recorded density hectare^{-1} values for *Pinus gerardiana,* ranging from 24 to 930 plants. In the present study density hectare^{-1} values were recorded for *S. laureola* which showed quite large values, ranging from 312 to 4437.5 (Fig. 4.3). A highest value was found in Bahrain, Swat while lowest value was recorded from Tajaka-Barawal, Upper Dir. As compared to other sites, Tajaka, Upper Dir was under intense biotic pressure because *S. laureola* is uprooted for commercial purposes and being grazed by goats during intense cold season. Because of these reasons this important plant is near to extinction in this area. Haq (1983) has reported *S. laureola* as endangered species in many localities of District Batagram, Pakistan. Similar situation might be occurring in other areas including the present research sites, as population size of this important plant is decreasing day by day due to habitat loss, over exploitation and overgrazing.

Regression analyses were carried out to find out Correlation of altitude with Density hectare^{-1}, importance values and importance value indices. Very less significant correlation (r^2 = 0.03393, P>0.001) was found between altitude and Density hectare^{-1}. Similarly weak correlation was observed between importance values (r^2= 0.1214, P>0.001) and importance values indices (r^2 = 0.3193, P>0.001). Significant correlation was found present between density hectare^{-1} and importance values (r^2 = 0.9636, P < 0.001) for *S. laureola* (Fig. 4.4). These results are ae well in accordance and supported by similar findings of Siddique *et al*. (2009) and Khan *et al*. (2010).

Soil analysis

Soil analysis of the six stands of S. *laurola* growing in understory was carried out for physical, chemical and biological properties. Soil profile, soil pH and various nutrients were detected as their presence in soil affect the growth and distribution of plants (Sharma & Kumar, 1991). Texture of the soils was found clay loamy in four sites while loamy in the

other two sites (Table 4.4). Nitrates contents were varied from 22.45 to 40.52 ppm. Phosphorus was found maximum in Bahrain Swat and Tjaka, Upper Dir while in other areas it has variable amounts. Similarly K, Fe, Zn and Cu were also detected in variable amounts in different localities. The pH of the soils ranged from 6.04 to 6.10 showing that *S. laureola* grows in weak acidic soil with organic contents ranging from 1.12 to 1.22 (Table 4.4). Similar type of soil analysis was also carried out by Khan *et al*. (2010), who investigated physicochemical characteristic of *Quercus baloot* forest soil in Chitral. The present results are also in lineage with Rashid *et al*. (2011), who characterized soils from various spots of the surroundings of Malam Jabba, District Swat.

4.2.2. Phytosociology of *Zanthxylum armatum*

Zanthoxylum armatum is a shrubby or small tree in nature, found in mixed community with other plants. Ethnobotanically it is considered very important in the study area, being used as hedge, as fuel, for offensive and defensive stick making and for various health problems especially in stomach problems (Barkatullah *et al*., 2009). Six localities in Khyber pukhtunkhwah, Pakistan (Table 4.5) were studied to explore its phytosociological relations. *Z. armatum* was found growing on the north and North West slopes at an elevation of 2800-4650 feet. This finding is well in accordance with the reports of Iqbal & Humayun (2005), Kala *et al*., (2006) and Gaur (2008), who also reported *Z. armatum* from this elevation range.

A total of 51 species including 12 trees, 14 shrubs and 25 herbs and grasses were recorded in association with *Z. armatum* in all the stands. *Ailanthus altissima*, *Ajuga bracteosa*, *Cynodon dactylon*, *Dicanthium annulatum, Dodonaea viscosa, Olea ferruginea* and *Rubus fruiticosus* were the constant species while *Artemisia scoparia* and *Conyza canadensis* were the mostly present species with *Z. armatum*. 12 species were found to be often present, 3 species were seldom and rest of the 25 species were the rare species found in only one stand (Table 4.6). More or less similar study on constancy character of vegetation was also conducted by Fakhar-i-Abbas *et al*. (2009) in Grey goral range of Pakistan and Azad Kashmir. Mian (2003) worked on phytosociological analysis and

habitate in Balochistan, Pakistan and Shah & Hussain (2009) on the phytosociology of Hayatabad, Peshawar.

On the basis of importance value ranking, *Z. armatum* was found dominant in one site, while in rest of the sites, it was the second dominat species among the shrubby plants (Table 4.7). Various numerical values for *Z. armatum* were worked out, which include average importance value (16.09) and average importance value index (5.38). Other shrubby species found in co-dominance were *Cotoneaster nummularia, Dodonaea viscosa, Gymnosporia royleana, Indigofera heterantha, Justicia adhatoda* and *Rubus fruiticosus* with variable importance values (Table 4.7). The herbs and grasses ranged in number from 12-14 in all the stands showing a pattern of rich ground flora. *Dicanthium annulatum* was recorded in five stands, was the most dominant ground specie with average importance value of 49.39, followed by *Heteropogon contortus,* recoreded in three stands having average IV of 30.34 and *Sonchus asper,* found in just one stand with IV of 24.90. *Datura innoxia*, *Plantago lanceolata, Oxalis carniculata* and *Parthenium hysterophorus* were also observed with comparatively high importance values (20.34, 23.19, 20.32 and 20.40 respectively). Although *Cynodon dactylon* was observed to be constant species with *Z. armatum,* but had a comparatively lower IV (16.10) as compared to other grasses. In the ground flora, just threppe species i.e. *Ajuga bracteosa, Cynodon dactylon* and *Dicanthium annulatum* were found 'constant species' according to Raunkiaer (1934) constancy classification. It was observed that ground flora was variable in all stands with variable importance values. 5-7 tree species were present in association with *Z. armatum*, in which *Ailanthus altissima* and *Olea ferruginea* were the constant species with average IV of 10.88 and 17.69 respectively. *Celtis australis*, *Melia azedarach* and *Reptonia buxifolia* were in class III of the constancy classes. *Ficus elastica*, *Ficus racemosa*, *Robinia pseudocacia* and *Zizypus oxyphylla* were the rare species. These results are well in accordance with the findings of Ahmed *et al*. (2009), Siddique *et al*. (2009) and Khan *et al*. (2010a), who also described associated flora and phytosociological postion of *Olea ferruginea*, *Pinus roxburghii* and *Quercus baloot* respectively.

Density hectare^{-1} values were also recorded for comparison in all the stands for *Z. armatum* which ranged from 560 to 1020. Highest value was found in Peto dara, lower Dir

while lowest value was recorded from Tor Warsak, District Buner (Fig 4.5). Similar results have also been reported by other workers. Ahmed *et al*., 1991 reported density values of *Pinus gerardiana* (24 to 930 trees ha^{-1}), Similarly Ahmed *et al*. (1990) reported density of 54 to 154ha^{-1} for Juniper forest of Rodhmallazi, Baluchistan. Siddique *et al*. (2009) reported variable density value for *Pinus roxburghii* in lesser Himalayan and Hindu Kush range of Pakistan. A comparison of the present study with the above workers proves that our study is well in accordance with previous studies.

Correlation of altitude with Density hectare-1, importance values and importance value indices was worked out through linear regression analysis. Little significant correlation was found between altitude and Density hectare-1(r^2= 0.3615, P>0.001). Weak correlation was found with Importance values (r^2= 0.0613, P>0.001) and importance values indices (r^2= 0.1676, P>0.001). Similarly there was no significant correlation present between density $hectare^{-1}$ and importance values (r^2= 0.0.01794, P>0.001) (Fig. 4.6). Similar results have been reported by that of Siddique *et al*. (2009) and Khan *et al*. (2010), which strengthen the findings.

Soil analysis

Physical and chemical characteristics of a soil are the indication of habitat for an organism. The forest land exhibit variation due to difference in available natural resources such as parent materials, vegetation litter and other physicochemical characteristics. Soil composition is also affected by anthropogenic activites leading to variation in soil texture, soil nutrients and pH etc (Rashid *et al*., 2011). In the present study soil profile, soil pH and various nutrients present in the soil are important parameters of soil analysis and effect the growth and distribution of plants (Sharma & Kumar, 1991). Soil analysis from six different habitat of *Z. armatum* was carried out for physical, chemical and biological properties. Texture of the soil was found to be loamy in three sites while clay loam in the other three sites (Table 4.8). Nitrates contents were varied from 19.53 to 35.89 ppm. Phosphorus and Potassium was maximum in Charkotli hills, Batkhela, while in other areas these have variable amount. Fe, Zn and Cu also showed variable values from all localities. pH of soil ranged from 6.00-6.10 clearly showing that soil in all site was weak acidic with organic

contents ranging from 1.00 to1.01 (Table 4.8). The present work shows that *Z. armatum* grows in loamy to clayey loam soils which are slightly acidic. Other workers like Khan *et al*., (2010), Rashid *et al*. (2011) and Ali *et al*. (2011) also conducted similar studies and reported that deforestation and soil erosion greatly affected soil nutrient composition both quantatively and qualitatively. The present findings are consistance with their reports.

As it was observed that biotic pressure and human activities have ruined the natural habitat, therefore population density of *Z. armatum* was seem to be unstable. It is suggested to undertake appropriate measures for conservation of this and the associated plants to save economy, environment and biodiversity.

4.3. Ethnobotany

Ethnobotanical knowledge characterizes traditional knowledge to establish priorities in the local communities (Ibrar *et al*., 2007) and establish an interaction of man and plants for sustainable development (Ahmad *et al*., 2006a). It makes the most important approach to study natural resource management by the people of mountains and remote areas (Iqbal & Hamayun, 2005). Now a day, these studies gain much popularty in the development of health care system in various parts of the world (Sardar & Khan, 2009). Plants are used since the dawn of civilization by human beings for readymade food, medicines for various ailments, fodder/forage for cattle, fuel, flower for celebration an decoration, sold to earn, honey collection, making agricultural tools, timber for construction and for many more useful items (Ahmad *et al*., 2006a; Iqbal & Hamayun, 2005). Plant uses in indigenous knowledge are multiple, diverse and play very significant role in human health services (Nair *et al*., 2005; Kufer *et al*., 2005) and also from the economic basis of people of remote areas (Barkatullah *et al*., 2009). 80% of the world population uses the plant for primary health care, as plants are easily approachable and have fewer side effects (Anonymous, 1998). In Pakistan traditional medicinal plant uses are in practice in remote and mountainous areas. The ethnobotanical information also helps ecologists, pharmocologists, taxonomists, watershed and wild life managers in their efforts for improving the economic status of the locals in the remote area (Ibrar *et al*., 2007). This integrated approach is also necessary for the conservation of medicinal plants as well as their habitat. Ethnobatanical information of the present research plants were collected from

inhabitants of the area where these plants (*Skimmia laureola* and *Zanthoxylum armatum*) grow.

4.3.1. Ethnobotany of *Skimmia laureola*

Local names. Nazr panra, Namair

Part used. Leaves, young twigs, fruit

Harvesting and collection. As *S. laureola* is perennial shrub growing at high altitudes, therefore young twigs with leaves are harvested throughout the year except winter, when it is covered under snow. They are plucked with hands and collected in sacs. The twigs are then tide into small bunches for sale. In winter these are sold in dry form. In hilly areas red fruits of *S. laureola* are collected and arranged into garland for ornamental purposes.

Local uses:

S. laureola is used extensively through out the area to ward of evils, as it is considered a sacred plant. Peoples keep this plant in homes in fresh or dry form and smoked occassionally to ward off evils from their homes. Many other workers such as Hamayun *et al*. (2006), Qureshi *et al*. (2009), Haq *et al*. (2010) and Sher *et al*. (2011) also reported that this plant is considerd as demon repelent for different parts of pakistan. In some areas, decoction of the leaves is used in treatment of common cold. Hamayun *et al*. (2006) and Qureshi *et al*. (2009) reported that this plant is used as antiviral agent which substantiate this use. These authors also reported that this plant is given to cattle in crushed form with flour as fodder to make milk thick and viscous and also as anthelmintic. Hamayun *et al*. (2006) and Sher *et al*. (2011) also reported that this plant is used as anthelmintic and for other cattle ailments. In the present research areas too, the plant is used for similar purposes. Most of the local hakims, pansaries and common aged people considerd the leaves of *Skimmia laureola* as a best hypoglycemic agent. For this purpose leaves are dried, powdered and swollowed with water. This property of *S. laureola* is not reported from any part of Pakistan. Fruit of *S. laureola* is beautiful bright red and is used for ornamental purposes. Fruit is also used as garland in some parts of India as reported by Ilorkar & Khatri (2003), which is line with our findings.

A large number of the people depend on mountains and forest resources for the livelihood, but over exploitation by man and over grazing imbalance the usage and production of natural wild plants (Salva *et al*., 2001). As this plant is mainly collected for commercial purpose and mostly collected by children and unskilled persons, they uproot the whole plant during collection, thus the population of this useful plant decreases day by day. If the local inhabitants are provided with information about its importance and scientific knowledge for its proper collection, then this plant can be used substantially for a long time.

4.3.2. Ethnobotany of *Zanthoxylum armatum*

Local name: Dambara

Part used: Leaves, Stem and fruit.

Harvesting and collection: Generally fruit is collected from April to June and packed in plastic bags and transported to the local market for sale. Wood and branches are cut specially in winter for fuel. Thick wood, which is covered with thick thorns, is harvested for making lethal sticks. Young twigs are harvested for making Maswak (toothbrush). Extensively used part was the fruit, which was dried, powdered and used as a spice in various dishes especially in chat and Choolay (boiled gram). It was considered a best stomachic and good for gastric problems. Some other workers reported it as best remedy for stomach and gastric problems, (Arshad & Ahmad, 2004; Abbasi *et al*., 2010; and Sher *et al*., 2011) which confirm its traditional uses. Unripe fruit is mixed with liquid yogurt (Lassi) and used as coolant agent in summer. Young twigs were made in to toothbrush for teeth cleaning and for gum diseases. Abbasi *et al*. (2010) and Sher *et al*. (2011) also reported same uses of this plant in other parts of the country. The wood is cut into thick stick locally called as "Dambarsoote" used weapon of offence and defence. Similar findings were also reported by Barkatullah *et al*. (2009). Leaves are not used locally, but Negi & Kanwal, (2009) reported that leaves are used for mouth wash.

Many people in the mountainous regions are dependent upon the collection and sale of plants for earning their livelihood without caring for conservation or future status of these plants (Sher & Al-Ymeni, 2010). This has resulted in extinction of many indigenous plants. The vegetation of all the selected areas (Table 4.1 & 4.5) is under intense biotic

pressure in the form of deforestation, improper collection, slashing, burning and overgrazing. These factors have also greatly affected the population size of *S. laureola* and *Z. armatum* in these areas. Strict measure involving the participation of local community should be planned to conserve important medicinal plants for more economic and sustainable use.

4.4. Market survey

Plant play significant role in the socioeconomic status of the local inhabitants as they provides livelihood for them. To know the market status of *S. laureola* and *Z. armatum, various* markets in the area were visited to record geographic source, purchase rate, sale rate and approximate annual consumption.

4.4.1. Market survey of *Skimmia laureola*

It was noticed during the market survey, that there were regular collectors of *S. laurola*, who collect this plant in their native areas and supply it to the nearest local market. Main markets for this plant in Malakand division Mingora, Buner and Upper Dir, from where these are supplied to other parts of the country. The purchase and sale prices of this important medicinal plant vary from place to place, season to season and on the destination from their place of collection (Table 4.9). Because of collection season and large supplies, low prices were observed during summer season as compared to winter. Local dealers in Mingora, Buner and Upper Dir purchase this plant in fresh form, packed in sacs and then supply it to small dealers and nearby towns. Some dealers make collection directly through their agents and supply it to Mardan, Peshawar, Rawalpindi, Islamabad, Lahore, Karachi and even abroad. Some collecters after collection and drying store the drug in plastic bags for higher rates later in winter, when it is not available in fresh form. Various purchase rate, sale rate and approximate annual consumption of *S. laureola* in different markets are given in Table 4.9, which shows that the average purchase price of *S. laureola* ranges from Rs. 47 to Rs. 160 and the average sale rate ranges from Rs. 80 to Rs 200 per kilogram, whereas the annual trade ranges from 35 to 550 kg in different markets of the area.

4.4.2. Market survey of *Zanthoxylum armatum*

Market survey was also conducted for Z. *armatum*, in different markets of the area. Generally it was observed that fruit of this plant is collected by the local inhabitants and supply it to the nearest local market. As *Z. armatum* grow in foothills, there for it is easily available and not transported to far off places. Main markets for this plant in Malakand division are Mingora, Buner, Batkhela, Temergara and Upper Dir. Very little amount is supplied to other parts of the country. Variation was observed in purchase and sale prices of this important medicinal plant from place to place and season to season and on the destination from their place of collection (Table 4.10). Low prices were observed during summer season due to large supplies as compared to winter. Local dealers in Mingora, Buner, Batkhela and Upper Dir purchase fruits of this plant in bulky amounts, packed in plastic bags and then supply it to small dealers and nearby towns. Some dealers make collection directly through their agents and supply it to local markets. Various purchase rate, sale rate and approximate annual consumption of *Z. armatum* in different markets of the research area are summarized in Table 4.10, which shows that the average purchase price of *Z. armatum* ranges from Rs. 110 to Rs. 165 and the average sale rate ranges from Rs. 160 to Rs 240 per kilogram, whereas the annual trade ranges from 75 to 360 kg in different markets of the area.

Market survey of both medicinal plants indicated that there is great scope for the expansion and improvement of the market value chain of both these medicinal plants, not only locally, but also in other parts of the country.

Table 4.1. Localities selected for phytosociological studies of *Skimmia laureola*.

S. No.	Locality	Aspect	Slop	Altitude(Feet)	Coordinates
1	Bahrain Swat	North facing	Moderate to high	8500-8600	35º 12′ 10.86″ N 72º 29′ 57.63″ E
2	Malam Jabba Swat	North facing	Moderate to high	7200-7300	34º 48′ 16.76″ N 72º 37′ 10.44″ E
3	Matiltan Kalam Swat	North facing	Moderate to high	8750-8800	35º 34′ 57.25″ N 72º 40′ 13.41″ E
4	Tajaka Barawal Upper Dir	North facing	Moderate to high	8700-8800	35º 00′ 07.77″ N 72º 07′ 11.72″ E
5	Jagam Upper Dir	North facing	Moderate to high	7000-7100	35º 04′ 45.27″ N 71º 50′ 40.14″ E
6	Patrak Kumrat Upper Dir	North facing	Moderate to high	7900-7950	35º 25′ 15.58″ N 72º 09′ 57.86″ E

Table 4.2. Summary of the phytosociological attributes of *Skimmia laureola* and associated flora in sampling areas. Species are listed in alphabetical order.

S. No	Species name	No, of STP	Cnstancy Class	Importance Value(IV)			Importance value Index (IVI)		
				Max.	Min.	Aver.	Max.	Min	Aver
1	*Abies pindrow* (Royle ex D.Don) Royle	4	IV	10.69	7.35	9.02	3.56	2.45	3.01
2	*Adiantum venustum* D.Don.	6	IV	14.33	10.33	12.33	4.78	3.44	4.11
3	*Anaphalis sp*	1	I	4.06	4.06	4.06	1.35	1.35	1.35
4	*Andrachne cordifolia* (wall. ex Decne.) Müll. Arg.	1	I	11.58	11.58	11.58	3.86	3.86	3.86
5	*Aquilegia pubiflora* Royle	1	I	15.56	15.56	15.56	5.19	5.19	5.19
6	*Arisaema propinquum* Schott	1	I	3.53	3.53	3.53	1.18	1.18	1.18
7	*Artemisia maritima* L.	2	II	4.06	2.57	3.32	1.35	0.86	1.11
8	*Barberis lyceum* Royle	4	IV	6.21	2.36	4.29	2.07	0.79	1.43
9	*Bergenia himalaic*a Boriss.	3	III	8.66	2.42	5.54	2.89	0.81	1.85
10	*Calamanthus* sp	1	I	7.38	7.38	7.38	2.46	2.46	2.46
11	*Caralluma tuberculata* Wall	2	II	12.39	1.40	6.90	4.13	0.47	2.30
12	*Cedrus deodara* (Roxb.) G.Don	1	I	13.48	13.48	13.48	4.49	4.49	4.49
13	*Cymbopogon distans* (Nees ex Steud)	1	I	13.49	13.49	13.49	4.50	4.50	4.50
14	*Diplachne fusca* (L.) P.Beauv.	2	II	20.03	8.63	14.33	6.68	2.88	4.78

15	*Epilobium angustifolium* L.	2	II	3.71	2.08	2.90	1.24	0.69	0.97
16	*Euphorbia wallichii* Hook. f.	1	I	8.38	8.38	8.38	2.79	2.79	2.79
17	*Fimbristylis squarrosa* Vahl	2	II	18.07	14.17	16.12	6.02	4.72	5.37
18	*Fragaria vesica* L.	6	V	46.34	30.37	38.36	15.4	10.1	12.79
19	*Galium aparine* L.	1	I	3.33	3.33	3.33	1.11	1.11	1.11
20	*Girardinia heterophylla* (Vahl) Decne.	1	I	13.16	13.16	13.16	4.39	4.39	4.39
21	*Heteropogon contortus* (L.) Beauv. ex Roemer & JA Schu.	2	II	18.72	16.10	17.41	6.24	5.37	5.80
22	*Impatience* sp.	1	I	8.10	8.10	8.10	2.70	2.70	2.70
23	*Indigofera heterantha* L.	6	V	18.55	10.74	14.65	6.18	3.58	4.88
24	*Isodon rugosus* Wall. ex Benth	6	V	9.14	4.50	6.82	3.05	1.50	2.27
25	*Origanum vulgare* L.	2	II	6.41	5.59	6.00	2.14	1.86	2.00
26	*Paeonia emodi* Wall. ex Royle	2	II	13.03	2.79	7.91	4.34	0.93	2.64
27	*Picea smithiana* (Wall) Boiss.	2	II	4.43	3.71	4.07	1.48	1.24	1.36
28	*Pinus wallichiana* A.B. Jacks	3	III	17.07	6.30	11.69	5.69	2.10	3.90
29	*Plantago lanceolata* L.	1	I	8.41	8.41	8.41	2.80	2.80	2.80
30	*Podophyllum hexandrum* Royale	5	V	4.18	3.29	3.74	1.39	1.10	1.25
31	*Polygonatum verticillatum* (Linn.) All.	2	I	8.62	8.62	8.62	2.87	2.87	2.87
32	*Pteridium aquilinum* (L.) Kuhn	6	V	38.25	21.08	29.67	12.7	7.03	9.89
33	*Ranunculus laetus* Wall. ex Hook.f. & Thoms.	3	III	12.91	7.90	10.41	4.30	2.63	3.47
34	*Rhus punjabensis* J. L. Stewart ex Brandis	2	II	2.15	0.82	1.49	0.72	0.27	0.50
35	*Rubus fruticosus* Agg.	4	IV	27.50	3.02	15.26	9.17	1.01	5.09
36	*Rumex dentatus* L.	3	III	6.94	6.52	6.73	2.31	2.17	2.24
37	*Saccharum bengalense* Retz.	2	II	11.18	10.11	10.65	3.73	3.37	3.55
38	***Skimmia laureola* (DC.) Sieb. & Zucc. ex Walp**	6	V	48.89	4.86	26.88	16.3	1.62	8.96
39	*Swertia chirayita* (Roxb. ex Fleming) Karsten	2	II	7.40	4.13	5.77	2.47	1.38	1.92
40	*Taxus baccata* L.	5	V	13.29	9.16	11.23	4.43	3.05	3.74
41	*Trifolium repens* L.	3	III	36.47	19.95	28.21	12.2	6.65	9.40
42	*Valeriana jatamansi* Jones	3	III	7.84	2.81	5.33	2.61	0.94	1.78
43	*Viburnum grandiflorum* Wall. ex DC.,	2	II	24.51	17.41	20.96	8.17	5.80	6.99
44	*Viola canescens* Wall ex Roxb.	1	I	5.37	5.37	5.37	1.79	1.79	1.79
45	*Viola odorata* L.	4	IV	35.21	11.71	23.46	11.7	3.90	7.82

Table 4.3. Dominant shrubby species on the bases of importance value (IV) in the selected localities of *Skimmia laureola*.

S.No.	Localities	Ist dominant	2nd dominant	3rd dominant	Position of *S. laureola*
1	Bahrain	*Skimmia laureola*	*Rubus fruiticosus*	*Indigofera heterantha*	1st
2	Malam Jabba	*Skimmia laureola*	*Viburnum grandiflorum*	*Isodon rugosus*	1st
3	Matiltan- Kalam	*Skimmia laureola*	*Viburnum grandiflorum*	*Isodon rugosus*	1st
4	Tajaka-Upper Dir	*Indigofera heterantha*	*Rubus fruiticosus*	*Skimmia laureola*	3rd
5	Jagam - Upper Dir	*Skimmia laureola*	*Rubus fruiticosus*	*Indigofera heterantha*	1st
6	Patrak-Upper Dir	*Skimmia laureola*	*Indigofera heterantha*	*Rubus fruiticosus*	1st

Table 4.4. Physicochemical analysis of the soil in different localities of *Skimmia laureola*

S.No.	Sample Description	Textural Class	No^{3-} (ppm)	P (ppm)	K (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)	pH	Organic matter
1	Bahrain, Swat	Clay loamy	40.52	11.29	98	16.40	1.70	3.02	6.04	1.21
2	Malam Jabba	Clay loamy	24.60	2.70	256	20.36	1.56	3.67	6.09	1.12
3	Matiltan Kalam,	Clay loamy	22.45	2.09	239	18.03	1.45	3.87	6.10	1.14
4	Tajaka Upper Dir	Loamy	36.21	9.20	197	17.42	1.62	3.12	6.07	1.22
5	Jagam Upper Dir	Loamy	27.62	3..70	253	22.33	1.53	3.62	6.08	1.21
6	Patrak Upper Dir	Clay loamy	24.55	3.21	241	19.42	1.48	3.88	6.08	1.22

Table 4.5. Localities selected for phytosociological studies of *Zanthoxylum armatum*.

S. No.	Locality	Aspect	Slop	Altitude(Feet)	Coordinates
1	Charkotli hills Batkhela	North facing	Moderate to high	2800-2850	34º 34′ 57.48″ N 71º 59′ 10.40″ E
2	Laram- lower Dir	North facing	Moderate to high	4600-4650	34º 47′ 15.07″ N 71º 59′ 25.23″ E
3	Maidan -Lower Dir	North facing	Moderate to high	3300-3350	34º 54′ 52.61″ N 71º 52′ 01.59″ E
4	Peto dara- lower Dir	North facing	Moderate to high	4250-4300	34º 49′ 36.40″ N 71º 54′ 07.42″ E
5	Panda khan kale - Kabal Swat	North facing	Moderate to high	3200-3250	34º 48′ 26.37″ N 71º 54′ 07.42″ E
6	Tor warsak District Buner	North facing	Plain	2950-3000	34º 33′ 43.00″ N 72º 18′ 40.14″ E

Table 4.6. Summary of the phytosociological attributes of *Zanthxylum armatum* and associated flora in sampling areas. Species are listed in alphabetical order.

S. No	Species name	No, of STP*	Constancy Class	Importance Value (IV)			Importance value Index (IVI)		
				Max.	Min.	Aver.	Max.	Min.	Aver.
1	*Achyranthus aspera* L.	3	III	15.97	12.14	14.06	4.05	2.99	3.52
2	*Ailanthus altissima* (Miller) Swingle.	5	V	19.45	2.31	10.88	6.48	0.71	3.60
3	*Ajuga bracteosa* Wall ex. Benth.	5	III	11.87	5.62	8.75	3.96	1.94	2.95
4	*Artemisia scoparia* Waldst. & Kit.	4	IV	12.65	4.71	8.68	3.87	1.43	2.65
5	*Barberis lyceum* Royle	1	I	7.49	7.49	7.49	2.50	2.50	2.50
6	*Calotropis procera* (Ait) R. Br	1	I	1.84	1.84	1.84	0.61	0.61	0.61
7	*Cannabis sativa* L.	1	I	21.97	21.97	21.97	7.32	7.32	7.32
8	*Celtis australis* L.	3	II	7.01	4.50	5.76	2.34	1.50	1.92
9	*Conyza canadensis* (L.) Cronquist	4	IV	5.44	9.10	7.27	1.81	1.34	1.58
10	*Cotoneaster nummularia* Fischer & C. A. Meyer	3	III	9.11	5.54	7.33	3.04	1.62	2.33
11	*Cynodon dactylon* (L.) Pers	5	V	28.65	3.54	16.10	9.59	3.04	6.32
12	*Datura innoxia* Miller	1	I	20.34	20.34	20.34	6.78	6.78	6.78
13	*Debregeasia saeneb* (Forsskal) Hepper & Wood,	2	I	6.92	4.81	5.87	2.31	1.60	1.96
14	*Dicanthium annulatum* (Forsk.) Stapf.	3	V	76.91	21.87	49.39	25.9	8.93	17.44
15	*Dodonaea viscosa* (L.) Jacq	5	V	29.19	8.63	18.91	9.73	2.88	6.31
16	*Dryopteris crenata* (Forssk.) Kuntze,	1	I	11.57	11.57	11.57	3.86	3.86	3.86

17	*Euphorbia prostrata* Aiton.	1	I	4.98	4.98	4.98	1.66	1.66	1.66
18	*Ficus elastica* Linn	1	I	4.70	4.70	4.70	1.57	1.57	1.57
19	*Fragaria indica* Andrew	3	III	22.03	22.09	22.06	7.97	6.50	7.24
20	*Geranium wallichianum* D.Don Ex Sweet.	2	I	15.65	6.74	11.20	5.22	2.16	3.69
21	*Grewia optiva* J. R. Drumm. ex Burret.	1	I	1.84	1.84	1.84	0.61	0.61	0.61
22	*Gymnosporia royleana* Wall. ex Lawson	4	IV	12.85	3.92	8.39	4.28	1.32	2.80
23	*Heteropogon contortus* (L.) Beauv. ex Roemer & JA Schultes	3	III	38.82	22.03	30.43	12.9	6.62	9.79
24	*Indigofera heterantha* L.	3	III	10.68	9.15	9.92	3.56	3.05	3.31
25	*Justicia adhatoda* L.	2	II	33.13	15.86	24.50	11.1	5.29	8.22
26	*Medicago minima* (L.) Bartal.	3	III	9.69	10.39	10.04	3.33	3.06	3.20
27	*Melia azedarach* L.	3	III	8.05	6.74	7.40	2.68	2.25	2.47
28	*Micromeria biflora* (Buch.-Ham. ex D. Don) Benth.	2	II	7.97	5.82	6.90	2.66	1.94	2.30
29	*Nerium indicum* - Mill.	3	III	5.41	2.21	3.81	1.67	0.74	1.21
30	*Olea ferruginea* Royle	5	V	17.69	1.68	9.69	5.90	0.56	3.23
31	*Otostegia limbata* (Bth.) Boiss	3	III	5.67	3.33	4.50	1.92	1.11	1.52
32	*Oxalis corniculata* L.	3	III	17.80	22.84	20.32	5.79	5.32	5.56
33	*Parthenium hysterophorus* L.	1	I	20.44	20.44	20.44	6.87	6.87	6.87
34	*Periploca aphylla* Dcne.	1	I	2.80	2.80	2.80	0.93	0.93	0.93
35	*Pinus roxburghii* Sarg.	1	I	15.79	15.79	15.79	5.20	5.20	5.20
36	*Plantago lanceolata* L.	1	I	23.19	23.19	23.19	7.70	7.70	7.70
37	*Reptonia buxifolia* (Falc.) A. DC.	3	II	13.60	3.75	8.68	4.53	1.57	3.05
38	*Robinia pseudocacia* L.	1	I	4.46	4.46	4.46	1.49	1.49	1.49
39	*Rubus fruiticosus* Agg.	6	V	13.44	4.93	9.19	4.48	1.64	3.06
40	*Salvia lanata* Roxb.	1	I	19.91	19.91	19.91	6.64	6.64	6.64
41	*Salvia moorcroftiana* Wall.	1	I	9.74	9.74	9.74	3.25	3.25	3.25
42	*Solanum nigrum* L.	3	III	12.88	8.91	10.90	4.29	3.04	3.67
43	*Sonchus asper* L.	1	I	24.90	24.90	24.90	8.30	8.30	8.30
44	*Sorghum halepense* (L) Pers	1	I	4.51	4.51	4.51	4.29	4.29	4.29
45	*Tribulus terestris* L.	1	I	17.51	17.51	17.51	5.84	5.84	5.84
46	*Urtica dioca* L.	1	I	4.24	4.24	4.24	1.41	1.41	1.41
47	*Verbascum thapsus* L.	1	I	5.13	5.13	5.13	1.71	1.71	1.71
48	*Viola odorata* L.	1	I	9.81	9.81	9.81	3.27	3.27	3.27
49	*Xanthium strumarium* L.	3	III	8.46	4.05	6.26	2.82	1.27	2.05
50	***Zanthoxylum armatum* DC**	6	V	19.60	12.57	16.09	6.56	4.19	5.38
51	*Zizyphus nummularia* (Burm. f.) Wight & Arn.	1	I	2.20	2.20	2.20	0.73	0.73	0.73
52	*Zizyphus oxyphylla* Edgew	1	I	2.01	2.01	2.01	0.67	0.67	0.67

*Number of stands present.

Table 4.7. Dominant shrubby species on the bases of importance value (IV) in the selected localities of *Zanthoxylum armatum*.

S. No.	Locality	Ist dominant	2nd dominant	3rd dominant	Position of *Z. armatum*
1	Charkotli hills Batkhela	*Rubus fruiticosus*	*Zanthoxylum armatum*	*Gymnosporia royleana*	2nd
2	Laram- lower Dir	*Dodonea viscose*	*Zanthoxylum armatum*	*Justicia adhatoda*	2nd
3	Maidan- Lower Dir	*Zanthoxylum armatum*	*Indigofera heterantha*	*Dodonea viscosa*	1st
4	Peto dara- lower Dir	*Dodonea viscose*	*Zanthoxylum armatum*	*Rubus fruiticosus*	2nd
5	Panda khan kale-Kabal Swat	*Dodonea viscose*	*Zanthoxylum armatum*	*Cotoneaster nummularia*	2nd
6	Tor warsak District Buner	*Dodonea viscose*	*Zanthoxylum armatum*	*Rubus fruiticosus*	2nd

Table 4.8. Physicochemical analysis of the soil in different localities of *Zanthoxylum marmatum*

S.No.	Sample	Textural Class	NO^{3-} (ppm)	P (ppm)	K (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)	pH	Organic matter
1	Charkotli hills Batkhela	Clay loamy	35.89	19.68	110	28.86	1.34	3.88	6.04	1.01
2	Laram- lower Dir	Loamy	19.53	10.33	54	15.34	1.38	3.53	6.01	1.00
3	Maidan- Lower Dir	Loamy	33.07	17.70	89	20.67	1.19	2.99	6.10	1.00
4	Peto dara- lower Dir	Loamy	23.53	8.33	70	44.01	1.92	3.09	6.00	1.00
5	Panda khan kale-Kabal Swat	Clay loamy	33.81	17.58	91	22.85	1.41	3.11	6.10	1.01
6	Tor warsak District Buner	Clay loamy	27.55	15.51	101	23.75	1.39	3.22	6.10	1.01

Table 4.9. Market value chain of minimum, maximum and average prices in Pakistani Rupees (PRs.) per kilogram of *Skimmia laureola* at different market points on the basis of data collected from local dealers, hakims and Pansaries.

S. No	Market	Status	Purchase (PRs./Kg)			Sale (PRs./Kg)			Annual trade (Kg) approximatly.
			Min.	Max.	Ave.	Min.	Max.	Ave.	
1	Mingora	Fresh	30.00	65.00	47.50	50.00	120.00	85.50	480
		Dry	50.00	75.00	62.50	80.00	170.00	125.00	220
2	Buner	Fresh	40.00	75.00	57.50	65.00	95.00	80.00	550
		Dry	65.00	95.00	80.00	75.00	120.00	97.50	320
3	Upper dir	Fresh	80.00	120.00	100.00	95.00	135.00	115.00	300
		Dry	120.00	145.00	132.50	140.00	165.00	152.50	75
4	Temergara	Fresh	65.00	105.00	85.00	85.00	125.00	105.00	300
		Dry	90.00	140.00	115.00	110.00	170.00	140.00	100
5	Batkhela	Fresh	70.00	100.00	85.00	100.00	160.00	130.00	80
		Dry	120.00	130.00	125.00	140.00	190.00	165.00	30
6	Dargai	Fresh	100.00	150.00	125.00	180.00	200.00	190.00	70
		Dry	140.00	180.00	160.00	180.00	220.00	200.00	35

Table 4.10. Market value chain of minimum, maximum and average prices in Pakistani Rupees (PRs.) per kilogram of *Zanthoxylum armatum* fruit at different market points on the basis of data collected from local dealers, hakims and Pansaries.

S. No	Market	Purchase (PRs./Kg)			Sale (PRs./Kg)			Annual trade (Kg) approximatly.
		Min.	Max.	Ave.	Min.	Max.	Ave.	
1	Mingora	110.00	150.00	130.00	160.00	200.00	180.00	360
2	Buner	115.00	150.00	132.50	170.00	210.00	190.00	175
3	Upper Dir	120.00	150.00	135.00	175.00	190.00	182.50	75
4	Temergara	120.00	145.00	132.50	160.00	195.00	177.50	130
5	Batkhela	115.00	155.00	135.00	155.00	220.00	187.50	120
6	Dargai	120.00	165.00	142.50	175.00	240.00	207.50	95



a





b c

Fig. 4.1. *Skimmia laureola* a. leaves b. fruits c. stem



a b

c d

Fig. 4.2. *Zanthoxylum armatum*. a. flowers b. fruits c. leaves d. stem

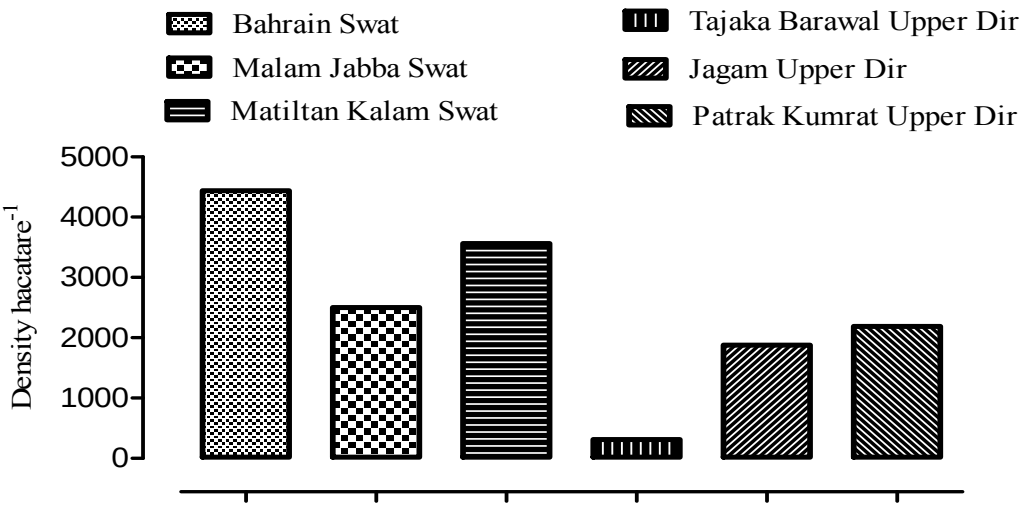


Fig. 4.3. Bars representing density per hactare of *Skimmia laureola* in Different localities.

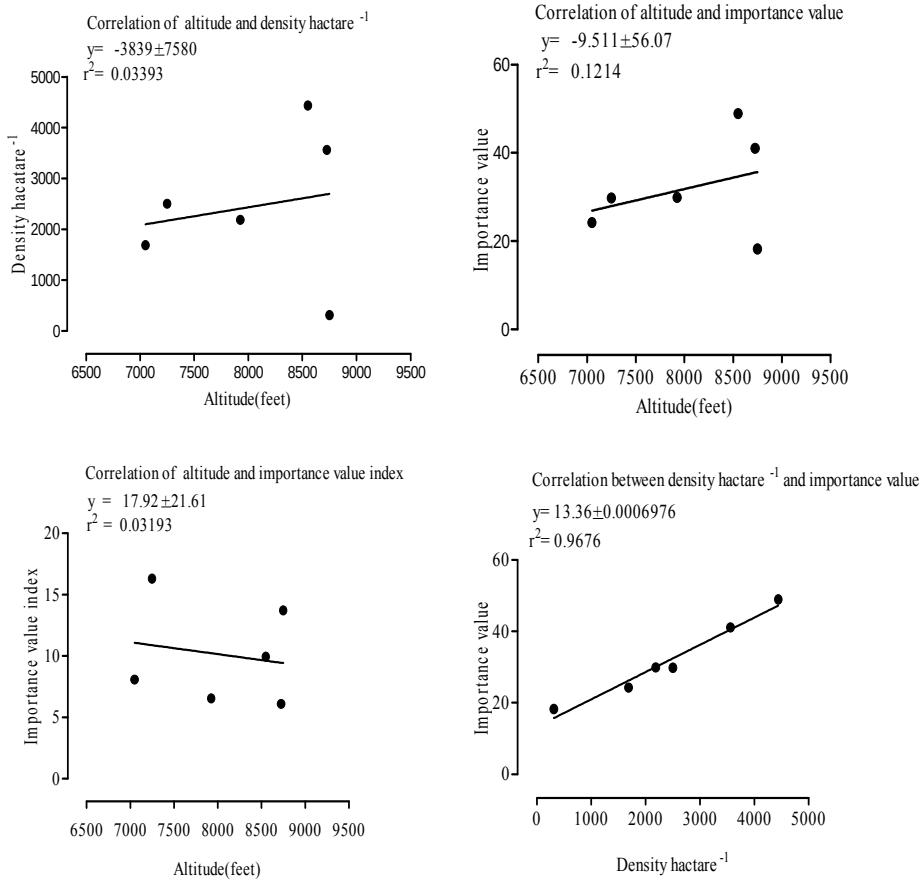


Fig. 4.4. Regression among some variables for phytosociological study *of Skimmia laureola.*

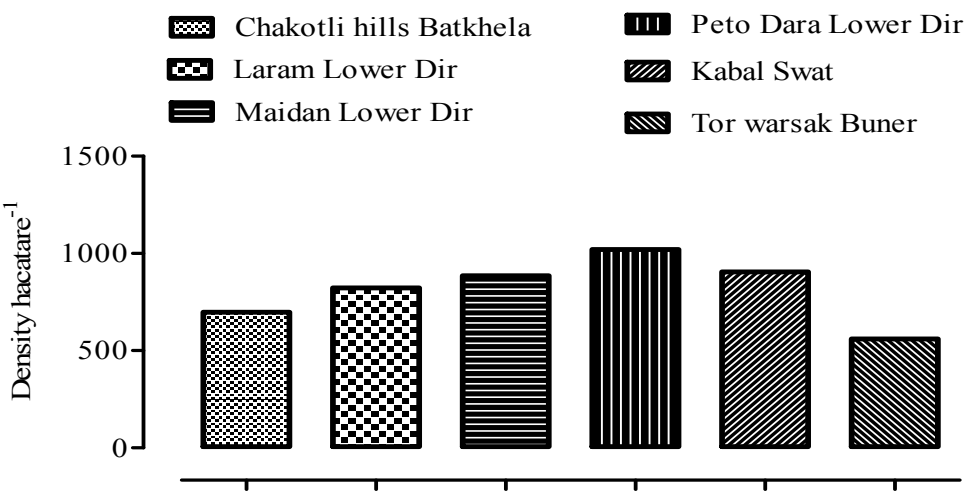


Fig. 4.5. Bars reperesenting density hectare^{-1} values of *Zanthoxylum armatum* in different localities.

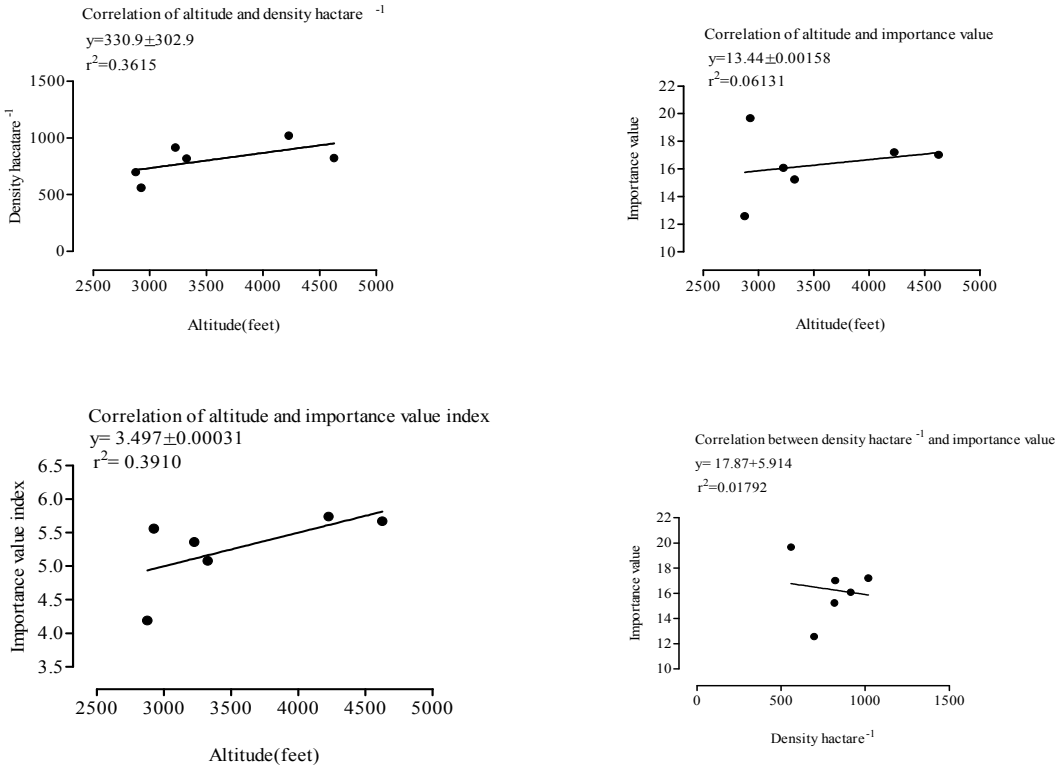


Fig. 4.6. Regression among some variables for phytosocialogical study of *Zanthoxylum armatum.*

4.5. Pharmacognosy

Pharmacognostic study is of utmost importance to correctly identify crude drugs (the starting materials for herbal products). For this purpose, emphasis is laid down on standardization of medicinal plants used in health problems. Inspite of addition of modern techniques, pharmacognostical techniques are still considered authentic for identification and standardization (Najafi & Deokule, 2010). Pharmacognosy is primarily study of physical, chemical, biochemical and biological properties of drugs of natural origin as well as the search for new drugs from natural sources (Tyler, 1999). It is a long-established discipline in finding, characterization, manufacturing and standardization of plant material in term of their morphological, anatomical and biochemical features (Kaplan, 2001; Kinghorn, 2002). In the present study Pharmacognosy of *Skimmia laureola* and *Zanthoxylum armatum* was carried out to standardized morphologic and histologic features of these drugs.

