# PHARMACOGNOSTIC STUDY OF SOME IMPORTANT SPECIES OF FAMILY ZYGOPHYLLACEAE AND EUPHORBIACEAE FROM PAKISTAN

By

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DEPARTMENT OF BOTANY
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PESHAWAR

2012

## DEDICATION

**Sincerely dedicated to**
**myparents, teachers and family**

UNIVERSITY OF PESHAWAR

PESHAWAR

# PHARMACOGNOSTIC STUDY OF SOME IMPORTANT SPECIES OF FAMILY ZYGOPHYLLACEAE AND EUPHORBIACEAE FROM PAKISTAN

A dissertation submitted in partial satisfaction

of the requirement for the degree of

Doctor of Philosophy

in

Botany

By

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**Dated:** 18-2-, 2013

June 19, 2012

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## ACKNOWLEDGEMENTS

All kind of praises is for Almighty Allah who has created the universe and bestowed the mankind with knowledge and wisdom to search for his secrets, who is the most merciful and bountiful.

I am very thankful to my research supervisor, Prof. Dr Farrukh Hussain for his guidance and support from initial conception of the research to the final preparation of the dissertation. His hard work valuable advice, tireless guidance and sincerity to his profession added real worth to this study. He broadened my vision and opened the doors of knowledge for me.

I would like to offer my gratitude to Prof. Dr. Mohammad Ibrar, Chairman, Department of Botany, University of Peshawar for his encouragement.

I wish to acknowledge Dr. Khanzadi Fatima Khattak, Chief Scientist, NIFA (Nuclear Institute for Food and Agriculture), Tarnab, Peshawar for her supervision and kind help in the proximate analysis of medicinal plant samples. I am really thankful to Dr. Abdul Sattar, Director, Entomology Section and Mr. Misbah who provided all the facilities to do insecticidal activity at NIFA (Nuclear Institute for Food and Agriculture), Tarnab Peshawar. I am thankful to Mr. Abid Ali Khan, Ph. D scholar at Centre of Biotechnology and Microbiology, University of Peshawar for his kind help in determining the antibacterial and antifungal activity of medicinal plants samples.

My sincere thanks are also to Prof. Dr. F. M. Sarim, Dr. Siraj ud Din, Miss Mussarat Jabeen, Mrs. Tanvir Burni, Mr. Zahir Mohammad, Mr. Naveed Akhtar, Dr. Lal Badshah, Mr. Barkatullah, Mr. Rehmanullah, Dr. Mohib Shah, Dr. Zaman Sher, Mr. Ishfaq Hameed for their thoughtful suggestions and beneficial critiques at various stages of my study.

I greatly acknowledge the finantial support provided by University of Peshawar to undertake this study. I am also very obliged to my wife who has always been source of inspiration and for her patience during this study. My kids missed me during this research. Thanks to them for their patience.

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## ABSTRACT

## PHARMACOGNOSTIC STUDY OF SOME IMPORTANT SPECIES OF FAMILY ZYGOPHYLLACEAE AND EUPHORBIACEAE FROM PAKISTAN

By

GHULAM DASTAGIR

Five species *Fagonia cretica* L., *Peganum harmala* L., *Tribulus terrestris* L., *Chrozophora tinctoria* (L.) Raf and *Ricinus communis* L.of family of family Zygophyllaceae and Euphorbiaceae were investigated for macroscopical, anatomical and Scanning Electron Microscope i.e., (SEM) features. All these species showed variability in colors, taste, fractures, odors, shapes and sizes in dry and fresh condition. The light microscopy and Scanning Electron Microscopy (SEM) revealed diagnostic anatomical features in roots, stems, leaves and fruits of these species. Anomocytic stomata were found in the leaves of *Fagonia cretica* L and *Peganum harmala* L while *Tribulus terrestris* L had staurocytic stomata on both the surfaces of the leaves. Stauracytic stomata were found in *Chrozophora tinctoria* and paracytic stomata in the leaves of *Ricinus communis* L.

*Fagonia cretica* L showed highest antibacterial activity against *Staphylococcus aureus* and lowest against *Salmonella typhi*. *Peganum harmala* L. showed highest inhibition against *Staphylococcus aureus* and *Klebsiella pneumoni*. *Chrozophora tinctoria* exhibited highest inhibition against *Pseudomonas aeruginosa* and low antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoni*. *Ricinus communis* showed highest inhibition against *Staphylococcus aureus*. The methanolic extracts of *Fagonia cretica* showed maximum inhibition against *Candida albicans*. *Peganum harmala* showed maximum inhibition against *Fusarium solani*. The maximum inhibition against *Aspergillus niger* was exhibited by *Tribulus terrestris* L. *Chrozophora tinctoria* and *Ricinus communis* was most inhibitory against *Aspergillus flavus*. The n-hexane extracts of *Fagonia cretica* L. was highly inhibitory against *Aspergillus niger*. In antifungal activity, maximum inhibition of *Candida albicans* was shown by *Peganum harmala* L. *Tribulus terrestris* L. exerted highest inhibition against *Aspergillus niger* and *Candida albicans*. The inhibition was maximum by *Chrozophora tinctoria* against *Candida albicans*. *Aspergillus niger* was inhibited the most by *Ricinus communis* L. Cytotoxic activity showed that the methanolic and n-hexane extracts of five tested plants had significant lethality against brine shrimps (*Artemia salina*). Phytotoxic activity indicated that the aqueous extract

depending upon concentration of *Fagonia cretica* L. was highly active with 49.99 % growth inhibition. The (%) growth inhibition of *Lemna minor* decreased with the dilutions. The methanolic and n- hexane extract of *Ricinus communis* L. was highly active having 61.09% and 33.33% growth inhibition. Comparing the two extracts the methanolic extract of *Fagonia cretica* L. and *Tribulus terrestris* L. were more inhibitory as compare to the n-hexane extract.

In insecticidal activity *Peganum harmala* L. against insects mortality was maximum while *Tribulus terrestris* L had the lowest mortality. All the doses (5 to 20%) of the plant extracts showed significant differences of mortality of *Tribolium castaneum* as compared to control. *Chrozophora tinctoria* showed the maximum insects mortality as compared to *Ricinus communis*. The termicidal activity showed that aqueous extracts (20g) of *Fagonia cretica* L., *Peganum harmala* L. and *Tribulus terrestris* L. caused significant mortality of *Heterotermes indicola* with significant differences among plants and control. All the tested concentrations (120,160,180 and 240 ml) of *Peganum harmala* L and *Tribulus terrestris* revealed significant mortality of *Heterotermus indicola.* with significant differences among themselves. The ANOVA values showed that aqueous extracts (20g) of *Chrozophora tinctoria* L. and *Ricinus communis* L. had significant mortality of *Heterotermus indicola* after 72 hrs. Plants and time were highly significant while their interactions were non- significant. The ANOVA results indicated that all the concentrations (120,160,180 and 240 ml) of *Chrozophora tinctoria* exhibited significant mortality of *Heterotermus indicola* with significant differences among themselves. Mortality was concentration and time dependent, it increased with concentration and the exposure time (24 to 72 hrs) in the present case.

The macro and micro minerals analysis in two seasons were performed for Ca, K, Mg, Na, Cr, Cu, Fe, Mn, Mo, Zn, Al. With few exceptions, most of the elements had higher concentrations in winter than summer in all the analyzed parts of the investigated plants. The average values showed that *Peganum harmala* L. excelled in many chemical parameters and has a good nutritive value, followed by *Fagonia cretica* L. that contained the highest fibre. The *Tribulus terrestris* L. had the highest protein and gross energy. The average values show that the moisture contents, ash contents, protein, fats, fibre, carbohydrate and gross energy was higher in *Chrozophora tinctoria* L. than *Ricinus communis* L. The moisture, fat, carbohydrate and gross energy increased in winter than summer in the studied plants. It is

concluded that sufficient levels of macro and micro nutrients and different energies were present in the investigated medicinal plants.

Phytochemical screening of the methanolic extract of *Fagonia cretica* L., *P. harmala, Chrozophora tinctoria* L. revealed the presence of saponins, pholobatannins, anthraquinones and alkaloids. Tannins and flavonoids were found in *Chrozophora tinctoria* L and *Ricinus communis* L. Glycosides were present in *Fagonia cretica* L. and *Peganum harmala* L. Terponoids and phenolics occurred in *Peganum harmala* L. Group tests revealed that the methanolic and n-hexane extract of *Fagonia cretica* L, *Peganum harmala* L., *Tribulus terrestris* L., *Chrozophora tinctoria* L. and *Ricinus communis* L. had OH, carbooxylic and phenolic groups. The analysis of essential oil study revealed that *Fagonia cretica* oils had 17 compounds that constituted 100% of the oil composition. Oxygenated monoterpenes (89.94%), was a major group of compounds. *Peganum harmala* L. oil had 18 phytochemicals. There were 10 compounds in *Tribulus terrestris* L. oil that consisted 100% of the total oil composition. Eight compounds were identified in *Chrozophora tinctoria* oils giving complete oil composition. It had oxygenated monoterpenes 86.93%, constituting of 2(4H) - Benzofuranone, 5, 6, 7, 7a tetrahydro-4, 4, 7a-trimethy 50.718%. *Ricinus communis* L. oil had 8 compounds with 100% of the oil composition.

The study will be helpful in the identification of the investigated plants to establish the quality and purity and will bring these plants to the business front and will help in the economic development of the country by preventing the purchase of foreign drugs and consequently the foreign exchange as well. The plants could be used singly as potential bioactive agents for the management of termites particularly by our poor farmers who cannot afford to buy expensive insecticides from markets. The investigated plants can add to the nutritional requirements of people and can be used as a source of nutrients. These plants could be potential sources of new antimicrobial, cytotoxic, phytotoxic and insecticidal agents. Our findings suggest that the essential oil from five plants have potential as antibacterial, antifungal and antioxidant agents and could be useful in the pharmaceutical, food and cosmetics industries. More research is needed to prove this.

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# CHAPTER-1
# INTRODUCTION

# CHAPTER 1

# INTRODUCTION TO TEST PLANTS

## i. Introduction to plants

**Family Zygophyllaceae**

**Scientific name**

1. *Fagonia cretica* L.



Fig. 1 *Fagonia cretica* L.

**Other Names**

English: Khorasan thorn. Pushto: Azghakhi, Damiya and Dhaman. Punjabi: Dama, Dramah, Shawkatul- Baiza. Arabic: Gamda. Bengali: Duralabha. Gujrati: Dhamaaso.

**Taxonomic Description**

It is annual to biennial to glabrous shrublet. Stem woody with prostrate branches. Lower leaves trifoliolate. Flowers pinkish-purple. Capsule long pubescent. Flowering period: throughout the year or April-June (Hussain *et al*. 2007).

**Distribution**

**Worldwide**

India, Iran, Aden, Eritrea, Athiopia, Sudan, Somalia and Kenya. The names *Fagonia cretica* and *Fagonia arabica* were applied to plants from south Asian subcontinent. The above named species occur only in the south Mediterranean region.

**Pakistan**

It is found in dry habitat throughout Pakistan from sea level to 1,478 meters.

**Medicinal importance**

It is used in curing piles, urinary disorders, dysentery, asthma, liver, stomach, typhoid, skin diseases, cancer and as a blood purifier (Marwat, *et al.*, 2008; Akhtar & Begum, 2009). *Fagonia* species are used as antitumor, antioxidant, analgesic, febrifuge and prophylactic against small-pox agents, fever, asthma, urinary discharges, toothache, kidney diseases (Alam, 2011).

**Ethnobotany**

Plant is wormicidal, leaves are used in snakebite. It is used as fodder and for fuel in Kurram Agency. It is used as cooling agent (Khan *et al.*, 2011)

**Constituents**

Abdel- Khaliq *et al.* (2000) reported flavonoids and triterpenoids saponins. Alam (2010) found variation in carbohydrates, saponins, tannins, unsaturated sterols, triterpenoids, alkaloids, cardiac and cyanogenic glycosides, flavonoids, coumarins, chlorides and sulphates. It had two new triterpenoid saponins, ursolic acid, ester (indicasaponin A), and two known triterpenoid saponins (Shaker *et al.*, 1999). It has four flavonoid compounds i.e., quercetin, isorhamnetin-α-3-O rhamnoside, quercetin 3-O-β-D-glucopyranosyl -(1"-6"')-β-D-glucopyranoside and quercetin 3-O-β-D-galactopyranosyl -(6"-1"')- α-L-2"' acetyl rhamnose-(3"'-1"") β-D-glucopyranoside . In addition to oleanolic acid, β-sitosterol-3-O-β-D-glucoside and stigmasterol 3-O-β-D-glucoside and flavonoids upto 3% were also present (Shehab *et al.*, 2011).

**Scientific name:**

**2. *Peganum harmala* L.** (Synonym *Peganum dauricum* Pall.). (Fig. 2).

**Other names** English Wild rue, Syrian rue, African-rue. Locally Harmal (Saraiki); Spalani (Pashto); Vern. Harmal, Ispand, Isband; German Steppenraute; Spanish Alharma, Gamarza; French Rue Sauvage



Fig. 2. *Peganum harmala* L.

**Taxonomic Description**

A perennial with corymbosely branched and labrous herb. Leaves sessile and pinnatisectly dissected. Flowers white or yellowish white and capsule 6-10 mm across. Seeds blackish-brown. Flowering period: April-October. or March, April, May, Fruiting Jul-Nov (Mahmoudian *et al.* 2002).

**Distribution**

**Worldwide**

India, Tibet, Pakistan westwards to North Africa, Europe and Russia. It is wild in semi arid areas in Egypt. Middle East, Mongolia and China

**Pakistan**

It occurs from sea level to 3,061 m slightly saline sands near oases, dry grasslands in desert areas.

**Medicinal importance**

Seed powder is used in asthma, colic, jaundice, analgesic and anthelmintic. Seeds are antiseptic, paralysis, gastrointestinal, urinary, epilepsy and menstrual problems, narcotic, hypotonic, antispasmodic, antiperiodic, emetic, alterative, lactagogue, in laryngitis, stimulant, abortifacient, healing wounds, diarrhea and indigestion It is brain tonic and used along with olive oil for ear problems (Qureshi *et al.*, 2007). It is used in lumbago and the plants alkaloids are protozoacidal agents, coronary dilators and for the nervous diseases. Harmine induces a fall in blood pressure and more toxic for protozoa than quinine. Harmaline had abortificant, narcotic, aphrodisiac, stimulant, sedative, emmanagogue, emetic, vermifuge and soporific properties (Bukhari *et al.*, 2008).

**Ethnobotany**

Smoke of seeds is considered as air purifier. Leaves and seeds are used to repel evils. Powdered seeds are mixed with mustard oil and it is rubbed on hair once a day as antilice agent. The smoke from burning seeds and leaves is believed as snake repellent and also used as protection against evil eyes (Attaullah *et al.* 2010).

**Constituents**

Arshad *et al.* (2008) reported flavonoids and anthroquinons. Sharaf *et al.* (1997) isolated acacetin 7-O-rhamnoside, 7-O-6-O-glucosyl-2-O-(3'''-acetylrhamnosyl) glucoside and 7-O-(2'''-O-rhamnosyl-2-O-glucosylglucoside), and the glycoflavone 2'''-O-rhamnosyl-2-O-glucosylcytisoside from aerial parts of *Peganum harmala*. Sarpeleh *et al.* (2009) and Bukhari *et al.* (2008) from seeds isolated alkaloids and other active compounds harmine, harmaline, harmal, harmalol, tetrahydroharmine and tetrahydroharmol. Nenaah (2010) and Aziz *et al.* (2010) reported β-carboline alkaloids and a novel carboline alkaloid (l-thioformyl-8-β-D-glucopyranoside-bis-2,3-dihydro-isopyridinopyrrol from aerial parts of *Peganum harmala* and it showed significant antibacterial activity against *Streptococcus pyogenus*. Khan *et al.* (2011) and Farouk *et al.* (2009) detected alkaloids, saponins, tannins, anthraquinonoes, flavonoids, flavons, flavonols, chalcones, terpenoids, phlobatannins and cardiac glycosides in *Peganum harmala*.

**3. Scientific name: *Tribulus terrestris* L.** (Fig. 3).



Fig. 3.*Tribulus terrestris* L.

**Other names**

English name: Caltrop, Puncture vine, Bullhead, Cat's-Head, Common Dubbletjie, Devil's-Thorn, Devil's-Weed, Goathead, Mexican Sandbur, Texas Sandbur, Tribulus, Local names: Bhakra, Gokhru; Tibbi name: Gokhru, Khar-e-khashak. Bhakra; Arabic: Chokaziz, Kutrah; French Tribule Terrestre; Indian Gokhru; Spanish: Abrojos, Espigón

**Taxonomic Description**

It is an annual or biennial, prostrate herb. Stem spreading with branches. Leaves paripinnate, lanceolate, ovate to elliptic-oblong. Flowers yellow. Fruit 1 cm in diameter. A highly variable species in leaf and flower size and fruit characters. It flowers throughout the year or June, July, August (Hussain *et al.*, 2011).

## Distribution

### Worldwide

Tropical and subtropical countries in Asia, Africa, S. Europe, North Australia and introduced in new world tropics. Turkey, Iran and Iraq

### Pakistan

It is found from sea level to 4,653 meters throught Pakistan in sandy soils of waste places and cultivated fields.

## Medicinal importance

Fruits are used in urinary bladder and leaves are used in colic and chronic cough (Marwat, *et al.*, 2008) Plant is used as a demulcent, tonic, and aphrodisiac. Fruits are used in urinary diseases, impotence, cough and heart diseases. In Sindh: Thumbtack-like plant nutlets are used against retention and itching in urine and improving male sexual strength. It has hypoglycaemic activity and is being used in the traditional medicine of China and India for centuries. Fruit restores depressed liver, improves acute conjunctivitis, headache and vertigo. Plant is antimicrobial, antihypertensive, diuretic and stimulates spermatogenesis and libido. In the modern medical practice the preparations and the dietary supplements containing extracts of *Tribulus terrestris* are being increasingly used for sexual impotence, edema, anorexia, skin diseases, vermifuge, rheumatoid arthritis and others (Semerdjieva, 2011).

