

**PHARMACOGNOSY OF *IPHIONA GRANTIOIDES* (BOISS.)
ANDERB. AND *PLUCHEA ARGUTA* BOISS. SUBSP. *GLABRA*
QAISSER OF FAMILY ASTERACEAE**



Ph. D. THESIS

BY

SHAHIDA NAVEED

DEPARTMENT OF BOTANY

UNIVERSITY OF PESHAWAR

May 2014

**PHARMACOGNOSY OF *IPHIONA GRANTIOIDES* (BOISS.)
ANDERB. AND *PLUCHEA ARGUTA* BOISS. SUBSP. *GLABRA*
QAISSER OF FAMILY ASTERACEAE.**



SHAHIDA NAVEED

**A THESIS SUBMITTED TO THE DEPARTMENT OF BOTANY,
UNIVERSITY OF PESHAWAR, PESHAWAR, PAKISTAN IN PARTIAL
FULFILLMENT FOR THE REQUIREMENT OF DEGREE OF DOCTOR OF
PHILOSOPHY IN BOTANY**

**DEPARTMENT OF BOTANY
UNIVERSITY OF PESHAWAR**

May 2014

DECLARATION

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

Shahida Naveed

CERTIFICATE OF APPROVAL

This Dissertation, entitled “**PHARMACOGNOSY *OFIPHIONA GRANTIOIDES* (BOISS.) ANDERB. AND *PLUCHEA ARGUTA* BOISS. SUBSP. *GLABRA* QAISER OF FAMILY ASTERACEAE** ” submitted by Shahida Naveed is hereby approved and recommended as partial fulfillment for the award of Degree of Doctor of Philosophy in Botany.

Professor Dr. Muhammad Ibrar
Research Supervisor, Department
of Botany, University of Peshawar.

External
Examiner

Professor Dr. Muhammad Ibrar
Chairman, Department of Botany,
University of Peshawar.

DEPARTMENT OF BOTANY
UNIVERSITY OF PESHAWAR

May 2014

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

DEDICATED TO

MY

PARENTS

MY

HUSBAND

AND

MY KIDS

AHSAN

ZUNAIRA

MOHSIN

LIST OF CONTENTS		
S .No	CONTENTS	P. NO
	Acknowledgement	i
	Abstract	iii
	Publications	x
	CHAPTER-1 INTRODUCTION	1-27
1.1	Pharmacognosy	1
1.2	Medicinal plants	2
1.3	Phytosociology	4
1.4	Ethnobotany	5
1.5	Pharmacognostic study	5
1.5.1	Identification and authentication of plant material	7
1.5.2	Anatomy/Histology	7
1.5.3	Phytochemical analysis	7
1.5.4	Extractive value	8
1.5.5	Fluorescence study	8
1.5.6.	Heavy metals analysis	8
1.6.	Pharmacology and bioassays	9
1.6.1	Antihelmintic activity	10
1.6.2	Antilice activity	11
1.6.3.	Antimicrobial activity	12
1.6.4.	Free Radicals and Antioxidants	13
1.6.4. a.	Antioxidant activity (DPPH Radical Scavenging activity)	13
1.6.5.	Cytotoxicactivity	14
1.6.6.	Hepatoprotective activity	16
1.6.7.	Insecticidal activity	17
1.6.8.	Phytotoxic activity	17
1.7	Family description of research plants	18
1.7.1	Tribe <i>Inuleae</i>	18
1.7.2.	Genus <i>Iphiona</i>	18

1.7.3.	<i>Iphiona grantioides</i>	18
1.7.4.	Taxonomic position <i>Iphiona grantioides</i> (Boiss.).	19
1.7.5.	Distribution of <i>Iphiona grantioides</i> (Boiss.) A. Anderb. in Pakistan	20
1.7.6.	Ethnomedicinal uses of <i>Iphiona grantioides</i> (Boiss.)A. Anderb.	20
1.8.1	Tribe <i>Plucheeae</i>	22
1.8.2.	Genus <i>Pluchea</i>	22
1.8.3.	<i>Pluchea arguta</i> (Boiss.).	23
1.8.4.	<i>Pluchea arguta</i> .subsp. <i>glabra</i> Qaiser	25
1.8.5.	Taxonomic position of <i>Pluchea arguta</i> subsp. <i>glabra</i> .	26
1.8.6.	Distribution in Pakistan	27
1.8.7.	Ethnomedicinal uses of <i>Pluchea arguta</i> subsp. <i>glabra</i>	27
	CHAPTER -2 REVIEW OF LITERATURE	28- 44
2.	Review of Literature	28
2.1.	Review of literature for <i>Iphiona grantioides</i>	28
2.1.1	Chemical constituents	28
2.1.2	Review of literature for other species of <i>Iphiona</i>	29
2.2.	Review of literature for <i>Pluchea arguta</i> subsp. <i>glabra</i>	30
2.3.	Review of literature of other species of the two genera i.e., <i>Iphiona</i> and <i>Pluchea</i> (asteraceae) .	30
2.4.	Pharmacognostic studies	36
2.4.1.	Ashing	36
2.4.2.	Extractive values	37
2.4.3.	Fluorescence study	37
2.4.4.	Phytochemical analysis	37
2.4.5	Heavy metals analysis	38
2.5	Biological activities	38
2.5.1.	Antihelmintic activity	38
2.5.2.	Antilice activity	39
2.5.3.	Antimicrobial activity	40
2.5.4.	Antioxidant activity	41

2.5.5.	Cytotoxic activity	42
2.5.6.	Hepatoprotective activity	42
2.5.7.	Insecticidal activity	43
2.5.8.	Phytotoxic activity	44
	CHAPTER - 3 MATERIALS AND METHODS	45- 85
3.1	Morphology of the two research plants	45
3.2	Phytosociology	45
3.2.1	Phytosociology of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	45
3.2.2	Community analysis	46
3.2.2.a.	Density	46
3.2.2.b.	Frequency (%)	47
3.2.2.c.	Cover of herbs	48
3.2. 2.d.	Importance Value Index	48
3.2.2.e.	Determination of Constancy	49
3.3.	Soil analysis	49
3.3. 1.	Soil sampling	49
3.3.2	Soil texture	49
3.3.3.	Soil pH	49
3.3.4.	Electrical conductivity	50
3.3.5.	Soil organic matter	50
3.3.6.	Total Nitrogen	50
3.3.7.	Phosphorus	50
3.3.8.	Potassium (K)	50
3.4.	Ethnobotany	50
3.5.	Pharmacognosy	51
3.5.1.	Pharmacognosy of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	51
3.5.2.	Macroscopical study	51
3.5.3.	Microscopic study of leaf, stem and root of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	53
3.5. 4.	Leaf surface study	53

3.5. 4.a.	Presence and absence of stomata/ Type of stomata	53
3.5.4.b.	Determination of stomatal Number (SN) and Stomatal index (SI)	54
3.5.4.c.	Vein islets and vein termination number	55
3.5.4.d.	Palisade Cell Ratio	55
3.5.5.	Study of physiochemical characteristics of the crud drugs of <i>Iphiona grantioides Pluchea arguta</i> subsp. <i>glabra</i>	56
3.5.5.a.	Ash analysis	56
3.5.5.b.	Determination of the moisture	58
3.5.6	Powder drug study of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	59
3.5.7	Phytochemical screening of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> .	59
3.5.8	Florescence study	66
3.5.9.	Extractive values in different solvents	67
3.6.	Heavy metals analysis	68
3.7.	Biological assays	69
3.7.1.	Antihelmintic activity.	70
3.7. 2.	Anti-lice activity	71
3.7.3.	Antibacterial activity	72
3.7.4.	Antifungal activity	73
3.7. 5.	Antioxidant (DPPH Free radical scavenging) activity	76
3.7.6.	Cytotoxic activity	78
3.7. 7.	Hepatoprotective activity	79
3.7.8.	Insecticidal activity	82
3.7.9.	Phytotoxicity	84
	CHAPTER- 4 RESULTS AND DISCUSSION	86- 201
4.1	Morphology of the research plants	86
4.1.1	Morphology of <i>Iphiona grantioides</i>	86
4.1.2	Morphology of <i>Pluchea arguta</i> .subsp. <i>glabra</i> Qaiser	89
4.2	Phytosociology	91
4.2.1	Phytosociology of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	91

4.3	Soil analysis	102
4.3.1	Soil sampling	102
4.3.2	Soil texture	102
4.3.3	Soil pH	102
4.3.4	Electrical conductivity	103
4.3.5	Soil organic matter (OM)	103
4.3.6	Total Nitrogen (N)	103
4.3.7	Available phosphorus (P)	103
4.3.8	Available potassium (K)	103
4.4	Ethnobotany	105
4.4.1	Ethnobotany of <i>Iphiona grantioides</i>	106
4.4.2	Ethnobotany of <i>Pluchea arguta</i> subsp. <i>glabra</i>	107
4.5	Pharmacognosy	107
4.5.1	Pharmacognostic studies of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> Qaiser	107
4.5.2	Macroscopical study of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	108
4.5.3	Microscopic study of leaf, stem and root of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> Qaiser	112
4.5.3.a.	Transverse section (T.S) of <i>Iphiona grantioides</i> stem	112
4.5.3.b	Transverse section (T.S) of stem of <i>Pluchea arguta</i> subsp. <i>glabra</i>	115
4.5.3.c.	Transverse section (T.S) of <i>Iphiona grantioides</i> leaf	116
4.5.3.d.	Transverse section (T.S.) of <i>Pluchea arguta</i> subsp. <i>glabra</i> . Qaiser leaf	120
4.5.3.e.	Transverse section (T.S.) of root of <i>Iphiona grantioides</i>	123
4.5.3.f.	Transverse section (T.S.) of root of <i>Pluchea arguta</i> subsp. <i>glabra</i>	124
4.5.4.	Leaf surface values of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	126
4.5.4.a.	Presence and absence of stomata and types of stomata in <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	126
4.5.4.b	Determination of Stomatal number and stomatal index of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	126

4.5.4.c.	Vein islet number and vein termination number of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> leaf	127
4.5.4. d.	Palisade ratio of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> leaf	127
4.5.5.	Physicochemical characteristics of the crude drug of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	130
4.5.6. a.	Powder drug study of different parts of <i>Iphiona grantioides</i>	132
4.5.6.b.	Powder drug study of <i>Pluchea arguta</i> subsp. <i>glabra</i>	136
4.5.7	Phytochemical screening	140
4.5.7.a.	Qualitative phytochemical screening of leaf, stem, root and flower of <i>Iphiona grantioides</i>	140
4.5.7.b.	Qualitative phytochemical screening of leaves, stem, root of <i>Pluchea arguta</i> subsp. <i>Glabra</i>	141
4.5.8	Fluorescence study	145
4.5.9	Extractive values	150
4.5.9.a.	Extractive values of <i>Iphiona grantioides</i>	150
4.5.9.b	Extractive values of <i>Pluchea arguta</i> subsp. <i>glabra</i>	150
4. 6	Heavy metals analysis	151
4.7	Bioassays (Biological activities) of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	158
4.7.1	Antihelmintic activity	158
4.7.2	Antilice activity	162
4.7.3	Antibacterial activity	165
4.7.4	Antifungal activity	169
4.7.5	Antioxidant activity	176
4.7.6	Cytotoxic activity	178
4.7.7	Hepatoprotective activity	182
4.7.8	Insecticidal activity	190
4.7.9	Phytotoxic activity	197
	Conclusion	202
	Recommendations	204
	References	205

LIST OF FIGURES		
Fig.No.	Title	Page No.
Fig.1.1	<i>Pediculus humanus capitis</i> (Human Head louse)	12
Fig.1.2.	<i>Artemia nauplii</i> (brine shrimps larvae)	15
Fig.1.3.	<i>Iphiona grantioides</i> Boiss.	21
Fig.1.3.b.	<i>Iphiona grantioides</i> Boiss.	22
Fig.1.4.	<i>Pluchea arguta</i> subsp. <i>glabra</i>	24
Fig.1.4.b.	<i>Pluchea arguta</i> subsp. <i>glabra</i>	25
Fig.4.1.	<i>Iphionao grantioides</i> (Boiss) Plant, growing in its natural saline habitate	87
Fig.4.2.	<i>Iphiona grantioides</i> , trichomes are clearly visible on leaves	87
Fig.4.3.	<i>Iphiona grantioides</i> (Boiss) .IR- <i>Iphiona</i> root	88
Fig.4.4.	<i>Pluchea arguta</i> subsp. <i>glabra</i> . Plant, growing in its natural saline habitate	89
Fig. 4.5.	<i>Pluchea arguta</i> subsp. <i>glabra</i> , glabrous leaves are clearly visible	90
Fig.4.6.	<i>Pluchea arguta</i> subsp. <i>glabra</i> . Roots	90
Fig.4.7.	Map of District Karak	92
Fig.4.8.	A research locality in Distt. Karak showing ecological status (Phytosociology) of associate plants of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> Qaiser	93
Fig.4.9.a.	T.S showing <i>Iphiona grantioides</i> stem. ctx- Cortex, g.t- Glandular trichome, Epi- Epidermis, h.der- Hypodermis. (10x)	113
Fig.4.9.b.	T.S. showing <i>Iphiona grantioides</i> stem, arranged in a ring. (10x)	113
Fig.4.9.c.	T.S. showing <i>Iphiona grantioides</i> stem. Vascular bundles are arranged in ring	114
Fig.4.9.d.	T.S. showing <i>Iphiona grantioides</i> stem. Vascular bundle.Xyl-Xylem, Phl- Phloem, Ca- Cambium (10 X)	114
Fig.4.10.	T.S. of <i>Pluchea arguta</i> subsp. <i>glabra</i> stem . epi- epidermis, vb- vascular bundle; Pi- Pith; X. xylem; Phl- Phloem; Cam- Cambium; Cor.-cortex	115

Fig.4.11.a.	T.S. of <i>Iphiona grantioides</i> leaf in the midrib region. epi-epidermis, cuti- cuticle,g.tri- glandular trichome, spg-meso-Spongy mesophyll, pal-meso- Pallisade mesophyll, ca- ox-calcium oxalate, VB-Vascular bundle, Xyl- Xylem,Phl-Phloem, Meso-V.B-Mesophyll- Vascular bundle	117
Fig.4.11.b.	T.S. of <i>Iphiona grantioides</i> leaf. Palisade cells	117
Fig.4.11.c.	<i>Iphiona grantioides</i> . Mid-vein.VB-Vascular bundle, Xyl- Xylem, Phl- Phloem,Scl-Sclerenchyma,Ca-ox-Calcium oxalate crystal	118
Fig.4.11.d.	T.S. of <i>Iphiona grantioides</i> leaf. Lateral vascular bundles in the mesophyll	118
Fig.4.11.e.	<i>Iphiona grantioides</i> . Leaf upper epidermis.An-sto-anisocytic stomata	119
Fig.4.11.f.	<i>Iphiona grantioides</i> leaf. Lower epidermis.An-sto-anisocytic stomata	119
Fig.4.11.g.	<i>Iphiona grantioides</i> leaf. Biseriate glandular trichome	120
Fig.4.12.a.	T.S of <i>Pluchea arguta</i> subsp. <i>glabra</i> leaf in midrib region.VB-vascular bundle, Xy-Xylem, Phl-Phloem, Sto-stomata, Epi-epidermis, Stkd. g- Stalked gland, ses. g- sessile gland	121
Fig.4.12.b.	T.S. of <i>Pluchea arguta</i> subsp. <i>glabra</i> .Vascular bundles.in the midrib reigon. Xy. vesl- Xylem vessel with spiral thickening	121
Fig.4.12.c.	<i>Pluchea arguta</i> subsp. <i>glabra</i> . Leaf upper epidermis. an.sto-anisocytic stomata	122
Fig.4.12.d.	<i>Pluchea arguta</i> subsp. <i>glabra</i> leaf lower epidermis . ses.g- sessile glands, dia.sto- Diacytic stomata, an.sto-Aniso-Stomata	122
Fig.4.13.a	T.S. of <i>Iphiona grantioides</i> Boiss. Root. Rhizo-epidermis, Co-cortex, Phl- phloem, Xyl-Xylem	123
Fig.4.13.b.	Transverse section (T.S.) of root of <i>Iphiona grantioides</i> Boiss. Pr.Xyl- Primary-xylem, Mt.Xyl-Meta Xylem, Phl-Phloem	124
Fig.4.14.a.	T.S.of <i>Pluchea arguta</i> subsp. <i>glabra</i> root. Cok-Cork, S. cor-Secondary cortex,Phl. fib-Phloem fiber,S.Phl-Secondary Phloem,Cam cambium, S.Med. R-Secondary medullary ray, S. Xyl-Secondary Xylem	125
Fig.4.14.b.	T.S of <i>Pluchea arguta</i> subsp. <i>glabra</i> . Root.Pr.Xyl-Primary Xylem, P.md.r- Primary medullary ray, Xyl.scl-Xylem Sclerenchyma,S.Md.R-Secondary medullary Ray	125

Fig.4.15.a.	<i>Iphiona grantioides</i> (Boiss.)a. Palisade cells arrangement under epidermal cells	128
Fig.4.15.b.	<i>Iphiona grantioides</i> Boiss. Veins arrangement in lamina. Ve.Isl.n- Vein islet number, Ve. ter. n- Vein termination number	128
Fig.4.16.a.	<i>Pluchea arguta</i> subsp. <i>glabra</i> pallisade cells under epidermal cells.epi.cell-epidermal cell, Pal. cell-Palisade Cell	129
Fig.4.16.b.	<i>Pluchea arguta</i> subsp. <i>glabra</i> . Ve.Isl.n- Vein islet number, Ve. ter. n-Vein termination number	129
Fig.4.17.a.	Powder drug study of <i>Iphiona grantioides</i> leaf	133
Fig.4.17.b.	Powder drug study of <i>Iphiona grantioides</i> stem	134
Fig.4.17.c.	Powder drug study of <i>Iphiona grantioides</i> root	135
Fig.4.18.a.	Powder drug study of <i>Pluchea arguta</i> subsp. <i>glabra</i> leaf	137
Fig.4.18.b.	Powder drug study of <i>Pluchea arguta</i> subsp. <i>glabra</i> stem	138
Fig.4.18.c.	Powder drug study of <i>Pluchea arguta</i> subsp. <i>glabra</i> root	139
Fig.4.19.	Anthelmintic activity of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp <i>glabra</i> against <i>Pheretima posthuma</i>	162
Fig.4.20.	Antilice activity of different parts of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i>	165
Fig. 4.21.	Antibacterial activity of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	168
Fig.4.22. 1-9	Antifungal activity of the crude ethanolic extract of the various parts of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> against different strains of pathogenic fungi	173-175
Fig. 4.23.	Antioxidant (DPPH radical scavenging) activity of crude extracts of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	177
Fig.4.24.	Cytotoxic activity of difference parts of <i>Iphiona grantioides</i> against brine shrimp larvae at three different doses; 10 µg/ml (■), 100 µg/ml (□) and 1000 µg/ml (▒)	181
Fig.4.25.	Cytotoxic activity of difference parts of <i>Pluchea arguta</i> subsp. <i>glabra</i> against brine shrimp larvae at three different doses; 10 µg/ml (■), 100 µg/ml (□) and 1000 µg/ml (▒)	181

Fig.4.26.	Comparison of different concentrations of serum markers AST, ALT and ALP in rabbits after administering different doses of plant extracts with control, silymarin and acetaminophen	185
Fig.4.27	Comparison of different concentrations of serum .Bilirubin in rabbits after administering different doses of plant extracts with control, silymarin and acetaminophen	186
Fig.4.28.	Liver of rabbit treated with normal diet (Group)	188
Fig.4.29.	Liver of rabbit treated with acetaminophen (Group-2)	188
Fig.4.30.	Liver of rabbit treated with Silymarin+ acetaminophen	189
Fig.4.31.	Liver of rabbit treated with ethanolic extract of IG	189
Fig.4.32.	Liver of rabbit treated with ethanolic extract of PA	190
Fig.4.33.	Liver of rabbit treated with ethanolic extract of IG+PA	190
Fig.4.34.	Insecticidal activity of difference parts of <i>Iphiona grantioides</i> (I) and <i>Pluchea arguta</i> subsp. <i>glabra</i> (P) against <i>Tribolium casteneum</i> at three different concentrations; 1% (■), 5% (□) and 10% (▨)	195
Fig.4.35.	Insecticidal activity of difference parts of <i>Iphiona grantioides</i> (I) and <i>Pluchea arguta</i> subsp. <i>glabra</i> (P) against <i>Sitophilus oryzae</i> at three different concentrations; 1% (■), 5% (□) and 10% (▨)	195
Fig.4.36.	Insecticidal activity of difference parts of <i>Iphiona grantioides</i> (I) and <i>Pluchea arguta</i> subsp. <i>glabra</i> (P) against <i>Callosobruchus analis</i> at three different concentrations; 1% (■), 5% (□) and 10% (▨)	196
Fig.4.37.	Insecticidal activity of difference parts of <i>Iphiona grantioides</i> (I) and <i>Pluchea arguta</i> subsp. <i>glabra</i> (P) against <i>Trogoderma granarium</i> at three different concentrations; 1% (■), 5% (□) and 10% (▨)	196
Fig.4.38.	Insecticidal activity of difference parts of <i>Iphiona grantioides</i> (I) and <i>Pluchea arguta</i> subsp. <i>glabra</i> (P) against <i>Rhyzopertha dominica</i> at three different concentrations; 1% (■), 5% (□) and 10% (▨)	197
Fig.4.39.	Phytotoxic activity of difference parts of <i>Iphiona grantioides</i> against <i>Lemna minor</i> at three different doses; 10 µg/ml (■), 100 µg/ml (□) and 1000 µg/ml (▨). Bars indicate ± two standard errors of the mean	201

Fig.4.40.	Phytotoxic activity of difference parts of <i>Pluchea arguta</i> subsp. <i>glabra</i> against <i>Lemna minor</i> at three different doses; 10 µg/ml (■), 100 µg/ml (□) and 1000 µg/ml (▨). Bars indicate ± two standard errors of the mean	201
-----------	--	-----

LIST OF TABLES		
Table 3.1	Conditions applied for detection of various elements	69
Table 3.2	Schedule of acetaminophen and plant extracts administration on a pretreatment basis	81
Table 3.3	Composition of medium for <i>Lemna minor</i> bioassay	85
Table 4.1	Localities selected for phytosociological studies of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	93
Table 4.2	Phytosociology of <i>Iphiona grantioides</i> , <i>Pluchea arguta</i> subsp. <i>glabra</i> and associated flora in the studied areas	94-98
Table 4.3	Dominant shrub plant species on the bases of importance value (IV) in the selected localities of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> in District Karak	99
Table 4.4	Descriptive statistics of soil parameters collected from different areas of District Karak	104
Table 4.5	Macroscopic features of leaf of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	110
Table 4.6	Macroscopic features of stem of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	111
Table 4.7	Macroscopical features of root of <i>Iphiona grantioides</i> Boiss. and <i>Pluchea arguta</i> subsp. <i>glabra</i>	111
Table 4.8	Leaf Constant values of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	130
Table 4.9	Ash contents of different parts of <i>Iphiona grantioides</i> and	131

	<i>pluchea arguta</i> subsp. <i>glabra</i>	
Table 4.10	Phytochemical investigation of different parts of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	143-144
Table 4.11	Fluorescence analysis of <i>Iphiona grantioides</i> flower powder with different reagents	146
Table 4.12	Fluorescence analysis of leaves of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> powder with different reagents	147
Table 4.13	Fluorescence analysis of stem of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> powder with different reagents	148
Table 4.14	Fluorescence analysis of root of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> powder with different reagents	149
Table 4.15	Extractive values of different parts of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> plants in different solvents	151
Table 4.16	Heavy metal profile of various parts of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	157
Table 4.17	Anthelmintic activity of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> against <i>Pheretima posthuma</i>	160-161
Table 4.18	Antilice activity of different parts of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i>	164
Table 4.19	Antibacterial activity of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> . The figures show zones of inhibition in mm	167
Table 4.20	Antifungal activity of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> against different fungal pathogens. The figures show percent (%) inhibitions	171-172
Table 4.21	Antioxidant (DPPH radical scavenging) activities of crude extracts of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	177
Table 4.22	Cyto-toxic activities of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>	179-180
Table 4.23	Effect of <i>Iphiona grantioides</i> ethanol extract (IGEE), <i>Pluchea arguta</i> subsp. <i>glabra</i> ethanol extract (PAEE) on liver enzyme	184

	profiles and Serum Bilirubin contents of rabbits	
Table 4.24	Insecticidal activity of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> ethanolic extract	193-194
Table 4.25	Phytotoxic activity of different parts of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i> ethanolic extract against <i>Lemna minor</i>	199-200

ACKNOWLEDGEMENT

In the name of Allah, the most Beneficent, the most Merciful. All praises are for “Almighty Allah”. The most Benevolent, the only to be Praised, whose blessing and exaltations flourished my thoughts and enabled me to improve my knowledge up to this stage. I thank **Allah** that in spite of all problems, which I encountered during Ph. D, enabled me to complete this dissertation. I offer my humble and sincerest words of thanks to **The Holy Prophet Hazrat Muhammad (S.A.W)** who is forever a torch of knowledge and guidance for humanity.

It is a matter of immense pleasure for me to express my deepest regards and feelings of gratitude to my research supervisor **Prof. Dr. Muhammad Ibrar, Chairman, Department of Botany, University of Peshawar**, for his meticulous mentorship, guidance, constructive criticism, illustrious advice and encouragement during my study and research work which helped me to complete this research work with full expression of my capabilities. I am particularly inspired by his affection and co-operative approach towards students.

I wish to express my deepest regards to **Professor Dr. Farrukh Hussain, Chairman, Department of Botany, Bacha Khan University, Charsadda**, for his support and constant encouragement all the way through my studies.

I am extremely thankful to Dr. Barkatullah, Department of Botany, University of Peshawar for his support, guidance and help throughout my studies.

After that, I am deeply grateful to Dr. Addur Rashid, Dr. Sirrajudin, Dr. Zahir Muhammad, Dr. Ghulam Dastagir, Dr. Tanvir Burni, Dr. Musarrat Jabeen, Dr. Lal Badshah, Dr. Khanzadi Fatima Khattak and Mr. Rehmanullah for their helping attitude.

I would like to take this opportunity to pay my debt of gratitude to Professor Dr. Ummar Sique Khattak (Chairman Department of Animal Health and Veterinary Sciences, KPAUP), Prof. Dr. Niamatullah Khattak (Center for Excellence in Geology, UOP), Dr.

Gayasudin & Mr. Abdur Rauf (Institute of Chemical Sciences, University of Peshawar), Mr. Arif (PCSIR, Peshawar) for helping me in the research work.

I pay high tribute to Dr. Abdu-Rauf Khattak, Dr. Rahim Khan Khattak and Dr. Abdu-Sattara Khattak for their constant encouragement throughout my studies.

I pay my thanks to dearest **Sundas Rauf** (neice), for her appreciation and moral support and for taking care of my kids study when I was busy in my research. May her soul rest in peace (Amin).

I am also thankful to Research Officer Aqeel Khattak (Gram Research Station, Ahmad Wala, Karak) and Associate Professor Dr. Ijaz Ahmad (Weed Science Department, Khyber Pukhtunkhwa Agriculture University Peshawar) for their help and support.

My deep regards are forwarded to Dr. Zahid Hanif, Mr. Ghulam Jelani and Mr. Imtiaz Ahmed.

I am also grateful to the contribution of office and para teaching staff of Department of Botany, University of Peshawar for helping me all the way throughout my study.

I am thankful to my driver Janan Ustad (Choudry Islam Janan), who made all my plant collection possible.

I am highly indebted to the Higher Education Department Govt. Khyber Pukhtunkhwa for granting me Non Objection Certificate (NOC) for conducting my higher studies.

Last but not the least I wish to express my deep sense of gratitude, from the core of my heart to my sweet and loving parents, my sweetest sisters (Mrs. Noreen Ahmad, Mrs. Qamer Wasee, Mrs. Mooni Khan, Mrs. Sana Gul and Mrs. Aleena Khan) and my dearest brother Yousaf Haroon, my kids (Ahsan, Zunaira and Mohsin) and my beloved husband Inayat Khattak (Kaka Abu), who remembered me in their prayers and without their support, I would not be here. Thank you very much for being by my side, believing in me, and putting up with me during the good and bad times.

I am also thankful to my Aday (mother in Law), Mrs. Shaheen Rauf, Muhammad Sajid Khattak, Muhammd Ibrar Khattak, Nouman Rauf and Faizan Rahim and my all family members for their cooperation and moral support that has finally made this success possible.

Shahida Naveed

**PHARMACOGNOSY OF *IPHIONA GRANTIOIDES* (BOISS.)
ANDERB.**

AND *PLUCHEA ARGUTA* BOISS. SUBSP. *GLABRA* QAISER

OF FAMILY ASTERACEAE

ABSTRACT

In the present study phytosociological, ethnobotanical, pharmacognostic, physicochemical and pharmacological study of *Iphiona grantioides* (Boiss.) Anderb. and *Pluchea arguta* (Boiss.) subsp. *glabra* Qaiser of family asteraceae was carried out.

Seven different localities in District Karak of Khyber Pukhtunkhwah, Pakistan were selected for phytosociological study of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* Qaiser (Fig. 4.6). Both the plants were widely growing in the highly saline area of district Karak i.e. Andai (along road side), Sour Daag, Noshpa, Bahader Khail (Near old tunnel), Naripanoos (Near algada), Malgeen (Salt Deposit) and Karat (Salt Deposit), Karak at an elevation ranging from 450 m to 900 m.

In the present study *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*, the two research plants, were present in close association of each other along with other 80 plants, in all the seven localities.

Iphiona grantioides was found dominant at two sites, while it was second dominant at Karat ghar site and third dominant at Naripanoos site, with average importance value (IV) 21.2, and maximum IV 49.9, at Chashmai area of Andai, while minimum IV was 7.1 at Malgeen area. Important value index (IVI) of *Iphiona grantioides* was 7.05 with maximum IVI 16.6 and minimum IVI 2.3. *Pluchea arguta* subsp. *glabra* was found in all the seven sites, mostly growing in close association with *Iphiona grantioides*. It was 1st dominant at Bahader Khail sit near the old tunnel, and second dominant at Chasmai and third dominant at Malgeen, Karat and Noshpa area, having the average IV of 13.2, maximum IV of 28.3 and minimum IV of 7.1. Important value index (IVI) of *Pluchea arguta* subsp. *glabra* was 4.4, maximum IVI 9.4 and minimum IVI 1.76.

Soil analysis revealed that all the soil samples analysed from different areas of District Karak were alkaline in reaction and strongly calcareous in nature. Organic matter, soil N and P were found deficient in most of area sampled. The sampled area soil had permanent salt with semi-arid climate.

Pharmacognostic study of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* Qaiser was carried out, which included macroscopical description of leaf, stem and root of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*, microscopic study which includes anatomical studies of leaf, stem and root, leaf surface study, determination of leaf constant values (Stomatal index, vein islet no. and Palisade ratio), physiochemical characteristics (Moisture contents and ash values), fluorescence study, phytochemical investigation and extractive values of powder drugs of leaf, stem and roots of both the plants were determined, while ethanolic extracts of different parts of the two plants were screened out for various bioassays.

The leaf of *Iphiona grantioides* is succulent in nature, pubescent, thickly covered with glandular trichomes and nonglandular hairs on both the surfaces. The leaf is amphistomatic, stomata being present on both the epidermises and the stomatal apparatus is of anomocytic type. Anatomical studies showed that both the (upper and lower) epidermises are covered with waxy cuticle and multicellular non glandular and glandular trichomes. Palisade cells have chloroplast and oil droplets while spongy mesophyll cells have cuboid crystals of calcium oxalate. In the midrib region the midvein is very prominent, having 1-2 vascular bundles, with sclerenchymatic cells on the lower side.

Similarly leaf of *Pluchea arguta* subsp. *glabra* Qaiser was sessile, spirally arranged, almost persistent and green, herbaceous, glabrous. Lamina was simple with serrate (dentate) margins. Leaf was bitter; unpleasant in taste with strong, pungent smell. Leaf anatomy of *Pluchea arguta* subsp. *glabra*. Qaiser revealed the presence of single layered upper and lower epidermises and glandular trichomes, which are sessile as well as with multicellular stalk (2-5 cells). The leaf has typical bifacial structure, with vascular system and mesophyll tissue comprises of palisade and spongy parenchymas cells containing the latter is oil globules. In the midrib region collenchymatous tissues is present below the both upper

and lower epidermises and there are 3 vascular bundles in the midrib. Vascular bundles are colletral and closed. Xylem vessels are with spiral wall thickening.

Palisade ratio of *Iphiona grantioides*, in the present study, ranged from 5 to 6 to 6.75. Vein islet and vein termination number were 8 to 10 (9) and 7 to 10 (8) per mm² respectively. The vein-islets were quite distinct as squaresh, elongated or polygonal shaped, internally provided with many forked and vascular branches, while stomatal number and stomatal index value for *Iphiona grantioides* was 120 to 150 (130) and 12 to 15 (13) per mm² respectively.

Pluchea arguta subsp. *glabra* leaf has the Palisade ratio from 6 to 7 (7.5). Vein islet and vein termination numbers were 10 to 12 (11) and 6 to 9 (8) per mm² respectively while stomatal number and stomatal index value was in the range of 110 to 160 (130) and 10 to 12 (11) per mm², respectively.

Ash analysis for crude powder drug of leaf, stem, root and flower of *Iphiona grantioides* was carried out. Highest value of total ash was recorded for stem (7.06%) and least for leaves (3.33%), while roots has 4.25% total ashes and flower has 3.38% total ash value. Acid insoluble ash was in the range of 1.54% (leaf), 1.425% (root), 1.27% (stem) and 0.35% (flower). Water soluble ash was in the range of 4.36 % (leaves), 3.34% (stem), 2.23 % (roots) and 1.82% (flower). Moisture contents was highest (9.16%) in root, 9.01 % (stem), 7.80% (leaf) and 6.12% (flower). Similarly Ash analysis of *Pluchea arguta* subsp. *glabra* for crude powder drug of leaf, stem and root was carried out. Stem has total ash value of 7.82 %, 6.12 % to 5.6%. Acid insoluble ash was in the range of 1.66%, 1.09 % and 1.49 %. Water soluble ash was in the range of 2.56%, 3.38% and 6.5%, while moisture contents was highest in leaves (8.87%), root (8.41 %) and lowest in stem (7.5%). Qualitative phytochemical screening of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* revealed that proteins, carbohydrates, tannins, alkaloids, flavonoids, saponins, glycosides, phytosterol and tri-terphenoids and volatile oil were detected in aqueous and ethanolic extracts of various parts of both the plants.

In the present study seven different solvents including ethanol, methanol, chloroform, acetone, distilled water, butanol and n-hexane were used for percent extractive values determination of different parts (leaf , flower, root and shoot f *Iphiona grantioides* and leaf, shoot and root of *Pluchea arguta* subsp. *glabra*). Highest extractive values were given by *Iphiona grantioides* leaf i.e., (Aqueous extract (46.2%), Ethanol (37%), Butane (36%), Hexane (22.8%), Ethyl acetate (13.75) followed by root extracts i.e., Acetone (20%), Chloroform (20%) , Ethanol (19 %) and aqueous extract (16.6%). Similarly highest extractive values were given by leaves of *Pluchea arguta* subsp.*glabra*. The values are as follow, Ethyl acetate (30%), Aqueous (27.2%), Ethanol (22.2 %), and Hexane (20.75%).

In the present study leaf, shoot, root and flower of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* were analyzed to determine the concentration of various heavy and toxic metals including Mn, Fe, Cu, Zn, Cr, Pb, Co, Ni and Cd in these plants. Obtained results showed that both the plants have all the elements within safer and permissible limits.

In the present study various bioassays were carried out to investigate the *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* for their curative values.

Crude ethanolic extracts of *Iphiona grantioides* (flower, stem,leaf,root) and *Pluchea arguta* subsp. *glabra* (stem,leaf,root) were tested against 4 human pathogenic fungal strains (*Trichophyton longifusus*, *Candida albicans*, *Microsporum canis*, *Candida glaberata*), 2 animal pathogenic fungi (*Aspergillus flavus* , *Aspergillus niger*) and the 3 Plant pathogenic types (*Fusarium solani*, *Mucor spp.* and *Alternaria spp.*) for antifungal activity. The flower and leaf extract of *Iphiona grantioides* was the most effective extracts ,as shown antifungal against all the nine species at all the three (125, 250 and 500 ug/ml) doses. Similarly the crude ethanolic extract (500 ug /ml) of stem, leaves and roots had inhibitory effects against the fungal strains in the order of leaves (43.6%) > stem (35%) > root (30%).

Antibacterial activity of ethanolic extract of various parts of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* was tested against *Escherichia coli*, *Staphylococcus viridans*,

Klebsiella pneumoniae, *B.licheniformis*, *Pasteurella multocida*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Micrococcus luteus*. The results of antibacterial activity of different parts of the two plants showed that ethanolic extract of *Iphiona grantioides* flower was most effective against the tested microorganism, with the highest inhibition zone of 21.23 mm (*Klebsiella pneumoniae*) followed by 19.67 mm (*Staphylococcus aureus*), 17.47 mm (*Pasteurella multocida*), 16.76 mm (*Bacillus licheniformis*), 15.67 mm (*Micrococcus luteus*), 15.00 mm (*Staphylococcus viridans*) while it exhibited moderate antibacterial effects against by producing zone of inhibition of 12.33 mm against *Bacillus Subtilis* and *Pseudomonas aeruginosa*. Ethanolic extract of leaves of *Iphiona grantioides* showed significant antibacterial effect against *Escherichia coli* (14 mm) while other extracts including leaf, stem and root, showed moderate antibacterial potential, in the range from 8.32 - 11.67 mm.

Ethanolic extract of *Pluchea arguta* subsp. *glabra* leaf was comparatively more potent than the other two extracts i.e., stem and root, in producing prominent zone of inhibitions that is 15.33 mm (*Escherichia coli*), 13.33 mm (*Pseudomonas aeruginosa*) and 12.00 mm (*Micrococcus luteus*) while the lowest zone of inhibition (10.33mm) was observed in *Micrococcus luteus* and *Pasteurella multocida* by root and stem extracts, respectively. leaf ethanolic extract of *Iphiona grantioides* has produced an excellent phytotoxic activity against *L. minor* at the highest tested concentration (1000 µg/ml) by causing (91.5%) inhibition of *Lemna minor*. Similarly flower extract also caused 87.2% inhibition. *Pluchea arguta* subsp. *glabra* leaf extract caused 73.2 % inhibition, while the stem caused 70.2% inhibition. Moderate inhibitions (61.7%) were observed for the ethanolic extracts of *Iphiona* and *Pluchea* roots. Lowest inhibition (59.6%) was caused by stem extract of *Iphiona grantioides* at concentration 1000 µg/ml).

Ethanolic extracts of flower, leaf of *Iphiona grantioides* showed excellent insecticidal (90%) activity against *Callosobruchus analis* and *Rhyzopertha dominica* at 10% dose concentration, respectively. Besides this, flower extract also significantly expressed insecticidal activity against *Sitophilus oryzae* (82%) and *Tribolium castaneum* (80%). Moderate lethal activity was shown by stem, leaf, flower and root extracts (in the range of 60% to 66%) against *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica*,

Trogoderma granarium and *Callosobruchus analis*. Week inhibitory activity (in the range of 22%-48%) was exhibited by stem, root and leaves extract against almost all the tested species except. Stem and roots of *Pluchea arguta* subsp. *glabra* displayed week inhibitory activity against *Tribolium castaneum* in the order of 28% and 62%, respectively. Ethanolic extract of *Pluchea arguta* subsp. *glabra* leaf exhibited good mortality activity against *Rhyzopertha dominica*, *Tribolium castaneum*, *Sitophilus oryzae*, *Trogoderma granarium* and *Callosobruchus analis* in the order of 100%, 90%, 80%, 80% and 70%, respectively. The extract obtained from the stem and root exhibited a moderate (50%- 60%) inhibitory activity against *Rhyzopertha dominica* and *Callosobruchus analis* and weak activity (10% - 40%) was shown by rest of the extracts against all tested insects.

Aerial parts of two plants were used for cytotoxic bioassay. The extracts displayed significant toxicity against brine shrimps.

The results of the present study showed that roots of *Pluchea arguta* subsp. *glabra* showed highest DPPH scavenging activity (82.89%) followed by ethanol extracts of leaf (77.3%) and flower (70.94 %) of *Iphiona grantioides*.

The anthelmintic activity of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* were very significant against the tested worms. Leaf extracts of *Iphiona grantioides* and *P. arguta* subsp. *glabra* at dose of 100 mg/ml caused death of the worm in 3.33 ± 0.57 and 2.16 ± 0.28 min, respectively. Moderate mortality time of root and stem of *Iphiona grantioides* was 12.43 ± 0.60 and 13.83 ± 0.76 , respectively. In addition, weak nematocidal activity of death time of 50 ± 5 was exhibited by flower extract of *Iphiona grantioides*.

The present study has exposed the enormous and varied medicinal properties of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* and both of these plants can be exploited for curative preparations on marketable level.

PUBLICATIONS

My research Paper **“HEPATOPROTECTIVE ACTIVITY OF TWO WILD PLANTS, IPHIONA GRANTIOIDES (BOISS.) ANDERB. AND PLUCHEA ARGUTA BOISS. SUBSP. GLABRA QAISER FOR ACETAMINOPHEN INDUCED TOXICITY IN RABBITS.”** Has been accepted in Pakistan Journal of Weed Science Research, Vol. 20 (3), 2014.

CHAPTER- 1

INTRODUCTION

1.1. Pharmacognosy

The term Pharmacognosy, derived from two Greek words "pharmakon" (drug) and "gnosis" (knowledge), was first introduced by an Austrian physician Schmidt in 1811 and in 1815 by a German medical student, C.A. Seydler in his work entitled 'Analectea Pharmacognostica' (Evans, 2002). Pharmacognosy is an applied science of medicines derived from natural resources and it studies the structure of physical, chemical and biological preparations, for the commercial purposes in a scientific and systematized way (Gokhale *et al.*, 2008). Pharmacognosy includes phytochemistry, phytochemical analysis and molecular biology as well as ethnobotany, ethnomedicine while ethnopharmacology has also become an important element in Pharmacognosy (Balunas & Kinghorn, 2005). Pharmacognosy is also defined as "the scientific study of crude drugs originated from four different natural resources namely animals, plants, metals and minerals" but almost 90% of the crude drugs are obtained from plant resources while the left behind are from other three sources (Joy *et al.*, 1998). Pharmacognosy study crude drugs from botanical, organoleptic, physical and pharmacological point of view, also to identify, Isolate active constituents or compounds and screen the crude drug for different biological assays, so advancement in new technologies has broaden its scope by linking various branches of biological sciences i.e., botany, pharamacology, pharmaceutics, also phytochemistry, various traditional systems of medicines, analytical chemistry, microbiology, plant tissue culture, biotechnology and genetic engineering, together (Rangari, 2002 ; Balunas & Kinghorn, 2005 and Jarald & Jarald, 2007). Pharmacognosy has played a milestone role in the discovery and development of new drugs and therapies from medicinal plants and is doing so even today (Sarker, 2012).

1.2. Medicinal plants

Living organism cannot exist on this Planet without plants. They are the source of almost everything, the living things depend on, either directly or indirectly. Plants are a fundamental part of all ecosystems. Since ancient times, plants have been one of the first and most available resources usable for treating illnesses, and the therapeutic effects of plants and their uses have been known by everybody (UNESCO, 1996).

According to the reports of World Health Organization (WHO), more than 80% of the world population relies on the traditional system of medicine, largely plant based, to meet their primary health care. In sub-continent, drugs of plant origin have been used widely from a very long time. According to the survey reports of W.H.O, traditional healers treat 90% patients in Bangladesh, 85% in Myanmar, 80% in India, 75% in Nepal, 65% in Sri Lanka and 60% in Indonesia. In Pakistan, 60% of the population, especially in villages is getting health care by Hakims (traditional practitioners), who recommends herbal preparations (Ahmed *et al.*, 2004; Gilani *et al.*, 2006).

Fossil records have also revealed the importance of plants as drugs since Middle Paleolithic Era i.e. more or less 60,000 years back (Fabricant & Farnsworth, 2001). Plants use as herbal medicines in different medicinal systems i.e., Greek, Chinese, Ayurvedic, Islamic, Homeopathic and Allopathic (Western) systems of medicines (Evans, 2002) shows the harmony among the nations over the use of plants as medicines.

All plants, as a result of normal metabolism produce primary metabolites (sugars, proteins, amino acids, chlorophyll) and secondary metabolites, which are responsible for most of the medicinal properties of plants. The biochemical have impact on the systems of other organism and majority of these molecules have shown great therapeutic role in the treatment of various Human and animal ailments (Kumar, 2004; Samuelsson, 2004; Kinghorn, 2002). As plants derived drugs are low cost, available

easily and safe in use, so they are still in use in developing countries of the world particularly in their rural communities (Srivastava *et al.*, 1996; Nair *et al.*, 2005).

Pakistan has a strong tradition of herbal treatments and its rural residents still depends mostly on native system of medicine for the cure of their health problems (Khattak *et al.*, 1985). Pakistan has diverse topographic strata, comprising of different climatic zones hosting a large variety of plant species. Pakistan has four phytogeographical regions, consisting of Irano-Turanian (46%), Sino-Himalayan (10%), Saharo-Sindian (9.5%), and Indian element (4.5%) and Pakistan has about 6,000 species of Angiospermic plants of which about 400 to 600 are considered very important medicinally (Hamayun *et al.*, 2005).

Ahmad *et al.*, (2008) reported more than 350 plants/plant parts, which are used by local Dawakhanas (labortries for herbal products) for the formulation of herbs based products. Pakistani flora is a rich source for providing new bioactive compounds for the welfare of human beings (Haq, 1983).

District Karak is situated in the southern part of province Khyber Pakhtunkhwa Pakistan. It is situated at 32° 47 to 33° 28 N and 70° 30 to 71° 30 E. District Karak is bounded by District Kohat and Hangu on the North, District Laki Marwat on South, Mianwali on South East, and District Bannu and Waziristan Agency on the West. The average altitude of the District is from 600 to 14,000 meter above the sea level. Total area of the district is 3,372 km². Maximum area of the district is highly saline and xeric and is dominated by Halophytes and Xerophytes, which are used by the local inhabitants for different purposes (Menzel & Lieth, 1999). Among these halophytes *Iphiona grantioides* (Boiss.) Anderb. and *Pluchea arguta* subsp. *glabra* Qaiser are commonly present along with other salt tolerant plants and in the present research work, these two plants will be pharmacognostically evaluated.

According to an estimate, the number of plants species on the earth is between 2, 50,000 to 5, and 00,000 (Borris, 1996). A comparatively very small proportion (1-10%) of these plants is used as food by both humans and other animal species, and

possibly, a major portion of these is used for medicinal purposes (Moermann, 1996). Active constituents of medicinal plants are mostly secondary metabolites, at least 12,000 of which have been isolated and identified, but even then this number is probably less than 10% of the total number of such compounds (Schultes, 1978), thus suggesting that a lot of work can be done in herbal medicine.

1.3. Phytosociology

The term "Phytosociology" was first used by Józef Paczoski in 1896 (Rabotnov, 1970). Phytosociology is the study of the distinctiveness, classification, association and distribution of plant communities. Plant community implies information of organization and composition of the component species. It is very helpful to gather such data in order to explain the population dynamics of each species studied and how they are related to the other species in the same community (Marielos, 2003). The survival and organization of a community reflects the plant type and habitat conditions under which they grow (Malik, 1986). The differences among various communities and plant association are due to exposure, microclimate, edaphic and biotic factors (El-Ghani & Amer, 2003). Vegetation is an ecological expression of an area showing complex inter-relationships among the various components including plant-plant, plant-animal and plant-physical environment interaction. Physiognomy of vegetation can be evaluated by estimating the combined influence of plant height and basal area, density and number of species on complexity index (Stone & Frayer, 1935).

Phytosociology is the quantitative study of vegetation and its major aim is to describe flora, explain its structure and categorize it in a meaningful way (Ilorkar & Khatri, 2003). Overexploitation of plant resources has resulted in the gradual degradation of vegetation and decrease in flora richness (Qureshi, 2008). Forests are unstable and degraded and would be vanished if not maintained properly. The original composition of the vegetation has been changed due to overgrazing and deforestation and there is a need for restoration of habitat (Siddiqui *et al.*, 2009).

In Pakistan, various plant communities of different parts have been studied phytosociologically, but very little information is available on the phytosociological attributes of a single species (Khan *et al.*, 2010). In this respect, some of reported previous work is on *Olea ferruginea* (Ahmad, 2007), *Picea smithiana* (Wahab *et al.*, 2008) and *Quercus baloot* (Khan *et al.*, 2010).

1.4. Ethnobotany

Ethnobotany arises from two words “ethnology”, which means study of society/traditions and “botany” (is the scientific study of plants), so it is the scientific study of the relationships that is present between humans and plants. Harshberger (1896) defined ethnobotany as “the study of the useful relationship between human beings and plants in their natural environment, as well as the medicinal uses of plants”. Ethnobotany is the relationship between plants and human cultures and is not limited to the use of plants only for food, clothing and protection but also includes their use for sacred ceremonies, decoration and health care (Schultes, 1992). Ethnobotanists aim to document, describe and clarify the complex relationships between cultures and (uses of) plants. They primarily focus on how plants are used, managed and perceived across human societies. It includes use of plants for food, clothing, medicine, construction, ritual, dye, cosmetics etc., (Acharya & Shrivastava, 2008). Ethnobotany is the knowledge of various ways and means by which folk people of a particular area uses plants/plant parts in their daily life according to their own customs and traditions (Igoli *et al.*, 2005; Tor-Anyiin *et al.*, 2005). The ethnobotanical information of plants is also very helpful for taxonomists, ecologists, pharmacologists, watershed and wild life managers in their efforts for improving the prosperity of an area (Ibrar *et al.*, 2007). Ethnobotanical studies have been proved very useful in the selection of medicinal plants in their selection for pharmacological screening (Igoli *et al.*, 2005).

1.5. Pharmacognostic study

Pharmacognostic study of crude drugs gives scientific information regarding the quality and purity of plant derived drugs (Sindhu, 2010) and give valuable information

regarding morphology, macroscopic, microscopic and physical characteristics of crude drugs (Sharma, 2004).

Plant derived products have been used as therapeutic alternatives in the health-care systems of several countries. Medicinal plants are important sources of bioactive compounds, as their easy access and low cost are the main reasons that justify their widespread application. The World Health Organization (WHO) has published several guidelines that aim at improving the quality, effectiveness and safety of the herbal medicinal preparations (WHO, 2002) and in order to make sure the safe use of these medicines, a necessary first step is the establishment of standards of quality, efficacy and safety (Bhat *et al.*, 2012).

Quality, purity and effectiveness of herbal medicines needs very care right from the identification, collection, extraction and purification process of the plants, season and area of collection and rationalizing the combination in case of polyherbal drugs (Patel *et al.*, 2006). The most important step in the standardization of the drugs is its identification /authentication and in this respect pharmacognostic study of the medicinal plants or crude drugs has proved very authentic (WHO, 2005). Bioassay is very important tool in the standardization of herbal drugs and can also become an important quality control method as well as for proper stability testing of the product (Bhutani *et al.*, 2003).

Crude drug materials are standardized by the following steps:-

- i. Stage of collection.
- ii. Parts of the plant collected.
- iii. Regional status.
- iv. Morphology, microscopical and histological analysis (cell walls, characteristic contents of cell, starch grains, crystals of calcium oxalate, presence /absence of trichomes, fibers, Xylem vessels (Shrikumar *et al.*, 2006). Pharmacognostic studies include the following histological parameters: leaf constants values (palisade ratio, vein islet number, vein termination number, types of stomata, stomatal number, and stomatal index), trichomes, quantitative microscopy, taxonomical identity, foreign matter,

organoleptic evaluation, extractive values, moisture content, ash values , UV light study (Wallis, 1985).

1.5.1. Identification and authentication of plant material

The first step in the standardization of crud drug is the identification of the concerned species in either form , fresh or dried , intact or powdered (Springfield *et al.*, 2005). This can be done by some pharmacognostic parameters i.e, Morphological description of the plants, anatomical study of the concerned parts of the plant and Biochemical characteristics (Anonymous,1998). Before starting any research work on a plant ,its positive identification is must. This identification can be done by actual collection of a plant or animal that has been positively identified, similarly by comparing the collected plant with a herbarium sheet and comparing the characters of the collected plant to a published description of the drug helps in identification (Tylor *et al.*, 2001).

1.5.2. Anatomy/Histology

Histological studies have been proved a helpful tool in identification and revealing the internal structure of plants. The pharmacognostical studies give valuable information about the morphology, macroscopic, microscopic and physical characteristics of crude drugs (Sharma, 2004). Pharmacognostic study gives the scientific information regarding the purity and quality of the plant drugs (Sindhu, 2010). The microscopic evaluation of crude drugs exclusively deals with the appearance of plant tissue in sectional view and powdered drug (William , 2000).

1.5.3. Phytochemical analysis

Phytochemical screening of medicinal plants has exposed the presence of numerous secondary metabolites including alkaloids, flavonoides, steroids, glycosides, saponins and tannins etc. Secondary metabolite serves as defense mechanism against predation by herbivores micro organisms and insects (Cowan, 1999). Phytochemicals are chemical compounds which can be isolated from plants. Phytochemicals have been in use, over a long history, as medicines. In common usage today, they are associated with health benefits

(Rouhi, 2003). Physicochemical studies verify the identity of plant and make certain the quality and purity of crude drug (Patel, 1998).

1.5.4. Extractive Values

Extraction is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures. The main purpose of extraction process is to obtain the therapeutically active portions of crude drugs (medicinal plant/parts) and to discard the unwanted material by treating with a selective solvent known as menstruum. During extraction process, solvents diffuse slowly into the plant material and solubilize the compounds with similar polarity. Extractive values of different solvents are very useful parameters for the evaluation of crude drugs as they indicate the nature of the chemical constituents present in a crude drug (Khandelwal, 2004).

1.5.5. Fluorescence study

Many drugs are used in powder form and almost all the crud drugs are dried and powdered for solvent extraction. The powder of plant tissues has specific fluorescence under UV light. This property is a useful tool for evaluation and for detection of adulteration, contamination as well as for identification of various chemical constituents of the crude drug (Gupta *et al.*, 2009). Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiations. The fluorescence analysis of powder drugs, treated with different chemical reagents, under visible & UV(254 & 366 nm) lights, show different color fluorescence, which help in evaluation of the drugs (Wallis,1985).

1.5.6. Heavy metals analysis

Various complex organic and inorganic compounds (carbohydrates, fats, proteins, vitamins, and minerals) and water are needed by human beings in their diet to meet their body requirements (Indrayan *et al.*, 2005). Every constituent plays an important role and deficiency of any one constituent may lead to abnormal developments of the body. Plants are the rich source of all the elements essential for human beings and the curative effect of

medicinal plants for the treatment of various diseases is based on the chemical compounds and different elements in them (Morabad *et al.*, 2012). Metallic elements and their compounds have been used for therapeutic and cosmetic effects on skin, since earliest times. Solution of aluminum acetate, copper sulphate and lotion of zinc solution are used as antiseptic, cleansing agents, skin disinfectant, soothing and cooling agents. Calcium, magnesium and manganese are required for the formation of the collagen and connective tissue. Similarly sulphur and phosphorus are used for the treatment of leprosy and scabies (Soderberge & Halimans, 1982; Sahito *et al.*, 2003).

Although herbal medicines have less side effects as compared to allopathic medicine but the presence of heavy metals in plants, which may be toxic when they are in higher amount, can cause health issues (Hussain *et al.*, 2006; Ibrar *et al.*, 2013b). According to the recommendation of WHO (1998) medicinal plants must be checked for the presence of heavy metals and other contaminations. Excess doses or prolonged intake of medicinal plants can lead to accumulation of trace elements which cause various health problems (WHO, 1992; Sharma *et al.*, 2009). In this perspective, elemental contents of the medicinal plants are very important and herbal preparations are needed to be screened for the presence of toxic heavy elements (Liang *et al.*, 2004 ; Arceusz *et al.*, 2010). Recent studies on the elemental analysis of medicinal plants have enhanced the consciousness about the heavy elements in plant products (Wong *et al.*, 1993 in China; Ajasa *et al.*, 2004 in Nigeria ; Caldas and Machado, 2004 in Brazil ; Sheded *et al.*, 2006 in Egypt ; Sharma *et al.*, 2009 in India ; Koe and Sari, 2009 ; Jabeen *et al.*, 2010 in Pakistan; Ibrar *et al.*, 2013b). These studies showed that essential metals, when ingested in high amounts, can result in toxicity whereas non-essential trace metals are toxic even in very low concentrations for human health.

1.6. Pharmacology and bioassays

Pharmacology (is derived from two Greek words, pharmakon, "poison" in classic Greek; "drug" in modern Greek; and logia/ logy "study of", "knowledge of") is the branch of medicine and biology concerned with the study of drug action on body functions (Vallance & Smart, 2006). A biological assay is a procedure for determining the purity,

concentration and biological activity of a substance (e.g., antibiotic, hormone, enzyme, vitamin, plant growth factor, or any other chemical) by measuring its effect on an organism, tissue, cell, enzyme physiology, compared to a standard preparation (Linton, 1983).

Bioassay, biological assay, or biological standardization is a type of experiment, conducted to measure the effects of a chemical on a living organism physiology and very important in the development of new drugs and in monitoring environmental pollutants. Bioassay is a procedure for determining the effective dose of a drug.

In the present study, the following biological assays were used for the evaluation of biological activities of ethanolic extract of the two research plants.

1. Antihelminthic assay.
2. Antilice assay.
3. Antimicrobial (Antibacterial and antifungal) assay.
4. Antioxidant assay (DPPH scavenging activity).
5. Cytotoxic assay (brine shrimp lethality test).
6. Hepatoprotective activity/assay.
7. Insecticidal assay.
8. Phytotoxic assay.

1.6.1. Antihelminthic activity

Helminth parasites are a major problem in animals throughout the world resulting in significant production losses as the performance of animals is inversely correlated with the intensity of helminth infections; moreover Helminth infections are among the most prevalent infections in humans, upsetting a huge population of the world. Majority of infections due to helminths are generally restricted to tropical regions and cause massive risk to health and contribute to the occurrence of Anemia, Undernourishment, Eosinophilia and Pneumonia (Bundy, 1994).

Resistance development to most of the commercially available antianthelmintics became a severe problem throughout the world (Waller & Thamsborg, 2004). In addition,

these drugs are high-priced, unreachable or poorly available to the resource-poor farmers of the developing countries (Hammond *et al.*, 1997). All these factors paved the way for herbal medicines as alternative anthelmintics (Fajmi & Taiwo, 2005; Egualé *et al.*, 2007). The plant kingdom is known to provide a rich source of botanical anthelmintics, antibacterials and insecticides (Kone *et al.*, 2012). Screening and proper evaluation of the claimed medicinal plants could offer possible alternatives that may be both sustainable and environmentally satisfactory (Githiori, 2004). A lot of work has been done in the field of plant anthelmintics including both ethno botanical surveys and scientific validation of their anthelmintic efficacies in many parts of the world (Gathuma *et al.*, 2004; Githiori, 2004; Iqbal *et al.*, 2004; Egualé *et al.*, 2007; Khurshid & Tantry, 2012).

1.6.2. Antilice activity

The head louse (*Pediculus humanus capitis*) is an obligate ectoparasite of humans (Fig.1.1). Head lice are insects without wings which spend their entire life on human scalp and feed wholly on human blood (Buxton, 1947). Among the various reasons of the regular spreading of head lice is certainly the increasing globalization with migration streams in all directions of the earth, leading to a closer contact between many individuals (Falagas *et al.*, 2008; Mumcuoglu *et al.*, 2009; Burgess, 2009; Abdel-Ghaffar *et al.*, 2010). This is important since the transmission of head lice occurs almost entirely by close hair-to-hair contact (Lee *et al.*, 2005). Second reason is the lack of effectiveness of antilice products that may be due to emergence of resistance by the head louse to synthetic compounds. So, the present logical problem is to find substances, which are neither toxic nor immunogenic to human beings, but which are completely active against all developmental stages of the life cycle of louse and this laid the foundation for research in exploring novel anti-lice agents from medicinal plants. Now researchers worldwide, are aimed to search for new substances, substitutes to synthetic ingredients, such as phytosconstituents obtained from plant sources, to eradicate the head louse problem.



Fig.1.1.*Pediculus humanus capitis* (Human Head louse) (2,570 × 1,720 pixels,
file size: 1.54 MB, MIME type: image/jpeg)

1.6.3. Antimicrobial activity

The indiscriminate use of antimicrobial drugs in the treatment of infectious diseases caused by pathogenic microorganisms has developed resistance in many bacteria and fungi against a wide range of antibiotics (Cowan, 1999). So it is necessitated that novel unconventional sources of antibiotics should be discovered or older antibiotics should be modified to overcome this health hazard (Tollefson & Miller, 2000). Substances those are toxic to the pathogenic microorganism but do no harm to the host cells are strong candidates for the development of antibiotics. Medicinal plants represent a rich source of antimicrobial agents as they contain secondary metabolites which have been found inhibitory to microorganisms including bacteria, fungi, viruses, parasites, in a number of ways. There is a continuous and vital need to discover new antimicrobial drugs with diverse chemical composition and new mechanisms of action for new and re-emerging infectious diseases (Rojas *et al.*, 2003). Therefore, researchers are keenly interested in herbal medicine to develop better drugs against microbial infections (Srinivasan *et al.*, 2001; Benkeblia, 2004).

1.6.4. Free Radicals and Antioxidants

Living organisms use oxygen for the attainment of energy in catabolism. However, oxygen-centered free radicals and other reactive oxygen species (ROS) are formed as byproducts during metabolic activities in cells. Free radicals are charged molecules, produced as natural by-products by our metabolism, some cell-mediated immune functions and also generated through environmental pollutants (Li & Trush, 1994), attack cells, break cellular membranes or react with the nucleic acids, proteins, and enzymes of the cells and causes oxidative stress, as a result the cells lose their structure, function and eventually result in cell dysfunction and may be the cause of accelerate aging process and diseases such as cirrhosis, diabetes, atherosclerosis, and cancer (Halliwell & Gutteridge, 1999). Normally, they are produced in an amount that is easily quenched by our antioxidant defense systems preventing them from causing deleterious effects in the body (Nose, 2000). But their excess leads to oxidative stress (Finkel & Holbrook, 2000).

The term ‘antioxidant’ refers to the activity of many vitamins, minerals and other phytochemicals to guard against the damage caused by reactive oxygen species (ROS). Plants (vegetables, fruits and herbal medicines, etc.) may contain a wide variety of antioxidants (free radical scavenging molecules) such as phenolic compounds (e.g. phenolic acids, flavonoids, stilbenes, quinones, coumarins, lignans, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which represent an important source of natural antioxidants (Zheng & Wang, 2001; Cai *et al.*, 2003). Epidemiological studies have shown that many of these antioxidant compounds possess anti atherosclerotic, anti-inflammatory, antimutagenic, antitumor, anticarcinogenic, antibacterial, and antiviral activities (Sala *et al.*, 2002).

1.6.4. a. Antioxidant (DPPH radical scavenging activity)

Various methods have been used to test the antioxidant activity but the most widely used methods are those that involve generation of free radical species which are then neutralized by antioxidant compounds (Arnao *et al.*, 2001). The DPPH radical scavenging

method is a standard procedure for the evaluation of anti-radical activity (Sanchez-Moreno *et al.*, 1998). Antioxidants react with 1, 1-Diphenyl-2-picrylhydrazyl, a Meta stable radical having highest absorbance at 517nm. When it accepts an electron on hydrogen radical to become more stable, the absorbance vanishes away. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form i.e., 1,1-Diphenylhydrazine with the loss of this violet colour although there would be expected to be a residual pale yellow color from the Picryl group still present (Molyneux, 2004).

The role of antioxidant compounds in mitigating oxidative damage to biological systems has been reported by Wang *et al.*, (2004) and Perez *et al.*, (2004). Various plants have been screened for antioxidant activity and reported to possess antioxidant potential (Fazal *et al.*, 2011; Uddin *et al.*, 2012 ; Uddin and Rauf , 2012 and Muruhan *et al.*, 2013).

1.6.5. Cytotoxic activity

Cancer or malignant disease is one of the major causes of death in humans. WHO (2002) reported that malignant neoplasm is the third (12.4%) leading cause of death worldwide after cardiovascular disease (30%) and infectious diseases (18.8%), which include HIV/AIDS (Mathers *et al.*, 2001). Cancer chemotherapy plays a significant role in the treatment of many malignancies and the objective of cancer chemotherapy is to kill cancer cells with as little damage as possible to normal cells (Halliwell & Gutteridge, 1988). So, discovery of any new anticancer drug must be related to novel molecular targets; i.e. they should be effective against specific types of cancer cells but less toxic to normal cells or have a unique mechanism of action for specific types of cancer (Pezzuto, 1997). Plants have a long history of use in the treatment of cancer (Hartwell, 1982). Natural products are important templates for chemotherapeutic agents. Studies on tumor inhibiting compounds originating from plants have yielded a remarkable collection of novel structures. The study of bioactive compounds from plants has required the development of bioassay techniques, especially in vitro methods which allow a large number of plant extracts to be screened for activity, especially cytotoxicity, against many types of cancer cell lines. Screening of Medicinal plant for cytotoxic activity against brine shrimp larvae is

the preliminary method for selection of active constituents from medicinal plants against cancer (Al-Fatimi *et al.*, 2007). The shrimp lethality assay was proposed by Michael *et al.*, (1956), and later developed by Vanhaecke *et al.*, (1981) and Sleet & Brendel (1983). It is based on the ability to kill laboratory-cultured *Artemia nauplii* brine shrimp larvae (Fig.1.2). The assay is considered a useful tool for preliminary assessment of toxicity (Solís *et al.*, 1993), and it has been used for the detection of fungal toxins (Harwig & Scott, 1971), plant extract toxicity (McLaughlin *et al.*, 1991), heavy metals (Martínez *et al.*, 1999) and pesticides (Barahona & Sánchez-Fortún, 1999) and newly synthesized compounds (Ansari *et al.*, 2006; Ahmad *et al.*, 2007 and Ahmad *et al.*, 2008).



Fig.1.2. *Artemia nauplii* (brine shrimps larvae)
http://i00.i.aliimg.com/img/pb/571/998/483/483998571_347.jpg

A number of other studies have demonstrated the use of the brine shrimp assay to screen plants popularly used as pesticides (Fatope *et al.*, 1993; Ibrar *et al.*, 2001), plants having ethnomedical uses related to cancer (Mongelli *et al.*, 1996), and tropical plants used medicinally (together with screening for larvicidal, fungicidal, and molluscicidal activity by Cepleanu *et al.*, 1994).

1.6.6. Hepatoprotective activity

Liver is the most important organ, which is the centre of metabolism of nutrients such as carbohydrates, lipids and proteins as well as excretion of waste metabolites. Moreover, it metabolises and excretes drugs and toxins absorbed by the digestive tract, thus provide protection against foreign substances by detoxifying and eliminating them (Saleem *et al.*, 2010). Liver is the main target organ for toxicity of xenobiotics and drugs, because hepatocytes, which make up the majority of the liver structure, are very active in the metabolism of exogenous chemicals, and this is one of the major reasons why the liver is a target for toxic substances (Timbrell, 2001). During the detoxification of drugs, reactive oxygen species (ROS) are produced which cause oxidative stress (Kohen & Nyska, 2002) which leads to the hepatic damage.

Hepatic disease is the main cause of morbidity and mortality of people, of all ages throughout the world. Some of the common liver disorders are viral hepatitis, metabolic liver disease; drug induced liver injury, gallstones, alcohol liver disease, non-alcoholic fatty liver disease and autoimmune liver disease etc. Drug/chemical-mediated hepatic injury is the common sign of drug toxicity and accounts for greater than 50% of acute liver failure cases (Lee, 2003). Conventional and allopathic drugs are sometimes insufficient or may cause serious side effects, when used in the treatment of liver diseases. Similarly existing medical treatments for liver diseases are often ineffective, and therefore efforts are being made to search for new effective medications (Seeff *et al.*, 2001). Pharmacologically effective agents from natural products have become a new trend by virtue of their few side effects. So drugs of herbal origin for liver diseases have been in use in Pakistan for a long time. Many plants have been evaluated through experimental tests and proved positive for possessing hepatoprotective potentials. Hepatoprotection or anti hepatotoxicity is the ability to prevent damage to the liver.