4.5.1. Pharmacognostic studies of *Skimmia laureola*

Leaf and stem bark of Skimmia *laureola* were evaluated for pharmacognostic features including macroscopy/morphology, microscopy and physicochemical study of powder drugs.

4.5.1.1. Macroscopy

Macroscopical feature like shape, color, odor, taste, dimentions etc of crude drugs are the primary pharmacognostic parameter for the correct identification. In the present study leaves and bark of *S. laureola* are described macroscpicaly, to established standard parameter. *S. laureola* leaf has darsiventral symmetry with prominent midrib (Fig. 4.7a, b). It is lanceolate to sub lanceolate in shape. The characteristic features of the leaf are presented in Table 4.11. Sugumaran & Vetrichelvan (2008) included macroscopic features for the pharmacognostical Standardization of *Bauhinia purpurea*. Similar studies were also conducted by Mathur *et al*. (2010) for *Amarathus spinosus* standardization. Sharma *et al*. (2010) described macroscopic evaluation, such as size, shape, colour, odor and taste etc as base for pharmacognostic evaluation. In present study *Skimmia laurola* bark was also also studied, having some distinguishing adaxial and abaxial features (Fig. 4.8a, b), summarized in Table 4.12. Some other workers have also carried out bark studies e.g Goyal *et al*. (2011)

studied stem bark of *Careya arborea* for pharmacognostic standardization; Ahmad & Urooj (2011) studied stem bark of *Ficus racemosa* for the same purpose. These studies strengthen our course of work.

4.5.1.2. Microscopy

Microscopy helps in exploring the internal structure of plant parts and is considered as a fascinating tool for correct taxonomy of the plants (Nancy & Dengler, 2002). Present microscopic study includes leaf anatomy, midrib anatomy, leaf surface values, stomatal study, bark anatomy and microscopy of crude powder drug of leaf and bark of *S. laureola*.

a. *Skimmia laureola* leaf anatomy

Transverse section of the leaf lamina through the midrib region of *Skimmia laurola* showed upper epidermis and lower epidermis with mesophyll region in between, containing vascular bundle (Fig 4.9). Upper epidermis was composed of compactly arranged, rectangular cells, arranged in double layer. These cells were 5.75-8.63µ in length with mean of 7.11±1.037 µ and 2.88-3.45 µ in width with a mean of 3.10±0.420µ. Upper epidermis was non stomatiferous and covered with thick cuticle. Msophyll region was easily distinguished into palisade and spongy mesophyll. Palisade mesophyll was single layered, composed of elongated rod like compactly arranged cells with a length of 12.65-15.53µ with mean value of 13.86±1.09 µ and width of 2.88-4.6 µ with an average of 3.19±0.670µ. Occasional calcium oxalate crystals were observed in these cells. The spongy mesophyll was consisted of rounded, oval or cylindrical cells with length of 12.08-14.95µ with mean value of 13.28±0.994 µ and width of 1.73-4.03 µ having an average of 3.19±0.878µ in width. Some of the cells were idioblast i.e. provide with calcium oxalate crystals (2.5 - 6.5 µ) and were loosely arranged with large intercellular spaces. These spaces seem to be schizogenous or lysogenous cavities. Collateral vascular bundles, consisting of parallel xylem elements including vessels and parenchymatous cells and phloem fibers were observed. Lower epidermis was stomatiferous with varied shaped (rectangular, elongated or irregular) cells. The length of these cells were 5.75 – 8.63 µ in length with a mean value of 6.67±1.021 µ and 2.30-3.455 µ in width with a mean of 2.88 ±0.469 µ.

b. *Skimmia laureola* leaf midrib anatomy

Adaxial surface of the midrib of *Skimmia laureola* was convex in appearance while the abaxial surface was wide semicircular in appearance. The most diagnostic and intrusting feature of the midrib was the complete absence of any kind of trichome or any other appendage. T.S of midrib was characterized by upper epidermis, hypodermis, phloem, xylem, cortex and lower epidermis (Fig 4.10). Upper epidermal cells were oval, rectangular or squirrel in shape, ranged from 2.76-4.86 µ with a mean of 3.66±0.967µ in size, and closely fitted end to end forming a single layer. A thin cuticle was present on the upper epidermis. Below the upper epidermis, hypodermis was present, composed of 2-3 layers of collenchymatous cells, with a thickness of 5.58-19.88 µ. These cells were round in shape, 2.76- 5.17 µ (with a mean of 3.24±1.00) in diameter. Next was a many layered ground tissue, composed of round shaped thin walled parenchymatous cells. These cells were characterized by having various types of isolated as well as aggregates of calcium oxalate crystal. The minimum diameter of these cells was 11.73- 22.08µ (with a mean of 18.08±3.485µ). Large Intercellular spaces were seen. Lysogenous oil cavity, looked to be formed by the dissolution of cells, were seen in this region. Vascular bundle appeared like an arc, in which xylum was present inner in position followed by phloem. Vascular bundle was surrounded by calcium oxalate containing collenchymatous (iodoblast) cells. Xylem was adaxial in position and appeared in radial rows of vesssels with a cell size ranging from 2.58- 5.15 µ (with mean diameter of 3.68±0.868). Phloem, abaxial in position was visible with round shaped cells with almost the same dimensions, as that of xylum. The lower epidermis of the midrib was composed of compactly arranged, rectangular shaped cells, ranging from 2.78-5.16 µ (with a mean of 3.78±0.988µ) in size.

c. Leaf surface values

Leaf epidermal studies are of great importance not only in pharmacognosy but also in phylogeny and taxonomy; therefore plant taxonomists pay much attention toward the leaf epidermal features to resolve the taxonomic problems (Taia, 2005). Various leaf surface features such as palisade ratio, vein islets number, vein termination number, stomatal number and stomatal index, which are the quantitative microscopic features, were carried out for *S. laureola* leaf (Table 13). Palisade ratio value recorded in the present study was

ranged from 6.75 to 7.75 to 9.00 (7.8±0.21) underneath pallisaed cells per epidermal cell (Fig. 4.11a). Vein iselet and vein termination number were 14-18 (15.4±0.63) and 9 - 13 (11.3±0.47) per mm^2 respectively. The vein-islets were quite distinct with squaresh, elongated or polygonal shaped thick walled areas, internally provided with many forked and unforked vascular branches (Fig 4.11b). Palisade ratio, vein islet and vein termination values were also reported for a number of other plants such as *Tylophora dalzellii* (Najafi & Deokule, 2010), *Acacia leucophloea* (Gupta *et al*., 2010) and *Lagenaria siceraria* (Shah *et al*., 2010). From these studies it is clear that different plant have variant values for these features. From the work of these and others it might be concluded that these feature play significant role in identification and authentication of leaf crude drugs.

d. Stomatal study

Stomata are special aperture in the epidermis especially in the leaves and used for exchange of gases i.e. oxygen and carbon dioxide between the plants and their environment, surrounded by two guard cells. Stomata may occur on both epidermises (amphistomatic leaf), or only on upper epidermis (epistomatic leaf) or mostly on the lower epidermis (hypostomatic leaf) (Perveen *et al*., 2007). Stomatal types are taxonomically considered important and provide effeicient bases for establishing phylogeny in the higher level of taxanomic hierarchy (Hameed *et al*., 2008). Stomatal index values may be used to distinguish between leaves of co-generic species (Evans, 2002)

The present study showed that *S. laureola* leaf is hypostomic i.e. stomata were found only on the lower epidermis (Fig. 4.12). The occurrence of stomata on lower epidermis appeared to be somewhat xerophytic trait (Esau, 1977). There were no proper orientations of the stomata to each other. Most of the stomata appeared to be parallel to each other while others were lying at right angle to each other. Still some were observed to be oriented forming an angle ranged from 100º to 140º. Various numerical values including stomatal Index, stomatal number, varience, co-efficient of variance, Standard deviation and standard error of stomatal number were worked out and summarized in table 4.13. Stomatal size and stomatal density are inversely proportional (Metacalfe & Chalk, 1950). This might be the reason for high stoamatal number (122.1±4.32) in *S. laureola* with relatively small size. Quantitative values like stomatal density, stomatal size, stomatal index, size of stomatal

pore are of utmost significance in differentiating the taxa at species and interspecies level (Nabin *et al*., 2000). Ferris *et al*., (2002) reported co-efficient of variance, stomatal density, stomatal index, epidermal cells area and number of epidermal cells per leaf in poplar. Similarly Hameed *et al*. (2008) carried out statistical evaluation including mean, standard deviation, variance and coefficient of variance of stomata in the epidermises of some member of family Polygonaceae and suggested a taxonomic uniformity of stomatal type. The present study is well in lineage with these workers.

Great diversity in stomatal arrangement was observed. Various types of somata with their frequency and other dimentions were also carried out (Table 4.14). Following main types of stomata were observed in the leaf of *S. laurola* including

1. Stephanocytic type, characterized by a rosset of four or more weakly identified subsidiary cells (Baranova, 1987). Sub types of this category, observed in the present study were a. Actinocytic, having radial elongation of subsidiary cells (Wilkinson, 1979) (Fig 4.13a). b. Actinostephanocytic having radial elongation of some cells or all has slight elongation (Carpenter, 2005) (Fig 4.13b). c. Incomplete stephanocytic bicyclic, which has incomplete outer ring of subsidiary cells (Carpenter, 2005) (Fig 4.13c). d. Staurocytic, defined by four or more subsidiary cells, having cell wall oriented differently to the stomatal pore, (Van Cotthem 1970) (Fig 4.13d).

2. Paracytic type, characterized by one or two lateral subsidiary cells, oriented parallel to the guard cells (Dilcher, 1974). In this category too, subtypes were observed as a. Brachy paracytic, having two lateral subsidiary cells, oriented parallel to guard cells (Carpenter, 2005)(Fig 4.13e). b. Amphibrachy paratetracytic, having four subsidiary cells ith two wider polar and two small lateral subsidiary cells (Chengqi *et al*., 2007) (Fig. 4.13f). c. Hemiparacytic, characterized by a single parallel cell. (Carpenter, 2005) (Fig. 4.13g).

3. Stomatal cluster, two stomata are arranged side by side without any common epidermal cell in between them (Tang *et al*., 2002) (Fig. 4.13h). This cluster was appeared to be brachy paratetra cytic. Stomatal cluster is considerd to be one of the special leaf epidermal features, reported in only 38 genera of vascular plants (Tang *et al*., 2002). Chengqi *et al*. (2007) reported similar pattern of stomatal apparatus in *camellia henryana*

and *C. tsingpienensis*. As this feature is very rare in vascular plants, it requires further exploitation to consider it as a texanomic fingerprint. In the present study actinostephanocytic stomata was the most occuring (70%) followed by amphibrachyparatetracytic (40%) and then actinocytic (30 %). Incomplete stephanocytic bicyclic (10%) and Hemiparacytic (10%) were the rarly occuring stomata present on the lower epidermis of *S. laureola*. Inspite of great variations in the frequencies, no significant differences were observed in the sizes of these stomata (Table 4.14).

Stomata modify the adaptation skills of plants, as they direct transpiration and photosynthesis. Stomata are also useful in taxonomic categorization and to detect future clues, to know how plants are affected by environmental factors. The number of stomata also affects the concentration of micro and macro elements in plants (Nabin *et al*., 2000; Brownlee, 2001). Very little work has been done on the stomatal studies of family Rutaceae. Ogunkunle & Oladele (1997) reported paracytic, hemiparacytic, brachy paracytic and brachyparatetracytic and anomocytic stomatal complexes with uniform size from abaxial leaf epidermises of various *Citrus* species (Rutaceae) and observed high stomatal number. Present study shows that *S.laureola* has an average stomatal number of 122.1±4.32 and Stomatal index of 12.96±0.14, which are also very high figures and suggest that this might be a distinguishing feature of this family.

e. Bark anatomy

S. laureola bark was differentiated into different layers (Fig 4.14). The outer most layer was cork (phellem). Cork was composed of a few layers of closely packed lignified thick walled rectangular cells, 4.73-5.85 µ (5.31±0.321 µ) in length and 3.38-4.05 µ (3.67±0.23 µ) in breadth. Below the cork was Cork cambium (phelogen) which is a continous layer of tangentially arranged small cells, ranged from 2.93-4.05 µ (3.47±0.354 µ) in length and 0.72-1.26 µ (0.92±0.462 µ) in breadth. These were thin walled, closely packed. Below the cork cambium was the secondary cortex (phelloderm) consisting of large parenchymatous cells with large intercellular spaces. The cells ranged from 2.61 – 6.10 µ (3.70±1.082µ) in length and 1.80- 3.60µ (2.34±0.553µ) in width. Some of the cells contained calcium oxalate aggregates. The medullary ray extended from lower region, appeared to consist of thin walled small cells having a diameter of 4.47±0.55 µ, mixed with

cortical cells. Below the secondary cortex was phloem, composed of variable sized and shaped cells. Some of these cells were crushed, intermixed with medullary cells, while others were intact and easily distinguishable. The mean diameter of the cells ranged from 3.32-8.44 µ (5.32±590 µ). Bast fibers were also seemed to present in phloem. Some other workers have also carried out anatomical studies of bark, e.g Ahmed & Urooj (2011) explored Cork, cork cambium and secondary cortex containing calcium oxalate crystals in the stem bark of *Ficus racemosa*. Similarly Kanth & Bhasker (2011) reported that periderm, cortex and secondary phloem were the general arrangement in the transverse section of stem bark of *Holoptelea Integrifolia*.

f. Powder drugs microscopy

Powder microscopy of *Skimmia laureola* leaf and bark were carried out. Presence of various structures in powder provide basis for standardization and authentication of crude drug powder.

I. Leaf powder microscopy

Leaf powder of *Skimmia laureola* appeared bright green in color with pleasant odor and astringent taste. Following were some of the structures observed under microscope (Fig. 4.15).

i. Numerous fragments of upper epidermis with irregular or polygonal shaped cells, closely packed, ranging from 12 µ to 17µ in length.
ii. Lower epidermal fragments of irregular shaped cells, 14 µ to 18 µ in length with anomocytic elliptical stomata, 21 µ to 26 µ in length.
iii. Fragments of epidermal cells, to which closely packed palisade and spongy mesophyl cells are attached.
iv. Vessels with attached parenchymatous cells.
v. Thick walled collenchymatous cells containing aggregates of calcium oxalate crystals with diameter of 4µ to 8µ.
vi. Broken fragments of spongy parenchyma
vii. Isolated starch grain 2µ to 6 µ in length.

viii. Isolated prismic, rectangular and aggregate crystals of calcium oxalate. 1.5 μ to 4 μ in diameter.

ix. Midrib cortical cells of the midrib with oil secretary cavity.

Some other workers have also carried out powder drug studies, e.g Maiti *et al*. (2002), who studied ten species of the family Solanaceae applying techniques of pharmacognosy and histochemistry for the differentiation and identification of these the species; Mishra *et al*. (2008) studied leaf powder of *Tridax procumbens* and Murti *et al*. (2010) carried out powder microscopy of *Calotropis procera* and reported the characteristic feature of the powders. The present microsopy of *Skimmia laureola* leaf also showed the above mentioned characteristic structures. One important feature of the powder drug was complete absence of any type of trichome.

II. Stem bark powder microscopy

Bark powder of *Skimmia laureola* appeared golden yellow in color with pleasant odor and astringent taste. Most frequently occurring fragment in the powder were (Fig. 4.16)

x. Closely packed dark brown color thick walled cork cell ranging from 37μ to 57 μ in length and 3-7 μ in width.

xi. Thick walled cortical cells with clusters of calcium oxalate crystals.

xii. Elongated fibres up to 180 μ, with attached polygonal phloem parenchyma cells.

xiii. Cork cells with closely packed thick walled phlem cells.

xiv. Scleriedes of various shapes i.e. elongated, rounded or triangular.

xv. Spiral vesssels up to 170 μ in length and 5-8μ in diameter.

xvi. Isolated crystals of calcium oxalate, 2 - 4 μ in diameter.

xvii. Starch grains, 4 -14 μ in diameter.

Bark powder drug study of some other plants has been carried out by other workers such as Reddy *et al*. (1999) has reported bark of *Wrightia tinctori*, Dev *et al*. (2010) reported bark powder of *Oroxylum indicum* and Singhal *et al*. (2010) has reported bark powder of *Geniosporum prostratum*.

4.5.1.3. Physicochemical characteristics

Various physicochemical characteristics of leaf and bark powder of *Skimmia laureola* have been worked out including Ash analysis, qualitative and quantitative

phytochemical screening, fluorescence astudy, extractive values, elemental analysis and proximate analysis.

a. Ash analysis

Ash analysis included Total Ash, Acid Insoluble Ash and Water Soluble Ash. Total ash value is important in the detection of adulteration or admixture of inorganic matter such as metallic salts and/or silica/clay during improper handling (Jarald & Jarald, 2007). Similarly acid insoluble ash values are carried out for drugs having calcium oxalate crystals and to which lime, sand or clay or other earthy matter is added (Wallis, 1985; Rangari, 2002). A water soluble ash value is important for detection of presence of water exhausted material in the genuine drug (Jarald & Jarald, 2007). In the present study Ash analysis for leaf and bark crude drug and exhausted drugs (with ethanol and n- hexane) of *Skimmia laureola* was worked out. It was observed that Total ash, Acid insoluble ash and water soluble ash were found 101.8 mg/g, 65.2mg/g and 296.8 mg/g respectively for SL, showing that these values were much higher than the SLEE and SLEH. Similarly SB has higher numerical values than their respective exhausted materials i.e SBEE and SBHE (Table 4.15). Fig 4.17a and 4.17b show comparison of percent values of Ash values for non-exhausted and exhausted powders of leaf and bark of *S. laureola*. The present study is well in accordance with those of Nayak *et al.,* 2010 (leaf of *Jatropha curcas*), Balakrishnan *et al.,* 2011 (*Amaranthus spinosus* Linn), Bisht *et al.,* 2011 (*Swertia chirayita*), Subha *et al.,* 2011 (*Acorus calamus*), Hussain *et al.,* 2011a (*Hygrophila auriculata*), Kumar *et al.,* 2011 (*Crocus sativus*) and Zunjar *et al.,* 2011 (*Carica papaya*) who also conducted ash analysis for standardization of various plants crude drugs.

b. Phytochemical screening

Each single plant contains several active constituents of pharmacological importance (Ming *et al.,* 2005). Therefore preliminary phytochemical screening is important and useful for isolation of pharmacologically active compounds present in the plants, (Sugumaran & Vetrichelvan, 2008). Alkaloids are the secondary metabolites with bitter/acrid taste providing safety to the plants against grazing and browsing. Most the alkaloids are physiologically and therapeutically active compounds (Tyler, 1999). Saponins, a special class of glycosides with soapy characteristics have proved to be a significant antifungal

agent (Fluck, 1973; Sodipo *et al*., 1991). Presence of tannins also suggests playing a major curative role in treatments of some human disorders (Asquith & Butler, 1986). Similarly Havsteen (2002) reported some interesting biological activities of flavonoids (phenolic compounds) like antimicrobial, antioxidant, anti-viral, cardioprotective and neuroprotective.

Both qualitative and quantitative phytochemical screenings were carried out using different extracts of the leaf and bark of *Skimmia laureola*. Qualitative phytochemical screening of the leaves of *Skimmia laureola* (Table 4.16) proved that carbohydrates were present in all aqueous, ethanolic and n-hexane extracts in the leaves. Alkaloids, flavonoids and tannins were detected in aqueous and Ethanolic extracts. Phytosterol, triterphenoids, fixed oil and volatile oil were screened out in the ethanolic and n-hexane extracts. Proteins were detected only in the hexane extract. More or less similar results were also obtained for the microchemical screening of the stem bark of *Skimmia laureola* (Table 4.17). Anthocyanine was the only constituent detected in the ethanolic extract of bark only. Magaji *et al*. (2007) reported that the methanolic extracts of the leaves, stem bark and root bark of *Securinega virosa* have almost same phytochemical constituents. Similarly Arjun *et al*. (2009) explored phytochemical composition of leaf of *Hygrophila spinosa*. Chirikova, *et al*. (2010) carried out preliminary phytochemical investigation of *Scutellaria baicalensis*. Kalyan *et al*. (2011) subjected ethanol extract of *Clitoria ternatea* seeds to preliminary phytochemical investigations. All these workers have emphasized the importance of phytochemical investigation in the pharmacognostic studies of crude drugs.

Quantitative analysis for bioactive constituents like alkaloids, sterol, saponins, tannins, phenols and flavonoids were also carried out in leaves and bark of *Skimmia lareola*. The results revealed the presence of bioactive constituents in leaves comprising of alkaloids (12.50±0.09 mg/g), sterols (81.30±0.61 mg/g) saponins (20.93±0.06 mg/ g), tannins (26.83±0.12mg/g), phenols (10.33±0.66 mg/g) and flavonoids (12.58±0.66 mg/ g). In the bark, these active constituents were found alkaloids (22.10±0.17 mg/g), sterols (56.73±0.25mg/g) saponins (22.63±0.12mg/ g), tannins (14.30±0.10 mg /g), phenols (14.68±1.33 mg/g) and flavonoids (16.66±0.33mg/ g) (Table 4.18). Comparison of these bioactive constituents in the leaf and bark of *Skimmia laureola* has been illustrated in Fig. 4.19. Our findings are well in lineage with that of Soares *et al*. (2003) for *Phyllanthus*

niruri, Okwu & Josiah (2006) for *Aspilia africana* and *Bryophyllum pinnatum* and Bhagwat *et al*. (2008) for *Euphorbia hirta*. It is obvious from both the qualitative and quantitative analysis, that this plant is a rich source of bioactive substances, which might be helpful in combating diseases.

c. Fluorescence study

Fluorescence study with UV is a very useful tool for evaluation of crude drugs. Cut surfaces of crude drugs, its powder or its extract, when viewed under UV light give characteristic fluorescence (Wallis, 1985). The fluorescence phenomenon is due to different chemical compositions, which is not observed in visible light (Ansari, 2006; Jarald & Jarald, 2007; Reddy & Chaturvedi, 2010). Fluorescence study is a helpful tool for authentication and standerdisation of crude drugs. Many workers tried out to authenticate crude drugs of plant origin, including Ismaeil & Sultana, 2008 (*Morinda umbellatai*), Sharma *et al.,* 2009 (*Holoptelea integrifolia*), Kalidass *et al.,* 2009 (root and stem of *Ichnocarpus frutescens*), Shrivastava & Leelavathi*,* 2010, (leaves of *Catunaregum spinosa*), Hussain *et al.,* 2011a (*Hygrophila auriculata*) and Kumar *et al.,* 2011 (*Crocus sativus*). Fluorescence analysis of the *S. laureola* leaf and bark powders alone as well as those treated with various reagents, was carried out using UV 256 and UV 300 and the results are presented in Table 4.19. Similarly extracts of these two parts in different solvents were also observed in UV light and the results are given in Table 4.20. This florescent study is a very useful physical evaluation method, which is quick, easy and reliable method for the detection of adultrations and admixtures.

d. Extractive values

Extractive values are primarily useful for the determination of exhausted or adulterated drugs (Singhal *et al*., 2010). Extractions with different solvents detect various types of adulteration and exhausted materials i.e. Alcohol and water soluble extractive values are indicative of the presence of the adulterants, defective processing and poor quality of the drug while petroleum ether soluble extractive value indicates lipid contents present in crude drug (Madhavan *et al*, 2009; Kokate, 1994). Different solvents including ethanol, methanol, chloroform, acetone, distilled water, butanol and n-hexane were used for percent extractive values determination of the leaf and bark of *S. laureola*. Highest

extractive value for leaf was found in methanol (21.80 %) while for bark highest value was obtained in chloroform (9.30 %). In other solvent both the parts showed variable values (Table 4.21). Recently, a lot of work has been done in this regard including Singhal *et al*., 2010 (*Geniosporum prostratum*), Balakrishnan *et al*., 2011 (*Amaranthus spinosus* Linn), Subha *et al*., 2011 (*Acorus calamus*), Hussain *et al*., 2011a (*Hygrophila auriculata* K.Schum), Kumar *et al*., 2011 (*Crocus sativus)* and Zunjar *et al*., 2011 (*Carica papaya)*. This suggests that extractive values determination is an important tool for evaluation of crude drugs and detection of adulterants.

e. Elemental analysis

Plants have the capacity to accumulate essential minerals in different parts, which are of utmost importance in human nutrition (Clemens *et al*., 2002). Some of the metals like Co, Cd etc which have no direct relation with the plant physiology, are also accumulated in some plants (Ajasa *et al*., 2004). In human, trace elements play a vital role both in prevention and treatment of various human diseases (Saeed *et al*., 2010). Unfortunately, plants also accumulate some toxic heavy metals due to environmental reasons, which create serious health hazards (En *et al*., 2003). In the present study leaf and bark of *S. laureola* were analyzed for elemental compositon. The presence and concentration of minerals is given in Table 4.22, which show that Zinc, Manganese and Chromium are important trace elements, which play a very significant role in glucose metabolism, hence known as hypoglycemic elements (Donsbach & Ayne, 1982). The present results show that Zn, Mn and Cr are present in fairly good amounts and may contribute to hypoglycemic effect of this plant.

Zinc (Zn): Zinc is considered one of the most abundant essential element found mostly in human muscles and bones. It is a component of many enzymes and insulin (Lokhande *et al*., 2010). In human body more than 300 Zn dependant proteins are found, which are important for bone strengthening, cell signaling, hormones release and in apoptosis (Saeed *et al*., 2010). Zn deficiency leads to disorder in glucose metabolism (Ibrar *et al*., 2003), weight loss (Lokhande *et al*., 2010) while its acute toxicity causes nausea, abdominal pain, diarrhoea and vomiting (Anonymous, 2001). Daily requirement of Zn for normal adult is 15mg while for pregnant and lactating women it is 20-25 mg (Prasad, 1982). Zn

concentration in the leaves and bark were 84.3±0.19 pm and 89.0±0.33 ppm, respectively. The permissible limit in plants is 50 ppm (Saeed *et al.,* 2010). The reported antidiabetic effect of *S.laureola* might be due to the presence of Zn in fairly large amounts, which enhance glucose metabolism in body. A similar conclusion was reported by Ibrar *et al*., 2003 for *Hedra helix*, which also contain the hypoglycmic elements in large amounts. This plant might be useful in acute Zn deficiency conditions. Saeed *et al*. (2010) and Sultan *et al*., (2010) also reported the presence of Zn in some medicinal plants and suggested to have a key role in plant as well as animal metabolism.

Manganese (Mn): Manganese is a trace element of significant importance. It is essential for glucose metabolism, normal body growth and reproductive function (Ibrar *et al*., 2003). Mn intoxication is responsible for Parkinsonism condition (Wang *et al*., 2008b). Its improper supply in diet leads to abnormal glucose utilization in the body (Donsbach & Ayne, 1982). Concentration of Mn in *S. laureola* leaves was 86.6±0.19 ppm while in bark it was 43.6±0.19 ppm, where as the permissible limit for plants is 200 ppm (Saeed *et al.,* 2010). Ibrar *et al*. (2003) reported that the hypoglycemic potential of *Hedera helix* might be due to the presence of fair amounts of trace elements including Mn. *S. laureola* leaves were locally reported to use traditionally for lowering glucose level. Mn may be one of the contributing factors for its alleged hypoglycemic property.

Chromium (Cr): Chromium is one of the the abundant elements on the earth, and play important role in body metabolism (Emsley & John, 2001). It is an important component of a complex known as "Glucose Tolerance Factor" which plays an essential role in insulin action by attaching it to cell membrane and ultimately enhancing glucose uptake capability of a cell (Robenson & Hurly, 1981; Ibrar *et al*., 2003). The deficiency of Cr leads to improper availability of this specific complex, resulting in defective functioning of insulin and abnormal glucose metabolism (Ottawa & Fell, 1986). Cr also plays important role in metabolism of carbohydrates, proteins, lipids and has also been proved to facilitate the action of insulin (Anonymous, 2001). Chromium based supplements are useful for weight loss (Lukaski *et al*., 2007). Concentration of Cr in leaves and bark of *Skimmia laureola* were recorded 1.47±0.33 ppm and 1.38±0.35ppm respectively which were in the permissible limit for Cr in plants (1.5 ppm) (Saeed *et al*., 2010). On the basis of present findings regarding

Zn, Mn and Cr (hypoglycemic elements), it can be concluded that *S. laureola* is a useful hypoglycemic plant and the findings of Ibrar *et al*. (2003) support this statement.

Copper (Cu): Copper is a micronutrient of significant importance, as synthesis of many human proteins is copper dependent (Huang & Failla, 2000). Cu deficiency also causes cellular level iron deficiency, as it affects Fe transport in body tissue, thereby causing a condition known as 'hypochromic microcytic anemia', similar to that caused by Fe deficiency (Arredondo & Nunez, 2005). In the present work Cu was found in a little larger amount in leaf and bark (11.33±0.19 ppm and 15.66±0.19 ppm respectively) than the normal permissible limit (10ppm) for plants. 340–900 μg /day of Cu is the recommended dietary allowance (RDA) (Saeed *et al*., 2010). So if otherwise not harmful, this plant can also be considered for Cu deficiency in body.

Iron (Fe): Iron**,** an important part of haemoglobin. It is the most abundant trace element in human body tissues (Arredondo & Nunez, 2005). Iron presence is important for the survival of all organism including plants, animals and microorganism. The role of Iron is associated with heamoglobin functioning and to the exchange of oxygen and carbon dioxide between lungs and tissues (Sigel, 1978). Iron deficiency is reported to be the most prevalent nutritional deficiency in humans, especially in females and is caused by insufficient dietary intake, excessive menstrual flow or multiple births. About 46% Children and 48% women of the world are suffering from anemia, the main cause of which is iron deficiency, also affects brain functioning (Beard, 2001). In our body most of the iron occurs as heamoglobin (57.6%) and non-heme iron complexes (33%), like ferritin and hemosiderin (James, 2005). In the present study, iron concentration was found to be 5.00±0.33 ppm in leaves and 4.83±0.35 ppm in bark, which are much lower than the permissible limit (36-241 ppm). Iron deficiency is also reported by Aremu *et al*. (2006) and Bakhsh *et al*. (2007) in some plants.

Nickel (Ni): Concentration of metals in plants is related to their concentration in soil (Al-Naseer *et al*., 2009). Nickel (Ni) is naturally present in the earth's crust abundantly and it is easily accumulated by plants. Nickel is thought to play a role in glucose metabolism due to its presence in pancreas and its role in insulin production (Cempel & Janicka, 2002). Skin allergy, liver disorders, kidney disorders, cardiovascular problems etc are some of the health

hazards of Nickel (Denkhaus & Salnikow, 2002; Saeed *et al*., 2010). In the present study Ni was not detected in the leaves while in bark it was 2.50±0.33 ppm, exceeding the standard permissible limits (1.5 ppm). More or less similar results for Nickel concentration were reported by Narendhirakannan *et al*. (2005) in the leaves of four traditional medicinal plants (*Murraya koenigii, Mentha piperita, Ocimum sanctum,* and *Aegle marmelos*). Similarly Ozcan *et al*. (2005) detected Ni concentration of young shoots, flower buds, caper berries (fruit), and seeds of *Capparis ovata* were found to be very low in all these parts of the plant.

Cobalt (Co): Cobalt is a trace element essential for the plant tubercles for nitrogen fixation. Vitamin B12 is the main source of Co, which can be synthesized by the animals as well as available in some animal food in the form of cobalamines (Lokhande *et al*., 2010). The recommended daily intake of Co is 0.13 mg (3 mg of vitamin B12) of which only 50% is absorbed in the intestine. Although Co is distributed throughout the body but highest concentration is usually found in bones, kidney and liver. Deficiency of vitamin B12 produces genetic defects and failure of gastric mucosa. Vitamin B12 is essential for normal functioning of all body cells, thyroid metabolism and is also of utmost importance for red blood cells maturation. Deficiency of vitamin B12 (Co) causes disorders related to abnormal functioning of red blood cells (Thunus *et al*., 1994). In the present study, high Co contents were found in leaves (2.00±0.33ppm) while in the bark it was below the level of detection. High Cobalt content with iron is regarded useful for anemia (Lokhande *et al*., 2010); therefore the leaves of *S. laureola* might be useful for curing blood related disorders.

Lead (Pb): Lead is a toxic element and its presence in plants may be due to environmental pollution. Negligible concentration of Pb in leaves and bark of *skimmia laureola* makes it safe for medicinal purposes. It is also indicative of the polluted environment in which the plant grows.

Sodium (Na): Sodium is macronutrient important for various metabolic process of human body. Most common dietary source is table salt. Na+ with K+ is crucial for life, as their movement play a very voluble role in excitation and transmission of nerve impulses during action potential of nerve cells (Saeed *et al*., 2010; Underwood, 1977). Na plays a very significant role in the distribution of intracellular and extracellular fluids, the overall balance

of which is dependent upon the concentration of Na+ Ions (Morris *et al*., 2008). Muscle cramps, dehydration, fatigue, mood changes, hair loss and hypotension are some of the symptoms of Na depletion (Harper *et al*., 1997). The present study showed adequate concentration of Na in the leaves (147.67±4.19 ppm) and in the bark (126.67±3.51 ppm) of *S.laureola* showing that it may be used in the disorders caused by sodium deficiency e.g dirrhoea. For plants, no international limit is available. Daily recommended intake of Na+ is 1–3.8 mg/day (Anonymous, 2001). Availability and almost same role of Na+ of some medicinal plants of KPK, Pakistan was also described by Hussain *et al*., 2011b. Similarly Saeed *et al*. (2010) reported almost similar results for *Polygonatum verticillatum*, as were found in *S. laureola*.

Potassium (K): Potassium is a macronutrient of both plants and animals and has a very crucial role in metabolism. The concentration of K ions concentration is mostly associated with action potential of nerve transmission in the human body (Saeed *et al*., 2010). The K^+ channels are important for multiple functions of both excitable and non-excitable cells. Cellular regulations affected by K+ concentration include membrane potential regulation, insulin and other hormonal secretion, signal transduction and immune response (Curran, 1998). For plants, no international limit for potassium ion concentration has been reported, however, for human being the average intake of Potassium is 3100 mg/day for adult men and 2300 mg/day for adult women (Anonymous, 2001). In the present study, 1833.00±2.19 ppm and 1066.7±1.51ppm K were detected in the leaves and bark of *S. laureola* respectively. These results signify this plant as a rich source of potassium. Jan *et al*. (2011) reported large quantity of K in *Cichorium intybus*. Similarly Ravi *et al*. (2011) reported 19.2% potassium per mg in the seeds of *Gloriosa superba*. These findings supported the present study.

Correlation of trace elements

Correlations exist among the trace elements in plants as suggested by some workers like, Herber & Stoeppler (1994), Kumar *et al*. (2005) and Lokhande *et al*. (2010) also suggested correlationship of essential elements like Fe, Zn, Co, Mn and Cr in plants.

Essential elements like Zinc, manganese and chromium play an important role in glucose metabolism; therefore they are named as hypoglycemic elements (Donsbach & Ayne, 1982).

Zn also plays an important role in wound healing and also act as a strong antioxidant like Mn (Zafar *et al*., 2010; Bibi *et al*., 2006). Similarly it is also reported in various research works that Zn, Mn and Cr are Important in maintaining glucose tolerance and insulin secretion (Choudhary & Bandyopadhyay, 1999; Ottawa & Fell, 1986; Zetic *et al*., 2001). Finding correlations and ratios among these trace elements are helpful in ascertaining their antagonistic and synergistic effects during metabolisms, thus providing bases for their availability in balanced manner. From the experimental data it was found that highly significant correlation was existed in Zn and Mn (r^2 =1.0000) in leaves of *S. laureola* (Fig. 4.19a). Contrary to leaves, the bark showed no correlation (r^2 = 0.0000) between Zn and Mn (Fig.4.20a). No correlations was observed for Cr with Zn and Mn in leaves while in bark a significant correlation was present between Zn and Cr with r^2 = 0.9231(Fig. 4.19b, c & 4.20b, c respectively). Similarly Zn/Mn, Zn/ Cr and Mn/Cr ratio were also determined in the leaves and bark of *S. laurola*. Bark has highest Zn/Mn ratio (20.41) than leaves (9.73). Zn/Cr ratio was found highest in bark as compared to that found in the leaves, while highest Mn/Cr ratio existed in leaves (Fig. 4.21).

For diabetics patients, 14.8–28.4 μg/g of Zn may be beneficial and it deficiency was found to be correlated with chronic malabsorption (O'Dell *et al*., 1997). Similarly iron is reported to facilitate carbohydrates, fats and proteins metabolism, and thereby controlling body weight, hence very beneficial in diabetes (Rajurkar & Pardeshi, 1997). Like Zn and Fe, Co is also physiologically active in metabolism and is required up to 3 μg per day for diabetic patients (Lokhande *et al*., 2010). In the light of above discussion, correlation and ratios among Zn, Fe and Co were also evaluated. Fe and Zn showed variant correlation. There were no correlation (r^2=0.000) in the leaves while highly significant correlation existed (r^2=0.9231) in the bark of *S. laureola* (Fig. 4.19d & 4.20d respectively). High Zn/ Fe iron ratio (18.42) was recorded in the bark as compared to leaves (16.86) (Fig. 4.20). Co was not detected in the bark while its correlation with iron in the leaves was found to be significant (r^2=0.9774) (Fig. 4.19e). Flow of ions especially of K^+ and Na^+ is of extreme

importance for life, as these regulate acid-base balance in the body fluids. Also their movement in the nerve cells control excitation and transmission of nerve impulse during action potential. The two ions exhibit maximum interdependence for their regulation (Ayoola *et al*., 2010; Lokhande *et al*., 2010). In the present study on *S. laureola*, it was found that K^+ and Na^+ correlation was insignificant in leaves (r^2=0.1842) (Fig. 4.19f), but was highly significant in the bark (r^2=0.8929) (Fig 4.19e). The result shows that K/Na ratio (12.47) in the leaves were larger than that of bark (8.42) (Fig. 4.21). The present results indicated that potassium contents were 12.47 times greater in leaves and 8.42 times greater in bark than sodium. The present results are somewhat inconsistant with that of Lokhande *et al*. (2010), who reported much greater ratio values for K/Na in some medicinal plants but are well in accordance with Njoku & Akumefula, (2007), who reported that K/Na ratio in *Spondias mombin* was 25.5.

f. proximate analysis

Proximate and nutrient analysis of plants is important for determination of nutritional and curative values. Medicinal plant species are utilized for curative purposes of various ailments. Besides medicinal value, proximate analysis are important, to understand nutritional worth of these plant as well (Pandey *et al*., 2006). In the present study proximate analysis of *Skimmia laureola* was conducted to evaluate its nutritional value.

Carbohydrates are considered the primary source of energy for all organisms, playing nutritional as well as structural role. High carbohydrates contents suggest suitability of plants as feed (Abighor *et al*., 1997). Bukhsh *et al*. (2007) reported high carbohydrates contents in the leaves of *Eruca sativa*. Similarly, Hussain *et al*. (2011b) carried out proximate analysis of *Aerva javanica* and *Calotropis proceraand* reported high carbohydrate content in these plants. The present proximate analysis also showed higher carbohydrates contents, both in leaf and stem, however, the contents were higher in leaf (Table 4.23).

It is imperative to increase protein production by utilizing all the available ways and means as they play both curative and nutritive role ((Shah & Khalil, 1988). Search for good quality proteins is increasing day by day due to rapid increase in population and health problems. It has been reported that the protein gap in Pakistan would continue to

increase unless well-planned measures are adopted to tackle the situation (Nisar *et al*., 2009). Protein contents in *S. laureola* leaves were 2.50±0.03 % in while 3.46±0.02 % in the bark (Table 4.23). The present findings are well supported by Hussain *et al*. (2010a), who reported high protien contents in *Spinacea oleracea* and *Trianthema portulacastrum* and Anwar & Rashid (2007), who found high protein contents in *Moringa oleifera*.

Plant materials containing 1- 2% fats, is considered useful to human beings, as excessive fat consumption results in cardiovascular disorders like atherosclerosis, cancer and aging (Antia *et al*., 2006). Fat contents, 2.35±0.02 % and 0.75±0.02% were detected in the leaves and bark of *S. laureola* respectively (Table 4.23). These contents of *S. laureola* are much lower as compared to those of *Amaranthus hybridus* (4.80%), *Baseila alba* (8.71%), *Calchorus africanum* (4.20%) and *Talinum triangulare* (5.90%) (Iniaghe *et al*., 2009), suggesting that in respect of fat contents this plant is safe both for medicinal as well as for nutritional purposes.

Crud fibres occur in higher amount in non starchy tissues (Agostoni *et al*., 1995) and are considered best the treatment of diseases like habitual constipation, diabetes, gastrointestinal disorders, obesity and cancer (Saldanha, 1995). In the present study, crude fibers contents were 20.83±0.25 % in the leaves and 25.50±0.17 % in the bark of *S. laureola* (Table 4.23). These values were much higher than those of *Acalypha hispada* (10.25%) and *Acalypha racemosa* (7.20%) (Hussain *et al*., 2011b). Similarly Naseem *et al*. (2006) reported 27.2 % crude fibers in *Crotalaria burhia*. Our findings suggest that crude fibers are well in the reported range and this plant might be an important source of crude fiber in diet or in drug.

High ash content is an indication of the mineral stuffing available in the plant materials (Antia *et al*., 2006). Observed ash values in the leaves and bark of *S. laureola* were 10.23±0.02 % and 8.89±0.07% (Table 4.23). These ash values indicated high deposit of minerals, already explored in the present study. These ash values found lower than that of *Talinum triangulare* (20.05%) (Iniaghe *et al*., 2009). However, these are higher than some

vegetables like *Hibiscus esculentus* (8.00%) and *Occimum graticimum* (8.00%) as reported by Akindahunsi & Salawu (2005).

Moisture contents were 2.21±0.02 % and 3.96±0.03 % in leaves and bark dry powder respectively (Table 4.23). These values are little inconsistent with the moisture contents values reported by Kochhar *et al*. (2006), Hameed *et al*. (2008) and Hussain *et al*. (2011b) in their work.

4.5.2. Pharmacognostic studies of *Zanthoxylum armatum*

Like *Skimmia laureeola*, leaf, stem bark and fruit of *Zanthoxylum armatum* were evaluated for pharmacognostic features including macroscopy, microscopy and physicochemical study of powder drugs.

4.5.2.1. Macroscopy

Macroscopic study included organoleptic evaluation like size, shape, color, odor and taste of the crude drugs, which provide basis for the primary pharmacognostic identification. Nayak *et al*. (2010) carried out detailed pharmacognostic study of *Jatropha curcas* leaf for pharmacognostic evaluation. Similarly Balakrishnan (2011) reported macroscopic features of *Amaranthus spinosus* (Amaranthaceae) in order to develop diagnostic parameters for quality control of leaf and stem. In the present study, leaf, stem bark and fruit of *Z. armatum* were studied and described macroscopically, to establish their standard parameters.

Z. armatum has compound bifacial leaf with prominent midrib, some time provided with spines. The leaflet is lanceolate in shape with slightly dentate margins and acute apex (Fig. 4.21). It has pleasant odor and astringent taste. Other macroscopical characteristic features of the leaf are summarised in Table 4.24.

Barks also have some diagonostic macroscopic characteristics such as size, shape, colour, odor and taste etc which provide base for pharmacognostic evaluation (Sharma *et al*., 2010). Various macroscopic features of the stem bark of the *Z. armatum* were worked out (Table 4.25). It is cylindrical irregular curved in shape, the outer surface of which was dark brown while the inner was light brown in color (Fig. 4.22). The present observations

are well in line with Goyal *et al*. (2011) for pharmacognostic standardization of the stem bark of *Careya arborea* and strengthened our studies.

Studies for fruit of *Z. armatum* were also carried out (Table 4.26). It is small drupes with spherical ovoid shape (Fig. 4.23). It is reddish brown in color and astringent in taste. Macroscopic features of Fruit are also reported by other workers e.g Kalhoro *et al*., 2002 for *Citrullus colocynthis* and Thomas *et al*., 2008 for ripe fruit of *Averrhoa carambola*.