## Ethnobotany

Plant is used as a fodder (Hussain *et al.*, 2007).

## Constituents

Conrad *et al.* (2004) reported furostanol saponin 1, named tribol, spirostanol saponins 2 and 3 and sitosterol glucoside,tribol(25R)-furost-5(6)-ene-3$\beta$,16,26-triol-3-O-$\alpha$-rhamnopyranosyl-(1→ 2)-[$\alpha$-rhamnopyranosyl-(1→ 4)]-$\beta$-glucopyranoside (1). Attaur Rehman (1983) studied 3-O-rhamnoside from flower extract of *Tribulus terrestris* quercetin. Hussain *et al.* (2011) and Rajkala *et al.* (2011) stated that high quality of the steroidal saponins and flavonoids are preferred for export to other

European countries and plant had steroidal saponins, alkaloids, flavonoids, harman, chlorogenin, diosgenin and its acetate, furostanolglycoside, spirostenol saponin, a dihydroxy spirosteroidal sapogenin, saponin C and G, Kaemferol, Kaemferol-3-glucoside, Kaemferol-3-rutinoside, Kaemferol-3- B-D (6-p-coumaroyl) - glucoside, glucose, amino acid, harmine, harmaline, herman, gitogenin.

**Family Euphorbiaceae**

**4. Scientific name *Chrozophora tinctoria*** (L.) Raf. (Fig. 4).

**Other names**

Plant is commonly known as dyers-croton, giradol or turnsole.



Fig. 4. *Chrozophora tinctoria* (L.) Raf

**Taxonomic Description**

*Chrozophora tinctoria* (L.) Raf. is an annual prostrate herb and common in arid soils. A common weed of fallow fields and dry waste places on sandy clay loam .Leaf-blades broadly ovate-rhombic to ovate-lanceolate. Stipules filiform. Male flowers subsessile; sepals lanceolate. Fruit rounded-trilobate. Seeds triangular-ovoid, tuberculate and grey (Radcliffe-Smith, 1986).

Flowering Period: Jan-Sept and April-Sept. Plant prefers light (sandy), medium (loamy), heavy (clay), acid, neutral and basic (alkaline) soils. It can grow in semi-shade (light woodland) or having no shade. It requires moist soil (Chopra *et al.*, 1956).

**Distribution**

**Worldwide**

Spain and N.W. Africa eastwards to Arabia and N.W. India. A common weed of fallow fields and dry waste places on sandy clay loam; 900 - 7500'/275 - 2300 m. In Turkey it grows at altitudes of 0-1650 m. It is native to Africa, (Algeria, Egypt, Libya, Morocco, Tunisia and Yemen) temperate and tropical Asia (Kuwait, Saudi Arabia, Afghanistan, Iran, Iraq, Israel, Lebanon, Syria, Turkey, Kazakhstan, Turkmenistan, India and Europe (Ukraine, Albania, Bulgaria, Greece, Italy, Malta,

France, former Yugoslavia, Portugal and Spain). It is found at 900 - 7500'/275 - 2300 m.

**Pakistan**

Peshawar, Punjab, Cholistan

**Medicinal importance**

It is used to treat warts, used as an emetic, cathartic, and for the treatment of fever in Iran (Delazar *et al*., 2006). The leaves are boiled in water and the obtained juice is given orally to relieve chest burning acting as stomachic in Kadhi areas of Khushab (Qureshi *et al*., 2011).

**Ethnobotany**

Economically, it is also important because its fruit is edible it has emetic and corrosive properties and resistant to termites (Caius, 1986). Its leaves is used for tanning and dyeing (Guarrera, 2006).

**Constituents**

Delazar *et al*. (2006) reported that plant is well known for producing dye substances and found flavonoids, alkaloids, coumarins, chromones, xanthones and phenylpropanoid glycosides and flavonoids, a new acylated flavone glucoside, named chrozophorin from the aerial parts of *Chrozophora tinctoria*. The aerial parts of *Chrozophora tinctoria* had flavonoids 1, 3 and 4, and plant also contained acacetin 7-*O*-rutinoside and flavonoid 5 a novel natural product. It also produced flavonoids 2-5 (apigenin derivatives with glycosylation at C-7).

**5. Scientific name *Ricinus communis* L.** (Fig. 5).



Fig. 5. *Ricinus communis* L.

**Other names**

English name: Castor Seed Erand (Hindko, Gujri), Arand (Punjabi, Urdu). Haran (Sindhi).

**Taxonomic Description**

It is an erect, single-stemmed or much-branched shrubby or tree like somewhat glaucous herb. Stem hollow, becoming woody at the base. Leaf-blades 7-9-lobed. Inflorescence 10-25 cm long with bracts while the bracteoles smaller. Male flowers 1 cm long and yellowish-green. Female flowers 3-5 mm. long. Fruit trilobate. Seeds shiny, greyish, silvery or beige streaked and flecked with brown. Flowering Period: Throughout the year (Radcliffe-Smith, 1986).

**Distribution:**

**Worldwide**

It probably originated in North-East Africa and spread to the Mediterranean, the Middle East and India. Today it is cultivated and growing wildly throughout the drier tropical, warm temperate and subtropical regions. Cultivated in Brazil, India, Russia, USA, Thailand, Ethiopia, and Romania. It is found at altitudes from sea level to about 3,000 m. The optimal altitude is 300-1800 m or wild up to 2400 m (Verma *et al.*, 2011).

**Pakistan**

It is widely found at 4000'/1220 in the Sub-Himalayan tract and in the plains, and naturalized near villages.

**Medicinal importance**

It is used for health care in India and used singly or in polyherbal formulation in Ayurveda and Homeopathy (Verma *et al.*, 2011). Leaves are analgesic and used in bronchial pneumonia (Attaur Rehman, 1983). It is expectorant, purgative, sedative and narcotic. Oil is used in rheumatism, paralysis, asthma, cough and constipation. Seeds are used against warts, freckles and seeds oil is used as hair tonic and in constipation. Leaves poultice is useful in boils and swelling (Barkatullah *et al.,* 2009). Seeds are used in sexual disorders and are considered to be poisonous when eaten excessively. Leaves are narcotic, poisonous and purgative. Castor oil is given in constipation before and after child birth to mother (Qureshi *et al.,* 2009). Decoction of ginger alongwith castor oil is very effective in flatulence and helps in backache. Fruits are roasted in Ghee and used for skin disease. Leaves warmed over fire and applied to breast of women acts as galactogogue i.e. increasing milk secretion. In leucorrhoea fresh leaves are crushed and boiled in 250 ml of milk. It is filtered and the filtrate is taken for one month. Heated and oiled leaves are placed on rheumatic joints, swellings and inflamed muscles (Shah & Khan, 2006). The oil and seeds are used for warts, cold tumors, indurations of the abdominal organs, whitlows, lacteal tumors, indurations of the mammary gland, corns and moles. Castor-oil is a cathartic and has labor-inducing properties. Ricinoleic acid has served in contraceptive jellies. Ricin, a toxic protein in the seeds, acts as a blood coagulant (Kensa & Yasmin, 2011). Castor oil helps to soften and lubricate the skin. A branded gel Gastro oil is useful against dermatitis and protective in occupational eczema and dermatitis. The oil is made into contraceptive jellies, foams and creams (Rizvi, 2007).

**Ethnobotany**

Plant is used as fuel wood, fodder, and hedge. Seeds are used to induce vomiting. It is emetic and narcotic, Seeds oil is used for candle making, in cosmetics. Fruit juice has cooling effect (Ahmed *et al.*, 2003; Sher *et al.*, 2011). Castor oil is used in family planning as an abortion agent (Kamal *et al.*, 2009). Bark and leaves are made into paste and applied on mammary glands to treat flogged breast (Badshah and Hussain, 2008). The oil from the seed has many uses - as an illuminant, in tanning as a leather-preservative, and in industry as a lubricant, especially for delicate machinery; the oil-cake is used for fertilizer and fuel.

**Constituents**

Verma *et al.* (2011) reported fixed oil and several amino acids. Fixed oil (45 to 55%) contained a mixture of triglycerides, triricinolein- 75% which on hydrolysis yields ricinoleic acid responsible for the cathartic effect. Leaves had ricinine, Ndemethylricinine, 3-O-$\beta$-D- rutinosides of Kaempferol and quercetin. Hundred gram leaves had, 24.8 g protein, 5.4 g fat, 57.4 g total carbohydrate, 10.3 g fiber, 12.4 g ash, 2,670 mg Ca, and 460 mg P content. Kensa & Yasmin (2011) detected tannins, saponins, alkaloids, carbohydrates, phenols, flavonoids, sterols, resins, oil, ricinoleic acid, ricin. Darmanin *et al.* (2009) identified monoterpenoids, 1, 8-cineole, camphor, pinene, and a sesquiterpenoid, caryophyllene in the leaf extract of *Ricinus communis*. Salimon *et al.* (2010) reported that the crude oil of Malaysian castor seeds had total lipid contents and high iodine value. The seed oil moisture content, acid value and free fatty acid percentage (% FFA) were 0.2%, 4.88 mg/g and 3.4%, respectively. The unsaturated fatty acids were 97.5%, ricinoleic acid 84% while other fatty acids were linoleic, oleic, palmitic, stearic, and linolenic. Five types of triacylglycerols were identified as triricinolein, diricinoleoylstearoylglycerol, diricinoleoyloleoyl-glycerol, diricinoleoyllinoleoylglycerol, and diricinoleoylpalmitoyl-glycerol, RRP (0.9%) respectively. Castor oil is one of the few naturally occurring glycerides with high purity, since the fatty acid portion is nearly 90% of ricinoleic. Kadri *et al:* (2011) reported seven constituents in the essential oil of *Ricinus communis* as thujone , 1,8-cineole, pinene, camphor and camphene.

# SUBJECT INTRODUCTION

**ii. Introduction to subject**

Medicinal plants are used in the therapy or as sources of certain substances and drug having remedial effects since prehistoric ages. Medicinal plants are of great value in drug supply. The world is rich in medicinal plants growing wild a cultivated forming a huge natural and economic wealth which must be protected, increased and industrialized for the sake of humanity, development of economy and wealth of peoples (Wallis, 2005).

Traditional Unani medicine is a part of our culture and Pakistan is one of those countries where traditional Unani medicine is popularly practiced among the large segment of its population. The physicians who practice this system are known as "Hakims". It originated in Greece, founded by old ancient Greek philosophers and was used by Muslims during the period of Islamic civilization. It was brought to the Indo-Pak subcontinent by Muslim scholars and practiced here for centuries. It also benefited from the Ayurvedic system of medicines. Traditional Unani medicine heavily depends on medicinal plants (Ahmad *et al.*, 2003).

Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. The first step towards ensuring quality of starting material is authentication. In recent years there has been a rapid increase in the standardization of selected medicinal plants and various workers have worked on the standardization of medicinal plants (Reddy *et al.*, 1999; Selvamani *et al.*, 2009; Shahla & Deokule, 2010). Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. According to World Health Organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken (Venkatesh *et al.*, 2004).

Morphology and histology makes the first step to get knowledge about the diagnostic features, which are ascertained through the study of the tissue and their arrangement, cell wall and cell contents (Youngkin, 1950). Plant morphology represents a study of the development, form, and structure of plants and by implication, an attempt to interpret these on the basis of similarity of plan and origin

plant evolution and paleobotany (Raven *et al*., 2005). Microscopy of the powder drug has the advantage of requiring only a small quantity of material. This study provides useful references not only for forensic scientists and other engaged in the evaluation of powder drug but also to the analysts who one concerned with standardization and authentication of prepared drug material (Jackson & Snowden, 1992). Microscopy allows the identification of herbal drugs and the detection of individual components of a mixture. It is highly important to ensure quality and purity of herbal medicines in order to maximize the efficacy and minimize adverse side effects. Moreover, microscopic analysis of the powdered herbal drugs is a mandatory technique on the official Pharmacopeia monographs. Sometimes, the characterization of histological sections of each herbal drug is very useful in the determination of the most relevant characteristics of each powder (Serrano *et al*., 2010). Leaves can have a wide range of anatomical and morphological structures that partially isolate stomata from the turbulent atmosphere. Traditionally, such stomatal protection has been considered as an adaptation to dry climates with the suggestion that they increase the effective boundary layer, decreasing stomatal conductance and thereby reducing stomatal transpiration. Some workers opinioned that careful and decisive anatomical studied of fewer morphological characters, in the framework of molecular phylogenies are productive to integrating the powers of morphological data with those of sequence information. Since the leaf epidermal studies are considered important in phylogeny and taxonomy, attention of plant taxonomists has been attracted toward the leaf epidermal anatomical studies to resolve the taxonomic problems and it has been shown that normal and vitreous leaves of *Datura insignis* differ in the frequency of normal and abnormal stomata (Hameed & Hussain, 2011). Stomata, trichome, mesophyll structure and vascular bundles in leaves provide information of taxonomic significance (Kaya *et al*., 2007). The stomata allow gases exchange. In green leaves they occur either on both surfaces or on only one, either the upper (epistomatic leaf) or more commonly on lower that is hypostomatic leaf (Abid *et al*., 2007). Due to the inconsistency of stomatal type in different taxa. Pant and Hkidwai, and Sen and Hennipmann, however, did not feel so sure about the effectiveness of stomatal characters in the grouping of different families (Chaudhry & Imran, 1997).

Nutrients play key role in animals and plants life. Carbohydrates are essential for the maintenance of plant and animal life and provide raw materials for many

industries (Ebun-Oluwa & Oshodi, 2007). Proteins, fat and carbohydrates contribute to caloric content of the dietary, minerals including trace elements, vitamins and water (Underwood, 1994). Energy is a key component within the body. All carbon containing compounds have a certain amount of energy. Plants are a primary source of medicines, fibers, food, shelters and other items in everyday use by humans with roots, stems, leaves, flowers, fruit and seeds providing food and medicines for humans (Hemingway, 2004). Impressive developments in the field of mineral elements have taken place in the chemical, biochemical and immunological areas of research. Deficiency of trace elements in human subjects can occur under the most practical dietary conditions and in many diseased statuses. In recent years, scientists and nutritionalists have started believing in the therapeutic role of metals in human health (Udayakumar & Begum, 2004).

In recent years, several authors reported many studies on the importance of elemental constituents of the herbal drug plants which enhanced the awareness about trace elements in these plants (Sheded *et al.,* 2006; Koe & Sari, 2009, Basgel & Erdemoglu, 2006; Ajasa *et al*., 2004).

Trace elements play both curative and preventing role in combating diseases. There is a vast scope to exploit the preventive medicinal aspects of various trace elements such as Ca, Cu, Co etc. Medicinal plants play the most important role in the traditional medicines. In most developing countries most of the flora remain virtually unexplored from point of view of the medicinal utilization through traditional Eastern system of medicines strongly upholds the use of these elements for curing many disease (Khanzada *et al*., 2008).

Phytochemicals could provide health benefits such as substrates for biochemical reactions, cofactors of enzymatic reactions, inhibitors of enzymatic reactions, absorbents/sequestrants that bind to and eliminate undesirable constituents in the intestine, stability of essential nutrients, fermentation substrates for beneficial oral, gastric or intestinal bacteria and selective inhibitors of deleterious intestinal bacteria. Such phytochemicals include terpenoids, phenolics, alkaloids and fiber (Cora *et al*., 2000). Phytochemical screening has been done for exploring the hidden potential of indigenous flora of Pakistan (Nisar *et al*., 2010; Zia-ul-Haq *et al*., 2007 a, ; Zia-ul-Haq *et al*., 2008 b; Zia-ul-Haq *et al* ., 2009 c).

Fluorescence test suggested the identification of plants chemical groups (alkaloids, quinones, saponins, polyphenols, anthranoids) by precipitation and colour reactions. A number of groups of products, including flavonoids, coumarins, cardenolides and steroids can be rapidly identified in plants by this method (Dhiraj *et al*., 2009).

Essential oils are produced by different plants to serve as plant defense mechanisms against predation by microorganisms, insects and herbivores or for reproductive purposes. They are used in the manufacture of perfumes, deodorants and soaps. They have antimicrobial and antioxidant properties and have also been the subject of study, particularly by the chemical, pharmaceutical, and food industries, because of their potential use in food. Antioxidants play important roles in preventing the diseases induced by reactive oxygen species (ROS) which result in oxidative damage to DNA, proteins and other macromolecules The use of medicinal plants and their essential oils in particular has increased due to the globalization and increase in international trade, but also because they are environmentally friendly and readily available (Gundidza *et al*., 2009; Kadri *et al*., 2011)

Globalization interferes with infectious disease control at the national level while microbes move freely around the world, unhindered by borders, human responses to infectious diseases and are conditioned by jurisdictional boundaries (Stepanovic *et al*., 2003).

There has been an increasing incidence of fungal infections in recent years, largely due to an increase of AIDS-related opportunistic fungal pathogens. The emergence of resistance strains of immunocompromised patents is still limited due to drug potency, poor solubility of drugs, emergence of resistant strains and drug toxicity. The screening of plants for antimicrobial activity has shown that plants represent a potential source of novel antibiotic prototypes (Aliero *et al*., 2006). Fungal infections remain a significant cause of mortality despite advances in medicine and the emergence of new antifungal agents. To overcome the alarming problem of microbial resistance to antibiotics, the discovery of novel active compounds against new targets is a matter of new urgency (Parekh & Chanda, 2008).