1.6.7. Insecticidal activity

Annual post-harvest losses of crops resulting from insect damages, microbial deterioration and others factors are estimated to be 10-25% of worldwide production (Matthews, 1993). These stored grain pests can only be controlled by the use of various

synthetic insecticides and fumigants. Access of everything is bad and it is true too in the case of widespread use of insecticidal that led to development of insect strains resistant to insecticides, toxic residues on stored grain, toxicity to consumers and increasing costs of application (Ribeiro *et al.*, 2003) . So it is an urgent need of the day to explore suitable alternative safe products for pest control. Screening of plant extracts for deleterious effect on insects is one of the approaches in the search of novel biological insecticides (Isman, 1995). Plants are a rich source of natuaceuticals that can be used to develop environment friendly products for insect control (Arnason *et al.*, 1989). These natural substances are naturally occurring insecticides that are derived from plants (Isman, 2000).

Many plants have demonstrated significant insecticidal activity against several insect pests (Isman, 2000; Carlini and Grossi-de-Sá , 2002; Rachid *et al.*, 2006; Uddin *et al.*, 2011a). The toxic effects of plant extracts or pure compounds on insects can be manifested in several ways including toxicity, mortality, antifeedant growth inhibitor, suppression of reproductive behaviour and reduction of fecundity and fertility. Botanicals used as insecticides currently comprise 1% of the world insecticide market (Rozman *et al.*, 2007).

1.6.8. Phytotoxic activity

Plants, being unable to move, have developed ways and means both physically and chemically for producing different toxic substances against the attacks of other predators such as insects, bacteria, and fungi, and to compete effectively with other plant species in their habitat for food and light. The defensive chemicals or secondary metabolites of plants can serve various types of functions. They can be antimicrobial to bacteria, fungi and viruses or herbicidal (Hussain *et al.*, 2010 b) to other plants. These chemicals with phytotoxic potentials are present in almost all plants and their tissues, including leaves, stems, roots, buds, flowers, seeds and bark. Under suitable environmental conditions these may be released in sufficient quantities to affect the growth of neighboring plants (Weston, 1996).

1.7. Family description of research plants

Asteraceae (Compositae) constitute the largest vascular plant family with ca.1700 genera and c. 25,000 species distributed in all continents, except Antarctica (Funk *et al.*, 2009). According to estimates it is assumed that there are 200,000-300,000 species of flowering plants in the world and one out of every 8-12 species of flowering plants is in the Compositae (about 10%). The family is characterized by florets arranged on a receptacle in centripetal heads and surrounded by bracts, anthers are fused in a ring and by the presence of achenes (cypselas) often with a pappus. The habit varies from annual and perennial herbs to shrubs, vines, or trees, although few are true epiphytes. The heads can have one to more than 1000 florets; chromosome numbers range from $n=2$ to high level polyploidy with $n=114$; and species grow in just about every type of habitat from forests to high elevation grasslands, however they are less common in tropical wet forests and more common in open areas (Funk *et al.*, 2005).

1.7.1. Tribe *Inuleae*

Inuleae has 38 genera and 480 species and is found in Northern Hemisphere including Europe, N. Africa, Arabia, S. W and C. Asia. In Pakistan this tribe is represented by 12 genera and 50 species.

1.7.2. Genus *Iphiona*

The genus *Iphiona* has 15 species widely distributed in Mediterranean, Tropical and South Africa, Mascarene Islands and Arabia extending to Central Asia (Anderberg, 1994; Qaiser & Abid, 2003). In Pakistan it is represented by two species viz., *I. aucheri* (Boiss.) Anderb. and *I. grantioides* (Boiss.) Anderb. Taxonomically these species are clearly Saharo-Sindian elements confined to Pakistan, Iran and Oman (Qaiser & Abid, 2005).

1.7.3. *Iphiona grantioides*

Iphiona grantioides is perennial herb, base often woody, branches from base, glandular and pilose, viscid, dense leafy. Leaves are fleshy, cuneate-linear or oblanceolate,

acute-apically 3-lobed, 3-5 *0.8-1.5 cm. Capitula is solitary terminal, radiate, 2-3 cm in diameter. Involucre is 8-12 (-14) mm long, few seriate, dense glandular, bracts linear-oblong, outer bracts herbaceous, 5-8*0.8-1.5 mm; inner membranous, linear, acuminate. Marginal florets are neuter, ligulate, 2-3-toothed. Central florets hermaphrodite, apically glandular. Pappus is with bristle only, 2-3 seriate, 22-72 in number, 6-8 mm long and golden yellow in colour.

1.7.4. Taxonomic position *Iphiona grantioides* (Boiss.) Anderb.

Taxonomic rank	Name of Taxonomic rank
Domain	Eukaryota - Whittaker & Margulis, 1978 - eukaryotes
Kingdom	Plantae - Haeckel, 1866 – Plants
Subkingdom	Viridaeplantae - Cavalier-Smith, 1981
Phylum	Tracheophyta - Sinnott, 1935 ex Cavalier-Smith, 1998 Vascular Plants
Subphylum	Euphyllophytina
Class	Spermatopsida - Brongniart, 1843
Subclass	Asteridae - Takhtajan, 1967
Superorder	Campanulanae - Takhtajan Ex Reveal, 1992
Order	Asterales - Lindley, 1833
Family	Asteraceae(Compositae) - Giseke, 1792
Subfamily	Asteroideae
Tribe	Inuleae
Genus	<i>Iphiona</i> - Boiss.
Specific epithet	grantioides - Boiss.
Botanical name	<i>Iphiona grantioides</i> (Boiss.) Anderb.
Flowering period	Throughout the year.
Vernacular Name	Zair gul in Pashto (Khan <i>et al.</i> , 2011), Naro, Kolmur (Sindhi) (Qureshi, 2012).

1.7.5. Distribution of *Iphiona grantioides* (Boiss.) Anderb. in Pakistan

Iphiona grantioides(Boiss.) Anderb. occur in dry arid and semiarid plains of Pakistan, growing on hilly slopes, stony grounds and dry river beds upto altitude of 700 m in Sindh, Balochistan and Khyber Pakhtunkhwa. In flora of Pakistan it is reported from the following localities of Pakistan. Dist. Bannu (C-6), Kohat Dist.(on the road tokohat from Bannu), Mianwali Dist.(Kalabagh), Kalat Dist. Larkana (Kerther Range) and Dadu Dist. Sakkur Dist, Makran Dist.(Pasni, Awaran, Jhal jao, Turbat, on the way from Bela to Awaran, on the way from sangad to omara, between Sunstar and Mund), Karachi Dist.(Malir, North Nazimabad, Manghopir hills, on the way to landhi, Karachi University Campus, Hyderabad Dist. near Kalri Lake (Qaiser & Abid ,2003).

1.7.6. Ethnomedicinal uses of *Iphiona grantioides* (Boiss.) Anderb.

Locally the plant is boiled in water and is given in snake bite (Atta-ur-Rehman *et al.*, 2006). Paste of the fresh plant is applied on wounds for fastening healing (Qureshi, 2012) .The leaves are stepped in water and used as a remedy for the treatment of asthma (Wazir *et al.*, 2007). It is also used as a fodder for camel and as soap for clothes washing.



Fig.1.3.a. *Iphionao grantioides* (Boiss.)Anderb. Plant. L- Leaf , F- Flower



Fig.1.3.b. *Iphiona grantioides* (Boiss.) Anderb.

1.8.1. Tribe *Plucheeae*

The tribe *Plucheeae* has 28 genera and 220 species. In Pakistan it is represented by 5 genera (*Pluchea*, *Blumeopsis*, *Doellia*, *Laggera* and *Pseudoconyza*) and 8 species (Qaiser & Abid, 2003).

1.8.2. Genus *Pluchea*

Pluchea is a genus of flowering plant in the family *Asteraceae*. Members of this genus are commonly known as camphorweeds, plucheads, or sometimes as "fleabanes". Some are known as sour bushes (*P. carolinensis* and *P. odorata*). There are at least 80 species in the genus, native to tropical and warm temperate areas, distributed in Asia, North and South America, Africa and Australia (Qaiser & Abid, 2003). Mostly they are resinous and bushy shrubs. This genus was named for the French naturalist Noël-Antoine Pluche (13 Nov. 1688 – 19 Nov. 1761), known as the abbé Pluche, was a French priest, known for his *Spectacle de la nature*, a most popular work of natural history (Srivastava & Shanker, 2012). *Pluchea* is represented in Pakistan by 5 species

(*P. arguta* subsp.*arguta*, *P. arguta* subsp. *glabra* Qaiser, *P. lanceolata* (DC.) C. B. Clarke, *P. ovalis* (Pers.) DC. , *P. pinnatifida* (Hook.f.), *P. wallichiana* DC.

1.8.3. *Pluchea arguta* (Boiss.)

An erect branched, stout shrub with pungent smell. Stem and branches terete, glutinous-pubescent to papillose or almost glabrous. Leaves quite variable, 1.5-3 (-3.5) x 0.3-0.8 (2.0) cm, sessile, alternate, sessile, attenuate at base, oblong-ob lanceolate or obovate, acute to subacute, coarsely serrate, dentate, teeth 5-7, sharp, densely glandulose to papillose or completely glabrous on both surfaces. Capitula ovoid or campanulate, 4-12 mm in diameter, solitary or few arranged in subcorymbose fashion; receptacle flat, naked; peduncle glandular hairy to almost glabrous; phyllaries 5-6-seriate, outer lanceolate, acute, serrate, pubescent, 2-3 x c. 0.5 mm, middle acute, pubescent, slightly longer than the outer ones, inner linear acute, entire, 5-7 x c. 0.5 mm. Florets pink or violet, marginal florets female, filiform, many; disc florets bisexual, fewer 6-20 (-30). Anthers bases are caudate. Cypselas small, linear to spindle shaped; pappus setae 6-7 (-10). A variable species, particularly, the leaf breadth and the indumentum. Densely glandular pubescent to almost glabrous forms are met with in our region. Two subspecies are recognized as:

1. *Pluchea arguta* subsp. *arguta*.

2. *Pluchea arguta* subsp. *glabra* Qaiser

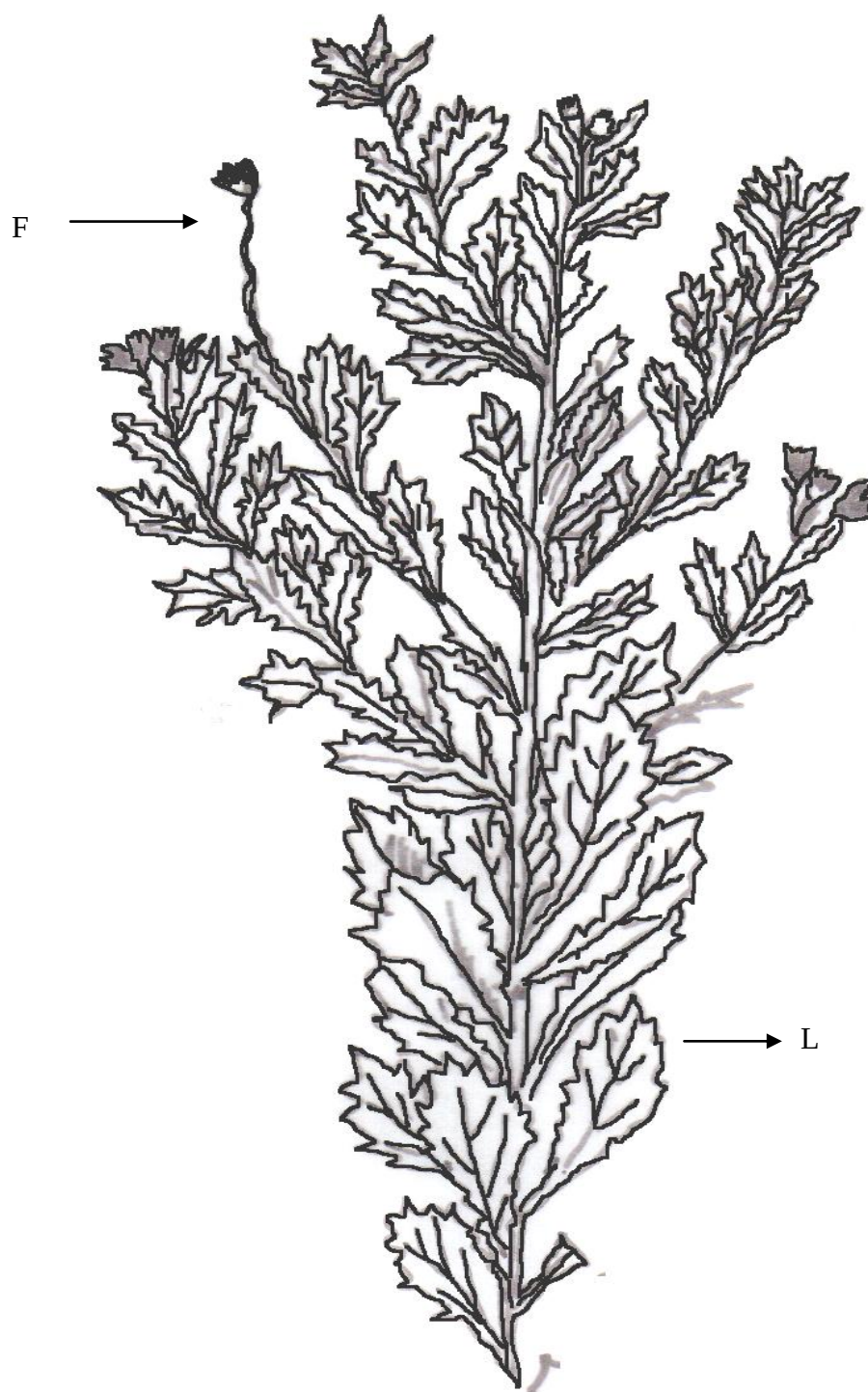


Fig. 1.4.a. *Pluchea arguta* subsp. *glabra*. F-Flower, L-Leaf.

1.8.4. *Pluchea arguta* subsp. *glabra* Qaiser

Pluchea arguta subsp. *glabra* is distributed throughout Pakistan while *P. arguta* subsp. *arguta* is confined to Sindh and Baluchistan only. *P. arguta* subsp. *arguta* differs from *P. arguta* subsp. *Glabra* (Fig. 1.4.a & 1.4.b) by having densely pubescent branches and leaves. The leaves are somewhat less dentate and the capitula are somewhat larger, 8-12 mm in diameter than the subsp. *glabra*. However, as far as the leaf dentation and the size of capitula are concerned, few intermediates are also met with, but the character of pubescence seems to be rather stable.



Fig.1.4.b. *Pluchea arguta* subsp. *glabra*

1.8.5. Taxonomic position of *Pluchea arguta* subsp. *glabra*.

Taxonomic rank	Name of Taxonomic rank
Domain	Eukaryota - Whittaker & Margulis, 1978 - eukaryotes
Kingdom	Plantae - Haeckel, 1866 - Plants
Subkingdom	Viridaeplantae - Cavalier-Smith, 1981
Phylum	Tracheophyta - Sinnott, 1935 ex Cavalier-Smith, 1998 Vascular Plants
Subphylum	Euphyllophytina
Class	Spermatopsida - Brongniart, 1843
Subclass	Asteridae - Takhtajan, 1967
Superorder	Campanulanae - Takhtajan Ex Reveal, 1992
Order	Asterales - Lindley, 1833
Family	Asteraceae(Compositae) - Giseke, 1792
Subfamily	Asteroideae
Tribe	<i>Plucheeae</i>
Genus	<i>Pluchea</i> - Cassini, 1817
Specific epithet	<i>arguta</i> - Boiss.
Botanical name	<i>Pluchea arguta</i> (Boiss.)subsp. <i>glabra</i> Qaiser
Flowering period	All the year round.
Vernacular Name	Kasstera

1.8.6. Distribution in Pakistan

Pluchea arguta Boiss. subsp. *glabra* Qaisar is mostly confined to arid areas of salt range hills in Punjab near Khoshab-Chakwal Road, Jhelum Dist, Rawalpindi Dist. In KP, Salt range Hills on Kohat-Bannu road, In Balochistan in Lasbella Distt., in Makran Distt. In sindh, in Dadu Distt., in Karachi Distt. near Clifton and in Hyderabad Distt. (Qaiser & Abid, 2002).

1.8.7. Ethnomedicinal uses of *Pluchea arguta* subsp. *glabra*

It is a small branched shrub; leaves are bright green with serrate margins. The aerial parts are glabrous and glandular (Qaiser, 2002). It is locally called “Kasstera” in Pashtoo. Decoction of the whole plant is used as a diuretic (Qureshi, 2012) and in Karak district the plant is used as camel fodder.

CHAPTER-2

REVIEW OF LITERATURE

2. Review of Literature

In the proposed study, pharmacognostic studies of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* were carried out. *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* belongs to the tribe *Inuleae* and *Plucheeae* of family *asteraceae*, respectively. The present review is focused on various reported studies related to these plants.

2.1. Review of literature for *Iphiona grantioides*

Iphiona grantioides (Boiss.) Anderb belongs to *Inuleae*, a cosmopolitan tribe of *asteraceae*. It grows on a wide range of soils including hilly slopes dry river beds, saline region and arid plains to 700m (Qaiser & Abid, 2003). *Iphiona grantioides* is a perennial herb, with woody base, 15-60 cm tall; the areal parts are glandular with hairy fleshy, lobed leaves and yellow flower (Qaiser & Abid, 2003). Ahmad & Ismail (1991 a) isolated triterpenes, sesquiterpenes, sterols and a new flavonol from the aerial parts of *Iphiona grantioides* (*Inula grantioides*), while Roeder *et al.*, (1994) isolated kaurane glycosides, terpinoides, two diterpenes glycosides, atractylolide, carboxyatractylolide and a non toxic pyrrolizidine from another species, *Iphiona aucheri*.

2.1.1. Chemical constituents

Burdi *et al.*, (1991) analysed the flowers of *Iphiona grantioides* for its sterol composition and isolated different fatty acids including myristic, pentadecanoic, palmitic, margaric stearic, arachidic behenic, tricosoic, lignoceric, hexadecanoic and heptacosic acids. Chromatographic separation of the acetone extract of the plant material resulted in the isolation of β -sitosterol, stigmasterol and β -sitosteryl glucoside. 11-Hentriacontene, pentatriacontene and a saturated hydrocarbon, pentatriacontane, were obtained for the first time from the flower of the plant. 5-hydroxy- 3, 6, 7, 2', 5' -pentamethoxyflavone 1, β -

Sitosterol, lupeol, taraxasterol, taraxasterol acetate, and triacontanol were also isolated for the first time from this plant (Burdi *et al.*, 1990; 1991).

Abid & Qaiser (2003) isolated flavonols, flavone o-glycoside, c-glycosyl flavones and tricin 5-glucoside from *Iphiona grantioides* and *I. aucheri*. In Sindh the plant is boiled in water and is given in snake bite (Atta-ur-Rehman).

The plant is used in Hepatitis, scabies and decoction of the whole plant has been reported effective when given in snake bite. Oil of the plant has been reported to possess strong antibiotic activity (Attur-ur- Rehman, 2006).

The leaves are stepped in water and used as a remedy for the treatment of asthma (Wazir *et al.*, 2007). Paste of the plant is applied on wounds for fastening healing (Qureshi, 2012).

2.1.2. Review of literature for other species of *Iphiona*

Iphiona aucheri (Boiss.) Anderb. is another species of genus *Iphiona* in Pakistan (Qaiser & Abid, 2003). Roeder *et al.*, (1994) reported *I. aucheri* to be responsible for camel poisoning in United Arab Emirates. Stewart *et al.*, (2000) isolated a number of substances from the aerial parts of *I. aucheri* in which atractyloside and carbxyatractyloside were identified as the toxic principle of the plant.

Martin *et al.*, (2001) reported the ethnobotanical uses of *Iphiona rotundifolia* and *Euphorbia somaliensis* from Somalia. Latex of *Euphorbia somaliensis* and an infusion of *Iphiona rotundifolia* and camel urine are used for controlling mange (Mange is a type of skin disease caused by parasitic mites).

Sharaby *et al.*, (2009) tested the ethanolic extract of *Iphiona scraba* and 10 other medicinal plants and some repellent oils for their effect on certain biological aspects of the insects. Results indicated that extracts of *Iphiona scraba* caused the maximum inhibition of egg hatchability.

Kakar *et al.*, (2012) investigated the crude methanol extracts (CME) of *Iphiona aucheri* and other three plants i.e. *Berberis baluchistanica*, *Seriphidium quettense*, *Ferula costata* against three Gram positive bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae*, and *S. pyogenes*) and four Gram negative bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). Plant extracts of all the four plants showed effectiveness against all the tested bacteria.

2.2. Review of literature for *Pluchea arguta* subsp. *glabra*

Recent chemical investigation of *Pluchea arguta* subsp. *glabra* reveals the presence of many sesquiterpenes and eudesmane type sesquiterpenes i.e., Argutin, Argutinin, Arguticin, 3-chloro-2-hydroxy-arguticin, Pluchecin, Pluchecin, 4-Epi-pluchecin, Odontinin, Odonticin, Odontin, Odonticin, 5-hydroxy-3, 6, 7, 2', 5'-Penta-methoxy-flavone, 3-chloro-2-hydroxy-arguticin, deacetoxy-3-chloro 2-hydroxy arguticin, 4,5-epi-cryptomeridiol, Pluchargutin, Plucharguticin, Terpinoides, Cuauhtemone ester and Argutenol (Ahmad *et al.*, 1986, 1987, 1988a, 1988b, 1988c, 1989a, 1989b, 1990, 1991a, 1991b, 1992a, 1992b, 1992c and 1995). Recent chemical investigation of *Pluchea arguta* (Boiss.) subsp. *glabra* reveals the presence of eudesmane sesquiterpene, pluchargen A (3-beta-[3'-chloro-2'-hydroxy-2'-methyl butyryl]-cuauhtemone) from the whole plant (Saba *et al.*, 2011).

Yogeshkumar (2009) studied analgesic and anti-inflammatory potentials of *Pluchea arguta*. Decoction of the whole plant is used as a diuretic (Qureshi, 2012) and the plant is used as a camel fodder in district Karak (present research area).

2.3. Review of literature of other species of the two genera i.e., *Iphiona* and *Pluchea* (Asteraceae).

Dried root of *Inula rhizocephala* var. *rhizocephaloids* (Clarke) Kitam. is used for treating colds, cough and chest complaints in Leh valley (India) by the local people (Chaurasia *et al.*, 1996).

Deriu *et al.*, (2008) investigated antimicrobial activity of essential oils from *Inula helenium*, belonging to the same tribe. Khan *et al.*, (2010) worked out secondary metabolites of *Inula britannica* and investigated biological activities of these two plants.

The essential oil of *Inula helenium* root contains eudesmane-type sesquiterpene lactones with alantolactone and isoalantolactone, thymol derivatives triterpenes, sterols and polysaccharide inulin. The sesquiterpene lactones showed cytotoxic and antiproliferative activities against human cancer cell lines. Furthermore, the compounds also inhibited plant growth (Lawrence *et al.*, 2001; Dirsch *et al.*, 2001; Konishi *et al.*, 2002).

Inula obtusifolia is useful for domestic animals suffering from diphtheria. It is also used for tuberculosis, chest problems and cough and as an antiseptic (Khan & Khatoon, 2008).

Diversified effects such as antidiabetic, hypolipidemia (Han *et al.*, 2004), antitumor, antifungal, antibacterial, hepatoprotective and antihepatitic (Cha *et al.*, 2006 and Wang *et al.*, 2007) were exhibited by dried leaves and roots of *Inula japonica* in modern pharmacological studies.

Inula helianthus-aquatica C. Y. Wu is a medicinal plant used to treat some cancers in traditional medicine of Yunnan, China. A sesquiterpene lactone namely Bigelovin has been isolated from this herb which acts as an inhibitor to eight cancer cell lines, prominent example being human monoblastic leukemia U937 cells (Zeng *et al.*, 2009).

Khan *et al.*, (2010) reviewed the bioactive phytochemicals and pharmacological potentials of *Inula britannica*. The plant is a rich source of secondary metabolites. Various compounds have been isolated from the flowers and aerial parts; mainly consist of terpenoids (sesquiterpene lactones and dimers, diterpenes and triterpenoids) and flavonoids. These compounds have shown various biological activities including hepatoprotective, antioxidant, anticancer, anti-inflammatory and neuroprotective activities.

Inula britannica is used for treating digestive disorders, bronchitis, and inflammation (Bai *et al.*, 2005). Its extracts have shown different biological activities i.e.,

antidiabetic, anti-inflammatory, antihepatitic, antibacterial and antitumor activities (Song *et al.*, 2000; Kobayashi *et al.*, 2002; Song *et al.*, 2002). Several sesquiterpene lactones have been isolated which have shown cytotoxic activity (Liu *et al.*, 2004; Rafi *et al.*, 2005).

Inula viscosa (L) Aiton. has been used for years in traditional medicine for its antipyretic, anti-inflammatory, antiphlogistic and antiseptic activities (Lev & Amar, 2000) and for treating diabetes (Yaniv *et al.*, 1987). *Inula viscosa* showed antifungal activity under in vitro conditions (Maoz *et al.*, 1999), while evidence of antifungal activity was reported by Cohen *et al* (2002). *I. viscosa* has also anti ulcerogenic effects (Alkofahi & Atta, 1999); causes abortion (prevents implantation of zygote in mammals (Al-Dissi *et al.*, 2001), prevents growth of pathogenic fungi (Maoz & Neeman, 2000) and has a strong antioxidant activity (Schinella *et al.*, 2002). There is also evidence for nematocidal/antihelminthic properties of *I. viscosa* (Oka *et al.*, 2001). *Inula viscosa* contains some pharmacologically active compounds including lactones, flavonoids, sesquiterpenes, azulenes, and essential oils (Marongiu *et al.*, 2003).

Nikomtat *et al.*, (2011) evaluated the methanol and dichloromethane extracts of *Inula cappa* (Ham. ex D. Don) DC. for inhibition of *herpes simplex* virus infection. Higher anti-herpes simplex virus (HSV) activity of *I. cappa* methanol extract was observed as compared to *I. cappa* dichloromethane extract. Within 30 hours, the effective inhibitory activity of methanol extract on HSV replication was higher than that of the dichloromethane extract. Both extracts had potent activities in reducing viral deoxyribonucleic acid (DNA) synthesis.

Gnanasekaran (2012) evaluated the hepatoprotective activity of the roots of *Inula racemosa* on the Chang cell line (normal human liver cells) by SRB assay (Sulphorhodamine B assay) and MTT assay [(3-(4,5 dimethylthiazole -2 yl)-2,5 diphenyl tetrazolium bromide) assay]. The results showed that the plant has a potential for hepatoprotectivity.

Talib *et al.*, (2012) reported that *Inula viscosa* (L.) showed antiproliferative properties in the cell lines in MTT assay and showed antimicrobial activities against *Bacillus cereus* and *Salmonella typhimurium*.

Juliani *et al.*, (2002) tested *Pluchea Arabica* (Boiss.) Qaiser & Lack for antibacterial activity and the results showed positive potential of the plant.

Suliman *et al.*, (2006) carried out steam GC–MS analysis of essential oils of the fresh twigs of *Pluchea arabica*. A total of 44 compounds were identified in the oil which exhibited antimicrobial activity against *Staphylococcus aureus* (ATCC 29213), *Candida albicans* (ATCC 10231) *Bacillus subtilis*, and inhibited the growth of all the three test species.

Methanol fraction of *Pluchea indica* root extract (PIRE) showed significant anti-inflammatory activity, in invivo test, against glucose oxidase induced paw oedema (Sen *et al.*, 2002).

Ghoshet *al.*, (2008) carried out in vitro antioxidant activities of methanolic root extract of tissue cultured *Pluchea indica* (L.) in the presence of (DPPH) radical. The extract showed effective reducing power and inhibition of β -carotene bleaching.

Jahangir *et al.*, (2005) studied the effect of *P. lanceolata* on the restoration of enzymes activity of antioxidant defence system (such as catalase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase) and on chromosomal aberrations. The results indicated that pre-treatment with *P. lanceolata* reduces the cadmium chloride induced oxidative stress and genotoxicity by altering antioxidant enzymes and reducing chromatid breaks and micronuclei formation.

Barros *et al* (2006) evaluated the antinociceptive and anti-inflammatory activities of the ethanolic extract from aerial parts of *Pluchea quitoc* DC. in mice and rats. The results showed that oral treatment with the extract (1–2 g/kg, p.o.) decreased the paw oedema induced by carrageenan in rats and also showed antinociceptive effects in the tail-flick test and in

acetic acid-induced writhing in mice, and inhibited both phases of pain (neurogenic and inflammatory) of the formalin test in rats.

Fernández and Torres (2006) carried out scavenging antioxidant activity of *Pluchea carolinensis* leaves.

Pramanik *et al.*, (2006) investigated the hypoglycemic and anti hyperglycemic activity of methanolic extract of *Pluchea indica* leaves in normal and in streptozotocin induced diabetic rats respectively. The extract showed reduction in blood glucose level in normal (35.12% and 36.01% for 200 and 400 mg/kg, p. o. (per os). respectively) and in streptozotocin induced diabetic rats (36.10% and 41.87% for 200 and 400 mg/kg, p.o. respectively). Acute toxicity study was also performed for the extract, which revealed that the extract is safe to use even at the doses of 3.2 gm/kg of body weight orally.

Pramanik *et al.*, (2007) established a genotype of *Pluchea indica* (L.) Less. through micro propagation, and evaluated the diuretic activity of the leaf extract of the tissue cultured plant in Wistar albino rats. Urinary excretion parameters were studied for evaluation of diuretic activity using Frusemide (20 mg/kg, p.o.) as standard. The extract showed significant diuretic activity at the doses of 100, 200 and 300 mg/kg. p.o (per os- Oral administration). An oral acute toxicity study for the extract was carried out and the LD₅₀ value was found to be 2,825 mg/kg body weight.

Marwah *et al.*, (2007) investigated the antioxidant activity of aqueous ethanol extract of the whole plant of *Pluchea arabica* and *P. crispa*, using in *in vitro* DPPH and phosphomolybdenum assay methods. The aqueous ethanol extract of *Pluchea arabica* showed 89–93%, DPPH antioxidant activity at a concentration of 50 µg /ml and the ethanol extract of *P. crispa* showed highest antioxidant capacity as gallic acid equivalents of 1790 mg/g in the phosphomolybdenum assay .

Gomes *et al.*, (2007) reported the anti-snake venom activity of root extract of *Pluchea indica* in experimental animals. The main active components isolated from the root extract were beta-sitosterol and stigmasterol. These active fraction (beta-sito sterol and stigma sterol)

significantly neutralized viper venom induced lethality, hemorrhagic, defibrinogenation, edema and Phospholipases A2 (PLA2s) activity.

Literature reviewed showed that *Pluchea arabica*, *P. ovalis*, and *P. indica* strongly inhibited different strains of fungi and Bacteria (Juliani *et al.*, 2002 and Pramanik & Chatterjee, 2008).

Roslida *et al.*, (2008) investigated the ethanolic extract of *Pluchea indica* leaf (PIL) for anti-inflammatory and antinociceptive activities by using carrageenan – induced oedema model and acetic acid induced writhing test. The leaf extract exhibited significant anti-inflammatory activity at an oral dose of 300 mg/kg, and i.p administration of PIL at a dose of 10, 30, 100 and 300 mg/kg produced significant inhibition of abdominal constriction when induced with 0.6% (v/v) acetic acid, in dose dependent manner.

Pluchea indica extract was screened for flavonoid and total phenolic contents and antioxidant activity. Results showed that extracts inhibited oxidation of linoleic acid and had DPPH, ABTS, and ferric cyanide antioxidant capacities. Therefore, the plant may add to dietary antioxidant intake (Andarwulan *et al.*, 2010). Khan *et al.*, (2010) carried out the phytochemical screening of *Pluchea lanceolata*. Bauer *et al.*, (2011) reported strong anti-neoplastic effect of *Pluchea odorata* extract in HL60 and MCF-7 cells and an anti-inflammatory response in HUVECs (human umbilical vein endothelial cells).

Arora *et al.*, (2011) evaluated the anti-asthmatic potential of *Pluchea lanceolata* ethylacetate fraction in in vitro animal model in isolated guinea pig tracheal chain preparation. The results showed ethyl acetate fraction showed significant anti-asthmatic activity (57.81 ± 1.22) at 100 μ g dose.

Cho *et al.*, (2012) investigated different concentrations of crude aqueous extracts of leaf and root of *Pluchea indica* (L.) for anticancer activities (antimigration, antiproliferation, and proapoptotic properties) on human malignant glioma cancer cells (GBM8401) and human (HeLa) cervical cancer cells and the underlying molecular mechanism. Both the extracts suppressed proliferation, viability, and migration of GBM8401 and HeLa cells.

2.4. Pharmacognostic studies

Pharmacognostic study of the crude drugs gives scientific information regarding the purity and quality of plant derived drugs (Sindhu, 2010) and provided important information about morphology, macroscopic, microscopic and physical characteristics of the crude drugs (Sharma, 2004).

Pharmacognostic parameters including determination of number of stomata mm^{-2} , stomatal index, total number of epidermal cells mm^{-2} , total number of vein-islets mm^{-2} , total number of vein termination mm^{-2} , Palisade ratio, Type and number of trichomes in leaf, parenchyma cell, cambium, xylem and phloem tissues of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabrawill* be studied in the present work. Physicochemical studies verify the identity of plant and make certain the quality and purity of crude drug (Patel, 2011), therefore the physiochemical studies of the research plants will also be carried out in order to obtain these parameters also.

2.4.1. Ashing

Determination of ash values is an important tool for finding adulterants in crude herbal drugs. Ash values are determined by various ways (total ash, acid insoluble ash and water soluble ash) in pharmacognosy (Jarald & Jarald, 2007). Several references are available for the determination of ash values, some of which are *Dodonaea viscosa* (Venkatesh *et al.*, 2008); *Pluchea lanceolata* (Khan, *et al.*, 2010); *Murraya koenigii* (Handral *et al.*, 2010); *Acacia leucophloea* (Gupta *et al.*, 2010); *Lagenaria siceraria* (Shah *et al.*, 2010); *Amaranthus spinosus* (Mathur *et al.*, 2010); *Lawsonia inermis* (Jain *et al.*, 2010); *Geniosporum prostratum* (Singhal *et al.*, 2010); *Tylophora dalzellii* (Najafi & Deokule, 2010); *Jatropha curcas* (Nayak & Patel, 2010); *Amaranthus spinosus* (Balakrishnan *et al.*, 2011); *Swertia chirayita* (Bisht *et al.*, 2011); *Acorus calamus* (Subha *et al.*, 2011), *Hygrophila auriculata* (Hussain *et al.*, 2011), *Crocus sativus* (Kumar *et al.*, 2011); *Carica papaya* (Zunjar *et al.*, 2011); *Bryophyllum pinnatum* (Patel *et al.*, 2011); *Azadirachta indica* (Gomase *et al.*, 2011); *Zizyphus xylopyrus* (Jain *et al.*, 2011); *Murraya koenigii* (Sindhu

&Sandeep,2012) ; *Melissa parviflora* (Bhat *et al.*, 2012) and *Callicarpa macrophylla* (Kumar *et al.*, 2012).

2.4.2. Extractive values

Extractive values are very useful parameters for the evaluation of crude drugs as they indicate the nature of the chemical constituents present in a crude drug (Khandelwal, 2004).The following account describes the recent work done on the extractive values of different medicinal plants i.e., Nayak *et al.*, 2010 (leaf of *Jatropha curcas*), Subha *et al.*, 2011 (*Acorus calamus*), Balakrishnan *et al.*, 2011 (*Amaranthus spinosus*), Kumar *et al.*, 2011 (*Crocus sativus*) , Hussain *et al.*, 2011a (*Hygrophila auriculata*), Zunjar *et al.*, 2011 (leaves of *Carica papaya*) and Barkatullah *et al.*,2012 (*Skimmia laureola* and *Zanthoxylum armatum*).In the present work the extractive values of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* will be worked out using different solvents.

2.4.3. Fluorescence study

Many drugs are used in powder form and almost all the crud drugs are dried and powdered for solvent extraction. The powder of plant tissues has specific fluorescence under UV light. This property is a useful tool for evaluation and for detection of adulteration, contamination as well as for identification of various chemical constituents of the crude drug (Gupta *et al.*, 2009). Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiations.The fluorescence analysis of powder drugs, treated with different chemical reagents, under visible & UV(254 & 366 nm) lights, show different color fluorescence.Saha *et al.*, (2011), Iqbal *et al.*, (2011) and Kalidass *et al.*, (2011) have carried out the fluorescence studies for various medicinal plants.On the same lines, fluorescence study of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* will be carried out in the present research work.

2.4.4. Phytochemical analysis

Phytochemical screening of medicinal plants has revealed the presence of numerous secondary metabolites, including alkaloids, steroids, flavonoides, glycosides, tannins and

saponins etc. Secondary metabolite serves as protection system against predation by herbivores, insects and microorganisms (Cowan, 1999). Most of the therapeutic properties of medicinal plants are due to the presence of these secondary metabolites in certain anatomic part of their body.

Phytochemical screening is an important step which leads to the isolation of new and novel compounds. Uddin *et al.*, (2011b) carried the phytochemical screening of different parts such as leaves, bark, roots of *Pistacia chinensis* var. *integerrima* and identified different classes of secondary metabolites. The study revealed the presence of alkaloids, terpenoids, flavonoids and tannins. Ibrar *et al.*, (2013a) investigated the active constituents of nut galls of wild olive tree. Barkatullah *et al.*, (2012) worked out the phytochemical constituents of *Viola canescens*. In the present study it is intended to carry out phytochemical screening of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*, in order to know the nature of secondary metabolites present in these plants.

2.4.5. Heavy metals analysis

The curative effect of medicinal plants for the treatment of various diseases is based on the chemical compounds and different elements in them (Morabad *et al.*, 2012). Although herbal medicines have less side effects as compared to allopathic medicine but the presence of heavy metals in plants, which may be toxic when present in higher amounts, can cause health issues (Hussain *et al.*, 2006). According to the recommendation of WHO (1998) medicinal plants must be checked for the presence of heavy metals and other contaminations. Recent work done on the heavy metal analysis of different medicinal plants includes Pytlakowska *et al.*, (2012); Djama *et al.*, (2012); Khan *et al.*, (2012), Khuda *et al.*, (2012) and Ibrar *et al.*, (2013b).

2.5. Biological activities

2.5.1. Antihelmintic activity

Parasites have been of concern to the medical field for centuries and the helminthes still cause considerable problems for human beings and animals, upsetting a huge

population of the world (Bundy, 1994). In spite of many advances made in understanding the mode of transmission and the treatment of helminths, still there are no efficient products to control certain helminths and the indiscriminate use of drugs has produced resistance in them (Coles, 1999). Several plants are reported, possessing anthelmintic properties such as *Inula helenium* (El Garhy & Mahmoud, 2002); *Artemisia brevifolia* (Iqbal *et al.*, 2004); *Calotropis procera* (Iqbal *et al.*, 2005); *Nicotiana tabacum* (Iqbal *et al.*, 2006a); *Butea monosperma* (Iqbal *et al.*, 2006b); *Ginger* (Iqbal *et al.*, 2006c); *Swertia chirata* (Iqbal *et al.*, 2006d); *Mentha piperita* and *Lantana camara* (Girme *et al.*, 2006); *Trachyspermum ammi* (Abdul Jabbar *et al.*, 2006); *Pongamia glabra* (Nirmal *et al.*, 2007); *Enhydra fluctuans* (Ghosha *et al.*, 2007); *Mimusops elongi* (Mali *et al.*, 2007); *Annona squamosa* (Choudhary, 2007); *Clitoria termatea* Linn. (Khadatkar *et al.*, 2008); *Vernonia guineensis* (Toyang *et al.*, 2012) and *Embelia ribes* (Choudhary, 2012). All these plants showed significant antihelmintic activity against various species of endoparasitic annelids of cattle. Similarly ethanol and aqueous extracts of stem of *Piper betle* Linn (Adate *et al.*, 2012), and crude methanolic extract and its subsequent solvent fractions of *Viola betonicifolia* (Naveed *et al.*, 2012) were tested against the adult species *Pheretima posthuma* and juvenile species of *Cephalobus litoralis* and *Helicotylenchus indicus*. Similarly methanolic extract of Nut galls from leaves of wild olive tree have been found possessing very significant antihelmintic activity (Ibrar *et al.*, 2013 a). As no such study is reported for *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*, so it is intended to find the antihelmintic potential of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* in the present study.

2.5.2. Antilice activity

Pediculus humanus capitis De Geer (Pediculidae) is an obligate ectoparasite which is found entirely on humans. Lice have been evolved with mankind and distributed all over the world (Burgess, 2004; Mehlhorn & Mehlhorn, 2009). The infestation with head lice is widespread throughout the world (Falagas *et al.*, 2008) and has been increasing since the start of the 1990s due to inefficiency of antilice products (Burgess, 2009). So, there is a tendency of finding and development of new alternative antilice products, especially those containing plant-derivative active ingredients. Till now various plants have been studied for their

effectiveness to eradicate head lice .e.g., Tiangda *et al.*, (2000) studied the hexane extract of seeds of *Annona squamosa* L. against head lice and found it to be very effective. Sunilsonet *al.*, (2009) got very effective results of *Pongamia pinnata* as antilice plant. Other antilice plants are *Dichrostachys cinerea* (Vijayalakshmi *et al.*, 2010); *Mucuna pruriens* (Singh *et al.*, (2011); *Syzygium aromaticum* (Asokanet *al.*, 2011); *Abrus precatorius* (Upadhyay *et al.*, 2011); *Dalmatian chrysanthemums* (Mullaicharam & Al-Matroushi, 2012) and *Myristica fragrans* Houtt (Surendra *et al.*, 2013).

As no antilice activity of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* has been worked out, so in the present study, these two plants will be screen out for antilice activity.

2.5.3. Antimicrobial activity

Many researchers have carried out antibacterial studies of various plants, as e.g., antibacterial activities of ethyl acetate, methanol, chloroform and acetone extracts of whole plant of *Artemisia absinthium* were studied by Erdogrul (2002). Aqil & Ahmad (2007) reported the antibacterial activity of 66 ethanolic plant extracts including *Seriphidium quettense*, against nine different strains of bacteria in which 39 plants extract showed highest antibacterial activity against bacteria. Similarly Ahameethunisa & Hopper (2010) examined six organic solvent extracts of *Artemisia nilagirica* against 15 different strains of bacteria in which the hexane and methanol extracts showed highest antibacterial activity. Zhao *et al.*, (2010) isolated four thymol derivatives from the roots of *Inula hupehensis* (a medicinal plant) which were tested for their antimicrobial activities against three bacteria and six plant pathogenic fungi.

Nisha *et al.*, (2010) reported that the ethanol extract of *Artemisia sieversiana* exhibited maximum zone of inhibition against *Escherichia coli* whereas chloroform extract produced maximum zone of inhibition against *B. subtilis*. Kakar *et al.*, (2012) tested the methanolic extracts of *Berberis baluchistanica*, *Iphiona aucheri*, *Ferula costata* and *Seriphidium quettense*, against three gram positive bacteria (*Staphylococcus aureus*, *Streptococcus pneumonia* and *Streptococcus pyogenes*) and four gram negative bacteria

(*Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*). Extracts from all the plants were found effective against all the tested bacteria. Rauf *et al.*, (2012) scrutinized the methanolic extract and their subsequent solvent fractions of three Pakistani medicinal plants for their antibacterial and phytotoxic potentials. As no such antibacterial activity has been reported on *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*, so in the present work the antibacterial potential of these two indigenous plants will be worked out.

2.5.4. Antioxidant activity

Oxidants are free radicals which are atoms or groups of atoms with an unpaired number of electrons, produced in biological systems from a wide range of sources, when oxygen interacts with certain molecules. Once formed these highly reactive atoms react with important cellular components mainly DNA and the cell membrane, may oxidize nucleic acids, proteins, lipids by starting a sequence reaction and cause degenerative disease. As a result cells may function inadequately or expire. Healthy body protect itself from free radical damage through the defense system of antioxidants. Antioxidants are compounds which trap free radicals (oxidants) and protect ourselves from various chronic diseases including cancer and heart disease. Natural antioxidants are present in fruits vegetables, grains, tea and red wine. Antioxidant compounds like phenolic acids, polyphenols and flavonoids, scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Many references of plants possessing antioxidant potential are available. Some of them are i.e., Greek aromatic plants (Couladis *et al.*, (2003); *Baccharis articulate*, *Baccharis spicata*, *B. trimera* and *B. usterii* (De Oliveira *et al.*, 2003, 2004); *Baccharis trimera* (Januario *et al.*, 2004); *Globularia alypum* (Es-Safi *et al.*, 2005) ; *Cissus multistriata* (James *et al.*, 2008); *Syzygium cumini* (Zhi *et al.*, 2008); *Stevia rebaudiana*, *Citrus sinensis* and *Saccharum officinarum* (Ahmad *et al.*, 2011); *Achillea millefolium*, *Acorus calamus*, *Arnebia nobilis*, *Fumaria indica*, *Gymnema sylvestre*, *Origanum vulgare*, *Paeonia emodi*, *Peganum harmala*, *Psoralea corylifolia*, *Rauvolfia serpentina* and *Vetiveria zizanioides* (Fazal *et al.*, 2011), *Lagenaria siceraria* (Mayakrishnan, 2012); *Elaeagnus umbellata* (Uddin *et al.*, 2012b) and *Citrullus colocynthis* (Benariba *et al.*,

2013).As no such study is done on the *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*, so it is planned in the present work to find out antioxidant potential of these two plants.

2.5.5. Cytotoxic activity

Various workers have studied medicinal plants for their cytotoxic potentials, Ara *et al.*, 2009 (Antimicrobial and cytotoxic activities of leaves and flowers extracts from *Lippia alba*), Haque *et al.*, 2009 (Antibacterial and cytotoxic activities of different parts of plant *Swietenia mahagony*), Ramachandran *et al.*, 2011 (Assessment of cytotoxic activity of *Agave cantula* using brine shrimp (*Artemia salina*) lethality bioassay), Ali *et al.*, 2011 (Acute toxicity, brine shrimp cytotoxicity and relaxant activity of fruits of *Callistemon citrinus* Curtis.), Rahman *et al.*, 2011 (Antidiabetic and cytotoxic activities of methanolic extract of *Tabernaemontana divaricata* (L.) leaves in alloxan induced mice), Mahmood *et al.*, 2012 (Biological activities of family Solanaceae), Barkatullah *et al.*, 2012 (Pharmacological study of *Viola canescens*), Rehmanullah *et al.*, 2012 (Phytotoxic, cytotoxic and insecticidal activities of *Calendula arvensis*), Amin *et al.*, 2012 (antimicrobial and cytotoxic activity of three bitter plants, *Enhydra fluctuans*, *Andrographis peniculata* and *Clerodendrum viscosum*), Venugopal *et al.*, 2012 (Mineral composition, cytotoxic and anticariogenic activity of *Scleropyrum pentandrum*), Killedar *et al.*, 2012 (Phytochemical investigation and cytotoxic screening of *Lagenaria siceraria* Standley. fruit using brine shrimp lethality assay models), Obayedullah *et al.*, 2013 (brine shrimp lethality bioassay of methanolic extracts of fourteen different edible vegetables). In the present research work, the cytotoxic potential of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* will be worked out in line with the reported works.

2.5.6. Hepatoprotective activity

Several studies have been carried out to assess the hepatoprotective potential of medicinal plants belonging to family asteraceae; *Inula britannica* (Song *et al.*, 2000), *Inula racemosa* (Gnanasekaran 2012 & Manipuri *et al.*, 2013); *Calendula officinalis* (Ali & Khan, 2006); *Cichorium intybus* (Butt *et al.*, 2012; Nallamilli *et al.*, 2013). Others plants

investigated for this purposes are *Azadirachta indica* (Chattopadhyay, 2003); *Pergularia daemia* (Sureshkumar & Mishra, 2007); *Leucas cephalotes* (Sailor *et al.*, (2010); *Butea monosperma* (Maaz *et al.*, 2010); *Flacourtia indica* (Gnanaprakash *et al.*, 2010); *Haloxylon salicornicum* (Ahmad & Eram, 2011); *Abutilon bidentatum* (Yasmin *et al.*,2011) *Chromolaena odorata* (Alisi *et al.*,2011) and other medicinal plants (Al hassan *et al.*,2012). As no hepatoprotective potential of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* has been worked out so far, so it is intended in the present work to find out hepatoprotective potential of these two plants.

2.5.7. Insecticidal activity

Insects of various kinds occur in large numbers in parcels of drugs. They mostly belong to *Lepidoptera* (Moths), various members of *Coleoptera* (beetles), *Orthoptera* (Cockroaches) and *Hymenoptera* (ants). Different insects cause great loses to our stored grains and various food and other articles in homes. The damage is mostly done by the larvae, which, as they feed, bore tunnels into the drugs, producing quantities of fine powder commonly called “pore-dust” (Wallis, 1985).

Liu *et al.*, (2006) carried out study to evaluate the insecticidal activity of isoalantolactone isolated from *Inula racemosa*, against rice weevil (*Sitophilus oryzae* (L.) (Coleoptera Curculionidae) and noted that the toxicity to rice weevil was dose dependent, whereas the repellency was not. Results indicated that isoalantolactone might be considered for control of weevils in stored wheat grains. Chaubey (2007) isolated and carried out analysis of essential oils from the dried fruits of three common spices, *Anethum graveolens*, *Nigella sativa* and *Trachyspermum ammi*, by hydrodistillation and tested its toxic, repellent, and developmental inhibitory activities against wheat flour insect pest (*Tribolium castaneum*). Essential oils of the three plants showed repellent effect on the adults of *T. castaneum* at low concentrations in the filter paper repellency assay. Fumigation with these essential oils caused the death of larvae and adults of *T. castaneum*. and inhibited the development of larvae to pupae and pupae to adults and also resulted in the deformities in the different developmental stages of the insect. Similarly insecticidal activity of various other plant have been reported e.g., *Crysanthemum* species (Haouas *et*

al.,2008); *Trichodesma indicum*, *Aconitum leave* and *Sauromatum guttatum* (Khan *et al.*, 2008); *Annona squamosa* (Kumar *et al.*, 2010); *Indigofera heterantha* (Uddin *et al.*, 2011a); *Azadirachta indica*, *Curcuma longa*, *Catharanthus roseus*, *Chrysanthemum trifurcatum* and *Gymnema sylvestre* with (Umadevi & Sujatha , 2013). In order to know the insecticidal properties of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*, it is planned to screen out these plants for insecticidal properties.

2.5.8. Phytotoxic activity

Weeds cause great reduction in productivity of our crops, as these compete with the other crops for nutrients, water, light and space. A large number of synthetic weedicides are being used to eradicate weeds from the fields. Though effective to great extent, these synthetic weedicides /herbicides cause various environmental hazards by polluting soil, air and water. There has been a continued effort to look for more safe, effective and cheap herbicides/ weedicides from natural (plant) sources.

There are several methods for the screening of plants/plants products for their phytotoxic behavior. The best phytotoxicity bioassay that has received much attention recently is the *Lemna minor* bioassay. *Lemna minor* is a small, free floating, fast-growing plant, easy to cultivate. These characteristics make it an ideal candidate for very useful, cheap and rapid toxicity testing. Studies for Phytotoxicity of different medicinal plants has been carried out by various workers i.e., Hussain *et al.*, 2010b (cytotoxicity and phytotoxicity of some selected medicinal plants of family Polygonaceae); Ahmad *et al.*, 2011 (screening of *Zizyphus jujuba* for antibacterial, phytotoxic and haemagglutination activities); Naveed & Saeed, 2011 (biological screening of *Viola betonicifolia* whole plant) and Ibrar *et al.*, 2013a (investigation of wild olive nut galls for phytotoxic activity). In the present study phytotoxic potential of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* will be determined through *Lemna minor* bioassay and a positive result will be in the list of already known phytotoxic plants, which can be used in some form as useful & safe weedicides/herbicides.

CHAPTER-3

MATERIALS AND METHODS

3.1. Morphology of the two research plants

The two research plants were studied in their natural habitat following Anderberg (1994), Qaiser & Abid (2003) and Qaiser & Abid (2005).

3.2. Phytosociology

Phytosociological studies for the both plants were carried out following Hussain (1989) Raunkiaer (1934) and Hussain (1989).

3.2.1. Phytosociology of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Six study sites were selected in Karak district for the current study. The sites were Malgeen (Salt Deposits), Nari Panoos, Bahader Khel (Old tunnel), Chushmai (Andi) Karak, Karat Ghar, Noshpa, Salt Range and Bannu-Kohat Rd, Sour Daag.

The field work was carried out from June 2010 to June 2011. In the first phase (June, 2010 – December 2010) soil samples were collected from the six study sites and analyzed in the laboratory for various physical and chemical parameters. In the next study phase (January, 2011 – June 2011) plant community structure was studied and plant specimens were collected from the six study sites and preserved according to the standard procedure, i.e. dried in plant presser with the help of blotting paper. Dried specimens were sprinkled with the powdered Naphthalene balls for protection against insects and fungal attacks, mounted on herbarium sheets. The specimens were identified with help of flora of Pakistan (Qaiser & Abid, 2003) and Mr. Ghulam Jelani, Curator, Herbarium, department of Botany, University of Peshawar, Pakistan, where voucher specimens number were assigned to the sheets and kept in the same herbarium for reference. Plants identification was also confirmed from Herbarium, Department of Botany, University of Karachi.

3.2. 2. Community analysis

Community analysis was carried out during rainy season when most of the plants were at the climax of their development. In every studied sites, 10 quadrats of 10 m × 10 m (100 sq m), 4 × 4m and 1m× 1m size were laid down randomly to study trees, shrubs and herbs of the area, respectively. The important quantitative analysis i.e., density, abundance and frequency of trees, shrubs and herbs were determined according to Hussain (1989).

3.2.2. a. Density

Density is an expression of the numerical strength of a species where the total number of individuals of each species in all the quadrats is divided by the total number of quadrats studied.

Density is calculated by the equation:

$$\text{Density} = \frac{\text{Total number of individuals of a species in all quadrats}}{\text{Total number of quadrats studied}}$$

Relative density

Relative density is the numerical strength of a species in relation to the total number of individuals of all the species and can be calculated as:

$$\text{Relative density} = \frac{\text{Number of individual of the species} \times 100}{\text{Number of individual of all the species}}$$

Ten density classes were made as follow, and the values of mid points were used for the calculations (Badshah, 2011).

Class	Range	Mid-point value
1	Up to 10	5
2	11-20	15
3	21-30	25
4	31-40	35
5	41-50	45
6	51-60	55
7	61-70	65
8	71-80	75
9	81-90	85
10	91-100	95

3.2. 2. b. Frequency (%)

Frequency means the expression of degree of occurrence of a species in terms of percentage occurrence. It was studied by sampling the study area at several places randomly and recording the name of the species that occurred in each sampling units. It is calculated by the equation:

$$\text{Frequency (\%)} = \frac{\text{Number of quadrats in which the species occurred}}{\text{Total number of quadrats studied}} \times 100$$

Relative frequency

The degree of dispersion of individual species in an area in relation to the number of all the species occurred.

$$\text{Relative frequency} = \frac{\text{Number of occurrence of the species} \times 100}{\text{Number of occurrence of all the species}}$$

3.2. 2. c. Cover of herbs

Cover means the vertical projection of foliage, shoot/ crown of a species to the ground surface expressed as fraction or percent of a surface area.

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

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

Plant cover was determined by the following 10 cover classes (Badshah, 2011).

Class	Range	Midpoint value
1	Up to 10	5
2	11-20	15
3	21-30	25
4	31-40	35
5	41-50	45
6	51-60	55
7	61-70	65
8	71-80	75
9	81-90	85
10	91-100	95

3.2. 2. d. Importance Value Index (IVI)

Overall importance of each species in the community structure is determined by the Importance Value Index. In calculating this index, the percentage values of the relative frequency, relative density and relative cover were summed up together and this value was designated as the Importance Value Index or IVI of the species (Curtis, 1956).

$$\text{Importance value (IV)} = \text{RD} + \text{RC} + \text{RF}$$

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

3.2. 2. e. 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

3.3. 1. Soil sampling

Soil samples were collected randomly from six study sites during June 2010 – December 2010. The soil samples were collected from the depth of 0-15cm, air dried and passed through a 2 mm mesh sieve to remove large sized particles and large root fragmen. The sieved soil was used for detail analysis.

3.3.2. Soil texture

The soil texture was determined by Bouyoucos hydrometer method (Brady, 1990).

3.3.3. Soil pH

Soil pH was measured by mixing 10 gram of soil sample with 50 ml of distilled water and stirred for 20 minutes in a 100 ml beaker using magnetic stirrer. The mixture was kept overnight and the reading was taken with the help of digital pH meter (Jackson, 1962).

3.3.4. Electrical Conductivity

Electrical conductivity of the soil was determined in 1: 5 soil water suspensions with EC meter.

3.3.5. Soil organic matter

Soil organic matter was determined by rapid dichromate oxidation technique. The organic matter in the soil was oxidized by chromic acid (Potassium dichromate plus conc. H_2SO_4), utilizing the heat of dilution of H_2SO_4 . The unreacted dichromate was determined by back titration with ferrous sulphate (Rayan *et al.*, 1997).

3.3.6. Total Nitrogen

Soil nitrogen was determined by Kjeldahl method of Bremner & Mulvaney (1982).

3.3.7. Phosphorus

Phosphorus was determined by following the method of Olsen & Sommers (1982).

3.3.8. Potassium (K)

Potassium was determined by using flame emission spectrophotometry (Rhoades, 1982).

3.4. Ethnobotany

Plants were grouped according to their common use in the area. The information about their use was collected from the local people of the area and Hakims.

3.5. Pharmacognosy

3.5.1. Pharmacognosy of *Iphiona grantioides* and *Pluchea arguta* subsp. *Glabra* Kaiser

The fresh specimens of both the plants (*Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*) were collected from district Karak. Each specimen was cleaned, washed, separated into leaf, branches and roots and shade dried for almost two weeks. Macroscopical characters were studied at the time of collection. The dried drugs were used for different tests i.e powder drug study, microchemical tests, nutritional analysis, elemental analysis and biological activities. After drying the specimens were grinded in electric grinder and sieved. The fine powder of each specimen was preserved in airtight bottles to keep it safe from deterioration and moisture. Fresh of both the plants were preserved and later used for section cutting, epidermal & stomatal studies and histological studies.

3.5.2. Macroscopical study

The morphological studies of various parts (root, stem, leaf, flower) of the two plants (*Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*) were carried out following (Trease and Evans, 1985; Evans, 2002).

Leaf parameters

- Colour
- Taste
- Odour
- Size
- Duration
- Insertion
- Phyllotaxis
- Presence and absence of petiole
- Presence and absence of stipule

Lamina

- a. Composition
- b. Incision
- c. Venation
- d. Margin
- e. Leaf apex
- f. Leaf base
- g. Surface
- h. Texture

Stem parameters

- Colour
- Odour
- Taste
- Shape
- Size
- Thickness
- Fracture
- Texture

Roots parameters

- Colour
- Odour

- Taste
- Shape
- Size
- Thickness
- Type of root
- Fracture
- Texture
- Rootlets

3.5.3. Microscopic studies of leaf, stem and root of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Thin transverse sections of various parts of the two research plants (*Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*) were made with the help of sharp blade. Transverse sections were made in a large number and only 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).

3.5.4. Leaf surface study

It includes presence and absence of stomata, type of stomata, stomatal number, stomatal index determination, Vein islets and vein termination number and Palisade cell ratio.

3.5.4. a. Presence and absence of stomata/ Type of stomata

Leaf of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* was study for the presence or absence of stomata on both the epidermises and the stomata types were following Dilcher (1974).

Procedure

A small piece (1sq mm) of the leaf was boiled, till clearing, in chloral hydrate solution in a test tube for few minutes. A part of upper and lower epidermis was peel out with the help of forceps and mounted in a drop of glycerin on a glass slide, and placed on the microscope stage. The epidermal cells were traced and studied for the presence or absence of stomata and the type of stomata was identified according to Dilcher (1974).

3.5.4. b. Determination of stomatal Number (SN) and Stomatal index (SI)

Stomatal number (SN), also called stomatal density, is the number of stomata present in 1 sq. mm of a leaf epidermis on each surface (upper and lower surface). Stomatal index (SI) is the % ratio of the number of stomata to the total number of epidermal cells (each stoma is counted as one cell) (Evans, 2002).

Procedure

A small piece (1sq mm) of the leaf was boiled, till clearing, in chloral hydrate solution in a test tube for few minutes. A part of upper and lower epidermis was peel out with the help of forceps and mounted in a drop of glycerin on a glass slide, and placed on the microscope stage. Stomata and epidermal cells were traced, and number of stomata present in 1 sq. mm was recorded. This practice was repeated ten times and average number of stomata per sq. mm present on each surface of the leaf was worked out and stomatal indices were calculated by the formula:

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

I = Stomata index,

S= No. of stomata per unit area,

E = No. of epidermal cells in the same unit area.

3.5.4. c. Vein islets and vein termination number

The smallest area of the photosynthetic tissue encircled by the final division of veins in leaf is known as vein islets number per sq. mm of the leaf. A vein termination is the ultimate free termination of vein-let and their number per sq.mm of the leaf. (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 and evaluation of leaf powder drug.

Procedure

Small piece of leaf lamina of about 2 sq.mm from tip, base, margins and mid regions were taken and boiled in a test tube containing concentrated chloral hydrate solution till clarity. The test tube was placed in a water bath (Evans, 2002). Latter, the piece was mounted on glass slide, in a drop of glycerin and examined under the power of microscope (5 X). Light was focused on the specimen through diaphragm adjustment. Starting from one side, all the vein islets were counted along with the veinlet termination number. Ten readings were taken for accurate calculation (Evans, 2002).

3.5.4. d. Palisade cell ratio

The average number of palisade cell present beneath each upper epidermal cell is called Palisade Cell Ratio (Evans, 2002). This value is also constant for a species like other constants and is not affected by geographical variation, therefore it is a diagnostic feature for characterization, standardization and identification of a particular species (Shruthi *et al.*, 2010). Palisade ratio is determined by counting the palisade cells beneath four continuous upper epidermal cells and then calculating the average.

Procedure

Small piece of leaf lamina of about 2 sq. mm, from the region between midrib and margins, were taken and boiled in a test tube containing concentrated chloral hydrate solution till clarification (Shruthi *et al.*, 2010). The plant pieces were mounted on microscopic glass slide, in a drop of glycerin, and examined under the microscope. The

microscope was adjusted in such a way that the epidermal cells and the palisade cells lying below them could be seen at the same time by a slight rotation of the fine adjustment of microscope. A number of groups of each of four epidermal cells was focused and then the underlying palisade cells under each were counted and traced, (Including the palisade cells in the count when more than half is covered in the area of epidermal cell). The average number of palisade cells beneath one epidermal cell was calculated by dividing with 4 to get the palisade ratio.

3.5.5. Study of physiochemical characteristics of the crud powder drug of *Iphiona grantioides Pluchea arguta* subsp. *glabra*

Crude Powder drug of *Iphiona grantioides* (flower, leaf, branch and root) and *Pluchea arguta* subsp. *glabra* (leaves branch and root) were studied for physiochemical characteristics. It includes the determination of ash values and moisture contents.

3.5.5. a. Ash analysis

Ash Analysis was carried out to detect adulteration or exhausted drugs. Analysis of ash includes total ash, acid insoluble, and water soluble ash.

Principal

The inorganic material left after ignition of organic matter at very high temperature is designated as ash. It is carbon free and includes the salts present in the crud drug. Ash may include the adulterants/contaminants added to the drugs. The ash is different from the original material as there may be losses due to volatilization or chemical interaction between the constituents (Jarald & Jarald, 2007). Ash analysis is very useful for the detection of adulterated and exhausted drugs (Wallis, 1985).

➤ **Total ash determination**

Equipment and glassware:

Analytical Balance, Muffle furnace, crucibles, desiccators, burner and tongs

Procedure

2-2.5 g of plant sample was taken in a clean, oven-dried silica crucible (W_1). The crucible was placed on burner and the plant material was burnt to make it smoke free. The partially ignited sample was then placed in the muffle furnace and the temperature was gradually raised to 600°C for several hours until the sample was turned in to white – gray ash. The furnace was turned off and when the temperature fell down to about 100°C , then the crucible along with the ash was taken out and placed in desiccators to cool. The crucible was weighed and again heated in the muffle furnace until a constant weight was obtained (W_2). The amount of ash was calculated according to the formula (Wallis, 1985; A. O. A. C., 2000).

$$\text{Ash \%} = \frac{W_2 - W_1}{\text{Wt. of sample}} \times 100$$

Weight of empty crucible = W_1

Weight of crucible with ash = W_2

Total ash (mg/g) = $W_2 - W_1$

➤ **Water soluble ash value**

Water insoluble ash is useful in detecting the presence of water exhausted materials in drugs (Jarald & Jarald, 2007).

Procedure

25 ml water was added to the crucible containing the total ash as obtained in the above procedure and boiled for 5 minutes. The insoluble matter was collected in an ash less

filter paper, washed with hot water and ignited in an electric furnace at 450 °C in crucible until it reaches to a constant value. The weight of insoluble matter was subtracted from the weight of the total ash to obtain the weight of water soluble ash.

➤ **Acid insoluble ash value**

Acid insoluble ash value is helpful analytical tool in drugs sample which have variable amount of Ca- Oxalate crystals or which are adulterated by clay, chalk, lime or sand (Wallis, 1985).

Procedure

Total ash was obtained as mentioned in section 3.6.ix.a. 25 ml of dilute 10% HCl was added and covered with watch glass and heated for 5 min. The watch glass was rinsed with 5 ml of hot distilled water and this liquid was also added to the crucible. The liquid was filtered through an ash less filter paper (Whatman No. 41) and the residue left on the ash less- filter paper was transferred to the crucible and ignited in the furnace to get a constant weight (W_3). The amount of acid insoluble ash was calculated according to the formula:

W_2 = Weight of crucible with ash.

W_3 = Weight of crucible with residue left after on the ash less filter paper.

Acid insoluble ash (mg/g) = $W_2 - W_3$

3.5.5. b. Determination of the moisture

Equipment and glassware: Electric oven, Petri dish, desiccators, electric balance.

Procedure

Moisture contents of the plant sample were determined by following A.O.A.C (2000). Plant sample (1-2 gram) was taken in a clean petri dish (W_1) and dried in an oven at 105°C, for 4-6 hours, until constant weight was obtained. The petri dish, after cooling for 30 minutes in the desiccators was weighed again (W_2) and the percent moisture was

calculated. The loss in weight after drying was the moisture contents of the sample (A.O.A.C. 2000).

$$\% \text{ Moisture} = \frac{W_1 - W_2}{\text{Wt. of the sample}} \times 100$$

Whereas

W_1 = weight of empty Petri dish

W_2 = weight of Petri dish + Sampl

3.5.6. Powder drug study of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Microscopic study includes the study of various tissues of (different parts) present in the powder drug while physical characteristics include color, taste and odour of the powder drug.

Procedure

Fine powder drug of each part was taken in a very small amount and macerated with Chloral hydrate, water and iodine solution in small beakers, separately. One drop of solution was taken on a clean and clear glass slide, warmed over the flame of spirit lamp for few seconds and observed under microscope, using 45 X and 10X objectives lenses for observation of different structures. The tissues observed were then sketched carefully (Wallis, 1985).

3.5.7. Phytochemical screening of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

The two plants were investigated for the presence/ absence of different phytochemicals, in order to establish a profile for the chemical composition of the given extract. Qualitative phytochemical analyses were carried out using the procedures of

Kokate (1994); Trease and Evans 1985; Harborne, 1998; Evans, 2002; Rangari, 2002; Khandelwal, 2004; Kumar & Kiladi, 2009 and Chitravadivu *et al.*, 2009.

➤ **Detection of Alkaloids**

50 mg of the solvent free extract was stirred with few ml of dilute Hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows.

- **Mayer's test**

To a few ml of filtrate, one or two drop of Mayer's reagent was added by the side of the test tube. A white or creamy precipitate indicated the test as positive (Khandelwal, 2004).

Mayer's reagent

Mercuric Chloride (1 – 35 g) was dissolved in 60 ml of water and Potassium Iodide (5.0 g) was dissolved in 10 ml of water. The two solutions were mixed and made up to 100 ml with water.

- **Wagner's test**

To a few ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish – brown precipitate confirmed the test as positive (Khandelwal, 2004).

Wagner's reagent

Iodine (1.27 g) and Potassium Iodide (2 g) was dissolved in 5 ml of water and made up to 100 ml with distilled water.