4.5.2.2. Microscopy

Microscopic evaluation is indispensable in the initial identification of plants materials by studying its anatomy as well as for identification of small fragments of crude or powder drugs. Other techniques like linear measurements, determination of leaf constants and quantitative microscopy are also used for drug evaluation (Jarald & Jarald, 2007). Microscopic evaluation including leaf surface values, stomatal study, anatomy of the leaf, anatomy of stem, anatomy of bark, anatomy of fruit and their powder drug microscopy of *Zanthxylum armatum* was carried out in the present study.

a. Leaf anatomy

Leaf lamina of *Zanthoxylum armatum* showed an upper epidermis followed by pallsade mesophyll, spongy mesophyle and then lower epidermis (Fig. 4.24). Vascular bundle, comprising of xylum and phloem was also present. Upper epidermis, covered with thin cuticle, was composed of rectangular shaped compactly arranged cells. These cells were 5.9-10.35µ with mean (8.33±1) in length, 362 µto 2.3-4.6 µ (with mean of 3.73±0.73 µ) in width. The upper epidermis was non stomatiferous. Palisade tissue in the meesophyll region was composed of compactly arranged cylindrical shaped cells. The palisade mesophyll was single layered. The cells were 10.35-14.38µ (with mean value of 12.19±1.52 µ) in length and 1.15-2.3 µ (with mean value of 1.95±0.555µ) in width. Calcium oxalate crystals were observed in these cells. The spongy mesophyll cells were rounded to oval to somewhat elongated with length, ranging from 3.45-5.18µ (with mean value of 3.85±0.609 µ) and width of 1.7-2.3 µ (having an average of 2.30±0.271µ) in width. Some of these cells also contained Ca-oxalate crystals and were loosely arranged with intercellular spaces. These cells might dissolve to form schizogenous or lysischizogenous cavities. Collateral vascular

bundles consist of xylem and phloem was also observed. Lower epidermis was stomatiferous with varied shaped (i.e. rectangular, elongated or irregular shaped) cells. The length of these cells was 4.60 – 6.33 µ (with a mean value of 5.69 ±0.571 µ) in length and 2.01-2.88 µ (with a mean of 2.31 ±0.227 µ) in width.

b. *Zanthoxylum armatum* leaf midrib anatomy

Adaxial surface of *Zanthoxylum armatum* leaf midrib was planoconvex while the abaxial surface was semicircular in appearance. The diagnostic feature of the midrib the complete absence of any kind of trichomes or any other appendage on it. T.S of midrib appeared to consist of upper epidermis, hypodermis, vascular bundle, cortex and lower epidermis (Fig 4.25). Upper epidermal was a single layered with oval to rectangular cells 1.72-2.30 µ (with mean of 2.05±0.275µ) in length and 1.5-2.0 µ (with a mean of 1.93±0.379µ) in width. The upper epidermis was covered by a thin cuticle. Below the epidermis, a 3-5layered hypodermis was present consisting of thick walled round cells 1.73-2.88 µ (with mean 2.16±0.35) in diameter. Next was many layered cortical region, of round thin walled parenchymatous cells with diameter of 2.88- 5.18 µ (with a mean of 4.07±0.75 µ). Shizogenous oil cavities were present in this region. Vascular bundle was Arc-shaped in which xylum was adaxial in position followed by phloem in the abaxial position. Vascular bundle was surrounded by iodoblast cells, containing various isolated as well as aggregates of calcium oxalate crystals. Xylem, appeared in radial rows with cell size of xylem vessels range from 2.07- 3.45 µ (with mean diameter of 2.62±0.618 µ). Phloem was characterized by rounded cells with almost the same dimensions, as that of xylum. The lower epidermis of the leaf was composed of rectangular cells, 2.30-4.03 µ (with mean of 3.34±0.890 µ) in size.

c. Leaf surface characters

Various quantitative microscopic features of leaf surface such as palisade ratio, vein islets number and vein termination number of *Z. armatum* were worked out in the present study (Table 4.27). The leaf has a Palisade ratio from 6.00 to 9.00 with average of 8.2±0.32 (Fig 4.27a). Vein iselet and vein termination number were 14-21 (16.8±0.64) and 17 - 21 (19.1±0.43) per mm^2 respectively. The vein-islets were quite distinct and were squaresh,

elongated, polygonal or irregular in shape, provided with many forked and unforked vascular branches (Fig. 4.27b). Leaf epidermal studies are of immense significance in finding phylogeny and taxonomy of closely related species and to resolve such problems, taxonomists have given prime importance to leaf epidermal features (Taia, 2005). Bhagwat *et al*. (2008) subjected *Euphorbia hirta* for pharmacognostic study including leaf surface features. Venkatesh *et al*. (2008) evaluated *Dodonaea viscosa* for such features in view of its medicinal importance, taxonomic confusion, microscopical and morphological characters and as referential information for identification of this crude drug. Other studies on *Tylophora dalzellii* (Najafi & Deokule, 2010) and *Acacia leucophloea* (Gupta *et al*., 2010) were also carried for Palisade, vein islet and vein termination values. These studies clearly indicated that these features showed variation in different spp. From the work of these and others it might be concluded that these feature are of utmost significance in identification and authentication of leaf crude drugs.

d. Stomatal study

Stomata are mostly present on the leaf epidermis. On the bases of its occurrence leaf may be amphistomatic (Stomata present on both epidermises), or epistomatic (stomata present only on upper epidermis) or hypostomatic (stomataonly present on the lower epidermis) (Perveen *et al*., 2007). Stomatal arrangement and types are considerd best taxonomic criteria and provide effeicient bases for exploring phylogenetic relationship in taxanomic hierarchy (Hameed *et al*., 2008). Some workers like Sen & Hennipman (1981) had the idea that they may not be so an effective tool in taxanomy because of their inconsistent arrangement in epidermises, however stomatal values like stomatal number and stomatal index are of great value in the evaluation of leaf origin crude drugs (Evans, 2002).

Z. armatum leaf was found to be hypostomatic as the stomata were found on the lower epidermis only (Fig. 4.28). The occurrence of stomata on lower epidermis is regarded to be a xerophytic trait (Esau, 1977). In *Z. armatum* orientation of stomata with respect to each other appeared inconsistent; some lie parallel, some at right angle and still other occurred at various angle to each other. Various numerical values of stomata including stomatal Index, stomatal number, varience, co-efficient of variance, Standard deviation and

standard error of stomatal number were worked out in (Table 4.27). The stomatal index was 12.32± 0.26, while the average stomatal number was 196.1±3.07 which is quite high as compared to other plants. Metacalfe & Chalk (1950) reported that stomatal size and stomatal density are inversely proportional to each other. A Similar situation was found in the present study, as stomatal size was small and its density was quite high.

Various features, like the stomatal size, stomatal index and stomatal pore size are of great significance in differentiating the taxa at specific and interspecific level (Nabin *et al*., 2000). Ferris *et al*. (2002) also reported the usefulness of co-efficient of variance, stomatal density, stomatal index, epidermal cell area and number of epidermal cells per leaf of poplar. Similarly Hameed *et al*. (2008) carried out statistical evaluation, including mean, standard deviation, variance and coefficient of variance of stomata in the epidermises of some members of family polygonaceae and suggested a texanomic uniformity of stomatal type. These studies support and strengthed the present study.

Epidermal study showed five different types of stomata in *Z.armatum* leaf with different frequencies and other dimentions. As given in Table 28, these types are

1. Anomocytic, characterized by four or more undifferentiated cells (Metacalfe & Chalk, 1950) (Fig.4.29a). 2. Stephanocytic type characterized by a rossete of four or more weakly identified cells (Baranova, 1987). Sub types of this type were also present, which were a. Actinocytic, having radial elongation of subsidiary cells (Wilkinson, 1979) (Fig. 4.29b). b. Actinostephanocytic having slight radial elongations of some or all cells (Carpenter, 2005) (Fig. 4.29c). c. Staurocytic, stomata surrounded by four or more cells, having differently oriented cell wall to the stomatal pore (Van Cotthem, 1970) (Fig. 4.29d). 3. Paracytic type, characterized by one or two lateral subsidiary cells, oriented parallel to the guard cells (Dilcher, 1974). This categogory had the following sub types a. Brachyparacytic, having two lateral epidermal cell, oriented parallel to guared cell (Carpenter, 2005) (Fig. 4.29e). b. Brachy paratetracytic having four subsidiary cells, of which two were polar and two lateral (Chengqi *et al*., 2007) (Fig. 4.29f). c. Hemiparacytic, characterized by a single parallel cell. (Carpenter, 2005) (Fig.4.29g). 4. Cyclocytic (laterocyclocytic), subsidiary cells arranged in the form of a circle around the stoma (Carpenter, 2005) (Fig 4.29h). 5. Stomatal

cluster, where two stomata are arranged side by side without any common subsidery cell in between them (Tang *et al*., 2002) (Fig. 4.29i). Stomatal cluster was considered to be a special leaf epidermal feature, reported only in 38 genera of vascular plants (Tang *et al*. 2002). Chengqi *et al*. (2007) reported similar pattern of stomatal apparatus in *Camellia henryana* and *C. tsingpienensis*. Stomatal cluster was very rare feature; therefore further work is required to consider it as a texanomic tool. Brachyparatetracytic stomata was the most frequent stoma (80%) followed by actinostephanocytic (40%) and then straucytic and brachy paracytic (30%). Hemiparacytic and stomatal cluster were the rarely occurring stomata (10%each) observed on the lower epidermis of *Z. armatum* leaf (Table 4.28). Inspite of great variation in the frequencies, no significant differences were observed in the length and breadth of these different stomata.

Adaptation skills of plants depend on stomatal arrangement on the epidermises, as transpiration and photosynthesis are closely related to Stomata. These are also useful in taxonomic categorization and detection of future clues for observing environmental factors. Micro and macro elements in plants were also closely related to stomatal density (Nabin *et al*., 2000; Brownlee, 2001). Very little work has been done on the stomatal study of family Rutaceae. Ogunkunle & Oladele (1997) reported paracytic, hemiparacytic, brachy paracytic, brachyparatetracytic and anomocytic stomatal complexes with uniform size from abaxial epidermises of various *Citrus* species (Rutaceae) and also proved that in spite of high stomatal density, they have relatively low transpiration rate as compared to species with low stomatal density. Similar observations were also recorded in the present study, showing that these might be a future finger printing in exploring phylogenetic relation of family Rutaceae.

e. Bark anatomy

Bark anatomy of *Z. armatum* showed different tissues identified as cork (Outer phellem), cork cambium (middle phelogen), cortex (Pheloderm), medullary rays, phloem, bast fibers and tracheads (Fig. 4.30). Cork was composed of a few layers of closely packed brown color lignified thick walled rectangular or squared shaped cell, ranged from 2.88-4.03 µ (3.39±0.571 µ) in length and 1.73-3.45 µ (2.01±0.729 µ) in breadth. Cork cambium is a continuous layer of small elongated rectangular thin walled parenchymatous cells, ranged

from 3.45-5.75 μ (4.72±473 μ) in length and 1.15-2.3 μ (1.78±0.424μ) in breadth. The cortex was composed of closely packed large parenchymatous cells. The length of the cells ranged from 3.45 – 5.18 μ (4.54±0.63 μ) in length and 1.15- 2.88 μ (1.84±0.706 μ) μ in width. In These cells various types of calcium oxalate crystals were deposited. Medullary rays were also present, passing through the phloem and were 2 to 5 cells wide. The phloem consisted of intact and crushed phloem elements. The bast fibers and large treachery cells were also observed. Ahmed & Urooj (2011) also carried out bark study of *Ficus racemosa* (Ahmed & Urooj, 2011), Similarly Kanth & Bashker (2011) reported periderm, cortex and secondary phloem as the general arrangement in the transverse section of stem bark of *Holoptelea Integrifolia*. The present study is supported by these reports.

f. Fruit anatomy

T.S of fruit showed two portion i.e. fruit wall and seed. Fruit wall is clearly distinguished into three layers, epicarp, mesocarp and endocarp (Fig 4.31). The outer most layer, epicarp is composed of closely arranged thick walled rectangular cells, 2.3-4.03μ (3.22±0.55 μ) in length and 0.75-1.13 μ (0.86±0.25 μ) in width. The middle layer, mesocarp is composed of irregular thin waled parenchymatous cells, 2.30-4.31 μ (3.31±0.70 μ) in length and 2.30-4.6 μ(3.74±0.82 μ) in width. This layer contained large schizogenous and lysoschizogenous oil cavities, about 30 μ in diameter. The inner layer of fruit wall, (endocarp) was composed of two layers. The outer layer is composed of small isodiametric or rectangular, thin walled parenchymatous cells, 4.60-7.48μ (6.04±1.24 μ) in length and 2.30-3.00 μ (2.69±0.33 μ) in width. The inner layer of mesocarp is composed of comparatively larger, thin walled rectigular cells, having a length of 8.05-13.8 μ (10.12±2.35 μ) and width of 3.45-5.18 μ (4.31±0.48 μ). There is a single, some what oval shped seed in the fruit. T.S of seed showed an outer pigmented layer of testa followed by a layer of thin walles small cells. The seed was non endospermic and contain small elongated embryo. This description is well supported by the fruit anatomy of Marzinek & Mourao, (2003) and Thomas *et al*. (2008) have described fruit anatomy of *Chorisia speciosa* and *Averrhoa carambola* respectively in similar manner.

g. Powder drugs microscopy

Powder drug microscopy of *Zanthoxylum armatum* leaf, stem bark and fruit were carried out.

I. Leaf powder drug microscopy

Z. armatum leaf powder was olive drab green with pleasant odor and some what astringent taste. Various fragments found in the leaf powder are shown in Fig. 4.32 and given below.

i. Upper epidermal fragments with irregular closely arranged thin walled cells, 19µ to 25µ, to which cylindrical palisade cells were attached from surface view and side view.
ii. Lower epidermis fragments with irregular, curved thick walled cells containing stomata.
iii. Spongy mesophyle cells in surface, 5µ in diameter.
iv. Vessels with elongated parenchyma cells.
v. Single and aggregated Calcium oxalate crystals, 2µ to 6µ in diameter.
vi. Rounded starch grains, 4µ to 8µ in diameter.
vii. Collenchymas cells with calcium oxalate crystals.
viii. Fibers from vein with attached parenchymatous cells.

These features of powder drug can be used as a future reference for identification of the powder drug of this plant. (Venkatesh *et al*., 2008) described the microscopic features of *Dodonaea viscosa*. Nayak *et al*. (2010) reported that rarly trichomes were present in the leaf powder of *Jatropha curcus* along with other fragments. Similarly Nikam *et al*. (2009) reported the powder drug study of *Sesbania sesban* leaf. The present findings in the leaf powder of *Z. armatum* were found a little different, as no trichomes were detected, in it.

II. Bark powder drug microscopy

Bark Powder of *Z.armatum* was grey brown in color with light pleasant odour and bitter taste (Fig. 4.33). Following fragments were detected in the bark.

i. Closely fitted, thick walled Cork cells.
ii. Collenchymatous cells containing aggregates of calcium oxalate crystals.

iii. Fragments of medulary rays.
iv. Isolated sclerids with calcium oxalate crystals.
v. Pitted Sieve elements, 40-45 μ in length.
vi. Thick walled phloem parenchyma, to which oval shaped medulary ray cells were attached.
vii. Fibers, 75 to 95 μ in length, to which parenchymatous cells are attached.
viii. Calcium oxalate crystals of various types i.e. cluster, prismatic, elongated, round and rectangular.
ix. Isolated and aggregated starch granules.

The present bark powder study could be valuable in the preparation of future monograph for appropriate evaluation and standardization of this medicinal plant. The present study is well in lineage with other workers, including Bhardwaj *et al*. (2010), who carried out bark powder study of *Tecomella undulata* and the study of Goyal *et al*. (2011) who carried out microscopic analysis of the bark of *Careya arborea*.

III. Fruit powder drug microscopy

Fruit is the main part used of *Z. armatum* and powder drug evaluation is essential to set parameters, which help in detection of adulteration. Fruit powder of Z. *armatum* was blackish brown with pungent smell and taste. Following are some of the fragments found in the fruit powder drug (Fig 4.34).

i. Group of sclerenchymatous cells from mesocarp.
ii. Thin walled closely packed parenchymatous cells.
iii. Vascular tissue, consisting of elongated fibres.
iv. Irregular, dark brown Parenchymatous cells
v. A fragment from fruit wall with broken oil gland.
vi. Inner epidermal cells of pericarp.
vii. Rounded Starch granules, 12-16μ in diameter.
viii. Sclerids from epicarp, irregulaer in shape.
ix. Epicarp cells elongated in shape with small parenchymatous cells attached.
x. Rounded or polygonal cells endocarp cells with calcium oxalate crystals.

Thomas *et al*., 2008, reported that trichomes and large lysigenous cavities are the characteristic features of the *Averrhoa carambola* fruit. Similarly starch grain and fibers in mesocarp are the distinguishing features of *Solanum xanthocarpum* fruit (Kadam *et al*., 2010). In the present study sclerids, starch grains, endocarp with calcium oxalate aggregates are some of the important fragments that might be helpful in authentication of *Z. armatum* fruit powder drug.

4.5.2.3. Physicochemical characteristics

Various physicochemical characteristics including Ash analysis, qualitative and quantitative phytochemical screening, fluorescence astudy, extractive values, elemental analysis and proximate analysis of leaf, stem bark and fruit powder of *Z. armatum* was carried out.

a. Ash analysis

Ash studies of powder drugs included total ash, acid insoluble ash and water soluble ash test for leaf, bark and fruit of *Z. armatum*. Ash analysis is important for detection of adulteration or the presence of inorganic matter such as metallic salts and/or silica during improper handling (Jarald & Jarald, 2007) as well as for detection of exhausted materials in the drugs (Wallis, 1985). Various ash values for genuine leaf, stem bark and fruit as well as, those exhausted with ethanol and n- hexane were carried out. Leaf, bark and fruit powder of *Z. armatum* have higher values of total ash, acid insoluble ash and water soluble ash than their respective powder exhausted with ethanol and n- hexane (Table 4.29). Fig. 4.35a,b,c provide a comparative look of percent ash values for exhausted and non exhausted leaf, bark and fruit powders of *Z. armatum*. A lot of workers such as Sugumaran & Vetrichelvan, 2008 (Leaves of *Bauhinia purpurea*), Nikam *et al*., 2009 (leaves of *Sesbania sesbana*), Shah *et al*., 2010 (*Lagenaria siceraria*), Mathur *et al*., 2010 (leaves of *Amaranthus spinosus*), Balakrishnan *et al*., 2011 (*Amaranthus spinosus*n), Bisht *et al*., 2011 (*Swertia chirayita*), Kumar *et al*., 2011 (*Crocus sativus*) and Zunjar *et al*., 2011 (leaves of *Carica papaya*) carried out ash analysis and tried out to standardize hebal crud drugs. The present ash studies of *Z. armatum* will be of immence importance for the evaluation of this valuable drug.

b. Phytochemical screening

Several active constituents of pharmacological importance may be available in a single plant (Ming *et al*., 2005). It is suggested that preliminary phytochemical screening is important and useful for isolation of pharmacologically active principles from a single plant (Sugumaran & Vetrichelvan, 2008). Alkaloids and saponins are the secondary metabolites, considered affective in curing diseases and protection from pathogens (Fluck, 1973; Sodipo *et al*., 1991). Similarly tannins play a major curative role in treatments of some human disorders (Asquith & Butler, 1986). Phenolic compounds are proved helpful in various biological activities (Havsteen, 2002). Both qualitative and quantitative phytochemical screening was carried out using different extracts of the leaf, bark and fruit of *Z. armatum*. Qualitative phytochemical screening of *Z. armatum* leaf is given in Table 4.30. Alkaloisds, flavonoids and tannins were found positive in all aqueous, Ethanolic and n-hexane extracts of the leaf. Carbohydrate was detected in aqueous and Ethanolic extracts while saponins were deteted only in aqueous extarct. Phytosterol, triterphenoids, fixed oil and volatile oil were detected in the ethanolic and n-hexane extracts. Proteins and glycoside were not detected in any of the extracts. Almost similar constituents were detected in the microchemical screening of the stem bark of *Z. armatum* (Table 4.31). In contrast to leaves, bark showed positive results for protein and glycosides. Fruit showed the presence of all screened compounds in one or the other extracts, while saponins, anthocyanins and glycoside were detected only in aqueous extract of the fruit of *Z. armatum* (Table 4.32). Thomas *et al*. (2008) carried out preliminary phytochemical analysis of *Averrhoa carambola* fruit. Bhagwat *et al*. (2008) screened out different extracts of *Euphorbia hirta* Patra *et al*. (2009) detected alkaloids, steroids, proteins, flavonoids, fats, oils, tannins, mucilage and organic acids in the leaves of *Hygrophila spinosa*. Shrivastava & Leelavathi (2010) carried out preliminary phytochemical investigation of *Catunaregum spinosa*. Kumar *et al*. (2011) detected alkaloids, flavonoids, carbohydrate glycosides, tannins, terpeniods, phenol, steroids and saponins in different petal extracts of *Crocus sativus* using various histochemical tests. The work of these previous workers suggests that preliminary phytochemical screening is of utmost significance for isolation of active constituents from plant materials and the present work is in accordance with the work of these researchers.

Bioactive constituents like alkaloids, sterol, saponins, tannins, phenols and flavonoids were quantitatively evaluated in leaf, bark and fruit of *Z. armatum*. The results revealed the presence of bioactive constituents in leaves comprising of alkaloids (15.60±0.10 mg/g), sterols (71.60±0.10 mg/g) saponins (21.57±0.12 mg/ g), tannins (34.43±0.21 mg/g), phenols (11.66±0.33 mg/g) and flavonoids (13.68±0.66 mg/ g). The bark contained alkaloids (19.60±0.10 mg/g), sterols (33.83±0.29 mg/g) saponins (14.78±0.10 mg/ g), tannins (28.62±0.13 mg /g), phenols (16.48±1.33 mg/g) and flavonoids (18.33±1.22mg/ g) while the fruits contained alkaloids (25.07±0.21mg/g), sterols (164.92±0.14 mg/g) saponins (28.60±0.10mg/ g), tannins (35.5±0.5 mg /g), phenols (21.68±0.44 mg/g) and flavonoids (22.8±1.33 mg/ g) (Table 4.33). Comparative statistics of these bioactive constituents in the leaves, bark and fruit of *Z. armatum* is shown in Fig 4.36. Similar studies have been conducted by Soares *et al*., (2003) for *Phyllanthus niruri,* Okwu & Josiah (2006) for *Aspilia africana* and *Bryophyllum pinnatum,* Thomas *et al*., (2008) for the fruit of *Averrhoa carambola* and Bhagwat *et al*., (2008) for *Euphorbia hirta*. The qualitative and quantitative analysis of *Z. armatum* showed that this plant might be a rich source of bioactive constituents for future use in pharmaceutical preparations.

c. Fluorescence study

Plants contain a variety of chemical constituents and thereby exhibit different fluorescence phenomenon in ordinary day light and under ultraviolet light. Some drugs have characteristic fluorescence under UV light which are not observable in visible range of light (Ansari, 2006; Jarald & Jarald, 2007; Reddy & Chaturvedi, 2010). Fluorescence analysis is considered helpful for authentication and standerdization of crude drugs. Many workers tried out to authenticate crude drugs of plant origin including Sharma *et al*., 2009 (various parts of *Holoptelea integrifolia*), Kalidass *et al*., 2009 (root and stem of *Ichnocarpus frutescens*) (Kumar & Kiladi, 2009), Mathur *et al*., 2010 (leaves of *Amaranthus spinosus*), Shrivastava & leelavathi, 2010 (leaves of *Catunaregum spinosa*), and Hussain *et al*., 2011a (*Hygrophila auriculata*). In accordance to these workers, fluorescence analysis of the *Z. armatum* leaf, stem bark and fruit powder as such and with various reagents as well as their extracts in various solvents has been carried out and the results are given in Table 4.34 and 4.35 respectively. The florescent study was carried out

using UV 256 and UV 300. Leaf, bark and fruit powders as such, those with various reagents and their various extracts exhibited variations in color. The fluorescence analysis can be used as a diagnostic tool for detecting adulterants, not only in the whole drugs, but also in their powders.

d. Extractive values

Determination of extractive value is useful in the detection of exhausted or adulterated drugs (Singhal *et al*., 2010). Extraction with different solvents detects various types of adulteration and exhausted materials. Alcohol and water soluble extractive values are indicative of the presence of the adulterants, defective processing and poor quality of the drug while petroleum ether soluble extractive value indicates lipid contents present in crude drug (Madhavan *et al*, 2009; Kokate, 1994). Percent extractive values of leaf, stem bark and fruit of *Z. armatum* were determined using different solvents including ethanol, methanol, chloroform, acetone, distilled water, butanol and n-hexane. Highest extractive value for leaves was fond in methanol (20.10 %), while for bark the highest value was in chloroform (8.5 %) and for fruit maximum extractive value was obtained with methanol (11.00%). In other solvents, all the parts showed variable values (Table 4.36). Recently, a lot of work has been done in this regard including Thomas *et al*., 2008 (fruit of *Averrhoa carambola*) Kalidass *et al*., 2009 (root and stem of *Ichnocarpus frutescens*), Nikam *et al*., 2009 (leaves of *Solanum sesbana*), Shah *et al*., 2010 (*Lagenaria siceraria*), Jain *et al*., 2010 (leaves of *Lawsonia inermis*), Balakrishnan *et al*., 2011 (*Amaranthus spinosus* Linn), and Zunjar *et al*., 2011(leaves of *Carica papaya* Linn.). This suggests that extractive values determination is an important tool for evaluation of drugs and for detection of variety of intentional and unintentional adulterantions in drugs.

e. Elemental analysis

Some of the trace elements play very crucial role in metabolism. Elemtal analysis of *Zanthxylum armatum* were carried out, wich detected the following elements. Results of elemental analysis are presented in Table 4.37.

Zinc (Zn): Zinc is a non toxic essential micro element, mostly accumulated in human muscles and bones. It is a component of many enzymes and insulin (Prasad, 1982;

Lokhande *et al*., 2010). Zn along with Mg and Cr are known as hypoglycemic elements (Donsbach & Ayne, 1982). There are more than 300 Zn dependant proteins and enzymes in human body. Zn plays a vital role in bone strengthening and functioning, cell signaling, hormones release and also in apoptosis (Saeed *et al*., 2010). Zinc is also considerd best for the growth and multiplication of cells, integrity of the skin, bone metabolism, taste sensation and eye sight (Thunus, 1994). Zn deficiency leads to defects in glucose metabolism (Ibrar *et al*, 2003), weight loss (Lokhande *et al*., 2010), nausea, abdominal pain, diarrhea and vomiting (Anonymous, 2001). 15mg of Zn is the daily intake recommendation for normal adults while for pregnant and lactating women the daily intake is 20-25 mg (Prasad, 1982). In *Z. armatum,* Zn was found 85.3±0.38ppm in leaves, 89.6±0.19 ppm in bark while 87.6±0.19ppm in fruit. With this high concentration of Zn, *Z. armatum* may have a curative role in the treatment of bleeding, boils, insect bites, wounds, and skin disease. The findings of Lokhande *et al*. (2010), Saeed *et al*. (2010) and Zafar *et al*. (2010), strengthen the present results and suggestions.

Manganese (Mn): Manganese is also an essential element for glucose metabolisms. This element also plays significant role in normal body growth and reproductive function. Parkinsonism condition is also because of Mn intoxication (Ibrar *et al*., 2003; Wang *et al*., 2008b). Its improper supply in diet leads abnormal glucose utilization in the body, leading to glycemia (Donsbach & Ayne, 1982) Mn concentration detected in the present study were 11.86±0.38ppm, 5.63±0.19 ppm and 2.50± 0.33 in the leaves, bark and fruit respectively, which were in the permissible range for plants (200 ppm). Ibrar *et al*. (2003) reported that the hypoglycemic potential of *Hedera helix* might be due to the presence of trace element including Mn. Jan *et al*. (2011) also reported that the leaves of *Cichorium intybus* contain maximum concentration. The presence of high Mn concentration suggested this plant might be a best source of Mn both for medicinal as well as for nutritional puposes.

Chromium (Cr): Chromium is one of the abundant elements on the earth, and play essential role in carbohydrate metabolism, in protein and cholesterol synthesis. Chromium also acts as an activator for several enzymes. High Cr content may be helpful in heart tonic preparation (Anke *et al* 1998; Emsley, 2001; Zetic *et al*., 2001). Glucose Tolerance Factor (GTF), (an inorganic compound, containing glutamic acid, cysteine and niacin) enhances the

removal of glucose from the blood. Cr is an important constituent of GTF, which helps in the potentiating of insulin. (Robenson & Hurly, 1985; Ibrar *et al*., 2003; Ravi *et al*., 2011). Cr deficiency leads to improper availability of this specific complex, results in defective functioning of insulin, increases blood sugar and cholesterol levels, hence leads to abnormal glucose metabolism (Ottaway & Fell, 1986; Zetic *et al*., 2001). From the results obtained, no significant difference has been observed in concentration of Cr in leaf, bark and fruit of *Z. armatum*. Although fruit (1.66±0.33ppm) has little high values than leaves (1.63±0.19ppm) and bark (1.60±0.33ppm). All the three parts crossed the permissible limit (1.5 ppm), suggesting this plant as a rich source of Cr. This plant might be advised as hypoglycemic agent for treating diabetic patients as well as its possible use in heart tonic preparations.

Copper (Cu): Copper is a micronutrient of significant importance, as many proteins are copper dependent in human body (Huang & Failla, 2000). Copper concentration in the present study exceeded the the recommended permissible limit (10ppm) in leaf (23.00±0.33 ppm) and in bark and (31.60±0.19 ppm). Fruit of *Z. armatum has* less concentration of Cu (10.00±0.33) as compared to leaf and bark. Cu plays significant role in oxidation-reduction reactions, energy production, connective tissues formation, neurotransmitter metabolism and myelin formation (Turnlund, 2006; Harris, 1997; Amina *et al*., 2003). Cu deficiency leads to cellular level iron deficiency as it affects Fe transport in the body tissue, thus creating a condition known as hypochromic microcytic anemia, similar to that caused by Fe deficiency (Arredondo & Nunez, 2005). 340–900 µg /day of Cu is the recommended dietary allowance (RDA) for human beings (Saeed *et al*., 2010). In the light of above facts, this plant might be helpful in curing hypochromic microcytic anemia and other disorders as it is a very rich source of Cu.

Iron (Fe): Iron is the most abundant trace element in human body tissues, constituting the core of heamoglobin molecule (Arredondo & Nunez, 2005). Fe is of utmost significance for the metabolic activities of all living organism. Hemoglobin, which has a key role in exchange of oxygen and carbon dioxide between lungs and the surrounding tissue fluid, is unable to perform its function without iron (Sigel, 1978). The most prevalent nutritional deficiency in humans is reported to be the iron deficiency, especially in females, caused by

insufficient dietary intake, excessive menstrual flow or multiple births. Iron deficiency causes anemia in children and women. Brain functioning is also negatively affected by iron deficiency (Beard, 2001). Our body takes most of the iron as haemoglobin (57.6%) and non-heme iron complexes (33%) like ferritin and hemosiderin (James, 2005). In the present study, iron concentration was detected to be 14.33±0.51ppm in leaf, 15.00±0.33 ppm in bark and 25.67±0.19ppm in fruit of *Z. armatum*, which seems to be in lower concentration than the permissible limit (36-241 ppm). It seems that this plant is not a good source of iron as compared to other minerals. Aremu *et al*. (2008), Bakhsh *et al*. (2007) and Hameed *et al*. (2008) reported iron in very low concentration than the normal permissible limit in the studied plants, strengthening our findings.

Nickel (Ni): Availability of minerals in plants are related to their concentration in the soil, in which they grow (Naser *et al*., 2009). Nickle is easily accumulated in the plants, as it is naturally present in the soil in large amounts. Due to its presence in pancreas and its relation to insulin production, it might paly some important role in glucose metabolism. Nickel deficiency is also responsible for liver disorders (Cempel & Janicka, 2002). Skin allergy, lever disorders, Kidney disorders, cardiovascular problems etc are some health hazards of Nickel (Denkhaus & Salnikow, 2002; Saeed *et al*., 2010). The permissible limit of Ni in plants is 1.5 ppm. The present work revealed that *Z. armatum* bark has maximum amount (8.00±0.33 ppm) of Nickle as compared to its leaves and fruit of *Z. armatum* (Table 35). All values exceeded the standard permissible limits i.e. 1.5 ppm (Markert, 1994). These findings are in lineage with that of Narendhirakannan *et al*. (2005), who reported a similar situation in the leaves of *Murraya koenigii, Mentha piperitae, Ocimum sanctum,* and *Aegle marmelos*. Our results shows inconsistency with Ozcan (2005), who reported very low Ni concentration in young shoots, flower buds, caper berries (fruit), and seeds of *Capparis ovate*.

Cobalt (Co): Cobalt is a trace element, essential for plants and present in the tubercles, thus help in nitrogen fixation. The main source of Co is vitamin B12, which can be synthesized by the animals as well as available in their food. Some of Co is also available to animals through cobalamines (Lokhande *et al*., 2010). The recommended daily intake of Co is 0.13 mg (3 mg of vitamin B12) of which only 50% is absorbed in the intestine. Although

Co is distributed throughout the human body but is abundantly present in bones, kidney and liver. Deficiency of vitamin B12 produces a genetic defect and failure of gastric mucosa. Vitamin B12 is essential for normal functioning of all body cells, thyroid metabolism and is of utmost importance for red blood cells maturation. Deficiency of vitamin B_{12} (Co) causes disorders related to abnormal functioning of red blood cells (Thunus *et al*., 1994). In the present study, Co contents were found higher in leaves (1.70±0.33ppm) than the bark (1.67±0.19 ppm) and fruit (not detected) in *Z. armatum*. High Cobalt content in synergistic action with iron is suggested for treating blood disorders, especially anemia (Lokhande *et al*., 2010). In the light of above facts, leaves and bark of this plant might be useful for curing blood related disorders.

Lead (Pb): Lead (Pb) is a toxic element and its poisoning have serious and even fatal consequences at any age, but young children are relatively more vulnerable. Children are more susceptible to the ill effects of lead, affecting their nervous system. Even in very low oxicity (less than 10 µg/dL) it can adversely affect the behavior and development of children (Ang & Lee, 2005). Lead toxicity includes inhibition of heam biosynthesis, which results in anemia (Meredith, 1978). It also affects the nervous system (Perlstein & Attala, 1966), cardiovascular system (Staessen, 1993) and reproductive system (Ang & lee, 2005). The permissible limit of Pb is 10 ppm (WHO, 1995). In the present study, lead was detected only in the leaves (0.22±0.69 ppm), which is quite lower than the permissible limit while it was not detected in the bark and fruit of *Z. armatum,* suggesting its safe usage, with respect to lead.

Sodium (Na): Sodium is a macronutrient, important for various metabolic process of human body.The most common dietary source is table salt. Na+ with K+ is crucial for life, as their movement play a very crucial role in excitation and transmission of nerve impulse during action potential of nerve cell (Saeed *et al*., 2010; Underwood, 1977). Adequate amount of sodium intake is necessary for optimal growth. It also plays a very significant role in the distribution of intracellular and extracellular fluid, the overall balance of which is dependent upon the concentration of Na+ Ions (Morris *et al*., 2008). Muscle cramps, dehydration, fatigue, mood changes, hair loss and hypotension are some of the symptoms of Na depletion (Harper *et al*., 1997). In the present study showed adequate concentration of Na in the

leaves(132.00±1.33ppm), in the bark (140.67±4.19ppm) and in fruit (141.33±2.19ppm) of *Z. armatum* showing that it may be suggested in the disorders caused by sodium deficiency. For plants, no international limit is available; however literature revealed that the daily recommended intake of Na+ is 1–3.8 mg/day (Anonymous, 2001). Availability and almost same role of Na+ were also described by Hussain *et al*. (2011b) of some medicinal plants of Khyber Pukhtoon Khuwa, Pakistan. Similarly Saeed *et al*. (2010) reported almost the same results for *Polygonatum verticillatum*. Our results are well in lineage with them.

Potassium (K): Potassium is a macronutrient of both plant and animals and has very crucial status in metabolism. K ions concentration is mostly associated with action potential of nerve transmission in the human body (Saeed *et al*., 2010). K ion channels are important for multiple functions of both excitable and non-excitable cells. Cellular regulations affected by K^+ concentration include membrane potential regulation, insulin and other hormones secretion, signal transduction and immune response (Curran, 1998). For plants, no international limit for potassium ion concentration has been reported. However, for human being the average intake of Potassium is 3100 mg/day for adult men and 2300 mg/day for adult women (Anonymous, 2001). In the present study, 916.7±2.19ppm, 570.0±3.33 ppm and 1636.7±2.19 ppm respectively were detected in the leaf, bark and fruit of *Z. armatum*. These results signify that this plant is a rich source of potassium. Jan *et al*. (2011) reported K in large quantity in *Cichorium intybus*. Similarly Ravi *et al*. (2011) reported 19.2 % potassium per mg in the seeds of *Gloriosa superba*. The present study is well in line with the work of these workers.

Correlation ship of trace elements

Correlation exists among the trace elements in plants (Da-lun *et al*., 1988). Other workers like, Herber & Stoeppler (1994), Kumar *et al*. (2005) and Lokhande *et al*. (2010) also suggested correlationship of essential elements like Fe, Zn, Co, Mn and Cr in plants. In the present study, correlations among various elements were carried out.

Essential elements like Zinc, manganese and chromium play an important role in glucose metabolism; therefore they are named as hypoglycemic elements (Donsbach & Ayne, 1982).

Zn like Mn plays important role in wound healing and also act as a strong antioxidant like (Zafar *et al*., 2010; Bibi *et al*., 2006). Similarly it is reported in various research works that Zn, Mn and Cr are Important in maintaining glucose tolerance and secretion of insulin (Choudhary & Bandyopadhyay, 1999; Ottawa & Fell, 1986; Zetic et *al*., 2001). To ascertain antagonistic and synergistic effects among these trace elements during metabolisms, it is mandatory to findout their correlations and matual ratios, thus providing bases for their availability in balanced manner. From the experimental data it was found that insignificant correlation existed in Zn and Mn (r^2 =0.2500) in the leaves of *Z. armatum* (Fig. 4.37a). Like leaves, the bark also exhibited very little correlation (r^2 =0.2500) between Zn and Mn (Fig. 4.38a), while somewhat significan correlation (r^2=0.7500) was detected in fruit for Zn and Mn (Fig. 4.39a). Similarly significant correlations were existed between Zn and Cr with r^2 values of 0.9463, 0.8292 and 1.000 respectively in leaf, bark and fruit of *Z. armatum*. Correlations between Mn and Cr, in bark showed significant r^2 value (0.5714) as compared to leaf (r^2 =0.4286) and fruit (r^2 =0.0000) (Fig. 4.37b, c; Fig. 4.38b, c; Fig. 4.39b, c).

Similarly Zn/Mn, Zn/Cr and Mn/Cr ratio were also determined in the leaves, bark and fruit of *Z. armatum*. Highest Zn/Mn ratio (35.04) was found in fruit followed by bark (15.91) and the lowest in leaf (7.19). Zn/Cr ratio was found higher in bark as compared to that found in leaves and fruit while highest Mn/Cr ratio existed in leaves (Fig. 4.40).

For diabetics patients, 14.8–28.4 µg/g of Zn may be beneficial and it deficiency was found to be correlated with poor glucose absorption absorption (O'Dell *et al*., 1997). Similarly iron is reported to facilitate carbohydrate, fat and protein metabolism and control body weight, thus very beneficial for diabetes (Rajurkar & Pardeshi, 1997). Cobalt is also physiologically active in metabolism like Zn ansd Fe and is required upto 3 µg per day for diabetic patients (Lokhande *et al*., 2010). Correlation and ratios among Zn, Fe and Co were also evaluated. There was a significant correlations between Fe and Co (r^2=0.5714) and Zn and Fe (r^2=0.8929) in the leaves of *Z. armatum* (Fig. 4.37d, e). Significant correlation were found between Fe and Zn in stem bark with r^2= 0.7500 as compared to Fe and Cr correlation (r^2= 0.7500) (Fig. 4.38d). No correlation existed among Fe, Zn and Cr in fruit of *Z. armatum* (Fig. 4.39d, e). High Zn/ Fe ratio was recorded in the bark (5.97) as compared to leaf (5.95)

and fruit (3.41). Similarly Fe/Co ratio was found higher in bark (8.98) as compared to leaf (8.43) (Fig. 4.40). Both K^+ and Na^+ ions flow is of extreme importance for life, as body fluid acid-base balance is largely regulated by these ions. Excitation and transmission of nerve impulses in nerve cells during action potential is also under the control of K^+ and Na^+ ions. The two ions exhibit maximum interdependence for their regulation (Ayoola *et al*., 2010; Lokhande *et al*., 2010). During the experimental work, it was found that K^+ and Na^+ correlation was insignificant in the leaves (r^2=0. 0854) while highly significant in bark (r^2=0.7500) and fruit (r^2=0.9146) of Z. *armatum* (Fig. 4.37f, 4.38f and 4.39f respectively). The result shows that K/Na ratio in the fruit (12.47) was highest followed by leaf (6.94) and bark (4.05) (Fig. 4.40). These results indicate that potassium contents were 12.47 times greater in leaves and 8.42 times greater in bark than sodium. These results are well supported by Njoku & Akumefula (2007), who reported that K /Na ratio in *Spondias mombin* was 25.5. Jan *et al*. (2011) also obtained more or less the same results for leaf, root and seed of *Cichorium intybus*.

f. Proximate analysis

Proximate and nutrient analyses of plants are important for determination of nutritional value. Various medicinal plant species are utilized for curing various ailments. Besides medicinal value, proximate analysis is also important, to ascertain nutritional worth of these plant as well (Pandey *et al*., 2006). In the present study too, the nutritional value of *Z. armatum* leaf, bark and fruit was evaluated and the results are presented in (Table 4.38).

Highest carbohydrate value was found in the fruit (59.59±0.06) followed by leaf (45.76±0.08) and bark (38.64±0.11) in the present study. Carbohydrates are considered the primary source of energy for all organisms, playing nutritional as well as structural role. High carbohydrates contents suggest suitability of the plant as feed (Abighor *et al*; 1997). Bukhsh *et al*. (2007) reported high carbohydrates contents in the leaves of *Eruca sativa*. Similarly *Aerva javanica* and *Calotropis procera* also has prominent amount of carbohydrates (Hussain *et al*., 2011b).

Protein contents were 16.16±0.05 % in leaf, 10.98±0.01% in bark and 7.74±0.04% in fruit of Z. *armatum*. It is imperative to increase protein production by utilizing all the available ways and means as they play both curative and nutritive role (Shah & Khalil,

1988). Search for good quality proteins is increasing day by day due to rapid increase in population and health problems. It has been reported that the protein gap in Pakistan would continue to increase unless well-planned measures are adopted to tackle the situation (Nisar *et al*., 2009). Some other workers have also carried out protein analysis of plants, as for example, Hussain *et al*. (2010a), who reported high protien contents in *Spinacia oleracea* and *Trianthema portulacastrum* and Anwar & Rashid (2007), who examined high protein contents in *Moringa oleifera*.

Plant materials containing 1- 2% fats, provide energy, which is considered to be significant to human beings, as excess fat consumption results in cardiovascular disorders like atherosclerosis, cancer and aging (Antia *et al*., 2006). The present analysis for fat contents showed 2.28±0.02 %, 1.22±0.05% and 8.90±0.03% fats in the leaf, bark and fruit respectively. Our findings for crude fat in the parts showed much lower values as compared to those of *Amaranthus hybridus* (4.80%), *Baseila alba* (8.71%), *Calchorus africanum* (4.20%) and *Talinum triangulare* (5.90%) (Iniaghe *et al*., 2009), suggesting that this plant is safe both for medicinal as well as for nutritional purposes.

Crud fibres are found in higher amount in non starchy materials (Agostoni *et al*., 1995) and are considered good for the treatment of diseases like diabetes, gastrointestinal disorders, obesity and cancer (Saldanha, 1995). In the present study, crude fiber contents were 21.50±0.17% in leaf, 30.83±0.10 % in bark and 10.50±0.17% in fruit of *Z. armatum*. Some other workers have also found crude fibers in plants as Hussain *et al*. (2011b) reported 10.25% fibres in *Acalypha hispida* and 7.29% in *A. racemosa*. Similarly Naseem *et al*. (2006) reported 27.2 % crude fibers in *Crotalaria burhia*. Our findings suggest that crude fibers amount is well in the reported range and this plant might an important source of diatry crude fibers.

High ash content is an indication of the mineral stuffing available in the plant materials (Antia *et al*., 2006). The ash values in *Z.armatum* were 11.18±0.09, 13.49±0.01% and 11.90±0.03 % in the leaf, bark and fruit respectively. Ash values ascertained high deposit of minerals in the plant tissue, already explored in the present study. Ash values studies have also been carried out on *Talinum triangulare* (20.05%) by Iniaghe e*t al*. (2009)

and *Hibiscus esculentus* (8.00%) and *Occimum graticimum* (8.00%) as reported by Akindahunsi & Salawu (2005).

Moisture contents in leaves were 2.90±0.10% in bark 5.03±0.06% in bark and 2.35±0.05% in the fruit. These values are little inconsistent with the moisture contents values reported by Kochhar *et al*., 2006, Hameed *et al*. (2008) and Hussain *et al*. (2011b) in their work.

Table 4.11. Macroscopic features of *Skimmia laureola* leaf.

S. No	Characteristic	Observation
1	Size	25 to 80mm in length and 16 to 25mm in width
2	Color	Upper surface dark green, Lower surface light green
3	Odor	Pleasant citrus leaf like
4	Taste	Light better
5	Phyllotaxis	Spiral forming terminal whorl
6	Insertion	Ramel i.e. inserted on the branches
7	Leaf base	Symmetrical
8	Petiole	Short petiole present
9	Lamina Composition	Simple lanceolate
10	Incision	No incision, margins entire
11	Venation	Reticulate and unicostate
12	Apex	Acute
13	Surface	Glabrous
14	Texture	Soft
15	Fracture of dry leaf	Short and smooth

Table 4.12. Macroscopic features of *Skimmia laureola* stem bark.

S. No	Characteristic	Observation
1	Shape	Irregular, curved
2	Dimension	0.5 to 2 cm in length and up to 1 cm in width
3	Outer surface	Yellowish green, rough texture, slightly ridged present
4	Inner surface	Smooth golden yellow in color.
5	Odor	Light pleasant
6	Taste	Astringent
7	Fracture	Uneven and fibrous
8	Thickness	Upto 1mm

Table 4.13. Leaf constant values of *Skimmia laureola* leaf.

S. No	Parameter	Range	Average
1	Palisade ratio	6.75 to **7.75** to 9.00	7.8±0.21
2	Vein islets number	14 to **16** to 18	15.4±0.63
3	Vein termination number	9 to **11** to 13	11.3±0.47
4	Stomatal number	105 to **127** to 141	122.1±4.32
5	Stomatal Index	12.47 to **12.93** to 14.10	12.96±0.14
6	Variance of stomatal number	186.79	-
7	Co-efficient of variance of stomatal number	11.19	-
8	Standard error of stomtal number	4.32	-
9	Standard deviation of stomtal number	13.66	-

Table 4.14. Stomatal diversity with frequency and quantitative features in the lower epidermis of *Skimmia laureola* leaf.

S. No	Stomatal type	Sub type	Frequency	Length (µ)	Breadth (µ)	Opening (µ)
1	Stephanocytic	Actinocytic	20	27.65±0.001	20.96±0.001	7.78±0.04
		Actinostephanocytic	70	26.72±0.002	21.03±0.001	2.1±0.02
		Staurocytic	30	27.70±0.001	20.78±0.002	0
		Incomplete stephanocytic bicyclic	10	27.52±0.000	20.88±0.000	0
2	Paracytic	Brachy paracytic	30	26.80±0.002	20.96±0.002	0
		Amphybrachy paratetracytic	40	26.72±0.001	20.92±0.002	7.78±0.67
		Hemiparacytic	10	27.70±0.000	21.03±0.000	7.80±0.00
3	Stoamatal cluster	-	10	27.52±0.000	20.78±0.000	7.71±0.00

Table 4.15. Ash analysis of leaf and stem bark of *Skimmia laureola*

S. No	Powder	Total ash (mg/g)	Acid insoluble ash (mg/g)	Water soluble ash (mg/g)
1	SL	101.8	65.2	296.8
2	SLEE	44.6	21.6	108.0
3	SLEH	36.2	11.4	213.6
4	SB	86.6	42.6	311.1
5	SBEE	26.6	27.8	120.0
6	SBEH	20.2	24.7	143.6

Table 4.16. Preliminary phytochemical screening of *Skimmia laureola* leaf.

S. NO	Constituents	Test name	Aqueous extract	Ethanolic extract	Hexane extract
1	Carbohydrates	Fehling,s test	+	+	+
		Molish,s Test	+	+	+
		Benedict,s test	+	+	+
2	Protien	Ninhydrine test	-	+	-
		Millon,s test	-	+	-
3	Alkaloids	Wagner,s test	+	+	-
		Mayer,s test	+	+	-
		Hager,s test	+	+	-
4	Phytosterol and Triterpenoids	Salkoskii ,s test	-	+	+
		Liebermann- Burchard Test	-	+	+
5	Phenol	Ferric chloride test	+	-	+
6	Flavonoids	Lead acetate test	+	+	-
		Alkali test	+	+	-
		Shinoda,s test	+	+	-
7	Tannins	Gilatin test	+	+	-
		Ferric chloride test	+	+	-
		Alkali test	+	+	-
8	Saponins	Frothing test	+	-	-
9	Anthocyanins	HCl test	-	-	-
10	Glycosides	Killaer kilani test	-	-	-
11	Fixed oil and fats	Spot test	-	+	+
		Alkali test	-	+	+
12	Volatile oil	Spot test	-	+	+

Table 4.17. Preliminary phytochemical screening of *Skimmia laureola* stem bark.