Bioactive compounds are often toxic to *Artemia salina* (leach) shrimp larvae. (Kivack *et al.*, 2001). Lethality assay has been used successfully to biomonitor the isolation of cytotoxic, antimalarial, insecticidal and antifeedent compounds from plants extracts (Krishuaraju *et al.*, 2006). The cytotoxicity bioassay against *Artemia salina* is a simple and inexpensive method to test cytotoxicity. It is also a very useful tool for the isolation of bioactive compounds (Ramachandran *et al.*, 2011).

Phytotoxic natural products may be utilized either directly or as lead compounds for the development of herbicides (Morimoto *et al.*, 2009). A number of secondary metabolites in plants can act as allelochemicals to other plants. Under certain conditions, these compounds are released into the environment by exudation from living plant tissues and decomposition of plant material and may affect the growth of the neighboring or successional plants Rice (1984) and Einhellig (1996). Using the *Lemna* assay, it is observed that natural antitumour compounds can inhibit *Lemna* growth. In addition, it was also discovered that some substances stimulate frond proliferation, and the assay may be useful to detect new plant growth stimulants. The commercial need for such natural, biodegradable, herbicides and plant growth stimulants may someday be filled with natural products detected by the simple and convenient *Lemna* bioassay (Atta-ur-Rahman, 1991).

Over the past 15 years, interest in botanical insecticides has increased as a result of environmental concerns and insect populations becoming resistant to conventional chemicals. Botanical insecticides are naturally occurring insecticides that are derived from plants (Isman, 2000). Many alternatives have been tested to replace methyl bromide fumigation for stored products and quarantine uses. There is an urgent need to develop safe alternatives that have the potential to replace the toxic fumigants, yet are effective, economical and convenient to use (Ayvaz *et al.*, 2010). Plants have been used throughout human history in cooking, medicines, cosmetics, and insecticides. A large number of plant essential oils may be potential sources of mosquito larvicides, because they constitute a rich source of bioactive components (Cetin *et al.*, 2004).

# CHAPTER-2
# REVIEW OF LITERATURE

# CHAPTER 2

# REVIEW OF LITERATURE

**i. Anatomical and powder drug study**

Koyuncu *et al.* (2008) reported that *Peganum harmala* had amphistomatic leaf. The pollens of *Peganum harmala* were tricolporate. The exine was striate-rugulate. Hidayat & Kusdianti (2009) stated stomatal characters were diverse in 13 species of family Euphorbiaceae. Monophyletic nature of Euphorbiaceae had been proved in this study.

The following research is available on the anatomical features of some plants in Pakistan.

Qureshi *et al.* (2008) worked on *Tragopogon dubius* and *T. gracilis* for morphology and anatomy. Basal leaves were sessile, whilst the basal, middle and upper leaf blade was linear lanceolate. The leaf epidermal anatomical feature and pollen grain characteristics were studied. The leaves showed anomotetracytic stomata.

Shaheen *et al.* (2009) worked on the micro-morphology and anatomy of glandular and glandular trichomes of leaves of *Sida alba*, *Sida alii, Sida abedin*, *Sida cordata*, *Sida mysorensis*, *Sida ovata, Sida spinosa* and *Sida yunnanensis*. Epidermal cells were polygonal to irregular. Glandular, eglandular, stellate, peltate trichomes were found in all taxa studied. *Sida alii* and *Sida spinosa* were distinct in having long-stalked and short-stalked capitate trichomes. Leaves were amphistomatic and amphitrichomic *Sida mysorensis* is characterized by multicellular uniseriate trichomes, but these were absent in other taxa.

Hayat *et al.* (2009) recorded variation in foliar trichomes from 15 taxa. Glandular trichomes, capitate glands had wide distribution on the both surfaces of leaves with notable variations. Non-glandular trichomes were most abundant.

Ahmad *et al.* (2009) reported morphology and leaf epidermal anatomy of *Asphodelus tenuifolius, Chenopodium album, Chenopodium murale, Convolvulus arvensis, Cyprus defformis, Amoen, Euphorbia helioscopia, Lathyrus aphaca,*

*Plantago major, Tribulus teristris* and *Vicia faba*. In leaf epidermal anatomy the type, shape and diameter of epidermal cell, type and diameter of stomata, length of stomatal complex, type of trichome (glandular non glandular) etc were studied. Five types of stomata were found while trichomes were present only in two species.

Yasmin *et al.* (2010) showed that the leaf anatomy of *Persicaria* species had variation in size and shape of epidermal cells, stomata, glandular and non glandular trichomes. Epidermal cell were polygonal. Five different stomatal patterns were observed. Two types of non glandular trichomes were observed in *Persicaria barbata*, *Persicaria stagnina* and *Persicaria orientalis*. Glandular trichomes were 1, 2 and 4-celled peltate, capitate and spheroidal.

Sahreen *et al.* (2010) reported variation in the type of stomata, shape and size of epidermal cells, trichomes and crystals of 16 species of *Silene*. Abaxial surface was different from the adaxial surfaces in all species. Diacytic stomata as the diagnostic character of family Caryophyllaceae. In *Silene* diacytic, anisocytic and anomocytic stomata were present. Epidermal cells of *Silene moorcroftiana* were larger in size, in *Silene indica* length of epidermal cell was 30µm. In *Silene villosa* glandular hairs were abundantly present.

Nisar *et al.* (2010) reported the morpho-anatomical study of fourteen accessions of *Lasiurus scindicus* and showed variation for leaf area, number of hairs, epidermal appendages, tomata, stomatal index and epidermal cells on upper surface of leaf. The accessions KWT1/1 and KWT1/2, collected from Khokhran Wala Toba, BC1/2, from Baghdad Campus, SWT, from Sulleh Wala Toba, showed high variation and appeared as the best genotypes..

Farooq *et al.* (2011) studied foliar epidermal anatomy of seven species of Poaceae and showed the presence rounded short cells, long cells, size of stomatal complex. Large cells with maximum length and largest stomatal complex were found in *Avena sp.* In genus *Agrostis, Phalaris and Koeleria* variations existed in morphology of silica bodies.

**ii. Antimicrobial activities**

Kianbakht & Jahanianiian (2003) evaluated the methanolic extracts of different parts of *Tribulus terrestris* against *Staphylococcus aureus, E. faecalis* and *Escherichia coli*. The methanolic extracts of all parts of the plant inhibited all the tested bacteria.

Usman *et al.* (2007) worked on the antimicrobial activity of leaf extract of *Tribulus terrestris* against *Salmonella typhi*, *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa* and *Candida albicans*. The minimal bactericidal concentration against *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa* and *Candida albicans* was found to be 1.563 mg $mL^{-1}$ while against *Salmonella typhi* it was 3.125 mg $mL^{-1}$. The extract inhibited all the test organisms at various concentrations.

Al-Bayati and Al-Mola (2008) worked on the antimicrobial activity of *Tribulus terrestris* against *Staphylococcus aureus*, *Bacillus subtilis*, *B. cereus*, *Corynebacterium diphtheriae*, *Escherichia coli*, *Proteus vulgaris*, *Serratia marcescens*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans*. Plants extracts showed antimicrobial activity against tested microbes.

Hussain *et al.* (2009) stated that *Tribulus terrestris* had considerable antibacterial activity against *Staphylococcus aureus*, *Escherichia coli, Proteus vulgaris, Pseudomonas aerugiuosa,* and *Klebsiella*.

Nenaah (2010) tested alkaloids of *Peganum harmala* and reported significant differences between compounds depending on the microorganism tested and the application method. These alkaloids produced effect on different fungi.

Edziri *et al.* (2010) tested ethyl acetate, chloroform, butanol, and methanol extracts of *Peganum harmala* against bacteria and the chloroform extract displayed good results.

Islam *et al.* (2010) reported antibacterial activity of leaf extracts of *Ricinus communis* against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoneae,*

*S. progens*. All the extracts showed significant antibacterial properties. The acetone extracts showed maximum zone of inhibition. These findings suggested *Ricinus communis* as an effective antibacterial agent.

Verma *et al.* (2011) stated that hexane and methanol extracts (200mg/ml) of roots of *Ricinus communis* showed good activity against pathogenic bacterial and fungal strains while aqueous extracts had no antimicrobial activity against any of the tested pathogens. Hexane extracts (200mg/ml) showed antimicrobial activity against *Candida albicans* and *Aspergillus niger* fungal strains. Methanolic extracts (200mg/ml) were effective against *Escherichia coli* and *A. niger*.

Salahudin *et al*. (2011) reported that aqueous extracts from from *Azadirachata indica*, *Foeniculum vulgare*, *Peganum harmala*, *Ricinus communis, Ocimum basilicum* and *Cichorium intybus* against *Staphylococcus aureus* showed antibacterial activity against *Staphylococcus aureus*. The stronger antibacterial activity was observed in *Peganum harmala* extract against *Staphylococcus aureus*.

Takhi *et al.* (2011) reported antimicrobial potential of *Peganum harmala* and *Ricinus communis*. The alkaloidic extract of *Ricinus communis* was effective against *Escherichia coli* ATTC 25922 and the MIC value determined is 0.02 mg/ml.

Jain & Nafis (2011) reported that the aqueous leaf and pericarp extract of *Ricinus communis* and *Punica granatum* were inhibitory against *Candida albicans*, *Candida glabrata* and *Candida tropicalis* with zone of inhibition ranging between 17mm and 32 mm followed by aqueous pericarp extract of *Punica granatum* that inhibited *Candida glabrata* and *Candida albicans* with zone of growth inhibition between 24 mm and 37mm. All the *Candida* spp except *Candida tropicalis* were resistant to the positive control, ketoconazole. Both the plants contained tannins, steroids and flavonoids.

In Pakistan work on the antimicrobial activity has been done which is reviewed as follows.

Hussain *et al*. (2010) reported the antimicrobial activity of the crude extract of *Polygonum persicaria*, *Rumex hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Polygonum plebejum* and *Rheum australe* against six bacterial species. Of which,

*Citrobacter frundii*, *Escherichia coli*, *Enterobacter aerogenes* and *Staphylococcus aureus* were the most susceptible to crude extract with. *Fusarium solani*, *Aspergillus flavus* and *Aspergillus niger* were more susceptible fungi to crude extracts.

Khattak *et al.* (2005) reported that the extracts of *Curcuma longa* and *Alpinia galanga* had no antibacterial activity.

Naseem *et al.* (2011) reported that *Mentha spicata* crude extract followed by ethyl acetate had lowest antibacterial activity. Similar results were reported for antifungal activity, for the same extracts and plants.

**iii. Cytotoxic activity**

Muthiah (2008a) reported that the aqueous and ethanolic crude extracts of *Hopea utilis* had strong cytotoxicity against the brine shrimps with LD50 values of 1.64 µg/ml and 1.34 µg/ml. Maridass (2008) found that ethanolic extracts of *Cinnamomum travancoricum, Cinnamomum walaiwarense, Cinnamomum wightii, Cinnamomum. Cinnamomum verum, Cinnamomum sulphuratum, Cinnamomum riparium,* and *Cinnamomum perrottetii* had strong cytotoxic activity against brine shrimps.

Manivasagan *et al.* (2009) tested twenty five strains of an actinobacteria, *Streptomyces* sp. PM-32 for their antimicrobial activityand it showed activity against the pathogenic microorganisms.

Sharififar *et al.* (2009) reported that essential oils from *Heracleum persicum*, *Nigella arvensis*, *Cinnamomum zeylanicum* and *Zingiber officinale* exhibit cytotoxicity and oils of *Heracleum persicum* and *Cinnamomum zeylanicum* had cytotoxicity as well.

Papiya *et al.* (2010) reported cytotoxic activity of *Berberis aristata* stem and rhizomes of *Hemidesmus indicus* on MCF7 breast cancer cell line. Flavonoids, terpenoids, alkaloids, phenols and sterols were detected. In Pakistan the studies on cytotoxic effects are reviewed as follows.

Zaidi *et al.* (2006) reported that methanolic extract of *Juniperus excelsa* showed high cytoxicity for brine shrimps. Hussain *et al.* (2007) reported the

cytotoxic, antitumor, and DNA damage assay of *Fagonia cretica.* Significant cytotoxicity was found against brine shrimps. Significant antitumor activity was found against all the tumor-inducing *Agrobacterium* strains tested (At6, At10 and At77), with maximum tumor inhibition (77.04%) against At10. Extract did not show any lethal activity against *Agrobacterium tumefaciens* strains, and no DNA damaging activity was observed. The results indicated a strong anti-cancerous potential of *Fagonic cretica.*

**iv. Phytotoxic activity**

Khan *et al.* (2002) reported that the *Abroma augusta* seed oil inhibited the growth of *Lemna aequinoctials* by 82% at 500 µm/ml.

Allan and Adkins (2005) reported that *Chamaesyce, hyssopifolia* and, *Melaleuca, Farnesiana, Ageratum, conyzoides* and *Alphitonia* showed phytotoxicity against *Lemna aequinoctialis*

Nisar *et al.* (2009) reported the phytotoxic effect of *Indigofera gerardiana.* Various fractions showed herbicidal activity against *Lemna minor. Indigofera gerardiana* was shown by *in-vitro* assays to be a potential source for natural herbicidal agents.

Uzair *et al.* (2009) stated that methanol extracts of *Euphorbia helioscopia* had radical-scavenging activity.

Ateeq-ur-Rehman *et al.* (2009) observed the phytotoxic activity of the methanol extract of *Thymus serpyllum* against *Lemna minor* L. and it also reduced radish seed germination and growth.

Hussain *et al.* (2009) working on the phytotoxic activity of *Nepeta juncea.* reported that n-hexane , n-butanol, chloroform and water fractions showed insignificant phytotoxic effect against *Lemna minor*. Nisar *et al.* (2010) worked on crude methanolic extracts of *Impatiens bicolor* along with n-hexane, dichloromethane, ethyl acetate, n-butanol and aqueous for phytotoxic activity. All fractions exhibited significant phytotoxicity. Khuda *et al.* (2012) reported the phytotoxic activity of

chloroform fractions from *Achyranthes aspera* and ethyl acetate fraction from *Duchesnea indica* showed significant phytotoxic activities.

**v. Insecticidal activity**

Kanvil *et al.* (2006) observed that extracts of *Saussurea lappa*, *Peganum harmala* and *Valeriana officinalis* showed repellening action against *Tribolium castaneum*. The plant extracts showed higher repellency during initial days and it gradually reduced with the passage of time *Valeriana officianalis* persisted better than *S. lappa* in petroleum ether while *Saussurea lappa* was better in ethanol extract.

Singh *et al.* (2008) observed that the acetone extracts of leaves and seeds from the *Tribulus terrestris* showed strong larvicidal activit against *Aedes culicifacies* species. It is confirmed that seed acetone extract of *Tribulus terrestris* was more effective than leaf extracts. The seed acetone extract showed strong repelient activity against adults mosquitoes.

Jbilou *et al.* (2007) observed that the methanol extract of *Peganum harmala* seeds inhibited the larval growth and food intake of *Tribolium castaneum*. All doses increased the duration of larval period and completely inhibited F1 progeny production. Furthermore, protein, lipid and glycogen contents reduced. Histological studies showed that *a* extracts caused a severe cytotoxicity on the epithelial cells of the midgut resulting in marked vacuolization of the cytoplasm and a large intercellular space.

In Pakistan the studies made on the insecticidal activity are reviewed as follows.

In Pakistan the studies made on the insecticidal activity are reviewed as follows.

Saljoqi *et al.* (2006) stated that all the ethanol extracts of *Melia azadarach*, *Myrtus communis*, *Mentha longifolia*, *Peganum harmala* and *Cymbopogon citrates* had repellent and lethal effects against rice weevil, *Sitophilus oryzae*. *Melia azadarach* showed mortality, followed by *Myrtus communis* (48.40%), *Mentha*

*longifolia* (47.40%) and *Melia azadarach* (46.80%), while *Peganum harmala* (16.80%) found less effective followed by *Cymbopogon citrates* (35.20%).

Sattar *et al.* (2005) working on the ecology of fruit tree termites. The results showed that 37 out of 117 apricot *Prunus armeniaca* L. variety Trevatt, trees were infested by termites, *Microtermes obesi,* and *Odontotermes lokanandi*. Both the species were interacting with each other. *Microtermes obesi* was dominant in the experimental field. Mean yield per trap ranged from 0.32-4.05g and 0.48- 1.47g for *M. obesi* and *O. okanandi* infested plots, respectively. It was found that termite activity was affected by atmospheric temperature and relative humidity.

**vi. Phytochemical screening**

Edeoga *et al.* (2005) reported saponins, alkaloid, terpenoid, flavonoids, phlobatannin from *Cleome nutidosperma,* and other medicinal plants. Delazar *et al.* (2006) reported five flavonoid glycosides from the methanol extract of the aerial parts of *Chrozophora tinctoria*. Ogunkunle & Ladejobi (2006) stated that *Senna tora, S. occidentalis*, *S. alata, S. podocarpa* and *S. siamea* had alkaloids, flavonoids, tannins, phlobatannins, saponins and anthraquinones.

Dinchev *et al.* (2008) identified steroidal saponins, protodioscin, prototribestin, pseudoprotodioscin, dioscin, tribestin, tribulosin and flavonoid rutin from *Tribulus terrestris*. These contents varied with region, sample collection, plant part, and stage of plant development. Reuben and Abdulrahman (2008) stated that *Croton zambesicus* had carbohydrates, and other constituents. Sule & Sani (2008) reported steroidal compounds and alkaloids from crude methanolic extract of *Ricinus communis*. White crystals of melting point 201-202 °C were isolated from the extracts.

Okoli *et al.* (2009) reported tannins, saponins, flavonoids, cardiac glycosides, alkaloids and steroids from *Bidens pilosa, Euphorbia heterophylla, Euphorbia hirta* and *Phyllanthus amarus*. Mughal *et al.* (2010) reported carbohydrates, proteins, alkaloids, phytosterol, phenol, flavonoids, tannins, saponins and phlobatannins from *Euphorbia prostrata, Euphorbia hirta, Euphorbia splendens, Ricinus communis* and *Jatropha integearrima* (Family: Euphorbiaceae). Kadri *et al.* (2011) reported hujone, 1, 8-cineole, pinene, camphor and camphene in aerial parts of *Ricinus communis*. The

literature available on the phytochemical screening of plants from Pakistan is summarized as follows:

Hussain *et al.* (2010) reported alkaloid, saponins, flavonoids, phenols, tannins, ascorbic acid, riboflavin, thiamine and niacin from *Sonchus eruca* and *Sonchus asper*.