- **Hager's test**

To a few ml of filtrate, 1 or 2 ml of Hager's reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive (Khandelwal, 2004).

Dragendorff's reagent**Preparation of stock solution**

Solution A: 1.7 g basic Bismuth nitrate in 100 ml water / Acetic acid (4:1).

Solution B: 40 g Potassium Iodide in 100 ml of water.

Mix reagents together as follows: 5 ml A + 5 ml B + 20 ml Acetic acid + 70 ml water.

The solution was stored in amber – coloured bottle.

Working solution

Stock solution (10 ml) was mixed with 20 ml of Acetic acid and made up to 100 ml with water.

- **Dragendorff's test**

To a few ml of filtrate, 1 – 2 ml of Dragendorff's reagent was added. A prominent yellow precipitate indicates the test as positive (Evans, 2002, Rangari, 2002).

➤ **Detection of Carbohydrates**

The extract (100 mg) was dissolved in 5 ml of water and filtered. The filtrate was subjected to the following tests.

- **Molish's test**

To 2 ml of filtrate, two drops of alcoholic solution of α -naphthol were added, the mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A violet ring indicates the presence of carbohydrates (Evans, 2002).

- **Fehling's test**

One ml of filtrate was boiled on water bath with 1ml each of Fehling solutions A and B. Red precipitates indicate the presence of sugar (Evans, 2002).

Fehling's solution

Fehling's solution A

Copper sulphate (34.66 g) was dissolved in distilled water and made up to 500 ml distilled water.

Fehling's solution B

Potassium sodium tartarate (173 g) and sodium hydroxide (50 g) was dissolved in water and made upto 500 ml.

- **Benedict's test**

To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A characteristic colored precipitate indicated the presence so sugar (Evans, 2002).

Benedict's reagent

Sodium citrate (173 g) and sodium carbonate (100 g) were dissolved in 800 ml distilled water and boiled to make it clear. Capper sulphate (17.3 g) dissolved in 100 ml distilled water was added to it.

➤ **Detection of Glycosides**

For detection of glycosides, 50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hrs on water bath, filtered and the hydrolysate (product of hydrolysis) was subjected to the following tests.

- **Keller-Killani test**

1ml of glacial acetic acid containing traces of FeCl_3 and 1 ml of concentrated H_2SO_4 was added to the extract carefully. Colour appearance will confirm the presence of glycosides in the root extracts (Harborne, 1998).

- **Borntrager's test**

1ml of benzene and 0.5 ml of dilute ammonia solution were added to the extract. A black brown colour was obtained which show the presence of glycosides in the root extracts.

➤ **Detection of Saponins**

- **Foam test**

50 mg of the extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 10-15 minutes. Formation of a two cm layer of foam indicates the presence of saponins (Trease and Evans 1985).

- **Sodium bicarbonate test**

Few drops of sodium bicarbonate (Na OH) were added to 10 mg of the extract and shaken well. Formation of honey comb like frothing indicates positive test for saponins.

➤ **Detection of Proteins and Amino Acids**

The extract (100 mg) was dissolved in 10 ml of distilled water and filtered through whatman No.1 filter paper and the filtrate was tested for proteins and amino acids.

- **Millon's test**

To 2 ml of filtrate, few drops of Millon's reagent were added. Appearance of white precipitate indicates the presence of proteins (Evans, 2002).

Millon's reagent

1 gram of Mercury was dissolved in 9 ml of fuming nitric acid. When the reaction was completed, equal volume of distilled water was added.

- **Biuret test**

An aliquot of 2 ml of filtrate was treated with one drop of 2 % copper sulphate solution. 1 ml of ethanol (95%) was added to this solution, followed by excess of potassium hydroxide pellets, pink colour in the ethanolic layer indicates the presence of proteins.

- **Ninhydrin test**

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of actone) were added to 2 ml of aqueous filtrate and boiled; a characteristic purple colour indicates the presence of amino acid (Kumar & Kiladi, 2009).

➤ **Detection of Phytosterols**

- **Libermann – Burchard's test**

50 mg of the extract was dissolved in 2 ml of acetic anhydride. To this solution, one or two drops of concentrated H_2SO_4 were added slowly along the side of the test tube. An array of colour changes shows the presence of phytosterols (Harborne, 1998).

- **Salkowski's reagent.**

Extract solution in chloroform was treated with few drops of concentrated H_2SO_4 and shaken well. Appearance of red colour in the lower layer will indicate the presence of

sterol while yellow color will show the presence of triterpenoids. Absence of colour in the upper layer indicates the absence of phytosterol and triterpenoids (Harborne, 1998).

➤ **Detection of Fixed Oils and Fats**

- **Grease Spot test**

Powder drug was taken in a small quantity and pressed between two filter papers; appearance of an oily stain indicated the presence of fats and oil (Evans, 2002).

- **Volatile oil detection test**

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

➤ **Detection of Calcium Oxalate**

The crystals may be identified as calcium oxalate, if these are soluble in HCl, and H_2SO_4 without effervescence (Trease and Evans 1985).

➤ **Detection of Tannin**

- **Ferric chloride test**

The presence of bluish black or greenish colour after treating the extract with dilute solution of ferric chloride indicates the presence of tannins (Trease and Evans 1985).

- **Gelatin test**

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

- **Lead acetate test**

Few drops of lead acetate solution (5%) were added to the alcoholic extract of the root. White precipitate was appeared which confirm the presence of Phenolic compounds (Kokate, 1994).

- **Alkali reagent test**

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

- **Detection of Flavonoids**

- **Shinoda test (Magnesium Hydrochloride reduction test)**

To the extract, few pieces of magnesium ribbon were added and concentrated Hydrochloric acid was 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).

- **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).

3.5.8. Florescence study

Florescence is the emission of different lights when treated with different chemical reagents and then observed in ordinary visible light and ultraviolet radiation. This technique is helpful in identification of powder drugs (Jarald & Jarald, 2007).

Material required

UV Lamp (254 nm and 366 nm), glass slides, different reagents

Reagents

50% HNO₃, 50% H₂SO₄, 50% HCl, NH₃ Solution, NaOH in Water, NaOH in ethanol, Picric acid, Iodine Solution, 10% FeCl₃ and Acetic acid.

Procedure

A pinch of each powder drug was taken on clean slide and treated with the various reagents and observed under visible day light and UV light of both short and long wave lengths (Evans, 2002).

3.5.9. Extractive values in different solvents

Extractive values are very useful parameters for the evaluation of crude drugs as they indicate the nature of the chemical constituents present in a crude drug (Khandelwal, 2004). In this regard polarity of a solvent is very important characteristic in extracting chemical constituents from powder drugs. For example water can extract water soluble glycosides and mucilaginous substances; tannins and resins are soluble in alcoholic solvents, while ether can extract oily and fatty substances. Moreover extractive values can detect contaminants and adulterants, if present in the crude drugs (Jarald & Jarald, 2007). So the powder drug of the two plants were extracted with different solvents including Acetone, Butane, Ethanol, Ethyl acetate, n- Hexane, Methanol and Water for extractive values determination.

Material required:

Analytical balance, beakers, funnel, filter papers, solvents of different polarity, air tight bottle, rotary evaporator

Extractive values of the two plants were determined by following the method of Ansari *et al.*, (2006). 5 gram of each powder drug was macerated in 200 ml of an organic solvent in air tight bottle, for 7-8 days period with occasional shaking. The extracts were filtered and the filtrate was dried to a semi solid residue in a rotary evaporator. The percent extractive value of the solvent was calculated as follow.

$$\% \text{ Extractive value} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times 100$$

3.6. Heavy metal analysis

Elemental analysis of powder drug of different parts of the two plants was carried out for heavy metals with atomic absorption spectrophotometer.

Chemical and equipments required

HNO₃, H₂SO₄, H₂O₂, HF, Perchloric acid, HCl, Pb, Cd, Co Mn (Sigma made) and Cu, Zn and Fe (Aldrich made). All the glass and plastic equipments were thoroughly cleaned with distilled water before use.

Sample preparation

For elemental analysis samples were prepared by wet digestion method (Perkin (1991)). One gram of each powder drug was taken in a small conical flask and 10 ml of concentrated HNO₃ was added and kept for 24 hours at room temperature, followed by the addition of 4 ml of Perchloric acid (HClO₄, 67%). After half an hour, the flasks were placed on hot plate and heated carefully till the production of red NO₂ fumes ceased and a clear solution (1 ml) was left. After cooling, the final volume was made up to 100 ml by addition of double distilled water and filtered through Whatman No. 42 filter paper. Each filtrate served as the stock solution for each sample. They were preserved in air tight bottles for elemental analysis through Flame Atomic Absorption Spectrophotometer (Polarized Zeeman Hitachi 2000) and Flame Photometer (Jenway PFP7, UK) in triplicate. Calibration standards of each metal was prepared by appropriate dilution of the stock solution (Saeed *et al.*, 2010a). The protocol for the operation is given in the table below.

Table.3.1. Conditions applied for the detection of various heavy metals elements

Elements	Flame type	Wavelength (nm)	Slit width (nm)	Acetylene flow(L/min)	Air Oxide Flow(L/min)	Cathods 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
Ni	Air/acetylene	232	0.2H	2.0	17	25
K	Air/acetylene	766.5	0.7H	2.0	17	12
Na	Air/acetylene	589	0.2H	2.0	17	0.8
Fe	Air/acetylene	248.3	0.2H	2.3	17	30

Statistical analysis

Arithmetic mean and standard deviation was used to analyze the data statistically (Saeed *et al.*, 2010 a).

3.7. Biological assays

Preparation of extracts

Different parts of the two plants were pulverized into fine powder using an electric grinder. Hundred grams of each plant parts was soaked in 300 ml of 70 % ethanol for 15 days. Thereafter each plant extract was filtrated through Whatman filter paper No.1823. This process was repeated for 3 times. The extracts were concentrated to a semi solid substance by evaporating in a rotatory evaporator at 40°C. The different extracts were

stored at 4°C for future use. Extracts of the two plants were used to carry out the following bioassays in order to know their therapeutic potentials.

3.7.1. Antihelmintic activity.

Bioassay was conducted to assess the antihelmintic potential of *Iphiona grantioides* and *Pluchea arguta* sub sp. *glabra* by following Deore *et al.*, (2009).

Requirements

Petri dishes, Analytical balance, DMSO, Distilled water, Standard Vermicide drug (Piperazine citrate), top watch, beaker, Saline Ringer Drip, Test worms, Piperazine citrate.

Procedure

The assay was performed on mature earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings (Deore *et al.*, 2009). Earthworms (3 to 7 cm in length and up to 0.2 cm in diameter) were collected from wet field soil and washed with normal saline. 50 ml solution of Piperazine citrate and different concentrations of plant extracts (25, 50 and 100 mg/ml each), dissolved in distilled water were taken in petridishes. Nine groups of more or less equal sized healthy earthworms consisting of five earthworms in each group were used. Group-1 served as negative control, received normal saline, while Group-2 served as positive control, received standard drug Piperazine citrate (10mg/ml). Group-3 to 9 served as extract test groups and received different concentrations (25, 50, 100 mg/ml) of ethanolic extracts of various parts of the two research plants. Observations were made for the time taken to paralysis or death of individual worms. Time for paralysis was noted when no movement of any type could be observed except when the worms were shaken vigorously. Death was confirmed when the worms lost their motility followed with fading away of their normal reddish body colour.

3.7. 2. Anti-lice activity

The head lice are ectoparasite of humans, especially in children and female of poor countries. Licitidal potential of the two plants, parts extract was assessed by following Yang *et al.*, (2003).

Requirments

Cleaned Petri plates (60 mm), Brush, Lice collecting comb, Plastic Jars, Filter paper, Lice etc

Collection of head lice

Adult *Pediculus humanus capitis* were collected from primary school children between the age group of 4-12 by combing through sections of the scalp using a clean comb, the lice were removed from the teeth of the comb very carefully, into plastic jars. Before the start of the experiment, the lice were fed by placing them on the bare foot of the volunteer for some time (Lee *et al.*, 2003).

Preparation of plant sample

Ethanolic extracts of various plant parts of the two plants were dissolved in distilled water to obtain three different concentrations (5%, 10%, and 20%).

Procedure

Ehanolic extracts of the two plants, parts were tested for antilice activity by filter paper diffusion method (Lee *et al.*, 2003; Yang *et al.*, 2003). Adult lice were identified and separated from nymph after careful selection under a dissecting microscope, and divided into 9 groups (5 lice each) and placed on a filter paper at the bottom of petri dish and kept uncovered. A 0.5 ml of each test sample was poured on the test organism and allowed to spread as a thin layer of 4 cm². Group-1 was treated with 0.5 ml of distilled water and served as negative control. Group-2 to 8 received 0.5 ml of different concentrations (5%, 10%, and 20%) of each ethanolic extract, respectively. Group-9 was treated with 0.5 ml of 5%, 10%, and 20% of Coopex (Antilice solution) as positive control. All the Petri dishes

were placed for 60 minutes in a dark chamber at $26 \pm 0.5^{\circ}\text{C}$ and $70 \pm 1\%$ humidity. After 60 minutes, petri dishes were taken out and 0.5 ml of distilled water was applied and again placed in the dark chamber under the condition mentioned above. After 18 hour, the petri dishes were taken out and lice were observed under a dissecting microscope for any possible movement. Absence of any movement was considered as dead. The activity was performed in triplicate for each concentration of the extracts. The data was expressed as mean value and the percentage mortality was calculated with the help of the following formula:

$$\text{Percentage mortality} = 100 - \frac{\text{No of lice alive in test} \times 100}{\text{No of lice alive in control}}$$

3.7.3. Antibacterial activity

The antibacterial study of the crude extracts was carried out by agar well diffusion method following Carron *et al.*, (1987).

Principle

The antimicrobial compounds present in the plant extracts are permitted to diffuse out into the medium and interact with the test organisms, in a freshly seeded plate. The resultant zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 36 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured on 6 mm petri plates (25-30 ml/plate) while still molten.

Nutrient broth (1L)

One liter of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000 ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and uncontaminated by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Bacterial Strains

Bacterial strains were obtained from the Microbiology Lab, department of Animal health and veterinary Sciences, Agriculture University, Khyber Pakhtunkhwa. Bacterial strains used were, *Bacillus licheniformis*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Micrococcus luteus*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, *Staphylococcus viridans*, *Staphylococcus aureus* and *Salmonella typhi*.

Procedure:

Various strains of bacteria were first cultured on nutrient broth and incubated for 24 hours before the start of experiment. Wells were bored in the media (nutrient agar) using 5 mm diameter of sterile metal cork borer and keeping a distance of 24 mm 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. The process was repeated three times turning the plate 60° between the streaking. About 100 µl of 3 mg /ml of respective plant extract, dissolved in DMSO was then added to the wells. Other wells were supplemented with DMSO and 10 µg Ciprofloxacin served as negative and positive control, respectively. Plates were incubated for 24 hours at 37 °C. They were then observed for zones of inhibition. All the experiments were conducted in triplicates.

3.7.4. Antifungal activity

Antifungal activity of the crude plant extracts was evaluated by following the method of agar tube dilution method developed by Atta-ur-Rehman *et al.*, (1991). Plant extracts were screened for their antifungal potential against various pathogenic fungal

strains including 4 human pathogenic strains i.e., *Trichophyton longifusus*, *Candida albicans*, *Microsporum canis*, *Candida glaberata*, 2 animal pathogenic fungi *Aspergillus flavus*, *Aspergillus niger* and the 3 plant pathogenic types *Fusarium solani*, *Mucor spp.* and *Alternaria spp.*

Material required for antifungal activity

Seven days old fungal cultures obtained from PCSIR labs Peshawar Pakistan, plant extracts, Sabouraud Dextrose Agar, Dimethyle Sulphoxide (DMSO), Screw cap test tubes, compound micropipette, autoclave, incubator, standard reference antibiotic (Miconazole).

Test fungi.

Human pathogens: *Trichophyton longifusus*, *Candida albicans*,
Microsporum canis, *Candida glaberata*,

Animal pathogens: *Aspergillus flavus*, *A. niger*, *Mucor spp*

Plant pathogens: *Fusarium solani*, *Alternaria spp.*

Preparation of SDA media

Sabouraud agar (SDA) is a type of agar containing peptones. It is used to cultivate dermatophytes and other types of fungi. It was prepared by Raymond Sabouraud in 1892, and it has the following composition.

40 g/L dextrose

10 g/L peptone

20 g/L agar

pH 5.6

For antifungal activity, media was prepared by dissolving 6.5 mg of SDA in 100 ml of distilled water, pH was adjusted to 5.6. 4ml of SDA (non- solidified media) was dispensed in each tube and the screw capped tubes were autoclaved for sterilization at 121 °C for 20 minutes.

Preparation of Stocksolutions

Stock solution was prepared by dissolving 24 mg of each crude extracts in 1 ml of sterile dimethyl sulfoxide (DMSO), separately. 12 mg of Miconazole was dissolved in one ml of DMSO to prepare a positive control solution and pure DMSO was used as negative control.

Loading of sample (plant extract stock solution) to SDA

Screw capped tubes were cooled up to 45 °C. Stock solutions of the plant extracts and standard antifungal drug were added to the non-solidified SDA with micropipette to give the final concentration of 400 ug / ml of the plant extracts and 200 ug / ml of the Miconazole. Tubes were then allowed to solidify in slanting position at room temperature. For each fungal species tubes were prepared in triplicate

Inoculation of media with fungus, incubation and measurement of fungal growth inhibition

Each tube was inoculated with a piece of inoculum (4 mm in diameter), of a seven days old culture of fungus. Media was supplemented with reference antifungal drugs (Miconazole) and DMSO, for positive and negative control, respectively. The tubes were incubated at 27-29 °C for 7-10 days. Cultures were examined during incubation and fungal growth in the media (tube) was determined by measuring linear growth (mm). Growth inhibition was calculated with reference to negative control (Umadevi *et al.*, 2003).

$$\% \text{ Mycelia inhibition} = \frac{G_n - G_t}{G_n} \times 100$$

Where

G_n = Mycelial growth in normal

G_t = Mycelial growth in test

3.7. 5. Antioxidant (DPPH Free radical scavenging) activity

A free radical is any atom or molecule that has a single unpaired electrons in its outer shell (Erbaş & Sekerci, 2011) and highly reactive (Herrling *et al.*, 2008) with a few exceptions. For most biological structures, free radical damage is closely associated with oxidative damage. Antioxidants are reducing agents, and limit oxidative damage to biological structures by passivating them from free radicals (Halliwell, 2012). DPPH (2, 2-diphenyl-1-picrylhydrazyl) is an organic compound, occurring in a dark-colored crystalline powder form, composed of stable free-radical molecules. DPPH is commonly used in antioxidant assay (Sharma, 2009). In the present study the potential of the different plant extracts was measured to act as the scavenger of the DPPH radical by following Kato *et al.*, (1988).

Principal

In the presence of a free radical scavenger, the free electron becomes paired which reduces the absorbance and following stoichiometric decolorization from deep violet to yellow colour occur.

Requirments

DPPH radical, Spectrophotometer, incubator, micropipette, Methanol, plant extracts, test tubes, etc.

Procedure

Stock solution was prepared by dissolving 25 mg of each plant extract in methanol and diluted up to 50 ml, then different micrograms solutions (20µg/ml, 40µg/ml, 50 µg/ml 60µg/ml, 80µg/ml, and 250µg/ml) were prepared by dilution method. 5ml solution of each extract was taken in a test tube and 1ml of 0.001M of DPPH solution was added to it. All these solutions were kept in dark for 30 minutes. 5 ml methanol was taken and 1ml of DPPH solution was added, for control. At the end of incubation period the mixtures were examined for the antioxidant activity using Optima UV-Visible spectrophotometer at wavelength of 517 nm. The antioxidant activity was performed in the following step wise manner.

1. The spectrophotometer was auto-zeroed with the blank (2.9 ml methanol + 0.1 ml plant extract) to neutralize the background color of the extract.
2. The control consisted of 1 ml DPPH + 1.9 ml methanol + 0.1 ml 50% ethanol while the sample had plant extract instead of ethanol.
3. The initial absorbance of the control and sample were taken at 517 nm.
4. The cuvettes were left in darkness for 15 min. DPPH changes from purple to yellow when it gets reduced by antioxidants.
5. The absorbance of the sample and control were measured after 15 min.
6. %DPPH was determined using formula:

$$\text{DPPH}\% = \frac{A_0 - A_s}{A_0} \times 100$$

Whereas A_0 is the absorbance of 0.001 M of DPPH solution only and A_s is the absorbance of the reaction mixture.

3.7.6. Cytotoxic activity

Cytotoxic activity was carried out to detect anticancer potential of the drug. The crude ethanolic extracts of the two plants were tested for cytotoxic activity by following the method of Atta- ur- Rehman *et al.*, (2001).

Requirement

Eggs of *Artemia salina* (Brine shrimps eggs), Sea Salt (38 g/L of D/W, pH 7.4), Hatching tray with perforated partition, Lamp to attract brine-shrimp larvae, Micro pipette (5, 50, 500 µl), Vials tray, vials, organic solvents (Ethanol).

Storage of *Artemia salina* eggs

The eggs were stored at low temperatures (4°C), thus they remain viable for years. They are available at fish pet shops.

Hatching of brine shrimp, eggs

The hatching tray was unequally divided by a perforated partition. Eggs were sprinkled over the solution in a small amount and covered with a dark carbon paper to create darkness. The tray was placed under a lamp. When the eggs hatched out; the larvae swam actively and migrated to the large illuminated portion of the tray.

Test sample preparation

Stock solution was prepared by dissolving 20 mg of each test sample in 2 ml of ethanol. From the stock solution 5, 50 and 500 µl was transferred to vials (3 vials /concentration). Solvents in all the vials were allowed to evaporate over night and the residue was resuspended in 2 ml of seawater. 10 larvae/vials, were placed using a Pasteur pipette. Nauplii were counted macroscopically in the stem of pipette against a light background. The final volume was made up to 5 ml with seawater this made the concentration 10, 100 and 1000 µg/ml respectively, and incubated at 25-27 °C for 24 hours under light. Other vials were provided with solvent (Ethanol) and reference cytotoxic drug

serving as negative and positive controls, respectively. After 24 hours, survivors in each treatment were counted with the aid of 3x magnifying glass.

Statistical analysis

LD₅₀ values with 95% confidence intervals were determined by analyzing the data with Finney computer program (Barkatullah *et al.*, 2011).

3.7. 7. Hepatoprotective activity

On the basis of ethnomedicinal information of these two research plants, the present study is designed to evaluate the hepatoprotective effect of *Iphiona grantioides* (Boiss.) Anderb. and *Pluchea arguta* subsp. *glabra* in rabbits. Liver injury was induced through oral administration of acetaminophen following the method of Yasmin *et al.*, (2011).

Chemicals/ Reagents

Acetaminophen and Silymarin were gifted by the Chairman Department of Animal Health and Veterinary Sciences, Khyber Pakhtunkhwa Agriculture University, Peshawar, Pakistan. Standard kits of serum AST, ALT, ALP and serum Bilirubin (Bio Apex) were purchased from the local market. All other reagents and solvents were of analytical grade and purchased from either Merck or Sigma.

Collection of plant sample and preparation of extract

Fresh plants of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* were collected from the highly saline area of district Karak, Khyber Pakhtunkhwa, Pakistan in March, 2011. The plant samples were authenticated by Curator Mr. Ghulam Jelani Department of Botany University of Peshawar and voucher specimens Bot.20030 (PUP) and Bot. 20031(PUP) of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*, were deposited in the herbarium of Botany Department, University of Peshawar, Pakistan, respectively. Cleaned and shade dried plant materials were pulverized into a fine powder and 500 g powder of each plant sample was macerated in 1 liter each of 70% ethanol for 15 days with occasional shaking. The extract was , then, filtered and concentrated to a dark

greenish semi solid residue (yield 20.5%) by a rotary evaporator at low temperature (45° C) and stored in a freezer (< 4°C) until needed.

Test animals

Adult healthy rabbits of local breed (*Oryctolagus cuniculus*), of either six (average weight 700 - 900 g) were obtained from PCSIR Laboratories Complex, Peshawar Pakistan, for the study. Rabbits were kept at animal house of the Department of Animal Health and Veterinary sciences, Khyber Pakhtunkhwa Agriculture University Peshawar, on a 12-h light/dark cycle under controlled conditions of temperature (25±1° C) and humidity (50±5° C) for one week. They were fed with standard diet and water *ad libitum*. NIH (National Institute of Health, 1985) guide lines for the care and use of the laboratory animals were followed, while performing all the experiments.

Experimental design

Preliminary acute toxicity of Plant material

Rabbits were divided into 9 groups (A- I) of 5 animals each. Group-A served as positive control and received saline water along with normal food and water. Groups (B,C D and E) received ethanolic extract of IG and groups (F,G,H and I) received ethanolic extract of PA through oral administration, in the form of aqueous suspension, in the dose of 0.5 g, 1.0 gm, 1.5 gm and 2.0 gm/ kg, respectively. The animals were observed after extract administration continuously for the first 4 hours in order to note any behavioral change and then for 48 hour to record the mortality (Jeyasekar *et al.*, 1997).

Experimental design of Induction of acetaminophen induced hepatotoxicity

Seventy two healthy rabbits were randomly divided into twelve groups (n = 6 in each group). Animals of group -1 (negative control) were administered with distilled water (1 ml/kg) daily for three days along with food and water. Group-2 (acetaminophen) received distilled water (1 ml /kg) once daily for three days and received acetaminophen (2 g/ kg body. wt.) on day 3. Group-3 received standard drug silymarin (100 mg/kg), orally, once daily for three days. Animals in group 4,5 and 6 were orally fed with ethanolic

extract of IG in three different doses (100, 300 and 500 mg/kg) as aqueous suspension once daily, for three days. Similarly rabbits in group 7, 8 and 9 were given ethanolic extract of PA in the same dosage as in case of IG. Test animals of group 10, 11 and 12 were given combined ethanolic extract of IG and PA in three doses in the ratio of 1:1 (50 mg IG: 50 mg PA, 150mg IG: 150 mg PA and 250 mg IG: 250 mg PA / kg b. wt.), once daily for three days. Animals in all groups (3-12) except 1 and 2 were given acetaminophen (2 g/kg) on day three, 30 minutes after the oral administration of Silymarin and plant extracts in either case. All the animals were anaesthetized 48 hours after the last treatment. Blood samples were collected via the vein puncture of animals ear and blood was kept in tubes (without anticoagulant), allowed to clot and serum was separated by centrifugation (2500 r.p.m. for 15 minutes) at 37°C and analyzed for various biochemical parameters (Prochezian &Ansari, 2005).

Table 3.2. Schedule of acetaminophen and plant extracts administration on a pre-treatment basis

Group 1 - Normal control (Distilled water (1 ml/kg)

Group 2 - Toxicant (Acetaminophen 2 g/kg)

Group 3- Standard drug (Silymarin 100 mg/kg) + acetaminophen

Group 4- Ethanol extract of IG (100 mg/kg) + acetaminophen

Group 5 - Ethanol extract of IG300 mg/kg) + acetaminophen

Group 6 - Ethanol extract of IG (500 mg/kg)+ acetaminophen

Group 7 - Ethanol extract of PA (100 mg/kg) + acetaminophen

Group 8 - Ethanol extract of PA (300 mg/kg) + acetaminophen

Group 9 - Ethanol extract of PA (500 mg/kg)+ acetaminophen

Group 10- Ethanol extract of IG (50 mg): PA (50 mg) /kg) + acetaminophen

Group 11 - Ethanol extract of IG (150 mg): PA (150 mg) /kg) + acetaminophen

Group 12 - Ethanol extract of IG (250 mg): PA (250 mg) /kg) + acetaminophen

Key = IG=*Iphiona grantioides*, PA=*Pluchea arguta* subsp.*glabra*

Liver function test

The biochemical parameters were assessed by the estimation of raised level of serum ALT, AST (Reitman & Frankle, 1957), ALP (Kind & King, 1954) and serum bilirubin (Mally & Evelyn, 1937) by using the commercially available test kits.

Histopathological examination

For histopathological examination, animals were sacrificed by cervical dislocation and the abdomen was cut open to remove the liver. A portion of the liver was cut into two to three pieces approximately of 6 mm size and fixed in 10% buffered formalin. After fixation, these tissues were processed in automatic tissue processor (RX-11B, Sakura, Japan) for histopathological studies. Tissues were embedded in paraffin wax in Paraffin Dispenser (TEC, SEMT-2 Sakura Japan) and thin section (3-5 μ m) were cut using rotary microtome (SRM 200, CW, Sakura, Japan). The mounted section on the glass slides were stained in automatic slide Stainer (DRS2000 J-B₂, Sakura Japan) using H&E staining as described by Bancroft (2008). The stained sections were made into permanent slides and examined under high resolution microscope (OLYMPUS C $\times$ 41, Japan) with photographic facility and photomicrographs were taken at resolution power (10 $\times$ 40 $\times$).

Data analysis

Data was expressed as a means $\pm$ SE. One-way analysis of variance (ANOVA) was used for the evaluation of difference between multiple groups followed by Duncan's multiple range test for individual comparisons using SAS 9.1 software (SAS Institute Inc., USA). Data was considered significant statistically when the *p* value was less than 0.05.

3.7.8. Insecticidal activity

Insects destroy our crops, grains and food items. So there is always search for new, and efficient and safe insecticides. Insecticidal activity of the crude extracts of different parts of the two research plants was carried out by impregnated filter paper method following Rashid *et al.*, (2009) and Naqvi & Parveen, (1991).

Requirements

Insects

Five insect species i.e. *Tribolium castaneum* (red flour beetle), *Rhyzopertha dominica*, *Trogoderma granarium*, *Callosobruchus analis* and *Sitophilus oryzae* (rice weevil) were obtained from Entomology department of Agriculture University of KP, Peshawar, Pakistan. Adult insects were fed with maize and incubated under controlled conditions of temperature and humidity.

Volatile organic solvent (Acetone), standard insecticide (Permethrin), Petri plates (9 cm in diameter), micropipette (1000µl), growth chamber, test samples (plant extracts), filter papers, glass vials and brush.

Preparation of test sample

The ethanol free semisolid extract of each plant part was dissolved in acetone and three different concentrations (1% w/v, 5% w/v and 10 % w/v) were made.

Rearing technique

The pests were reared in the laboratory under controlled conditions of temperature and humidity, in plastic bottles containing sterile breeding media. Insects of uniform age and size were selected for the experiment.

Procedure

Petri plates were cleaned and sterilized; filter papers were cut according to the size of the petri plates (90 cm) and placed in the plates. Plant sample was loaded over the filter paper with the help of micropipette and left for 24 hours. After complete evaporation of the solvents, 10 healthy insects of each species were put in each plate with the help of a clean brush. The plates were incubated in the growth chamber at 27⁰C with 50% relative humidity for 24 hours without food. Positive (Permethrin) and negative control (using volatile solvent, Acetone, instead of plant extract) were run along the plant extract assay.

Insects were observed under a dissecting microscope for mortality and death was confirmed when they did not react to a mechanical stimulus.

The survival of the insects was assessed by counting the number of survived insects of each species and the percentage mortality was calculated with the help of the following formula:

$$\text{Percentage mortality} = 100 - \frac{\text{No of insects alive in test} \times 100}{\text{No of insects alive in + control}}$$

3.7.9. Phytotoxicity

Phytotoxic activity is generally carried out to know the herbicidal potential of a plant. Phytotoxic activity of ethanolic extract of *Iphiona grantioides* (flower, leaves, branches and roots) and *Pluchea arguta* subsp *glabra* (leaves, branches and roots) was carried out against the *Lemna minor* following Atta-ur- Rehman *et al.*, (2001).

Requirments

Lemna minor (test plants), distilled water, flask -250 ml, micropipette (10-100µl and 100-1000µl), plant extracts (test samples), filter papers, glass vials, E-medium, brush and standard phytotoxic drug (Atrazine) etc.

E-medium preparation

E-Medium was prepared by mixing different minerals salts in a fixed ratio in distilled water (Table.3.3) for the growth of *Lemna minor*. The volume was finally made up to 1000 ml. pH was adjusted between 5.5 to 6.00 by adding KOH. The medium was autoclaved at 121 °C for 15 minutes.

Procedure

Stock solution was prepared by dissolving 30.0 mg of each extract in 1 ml of ethanol. Glass vials, three for each concentration, were inoculated with 1000, 100 and 10 µl

of the stock solution to give the final concentration of 1000, 100 and 10 ppm, respectively. The solvent was allowed to evaporate overnight under sterile conditions. To each vial, 20 ml of E medium and 10 plants of *Lemna minor* containing a rosette of three fronds were added. Other vials (3 for each) were supplemented with E- medium alone and standard drug (Atrazine), which served as negative and positive control, respectively. Vials were kept in the growth cabinet for seven days. The number of fronds per vials was counted and recorded on the day seven. The percent growth inhibition was recorded with reference to the negative control using the following formula:

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

Statistical analysis

Finney computer program was used for data analysis to determine FI_{50} values with 95% confidence intervals (Barkatullah *et al.*, 2011).

Table. 3.3. E-medium Composition for <i>Lemna minor</i> bioassay		
Constituents	Chemical Name	Quantity (g.L ⁻¹)
Potassium DihydrogenPhosphate	KH ₂ PO ₄	0.68
Calcium Nitrate	KNO ₃	1.515
Magnesium Sulphate	Mg SO ₄ .7H ₂ O	0.492
Boric Acid	H ₃ BO ₃	0.00286
Manganese Chloride	Mn Cl ₂ .4H ₂ O	0.00362
Ferric Chloride	FeCl ₃ .6H ₂ O	0.00540
Zinc Sulphate	ZnSO ₄ .5H ₂ O	0.00022
Copper Sulphate	CuSO ₄ .5H ₂ O	0.00022
Sodium Molybdate	Na ₂ MO ₄ .2H ₂ O	0.00012
Ethylene Diamine Tetra Acetic Acid	EDTA	0.01120

CHAPTER -4

RESULTS AND DISCUSSION

4.1. Morphology of the two research plants

4.1.1. Morphology of *Iphiona grantioides*

Iphiona grantioides is a perennial herb, with soft aerial parts and woody base, branches are glandular, pilose and viscid, densely covered with trichomes and leaves (Fig.4.1). Leaves are fleshy, cuneate-linear or oblanceolate, acute, apically 3-lobed. Leaf is from 3 cm to 5cm length and width is from 0.8cm to 1.5cm (Fig. 4.2).

Capitulum is solitary terminal, radiate, 2-3 cm in diameter. Involucre is 8-12 or 14 mm long, few seriate, dense glandular, bracts linear-oblong, outer bracts herbaceous; inner membranous, linear, acuminate, 5-8 mm long to 0.8-1.5 mm wide. Marginal florets are neuter, ligulate, 2-3-toothed. Central florets hermaphrodite, apically glandular. Pappus is with bristle only, 2-3 seriate, 22-72 in number, 6-8 mm long and golden yellow in color.

Root is tape root, slightly fleshy, aromatic, curved, provided with rootlets (Fig.4.3). These observed features are of taxonomic importance and could be used for proper identification of *Iphiona grantioides*. Anderberg (1994), Qaiser & Abid (2003) and Qaiser & Abid (2005) described the plant with more or less similar features, which confirmed the present observation.

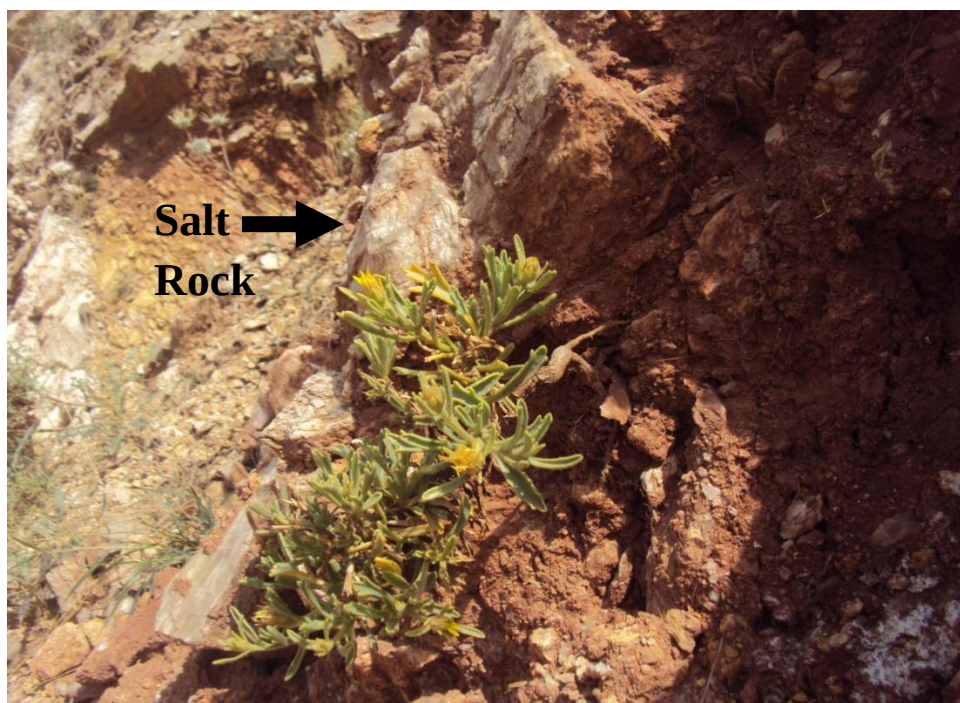


Fig.4.1. *Iphionao grantioides* (Boiss) Plant, growing in its natural saline habitat.



Fig.4.2. *Iphiona grantioides*, trichomes are clearly visible on leaves.



Fig.4.3. *Iphiona grantioides* (Boiss). R- root

4.1.2. Morphology of *Pluchea arguta* .subsp. *glabra* Qaiser

Pluchea arguta .subsp. *glabra* is an erect branched, stout shrub with pungent smell. Stem and branches terete and glabrous. Leaf is from 1.5 cm to 3cm in length and 0.3 to 2.0 cm in width, sessile, alternate, attenuate at base, oblong-oblongate or obovate, acute to subacute, coarsely serrate, dentate, teeth 5-7, sharp, glabrous on both surfaces (Fig. 4 . 4).

These features are of taxonomic characterization and could be used for proper identification of *Pluchea arguta* .subsp. *glabra* Qaiser. Root is tape root, provided with rootlets. The root is not straight but slightly twisted, hard and woody, of dark brown colour in fresh form and has slight pungent odour (Fig.4.6). Our observation of morphological characteristics of *Pluchea arguta* subsp. *glabra* are in accordance with Qaiser (2002), who gives the same taxonomic details of *Pluchea arguta* subsp. *glabra*.



Fig. 4.4. *Pluchea arguta* subsp. *glabra*. Plant growing in its natural saline habitate



Fig. 4.5. *Pluchea arguta* subsp. *glabra*. Glabrous leaves are clearly visible



Fig.4.6. *Pluchea arguta* subsp. *glabra* root

4.2. Phytosociology

Phytosociology is the quantitative study of vegetation and its principal aim is to describe vegetation, explain or predict its structure and classify it in a meaningful way (Ilorkar & Khatri, 2003). Overexploitation of natural resources resulted in a gradual decrease in flora richness and vegetation degradation (Qureshi, 2008). The original vegetation structure has been distorted due to deforestation and overgrazing and there is a need for restoration of the 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 workers carried out phytosociological studies of *Olea ferruginea* (Ahmad, 2007), *Picea smithiana* (Wahab *et al.*, 2008), *Quercus baloot* (Khan *et al.*, 2010) and *Skimmia laureola* and *Zanthoxylum armatum* (Barkatullah, 2012).

In the present study phytosociology of two ethno botanically important plants *Iphiona grantioides* and *Pluchea arguta* sub sp. *glabra* Qaiser from saline area of district Karak was carried out.

4.2.1. Phytosociology of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Seven different localities in district Karak of Khyber Pukhtunkhwah, Pakistan (Table.4.1) were selected for phytosociological study of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* Qaiser (Fig. 4.6). Both the plants were widely growing in the highly saline area of district Karak i.e. Andai, Sour Daag, Noshpa, Bahader Khail (Near old tunnel), Naripanoos (Near algada), Malgeen (Salt Deposit) and Karat (Salt Deposit), Karak at an elevation ranging from 450 m to 900 m (Table 4.1). Wazir *et al.*, (2007) and Khan *et al.*, (2011) reported the ethnobotanical uses of these plants from district Karak while Qureshi (2012) and Atta -ur-Rehman *et al.*, (2006) also reported these plants from Sind and Ziarat (Balochistan) respectively, from almost the same altitudes thus confirming a wide range of distribution of these plants in arid and saline regions of Pakistan the present findings.

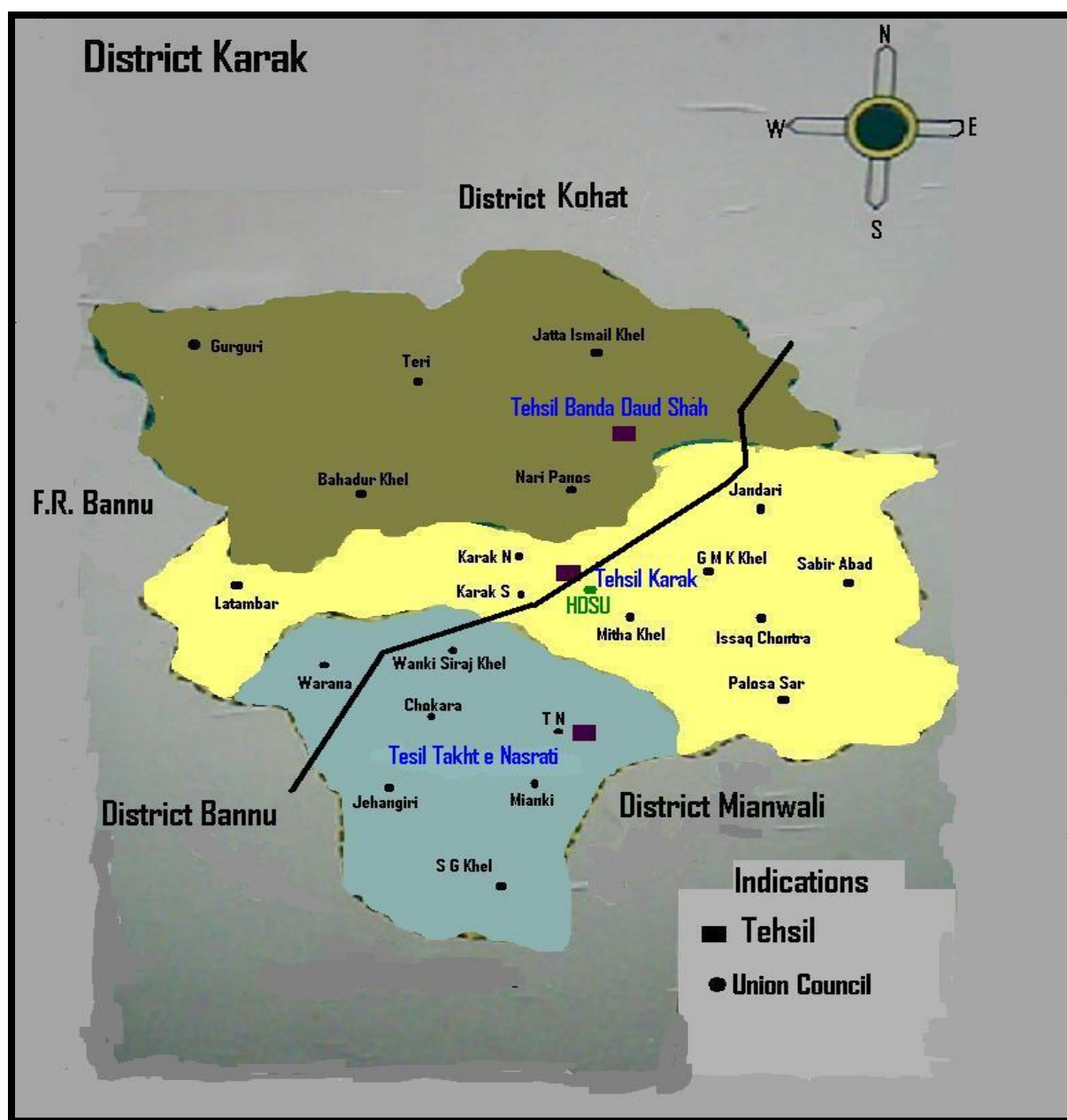


Fig.4.7.Map of District Karak

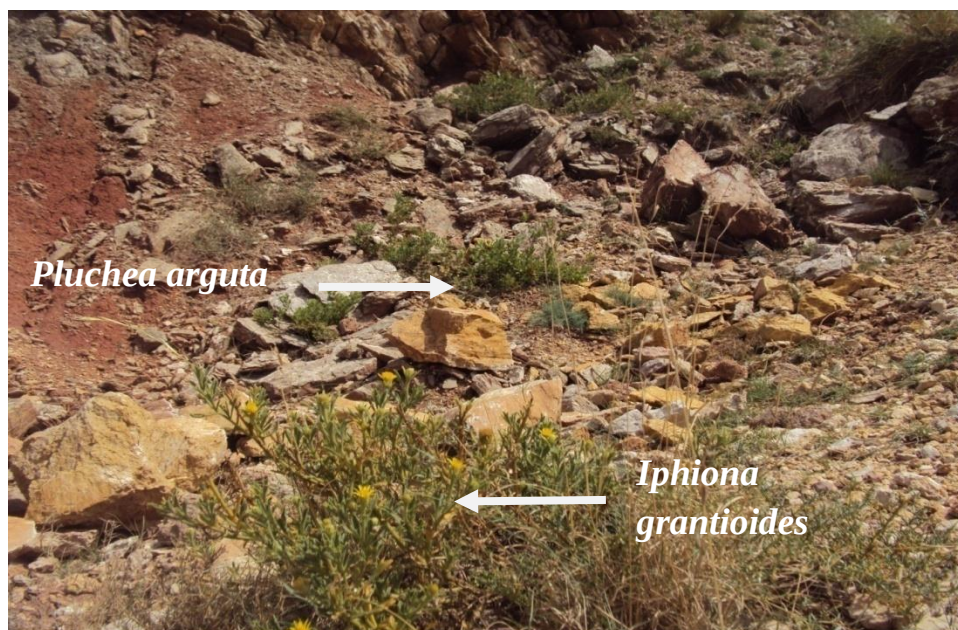


Fig.4.8. A locality in district Karak showing ecological status (Phytosociology) of associate plants of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Table. 4.1. Localities selected for phytosociological studies of <i>Iphiona grantioides</i> and <i>Pluchea arguta</i> subsp. <i>glabra</i>			
S. No.	Locality	Altitude(Feet)	Coordinates
1.	Chushmai (Andi) Karak	450 m(1476.38 ft)	33° 7' 56.29" N 71° 5' 40.28" E
2.	Bannu-Kohat Road,Sour Daag ,Karak	580 m(1902.89)	33°6'33N 70°52'3 E
3.	Noshpa, Salt Range, Karak	750 m (2460.63 ft)	33° 7' 28.29" N71° 3' 11.50" E
4.	Naripanoos Algada,Karak	678 metres (2227 feet)	33°10'57"N 71°9'46"E
5.	Bahader Khail, Karak	548 metres (1797.9 ft)	33° 10' 57 N 70° 57' 15 E

6.	Malgeen (Salt Deposit of Karak	500(1640.42 ft)	33° 7' 39.46" N 71° 5' 7.92" E
7.	Karat Ghar Salt Deposit, Karak	900 m (2952.76 ft)	33° 7' 17.30" N 71° 5' 5.00" E

Table. 4.2. Phytosociology of *Iphiona grantioides*, *Pluchea arguta* subsp. *glabra* and associated flora in the studied areas

S.No	Species Name	No. of STP	Consistency	IV			IVI		
				Min	Max	Aver	Min	Max	Aver
1	<i>Acacia jacquemontii</i> Benth.	7	V	8.1	14.5	10.82	2.7	4.8	3.58
2	<i>Acacia modesta</i> Wall.	1	I	-	7.6	7.6	-	2.6	2.6
3	<i>Acacia nilotica</i> subsp. <i>nilotica</i> L. Delile	4	IV	8.72	13.4	11.0	2.9	4.4	4.2
4	<i>Achyranthus aspera</i>	3	III	4.9	7	5.95	1.63	2.3	1.96
5	<i>Alhaji maurorum</i> Medic	2	II	5.7	9.5	7.6	1.9	3	2.45
6	<i>Amaranthus graecizans</i> L.	1	I	-	5.8	5.8	-	1.8	1.8
7	<i>Amaranthus viridus</i>	4	IV	6.2	9.7	7.98	2	3.2	2.6
8	<i>Aristida mutabilis</i> Trin & Rupr.	2	II	4	13.8	8.9	1.3	4.6	2.95
9	<i>Arva javanica</i>	4	IV	5.29	28	13.7	1.76	9.3	4.5
10	<i>Asphodelus tenuifolius</i>	2	II	11.09	45.12	28.1	9.3	15.04	12.17
11	<i>Astragalus spinosa</i>	2	II	-	10	10	-	3.48	3.48
12	<i>Boerhvia diffusa</i>	2	II	4.65	6.61	5.63	2.2	1.55	1.87
13	<i>Calligonum polygonoides</i>	2	II	4.8	7.6	6.2	1.6	3.1	2.35
14	<i>Calotropis procera</i>	4	IV	15.4	8.4	11.97	2.8	5	3.95
15	<i>Capparis decidua</i> (Forssk.) Edgew.	7	V	2.7	8.8	6.44	0.9	2.9	2.13

16	<i>Carthamus oxycantha</i> Bieb.	1	I	-	12.67	12.67	-	4.22	4.22
17	<i>Centaurea calcitrapa</i> ,	1	I	-	12.5	12.5	-	4.1	4.1
18	<i>Cenchrus ciliaris</i> L.	4	IV	6.97	10.6	8.31	2.32	3.5	2.7
19	<i>Citrulus colocynthes</i>	1	I	-	1.86	1.86	-	0.62	0.62
20	<i>Chenopodium album</i> L.	2	II	5	9.5	7.25	1.6	3.1	2.35
21	<i>Chenopodium murale</i> L.	2	II	4.9	4.9	4.9	1.6	1.6	1.6
22	<i>Chrozophora tinctoria</i> (L.) Raf	1	I	-	6.4	6.4	-	2.1	2.1
23	<i>Cleome brachycarpa</i> Vahl. ex DC.	1	I	-	8.4	8.4	-	2.5	2.5
24	<i>Cleome viscosa</i> L.	1	I	-	10.51	10.51	-	3.50	3.50
25	<i>Cocculus pendulus</i>	1	I	-	5.7	5.7	-	1.9	1.9
26	<i>Convolvulus arvensis</i>	2	II	11	12.23	11.6	3	4.07	3.5
27	<i>Convolvulus microphyllus</i> Sieb.ex Spreng.	2	II	4.9	6	5.45	1.6	2	1.8
28	<i>Coronopus didymus</i> (L.) Smith	1	I	-	6.4	6.4	-	3	3
29	<i>Conyza canadensis</i> (L.) Cronquist	2	II	5.4	9.3	7.3	1.8	3.1	2.45
30	<i>Cymbopogon jwarancusa</i> (Jones) Schultes	2	II	11	20.27	15.6	3.7	6.75	5.2
31	<i>Cynodon dactylon</i> (L.) Pers.	3	III	7.5	26.6	14.46	2.5	8.8	4.81
32	<i>Cyprus rotundus</i> L.	3	III	5.7	6.88	6.3	1.9	2.29	2.09
33	<i>Datura innoxia</i> ,	1	I	-	8.8	8.8	-	2.9	2.9

34	<i>Desmostachya bipinnata</i> (L.) Stapf.	3	III	3.98	20.2	11.26	1.32	6.7	3.74
35	<i>Dichanthium annulatum</i> (Forssk.) Stapf.	1	I	-	10	10	-	3.33	3.33
36	<i>Dodonaea viscosa</i> Jacq.	2	II	8	20	14	2.6	6.6	4.6
37	<i>Eragrostis minor</i> Host	2	II	-	6.6	6.6	-	2.2	2.2
38	<i>Euphorbia helioscopia</i> L.	2	II	7.4	21.8	14.7	2.4	7.2	4.86
39	<i>Euphorbia prostrata</i> Ait.	2	II	-	12.1	12.1	-	4	4
40	<i>Fagonia critica</i> L.	3	III	6.9	12.7	9.8	2.3	4.2	3.2
41	<i>Ficus carica</i> L.	1	I	-	11.5	8.29	-	11.5	2.7
42	<i>Fumaria indica</i> (Hausskn.) Pugsley	1	I	-	13.1	9.2	-	13.1	3
43	<i>Grewia tenax</i> Forsk.	3	III	12.2	12.8	12.5	4	4.2	4.1
44	<i>Haloxylon multiflorus</i> Bunge.	1	I	-	11.5	11.5	-	3.8	3.8
45	<i>Heliotropium europaeum</i> L.	1	I	-	13.1	13.1	-	4.3	4.3
46	<i>Imperata cylindrica</i> L.	1	I	-	8.7	8.7	-	2.9	2.9
47	<i>Indigofera spinosa</i>	1	I	-	11.5	11.5	-	3.8	3.8
48	<i>Iphiona grantioides</i>	7	V	7.1	49.9	21.2	2.3	16.6	7.05
49	<i>Justicia adhatoda</i>	1	I	-	17.1	17.1	-	5.7	5.7
50	<i>Launea nudicaulis</i> (L.) Hook.f.	1	I	-	14	14	-	4.6	4.6
51	<i>Launaea procumbens</i>	1	I	-	13.8	13.8	-	4.6	4.6
52	<i>Maytenus royleanus</i>	2	II	9.8	10	9.9	3.2	3.3	3.2

53	<i>Medicago minima</i> (L.) Grufb	2	II	7.5	11.2	9.35	2.5	3.7	3.1
54	<i>Monothea buxifolia</i>	4	IV	6	10	8.4	2	3.3	2.8
55	<i>Otostegia limbata</i>	2	II	4.3	10.3	7.3	1.4	3.4	2.4
56	<i>Peganum harmala</i> L.	1	I	-	33.6	33.6	-	11	11
57	<i>Pentatropis spiralis</i> (perwatay)	2	II	4.3	13.1	8.76	1.43	13.1	2.87
58	<i>Periploca aphylla</i> (Decne.) Edgew.	4	IV	6.5	9.9	7.74	2.1	3.3	2.58
59	<i>Poa annua</i> L.	2	II	10.4	16.79	13.5	3.4	11.2	7.3
60	<i>Phoenix dactylifera</i> L.	3	III	7.2	11.3	8.74	2.4	3.7	2.89
61	<i>Pluchea arguta</i> subsp. <i>glabra</i>	7	V	7.1	28.3	13.2	9.4	2.4	4.4
62	<i>Polygonum plebejum</i> R. Br.	1	I	-	9.3	9.3	-	3.1	3.1
63	<i>Pulicaria glutinosa</i>	6	V	8.7	19.9	11.8	2.9	6.6	3.9
64	<i>Rhazya stricta</i> Dcne	1	I	-	36	36	-	12	12
65	<i>Saccharum munja</i> Roxb.	2	II	5.8	8.1	6.95	1.9	2.7	2.31
66	<i>Saccharum spontaneum</i> L.	4	IV	2.56	10.4	7.53	0.85	3.4	2.4
67	<i>Salsola foetida</i> Del.ex Spreng.	3	III	6.2	10	7.6	2.0	3.4	2.56
68	<i>Salvadora oleoides</i> Decne.	4	IV	8.9	12.8	10.55	1.7	4.2	3.48
69	<i>Salvadora percica</i>	1	I	-	5.1	5.1	-	1.7	1.7
70	<i>Salvia aegyptiaca</i>	1	I	-	5.7	5.7	-	1.9	1.9
71	<i>Setaria verticillata</i> (L.) P. Beauv	1	I	-	12.2	12.2	-	4	4
72	<i>Solanum surattense</i> Burm. f.	3	III	3.9	17	8.62	1.32	5.6	2.84

73	<i>Sporobolus virginicus</i> grass	1	I	-	11	11	-	3.7	3.7
74	<i>Suaeda fruticosa</i> Forssk. ex J. F. Gmelin	4	IV	2.56	18	8.39	0.85	6	2.77
75	<i>Tamarix aphylla</i> (L.) Karst	6	V	22.9	5.6	10.86	7.6	1.8	3.8
76	<i>Tamarix dioica</i> Roxb ex. Roth	1	I	-	18	18	-	6	6
77	<i>Tecomella undulate</i> (Sm.) Seem	1	I	-	7.4	7.4	-	2.4	2.4
78	<i>Tribulus terrestris</i> L.	3	III	11.78	19	14.7	3.9	6.3	4.7
79	<i>Withania coagulans</i> Dunal	5	IV	2.2	12.8	7.92	0.7	4.2	2.6
80	<i>Xanthium strumarium</i> L.	2	II	7.6	13.9	10.7	2.5	4.6	3.5
81	<i>Zizyphus mauritiana</i> Lam.	5	IV	5.3	13.4	9.62	1.7	4.4	3.16
82	<i>Zizyphus nummularia</i> Burm. (f.) Wight & Arn.	4	IV	7.9	12.8	9.7	2.6	4.2	3.1

Table 4.3 Dominant shrub plant species on the bases of importance value (IV) in the selected localities of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* in district Karak

S.No.	Locality	Ist dominant	2nd dominant	3rd dominant	Position of <i>Iphiona grantioides</i>	Position of <i>Pluchea arguta</i> subsp. <i>glabra</i>
1	Chushmai (Andi) Karak	<i>Iphiona grantioides</i>	<i>Pluchea arguta</i> subsp. <i>glabra</i>	<i>Calotropis procera</i>	1 st	2 nd
2	Bannu-Kohat Road,Sour Daag ,Karak	<i>Suaeda fruticosa</i> Forssk.ex Roth	<i>Calotropis procera</i>	<i>Arva javanica</i> (Burm.f.) Juss.ex J.A.Schulets	6 th	Less dominant
3	Noshpa, Salt Range, Karak	<i>Iphiona grantioides</i>	<i>Tamarix dioica</i> Roxb. ex. Roth	<i>Pluchea arguta</i> subsp. <i>glabra</i>	1 st	3 rd
4	Naripanoos Algada,Karak	<i>Rhazya stricta</i> Dcne	<i>Peganum harmala</i> L.	<i>Iphiona grantioides</i>	3 rd	Less dominant
5	Bahader Khail, Karak	<i>Pluchea arguta</i> subsp. <i>glabra</i>	<i>Withania coagulans</i> Dunal, <i>Salvadora oleoides</i> Decne. and	<i>Haloxylon multiflorus</i> Bunge,	5 th (Less dominant)	1 st

			<i>Grewia tenax</i> (Forsk)	<i>Indigofera spinosa</i>		
6	Malgeen (Salt Deposit of Karak	<i>Calotropis procera</i>	<i>Pentatropis spiralis</i> (perwatay)	<i>Pluchea arguta</i> subsp. <i>glabra</i>	9 th	3 rd
7	Karat Ghar Salt Deposit, Karak	<i>Justicia adhatoda</i>	<i>Iphiona grantioides</i>	<i>Pluchea arguta</i> subsp. <i>glabra</i>	2 nd	3 rd

In the present study *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*, the two research plants, were present in close association of each other along with other 80 plants, in all the seven localities. Two species *Acacia jacquemontii*, *Capparis deciduas* (Forssk.) Edgew. were present constantly in all stands; *Pulicaria glutinosa*, *Tamarix aphylla* (L.), *Ziziphus mauritiana*, *Ziziphus nummularia* were mostly present species while *Suaeda fruticosa* Forssk. ex J. F. Gmelin, *Acacia nilotica* subsp. *nilotica* (L.) Delile, *Arva javanica*, *Calotropis procera*, *Cenchrus ciliaris* L., *Monothea buxifolia*, *Periploca aphylla* Decne., *Saccharum spontaneum* L., *Salvadora oleoides* Decne., *Withania coagulans* were the most often species present in association with *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* Qaiser. The associated flora also included 23 seldom and 27 rare species (Table 4.2).

Dominant plants in all the sites were determined on the basis of importance value (IV) (Barkatullah 2012). *Iphiona grantioides* was found dominant at two sites, while it was second dominant at Karat ghar site and third dominant at Naripanoos site (Table.4.3), with average importance value (IV) 21.2, and maximum IV 49.9, at Chashmai area of Andai, while minimum IV was 7.1 at Malgeen area. Important value index (IVI) of *Iphiona grantioides* was 7.05 with maximum IVI 16.6 and minimum IVI 2.3.

Pluchea arguta subsp. *glabra* was found in all the seven sites, mostly growing in close association with *Iphiona grantioides*. It was 1st dominant at Bahader Khail sit near the old tunnel, and second dominant at Chasmai and third dominant at Malgeen, Karat and Noshpa area, having the average IV of 13.2, maximum IV of 28.3 and minimum IV of 7.1 (Table 4.3). Important value index (IVI) of *Pluchea arguta* subsp. *glabra* was 4.4, maximum IVI 9.4 and minimum IVI 1.76 (Table 4.3). Associated shrubs found in co-dominance with *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* were *Pulicaria glutinosa*, *Saccharum spontaneum* L., *Saccharum munjka* L., *Suaeda fruticosa* Forssk. ex J. F. Gmelin, *Otostegia limbata*., *Salsola foetida* Del.ex Spreng., *Zizyphus nummularia* Burm. (f.) Wight & Arn., *Calotropis procera*, *Periploca calophylla* (Wight) Falc., *Capparis decudua* (Forssk.) Edgew., *Withania coagulans* Dunal, *Aerva javanica*

(Burm. f.) Juss. ex J.A., *Tamarix dioica* Roxb ex. Roth , *Salvadora oleoides* , *Astragalus spinosus*, *Ficus carica* L., *Calligonum polygonoides*, *Pentstemon spiralis*, *Rhazya stricta* Dcne, *Dodonaea viscosa* Jacq., *Xanthium strumarium* L., *Eragrostis minor* Host, *Aristida mutabilis* Trin & Rupr., *Conyza canadensis* (L.) Cronquist, *Launea nudicaulis* (L.) Hook.f., *Euphorbia prostrata* Ait., *Amaranthus graecizans* L., *Chrozophora tinctoria* (L.) Raf, *Amaranthus viridis* L., *Cymbopogon jwarancusa* (Jones) Schultes, *Sporobolus virginicus* , *Grewia tenax*(Forsk), *Haloxylon multiflorus* Bunge, *Indigofera spinosa*, *Alhagi maurorum* Medic., *Justicia adhatoda* and *Maytenus royleanu* with variable importance values (Table 4.2).

Among the herbaceous flora, herbs with highest IV values were *Asphodelus tenuifolius* (IV28.1), *Tribulus terrestris* L. (Average IV 14.7 and maximum IV 19), *Pulicaria glutinosa* (Average 11.8, maximum IV 19.9), *Peganum harmala* L. (Maximum IV 33.6), *Cymbopogon jwarancusa* (Jones) Schultes (Average IV 15.6 and maximum IV 20.27), *Justicia adhatoda* (Average IV 17.1 and maximum IV 17.1). *Cenchrus ciliaris* L. and *Amaranthus viridis* were the only species that fell in “mostly present” class of Raunkiaer’s classes of constancy. Most of the herbs were classified as seldom and rare species having narrow range of distribution with *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*.

Nine trees species i.e., *Acacia jacquemontii* Benth., *Tamarix aphylla* (L.) Karst, *Zizyphus mauritiana* Lam., *Monothea buxifolia*, *Acacia nilotica* subsp. *nilotica* (L.) Delile, *Phoenix dactylifera* L., *Tecomella undulate* (Sm.) Seem, *Salvadora persica* and *Acacia modesta* Wall, were found in all localities in association with *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* , in which *Acacia jacquemontii* Benth. was the only constant tree species occurring in all seven stands. *Tamarix aphylla* (L.), was the second constant tree species present in all the sites, except one stand, had the highest importance values of 22.9 among the tree species. Similar phytosociological studies have also been conducted for the local flora of Tehsil Takht –e- Nasrati of District Karak and by Khan (2012) and Badsha (2011) for District Tank with more or less similar phytosociological results for the plants found in association with the two research plant, respectively.

Similarly Barkatullah (2012) has conducted phytosociological studies of *Skimmia laureola* and *Zanthoxylum armatum*.

4.3. Soil analysis

4.3.1. Soil Sampling

Soil, collected from growing sites of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* Qaiser was analyzed for chemical, physical, and biological properties. Soil texture, soil pH and various nutrients were detected as they affect the growth and distribution of plants (Sharma & Kumar, 1991). The results obtained are presented in the Table 4.4.

4.3.2. Soil Texture

All the soil samples analysed from different areas of district Karak were alkaline in reaction and strongly calcareous in nature. Organic matter, soil N and P were found deficient in most of area sampled. The sampled area soil had permanent salt with semi-arid climate. Therefore, the soil can be reclaimed by management practices and growing salt tolerant plants.

In general soil of the studied sites was loamy in nature. Three places had sandy-clay loam while four sites had sandy-loam, silt-loam, sandy-silt loam and silt-clay loam respectively.

4.3.3. Soil pH

Soil pH of the surface soil ranged from 7.40 to 8.95 with a mean value of 7.98 being higher in Bahader Khel salt range, while its lower value was found in soil from Sour Daag area (Table 4.4). Variation in pH was low in the area. Almost all of the soil samples were alkaline in reaction.

4.3.4. Electrical conductivity

Electrical conductivity of the soil ranged from 4.30 to 17.65 dS m⁻¹ (Table 4.4) with a mean value of 10.98 dS m⁻¹. The higher and lower values were found in Karat Ghar and Chushmai area, respectively. All of the samples collected from different locations were found saline considering the cutoff value of 4 dS m⁻¹.

4.3.5. Soil Organic matter (OM)

Organic matter content of the soil ranged from 0.72 to 1.66 % with a mean value of 1.14 % being higher in Sour Daag area. Twenty eight percent samples were deficient, 43 % marginal and 29 % samples were found adequate in organic matter.

4.3.6. Total nitrogen (N)

Total N in the soil ranged from 0.013 to 0.041 % with a mean value of 0.0226 % being higher in Noshpha area (Table 4.4). All of the samples collected from different area were deficient in total N.

4.3.7. Available phosphorus (P)

Available P in soil ranged from 1 to 4 mg kg⁻¹ with an average of 1.143 mg kg⁻¹ (Table 4.4). The higher value was found in Noushpa while Malangeen and Sour Daag area had lower P value. About 14 % samples were deficient while all other samples were deficient in available phosphorus. Phosphorous content showed very high variation.

4.3.8. Available Potassium (K)

Available Potassium in the surface soil ranged from 100 to 210 mg kg⁻¹ with an average value of 155.7 mg kg⁻¹ (Table 4.4). The higher value was found in Karat Ghar and its lower value was noted in Bahader Khel area. Regarding categorization of available K, only 29 % samples were marginal and rest of the samples were adequate in Potassium.

Table 4.4. Descriptive statistics of soil parameters collected from different areas of district Karak

Samples No & Locality	pH	EC dS m ⁻¹ (1:2)	Textural Class	Organic Matter (%)	Salinity	N (%)	P (%)	K (%)	Na (%)
1. Malgeen (Salt Deposit s)	7.74	15.88	Sandy – Clay- loam	1.52	7.7	0.014	1	130	0.3
2. Nari panoos	7.82	16.32	Sandy-Red Clay- loam	1.14	7.1	0.023	3	120	0.2
3. Bahader Khail(Old tunnel)	8.95	10.48	Sandy- loam	0.72	7.2	0.031	1	100	0.6
4. Chushmai (Andi) Karak	7.50	4.33	Sandy-Red Clay- loam	1.04	7.9	0.013	2	160	1.1
5. Karat Ghar	7.64	17.65	Silt-Clay- loam	1.20	7.3	0.021	3	210	1.2
6. Noshpa, Salt Range	8.82	4.77	Sandy-Silt -loam	0.72	5.2	0.041	4	190	0.6
7. Bannu-Kohat Rd, SourDaag	7.4	7.4	Silty- loam	1.66	7.1	0.015	1	180	0.3

The present results are also in lineage with Khan *et al.*, (2012), who characterized soils from various spots of the surroundings of tehsil Takhti Nasrati, district Karak.

The present study of ecological distribution revealed that the two research plants are xerophytic in nature, they occurred in dry and saline soil conditions of district Karak. They have been found in close association with other xeric plants which are characteristics of dry and saline area. The pharmacological potential of these was investigated and in almost all studies they showed effectiveness, which advocate their use as a useful medicinal plants, however population of these plants in their natural habitate was not rich as it is clear from their IVI and IV values. It is therefore suggested that proper curative measures should be taken by the relevant departments for the improvement of, which in turn will improve their growth and conservation. It, in turn, will improve the economy of the local people, and will increase the biodiversity of the area.