S. NO	Constituents	Test name	Aqueous extract	Ethanolic extract	Hexane extract
1	Carbohydrates	Fehling,s test	+	+	+
		Molish,s Test	+	+	+
		Benedict,s test	+	+	+
2	Protien	Ninhydrine test	-	+	-
		Millon,s test	-	+	-
3	Alkaloids	Wagner,s test	+	+	-
		Mayer,s test	+	+	-
		Hager,s test	+	+	-
4	Phytosterol and Triterpenoids	Salkoskii,s test	+	+	+
		Liebermann- Burchard Test	+	+	+
5	Phenol	Ferric chloride test	+	-	+
6	Flavonoids	Lead acetate test	+	+	-
		Alkali test	+	+	-
		Shinoda,s test	+	+	-
7	Tannins	Gilatin test	+	+	+
		Ferric chloride test	+	+	+
		Alkali test	+	+	+
8	Saponins	Frothing test	+	-	-
9	Anthocyanins	HCl test	-	+	-
10	Glycosides	Killaer kilani test	+	-	-
11	Fixed oil and fats	Spot test	-	+	+
		Alkali test	-	+	+
12	Volatile oil	Spot test	-	+	+

Table 4.18. Quantitative chemical analysis of *Skimmia laureola*. All values are mean± SEM of three determinations. All values are expressed in mg/g.

S. No	Extract	Flavonoids	Phenol	Alkaloids	Tannins	Saponin	Sterol
1	SLE	12.58±0.66	10.33±0.66	12.50±0.09	26.83±0.12	20.93±0.06	81.30±0.61
2	SBE	16.66±0.33	14.68±1.33	22.10±0.17	14.30±0.10	22.63±0.12	56.73±0.25

Table 4.19. Fluorescence analysis of *Skimmia laureola* leaf and stem bark powder with different reagents.

S. No	Reagents	Visible light	UV 256	UV310
1	SL Powder as such	Grass green	Dark brown	Yellow green
2	SL powder +50 %HNO_3	Golden brown	Yellow	Gray
3	SL powder+Picric acid	Yellowish green	Dark yellow	Brown
4	SL powder +NH_3	Sea green	Yellowish green	Gray
5	SL powder+H_2SO_4	Light green	Yellowish green	Light brown
6	SL powder+NaOH	Yellowish green	Yellowish green	Light brown
7	SL powder+HCl	Brown	Brown	Black
8	SLpowder+NaOH+ Ethanol	Yellowish brown	Brown	Yellowish brown
9	SL powder+Iodine	Light green	Light green	Grey
10	SL powder+$FeCl_3$	Blakish brown	Brown	Greenish
11	SB Powder as such	Golden reddish yellow	Maroon brown	Yellowish brown
12	SB powder +50 %HNO_3	Golden brown	Yellow	Yellowish brown
13	SB powder+Picric acid	Golden Yellow	Dark yellow	Bluish yellow
14	SBpowder +NH_3	Golden yellow	Light yellow	Brown
15	SB powder+H_2SO_4	Light yellow	Light yellow	Yellowish blue
16	SB powder+NaOH	Light yellow	Light yellow	Brown
17	SB powder+HCl	Reddish Brown	Yellowish Brown	Brown
18	SBpowder+NaOH+ Ethnol	Dark yellow	Light brown	Grey
19	SB powder+Iodine	Yellow	Yellow	Bluish brown
20	SBpowder+$FeCl_3$	Dark brown	Dark brown	Black

Table 4.20. Fluorescence analysis of *Skimmia laureola* leaf and stem bark extracts.

S.No	Part	Solvent	Day light	UV 256	UV 310
1	Leaves	Ethanol	Blackish green	Dark green	Dark green
		Methanol	Dark Green	Greenish brown	Forest green
		Chloroform	Bluish green	Brownish green	Bluish green
		Acetone	Blackish brown	Bluish black	Yellowish green
		Water	Chocolate color	Lawn green	Greenish brown
		Hexane	Greenish brown	Blackish green	Bluish green
		Butanol	Brownish green	Dark green	Bluish green
2	Bark	Ethanol	Dark orange	Yellowish green	Yellowish green
		Methanol	Golden yellow	Yellowish green	Yellowish green
		Chloroform	Dark green	Yellowish green	Yellowish green
		Acetone	Greenish orange	Gold	Orange
		Water	Brick Red	Yellowish green	Yellowish green
		Hexane	Bluish green	Brownish green	Yellowish green
		Butanol	Yellowish green	Forest green	Olive drab

Table 4.21. Percent extractive values of leaf and stem bark of *Skimmia laureola* with different solvents.

S. No	Part	Solvent	Percent extracts
1	Leaf	Ethanol	20.20
		Methanol	21.80
		Chloroform	12.00
		Acetone	6.30
		Water	15.40
		Hexane	4.30
		Butanol	9.20
2	Bark	Ethanol	6.10
		Methanol	7.50
		Chloroform	9.30
		Acetone	4.40
		Water	3.90
		Hexane	4.10
		Butanol	6.50

Table 4.22. Concentration of various elements in leaf and stem bark of *Skimmia laureola*. All values are mean±SEM of three values.

Sample	Zn (ppm)	Mn (ppm)	Cr (ppm)	Cu (ppm)	Fe (ppm)	Ni (ppm)	Co (ppm)	Pb (ppm)	Na (ppm)	K (ppm)
SL	84.3±0.19	8.66±0.19	1.47±0.33	11.33±0.19	5.00±0.33	ND	1.64±0.1	0.67±0.19	147.67±4.19	1833.00±2.19
SB	89.0±0.33	4.36±0.19	1.38±0.35	15.6±0.19	4.83±0.35	2.50±0.33	ND	0.47±0.33	126.67±3.51	1066.7±1.51

Table 4.23. Proximate analysis of *Skimmia laureola* leaf and stem bark. All values are mean±SEM of three values.

Sample	Carbohydrates	Proteins	Fats	Fiber	Ash	Moisture
SL	62.96±0.05	2.50±0.03	2.35±0.02	20.83±0.25	10.23±0.02	2.21±0.02
SB	57.62±0.15	3.46±0.02	0.75±0.02	25.50±0.17	8.89±0.07	3.96±0.03

Table 4.24. Macroscopic features of *Zanthoxylum armatum* leaf.

S. No	Characteristic	Observation
1	Size	20 to 80mm in length and 12 to 25mm in width
2	Color	Upper surface dark green, Lower surface light green
3	Odor	Pleasant
4	Taste	Astringent
5	Phyllotaxis	Spiral
6	Insertion	Ramel i.e. inserted on the branches
7	Leaf base	Symmetrical
8	Lamina Composition	Compound, Leaflets lanceolate
9	Petiole	Leaf stalk winged, Leaflet sessile
10	Incision	Dentate slight incision present
11	Venation	Reticulate and unicostate
12	Apex	Acute
13	Surface	Glabrous, thorny
14	Texture	Soft
15	Fracture	Short and smooth

Table 4.25. Macroscopic features of *Zanthxylum armatum* stem bark

S. No	Characteristic	Observation
1	Shape	Irregular, cylindrical curved
2	Dimension	2 to 5 cm in length and up to 3 cm in width
3	Outer surface	Dark brown in color, rough texture, hard spines with corky base present
4	Inner surface	Light brown in color, scaly with ridges, cavities may present
5	Odor	Light pleasant
6	Taste	Bitter
7	Fracture	Uneven and fibrous
8	Thickness	1-3 mm

Table 4.26. Macroscopic features of *Zanthxylum armatum* fruit.

S. No	Characteristic	Observation
1	Kind	Small drupes, pedicel present
2	Dimensions	6 to 10 mm in diameter
3	Shape	Spherical ovoid
4	Insertion	Ramal i.e. present on the braches
5	Dehiscence	Splits into two halves at maturity i.e. bivalvate
6	Color	Reddish brown with oil gland spots
7	Taste	Astringent
8	Odour	Pungent
9	Texture	Soft
10	Marking	Ridges present
11	Sutures	Longitudnal
12	Seeds	One, black in color, hard in texture, ovoid in shape, raphe distinguished, micropyle present

Table 4.27. Leaf constant values of *Zanthoxylum armatum* leaf.

S. No	Parameter	Range	Average
1	Palisade ratio	6.00 to **7.75** to 9.00	8.25±0.32
2	Vein islets number	14 to **18** to 21	16.8±0.64
3	Vein termination number	11 to 13 to 15	13.1±0.43
4	Stomatal number	175 to **197** to 210	196.1±3.07
5	Stomatal Index	10.28 to **12.33** to 13.58	12.32±0.26
6	Variance of stomatal number	94.22	
7	Co-efficient of variance of stomatal number	4.95	
8	Standard error of stomtal number	3.07	
9	Standard deviation of stomtal number	9.71	

Table 4.28. Stomatal diversity with frequency and quantitative features in the lower epidermis of *Zanthoxylum armatum* leaf.

S. No	Type of stomata	Sub type	Frequency	Length (μ)	Breadth (μ)	Opening (μ)
1	Anomocytic	-	20	25.08±0.001	12.3±0.001	0±0
2	Stephanocytic	Actinocytic	20	25±0.000	11.7±0.000	1±0.001
		Actinostephanocytic	40	24.7±0.001	12.2±0.001	1±0.001
		Staurocytic	30	24.78±0.001	12.2±0.001	1±0.001
3	Cyclocytic	Laterocyclocytic	20	25.2±0.003	11.9±0.001	1±0.001
4	Paracytic	Brachy paracytic	30	26±0.001	12.3±0.001	0±0
		Brachy paratetracytic	90	25±0.001	11.7±0.001	0±0
		Hemiparacytic	10	24.7±0.00	12.5±0.00	0±0
5	Stomatal cluster	-	10	24.78±0.00	12.3±0.00	1±0

Table 4.29. Ash analysis of *Zanthoxylum armatum* leaf, stem bark and fruit.

S. No	Powder	Total Ash	Acid insoluble Ash	Water soluble Ash
1	ZL	110.4	51.3	320.5
2	ZLEE	52.0	23.5	136.5
3	ZLEH	64.4	20.3	231.0
4	ZB	134.6	69.7	361.3
5	ZBEE	64.8	18.5	102.8
6	ZBEH	29.0	24.7	143.6
7	ZF	118.0	94.2	237.5
8	ZFEE	35.6	20.4	111.1
9	ZFEH	32.0	16.0	142.9

Table 4.30. Preliminary phytochemical screening of *Zanthoxylum armatum* leaf.

S. NO	Constituents	Test name	Aqueous extract	Ethanolic extract	Hexane extract
1	Carbohydrates	Fehling,s test	+	+	-
		Molish,s Test	+	+	-
		Benedict,s test	+	+	-
2	Protien	Ninhydrine test	-	-	-
		Millon,s test	-	-	-
3	Alkaloids	Wagner,s test	+	+	+
		Mayer,s test	+	+	+
		Hager,s test	+	+	+
4	Phytosterol and Triterpenoids	Salkoskii ,s test	-	+	+
		Liebermann- Burchard Test	-	+	+
5	Phenol	Ferric chloride test	-	+	-
6	Flavonoids	Lead acetate test	+	+	+
		Alkali test	+	+	+
		Shinoda,s test	+	+	+
7	Tannins	Gilatin test	+	+	+
		Ferric chloride test	+	+	+
		Alkali test	+	+	+
8	Saponins	Frothing test	+	-	-
9	Anthocyanins	HCl test	+	+	-
10	Glycosides	Killaer kilani test	-	-	-
11	Fixed oil and fats	Spot test	-	+	+
		Alkali test	-	+	+
12	Volatile oil	Spot test	-	+	+

Table 4.31. Preliminary phytochemical screening of *Zanthoxylum armatum* stem bark.

S. NO	Constituents	Test name	Aqueous extract	Ethanolic extract	Hexane extract
1	Carbohydrates	Fehling,s test	+	+	+
		Molish,s Test	+	+	+
		Benedict,s test	-	+	-
2	Protien	Ninhydrine test	+	-	-
		Millon,s test	+	-	-
3	Alkaloids	Wagner,s test	+	+	-
		Mayer,s test	+	+	-
		Hager,s test	+	+	-
4	Phytosterol and Triterpenoids	Salkoskii ,s test	-	+	+
		Liebermann- Burchard Test	-	+	+
5	Phenol	Ferric chloride test	+	+	-
6	Flavonoids	Lead acetate test	+	+	-
		Alkali test	+	+	+
		Shinoda,s test	+	+	+
7	Tannins	Gilatin test	+	+	+
		Ferric chloride test	+	+	+
		Alkali test	+	+	+
8	Saponins	Frothing test	+	-	-
9	Anthocyanins	HCl test	-	+	-
10	Glycosides	Killaer kilani test	+	-	-
11	Fixed oil and fats	Spot test	-	+	+
		Alkali test	-	+	+
12	Volatile oil	Spot test	-	+	+

Table 4.32. Preliminary phytochemical screening of *Zanthoxylum armatum* fruit.

S. NO	Constituents	Test name	Aqueous extract	Ethanolic extract	Hexane extract
1	Carbohydrates	Fehling,s test	+	+	+
		Molish,s Test	+	+	+
		Benedict,s test	-	+	-
2	Protien	Ninhydrine test	+	+	-
		Millon,s test	+	+	-
3	Alkaloids	Wagner,s test	+	+	-
		Mayer,s test	+	+	-
		Hager,s test	+	+	-
4	Phytosterol and Triterpenoids	Salkoskii ,s test	-	+	+
		Liebermann- Burchard Test	-	+	+
5	Phenol	Ferric chloride test	+	+	-
6	Flavonoids	Lead acetate test	+	+	-
		Alkali test	+	+	-
		Shinoda,s test	+	+	
7	Tannins	Gilatin test	+	+	+
		Ferric chloride test	+	+	-
		Alkali test	+	+	-
8	Saponins	Frothing test	+	-	-
9	Anthocyanins	HCl test	+	-	-
10	Glycosides	Killaer kilani test	+	-	-
11	Fixed oil and fats	Spot test	-	+	+
		Alkali test	-	+	+
12	Volatile oil	Spot test	-	+	+

Table 4.33. Quantitative chemical analysis of *Zanthoxylum armatum*. All values are mean ± SEM of three determination. All values are expressed in mg/g.

S.No	Extract	Flavonoids	Phenol	Alkaloids	Tannins	Saponin	Sterol
1	ZLE	13.68±0.66	11.66±0.33	15.60±0.10	34.43±0.21	21.57±0.12	71.60±0.10
2	ZBE	18.33±1.22	16.48±1.33	19.60±0.10	28.62±0.13	14.78±0.10	33.83±0.29
3	ZFE	22.8±1.33	21.68±0.44	25.07±0.21	35.5±0.5	28.60±0.10	164.92±0.14

Table 4.34. Fluorescence analysis of leaf, stem bark and fruit powder of *Zanthoxylum armtum* with different reagents

S. No	Reagents	Day light	UV 256	UV310
1	ZF powder as such	Blackish brown	Dark slate blue	Dark brown
2	ZF powder +50 %HNO_3	Dark brown	Dark brown	Dark gray
3	ZF powder+Picric acid	Brownish	Brownish	Dark gray
4	ZF powder +NH_3	Dark yellow	Brown	Dark gray
5	ZF powder+H_2SO_4	Grey	Light brown	Black
6	ZF powder+NaOH	Yellowish brown	Yellow brown	Canker Brown
7	ZF powder+HCl	Yellowish brown	Brown	Brown
8	ZFpowder+NaOH+ Ethanol	Brown	Grayish	Grayish Brown
9	ZF powder+Iodine	Yellowish brown	Grayish brown	Brown
10	ZF powder+$FeCl_3$	Grey brown	Brown	Canker Brown
11	ZL powder as such	Olive Drab	Brown	Dark Olive green
12	ZL powder +50 %HNO_3	Reddish brown	Brown	Dark brown
13	ZL powder+Picric acid	Greenish	Yellow	Brown
14	ZL powder +NH_3	Grey	Greenish	Blackish brown
15	ZL powder+H_2SO_4	Grey	Grey	Grey
16	ZL powder+NaOH	Dark brown	Green	Brown
17	ZL powder+HCl	Brown	Brown	Black
18	ZLpowder+NaOH+ Ethanol	Blackish	Blackish brown	Black
19	ZL powder+Iodine	Grayish	Brown	Grayish brown
20	ZL powder+$FeCl_3$	Light black	Dark green	Dark green
21	ZB powder as such	Dark Golden red	Yellowish brown	Brown
22	ZB powder +50 %HNO_3	Golden brown	Brown	Brown
23	ZB powder+Picric acid	Yellow	Grey	Brown
24	ZB Powder +NH_3	Yellow	Brown	Grayish Brown
25	ZB pwder+H_2SO_4	Yellow	Yellowish brown	Brown
26	ZB powder+NaOH	Yellowish brown	Dark brown	Yellowish Brown
27	ZB powder+HCl	Dark brown	Reddish brown	Black
28	ZBpowder+NaOH+ Ethanol	Blackish brown	Dark brown	Dark brown
29	ZB powder+Iodine	Brown	Brown	Black
30	ZB powder+$FeCl_3$	Yellowish brown	Green	Bluish brown

Table 4.35. Fluorescence analysis of leaf, stem bark and fruit extracts of *Zanthoxylum armatum*.

S.No	Part	Solvent	Day light	UV 256	UV310
1	Leaf	Ethanol	Dark green	Dark green	Greenish brown
		Methanol	Blackish Brown	Blackish Brown	Dark Brown
		Chloroform	Dark green	Blue green	Greenish brown
		Acetone	Blackish brown	Bluish black	Yellowish green
		Water	Dark Brown	Bluish black	Dark blue
		Hexane	Yellow green	Brownish green	Brownish green
		Butanol	Yellow green	Forest green	Olive drab
2	Bark	Ethanol	Brownish red	Brownish green	Brownish green
		Methanol	Maroon greenish	Forestgreen	Olive green
		Chloroform	Forest green	Dark green	Yellowish green
		Acetone	Greenish brown	Lawn green	Lawn green
		Water	Blackish Brown	Bluish Green	Bluish blak
		Hexane	Dark green	Greenish brown	Dark green
		Butanol	Dark orange	Dark green	Dark green
3	Fruit	Ethanol	Orange red	Brownish green	Yellow green
		Methanol	Reddish orange	Dark Olive Green	Olive Drab
		Chloroform	Forest green	Dark green	Olive drab
		Acetone	Greenish brown	Olive green	Blue green
		Water	Brownish red	Light green	Greenish brown
		Hexane	Yellow green	Dark olive green	Brownish green
		Butanol	Lawn green	Lawn green	Yellowish green

Table 4.36. Percent extractive values of leaf, bark and fruit of *Zanthoxylum armatum* with different solvents.

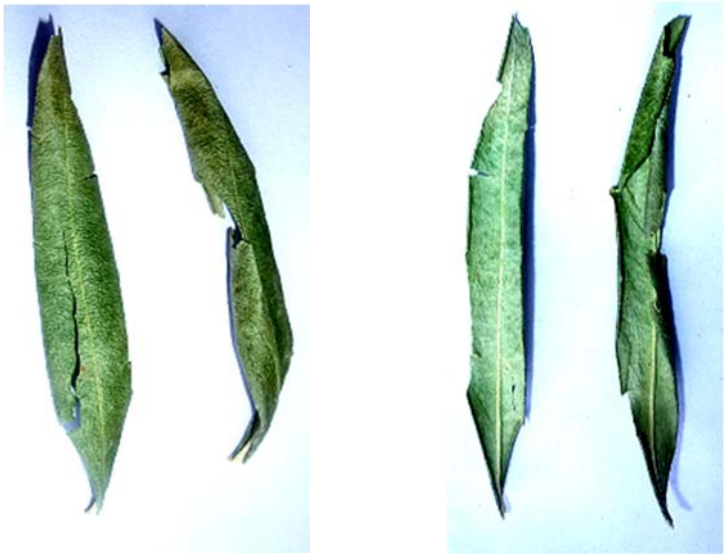
S. No	Part	Solvent	% extracts
1	Leaves	Ethanol	19.70
		Methanol	20.10
		Chloroform	6.10
		Acetone	5.20
		Water	14.80
		Hexane	4.80
		Butanol	7.10
2	Bark	Ethanol	7.20
		Methanol	7.40
		Chloroform	8.50
		Acetone	2.60
		Water	8.00
		Hexane	8.10
		Butanol	3.30
3	Fruit	Ethanol	10.80
		Methanol	11.00
		Chloroform	6.10
		Acetone	7.30
		Water	9.30
		Hexane	9.40
		Butanol	9.00

Table 4.37. Concentration of various elements in different parts of *Zanthoxylum armatum*. All values are mean±SEM of three values.

Sample	Zn(ppm)	Mn(ppm)	Cr(ppm)	Cu (ppm)	Fe(ppm)	Ni(ppm)	Co (ppm)	Pb (ppm)	Na (ppm)	K (ppm)
ZL	85.3±0.38	11.86±0.38	1.63±0.19	23.0±0.33	14.33±0.51	7.20±0.33	1.70±0.33	0.22±0.69	132.00±1.33	916.7±2.19
ZB	89.6±0.19	5.63±0.19	1.60±0.33	31.6±0.19	15.00±0.33	8.00±0.33	1.67±0.19	ND	140.67±4.19	570.0±3.33
ZF	87.6±0.19	2.50±0.33	1.66±0.33	10.0±0.33	25.67±0.19	2.37±0.19	ND	ND	141.33±2.19	1636.7±2.19

Table 4.38. Proximate analysis of different parts of *Zanthoxylum armatum*. All values are mean±SEM of three values.

Sample	Carbohydrates	Proteins	Fats	Fiber	Ash	Moisture
ZL	45.76±0.08	16.16±0.05	2.28±0.02	21.50±0.17	11.18±0.09	2.90±0.10
ZB	38.64±0.11	10.98±0.01	1.22±0.05	30.83±0.10	13.49±0.01	5.03±0.06
ZF	59.59±0.06	7.74±0.04	8.90±0.03	10.50±0.17	11.90±0.03	2.35±0.05



a b

Fig. 4.7. Macroscopic features of *Skimmia laureola* leaf. a. Adaxial surface b. Abaxial surface



a b

Fig. 4.8. Macroscopic features of *Skimmia laureola* bark. a. Adaxial surface b. Abaxial surface

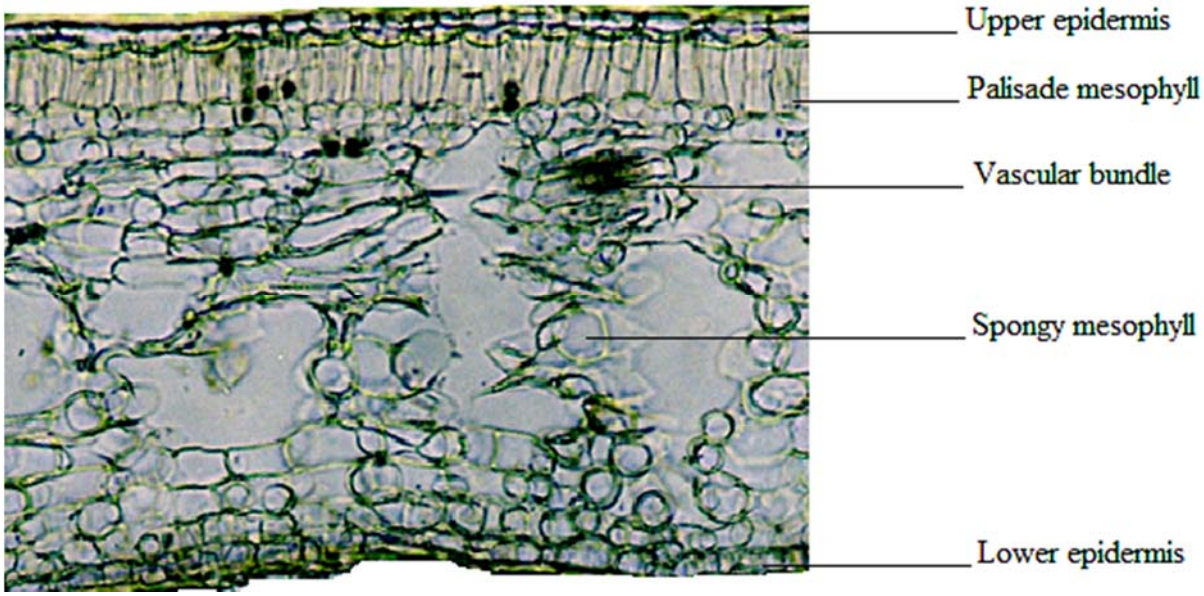


Fig. 4.9. T. S. of *Skimmia laureola* leaf lamina.

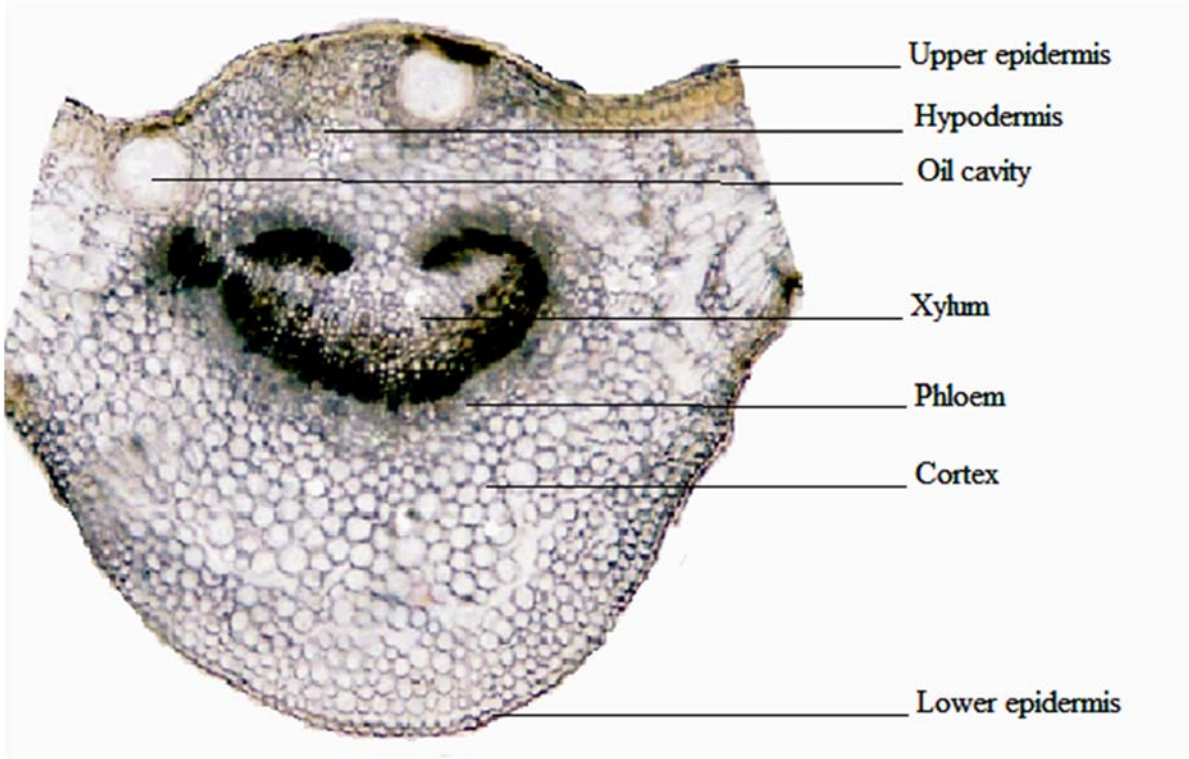
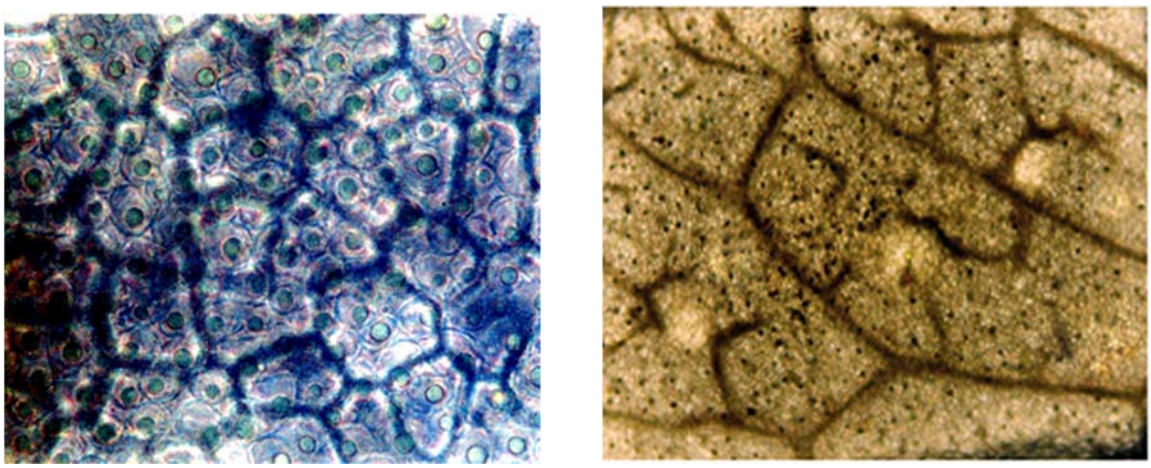
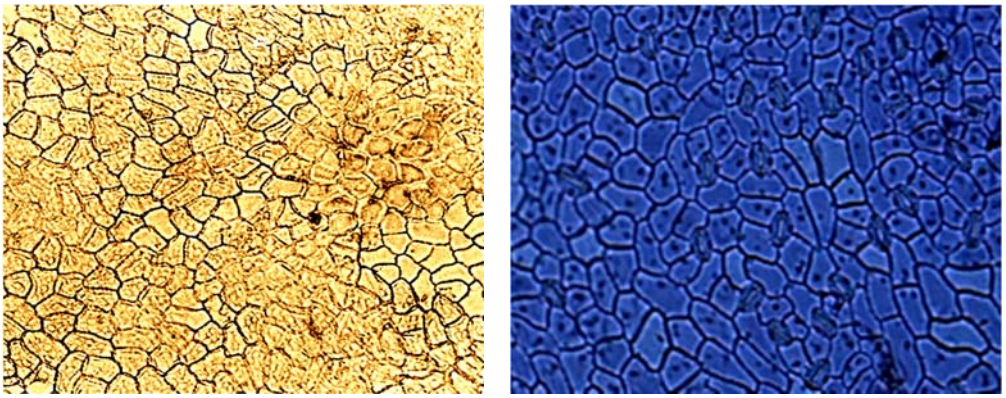


Fig. 4.10. T.S. of *Skimmia laureola* leaf midrib



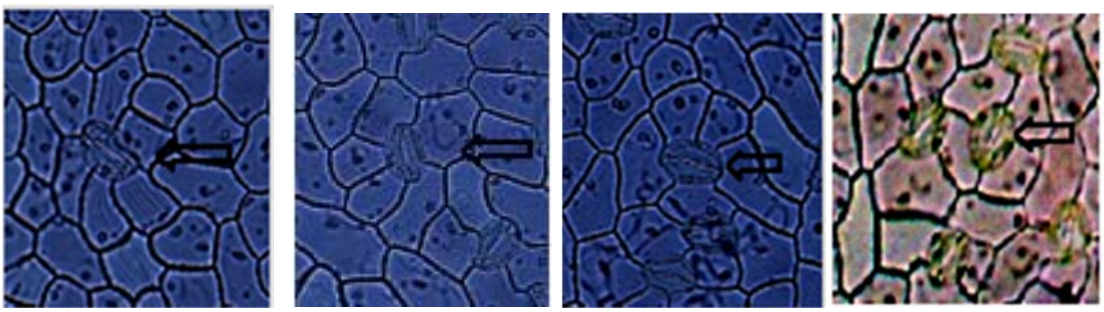
a b

Fig. 4.11. *Skimmia laureola* leaf. a. Palisade cells arrangement under epidermal cells b.veins arrangement in lamina.

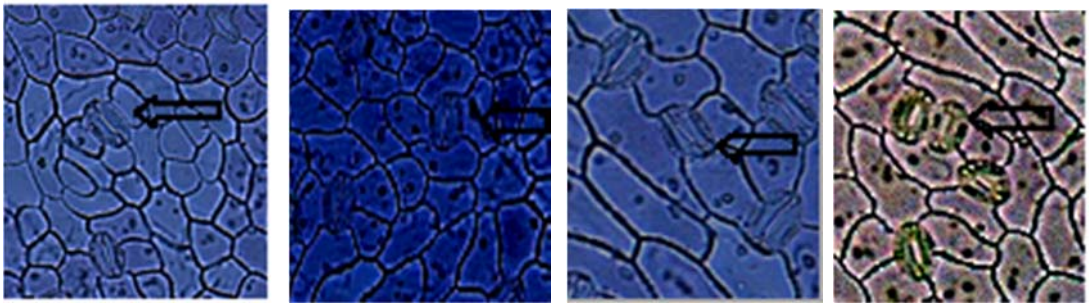


a b

Fig. 4.12. *Skimmia laureola* leaf. a. Upper epidermis b. lower epidermis.



a b c d



e f g h

Fig. 4.13. Various types of stomata in the lower epidermis of *Skimmia laureola* leaf
a. Actinocytic; b. Actinostephanocytic; c. Incomplete stephanocytic bycyclic; d. Staurocytic; e. Brachyparacytic; f. Amphibrachy paratetracytic; g. Hemiparacytic; h. Stomatal cluster.

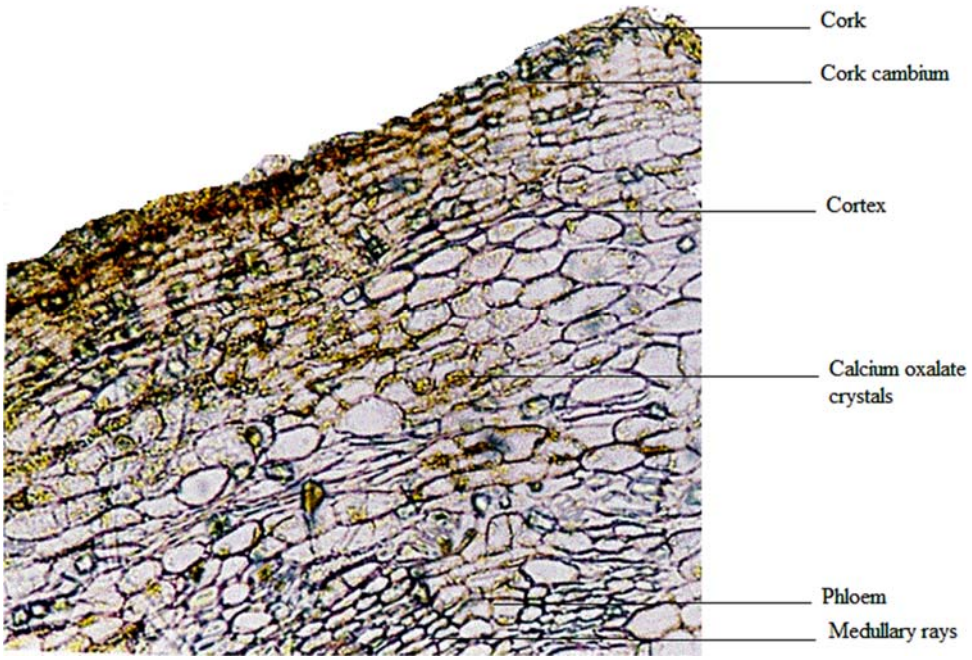


Fig. 4.14. T. S. of *Skimmia laureola* stem bark

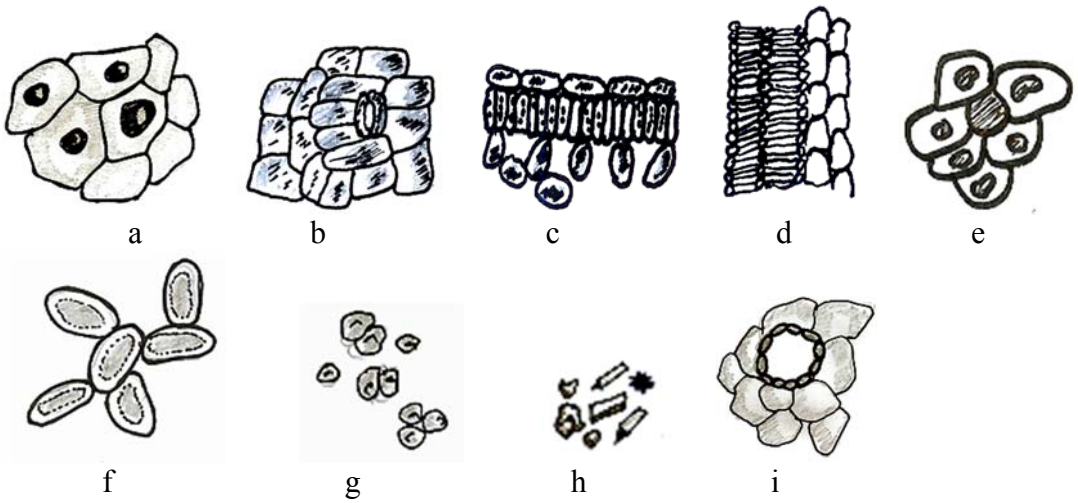


Fig. 4.15. *Skimmia laureola* leaf powder a. Upper epidermis; b. lower epidermal fragments with stoma; c. fragments of upper epidermal cells with mesophyl cells; d. vessels with attached parenchymatous cells; e. Collenchymatous cells with calcium oxalate crystals; f. spongy parenchyma ; g. starch grains; h. calcium oxalate crystals; i. secretary cells from midrib region.

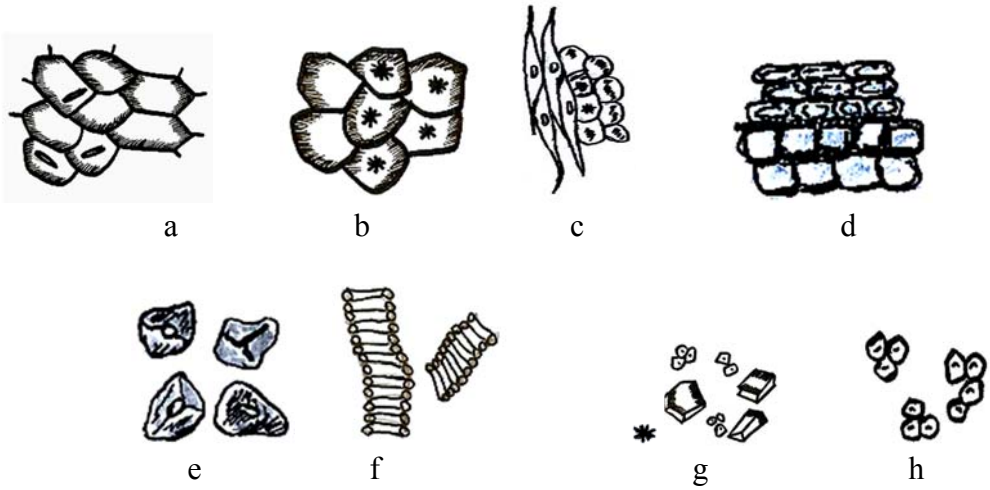


Fig. 4.16. *Skimmia laureola* stem bark powder. a. cork cells; b. cortical cells with calcium oxalate crystals; c. elongated fiber with phloem parenchyma; d. cork cells with attached phellem cells; e. sclerieds; f. spiral vessels; g. isolated crystals of calcium oxalates; h. starch grains.

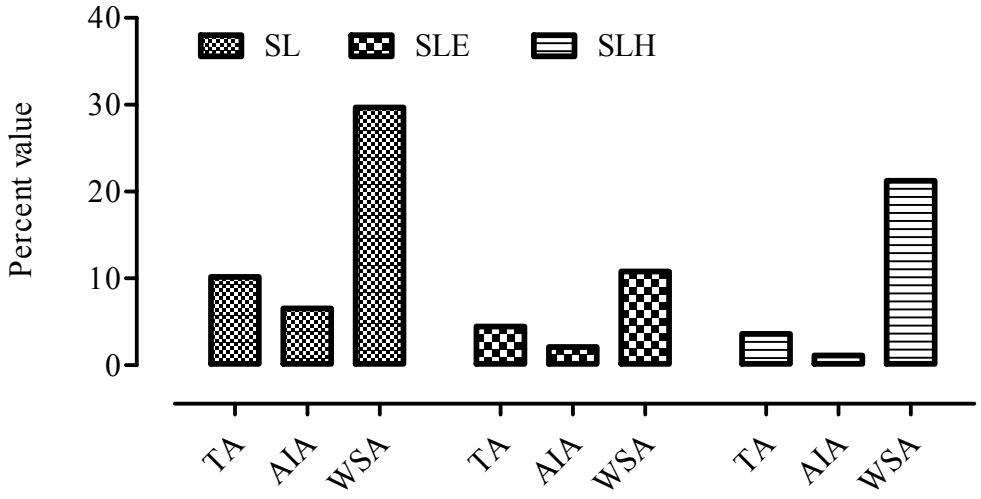


Fig. 4.17a. Comparison of total ash (TA), acid insoluble ash (AIA) and water soluble ash (WSA) percent values of *Skimmia laureola* leaf powder non exhausted (SL) and exhausted with ethanol (SLE) and n-hexane (SLH).

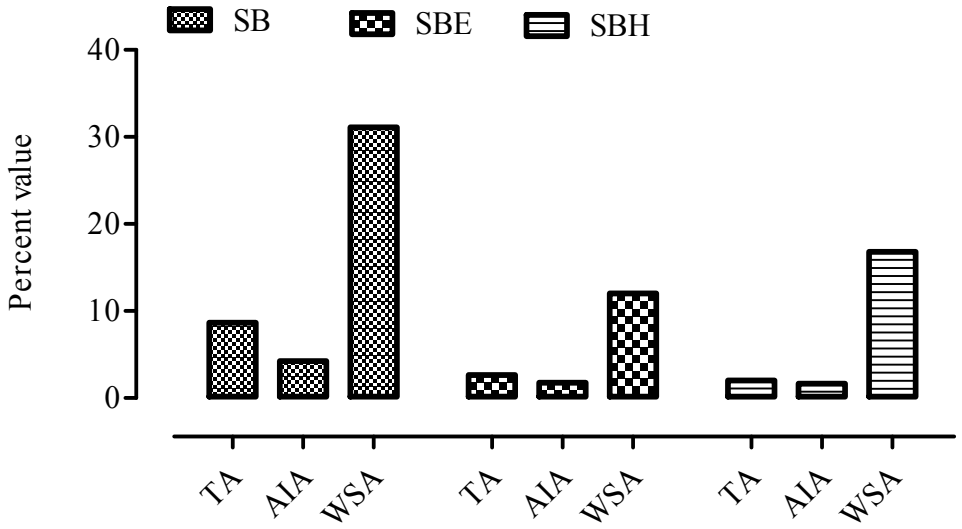


Fig. 4.17b. Comparison of total ash (TA), acid insoluble ash (AIA) and water soluble ash (WSA) percent values of *Skimmia laureola* bark powder non exhausted (SB) and exhausted with ethanol (SBE) and n-hexane (SBH).

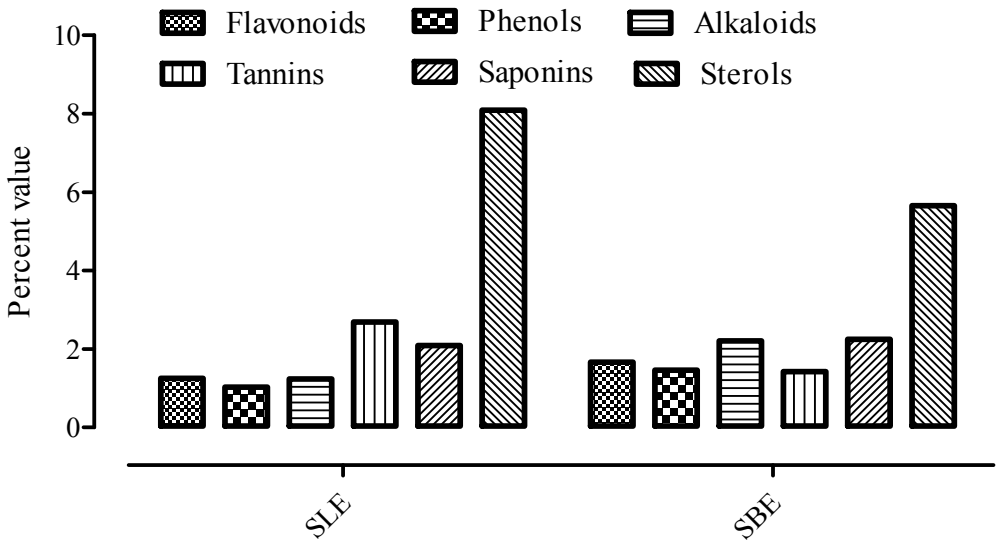


Fig. 4.18. Bars representing percent values of flavonoids, phenols, alkaloids, tannins, saponins and sterols in the ethanolic extracts of *Skimmia laureola* leaf (SLE) and bark (SBE).