Shahwar *et al.* (2010) reported total phenolic contents from extract of plants of family Euphorbiaceae in petroleum ether, chloroform, ethyl acetate and methanol/n-butanol. Maximum phenolic contents were in n-butanol extract of *Ricinus communis*.

Khan *et al.* (2011) reported the phytochemical screening of twenty different medicinal plants, which showed the presence of reducing sugar, anthraquinones, terpenoids, flavonoids, saponins, tannins, alkaloids and cardiac glycosides were present. However in some cases the reducing suger and the tannins were absent. Hussain *et al.* (2011) reported alkaloid, flavonoid, saponin, phenol and tannin contents from *Lepidium sativum, Nerium oleander, Ranunculus repens, Tecoma stans* and *Urtica dioca*. Phytochemical inveigation of plant samples determines that alkaloid (63.6%) and flavonoid (0.91%) were highest in *N. oleander,* saponin (11%) and phenol (0.031) in *T. stans,* tannin (0.61%) in *L. sativum*.

**vii. Nutritional and mineral composition**

Abolaji *et al.* (2007) working on nutritional qualities of *Xylopia aethiopica, Blighia sapida* and *Parinari polyandra*, commonly used by pregnant women of western part of Nigeria, detected reasonable amounts of Ca, Zn and Na. Abou-Arab *et al.* (2010) observed that *Jatropha curcas* seeds had good source of protein (32.88%), oil (27.36%) and carbohydrates (30.11%). The seeds are rich in micro-elements, including Mn, Fe, and Zn, which recorded 28.37, 0.38 and 47.13 mg/kg, respectively. In macro-elements, like K, Ca, Na, Mg and P, also recorded 103.13, 34.21, 8.44, 109.89 and 185.17 mg/kg respectively. The seeds contained 52.59 mg/100 g, 25.58 mg/g, 39.95 mg/100 g and 1.51 g/100 g of phytic acid, trypsin inhibitor activity, total phenols and saponins, respectively. They concluded that seeds were nutritionally promising because of its high nutrient content and low antinutrient level. Gowrishankar *et al.* (2010) detected Cl, K, Ca, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, Br, and Sr in *Tinospora cordifolia*, *Ocimum sanctum, Moringa oleifera,* and *Phyllanthus niruri* with varying concentrations. The results showed very high

concentrations of Cl, K, and Ca in all the leaf samples, appreciable levels of Mn in all plants, high Zn content in *Tinospora cordifolia*, and the aqueous extract of *Moringa* leaves compared to others and relative higher concentrations of Cr in all the plants.

Sabir & Riaz (2005) reported that *Elaeagnus umbellata* berries showed variation in vitamin C (13.8-16.9 mg/100 g), seed oil (5.7-6.1%), oil in pulp (7.6-8.1%), reducing sugar (6.8-8.4%), non-reducing sugar (1.4-2.2%), protein (2.5-5.1%) and chlorophyll content (5.3-6.8%) in leaves, while the minerals composition showed high contents of potassium, calcium, magnesium, iron and phosphorus. Ahmed & Javed (2007) analysed *Amaranthus viridis, Hedera helix* and *Urtica dioca* for ash, total protein, fats and carbohydrates. Protein contents in *A. viridis* were 34.2%, *H. nepalensis* 16.2% and *Urtica dioica* 22%. Fats in *Amaranthus viridis* were 5.3%, *H. nepalensis* 35.1% and in *Urtica dioica* 4%. Carbohydrates in *A viridis, H. nepalensis and U. dioica* are 44.1, 10 and 30%, respectively. Bukhsh *et al*. (2007) stated that *Carthamus oxycantha, Eruca sativa* and *Plantago ovata* contained trace elements like Cu, Fe, Mn, Mg, Cr, Zn, Mo, K, P, Ca and Na. Khanzada *et al*. (2008) reported fatty acid, elements and total protein from *Calotropis procera* from Sindh with leaves, roots and bark being exported as popular medicine to fight many human and animal diseases. Bhatt *et al*., (2008) reported that nutritional value of the aerial parts of *Artemesia maritima* contained high amount of vitamin C followed by phenolics and total carbohydrate.

Hussain *et al*. (2009) investigated *Coriandrum sativum*, *Amomum subulatum, Ziziphus vulgaris*, *Punica granatum, Pistacia vera, Mentha sylvestris, Sphaeranthus hirtius,* and *Cassia angustifolia* for proximate and micronutrient analysis. *Pistacia vera* showed higher concentrations of fat, protein and energy values compared to the other species. Fabulous concentration level of Fe was revealed in *S. hiritus,* considerable amount in *Mentha sylvestris*. Zinc was highest in *M. sylvestris* while the Co concentration was highest in *S. hiritus*. Saeed & Sabir (2005) reported sixteen trace elements (P, Ca, Mg, K, Na, Fe, Cu, Ni, Co, Ba, Zn, Cr, Mn, Sr, Cd and Al) from the fruit pulp of *Ficus carica*. Sabir *et al*. (2005) reported high contents of potassium (140-360 ppm), sodium (20-80 ppm), calcium (70-98 ppm), magnecium (150-240 ppm), iron (40-150 ppm), and phosphorus (110-133 ppm) in *Hippophae rhamnoides turkestanica* berries from Pakistan origin.

**Basis of choosing these plants**

The selection of these plants was based on ethno-botanical and ethno medicinal traditional knowledge of plants, as plants were used for the treatment of different diseases particularly in KPK. Apart from medicinal uses, the investigated plants were used as fodder, wormicidal, for fuel, air purifier, as antilice agent, protection against evil eyes, food, for corrosive properties, resistant to termites, tanning and dyeing.

Limited references were available on the insecticidal, antimicrobial activity, stomatal and phytochemical study of *Tribulus terrestris* and *Ricinus communis*. No references were available on macroscopical, scanning electron microscope (SEM), phytotoxic, cytotoxic, termicidal, antifungal, elemental, nutritional, essential oil and fluorescence characters of *Fagonia cretica* L., *Peganum harmala* L., *Tribulus terrestris* L., *Chrozophora tinctoria* (L.) Raf. and *Ricinus communis*.

# AIMS AND OBJECTIVES

This study had the following aims and objectives.

1. To document the traditional knowledge of important species of family Zygophyllaceae and Euphorbiaceae found in KPK.
2. To study the pharmacognostic and botanical features of plants and powdered drug (tissues in fragments form).
3. To assess the antibacterial activity.
4. To evaluate the antifungal activity.
5. To study the cytotoxic effect.
6. To find out the phytotoxic potential.
7. To evaluate the insecticidal activity.
8. To screen the plants microchemically.
9. To investigate the nutritional value.
10. To carry out mineral analysis of therapeutic importance.
11. To extract the essential oils and identify different phytochemicals.
12. To study the fluorescence character of different parts of plants.

# CHAPTER-3

# MATERIALS AND METHODS

# CHAPTER 3

# MATERIALS AND METHODS

### i. Collection and Preservation

Fresh plants of *Fagonia cretica* L., *Peganum harmala* L., *Tribulus terrestris* L., *Chrozophora tinctoria* (L.) Raf. and *Ricinus communis* L., were collected from Peshawar and Attock Hills. *Fagonia cretica* was collected in June 2009 (Flowering stage); *Peganum harmala* in June 2009 (Fruiting stage); *Tribulus terrestris* in June 2009 (Flowering and fruiting stage); *Chrozophora tinctoria* in August 2009 (Flowering and fruiting stage); *Ricinus communis* in August 2009 (Fruiting stage) and in December 2009 in (Flowering stage). Each specimen was cleaned, washed, separated and dried in shade for 15 days. These specimens were used for pharmacognostic, anatomical, phytochemical, nutritional, elemental analysis, oil extraction, florescence characters and biological activities. The specimens were powdered and meshed through 60 mm and preserved in airtight bottles. Macroscopical, anatomical and stomatal features were studied from fresh specimens.

### ii. Macroscopical Study

Macroscopical or organoleptic study was carried out in fresh and dried states of the plant following standard techniques (Trease & Evans, 1992; Bhattacharya *et al.*, 2009; Evans, 2009). Root features included colour, odour, taste, size, shape, fracture, texture and position. Stem features included branches, colour, odour, taste, size, shape, fracture and texture. Leaf features included colour, odour, taste, size, shape, fracture, margin, venation, surface, apex, type, petiole condition, incision and base. Fruit features included colour, odour, taste, size, shape, texture, kind, marking, seeds number and position.

### iii. Anatomical Study

The anatomical sections of roots, stems, leaves and fruits were cut with free hand sectioning and thin sections were stained after Puruis (1966) . Measurements of various tissues were taken. Mean (average value was calculated from five readings), frequently, minimum, and maximum occurred values were recorded. Photomicrographs were taken with the help of microscopic camera. The statistical

evaluation for various anatomical features of each part of the five plants and stomatal study was done following methods of Bluman (2009) and Sokal & Rohlf (1995).

**iv. Scanning Electron Microscopy**

The microscopic study of different tissues of various parts of *Fagonia cretica Peganum harmala, Tribulus terrestris, Chrozophora tinctoria,* and *Ricinus communis* were carried out at Centralized Resourse Laboratory, Department of Physics, University of Peshawar using Scanning Electron Microscope (SEM) , Model (JSM-5910), company, (JEOL), Japan. The images were taken and tissues were identified following Kahraman *et al.* (2009); Korejo *et al.* (2010). Only those parts were considered for SEM study that had been used medicinally and the literature review proved their therapeutic value.

**v. Stomatal Study**

The fresh leaves were immersed in water to prevent desiccation. Peels from the abaxial and adaxial surfaces were obtained and cleaned with distilled water. They were mounted in Canada balsam for examination (Wallis, 2005; Evans, 2009; Robert *et al*. 2009; Ozdemir *et al*., 2008). The type of stomata, stomatal distribution, number of stomata, number of epidermal cells, stomatal index, size of stomatal pore, size of guard cells, open and close stomata were observed. Average value was calculated from ten readings (Wallis, 2005). The average number of palisade cells beneath each upper epidermal cell is termed the palisade ratio (Venkatesh *et al*., 2004; Betty *et al*., 2008 ; Evans, 2009). Pieces of leaf about 2 $mm^2$ or powder were cleaned by boiling with chloral hydrate solution, mounted and examined with a 4 mm objective. Epidermal cells and the palisade cells lying below them were traced. First a number of groups each of four epidermal cells were traced and their outlines inked in to make them more conspicuous. The palisade cells lying beneath each group were then focused and traced. The palisade cells in each group were counted. The cells more than half covered by the epidermal cells were counted. The value obtained was divided by 4 to get palisade ratio of that group (Betty *et al*., 2008).

**vi. Biological activities**

Five types of bioassays including antibacterial, antifungal, phytotoxic, cytotoxic and insecticidal were done. The antibacterial and antifungal activity data were subjected to T-test following (Cox, 1967). The phytotoxic, cytotoxic and

insecticidal activity data were subjected to two way analysis of variance (ANOVA) and differences between samples were determined by F-test using the Statistical Analysis System (SAS, 1999) program. P values < 0.05 were regarded as significant level (Khan *et al.,* 2008). Mortality in cytotoxic and insecticidal activity was corrected using Abbott's formula (Abbott *et al.,* 1925)

**vii. Antibacterial activity**

Both gram negative and gram positive strains *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa* were used for testing. Nutrient agar medium was used for the growth of bacteria for agar diffusion method (Mariam *et al*., 1993) and nutrient broth medium for serial dilution method (Spooner & Sykes, 1972). Fifty grams of each plant sample was soaked in 250 ml 70 % methanol and n-hexane for 72 hours. All plant extracts were filtered through Whatman filter paper No. 1823. This process was repeated 3 times. Each extract was concentrated using rotatory evaporator. These extracts were stored at 4° C prior to use. The standard drug (Imepinem) was also used. The antibacterial study of the crude extracts was carried out by agar well diffusion method (Carron *et al*., 1987).

**viii. Antifungal activity**

Antifungal activity of the crude extract was evaluated by agar tube dilution method following Atta-ur-Rehman *et al.* (1991). *Aspergillus niger*, *Aspergillus flavus*, , *Fusarium solani*, *Candida albicans* and *Candida glabarata* were used as test species in this study. Mucanazole was used as a standard drug.

**ix. Cytotoxic activity**

Fifty g of each plant sample was soaked in 250 ml 70 % methanol and n-hexane for 72 hours and passed through Whatman filter paper No. 1823. This process was repeated three times. Evaporating in a rotatory evaporator at 40 °C, concentrated the extracts. These extracts were stored at 4°C prior to use. The methanolic and n-hexane plant extracts i.e., test sample (10 mg) were dissolved in 1 ml of dimethylsulphoxide (DMSO) and from this stock solution transferred five concentrations i.e., 10 µl, 50 µl, 100 µl, 300 µl and 1000 µl to sterilised vials. Three replicates were taken.

The eggs of brine shrimps were stored at low temperatures (4 °C) to maintain viability. Half-filled the hatching tray (a rectangular dish (22x32 cm)) with filtered

brine solution was then sprinkled on 50 mg brine shrimp eggs and incubated at 37 °C. After 2-days hatching and maturation as nauplii placed 10 larvae/vials, using a Pasteur pipette. The volume was made to 10 ml with seawater and incubated at 25-27 °C for 24 hours under illumination. Supplement other vials with solvent, serving as negative controls, respectively. The cytotoxic activity of the crude extracts of the plants was carried out following the method of Meyer *et al.* (1982). Five concentrations 10 µl, 50 µl, 100 µl, 300 µl and 1000 µl of potassium dichromate were used as positive control. After 24 hours, the vials were observed and the number of servivors in each vial were counted following (Padmaja *et al.*, 2002 ; Moshafi *et al.*, 2009).

**x. Phytotoxic activity**

*Lemna minor* was used as test organism. The experiment condition included Light intensity (9000 Lux), Day length (12 hours), incubation condition (28° + 1 ° C, 56 + 1% rh). Ten plants per flask were used. Number of plants per dose = Single with a rosette of three fronds. Phytotoxic activity of the extracts was carried out against the *Lemna minor* following McLaughlin *et al.* (1991) & Nisar *et al.* (2011).

**Preparation of aqueous extract experiment for phytotoxic activity.**

Ten gram of *Fagonia cretica, Peganum harmala, Tribulus terrestris, Chrozophora tinctoria* and *Ricinus communis* were soaked in 90 ml of distilled water and the same plants were soaked in 80 ml of distilled as two standard solutions of distilled water in conical flasks. The flasks were covered with aluminum foil and allowed to stand at 25 ° C for 48 hrs. The extracts were filtered by Whatmann filter paper. The aqueous extracts were further diluted. Serial dilutions of extracts (10 ml + 90 ml, 30 ml+70 ml, 50 ml+50 ml, 90 ml +10 ml ) plants extracts + distilled water, respectively were made of the stock solutions and tested for phytotoxicity against *Lemna minor.*

**Preparation of methanolic and n-hexane extract experiment for phytotoxic activity.**

Preparation of E-medium: 1. E-medium was prepared by mixing various constituents in 1000 ml distilled water and pH was adjusted between 5.5-6.0 by adding KOH pellets. The constituents and their respective proportions are : 1. Potassium dihydrogen phosphate (0.68gm/L). 2. Potassium nitrate (1.515gm/L). 3.

Calcium nitrare (1.180gm/L). 4. Magnesium sulphate (0.492gm/L). 5. Boric acid (0.00286gm/L). 6. Magenous chloride (0.00362gm/L). 7. Ferric chloride (0.00540gm/L). 8. Zinc sulphate (0.00022gm/L). 9. Copper sulphate (0.00022gm/L) 10. Sodium molybdate. 11. Ethylene diamino tetracetic acid. The medium was autoclaved at 121°C for 15 min. Fifteen (15 mg) of extract was dissolved in 1.5 ml of solvent (Methanol/n-hexane) serving as stock solution. Three sterilized flasks were incubated with 10 µl, 100 µl and 1000 µl of solution pipetted from the stock solution and added to the flasks containing *Lemna* plants. Twenty ml of E-medium was added to each flask. Other flasks were supplemented with E-medium only as control. Plants were examined daily during incubation. Flasks were placed in growth cabinet for 7 days. The number of fronds / flask were counted and recorded on seventh day. Results were analysed as growth regulation in percentage (Nisar *et al*., 2011).

**xi. Insecticidal activity**

**Insecticidal activity of *Tribolium castaneum***

The insecticidal activity was done following Khan *et al.* (2008).

Twenty grams (20g) of ground plant material was soaked in 80 ml of distilled water for 48 hrs. They were filtered using standard filter paper. Different dilutions of the plant extracts were made 20%, 15%, 10% and 5%. using following formula. M1C1= M2C2,

M1: Known volume of standard solution.

C1: Concentration (%) of standard solution.

M2: Wanted volume.

C2: Wanted concentration (%).

Each extract was then used for insecticidal activity against *Tribolium castaneum* Herbst., stored insect at NIFA (Nuclear Institute for Food and Agriculture) Tarnab, Peshawar.