4.4. Ethnobotany

Ethnobotany is the customary knowledge about local plant resources, which are helpful in establishing priorities in the local communities (Ibrar *et al.*, 2007) and establish an interaction of man and plants for sustainable development (Ahmad *et al.*, 2006). It makes the most important approach to study natural resource management by the people of mountainous and distant areas (Iqbal & Hamayun, 2005). Now a day, these studies gained popularity in the development of health care system in various parts of the world (Sardar & Khan, 2009). Plants are in use of human beings since the dawn of civilization for fodde, shelter, for curing various ailments, forage for cattle, fuel, flowers for celebration and decoration, timber for construction and for many other useful objects (Iqbal & Hamayun, 2005; Ahmad *et al.*, 2006). In traditional knowledge plants uses are numerous, varied and play very important role in improving human health (Nair *et al.*, 2005) and economic conditions of the local people (Barkatullah *et al.*, 2009). Plants are easily available and have fewer side effects, so 80% of the world population relies on medicinal plants for primary health care. In Pakistan the people living in hard areas prefer to use traditional medicinal plant. The ethnobotanical information is also very

helpful for ecologists, taxonomists, pharmacologists, watershed and wild life managers in their efforts for improving the financial condition of the local people in the remote areas (Ibrar *et al.*, 2007) and for the conservation of medicinal plants as well as their habitat. Ethnobotanical informations of the present research plants were collected from inhabitants of the area where these plants (*Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* Qaiser) grow wild.

4.4.1. Ethnobotany of *Iphiona grantioides*

Local names Zair gul in Pashto (Khan *et al.*, 2011), Naro in Balochi (Qureshi, 2012) and Khol Meer (Sindhi) (Attur-ur- Rehman, 2006).

Part used: leaves and whole plant

Local uses

The leaves are stepped in water and used as a remedy for the treatment of Asthma (Wazir *et al.*, 2007). The paste of the plant is applied on wounds for fastening healing (Qureshi, 2012). Leaves are used against itching, scabies, haepatitis and against snake bite (Attur-ur- Rehman, 2006) by following the given methods.

1. Method of use for hepatitis:

Fresh leaves of plant are kept in water for overnight and in the morning, these are crushed, filtered and the filterate is dranked.

2. Method of use for itching:

Paste of the fresh plant is made and applied on the affected area of skin.

3. Method of use for snake sting:

About 10g of fresh whole plant is grounded and to it is added 1 glass of water and it is drink and also the paste of whole plant is applied on the affected area of the skin.

4.4.2. Ethnobotany of *Pluchea arguta* subsp. *glabra*

Local names: Kasteera (Pashtoo)

Part used: Leaves, Whole plant

Local uses: Decoction of the whole plant is used as a diuretic and as a camel fodder (Qureshi, 2012).

Similar ethnobotanical studies have been carried out by various other workers in Pakistan, particularly in Khyber Pakhtoonkhwa, e.g., Barkatullah *et al.*, (2009), Jan *et al.*, (2010); Ahmad *et al.*, (2011); Khan *et al.*, (2011) Barkatullah *et al.*, (2011, 2012); Khan *et al.*, (2013) and Sher *et al.*, (2014). The present ethnobotanical studies will open new avenues for researchers to significantly investigate the phytochemical and Pharmacological properties of these plants, and in the present study too various bioassays have been carried out to ascertain the medicinal potential of these two plants.

4.5. Pharmacognosy

4.5.1. Pharmacognosy of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Qaiser

Pharmacognostic study of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* Qaiser was carried out, which included macroscopical description of leaf, stem and root of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*, microscopic study which includes anatomical studies of leaf, stem and root, leaf surface study, determination of leaf constant values (Stomatal index, vein islet number and Palisade ratio), physiochemical characteristics (moisture contents and ash values), fluorescence study, phytochemical investigation and extractive values of powder drugs of leaf, stem and roots of both the plants were determined, while ethanolic extracts of different parts of the two plants were screened out for various bioassays.

4.5.2. Macroscopical study of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Macroscopic characteristics like shape, odor, color and taste of crude drugs are the chief pharmacognostic parameters for the accurate identification. In the present study leaf, stem and root of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* Qaiser are described macroscopically, to establish standard parameter (Fig. 4). The characteristic features of the leaf, stem and roots of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* Qaiser are summarized in Table 4.5, Table 4.6 & Table 4.7.

The leaf of *Iphiona grantioides* shows succulent nature, pubescent, thickly covered with glandular trichomes and nonglandular hairs on both the surfaces. Apex is acute to subacute and base is decurrent. Lamina is divided into three lobes at the apex region and provided with reticulate and unicostate venation. Similarly leaf of *Pluchea arguta* subsp. *glabra* Qaiser was sessile, spirally arranged, almost persistent and green, herbaceous, glabrous. Lamina was simple with serrate (dentate) margins. Leaf was bitter; unpleasant in taste with strong, pungent smell. Leaf base was attenuate, apex acute to subacute, provided with reticulate and unicostate venation (Table 4.5).

As given in Table 4.6, the stem of *Iphiona grantioides* is light green, cylindrical in shape, with 0.2 cm- 1.5 cm in diameter, thickly covered with hairs and trichome. Smell and taste was strong aromatic and slight sour, respectively. Similarly stem of *Pluchea arguta* subsp. *glabra* is fresh green, cylindrical, 0.3 cm- 2 cm in diameter. Taste and odour is bitter and pungent, respectively.

Root of *Iphiona grantioides* is a tap root. It is cylindrical and slightly curved. Rootlets are present. It is creamish brown in colour with aromatic smell and slight sour taste, while root of *Pluchea arguta* subsp. *glabra* is light brown in colour. It is also cylindrical, slightly curved with rootlets, present. Taste is slight bitter and smell is weak aromatic. The observed characters for the roots of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* are given in the Table 4.7.

Bercu *et al.*, 2012 gives almost the same results of leaf, stem and roots for *Aster tripolium* from saline area, a plant belonging to the family asteraceae. Toma *et al.*, 2010

investigated some Romanian species of genus *Inula* and described the leaves, stem and roots, with some what similar characteristic .Our findings are in line with the study of Khan *et al.*, (2010) who reported almost the same results for *Pluchea lanceolata*. Sugumaran & Vetrivelvan (2008) investigated the macroscopic characteristic for the pharmacognostic standardization of *Bauhinia purpurea*. Mathur *et al.*, (2010) conducted similar studies for *Amaranthus spinosus* standardization. According to Sharma *et al.*, (2010) macroscopic evaluation including size, shape, colour, odor and taste etc are bases for pharmacognostic evaluation. The present results and observation will set evaluation parameters for correct identification of these parts of the two plants.

Table 4.5. Macroscopic features of leaf of *Iphiona grantioides* and *Pluchea arguta* subsp.*glabra*

S. No	Characteristic	Observation (<i>Iphiona grantioides</i>)	Observation (<i>Pluchea arguta</i> subsp. <i>glabra</i>)
1	Size	L ength3– 5cm, Width = 0.8 -1.5 cm	Length = 1.5 cm to 3cm, Width = 0.3-0.8 (2.0) cm
2	Duration	Almost Persistent	Almost persistant
3	Colour	fresh light green colour	fresh green colour
4	Taste	Light Sour	Bitter, unpleasant
5	Odour	Strong aromatic, slightly pungent	Strong , pungent
6	Phyllotxis	Spiral	Spiral ,alternate
7	Insertion	Ramel and Cauline	Ramel and Cauline
8	Leaf base	Decurrent	Attenuate
9	Petiole, stipule	Sessile/ exstipulte	Sessile
10	Lamina Composition	Simple and Spathulate	Simple and Ovate
11	Incision	Pinnatifid, 3 lobed	Margin serrate ,dentate,
12	Venation	Reticulate and unicostate	Reticulate and unicostate
13	Apex	Acute to subacute	Acute to subacute
14	Surface	Pubescent	Glabrous
15	Texture	Succulent	Herbaceous
16	Fracture of dry leaf	Short and smooth	Short and smooth

Table 4.6. Macroscopic features of stem of *Iphiona grantioides* and *Pluchea arguta subsp.glabra*

S.No.	Features	Observation (<i>Iphiona grantioides</i>)	Observation (<i>Pluchea arguta subsp.glabra</i>)
1	Colour	Light green	Fresh green
2	Odour	Strong, aromatic	Strong, Pungent
3	Shape	Cylindrical with hairs & trichomes	Quadrangle
4	Taste	Slight Sour	Bitter, unpleasant
	Thickness	0.2 cm- 1.5cm in diameter	0.3 cm- 2 cm in diameter
6	Fracture	Short	Short
7	Fracture surface	Uneven & fibrous	Uneven & fibrous
8	Texture	Herbaceous, older woody	Herbaceous,older woody

Table 4.7. Macroscopical features of root of *Iphiona grantioides* (Boiss.) Anderb. and *Pluchea arguta subsp.glabra*

S.No	Features	Observation (<i>Iphiona grantioides</i>)	Observation (<i>Pluchea arguta subsp.glabra</i>)
1	Colour	Creamish brownish	Light brown
2	Odour	Strong Aromatic	Strong Aromatic
3	Taste	Slight sour	Slight sour
4	Shape	Cylindrical, slightly curved	Cylindrical, slightly curved
5	Rootlets	Present	Present
6	Type of root	Tape root	Tape root
7	Fracture	Short	Short
8	Texture	Tough	Tough
9	Fracture surface	Fibrous, uneven	Fibrous, uneven

4.5.3. Microscopic study of leaf, stem and root of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Microscopic study includes anatomy of leaf, stem and root of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*), leaf constant values (stomatal index, palisade ratio, vein islet number and vein termination number) and powder drug study of various parts of these plants was carried out.

4.5.3. a. Transverse section (T.S) of *Iphiona grantioides* stem

The T.S of stem is showed in Fig.4.9. (a - d). The epidermis is single layered, covered by multi-cellular glandular and non glandular trichomes. The cortex is differentiated into hypodermis and general cortex. The hypodermis is many layered, chlorenchymatous (parenchyma cells contain chloroplast) (Fig.4.9.a). General cortex is composed of multilayered parenchymatous cells. Endodermis is the inner most layer of the cortex surrounding the stele. The outer most layer of the stele is single layered pericycle, surrounding the vascular bundles (Fig. 4.9.b). Vascular bundles are many in number, closely present, arranged in a ring (4.9.c). Each vascular bundle is of conjoint, collateral and open type (Fig. 4.8.d). Medullary rays are present in the form of narrow strips; separating the vascular bundles. The pith is large and parenchymatous.

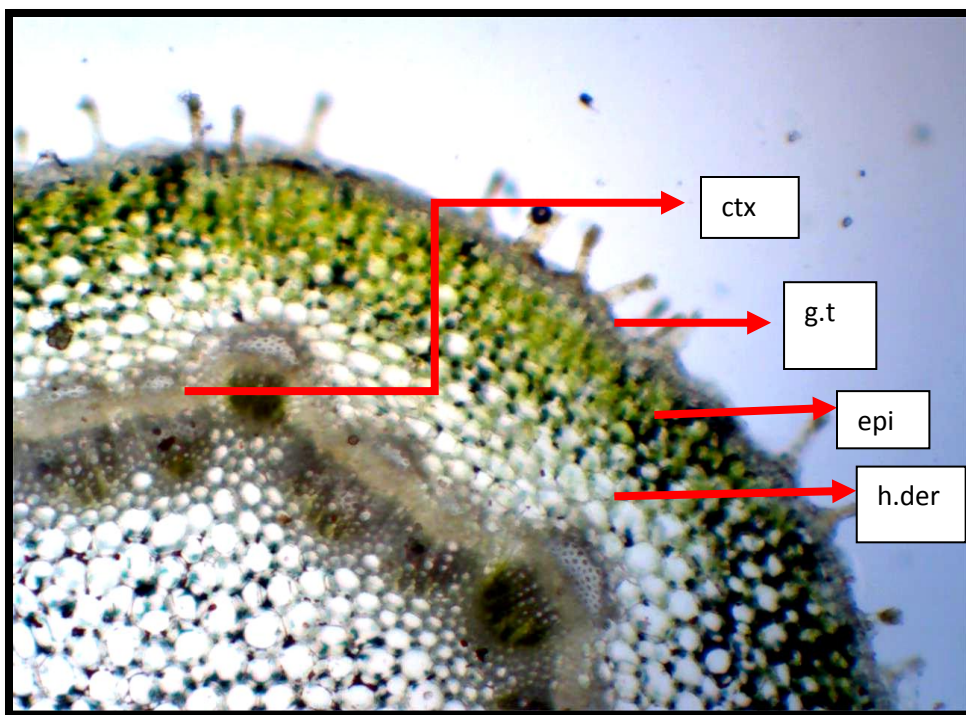


Fig. 4.9.a. T.S showing *Iphiona grantioides* stem.ctx- Cortex, g.t- Glandular trichome, epi- Epidermis, h.der- Hypodermis.(10x).

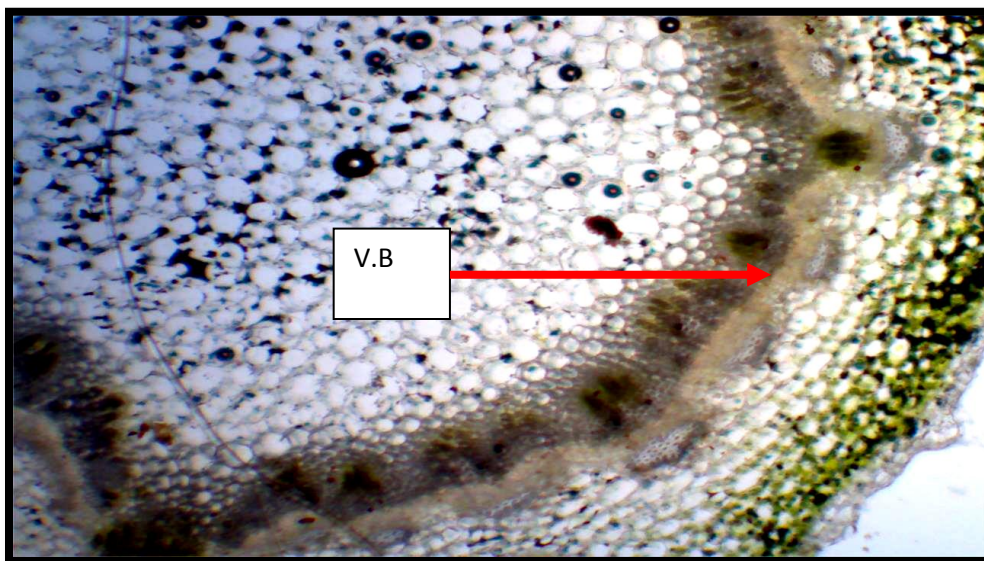


Fig.4.9.b. T.S showing *Iphiona grantioides* stem, arranged in a ring (10x)

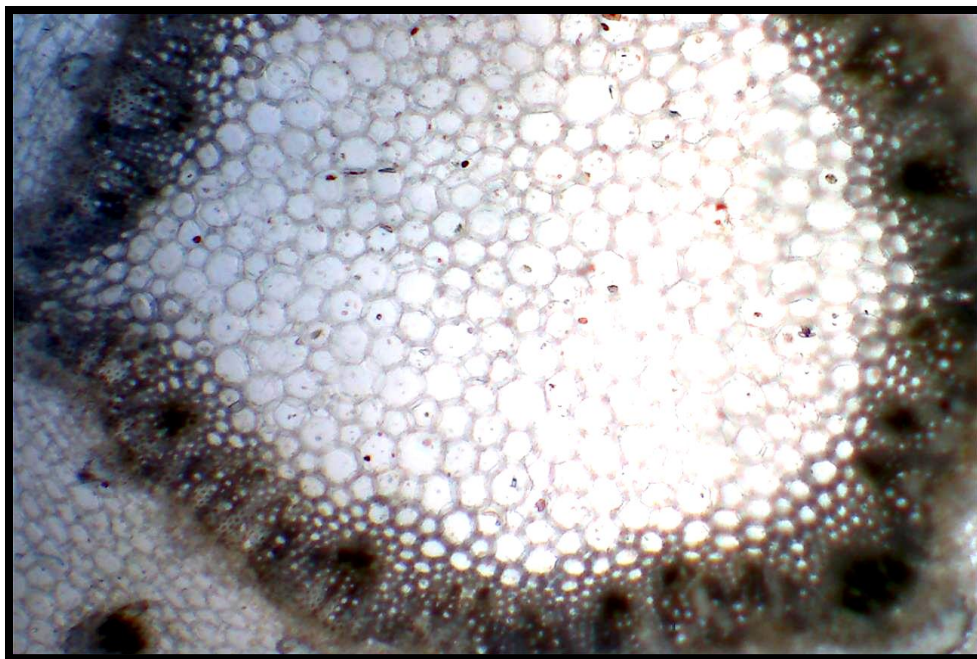


Fig.4.9. c. T.S showing *Iphiona grantioides* stem. Vascular bundles are arranged in ring.

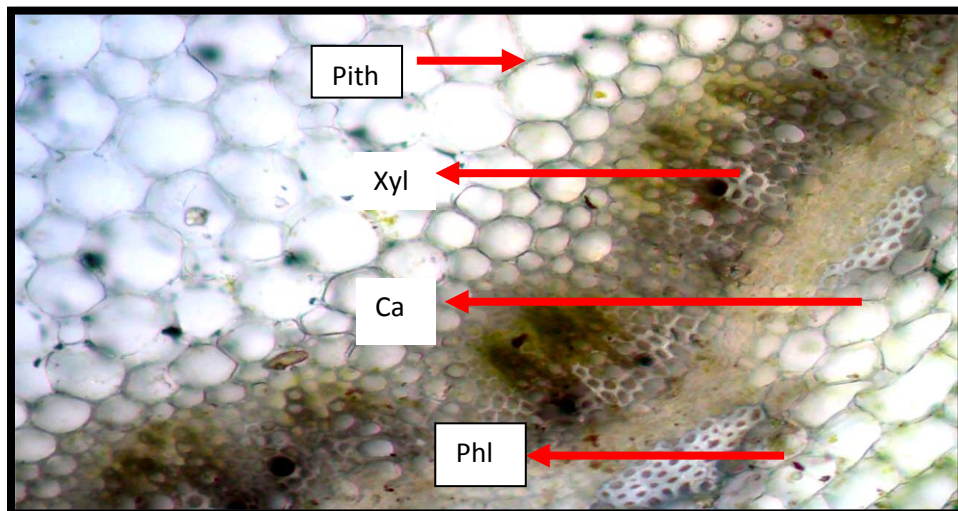


Fig.4.9.d. T.S. showing *Iphiona grantioides* stem. Vascular bundle. Xyl-Xylem, Phl-Phloem, Ca- Cambium (10 X).

4.5.3. b. Transverse section (T.S) of stem of *Pluchea arguta* subsp. *glabra*

The T.S of stem showed that epidermis is single layered in thickness, interrupted by the presence of stomata and covered by cuticle. Below the epidermis is 1-2 cell thick chlorenchymatous hypodermis, followed by few celled thick parenchymatic cortex with intercellular spaces. Next layer is of endodermis, followed by pericycle surrounding vascular bundles. Vascular bundles are many in number, arranged in a ring. Vascular bundles are conjoint, collateral, and open. Medullary rays are present, separating the vascular bundles. In the center a large pith with intercellular spaces is present (Fig. 4.10).

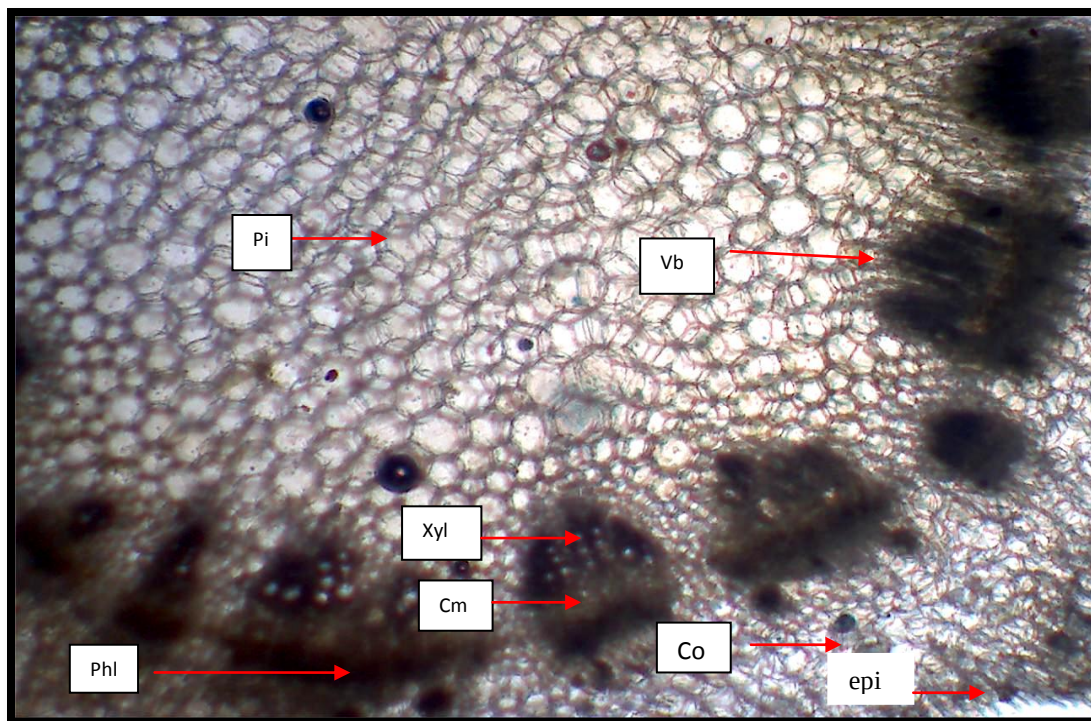


Fig.4.10 .T.S.of *Pluchea arguta* subsp. *glabra* stem .epi- epidermis, vb- vascular bundle; Pi-Pith; X. xylem; Phl- Phloem; Cam- Cambium; Cor-cortex.

4.5.3. c. Transverse section (T.S) of *Iphiona grantioides* leaf

The present study is intended to identify, describe and document the leaf epidermal characters that are diagnostic and taxonomically important in the family asteraceae occurring in Pakistan.

Transverse section of (T.S.) of *Iphiona grantioides* leaf is shown in the Fig. 4.11.a-g, which show that both the (upper and lower) epidermises are composed of a single layer of closely packed epidermal cells, outer cell walls are covered with waxy cuticle which is more thicker on adaxial side as compare to abaxial side. Both the epidermises have multicellular non glandular and glandular trichomes. Non glandular trichomes were uniseriate with long pointed apical cell while glandulars hairs are biseriate with multicellular stalk and multicellular capitate head (Fig.4.11-g).

Underneath the upper epidermis is present the heterogeneous mesophyll tissue, which is composed of multicellular palisade tissue and spongy parenchyma tissue beneath. Palisade cells have chloroplast and oil droplets while spongy mesophyll cells have cuboid crystals of calcium oxalate. In the midrib region the midvein is very prominent, having 1-2 vascular bundles, with sclerenchymatic cells on the lower side.

Mesophyll tissue is traversed by small vascular bundles, which are present towards the upper epidermis near the wing of the leaf which supplies food and water supply to the lateral sides. Meric (2009) has also reported Ca Oxalate from some species of the tribe *Inuleae*. The leaf is amphistomatic, stomata being present on both the epidermises and the stomatal apparatus is of anomocytic type (Fig. 4.11.e and f).

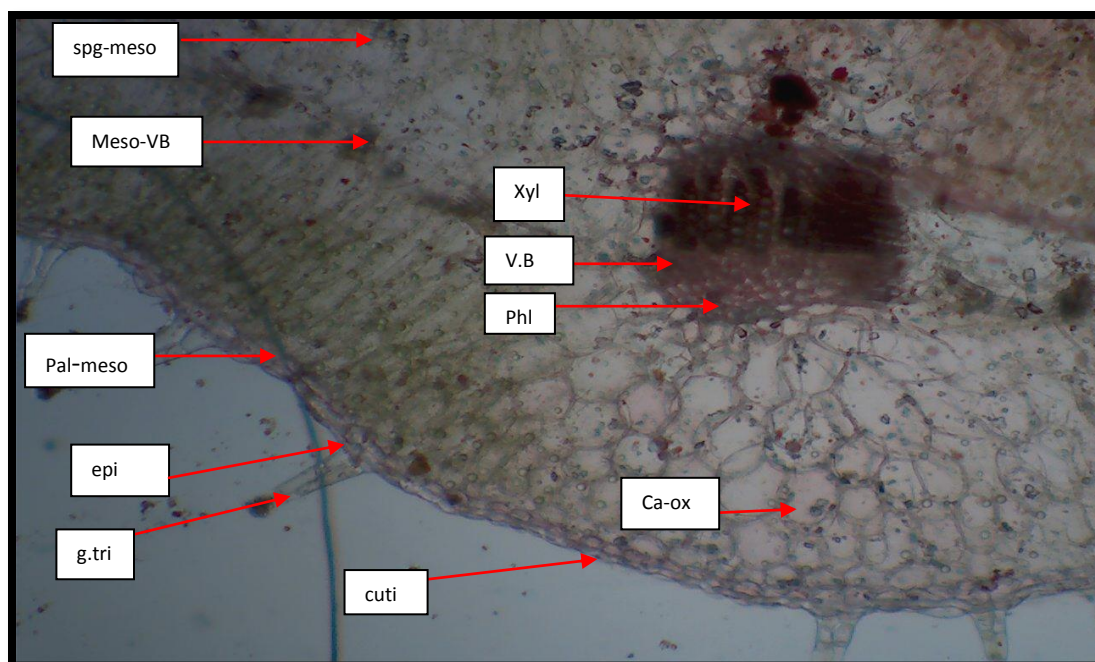


Fig.4.11.a. T.S. of *Iphiona grantioides* leaf in the midrib region. epi-epidermis, cuti-cuticle, g.tri-glandular trichome, spg-meso-Spongy mesophyll, pal-meso- Palisade mesophyll, ca-ox-calcium oxalate, VB-Vascular bundle, Xyl-Xylem, Phl-Phloem, Meso-V. B-Mesophyll- Vascular bundle



Fig.4.11.b. T.S. of *Iphiona grantioides* leaf. Palisade cells

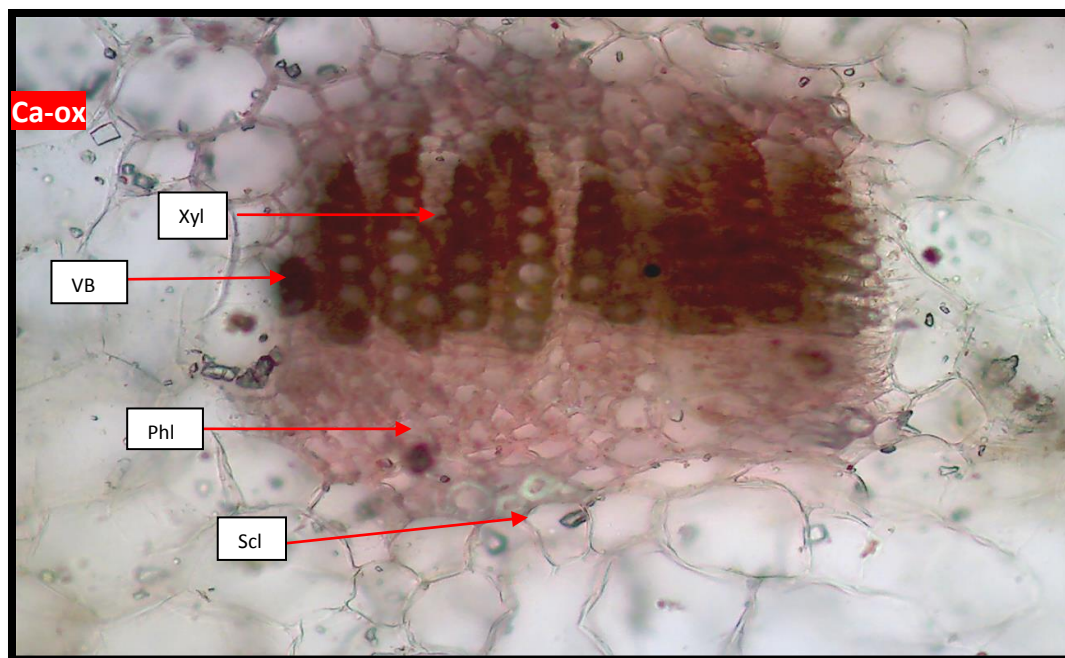


Fig.4.11.c.*Iphiona grantioides* leaf. Mid-vein.VB-Vascular bundle, Xyl-Xylem, Phl-Phloem,Scl-Sclerenchyma,Ca-ox-Calcium oxalate crystal

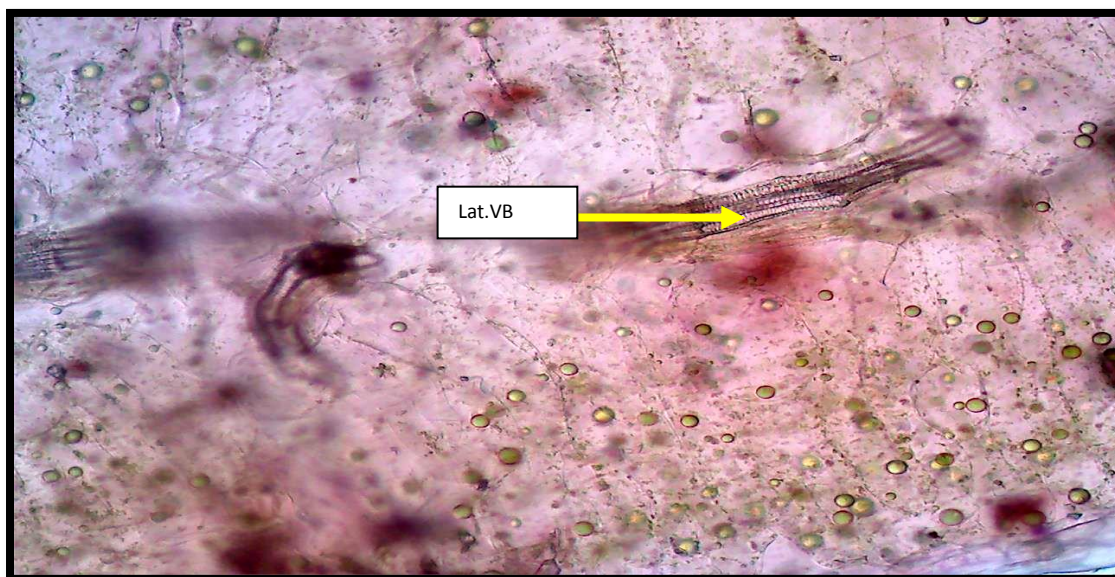


Fig. 4.11.d.T.S. of *Iphiona grantioides* leaf.Lat.VB- Lateral vascular bundles in the mesophyll

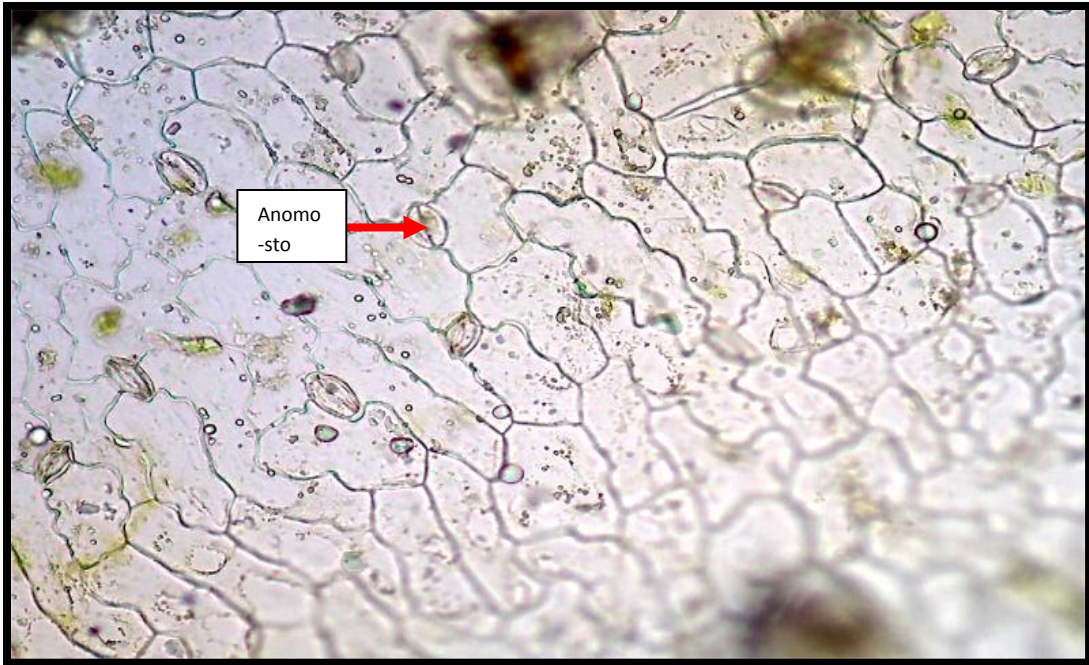


Fig.4.11. e. *Iphiona grantioides* leaf upper epidermis. An-sto-anomocytic stomata

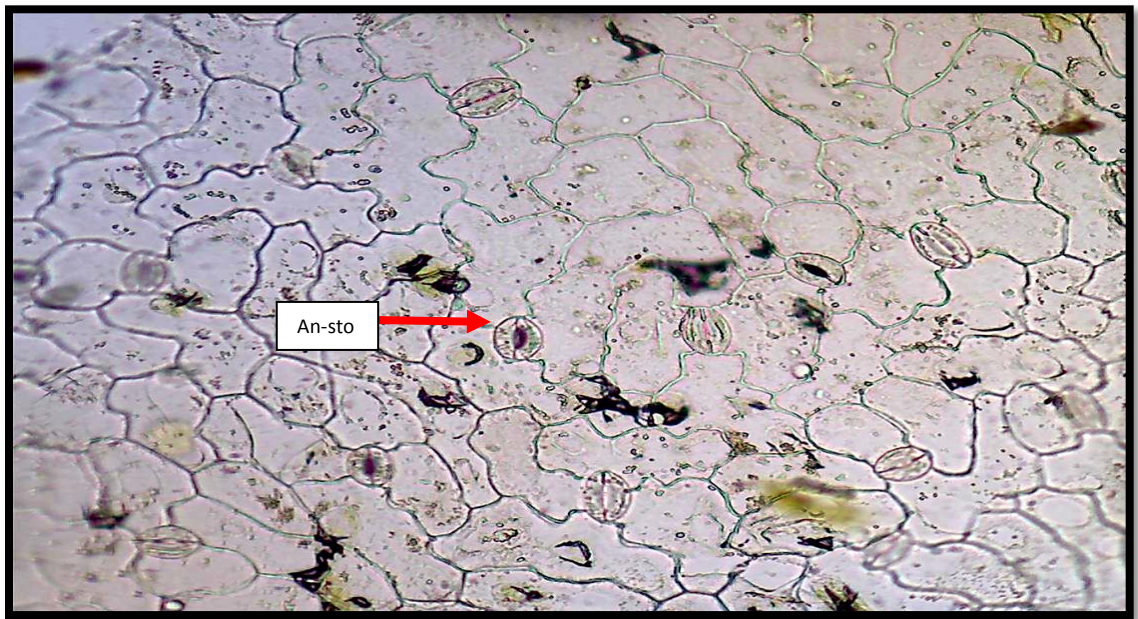


Fig.4.11.f. *Iphiona grantioides* leaf lower epidermis. An-sto-anomocytic stomata

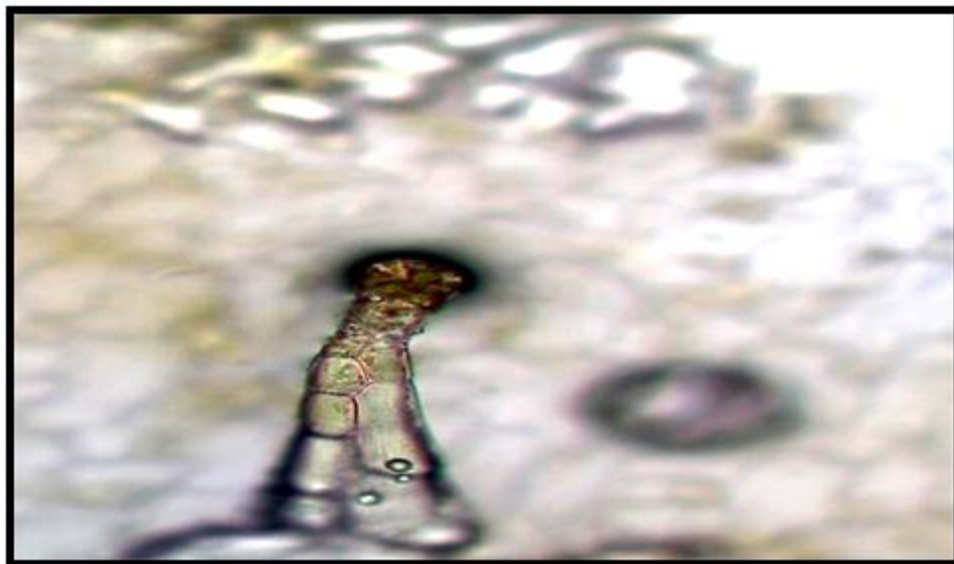


Fig.4.11.g. *Iphiona grantioides* leaf. Biseriate glandular trichome

4.5.3. d. Transverse section (T.S.) of *Pluchea arguta* subsp.*glabra*. Qaiser leaf

Transverse section revealed the presence of single layered upper and lower epidermises covered by thick cuticle on the outer side. The leaf has glandular trichomes, which are sessile as well as with multicellular stalk (2 - 5 cells). Sessile trichomes are surrounded by 5 - 9 epidermal cells (Fig.4.12.a - d). In the lamina the vascular system is embedded and remaining mesophyll comprises of palisade and spongy parenchymas. The latter is partially filled with oil globules. In the midrib region collenchymatous tissues lie under both the upper and lower epidermises, but it is strongly developed on the upper side. In the midrib region, there are 3 vascular bundles; the central one is comparatively larger than the other two. Vascular bundles are collateral and closed. Xylem lies toward the upper epidermis and phloem toward the lower epidermis. Xylem vessels are with spiral wall thickening (Fig.4.12.b).

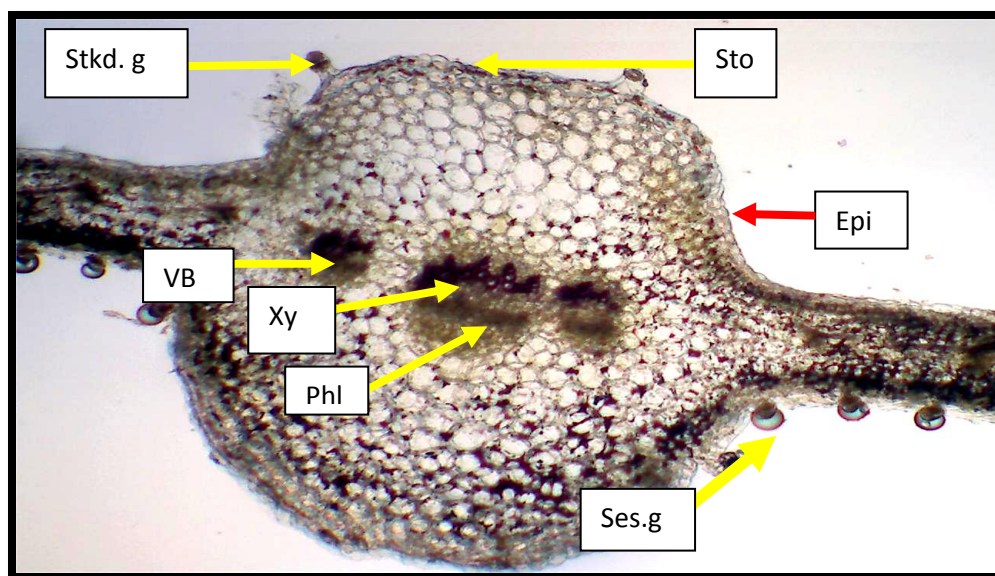


Fig. 4.12.a. T.S. of *Pluchea arguta* subsp. *glabra* leaf in midrib region. VB-vascular bundle, Xy-Xylem, Phl-Phloem, Sto-stomata, Epi-epidermis, Stkd.g-Stalked gland, Ses.g- Sessile gland



Fig.4.12.b. T.S. of *Pluchea arguta* subsp. *glabra*. Vascular bundles in the midrib region. Xy.vesl-Xylem vessel with spiral thickening

The stomatal apparatus is of Anomocytic type (3 or more epidermal cells surrounding guard cell having one cell distinctly smaller than the other two cells) and few diacytic type (surrounded by two cells enclosing guard cells and at right angle to its long

axis) (Dilcher, 1974). The epidermal cells are somewhat rectangular in shape, but different in size and with straight walls (Fig.4.12.c, d.)

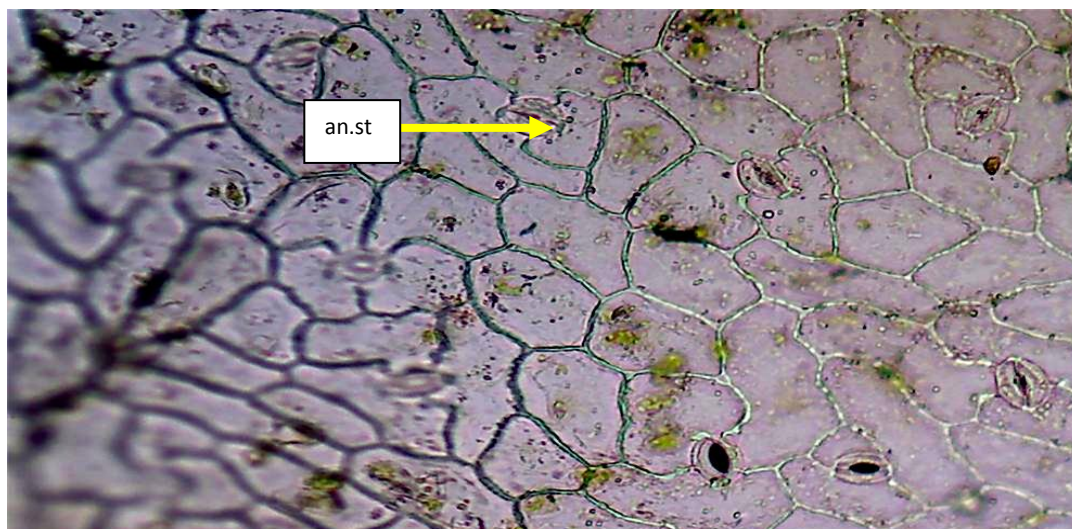


Fig. 4.12.c. *Pluchea arguta* subsp. *glabra*. Leaf upper epidermis. an. sto-Anisocytic stomata

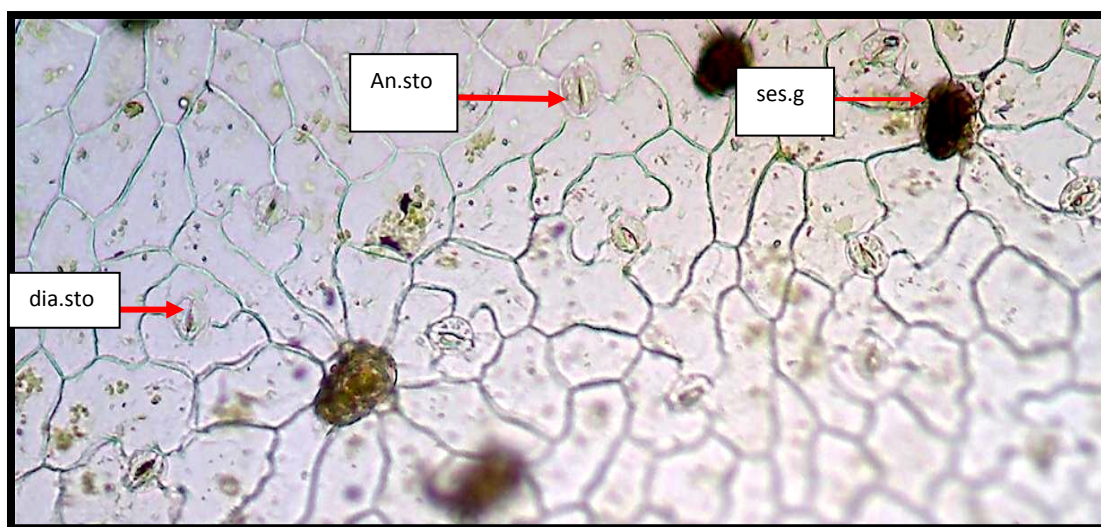


Fig.4.12. d. *Pluchea arguta* subsp. *glabra*. leaf lower epidermis .ses.g- sessile glands, dia.sto-Diacytic stomata, an. sto- Anomocytic stomata

4.5.3. e. Transverse section (T.S.) of root of *Iphiona grantioides*

Transverse sections of the root reveal that externally is the rhizodermis, followed by multicellular hypodermis of cortex. The general cortex is composed by paranchymatous cells. Endodermis surrounds the stele, located in the center. The stele is composed of pericycle and conductive tissue. Sclerenchymatous medullary rays are present between the xylem elements. Central part is occupied by primary xylem. Large xylem vessels are arranged radially, interconnected by a sclerenchymatous parenchyma cells and xylem fibres with mechanical role (Fig.4.13.a-b). The phloem is present, alternating with xylem, consisting of phloem vessels.

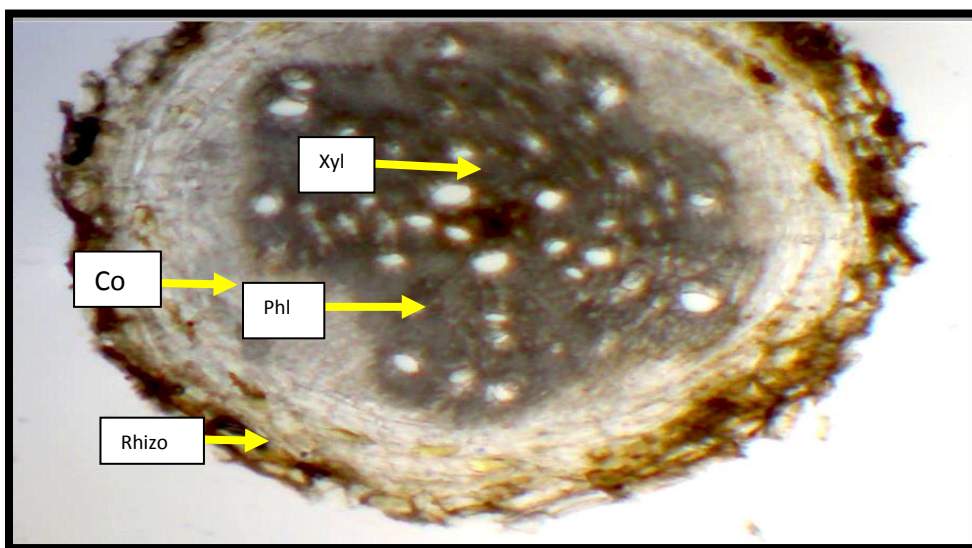


Fig.4.13.a. T.S. of *Iphiona grantioides* (Boiss.) root. Rhizo-epidermis, Co-cortex, Phl-phloem, Xyl-Xylem.

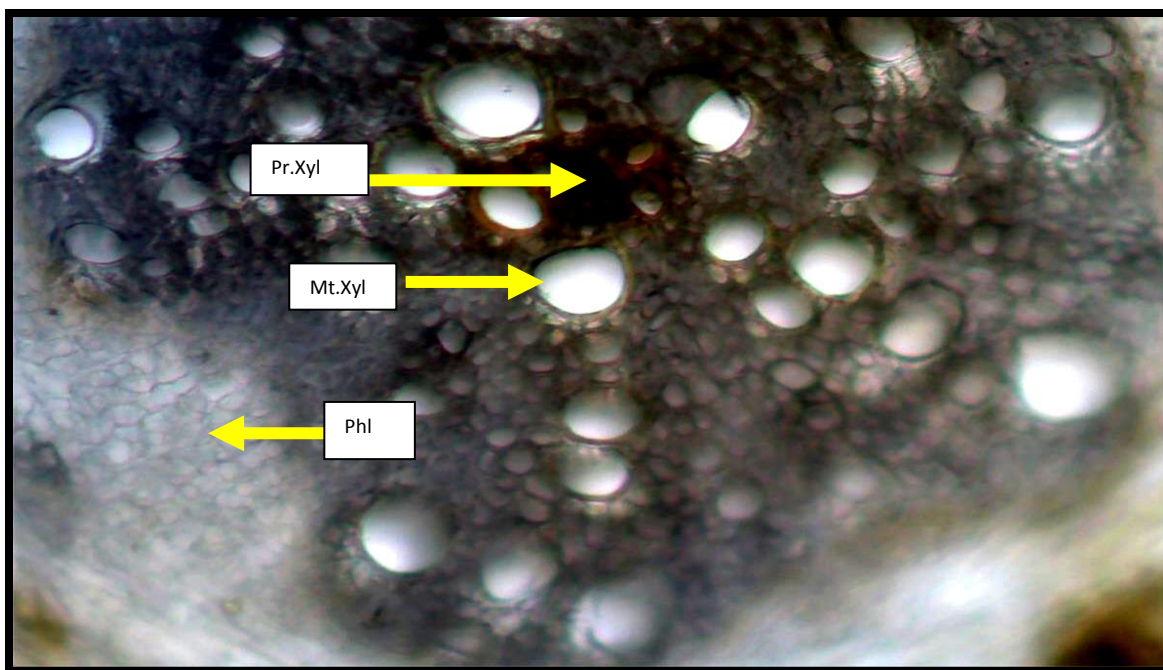


Fig.4.13.b. Transverse section (T.S.) of root of *Ipheion grantioides* Boiss. Pr.Xyl- Primary-xylem, Mt.Xyl- Meta Xylem, Phl- Phloem

4.5.3. f . Transverse section (T.S.) of root of *Pluchea arguta* subsp. *glabra*

Cross sections of the root of *plucheaarguta* subsp. *glabra* showed a secondary structure. The following anatomical features were prominent. In root, showing secondary growth, the epidermis or rhizodermis is replaced by cork due to the activity of cork cambium that produces secondary cortex or Phelloderm on the inside. Below the cortex is the secondary conductive tissues of the root, consisting of secondary phloem on the outside and secondary xylem tissues on inside, separated by cambium tissue. The secondary phloem is surrounded by sclerenchymatous fibers, also known as phloem fibers. Secondary xylem is represented by large, well developed, wide lignified vessels and accessory tissues while primary xylem tissue occupied the center of the root. Primary Xylem Vessels and secondary xylem vessels are separated by numerous multiseriate primary and secondary medullary rays, respectively (Fig.4.14.a, b)

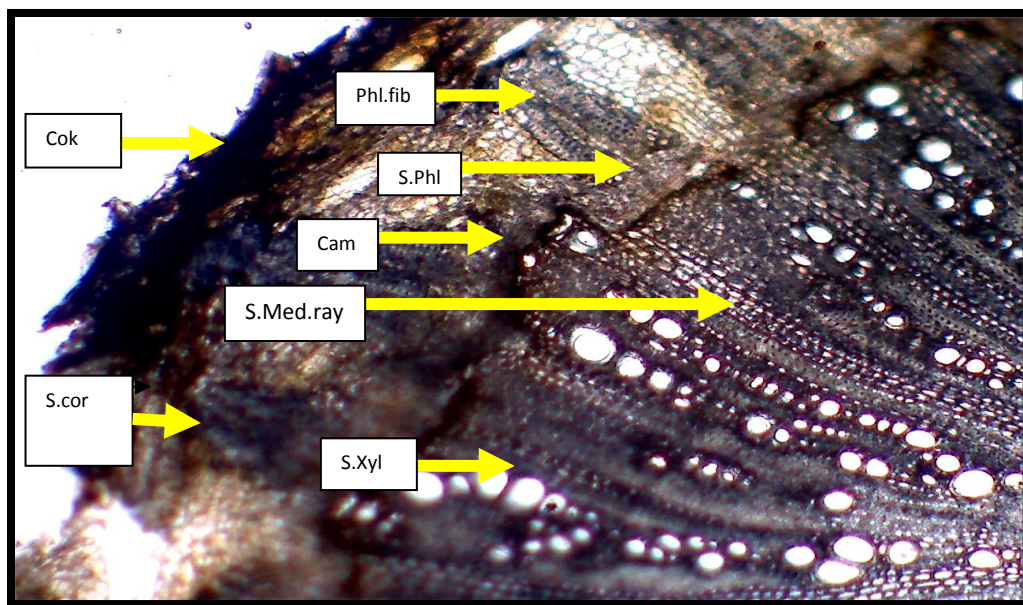


Fig. 4.14.a. T.S. of *Pluchea arguta* subsp. *glabra* root. Cok-Cork, S. cor-Secondary cortex, Phl.fib-Phloem fiber, S.PhI-Secondary Phloem, Cam-cambium, S.Med.R-Secondary medullary ray, S.Xyl-Secondary Xylem

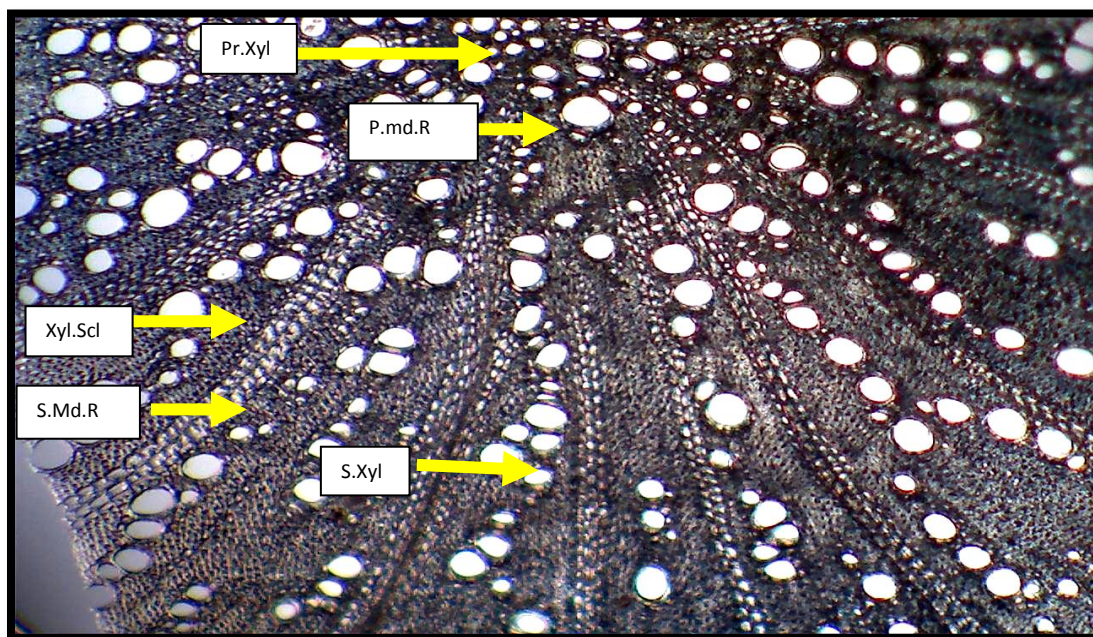


Fig. 4.14.b. T.S. of *Pluchea arguta* subsp. *glabra*. Root. Pr.Xyl-Primary Xylem, P.md.r-Primary medullary ray, Xyl.scl-Xylem Sclerenchyma, S.Md.R-Secondary medullary Ray. S.Xy-Secondary Xylem.

4.5.4. Leaf surface study of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Studies of the leaf epidermal characteristics are of great importance in pharmacognosy as well as in phylogeny and taxonomy; therefore plant taxonomists give more importance to the leaf epidermal features to solve the taxonomic problems (Taia, 2005). The following leaf surface features such as palisade ratio, vein islets number, vein termination number, stomatal number and stomatal index were carried out for *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* leaves.

4.5.4. a. Presence and absence of stomata and types of stomata in *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Stomatal types are considered very important taxonomically and provide efficient basis for establishing phylogeny in the higher level of taxonomic hierarchy (Hameed *et al.*, 2008). Stomata occurs mostly on leaf epidermis (on both epidermises -amphistomatic leaf, on upper epidermis only-epistomatic leaf or lower epidermis only -hypostomatic leaf) (Perveen *et al.*, 2007).

Leaf of *Iphiona grantioides* is amphistomatic, stomata being present on both the epidermises and the stomatal apparatus is of anomocytic type. Stomatal apparatus of *Pluchea arguta* subsp. *glabra* is anomocytic type (3 or more epidermal cells surrounding guard cell having one cell distinctly smaller than the other two cells) and few diacytic type (surrounded by two cells enclosing guard cells and at right angle to its long axis)(Dilcher, 1974).

4.5.4. b. Determination of Stomatal number and stomatal index of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Stomatal number and stomatal index value for *Iphiona grantioides* was 120 to 150 (**130**) and 12 to 15 (**13**) per mm² respectively, while in case of *Pluchea arguta* subsp. *glabra* stomatal number and stomatal index value was in the range of 110 to 160 (**130**) and 10 to 12 (**11**) per mm², respectively.

4.5.4. c. Vein islet number and vein termination number of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* leaf

Vein islet and vein termination number were 8 to 10 (**9**) and 7 to 10 (**8**) per mm² respectively, in case of *Iphiona grantioides*. The vein-islets were quite distinct as squarish, elongated or polygonal shaped, internally provided with many forked and vascular branches (Fig 4.15.b). Vein islet and vein termination numbers of *Pluchea arguta* subsp. *glabra* were 10 to 12 (**11**) and 6 to 9 (8) per mm² respectively (Fig 4.15.b).

4.5.4. d. Palisade ratio of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* leaf

The palisade ratio for *Iphiona grantioides* was ranged from 5 to 6 to 6.75 (Fig. 4.15.a, Table.4.8) and *Pluchea arguta* subsp. *glabra* leaf has the Palisade ratio from 6 to 7 (7.5) (Fig. 4.16.a, Table.4.8).

Palisade ratio, vein islet number and vein termination values were also reported for a number of other plants such as *Skimmia laureola* (dc.) siebold. & zucc. ex walp. and *Zanthoxylum armatum* dc., Barkatullah (2012), *Acacia leucophloea* (Gupta *et al.*, 2010) and *Lagenaria siceraria* (Shah *et al.*, 2010).

Stomatal index values are very important and used to distinguish between leaves of co-generic species (Evans, 2002). Hameed *et al.* (2008) carried out stomatal study of some members of family Polygonaceae and Solanaceae and suggested a taxonomic uniformity of stomatal type.

It becomes clear from these studies that different plant have different values for these features. So it may be concluded from the work of these and other workers, that these features play important role in identification and verification of crude drugs of leaves.

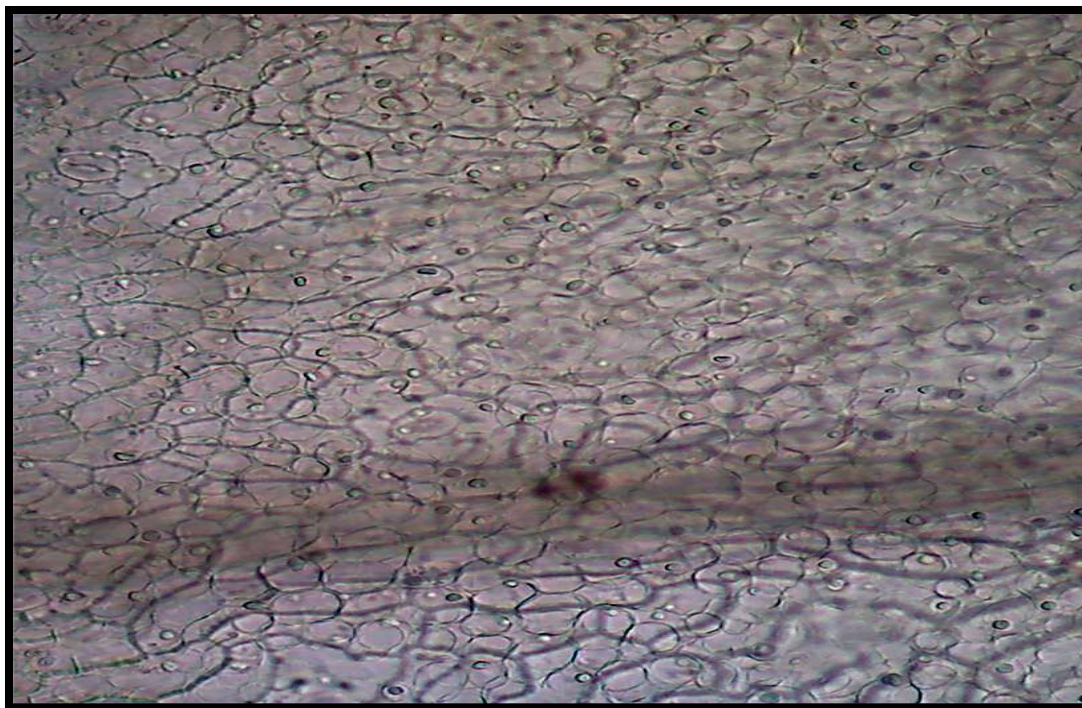


Fig.4. 15. a. *Iphiona grantioides* Boiss.a. Palisade cells arrangement under epidermal cells

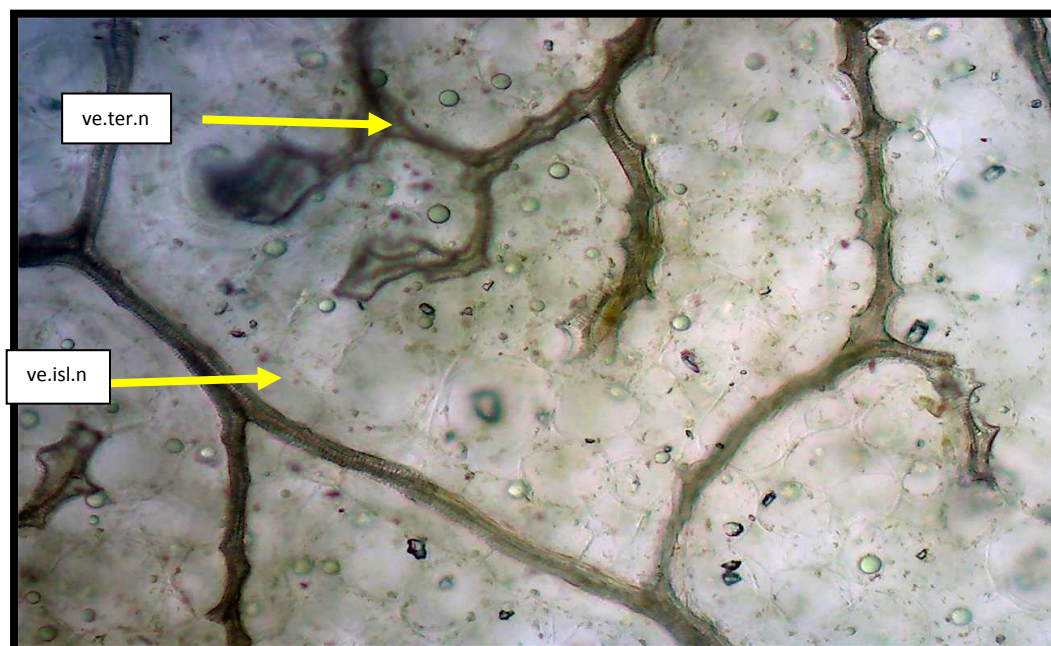


Fig.4.15.b. *Iphiona grantioides* Boiss.Veins arrangement in lamina. Ve.Isl.n- Vein islet number, Ve. ter.n- Vein termination number

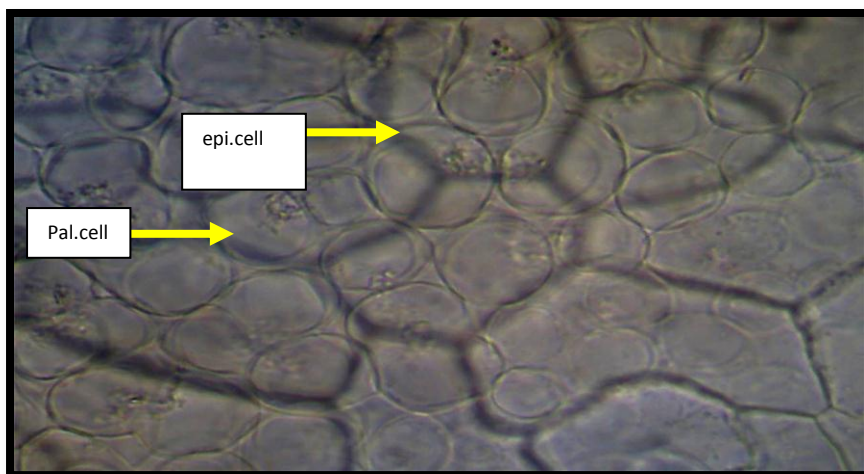


Fig. 4.16.a. *Pluchea arguta* subsp.*glabra* palisade cells under epidermal cells.epi.cell-epidermal cell, Pal.cell-Palisade Cell

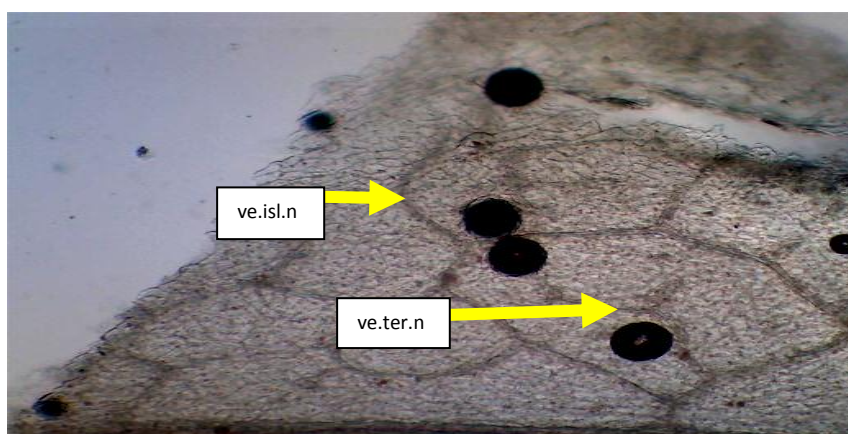


Fig.4.16.b. *Pluchea arguta* subsp.*glabra*. Ve. Isl.n- Vein islet number, Ve. ter.n- Vein termination number

Table 4. 8. Leaf Constant values of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

S. No	Plant species	Parameter	Range	Average
1	<i>Iphiona grantioides</i>	Palisade ratio	5 to 6 to 6.75	5.91
		Vein islets number	8 to 10	9
		Vein termination number	7 to 10	8
		Stomatal number	120 to 150	130
		Stomatal Index	12 to 15	13
2	<i>Pluchea arguta</i> subsp. <i>glabra</i>	Palisade ratio	6 to 7 to 7.5	6.83
		Vein islets number	10 to 12	11
		Vein termination number	6 to 9	8
		Stomatal number	110 to 160	130
		Stomatal Index	10 to 12	11

4.5.5. Physicochemical characteristics of the crude drug of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Various physicochemical characteristics of the powder drug of different parts of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* were carried out.

Ash analysis and moisture contents of *Iphiona grantioides*

In the present study ash analysis for crude powder drug of leaf, stem, root and flower was carried out. It was observed that highest value of total ash was recorded for stem (7.06%) and least for leaves (3.33%), while roots has 4.25% total ashes and flower has 3.38% total ash value. Acid insoluble ash was in the range of 1.54% (leaf), 1.425% (root), 1.27% (stem) and 0.35% (flower). Water soluble ash was in the range of 4.36% (leaves), 3.34% (stem), 2.23% (roots) and 1.82% (flower). Moisture contents was highest (9.16%) in root, 9.01% (stem), 7.80% (leaf) and 6.12% (flower) (Table 4.9).

➤ **Ash analysis and moisture contents of *Pluchea arguta* subsp.*glabra***

Ash analysis for crude powder drug of leaf, stem and root was carried out. Stem has total ash value of 7.82 %, 6.12 % to 5.6%. Acid insoluble ash was in the range of 1.66%, 1.09 % and 1.49 %. Water soluble ash was in the range of 2.56%, 3.38% and 6.5%, while moisture contents was highest in leaves(8.87%), root (8.41 %) and lowest in stem (7.5%)(Table 4.9).