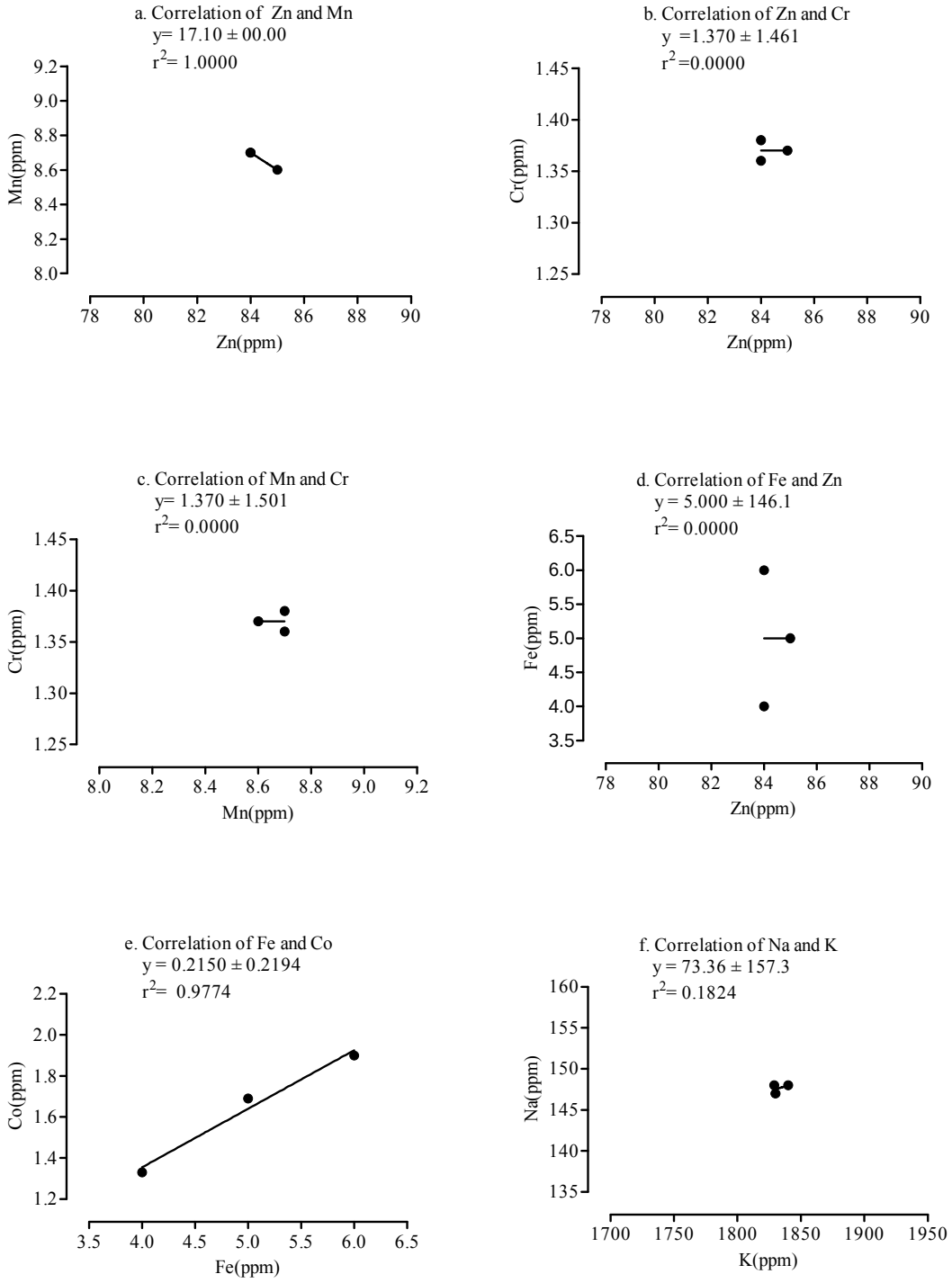


Fig. 4.19. Correlations of various trace elements in *Skimmia laureola* leaf.

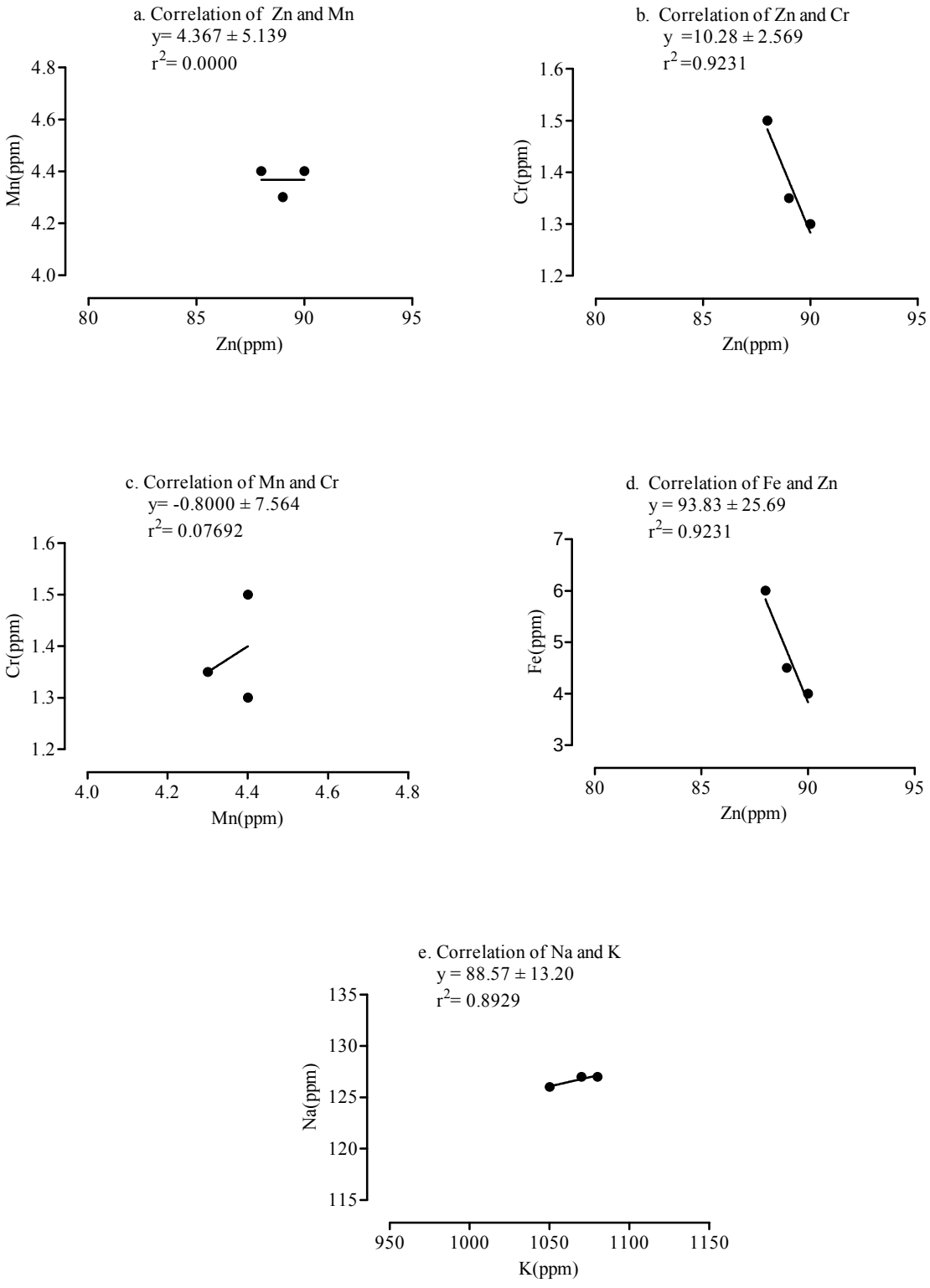


Fig. 4.20. Correlations of various trace elements in *Skimmia laureola* stem bark.

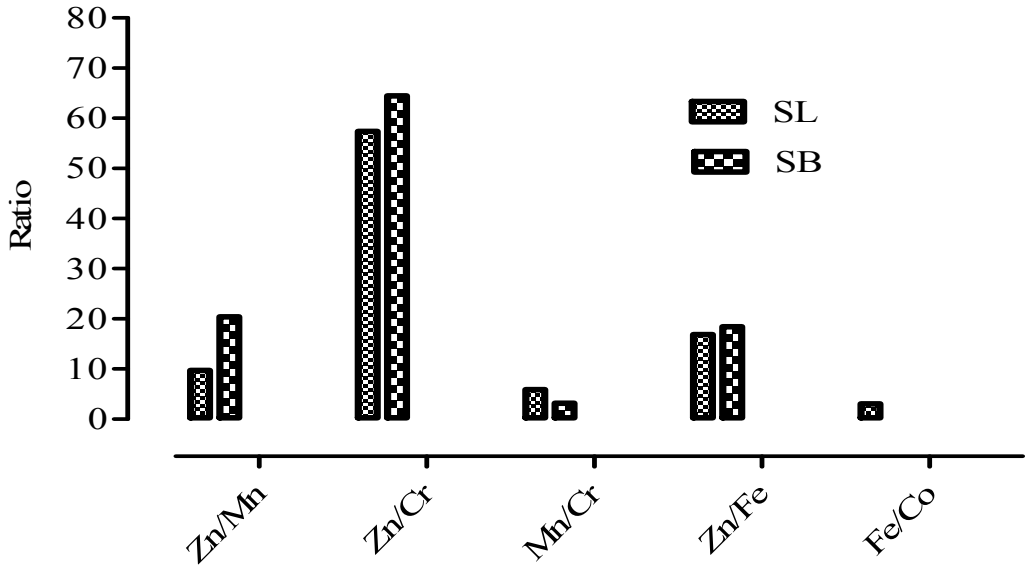


Fig. 4.21. Bars representing ratios among various elements in *Skimmia laureola* leaf (SL) and stem bark (SB).



a b

Fig. 4.22. *Zanthoxylum armatum* leaf; a. Adaxial surface, b. Abaxial surface.



a b

Fig. 4.23. *Zanthoxylum armatum* stem bark; a. Adaxial surface, b. Abaxial surface.



Fig. 4.24. *Zanthoxylum armatum* fruit.

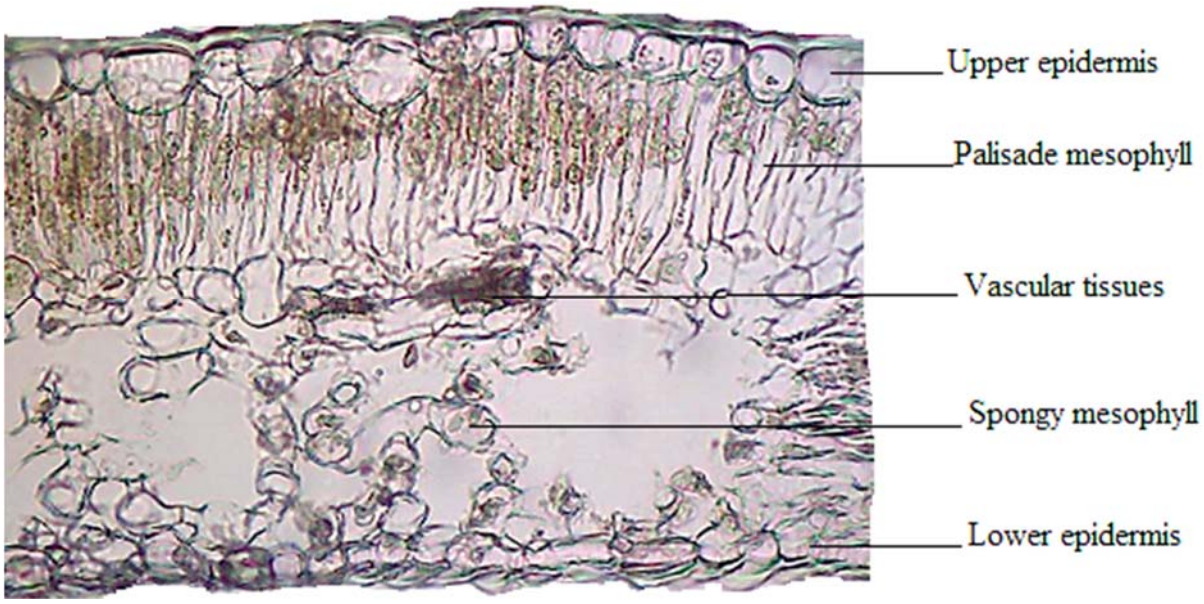


Fig. 4.25. T. S. of *Zanthoxylum armatum* leaf lamina.

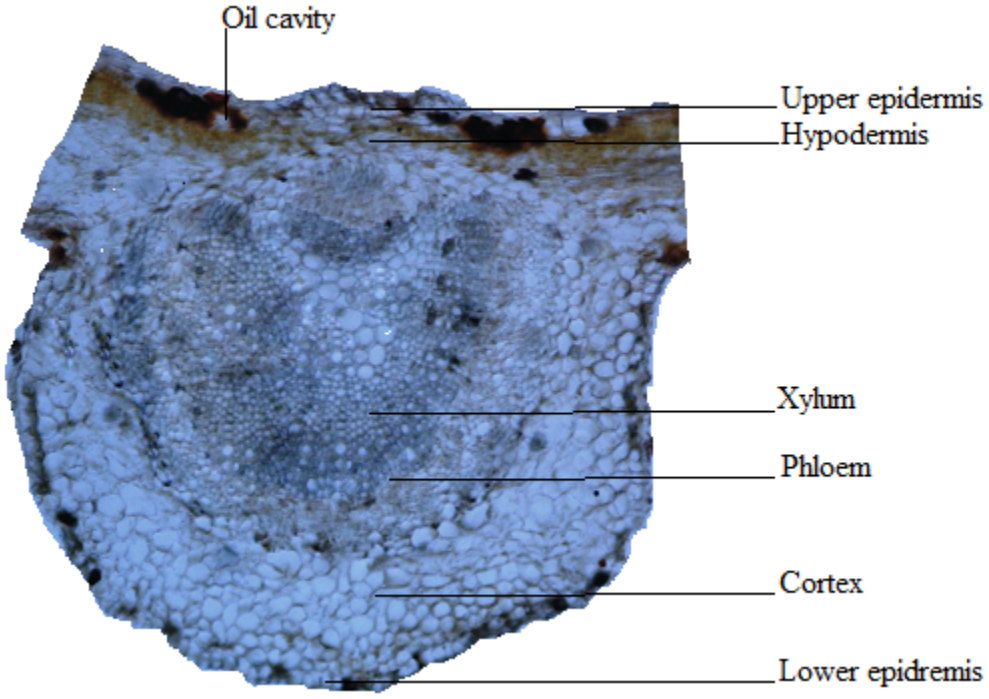
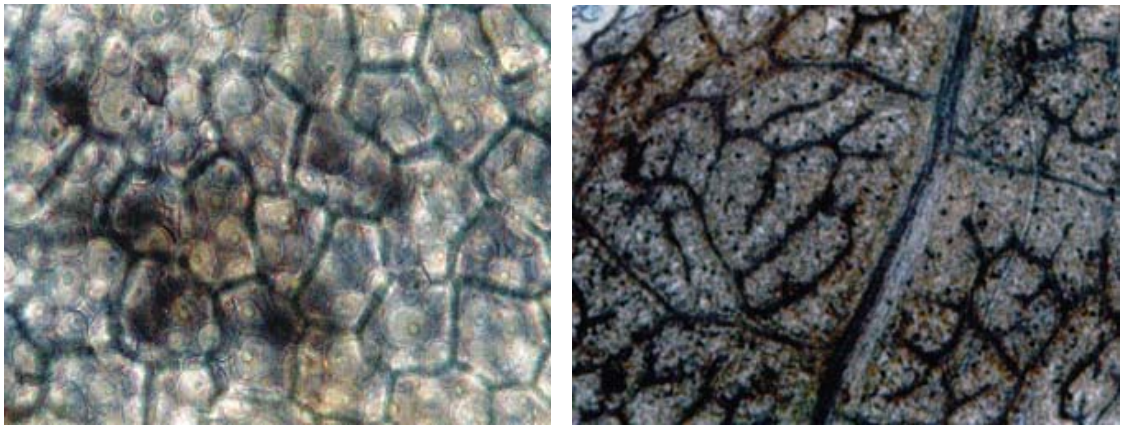
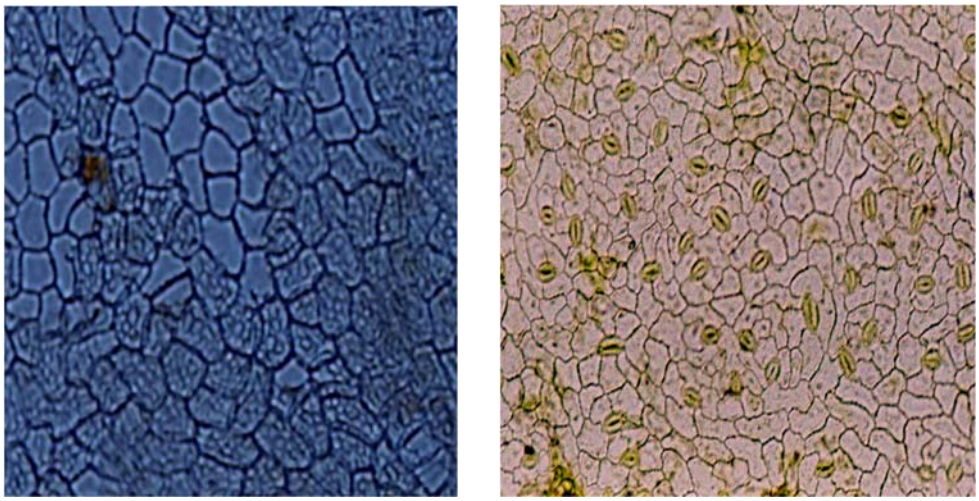


Fig. 4.26. T. S. of *Zanthoxylum armatum* leaf midrib



a b

Fig. 4.27. *Zanthoxylum armatum* leaf; a. Palisade cells arrangement under epidermal cells, b. veins arrangement in lamina.



a b

Fig.4.28. *Zanthoxylum armatum* leaf a. upper epidermis b. lower epidermises.

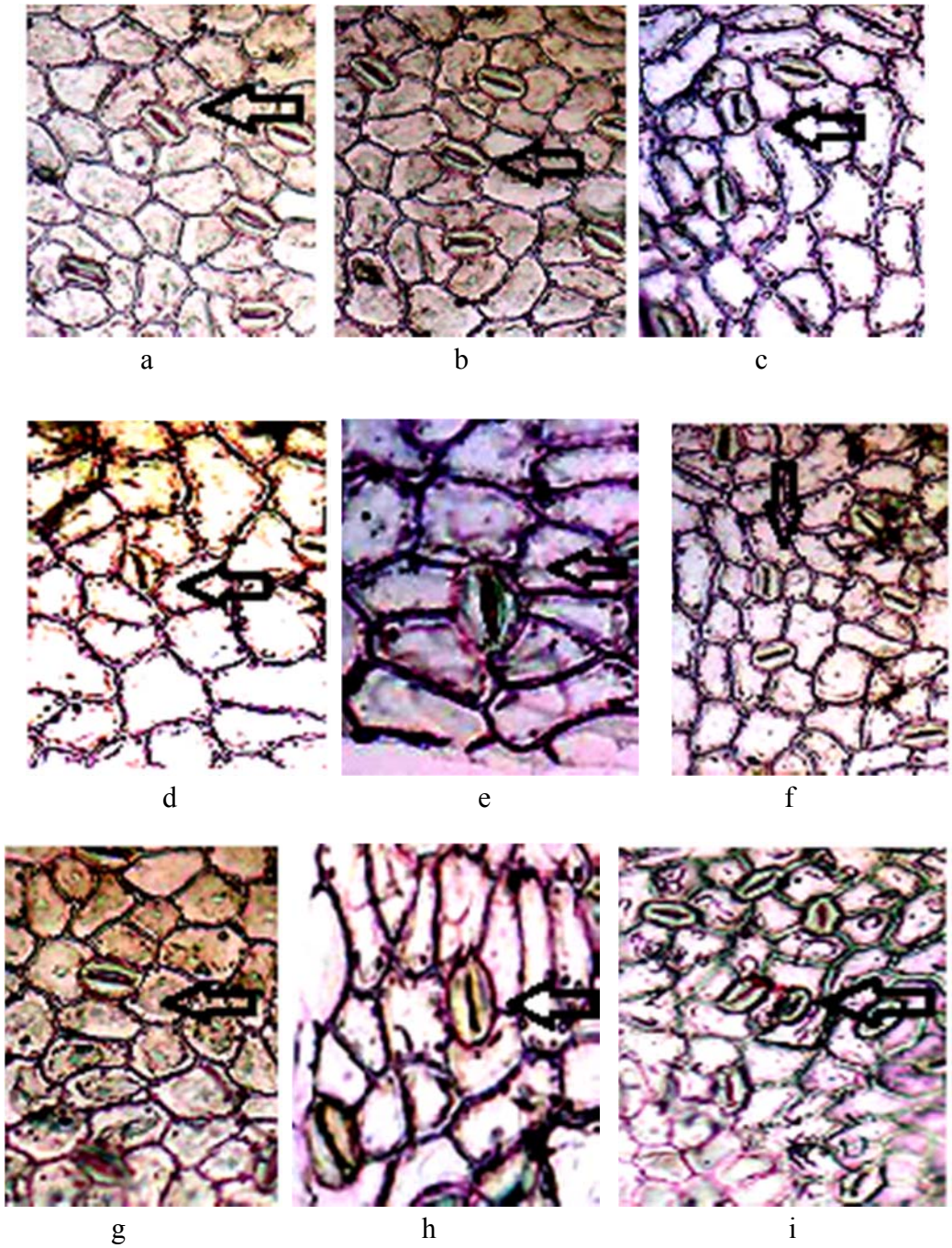


Fig. 4. 29. Various types of stomata in the lower epidermis of *Zanthoxylum armatum* leaf a. Anomocytic; b. Actinocytic ; c. Actinostephanocytic; d. Staurocytic cytic; e. Laterocyclocytic; f. Brachyparacytic; g. Brachy paratetracytisc; h. Hemiparacytic; i. Stomatal cluster.

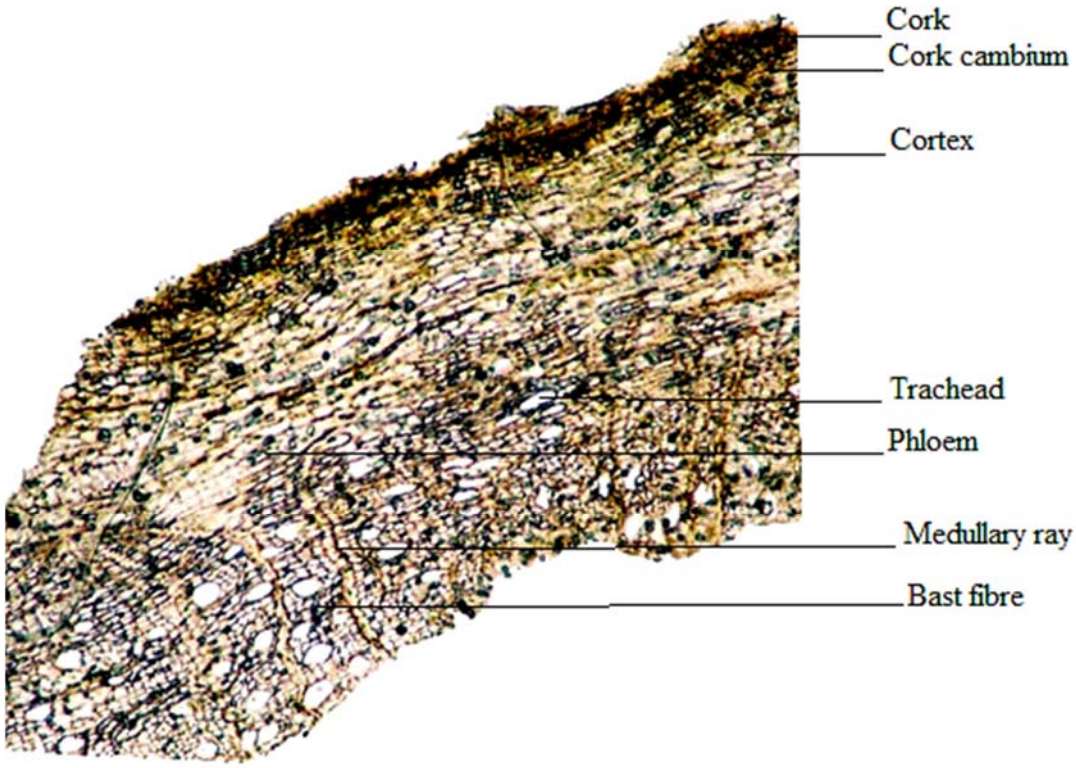
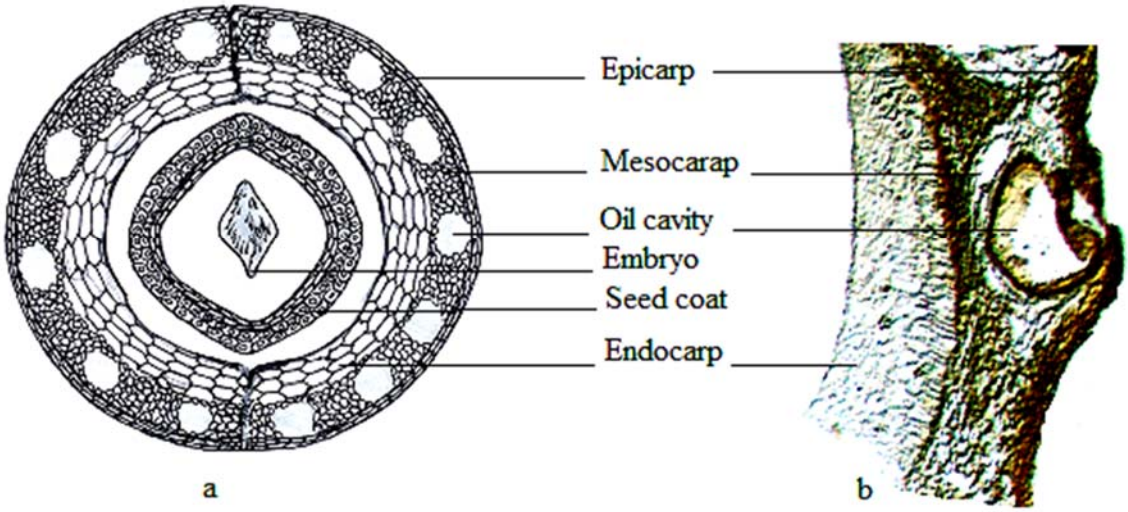


Fig. 4.30. T. S. of *Zanthoxylum armatum* bark.



Fi. 4.31. *Zanthoxylum armatum* fruit. a. T. S of fruit; b. T. S. of fruit wall.

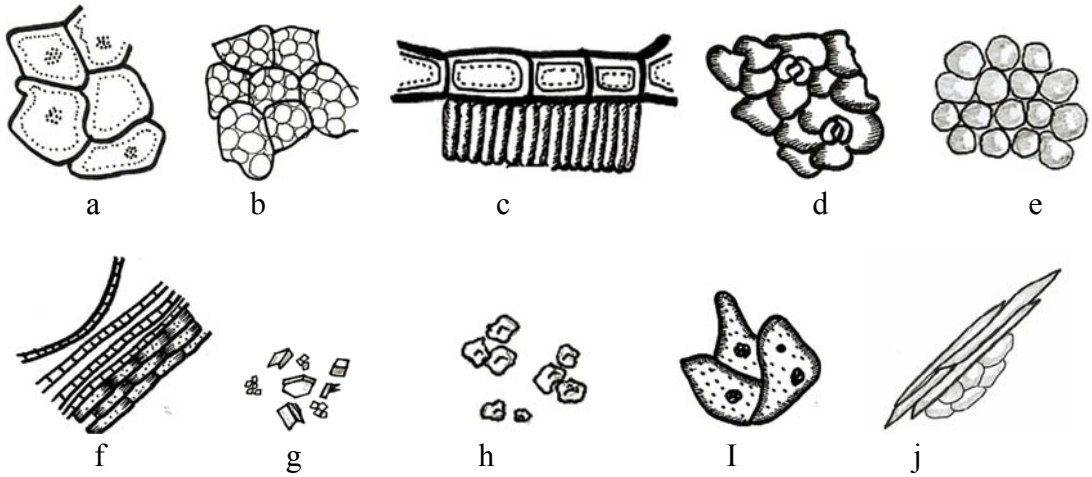


Fig. 4.32. *Zanthoxylum armatum* leaf powder. a. upper epidermal cells; b. upper epidermal cells, with palisade cells attached, surface view; c. upper epidermal cells with palisade cells attached, side view; d. lower epidermal cells with stomata; e. spongy mesophyl cells in surface view; f. vessels with elongated parenchymatous cells; g. single and aggregate crystals of calcium oxalate; h. starch grains; i. collenchymatous cells with calcium oxalate crystals; j. fibers from vein with parenchymatous cells.

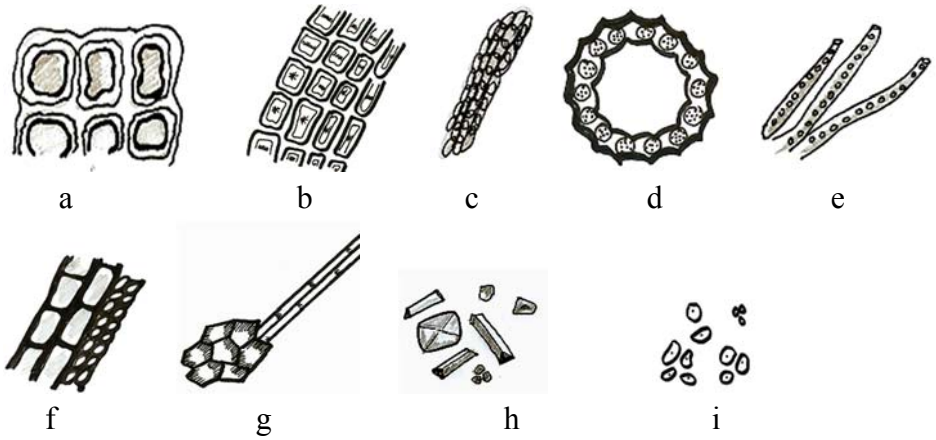


Fig. 4.33. *Zanthoxylum armatum* bark powder fragments. a. closely fitted cork cells; b. collenchymatous cells with calcium oxalate crystals; c. fragments of medullary rays; d. isolated sclerieds; e. pitted sieve elements; f. phloem parenchyma with attached medullary ray cells; g. fibers with attached parenchymatous cells are attached; h. different types of calcium oxalate crystals; i. starch grains.

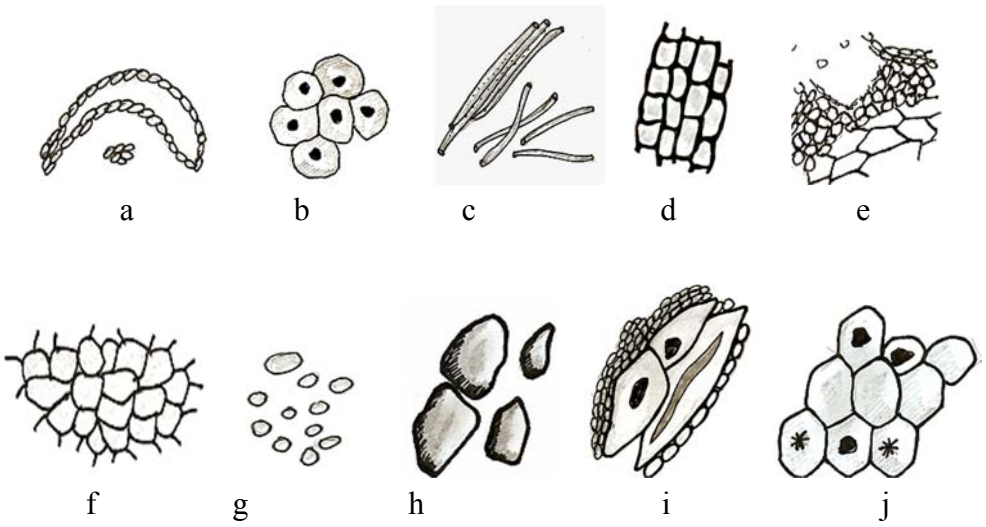


Fig. 4.34. *Zanthoxylum armatum* fruit powder. a. group of parenchymatous cells from mesocarp; b. a group of parenchymatous cells of mesocarp; c. fibers; d. thick walled parenchymatous cells from endocarp.; e. a fragment from fruit wall with broken oil gland; f. Inner epidermal cells of pericarp; g. starch grains; h. Sclerids from epicarp; i. elongated sclerieds, with small parenchymatous cells from epicarp; j. endocarp cells with calcium oxalate crystals.

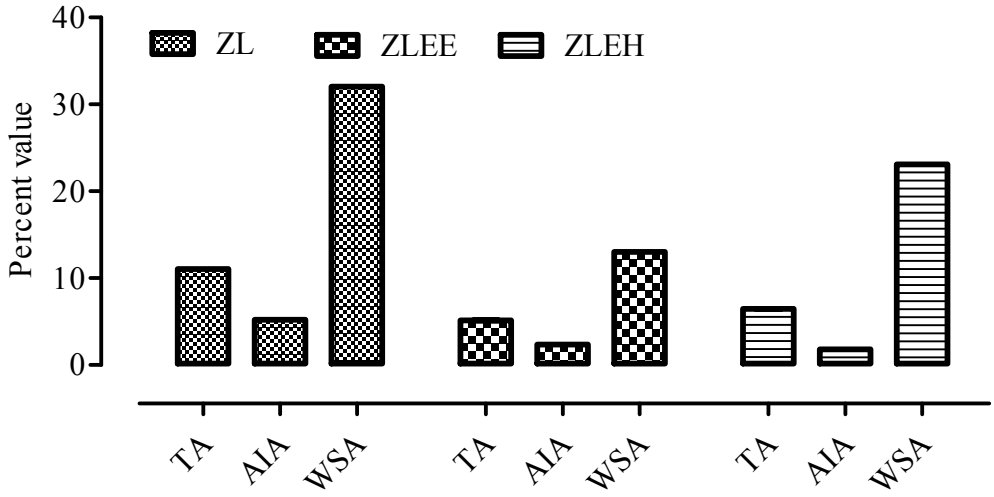


Fig. 4.35a. Comparison of total ash (TA), acid insoluble ash (AIA) and water soluble ash (WSA) percent values of *Zanthoxylum armatum* leaf powder; non exhausted (ZL) and exhausted with ethanol (ZLEE) and n-hexane (ZLEH).

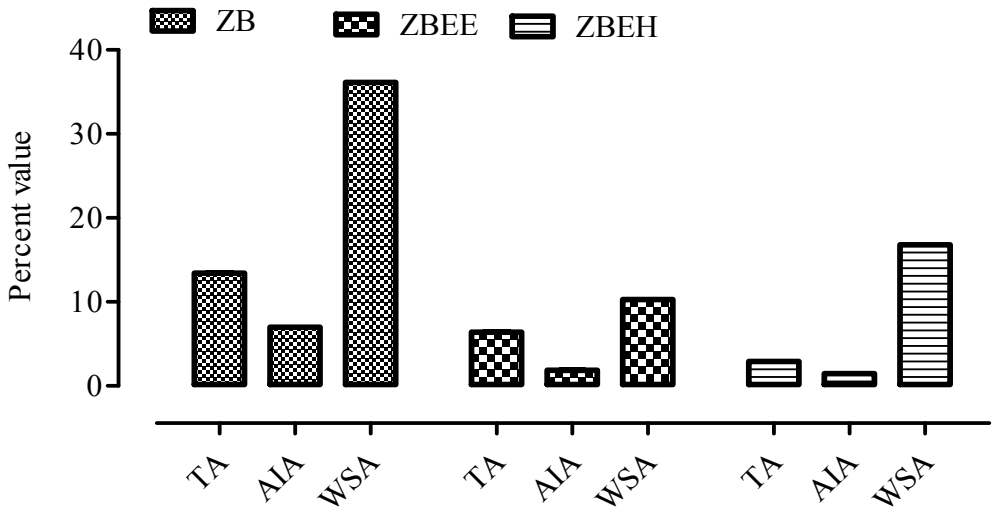


Fig. 4.35b.Comparison of total ash (TA), acid insoluble ash (AIA) and water soluble ash (WSA) percent values of *Zanthoxylum armatum* bark powder; non exhausted (ZB)and exhausted with ethanol(ZBEE) and n-hexane (ZBEH).

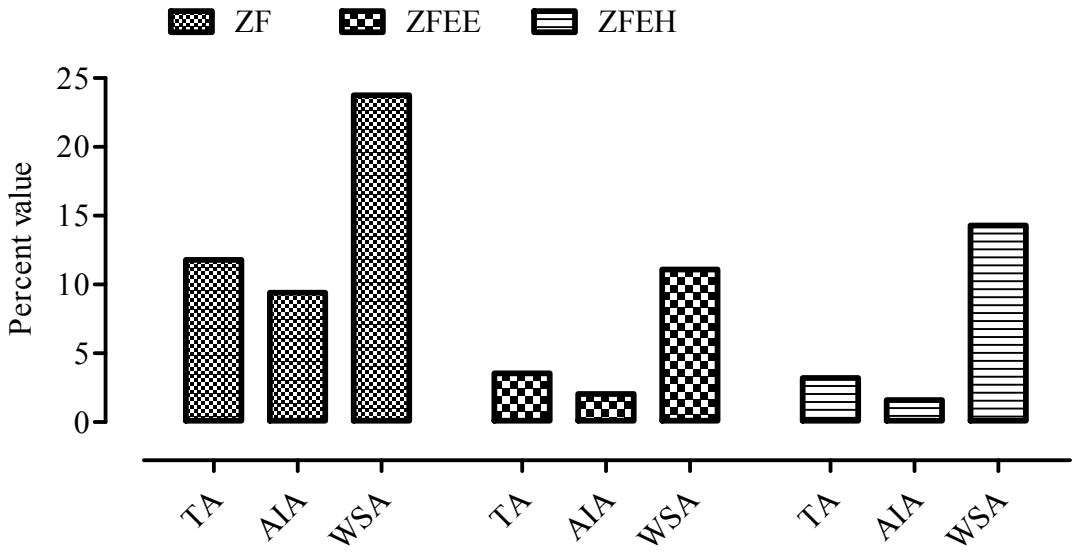


Fig. 4.35c. Comparison of total ash (TA), acid insoluble ash (AIA) and water soluble ash (WSA) percent values of *Zanthoxylum armatum* fruit powder non exhausted (ZF) and exhausted with ethanol(ZFEE) and n-hexane (ZFEH).

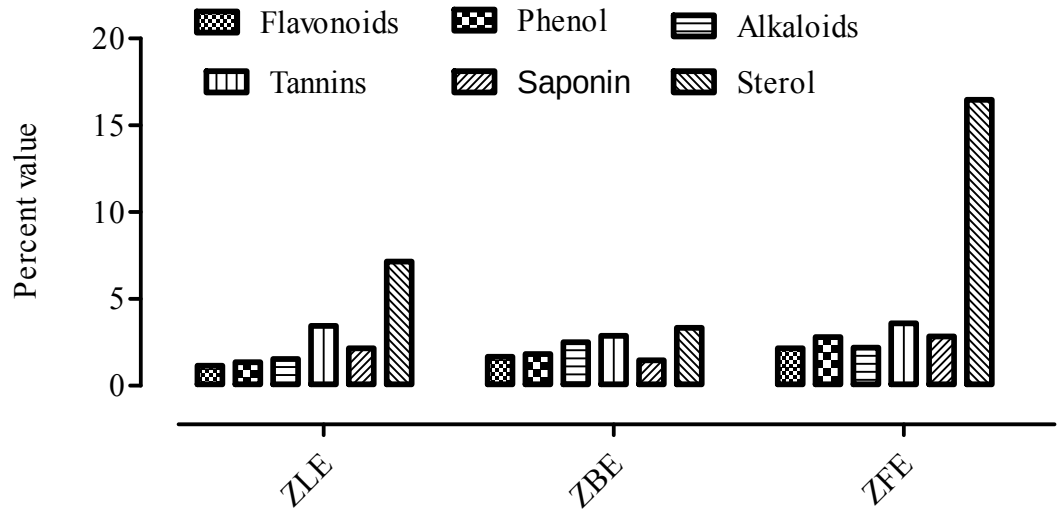


Fig. 4.36. Bars represent percents values of flavonoids, phenols, alkaloids, tannins, saponins and sterols in ethanolic extracts *Zanthoxylum armatum* leaf (ZLE), bark (ZBE) and fruit (ZFE).

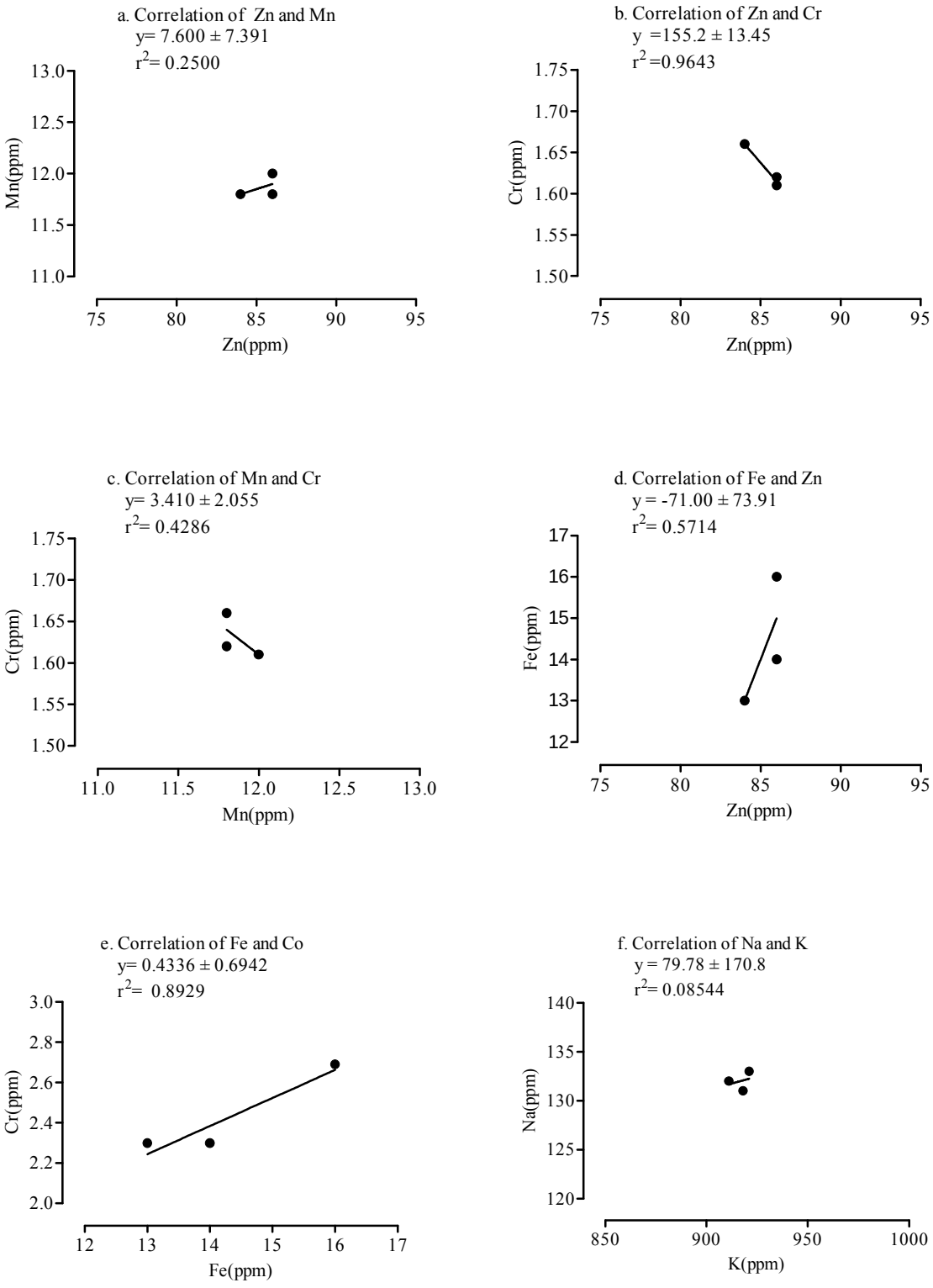


Fig. 4.37. Correlations of various trace elements in *Zanthoxylum armatum* leaf.

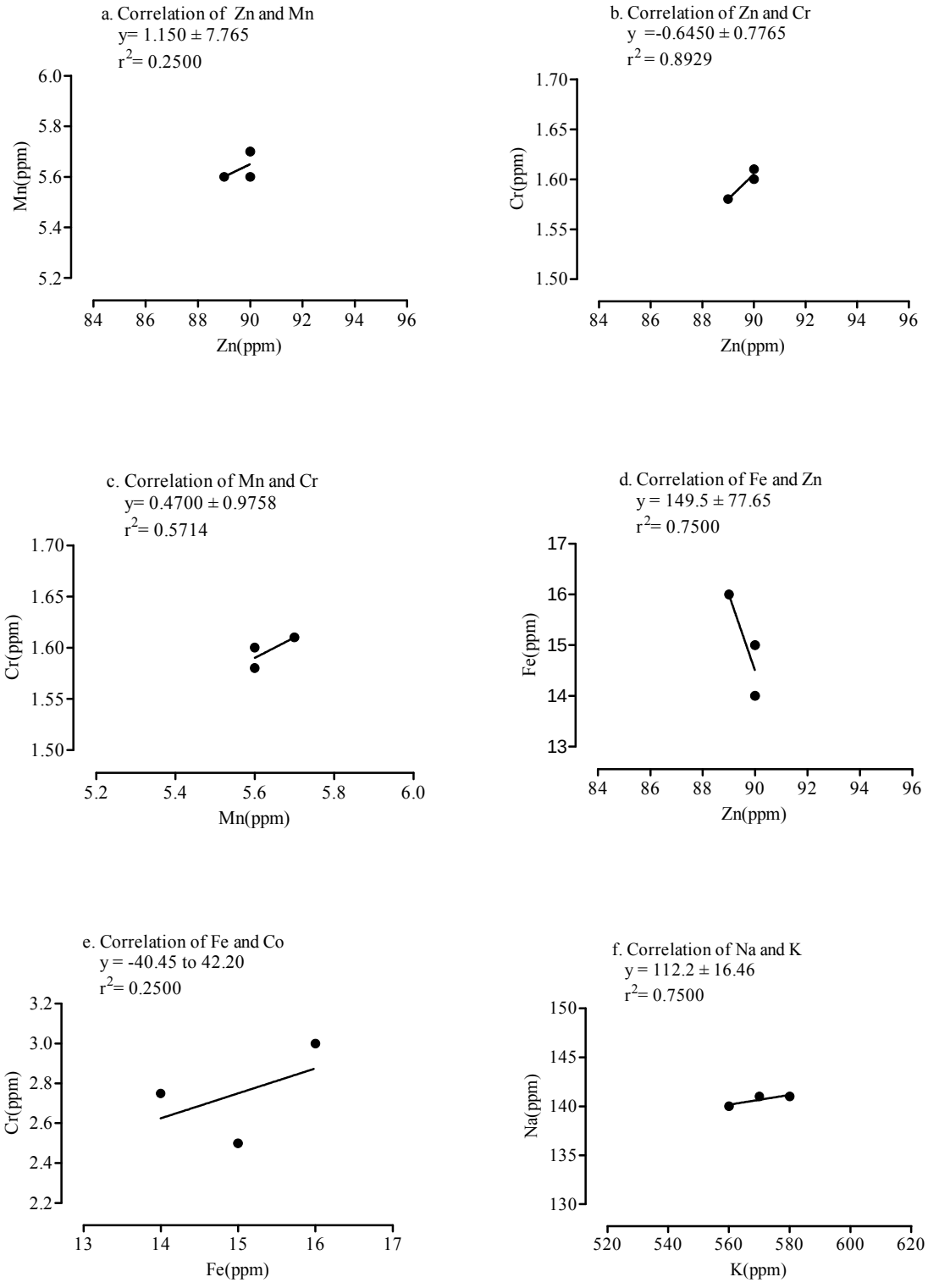


Fig. 4.38. Correlations of various trace elements in *Zanthoxylum armatum* stem bark.