Day-1 : The filter papers were cut according to the size of petri plate (9 cm or 90 mm) and were placed in the plates. The test sample was poured over the filter paper with the help of micropipette. The plates were left for 24 hrs to evaporate the solvent completely. Day-2: Next day (after the evaporation of solvent) *Tribolium castaneum* (10 healthy insects) were kept in each plate (test and control) with the help

of a clean brush. The healthy and active insects of same size and age were selected. The plates were incubated at 27 °C for 24 hours with 60% relative humidity in growth chamber. Day-3: The number of the survived insects were counted. The percent inhibition or percentage mortality was calculated with the help of following formula:

$$\text{Mortality (\%)} = 100 - \frac{\text{No of insects alive in test}}{\text{No of insects alive in control}} \times 100$$

**xii. Termicidal activity**

Popular wood stakes (2.5 thick x 4 width x 28 length cm) were buried in soil near the base of each tree at NIFA (Nuclear Institute for Food and Agriculture) Tarnab, Peshawar. The stakes were checked fortnightly. When a stake was found infested by termites, a NIFA-TERMAP was installed on that point. The stakes as well as NIFA-TERMAP were checked fortnightly. If traps were found infested by termites it was replaced with a new one. The infested traps were brought to the laboratory to separate the termites from the soil and debris. The termites thus collected were weighed. The number of soldiers, workers, and winged padded was also determined in 0.5 g termite sample. The total number of termites was obtained by multiplying the number with the total weight. From each trap, 5-10 workers and soldiers were preserved in 80% alcohol for identification of the species. Identification of termites was done by using the key of Chaudhry *et al.* (1972); Sattar *et al.*, (2007).

**Preparation of Extracts**:

Twenty g whole plant was ground and soaked in 80 ml of distilled water for 24 hours. After 24 hours the plant extract was filtered and kept in refrigerator. Three replicates were taken. The petri dishes were first topped by double filter paper and moistened with plant extracts. Control contained only distilled water. The termites before application were separated and counted under magnifying lens for exact counting. In each petri dish 25 healthy, active termites were placed. All the petri dishes were kept in desiccators and kept in air conditioned room having temperature of 25 °C at 75% relative humidity. The petri dishes were observed after 24, 48, 72, hours or up to 8 days. The number of survived termites were recorded.

**xiii Chemical Evaluation**

**xiv. Phytochemical Screening**

Chemical tests for alkaloids, mucilage, anthraquinone, saponins, fat and oil ,tannin, flavonoids, steroids, terpenoids, cardiac glycosides, phlobatannins and chemical group tests namely phenolic, carboxylic acid, Lucas test for alcohol, potassium permanganate test for unsaturation or hydroxyl group, Tollen's test for aldehyde and/or ketone groups were carried out on the methanolic and n-hexane extracts using standard procedures to identify the constituents as described by Harborne (1973), Ngoci *et al.*( 2011) and Edeoga *et al.* (2005).

**xv. Proximate composition**

Plant parts were powdered for proximate composition study. Ten g of the powdered sample was processed for various parameters such as crude proteins, crude fiber, crude fat or ether extracts, ash contents, moisture contents, carbohydrate contents, gross energy and the methods used for these parameters were after the Association of Official Analytical Chemists methods (A.O.A.C. 2000) and Garrett & Johnson (1983). The formula used for Gross Energy is as follows

GE (Kcal/g) = 5.72 x (protein) + 9.5 (fat) + 4.79 (fiber) + 4.03 (carbohydrate) = Gross Energy Value.

The data were subjected to two way analysis of variance (ANOVA) and differences between samples were determined by F-test using the Statistical Analysis System (SAS, 1999) program. P values $< 0.05$ were regarded as significant level (Khan *et al.,* 2008).

**xvi. Mineral Composition**

A small amount i.e., 0.5 g of the powdered plant material of each plant was taken in a 50 ml conical flask and 10 ml nitric acid ($HNO_3$) was added in it. Next day, 4 ml perchloric acid ($HClO_4$) was added in it and it was boiled on a hot plate in a fume hood. After few minutes the yellowish color of plant material changed into white fumes and it indicated that digestion was completed. The flasks were removed, cooled down and 100 ml distilled water was added. It was filtered with filter paper (Whatmann No. 42) and filterates were collected in labeled plastic bottles. These solutions were analysed for the elements of interest utilizing Atomic Absorption

Spectrometer (Zafar *et al.*, 2010). The results of elemental analysis were subjected to two way analysis of variance (ANOVA) and differences between samples were determined by F-test using the Statistical Analysis System (SAS, 1999) program. P values < 0.05 were regarded as significant level (Khan *et al.,* 2008).

**xvii. Analysis of essential oils**

Sample Preparation

Dilute approximately 40 mg of oil samples, weighed accurately up to 0.1 mg, with 2 ml of dichloromethane and filtered through 0.45 µm membrane filter and injected 1 µl to GC-MS using auto injection system.

The obtained essential oil from the aerial parts of *Fagonia cretica*, *Peganum harmala, Tribulus terrestris*, *Chrozophora tinctoria* and *Ricinus communis* was analyzed using Shimadzu Model QP 2010 plus GC-MS. The separation was performed on a column (30 m × id 0.25 mm, thickness 0.25 $\mu$m 95% Dimethyl-5% diphenyl polysilphenylene; DB-5MS, Agilent technologies, USA) using Helium as the carrier gas with a splitting ratio of 1:50. The injector and the interface temperature were 240 ºC and 240 ºC, respectively. The *m/z* range was 40-500. The relative amounts (RA) of individual constituents of the oil are expressed as the percent peak area relative to the total peak area (Essien, *et al.* 2008) .

**xviii. Fluorescence Analysis**

Plant parts were crushed using grinding machine and powdered samples were treated further. The colors of the powdered drugs of all the specimens were observed in daylight and under UV short wavelength (254 nm) and long wavelength (366 nm) by applying ammonia, picric acid, chloroform, ferric chloride, ether, ethanol, hydrochloric acid, distilled water iodine, acetic acid, sulphuric acid, concentrated nitric acid and butanol following the methods of Kokashi *et al.* (1958), Bhattacharya *et al.*(2009); Shahla & Deokule (2010).

# RESULTS AND DISCUSSION

# CHAPTER-4

# MACROSCOPICAL FEATURES

# CHAPTER 4

# MACROSCOPICAL FEATURES

**Root**

The root color was variable. It was yellow in *Fagonia cretica* and *Tribulus terrestris;* brown in *Peganum harmala;* light blue in *Chrozophora tinctoria* and yellow brown in *Ricinus communis* in the fresh state. In dry condition the root color was yellow in *Fagonia cretica* and *Tribulus terrestris;* brown in *Peganum harmala;* yellow brown in *Chrozophora tinctoria* and *Ricinus communis* (Table 1). These findings agree with those of Madhavan *et al.* (2010), Jain *et al.* (2011), Paul *et al.* (2011) and Rajendran *et al.* (2007) who reported similar colours of roots of *Asparagus gonoclades, Amaranthus spinosus*, *Acacia leucophloea* and *Ipomoea quamoclit*.

In the present study the root odour also varied among the tested species. It was walnut like in *Peganum harmala*; indistinct in *Fagonia cretica* and *Tribulus terrestris* ; irritant in *Chrozophora tinctoria* and agreeable in *Ricinus communis* in the fresh condition. In the dry state the root odour was similar to that of fresh material. Similar results were reported by (Jayalakshmi *et al.*, 2010; Gahlot *et al.,* 2011; Aneja 2011; Kumar *et al.*, 2010 and Rajendran *et al.*, 2007) in *Flemingia chappar*, *Amaranthus tricolor*, *Hibiscus micranthus*, *Ipomoea quamoclit* and *Imperata cylindrica* root.

The tastes of the herbs are important indicators of their properties. Sweet taste is nutritive, pungent is dispersing, sour is digestive and bitter is cooling and detoxifying (Tierra, 1990). The tastes of roots of the investigated plants were variable. It was bitter in *Fagonia cretica* and *Peganum harmala;* agreeable in *Tribulus terrestris* and *Ricinus communis;* and irritant in *Chrozophora tinctoria* in the fresh state.

The odour of the dried material was similar to that of fresh material in the present case (Table 1). These results agree with Rajan *et al.* (2011), Rathore *et al.* (2011), Sudhakar *et al.* (2011) and Nasreen & Radha (2011) who observed bitter and agreeable

tastes in roots of *Hemidesmus indicus*, *Fagonia schweinfurthii*, *Pimpinella tirupatiensis* and *Withania somnifera*.

The average length of roots was highest in *Chrozophora tinctoria* and lowest in *Fagonia cretica* in the fresh state. These results disagree with Rathore *et al*., (2011) who reported increased root length and thickness in *Fagonia schweinfurthii*. The average length of roots of all the investigated plants was higher in fresh condition than dried. The average thickness of roots was maximum in *Fagonia cretica* and minimum in *Chrozophora tinctoria* in the fresh state. The average breadth of roots was highest in *Peganum harmala* and lowest in *Tribulus terrestris* in the dry condition (Table 1). Many scientists (Kadam *et al*., 2012; Jain *et al*., 2011; Madhavan *et al*., 2010; Rajendran *et al*., 2007) reported increased thickness of roots of medicinal plants and their results disagree with the present case.

Fracture of the root was flexible in *Fagonia cretica*; *Peganum harmala*; *Tribulus terrestris*; *Chrozophora tinctoria* and *Ricinus communis* in the fresh state. In the dry condition the fractures were hard in all the plants except in *Tribulus terrestris* (Table 1). These findings agree with Prabhu *et al*. (2011), Srivastava *et al*. (2001), Singh *et al*. (2009) and Singh *et al*. (2012) who reported hard fractures of roots in *Berberis lycium*, *Cicer arietinum*, *Viburnum punctatum* and *Cryptolepsis sanguinolenta*.

**Stem**

The stem was green in all the plants except *Ricinus communis* having brownish black in the fresh state. It was green in *Fagonia cretica*. This agrees with Patel *et al*. (2012) who reported similar results in *Fagonia cretica*. *Tribulus terrestris* showed yellow color in *Peganum harmala* and *Chrozophora tinctoria* and brownish black in *Ricinus communis* when dried (Table 1). Similar colors variation was also recorded by Rajendren *et al*. (2007), Garg *et al*. (2011), Sher *et al*. (2011) and Kumar & Kiladi (2009) in stems of different medicinal plants.

Odours have a great influence on the digestive system and some pleasant odours stimulate the appetite while upleasant ones impair it. Odours have been used as a

diagnostic tool in the identification of the medicinal plants (Mahmud *et al*., 2010). The odour was indistinct in all the plants in fresh and dried conditions except in *Ricinus communis* that had chlorophyllous odour. These findings agree with Sen *et al*. (2011), Sinha *et al*. (1986) and Sher *et al*. (2011).

Taste both in fresh and dried condition was agreeable in *Peganum harmala* and *Chrozophora tinctoria*; indistinct in *Tribulus terrestris* and *Ricinus communis* and chlorophyllous in *Fagonia cretica* (Table 1). These results agree with Sinha *et al*. (1986), Prakash *et al*. (1986) who reported indistinct tastes of stem of *Alternanthera sessilisi* and *Polygonum glabrum*. The average length of the stem was highest in *Fagonia cretica* and lowest in *Tribulus terrestris* in both the fresh and dried condition. The average breadth of the stem was highest in *Ricinus communis* and lowest in *Fagonia cretica* in both the fresh and dried condition (Table 1). Identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive (Uthayakumari & Sumathy, 2011). The fracture of plant parts is an essential parameter for distinction of plant species. The stem fracture was flexible in *Fagonia cretica* and *Peganum harmala*; hard in *Tribulus terrestris* and *Chrozophora tinctoria*; soft in *Ricinus communis* in the fresh condition. When dried stem fracture was hard in *Fagonia cretica*, *Peganum harmala* and *Ricinus communis*; very hard in *Tribulus terrestris* and *Chrozophora tinctoria* (Table 1). These findings are in agreement with by Sudhakar *et al*. (2012) and Jarald *et al*. (2008) who reported hard fracture in *Syzygium alternifolium*.

**Leaf**

The colors of plant drugs have always served as useful key in faster and early identification of plant material and also served an important standardization parameter (Soni *et al*., 2011). The color of leaf in all the plants was green in fresh condition. When dried it was yellow in *Fagonia cretica*, *Peganum harmala*, *Ricinus communis*; green in *Tribulus terrestris* and *Chrozophora tinctoria*. These findings are supported by other wprkers (Ushir *et al*., 2010; Baranwal *et al*., 2012; Prajapati *et al*., 2008; Venkatesh *et al*., 2008).

In the present study the leaf odor was indistinct both in fresh and dried form in *Fagonia cretica* and *Tribulus terrestris*; agreeable in *Peganum harmala*; spicy and black pepper like in *Chrozophora tinctoria*; bitter and pungent in *Ricinus communis* (Table 2). Similar results were also recorded by Sen *et al.* (2011) who noticed indistinct odour in *Phyllanthus fraternus* (Euphorbiaceae) and *Convolvulus pluricaulis* leaves.

The taste of leaf in fresh and dried state was bitter in *Fagonia cretica, Peganum harmala*; indistinct in *Tribulus terrestris*; spicy and black pepper like in *Chrozophora tinctoria*. These findings agree with Nayak & Patel (2010), Solanki *et al.* (2011), Shruthi *et al.* (2010), Jain *et al.* (2010), Prajapati *et al.* (2008), Mukherjee *et al.* (2011) and Sen *et al.* (2011).

The average length of leaves was longest in *Ricinus communis* and lowest in *Fagonia cretica* in both the fresh and dried condition. In fresh and dried condition the average breadth of leaves was maximum in *Ricinus communis* and lowest in *Peganum harmala* (Table 2).

The shape of leaf both in fresh and dried state was linear in *Peganum harmala* and *Tribulus terrestris*; lanceolate in *Fagonia cretica*; ovate lanceolate in *Chrozophora tinctoria* and palmate in *Ricinus communis*. Similar findings were also recorded by Shruthi *et al.* (2010), Mehmud *et al.* (2010), Mathur *et al.* (2010), Prajapati & Patel (2010), Prajapati *et al.* (2008) and Chetan *et al.* (2011) who observed shape variation in leaves of different medicinal plants. Plants had natural variation in their form and structure and within single individual, parts are repeated which might differ both in form and structure from other similar parts. This variation is easily seen in leaves compared to stems and flowers that may show similar variation. There are three primary causes of this variation i.e., positional effects, environmental effects, and juvenility.

Fracture of the leaf was brittle in *Fagonia cretica, Peganum harmala, Tribulus terrestris* and *Chrozophora tinctoria*; flexible in *Ricinus communis* in fresh condition while in the dry condition while it was brittle in *Fagonia cretica, Peganum harmala, Tribulus terrestris*; papery in *Chrozophora tinctoria* and *Ricinus communis* in the dry

condition (Table 2). These findings are in accordance with that of Tapankumarmaity *et al.* (2000) who reported similar fractures in *Cassia tora.*

The apex of leaf was cuspidate in *Fagonia cretica, Peganum harmala*; pointed in *Tribulus terrestris*; acute in *Chrozophora tinctoria* and acuminate in *Ricinus communis.* These findings agree with Singh *et al.* (2010), Mohammad *et al.* (1999), Patil & Deokule (2010), Prabhu *at al.* (2011), Khyade & Vaikos (2009) and Shruthi *et al.* (2010). The surface of leaf was smooth in *Fagonia cretica, Peganum harmala* and *Tribulus terrestris*; hairy in *Chrozophora tinctoriai* and smooth irregular in *Ricinus communis*. Similar results were reported by other workers (Mohammad *at al.*, 1999; Bairagi *et al.*, 2012; Shruthi *et al.*, 2010; Srivastava *et al.*, 2007 and Sandeep *et al.*, 2010) who recorded smooth and hairy leaf surface in different medicinal plants.

**Fruit**

Macroscopical study is a technique based on morphology and sensory profiles of drugs. The macroscopical characters of the plants can serve as diagnostic parameters (Sumalatha *et al.*, 2012). The diameter of fruits was maximum in *Ricinus communis* and lowest in *Fagonia cretica* both in fresh and dried conditions.

The shape of the fruit was subglobose in *Fagonia cretica*, rounded in *Peganum harmala*; spiny in *Tribulus terrestris*; rounded-tribolate in *Chrozophora tinctoria* and cylindrical in *Ricinus communis* both in fresh and dried states. These results agree with the findings of Selvadurai *et al.* (2012) who reported similar fruit shapes in medicinal plants. Literature review suggests that there is a lot of variation in the fruit colors of medicinal plants. Fruits colors differentiation would be of immense value in identification and authentication of plant drug and can prevent its adulteration.

The fruits colour varied among the tested plants. It was green in *Fagonia cretica, Tribulus terrestris*, *Chrozophora tinctoria*; yellow in *Peganum harmala*, and spotted in *Ricinus communis* in fresh conditions. In dry condition it became yellow in *Fagonia cretica* and *Peganum harmala*; green in *Tribulus terrestris*; bluish black in *Chrozophora tinctoria* and spotted in *Ricinus communis.* This color variation i.e., yellow and buish lack

is due to the fruits maturity and this agrees with the findings of Kadam *et al.* (2010) who recorded similar results in the fruits of *Solnum xanthocarpum*. These results agree with those of Sudhakar *et al.* (2012); Deepika *et al.* (2011); Singh *et al.* (2010) and Srivastava *et al.* (2007) who observed similar fruit colors in some selected medicinal plants.

The fruit odour remained indistinct in *Tribulus terrestris*, *Chrozophora tinctoria* and *Ricinus communis*; odourless in *Fagonia cretica* and tobacco-like in *Peganum harmala* in the fresh and dried conditions. These findings are similar to that of Singh and Sharma (2010); Kishorkumar and Ajaykumar, (2012). The macroscopic studies revealed important characteristics, like typical tongue sensitizing aromatic taste and characteristic odour of fresh and dried fruits, which are useful diagnostic characters (Singh & Sharma, 2010).

The taste of fruit was bitter in *Fagonia cretica*, *Peganum harmala*, *Chrozophora tinctoria* and *Ricinus communis;* unpleasant in *Tribulus terrestris* in the fresh and dry condition (Table 2). These findings agree with Kadam *et al.* (2010), Singh & Sharma (2010) and Mohan & Sanjay (2010). The fruits were found at lower position in *Fagonia cretica*; at top position in *Peganum harmala* and *Ricinus communis*; at upper position in *Tribulus terrestris* and hanging in *Chrozophora tinctoria* in the present study.