Ash value determination is an important tool for the detection of adulterants or a mixture of inorganic matter such as metallic salts, silica or clay during improper handling (Jarald & Jarald, 2007). Likewise acid insoluble ash values are carried out for drugs having calcium oxalate crystals or sand, clay or other earthy material 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).

Table 4.9. Ash contents of different parts of *Iphiona grantioides* and *pluchea arguta* subsp.*glabra*

Sample	Total ash % W/W		Water Soluble % W/W		Acid Insoluble % W/W		Moisture cont % W/W	
	<i>Iphiona grantioides</i>	<i>pluchea arguta</i> subsp. <i>glabra</i>	<i>Iphiona grantioides</i>	<i>pluchea arguta</i> subsp. <i>glabra</i>	<i>Iphiona grantioides</i>	<i>pluchea arguta</i> subsp. <i>glabra</i>	<i>Iphiona grantioides</i>	<i>pluchea arguta</i> subsp. <i>glabra</i>
Flower	3.38%		1.82%		0.35%		6.12%	
Roots	4.25%	5.6%	2.23%	3.38%	1.42%	1.66%	9.16%	8.41%
Leaves	3.33%	7.82%	4.36%	6.5%	1.54%	1.49%	7.80%	8.87%
Stem	7.06%	6.12%	3.34%	2.56%	1.27%	1.09%	9.01%	7.5

4.5.6. a. Powder drug study of different parts of *Iphiona grantioides*

➤ Leaf powder

Leaf powder of *Iphiona grantioides* appeared bright green in color with pleasant aromatic odor and acrid taste. The powder study of leaf showed fragments of upper and lower epidermis along with anomocytic type of stomata, fragments of upper epidermis with cuticle and mesophyll cells, abundance of simple multicellular and glandular trichomes. The powder also showed transversely cut fragments of lamina with a row of palisade cells underneath upper epidermis, fragments of phloem fibres, spiral/annular xylem vessels wall thickening, and numerous isolated cuboid shaped crystals of Ca oxalate and in the mesophyll cells of leaf. Patches of parenchyma cells with oil droplets (Fig 4.17.a).

➤ Stem powder

Stem powder of *Iphiona grantioides* appeared dull green in color with mild aromatic odor and slight acrid taste. The powder study of stem showed rectangular epidermal and oval shaped cortical cells, vessels with pitted, reticulate, annular and spiral thickening, sclerenchymatous cells and glandular/ multi-cellular trichomes (Fig.4.17.b).

➤ Root powder

Powder drug of the root showed the presence of some cork cells, fragments of cortex, phloem fibers and xylem vessels and thick walled paranchymatous cells (Fig.4.17.c). Root powder was light in colour with a slight aromatic taste and slight characteristic odour.

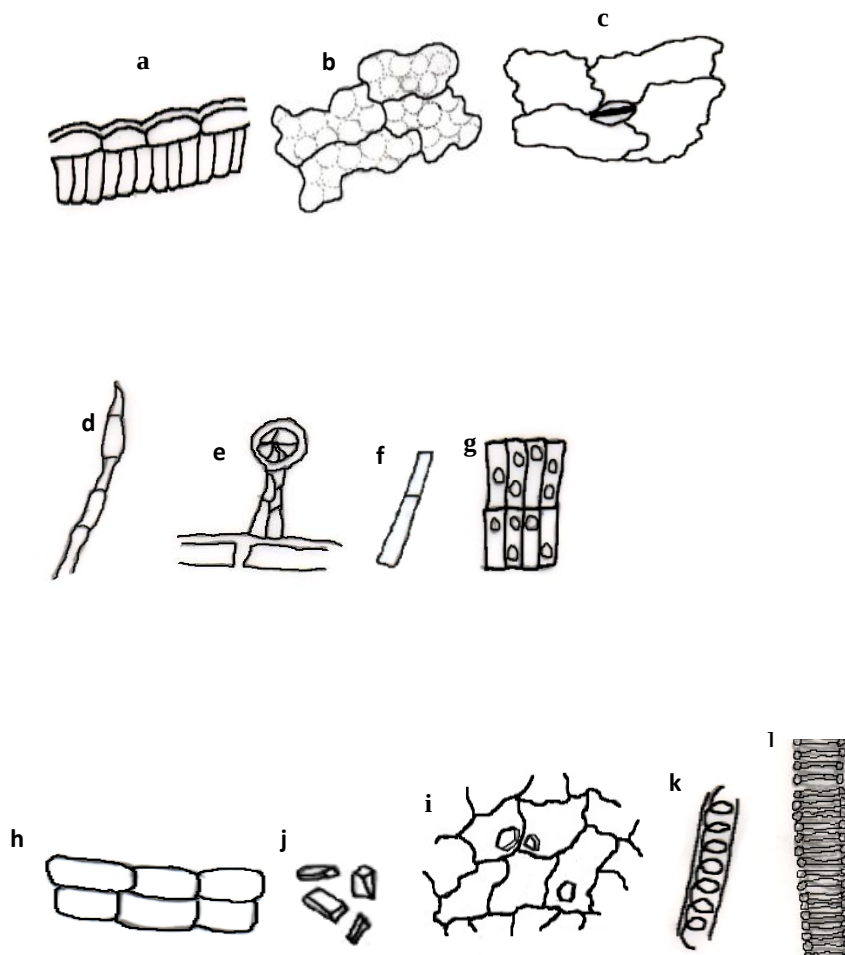


Fig.4.17.a.Powder drug study of *Iphiona grantioides* leaf. a.fragments of upper epidermal cells with cuticle and mesophyll cells; b.epidermal cells with mesophyll cells attached below; c. epidermal cells with anisocytic stomata; d. nonglandular multicellular hair; e. glandular trichome; f. fragment of a trichome ; g. Parenchyma cells with oil droplets h.epidermal and palisade cells; i. isolated crystals of calcium oxalates; j. Spongy mesophyll cells with crystals of calcium oxalates; k.vessels with spiral thickening; l. vessels with annular thickening.

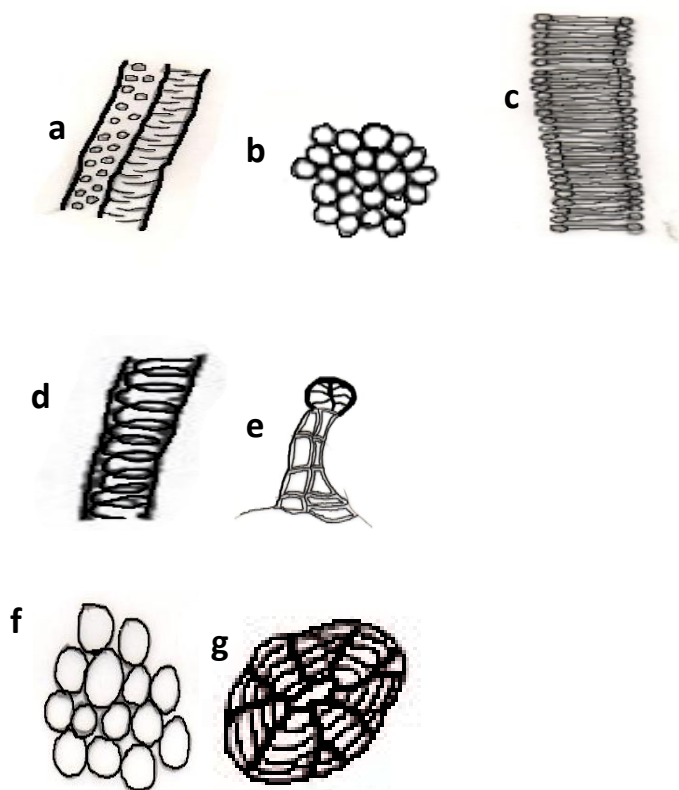


Fig.4.17.b. Powder drug study of *Iphiona grantioides* stem. **a.** Vessels with pitted and reticulate thickening ; **b.** cortical parenchyma cells; **c.** annular vessels; **d.** vessels with spiral thickening; **e.** glandular trichome; **f.** rectangular epidermal cells ; **g.** sclerenchymatous cells.

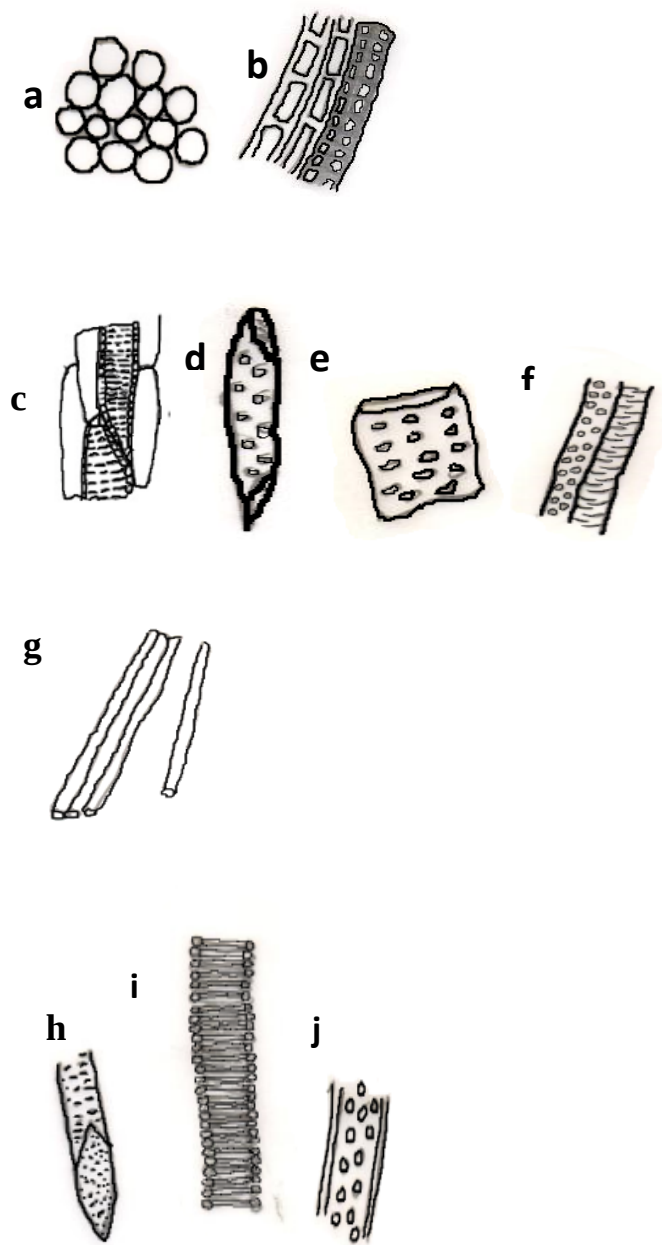


Fig.4.17.c. Powder drug study of *Iphiona grantioides* root. **a.** fragments of cortex; **b.** cork cells; **c.** xylem vessels with pits and lignified paranchymatous cells, attached; **d.e. & f.**vessels with reticulate and pitted thickenings; **g.** fibers.**h.i&j.** vessels with annular and pitted thickenings.

4.5.6. b. Powder drug study of *Pluchea arguta* subsp. *glabra*

➤ Leaf powder

Leaf powder of *Pluchea arguta* subsp. *glabra* was dark green in colour, with pungent odour and unpalatable taste. Microscopic study of the leaf powder showed pieces of upper and lower epidermises in surface view with anomocytic type of stomata, many simple multicellular and non glandular hairs. The powder also showed transversely cut fragments of lamina with a row of palisade underneath the upper epidermis, fragments of phloem fibres, spiral/annular xylem vessel (Fig.4.18.a).

➤ Stem powder

Colour, odour and taste of the stem powder of *Pluchea arguta* subsp. *glabra* is dull green, slight pungent and slight bitter, respectively. The powder study of stem showed epidermal cells, paranchymatous pith cells, annular/spiral vessel and sclerenchymatous cells (Fig.4.18.b).

➤ Root powder

Colour, odour and taste of the root powder of *Pluchea arguta* subsp. *glabra* is light brown, odour less and unpalatable, respectively. Further the powder drug of the root of *Pluchea arguta* subsp. *glabra* revealed the presence of some cork cells, fragments of cortex, phloem fibers, xylem vessels and paranchymatous cells (Fig.4.18.c).

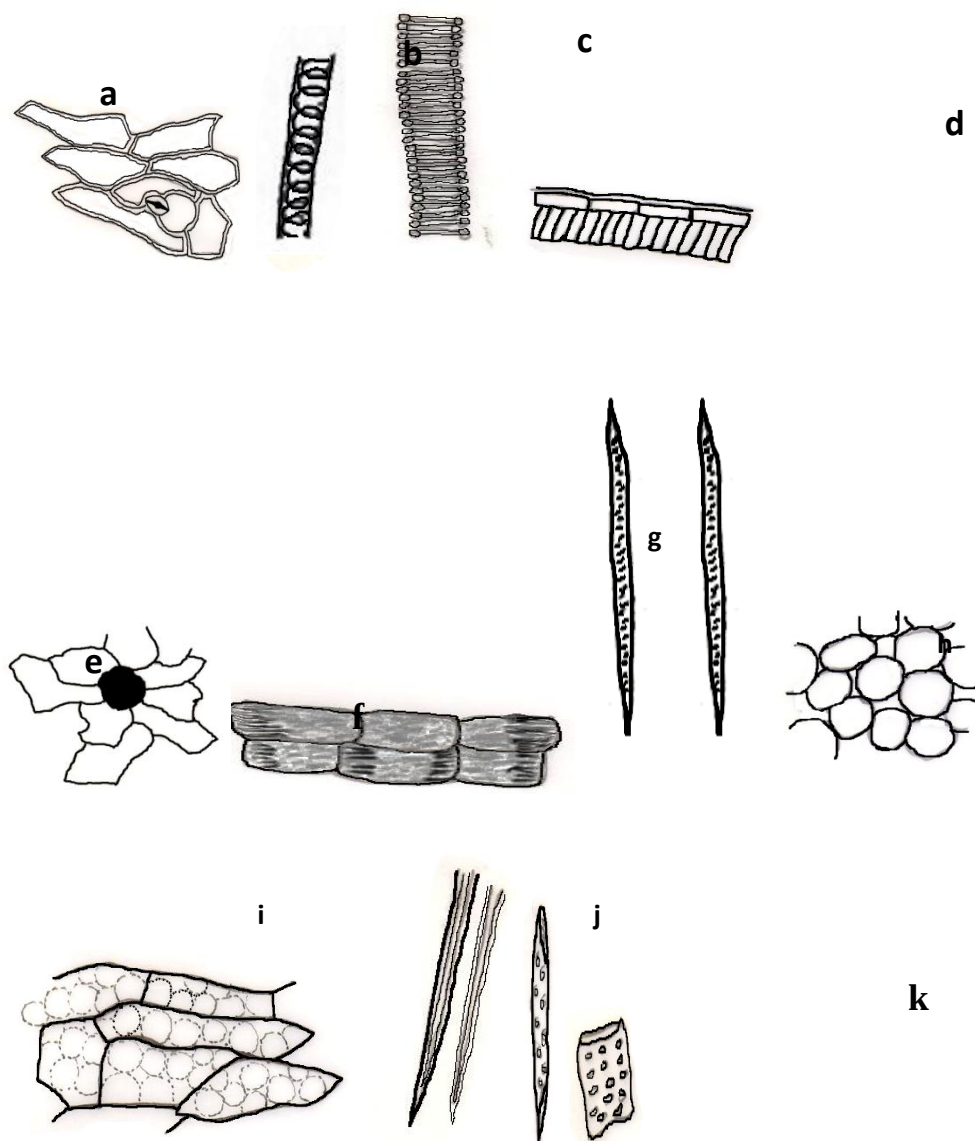


Fig.4.18. a. Powder drug study of *Pluchea arguta* subsp. *glabra* leaf. a. fragments of epidermal cells with anomocytic stomata. b. & c. vessels with annular and spiral thickening; d. fragments of epidermal cells with mesophyll cells attached; e. sessile glands attached to epidermal cells; f. epidermal cells with mesophyll cell attached beneath; g. tracheids ; h. Cortical cells; i. Epidermal cells; j. thick walled fibers; k. Pitted vessels

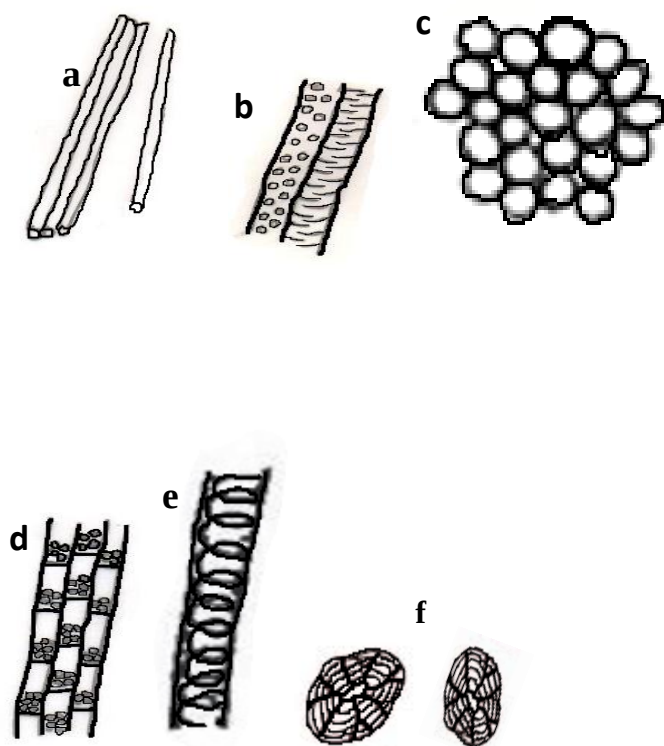


Fig.4.18.b. Powder drug study of *Pluchea arguta* subsp. *glabra* stem. **a.** Fiber; **b.** Vessels with annular wall thickening and pits; **c.** parenchyma cells, **d.** cells with starch grains, **e.** vessels with spiral wall thickening; **f.** sclerenchymatous cells.

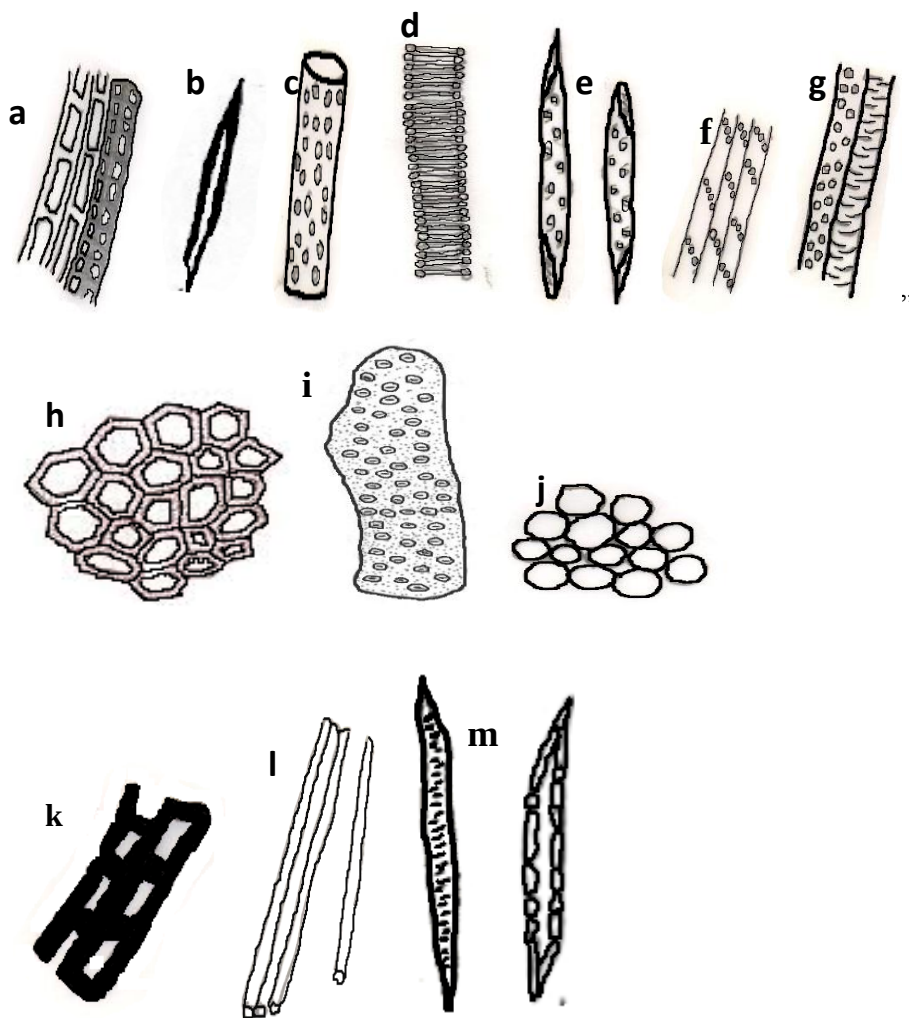


Fig.4.18.c. Powder drug study of *Pluchea arguta* subsp. *glabra* root. **a.** Patches of cork cells; **b.** fibers; **c.d. e. f. & g.** vessels with different wall thickening; **h.** Sclerenchymatous cells; **i.** Pitted vessels; **j.** Parenchymatous cells; **k.** fragments of cork cells; **l.** bundles of fibers; **m.** thick walled fibers.

Powder drug studies have been carried out by some other workers e.g Maiti *et al.* (2002) carried out the pharmacognosy and histochemistry of several plants belonging to family. *Solanaceae* for the identification of these plants; Mishra *et al.*, (2008) worked out the leaf powder of *Tridax procumbens* while Murti *et al.*, (2010) carried out the microscopic study of powder of *Calotropis procera*. The present study is well in accordance with those of Nayak *et al.*, 2010 (*Jatropha curcas*), Balakrishnan *et al.*, 2011 (*Amaranthus spinosus* Linn), Subha *et al.*, 2011 (*Acorus calamus*), Bisht *et al.*, 2011 (*Swertia chirayita*), Zunjar *et al.*, 2011 (*Carica papaya*), Hussain *et al.*, 2011 (*Hygrophila auriculata*) and Kumar *et al.*, 2011 (*Crocus sativus*) who also conducted ash analysis for standardization of various plants crude drugs.

4.5.7. Phytochemical screening

Plant species with medicinal properties have been playing a fundamental role in the efforts for drug discovery all over the world. 80% populations in developing countries are dependent on plants for their primary health care, and in spite of the significant progress in the field of synthetic organic chemistry of the twentieth century, more than 25% of prescribed medicines in developed countries are derived directly or indirectly from plant sources (Newman *et al.*, 2000).

Preliminary phytochemical screening is important and useful for isolation of pharmacologically active compounds present in the plants, (Sugumaran & Vetrichelvan, 2008). This serves as an important tool for the quality assurance of plant for future studies. Till now almost all investigated plants showed to contain different active constituents of pharmacological importance in the form of secondary metabolites (Ming *et al.*, 2005).

4.5.7.a. Qualitative phytochemical screening of leaf, stem, root and flower of *Iphiona grantioides*

Qualitative phytochemical screening of the leaves, branch, roots and flowers of *iphiona grantioides* (Table 4.10) revealed that Proteins ,carbohydrates and tannins were present in both aqueous and ethanolic extracts of all the parts. Alkaloids were detected

in both aqueous and ethanolic extracts of leaf and stem. Flavonoids were present in both type of extracts of all parts except root. Saponins and glycosides were present in all extracts; however Glycosides were not detected in Borntrager test. Phytosterol and triterphenoids were present in all the parts of *Iphiona grantioides*, while spot test for fixed oil gave negative result. Volatile oil were only detected in the ethanolic and aqueous extracts of flower and leaf of *Iphiona grantioides*. The result showed that *Iphiona grantioides* is rich in bioactive compounds and hence is a potential source of therapeutic properties.

4.5.7. b. Qualitative phytochemical screening of leaves, stem, root of *Pluchea arguta* subsp. *glabra*.

More or less similar results were also obtained for the Phytochemical screening of leaf, stem and root of *Pluchea arguta* subsp. *glabra* (Table 4.14). Proteins, carbohydrates and tannins were present in both aqueous and ethanolic extracts of all parts; however alkaloids were detected only in aqueous extract of *Pluchea arguta* subsp. *glabra*. Flavonoids and Saponins were present in all parts in both types of extracts. Glycosides were present in stem and leaves but were absent in both extracts of root. Phytosterol, triterphenoids and volatile oil were present only in leaves of *Pluchea arguta* subsp. *glabra*. Spot test for fixed oil gives negative result for all tested parts. So just like *Iphiona grantioides*, *Pluchea arguta* subsp. *glabra* is also a source of therapeutically important metabolites.

Khan *et al.*, (2010) reported that the ethanolic extracts of the leaf, stem and root of *Pluchea lanceolata* have almost the same phytochemical constituents as present in the present research plants. Arjun *et al.*, (2009) also explored phytochemical composition of leaf of *Hygrophila spinosa*. Chirikova *et al.*, (2010) investigated preliminary phytochemical screening of *Scutellaria baicalensis*. Kalyan *et al.*, (2011) subjected ethanol extract of *Clitoria ternatea* seeds to preliminary phytochemical investigations. All these works emphasize on the importance of phytochemical screening for obtaining first hand information about metabolites of pharmacological significance.

The phytochemicals detected in our extracts are well known for various pharmacological activities. For instance alkaloids are well known for antibacterial, antimalarial, cytotoxic and anticancerous properties (Wirasathien *et al.*, 2006). Similarly saponins are the common insecticidal, antibiotic and fungicidal agents (Sparg *et al.*, 2004). Flavonoids are phenolic compounds are extensively distributed in the plant kingdom (Nijveldt *et al.*, 2001) and have been proved to have antibacterial, anti-inflammatory, antiallergic, antineoplastic, antiviral, antidiarrhoeal anti-thrombotic antioxidant and vasodilatory properties (Miller, 1996). Tannins have shown antiviral (Lin *et al.*, 2004), antibacterial (Akiyama *et al.*, 2001) and antioxidant potential (Yokozawa *et al.*, 1998). Tannins were isolated from oriental medicinal herbs and evaluated for their antioxidant ability with a 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and the results showed that tannins are active free-radical scavengers (Yokozawa *et al.*, 1998). Similarly hydrolyzable tannins might cause double-strand and single-strand breakages in DNA (Shirahata *et al.*, 1985). In the past few years tannins have also been studied for their anticancer potential (Bhagavathi, 1999). Cardiac glycosides have been studied for their cytotoxic and Na-K-ATPase inhibitory properties (Joseph *et al.*, 2005). All these compounds are commonly present in medicinal plants and well known for their pharmacological activities.

Furthermore the present study revealed that different extracts of same plants may have different phytochemicals suggesting that there may be variation in phytochemistry of different tissues. Evans *et al.*, (2002), also reported that saponins were present in small quantity in roots but absent from the leaves and inflorescence of *Euphorbia hirta*.

Table 4.10. Phytochemical investigation of different parts of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

			Ethanol Crude Extract							Aqueous Crude Extract						
1	Constituents	Test name	IF	IL	IB	IR	PL	PB	PR	IF	IL	IB	IR	PL	PB	PR
	Carbohydrates	Molish	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Benedict	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Fehling	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Protein	Ninhydrin	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Bieruit	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Millon's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	Tanins	Lead Acetate	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		FeCl ₃	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Gilatin test	+	+	+	+	+	+	+	+	+	+	+	+	+	+

		Alkali Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	Alkaloi des	Dragon Droff	–	+	–	+	–	–	–	–	+	–	+	–	–	–	–
		Hager	+	+	+	+	+	–	–	+	+	+	+	–	–	–	–
		Wager	+	+	+	+	–	–	–	+	+	+	+	–	–	–	–
		Mayer test															
5	Flavon oides	Shinoda test	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+
		Alkali Test	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+
6	Sapnins	Forth Formation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		NaHCO ₃		+	–	–	+	–	–	–	+	–	–	+	–	–	–
7	Glycosi des	Killaer kilani test	+	+	+	–	+	+	–	+	+	+	–	+	+	–	–
		Borntrager	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+

8	Phytosterol/ Triterpenoides	Salkowski's test Liebermann – Burchard's test	+	+	+	+	+	–	–	+	+	+	+	+	–	–
9	Fixed Oil	Spot Test	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10	Volatile Oils		+	+	–	–	+	–	–	+	+	–	–	+	–	–
Key = IF = <i>Iphiona grantioides</i> flower PL= <i>Pluchea arguta</i> subsp. <i>glabra</i> leaf IL = <i>Iphiona grantioides</i> leaf PS = <i>Pluchea arguta</i> subsp. <i>glabra</i> stem IS = <i>Iphiona grantioides</i> stem PR= <i>Pluchea arguta</i> subsp. <i>glabra</i> root IR = <i>Iphiona grantioides</i> root																

4.5.8. Fluorescence study

Fluorescence study with UV light is a very useful tool for evaluation of crude drugs. Crude extract or powder drugs, when viewed under UV light give characteristic fluorescence (Wallis, 1985). The fluorescence phenomenon is the emission of light of different wave lengths, under the influence of UV light, due to different chemical constituents, otherwise not observable in ordinary visible light (Ansari, 2006; Jarald & Jarald, 2007; Reddy & Chaturvedi, 2010). Fluorescence study helps for authentication and standardization of crude drugs. Several Crude drugs of plant origin have been authenticated through the fluorescence study technique e.g., *Morinda umbellata* (Ismaeil & Sultana 2008), *Holoptelea integrifolia* (Sharma *et al.*, 2009), root and stem of *Ichnocarpus frutescens* (Kalidass *et al.*, 2009), leaves of *Catunaregum spinosa* (Shrivastava & Leelavathi, 2010), *Hygrophila auriculata* (Hussain *et al.*, 2011a) and *Crocus sativus* (Kumar *et al.*, 2011).

Powder drug of *Iphiona grantioides* (flower, leaf, stem, and root) and *Pluchea arguta* subsp. *glabra* (leaf, stem, and root) were such and after treatment with various solvents was subjected to fluorescence analysis. Observations were made under visible light and under UV light of short wave length and long wave length. Results are presented in Table 4.11- Table 4.14. Powder drug of the two plants exhibited fluorescence. Fluorescence study is very useful, quick, easy and unfailing method for the detection of adulterations.

Table 4.11. Fluorescence analysis of *Iphiona grantioides* flower powder with different reagents

S.No.	Reagents	Visible light	UV256	UV 310
1	Powder as such	Light Yellow	Brown Yellow	Light Brown
2	Powder+50% HNO ₃	Orange	Yellow Orange	Reddish Brown
3	Powder+ Picric Acid	Intense Yellow	Light Brown	Light Brown
4	Powder+NH ₃	Yellow	Light Green	Dull Green
5	Powder+H ₂ SO ₄	Black	Yellow Brown	Green Yellow
6	Powder+ NaOH	Orange	Reddish Brown	Yellow Red
7	Powder+ Actic Acid	Buff White	Creamy Yellow	Light Brown
8	Powder+ HCL	Light Yellow	Brown Yellow	Yellowish Green
9	Powder+ NaOH+ Ethanol	Yellow Orange	Yellowish Orange	BrownishRed
10	Powder+ Iodine	Creamy	Brown	Light Brown
11	Powder+ FeCl ₃	Black	Dark brown	Blakish brown

Table.4.12. Fluorescence analysis of leaves of *Iphiona grantioides* and *Pluchea arguta* subsp.*glabra* powder with different reagents

S.No .	Reagents	<i>Iphiona grantioides</i>			<i>Pluchea arguta</i> subsp. <i>glabra</i>		
		Visible light	UV256	UV 310	Visible light	UV256	UV 310
1	Powder as such	Dark Green	Olive Green	Light Green	Dark green	Light dull green	Brownish green
2	Powder+ 50% HNO ₃	Light Yellow	Light Yellow	Orange	Orange	Yellow	Orange yellow
3	Powder+ Acetic acid	White	Light yellow	White creamy	white	Cream colour	Creamish yellow
4	Powder+ Picric Acid	Parrot Green	yellow	Light Yellow	Intense yellow	Dark Yellow	Yellow
5	Powder+ NH ₃	yellow	Light yellow	yellow	Orange	orange	Rust color
6	Powder+ 50% H ₂ SO ₄	Light Green	Dull green	Greenish Bblack	Blakish green	Black	Black
7	Powder+ NaOH	Yellow Green	Light yellow	Cream	Yellow	Intense Yellow	Yellow
8	Powder+ 50%HCl	Yellow Green	brown	Light Brown	Dark green	Black green	Black green
9	Powder+NaOH+Ethaol	Yellow Green	Light yellow	Dull yellow	Yellow	Yellow	Yellow brown
10	Powder+ Iodine	GreenishBrown	Brown	Cream Yellow	White	white	Creamish
11	Powder+ FeCl ₃	Blackish Brown	Black	Black	Black green	Black	Blackish green

Table.4.13. Fluorescence analysis of stem of *Iphiona grantioides* and *Pluchea arguta* subsp.*glabra* powder with different reagents.

S.No	Reagents	<i>Iphiona grantioides</i>			<i>Pluchea arguta</i> subsp. <i>glabra</i>		
		Visible light	UV256	UV 310	Visible light	UV256	UV 310
1	Powder as such	Dull Light Green	Light Brown	Dull green	Dull green	Light green	Green
2	Powder+ Picric Acid	Intense Yellow	Brotwn	Yellow	Intense yellow	Yellow	Yellow
3	Powder+ NH ₃	Yellow	Light yellow	Cream	yellow	Yellow	Yellow
4	Powder+ H ₂ SO ₄	Greenish Brown	Black green	Dull Brown	Black brown	Brown	Black green
5	Powder+ NaOH	Intense Yellow	yellow	Litgh Yellow	Light Yellow	Light Yellow	Dull yellow
6	Powder + Acetic Acid	Buff Cream	cream	Creamy	White	Cream	Cream
7	Powder+ HCl	Light Cream	Light yellow	White	Black green	Black	Black
8	Powder+ NaOH+Ethanol	Yellow	Light yellow	yellow	Slight yellow	Yellow	Light yellow
9	Powder+ Iodine	Cream	Light brown	Cream brown	Buff colour	White	Cream
10	Powder+ FeCl ₃	Black	brown	Brown Black	Green Black	Black	Black
11	Powder+50% HNO ₃	Light Yellow	yellow	Light Orange	Yellow	Yellow	Yellow

Table. 4.14. Fluorescence analysis of root of *Iphiona grantioides* and *Pluchea arguta* subsp.*glabra* powder with different reagents.

S.No	Reagents	<i>Iphiona grantioides</i>			<i>Pluchea arguta</i> subsp. <i>glabra</i>		
		Visible light	UV256	UV 310	Visible light	UV256	UV 310
1	Powder as such	Light brown	brown	Light brown	Light brown	brown	Light Brown
2	Powder+ Acetic Acid	Creamish	Cream	Whitish	Cream colour	Light brown	Buff Colour
3	Powder+ NH ₃	Light yellow	cream	Light yellow	Yellow	Yellow	Yellow
4	Powder+ H ₂ SO ₄	Creamish Brown	Light brown	Brown	Brown	brown	Dull brown
5	Powder+ NaOH	Dull Brown	brown	LightBrown	Brown Yellow	Light yellow	Brown
6	Powder+ HCl	Light Brown	Light yellow	Dull Black	Light yellow	cream	Cream
7	Powder+ NaOH+Ethanol	Light yellow	cream	Yellow	Light yellow	Cream	Cream
8	Powder+ Iodine	Creamy	Buff colour	White	White	White	Buff White
9	Powder+ FeCl ₃	Brown	Brown	Light Brown	Brown	Light brown	Light Brown
10	Powder+50% HNO ₃	Yellow Orange	Light yellow	Creamy	Light yellow	Light yellow	Light yellow
11	Powder+ Picric Acid	Yellow	yellow	Greenish Yellow	Light Yellow	Light Yellow	Creamy

4.5.9. Extractive values

Extractions of crude powder drug with different solvents gives different extractive values. Extractive value is one of the useful methods for evaluation of crude drugs, and provides guidance about the most suitable solvent to be used for extraction, and also helps in detecting various types of adulteration and exhausted materials i.e. Water and alcohol soluble extractive values can be used for the detection of adulterants, defective processing and poor quality of the drug while petroleum ether soluble extractive value indicates lipid contents present in the crude drugs (Madhavan *et al.*, 2009; Kokate, 1994).

4.5.9. a. Extractive values of *Iphiona grantioides*

In the present study seven different solvents including ethanol, methanol, chloroform, acetone, distilled water, butanol and n-hexane were used for percent extractive values determination of different parts (leaf , flower, root and shoot f *Iphiona grantioides* and leaf, shoot and root of *Pluchea arguta* subsp. *glabra*). Results obtained are presented in the Table 4.15. Highest extractive values were given by *Iphiona grantioides* leaf i.e., (Aqueous extract (46.2%), Ethanol (37%), Butane (36%), Hexane (22.8%), Ethyl acetate (13.75) followed by root extracts i.e., Acetone (20%), Chloroform (20%), Ethanol (19 %) and Aqueous extract (16.6%).

4.5.9. b. Extractive values of *Pluchea arguta* subsp.*glabra*.

Solvents of different polarity were used for the determination of extractive values for *Pluchea arguta* subsp. *glabra*. Highest extractive values are Ethyl acetate (30%), Aqueous (27.2%), Ethanol (22.2 %), Chloroform (20%) and Hexane (20.75%). In other solvent both the parts showed variable values (Table 4.15).

Many workers have carried out studies for the determination of extractive values of different medicinal plants including Singhal *et al.*, 2010 (*Geniosporum prostratum*), Balakrishnan *et al.*, 2011 (*Amaranthus spinosus* Linn), Subha *et al.*, 2011 (*Acorus calamus*), Hussain *et al.*, 2011 (*Hygrophila auriculata* K.Schum), Kumar *et al.*, 2011 (*Crocus sativus*) and Zunjar *et al.*, 2011 (*Carica papaya*). So it is suggested that

determination of extractive values is an important tool for evaluation of crude drugs, detection of adulterants and for selection of suitable solvent for extraction.

Table 4.15. Extractive values of different parts of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* plants in different solvents

S.No	Extractive Values (mg)	IF	IL	IS	IR	PL	PS	PR
1	Aqueous	5.25	46.2	31.8	16.6	27.2	22.8	4.2
2	Acetone	2.6	7.5	4.25	20	1.75	5	35.5
3	Butane	8.8	36	2.4	7.6	3.6	13.2	2.5
4	Chloroform	2.75	8.25	0.75	20	5	3.5	5.5
5	Ethanol	22.25	37	11.25	19	22.2	13.4	8.75
6	EthylAcetate	13.75	10	2.75	13.75	30.25	22.4	2.5
7	Hexane	2.75	22.8	1.27	2.5	20.75	22.75	0.25
Key = IF = <i>Iphiona grantioides</i> flower PL= <i>Pluchea arguta</i> subsp. <i>glabra</i> leaf IL = <i>Iphiona grantioides</i> leaf PS = <i>Pluchea arguta</i> subsp. <i>glabra</i> stem IS = <i>Iphiona grantioides</i> stem PR= <i>Pluchea arguta</i> subsp. <i>glabra</i> root IR = <i>Iphiona grantioides</i> root								

4.6. Heavy metal analysis

Plants have the ability to accumulate such essential elements in their different parts, which are of highest importance in human nutrition (Clemens *et al.*, 2002). There are also some toxic heavy metals like Co, Cd etc., which are not directly required by the plants, but still they are accumulated in some plants due to environmental pollutions (Ajasa *et al.*, 2004) which create serious health hazards, when the plants are consumed by the humans and animals (En *et al.*, 2003). In human body, trace elements play a vital role both in prevention and treatment of different human diseases (Saeed *et al.*, 2010 a). In the present study leaf, shoot, root and flower of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* were analyzed to determine the concentration of various heavy and toxic metals in these plants. The results obtained for these and some other metals are given in Table 4.16.

Nickel (Ni)

In the present study, Ni was present in the range of 0.102 ± 0.0232 ppm - 0.287 ± 0.0211 ppm in *Iphiona grantioides* and 0.398 ± 0.0066 ppm in *Pluchea arguta* which are within the standard permissible limits (1.5 ppm) (Table 4.16). Daily intake of Ni should be less than 1 mg beyond which it is toxic (McGrath & Smith, 1990).

Nickel is a chemical element with the chemical symbol Ni and atomic number 28. Nickel belongs to the transition metals and is naturally present in the earth's crust abundantly and is easily accumulated by plants. Nickel role is very important as an active site of some enzymes (Sydor & Zambie, 2013). Intake of Nickel (Ni) in large amount can cause contact dermatitis and cancer of different organs, disorders of liver and kidney and cardiovascular problems (Ibrar *et al.*, 2013 b). More or less similar results for Nickel concentration were reported by Ibrar *et al.*, 2013 b; Saeed *et al.*, 2011; Saeed *et al.*, 2010a (*Polygonatum verticillatum*); Saeed *et al.*, 2010b. Similarly Ozcan (2005) detected Ni presence in young shoots, flower buds, berries (fruit), and seeds of *Capparis ovata* but were found to be very low in all these parts of the plant. So these plants can be used safely without any fear of accumulation of Ni beyond the allowable limit.

Zinc (Zn)

Zinc concentration in *Iphiona grantioides* was in the range of 2.234 ± 0.0151 ppm and 3.517 ± 0.0095 ppm in branch and leaves and 4.730 ± 0.0010 ppm - 2.572 ± 0.3881 ppm in branches and leaves of *Pluchea arguta* subsp. *glabra*, respectively. Zinc is one of the most essential elements found in human muscles and bones. In human body more than 300 Zn dependent proteins are found, which are important for bone strengthening, cell signaling, hormones release and in apoptosis (Saeed *et al.*, 2010 a). The permissible limit of Zn in medicinal plants is $50 \mu\text{g/g}$ (Markert, 1994) and the dietary limit of Zn is 100 ppm (Jones, 1987). Zn deficiency causes Copper deficiency due to its displacement character and weakness, rapid breathing, profuse sweating (Anonymous, 2001). Its deficiency leads to disorder in glucose metabolism (Ibrar *et al.*, 2003) and weight loss (Lokhande *et al.*, 2010) while its acute toxicity causes nausea, abdominal pain, diarrhoea and vomiting (Anonymous, 2001). Saeed *et al.* (2010a) and Sultan *et al.*, (2010) reported

the presence of Zn in some medicinal plants and suggested to have a key role in plant as well as animal metabolism. Our findings showed that Zn amount was below the permissible limit so this plant could be useful Zn deficiency conditions.

Manganese (Mn)

According to our findings, concentration of Mn in *Iphiona grantioides* [(leaves) 8.842 ± 0.0396 ppm- (stem) 3.141 ± 0.0199 ppm] and *Pluchea arguta* subsp. *glabra* [(roots) 2.317 ± 0.0316 ppm - (leaves) 7.273 ± 0.0343 ppm] was within the permissible limits. Permissible limit of Mn is 200 ppm in plants (Saeed *et al.*, 2010 a) and adequate daily dietary intake in adults is 11 mg/day (Pendias & Pendias, 1992). Manganese is of significant importance in living organisms and function as cofactors for a large variety of enzymes. It is essential for glucose metabolism, normal body growth and reproductive function (Ibrar *et al.*, 2003). Mn intoxication is responsible for Parkinsonism condition (shaking or tremors) (Wang *et al.*, 2008). Deficiency of Mn in human causes myocardial infarction and other cardiovascular diseases, also disorder of bony cartilaginous growth in infants & children and may lead to immuno- deficiency disorder and rheumatic arthritis in adults (Smith, 1990). Similar results are also reported by Ibrar *et al.*, (2003) for Mn contents in *Hedera helix*. The results clearly showed that both the plants are safe to be used with reference to Mn.

Chromium (Cr)

The results obtained in the present study showed a concentration range of 0.044 ± 0.0254 - 1.345 ± 0.0022 µg/g in various samples as shown in Table.4.16. The daily intake of Cr 50-200 µg has been recommended for adults by US National Academy of Sciences (Watson, 1993). Thus the concentrations are within the permissible limit for Cr in plants (1.5 ppm) (Saeed *et al.*, 2010 b). Chromium is one of the abundant elements on the earth, and play important role in body metabolism (Emsley & John, 2001). It is an integral part of a complex known as “Glucose Tolerance Factor (GTF)” which plays an essential role in insulin action by attaching it to cell membrane and ultimately enhancing glucose uptake capability of a cell (Ibrar *et al.*, 2003). Chromium based supplements are

useful for weight loss (Lukaski *et al.*, 2007). Inhaling high level of chromium (Cr) may lead to irritation of the nose, asthma, shortness of breath and wheezing. It is also responsible for allergic reactions (Anonymous, 2008).

Copper (Cu)

The range of Cu in present study was 0.581 ± 0.00 ppm (*Iphiona grantioides* root), 0.412 ± 0.00 (*P.arguta* leaf), 0.327 ± 0.00 (*P.arguta* root), 0.2744 ± 0.00 (*P.arguta* stem), 0.226 ± 0.00 ppm (*I. grantioides* leaves), 0.185 ± 0.00 (*I. grantioides* stem) and 0.147 ± 0.00 (*I. grantioides* flower) (Table 4.16). The permissible limit of Cu is 10 µg/g in plants (Markert, 1994) and 340–900 µg /day of Cu is the recommended dietary allowance (RDA) (Saeed *et al.*, 2010b). Cu is an essential trace metals found in human and animal bodies required for normal enzymatic function (Huang & Failla, 2000). It is involved in oxidation- reduction reactions, energy production, connective tissues formation, iron metabolism, neurotransmitter synthesis, and metabolism of neurotransmitters and formation of myelin (Amin, *et al.*, 2003). Cu deficiency 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). The results suggest that both the plants are safe to use as far as concentration of Cu is concerned.

Iron (Fe)

In the present study, iron concentration was 10.50 ± 0.086 ppm in flower, 11.72 ± 0.026 ppm in leaf, $13.090.09 \pm$ ppm in stem and 20.47 ± 0.09 ppm in root while in *Pluchea arguta* subsp. *glabra* the concentrations were 23.60 ± 0.17 ppm in root, 16.35 ± 0.057 ppm in leaf and 7.492 ± 11.62 ppm in stem ,which is within the the permissible limit (36-241 ppm). The recommended dietary allowance (RDA) of Iron in adult male is 8 mg per day, while it is 7-10 mg per day in children (Anonymous, 2004). Iron is a chemical element with the symbol Fe (from Latin: ferrum) and atomic number is 26. Iron is an essential trace element found in nearly all living organisms. In higher organisms Iron containing proteins are hemoglobin, myoglobin and cytochrome

P450. Iron is the most abundant trace element in tissues of human body (Arredondo & Nunez, 2005). Iron deficiency is most common in females and children than male throughout the world. Fe deficiency slow down mental activities (Beard, 2001). Low Fe content causes gastrointestinal infection, nose bleeding and myocardial infarction (Hunt, 1994). The present data indicated that Iron is in sufficient amount in our tested samples and this might be the reason for hepatoprotective effect of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*.

Cobalt (Co)

The concentration of Co in the present study was 0.034 ± 0.00 ppm (leaf), 0.067 ± 0.00 pm (root), 0.054 ± 0.01 ppm (stem) and 0.029 ± 0.00 ppm (flower) in *Iphiona grantioides*, while it was 0.103 ± 0.00 ppm (leaf), 0.104 ± 0.004 ppm (stem) and 0.094 ± 0.0048 ppm (root) in *Pluchea arguta* subsp. *glabra* (Table.4.16). In plants permissible limit of Co is 0.2 ppm (Markert, 1994). It means that all the samples of crude drugs contain Co within the standard limits (0.2ppm). Cobalt is a trace metals found in plants, animals, rocks, and water. It is a part of vitamin B12, which is essential for human health. Cobalt is useful in the treatment of anaemia in pregnant women. However, high concentrations of cobalt may damage human health.

Lead (Pb)

In our present study Pb concentration was (0.0113 ± 0.0271 - 0.122 ± 0.0239 ppm in leaves and flower of *Iphiona grantioides* while it was 0.127 ± 0.0037 - 0.245 ± 0.0187 ppm in roots and branches of *Pluchea arguta* subsp. *glabra*, respectively. Lead is present within the range of permissible limits (10 µg/g) designed by World Health Organization (Issac & Jonson, 1975). Lead is a toxic element and its presence in plants may be due to environmental pollution. Pb poisoning is serious at any age, but children are relatively more vulnerable (Asiri, 2006). Pb toxicity causes anemia due to inhibition of heme biosynthesis and affects multiple body systems (Ang & Lee, 2006). According to our present findings, both the crude drugs are safe for human use as herbal remedy.

Cadmium (Cd)

Like Lead, cadmium is a non-essential trace element having functions neither in humans' body nor in plants. They induce various toxic effects in humans at low doses. Cadmium accumulates in human body and damages mainly the kidneys and liver. The lowest level of Cd which can cause yield reduction is 5-30 ppm, while the maximum acceptable concentration for food stuff is around 1 ppm (Neil, 1993). Surprisingly no Cd was detected in plant samples (Below detection limit).

Table 4.16. Heavy metal profile of various parts of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Metal	IF (ppm)	IL (ppm)	IS (ppm)	IR (ppm)	PL (ppm)	PS (ppm)	PR (ppm)
Mn	1.083±0.04	1.842±0.039	1.141±0.01	2.012±0.05	7.273±0.03	4.912±0.012	2.317±0.03
Fe	10.50±0.086	11.72±0.026	13.09±0.09	20.47±0.09	16.35±0.05	7.492±11.62	23.60±0.17
Cu	0.147±0.00	0.226±0.00	0.185±0.00	0.581±0.00	0.412±0.00	0.2744±0.00	0.327±0.00
Zn	2.412±0.01	3.117±0.00	2.234±0.01	3.544±0.01	0.572±.388	4.730±.00	3.050±0.02
Cr	0.041±0.00	0.021±0.00	0.030±0.00	0.061±0.00	0.045±0.00	0.061±0.00	0.044±0.02
Pb	0.122±0.02	0.0113±0.02	0.051±0.03	0.066±0.03	0.176±0.03	0.245±0.018	0.127±0.00
Co	0.029±0.00	0.034±0.00	0.054±0.01	0.067±0.00	0.103±0.00	0.104±0.004	0.094±0.00
Ni	0.111±0.04	0.102±0.02	0.149±0.00	0.287±0.02	0.304±0.03	0.398±0.006	0.325±0.01
Cd	ND	ND	ND	ND	ND	ND	ND
Key = IF = <i>Iphiona grantioides</i> flower PL= <i>Pluchea arguta</i> subsp. <i>glabra</i> leaf IL = <i>Iphiona grantioides</i> leaf PS = <i>Pluchea arguta</i> subsp. <i>glabra</i> stem IS = <i>Iphiona grantioides</i> stem PR= <i>Pluchea arguta</i> subsp. <i>glabra</i> root IR = <i>Iphiona grantioides</i> root							

According to the recommendations of World Health Organization, medicinal plants, which provide raw materials for the final products, must be checked for the presence of toxic heavy metals and it also regulates the maximum allowable limits of toxic metals (WHO, 1998). Medicinal plants may very easily be contaminated during growth, development, collection, processing and after conversion into dosage form the heavy metals present in plants may finally enter into the human body and disturb the whole body normal functioning i.e., central nervous system, liver, lungs, heart, kidney and brain, leading to hypertension, abdominal pain, intestinal ulcer, skin eruptions, and different types of cancers. As it is clear from the results (Table. 4.16), that both the plants, in the present study, have all the elements within safer and permissible limits.

4.7. Bioassays (Biological activities) of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Bioassays are pharmacological tools of utmost importance for screening out of different plant extracts for the treatment of various ailments (Srirama *et al.*, 2007). In the present study various bioassays were carried out to investigate the *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* for their curative values.

4.7.1. Antihelmintic activity

The anthelmintic drugs that are in common use for the treatment of nematodes have little efficacy when applied in a single dose, moreover, the clinical importance has been reduced by the resistance of different nematodes to such drugs (McCarthy, 2005). Extensive efforts have been made to find out constituents efficient and secure nematicidal drugs. For the last some period of time, medicinal plants have been screened to find out constituents with nematicidal activity because the botanic sources are relatively safer than artificial agents. In this perspective a bioassay was performed on mature earthworm, *Pheretima posthuma* due to its anatomical and physiological similarity with the intestinal parasitic round worms of human beings (Deore *et al.*, 2009).

On the whole antihelmintic activity of the two research plants was exhibited in a concentration dependent manner. The antihelmintic activity of *Iphiona grantioides* and

Pluchea arguta subsp. *glabra* were very significant against the tested worms. Leaf extracts of *Iphiona grantioides* and *P.arguta* subsp. *glabra* at dose of 100 mg/ml caused death of the worm in 3.33 ± 0.57 and 2.16 ± 0.28 min, respectively as shown in Table 4.17, Fig.4.19, which are almost similar to the same effect produced by drug, Piperazine Citrate. Moderate mortality time of root and stem of *Iphiona grantioides* was 12.43 ± 0.60 and 13.83 ± 0.76 , respectively. In addition weak nematocidal activity of death time of 50 ± 5 was exhibited by flower extract of *Iphiona grantioides*. While rest of the extracts of stem and roots of *Pluchea arguta* subsp. *glabra* expressed insignificant activity. The current finding can be credited to the presence of tannins, flavonoids and polyphenols in the *Pluchea arguta* subsp. *glabra* and *Iphiona grantioides*. Based on the results, it can be concluded that the two plants could be a significant natural source of vermicides. Further detail studies on the extract may lead to the isolation of pure molecules with more prominent efficacy and safety.

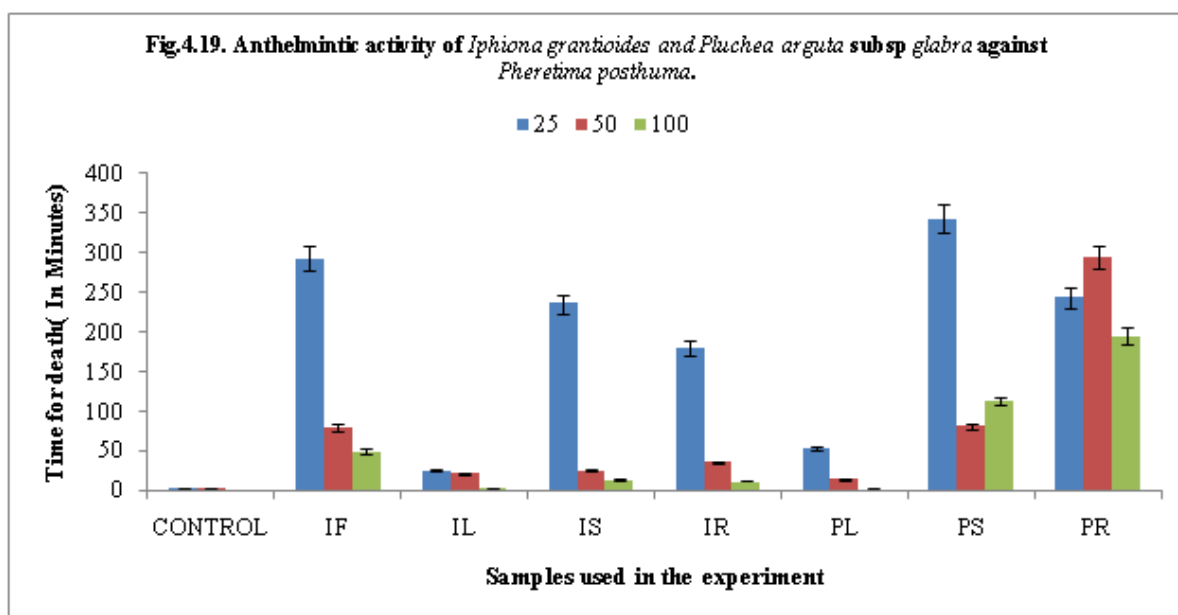
Furthermore the work of previous researchers on different plants, investigated for antihelmintic activity i.e., *Inula helenium* (El Garhy & Mahmoud, 2002), *Artemisia brevifolia* (Iqbal et al., 2004), *Calotropis procera* (Iqbal et al., 2005), *Nicotiana tabacum* (Iqbal et al., 2006a) and *Butea monosperma* (Iqbal et al., 2006b), are in line with our findings. Similarly ethanol and aqueous extracts of stem of *Piper betle* Linn (Adate et al., 2012) and crude methanolic extract and its subsequent solvent fractions of *Viola betonicifolia* (Naveed et al., 2012), methanolic extract from nut galls of wild olive (Ibrar et al., 2013 a) and ethanolic extracts of leaves of *Dichrocephala integrifolia* (Wabo et al., 2013) expressed significant nematocidal activity against *P.posthuma*, in a dose dependent manner. All these findings strengthen our findings.

Table 4.17. Antihelmintic activity of *Iphiona grantioides* and *Pluchea arguta* subsp *glabra* against *Pheretima posthuma*

S.No	Sample	Concentration(mg/ml)	Time for paralysis(min)	Time for Death (min)
1	Piperazine citrate	25	3 ± 1	2.93 ±0.49
		50	2.8 ±0.76	2.53 ±0.15
		100	1 ± 0	2 ±0.5
2	IF	25	160 ± 10	293. 33 ±51.3
		50	81.66 ± 10.40	80 ±10
		100	4 ± 1	50 ±50
3	IL	25	45 ± 50	25 ±50
		50	22.33 ± 0.57	22.33 ±2.52
		100	7.33 ± 0.57	3.33 ±0.57
4	IS	25	88.33 ± 2.88	236 ±41.63

		50	60 ± 50	25 ± 50
		100	10.5 ± 0.5	13.83 ± 0.76
5	IR	25	75.96 ± 9.55	180 ± 26.45
		50	9.66 ± 0.76	35.5 ± 0.5
		100	17.76 ± 0.25	12.43 ± 0.60
6	PL	25	6.16 ± 0.28	53.33 ± 5.77
		50	5.16 ± 0.28	14.1 ± 0.85
		100	3.66 ± 0.57	2.16 ± 0.28
9	PS	25	166.66 ± 15.27	343.33 ± 50.33
		50	175.66 ± 24.17	81.66 ± 10.40
		100	60.33 ± 4.51	113.33 ± 11.54
10	PR	25	182.66 ± 14.18	195.66 ± 4.93
		50	131.66 ± 32.53	295 ± 50

		100	113.33 ± 32.14	243.33 ± 40.41
Key: IF = <i>Iphiona grantioides</i> flower IL = <i>Iphiona grantioides</i> leaf IS = <i>Iphiona grantioides</i> stem IR = <i>Iphiona grantioides</i> root PL= <i>Pluchea arguta</i> subsp. <i>glabra</i> leaf PS = <i>Pluchea arguta</i> subsp. <i>glabra</i> stem PR= <i>Pluchea arguta</i> subsp. <i>glabra</i> root				

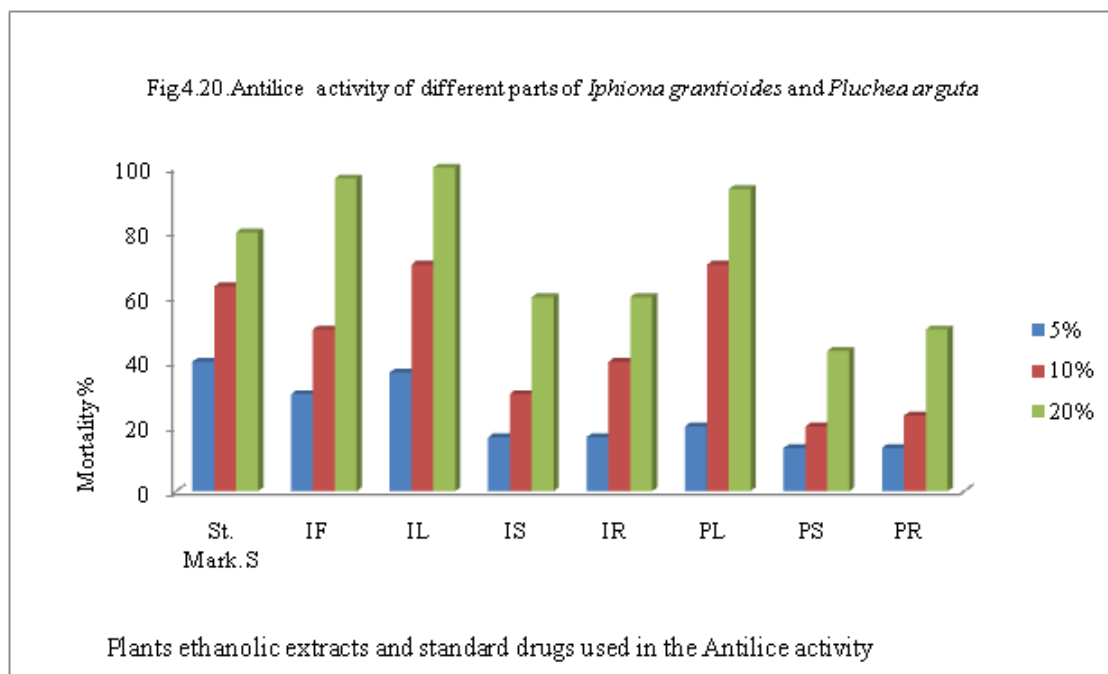


4.7.2. Antilice activity

Antilice activity of the ethanolic extract of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* Kaiser were investigated against the common head lice (*Pediculus humanus capitis*). The results indicated that various parts of both the plants have significant antilice potential. The results (Table 4.18) and Fig.4.20 showed the excellent antilice activity was recorded in case of *Iphiona grantioides* leaf (100%) followed by flower (96.67%) and leaf (93.33%) of *Pluchea arguta* subsp. *glabra*. Moderate antilice activity was showed by roots (60%) of *Iphiona grantioides* and stem of *Pluchea arguta* subsp. *glabra*. Fifty percent lice mortality was exhibited by roots while weak activity was shown by stem (43.33%) of *Pluchea arguta* subsp. *glabra*.

Our findings are further confirmed by the work of Tiangda *et al.*, 2000 (*Annona squamosa* L.seeds); Sunilson *et al.*, 2009 (*Pongamia pinnata* leaves); Vijayalakshmi *et al.*, 2010 (*Dichrostachys cinerea* Wight & Arn); Singh *et al.*, 2011 (*Mucuna pruriens* seeds); Asokan *et al.*, 2011 (*Syzygium aromaticum*); Upadhyay *et al.*, 2011 (*Abrus precatorius* seeds); Mullaicharam & Al-Matroushi, 2012 (*Dalmatian chrysanthemums*) and Surendra *et al.*, 2013 (pulp of *Myristica fragrans* Houtt.), whose work reported a number of medicinal plants, processing antilice potential and as an effective alternative for treating human head lice. The findings of this study showed excellent antilice activity of leaves

and flower of *Iphiona grantioides* and leaves of *Pluchea arguta* subsp. *glabra*, which may be due to the presence of flavonoides, tannis and polyphenols derivatives, which might be responsible for the enhanced penetration and bioavailability of chemical components into the body of louse. Natural extracts from medicinal plants have been noticed for its safe and effective use, and emergence of resistance patterns were minimal due to their different modes of action (Breuer *et al.*, 2003; Isman. 1996), which greatly supports the possible safe use of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* extracts as potent antilice agents as they contain chemical constituents responsible for licicidal property and the constituents are predominately soluble in ethanol.



4.7.3. Antibacterial activity

Antibacterial activity of ethanolic extract of various parts of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* was tested against *Escherichia coli*, *Staphylococcus viridans*, *Klebsiella pneumoniae*, *B. licheniformis*, *Pasteurella multocida*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Micrococcus luteus*. The results of antibacterial activity of different parts of the two plants are shown in the Table 4.19 and Fig.4.21. The results show that ethanolic extract of *Iphiona grantioides* flower was most effective against the tested microorganism, with the highest inhibition zone of 21.23 mm (*Klebsiella pneumoniae*) followed by 19.67 mm (*Staphylococcus aureus*), 17.47 mm (*Pasteurella multocida*), 16.76 mm (*Bacillus licheniformis*), 15.67 mm (*Micrococcus luteus*), 15.00 mm (*Staphylococcus viridans*) while it exhibited moderate antibacterial effects against by producing zone of inhibition of 12.33 mm against *Bacillus Subtilis* and *Pseudomonas aeruginosa*. Ethanolic extract of leaves of *Iphiona grantioides* showed significant antibacterial effect against *Escherichia coli* (14 mm) while other extracts including leaf, stem and root, showed moderate antibacterial potential, in the range from 8.32 - 11.67 mm. Our findings are further

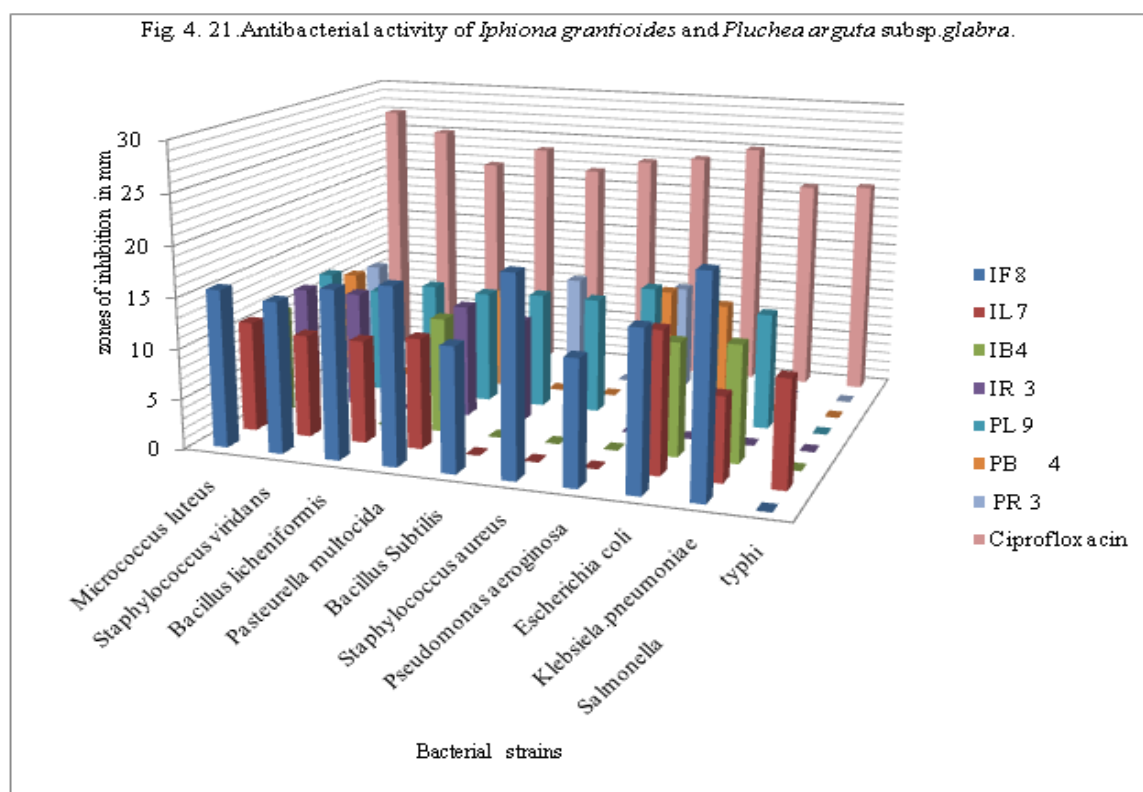
reaffirmed by the study of Attur-ur- Rehman, (2006), who reported that the oil obtained from *Iphiona grantioides* has possess strong antibiotic activity. Similarly the crude methanol extracts (CME) of *Iphiona aucheri* and other three plants were found ineffective against three Gram positive bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae*, and *S. pyogenes*) and four Gram-negative bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) (Kakar *et al.*, 2012).

Pluchea arguta subsp. *glabra* has, also showed antibacterial activity against the same strains of bacteria. Ethanolic extract of *Pluchea arguta* subsp. *glabra* leaf was comparatively more potent than the other two extracts i.e., stem and root, in producing prominent zone of inhibitions that is 15.33 mm (*Escherichia coli*), 13.33mm (*Pseudomonas aeruginosa*) and 12.00 mm (*Micrococcus luteus*) while the lowest zone of inhibition (10.33mm) was observed in *Micrococcus luteus* and *Pasteurella multocida* by root and stem extracts, respectively. Our findings are in line with the results of Pramanik *et al.*, (2008), who investigated the antimicrobial activities of *Pluchea indica* (L.) Less against bacillary dysentery and conclude that it has a significant potential against the dysentery causing bacteria.