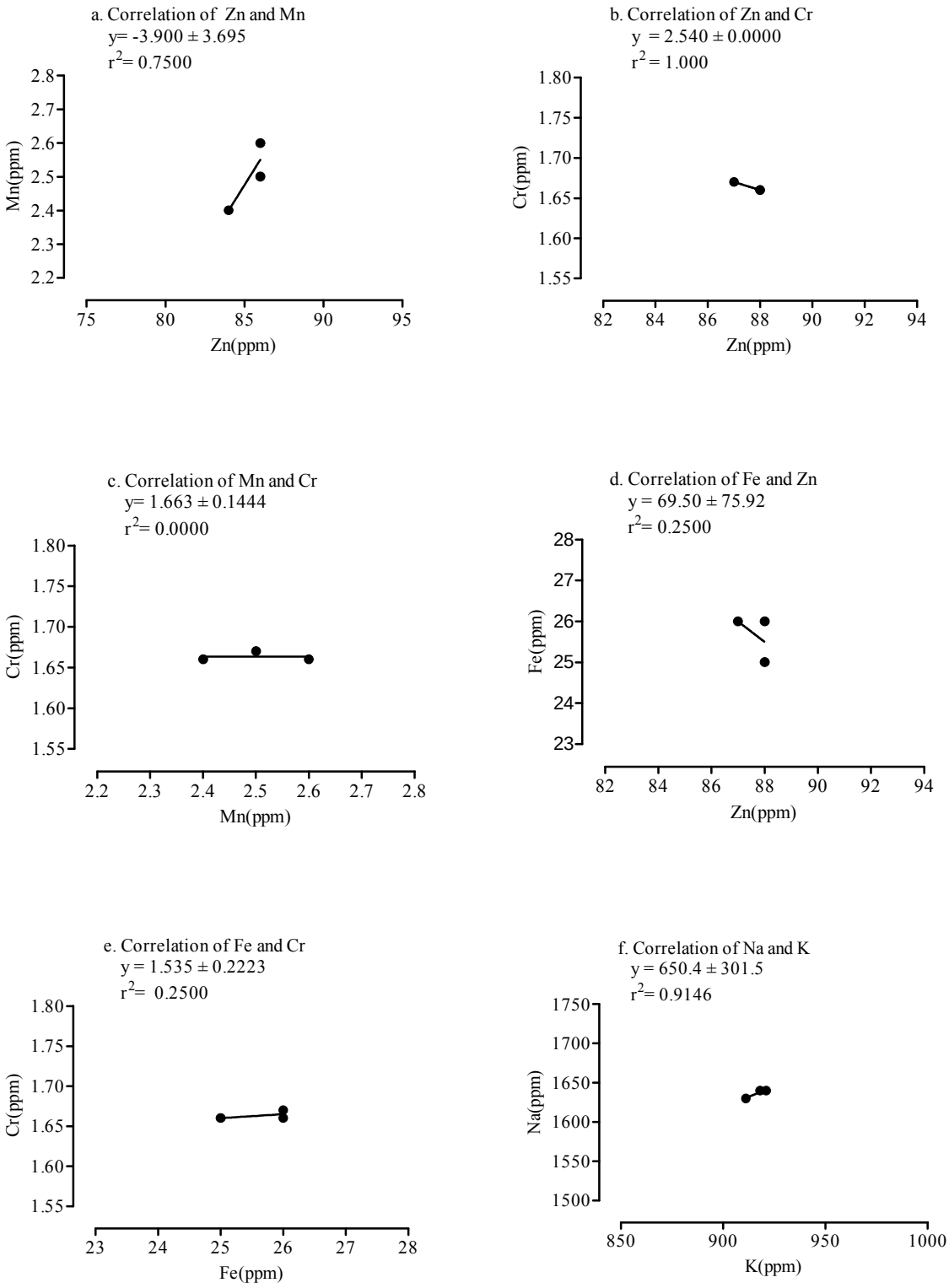


Fig. 4.39. Correlations of various trace elements in *Zanthoxylum armatum* fruit.

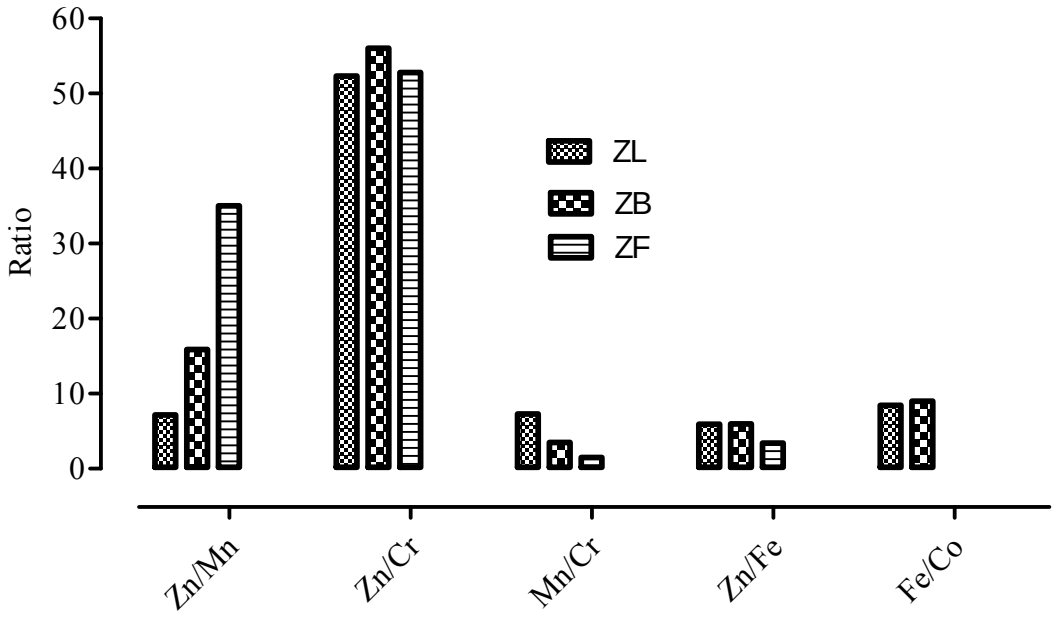


Fig. 4.40. Bars representing ratios among various elements in leaf (ZL), bark (ZB) and fruit (ZF) of *Zanthoxylum armatum*.

4.6. Physicochemical analysis of oil

Studies of various physicochemical characteristics identify the practical importance and provide bases for suitability and utility of various oils of plants origin in daily life. Physicochemical properties of oil like color, odor, density, specific gravity, refractive index, optical rotation, acid value, iodine value, saponification value etc indirectly tells about the quality of both essential and fixed oils (Parthiban *et al*., 2011).

In the present study essential oils obtained from the leaf of *S.laureola* (SVO) and *Z. armatum* (ZVO) and fixed oil from the fruit of *Z. armatum,* extracted with different solvent i.e n-hexane (ZHO) and petroleum ether (ZEO) were evaluated for physicochemical characteristics (Table 4.39). The essential oils were colorless to light apple white in appearance. The fixed oil i.e. (ZHO and ZEO) were brownish yellow and golden yellow respectively. Khoddami *et al*. (2011) also reported that there is a color variation in the fixed oil of *Nigella* seeds extracted through n- hexane and petroleum ether, which strengthenes the present result. Like other essential oils (Al-Rehaily *et al*., 2003; Waheed *et al*., 2011; Parthiban *et al*., 2011), both the volatile had pleasant odour while fixed oil had unpleasant odor. The percent yield of oils in the present study was 0.395±0.031, 0.198±0.018, 9.40±0.054 and 9.97±0.032 for SVO, ZVO, ZHO and ZEO respectively. Essentai oil yield of SVO in the present study was less than the reported yield in Nepal which ranged from 0.93 to 1.12 % (Bhattarai & Karki, 2006). The difference appeared, might be due to ecological factors. Similarly fixed oil showed variation in the yield, when extracted with different organic solvents.

Of the two volatile oil, it was found that SVO had a higher optical rotation (+37.3^{o}) than the ZVO (+34.9^{o}). The fixed oil extracted with n-hexane had a high optical activity as compared to the oil extracted with Petroleum ether. All the samples of fixed in the study have almost the same numerical values for refractive index ranged from 1.4171 to 1.4194 showing richness in long fatty acid chain in all samples (Pearson, 1976). Similar studies have also carried out by Khan (2010) on various citrus species and Pujiarti *et al*. (2011) on *Melaleuca leucadendron*.

Specific gravity values of oils are less than 1 for most of the oils except few containing oxygenated aromatic compounds (Osagie *et al*., 1986). In the present study,

fixed oils from fruit of Z. armatum have high specific gravity values as compared to essential oils from the leaves of both plants. ZHO has a specific gravity of 0.886±0.001 followed by ZEO (0.871±0.001), ZVO (0.816±0.001) and SVO (0.792±0.001). These findings were found well in lineage with the values determined for seed oils of some plants from Congo (Minzangi *et al*., 2011). Our results are little inconsistent with Elert (2000), who described that most of the oils are characterized with specific gravity ranges from 0.9100 to 0.9400.

Acid value is an indirect method for determination of free fatty acid of amount in oil samples and its edibility (Akbar *et al*., 2009). Oil with low free fatty acids has more significant usage (Coenen, 1976). The Total acid number (TAN) values recorded in the present study were 1.78±0.01, 1.98±0.01, 1.45±0.02 and 1.58±0.01 for SVO, ZVO, ZHO and ZEO respectively. These values were found in the permissible limits i.e. 10 mg KOH/g of oil (Codex Alimentarious Commision, 1982) and found to be suitable for dietary purposes, as they contain lower fatty acid contents (Essein & Amadi, 2009). Values obtained in the present study were found lower than seed oil of *Jatropha curcas* (Akbar *et al*., 2009) and seeds oil of five Nigerian species (Akubugwo *et al*., 2007).

Carbon residue values for SVO were found 2.65%, less than for ZVO (2.80 %). For fixed oils these values werer found smaller than essential i.e. 2.21% for ZHO and 2.35% for ZEO. Absolute viscosity was found quite higher for SVO in all samples. Similarly other values related to viscosity like viscosity index and kinematic viscosity were also calculated showing variant results (Table 4.39). Similar values were also determined for different *Citrus* seed oils (Anwar *et al*., 2008) and *Jatropha curcas* oil (Adebayo *et al*.,2011), which strengthened the present study.

Saponification values determined were found greater for fixed oil of *Z. armatum* fruit as compared to SVO and ZVO. The oil samples have saponification values of 109.00±0.67, 105.33±0.84, 163.33±1.02 and 151.67±0.69 mg/KOH/g for SVO, ZVO, ZHO and ZEO respectively. The saponification values for ZHO and ZEO are more or less in lineage with the work done by previous workers like Anwar & Rashid (2007). Essein & Amadi (2009), Minzangi *et al*. (2011) and Akinyeye *et al*. (2010). Essential oils

saponification values were found in accordance with that of Ejikeme *et al*. (2010), thus strengthening our findings. All the oil samples are found in the range reported for plants (lower than the 188 – 196) (Pearson, 1976) but not useful in soup industry as these values are much lesser than the required value (±300) (Alabi, 1993).

The iodine value is the indicative of fats and oils unsaturation. Fats and oils with higher unsaturation show high iodine value (Kyriakidis & Katsiloulis, 2000; Knothe, 2002). The iodine valve observed for the oil samples in the present study were 118±0.11, 102±0.11, 157±0.65 and 145±0.33 for SVO, ZVO, ZHO and ZEO respectively. These values were found in the permissible range for semi-drying of oil (100-300) (Kagwachie & Anozie, 1995). Recorded data for iodine number showed that all the oil samples have high unsaturated fatty acids contents (Anwar *et al*., 2005).The present study also showed inconsistency with that of Essein & Amadi (2009), Mohanty *et al*. (2010) and Akinyeye *et al*. (2011), who studied various oil samples of plant origin and reported smaller iodine value for these oils.

4.7. GC-MS Analysis.

GC- MS Analysis of essential oils obtained from the leaves of *S.laureola* (SVO) and *Z. armatum* (ZVO) through hydrodistilation and of the fixed oil from the fruit of *Z. armatum,* extracted with n- hexane (ZHO) and petroleum ether (ZEO) were evaluated for their chemical composition.

4.7.1. GC-MS Analysis of essential oil of *Skimmia laurola* leaf.

The essential oils from the leaves of *Skimmia laureola* (SVO) were extracted through hydro distillation and analysed for chemical composition through GC-MS (Fig. 4.41). Various identified components with their respective chemical nature, percent concentration, retention time and charge to mass ratio are given in Table 4.40. A total 31 different components were identified including Bergamot mint oil (53.41%), hydrocarbon monoterpenes (11.87 %), alcoholic monoterpenes (22.06 %), acetate monoterpenes (12.12 %), aldehyde monoterpenes (0.13 %), oxygenated monoterpenes (0.07 %) and sesquiterpenes (0.42 %). Bergamot mint oil is a mixture of acetate and alcoholic monoterpenes (Linalool and linalyl acetate) was the major constituents (53.41%). beta-

Linalool (14.19 %) was the second largest components, which showed inconsistency with Shah *et al*. (2003), who reported 37 to 64% linalool in *skimmia* essential oil in different seasons. Al-Rehaily *et al*. (2003) has reported 12.68% linlool for *Rosa abyssinica,* Mumtaz *et al*., (2007) reported 6.9% for *R*. *centifolia* and Shabbir *et al*. (2009) 0.222% for R. *centifolia*. Linalool has been considerd as refreshing and fragrant agent, while linalyl acetate has pleasant, sweet, and fruity characteristics (Zaks *et al*., 2008). Due to the presence of bergamot mint oil and linalool in large amount, SVO might have strong antibiotic potential. They may also be effective in reducing bacterial contamination in various food systems (Fisher & Phillips, 2006).

Other components detected in the SVO were geraniol acetate (9.16 %) and p-meth-1-en-8-ol (6.67 %). beta- Myrcene (3.69 %), Trans-beta-Ocimene (2.48 %) and alpha- pinene (2.42 %) are the major monoterpenes of hydrocarbon nature. Mnor components detected were Nerol acetate (1.82 %), cis-beta-Ocimene (1.69 %), Sabinene (1.06 %), p-meth-1-en-8-ol, acetate (1.05 %) and cis-Geraniol (1.03 %). Very small amount of oxygenated monoterpenes were present as compared to other constituents. Gamma elemine (0.21 %) was the major sesquiterpenes, although total amount of sesquiterpenes were found to be very low. this observation was found contrary to that of Laribi *et al*. (2011), who reported sesquiterpenes hydrocarbons the major constituents in the essential oil of *S. pandurifolius*. Comparison of the present data with reported work showed that monoterpene hydrocarbons were the major constituents (Mirza & Baher, 2008; Belaunde *et al*., 2007; Fernandez-Zuniga *et al*., 1996). These oils also have resemblances in having almost similar monterpenes as in the essential oil of *Mentha aquatica* (Zaks *et al*., 2008), *Lavandula officinalis* and *Sallvia sclarea* (Bauer *et al*., 1990). Sesquiterpenes like caryophyllene and germacrene D, reported in the present study were also reported in *Mentha aquatica* (Zaks *et al*., 2008).

Essential are therapeutically active in large number of ailments, also used extensively as spices, condiments and flavoring agents in a variety of foods, beverages and icecream and in hundreds of coamatics. Some of the oil like eugenol (Clove) has antiseptic and antimicrobial properties. Therefore these oils have a wide range of its utility as therapeutic agents as well as have a beneficial role in enhancing food qualities (Asalvat *et al*., 2001; Baker *et al*., 1994; Pauli, 2001). SVO is enriched in certain

monoterpenes, which are reported to be extensively used in pharmaceutical as well as in flovours, foods, cosmetics industries (Bhat *et al*., 2002). Therefore this oil might be useful in providing beneficiary support to human health. This oil may be commercialized due to high contents of bergamot mint oil. The oil may also useful in fragrance and flavouring industries, because linalool and linalyl acetate are present individually as well as in mixture form in the bergamot oil, are used as fragrance and flavoring agents.

4.7.2. GC-MS Analysis of essential oil of *Zanthoxylum armatum* leaf

Eessential oil of *Zanthoxylum armatum* leaf (ZVO) were hydro distillatilled and GC-MS analysis was carried out for identification of various components (Fig. 4.41). The components with their respective terpenes nature, percent concentration, retention time and charge to mass ratio are presented in Table 4.42. Out of the total 34 identified components, monoterpenes alcohol was the largest component (56.57 %), followed by monoterpenes hydrocarbins (14.99%), Bergamot mint oil (12.73 %), monoterpenes oxygenated (11.39 %), monoterpenes acetate (1.9 %), monoterpenes aldehyde (0.05 %), and sesquiterpenes (2.67 %). These results are inconsistent with that of Waheed *et al*. (2011), who reported much higher percentage of oxygenated monoterpenes (37.23%) and sesquiterpenes (10.83 %) and lesser percentage of alcoholic monoterpenes from seed essential oil of *Z. armatum*, thus showing compositional variation in leaves and seeds. Among the monoterpene alcohol, beta- Linalool (53.05 %) was the major constituents. Some other workers have also reported linalool in other plants as for example in *Rosa abyssinica* (12.68%) (Al-Rehaily *et al*., 2003), *Rosa abyssinica* (12.68%)*, R. centifolia* (6.9 %) (Mumtaz *et al*., 2007), *Blumea balsamifera* (1.13 %) (Bhuiyan *et al*., 2009) and *R. centifolia* (0.222%) (Shabbir *et al*., 2009). Linalool has a refreshing and fragrant properties and (Zaks *et al*., 2008). It has also been found effective in reducing bacterial contamination to avoid food decay (Fisher & Phillips, 2006). As beta- linalool was found in large amount in ZVO, the oil may have strong therapeutic as well as preservative potentials. Bergamot mint oil was the second largest components (12.73%), which is a mixture of two monoterpenes i.e. Linalool and linalyl acetate and has the same therapeutic and industrial potential as that of lavender oil (Bauer *et al*., 1990). Other significant components detected in ZVO were monoterpenes of hydrocarbon including alpha-Limonene diepoxide (11.39%), alpha- pinene (4.08%), beta- Myrcene (3.69%) and

D. limonene (3.1%). α-pinene has strong bactericidal and bacteriostatic potentials (Cowan, 1999). As ZVO containing good amount of α-pinene, it might be used as a good antibacterial as well as bacteriocidal in various formulations.

Minor components detected were the monoterpenes including p-meth-1-en-8-ol (2.47%), Geraniol acetate (1.32%) cis-beta-Ocimene (1.29%), Sabinene (1.24%) and p-meth-1-en-8-ol, acetate (1.05%) and sesquiterpenes i.e. Carryophyline (1.32%) and Germacrene- D (1.01%). Laribi *et al*. (2011) has also reported sesquiterpene hydrocarbons as the major constituents in the essential oil of *S. pandurifolius*. The present analysis showed that monoterpene hydrocarbons were the major constituents of the volatile oil. Other workers (Fernandez-Zuniga *et al*., 1996; Bhat *et al*., 2002; Belaunde *et al*., 2007; Mirza & Baher, 2008; Zaks *et al*., 2008) has also reported similar results. Sesquiterpenes (caryophyllene, and germacrene D) reported in the present study are also in lineage with the that of Al-Rehaily *et al*. (2003), Mumtaz *et al*. (2007), Zaks *et al*. (2008) and Bhuiyan *et al*. (2009).

Therapeutic potential of some plants are due to the presence of essential oil (Asalvat *et al*., 2001). Essential oils are also useful as flavoring agent in food industry and as anti microbial agents in pharmaceutical preparations (Baker *et al*., 1994; Pauli, 2001). Volatile oil have a very important role as flavering agent in foods, beverages, cosmatics and pharmaceutical preparation as well as an important curtive role in many stomach disorders, rheumatism and nercvine disorders (Bhat *et al*., 2002). ZVO oil might be supportive in providing beneficiary effects on human health. linalool and linalyl acetate, which are present free as well as in mixture form in bergamot oil, are used as fragrances and flavoring agents (Zaks *et al*., 2008). As beta linalool was found to be the major component of this oil, it can be patented and commercialized for economic benefit of the local people.

4.7.3. GC-MS analysis of Fixed oils of *Zanthoxylum armatum* fruit

Fixed oil extracted with n-hexane (ZHO) and petroleum ether (ZEO) from the fruit of *Z. armatum* were analysed by GC- MS. Fig 4.43a & b show the GC- MS chromatogram of fatty acids compositon. The fatty acids with their carbon numbers,

status, percent concentration, retention time and charge to mass ratio of ZHO and ZEO are presented in Table 4.42 & 4. 43 respectively. Fourteen components were identified in ZHO methyl esters, in which the proportion of saturated and unsaturated oils was found 93.42 % and 6.58% respectively. Palmic acid, (83.06%) was the major fatty acid followed by Stearic acid (7.33%), Oliec acid (3.51%), Palmitoleic acid (2.07%) and Eladic caid (1.28 %). Ther emaining fatty acids were found to be less than 1%.

Similarly 14 fatty acids were also identified in ZEO, consisting of 47.4 % saturated and 52.79 % unsaturated compounds. All were methyl esters except Behinic acid, which was ethyl ester. The major constitutes of the ZEO were Oliec acid (30.16%), Palmic acid, (25.09%), Linolic acid (17.71%), Stearic acid (17.50%), Eladic caid (3.28%), Behinic acid (2.97%) and Arachidic acid (1.23%). Palmitoleic acid is a skin protectant ingredient and reported to have a leading role in healing wounds and treating burns. It is also considered as UV- blocker and tsissue regeneration promoter (Abed, 2007). It suggests that the fruit oil of *Z. armatum* might be helpful in combating skinediseases, burns and wounds.

Mutual comparison of both fixed oils of *Z. armatum* fruit (ZHO & ZEO) showed variation in fatty acids composition, both in saturation and unsaturation and also in specific type of fatty acids. ZHO was found to be rich in saturated fattyacids while in ZEO, proportion of both saturated and unsaturated fattyacids was almost equal. The major constituent of ZHO was palmic acid (saturated) while that of ZEO was oleic acid (Unsaturated). Some fatty acids like Tridecanoic acid, Arachidic acid, Eicosadienoic acid, Behinic acid and Tetracosanoic acid were not observed in ZHO while Hexanoic acid, Capric caid, Lauric acid, octadecanoic acid and Linolinic acid were not detected in ZEO. Unsaturated fatty acids were much higher in ZHO than ZEO. It is reported that unsaturated fatty acids like linoleic acid and linolenic acids have a beneficial and curative role in human health, especially for high cholesterol level in blood and cardiovascular problems (Goyle *et al*., 2002; Abed, 2007). It can be conclude that this oil might be helpful in controlling cardiovascular problems due to its high contents of linoleic acid.

Saturated fatty acids can increase the risk of heart diseases from Atherosclerosis, cauesd by high cholesterol level in the blood due to the some saturated fatty acids, especially myristic acid (Appel *et al*., 1997). *Z. armatum* fruit fixed oil contains very less amount of such fatty acids thus may not pose threat to human health.

Table 4.39. Physicochemical characteristics of S VO, ZVO, ZHO and ZEO. All values are mean±SEM of three values.

S. No	Characteristics	SVO	ZVO	ZHO	ZEO
1	Colour	Colorless to light apple white	Colorless to light apple white	Brownish yellow	Golden yellow
2	Odour	Pleasant	Pleasant	Unpleasant	Unpleasant
3	% oil yield	0.395±0.031	0.198±0.018	9.40±0.054	9.97±0.032
4	Optical activity	+37.3 º	+34.9 º	+80.5 º	+80.9 º
5	Refractive index at 27ºC	1.4194±0.0001	1.4180±0.0001	1.4176±0.0001	1.4171±0.0001
6	Specific gravity at 27ºC	0.792±0.001	0.816±0.001	0.886±0.001	0.871±0.001
7	Carbon residue (%)	2.6633±0.051	2.8133±0.0051	2.2100±0.0033	2.3467±0.0051
8	Absolute viscosity	105.30±0.01	112.23±0.10	88.71±0.08	97.98±0.02
9	Kinematic viscosity	90.20±0.07	93.73±0.08	80.70±0.03	86.80±0.03
10	Total acid number (TAN) mg KOH/g	1.78±0.01	1.98±0.01	1.45±0.02	1.58±0.01
11	Iodine number	118±0.11	102±0.11	157±0.65	145±0.33
12	Saponification value (mg KOH/g oil)	109.00±0.67	105.33±0.84	163.33±1.02	151.67±0.69

Table 4. 40. GC-MS profile of the *Skimmia laureola* leaf essential oil (SVO).

S. No	Name	Compound type	% composition	r/time	m/z
1	alpha- pinene	Monoterpene hydrocarbon	2.42	8.889	93
2	Camphene	Monoterpene hydrocarbon	0.01	9.575	93
3	Sabinene	Monoterpene hydrocarbon	1.06	10.594	93
4	Beta – Pinene	Monoterpene hydrocarbon	0.08	10.779	93
5	beta- Myrcene	Monoterpene hydrocarbon	3.69	2.21	93
6	beta – phellandrene	Monoterpene hydrocarbon	0.34	13.258	40
7	Cineole	Monoterpene hydrocarbon	0.14	13.323	43
8	Trans-beta-Ocimene	Monoterpene hydrocarbon	2.48	13.587	93
9	cis-beta-Ocimene	Monoterpene hydrocarbon	1.69	14.079	93
10	3-Carene	Monoterpene hydrocarbon	0.01	14.59	93
11	gamma turpentine	Monoterpene hydrocarbon	0.01	14.59	93
12	Terpinolene	Monoterpene hydrocarbon	0.12	15.893	93
13	.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl] oxiranemethanol	Monoterpene alcohol	0.04	15.997	59
14	beta- Linalool	Monoterpene alcohol	14.19	16.733	71
15	1-Terpinene-4-ol	Monoterpene alcohol	0.13	19.548	71
16	p-meth-1-en-8-ol	Monoterpene alcohol	6.67	19.974	59
17	n-octyle acetate	Monoterpene acetate	0.06	20.384	43
18	cis-Geraniol	Monoterpene alcohol	1.03	20.705	41
19	Brgamot mint oil	Mixture of acetate and alcohol monoterpenes	53.41	21.327	93

20	Alpha-Citral	Monoterpene aldehyde	0.13	21.686	69
21	Bornyl acetate	Monoterpene acetate	0.03	22.059	95
22	cis-Limonene oxide	Monoterpene oxygenated	0.03	22.941	43
23	3-Nonanol,1,2;6,7-dipoxy-3,7-dimethyle acetate	Sesquetepene	0.06	23.152	43
24	p-meth-1-en-8-ol, acetate	Monoterpene acetate	1.05	23.227	121
25	Nerol acetate	Monoterpene acetate	1.82	23.401	69
26	Geraniol acetate	Monoterpene acetate	9.16	23.739	69
27	Caryophyllene	Sesquiterpene	0.04	24.543	41
28	alpha-Limonene diepoxide	Monoterpene oxygenated	0.04	25.567	43
29	gamma-Elemene	Sesquiterpene	0.21	25.701	161
30	trans-Nerolidol	Sesquiterpene	0.08	25.506	69
31	Caryophyllene oxide	Sesquiterpene	0.03	27.22	41

Table 4.41. GC-MS profile of *Zanthoxylum armatum* leaf essential oil (ZVO).

S. No	Name	Compound type	Concentrtation	R/time	m/Z
1	alpha-phelendrene	Monoterpene hydrocarbon	0.02	8.617	
2	alpha- pinene	Monoterpene hydrocarbon	4.08	8.914	93
3	Camphene	Monoterpene hydrocarbon	0.03	9.59	93
4	Sabinene	Monoterpene hydrocarbon	1.24	10.61	93
5	Beta – Pinene	Monoterpene hydrocarbon	0.16	10.795	93
6	beta- Myrcene	Monoterpene hydrocarbon	3.69	11.411	93
7	D-Limonene	Monoterpene hydrocarbon	3.1	13.219	68
8	beta – phellandrene	Monoterpene hydrocarbon	0.41	13.282	40
10	Trans-beta-Ocimene	Monoterpene hydrocarbon	0.24	13.602	93
11	cis-beta-Ocimene	Monoterpene hydrocarbon	1.29	14.094	93
12	3-Carene	Monoterpene hydrocarbon	0.15	14.608	93
13	gamma turpentine	Monoterpene hydrocarbon	0.16	14.608	93
14	Terpinolene	Monoterpene hydrocarbon	0.32	15.921	93
15	alpha-Methyl-alpha-[4-methyl-3-pentenyl] oxirane methanol	Monoterpene alcohol	0.03	15.994	59
16	beta- Linalool	Monoterpene oxygenated	53.05	16.813	71
17	1-Terpinene-4-ol	Monoterpene alcohol	0.51	19.56	71
18	p-meth-1-en-8-ol	Monoterpene alcohol	2.47	19.997	59
19	n-octyle acetate	Monoterpene acetate	0.01	20.4	43
20	Cis Geraniol	Monoterpene alcohol	0.39	20.711	41
21	Brgamot mint oil	Mixture of acetate and alcohol monoterpenes	12.73	21.31	93
22	Alpha-citral	Monoterpene aldehyde	0.05	21.693	69
23	3-Nonanol,1,2;6,7-	Sesquiterpene	0.03	23.239	43

	dipoxy-3,7-dimethyle acetate				
24	p-meth-1-en-8-ol, acetate	Monoterpene acetate	0.03	23.239	121
25	Nerol acetate	Monoterpene acetate	0.54	23.409	69
26	Geraniol acetate	Monoterpene acetate	1.32	23.744	69
27	Caryophyllene	Sesquiterpene	1.39	24.553	41
28	beta-Farnesene	Sesquiterpene	0.02	24.85	69
29	alpha-Limonene diepoxide	Monoterpene oxygenated	11.39	25.58	43
30	Germacrene D	Sesquiterpene	1.01	25.496	161
31	trans-Nerolidol	Sesquiterpene	0.14	26.516	69
32	Caryophyllene oxide	Sesquiterpene	0.08	26.962	41
33	p-Cimene	Monoterpene hydrocarbon	0.1	32.842	40
34	2-Furanmetahnol	Monoterpene alcohol	0.12	33.035	40

Table 4.42. GC- MS profile of n-hexane extracted fixed oil of *Z. armatum* fruit (ZHO).

S. No	C #	Name	Status	Concentration	R. time	m/Z
1	C6	Hexanoic acid	Saturated	0.08	3.065	87
2	C10	Capric caid	Saturated	0.05	6.781	87
3	C12	Lauric acid	Saturated	0.09	8.25	87
4	C14	Myrestic acid	Saturated	0.37	10.951	87
5	C15	Penta decanoic acid	Saturated	0.22	12.609	87
6	C16	Palmic acid	Saturated	83.06	14.612	87
7	C16	Palmitoleic acid	Saturated	2.07	15.232	97
8	C17	Margaric acid	Saturated	0.15	16.899	87
9	C18	Stearic acid	Saturated	7.33	19.589	87
10	C18	Oliec acid	Unsaturated	3.51	20.222	97
11	C18	Eladic caid	Unsaturated	1.28	20.325	97
12	C18	octadecanoic acid	Unsaturated	0.5	20.543	97
13	C18	Linoleic caid	Unsaturated	0.95	21.731	95
14	C18	Linolinic acid	Unsaturated	0.34	24.29	95

Table 4.43. GC- MS profile of Pet. Ether extracted fixed oil of *Z. armatum* fruit (ZEO).

S.No	C #	Name	Status	Concentration	R. time	m/z
1	C13	Tridecanoic acid	Saturated	0.2	9.606	87
2	C14	Myrestic acid	Saturated	0.24	10.953	87
3	C15	Penta decanoic acid	Saturated	0.05	12.609	87
4	C16	Palmic acid	Saturated	25.09	14.607	87
5	C16	Palmitoleic acid	Unsaturated	0.14	15.14	97
6	C17	Margaric acid	Saturated	0.12	16.9	87
7	C18	Stearic acid	Saturated	17.5	19.608	87
8	C18	Oliec acid	Unsaturated	30.16	20.178	97
9	C18	Eladic caid	Unsaturated	3.28	20.333	97
10	C18	Linoleic caid	Unsaturated	17.71	21.754	95
11	C20	Arachidic acid	Saturated	1.23	27.165	87
12	C20	Eicosadienoic acid	Unsaturated	0.6	29.627	95
13	C22	Behinic acid	Saturated	2.97	34.284	87
14	C24	Tetracosanoic acid	Unsaturated	0.9	40.623	87

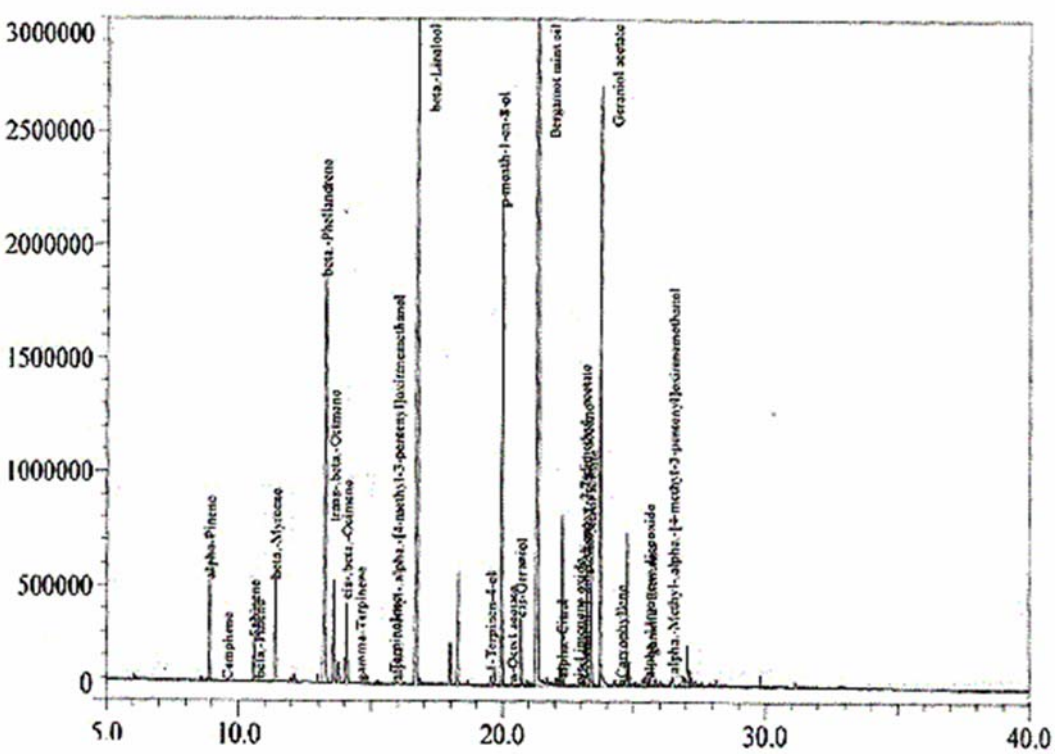


Fig. 4.41. Typical GC-MS chromatogram of *Skimmia laureola* leaf essential oil (SVO) showing the separation of chemical components.

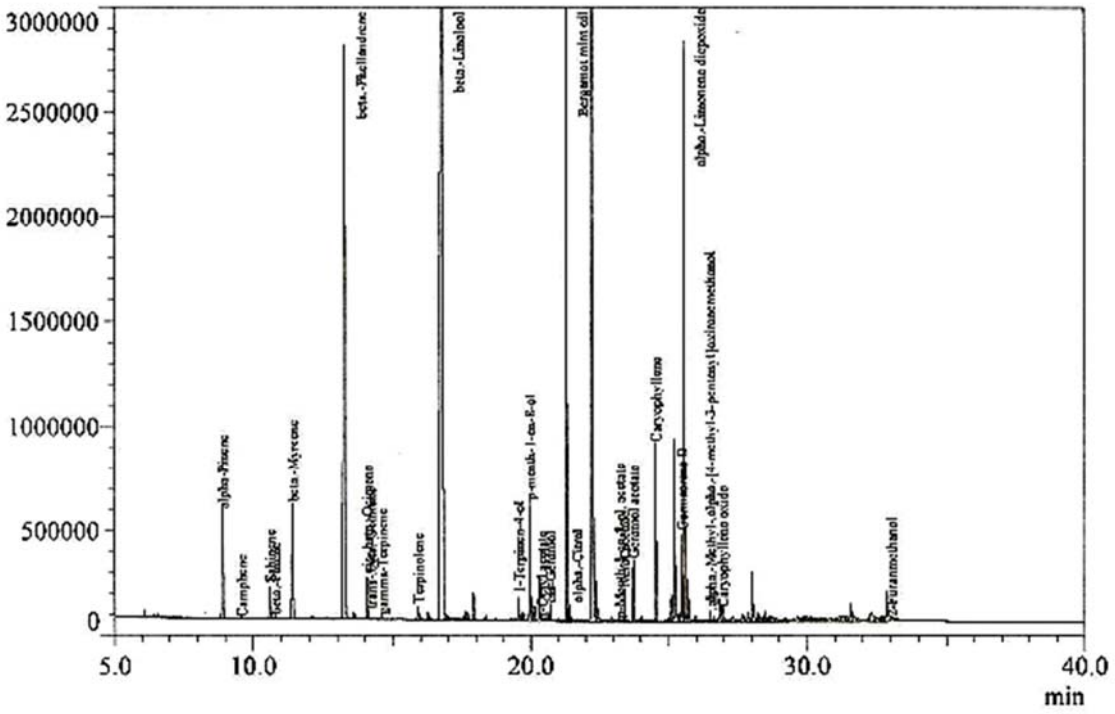


Fig. 4.42. Typical GC-MS chromatogram of *Zanthoxylum armatum* leaf essential oil (ZVO) showing the separation of chemical components.

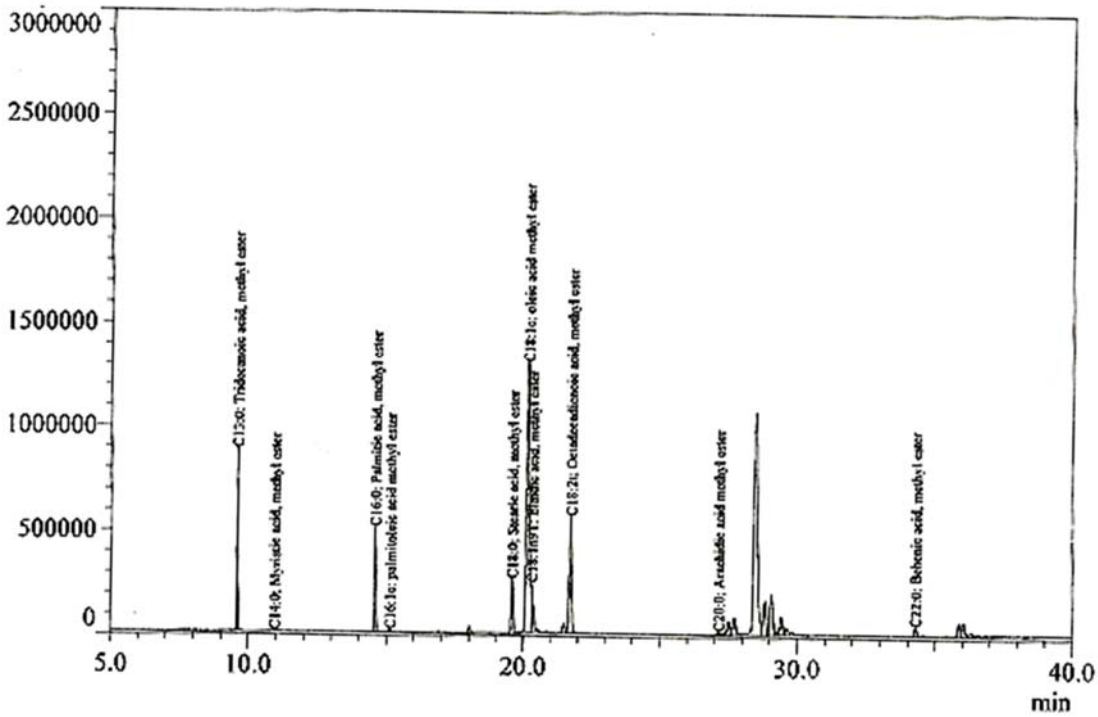


Fig. 4.43a. Typical GC-MS chromatogram of *Zanthoxylum armatum* fruit fixed oil, extracted with n-hexane (ZHO) showing the separation of chemical components.

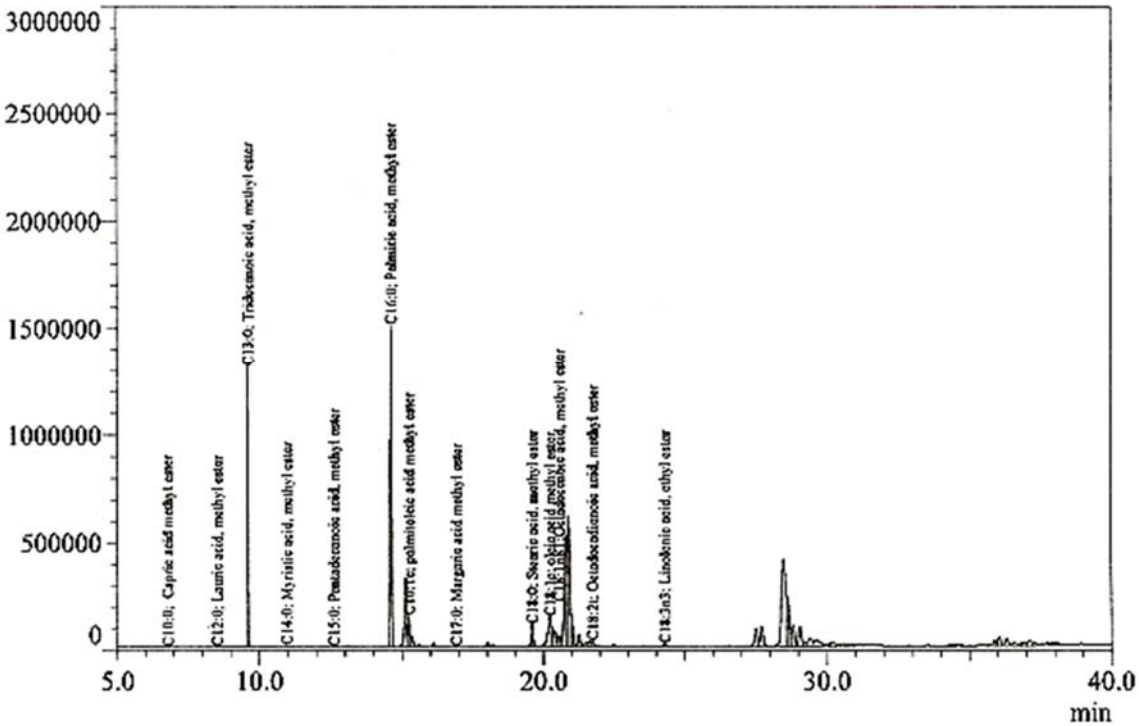


Fig. 4.43b . Typical GC-MS chromatogram of *Zanthoxylum armatum* fruit fixed oil, extracted with petroleum ether (ZEO) showing the separation of chemical components.

4.8. Pharmacology

Pharmacology presents various scientific techniques like screening of extracts, fractions and compounds of plants origin in the form of bioassays. (Nelms, 1997). Different bioassays suggest advantages to screen out various plant extracts for the treatment of various ailments (Srirama *et al.,* 2007). In the present study *Skimmia laureola* and *Zanthoxylum armatum* were screened out for various bioassays, in order to investigate their therapeutic value.

4.8.1. Biological activities of *Skimmia laureola*

The following biological activities of *S. laureola*. were carried out.

4.8.1.1. Acute toxicity test

Acute toxicity test through medicinal plants and crude drugs are evaluated for its possible adverse effects. On the bases of this test, the drugs may or may not be recommended for safe consumption to cure human health problems. Although new modern techniques have been developed because of more modern approaches to toxicology (Combe *et al.,* 2004), but the use of animals in toxicity study evaluations in acute systemics is still preferred (Anonymous, 2000). In the present study ethanolic extract of *S. laureola* leaf at doses of 500, 1000 and 2000 mg/kg body weight was evaluated for toxicological effects, using mice as test animals. No mortality or morbidity was observed for the first four hours and then even for the next 24 hours, showing that the plant is safe for human use (Table 4.44). Our study was found well in lineage with some previous workers. Magaji *et al*. (2007) evaluated the leaf and stem bark of *Securinega virosa* for toxicity and found it safe for consumption. The ethanol extract of *Trigonella foenum-graecum* seeds at a dose up to 3 g/kg body weight was also reported to be safe (Mowla *et al.,* 2009). Similarly Udem & Asogwa (2010) reported no mortality or morbidity for the leaf aqueous extract of *Ipomea batatas* even at the highest dose of 1,600 mg/kg. The present acute toxicity test suggests that *S.laureola* can be used to carry out further bioassays to know its therapeutic usefulness.

4.8.1.2. Antipyretic activity

Pyrexia or hyperthermia is a secondary blow of malignancy, infectivity or other ill situations. (Chattopadhyay *et al.,* 2005). Body temperature (regulated by very narrow range between heat gain and heat loss and set a point at which body temperature is maintained) is controlled by the hypothalamus. Elevation of this set point causes pyrexia and various synthetic drugs like paracetamol are then used to normalize body temperature (Barkatullah *et al*., 2011). Hyperthermia due to brewer yeast intake is well known and convenient method for assessment of antipyretic potential of medicinal plants or other agents (Amole & Onbanjo, 1999). In the present study, crude ethanolic extracts of *S. laureola* leaf (SLE) was screened for its potential as on antipyretic agent (Table 4.45). Maximum observed antipyretic action of SLE was 72.31 and 52.84% at the third hour and remained significant till the fifth hour after administration of extract at the doses of 300, 200 and 100 mg/kg body weight respectively (Fig. 4.44). At low dose no promising antipyretic activity was observed. At a dose of 200 mg/kg, significan reduction in body temperature was observed from second hour to fifth hour while at a dose of 300mg/kg, fall in body temperature was observed at the first hour, after which highly significan hypothermia was recorded till the end of experiment. At higher doses, its effecacy was found to almost paracetamol. Hesperidin and coumarinolignans isolated from *Zanthoxylum* species (Rutaceae), have been reported to be antipyretic, analgesic and anti-inflammatory agent (Santos & Moreno, 2004; Liu *et al*., 2007; Chen *et al*., 2008). As *S. laureola* belongs to this family, these compounds might also be present in this plant, which were responsible for the antipyretic effect of SLE. Like the other antipyretic drugs, SLE might be inhibitory to postaglandin- biosynthesis and this may be the possible mechanism of its antipyretic action. Some other workers have also carried similar studies. Padhan (2010) evaluated alcoholic extract of *Capparis zeylanica* for possible antipyretic potential using brewer's yeast induced pyrexia in albino rats. Chomchuen *et al*. (2010) reported significant antipyretic activity of ethanolic extract of *Ficus racemosa* against brewer's yeast-induced fever in rats. Padhan *et al*. (2010) reported significant antipyretic potential of alcoholic extract of *Capparis zeylanica* in yeast induced hyperthermic rats. Similarly Barkatullah *et al*. (2011) also reported similar findings for *Zanthoxylum armatum*.