There is no macroscopical or organoleptic study on the presently studied traditional drugs, and this investigation was carried out to lay down standards which could be useful to detect the authenticity of these useful plants. Macroscopical features examined might serve as tool for identification of the plants.

The process of standardization can be achieved by stepwise pharmacognostic studies. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics (Kalidass & Mohan, 2010). w

Table 1. Comparative macroscopicol features of root and stem of some members of family Zygophyllaceae and Euphorbiaceae.

| Family | Color | Odour | Taste | Size (cm) | Shape | Fracture | Texture |
|---|---|---|---|---|---|---|---|
| **Zygophyllaceae** | | | | | | | |
| | F D | F D | F D | F D | F D | F D | F D |
| **Root** | | | | | | | |
| *Fagonia cretica* L. | Y Y | In In | Bt Bt | L= 10.15, L= 9.0, B= 8.2 B= 0.71 | Co Co | Fl Hr | Bri Hr |
| *Peganum harmala* L. | Br Br | In In | Bt Bt | L= 15.0, L= 13.25, B= 1.76; B= 1.63 | Tw Tw | Fl Hr | Bri Hr |
| *Tribulus terrestris* L. | Y Y | In In | Ag Ag | L=10.3 ; L= 9.29 B= 0.36 ; B= 0.22 | Co Co | Fl Fl | Bri Hr |
| **Euphorbiaceae** | | | | | | | |
| *Chrozophora tinctoria* L.(Raf). | Lb Lb | Iri Iri | Swt Swt | L=24.8 ; L= 22.5 B= 1.03; B= 0.8 | Fu Fu | Sf Hr | Bi Hr |
| *Ricinus communis* L. | YBr YB | Ag Ag | Ag Ag | L=11.5; L=11.3 B=2.25; B = 0.6 | tw tw | Fl Hr | Hr Hr |
| **Zygophyllaceae** | | | | | | | |
| **Stem** | | | | | | | |
| *Fagonia cretica* L. | Gr Gr | In In | Ch Ch | L= 0.89, L= 0.87, | Cy Cy | Fl Hr | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | B= 0.24; B= 0.13 | | | |
| *Peganum harmala* L. | Br Br | W W | Bt Bt | L= 2.21 ; L= 1.5<br>B= 0.2; B= 0.1 | Tw Tw | Fl Hr | |
| *Tribulus terrestris* L. | Gr Gr | In In | In In | L=1.8 ; L= 1.6 ;<br>B= 0.6 ; B= 0.5 | Cy Cy | Hr To | |
| **Euphorbiaceae** | | | | | | | |
| *Chrozophora tinctoria* L.(Raf). | Gr y | In In | Ag Ag | L = 4.8 ; L = 4.1<br>B= 3.3; B=2.7 | Cy Cy | Hr To | |
| *Ricinus communis* L. | Bb Bb | Ch Ch | In In | L=12.25 ; L= 9.35<br>B= 5.15; B= 4.3 | Cy Cy | Sf Hr | |

Key : Ag = Ageeable;B = Breadth; Br = Brown; Bt = Bitter; Bri = Brittle; Cy = CyIendrical; Co = Conical;Ch = Chorophyllous; Fl = Flexible; Fu = Fusiform;Gr = Green; Hr = Hard; In = Indistinct; Iri = Irritant; Lb = Light blue; L = Length; Sf = Soft; To = Tough; Twi = Twisted; W = Walnut like; Y=Yellow.

Table 2. Comparative macroscopicol features of leaf and fruit of some members of family Zygophyllaceae and Euphorbiaceae.

| Family | Color | Odour | Taste | Size (cm) | Shape | Fracture | Venation | Surface | Apex |
|---|---|---|---|---|---|---|---|---|---|
| **Zygophyllaceae** | | | | | | | | | |
| **Leaf** | F D | F D | F D | F D | F D | F D | F D | F D | F D |
| *Fagonia cretica* L. | G Y | In In | Bt Bt | L= 10.15,L= 9.0, B= 8.2, B= 0.71 | Ln Ln | Bri Bri | -- -- | S S | C C |
| *Peganum harmala* L. | G Y | Ag Ag | Bt Bt | L=15.0,,L,=13.25, B= 1.76 B=1.63 | L L | Bri Bri | Pp Pp | S S | C C |
| *Tribulus terrestris* L. | G G | In In | In In | L=10.3, L= 9.29 B= 0.36, B= 0.22 | L L | Bri Bri | Re Re | S S | P P |
| **Euphorbiaceae** | | | | | | | | | |
| *Chrozophora tinctoria* L.(Raf). | G G | Sp Sp | Sp Sp | L=24.8, L= 22.5 B= 1.03, B= 0.8 | Ov Ov | Bri Pp | Re Re | Hy Hy | Ac Ac |
| *Ricinus communis* L. | G Y | Bt Bt | Bt Bt | L=11.5, L= 11.3 B= 2.25, B= 0.6 | Pl Pl | Fl Pp | Re Re | Sl Sl | Acu Acu |
| Family | Kind | Size | Shape | Marking | Color | Odor | Taste | Position | |
| **Fruit** | | | | | | | | | |
| **Zygophyllaceae** | F D | F D | F D | F D | F D | F D | F D | F D | |
| *Fagonia cretica* L. | C C | 4.3 4.3 | Sg Sg | Us Us | Gr Y | Ol Ol | Bt Bt | L L | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Peganum harmala L.* | C C | 6.8 5.8 | R R | -- -- | Y Y | Tb Tb | Bt Bt | T T | |
| *Tribulus terrestris* L. | C C | 7.9 6.4 | -- -- | Hy Hy | Gr Gr | In In | Up Up | U U | |
| **Euphorbiaceae** | | | | | | | | | |
| *Chrozophora tinctoria* (Raf) L. | C C | 6.8 5.8 | -- -- | Ir Ir | Gr Bb | In In | Bt Bt | H H | |
| *Ricinus communis* L. | C C | 13.1,10.6 | Cy Cy | S S | Br Br | In In | Ag Ag | T T | |

Key : A = Alternate; Acu = Acuminate; Ag = Ageeable; B = Breadth; Bb = Bluish black; Br = Brown; Bt = Bitter; Bri = Brittle; C = Capsule; Cy = Cylindrical; Co = Conical; Ch = Chorophyllous; Fl = Flexible; Fu = Fusiform; Gr = Green ; Hy = Hairy; F = Flexible; Fu = Fusiform; Gr = Green; H = Hanging; Hr = Hard; In = Indistinct; Gr = Green; Hy = Hairy; Fl = Flexible; Fu = Fusiform; Gr = Green; H = Hanging; Hr = Hard; In = Indistinct; Ir = Irregular; Iri = Irritant; L = Lower; Lb = Light blue; Ln Linear; Ov = Oval; O = Opposite; Pl = Palmate; P = Pointed; Pap = Papery; L = Length; Re = Reticulate; S = Smooth; Sg = Subglobose; SI = Smooth Irregular; Sp = Spiny; Sf = Soft; T = Top; Tb = Tobacco; To = Tough; Twi = Twisted; US = Unsmooth; U = Unifoliate; W = Walnut like; Y = Yellow

# CHAPTER-5
# ANATOMICAL FEATURES

# CHAPTER 5

# ANATOMICAL FEATURES

Anatomical features were explored separately in roots, stems, leaves and fruits of the investigated plants. Two methods were used. First hand made transverse sections were studied through light microscopy and further refined structures were investigated through Scanning Electron Microscopy (SEM). The results are narrated and discussed for each part as follows:

## A. Root anatomy

### i. Comparative root features

The cellular measurements of roots were given in (Table 3). Epidermis and root hairs were ruptured in *Peganum harmala* (Fig. 15). Similar results were reported by Mohan *et al.* (2010) who observed ruptured epidermis in root of *Elephantopus scaber.* They were absent in *Fagonia cretica*, *Tribulus terrestris* and *Ricinus communis*. *Chrozophora tinctoria* showed that the epidermis consisted of single layer of rectangular epidermal cells. The length of epidermal cells was (4.5µm) and width was (1.8µm) was (Fig. 8). These findings agree with Bratati & Datta (1990) and Manisha *et al.* (2011) who reported single layered epidermis in roots of *Phyllanthus amarus* and *Bacopa monnieri* while it disagrees with Baslar (2000), who reported that epidermis of root had been destroyed by the development of secondary structure in *Chrozophora tinctoria*. *Chrozophora tinctoria* showed conical shaped root hairs having the length of (126µm) and width of (2.7µm). The findings agree with Madhavan *et al.* (2010), Pakianathan *et al.* (1998) and Kumar *et al.* (2010) who also reported root hairs in *Hibiscus micranthus* roots. Bark was ruptured in *Peganum harmala* and absent in *Chrozophora tinctoria*. The length of cork cells was highest in *Fagonia cretica* and lowest in *Ricinus communis*. The width of cork cells was highest in *Ricinus communis* and lowest in *Fagonia cretica* and *Tribulus terrestris* (Figs. 10, 12. The total width of cork cells was maximum for *Fagonia cretica* and *Ricinus communis* and least for *Tribulus terrestris*. The number of cortical

cells layers were variable. It was three to five in *Fagonia cretica* (Fig. 9); three in *Tribulus terrestris*; five to seven in *Chrozophora tinctoria* (Figs. 8, 12); nine to eleven in *Ricinus communis* (Table 3). It is supported by Nikolova & Vassilev (2011), Jhade *et al.* (2011), Sudhakar *et al.* (2011) and Behera & Raina (2011) who also reported variability in number of cortical cells in other medicinal plants. The shape of cortical cells was rectangular in *Fagonia cretica, Chrozophora tinctoria*, *Ricinus communis* and squarish in *Tribulus terrestris*. These results agree with Bhaskar & Balakrishnan (2010) who found similar shaped cortical cells in *Pergularia daemia* root. The maximum length of cortical cells was recorded in *Fagonia cretica* and lowest in *Chrozophora tinctoria*. The width of cortical cells was highest in *Fagonia cretica* and *Tribulus terrestris* and lowest in *Chrozophora tinctoria*. The cortical cells were ruptured in *Peganum harmala*. There were cluster and stellate calcium oxalate crystals in *Tribulus terrestris, Chrozophora tinctoria* and *Ricinus communis* while calcium oxalate crystals were not recorded in *Fagonia cretica* and *Peganum harmala*. The present findings are supported by Malode & Manik (2009), and Kumar *et al.* (2010), who reported similar calcium oxalate crystals in roots of *Tribulus terrestris, Hibiscus rosa sinensis, Abelmoschus esculenta* and *Withania somnifera*. The length of crystals was maximum in *Chrozophora tinctoria* and lowest in *Tribulus terrestris*. The width of crystals was maximum in *Chrozophora tinctoria* and *Ricinus communis* and lowest in *Tribulus terrestris*. The roots of *Tribulus terrestris* had space of 19.4µm in width between cortical cells and endodermis. Endodermis was absent in *Peganum harmala* which is in line with Mbagwu *et al.* (2007) who also reported absence of endodermis in the roots of *Salanum macrocarpum* and *Salanum nigrum*. Endodermis was not clear in *Chrozophora tinctoria* and *Ricinus communis*. This agrees with Behera & Raina (2011) and Nataraj *et al.* (2011) who reported that endodermis was indistint in *Gentiana kurroo* and *Maerua oblongifolia* roots. Endodermis was single layered and found in *Fagonia cretica* and *Tribulus terrestris*. Endodermal cells were rounded with similar length and width in both these plants. The results agree with Kumar *et al.* (2012), Manisha *et al.* (2011) and Patil & Deokule (2010) who recorded similar findings in different medicinal plants. The total width of the endodermis was maximum in *Fagonia cretica* and minimum in *Tribulus terrestris*. There was variation in composition of vessels, they were single, two or four celled. Single celled vessels were

oval or rounded in *Fagonia cretica* (Fig. 9), *Peganum harmala* (Fig. 14), *Tribulus terrestris* (Fig. 11), *Chrozophora tinctoria* and *Ricinus communis*. The length of the single vessel was maximum in *Peganum harmala* and lowest in *Fagonia cretica*. The width was maximum in *Tribulus terrestris* and lowest in *Ricinus communis*. Two and four celled vessels were present in *Peganum harmala* and two celled vessels are found in *Chrozophora tinctoria* (Fig. 7) and *Ricinus communis*. The length and width of the two celled vessels was maximum in *Peganum harmala* and lowest in *Ricinus communis* (Table 3). The larger vessels were found near pith region while the smaller vessels were found near vascular region in *Fagonia cretica*. Similar findings were also reported by Srivastava & Srivastava (1988) and Panda *et al.* (2010) in *Catharanthus roseus*, *Inula racemosa* and *Chlorophytum borivilianum* roots. Medullary ray cells were not clear in *Fagonia cretica* and were observed in other plants. The results are supported by Koyuncu *et al.* (2008), who observed medullary ray in the roots of *Peganum harmala*. This point has also been stressed by (Metcalfe & Chalk, 1972). The cells were square shaped in *Peganum harmala, Tribulus terrestris* and *Ricinus communis* (Figs. 6, 12); elongated in *Chrozophora tinctoria* (Fig. 7). The length of medullary ray cells was maximum in *Ricinus communis* and lowest in *Tribulus terrestris*. The width was also maximum in *Ricinus communis* and lowest in *Tribulus terrestris* and *Chrozophora tinctoria*. The total length of medullary ray cells was highest in *Ricinus communis* (Fig. 6) and lowest in *Tribulus terrestris*. Total length of medullary ray cells was not clear in *Fagonia cretica*. Pith cells had maximum length in *Chrozophora tinctoria* and lowest in *Peganum harmala* (Fig. 13) and *Ricinus communis*. The width of pith cells was highest in *Fagonia cretica* and lowest in *Tribulus terrestris* and *Ricinus communis*. The total width of pith region was not recorded due to the presence of vessels (Table 3). This is in line with Sultan *et al.* (2010) and Baran & Özdemir (2006) who found that no pith, as center of root remained covered with xylem vessels. The shape of the pith cells was rounded in *Fagonia cretica, Tribulus terrestris* (Fig. 11), *Chrozophora tinctoria* and *Ricinus communis* and the pith cells shape was not clear in *Peganum harmala*. The pith region of *Fagonia cretica, Peganum harmala Tribulus terrestris, Chrozophora tinctoria* and *Ricinus communis* roots was occupied by the vessels (Figs.7, 9; Table 3).

**ii. Statistical evaluation of root features**

**Comparative study based on mean value**

The average epidermal cells length was highest in *Fagonia cretica* and lowest in *Ricinus communis*. The average epidermal cells width was highest in *Ricinus communis* and lowest in *Chrozophora tinctoria*. The average cortical cells length was highest in *Fagonia cretica* and lowest in *Tribulus terrestris*. The average cortical cells width was highest in *Fagonia cretica* and lowest in *Chrozophora tinctoria*. The average endodermal cells length was (7.56µm) and average width was (2.7µm) in *Fagonia cretica* (Fig. 9). Single celled vessels comparison showed that the average xylem vessels length was highest in *Peganum harmala* and *Tribulus terrestris* and lowest in *Fagonia cretica* and *Chrozophora tinctoria*. The average xylem vessels width was highest in *Tribulus terrestris* and lowest in *Fagonia cretica*. Two celled vessels comparison showed that the average xylem vessels length and width was highest in *Peganum harmala* and lowest in *Ricinus communis*. The average length of medullary ray cells was highest in *Ricinus communis* and lowest in *Tribulus terrestris*. The average width of medullary ray cells was highest in *Ricinus communis* and lowest in *Peganum harmala* and *Tribulus terrestris* (Table 4).

**Comparative study based on coefficient of variation**

The coefficient of variation of epidermal cells length was highest in *Chrozophora tinctoria* and lowest in *Fagonia cretica*. The coefficient of variation of epidermal cells width was highest in *Chrozophora tinctoria* and *Ricinus communis* and lowest in *Fagonia cretica*. The coefficient of variation of cortical cells length was highest in *Fagonia cretica* and lowest in *Tribulus terrestris*. The coefficient of variation of cortical cells width was highest in *Fagonia cretica* and lowest in *Chrozophora tinctoria*. The coefficient of variation of endodermal cells diameter was (9.52) in *Fagonia cretica*. Single celled vessels comparison showed that the coefficient of variation of xylem vessels length was highest in *Fagonia cretica* and lowest in *Ricinus communis*. The coefficient of variation of xylem vessels width was highest in *Fagonia cretica* and lowest in *Chrozophora tinctoria*. Two celled vessels comparison showed that the coefficient of

variation of xylem vessels length was highest in *Chrozophora tinctoria* and lowest in *Ricinus communis*. The coefficient of variation of xylem vessels width was highest in *Peganum harmala* and lowest in *Chrozophora tinctoria*. The coefficient of variation of medullary ray cells length was highest in *Peganum harmala* and *Chrozophora tinctoria* and lowest in *Tribulus terrestris* and *Ricinus communis*. The coefficient of variation of medullary ray cells width was highest in *Peganum harmala* and lowest in *Tribulus terrestris* and *Chrozophora tinctoria* (Table 4).

**iii. Scanning Electron Microscopy (SEM) comparative study of root.**

This study was carried out to find out some comparative diagnostic features of roots.

**1. Trichomes**

The light microscopic study revealed that the trichomes were not found in the T.S of roots. However, SEM study showed two types of trichomes as follows:

**Non-glandular trichomes**

A trichome was present in *Chrozophora tinctoria* having length of (220 µm) and width of (50µm) (Fig. 24). It agrees with Badmus & Afolayan (2010) who reported similar trichomes. he non-glandular trichomes were long and unicellular in *Chrozophora tinctoria*, *Ricinus communis* and *Peganum harmala*. The length of non-glandular trichomes was highest (230µm) in *Ricinus communis* (Fig. 26) and lowest in *Peganum harmala* (20µm) while the width was highest in *Chrozophora tinctoria* (50µm) and lowest in *Ricinus communis* (15 µm) (Figs. 24, 26).