Table 4.19. Antibacterial activity of *Iphiona grantioides* and *Pluchea arguta* subsp.*glabra*
(The figures show zones of inhibition in mm)

S.No	Bacterial strain	IF	IL	IS	IR	PL	PB	PR	Ciprofloxacin
1	<i>Micrococcus luteus</i>	15.67	11	10.67	11.67	12.00	10.67	10.33	27.00
2	<i>Staphylococcus viridians</i>	15.00	10.2	×	11.67	10.67	×	×	25.00
3	<i>Bacillus licheniformis</i>	16.67	10.21	×	×	11.67	×	×	21.67
4	<i>Pasteurella multocida</i>	17.47	11	11.50	11.33	11.33	10.33	×	23.67
5	<i>Bacillus Subtilis</i>	12.33	×	×	10.33	11.67	×	10.67	21.67
6	<i>Staphylococcus aureus</i>	19.67	×	×	×	11.67	×		23.00
7	<i>Pseudomonas aeruginosa</i>	12.33	×	×	×	13.33	11.67	10.67	23.67
8	<i>Escherichia coli</i>	15.67	14	11.33	×	15.33	10.67	×	25.00
9	<i>Klebsiella pneumoniae</i>	21.23	8.32	11.67	×	11.67	×	×	21.33
10	<i>Salmonella typhi</i>	×	10.6	×	×	×	×	×	21.67

	Key = IF = <i>Iphiona grantioides</i> flower IL = <i>Iphiona grantioides</i> leaf IS = <i>Iphiona grantioides</i> stem IR = <i>Iphiona grantioides</i> root PL= <i>Pluchea arguta</i> subsp. <i>glabra</i> leaf PS = <i>Pluchea arguta</i> subsp. <i>glabra</i> stem PR= <i>Pluchea arguta</i> subsp. <i>glabra</i> root
--	---



In the present study, flavonoids may be the cause of antibacterial potential of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*: Flavonoids have been reported for their significant antibacterial effects against pathogenic bacteria (Cushnie & Lamb *et al.*, (2005); Akroum *et al.*, (2009). Similarly sesquiterpenes are important constituents of *Pluchea arguta* subsp. *glabra* which are also known for their bactericidal effect on *Staphylococcus aureus* (Claeson *et al.*, 1992). *Inula britannica* has been reported for its antibacterial activities due to presence of flavonoides (Song *et al.*, 2002).

The pathogens investigated for antibacterial activity in the present study, were also significantly inhibited by methanolic, chloroform and aqueous extracts of aerial parts of *Buddleja asiatica* Lour. (Shivani *et al.*, 2011), which also contains triterpenoids, steroids, polyphenols, flavonoids (Okoro *et al.*, 2010). Similarly Syahriel *et al.*, (2012) also investigated the broad spectrum antimicrobial activity of seven different solvents of *Cynodon dactylon* (L.) Pers. against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus*

pyogenes, and *Streptococcus pneumonia*. All the extracts were reported to contain alkaloids, cardiac glycosides, Phenolic compounds, flavonoides, tannins, terpenoides, saponins, carbohydrates and protiens. Similarly Morshed *et al.*, (2012) screened the antibacterial activities of various solvents fractions of methanolic extracts of the whole plant of *Paederia foetida* (family Rubiaceae) against same pathogens .The ethyl acetate, chloroform and n-hexane fractions exhibited moderate to less moderate activity against *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina lutea*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Salmonella typhi*, *Shigella boydii*, *Shigella dysenteriae*, *Vibrio mimicus*, *Vibrio parahemolyticuss*.The plant contain carbohydrates, proteins, amino acids,tannins, phenolics, flavonoids, steroids, mucilage and saponins (Kumar *et al.*, 2009). So it can be concluded from the present findings that *Iphiona grantioides* (flower and leaves) and *Pluchea arguta* subsp. *glabra* leaf have significant antifungal and significant to moderate antibacterial properties against important human pathogenic bacteria and fungi.

4.7.4 . Antifungal activity

Crude ethanolic extracts of *Iphiona grantioides* (flower, stem,leaf,root) and *Pluchea arguta* subsp. *glabra* (stem,leaf,root) were tested against 4 human pathogenic fungal strains (*Trichophyton longifusus*, *Candida albicans*, *Microsporum canis*, *Candida glaberata*), 2 animal pathogenic fungi (*Aspergillus flavus*, *Aspergillus niger*) and the 3 Plant pathogenic types (*Fusarium solani*, *Mucor spp.* and *Alternaria spp.*) for antifungal activity. The results are given in Table 4.20 and Fig.4.22 (1-9).

The results showed that in case of *Iphiona grantioides* the most efficient extracts with highest percent inhibition values were in the order of i.e., leaves (54%) flower (53%) stem (27.56%) > root (19%) and the order of effectiveness of these extracts by inhibiting the number of tested pathogens was almost same for all the extracts i.e., leaves (inhibited 9 pathogens), flower (inhibited 9 pathogens), stem (inhibited 9 pathogens) and root (inhibited 8 pathogens). It is clear from Table 4.20, that flower and leaf extract of *Iphiona grantioides* was the most effective extracts, as shown antifungal against all the nine species at all the three (125, 250 and 500 ug/ml) doses.

In case of *Pluchea arguta* subsp. *glabra*, results of antibacterial activities of ethanolic extracts of various parts (leaves, stem and root) also showed promising effects activity against the tested fungal pathogens (Table 4.20). Different concentrations of the extract (125, 250, 500 ug /ml) were tested against *Trichophyton longifusus*, *Candida albicans*, *Microsporum canis*, *Candida glaberata*, *Aspergillus flavus*, *Aspergillus niger* , *Fusarium solani*, *Mucor* spp. and *Alternaria* spp. The crude ethanolic extract (500 ug /ml) of stem, leaves and roots had inhibitory effects against the bacterial strains in the order of leaves (43.6%) stem (35%) > root (30%). In this case the plant extract was most efficient as it inhibited all the nine tested fungal species.

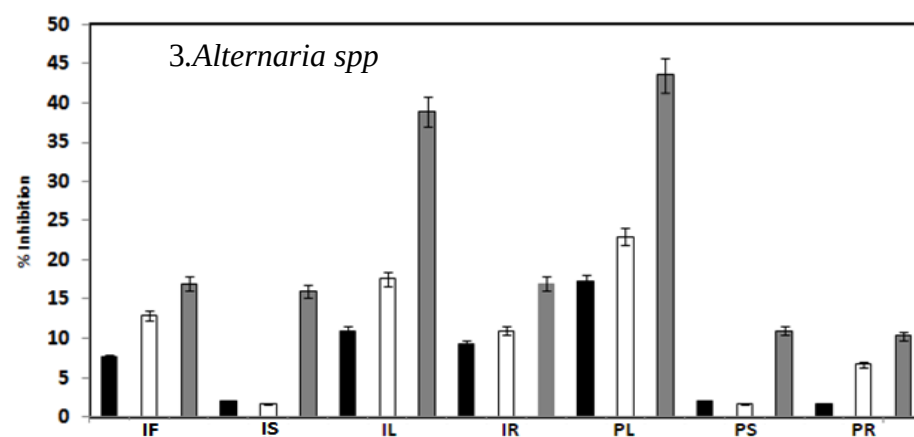
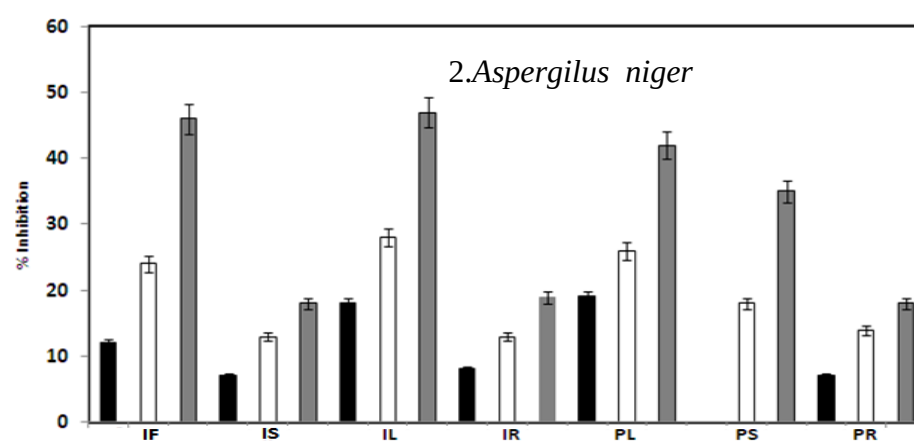
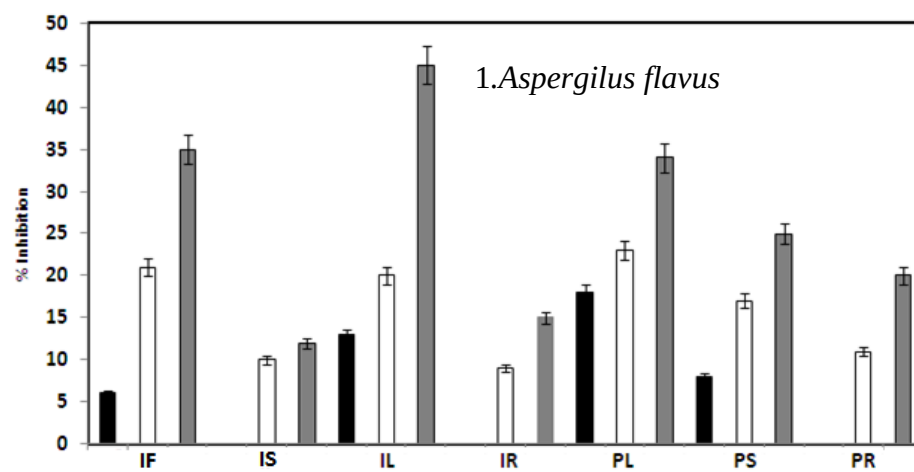
The present study showed that flower and leaves of *Iphiona grantioides* and leaves of *Pluchea arguta* subsp. *glabra* have a wide range of antifungal activities and both can be used as strong potential sources of antifungal drugs not only for human and cattle diseases , but can also be used as useful, safe pesticides in agricultural practices.

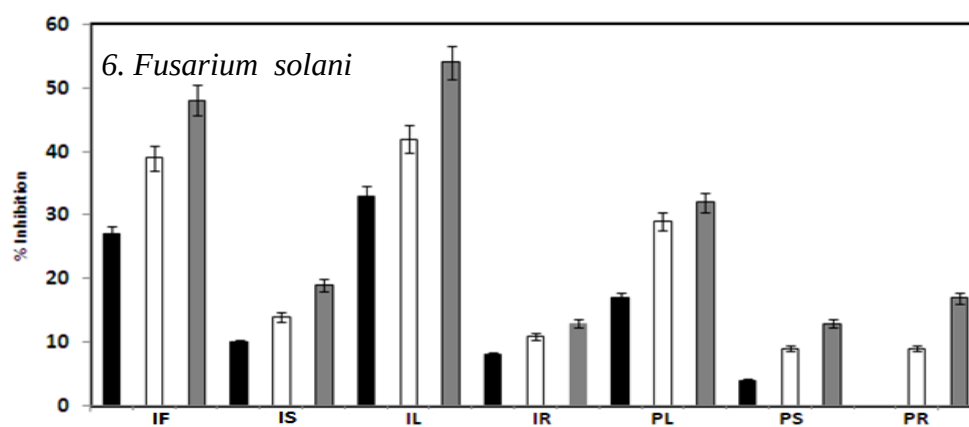
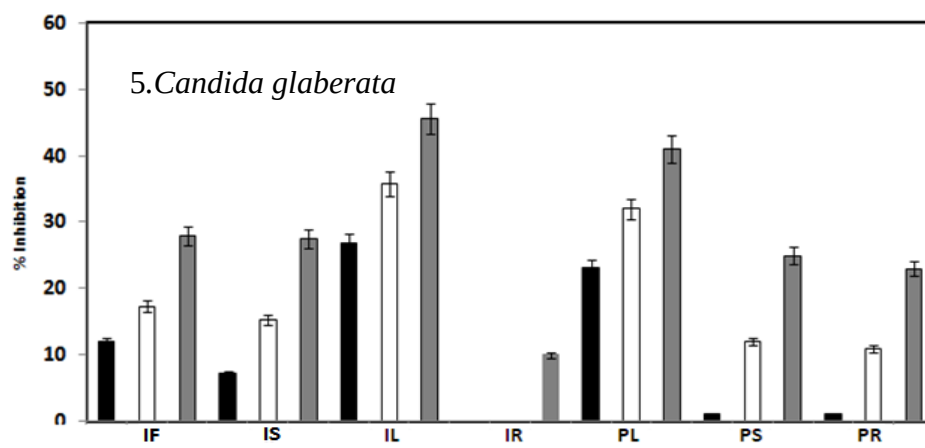
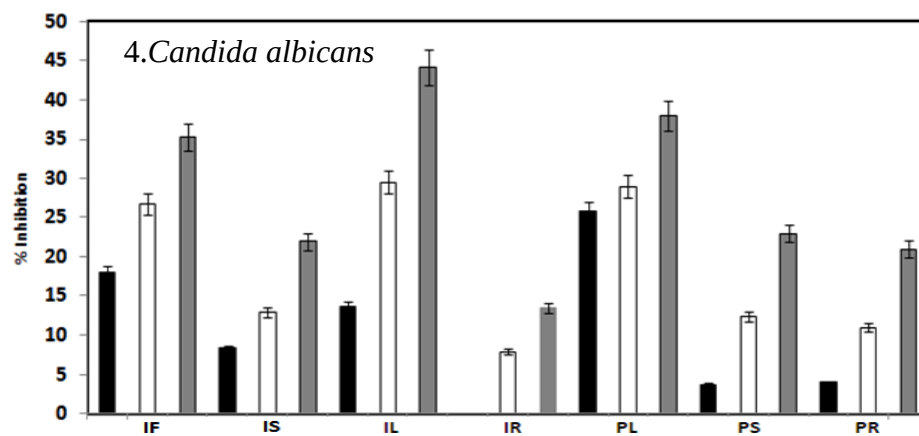
Preliminary phytochemical screening reveals the presence of alkaloids, tannins, glycosides, saponins, terpenes and flavonoids. These compounds are believed to be responsible for the observed anti fungal effects. Some workers e.g., Hussain *et al.*, (2009); Uddin *et al.*, (2010) ; Rajeswari *et al.*, (2011); Mansour *et al.*, (2012) have also attributed the observed antifungal effects of plant extracts to the presence of these secondary metabolites. Thus, it can be deduced that the moderate to good fungal activities may be credited to the phytoconstituents present in the extracts.

Table 4.20. Antifungal activity of *Iphiona grantioides* and *Pluchea arguta* subsp.*glabra* against different fungal pathogens
(The figures show percent (%) inhibitions)

Extracts	Concentration ug/ml	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Alternaria spp</i>	<i>Candida albicans</i>	<i>Candida glaberata</i>	<i>Fusarium solani</i>	<i>Microsporu m canis</i>	<i>Mucor spp</i>	<i>Trichophyto n longifusis</i>
IF	125	6	12	7.6	18	12	27	1	12	28
	250	21	24	13	26.7	17.3	39	13	24	35
	500	35	46	17	35.3	28.00	48	32	36	53
IS	125	-	7	2	8.3	7.23	10	-	-	7
	250	10	13	1.6	13	15.3	14	-	-	-
	500	12	18	16	22	27.56	19	17	8	8
IL	125	13	18	11	13.7	26.9	33	16	16	13
	250	20	28	17.6	29.5	35.76	42	26	23	19
	500	45	47	38.9	44.2	45.67	54	34	30	29
IR	125	-	8	9.3	-	-	8	-	2	-
	250	9	13	11	7.9	-	11	-	5	-
	500	15	19	17	13.5	10	13	7	6	-
PL	125	18	19	17.3	25.8	23.2	17	11	9	10
	250	23	26	23	29	32	29	19	16	11
	500	34	42	43.6	38	41	32	23	11	16
PS	125	8	-	2	3.7	1	4	5	-	-

	250	17	18	1.6	12.4	12	9	8	3	-
	500	25	35	11	23	25	13	10	8	8
PR	125	-	7	1.7	4	1	-	16	12	-
	250	11	14	6.7	11	11	9	19	18	11
	500	20	18	10.3	21	23	17	26	30	16
Miconazole	(0.5mg/ml)	42±0.5	27	32	42	53	32	43	45	37
Key = IF = <i>Iphiona grantioides</i> flower PL= <i>Pluchea arguta</i> subsp. <i>glabra</i> leaf IL = <i>Iphiona grantioides</i> leaf PS = <i>Pluchea arguta</i> subsp. <i>glabra</i> stem IS = <i>Iphiona grantioides</i> stem PR= <i>Pluchea arguta</i> subsp. <i>glabra</i> root IR = <i>Iphiona grantioides</i> root										





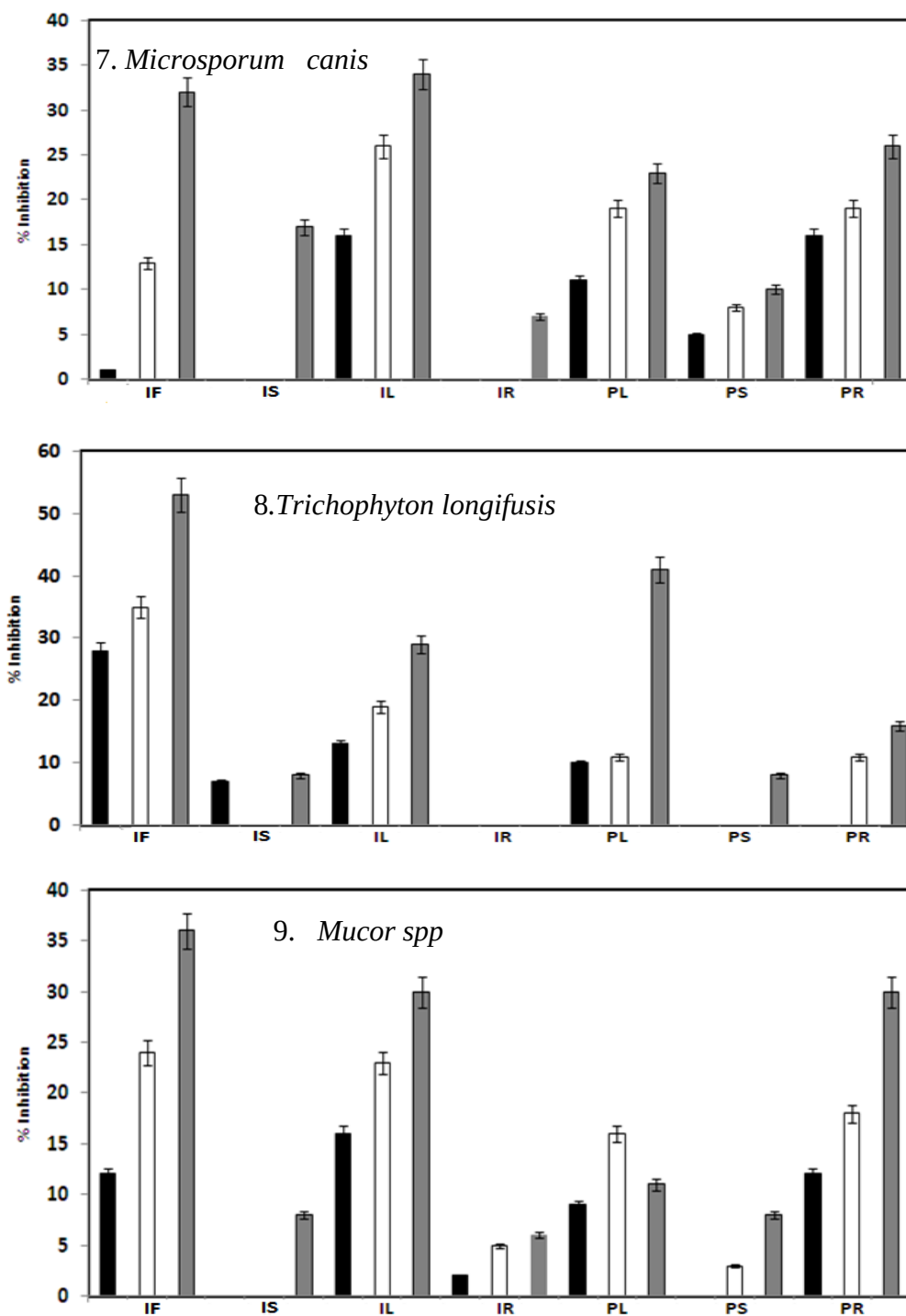


Fig.4. 22(1-9). Antifungal activity of the crude ethanolic extract of the various parts of *Iphiona grantioides* and *Pluchea arguta* subsp.*glabra* against different strains of pathogenic fungi

1. *Aspergillus flavus*, 2. *Aspergillus niger*, 3. *Alternaria spp*, 4. *Candida albicans*, 5. *Candida glaberata*, 6. *Fusarium solani*, 7. *Microsporium canis*, 8. *Trichophyton longifusus*, 9. *Mucor spp*

4.7.5. Antioxidant activity

The DPPH test is a non-enzymatic method currently used to provide basic information on the ability of extracts to scavenge free radicals. Ethanolic extracts of *Iphiona grantioides* (flower, leaf, stem and root) and *Plucheaarguta* subsp. *glabra* (leaf, stem and root) were investigated for their free radical scavenging capacity. DPPH (1, 1-Diphenyl-2 picrylhydrazyl) was used in the experiment. The bleaching of DPPH colour in the experiment by *Iphiona grantioides* and *Plucheaarguta* subsp. *glabra*-extracts indicated the scavenging capacity of extracts. Results are presented in Table 4.21. and Fig. 4.23. The results of the present study showed that roots of *Plucheaarguta* subsp. *glabra* showed the highest DPPH scavenging activity (82.89%) followed by ethanol extracts of leaf (77.3%) and flower (70.94 %) of *Iphiona grantioides*. In the present study antioxidant activity was conducted for *Iphiona grantioides* and *Plucheaarguta* subsp. *glabra* and from the result it is concluded that leaves of *Plucheaarguta* subsp. *glabra* and leaves and flower of *Iphiona grantioides* could be a rich source of antioxidants, however before these two plants are used as antioxidants, more research work is needed to explore, isolate and purify the active chemical compounds responsible for antioxidant activity. The findings of Ghosh *et al.*, (2008) are in line with our findings who investigated the antioxidant activity of the methanolic root extract of tissue cultured *Pluchea indica* (L.) in the presence of (DPPH) radical. The methanolic root extract showed effective reducing power, and inhibition of β -carotene bleaching. Similarly Fernández and Torres (2006) used the scavenging activity of the hydroxyl radical produced in Fenton reaction to evaluate the antioxidant activity of *Pluchea carolinensis* leaves. Andarwulan *et al.*, (2010) suggested that the plant may contribute to dietary antioxidant intake of *Pluchea indica* as its extracts inhibited linoleic acid oxidation and had the DPPH, ABTS, and ferric cyanide antioxidant capacities.

Key :

IF = *Iphiona grantioides* flower

IL = *Iphiona grantioides* leaf

IS = *Iphiona grantioides* stem

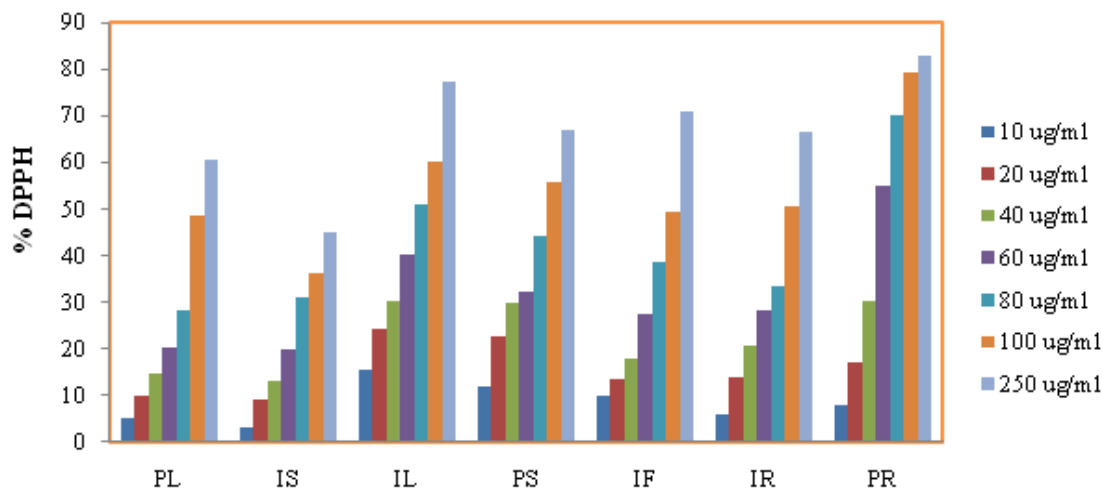
IR = *Iphiona grantioides* root

PL = *Pluchea arguta* subsp. *glabra* leaf

PS = *Pluchea arguta* subsp. *glabra* stem

PR = *Pluchea arguta* subsp. *glabra* root

Fig. 4.23. DPPH radical scavenging activities of crude extracts of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*



4.7.6. Cytotoxic activity

Aerial parts of two plants were used for this bioassay and the results are presented in the Table 4.22. Test concentration of all extracts was at 10, 100 and 1000 µg/ml. According to the definition by Meyer *et al.*, (1982), who classified crude extracts and pure substances into toxic (LD 50 value < 1000 µg/ml) and non-toxic (LD 50 value 1000 µg/ml), majority of the extracts tested showed good brine shrimp cytotoxic activity, in brine shrimp bioassay.

The extracts displayed significant toxicity against brine shrimps having the LD 50 values lesser than 1000 µg/ml. As shown in the Table 4.22, the LD 50 values for all most all the crude extracts of *Iphiona grantioides* (flower, leaf, stem & root) and *Pluchea arguta* subsp. *glabra* (root, stem and leaf) were found to be 34.65, 242.83, 6.21, 29.92 µg/ml and 0.02, 0.03 and 84.66 µg/ml respectively. The results are presented graphically in Fig.4.24 and Fig.4.24. Other research workers like Mahmood *et al.*, 2012 (Solanaceae); Ali *et al.*, 2011 (*Callistemon citrinus* Curtis.); Ara *et al.*, 2009 (*Lippia alba*); Haque *et al.*, 2009 (*Swietenia mahagony*) and Barkatullah *et al.*, 2012 (*Viola canescens*) also carried out brine shrimp lethality bioassay.

The brine shrimp lethality test was first developed as a convenient method for the detection of insecticidal residues but later, the test was applied for the screening of mycotoxins and bioassay guided fractionation of toxic fungal cultures. This bioassay has been modified now, to offer a quick and low-cost method for the screening of plant extracts for general toxicity and bioassay guided fractionation of cytotoxic and antiplasmodial extracts. Significant correlations with brine shrimp toxicity have been shown for cytotoxicity and antitumor activity. To day the brine shrimp bioassay is an efficient ,rapid and inexpensive test that require only a small amount of sample and has led to the discovery of some new pesticides and anticancer drugs from plants(McLaughlin *et al.*, 1991). The present investigation revealed that ethanolic extract of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* are safe and sound enough to be used in traditional system of medicine and could be a good source of valueable antitumor and herbicidal drugs.

Table 4.22. Cytotoxic activities of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*

Dose µg/ml	Plant part used	T. No. of shrimps	Shrimps survive	Shrimps Dead	Percent Dead	% to Probit	Intercept	Slope	R-square	LD ₅₀
10	control	30	21	9	30.0	2.252	2.094	0.162	0.999	110.2 4
100		30	15	15	50.0	2.424				
1000		30	7	23	76.7	2.576				
10	IL	30	23	7	23.3	2.171	2.023	0.168	0.957	242.8 3
100		30	16	14	46.7	2.400				
1000		30	11	19	63.3	2.507				
10	IF	30	19	11	36.7	2.319	2.151	0.177	0.991	34.65
100		30	10	20	66.7	2.525				
1000		30	0	30	100.0	2.674				
10	IB	30	15	15	50.0	2.424	2.358	0.083	0.886	6.21
100		30	8	22	73.3	2.559				

1000		30	6	24	80.0	2.591				
10	IR	30	18	12	40.0	2.348	2.184	0.163	1.000	29.92
100		30	11	19	63.3	2.507				
1000		30	0	30	100.0	2.674				
10	PL	30	19	11	36.7	2.319	2.082	0.177	0.750	84.66
100		30	19	11	36.7	2.319				
1000		30	0	30	100.0	2.674				
10	PB	30	8	22	73.3	2.559	2.513	0.057	0.909	0.03
100		30	2	28	93.3	2.648				
1000		30	0	30	100.0	2.674				
10 PR	PR	30	8	22	73.3	2.559	2.517	0.057	0.832	0.02
100		30	1	29	96.7	2.661				
1000		30	0	30	100.0	2.674				
Key = IF = <i>Iphiona grantioides</i> flower PL= <i>Pluchea arguta</i> subsp. <i>glabra</i> leaf										

IL = *Iphiona grantioides* leaf PS = *Pluchea arguta* subsp. *glabra* stem

IS = *Iphiona grantioides* stem PR= *Pluchea arguta* subsp. *glabra* root

IR = *Iphiona grantioides* root

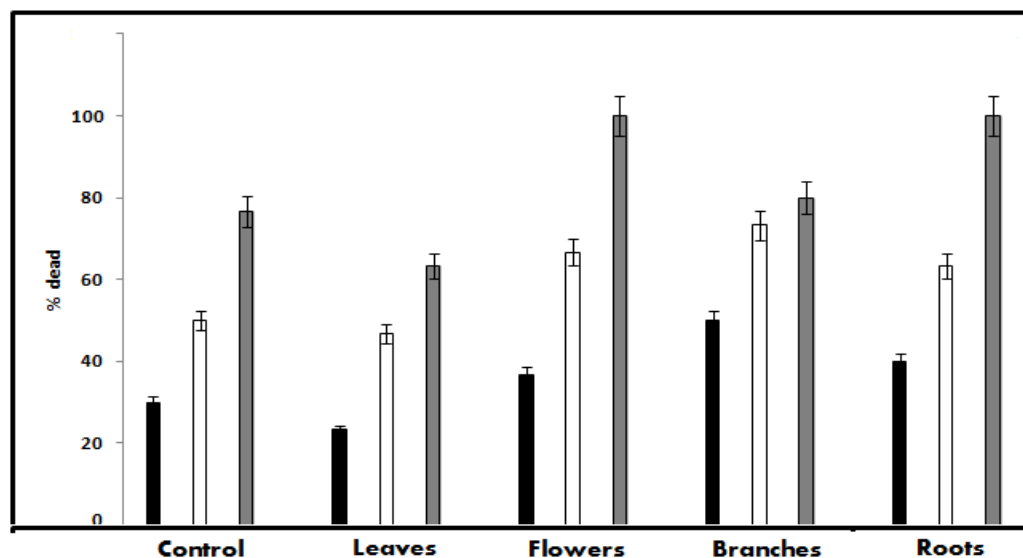


Fig.4.24. Cytotoxic activity of different parts of *Iphiona grantioides* against brine shrimp larvae at three different doses; 10 µg/ml (■), 100 µg/ml (□) and 1000 µg/ml (▒). Bars indicate $\pm$ two standard errors of the mean.

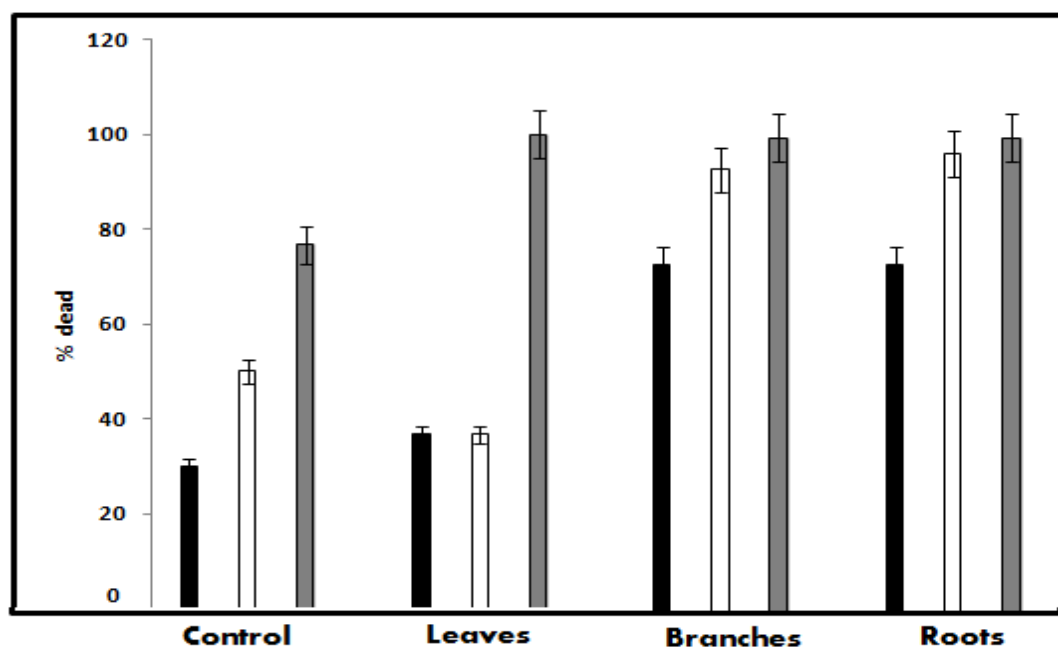


Fig.4.25. Cytotoxic activity of difference parts of *Pluchea arguta* subsp. *glabra* against *brine shrimp* larvae at three different doses; 10 µg/ml (■), 100 µg/ml (□) and 1000 µg/ml (▣). Bars indicate $\pm$ two standard errors of the mean.

4.7.7. Hepatoprotective activity

Acute toxicity of plant

Animals of all the groups were found alive after 24 hours observation and no behavioural changes were observed, that proved that the plant material was found safe upto an oral dose of 3.0 gm /kg.

Acetaminophen induced hepatotoxicity

Results of the hepatocurative activity of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* are presented in the Table 4. 23.

The present study was designed to evaluate the hepativeprotective effect of ethanol extract of *Pluchea arguta* subsp. *glabra* and *Iphiona grantioides* separately and in combination. As compared to control (Group -1), acetaminophen administration in Group - 2 significantly ($p < 0.05$) increased AST, ALT, ALP and Serum Bilirubin levels. In all the test groups (Group 3-12), where ethanolic extracts of *Iphiona grantioides* (IGEE) and *Pluchea arguta* subsp. *glabra* (PAEE) were given in three different (200,350,500 mg/kg.b.wt.) doses, have significantly ($p < 0.05$) decreased these parameters towards normalization in a dose dependant manner. Results are shown in Table 4.23. and Fig.4.26 and Fig.4.27.

Values of AST, ALT, ALP and S. Bilirubin in rabbits of control group were found to be 37.035 ± 3.581^F I.U / L, 31.91 ± 2.175^I I.U / L, 54.22 ± 3.611^G I.U/L and 0.063 ± 0.004^F mg/dl (n= 6) respectively, while a toxic dose of acetaminophen (2 g /kg) significantly raised ($p < 0.05$) the respective serum values to 188.17 ± 7.703^A I.U / L, 273.83 ± 6.231^A I.U / L, 191.62 ± 3.360^A I.U / Land 2.190 ± 0.164^A mg/dl in animals of group-2. Serum values of AST, ALT, ALP and S. Bilirubin in animals of rest of the groups (4-12) treated with ethanolic extracts of IG, PA and combined extract of IG+PA, in three different doses (100 , 250 and 500 mg/kg. body. wt.) were decreased in a dose

dependent manner. Ethanolic extract of IG, PA and combined extract of IG+PA in the dose of 100 mg/kg and 250 mg/kg, slightly and moderately decreased the AST, ALT, ALP and S. Bilirubin values, while for the dose of 500 mg/kg for IG extract, the respective values were 59.917 ± 2.556^E I.U/L, 68.54 ± 1.466^F I. U/L, 154.16 ± 1.809^E I.U/L and 0.833 ± 0.033^{DE} mg/dl respectively. The respective values for 500 mg/kg PA extract were 59.078 ± 2.174^E I.U / L, 63.59 ± 2.627^{FG} I.U / L, 155.89 ± 1.033^E I.U / L and 0.850 ± 0.022^{DE} mg/dl, respectively while serum values for combined extract of IG+PA were 60.017 ± 2.849^E , 60.245 ± 1.523^{GH} , 153.04 ± 1.015^E and 1.007 ± 0.0201^{CD} respectively, which were significantly lower ($p < 0.05$) than the values of toxic group and very close to the serum values of Silymarin treated group which were 52.850 ± 6.257^E (AST), 54.625 ± 3.948^H (ALT), 124.88 ± 2.030^F (ALP) and 0.7550 ± 0.044^E (S. Bilirubin).

Results of histopathology

In group-1, Normal hepatocytes showed fairly well preserved lobular texture. No cellular infiltration or circulatory disturbances were observed. Nuclei appeared normal and were centrally placed. No abnormal morphological pattern and cellular accumulations were observed in the parenchyma (Fig. 4.28)

In group-2 (Intoxicated acetaminophen group), sinusoidal spaces were dilated due to shrinkage of hepatocytes. A severe degree of cellular infiltration around the blood vessels was observed in some sections (Fig.4.29). There was severe vacuolation, suggesting early stage cellular injury in the cytoplasm of hepatocytes.

Table 4.23. Effect of *Iphiona grantioides* ethanol extract (IGEE) and *Pluchea arguta* subsp. *glabra* ethanol extract(PAEE)on liver enzyme profiles and Serum Bilirubin contents of rabbits

S. No	Groups	AST	ALT	ALP	S. Bilirubin
1	Control	37.035± 3.581 ^F	31.91±2.175 ^I	54.22±3.611 ^G	0.53± 0.004 ^F
2	Silymarin+ Acetaminophen	52.850± 6.257 ^E	54.625±3.948 ^H	124.88±2.030 ^F	0.7550±0.044 ^E
3	Acetaminophen 2 g/kg	188.17± 7.703 ^A	273.83±6.231 ^A	191.62±3.360 ^A	2.190±0.164 ^A
4	IGEE 100 mg + Acetaminophen	122.50± 473 ^B	132.06±2.213 ^C	180.89±2.295 ^B	2.048±0.039 ^A
5	IGEE 300 mg+ Acetaminophen	85.215± 2.576 ^D	84.28±1.375 ^{DE}	168.23± 4.047 ^{CD}	1.0417±0.009 ^{CD}
6	IGEE 500 mg+ Acetaminophen	59.917± 2.556 ^E	68.54±1.466 ^F	154.16± 1.809 ^E	0.833±0.033 ^{DE}
7	PA 100 mg+ Acetaminophen	129.83± 2.614 ^B	151.43± 2.661 ^B	172.99± 1.780 ^C	1.703±0.206 ^B
8	PA 300 mg+ Acetaminophen	102.98± 2.573 ^C	90.43±1.117 ^D	164.36± 1.157 ^D	1.025±0.004 ^{CD}
9	PA 500 mg+ Acetaminophen	59.078± 2.174 ^E	63.59±2.627 ^{FG}	155.89±1.033 ^E	0.850±0.022 ^{DE}
10	IG+PA 100mg+ Acetaminophen	108.75± 3.024 ^C	130.89±2.199 ^C	184.88± 1.724 ^B	1.183±0.046 ^C

11	IG+ PA 300 mg+ Acetaminophen	77.12±5.785 ^D	77.348±2.294 ^E	164.43±1.480 ^D	1.035±0.008 ^{CD}
12	IG+PA 500 mg+ Acetaminophen	60.017± 2.849 ^E	60.245±1.523 ^{GH}	153.04± 1.015 ^E	1.007±.0201 ^{CD}

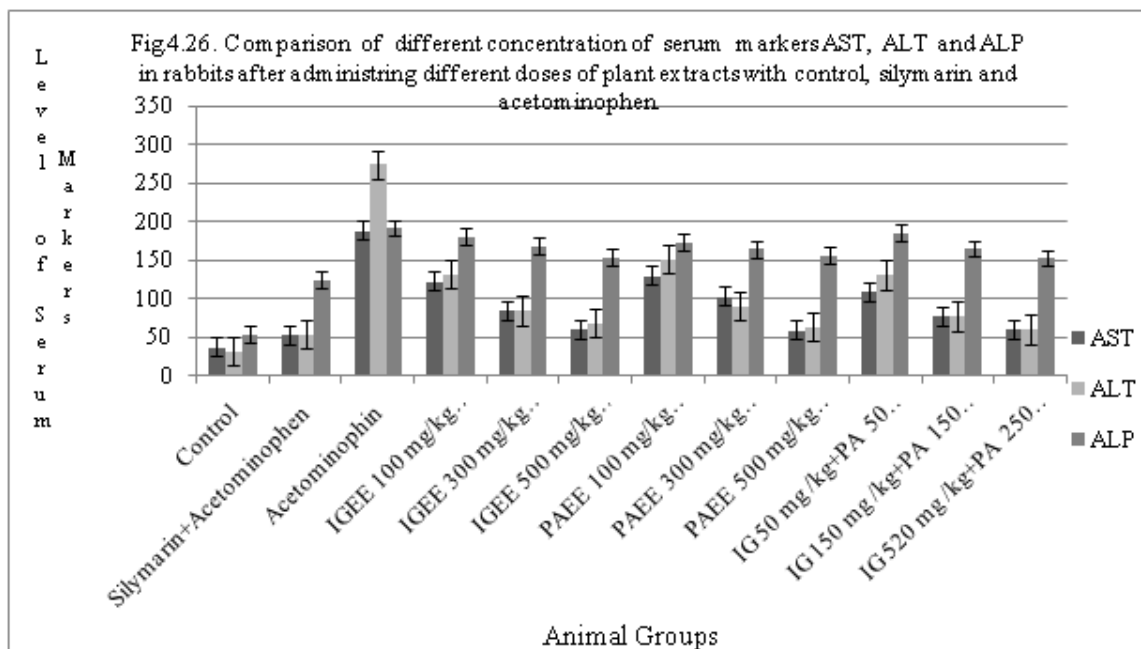
Means with different superscript letters are significantly different (P<0.05).

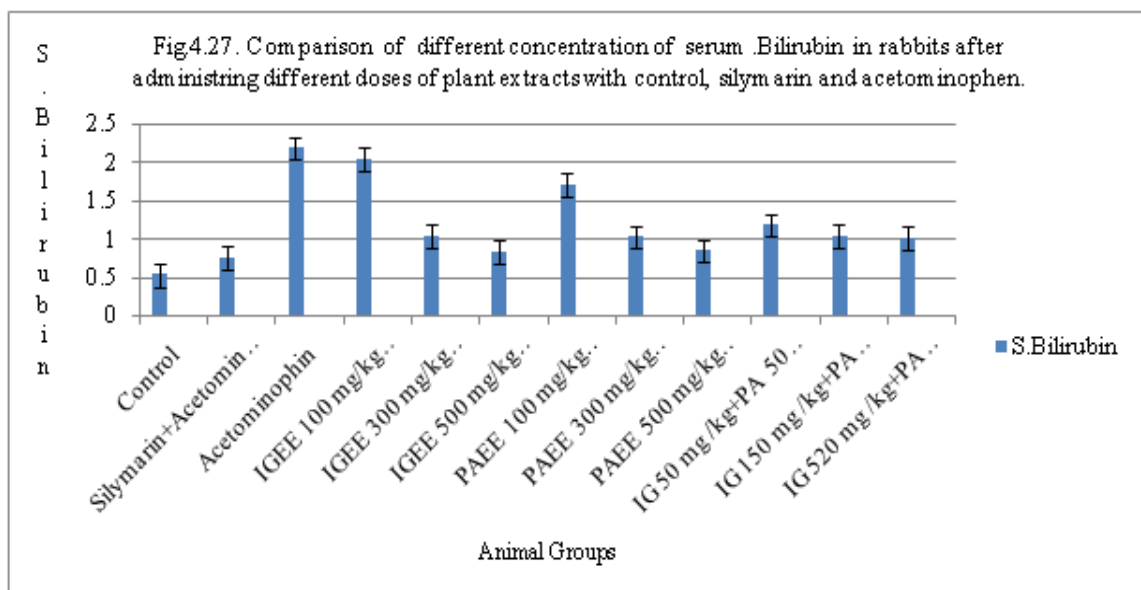
The Silymarin treated group showed less disruption and deterioration of hepatocytes, representing noticeable restoration activity (Fig.4.30).

While the group, treated with ethanolic extract of IG, the protective effect of *Iphiona grantioides* (Boiss) was evident by decrease in the changes as observed in the rabbits of group-2. A cellular infiltration of moderate degree along with mild to moderate degree of leukocytic infiltration was observed around the blood vessels. The vaculation of lesser degree than rabbits in group B was observed (Fig.4.31).

In the group treated with ethanolic extract of PA, the histological picture of the liver was almost similar to the rabbits in group treated with IG extract, showing that *Pluchea arguta* subsp. *glabra* Qaiser has similar healing effects on hepatocytes (Fig. 4.32)

Rabbits of the group treated with combined extract of IG+PA, exhibited almost same liver histology as in groups treated with IG and PA extract. The sinusoidal spaces were normal and hepatocytes showed well preserved foamy structure. The blood vessels showed no cellular infiltrations in their surroundings (Fig.4.33).





Acetaminophen (N-acetyl-p-aminophenol para-acetylaminophenol or APAP) is most widely used analgesics (Larson, 2005) but when taken in overdoses, it results in liver injury (Litovitz *et al.*, 2002) due to its toxic metabolite known as N-acetyl-p-benzoquinoneimine (NAPQI) (Bonkovsky *et al.*, 1994). Under normal conditions, NAPQI, produced by isoenzymes of cytochrome P450 system, is completely detoxified by glutathione through conjugation, however in case of overdosage of acetaminophen, excess amount of NAPQI is produced that reacts with cellular membrane molecules of hepatocytes, resulting in their damage and death, resulting in acute hepatic necrosis and increased biomarkers level (Dai & Cedebaum, 1995).

ALT, is an enzyme which is specific to liver cells, with lesser amounts in other body tissue, however AST and ALP (enzymes) are mostly found in kidney, heart, skeletal muscles and liver too). ALT is a relatively specific indicator of acute liver cell damage. Human ALT levels in serum are used in clinical diagnosis of liver and heart diseases. Similarly Serum bilirubin is also associated with liver cell damage. The enzymes are higher than normal in serum when there is liver necrosis (Price & Stevens, 2003).

In the present study, acetaminophen intoxication raised the levels of biomarkers i.e., AST, ALT, ALP as well as Serum bilirubin in healthy rabbits, which was the indication of acute hepatocellular damage and biliary obstruction.

The effectiveness of any hepatoprotective drug is basically dependent on its ability in reducing the harmful effects or maintaining the normal liver physiology that has been disturbed by a hepatotoxin. In the present study the ethanol extract of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* significantly ($p < 0.05$) decrease the elevated level of AST, ALT, ALP level and Serum bilirubin in rabbits (Table.2) in a dose dependent order. Same results are also reported by Ali & Khan (2006); Mazz *et al.*, (2010) and Yasmin *et al.*, (2011), who reported that *Calendula officinalis*, *Butea monosperma* and *Abutilon bidentatum* also processes hepatoprotective potential, respectively.

There are many hypothesis to explain the drug induce liver injury. The toxicity produced by acetaminophen following NAPQI generation is chiefly due to oxidative stress and can effectively be ameliorated by anti oxidants (Harman, 1985). Calcium contents in the liver cells are increased during experimental hepatic damage (Moor *et al.*, 1985) and Ca^{++} channel blocking agents i.e., nifedipine, diltiazem and verapamil were found to inhibit the development of hepatic damage (London *et al.*, 1986; Thibault *et al.*, 1991). Some hepatoprotective drugs have been proved to have Ca^{++} channel blocking constituents (Gilani *et al.*, 1994). The other possible mechanism is through microsomal drug metabolizing enzyme (MDME) inhibition. The inhibitors of MDME can impair the bioactivation of acetaminophen into its reactive metabolite and hence provide protection against the prevailing hepatocellular damage and since MDME inhibitory activity is reported to be common in medicinal plants (Shin, 1989), so both the plants *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* must be investigated for possessing any such activity or not.

In the present study, hepatoprotective potential of both the plants may be due to the presence of flavonoids and phenolic compounds, which are known for antioxidant and hepatoprotective activities (Di Carlo *et al.*, 1999), that might enhanced the regeneration ability of the liver either by enhancing the functioning of the hepatic glutathione antioxidant system (Ip *et al.*, 1996) or the regeneration of parenchymal cells and thus protecting against membrane fragility, decreasing the leakage of marker enzymes into the circulation, interfering with the microsomal activation of acetaminophen or accelerating detoxification (Bishayee *et al.*, 1995).

The plant material is safe as is apparent by the lack of any symptoms of acute toxicity at an oral dose of as high as 3.0 gm / kg.

Results of histopathological studies provided supportive evidence for biochemical analysis. Histopathological examination of the liver sections confirmed that normal cellular construction was damaged with acetaminophen treatment showing necrosis with loss of cellular boundaries (Fig.4.29). However, the liver sections of the plant extract treated rabbits (Groups 4-12) showed decrease of hepatocyte damage (Fig. 31-33).

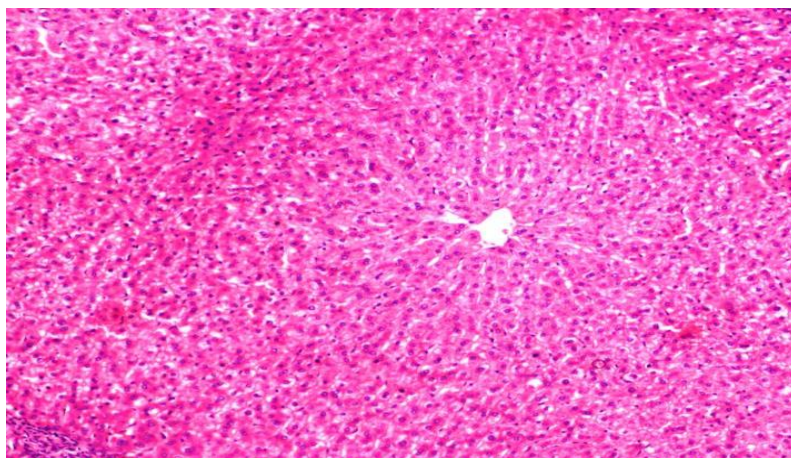


Fig.4.28. Liver of rabbit treated with normal diet (Group-1)

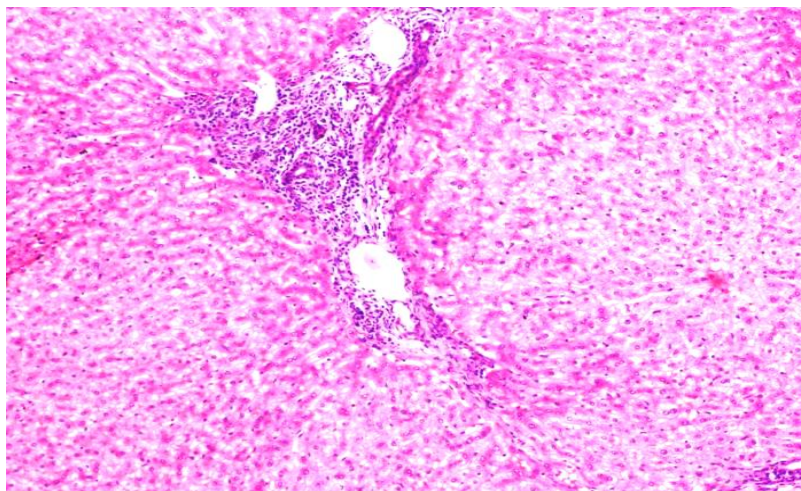


Fig.4.29. Liver of rabbit treated with acetaminophen (Group-2)

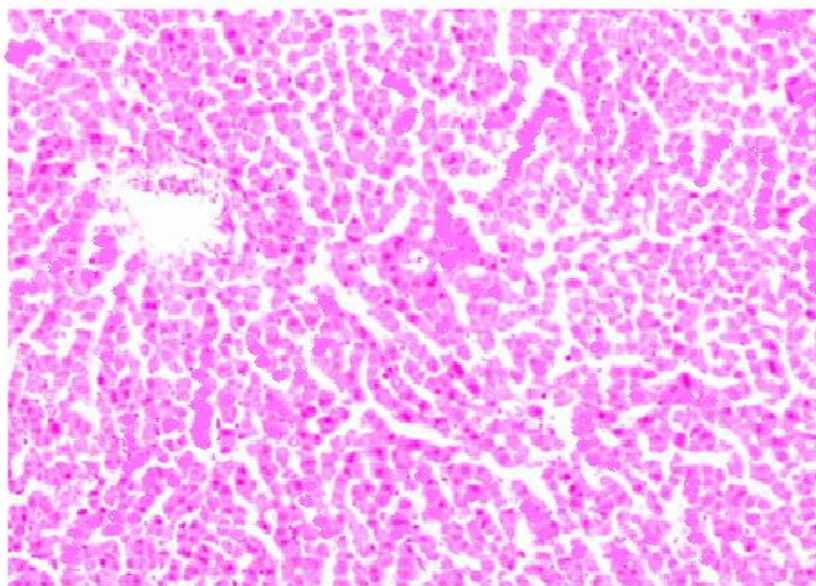


Fig.4.30. Liver of rabbit treated with Silymarin+ acetaminophen

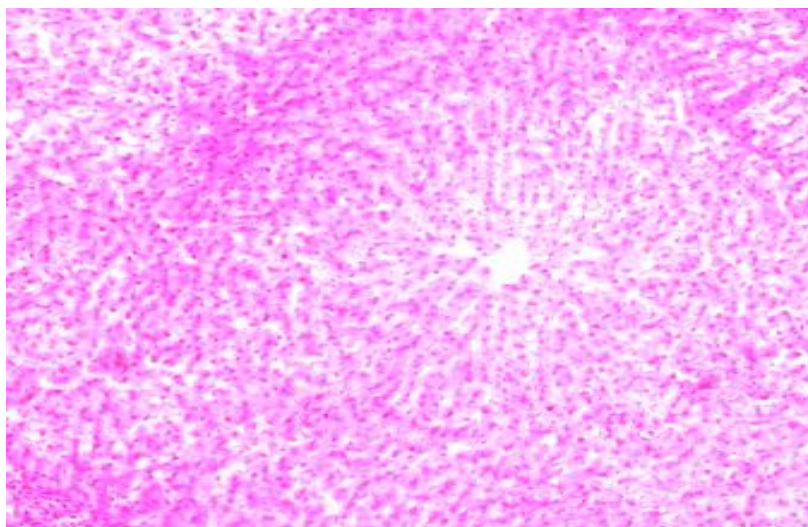


Fig.4.31. Liver of rabbit treated with ethanolic extract of IG

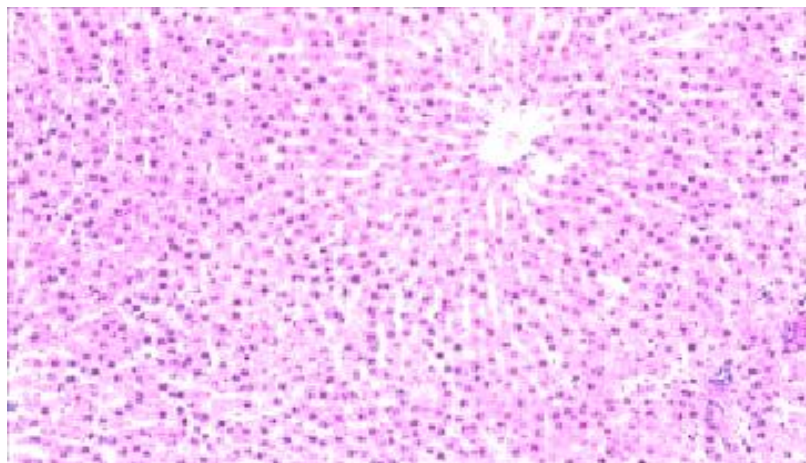


Fig.4.32. Liver of rabbit treated with ethanolic extract of PA

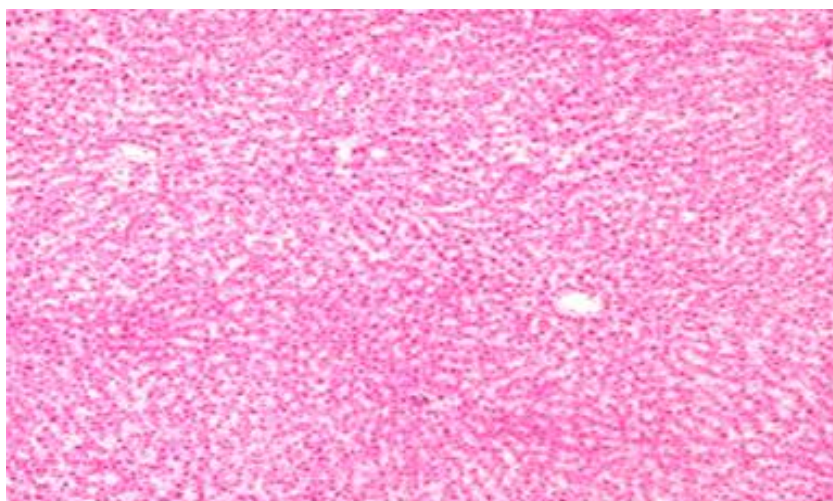


Fig.4.33. Liver of rabbit treated with ethanolic extract of IG+PA (IG= *Iphiona grantioides*, PA= *Pluchea arguta* subsp *glabra*).

4.7.8. Insecticidal activity

Results of insecticidal activity of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* against *Tribolium castaneum* (Red flour beetle- pest of stored grains, flour, cereals, nuts, beans, biscuits and pasta- Grünwald *et al.*, 2013), *Sitophilus oryzae* (Pest of wheat, rice and maize), *Callosobruchus analis* (Graham bean weevil), *Trogoderma granarium* (Khapra beetle pests of grain products and seeds - Stibick *et al.*, 2007), *Rhyzopertha dominica* (stored grain borer- pest of stored cereal grains- Peter, 2012) are

shown in the Table 4.24 and Fig.4.34.and 4.38. Different pests respond differently because of their variability in morpho-histology, physiology and genomics. Ethanolic extracts of flower, leaf of *Iphiona grantioides* showed excellent insecticidal (90%) activity against *Callosobruchus analis* and *Rhyzopertha dominica* at 10% dose concentration, respectively. Besides this, flower extract also significantly expressed insecticidal activity against *Sitophilus oryzae* (82%) and *Tribolium castaneum* (80%). Moderate lethal activity was shown by stem, leaf, flower and root extracts (in the range of 60% to 66%) against *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Trogoderma granarium* and *Callosobruchus analis*. Week inhibitory activity (in the range of 22%-48%) was exhibited by stem, root and leaves extract against almost all the tested species except. Stem and roots of *Pluchea arguta* subsp. *glabra* displayed week inhibitory activity against *Tribolium castaneum* in the order of 28% and 62%, respectively. Ethanolic extract of *Pluchea arguta* subsp. *glabra* leaf exhibited good mortality activity against *Rhyzopertha dominica*, *Tribolium castaneum*, *Sitophilus oryzae*, *Trogoderma granarium* and *Callosobruchus analis* in the order of 100%, 90%, 80%, 80% and 70%, respectively. The extract obtained from the stem and root exhibited a moderate (50%- 60%) inhibitory activity against *Rhyzopertha dominica* and *Callosobruchus analis* and weak activity (10% - 40%) was shown by rest of the extracts against all tested insects.

The present finding are in line with the results reported by Liu *et al.*, 2006 (*Inula racemosa*, against rice weevil (*Sitophilus oryzae* (L.)); Chaubey 2007(*Trachyspermum ammi*, *Anethum graveolens* and *Nigella sativa* extracts against *Tribolium castaneum*); Haouas *et al.*, 2008 (*Crysanthemum* extract against *Tribolium confusum*); Khan *et al.*, 2008; Kumar *et al.*, 2010; Mostafa *et al.*, and Umadevi & Sujatha ,2013 (*Azadirachta indica*, *Curcuma longa*, *Catharanthus roseus*, *Chrysanthemum trifurcatum* and *Gymnema sylvestre* with)).

The interest in finding suitable insecticides of plant origin as alternatives to the artificial ones, which possess well known undesirable effects on agroecological systems, has been increased in the last few decades in integrated pest managing program. This interest has led to the rising number of investigation which have discovered the potential of secondary plant metabolites as pest control agents (Pavela, 2004) numerous plants

have been confirmed to possess pesticidal compounds, either as a chemical defense mechanism against predation or infection (Ciccia *et al.*, 2000). Botanical insecticides are relatively safe and biodegradable, and are readily available sources of natural insecticides (Shafeek *et al.*, 2004). Consequently, the use of plant allelochemicals has been accepted as a promising approach for pest management. Therefore, in recent years, agrochemical companies have focused on the study of natural products for the development of new insecticides (Addor, 1995). During the present investigations, ethanolic extract of flower of *Iphiona grantioides* and leaf extract of *Pluchea arguta* subsp. *glabra* have shown considerable insecticidal activity mainly against *Tribolium castaneum* and *Callosobruchus analis*, respectively. Therefore the studied plants could be new sources of stored product pest control especially if used in a little higher concentrations than the tested one.

Table 4.24. Insecticidal activity of ethanolic extract of *Iphiaona grantioides* and *Pluchea arguta* subsp. *glabra*

Samples	Dose(Percent)	<i>Tribolium.castaneum</i>			<i>Sitophilus oryzae</i>			<i>Callosobruchus analis</i>			<i>Trogoderma granarium</i>			<i>Rhyzopertha dominica</i>		
		No. of survivors	No. of died	% Mortality	No. of survivors	No. of died	% Mortality	No. of survivors	No. of died	% Mortality	No. of survivors	No. of died	% Mortality	No. of survivors	No. of died	% Mortality
IF	1	8	2	20	8.2	1.8	18	7	3	30	7	3	30	9	1	10
	5	6	4	40	6.2	3.8	38	5	5	50	6	4	40	6	4	40
	10%	2	8	80	1.8	8.2	82	1	9	90	4	6	60	4	6	60
IL	1%	7.4	2.6	26	8.6	1.4	14	8	2	20	9	1	10	9	1	10
	5%	5.8	4.2	42	7.8	2.2	22	5.2	4.8	48	6.8	3.2	32	6	4	40
	10%	2.2	7.8	78	5.2	4.8	48	2.5	7.5	75	3.4	6.6	66	1	9	90
IB	1%	8.5	1.5	15	10	0	0	8.8	1.2	12	9	9	10	9	1	10
	5%	6.8	3.2	32	8	2	20	7	3	30	8	2	20	7	3	30

	10%	3.5	6.5	65	4	6	60	5	5	50	6	4	40	4	6	60
IR	1%	9	1	10	10	0	0	9	1	10	10	0	0	9	1	10
	5%	8.4	1.6	16	8.8	1.2	12	7	3	30	2.7	7.3	73	8	2	20
	10%	7.2	2.8	28	7.8	2.2	22	4	6	60	6	4	40	7	3	30
PL	1%	7.6	2.4	24	8	2	20	7.5	2.5	25	7	3	30	9	1	10
	5%	5.4	4.6	46	6	4	40	4.2	5.8	58	4	6	60	6	4	40
	10%	2	9	90	2	8	80	3	7	70	2	8	80	0	10	100
PB	1%	10	0	0	9	1	10	9	1	10	9	1	10	9	1	10
	5%	8.8	1.2	12	8	2	20	8.2	1.8	18	8	2	20	8	2	20
	10%	7.2	2.8	28	5.5	4.5	45	7	3	30	6	4	40	5	5	50
PR	1%	10	0	0	10	0	0	10	0	0	10	0	0	10	0	0
	5%	8.4	1.6	16	8	2	20	6	4	40	10	0	0	9	1	10
	10%	7.4	2.6	26	6	4	40	4	6	60	8	2	20	6	4	40

Key = IF = *Iphiona grantioides* flower

PL= *Pluchea arguta* subsp. *glabra* leaf

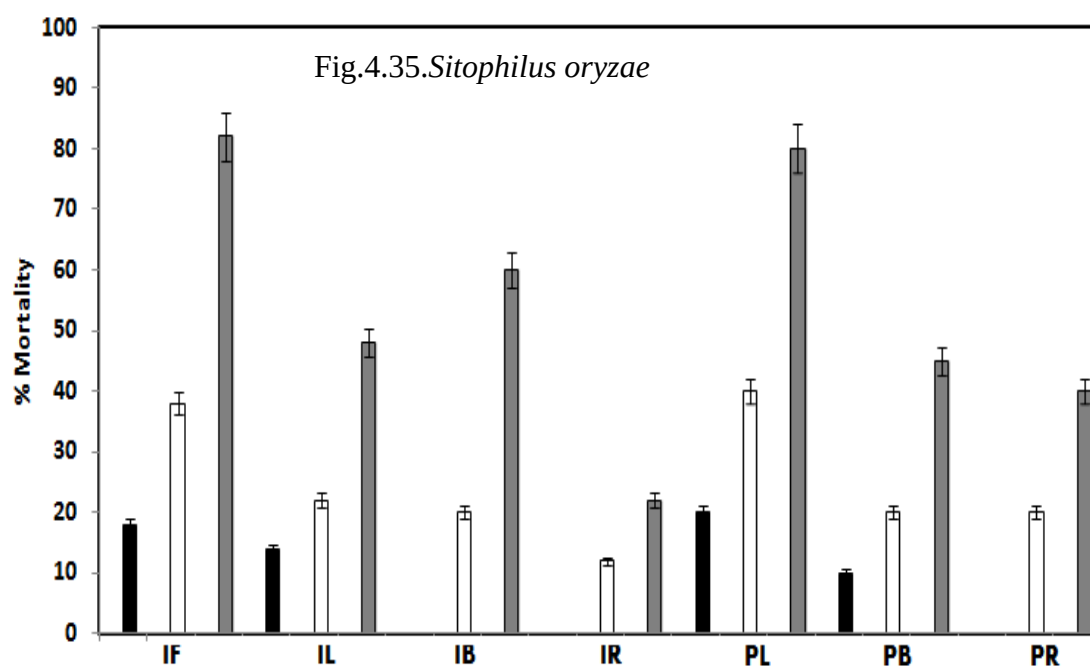
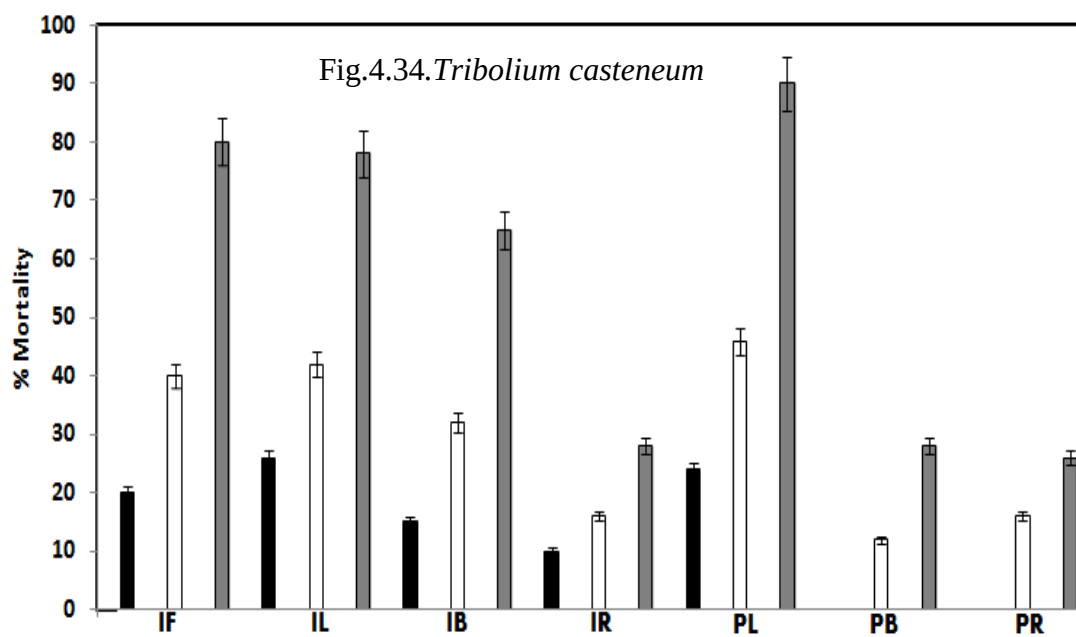
IL = *Iphiona grantioides* leaf

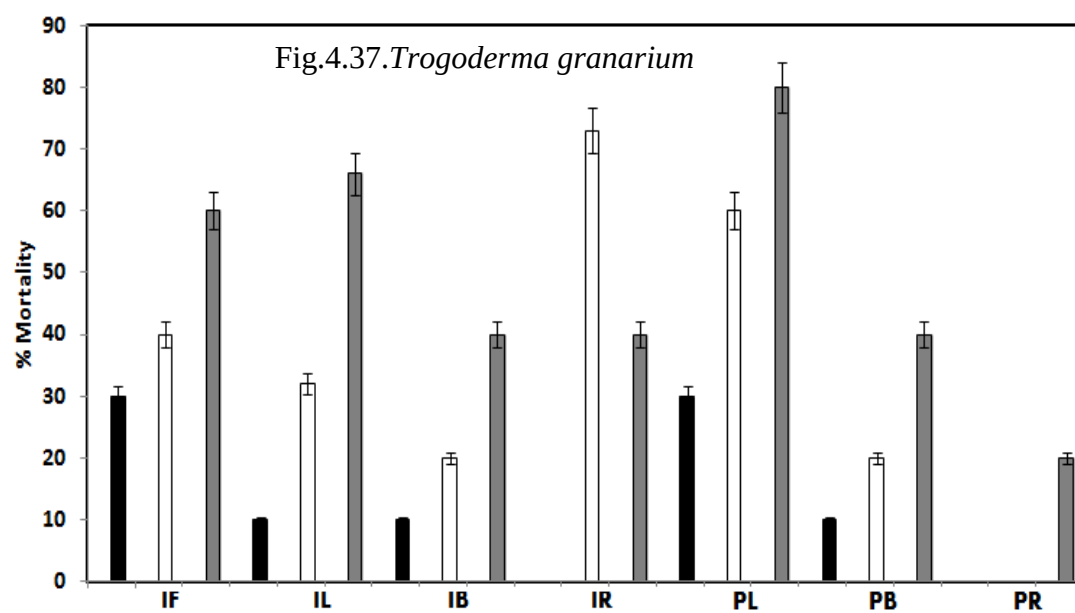
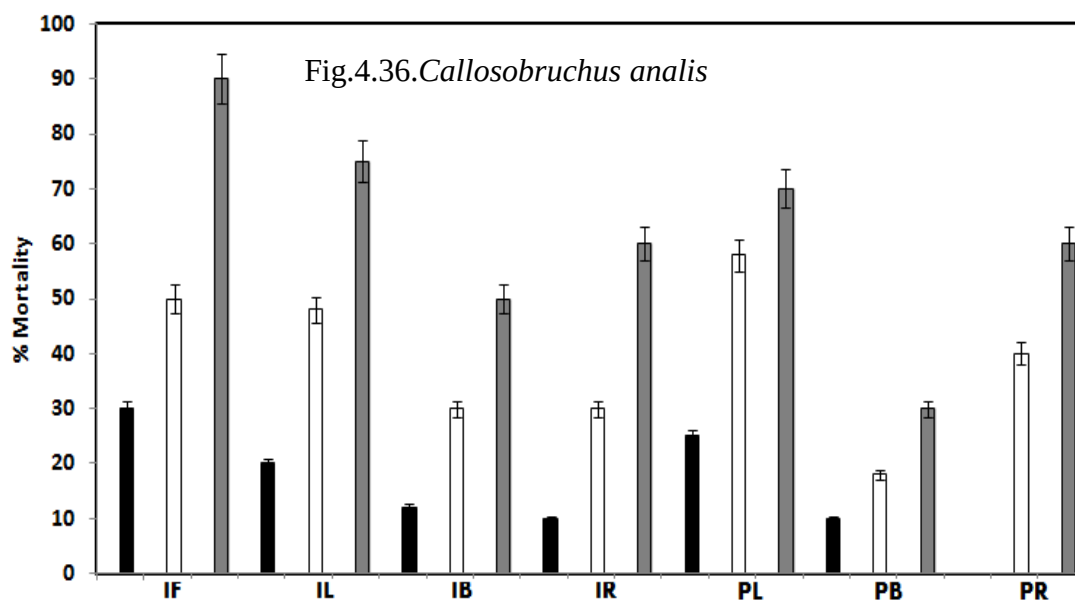
PS = *Pluchea arguta* subsp. *glabra* stem

IS = *Iphiona grantioides* stem

PR= *Pluchea arguta* subsp. *glabra* root

IR = *Iphiona grantioides* root





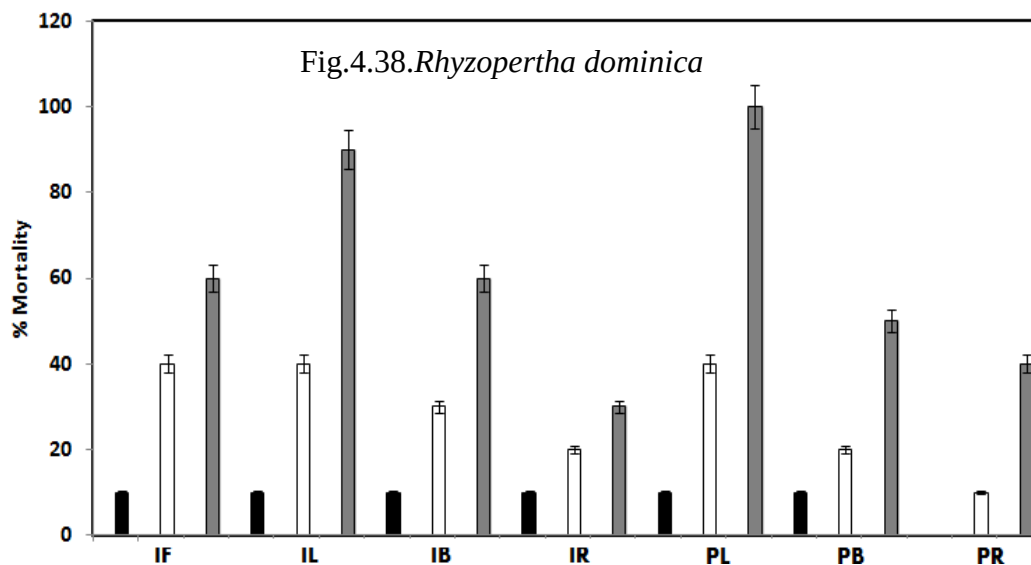


Fig.4.34- 4.38. Insecticidal activity of different parts of *Iphiona grantioides* (I) and *Pluchea arguta* subsp. *glabra* (P) against different pests at three different concentrations; 1% (■), 5% (□) and 10% (▨). Fig.4.34. *Tribolium castaneum*, Fig.4. 35. *Sitophilus oryzae*, Fig.4.36. *Callosobruchus analis*, Fig.4.37. *Trogoderma granarium*, Fig.4.38. *Rhyzopertha dominica*

F=Flowers, L= Leaves, B=Branches and R=Roots. Bars indicate $\pm$ two standard errors of the mean.