4.8.1.3. Antidiabetic activity

Diabetes mellitus is one of the leading serious metabolic disorder characterized by persistent hyperglycemic condition and conflicts in the metabolism of carbohydrate, protein and fat due to absolute or relatively deficient availability of insulin. It is one of the leading causes of mortility in developing countries (Jia *et al.,* 2004; Grover & Yadav, 2004; Anonymous, 2008). A large number of medicinal plants including *Cassia kleinii* (Babu *et al*., 2003), *Caesalpinia bonducella* (Chakrabarti *et al.,* 2005), *Ceiba pentandra* (Djomeni *et al.,* 2006), *Capparis sepiaria* (Selvamani *et al.,* 2008), *Aegle marmelos* (Arumugam *et al.,* 2009) and *Clitoria ternatea* (Kalyan *et al.,* 2011) have been reported to posses antidiabetic properties. In the present study hypoglycemic effect of SLE at three different doses (100, 200 and 300 mg/kg body weight) were evalvated in comparison with standerd drug glibenclmide in Alloxan induced hyperglycemic rabbits (Table 4.46). Alloxan (β- cytotoxin) damage β- cells of pancrease through the dismutation of hydrogen per oxide by releasing free radicals, thus creating hyperglycemic condition (Szkudelski, 2001; Raju *et al*., 2004). Alloxane caused 90 % increase in blood glucose level in alloxinised rabbit as compared to normal rabbit. This was lower than the reported one i.e. 164% in *Chamaerops humilis* leaves (Gaamoussi *et al*., 2010). The glibenclamide reduced blood glucose level from 165.25±6.5 at 0 hour to a reduction of 90.5±5.97 at 8th hour interval (94.16±0.22%). The effect of SLE was found to be dose dependent. Like the standard drug, SLE induced reduction of blood glucose level after 2 hour of dose administration. All the doses showed significant reduction ($p<0.05$) in glucose level at 6th hour post administration (Fig. 4.45). 300 mg/Kg body weight dose of SLE showed maximum reduction at 6 hour (80.54±0.04%) but contrary to the standard drug, its effect was seen decreasing after 6th hour, although it maintained the low blood glucose level till 8 hour of post dose administration. After 8 hours, the blood glucose level again increased, suggesting that regular dosing of the extracts might be obligatory after 8 hours. Glibenclamide reduces blood glucose level by suppressing hepatic gluconcogenesis; inhibit glucose absorption from intestine or stimulating the glycolysis (Murugan & Reddy, 2009). As SLE was found to have the same effect like glabinclamide, it might have some active constituents with similar mode of action, as the standard drug for glycemia reduction. The present study rationalizes the traditional use of

S.laureola leaves as antidiabetic drug, as many people use this plant as a remedy for diabetis. Our study is also well in accordance with the previous work done on hypoglycemic medicinal plants e.g. .hypoglycemic effect of *Hemidesmus indicus* in alloxane induced rats (Gayathri & Kannabira, 2008), significant decrease of blood glucose level in diabetic rabbits by *Aegle marmelos* (Arumugam *et al*., 2008) and *Citrus medica* seed as antidiabetic agent (Sah *et al*., 2011).

4.8.1.4. Antispasmodic activity

Diarrhea and other gastrointestinal disorders are the most serious issue in child health care system, as 1000 million causalities per yaer of children under the age of 5 years has been reported in developing countries (Carlos & Saniel, 1990). In spite of technology expansions, developing countries are still relying on herbal drugs for treatment of diarrheal diseases. (Ojewole, 2004; Agunu *et al*., 2005). The world health organization (WHO) has highly respected the conventional medical practices for treatment and precautionary measure of diarrheal diseases (Atta & Mouneir, 2004). For evaluation of antispasmodic/antidiarrheal potentials of plants, studies on isolated organ(s) are valuable *in-vitro* techniques, as these are cheaper and conducted in short span of time (Baker *et al*., 1995; Enna *et al*., 2002). In the Present study, ethanolic and *n*-hexane extracts of leaf (SLE, SLH), bark (SBE, SBH) and leaf essential oil (SVO) of *S. laureola* were evaluated for antispasmoidc potential on the isolated rabbit jejunum in quests to search for cheaper, safer and accessible sources of anti diarrheal drugs with scientific proofs for its ethnopharmacological use as an antispasmodic drug. All the samples were tested against spontaneous and potassium chloride induced contracted smooth muscle of the isolated rabbit jejunum (Fig. 4.46 & 4.47). For all samples, a dose dependant effects has observed in both spontaneous and potassium chloride induced contractions of the isolated jejunum preparations. To find out the possible mode of action, the tissue was pretreated with high concentration of KCl (80 mM in final bath solution) which caused depolarization and kept the tissue in a position of sustained contraction (Farre *et al,* 1991). The drugs were then applied in cumulative manner to obtain a dose dependant curve and relaxation results were expressed as percent of KCl induced contraction (Van Rossum, 1963).

The effect of SLE was dose-dependent in both spontaneous and potassium chloride induced contractions of the isolated jejunum preparations. Significant spasmolytic effect started from 0.3 mg/ml and 100% contraction was observed at 10 mg/ml. The SLH inhibited the depolarization of the smooth muscles of the jejunum, caused by high concentration of potassium chloride solution (80 mM) (Ahmad *et al*., 2009c). The antispasmodic effect observed was spasmogenic at a dose of 0.1-3 mg/ml, while at a high doses i.e 5-10 mg/ml, it coused relaxation in both spontaneous and KCl induced contration of the rabit jejunum. This aspect of SLH can further be explored. The SBE also produces spansmolytic effect in both spontaneous as well as in the potassium induces contractions, but its effect was more pronounced than leaf extracts. In case of SBH, the relaxation of smooth muscle of spontaneous and potassium induced contraction was less than the other tested samples.

Similarly the effect of SVO was also tested against spontaneous and Potassium chloride induced contracted smooth muscles of isolated rabbit jejunum. It is evident from Fig. 4.46 that a dose dependent response was produced in both the cases. In comparison to control, SVO significantly relaxed the contracted smooth muscles in both cases. The spasmolytic effect of the oils started from 0.03 mg/ml and was 100 % at the dose of 10 mg/ml. EC_{50} values, for both spontaneous and KCl induced contractions for all samples of *S. laureola* are given in Table 4.47, which shows that SVO was the most efficient, causing 100 % relaxation of the smooth muscles at a very low concentration.

The contraction of smooth muscle of rabbit jejunum is due to increased concentration of the free calcium in cytoplasm, which stimulates the chemical mediators, responsible for the contraction. This increase in calcium level may be either due to influx via voltage dependent calcium channels or direct release of calcium from endoplasmic reticulum (calcium store). Thus a periodic depolarization is created due to high speed action potential. When there is increase potassium concentration, the contraction of the smooth muscle will increase due to rapid action potential. When the calcium channel is blocked through calcium channel blocker agent, the contracted smooth muscle will relax (Ali & Shah, 2011). In our results the extracts relaxed the contracted muscle, therefore it is suggested that the possible mode of action of this plant is either blocking the release of

stored calcium from the sarcoplamic reticulum or blocking the calcium channel. Ahmad *et al*. (2009c) carried out antispasmodic activities on isolated rabbit jejunum testing the crude methanolic extract of *Tylophora hirsuta* and proved Ca channel blockage activity. The methanolic- aqueous extract of the aerial part of *Z. armatum* has been tested for their muscles relaxation in gut, air passage way and in cardiovascular system (Gillani *et al*., 2010). Azam *et al*. (2011) evaluated antispasmodic action of the crude methanol extract of the aerial parts (leaves and stem) of *Myrsine africana* on spontaneous rabbit's jejunum preparations at different concentrations. These results of these workers strengthened the present findings.

4.8.1.5. Cytotoxicity

Plants secondary metabolites are tested to explore new medicinal drugs which have potentials for curing and healing various health problems including cancer (Amara *et al.,* 2008; Barkatullah *et al*., 2011). Brine Shrimp lethality test is a preliminary exploration for the detection and development of such anti-cancer drugs. Tests were carried out to investigate preliminary cytotoxic potential of the crude ethanolic and n-hexane extract of leaves, bark and leaf essential oil of *S. laureola* were testify to evaluate them for cytotoxicity. Significant dose dependant cytoxicity was observed for all the samples used (Table 4.48). The SLE, SLH and SVO showed outstanding cytotoxic results with LD_{50} values of 5.34, 7.44 and 11.01 µg/ml respectively. Significant mortality was found at 10µg/ml while highly significant results were observed at 100 and 1000 µg/ml dose of the three samples. SBE and SBH were found not as effective as SLE, SLH and SVO, as they have high LD_{50} values i.e 180 and 115 respectively. Similar work on other plants has been reported including workers *Hibiscus sabdariffa (*Tolulop, 2007), *Thymus serpyllum* (Ateeq-ur-Rehman *et al.,* 2009) and *Polygonatum verticillatum* (Saeed *et al*., 2009). The present study suggested that some active constituents might be present in this plant that has cytotoxic potential. It is recommended that to carryout further detailed investigations for identification and quantification of specific cytotoxic compounds in *S. laureola*.

4.8.1.6. Phytotoxicity

Pakistan like other developing countries is facing problem of weed growth with the cereal plants, due to which there is great loss to crops both qualitatively and quantitatively. The extent of weed damage was found to be more shocking as compared to pests and pathogens, but its effects usually remains unnoticed. Weeds reduce productivity, because of competition for the available natural resources such as sunlight, water and minerals etc. Also weeds might provide habitat for insects which damage the crops by eating them or spreading diseases. Weeds controls through synthetic drugs has caused various human health problems and soil water pollution (Barkatullah *et al*., 2011). So weeds control through harmless means is indispensable, to increase yield of various crops and to protect environments. Phytotoxicity screening of crude ethanolic and n-hexane extract of leaf, bark and leaf essential oil of *S. laureola* were carried out using *Lemna minor* as test species. A significant dose dependant phyto inhibition was observed for all the tested samples (Table 4.49). SBE showed 100% inhibition at 1000 µg/ml followed by SBH (94.23±1.23), SLE (90.38±1.23) and SLH (88.46±2.77) having FI_{50} of 25, 1.38, 4.54 and 8.67 µg/ml respectively. Essential oil of the leaves was also found to be phytotoxic FI_{50} with 46.09µg/ml, but was less effective than the other extracts. These results indicate that *S. laurola* has great potentials as herbicides or weedicides. Further study is needed to explore its phytotoxic mechanism and also to identify and quantify the phytotoxic constituents. It might be helpful to investigate its efficacy in detail as a weeds, pests and disease control agent. Some previous workers like Zaidi *et al*., 2008 (*Arceuthobium oxycedri*), Onocha & Ali, 2010 (*Phyllanthus muellerianus*) and Ahmad *et al*., 2011 (*Zizyphus jujube*) also carried out phytotoxic studies using *Lemna minor* as test species and have reported similar results,as obtained in the present work on *S. laureola*.

4.8.1.7. Antibacterial activity

Because of the adverse side effects of antibiotics and development of resistance by pathogens to antibiotics, scientists are trying to explore new safe sources of effective antimicrobial drugs. For this purpose plants are being screened out and proved to be a potential source of novel compounds of known advantageous features (Maurer-Grimes *et al*., 1996; Rabe and van Staden, 1997; Marchese and Shito, 2001). The present study was also carried out, to explore such antimicrobial agents. All the samples tested in the

present study were found with inhibitory effect against various gram positive and gram negative bacterial strains. The ethanolic and n-hexane extracts of leaf, bark and leaf essential oil of *S. laureola* were tested for their antibacterial potential against various bacterial strains (*Micrococcus leutus, Escherichia coli, Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Bacillus subtilis,* and *Streptococcus viridines*) (Table 4.50). The results show that different bacterial strains have variable dose dependant susceptibility to the extracts used. Ciprofloxacin was used as standard drug and the antibacterial potentials of the tested samples were compared. The SLE extract was found to be effective against all the bacterial strains except *S. aureus*. Highest inhibition was observed against *M. leutus* (20.33±0.33 mm) followed by *P. multocida* (18.33±0.58 mm) and *P. aeruginosa* (16.00±0.77mm). Growth of *E.coli*, *B. subtilis* and *S. viridans* was also inhabited to lesser extent than the other species. SLH was also found to be inhibitory like SLE, but *P. aeruginosa* was found resistant to it. The SBE extract showed highly significant efficacy against five strains including *E. coli* (22.33±0.33mm), *M. leutus* (17.67±0.33 mm), *P. multocida* (17.67±0.58mm), *P. multocida* (17.67±0.58mm) and *B. subtilis* (13.67±0.33). Interestingly *E. coli* was found to be the most susceptible strain to SBE as compared to the other suseptible *M. leutus*. SBH extract was found inhibitory against five of seven test species. Maximum Zone of inhibition was found for *M. leutus* (18.33±0.33) followed by *P. multocida* (17.67±0.58 mm), *B. subtilis* (17.67±0.58 mm). Other species were also affected but less than these three. *S. aureus* and *P. aeruginosa* were the resistant species to SBH. From the above results it is evident that *S. aureus* and *P. aeruginosa* were the resistant species as compared to other strains. Antibacterial activities of the other plants have also been reported. Abo & Olugbuyiro (2004) reported *S. aureus, P. aerugniosa* and *E. coli* were found susceptible to the leaf extract of *Flabelleria paniculata*. Similarly different extracts of leaves of *Solanum tomentosum* were found inhibitory to the growth of *S. aureus* and *E. coli* (Aliero & Afolayan, 2006). Kumaraswamy *et al*., (2008) found *Betula utilis* as a useful antibacterial agent against some Human Pathogenic Bacteria. Aqueous and ethanol extracts of *Ocimum gratissimum and Piper guineense* leaves were found successful inhibitory against *E. coli* and *S. aureus* (Nwinyi *et al.,* 2009). The present study, like other workers might be helpful in exploring potential sources of safe antibiotics.

Oils from plants have been used extensively for therapeutic purposes against various diseases including strong antibacterial and antifungal infections (Cleff *et al*., 2010). In the present study, SVO was also evaluated for antibacterial potential and was found effective against all the tested bacterial strains. *M*. *leutus* and *S*. *viridans* were found more susceptible as compared to other test species (Table 4.50). Prabuseenivasan *et al*. (2006) reported that essential oils from Cinnamon, Clove, Geranium, Lemon, Lime, Orange and Rosemary oils exhibited significant inhibitory effect on gram-negative bacteria **(***E. coli*, *P. aeruginosa* and *P. vulgaris***)** and gram-positive bacteria *B*. *subtilis* and *Staphylococcus aureus*. Similarly *Citrus* peel oil is reported to have with strong antimicrobial potential against various pathogenic bacterial strains (Kirbaslar *et al*., 2009). The present study suggests that this oil has strong antibacterial potential.

The Mininmum inhibitory concentration (MIC) for the tested samples ranged from 0.65- 5 mg/ml (Table 4.51). It was observed in most of the cases that all the samples were highly effective even in low concentrations 0.65 mg/ml or 1.25 mg/ml against most of the bacterial strains. SVO was appeared to be the most powerful antibacterial agent, as it was efficient in growth inhibition of all bacterial strains. The MIC values ranged from 0.65- 2.5 mg/ml with the lowest values for *M.leutus* and *S.viridians* respectively. The highest MIC value was observed for *B*. *subtilis* (2.5 mg/ml). Results shows that *M*. *leutus* was found most susceptible species with low MIC values as in most cases while *B*. *subtilis* and *S*. *viridans* were found most resistant bacterial strains with comparatively high MIC values. These results were very promising especially that of SVO, which has a very low MIC. MIC values for most of the pathogens in the present study were also very low, thus providing that this plant has strong antibacterial potentials. MIC values found in the present study were almost in the same range as reported for essential oil of *Euclyptus globules* (Damjanovic-Vratnica, 2011).

Excessive bacteria growth of bacteria in teeth is the potent source of teeth decay and gingival diseases e.g. Halitosis (Bad breath) (Yaegak & Coil, 1999). Bad breath and bad body smell were due to skin infections, caused by *M. luteus* and *B.subtilis*. Similarly *E*. *coli* and *P*. *aeruginosa* are the pathogens responsible for urinary tract infections, as their accumulation damages tissue in the urinary tract (Debnath *et al*., 2011). The present

study also suggests that *S.laureola* can be helpful in resolving the above and many more bacterial infections.

4.8.1.8. Antifungal avtivity

Herbal drugs have no or very less side effects, therefore considered safe and best alternative of antibiotics (Sofowora, 1993). Now a days, scientist have much tendency for exploring folk medicine as affective remedy for microbial infections (Srinivasan *et al.,*2001). Plants have been found to be a potential source of active constituents, having the capabilities of inhabiting human fungal pathogenes (Khan *et al.,* 2004). Ethanolic and n-hexane extracts of leaf, stem bark and leaf essential oil of *S. laureola* were evaluated for antimycotic potential against some fungal strains like *Trichophyton longifusis, Candida albicans, Fusarium solani, Microsporum canis, Aspergillus flavus* and *Candida glabrata,* the results are shown in Table 4.52, which show a dose dependent activity. SLE was found effective against *A. flavus* (70.97±0.58%) followed by *C. albicans* (58.82±0.58 %), and *M. canis* (53.85±0.33%) at a 500 ug/ml dose. *F. solani* was observed to be resistant to SLE at lower concentration, but showed some inhibition at higher concentration. SLH showed inhibitiory effects against three fugal strains at lower (125 ug/ml) concentration. *C. albicans, F.solani* and *A. flavus* showed resistant to SLH at lower concentration, but were suppressed at higher concentration. SBE and SBH were also found to be inhibitrory at higher dose only, but were effective as compared to the two leaf extracts (Table 4.52). Similar antifungal studies have been carried out by Khan *et al*. (2004) for *Tamarix dioica* extract, Khan & Khan (2007) for *Rhazya stricta* and Segismundo *et al*. (2010) for the leaf extracts of *Gouania javanica*. All these studies suggested that plants including *S. laureola* has strong antimycotic potentials.

SVO was also evaluated for antifungal activity (Table 4.52). All the tested fungal strains were inhibited by SVO. Concentration dependent effect was observed against all fungi. Maximum inhibitory zone was observed for *C. albicans* (67.32± 0.90%) followed by *A. flauus* (64.45± 1.98%) and *T. longifusis* (62.66± 1.34%) at a dose of 125µg/ml. Other fungal strains were also affected at a dose of 125µg/ml. Essential oils can be used as curing agent for mycotic infections (Bansod & Rai, 2008). The present antifungal study of SVO were found in agreement with the previous workers likes

Sridhar *et al*. (2003), who studied essential oils from *Cymbopogan spp* and Essien *et al*. (2008), who reported that essential oil of *Citrus medica* to have antimycotic potentials and suggest that SVO can be effectively formulated in various dermal/skin preparation.

4.8.2. Biological activities of *Zanthoxylum armatum*

Z. armatum was screened out for the following activites

4.8.2.1. Acute toxicity study

Acute toxicity study is a technique of utmost importance, through which medicinal plants and crude drugs are evaluated for their potential adverse effects and for safe consumption against various health problems. Although several different new techniques have been developed the test animals (mice) were carried out to evaluate for more modern approach to toxicology (Combe *et al*., 2004), but the use of animals in safety and other toxicological evaluations in acute systemic toxicity testing is still in practice (Anonymous, 2000). In the present study, ethanolic extracts of leaf and fruit (ZLE & ZFE) of *Z. armatum* at a dose of 500, 1000 and 2000 mg/kg body weight against (mice) were carried out to evaluate acute toxicity of this plant. No mortality or any other abnormality was observed in the test animals even after 24 hours, showing that the plant is safe for human use (Table 4.53). Most of the herbs and crude drugs are safe and free of acute toxicity. Other workers have also reported absence of acute toxicity in their studies on medicinal plants Magaji *et al*. (2007) worked out toxicity study for the leaf and stem bark of *Securinega virosa* and found to be safe for consumption. Mowla *et al*. (2009) reported no acute toxicity for ethanol extract of *Trigonella foenum-graecum* seeds at a dose up to 3 g/kg body weight. Similarly Udem & Asogwa (2010) reported that no death was recorded for leaf aqueous extract of *Ipomoea batatas* even at the highest dose of 1,600 mg/kg. The present study also showed similar results as regarding acute toxicity of *Z. armatum*.

4.8.2.2. Antipyretic activity

Causes and mechanism of pyrexia has been discussed in the beginning of Section 4.8.1.2. In the present study, ethanolic extracts of leaf (ZLE) and fruit (ZFE) were screened out for possible antipyretic potential (Table 4.54). Both the parts showed a dose dependent antipyretic action. ZLE also showed a percent inhibition of 85.42, 77.19 and

46.18 at the dose of 300, 200 and 100 mg/kg, respectively. The maximum antipyretic action was observed at the 3rd hour and remained significant up to 5 hours (Fig. 4.48). Maximum antipyretic action of ZFE was 83.84, 80.70 and 44.18% observed at the third hour and maintained significant till the fifth hour after administration of 300, 200 and 100 mg/kg body weight doses respectively (Fig. 4.49) A compound Hesperidin, isolated from one *Zanthoxylum* species, has been reported as an antipyretic, analgesic and anti-inflammatory agent (Santos & Moreno, 2004). Similarly coumarinolignans present in *Z. avicennae* was also reported to have anti-inflammatory potential (Liu *et al*., 2007; Chen *et al*., 2008). The present study was carried out with the same genus, so these compounds might be present in this plant too and was responsible for the antipyretic effect. ZLE and ZFE might have affected prostaglandin biosynthesis, which has a thermo-regulatory effect (Milton, 1982). The inhibition of prostaglandin synthesis by these extracts could be the possible mechanism of antipyretic action. The present study is also well lineage in with some previous workers. Padhan (2010) evaluated the crude alcoholic extract of *Capparis zeylanica* for antipyretic activity using brewer's yeast induced pyrexia in Wister strain albino rats. Chomchuen *et al*. (2010) investigated significant antipyretic activity of the ethanolic extract of *Ficus racemosa* against brewer's yeast-induced fever in rats. Similarly Padhan *et al*. (2010) studied antipyretic activity of alcoholic extract of *Capparis zeylanica* and showed a significant ($P<0.01$) dose dependent antipyretic effect in yeast induced hyperthermia in experimental rats. Therefore these studies support the present findings.

4.8.2.3. Antispasmodic activity

Developing countries have been facing morbidity and mortality of childhood due to diarrhea and other gastrointestinal problems. An estimated frequently 10 million deaths per year in children under age of 5 has been reported (Carlos & Saniel, 1990). In these countries people are still rely on the herbal drugs for control of diarrhoea despite of enormous development of all antidiarrhoeal medicines in the world (Ojewole, 2004; Agunu *et al*., 2005). The world health organization (WHO) highly appreciated the conventional medical practices for treatment and precautionary measure of diarrhoeal diseases (Atta & Mouneir, 2004). Studies on isolated organ(s) are valuable means to evaluate such pharmacological activity of a potential drug. These *in vitro* techniques are

cheaper and conducted in short span of time (Baker *et al*., 1995; Enna *et al.,* 2002). In the Present study, ethanolic and *n*-hexane extracts of leaves (ZLE, ZLH), bark (ZBE, ZBH) and fruits (ZFE, ZFH) and essential oil of leaf (ZVO) of *Z. armatum* were evaluated on the isolated rabbit jejunum for possible antidiarrheal effect, which may be a cheaper and accessible source for treatment of diarrhea and will also provide a scientific proof for its ethnopharmacological use as an antispasmodic drug. All the samples were tested against spontaneous and potassium chloride induced contracted smooth muscle of the isolated rabbit jejunum. The results of this all bioassays are presented in Fig. 4.50 & 4.51. All samples produced antidiarrheal effect in a dose dependent manner. To determine the possible mode of action, the tissue was pre treated with high concentration of KCl (80 mM in final bath solution). KCl cause depolarization and keep the tissue in a position of sustained contraction (Farre *et al*., 1991). The test samples were then applied in cumulative manner to obtain a dose dependant curve and relaxation results were expressed as % of KCl induced contraction (Van Rossum, 1963).

The effect of ZLE was dose dependent in both spontaneous and potassium chloride induced contractions. Significance antispasmodic effect started from 0.3 mg/ml and maximum contraction was observed at 10 mg/ml. The ZLH inhibited the depolarization of the smooth muscles of the jejunum, caused by high concentration of potassium chloride solution (80 mM) (Ahmad *et al*., 2009c). The relaxant effect of the ZLH seems to be spasmolytic in the spontaneous tissue treatment. The ZBE produced spansmolytic effect in both spontaneous as well as in the KCl induced contraction. Significant effects were seen from 3 -10 mg/ml concentration. In case of ZBH, the relaxation of smooth muscle of potassium induced contraction was more than the spontaneous. The antispasmodic effect was observed at concentration of 3 mg/ml, while the most significant effect was observed at 10 mg/ml. The ZFE produced strange effect in both the cases (spontaneous and in potassium induced contraction). This extract was just like control up to 1 mg/ml and at higher concentrations, a good right ward shift was produced as shown in Fig. 4.50. The antispasmodic effect was found maximum at highest concentration. To conform the spasmolytic effect of ZFE was due to possible calcium channel blockade, another series of experiments were performed, in which a pre treated atropine isolated tissues was treated with KCl (80 mM) that opens the voltage operated

calcium channels, releasing extra-cellular calcium into the cytosol creating depolarization of the tissue (Gilani *et al*., 2005). ZFE was found to relax the KCl depolarized tissue and was found to be calcium channel blocking activity at a dose range of 0.1 -5.0 mg/ml (Fig. 21) (Ahmad *et al*., 2009c).

The n-hexane extract of the fruits produced 100% spansmolytic effect at 1 mg/ml in spontaneous treatment while in case of potassium induced contraction a gradual right ward shift was observed. ZVO was also tested against spontaneous and Potassium chloride induced contracted smooth muscle of isolated rabbit jejunum. It is evident from Fig. 4.50, that a dose dependent response was produced in the both cases. As compared to control, ZVO significantly relaxed the contracted smooth muscles in both the cases. The spasmolytic effect of the oils started from 0.03 mg/ml and showed 100 % effect at 10 mg/ml dose. EC_{50} values for both spontaneous and KCl induced contraction for all samples of *Z. armatum* are given in Table 56, which show that of all the samples, ZFH was the most efficient, causing 100 % relaxation of the smooth muscles.

The contraction of smooth muscle of rabbit jejunum is due to increase concentration of the free calcium in cytoplasm, which stimulates the chemical mediators which are responsible for contraction. This increase in calcium level may be either due to influx via voltage dependent calcium channels or direct release of calcium from endoplasmic reticulum (calcium store). Thus a periodic depolarization is created due to high speed action potential. When there is increase potassium concentration, the contraction of the smooth muscle will increase due to rapid action potential. When the calcium channel is blocked through calcium channel blocker agents, the contracted smooth muscle will relax (Ali & Shah, 2011). In the present study, the extracts relaxed the contracted muscle, suggesting that the possible mode of action of this plant is either blocking the release of stored calcium from the sarcoplamic reticulum or blocking the calcium channel. Similar studies have also carried out by other workers. Ahmad *et al*., (2009c) carried out antispasmodic activities on isolated rabbit jejunum testing the crude methanolic extract of *Tylophora hirsuta*. The methanolic- aqueous extract of the aerial part of *Z.armatum* has been tested for muscles relaxation effect in gut, air passage way and in cardiovascular system (Gilani, 2010). Azam *et al*. (2011) evaluated antispasmodic

action of the crude methanol extract of the aerial parts (leaves and stem) of *Myrsine africana* on spontaneous rabbit's jejunum preparations at different concentrations. The present study is also well in lineage with these workers.

4.8.2.4. Cytotoxicity

Biogenic compounds from plants are used to discover new medicinal drugs which have an affective role in protection and treatment against various ailments including cancer (Amara *et al*., 2008). Brine Shrimp Toxicity bioassay is preliminary study for the detection and development of anti cancer drugs. Brine shrimp lethality tests were carried out to investigate preliminary cytotoxic potential of the crude ethanolic and n-hexane extract of leaf, bark, fruit and leaf essential oil of *Z. armatum*. All the samples showed dose dependant cytoxicity was observed (Table 4.57). The ZBE, ZBH and ZVO showed outstanding mortality rate (100 %) at a dose of 1000µg/ml with 16.79, 17.06 and 15.90 LC_{50} values respectively. ZLE (LC_{50} = 20.00) and ZLH (LC_{50} = 19.04) also have a significant mortility rate at 100 and 1000 µg/ml. ZFE and ZFH also showed significan mortility rate with comparatively large LC_{50} values. The present study showed that all samples of *Z. armatum* have much lesser LD_{50}, suggesting that some active constituents might be present in this valuable plant. Other workers like Tolulop, 2007 (*Hibiscus sabdariffa*) and Ateeq-ur-Rehman *et al*., 2009 (*Thymus serpyllum*) also carried out Brine shrimp lethality test. It is needed to carry further detailed investigations for identification and quantification of pharmacologically bioactive specific constituents from *Z. armatum*.

4.8.2.5. Phytotoxicity

In all countries including Pakistan, there is a great reduction in crop yield due to weeds. The extent of losses caused by weeds was found to be more as compared to insects and other diseases but their effects are usually ignored (Barkatullah *et al*., 2011). They reduce crops yield as a result of competition for various available natural resources such as sunlight, water and minerals. Weeds provide habitat for insects which damage the crops by spreading diseases. A weeds control through synthetic herbicides causes various environmental and human health problems. So weeds control through harmless means has become indispensable to increase yield of various crops. Some of the previous workers like Zaidi *et al*., 2008 (*Arceuthobium oxycedri*), Onocha & Ali, 2010

(*Phyllanthus muellerianus*) and Ahmad *et al.,* 2011 (*Zizyphus jujube*) also carried out phytotoxic studies using *Lemna minor* as test species. They showed that these plants inhibited the growth of test species, showing some phytotoxic potential. In this regard phytotoxic screening of crude ethanolic and n-hexane extract of leaf, bark, fruit and leaf essential oil of *Z. armatum* were carried out using *Lamna minor* as test species. All the parts showed significant dose dependant phyto inhibition (Table 58). The ZFH showed 100% inhibition at 1000 µg/ml which is an outstanding action having FI_{50} of 3.69 µg/ml. The ZBE, ZFE and ZLH also showed excellent percent inhibition i.e. 94.86±2.22, 94.15±2.28, 90.99±1.36 with FI_{50} values of 7.98, 9.24 and 19.13 µg/ml respectively. These results suggested that all parts have some active principles with phytotoxic potential and *Z. armatum* can be a good herbicides or weedicides. Further study is needed to exploit its phytotoxic mechanism and also to identify and quantify the bioactive constituents. It might be helpful to investigate its efficacy in detail as a weeds, pests and disease control agent.

4.8.2.6. Antibacterial activity

Adverse side effects of antibiotics have been reported causing some uncommon serious infections, which forced scientists to explore new sources of potent antimicrobial drugs. For this purpose plants have been screened out for safe antimicrobial compound that proved to be a potential source of novel compounds of known beneficial features (Maurer-Grimes *et al.,* 1996; Rabe & Van Staden, 1997; Marchese & Shito, 2001). Present study was also carried out for investigation of *Z. armatum* for such antimicrobial agents. Ethanolic and n-hexane extracts of leaf, bark, fruits and leaf essential oil of *Z. armatum* were tested against various gram positive and gram negative bacteria i.e. *Micrococcus leutus, Escherichia coli, Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Bacillus subtilis,* and *Streptococcus viridines* as shown in Table 4.59. The antibacterial actions of the extracts were compared with ciprofloxacin as standard drug. It was observed that all the extract have inhibitory effect against various gram positive and gram negative bacterial strains but the antibacterial potential was greatly varied among the extracts. ZLE extract was found to be active against four out of seven tested bacterial strains, showing highest inhibition against *M. leutus* (18.00±0.71 mm), *E. coli* (17.00±0.57mm), *P. multocida* (18.00±0.71 mm) and *B. subtilis*

(15.33±0.81mm). ZLH was also inhibitory to the same four bacterial strains but was less effective as compared ZLE. Result showed that both ZLE and ZLH were ineffective against *S. aureus, P. aeruginosa* and *S. viridines*. ZFE extract showed highly significant efficacy against five out of seven strains including *M. leutus, S. aureus, P. multocida, P. aeruginosa* and *B. subtilis*, among which the first two were found most susceptible. *E. coli* and *S. viridines* were found resisitant to ZFE. *S. aureus* was the most resistant strain against most of the samples tested in the present study, but was inhabited by ZFE having 17.33±0.33 mm zone of inhibition. ZFH extract was found inhibitory against all the test species except *S. aureus*. Maximum Zone of inhibition was found for *M. leutus* (19.67±0.33mm), *E. coli* (17.00 ±0.58mm) and *P. multocida* (17.67±0.33mm). The other species were also affected but to lesser extent than these three strains. Although ZBE was found inhibitory to all test species except *S. aureus*, but its effect was not so pronounced as compared to other samples tested. ZBH was most active against *M. leutus* (20.33±0.41 mm) as compared to other test species. From the above results it is evident that *M. leutus, P.multocida* and *B. subtilis* were most sensitive strains, inhibited by all the samples. Other bacterial strains (*P.aeruginosa*, *S. aureus* and *S. viridians*) also showed inhibition to different extract. Other workers have also conducted similar antibacterial studies e.g Abo & Olugbuyiro (2004) reported that leaf extract of *Flabelleria paniculata* was inhibitory against *S. aureus, P. aeruginosa* and *E. coli*. Aliero & Afolayan, (2006) reported various extracts of leaves of *Solanum tomentosum* inhibited the growth of *S. aureus and E. coli*. Similarly Kumaraswamy *et al*. (2008) proved *Betula utilis* as useful antibacterial agent against Some Human Pathogenic Bacteria. Nwinyi *et al*. (2009) screened out successfully aqueous and ethanol extracts of *Ocimum gratissimum and Piper guineense* leaves for antibacterial activity against *E. coli* and *S. aureus*. The present study on *Z.armatum* has also showed very encouraging antibacterial potential.

Volatile oils have been extensively used for therapeutic purposes and are found strong antibacterial and antifungal agents (Cleff *et al*., 2010). Antibacterial potential of ZVO was evaluated in the present study and it was found effective against all tested bacterial strains. *B. subtilis,* and *S. viridans* were found more susceptible as compared to other strain tested. The maximum effect was observed against *M. leutus* followed by *Streptococcus viridans* (17.67±0.58 mm) and *B. subtilis* (15.83±0.41mm). *S. viridans* and

S. aureus which were found resistant against the other samples tested, affected significantly by ZVO. Essential oils from other plats like Cinnamon, Clove, Geranium, Lemon, Lime, Orange and Rosemary oils were found inhibitory to gram-negative bacteria (*E. coli*, *P. aeruginosa*, *P. vulgaris*) and gram-positive bacteria (*B. subtilis* and *S.aureus*) (Prabuseenivasan *et al.,* 2006). C*itrus* peel oils have strong antimicrobial activity against various bacterial strains (Kirbaslar *et al.,* 2009). Our study is well lineage with them.

Minimum inhibitory concentration (MIC) values were also determined for the test samples, which ranged from 0.65- 5µg/ml (Table 60). It is evident that ZLE, ZFE and ZBH were highly effective even at low concentrations against most of the bacterial strains having MIC value of 0.65µg/ml or 1.25µg/ml. ZBE was found less effective with MIC value of 5 µg/ml. ZVO appeared to be the most potent antibacterial agent, as it was effective in inhabiting growth of all bacterial strains with very low MIC values ranging from 0.65- 1.25 µg/ml with the lowest value for *M. leutus* and *S. viridians*. Over all results showed that *M. leutus* was the most susceptible species, as in most of the cases it has a low MIC value while *B. subtilis* was the most resistant bacterial strain with highest MIC values. MIC values for most of the samples in the present study were very encouraging and further research will enhance opportunity for exploiting this important plant as a strong antibacterial agent, especially the volatile oil may become an effective drugs especially for treating halitosis (bad breath) caused by excessive growth of bacteria (Yaegak & Coil, 1999), urinary tract infection caused by *E. coli* and *P. aeruginosai* and skin infection caused by *M. luteus* and *B.subtilis* (Debnath *et al*., 2011). These results are also found very promising as these extract especially the essential oil were found efficient in quite lower concentration. MIC values found in the present study was almost in the same range as reported for Essential oil from *Euclyptus globules* (Damjanovic-Vratnica, 2011).

4.8.2.7. Antifungal

Herbal drugs are now considered as the best alternative of antibiotics with no or very less side effects (Sofowora, 1993). Researchers are therefore increasingly turning their attention to folk medicine, looking for better drugs against microbial infections

(Srinivasan *et al*., 2001). Various types of fungal pathogens have been reported to cause severe type of diseases in human beings. Plants have been found to be a potential source of active constituents for curing such fungal infections. (Khan *et al*., 2004). In the present study ethanolic and n-hexane extracts of *Z. armatum* leaf, stem bark, fruit and leaf essential oil from leaves were evaluated for antimycotic potential against various fungal strains like *Trichophyton longifusis, Candida albicans, Fusarium solani, Microsporum canis, Aspergillus flavus* and *Candida glabrata*as. The results are shown in table 61. A general trend of dose dependency was observed i.e. effect became more pronounced with increasing concentration of the various tested samples. ZLE was found effective against *F. solani* (35.33±0.41 %) followed by *C. albicans* (334.33±0.43 %) and *A. flavus* (32.44±0.33 %) at dose of 125ug/ml. *T. longifusis* was observed to be resistant to ZLE at lower concentration. ZLH was active against *C. albicans* (26.98±0.33 %) followed by *T. longifusis* (20.93±0.41 %) but showed week effect against remaining fungal strains at a concentration of 125µg/ml. *F. solani* (66.67±0.33 %), *C. albicans* (56.33±0.71 %) and *M. canis* (56.33±0.71%) were sensitive to ZBE at 125µg/ml dose as shown in Table 3. ZBH showed weak activity against all fungal species however with increasing dose the inhibition was found significant. ZFE was active against *A. flavus* (44.29±0.66%) and *T.longifusis* (35.5±0.57%) while ZFH was found weak at 125µg/ml and effective at higher dose. Other workers have also carried out similar antifungal investigation on plants. *Tamarix dioica* (Khan *et al*., 2004), *Rhazya stricta* (Khan & Khan, 2007) and *Gouania javanica* (Segismundo, *et al*., 2008) were found inhibitory to more or less the same fungal strains as tested in the present study. All these studies suggesting that plants including Z. *armatum* has strong antimycotic potentials.

Antifungal activity of ZVO as a percent inhibition of mycelia growth showed a concentration dependent effect against all the fungi. Best anti fungal effect was observed against *C. albicans* (66.67±0.57) followed by *A. flauus* (55.33±0.57) and *F. solani* (46.33±0.33). Other fungal strains were also affected at 125µg/ml concentration. Over all results showed that all fungal strains were inhibited by the ZVO. Essential oils from other plants like *Cymbopogan* spp (Sridhar *et al*., 2003) and *Citrus medica* (Essien *et al*., 2008) were found inhibitory to pathogenic fungal strains. Plants oils including essential oil from *Z. armatum* might be a rich source of antifungal preparation.

Table 4.44. Acute toxicity test of *Skimmia laureola* leaf in mice, monitored for 24 h.

Group	Dose(mg/Kg)	Dead	Survived	Gross effect
Saline	10	-	All	-
SLE	500	-	All	-
	1000	-	All	-
	2000	-	All	-

Table 4.45. Antipyretic effect of ethanolic extract of *Skimmia laureola* leaf (100, 200 and 300 mg/kg i.p.) and pracetamol (150 mg/kg).

Treatment	Dose mg/kg	Initial rectal temperature (C^o)		Rectal temperature (C^o) after administration of drug				
		Normal (A)	After 24 h (B)	1 h (C1)	2 h (C2)	3 h (C3)	4 h (C4)	5 h (C5)
Normal saline	10 mL	36.66±0.21	38.93±0.23	38.81±0.12	38.88±0.13	38.88 ± 0.22	38. 78 ± 0.18	38.75 ± 0.25
Paracetamol	150	37.10± 0.08	38.8± 0.04	37.51± 0.01	37.46± 0.03	37.32± 0.02	37.35± 0.28	37.45± 0.04
SLE	100	37.10± 0.17	38.37±.0.06	38.10 ± 0.44	38.13± 0.12	38.00±0.10	38.00±0.10	38.00±0.10
	200	36.87± 0.31	38.63± 0.05	38.07± 0.38	37.83*± 0.15	37.70* ± 0.17	37.73*± 0.15	37.8* ± 0.17
	300	37.17± 0.31	38.47± 0.21	37.98* ± 0.44	37.93*± 0.23	37.53**± 0.35	37.60**± 0.10	37.63**± 0.15

Values are reported as mean ± SEM for group of six animals. The data was analyzed by one way ANOVA followed by Dunnett's test. *Significant at P < 0.05, **P<0.01. ** Highly significant at P<0.01.

Table 4.46. Hypoglycemic effect of ethanol extract of *Skimmia laureola* leaf on blood glucose level of alloxan-induced diabetic rabbits.

	Normal	After 7 days(0 h)	2 h	4 h	6 h	8 h	12 h
Treatment		Mean blood glucose level mg/dL ± SEM					
Non treated (Control)	85±0.02	87±0.12	87±0.14	8.7±0.02	85.75±20.14	86±0.03	85.25±0.11
Treated control (Glibenclamide)	86±0.10	165.25±0.0	141.5±0.04**	107.75±0.05**	97.5±0.02**	90.5±0.00**	120.25±0.00**
SLE 100 mg/kg	87.14±1.28	162±3.36	148.25±6.94*	126.25±9.14**	103.75±4.57**	129±4.96*	151.5±6.24
SLE 200 mg/kg	86.75±1.02	160.75±6.94	147±3.55*	121±2.16**	97.25**±4.2	120.75± 4.78**	138.5±6.45
SLE 400 mg/kg	83±2.33	161.5±3.69	145.75±2.87*	116.25±3.59**	95.25±5.6**	111.25±2.87**	128.5±3.31*

Values are reported as mean ± SEM for group of five animals. The data was analyzed by one way ANOVA followed by Dunnett's test. * Significant at $P < 0.05$, ** $P<0.01$. ** Highly significant at $P<0.01$.

Table 4.47. EC_{50} (half maximal effective concentration values) of ethanolic and n-hexane extracts of leaf, bark and leaf essential oil of *Skimmia laureola.*

Part used	Extract	EC_{50} value for spontaneous Rabbit jejunum	EC_{50} value for KCl induced contraction
Leaves	SLE	1.00	2.54
	SLH	10.00	8.12
	SVO	0.447	0.061
Bark	SBE	2.60	4.60
	SB H	8.00	8.60

Table 4.48. Cytotoxicity of ethanolic and n-hexane extracts of leaf, bark and leaf essential oil of *Skimmia laureola*.

Name	No of shrimps	Percent mortality			LD_{50}
		10 ppm	100 ppm	1000 ppm	
Control	30	-	-	-	-
SBE	30	26.43± 1.89	31.36±2.44	55.02± 2.02	180
SBH	30	22.78± 0.86	48.99±1.11	70.12± 0.96	115
SLE	30	66.33± 0.68	93.17±1.23	96.67± 1.67	5.34
SLH	30	70.27± 1.22	92.17±1.30	95.23± 1.23	7.44
SVO	30	73.33±5.77	76.67±5.77	100.00±0.00	11.01

Table 4.49. Phytotoxicity of ethanolic and n-hexane extracts of leaf , bark and of leaf essential oil of S*kimmia laureola*.

Name	Percent growth inhibition			FI_{50}
	10µg/ml	100µg/ml	1000 µg/ml	
Control (Atrazine 100 µg/ml)	87.82±1.11	87.82±1.11	87.82±1.11	8.87
SBE	42.30±0.87	55.76±2.84	100±0.00	25.00
SBH	65.38±	57.69±1.34	94.23±1.23	1.38
SLE	55.76±	67.30±2.18	90.38±1.23	4.54
SLH	50.00±	63.46±2.38	88.46±2.77	8.67
SVO	42.23±2.18	59.40±4.55	66.55±1.07	46.09

Table 4.50. Antibacterial activities of the ethanolic and n-hexane extracts of leaf, bark and of leaf essential oil of *Skimmia laureola*. All values are mean ± SEM of three determinations.

Treatment	*Micrococcus leutus*	*Escherichia coli*	*Staphylococcus aureus*	*Pasteurella multocida*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Streptococcus viridans*
	Zone of inhibition (mm)						
DMSO (Neg. cont.)	-	-	-	-	-	-	-
Cifrofloxacin (Positive cont.)	28	30	24	32	44	28	22
SLE	20.33±0.33	10.67±0.33	-	18.33±0.58	16.00±0.77	10.67±0.33	7.67±0.58
SLH	21.67±0.33	19.67±0.33	-	16.0±0.77	-	13.67±0.33	10.67±0.87
SBE	17.67±0.33	22.33±0.33	9.33±0.58	17.67±0.58	-	13.67±0.33	15.67±0.58
SBH	18.33±0.33	15.67±0.58	-	17.67±0.58	-	17.67±0.58	10.00±0.58
SVO	20.00±0.33	11.00±0.58	13.00±0.88	15.83±0.41	10.33±0.58	10.83±0.41	15.33±0.41

Table 4.51. MIC (Minimum inhibitory concentration) values of ethanolic and n-hexane extracts of *Skimmia laureola* leaf, stem bark and leaf essential oil.

	Micrococcus leutus	*Escherichia coli*	*Staphylococcus aureus*	*Pasteurella multocida*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Streptococcus viridans*
	Minimum inhibitory concentration MIC (mg/ml)						
SLE	1.25	5.0	-	0.65	-	5.0	5.0
SLH	1.25	2.5	-	1.25	1.25	5.0	5.0
SBE	0.65	0.67	5.0	1-25	-	2.5	2.5
SBH	1.25	2.5	-	1.25	-	1.25	5.0
SVO	0.65	1.25	1.25	1.25	1.25	2.5	0.65

Table 4.52. Antifungal activities of ethanolic and n-hexane extracts of *Skimmia laureola* leaf, bark and leaf essential oil.