**Stellate trichomes**

These trichomes were found in *Fagonia cretica* and *Peganum harmala* only. (Fig. 16). The shape was also variable, some trichomes showed the curved ends or straight and tapering end. These trichomes had a broad base and the base width was (40 µm) (Fig. 16). They were variable in length and width. The length varied from (75 to 500 µm) while width varied from (2.5 to 3.9µm). The mean length varied from (215.63 to

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311.66 µm) and mean width varied from (3.9 to 12.5µm) (Fig. 29). Some fragments of trichomes appeared to be a new type of trichome. The individual trichomes were four in number and they varied from (25-60 µm) in length and (10-15µm) in width, respectively (Fig. 20). Senakun & Chantaranotha (2010) reported similar results in *Croton* (Euphorbiaceae) using SEM that supports the present findings.

**Parenchyma cell**

The light microscopy of root of *Peganum harmala* showed parenchyma tissues to be ruptured while SEM study revealed elongated parenchyma cells associated with glandular trichomes with a rounded head. The length of the parenchyma cell was (70µm) (Fig. 17). The parenchyma cells were also associated with a conical trichome which had pointed and tapering end. It was (20µm) in length (Fig. 18). Some parenchyma cells were polygonal in shape having numerous crystals. There had intercellular spaces (Fig. 21). In *Tribulus terrestris* broken fragments of elongated parenchyma cells were observed that contained calcium oxalate crystals (Figs. 22-23). A fragment of elongated parenchyma cells had length of (100µm) and width (40µm). In *Ricinus communis* a fragment of elongated parenchyma cells was found (Fig. 28).

**Vessels**

Numerous fragments of vessels were present in *Peganum harmala* and a fragment showed small pits at the upper part of the vessel (Fig. 19). In *Chrozophora tinctoria* long and short vessels were found. The length varied from (50-100µm) and width from (40 to 50µm). The fragment of a vessel showed some prominent pits, having length less than (10µm). These pits had a regular pattern. The outer sides of this were irregular and somewhat wavy (Fig. 25). In another fragment of vessel, the pits were prominent, with length of (2.5µm). In *Ricinus communis* a part of a fragmented vessel exhibited prominant pits and somewhat stretched (Fig. 27, 30). The length of the fragment was (100µm) and the width was (60µm). A crystal was found and each side was (20µm) (Fig. 31).

Table 3. Comparative anatomical features of roots of plants of family Zygophyllaceae and Euphorbiaceae.

| Anatomical features | Aspects | *Fagonia cretica* L. | *Peganum harmala* L. | *Tribulus terrestris* L. | *Chrozophora tinctoria* L.(Raf) | *Ricinus communis* L. |
|---|---|---|---|---|---|---|
| Epidermis | Layer | Ab | Rup | Ab | Single | Ab |
| | Shape | Ab | Rup | Ab | Rectangular | Ab |
| | Size (µm) | Ab | Rup | Ab | L=4.5, W=1.8 | Ab |
| | T. W (µm) | Ab | Rup | Ab | Ab | Ab |
| Root hairs | Shape | Ab | Rup | Ab | Fiber shaped, conical | Ab |
| | Size (µm) | Ab | Rup | Ab | L=126, W=2.7 | Ab |
| Bark | Layer | Six | Rup | Six to seven | Ab | Six |
| | Shape | Rectangular | Rup | Elongated rectangular | Ab | Elongated rectangular |
| | Size (µm) | L=4.32,W=2.7 | Rup | L=4.14, W=2.7 | Ab | L=4.05, W=2.88 |
| | T. W(µm) | T.W=9.18 | Rup | T.W=4.14 | Ab | 9.18 |
| Cortical cells | Layer | Three to five | Rup | 3-Layers | Five to seven | Nine to eleven |
| | Shape | Rectangular | Rup | Square | Rectangular | Rectangular |
| | Size (µm) | L=7.56, W=3.6 | Rup | L=3.78, W=3.6 | L=3.6,W=1.8 | L=6.66,W=2.88 |
| | T. W(µm) | T.W=12.42 | Rup | T.W=7.47 | T.W=12.06 | 30.66 |
| Calcium oxalate crystals | Shape | Ab | Ab | Clusters, stellate | Clusters | Clusters |
| | Number | Ab | Ab | 60 | 257 | |
| | Size (µm) | Ab | Ab | L=2.7, W=2.7 | L=3.6, W=2.88 | L=3.24, W=2.88 |
| Space | Size (µm) | Ab | Ab | T.W=19.40 | Ab | Ab |
| Endodermis | Layer | Single | Ab | Single | Nc | Nc |
| | Shape | Rounded, oval | Ab | Round | Nc | Nc |
| | Size (µm) | L=1.8, W=1.8 | Ab | L=1.8, W=1.8 | Nc | Nc |
| | T. W(µm) | 7.56 | Ab | 1.8 | Nc | 3.24 |
| Xylem 1 celled | Shape | Oval and rounded | Oval and rounded | Oval and rounded | Oval and rounded | Oval and rounded |
| | Size (µm) | L=7.92, W=5.76 | L=10.99, W=8.82 | L=10.86, W=9.14 | L=8.85, W=7.92 | L=9.18, W=7.02 |
| 2-celled | shape | Ab | Oval and rounded | Ab | Oval and rounded | Oval and rounded |
| | Size (µm) | Ab | L=14.4, W=8.82 | Ab | L=11.7, W=7.02 | L=10.08, W=4.14 |
| 4-celled | Shape | Ab | Oval and rounded | Ab | Ab | Ab |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Size (µm) | Ab | L=18.7 ,W=8.82 | Ab | Ab | Ab |
| | | Ab | Nc | Ab | Ab | Ab |
| Phloem | Shape | Nc | Nc | Nc | Nf | Squarish |
| | size | Nc | Nc | Nc | Nf | Nil |
| Medullary ray cells | Shape | Nc | Square | Square | Elongated | Squarish |
| | Size (µm) | Nc | L=2.52 ,W=2.43 | L=1.8, W=1.8 | L=2.52, W=1.8 | L=3.6, W=2.79 |
| | T. L(µm) | Nc | T.L=75.06 | T.L=53.1 | T.L=83.16 | 92.7 |
| Pith | Shape | Rounded | Nc | Rounded | Rounded, polygonal | Rounded |
| | Size (µm) | L=1.35, W=7.35 | Nc | L=1.8, W=1.8 | L=2.7, W=2.7 | L=1,8, W=1.8 |
| | T. W(µm) | OV | Nc | T.W=OV | T.W=10.08 | OV |

Key : Ab= Absent; Nc = Not clear; Nf= Not found; L=Length;W=Width; T=Total: Rup=Ruptured; ov = Occupied by vessels

Table 4. Statistical data of root anatomy of some plants of family Zygophyllaceae and Euphorbiaceae.

| Plants | Anatomical Features | Aspects | X | S.D | Var | Coef.V | S. E | CIM 1 | CIM 2 |
|---|---|---|---|---|---|---|---|---|---|
| **Zygophyllaceae** | | | | | | | | | |
| 1. *Fagonia cretica* L. | | | | | | | | | |
| | Root hair | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | Cork cells | L | 4.32 | 0.36 | 0.13 | 8.33 | 8.33 | 23.17 | -14.53 |
| | | W | 2.7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Cortical cells | L | 7.56 | 1.56 | 2.46 | 20.59 | 0.70 | 9.13 | 5.99 |
| | | W | 3.6 | 0.57 | 0.32 | 15.81 | 0.25 | 4.18 | 3.02 |
| | Calcium ox cry | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | Endodermis | L | 1.8 | 0.72 | 0.52 | 9.52 | 0.32 | 8.29 | 6.83 |
| | | W | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Xylem | L | 5.76 | 2.56 | 6.54 | 32.30 | 1.14 | 10.51 | 5.33 |
| | | W | 5.4 | 1.61 | 2.59 | 29.81 | 0.72 | 7.03 | 3.77 |
| | Phloem | L | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | | W | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | Medullary ray | T. L | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | | L | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | | W | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | Pith | L | 1.35 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | W | 7.35 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2. *Peganum harmala* L. | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Root hair | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | Cork cells | L | Rup | Rup | Rup | Rup | Rup | Rup | Rup |
| | | W | Rup | Rup | Rup | Rup | Rup | Rup | Rup |
| | Cortical cells | L | Rup | Rup | Rup | Rup | Rup | Rup | Rup |
| | | W | Rup | Rup | Rup | Rup | Rup | Rup | Rup |
| | Calcium ox cry | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | Endodermis | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | Xylem | L | 10.98 | 1.44 | 2.07 | 16.33 | 0.64 | 10.98 | 9.52 |
| | 1-celled | W | 8.82 | 1.55 | 2.40 | 17.56 | 0.69 | 10.39 | 7.25 |
| | 2-celled | L | 14.4 | 1.80 | 3.24 | 12.50 | 0.80 | 16.22 | 12 58 |
| | | W | 8.82 | 0.88 | 0.78 | 10.00 | 0.39 | 9.71 | 7.93 |
| | 4-celled | L | 38.7 | 4.67 | 21.84 | 24.49 | 2.09 | 23.81 | 14.35 |
| | | W | 8.82 | 2.00 | 4.02 | 22.73 | 0.90 | 10.85 | 6.79 |
| | Phloem | L | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | | W | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | Medullary ray | T. L | 75.06 | 4.24 | 18.01 | 5.65 | 1.90 | 79.35 | 70.77 |
| | | L | 2.52 | 0.36 | 0.13 | 14.29 | 0.16 | 2.88 | 2.16 |
| | | W | 2.43 | 0.36 | 0.13 | 14.81 | 0.16 | 2.79 | 2.07 |
| | Pith | L | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | | W | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| 3. *Tribulus terrestris.* L. | | | | | | | | | |
| | Root hair | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | Cork cells | L | 4.14 | 0.44 | 0.19 | 10.65 | 0.20 | 4.59 | 3.69 |
| | | W | 2.7 | 0.08 | 0.01 | 3.01 | 0.04 | 2.74 | 2.58 |
| | Cortical cells | L | 3.78 | 0.36 | 0.13 | 9.52 | 0.16 | 4.14 | 3.42 |
| | | W | 3.6 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Calcium ox cry | L | 2.7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | W | 2.7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Space | Thickness | 19.44 | 1.85 | 3.43 | 9.53 | 0.83 | 21.31 | 17.57 |
| | Endodermis | L | 1.8 | 0.72 | 0.52 | 9.52 | 0.32 | 8.29 | 6.83 |
| | | W | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Xylem | L | 10.86 | 0.57 | 0.33 | 5.26 | 0.26 | 11.44 | 10.28 |
| | | W | 9.14 | 0.57 | 0.32 | 6.32 | 0.25 | 9.58 | 8.42 |
| | Phloem | L | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | | W | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | Medullary ray | T. L | 53.1 | 1.56 | 2.43 | 2.94 | 0.70 | 54.68 | 51.52 |
| | | L | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | W | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Pith | L | Ov | Ov | Ov | Ov | Ov | Ov | Ov |
| | | W | Ov | Ov | Ov | Ov | Ov | Ov | Ov |
| **Euphorbiaceae** | | | | | | | | | |
| 4. *Chrozophora tinctoria* (L.) Raf. | | | | | | | | | |
| | Root hair | L | 126 | 27.89 | 777.60 | 22.13 | 12.47 | 154.21 | 97.79 |
| | | W | 2.7 | 0.57 | 0.32 | 21.08 | 0.25 | 3.28 | 2.12 |
| | Epidermal cells | L | 4.5 | 0.57 | 0.32 | 12.65 | 0.25 | 5.08 | 3.92 |
| | | W | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cortical cells | L | 3.6 | 0.57 | 0.32 | 15.81 | 0.25 | 4.18 | 45.42 |
| | | W | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Calcium ox cry | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | Endodermis | L | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | | W | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | Xylem 2-celled | L | 11.7 | 0.85 | 0.72 | 12.11 | 0.38 | 12.70 | 10.98 |
| | | W | 7.02 | 0.36 | 0.13 | 5.13 | 0.16 | 7.38 | 6.66 |
| | 1- celled | L | 8.85 | 0.70 | 0.49 | 8.15 | 0.31 | 9.26 | 7.84 |
| | | W | 7.92 | 0.36 | 0.13 | 4.55 | 0.16 | 8.28 | 7.56 |
| | Phloem | L | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | | W | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | Medullary ray | T. L | 83.16 | 12.33 | 152.15 | 14.83 | 5.52 | 95.64 | 70.68 |
| | | L | 2.52 | 0.36 | 0.13 | 14.29 | 0.16 | 2.88 | 2.16 |
| | | W | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Pith | L | 2.7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | W | 2.7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *5.Ricinus communis* L. | | | | | | | | | |
| | Root hair | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | Epidermal cells | L | 4.05 | 0.57 | 0.32 | 14.05 | 0.25 | 4.63 | 3.47 |
| | | W | 2.88 | 0.36 | 0.13 | 12.50 | 0.16 | 3.24 | 2.52 |
| | Cortical cells | L | 6.66 | 0.72 | 0.52 | 10.81 | 0.32 | 7.39 | 5.93 |
| | | W | 2.88 | 0.36 | 0.13 | 12.50 | 0.16 | 3.24 | 2.52 |
| | Calcium ox cry | L | 3.24 | 0.44 | 0.19 | 13.61 | 0.20 | 3.69 | 2.79 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | W | 2.88 | 0.36 | 0.13 | 12.50 | 0.16 | 3.24 | 2.52 |
| | Endodermis | L | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | | W | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | Xylem | L | 9.8 | 0.44 | 0.20 | 4.82 | 0.20 | 9.64 | 8.75 |
| | 1-celled | W | 7 | 0.57 | 0.32 | 7.91 | 0.25 | 7.78 | 6.62 |
| | 2-celled | L | 10.08 | 1.19 | 1.43 | 0.12 | 0.53 | 11.29 | 8.87 |
| | | W | 4.14 | 0.44 | 0.19 | 10.65 | 0.20 | 4.59 | 3.69 |
| | Phloem | L | 2.7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | W | 2.7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Medullary ray | T. L | 92.7 | 10.50 | 110.16 | 11.32 | 4.69 | 103.32 | 82.08 |
| | | L | 3.6 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | W | 2.79 | 0.18 | 0.03 | 6.45 | 0.08 | 2.97 | 2.61 |
| | Pith | L | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | | W | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Key: Ab = Absent; Ox = Oxalate; Cry = Crystals; X = Mean; Nc = Not clear; Ov = Occupied by vessels; Var = Variance; Coef.V = Coefficient of variance; S.E.= Standard Error; S.D = Standard deviation; T.L Total length; CIM =Confidence interval for mean

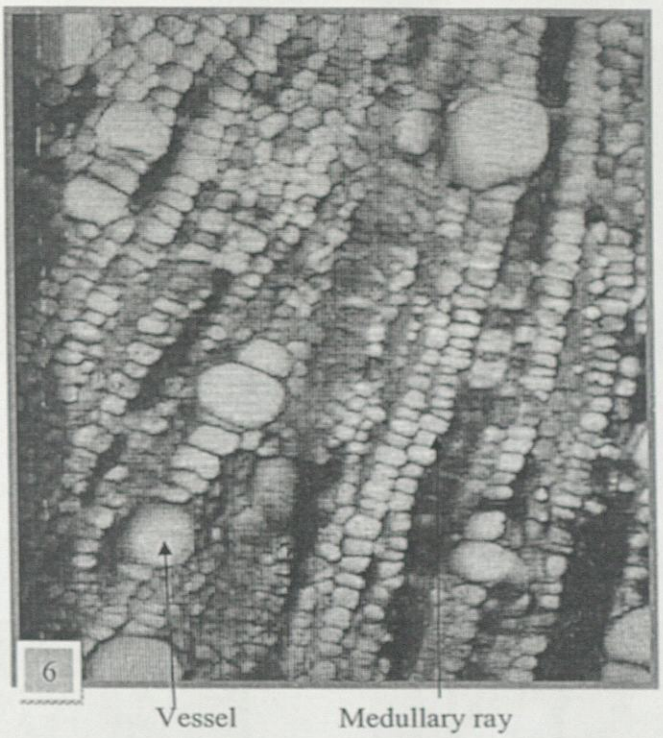



Fig. 6. T.S of root of *Ricinus communis*.

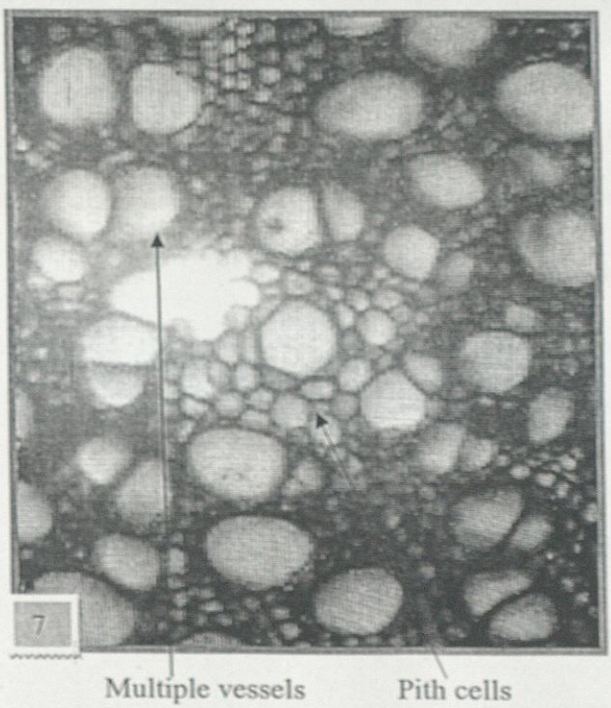
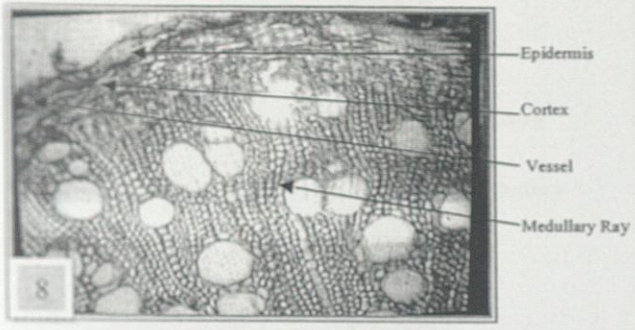



Fig. 7. T. S of root of *Chrozophora tinctoria*.