4.7.9. Phytotoxic activity

Results of the phytotoxic activity of *Iphiona grantioides* and *Pluchea arguta* are shown in Table.4.25 and Fig.4.39 – Fig. 4.40, respectively. The results indicate that ethanolic extracts of leaf and flower of *Iphiona grantioides* have a phytotoxic effect in higher tested concentrations while the rest of the tested extracts have moderate herbicidal activity against *Lemna minor*. It is clear from Table.4.26, that leaf ethanolic extract of *Iphiona grantioides* has produced an excellent phytotoxic activity against *L. minor* at the highest tested concentration (1000 μ g/mls) by causing (91.5%) inhibition of *Lemna minor*. Similarly, flower extract also caused 87.2% inhibition.

Pluchea arguta subsp. *glabra* leaf extract caused 73.2% inhibition, while the stem caused 70.2% inhibition. Moderate inhibitions (61.7%) were observed for the

ethanolic extracts of *Iphiona* and *Pluchea* roots. Lowest inhibition (59.6%) was caused by stem extract of *Iphiona grantioides* at concentration 1000 ug/ml).

The present results for phytotoxic activity are in line with similar phytotoxic studies, carried out by various workers e.g., Hussain *et al.*, 2010(Polygonaceae); Ahmad *et al.*, 2011(*Zizyphus jujuba*), Naveed & Saeed, 2011(*Viola betonicifolia*), Ibrar *et al.*, 2013a (Wild olive nut galls) and Rehmanullah *et al.*, 2012 (*Calendula arvensis* L.)

There is a need to discover new herbicides since the number of herbicide-resistant weeds is increasing and conventional synthetic herbicides are becoming less and less effective against the resistant weed biotypes (Bhowmik & Inderjit, 2003). In addition the synthetic herbicides also cause ecological and health problems. New herbicides from natural sources are at present receiving much attention which could be efficient suitable and harmless alternatives to the currently used synthetic agrochemicals. The natural products are generally effective, eco-friendly and thus pose less threat to the environment. The present results of phytotoxic activity indicates that the *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* possess considerable phytotoxic activity against *L. minor* and therefore might be useful as cheap natural herbicides and could be sources of bioactive agrochemicals.

This preliminary study is a starting point for further purification and characterization of the active principles responsible for insecticidal and phytotoxic activities.

Table 4.25. Phytotoxic activity of different parts of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* ethanolic extract against *Lemna minor*

Solvents	Concentrations (µg/ml)	No of fronds in test	No of fronds in control	% inhibition	Intercept	Slope	R-Square	FI ₅₀
IF	10	29	47	38.3	2.168	0.145	0.944	58.74
	100	24	47	48.9				
	1000	6	47	87.2				
IL	10	28	47	40.4	2.176	0.144	0.875	52.61
	100	25	47	46.8				
	1000	4	47	91.5				
IR	10	36	47	23.4	2.036	0.163	0.922	240.39
	100	24	47	48.9				

	1000	18	47	61.7				
IS	10	31	47	34.0	2.191	0.096	0.985	271.07
	100	27	47	42.6				
	1000	19	47	59.6				
PL	10	37	47	21.3	1.943	0.201	0.999	245.80
	100	28	47	40.4				
	1000	14	47	70.2				
PR	10	36	47	23.4	1.981	0.163	0.917	527.44
	100	33	47	29.8				
	1000	18	47	61.7				
PS	10	37	47	21.3	1.902	0.207	0.930	336.56
	100	33	47	29.8				
	1000	13	47	72.3				

Key = IF = *Iphiona grantioides* flower

PL= *Pluchea arguta* subsp. *glabra* leaf

IL = *Iphiona grantioides* leaf

PS = *Pluchea arguta* subsp. *glabra* stem

IS = *Iphiona grantioides* stem

PR= *Pluchea arguta* subsp. *glabra* root

IR = *Iphiona grantioides* root

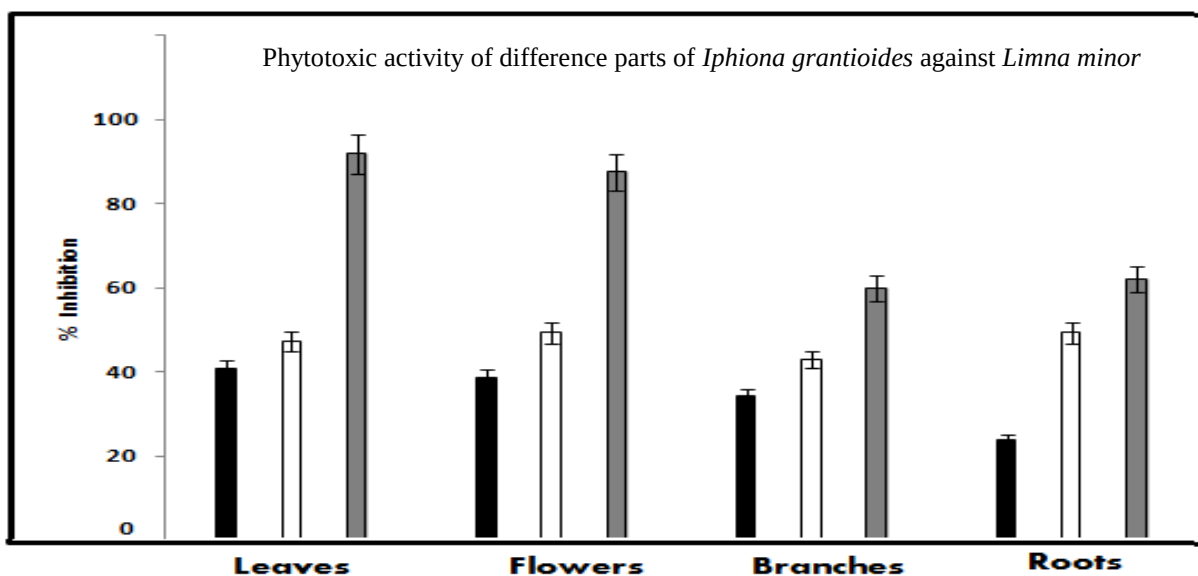


Fig.4.39 Phytotoxic activity of difference parts of *Iphiona grantioides* against *Lemna minor* at three different doses; 10 µg/ml (■), 100 µg/ml (□) and 1000 µg/ml (▨) Bars indicate $\pm$ two standard errors of the mean

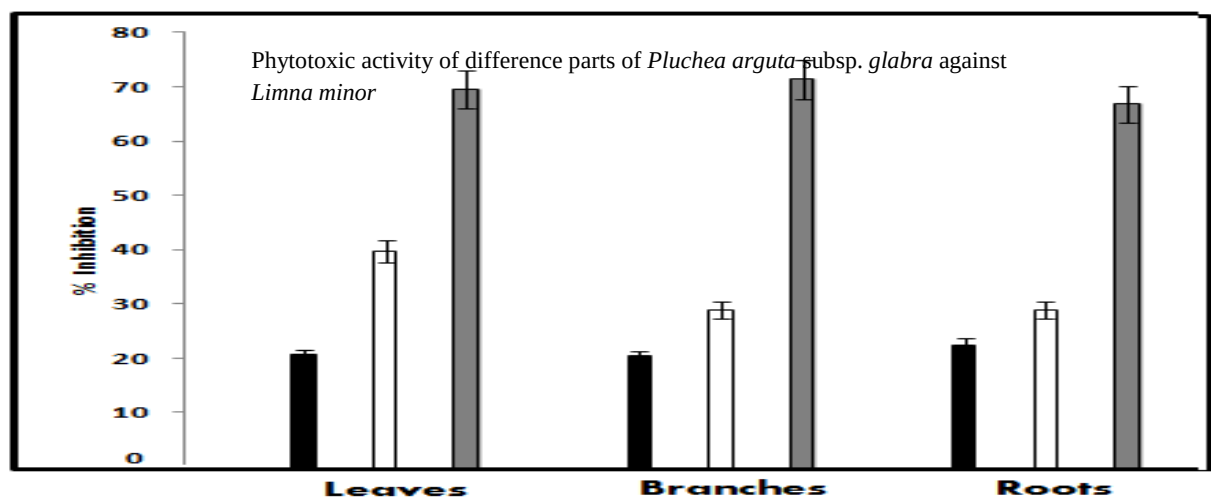


Fig.4.40 Phytotoxic activity of difference parts of *Pluchea arguta* subsp. *glabra* against *Lemna minor* at three different doses; 10 µg/ml (■), 100 µg/ml (□) and 1000 µg/ml (▨) Bars indicate $\pm$ two standard errors of the mean

Conclusion

Phytosociological study of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* Qaiser was carried out at seven different saline localities in district Karak of Khyber Pukhtunkhwah, Pakistan, at an elevation ranging from 450 m to 900 m. The two research plants, were present in close association of each other along with other 80 plants, in all the seven localities. The present study revealed that the two plants are rather sparse in their distribution in the area

The description of macro-micro morphological features of *Iphiona grantioides* and *Pluchea arguta* sub sp. *glabra* distinguishes these plants from other members of their respective genera.

Chemomicroscopy, numerical data and quantitative leaf microscopy are parameters that are unique to these plants and useful parameters required in their standardization.

Qualitative phytochemical screening of various parts of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* revealed the presence of active secondary metabolites including proteins, carbohydrates, tannins, alkaloids, flavonoids, saponins, glycosides, phytosterol ,tri-terpenoids and volatile oil in both the plants, which were attributed with strong pharmacological behavior.

Bearing in mind the results of biological assays it can be concluded that ethanolic extract of various parts of the two plants appeared as an important source for the innovation of new antimicrobial drugs.

The results obtained by in vitro studies are noteworthy regarding cytotoxic, phytotoxic, antilice, antihelmintic and insecticidal activities of both the plants samples attributed due to the flavonoid and phenolic contents and it can be concluded that these plants could be exploited for herbal drugs exploration for the health care of mankind.

Due to the increased interest in discovering natural antioxidants, many research groups have examined plants for exploiting new potential sources of natural antioxidants. Both the asteraceae plants under the present study were tested for

antioxidant activity. These plants showed highest antioxidant potential which indicates that most of the active constituents of these plants might be flavonoids and phenolic compounds and that these plants may be helpful in controlling complications during degenerative diseases.

The in vivo studies of ethanolic extracts of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* showed significant hepatoprotective activity. The hepatic injuries induced were restored to normal by aqueous ethanolic extracts of both the plants. It is concluded from the results that *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* contains some specific constituents that may be potentially effective against liver injury. The present study, although initial, has provided a strong evidence of the hepatoprotective potential of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*. Further studies on these plants should be conducted for the isolation, structure determination and pharmacology of the hepatocurative/hepatoprotective constituents of these two plants and the two plants may be used for the development of new drugs for the treatment of various hepatic diseases.

Recommendations

In light of the valuable conclusions in the present research work, the following recommendations are presented for future research work.

The ecological and traditional knowledge about *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* will be useful for conservation and its profitable cultivation, which will be helpful in improving the financial and social conditions of the residents of the area.

Morphological and anatomical information of these plants should be exploited for standardization of various characteristics of the parts used as crude drug.

The pharmacognostic parameters and physicochemical analyses of the various parts of the two plants will be helpful for identification, quality, purity and detection of adulteration in these drugs.

Phytochemical and heavy metals composition of biological importance will be helpful in exploring these plants for future research work.

Pharmacological activities like antihelmintic, antilice, antibacterial, antifungal, antioxidant, cytotoxic, phytotoxic, insecticidal and hepatoprotective potentials of these two plants showed that these plants should be further exploited for isolating various active constituents for pharmacological preparations for treating various ailments.

REFERENCES

- Abid, A.; Qaiser, M. Chemotaxonomic study of *Inula* L. (S.Str.) and its allied genera (*Inuleae* – *Compositae*) from Pakistan and Kashmir. *Pak. J. Bot.* **2003**, *35*(2), 127- 140.
- Abdel-Ghaffar, F.; Semmler, M.; Al-Rasheid, K.; Klimpel, S.; Mehlhorn, H. Comparative in vitro tests on the efficacy and safety of 13 anti-head-lice products. Comparative in vitro tests on the efficacy and safety of 13 anti-head-lice products. *Parasitol Res.* **2010**, *106*(2), 423-429.
- Abdul Jabbar.; Iqbal, Z.; Khan, M. N. In vitro anthelmintic activity of *Trachyspermum ammi* seeds. *Pharmacognosy Magazine.* **2006**, *2*(6), 126.
- Acharya, D.; Shrivastava, A. Indigenous Herbal Medicines: Tribal Formulations and Traditional Herbal Practices, Aavishkar Publishers Distributor, Jaipur, India. **2008**, ISBN, 978- 81-7910-252-7. pp 440.
- Adate, S.P.; Parmesawaran, S.; Chauhan, Y. In vitro anthelmintic activity of stem extracts of *Piper betle* Linn. against *Pheritima posthuma*. *Phcog J.* **2012**, *4*(29), 61.
- Addor, R.W. Insecticides In : Godfrey, C.R.A.(ed), Agrochemicals from natural products, Marcel Dekker Inc, New York. **1995**.
- Ahameethunisa, A. R.; Hopper, W. Antibacterial activity of *Artemisia nilagirica* leaf extracts against clinical and phytopathogenic bacteria. *BMC Complementary and Alternative Medicine.* **2010**, *10*(1), 6.
- Ahmad , V .U.; Fizza ,K. 4-Epi-plucheinol, a new sesquiterpene from *Pluchea arguta*. *Phytochemistry.* **1986**, *25*(4), 949-950.
- Ahmad , V. U.; Fizza ,K. Arguticin, A new sesquiterpene from *Pluchea arguta*. *Liebigs Ann.Chem.* **1987**, (7), 643.

- Ahmad , V. U.; Fizza ,K.Arguticinin, A sesquiterpene from *Pluchea arguta*. *Phytochemistry*.**1988a**,27, 1861.
- Ahmad , V. U.; Fizza ,K. Argutin, A new cuauhtemone ester from *Pluchea arguta* Boiss..*Planta Medica*. **1988b**, 54(5), 462.
- Ahmad , V. U.; Fizza, K. Structure of new eudesmane sesquiterpene argutinin from *Pluchea arguta*(Boiss.). *Z. Naturforsch.***1988c**, 43b, 911-913.
- Ahmad , V. U.; Fizza , K.; Amber, A.U.R. Odontin and Odonticin, two new eudesmane sesquiterpenes from *Pluchea arguta* Boiss, *J. Nat. Prod.* **1989a**, 52(4), 861.
- Ahmad , V. U .; Fizza ,K.; Sultana, A. Isolation of two sesquiterpenes from *Pluchea arguta*. *Phytochemistry*. **1989b**, 28(11), 3081.
- Ahmad , V. U.; Fizza ,K.; Sultana,A. Two new terpenoids from *Pluchea arguta* Boiss, *Z. Naturforsch.* **1990**, 45b, 385.
- Ahmad , V. U.; Fizza,K.; Khan, M.A.; Ahmad, T. F. Two new sesquiterpenes from *Pluchea arguta*. *Phytochemistry*. **1991a**, 30(2), 689.
- Ahmad, U.V.; Ismail, N. 5-Hydroxy-3, 6, 7, 2', 5'-Pentamethoxyflavone from *Inula grantioides*. *Phytochem.* **1991b**, 30(3), 1040-1041.
- Ahmad, V. U.; Farooqui, T. A.; Fizza, K.; Sultana, A.; Khatoon, R. Three new eudesmane sesquiterpenes from *Pluchea arguta*. *J.Nat. Prod.* **1992a**, 55(6), 730.
- Ahmad ,V. U.; Farooqui, T. A.; Fizza, K.; Sultana, A.; Khatoon, R.; Ismail, N. Two new eudesmane sesquiterpenes from *Pluchea arguta*. *Nat. Prod. Lett.* **1992b**, 1(3), 225.

- Ahmad ,V. U.; Farooqui, T. A.; Fizza, K.; Sultana, A.; Khatoon, R.; Ismail, N. Two sesquiterpenes from *Pluchea argua*, *Phytochemistry*. **1992c**, 31, 2888.
- Ahmad, V. U.; Khatoon, R. Two new sesquiterpenes from *Pluchea arguta*. *Sci.Pharm*. **1995**, 63, 231.
- Ahmad, S.; Ali, A.; Beg, H.; Dasti, A. A.; Shinwari, Z. K. Ethnobotanical studies on some medicinal plants of booni valley, District Chitral, Pakistan. *Pak J. Weed Sci. Res*. **2006**,12 (3), 183-190.
- Ahmad, B.; Khan, I.; Bashir, S.; Azam, S.; Hussain, F. Screening of *Zizyphus jujuba* for antibacterial, phytotoxic and haemagglutination activities. *African Journal of Biotechnology*. **2011**, 10(13), 2514-2519.
- Ahmad, M.; Eram, S. Hepatoprotective studies on *Haloxylon salicornicum*: A plant from Cholistan desert. *Pak. J. Pharm. Sci*. **2011**, 24(3), 377-382.
- Ahmad, N.; Fazal, H.; Abbasi, B. H.; Rahman,I.U.; Anwar, S.; Khan, M. A.; Basir, A.; Inayat,H.; Zameer, R.; Khalil, S. A.; Khan, K.Y. DPPH-scavenging antioxidant potential in regenerated tissues of *Stevia rebaudiana* , *Citrus sinensis* and *Saccharum officinarum*. *Journal of Medicinal Plants Research*. **2011**,5(14), 3293-3297.
- Ahmad, I.; Ibrar, M.; Barkatullah.; Ali, N. Ethnobotanical study of Tehsil Kabal, Swat District, KPK, Pakistan. *Journal of Botany*. **2011**, 2011, pp.9 pages.
- Ahmad, M.S.; Hussain, M.; Hanif, M.; Ali, S.; Mirza, B. Synthesis, chemical characterization and biological screening for cytotoxicity and antitumor activity of organotin (IV) derivatives of 3, 4-Methylenedioxy, 6-nitrophenylpropenoic acid. *Molecules*.**2007**, 12, 2348-2363.
- Ahmad, S. S. Medicinal wild plants from Lahore-Islamabad Motorway (M-2). *Pak. J. Bot*. **2007**, 39 (2), 355-375.

- Ahmad, K.; Khan, Z. I.; Ashraf, M.; Hussain, M.; Ibrahim, M.; Valeem, E. E. Status of plant diversity at Kufri (Soone Valley) Punjab, Pakistan and prevailing threats therein. *Pak. J. Bot.***2008**, *40* (3), 993-997.
- Ahmed, E.; Arshad, M.; Ahmad, M.; Saeed, M.; Ishaque, M. Ethanopharmacological survey of some medicinally important plants of Galliyat areas of NWFP, Pakistan. *Asian J. Plant. Sci.***2004**, *3*(4), 410-415.
- Ahsan, M.R.; Islam, K.M.; Bulbu, I.J. Hepatoprotective activity of Methanol Extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. *Eur. J. Sci Res.***2009**, *37*(2), 302-310.
- Ajasa, M.A.; Bello, O.M.; Ibrahim, O.M.; Ogunwande, A.I.; Olawore, O.N. Heavy trace metal and macronutrients status in herbal plants of Nigeria. *Food Chem.* **2004**, *85*, 67-71.
- Akiyama, H.; F, Kazuyasu.; Yamasaki, O.; Oono, T.; Iwatsuki, K. Antibacterial action of several tannins against *Staphylococcus aureus*. *J. antimicrob chemotherapy.* **2001**, *48*, 487-491.
- Akroum, S.; Satta, D.; Lalauoui, K. Antibacterial, antioxidant, cytotoxic activities and phytochemical screening of some Algerian plants, European. *Journal of Science Research.* **2009**, *31*(2), 289-295.
- Al-Fatimi, M.; Wurster, M.; Schröder, G.; Lindequist, U.; Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *J. Ethnopharmacol.* **2007**, *111*, 657-66.
- Al-Dissi, N. M.; Salhab, A.S.; Al-Hajj, H.A. Effects of *Inula viscosa* leaf extract on abortion and implantation in rats. *J. of Ethnopharmacology.* **2001**, *77*, 117-121.
- Al Hassan, A.J.; Sule, M.S.; Atiku, M.K.; Wudil, A.M.; Dangambo, M.A. Mashi, J.A.; Zaitun, M.; Uba, G.K. Effect of *Calitropis procera* aqueous root extract against CCl₄ induced liver toxicity in rabbits. *Bayero Journal of Pure and Applied Sciences.* **2012**, *5* (1), 38 – 43.

- Ali, J.; Khan, H.A. Preventive and curative effects of *Calendula officinalis* leaves extract on acetaminophen induced hepatotoxicity. *JPMI*. **2006**, *20* (4), 370-373.
- Ali, N.; Ahmed, G.; Shah, S.W.A.; Shah, I.; Ghias, M.; Khan, I. Acute toxicity, brine shrimp cytotoxicity and relaxant activity of fruits of *Callistemon citrinus* Curtis. *BMC Complementary and Alternative Medicine*. **2011**, *11*(99), 1-8
- Alisi, C.S.; Okike, G.; Onyeze, C.; Ojiako, O.A.; Osuagwu, C.G. Evaluation of the Protective Potential of *Chromolaena odorata* Linn. Extract on Carbon Tetrachloride- Induced Oxidative Liver Damage. *International Journal of Biochemistry Research Review*. **2011**, *1*(3), 69-81.
- Alkofahi, A.; Atta, A.H. Pharmacological screening of the anti- ulcerogenic effects of some Jordanian medicinal plants in rats. *Journal of Ethnopharmacology*. **1999**, *67*, 341–345.
- Amin, L. R.; Murimboh, J.; Hassan, N. M.; Mandal, R.; Younes, M. E. B.; Chakrabarti, C. L.; Back, M.H. Kinetic Speciation of Co (II), Ni (II), Cu (II), and Zn (II) in Model Solutions and Freshwaters: Stability and the d Electron Configuration. *Environ. Sci. Technol.* **2003**, *37* (1), 68-74.
- Amin, M. R.; Mondol, R.; Habib, M. R.; Hossain, M. T. Antimicrobial and Cytotoxic Activity of Three Bitter Plants-*Enhydra fluctuans*, *Andrographis Peniculata* and *Clerodendrum Viscosum*. *Advanced Pharmaceutical Bulletin*. **2012**, *2*(2), 207-211.
- Andarwulan, N.; Batari, R.; Sandrasari, D.A.; Bolling, B.; Wijaya, H. *Food Chemistry*. **2010**, *121*, 1231-1235.
- Anderberg, A. Tribe *Inuleae*. In *Asteraceae*; Cladistics and Classification; Bremer, K. Ed.; **1994**, pp. 273-291.
- Ang, H.H.; Lee, K.L. Contamination of mercury in tongkat Ali hitam herbal preparations. *Food Chemistry and Toxicology*. **2006**, *44*, 1245-1259.
- Anonymous. Report of the ASM Task Force on Antibiotic Resistance. The American

Society for Microbiology, Public and Scientific Affairs Board. **1995**.

Anonymous. Macroscopic and microscopic Examination: Quality Control Methods for Medicinal Plant Materials, WHO, Geneva. **1998**. WHO/EDM/TRM/2000.1: 184

Anonymous. Dietary reference intakes for vitamin A, vitamin K, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc, Food and Nutrition Board, Institute of Medicine. Washington D.C.National Academy Press. **2001**, pp. 290- 442.

Anonymous. Potassium. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride and Sulfate. Washington, D. C. :National Academies Press; **2004**, 173-246.

Anonymous. (ATSDR). Toxicological Profile for Chromium (Draft for Public Comment). Atlanta, GA: U.S. Department of Public Health and Human Services, Public Health Service. **2008**.

Ansari, F.L.; Nazir, S.; Noureen, H.; Mirza, B. Combinatorial synthesis and antibacterial evaluation of anindexed chalcone library. *Chem. Biodiverse*. **2006**, 2, 1656- 1664.

Ansari, M.; Ahmad, M. J.; Ansari, S. H. Pharmacognostic evaluation of the stem barks of *Balanites aegyptica* Delile “Hingot”. *Hamdard Medicus*. **2006**, 50 (1), 82-94.

A.O.A.C. (Association of Official Analytical Chemists). Official methods of analysis.Gaithersburg,Washington,USA. **2000**.

Aqil, F.; Ahmad, I. Antibacterial properties of traditionally used Indian medicinal plants. *Methods and findings in experimental and clinical pharmacology*. **2007**, 29(2), 79-92.

- Arceusz, A.; Radecka, I.; Wesolowski, M. Identification of diversity in elements content in medicinal plants belonging to different plant families. *Food Chem.* **2010**, *120*, 52- 58.
- Arjun, P.; Shivesh, J.; Murthy, P. N. Pharmacognostical Standardization of Leaves of *Hygrophila spinosa* T. Anders. *Pharmacog. Jour.* **2009**, *1*(2), 82-87.
- Arredondo, M.; Nunez, T. M. Iron and Copper metabolism. *Mol. Aspects Med.* **2005**, *26*, 313- 327.
- Arnason, J.T.; Philogene, B.J.R.; Morand, P. Insecticides of plants origin. *American Chemical Society Symposium Series*. **1989**, Vol.387. Washington.
- Arnao, M.B.; Cano, A.; Acosta, M. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.* **2001**, *73*, 239-244.
- Ara, N.; Nur, M.H.; Amran, M.S.; Wahid, M.I.; Ahmed, M. In vitro antimicrobial and cytotoxic activities of leaves and flowers extracts from *Lippia alba*. *Pak. J. Biol Sci.* **2009**, *12*(1), 87-90.
- Arora, R.; Gill, N.S.; Dhingra, V. K.; Rana, A.C.; Rayat. Evaluation of anti-asthmatic potential of ethyl acetate fraction of *Pluchea lanceolata*. *Pharmacologyonline*. **2011**, *2*, 1126-1133.
- Asiri, A.Y. Lead Toxicity of an Infant from home made remedy. *Saudi Pharmaceutical Journal.* **2006**, *14*, 132-135.
- Asokan, B.; Rahuman, A.; Chinnaperumal, K.; Gandhi, E.; Abduz, Z.A.; Chidambaram, J.; Thirunavukkarasu, S.; Marimuthu, S. Contact and fumigant toxicity of hexane flower bud extract of *Syzygium aromaticum* and its compounds against *Pediculus humanus capitis*. *Parasitology research*. **2011**, *109*(5), 1329-1340.
- Atta-ur-Rehman. *Studies in Natural Product Chemistr.* Elsevier Science Publishers Netherlands, **1991**, *9*, 383-384.

- Atta-ur-Rahman.; Choudhary, M.I.; William, J.T. Bioassay techniques for drug Development; Harward academic Publisher: **2001**, pp.67-68.
- Atta-ur-Rehman.; Iqbal, M.C.; Saifullah, B. Monograph medicinal plants of sindh indigenous knowledge and scientific facts .Study Sponsored byDepartment of Planning and Development Government of Sindh, **2006**, pp.101-102.
- Badshah, L. Ecological evaluation of plant resources and vegetation structure of district Tank, Pakistan. Ph.D. Thesis, Department of Botany, University of Peshawar, Khyber Pakhtunkhawa, Pakistan, **2011**.
- Bai, N.; Lai, C.S.; He, K.; Zhou, Z.; Zhang, L.; Quan, Z.; Zhu, N.; Zheng, Q.Y.; Pan, M.H.; Ho, C.T.Sesquiterpene Lactones from *Inula britannica* and their cytotoxic and apoptotic effects on human cancer cell lines. *J. Nat. Prod.* **2005**, 69, 531-535.
- Balakrishnan, S.; Natarajan, B.; Arumugam, B.; Hariram, B. V. Pharmacognostical Evaluation of *Amaranthus spinosus* L. *Pharmacon. J.* **2011**, 3(19), 13-18.
- Balunas, M.J.; Kinghorn, A.D. Drug discovery from medicinal plants *Life Sci.***2005**, 78, 31-41.
- Bancroft, J. D.; Gamble, M. Theory and Practice of Histological Techniques. Churchill Livingstone; 6th Ed. **2008**.
- Barkatullah .; Ibrar, M.;Hussain, F. Ethnobotanical studies of plants of Charkotli Hills, Batkhela District, Malakand, Pakistan. *Front. Biol. China.***2009**,4 (4), 539-548.
- Barkatullah.; Ibrar, M. Plants profile of Malakand Pass Hills, District Malakand,Pakistan. *African Journal of Biotechnology.* **2011**, 10, 16521-16535.
- Barkatullah .; Ibrar, M.; Naveed, M. Evaluation of *Zanthoxylum armatum* DC for in-vitro and in-vivo pharmacological screening. *African Journal of Pharmacy and Pharmacology.* **2011**, 5(14), 1718-1723.

- Barkathullah, Ibrar, M.; Jelani, G.; Rehamnnullah .; Ehsan, M. Phytosociological and ethnobotanical attributes of *Skimmia laureola* (dc.) siebold. & zucc. ex walp. and *Zanthoxylum armatum* DC. *Int. J. Biosciences*. **2012**, 2,46-56.
- Barkatullah. Pharmacognosy of *Skimmia laureola* (dc.) siebold. & zucc. ex walp. and *Zanthoxylum armatum* dc. Family *Rutaceae*. Ph. D. Theses, **2012**.
- Barkatullah .; Ibrar, M.; Ali,N.; Muhammad, N.; Ehsan, M. In-vitro pharmacological study and preliminary phytochemical profile of *Viola canescens* Wall. Ex Roxb. *African Journal of Pharmacy and Pharmacology*.**2012**, 6(15), 1142 – 1146.
- Barahona, M.V.; Sánchez-Fortún , S. Toxicity of carbamates to the brine shrimp *Artemia salina* and the effect of atropine, BW284c51, iso-OMPA and 2-PAM on carbaryl toxicity.*Env.Pollut*. **1999**, 104, 469-476.
- Barros, I.M.; Lopes, L.D.; Borges, M.O.; Borges, A.C.; Ribeiro, M.N.; Freire, S.M. Anti-inflammatory and anti-nociceptive activities of *Pluchea quitoc* (DC.) ethanolic extract. *J Ethnopharmacol* . **2006**, 106 (3), 317-20.
- Bauer, S.; Singhuber, J.; Seelinger, M. Separation of anti-neoplastic activities by fractionation of a *Pluchea odorata* extract. *Frontiers in Bioscience*. **2011**, 3, 1326–1336.
- Benariba, N.; Djaziri, R.; Bellakhdar, W.; Belkacem, N.; Kadiata, M.; Malaisse, W. J.; Sener, A. Phytochemical screening and free radical scavenging activity of *Citrullus colocynthis* seeds extracts. *Asian Pac J Trop Biomed*. **2013**, 3(1), 35-40.
- Beard, J. L. Iron biology in immune function, muscle metabolism and neuronal functioning. *J. Nutr*. **2001**, 131, 568–579.
- Benkeblia, N. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *Lebensm. Wiss Technol*.**2004**, 37, 263-268.

- Bercu, R.; Făgăraș, M.L.; Broască .Anatomical features of *Aster tripolium* (Asteraceae) to saline environments . *Annals of RSCB*. **2012**, *Xvii* (1), 271-277.
- Bhat, J.U.; Nizami, S. Q.; Parray, M.; Aslam , N.; Fahamiya, A.; Siddiqui, M.; Mujeeb, R.; Khanam, M.; Khan, M. A.Pharmacognostical and phytochemical evaluation of *Melissa parviflora* and HPTLC finger printing of its extracts *J. Nat. Prod. Plant Resour.* **2012**,*2* (1), 198-208.
- Bhagavathi, F.; Burapadja, S.; Bunchoo, A. Antimicrobial activity of Tannins from *Terminalia citrina*. *Planta Medica*. **1999**, *61* (4), 365- 366.
- Bhawna, S.; Kumar, S.U. Hepatoprotective activity of some indigenous plants. *Int. J. Pharm. Tech. res.* **2009**, *4*, 1330-1334.
- Bhowmik, P.C.; Inderjit. Challenges and opportunities in implementing allelopathy for natural weed management. *Crop Protec.* **2003**, *22*, 661-671
- Bhutani, K.K. "Herbal medicines an enigma and challenge to science and directions for new initiatives", *Indian Journal of Natural Products*.**2003**,*19* (1), pp.3-8.
- Biour, M.; Poupon, R.; Grange, J.D.; Chazouilleres, O.; Jaillon, P. Drug induced Hepatotoxicity. Eleventh update of the bibliographic database on liver injuries and responsible drugs). *Gastroenterol. Clin. Biol.* **1998**, *22*, 1004-44.
- Bisht, D.; Gupta, M.S.; Srivastava, B. D.; Rawat, A. K. S. Comparative pharmacognosticevaluation of three species of *Swertia* L. (Gentianaceae). *Pharma. J.* **2011**, (19), 7-12.
- Bishayee, A.; Sarkar, A.; Chatterjee, M . Hepatoprotective activity of carrot (*Daucus carota* L.) against carbon tetrachloride intoxication in mouse liver. *J. Ethnopharmacol.***1995**, *47*(2), 69-74.
- Bonkovsky, H.L.; Kane, R.E.; Jones, D.P.; Galinsky, R.E.; Banner, B. Acute hepatic and renal toxicity from low doses of acetaminophen in the absence of alcohol abuse or malnutrition: evidence for increased susceptibility to drug toxicity

due to cardiopulmonary and renal insufficiency. *Hepatology*. **1994**, 19, 1141-1148.

Borris, R.P. Natural productions research: perspectives from a major pharmaceutical company, *J. Ethnopharmacol.* **1996**, 51, 29-38.

Brady, N. C. The Nature and Properties of Soils. 10th ed. Macmillan Publishing Co. New York, NY; **1990**: 621 p.

Breuer, M.; Hoste, B.; De Loof, A.; Naqvi, S.N.H. Effect of *Melia azedarach* extract on the activity of NADPH-cytochrome c reductase and cholinesterase in insects. *Pestic Biochem Physiol.* **2003**, 76, 99-103.

Bremner, J. M.; Mulvaney, C.S. Nitrogen - Total. In: Methods of Soil Analysis (A. L. Page et al., ed.). Agronomy Monograph 9, Part 2, 2nd ed. American Society of Agronomy, Madison, WI. **1982**: 595-624.

Bundy, D. A. Immunoepidemiology of intestinal helminthic infection I: The global burden of intestinal nematode disease. *Trans Royal Soc Trop Med Hyg.* **1994**, 8, 259-261.

Burdi, D. R.; Hasan, M.; Ahmad, V. U. Fatty acids from *Inula grantioides*. *Pak. J. of Pharmaceutical Sciences.* **1990**, 3(2) 33-37.

Burdi, D.R.; Hasan, M.; Ahmad, V.U. Sterols and a Glycoside from the flowers of *Inula grantioides*. *Pak. J. of Pharm. Sciences.* **1991**, 4(2), 131-136.

Burgess, I.F. Human lice and their control. *Annu Rev Entomol.* **2004**, 49, 475–481.

Burgess, I.F. Current treatments for *Pediculosis capitis*. *Curr Opin Infect Dis.* **2009**, 22, 131–136.

Butt, K.; Yunas, S.; Sheikh, R. M. Hepatoprotective effect of *Cichorium intybus* on paracetamol-induced liver damage in albino rats. *Libyan Agriculture Research Center Journal International.* **2012**, 3 (2), 60-63.

- Buxton, P. A. "The biology of *Pediculus humanus*". *The Louse*; an account of the lice which infest man, their medical importance and control (2nd ed.). London:Edward Arnold.**1947**,pp. 24–72.
- Cai, Y.Z.; Sun, M.; Corke, H. Antioxidant activity of betalains from plants of the *Amaranthaceae*.*J.Agricult. Food. Chem.* **2003**,51(8), 2288–2294.
- Caldas, E. D.; Machado, L. L. Cadmium, mercury and lead in medicinal herbs in Brazil. *Food Chem.Toxicol.***2004**, 42, 599-603
- Carlini, C. R.; Grossi-de Sá, M.F. Plant toxic proteins with insecticidal properties. A review on their potentialities as bio insecticides.*Toxicon.* **2002**, 40, 1515-1539.
- Carron, R. A.; Maran, J. M.; Montero,L.; Fernandozaigo.; Dominguez, A. A. *Plantas Medicinales Phytotherapic*.**1987**, 21, 195-202.
- Cepleanu, F.; Hamburger, M.O.; Sordat, B.; Msonthi, J.D.; Gupta, M.P.; Saadou, M.; Hostettmann, K.Screening of tropical medicinal plants for molluscicidal, larvicidal, fungicidal and cytotoxic activities and brine shrimp toxicity.*Int. J. Pharmacog.***1994**, 32, 294-307.
- Cha, M.R.; Kim, J.Y.; Hwang, J.H. Cytotoxic activity of the *Inula japonica* extracts against several human cancer cell lines in vitro. *Korean J Pharmacogn.***2006**, 37,130–135.
- Chaffey, N. J. Putting plant anatomy in its place. *Trends in Pl. Sci.* **2001**, 6, 439-440.
- Chattopadhyay, R. R. Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract: part II. *J. Ethnopharmacol.* **2003**, 89, 217–219.
- Chaubey, M.K. Insecticidal activity of *Trachyspermum ammi* (Umbelliferae), *Anethum graveolens* (Umbelliferae) and *Nigella sativa* (Ranunculaceae) essential oils against stored-product beetle *Tribolium castaneum* Herbst

- (Coleoptera: Tenebrionidae). *African Journal of Agricultural Research*. **2007**, 2 (11), 596-600.
- Chaurasia, O.P.; Singh, B.; Sareen, S.K. Cold Desert Flora Vol. I, Leh valley, **1996**, Desidoc, Dehli pp. 170.
- Chirikova, N. K.; Olennikov, D. N.; Tankhaeva, L. M. Pharmacognostic Study of Aerial Parts of Baikal Skullcap (*Scutellaria baicalensis* Georgi). *Russ. J. Bioorg. Chem.* **2010**, 36 (7), 909-914.
- Chitravadivu, C.; Manian, S.; Kalaichelvi, K . Qualitative Analysis of Selected Medicinal Plants, Tamilnadu, India. *Middle-East J. Sci. Res.* **2009**, 4 (3), 144-146.
- Cho, J.J.; Cho, C.L.; Kao, C-L.; Chen, C-M.; Tseng, C-N.; Lee, Y-Z.; Liao ,L-J.; Hong, Y-R. Crude aqueous extracts of *Pluchea indica* (L.) Less. inhibit proliferation and migration of cancer cells through induction of p53-dependent cell death. *BMC Complementary and Alternative Medicine* .**2012**, 12, 265.
- Choudhary, G.P.”Anthelmintic activity of *Annona squamosa*.” *Asian Jour. Of Chemistry*.**2007**,19(1), 799-800.
- Choudhary, G.P. “Anthelmintic activity of Fruits of *Embelia ribes* burm” *IJPCS*.**2012**,1(4),1336-1337.
- Ciccia, G.; Coussio, J.; Mongelli, E. Insecticidal activity against *Aedes aegypti* larvae of some medicinal South American plants. *J. Ethnopharmacol.* **2000**, 72, 185-189.
- Claeson, P.; Radstrom, P.; Skold, O.; Nilsson, A.; Hoglund, S. Bactericidal effect of the sesquiterpene t-cadinol on *Staphylococcus aureus*. *Phytother Res.***1992**,6, 94–98.
- Clemens, S.; Palmgren, M. G.; Krämer, U. A long way ahead: understanding and engineering plant metal accumulation. *Tren. in Pl. Sci.* **2002**, 7, 309–315.

- Cohen, Y.; Baider, A.; Ben-Daniel, B.H.; Ben- Daniel, Y. Fungicidal preparations from *Inula viscosa*. *Plant Protection Science*. **2002**, 38, 629–630.
- Coles, G.C. Anthelmintic resistance and the control of worms. *Journal of Medicinal Microbiology*. **1999**, 48, 323-325.
- Couladis, M.; Tzakou, O.; Verykokidou, E.; Harvala , C. Screening of some Greek Aromatic plants for antioxidant activity. *Phytother. Res.* **2003**, 17, 194-195.
- Cowan, M.M. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*. **1999**, 12, 564-582.
- Curtis, J. T.; Cottam, G. Plant ecology works book. Laboratory field reference manual. Burgess publishing co. Minnisota. **1956**.
- Cushnie, T.P.; Lamb, A.J. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents*. **2005**, 26, 343–356.
- Dai, Y.; Cederbaum, A.I. "Cytotoxicity of acetaminophen in human cytochrome P450E1- transfected HepG2 cells". *The Journal of Pharmacology and Experimental Therapeutics*. **1995**, 273(3), 1497– 505.
- Deore, S.L.; Khadabadi, S.S.; Kamdi, K.S.; Ingle, V.P.; Kawalkar, N.G.S.; Patil, U.A. Vyas ,A, *Int. J. Chem. Tech. Res.* **2009**, 2, 177-179.
- DiCarlo, G. N.; Mascolo, A.A.; Izzo .; Capasso, F. Flavonoids: Old and new aspects of a class of natural therapeutic drugs. *Life.Sci.* **1999**, 65, 337-353.
- De Oliveira, S.Q.; Dal-Pizzol, F.; Gosmann, G.; Guillaume, D.; Moreira, J.C.F.; Schenkel, E.P. Antioxidant activity of *Baccharis articulata* extracts: Isolation of a new compound with antioxidant activity. *Free Radic Re.* **2003**, 37, 555-559.
- De Oliveira, S.Q.; Dal-Pizzol, F.; Moreira, J.C.F.; Schenkel, E.P.; Gosmann, G. Antioxidant activity of extracts of *Baccharis spicata*, *Baccharis trimera* and *Baccharis usterii*. *Acta Farm Bonaerense*. **2004**, 23, 365-368.

- Deriu, A.; Zanetti, S.; Sechi, L. A. Antimicrobial activity of *Inula helenium* L. essential Oil against Gram-positive and Gram-negative bacteria and *Candida* spp. *Int. J. of Antimicrobial Agents*. **2008**, 581–592.
- Dilcher, D. L. Approaches to the identification of angiosperm leaf remains. *Bot. Rev.* **1974**, 40,(1), 1–157.
- Dirsch, V. M.; Stuppner, H.; Vollmar, A. M. Cytotoxic sesquiterpene lactones mediate their death -for rapid growth and bioassays with tobacco tissue inducing effect in leukemia T cells by triggering apoptosis. *Planta Med.* **2001**, 67, 557-559.
- Djama, A.A. D.; Goffri, M.C. K.; Koua, A. A.; Ofosu, F.G.; Aboh, I.J.K. Heavy Metal Analysis of Some Anti-Diabetic medicinal plants in Côte d'Ivoire. *Current Research Journal of Biological Sciences*. **2012**, 4(5), 633-637.
- Eguale, T.; Tilahun, G.; Debella, A.; Feleke, A.; Makkonen, E. *Haemonchus contortus* : In vitro and in vivo anthelmintic activity of aqueous and hydroalcoholic extracts of *Hedera helix*. *Exp Parasitol.* **2007**, 52, 99-109.
- El-Ghani, M. M.A.; Amer, W.M. Soil-vegetation relationships in a coastal desert plain of southern Sinai, Egypt. *Journal Arid Environments*. **2003**, 55, 607-628.
- El Garhy, M.F.; Mahmoud, L.H. Anthelmintic efficacy of traditional herbs on *Ascaris lumbricoides*. *J. Egyptian Soc. Parasitol.* **2002**, 32, 893–900.
- Emsley, J. "Chromium". *Nature's Building Blocks: An A-Z Guide to the Elements*. Oxford, England, UK: Oxford University Press. **2001**, 495–498.
- En, Z.; Vasilov, A.; Tsipin, V.; Tillaev, T.; Jumaniyazova, G. Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment. **2003**, 505, 462.

- Erbas, M.; Sekerci, H. Importance of free radicals and occurring during food processing. *Serbest Radikallerin Onemi Ve Gida Isleme Sirasinda Osumu*. **2011**, 36(6), 349-56.
- Erdogrul, O.T. Antibacterial activities of some plant extracts used in folk medicine. *Pharmaceutical Biology*. **2002**, 40(4), 269-273.
- Es-Safi, N.E.; Khlifi, S.; Kerhoas, L.; Kollmann, A.; Abbouyi, A. El.; Ducrot, P. H. Antioxidant constituents of aerial parts of *Globularia alypum* growing in Morocco. *J. Nat.Prod.* **2005**, 68, 1293-1296.
- Evans, W.C. Text book of Pharmacognosy. 14th ed. Saunders; Singapore: Harcourt Brace Asia; pp. 329, 298-299. **1997**.
- Evans, W. C. Plants in medicine: the origins of pharmacognosy. In: Tease and Evans's Pharmacognosy. 15th ed. W. B. Saunders, London, U. K. **2002**, pp: 3 – 4.
- Evans, W. C. Pharmacognosy. 15th ed. English Language Book, Society Baillere Tindall, Oxford University Press. **2002**.
- Evans, C.E.; Bansa, A.; Samuel, O.A. Efficacy of some medicinal plants against *Salmonella typhi*: an in vitro study. *J Ethnopharmacol.* **2002**, 80, 21-24.
- Evan, W.C. Trease and Evans Pharmacognosy. New Delhi: Elsevier Publication, A Division of Reed Elsevier India Private Limited; **2006**. p. 3-4.
- Fabricant, S. D.; Fransworth, R. N. The value of plants used in Traditional Medicine for Drug Discovery. *Environmental Health Perspectives*. **2001**, 109, 69 – 75.
- Fajimi, A.K.; Taiwo, A.A. Herbal remedies in animal parasitic diseases in Nigeria: A review. *Afr. J. Biotechnol.* **2005**, 4(4), 303-307.
- Falagas, M.; Matthaïou, D.; Rafailidis, P.; Panos, G.; Pappas, G. Worldwide prevalence of head lice. *Emerg Infect Dis.* **2008**, 14, 1493– 1494.

- Fatope, M.O.; Ibrahim, H.; Takeda, Y. Screening of higher plant reputed as pesticides using the brine shrimp lethality assay. *Pharm boil.* **1993**, *31*, 250-254.
- Fazal, H.; Ahmad, N.; Khan, M.A. Physico-chemical, phytochemical evaluation and dpph-scavenging antioxidant potential in medicinal plants used for herbal formulation in Pakistan. *Pak. J. Bot.* **2011**, *43*, 63-67.
- Fernández , F.; Torres, M. Evaluation of *Pluchea carolinensis* extracts as antioxidants by the epinephrine oxidation method. *Fitoterapia.* **2006**, *77* (3), 221–226.
- Finkel, T.; Holbrook, N.J. Oxidants, oxidative stress and biology of ageing. *Nature.* **2000**, *408*, 239–247.
- Funk, V. A.; Bayer, R.J.; Keeley, S.; Chan, R.; Watson, L.; Gemeinholzer, B.; Schilling, E.; Panero, J.L.; Baldwin, B.G.; Garcia-Jacas, N.; Susanna, A.; Jansen, R.K. Everywhere but Antarctica: Using a supertree to understand the diversity and distribution of the Compositae. *Biol. Skr.* **2005**, *55*, 343-374. ISSN 0366-3612.
- Funk, V.A.; Susanna, A.; Stuessy, T.F.; Bayer, R.J. Systematics, Evolution and Biogeography of Compositae. IAPT (Vienna. International Association for Plant Taxonomy). **2009**.
- Gathuma, J.M.; Mbaria, J.M.; Wanyama, J.; Kaburia, H.F.; Mpoke, L.; Mwangi, J.N.; Samburu, T. H. Efficacy of *Myrsine africana*, *Albizia anthelmintica* and *Hilderbrandtia sepalosa* herbal remedies against mixed natural sheep helminthosis in Samburu district, Kenya. *J Ethnopharmacol.* **2004**, *91*(1), 7-12.
- Ghosh, T.; Maity, T. K.; Swain, P. K.; Bose, A. Anthelmintic and antimicrobial activity of *Enhydra fluctuans* Lour. aerial part. *Pharmacognosy Magazine.* **2007**, *11* (Suppl.), 204.
- Ghosh, S.; Pramanik, K.C.; Maheswari, U.; Chatterjee, T.K. *Pharmacognosy Magazine* **2008**, *4* (16), p.174.

- Gilani, A.H.; Janbaz, K.H.; Latif, A.; Zaman, M.; Suria, A.; Ahmad, H.R. Possible presence of Ca^{++} channel blockers in *Rubia cardifolia* and idiginous medicinal plants. *J Pak Med Assoc.* **1994**, *44*, 82.
- Gilani, S. A.; Qureshi, R. A.; Gilani, S. J. Indigenous uses of some important ethnomedicinal herbs of Ayubia National Park, Abbottabad, Pakistan. *Ethnobot. Llets.* **2006**, *10*, 285- 293
- Girme, A, S.; Bhalke, R.D.; Ghogare .; Tambe, P.B. *Dhaka University Journal of pharmaceutical sciences.* **2006**, *5(1-2)*, 5-7.
- Githiori, J.B. Evaluation of anthelmintic properties of ethnoveterinary plant preparations used as livestock dewormers by pastoralists and smallholder farmers in Kenya. Doctoral Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden, **2004**.
- Gnanaprakash, K.; Madhusudhana, C.C.; Ramkanth, S.; Alagusundaram, M. Aqueous Extract of *Flacourtia indica* Prevents Carbon Tetrachloride Induced Hepatotoxicity in Rat. *International Journal of Biological and Life Sciences* .**2010**, *6(1)*, 51-55.
- Gnanasekaran, D.; Umamaheswara, R. C.; Jaiprakash, B.; Narayanan, N.; Ravi, K.Y. Elizabeth, H. In vitro hepatoprotective activity of *Inula racemosa* (roots) against CCl_4 induced toxicity. *Int. J. of Res. and Rev. in Pharm. and Applied science.* **2012**, *2(3)*, 578-587.
- Gomes, A.; Saha, A.; Chatterjee, I.; Chakravarty, A.K. Viper and cobra venom neutralization by beta-sitosterol and stigmasterol isolated from the root extract of *Pluchea indica* Less. (Asteraceae) .*Phytomedicine: international journal of phytotherapy and phytopharmacology.* **2007**, *14(9)*, 637-43.
- Gomase, P.V.; Shire, P. S.; Nazim, S.; Choudhari, A. B.; Shaikh, S.; Khairnar, A. Phytochemical evaluation and Analgesic activity of fresh juice of young stem

(tender) bark of *Azadirachta indica*. *Der. Pharmacia. Lettre*. **2011**, 3(1), 407-415.

Gokhele, S. B.; Kokate, C. K.; Purohit, A. P. A text book of Pharmacognosy. Nirali Prakashan, Punne India. **2008** ,pp 12.

Gupta, R.; Gupta , A. K.; Aiswarya, G. Pharmacognostical Investigations on *Acacia leucophloea* Leaf, *Res. J. Pharma. Biol. & Chem.Sci.* **2010**, 1 (4), 360- 365.

Gupta, V.; Bansal, P.; Grag, A.; Meena, A. K . *Int.J.Pharm Clin Res.***2009**, 1(3), 124-126.

Grünwald, S.; Iris, V. A.; Ana-Maria, G.; Ludmila, B.; Michael, B.; Uwe, W. "The Red Flour Beetle *Tribolium castaneum* as a Model to Monitor Food Safety and Functionality". *Adv Biochem Eng Biotechnol.***2013**, 135, 111–122

Halliwell, B.; Gutteridge, J.M.C. Free radicals in biology and medicine. 2nd ed. pp. 481, **1988**, Oxford, Charendon Press.

Halliwell, B.; Gutteridge, J.M.C.The definition and measurement of antioxidant in biologicalsystems.*Free Radic Biol and Med.***1999**, 18(1),125–126.

Halliwell B. "Free radicals and antioxidants: updating a personal view. *Nutrition Reviews.* **2012**, 70(5), 257-65.

Hamayun, M.; Khan, S. A.; Iqbal, I.; Rehman, G.; Hayat, T.; Khan, M. A. Ethnobotanical Profile of Utror and Gabral Valleys, DistrictSwat, Pakistan. *Ethnobotanical Leaflets.* **2005**, 1, Article 9.

Hameed, I.; Dastagir, G.; Hussain, F.Nutritional and elemental analyses of some selectedmedicinal plants of the family Polygonaceae. *Pak. J. Bot.* **2008**, 40 (6), 2493- 2502.

Hammond, J.A.; Fielding , D.; Bishop, S.C.Prospects for plant anthelmintics in tropical veterinary medicine. *Vet. Res. Commun.* **1997**,21, 213-28.

- Han, M.; Wen, J.K.; Zheng, B.; Zhang, D.Q. Acetylbritannilactone suppresses NO and PEG2 synthesis in RAW 264.7 macrophages through the inhibition of NOS and Cox-2 gene expression. *Life Sci.* **2004**, *74*, 675- 684.
- Handa, S.S.; Sharma, A .; Chakraborti, K.K. *Fitoterapia.* **1986**, *57*, 307-351.
- Handral, H.K.; Jha, P. K.; Shruthi, S.D. Pharmacognostic and phytochemical studies on th leaves of *Murraya koenigii*. *Pharmacophore.* **2010**, *1* (3), 231-238
- Haouas, D.; Ben Halima-Kamel, M.; Ben Hamouda, M.H. Insecticidal activity of flower and leaf extracts from *Chrysanthemum* species against *Tribolium confusum*. *Tunisian Journal of Plant Protection.* **2008**, *3*, 87-93.
- Haque, M.; Ullah, M.O.; Nahar, K.; In vitro antibacterial and cytotoxic activities of different parts of plant *Swietenia mahagony*. *Pak J Biol Sci.***2009**, *12* (7), 599-602.
- Haq, I. Medicinal plants. Hamdard Foundation Press, Pakistan. **1983**.
- Harborne, J. B. Phytochemical methods (3rd Edn). Chapman and Hall: New York, **1998**.
- Harman, A.W. The effectiveness of antioxidants in reducing Paracetamol damage subsequent to Paracetamol activation. *Res Commun Chem Pathol Pharmacol.* **1985**, *49*, 215.
- Harshberger, J.W. The purpose of ethnobotany. *Bot. Gaz.* **1896**, *21*, 146-158.
- Hartwell, J.L. Plants used against cancer: a survey. Lawrence, MA. Quarterman Publications, Massachusetts. **1982**, pp 438-439.
- Harwig, J.; Scott, P. Brine shrimp (*Artemia salina* L.) larvae as a screening system for fungal toxins. *Appl Microbiol.* **1971**, *21*, 1011-1016.

- Herrling, T.; Jung, K.; Fuchs, J. "The role of melanin as protector against free radicals in skin and its role as free radical indicator in hair. *Spectrochimica Acta Part A: Molecular & Biomolecular Spectroscopy* . **2008**, 69(5), 1429-35.
- Huang, Z. L.; Failla, M. L. Copper deficiency suppresses effector activities of differentiated U937 cells. *J. Nutr.* **2000**, 130, 1536-1542.
- Handral, H.K.; Jha, P. K.; Shruthi, S.D. Pharmacognostic and phytochemical studies on the leaves of *Murraya koenigii*. *Pharmacophore*. **2010**, 1 (3), 231-238
- Hunt, J.R. Bioavailability of Fe, Zn and other Trace Minerals for Vegetarian Diets. *Am. J. Clin. Nutr.* **1994**, 78, 633-39.
- Hussain, F. Field and Laboratory Manual for Plant Ecology; University Grants Commission: Islamabad, Pakistan, **1989**.
- Hussain, F.; Ahmad, B.; Ilahi, I. Allelopathic Effects of *Cenchrus ciliaris* L. and *Bothriochloa pertusa* (L.) a. Camus. *Pak. J. Bot.* **2010a**, 42(5), 3587-3604.
- Hussain, F.; Hameed, I.; Dastagir, G.; Nisa, S.; Khan, I.; Ahmad, B. Cytotoxicity and Phytotoxicity of some selected medicinal plants of family Polygonaceae. *African Journal of Biotechnology*. **2010b**, 9(5), 770-774.
- Hussain, I.; Khan, F.; Khan, I.; Khan, L. Determination of heavy metals in medicinal plants. *J Chem. Soc. Pak.* **2006**, 28, 347-351.
- Hussain, J.; Rehman, N. U.; Khan, A. L.; Hamayun, M.; Hussain, S. M.; Shinwari, Z. K. Proximate and essential nutrients evaluation of selected vegetables species from Kohat region, Pakistan. *Pak. J. Bot.* **2010**, 42 (4), 2847-2855.
- Hussain, J.; Jamila, N.; Gilani, S. A.; Abbas, G.; Ahmed, S. Platelet aggregation, antiglycation, cytotoxic, phytotoxic and antimicrobial activities of extracts of *Nepeta juncea*. *African Journal of Biotechnology*. **2009**, 8(6), 935-940.

- Hussain, M. S.; Fareed, S.; Ali, M. Preliminary phytochemical and pharmacognostical screening of the Ayurvedic drug *Hygrophila auriculata* (K. Schum) Heine. *Pharmacon Jour.* **2011**, 3 (23), 28-40.
- Ibrar, M.; Ilahi, I.; Hussain, F. The cytotoxic potential of Ivy (*Hedra helix* L.) leave. *Pak.J.Bot.* **2001**, 33, 697-702.
- Ibrar, M.; Ilahi, I.; Hussain, F. Hypoglycemic activity of *Hedera helix* L. leaves and possible mechanism of action. *Pak. J. Bot.* **2003**, 35 (5), 805-809.
- Ibrar, M.; Hussain, F.; Amir, S. Ethnobotanical studies on plant resources of Ranyal Hills. District Shangla, Pakistan. *Pak. J. of Bot.* **2007**, 39(2), 329-337.
- Ibrar, M.; Muhammad, N.; Shah, W.; Barkatullah. Evaluation of trace and toxic heavy metals in selected crude drugs used in Khyber Pukhtonkhawa, Pakistan. *Pak.Bot.* **2013 b**, 45(1), 141-144.
- Ibrar, M.; Naveed, S.; khattak, I.; Ahmad, I. Phytochemical investigation of wild olive leaf nut galls from the district of Karak, KPK, Pakistan. *Topclass Journal of Herbal Medicine.* **2013a**, 2(1), 7-12.
- Igoli, J.O.; Ogaji, O.G.; Tor-anyiin, T.A.; Igoli, N.P. Traditional medicine practice amongst the Igede people of Nigeria. Part II. *Afr. J. Trad.* **2005**, 2, 134-152.
- Ilorkar, V. M.; Khatri, P. K. Phytosociological study of Navegaon National Park (Maharashtra). *Indian Forester* . **2003**, 129, 377-387.
- Ip, S.P.; Poon, M.K.T.; Che, C.T.; Ng, K.H.; Kong, Y.C.; Ko, K.M. Schisandrin B protects against carbon tetrachloride toxicity by enhancing the mitochondrial glutathione redox status in mouse liver. *Free. Radical. Bio. Med.* **1996**, 21, 709-12.
- Indrayan, A.K.; Sharma, S.; Durgapal, D.; Kumar, N.; Kumar, M. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. *Current Sci.* **2005**, 89, 1252-1255.

- Iqbal, H.; Khan, F. A.; Khan, H.; Rehman, S.U.; Badrullah. Antimicrobial, Phytochemical and Fluorescence Studies on *Tribulus terrestris*. *Journal of Pharmacy Research*. **2011**, 4(5), 1556.
- Iqbal, Z.; Lateef, M.; Ashraf, M.; Jabbar, A. Anthelmintic activity of *Artemisia brevifolia* in sheep. *J. Ethnopharmacol.* **2004**, 93, 265–268.
- Iqbal, Z.; Lateef, M.; Jabbar, A.; Muhammad, G.; Khan, M.N. Anthelmintic activity of *Calotropis procera* (Ait.) flowers in sheep. *J. Ethnopharmacol.* **2005**, 102, 256–261.
- Iqbal, Z.; Lateef, M.; Jabbar, A.; Ghayur, M.N.; Gilani, A.H.; In vitro and in vivo anthelmintic activity of *Nicotiana tabacum* L. leaves against gastrointestinal nematodes of sheep. *Phytoth. Res.* **2006a**, 20, 46–48.
- Iqbal, Z.; Lateef, M.; Jabbar, A.; Ghayur, M.N.; Gilani, A.H. In vivo anthelmintic activity of *Butea monosperma* against Trichostrongylid nematodes in sheep. *Fitoterapia*. **2006b**, 77, 137–140.
- Iqbal, Z.; Lateef, M.; Akhtar, M.S.; Ghayur, M.N.; Gilani, A.H. In vivo anthelmintic activity of ginger against gastrointestinal nematodes of sheep. *J. Ethnopharmacol.* **2006c**, 106, 285–287.
- Iqbal, Z.; Lateef, M.; Khan, M.N.; Jabbar, A.; Akhtar, M.S. Anthelmintic activity of *Swertia chirata* against gastrointestinal nematodes of sheep. *Fitoterapia*. **2006d**, 77, 463–465.
- Iqbal, I.; Hamayun, M. Studies on the Traditional Uses of Plants of Malam Jabba Valley, District Swat, Pakistan. *Ethnobotanical Leaflets*. **2005**, 1, Article 32. Available at: <http://opensiuc.lib.siu.edu/ebf/vol2005/iss1/32>.
- Ismail, T. S.; Sulthana, A.P. Standardization of *Morinda umbellata* Linn. - An Effective Crude Drug for Diarrhoea. *E-Journal of Chemistry*. **2008**, 5(3), 652-658.

- Isman, M.B. Leads and prospects for the development of new botanical insecticides in: Roe, R.M. Khur, R.J. (Eds) *Reviews in Pesticide Toxicology* .**1995**, 3, 1-20.
- Isman, M.B. Barriers to the commercialization of new botanical insecticides. Biopesticides for crop protection. In: *Proceedings of the '96 Agricultural Biotechnology Symposium*. Suwon, Korea. **1996**.
- Isman, M.B. Plant essential oils for pest and disease management. *Crop Prot.* **2000**,*19*, 603-608.
- Issac, R.A.; Jonson, W.C. Collaborative study of wet and dry ashing techniques for the metalsal analysis of plant tissue by atomic absorption spectrophotometry. *Journal of American Pharmaceutical Association*. **1975**, 58, 436-440.
- Jabeen, S.; Shah, M.T.; Khan, S.; Hayat, M.Q. Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan. *J. Med.Plants Res.***2010**, 4, 559-566.
- Jackson, M.L. *Soil chemical Analysis*. Constable & Co., Ltd., London. **1962**, p. 406-407.
- Jain, S.; Sharma, C.; Khatri, P.; Jain, A.; Vaidya, A. Pharmacognostic and phytochemical investigations of the leaves of *Zizyphus xylopyrus* (retz) willd .*Int J Pharm Pharm Sci.***2011**,*3*(2), 122-125.
- Jain, V.C.; Shah, D. P.; Sonani, N.G.; Dhakara, S.; Patel, N.M. Pharmacognostical and Preliminary Phytochemical investigation of *Lawsonia inermis* l. leaf. *Rom. J. Biol– Plant Biol.* **2010**, *55*(2), 127–133.
- Jahangir, T.; Khan, T.H.; Prasad, L.; Sultana, S.; *Pluchea lanceolata* attenuates cadmium chloride induced oxidative stress and genotoxicity in Swiss albino mice. *J. Pharm. Pharmacol.* **2005**, *57*(9), 1199-204.