Treatment	Concentration	Percent Inhibition of mycelial growth					
		Trichophyton longifusis	*Candida albicans*	*Fusarium solani*	*Microsporu mcanis*	*Aspergillusf lavus*	*Candida glabrata*
SLE	125µg/ml	20.33±0.58	32.44±0.41	-	30.37±0.41	64.45±0.47	28.00±0.58
	250µg/ml	34.34±0.58	41.33±0.33	-	32.33±0.43	67.77±0.71	43.00±0.41
	500µg/ml	38.24±0.33	45.16±0.58	7.41±0.58	53.85±0.33	70.97±0.58	58.82±0.58
SLH	125µg/ml	12.00±0.71	-	-	34.76±0.33	-	21.33±0.58
	250µg/ml	34.45±0.71	-	20.12±0.33	44.33±0.58	56.93±0.58	29.21±0.33
	500µg/ml	50.00±0.57	3.23±0.87	22.22±0.33	46.15±0.58	64.52±0.58	52.94±0.58
SBE	125µg/ml	12.33±0.47	-	-	8.66±0.58	-	7.66±0.66
	250µg/ml	32.33±0.47	-	24.33±0.58	12.65±0.71	-	12.44±0.33
	500µg/ml	38.24±0.58	-	29.63±0.41	30.77±0.71	3.23±0.71	38.24±0.58
SBH	125µg/ml	-	6.63±0.33	-	7.88±0.71	-	-
	250µg/ml	23.45±0.58	10.67±0.33	12.0±0.58	8.33±0.58	10.38±0.33	12.33±0.87
	500µg/ml	32.35±0.58	38.71±0.33	29.6±0.41	34.62±0.58	9.68±0.41	26.47±0.87
SVO	125µg/ml	62.66± 1.34	67.32±0.90	45.64± 1.40	30.37± 1.97	64.45± 1.98	28.00± 1.14
	250µg/ml	63.45± 1.45	77.0± 1.56	56.7± 1.21	32.33± 1.17	67.77± 1.88	61.33± 1.40
	500µg/ml	67.65± 1.40	83.87±1.98	62.96± 1.11	50.00± 1.60	70.97± 1.66	82.35± 1.49

Table 4.53. 24 hours acute toxicity test of *Zanthoxylum armatum* leaf and fruit in mice.

Group	Dose(mg/Kg)	Dead	Survived	Gross effect
Saline	10	-	All	-
ZLE	500	-	All	-
	1000	-	All	-
	2000	-	All	-
ZFE	500	-	All	-
	1000	-	All	-
	2000	-	All	-

Table 4.54. Anti pyretic ffect of ethanolic crude extract of *Zanthoxylum armatum* fruits , leaf (100, 200 and 300 mg/kg i.p.) and paracetamol (150mg/kg).

Treatment	Dose mg/kg	Initial Rectal temperature (C^o)		Rectal temperature (C^o) after administration of drug				
		Normal (A)	After 24 h (B)	1 h (C1)	2 h (C2)	3h (C3)	4 h (C4)	5 h (C5)
Normal Saline	10ml	36.66± 0.21	38.93± 0.23	38.81 ± 0.12	38.88 ± 0.13	38.88 ± 0.22	38. 78 ± 0.18	38.75 ± 0.35
Paracetamol	150	37.10± 0.08	38.8± 0.04	37.51**±0.01	37.46**±0.03	37.32**±0.02	37.35**±0.28	37.45**±0.04
ZFE	100	37.17± 0.28	38.83± 0.29	38.57*±0.12	38.30*± 0.17	38.10*±0.10	38.17*±0.06	38.20*±0.20
	200	36.73± 0.21	38.63±0.55	38.30*±0.35	37.60*± 0.35	37.10**±0.10	37.17**±0.15	37.30**±0.06
	300	37.17± 0.31	38.47 ±0.21	38.17*±0.15	37.23**±0.32	37.10**±0.10	37.17**±0.55	37.17**±0.44
ZLE	100	37.11±0.17	38.77± 0.25	38.43*±0.12	38.13* ± 0.12	37.00* ± 0.10	37.00*±0.10	38.07*± 0.21
	200	36.63± 0.15	38.53 ±0.47	38.27*± 0.32	37.47**±0.31	37.07**±0.06	37.10**±0.10	37.20**±0.21
	300	36.93± 0.06	38.53 ±0.15	37.97**±0.06	37.40**±0.10	37.17**±0.23	37.27**±0.32	37.37**±0.40

Values are reported as mean ± SEM for group of six animals. The data was analyzed by one way ANOVA followed by Dunnett's test. *Significant at P < 0.05, **P<0.01. ** Highly significant at P<0.01.

Table 4.55. EC_{50} ((half maximal effective concentration values) of ethanolic and n- hexane extract of leaf, bark, fruit and of leaf essential oil of *Zanthoxylum armatum*.

Part used	Extract	EC_{50} value spontaneous Rabbit jejunum	EC_{50} value for KCl induced contraction
Leaves	ZLE	0.23	6.81
	ZLH	6.13	4.45
	ZVO	0.22	0.73
Bark	ZBE	4.00	6.00
	ZBH	5.5	4.5
Fruit	ZFE(with out atropine)	1.69	7.49
	ZFE(with atropine)	8.86	3.18
	ZFH	0.74	5.00

Table 4.56. Cytotoxicity of ethanolic and n-hexane extracts of leaf, fruit, stem bark and of leaf essential oil of *Zanthoxylum armatum*. All values are expressed as mean±SEM of three determinations.

Treatment	No, of shrimps	Percent mortality			LD_{50} values
		10µg/ml	100µg/ml	1000µg/ml	
N/ control	30	0.0.±0.00	0.0.±0.00	0.0.±0.00	-
ZLE	30	42.67±5.77	76.67±5.77	94.00±0.00	20.00
ZLH	30	46.67±5.77	60.00±10	93.33±5.77	19.34
ZFE	30	30.66±5.77	70.33±5.77	90.00±0.00	65.05
ZFH	30	20.67±5.77	55.33±5.77	77.00±0.00	63.30
ZBE	30	90.00±10.00	96.67±5.77	100.00±0.00	16.79
ZBH	30	93.33±5.77	90.00±10.00	100.00±0.00	17.06
ZVO	30	83.33±0.00	100.00±0.00	100.00±5.77	15.90

Table 4.57. Phytotoxicity of ethanolic and n-hexane extracts of *Zanthxylum armatum* leaf, fruit, stem bark and leaf essential oil. Data is expressed as mean ±SEM of three determinations.

Name	Percent growth inhibition			FI_{50}
	10µg/ml	100µg/ml	1000µg/ml	
N/ control	0.00±0.00	0.00±0.00	0.00±0.00	-
P.control	87.82±1.11	87.82±1.11	87.82±1.11	8.87
ZLE	68.61±0.69	66.59±3.09	78.72±2.96	10.89
ZLH	48.66±1.15	52.43±4.45	90.99±1.36	19.13
ZFE	56.23±3.64	79.46±0.79	94.15±2.28	9.24
ZFH	62.72±2.03	89.76±2.80	100.00±0	3.69
ZBE	44.06±3.87	87.77±1.39	94.86±2.22	7.98
ZBH	26.93±0.14	49.95±1.85	62.73±3.23	19.59
ZVO	49.26±2.18	61.98±3.88	65.78±1.34	13.20

Table 4.58. Antibacterial activity of ethanolic and n-hexane extracts of leaf, fruit, bark and leaf essential oil of *Zanthoxylum armatum* DC. All values are mean ± SEM of three determinations.

	Micrococcus leutus	*Escherichia coli*	*Staphylococcus aureus*	*Pasteurella multocida*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Streptococcus viridans*
	Zone of inhibition (mm)						
DMSO(Neg. cont)	-	-	-	-	-	-	-
Cifrofloxacin(P.C)	28	30	24	32	44	28	22
ZLE	18.00±0.57	17.00±0.57	-	18.00±0.57	-	15.33±0.66	-
ZLH	11.67±0.33	11.67±0.33	-	8.67±0.66	-	9.33±0.87	-
ZFE	21.33±0.33	-	17.33±0.33	18.33±0.33	14.67±0.41	11.67±0.33	-
ZFH	19.67±0.33	17.00±0.58	-	17.67±0.0.33	10.33±0.33	11.67±0.41	7.67±0.41
ZBE	13.33±0.33	14.33±0.33	-	9.67±0.58	9.33±0.33	10.33±0.33	7.67±0.33
ZBH	20.33±0.41	11.33±0.41	12.67±0.58	15.33±0.33	-	11.33±0.33	10.00±0.58
ZVO	23.33±0.58	14.83±0.41	13.33±0.58	13.67±0.58	11.67±0.41	15.83±0.41	17.67±0.58

Table 4.59. MIC value of ethanolic and n-hexane extracts of *Zanthoxylum armatum* leaf, fruit, bark and leaf essential oil.

	Micrococcus leutus	*Escherichia coli*	*Staphylococcus aureus*	*Pasteurella multocida*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Streptococcus viridans*
	Minimum inhibitory concentration MIC (mg/ml)						
ZLE	1.25	1.25	-	0.65	-	2.5	-
ZLH	5.0	-	-	5.0	-	-	-
ZFE	0.65	-	0.65	0.65	1.25	1.25	-
ZFH	0.65	0.65	-	1.25	2.5	2.5	-
ZBE	5.0	5.0	-	-	-	5.0	-
ZBH	1.25	2.5	2.5	1.25	1.25	1.25	-
ZVO	0.65	1.25	1.25	0.65	1.25	1.5	0.65

Table 4.60. Antifungal activities of ethanolic and n-hexane extracts of leaves, fruit, bark and leaf essential oil of *Zanthoxylum armatum*. All values are mean± SEM of three determinations.

Extract	Concentration	Percent Inhibition					
		Trichophyton longifusis	*Candida albicans*	*Fusarium solani*	*Microsporum canis*	*Aspergillus Flavus*	*Candida glabrata*
ZLE	125µg/ml	-	34.33±0.43	35.33±0.41	8.66±0.47	32.44±0.33	7.33±0.66
	250µg/ml	-	47.32±0.33	45.33±0.41	12.65±0.47	44.56±0.71	7.66±0.41
	500µg/ml	8.82±0.58	70.97±0.33	59.26±0.57	46.15±0.33	45.16±0.87	20.59±0.58
ZLH	125µg/ml	20.93±0.41	26.98±0.33	12.45±0.57	7.88±0.71	19.32±0.87	19.22±0.71
	250µg/ml	30±0.57	39.33±0.41	37.33±0.71	8.33±0.87	42.34±0.33	36.76±0.57
	500µg/ml	32.35±0.71	45.16±0.44	40.74±0.71	26.92±0.87	54.84±0.47	44.12±0.57
ZBE	125µg/ml	34.5±0.57	56.33±0.71	66.67±0.33	54.33±0.33	44.29±0.47	12.56±0.57
	250µg/ml	53.33±0.57	67.33±0.71	70.33±0.33	61.22±0.47	56.97±0.33	34.77±0.33
	500µg/ml	55.88±0.71	74.19±0.57	74.07±0.57	69.23±0.87	61.29±0.41	67.65±0.57
ZBH	125µg/ml	29.33±0.33	18.33±0.48	23.78±0.87	7.33±0.33	23.33±0.58	19.33±0.71
	250µg/ml	50.63±0.33	44.33±0.21	49.33±0.87	18±0.17	56±0.58	32.76±0.71
	500µg/ml	70.59±0.71	70.97±0.41	59.26±0.87	26.92±0.33	58.06±0.47	44.12±0.33
ZFE	125µg/ml	35.5±0.57	32.31±0.57	21.76±0.33	-	44.29±0.66	13.46±0.47
	250µg/ml	54.33±0.57	57.43±0.77	41.22±0.57	10.54±0.47	56.97±0.33	34.77±0.48
	500µg/ml	55.88±0.41	58.06±0.41	48.15±0.41	11.54±0.17	64.52±0.21	44.12±0.33
ZFH	125µg/ml	29.33±0.71	18.33±0.71	23.78±0.87	7.33±0.17	23.33±0.47	19.33±0.58
	250µg/ml	50.63±0.41	42.33±0.41	31.66±0.47	47.33±0.33	24.74±0.47	32.76±0.58
	500µg/ml	52.94±0.33	58.06±0.57	37.04±0.41	57.69±0.33	29.03±0.47	52.94±0.41
ZVO	125µg/ml	30.43±0.33	66.67±0.57	46.33±0.33	34.76±0.87	55.33±0.57	21.33±0.71
	250µg/ml	54.33±1.02	76.33±0.71	67.00±0.41	76.33±0.87	56.93±0.81	29.21±0.71
	500µg/ml	70.59±0.71	83.87±0.71	70.37±0.41	84.62±9.41	67.74±0.47	79.41±0.58

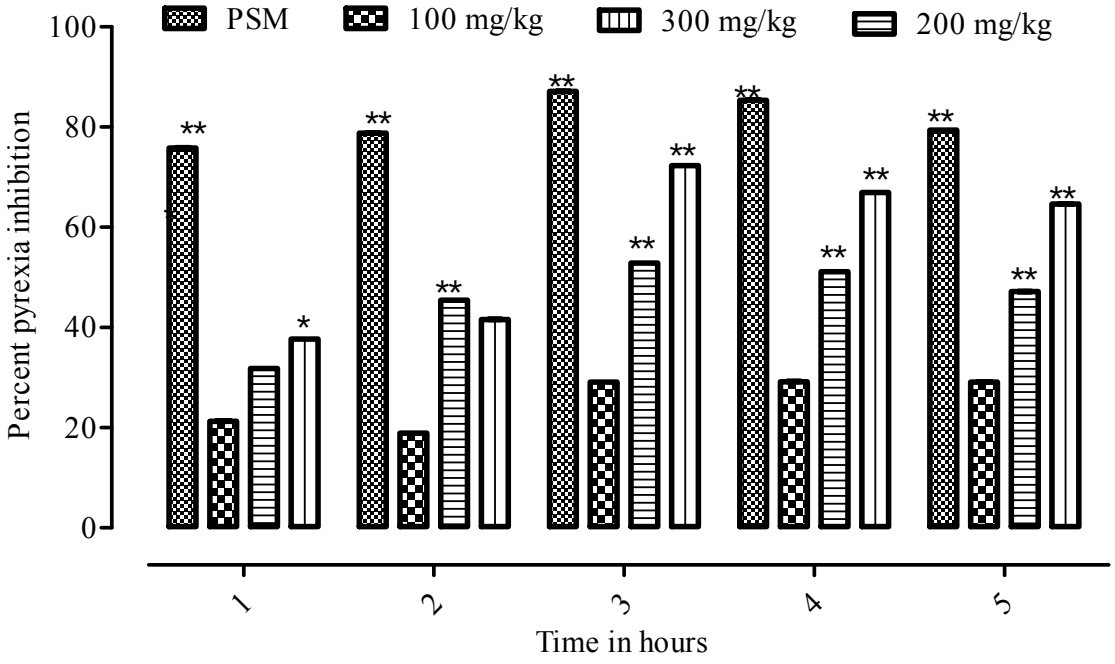


Fig. 4.44. Antipyretic effect of SLE (100, 200 and 300 mg/kg) and (PSM) paracetamol (150mg/kg) on brewer yeast induced pyrexia in mice after 1, 2, 3, 4 and 5h.

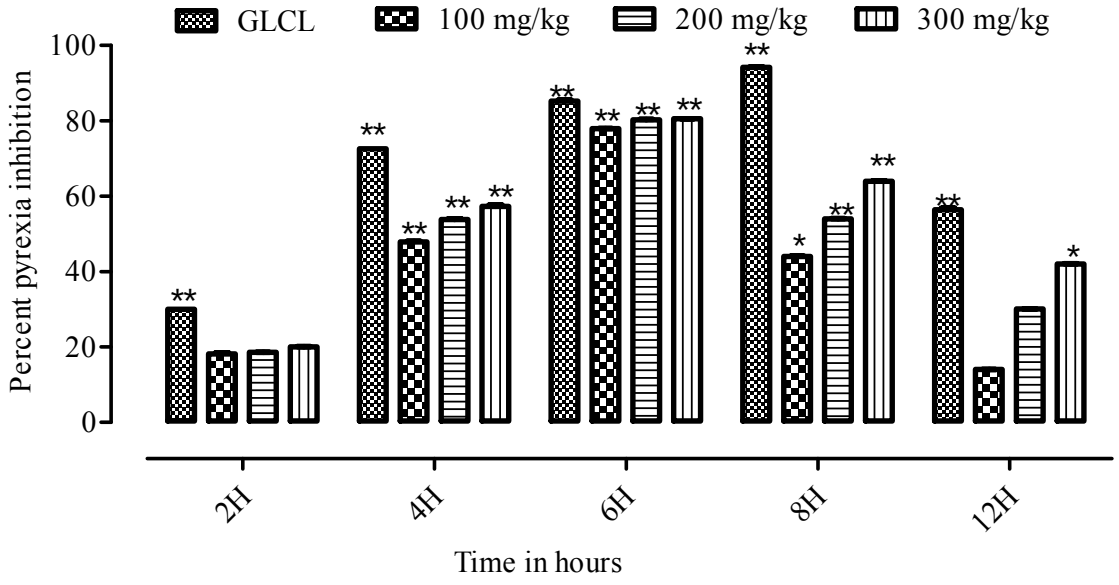


Fig. 4.45 . Hypoglycemic effect of SLE on alloxan-induced diabetic rabbits treated with extracts of SLE (100, 200 and 300 mg/kg) and glibinclamide (GLCL) (100 mg/kg) after 2, 4, 6, 8 and 12h. Bar representing percent ihibition of blood glucose level.

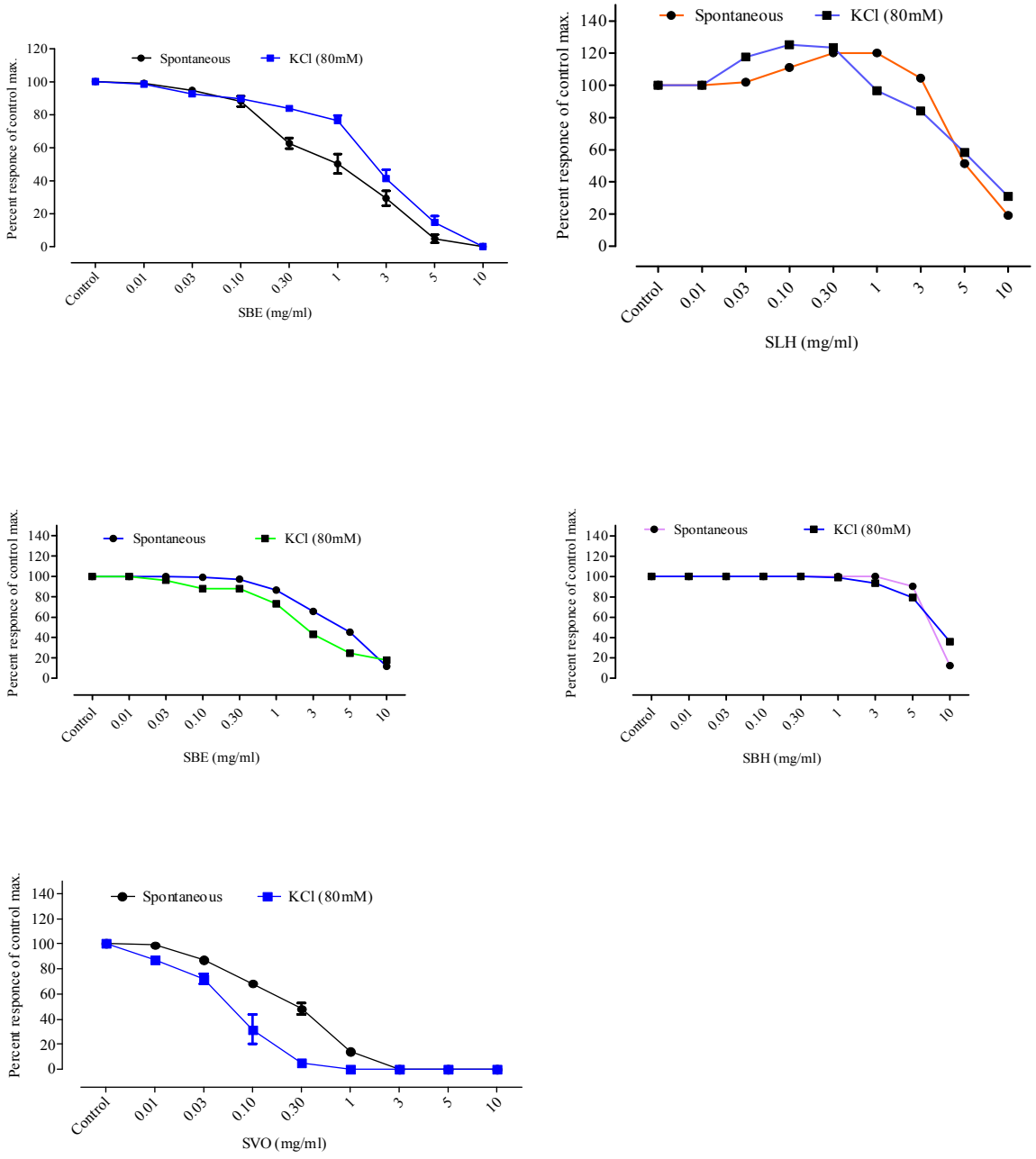
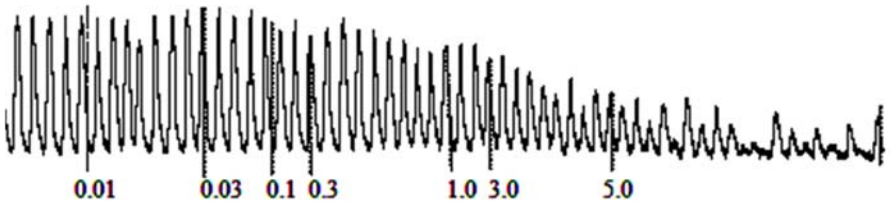
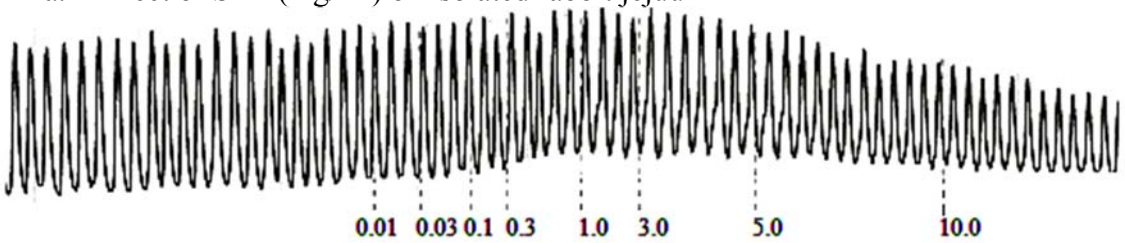


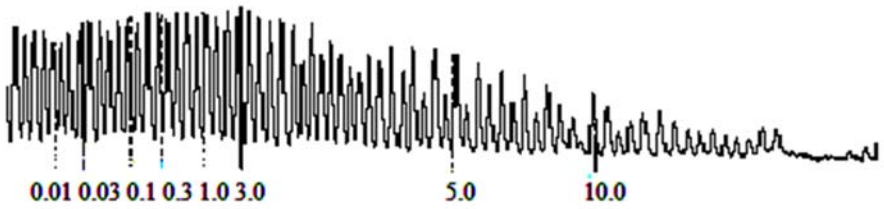
Fig. 4.46. Dose response curve of the SLE, SLH, SBE, SBH and SVO on isolated rabbit's Jejunum preparations. All values are Mean ±SEM, n = 5).



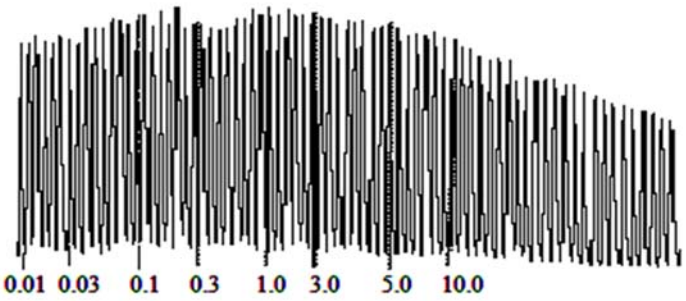
a. Effect of SLE (mg/ml) on isolated rabbit jejuum



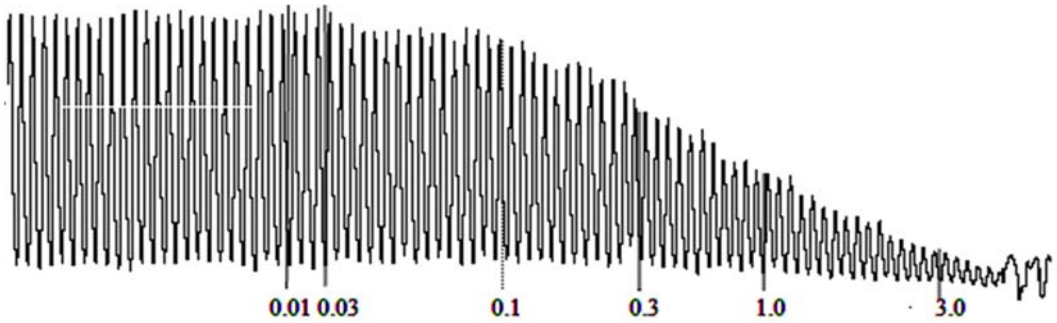
b. Effect of SLH (mg/ml) on isolated rabbit jejuum



c. Effect of SBE (mg/ml) on isolated rabbit jejuum



d. Effect of SBH (mg/ml) on isolated rabbit jejuum



e. Effect of SVO (mg/ml) on isolated rabbit jejuum

Fig. 4.47. Antispasmodic effect of *Skimmia laureola*.

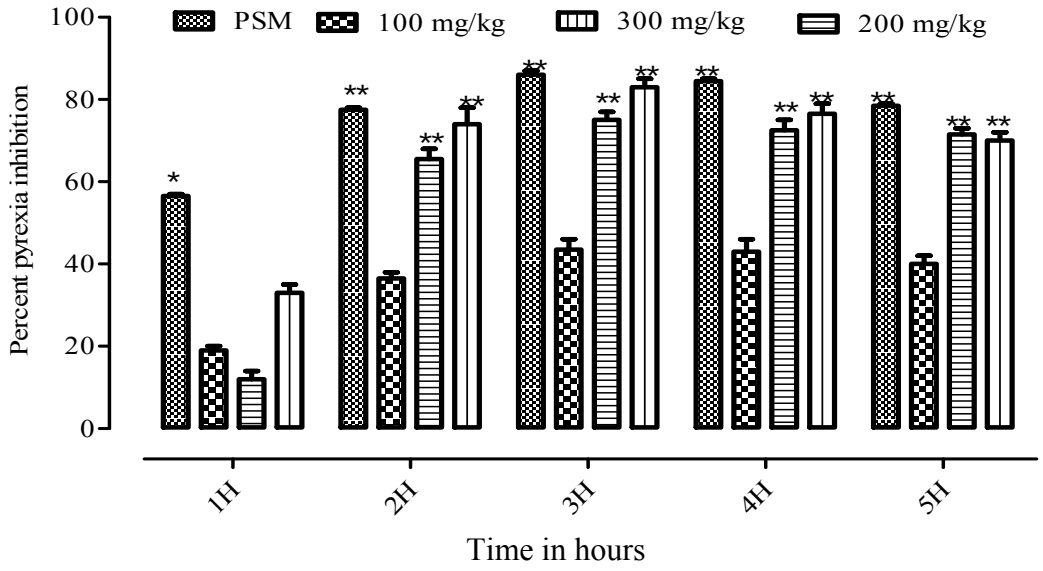


Fig. 4.48. Effect of ZLE on brewer yeast pyrexia in mice treated with extract of 100, 200 and 300 mg/kg and paracetamol (PSM) (150 mg/kg) after 1, 2, 3, 4 and 5h. Bar represent the percent ihibition of pyrexia.

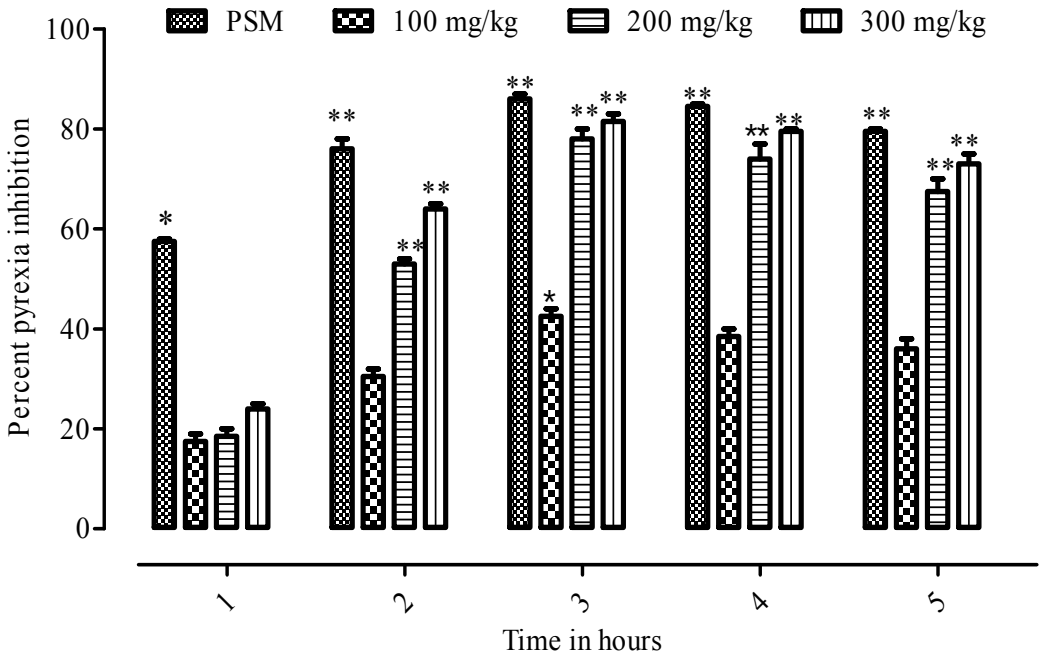


Fig. 4.49. Effect of ZFE on brewer yeast induced pyrexia in mice treated with extract of 100, 200 and 300 mg/kg and paracetamol (PSM) (150 mg/kg) after 1, 2, 3, 4 and 5h. Bar represent the percent ihibition of pyrexia.

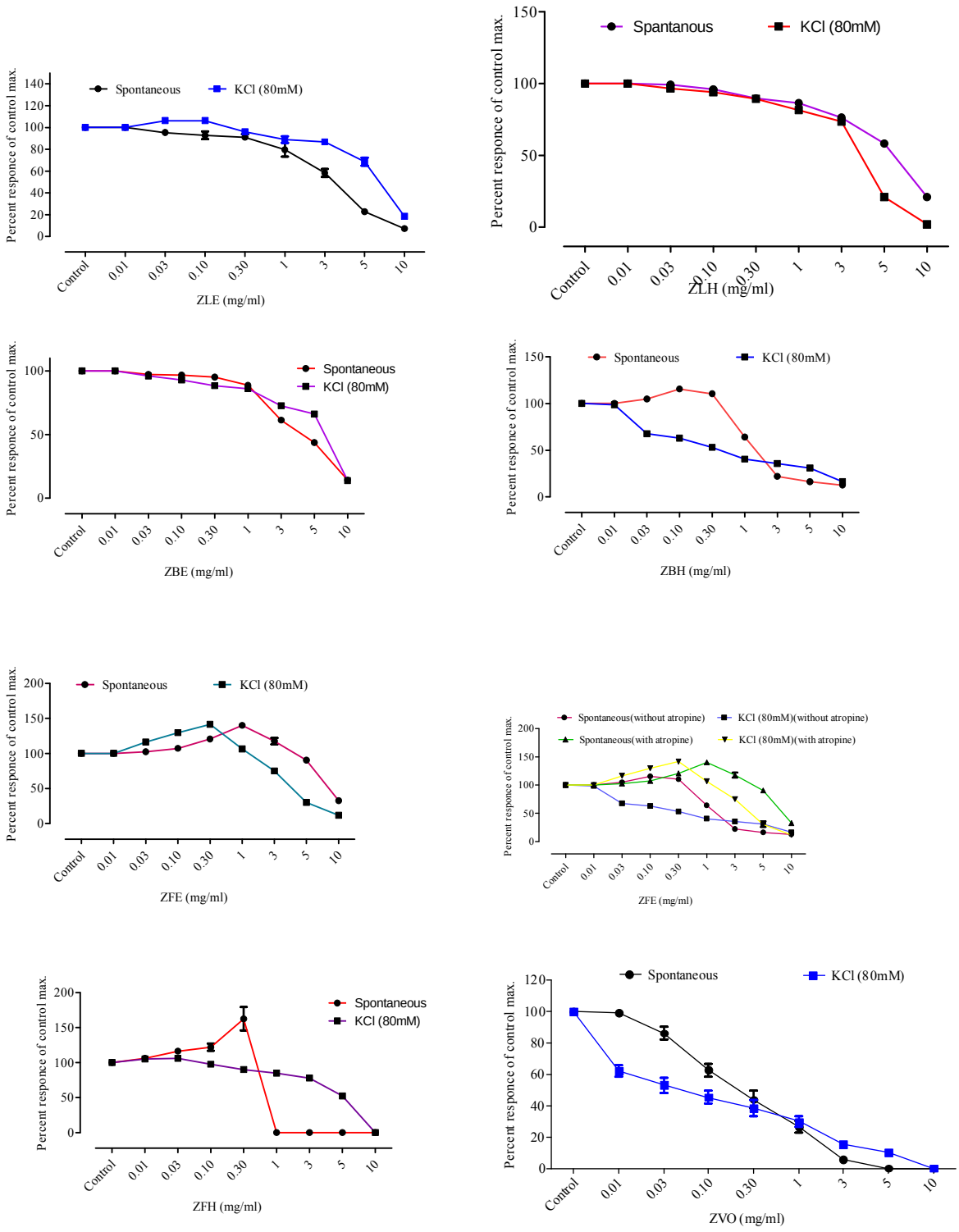
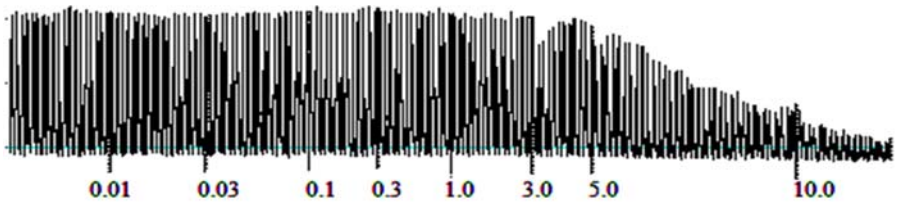
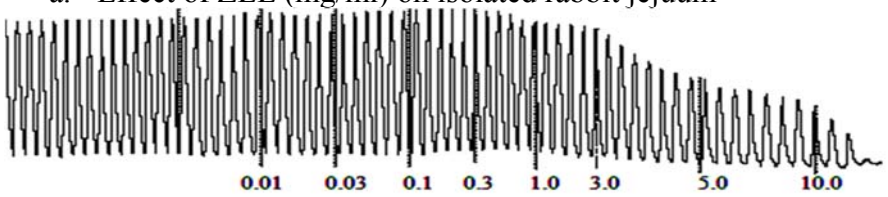


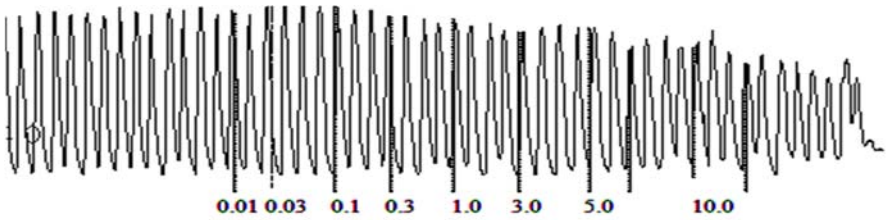
Fig. 4.50. Dose response curve of the ZLE, ZLH, ZBE, ZBH, ZFE, ZFH, and ZVO on isolated rabbit's jejunum preparations. All values are Mean ±SEM, n = 5).



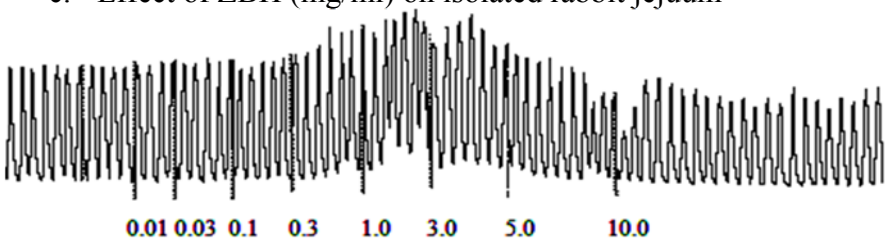
a. Effect of ZLE (mg/ml) on isolated rabbit jejuum



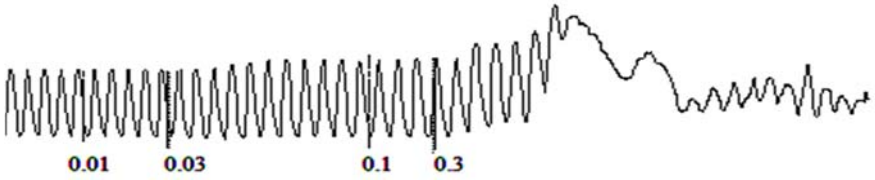
b. Effect of ZBE (mg/ml) on isolated rabbit jejuum



c. Effect of ZBH (mg/ml) on isolated rabbit jejuum



d. Effect of ZFE (mg/ml) on isolated rabbit jejuum



e. Effect of ZFH (mg/ml) on isolated rabbit jejuum



f. Effect of ZVO (mg/ml) on isolated rabbit jejuum

Fig. 4.51. Antispasmodic effect of *Zanthoxylum armatum*.

CONCLUSIONS

- *Skimmia laureola* grows gregariously in laomy or clay loamy soils at high altitude ranging from 2400- 3400 meters toward nort facing slope. It was found dominant in five out of six localities studied. *S. laureola* was found growing in association with a total of 44 plants, out of which Seven species including *Adiantum venustum, Fragaria vesica, Indigofera heterantha, Isodon rugosus, Podophyllum hexandrum, Pteridium aquilinum* and *Taxus baccata* were found to be the constant species in all stands. Density hectare^{-1} values for *S. laureola* were ranging from 312 to 4437.5.
- Locally *S. laureola* is known as "Nazarpanra" and is extinsively used through out the area to ward off bad evils. It is also considerd a best hypoglycemic agent. Market servey shows that the average purchase price of *S. laureola* ranges from Rs. 47.00 to Rs. 160.00 and the average sale rate ranges from Rs. 80.00 to Rs 200.00 per kilogram, whereas the annual trade ranges from 35 to 550 kg.
- *Zanthoxylum armatum* grows on the North and North West slopes in the foothills at an elevation of 850 - 1600 meters. Phytosocialogical studies in six different localities showed total of 51 species including 12 trees, 14 shrubs and 25 herbs and grasses in association with *Z. armatum,* in which *Ailanthus altissima*, *Ajuga bracteosa*, *Cynodon dactylon*, *Dicanthium annulatum, Dodonaea viscosa, Olea ferruginea* and *Rubus fruiticosus* were the constant species while *Artemisia scoparia* and *Conyza canadensis* were the mostly present species. Density hectare^{-1} values recorded in all stands for *Z. armatum*, ranged from 560 to 1020.
- Locally *Z. armatum* is known as "Dambara" and ethnobotanically it is used as fuel, as fencing plants and for Maswak (toothbrush) making. Its fruit is extensively used as a spice in various dishes especially in chat and Choolay (boiled gram). The average purchase price of *Z. armatum* ranges from Rs. 110.00 to Rs. 165.00 and the average sale rate ranges from Rs. 160.00 to Rs. 240.00 per kilogram, whereas the annual trade ranges from 75 to 360 kg in different markets of the area.
- Pharmacognostic evaluation shows that *S. laureola* leaf is punctuate with glabrous surfaces, the transverse section through the midrib region showed bifacial arrangement. The midrib region showed prominent oil cavities.

- Leaf surface features of *S. laureola* showed that it has a palisade ratio of 7.8±0.21, vein islets number 15.4±0.63 per mm^2, vein termination number 19.1±0.43 per mm^2, stomatal number 196.1±3.07 per mm^2 and stomatal index 12.96±0.14. Stomata showed variation in their arrangement showing eight different types, in which actinostephanocytic was the most frequently occurring. Stomatal cluster (very rarely occurring phenomena) was also observed.
- Leaf of *Z. armatum* is compound, punctate with glabrous surfaces, anatomically the leaf is bifacial. The midrib region showed prominent oil cavities. Leaf surface values showed a palisade ratio of 8.2±0.32, vein islets number 16.8±0.64 per mm^2, vein termination number 11.3±0.47 per mm^2, stomatal number 122.1±4.32 per mm^2 and stomatal index 12.32± 0.26 were calculated.
- T.S of *Z. armatum* fruit shows two portion i.e. fruit wall and seed. Fruit wall is clearly distinguished into three layers, epicarp, mesocarp and endocarp. The seed was non endospermic and contains elongated embryo.
- Leaf powder of *Skimmia laureola* appeared bright green in color with pleasant odor and astringent taste while bark powder of this plant appeared golden yellow in color with pleasant odor and astringent taste. Powder microscopy of the parts studied of both plants showed characteristic fragments.
- *Z. armatum* leaf powder was olive drab green with pleasant odor and somewhat astringent taste. Bark powder was grey brown in color with light pleasant odor and bitter taste, while fruit powder of Z. *armatum* was blackish brown with pungent odor and taste. The most striking feature of the powder drug study is the absence of any kind of trichomes in both the plants.
- Total ash, Acid insoluble ash and water soluble ash were determind as 101.8 mg/g, 65.2mg/g and 296.8 mg/g respectively for SL, 86.6 mg/g, 42.6 mg/g and 311.1 mg/g for SB, 110.4 mg/g , 51.3 mg/g and 320.5 mg/g for ZL, 134.6 mg/g, 69.7 mg/g an d361.3 mg/g for SB, 118.0 mg/g, 94.2 mg/g and 237.5 mg/g for ZF. These values were found much higher as compared to the respective samples exhausted with ethanol and n- hexane.
- Qualitative preliminary phytochemical screenings of *S. laureola* and *Z. armatum*, using aqueous, ethanolic and n- hexane extracts, showed the the presence of

carbohydrates, proteins, alkaloids, phytosterols, Triterpenoids, phenols, Flavonoids, tannins, anthocyanins, saponins, glycosides, fixed oil and volatile oil in aqueous, Ethanolic and n- hexane extracts. Quantitatification of flavonoids, phenol, alkaloids, tannins, saponin and sterol were also carried out in the studied parts.

- Zn, Mn and Cr were present in fairly good amounts and may contribute to hypoglycemic effects of these plants. Most of the other important bio elements were also detected in a both plants in the permissible limits. Correlations among various elements were also determined showing interdependency of these elements. Similarly proximate analysis of both plants showed carbohydrate, proteins, fibers, fats and moisture contents found in fairly large amount.

- SLE, ZLE and ZFE at doses of 500, 1000 and 2000 mg/kg body weight were evaluated for toxicological effects. No mortality or morbidity was observed even for the next 24 hours, showing that the plants are safe for human use.
- SLE, ZLE and ZFE showed maximum antipyretic action of 72.31% 83.84%, 80.70 and 85.42 % respectively at the third hour and remained significant till the fifth hour after administration of 300, 200 and 100 mg/kg body weight doses respectively.
- The hypoglycemic effect of SLE was found to be dose dependent. Like the standard drug, SLE induced reduction of blood glucose level after 2 hour of dose administration. All the doses showed significant reduction ($p < 0.05$) in glucose level at 6th hour post administration. 300 mg/Kg body weight dose of SLE showed maximum reduction (80.54±0.04%) at 6th hour.
- Ethanolic and *n*-hexane extracts of leaf (SLE, SLH), bark (SBE, SBH) and leaf essential oil (SVO) of *S. laureola* and ethanolic and *n*-hexane extracts of leaf (ZLE, ZLH), bark (ZBE, ZBH), fruit (ZBE, ZBH) and leaf essential oil (ZVO) of *Z. armatum* were evaluated for antispasmoidc potential on isolated rabbit jejunum in quests to search for cheaper, safer and accessible sources of anti diarrheal drugs with scientific proofs. EC_{50} values, for both spontaneous and KCl induced

contractions for all samples showed that SVO and ZVO were the most efficient ones, causing 100 % relaxation of the smooth muscles at a very low concentration.

- The SLE, SLH and SVO of *S. laurola* and ZBE, ZBH and ZVO of *Z. armatum* showed outstanding cytotoxic results with LD 50 values of 5.34, 7.44, 11.01, 16.79 17.06 and 15.90 µg/ml. respectively.
- *Lemna minor* phytotoxicity asssay of SBE showed 100% inhibition at 1000 µg/ml followed by SBH, SLE and SLH with FI_{50} of 25, 1.38, 4.54 and 8.67µg/ml respectively. The ZBE, ZFE and ZLH also showed excellent inhibition of the same test species with FI_{50} values of 7.98, 9.24 and 19.13 µg/ml respectively.
- Antibacterial bioassays showed that all the samples were effective against the test bacterial strains. SVO and ZVO were highly effective against all the strains (*Micrococcus leutus, Escherichia coli, Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Bacillus subtilis,* and *Streptococcus viridines*). Other extracts showed variant inhibitory effects against all or some of the test bacterial strains.
- A dose dependent antifungal potential against the test species (*Trichophyton longifusis, Candida albicans, Fusarium solani, Microsporum canis, Aspergillus flavus* and *Candida glabrata*) was found for all the samples. SVO inhabited the entire test strains in which maximum inhibitory zone were observed for *C. albicans* (67.32± 0.90%), followed by *A. flauus* (64.45± 1.98%) and *T. longifusis* (62.66± 1.34%) at a dose of 125µg/ml. Best anti fungal effect was observed for ZVO against *C. albicans* (66.67±0.57) followed by *A. flauus* (55.33±0.57) and *F. solani* (46.33±0.33). Other fungal strains were also affected at 125µg/ml concentration.

RECOMMENDATIONS

In light of the valuable findings in the present research work, the following recommendations are presented for perusal.

- The ecological and folklore knowledge about *Skimmia laureola* and *Zanthoxylum armatum* for conservation and commercial scale cultivation, which will greatly improve the economic conditions of the inhabitants of the area.
- The information about morphological and anatomical characteristics of these plants should be exploited for standardization of various characteristics of the parts used as crude drug.
- The pharmacognostic parameters of the part used are to be utilized for positive identification, quality and detection of adulteration in these drugs.
- Physicochemical analyses will be helpful in characterization of powder drugs.
- Determine the various nutritional components present in the drugs, which can be used for other purposes also.
- Phytochemical and trace, micro and macro elements composition of biological importance will be helpful in exploring these plants for future research work.
- Pharmacological potentials like antipyretic, hypoglycemic, antispasmodic, cytotoxic, phytotoxic, antibacterial and antifungal of these plants show that these plants should be exploited in future for extracting various active constituents for their pharmacological preparations. These findings might be exploited as cheaper effective remedies for various ailments.
- Physicochemical characteristics of essential and fixed oils from these two plants provide a guideline for their potential use in food, pharmaceutical and perfumery industries.

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