- James, O.; Okafor.; Nnacheta, P. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata* .*Afr. J. of Biotechnology* . **2008**, 7(17), 3129-3133.
- Jan, G.; Khan¹, M.A.; Gul, F.; Ahmad, M.; Jan, M.; Zafar, M. Ethnobotanical study of weeds of Dir Kohistan valley, Khyber Pakhtoonkhwa, Pakistan. *Pak. J. Weed Sci. Res.* **2010**, 16(1), 81-88.
- Januário, A.H.; Santos, S. L.; Marcussi, S.; Mazzi, M. V.; Pietro, R.C.L.R.; Sato, D.N.; Ellena, J.; Sampaio, S. V.; França, S. C.; Soares, A. M. Neo-clerodane diterpenoid, a new metalloprotease snake venom inhibitor from *Baccharis trimera* (Asteraceae): anti-proteolytic and anti-hemorrhagic properties. *Chem. Biol. Interact.* **2004**, 150(3), 243-251.
- Jarald , E.E.; Jarald, S.E. A text book of pharmacognosy and phytochemistry (1st Ed.) CBS Publisher and distributors, New Delhi, India. **2007**.pp. 6.
- Jeyasekar, P.; Mohanan, P.V.; Rathinak, K. Hepatoprotective activity of ethyl acetate extract of *Acacia catechu*. *Indian J. Pharmacol.* **1997**, 29, 426-428.
- Jones, J.W. Determination of Trace Elements in Food by Inductively Coupled Plasma Atomic Emission Spectrometry, Elements in Health and Disease. **1987**.
- Joseph, M.L.; Noe, R.P.; Ilia, A.; Michael, F.H.; Jon, S.T. Enhancing the anticancer properties of cardiac glycosides by neoglycorandomization. *P.N.A.S.* **2005**, 102, 12305–12310.
- Joy, P. P.; Thomas, J.; Mathew, S.; Skaria, B.P. In Bose TK *et al.* (eds). Medicinal Plants. Naya Prokash publishers, Calcutta, Trop. Horticult. **1998**, 2, 449-632.
- Juliani, Jr.; Biuruun, H.R.; Koroch, F.; Oliva, A.R.; Demo, M.M.; Trippi, V.S. *Planta Medica*. **2002**, 762, 68-71.
- Kalidass, C.; Mohan ,V.R.; Sivalingam, R. Pharmacogostical Studies of a multipurpose medicinal plant *Leptadenia reticulata* (RETZ.) WIGHT. & ARN. *Pharmacology* .**2011**, 3, 713-718.

- Kalyan, B. V.; Kothandam, H.; Palaniyappan, V.; Praveen, A. R. Hypoglycaemic Activity of Seed Extract of *Clitoria ternatea* Linn in Streptozotocin- Induced Diabetic Rats. *Pharmacon. J.* **2011**, 3 (19), 45-48.
- Kakar, S.A.; Tareen, R.B.; Kakar, M.A.; Jabeen, H.; Kakar ,S.U.R.; Al-kahraman, Y. M.S.A.; Shafee, M. Screening of antibacterial activity of four medicinal plants of Balochistan-Pakistan . *Pak. J. Bot.***2012**, 44, 245-250.
- Kato, K.; Terao, S.; Shimamoto, N.; Hirata, M. Studies on scavengers of active oxygen Species. 1. Synthesis and biological activity of 2-Oalkylascorbic acids. *J. Med. Chem.***1988**, 31,793-798.
- Khadtatkar, S. N.; Manwar, J. V.; Bhajipale, N. S. In-vitro anthelmintic activity of root of *Clitoria ternatea* Linn. *Phcog Mag.* **2008**,4(13), P-149.
- Khandelwal, K. R. Practical Pharmacognosy, Techniques and experiments (12th Edn). Nirali Prakashan, Punne India. **2004**, pp.157.
- Khan,A.L.; Hussain , J.; Hamayun , M .; Gilani, S.A.; Ahmad , S.; Rehman , G.; Kim Y.H.; Kang, S.M.; Lee, I.J.Secondary Metabolites from *Inula britannica* L. and their biological activities.*Molecules*, **2010**, 15, 1562-1577.
- Khan,H.; Khan, M.A.; Tariq, S.A.; Saeed, M.; Muhammad, N.; Gul, F.; Inayat, H. Analysis of metal content of samples of *Gloriosa superba* L (Colchicaceae) collected from two different locations in Pakistan. *Tropical Journal of Pharmaceutical Research.***2012**, 11(4), 631-636.
- Khan, M.; Musharaf, S.; Shinwari, Z. K.Ethnobotanical importance of halophytes of Noshpho salt mine, District Karak, Pakistan. *Research In Pharmaceutical Biotechnology.***2011**, 3(4), pp. 46-52.
- Khan, M. Dimension and Composition of plant life in Tehsil Takht-e-Nasrati, district Karak, Khyber Pakhtun Khawa, Pakistan. Ph.D. thesis, Department of Botany, University of Peshawar, Khyber Pakhtun Khawa, Pakistan; **2012**.

- Khan, N.; Ahmed, M.; Wahab, M.; Nazim, K.; Ajaib, M. Phytosociology, structure and physiochemical analysis of soil in *Quercus baloot* Griff. Forest District Chitral, Pakistan. *Pak. J. Bot.* **2010**, *42* (4), 2429-2441.
- Khan, S.W.; Khatoon, S. Ethnobotanical studies on some useful herbs of Haramosh and Bugrote valleys in Gilgit, Northern areas of Pakistan. *Pak.J.Bot.* **2008**, *40*, 43-58.
- Khan, S.; Rawat, R.; Rawat, A.K.S.; Shirwaiker, A. A report on the quality control parameters of aerial parts of *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae. *Brazilian Journal of Pharmacognosy*.**2010**, *20*(4), 563-567.
- Khan, T.; Ahmad, M.; Khan, R.; Khan, H.; Choudhary, M.I. Phytotoxic and insecticidal activities of medicinal plants of Pakistan, *Trichodesma indicum*, *Aconitum leave* and *Sauromatum guttatum*. *J.Chem.Soc.Pak.* **2008**, *30*, 251-255.
- Khan, R.U.; Mehmood, S.; Khan, S. U.; Jaffar, F. Ethnobotanical study of food value flora of district Bannu Khyber Pakhtunkhwa, Pakistan. *Journal of Medicinal Plants Studies*. **2013**, *1*(4), 93-106.
- Khattak, S.G.; Gilani, S.N.; Ikram, M. Antipyretic studies on some indigenous Pakistani Plants. *J.Ethnopharmacol.* **1985**, *14*, 45.
- Khoshnoud, H.; Ghiyasi, M.; Amirnia, R.; Fard, S.S.; Tajbakhsh, M.; Salehzadeh, H.; Alahyary, P. *Pakistan Journal of Biological Sciences*.**2008**, *11*(10), 1380-1384.
- Khoshnoud, H.; Khayamy, M. Insecticidal effects of ethanolic extract from *Verbascum cheiranthifolium* Boiss. against two stored –Product insect pests species. *J. of Biological Sciences*, **2008**, *8*(1), 191-195.
- Khuda, F.; Iqbal, Z.; Zakiullah; Khan, A.; Nasir, F.; Muhammad, N.; Khan, J.A.; Khan, M.S. Metal analysis, phytotoxic, insecticidal and cytotoxic activities of selected medicinal plants of Khyber Pakhtunkhwa. *Pak. J. Pharm. Sci.* **2012**, *25*(1), 51-58.

- Khurshid, A.T.; Tantry, M. A. Preliminary Studies on Plants with Anthelmintic Properties in Kashmir—The North-West Temperate Himalayan Region of India. *Chinese Medicine* .**2012**,**3**,106-112.
- Killedar, S. G.; Mahamuni, S. S.; More, H. N.; Gadakari, S. S.; Nale, A. B.; Pawar, A. A. Phytochemical investigation and cytotoxic screening of *Lagenaria siceraria* fruit using brine shrimp lethality assay models. *IJPCBS*. **2012**, *2*(4), 422-426.
- Kim, S.I.; Park, C.; Ohh, M.H.; Cho, H.C.; Ahn, Y.J. Contact and fumigant activities of aromatic plants extracts and essential oils against *Lasioderma serricorne*(Coleoptera: Anobiidae).*Journal of Stored Products Research*. **2003**, *39*, 11-19.
- Kind, P.R.N.; King, E.J. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *J. Clin. Pathol*. **1954**, *7*, 322-326.
- Kinghorn, A. D. The role of pharmacognosy in modern medicine. Informa health care. **2002**, *3*(2), 77-79.
- Kobayashi, T.; Song, Q.H.; Hong, T.; Kitamura, H.; Cyong, J.C. Preventative effects of the flowers of *Inula britannica* on auto immune diabetes in C57BL/KsJ mice induced by multiple low doses of streptozotocin. *Phytother.Res*. **2002**, *16*, 377-82.
- Koe, H.; Sari, H. Trace metal contents of some medicinal, aromatic plants and soil samples in the Mediterranean region, *Turkish J. Appl. Chem. Res.***2009**, *8*, 52-57.
- Kohen, R.; Nyska, A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicologic Pathology*. **2002**, *30*, 620-650.
- Kokate, C. K. Practical Pharmacognosy. 4th Ed. Vallabh Prakashan, New Delhi: **1994**. 120-156.

- Kokate, C.K.; Purhoit, A.P.; Gokhale, S.B. Text book of Pharmacognosy; Nirali Prakashan: New Delhi, Pune: **2006**, vol. 36th, pp. 1
- Koné, W.M.; Vargas, M.; Keiser, J. Anthelmintic activity of medicinal plants used in Côte d'Ivoire for treating parasitic diseases. *Parasitol Res.* **2012**, *110*(6), 2351- 2362.
- Konishi, T.; Shimada, Y.; Nagao, T.; Okabe, H.; Konoshima, T. Antiproliferative sesquiterpenelactones from the roots of *Inula helenium*. *Biol Pharm Bull.* **2002**, *25*(10), 1370-1372.
- Kumar, B. J. R.; Kiladi, C. P. Preliminary Phytochemical and Pharmacognostic Studies of *Holoptelea integrifolia* Roxb. *Ethnobotanical Leaflets.* **2009**, *13*, 1222-1231.
- Kumar, J.A.; Rekha, T.; Devi, S.S.; Kannan, M.; Jaswanth, A.; Gopal, V. Insecticidal activity of ethanolic extract of leaves of *Annona squamosa*. *J. Chem. Pharm. Res.* **2010**, *2*(5), 177-180.
- Kumar, P. Green healers. In Trivedi, C. P. editor: Medicinal plants utilization and conservation. 1st ed. Aavishkar publishers, Jaipur, India. **2004**, 95 – 103.
- Kumar, V.; Pankajkumar, S. Y.; Pratap, S. U.; Bhat, H.R.; Zaman, M, K. Pharmacognostical and Phytochemical study on the leaves of *Paederia foetida* linn. *International Journal of Pharm Tech.* **2009**, *1*(3), 918-920.
- Kumar, V.; Bhat, Z. A.; Kumar, D.; Shah, M. Y.; Chashoo, I. A.; Khan, N. A. Physicochemical and Preliminary Phytochemical Studies on Petals of *Crocus sativus* 'Cashmerianus'. *Pharmacon Jour.* **2011**, *3*(23), 46-49.
- Kumar, Y.V.; Swaha, S.; Arjun, P. Pharmacognostical studies of *Callicarpa macrophylla* Stem. *Int. J. Phytotherapy Research.* **2012**, *2* (1), 35-41.
- Larson, A.M.; Polson, J.; Fontana, R.J. Acetaminophen-induced acute liver failure: results of a United States multi-center, prospective study. *Hepatology.* **2005**, *42*, 1364–1372.

- Lawrence, N.J.; McGown, A.T.; Nduka, J.; Hadf, J.A.; Prtichard, R.G. Cytotoxic Michael-type amine adducts of α -methylene lactones, alantolactone and isoalantolactone. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 429-431.
- Lee, M.; Edman, J.; Mullens, B.; Clark, M.; Transmission potential of the human head louse, *Pediculus capitis* (Anoplura: Pediculidae). *Int J Dermatol.* **2005**, *44*, 811–816.
- Lee, M.T.; Yoon, K.S.; Edman, J.D.; Mullens, B.A.; Clark, J.M. *J. Medical Entomol.* **2003**, *40*(5), 628- 635.
- Lee, S.E.; Peterson, C.J.; Coats, J.R. Fumigation toxicity of monoterpenoids to several stored-product insects. *Journal of Stored Products Research.* **2003**, *39*, 77-85.
- Lev, E.; Amar, Z. Ethnopharmacological survey of traditional drugs sold in Israel at the end of the 20th century. *Journal of Ethnopharmacology.* **2000**, *72*, 191–205.
- Liang, J.; Wang, Q.Q.; Huang, B.L. Concentration of hazardous heavy metals in environmental samples collected in Xiaman, China as determined by vapor generation non-dispersive atomic fluorescence spectrometry. *Analy. Sci.* **2004**, *20*, 85.
- Li, Y.; Trush, M.A. Reactive oxygen dependent DNA damage resulting from the oxidation of phenolic compounds by a copper redox cycle. *Cancer Res.* **1994**, *54*, 1895- 1898.
- Linton, A.H. Antibiotics: assessment of antimicrobial activity and Resistance. (Russell AD and Quernel LB eds). London: Academic Press. **1983**, pp 19-30.
- Lin, L.U.; Shu-wen, L.; Shi-bo, J.; Shu-guang, W. Tannin inhibits HIV-1 entry by targeting gp 41. *Acta Pharmacol Sin.* **2004**, *25*, 213-218.
- Litovitz, T.L.; Klein-Schwartz, W.; Rodgers, G.C. Jr.; Cobaugh, D.J.; Youniss, J.; Omslaer, J.C.; May, M.E.; Woolf, A.D.; Benson, B.E. Annual report of the

- American Association of Poison Control Centers Toxic Exposure Surveillance System. *Am J Emerg Med.* **2002**, 20,391–452.
- Liu , C. H.; Mishra, A.K.; Tan, R.X. Repellent, insecticidal and phytotoxic activities of Isoalantolactone from *Inula racemosa*. *Crop Protection.* **2006**, 25(5), pp. 508–511.
- Liu, S.X.; Liu, H.; Yan, W.; Zhang, L.; Bai, N.S.; Ho, C.T. B. *Med. Chem. Lett.* **2004**, 14, 1101-1104.
- Lokhande, R.; Singare, P.; Andhale, M. Study on Mineral content of Some Ayurvedic Indian Medicinal Plants by Instrumental Neutron Activation Analysis and AAS Techniques. *Health Sc. Jour.* **2010**, 4 (3), 157-168.
- Lukaski, H. C.; Siders, W. A.; Penland, J. G. Chromium picolinate supplementation in women: effects on body weight, composition, and Iron status. *Nutrition.* **2007**, 23, 187- 195.
- Maaz, A.; Bhatti, A.S.A.; Mayam, S.; Afzal, S.; Ahmad, M .; Gilani, A.N. Hepative evaluation of *Butea monosperma* against liver damage by paracetamol in Rabbits. *Special editions annals.* **2010**, 16(1), 73-76.
- Madhavan, V.; Hema, B.; Guru, D.M.R.; Yoganarsimhan, S.N. Pharmacognostical evaluation of *Drosera burmannii* Vahl (Droseraceae). *Indian J. Trad. Knowledge.* **2009**, 8(3), 326-333
- Mahmood, A.; Mahmood, A.; Mahmood, M. In vitro Biological Activities of Most Common Medicinal Plants of Family Solanaceae. *World Applied Sciences Journal.* **2012**, 17(8), 1026-1032.
- Mali, R.G.; Mahajan, S.G.; Mehta, A.A. In-vitro anthelmintic activity of stem bark of *Mimusops elengi* Linn. *Phcog Mag.* **2007**, 3(10), P- 73.
- Malik, Z. H “Phytosociological studies on the vegetation of Kotli.Hills AJ&K”, M.Phil. Thesis, University of Peshawar, Peshawar. **1986**.

- Mally, H.T.; Evelyn ,K.A. Estimation of serum bilirubin level. *J. Biol. Chem.* **1937**, *119*, 481.
- Manipuri, P.; Indala, R.; Jagaralmudi, A.; Kumar, R. Hepatoprotective Effect of *Inula racemosa* on Hepatic Ischemia/ reperfusion Induced Injury in Rats. *J Bio analysis & Biomedicine.* **2013**, *5(2)*, 022-027.
- Mansour-D,H.; Kahlouche-R ,F.; Djerrou, Z.; Serakta-D,M.; Hamimed,S.; Trifa,W.; Djaalab,T.; Hamdi, P.Y. In vitro evaluation of antifungal effects of *Lawsonia inermis*, *Pistacia lenticus* and *Juglans regia*.*Int.J. Med.Arom.Plants.***2012**,*2*,263-268.
- Maoz, M.; Kashman, Y.; Neeman, I. Isolation and identification of a new anti-fungal sesquiterpene lactone from *Inula viscosa*. *Planta Medica.***1999**, *65*, 281–282.
- Maoz, M.; Neeman, I. Effect of *Inula viscosa* extract on chitin synthesis in dermatophytes and *Candida albicans*. *Journal of Ethnopharmacology.***2000**, *71*, 479–482.
- Marielos, P-C. Changes in forest structure and species composition during secondary forest succession in the Bolivian Amazon.*Biotropica* .**2003**, *35(4)*,450-461.
- Markert, B. ‘Plants as biomonitors-Potential advantages and problems’, In: Biogeochemistry of Trace Metalss. (Eds.): D.C. Adriano, Z.S. Chen and S.S. Yang. Science and Technology Letters. Northwood, NY, **1994**. pp. 601-613.
- Marongiu, B.; Piras, A.; Pani, F.; Porcedda, S.; Ballero, M. Extraction, separation and isolation of essential oils from natural matrices by supercritical CO₂. *Flavour and Fragrance Journal.* **2003**, *18*, 505–509.
- Martínez, M.; Ramo, J.D.; Torreblanca, A.; Díaz-Mayans, J. Effect of cadmium exposure on zinc levels in the brine shrimp *Artemia partenogenética*. *Aquaculture.* **1999**,*172*, 315-325.

- Martin, M.; McCorkle, M.C.; Mathias, E. *Ethnoveterinary Medicine. An annotated bibliography of community animal healthcare*. ITDG Publishing, London; **2001**. ISBN: 1853395226, pp. 611.
- Marwah, R.G.; Fatope, M.O.; Mahrooqi, R.A.; Varma, G.B.; Abadi, H.A.; Al-Burtamani, S.K.S. *Food Chemistry*. **2007**, *101*, 465-468.
- Mathers, C.D.; Murray, C.J.L.; Lopez, A.D.; Boschi-Pinto, C. Cancer incidence, mortality and survival by site for 14 regions of the world. Geneva, World Health Organization (GPE Discussion Paper No. 13). **2001**.
- Mathur, J.; Khatri, P.; Samanta, K. C.; Sharma, A.; Mandal, S. Pharmacognostic and preliminary phytochemical investigations of *Amaranthus spinosus* (Linn.) leaves. *Int. J. Pharm. Sci.* **2010**, *2(4)*, 121- 124.
- Matthews, G.A. Insecticide application in the stores. In Matthews, G.A. and Hislop, E.C. (eds.). *Application technology for crop protection*. CAB, London, **1993**, pp. 305-315.
- Mayakrishnan, V.; Veluswamy, S.; Sundaram, K. S.; Kannappan, P.; Abdullah, N. Free radical scavenging potential of *Lagenaria siceraria* (Molina) Standl fruits extract. *Asian Pacific Journal of Tropical Medicine*. **2012**, 20-26.
- Mccarthy, J. Is anthelmintic resistance a threat to the program to eliminate lymphatic filariasis. *Am. J. Trop. Med. Hyg.* **2005**, *73*, 232-233.
- McGrath, S.P.; Smith, S. Chromium and nickel in heavy metals in soils. B.J. Alloway (ed.). Blackie, Glasgow; **1990**: 125.
- McLaughlin, J.L.; Chang, C.J.; Smith, D.L. "Bench-top" bioassays for the discovery of bioactive natural products: an update. In: *Studies in Natural Product Chemistry* (Rahman A eds.). New York: Elsevier Science Publishing Company Inc. **1991**, pp 383-409.
- Mehlhorn, B.; Mehlhorn, H. *Louse alarm*. Düsseldorf University Press, Düsseldorf. **2009**.

- Menzel, U.; Lieth, H. Halophyte Database Vers. 2.0. Halophyte Uses in different climates I: Ecological and Ecophysiological Studies. Progress in Biometeorology, Vol.13. pp. 77-88. Edited by H. Lieth, M. Moschenko, M. Lohman, H. -W. Koyro and A. Hamdy. Backhuys Publishers. The Netherlands. **1999**.
- Meric, C. Calcium oxalate crystals in some species of the tribe Inuleae (Asteraceae). *Acta biologica cracoviensia Series Botanica*. **2009**, 51(1), 105–110.
- Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobson, L. B.; Nichols, D. E.; McLaughlin, J. L. Brine shrimp: a convenient general bioassay for active plants constituents. *Planta Med.* **1982**, 45, 31-34.
- Miller, A.L. Antioxidant Flavonoids: Structure, Function and Clinical Usage. *Alt Med Rev.* **1996**, 1, 103-111.
- Ming, D. S.; Hillhouse, B. J.; Guns, E. S.; Eberding, A.; Xie, S.; Vimalanathan, S.; Towers, G. H. N. For Bioactive compounds from *Rhodiola rosea* (Crassulaceae). *Phytotherapy Res.* **2005**, 19 (9), 740-743.
- Michael, A.S.; Thompson, C.G.; Abramovitz, M. *Artemia salina* as a test organism for a bioassay. *Science*. **1956**, 123, 464
- Mishra, S. B.; Sangameswaran, B.; Prajapati, K.; Singh, D.; Dubey, P. Pharmacognostical and Preliminary Phytochemical Studies of Leaves of *Tridax procumbens* L. *Ethnobotanical Leaflets*. **2008**, 12 (15), 1283-89.
- Moermann, D.E. An analysis of the food plants and drug plants of native North America. *J. Ethnopharmacology*. **1996**, 52, 1-22.
- Morabad, R.B.; Patil, S.J.; Tapash, R.R. First series transition elemental analysis in some therapeutically important medicinal plants by AAS method. *J. Mater. Environ.* **2012**, 4 (2), 171-176.
- Morshed, H.; Islam, M. S.; Parvin, S.; Ahmed, M. U.; Islam, M. S.; Mostofa, M. A. G.; Sayeed, M. S. B. Antimicrobial and Cytotoxic Activity of the Methanol

- Extract of *Paederia foetida* Linn.(Rubiaceae). *Journal of Applied Pharmaceutical Science*. **2012**, 02 (01), 77-80.
- Molyneux, P. The use of the stable free radical diphenyl picrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol.* **2004**, 26(2), 211-219.
- Mongelli, E.; Martino, V.; Coussio, J.; Ciccia, G. Screening of Argentine medicinal plants using the brine shrimp microwell cytotoxicity assay. *Pharm boil.* **1996**, 34, 249-254.
- Mostafa, M.; Hossain, H.; Hossain, M. A.; Biswas, P. K.; Haque, M. Z. Insecticidal activity of plant extracts against *Tribolium castaneum* Herbst. *J. Adv. Sci. Res.* **2012**, 3(3), 80- 84.
- Mullaicharam, A.R.; Al-Matroushi, M.K.M. Comparative in vitro Tests on the Efficacy of Anti-Head-Lice Products Containing Stem Extract of Pyrethrum. *J. of Biomed. and Pharm.Res.* **2012**, 1 (3), 36-38.
- Murti, Y.; Yogi, B.; Pathak, D. Pharmacognostic standardization of leaves of *Calotropis procera* (Ait.) R. Br. (Asclepiadaceae). *Int. J. Ayurveda Res.* **2010**, 1 (1), 14–17.
- Muhammad, N.; Saeed, M. Biological screening of *Viola betonicifolia* Smith whole plant. *African Journal of Pharmacy and Pharmacology*. **2011**, 5(20), pp. 2323-2329,
- Mumcuoglu, K.; Gilead, L.; Ingber, A. New insights in pediculosis and scabies. *Expert Review Dermatol.* **2009**, 4, 285–302.
- Muruhan, S.; Selvaraj, S.; Viswanathan, P. K. In vitro antioxidant activities of *Solanum surattense* leaf extract. *Asian. Pac. J. Trop. Biomed.* **2013**, 3(1), 28-34.

- Nair, R. T.; Kalariy, J.; Chanda, S. Antibacterial activity of some selected Indian medicinal flora. *Turkish J. Biol.* **2005**, 29, 41-47.
- Najafi, S.; Deokule, S. S. Pharmacognostic study of *Tylophora dalzellii* Hook.f. *J. Med. Pl. Res.* **2010**, 4 (5), 403-406.
- Nallamilli, B.R.; Phani, K. Ch.S.; Veer Reddy, K.; Lakshmi, P.M.; Maruthi, V.; Sucharita, P. Hepatoprotective activity of *Cichorium intybus* (Linn.) root extract against carbon tetrachloride induced hepatotoxicity in albino Wistar rats. *Drug Invention Today.* **2013**, 5(4), 311–314.
- Naqvi, S.N.H.; Perveen, F.; Toxicity and Residual effect of *Nerium indicum* crude extract as compared with Coopex against adults of *Tribolium castaneum*. *Pak.J.Entomology.* **991**, 6, 35- 44.
- National Institute of health. Guide for the care and use of laboratory animals. NIH contact no. NOI-RR-2-2135. NIH, Bethesda, MD, pp. 11-28. **1985**.
- Naveed, M.; Saeed, M. Biological screening of *Viola betonicifolia* Smith whole plant. *African Journal of Pharmacy and Pharmacology.* **2011**, 5 (20) 2323-2329.
- Naveed, M.; Saeed, M.; Khan, H.; Qayum, M.; Barkatullah.; Badshah, A. Evaluation of *Viola betonicifolia* for anthelmintic activity. *African Journal of Pharmacy and Pharmacology.* **2012**, 6(10), 698-701.
- Nayak, B.S.; Patel, K. N. Pharmacognostic studies of the *Jatropha curcas* leaves. *Int. J. Pharm. Tech. Res.* **2010**, 2(1), 140-143.
- Neil, P.O. Minor element and environmental problems. *Envir.Chem.* 2nd ed. p 96. **1993**.
- New Wall, C.A.; Anderson, L.A.; Phillipsan, J.D. Herbal medicines- A guide for healthcare of Clinical Pathology. The Pharmaceutical Press, London, **1996**, 45, 493-496.

- Newman, D.J.; Cragg, G.M.; Snader, K.M. The influence of natural products upon drug discovery. *Nat Prod Rep* .**2000**, 17, 215–234.
- Nijveldt, R.J.E.; Van Nood ,D.E.C.; Van Hoorn, P.G.; Boelens, K.; Van Norren.; Van Leeuwen, P.A.M.Flavonoids: a review of probable mechanisms of action and potential applications. *Am J Clin Nutr*. **2001**, 74, 418–25.
- Nikomtat, J.; Meepowpan, P.; Tragoolpua, Y.Inhibition of *Inula cappa* (Ham. ex D. Don) DC. extracts on herpes simplex virus infection in vitro. *African J. of Microbiol. Res.***2011**, 5(24), 4049-4058.
- Niranjan, R. M.; Kanaki, S. Phytochemical Standardization of Herbal Drugs and Polyherbal Formulations. *Bioactive Molecules and Medicinal Plants*.**2008**, pp. 349-369.
- Nirmal, S.A.; Malwadkar, G.; Laware, R.B. ‘Anthelmintic activity of *Pongamia glabra*’ ; *Songklanakarin J. Sci. Technol*. **2007**,29 (3), 755-757.
- Nisha, M.C.; Subramanian, M.S.; Prathyusha, P.; Santhanakrishnan, R. Comparative studies on antimicrobial activity of *Artemisia sieversiana* Ehrhart. Ex. Willd., and *Origanum vulgare* L. *Int. J. of Pharm. Tech Research*.**2010**, 2(2), 1124-1127.
- Nose, K. Role of reactive oxygen species in regulation of physiological functions. *Biol Pharmaceut Bull*. **2000**, 22, 897–903.
- Obayedullah , M.; Mahmuda, H.; Kaniz, F. U.; Hasanat, A.; Zulfiker, M.; Elichea, S. A.; Hamid, M. B. K.Anti-bacterial activity and brine shrimp lethality bioassay of methanolic extracts of fourteen different edible vegetables from Bangladesh. *Asian. Pac. J. Trop.Biomed*.**2013**,3(1), 1-7.
- Oka, Y.; Ben-Daniel, B.H.; Cohen, Y. Nematicidal activity of powder and extracts of *Inula viscosa*. *Nematology*.**2001**, 3, 735–742.

- Okoro, I.O.; Osagie, A.; Asibor, E.O. Antioxidant and antimicrobial activities of polyphenols from ethnomedicinal plants of Nigeria. *Afr. J. Biotechnol.* **2010**, *9*, 2989-2993.
- Oslen, S. R.; Sommers, L.E. Phosphorus. In: *Methods of Soil Analysis, Part 2*(2nd Ed.), Madison, WI, USA, pp.406-407. **1982**.
- Ozcan, M. Mineral composition of different parts of *Capparis ovata* Desf. var. *canescens* (Coss.) Heywood growing wild in Turkey. *J. Med. Food.* **2005**, *8* (3), 405- 407.
- Pandey, M.; Abidi, A.B.; Singh, S.; Singh, R.P. Nutritional Evaluation of Leafy Vegetable Paratha. *J. Hum. Ecol.* **2006**, *19* (2), 155-156.
- Patel, R.; Buckton, G.; Rawlins, D.A.; Storey, D.E. The physico-chemical basis of enhanced drug absorption: Calorimetric studies of drug/surfactant interactions. *Journal of Pharmacy and Pharmacology* . **1998**, Vol. 50(S9), 56.
- Patel, K.; Kumar, N.; Sharma, N.K. Pharmacognostic & Phytochemical Evaluation of *Bryophyllum Pinnatum* Leaves. *J. Adv. Sci. Res.* **2011**, *21*, 42-49.
- Patel, P.M.; Patel, N.M.; Goyal, R.K. "Quality control of herbal products", *The Indian Pharmacist*. **2006**, *5*(45), pp.26-30.
- Pavela, R. Insecticidal activity of certain medicinal plants. *Fitoterapia*. **2004**, *75*(7-8), 745-749.
- Pendias, A.K.; Pendias, H. Trace Elements in Soils and Plants. 2nd ed. Boca Raton FL: CRC Press, **1992**. p. 365.
- Perkin, E. AY-5 Analysis of plant tissue wet digestion. Operational manual: Atomic Absorption Spectrophotometer. Analytical Instrument, Perkin Elmer Corp. USA, **1991**, 141-142.
- Perez, R.M.G.; Vargas, R.S.; Martinez, F.J.M.; Cordova, I.R. Antioxidant and free radical veronicaefolia. *Phytother. Res.* **2004**, *18*, 428–430.

- Perveen, A.; Abid, R.; Fatima, R. Stomatal types of some dicots within flora of Karachi, Pakistan. *Pak. J. Bot.* **2007**, *39*(4), 1017-1023.
- Perveen, A.; Sarwar, G. R.; Hussain, I. Plant biodiversity and phytosociological attributes of Dureji (Khirthar Range). *Pak. J. Bot.* **2007**, *40* (1), 17-24.
- Peter, A. Edde. A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. *Journal of Stored Products Research*. **2012**, *48*, 1-18.
- Pezzuto, J.M. Plant-derived anticancer agents. *Biochem. Pharmacol.* **1997**, *53*, 121-133.
- Pramanik, K. Ch.; Bhattacharya, P.; Biswas, R.; Bandyopadhyay, D.; Mishra, M.; Chatterjee, T.K. Hypoglycemic and antihyperglycemic activity of leaf extract of *Pluchea indica* Less. *Oriental Pharmacy and Experimental Medicine*. **2006**, *6*(3), 232-236.
- Pramanik, K. C.; Biswas, R.; Mitra, A.; Bandyopadhyay, D.; Mishra, M.; Chatterjee, T. K. culture of the plant *Pluchea indica* (L.) Less. and evaluation of diuretic potential of its leaves. *Oriental Pharmacy and Experimental Medicine*. **2007**, *7*(2), 197-204.
- Pramanik, K.C.; Chatterjee, T. K. . *Pharmacognosy Magazine*. **2008**, *4*(14), 78-84.
- Price, N. C.; Stevens, L. Fundamental of Enzymology, 3rd ed. Oxford University Press, Oxford. **2003**; Pp. 404 – 406.
- Prochezian, E.; Ansari, S.H. Hepatoprotective activity of *Abutilon indicum* on experimental liver damage in rats. *Phytomed*. **2005**, *12*, 62-64.
- Pytlakowska, K.; Kita, A.; Janoska, P.; Połowniak, M.; Kozik, V. Multi-element analysis Of mineral and trace elements in medicinal herbs and their infusions. *Food Chem.* **2012**, *135*(2), 494-501.
- Qaiser, M.; Abid, R. Contribution to *Asteraceae* from Pakistan and adjoining areas- Some new taxa, Combination and new records. *Pak. J. Bot.* **2002**, *34*(4), 367-373.

- Qaiser, M.; Abid, R.; Flora of Pakistan. Asteraceae (II) Inuleae, Plucheeae and Gnaphalieae; Editors, S.I. Ali and M. Qaiser., Eds.; Department of Botany, University of Karachi and Missouri Botanical Press. Missouri Botanical Garden St.Louis, Missouri, U.S. A, **2003**, 210, 1-215.
- Qaiser, M.; Abid, R. Distribution pattern of *Inula* L. (S.Str.) and its allied genera from Pakistan and Kashmir. *Pak. J. Bot.* **2005**, 37(3), 551- 558.
- Qureshi, R. U. Vegetation assessment of sawan wari of Nara desert, Pakistan. *Pak. J. Bot.* **2008**, 40(5), 1885-1895.
- Qureshi, R. Medicinal flora of Hingol National Park, Baluchistan, Pakistan. *Pak. J. Bot.* **2012**, 44(2), 725-732.
- Rabotnov, T.A. Phytocoenology. In: The Great Soviet Encyclopedia, 3rd ed. **1970**.
- Rachid, J.; Ennabili, A.; Sayah, F. Insecticidal activity of four medicinal plant extracts against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *African Journal of Biotechnology*. **2006**, 5(10), 936-940.
- Rafi, M.M.; Bai, N.S.; Ho, C.T.; Rosen, R.T.; White, E.; Perez, D.; Di Paola, R.S. A sesquiterpene lactone from *Inula britannica* induces antitumor effects dependent on Bcl-2 phosphorylation. *Anticancer Res.* **2005**, 25, 313-318.
- Rahman, M. M.; Sayeed, M. A.; Biplab, K. P.; Siddique, S. A. Antidiabetic and cytotoxic activities of methanolic extract of *Tabernaemontana divaricata* (L.) leaves in alloxan induced mice. *Asian J Pharm Clin Res.* **2011**, 5(1), 49-52.
- Raghuvanshi, V. S.; Singh, J.K.; Singh, M.; Paul, R.; Rana, K.S. Insecticidal activity of *Spilanthes acmella* Murr. against *Chilo partellus* (Swinhoe.). International Society for Zoological Research (ISZR). *International Journal of Entomology*. **2010**, (1), 13-18.
- Rajeswari, D. V.; Gajalakshmi, S.; Jayanthi, P.; Vijayalakshmi, S. Pharmacological Activities of *Dodonaea viscosa*: A Perspective Review. *Journal of Pharmacy Research*. **2011**, 4(10), 3331-3333.

- Ramachandran, S.; Vamsikrishna, M.; Gowthami, K.V.; Heera, B.; Dhanaraju, M.D. *Asian Journal of Scientific Research* . **2011**,4(1), 90-94,
- Rangari, V.D. Pharmacognosy and phytochemistry. 1st Edn., New Delhi: Carrier Publication; **2002**. p.100-101.
- Rashid, R.; Farah, M.; Mirza, M.N. Biological screening of *Salvia cabulica*. *Pak. J. Bot.* **2009**, 41(3), 1453-1462.
- Rauf, A.; Muhammad, N.; Khan, A.; Uddin, N.; Atif, M.; Barkatullah. Antibacterial and Phytotoxic Profile of Selected Pakistani Medicinal Plants. *World Applied Sciences Journal*. **2012**, (4), 540-544.
- Raunkiaer, C. The life form of plants and statistical plants Geography being the collected Papers of C.Raunkiaer. Clarendon press, Oxford. **1934**.
- Rayan, J.N.; Harvey, R.W.; Metge, D.W.; Larson, J.E. Transport of bacteriophage PRDI and silica colloids in a sewage-contaminated aquifer. Eos, Transtion of the American Geophysical Union 86, F231. Presented at the Fall Meeting of the American Geophysical Union. **1997**.
- Reddy, M.; Chaturvedi, A.A. Pharmacognostical studies of *Hymenodictyon orixence* (Roxb.) Mabb. leaf. *Int. J. Ayurveda Res.* **2010**, 1(2), 103-5.
- Reitman, S.; Frankel, S. A calorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. *America Journal of Clinical Pathology*. **1957**, 28, 56-62.
- Ribeiro, B.M.; Guedes, R.N.C.; Oliveira, E.E.; Santos, J.P. Insecticide resistance and synergism in Brazilian populations of *Sitophilu zeamais* (Coleoptera: Curculionidae). *J. Stored Prod. Res.* **2003**, 39, 21-31.
- Rehmanullah.; Ibrar, M.; Shah, S.; Hameed, I. Phytotoxic, cytotoxic and insecticidal activities of *Calendula arvensis* L. *E3 Journal of Biotechnology and Pharmaceutical Research* . **2012**, 3(6), 104-111.

- Rhoades, J.D. Soluble salts. In: Methods of Soil Analysis, Part 2 (2nd ed.), Madison, WI, USA, pp.169-173. **1982**.
- Roeder, E.; Bourauel, T.; Meier, U.; Wiedenfeld, H. Diterpene glycoside from *Iphiona aucheri*. *Phytochemistry*. **1994**, 37(2), 353-5.
- Rojas, R.; Bustamante, B.; Bauer, J.; Fernández, I.; Albán, J.; Lock, O. Antimicrobial activity of selected Peruvian medicinal plants. *J Ethnopharmacol*. **2003**, 88, 199-204.
- Roslida, A.H.; Erazuliana, A. K.; Zurain, A. Anti-Inflammatory and antinociceptive activities of the ethanolic extract of *Pluchea indica* (L) LESS leaf. *Pharmacologyonline*. **2008**, 2, 349-360.
- Rouhi, A.M. Betting on natural products for cures. *Chem. Eng. News*. **2003**, 81, 93-103.
- Rozman, V.; Kalinovic, I.; Korunic, Z. Toxicity of naturally occurring compounds of *Lamiaceae* and *Lauraceae* to three stored-product insects. *Journal of Stored Products Research*. **2007**, 43, 349-355.
- Saba, N.; Khatoon, R.; Ahmad, V.U.; Khan, S.S. *Nat. Prod. Commun.* **2011**, 6(1), 1-2.
- Saeed, A.E.M.; Bashir, R.S.M. Physiochemical analysis of *Ximenia americana* L. seed oil and Structure elucidation of some chemical constitution of its seed oil and fruit pulp. *Jour. Pharmco. & Phytoth.* **2010**, 2(4), 49-55.
- Saeed, M.; Khan, H.; Khan, M.A.; Khan, F.U.; Khan, S.A.; Muhammad, N. Quantification of various metals accumulation and cytotoxic profile of aerial parts of *Polygonatum verticillatum*. *Pak. J. Bot.* **2010a**, 42(6), 3995-4002.
- Saeed, M.; Muhammad, N.; Khan, H.; Khan, S.A. Analysis of toxic heavy metal in branded Pakistani herbal products. *Journal Chemistry Society Pakistan*. **2010b**, 32, 471-475.

- Saeed, M.; Muhammad, N.; Khan, H. Assessment of heavy metal content of branded Pakistani herbal products', *Tropical Journal of Pharmaceutical Research*. **2011**, 10(4), 499-506.
- Sahito, S.R.; Memon, M.A.; Kazi, T.G.; Kazi, G.H. Evaluation of mineral contents in medicinal plant *Azadirachta indica* (neem). *J.Chem.Soc. Pak.* **2003**, 25 (2), 139-143.
- Sailor, G.U.; Dudhrejiya, A.V.; Seth, A. K.; Maheshwari, R. ; Shah, N.; Aundhia, C. Hepatoprotective effect of *Leucas cephalotes* spreng on CCl₄ induced liver damaged in rats. *Pharmacologyonline*. **2010**, 1, 30-38.
- Sala, A.; Recio, M.D.; Giner, R.M.; Manez, S.; Tournier, H.; Schinella, G.; Rios, J.L. Anti-inflammatory and antioxidant properties of *Helichrysum italicum*. *J Pharm and Pharmacol.* **2002**, 54(3), 365–371.
- Saleem, T.S.M.; Chetty, C.M.; Ramkanth, S.; Rajan, V.S.T.; Kumar, K.M.; Gauthaman, K. Hepatoprotective herbs - a review. *International Journal of Research in Pharmaceutical Sciences*. **2010**, 1, 1-5.
- Samuelsson, G.; Drugs of Natural Origin - A Textbook of Pharmacognosy; Swedish Pharmaceutical Society, Swedish pharmaceutical Press: Stockholm, Sweden, **2004**; Rev. Ed.
- Sa,nchez-Moreno, C.; Larrauri, J.A.; Saura-Calixto, F. New parameter for evaluation of free radical scavenging capacity of polyphenols, 2nd International Electronic Conference on Synthetic Organic Chemistry (ESCOC-2), <http://www.mdpi.org/escoc/>, Sep. 1-30, **1998** [dp130]; http://ecsoc2.hcc.ru/DP_TOP1/dp130/dp130.htm
- Santos, F.A.; Bastos, E.M.A.; Uzeda, M.; Carvalho, M.A.R.; Farias, L.M.; Moreira, E.S.A.; Braga, F.C. Antibacterial activity of *Brazilian propolis* and fractions against oral anaerobic bacteria. *Journal of Ethnopharmacology*. **2002**, 80, 1-7.
- Sardar, A. A.; Khan, Z. Ethnomedicinal studies on plant resources of Tehsil Shakargarh, District Narowal, Pakistan. *Pak. J. Bot.* **2009**, 41(1), 11-18.

- Sarker, S.D. Pharmacognosy in modern pharmacy curricula. *Phcog. Mag.***2012**,*8*, 91-92.
- Schinella, G.R.; Tournier, H.A.; Prieto, J.M.; Mordujovich, P.; Rios, J.L. Antioxidant activity of anti-inflammatory plant extracts. *Life Sci.* **2002**, *70*, 1023–1033.
- Schultes, R.E. The kingdom of plants, In W.A.R. Thomson (ed.), *Medicines from the earth*. Mc Graw-Hill Book Co., New York, N.Y. **1978**, p. 208.
- Schultes, R.E. Ethnobotany and technology in the Northwest Amazon: A partnership. In *Sustainable harvested and marketing of rain forest products*, Eds. Plotkin and Famolare, Island Press, CA, **1992**, pp: 45-76.
- Seeff, L.B.; Lindsay, K.L.; Bacon, B.R.; Kresina, T.F.; Hoofnagle, J.H. Complementary and alternative medicine in chronic liver disease. *Hepatology.* **2001**, *34*, 595-603.
- Sen, T.; Dhara, A.K.; Bhattacharjee, S.; Pal, S.; Chaudhuri, A.K. Antioxidant activity of the methanol fraction of *Pluchea indica* root extract. *Phytother. Res.***2002**, *16*, 331-335.
- Shafeek, A.; Prasanthi, R. P. J.; Reddy, G. K.; Chetty, G. S.; Reddy, G. R. Alterations in acetylcholinesterase and electrical activity in the nervous system of cockroach exposed to the neem derivative, azadirachtin. *Ecotoxicology and Environmental Safety.* **2004**, *59*, 205 - 208.
- Shah, B. N.; Shah, A. K.; Desai, R.V. Phytopharmacological profile of *Lagenaria siceraria*: A review. *Asi. J. Pl. Sci.* **2010**, *9*(3), 152-157.
- Saha, D.; Pahari, S.K.; Maity, T.; Sur, D.; Kayal, S.; Ghindora, G.L.; Dhirehe, U.K. Pharmacognostic Studies of Leaf of *Lippia alba*. *Asian J. Pharm. Res.* **2011**, *1*(1), 17-18.
- Sharaby, A.; Rahman, H. A.; Moawad, S. Biological effects of some natural and chemical compound on the potato tuber moth, *Phthorimaea operculella* Zell. (Lepidoptera: Gelechiidae). *Saudi. J. Biol. Sci.* **2009**, *16*(1), 1–9.

- Sharma, C. M.; Kumar, A. Community structure of some Natural forest stands in landowne forest range of Garhwal Himalaya. *J. Trop. For. Sci.* **1991**, 5 (1), 8-12.
- Sharma, K.R.; Agrawal, M.; Marshall, M.F. Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. *Food Chem. Toxicol.* **2009**, 47, 583-591.
- Sharma, O. P.; Bhat, T. K. DPPH antioxidant assay revisited Food Chemistry. **2009**, 113(4), 1202–1205.
- Sharma, S.; Khatri, P.; Samanta, K. C.; Sharma, A.; Panday, A.; Jakhetia, V.; Agarwal, P. Standardization of *Morus alba* stem bark. *J. Med. & Arom. Pl. Sci.* **2010**, 28, 31-36.
- Sharma, S. K.; Ali, M.; Gupta, J. *Phytochemistry and Pharmacology*. **2002**, 2, 253-270.
- Sharma, S.K. Recent approach to herbal formulation development and standardization. **2004**. <http://pharmainfo.net>.
- Sharma, S.; Lakshmi, K. S.; Patidar, A.; Chaudhary, A.; Dhaker, S. Studies on anti-inflammatory effect of aqueous extract of leaves of *Holoptelea integrifolia*, Planch. in rats. *Ind. J. Pharm.* **2009**, 41 (2), 87-88.
- Shivani, J.; Devendra, M.; Khetwal, K.S.; Ganga, B. Antimicrobial Evaluation of *Buddleja. asiatica* Lour. Leaves and Flowers Extract. *Research Journal of Phytochemistry*. **2012**, 6, 92-95.
- Shruthi, S. D.; Ramachandra, Y. L.; Rai, S.P.; Jha, P.K. Pharmacognostic evaluation of the leaves of *Kirganelia reticulate* Bail. *The Asian and Aust. J. Plant Sci. Biotec.* **2010**, 4 (1), 62-65.
- Sheded, G.M.; Pulford, I.D.; Hamed, I.A. Presence of major and trace elements in seven medicinal plants growing in the South-Eastern Desert. *Egypt. J. Arid Env.* **2006**, 66, 210- 217.

- Sher, Z.; Hussain, F.; Ibrar, M. Traditional knowledge on plant resources of Ashezai and Salarzai Valleys, District Buner, Pakistan. *African Journal of Plant Science*. **2014**, *8*(1), pp. 42-53.
- Shin, K.H. Hepatic drug metabolizing enzyme inhibitors from herbal medicines. *Procend Int. Symp Rece Adv Natu Prod Res*, **1989**; 176.
- Shinwari, Z. K.; Watanabe, T.; Rehman, M.; Yoshikawa, T. A pictorial guide to medicinal plants of Pakistan. Published by KUST, Kohat. **2006**, pp. 400.
- Shirahata, S.; Murakami, H.; Nishiyama, K.; Sugata, I.; Shinohara, K.; Nonaka, G.; Nishioka, I.; Omura, H. DNA breakage by hydrolyzable tannins in the presence of cupric ions. *Agric Biol Chem*. **1985**, *49* (4), 1033-1040.
- Shirin, K.; Imad, S.; Shafiq, S.; Fatima, K. Determination of major and trace elements in the indigenous medicinal plant *Withania somnifera* and their possible correlation with therapeutic activity. *J. Saudi Chem. Soc.* **2010**, *14*, 97-100.
- Shrikumar, S.; Maheshwari, U.; Sughanti, A.; Ravi, T.K. WHO guidelines for standardization of herbal drugs. *Pharminfo.net*. **2006**, *2*, 78-81.
- Shrivastava, S.; Leelavathi, S. Preliminary phytochemical evaluation of leaf extracts of *Catunaregum spinosa* Thumb. *Int. J. Pharma. Sci. Rev. & Res.* **2010**, *3*(2), 114-118.
- Siddiqui, M. F.; Ahmed, M.; Wahab, M.; Khan, N.; Uzair, K. M.; Nazim, K.; Hussain, S. S. Phytosociology of *Pinus roxburghii* Sargent. (chir pine) in lesser Himalayan and Hindu Kush range of Pakistan. *Pak. J. Bot.* **2009**, *41* (5), 2357-2369.
- Sindhu, R. K.; Vasudeva, N.S.; Sharma, K. Pharmacognostical and preliminary phytochemical investigations on *Verbesina encelioides* roots. *Journal of Herbal Medicine and Toxicology*. **2010**, *4*, 113-118.

- Sindhu, R. K.; Sandeep, A. .Phytochemical and Pharmacognostical Studies on *Murraya koenigii* (L.)spreng. Roots .*Drug Invention Today*, **2012**, 4(1), 325-333.
- Singh, D.; Gopal, G.; Vishal, G. In vitro screening of *Mucuna pruriens* seeds extract for antilice activity. *Pharma and Bio Sciences*. **2011**, 2(3), 107-110.
- Singhal, A. K.; Bhati ,V. S.; Kumar, S. V. Pharmacognostic study of aerial aarts of plant *Geniosporum prostratum* (L.) Benth. *J. Sci. Specul. & Res*. **2010**, 1 (1), 19-24.
- Sinha, R. K.; Sinha, S. Ethnobiology; Role of Indigenous and Ethnic Societies in Biodiversityconservation, Human Health Protection and Sustainable Development ; Surabhi: Jaipur, **2001**,pp. 335.
- Sleet, R.B.; Brendel, K.Improved methods for harvesting a synchronous populations of *Artemia naup* for use in developmental toxicology. *Ecotoxicol. Env. Safety*. **1983**, 7,435-446.
- Smith, K.A. 1990. Manganese and cobalt in heavy metals in soils. In B.J. Alloway (ed.). Blakie, Glasgow, p.197.
- Soderberg, T.; Halimans, G.Treatment of leprosy with adhesive Zinc tapes. *Lep. Rev.***1982**, 53(4), 271.pp.
- Solís, P.N.; Wright, C.W.; Anderson, M. M.; Gupta, M.P.; Phillipson, J.D. A microwell cytotoxicity assay using *Artemia salina*. *Plant. Med*. **1993**, 59,250-252.
- Somer, E. The toxic potential of trace metalsin foods: A review. *J. Food Sci.***1983**, 39, 215- 217.
- Song, Q.H.; Kobayashi, T.; Iijima, K.; Hong, T.; Cyong, J.C. Hepatoprotective effects of *Inula britannica* on hepatic injury in mice. *Phytother. Res.***2000**, 14, 180-186.

- Song, Q.H.; Kobayashi, T.; Hong, T.; Cyong, J.C. Effects of *Inula britannica* on the production of antibodies and cytokines and on T cell differentiation in C57BL/6 mice immunized by ovalbumin. *Am. J. Chin. Med.* **2002**, *30*, 297-305.
- Sparg, S.G.; Light, M.E.; Staden, J. Biological activities and distribution of plant saponins. *J Ethnopharmacol.* **2004**, *29*, 219-243.
- Springfield, E.P.; Eagles, P.K.F.; Scott, G. Quality assessment of South African Herbal Medicines by means of HPLC fingerprinting. *J. Ethnobotany.* **2005**, *101(1-3)*, 75-83.
- Srirama, R.; Ramesha, B. T.; Ravikanth, G.; Shaanker, R.; Ganeshaiah, K. N. Are plants with anti-cancer activity resistant to crown gall? A test of hypothesis. *Current Sci.* **2007**, *95 (10)*, 99-106.
- Srinivasan, D.; Nathan, S.; Suresh, T.; Perumalsamy, P.L. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J Ethnopharmacol.* **2001**, *74*, 217- 220.
- Srivastava, J., Lambert, J., and Vietmeyer, N. "Medicinal Plants: An Expanding Role in Development". World Bank Technical Paper. No. 320. **1996**.
- Srivastava, P.; Shanker, K. *Pluchea lanceolata* (Rasana): Chemical and biological potential of Rasayana herb used in traditional system of medicine. *Fitoterapia.* **2012**, *83(8)*, 1371-1385.
- Stewart, J.; Michael.; Steenkamp.; Vanessa. The biochemistry and toxicity of atractyloside. review. *Therapeutic Drug Monitoring.* **2000**, *22(6)*, 614-649.
- Stibick, J. New Pest Response Guidelines: Khapra Beetle. USDA-APHIS-PPQ- Emergency and Domestic Programs, Riverdale, Maryland. **2007**.
- Stone, W. C.; Frayer, J. R. A. "A botanical study of pasture mixtures," *Sci. Aric.* **1935**, *15*, 777- 805.

- Subha, T. S.; Gnanamani, A.; Mandal, A. B. Pharmacognostic evaluation of *Acorus calamus* L. *Pharmacon J.* **2011**, 3(23), 24-27.
- Sugumaran, M.; Vetrichelvan, T. "Studies on Some Pharmacognostic Profiles of *Bauhinia purpurea* Linn. Leaves (Caesalpinaceae)," *Ethnobotanical Leaflets.* **2008**, 1, Article 56.
- Suliman, F.E.O.; Fatope, M.O.; Al-saidi, S.H.; Al-Kindy, S.M.Z.; Marwah, R.G. Composition and antimicrobial activity of the essential oil of *Pluchea arabica* from Oman. *Flavour Fragr J.* **2006**, 21, 469-471.
- Sultan, J.I.; Rahim, I.U.; Javaid, A.; Bilal, M.Q.; Akhtar, P.; Ali, S. Chemical composition, mineral profile, palatability and In vitro digestibility of shrubs. *Pak. J. Bot.* **2010**, 42(4), 2453-2459.
- Sunilson, A.; Samuel, J.J.; Radhamani, S.; Gopinath, R.; Kalusalingam, A.; Kumari, A.G.; Vimala, A.; Husain, H.J.A. In Vitro Screening of Anti-lice Activity of *Pongamia pinnata* Leaves. *Korean J. Parasitol.* **2009**, 47(4), 377-380.
- Surendra, K. M.; Reshma, M.R.; Nusrath, C.; Pulikal, D.A.; Sabeer, P.A.; Babu, G. In vitro Screening of *Myristica fragrans* Houtt Fruit Pulp for Anti-Lice Activity. *Res. J. of Pharm. Bio. and Chem. Sci.* **2013**, 4(2), 562-566.
- Sureshkumar, S.V.; Mishra, S.H. Hepatoprotective activity of extracts from *Pergularia daemia* Forsk. against carbon tetrachloride induced toxicity in rats. *Pharmacognosy Magazine.* **2007**, 3(11), 187-191.
- Syahriel, A.; Gobilik, J.; Chong, K.P. Preliminary phytochemical study and antimicrobial activity from various extract of *Cynodon dactylon* (L.) Pers. (Bermuda) against selected pathogens. *Int. J. Pharmacy and Pharmaceutical Sciences.* **2012**, 4(5), 227-230.
- Sydor, A. M.; Zambie, D. B. "Chapter 11 Nickel Metallomics: General Themes Guiding Nickel Homeostasis". In Banci, Lucia (Ed.). *Metallomics and the Cell. Metal Ions in Life Sciences* 12. Springer. **2013**. doi:10.1007/978-94-007-5561-10_11.

- Taia, W. K. Modern trends in plant taxonomy. *Asian J. Pl. sci.* **2005**, 4 (2), 184-206.
- Taiga, A.; Suleiman, M.N.; Aina, D.O.; Sule, W.F.; Alege, G.O. Proximate analysis of some dry season vegetables in Anyigba, Kogi State, Nigeria. *Afr. J.*, **2008**, 7(10), 1588-1590.
- Talib, W. H.; Musa, H.; Zarga, A.; Mahasneh, A.M. Antiproliferative, Antimicrobial and Apoptosis Inducing Effects of Compounds Isolated from *Inula viscosa*. *Molecules*. **2012**, 17, 3291-3303.
- Tiangda, C.; Wandee, G.; Sookvanichship, N.; Limchalearn, A. Anti head lice effect of *Annona squamosa* seeds extract. *Southeast asian J. of tropical medicine and public health*. **2000**, 31(1), 174-177.
- Timbrell, J. Introduction to Toxicology. 3rd ed. Taylor and Francis, New York, **2001**. pp. 57-71.
- Tollefson, L.; Miller, M. A. Antibiotic use in food animals: controlling the human health impact. *J. A.O.A.C. Int.* **2000**, 83, 245-254.
- Toma, C.; Grigore, M.N.; Afemei, M.; Stănescu, I-E. Histo-anatomical considerations on some Romanian *Inula* L. species, with pharmacological action. *Analele științifice ale Universității "Al. I. Cuza" Iași* Tomul LVI, fasc. 1, s. II a. Biologie vegetală, pp: 5-13, **2010**.
- Tor-Anyiin, T.A.; Shaato, R.; Oluma, H.O.A. Ethnobotanical survey of antimalarial medicinal plants amongst the Tiv people of Nigeria. *Journal of herbs, spices and medicinal plants*. **2005**, 10, 61-74.
- Toyang, N.J.; Ateh, E.N.; Keiser, J.; Vargas, M.; Bach, H.; Tane, P.; Sondengam, L.B.; Davis, H.; Bryant, J.; Verpoorte, R. Toxicity, antimicrobial and anthelmintic activities of *Vernonia guineensis* Benth. (Asteraceae) crude extracts. *J. Ethnopharmacol.* **2012**, 18, 144(3), 700-704.
- Trease, G.E.; Evans, W.C. Pharmacognosy; Bailliere Tindall: London, 12, **1985**.

- Tyler ,V.E.Phytomedicines: back to the future.*Journal of Natural Products*.**1999**, 62, 1589- 592.
- Taylor, R.B.; Shakoor, O.; Behrens, R.H.; Everard, M.; Low, A.S.; Wangboonskul, J.; Reid, R.G.; Kolawole, J.A. Pharmacopoeial quality of drugs supplied by Nigerian pharmacies. *Lancet*. **2001**357(9272), 1933-1936.
- Uddin, G.; Rehman, T.U.; Arfan, M.; Liaqat, W.; Waliullah .; Rauf, A.; Khan, I.; Mohammad , G.;Choudhary, M.I. Antimicrobial, insecticidal and phytotoxic activities of *Indigofera heterantha* roots. *Journal of Medicinal Plants Research* . **2011 a**, 5(24), 5835-5839.
- Uddin, G.; Rauf, A.; Rehman, T.U.; Qaisar, M .Phytochemical Screening of *Pistacia chinensis* var. *integerrima*. *Middle-East J Sci Res*. **2011b**, 7(5), 707-711.
- Uddin, G.; Rauf, A.; Arfan, M.; Ali, M.; Qaisar, M.; Saadiq, M.; Atif, M.Preliminary phytochemical Screening and antioxidantactivityof *Bergenia Caliata*. *Middle-East Journal ofScientific Research* .**2012a**,11, 1140-1142.
- Uddin, G.; Rauf, A.Phytochemical screening and biological activity of the aerial parts of *Elaeagnus umbellate*.*Scientific Research and Essays*. **2012b**, 7(43), 3690- 3694.
- Uddin, N.; Rahman, A.; Ahmed, N.U.; Rana, S.; Akter, R.; Chowdhury, M.A. Antioxidant,cytotoxic and antimicrobial properties of *Ecliptaalba* ethanol extract. *Int J Biol Med Res*. **2010**, 1(4), 341-346.
- Umadevi, S.; Mohanta, G. P.; Chelladurai,V.; Manna, P. K.; Manavalan,R. Antibacterial and antifungal activity of *Andrographis echiodes*. *J. Nat. Remedies*.**2003**, 3,185-188.
- Umadevi, M.; Sujatha, K. Evaluation of insecticidal activity and phytochemical analysis of five different plant crude extracts against *Tribolium castaneum* (Herbst.) *Int. J. of Res. in Pharm. and Biomedical Sciences* . **2013**, 4 (2), 497-503.

- Upadhyay, S.; Ghosh, A. K.; Singh, V. Anti-lice activity of *Abrus precatorius* Linn. (Fabaceae) seeds oil. *Egypt. Dermatol. OnlineJ.* **2011**, 7 (2), p.1-6.
- UNESCO. Culture and Health, orientation Texts- World Decade for Cultural Development 1988-1997, Document CLT/DEC/PRO-**1996** Paris, France, 129. PMID 16402118.
- Vallance, P.; Smart, T.G. "The future of pharmacology". *British Journal of Biotechnology.* **2006**,5(10), 936-940.
- Vanhaecke, P.; Persoone, G.; Claus, C.; Sorgeloos, P. Proposal for a short-term toxicity test with *Artemia nauplii*. *Ecotoxicol Env Safety.* **1981**,5,382-387.
- Venkatesh, S.; Reddy, Y. S. R.; Ramesh, M.; Swamy, M. M.; Mahadevan, N.; Suresh, B. Pharmacognostical studies on *Dodonaea viscosa* leaves. *Afr. J. Pharm.Pharmacol.* **2008**, 2(4), 083-088.
- Venugopal, T. M.; Swathi, D.; Suchitha, Y.; Prashith, K. T. R.; Mallikarjun, N.; Soundarya, S.; Eyasu, E.; Raghavendra,H.L. Mineral Composition, Cytotoxic and Anticariogenic Activity of *Scleropyrum pentandrum* (Dennst.) Mabb. *Int. J. Drug Dev. & Res.* **2012**, 4(1), 168-174.
- Vijayalakshmi, M.; Periyannayagam, K.; Lakshmana, P.S. Invitro Antilice activity of *Dichrostachys cinerea* (L.) Wight & Arn. *Int. J. of Pharm.Tech. Res.* **2010**, 2(4), 2210- 2213.
- Wabo, P.J.; Payne,V. K.; Gertrude, M. T.; Claire, K.M.; Jeannette, Y.; Ngangout, A.M.; Mbida,M.; Bilong, B.C.F. In vitro anthelmintic efficacy of *Dichrocephala integrifolia* (Asteraceae) extracts on the gastro-intestinal nematode parasite of mice: *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae) .*Asian Pac J Trop Biomed.* **2013**, 3(2), 100-104.
- Wahab, M.; Ahmed, M.; Khan, N. Phytosociology and dynamics of some pine forests of Afghanistan. *Pak. J. Bot.* **2008**, 40 (3), 1071-1079.

- Waller, P. J.; Thamsborg, S.M. Nematode control in green ruminant Production systems. *Trends Parasitol.* **2004**, *20*, 493-7.
- Wallis, T. E. Text book of pharmacognosy; 5th ed. CBS Publisher and Distributors, Darya Ganj New Delhi: **1985**.
- Wang, S.Y.; Wu, J.H.; Cheng, S.S.; Lo, C.P.; Chang, H.N.; Shyur, L.F.; Chang, S.T. Antioxidant activity of extracts from *Calocedrus formosana* leaf, bark and heartwood. *J. Wood. Sci.* **2004**, *50*, 422-426.
- Wang, C.M.; Jia, Z.J.; Zheng, R.L. The effects of 17 sesquiterpenes on cell viability and telomerase activity in the human ovarian cancer cell line HO- 8910 [J]. *Planta Med.* **2007**, *73*, 180-184.
- Wang, D.; Du, X.; Zheng, W. Alteration of saliva and serum concentrations of manganese, Copper, Zinc, Cadmium and Lead among career welders. *Toxicology Letters.* **2008**, *176*, 40-47.
- Watson, D. Safety of chemicals in food, chemical contaminants. Published by Ellis, New York p 109. **1993**.
- Wazir, S.M.; Saima, S.; Dasti A. A.; Subhan, M. Ethnobotanical importance of salt range species of district Karak, Pakistan. *Pak. J. Pl. Sci.* **2007**, *13(1)*, 29-31.
- Wirasathien, L.; Boonarkart, C.; Pengsuparp, T.; Suttisri, R. Biological activities of alkaloids from *Pseuduvaria setosa*. *Pharm boil.* **2006**, *44*, 274-278.
- WHO–World Health Organization, Traditional Medicine, (**1998**) Available at: www.who.int/medicines/factsheets/fs134/en/ > access in: 07/ 08/2003.
- Weston, L.A. Utilization of allelopathy for weed management in agro ecosystems. *Agron. J.* **1996**, *88*, 860-866.
- WHO. http://www.who.int/whr/2002/whr_2002-annex2.pdf.

- WHO. Traditional Medicine strategy **2002-2005**. WorldHealthOrganization, http://whqlibdoc.who.int/hq/2002/who_edm_trm_2002.1.pdf. Accessed 5 Jul 2011. WHO. Cadmium. Environmental Health Criteria. **1992**,134, Geneva.
- WHO. Quality Control Methods for Medicinal Plant Materials, Revised, Geneva. **2005**.
- William, C. D. Integrative Plant Anatomy. Copyright © Elsevier Inc. ISBN: 978-0-12-215170-5. **2000**.
- Wong, M.K.; Tan, P.; Wee, Y.C. Heavy metals in some of Chinese herbal plants. *Biol. Trace Elem. Res.* **1993**,36, 135-142.
- Yang, Y.C.; Lee, S.H.; Lee, W.J.; Ovicidal and adulticidal effects of *Eugenia caryophyllata* bud and leaf oil compounds on *Pediculus capitis*. *J Agr Food Chem.* **2003**,51(17),4884– 4888.
- Yaniv, Z.; Dafni, A.; Friedman, L.; Palevitch, D. Plants used for the treatment of diabetes in Israel. *J. of Ethnopharmacology.* **1987**, 19,145–151.
- Yasmin, S.; Kashmiri, M. A.; Anwar, K. Screening of aerial parts of *Abutilo bidentatum* for hepatoprotective activity in rabbits. *J. Medicinal Plants Research.* **2011**, 5(3), 349- 353.
- Yogeshkumar, V. Screening of some medicinal Plants for Antimicrobial Properties – Phytochemical and Pharmacological Studies of a Selected Medicinal Plant. PhD Thesis, Saurashtra University, India. **2012**.
- Yokozawa, T.; Dong, E.; Nakagawa, T.; Kashiwagi, H.; Nakagawa, H.; Takeuchi, T.; Chung, H.Y. In vitro and in vivo studies on the radical-scavenging Activity of tea. *Journal of the Agricultural and Food Chemistry.* **1998**, 46, 2143-2150.
- Zhao, J.; Li, Y.; Liu, Q.; Gao, K. Antimicrobial activities of some thymol derivatives from the roots of *Inula hupehensis* . *Food Chemistry.* **2010**, 120(2), 512–516.

- Zeng, G.Z.; Tan, N.H.; Ji, C.J.; Fan, J.T.; Huang, H.Q.; Han, H.J.; Zhou, G.B. Apoptosis Inducement of Bigelovin from *Inula helianthus-aquatica* on Human Leukemia U937 Cells. *Phytother. Res.* **2009**, *23*, 885–891.
- Zheng, W.; Wang, S.Y. Antioxidant activity and phenolic compounds in selected herbs. *J.Agricul.Food. Chem.* **2001**, *49*(11), 5165–5170.
- Zhi , P. R.; Zhang, L. L.; Lin, Y. M.Evaluation of the antioxidant activity of *Syzygiumcumini* Leaves.*Molecules.* **2008**,*13*, 2545-2556.
- Zunjar, V.; Mammen, D.; Trivedi, B.M.; Daniel, M. Pharmacognostic, Physicochemical and Phytochemical Studies on *Carica papaya* Linn. leaves. *Pharmacon. Jour.* **2011**, *20*, 5-8.