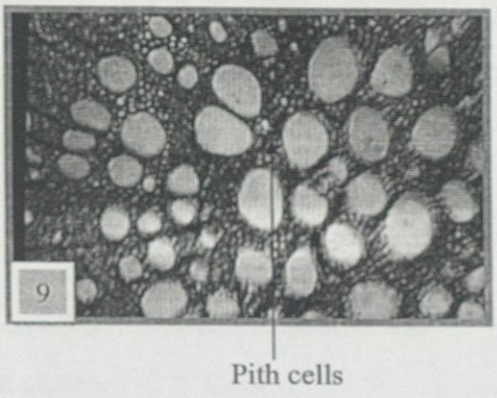
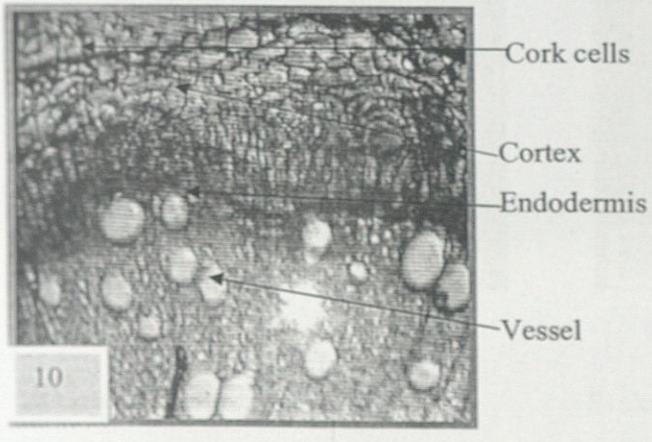




Fig. 8. T.S of root of *Chrozophora tinctoria*




Figs. 9-10. T.S of root of *Fagonia cretica*.

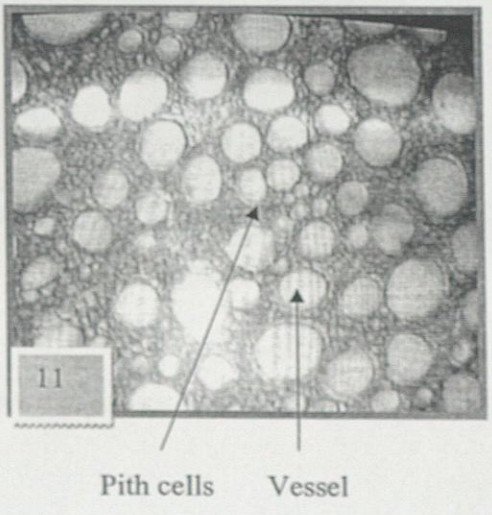



Fig. 11. T.S of root of *Tribulus terrestris* .

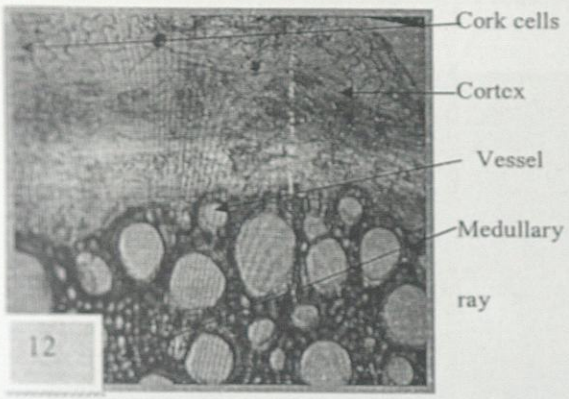



Fig. 12. T.S of root of *Tribulus terrestris*.

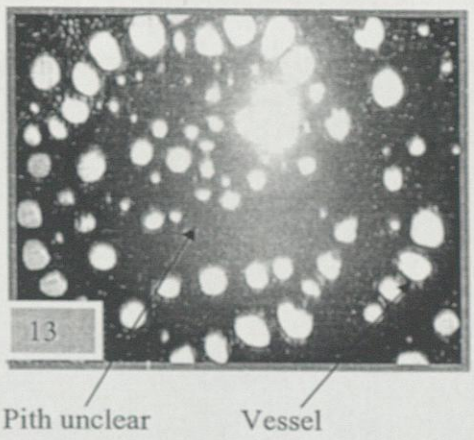
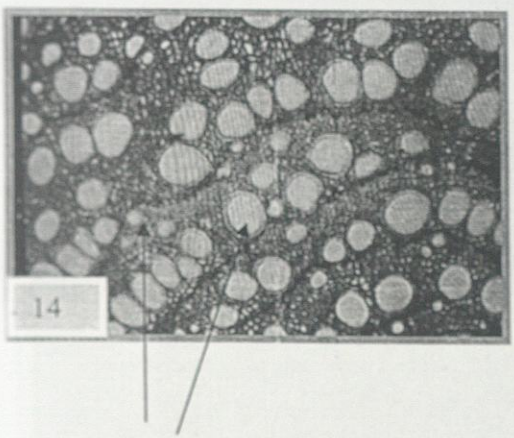
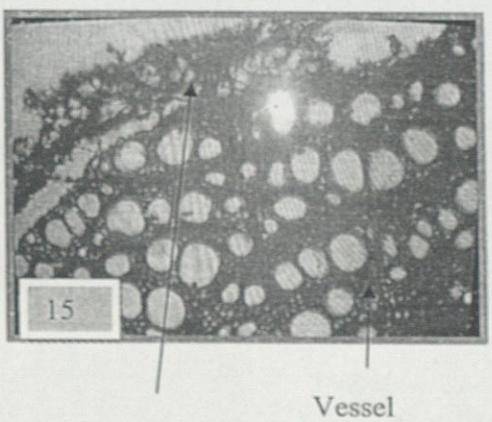



Fig. 13. T.S of root of *Peganum harmala*.




Figs. 14-15. T.S of root of *Peganum harmala*.

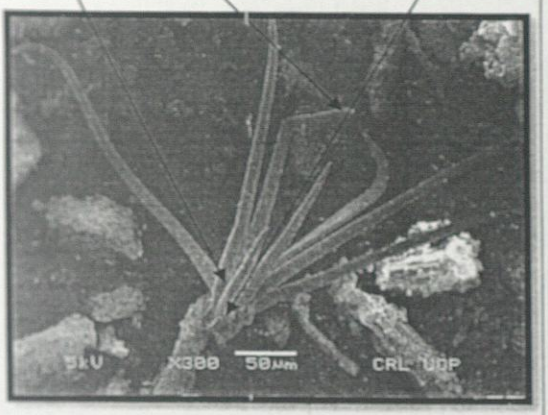



Fig. 16. SEM micrograph of stellate trichome in root of *Peganum harmala*.

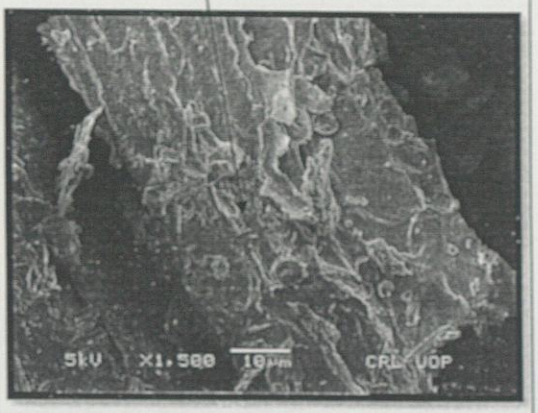
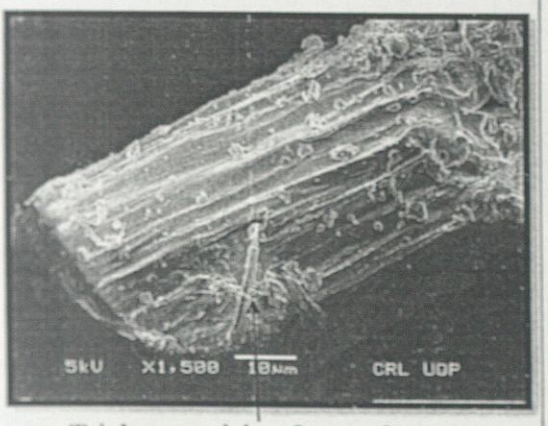
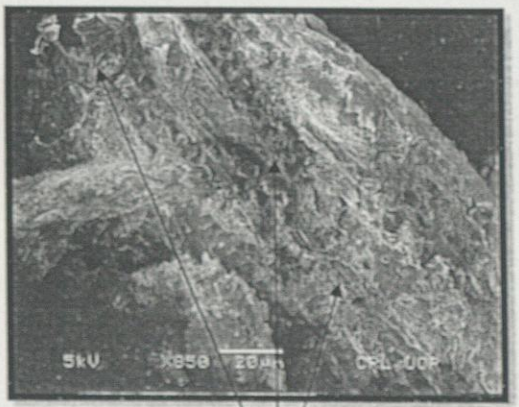



Fig. 17. SEM micrograph of parenchyma cells in root of *Peganum harmala*.




Trichome arising from a fragment

Fig. 18. SEM micrograph of trichome in root of *Peganum harmala*.




Pits

Fig. 19. SEM micrograph of vessels with small pits in root of *Peganum harmala*.

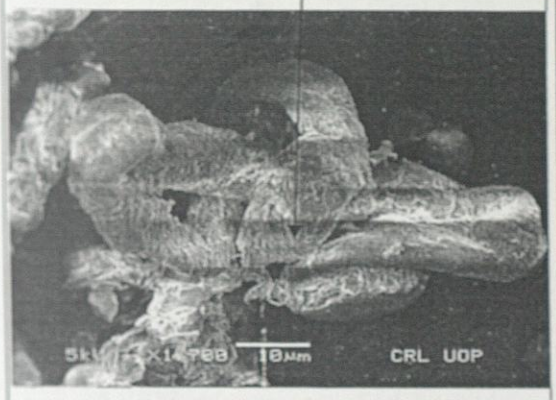



Fig. 20. SEM micrograph of trichome in root of *Peganum harmala*.

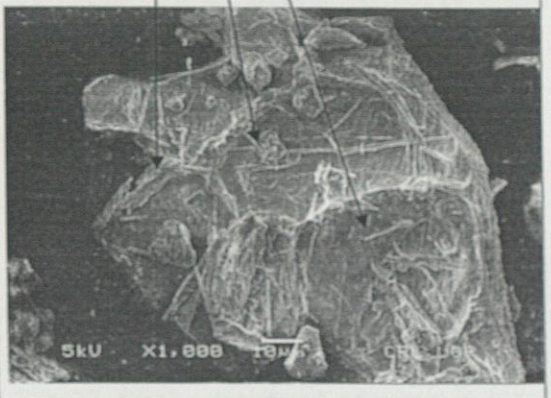



Fig. 21. SEM micrograph of parenchyma cells with crystals in root of *Peganum harmala*.

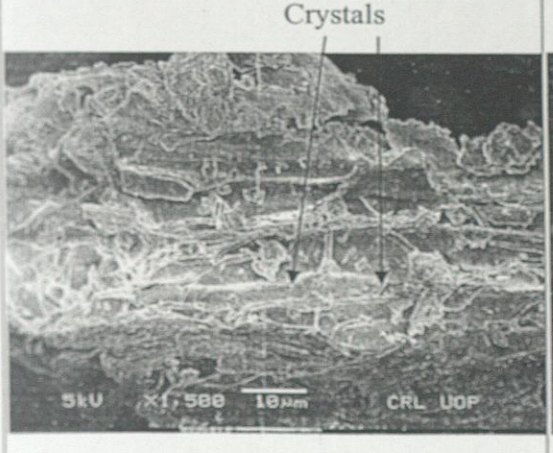



Fig. 22. SEM micrograph of parenchyma cells with crystals in root of *Tribulus terrestris*.

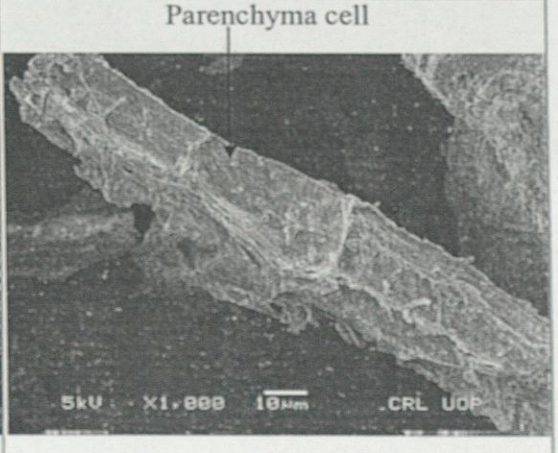



Fig. 23 SEM micrograph of parenchyma cells of root of *Tribulus terrestris*.

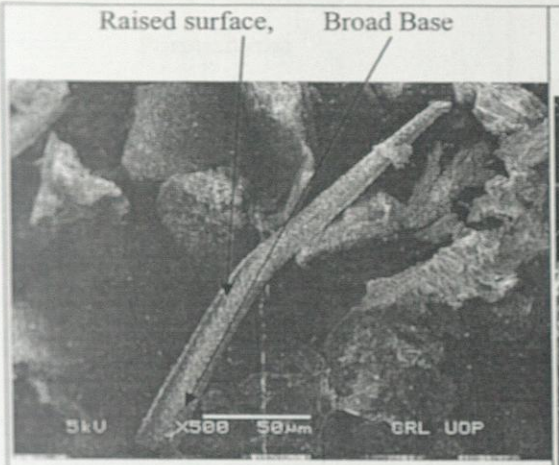



Fig. 24 SEM micrograph of trichome in root of *Chrozophora tinctoria*.

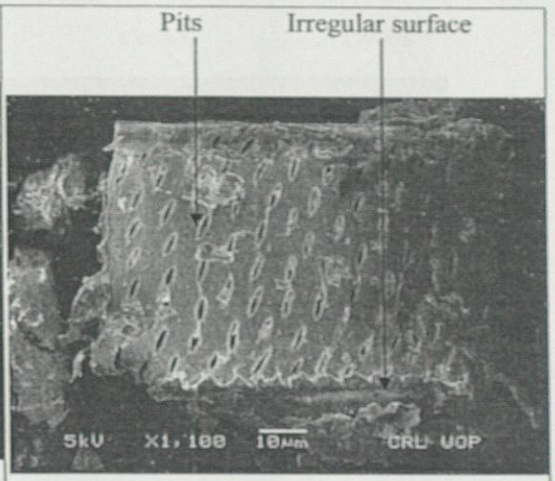



Fig. 25. SEM micrograph of vessel in root of *Chrozophora tinctoria*.

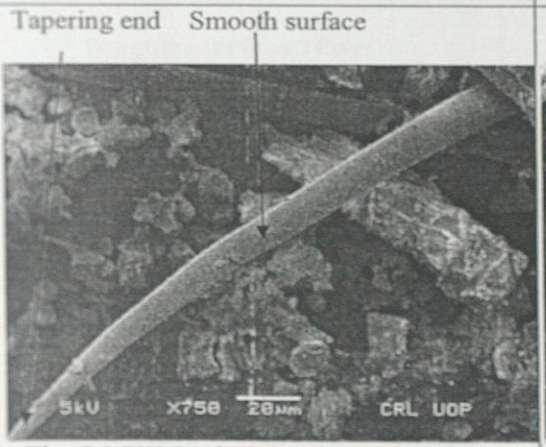



Fig. 26 SEM micrograph of trichome in root of *Ricinus communis*.

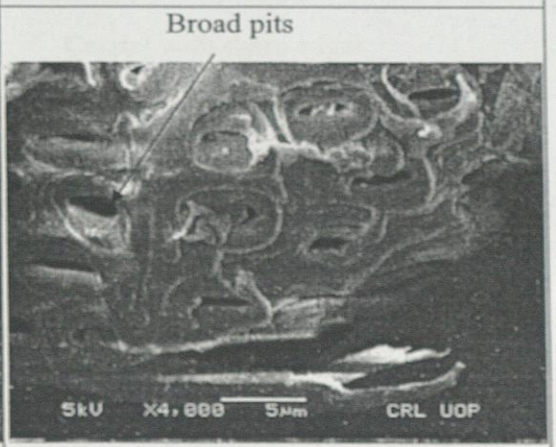



Fig. 27. SEM micrograph of vessel in root of *Ricinus communis*.

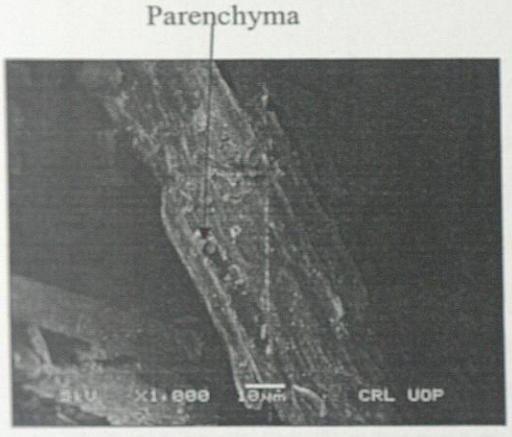



Fig. 28. SEM micrograph of parenchyma cells in root of *Ricinus communis*.

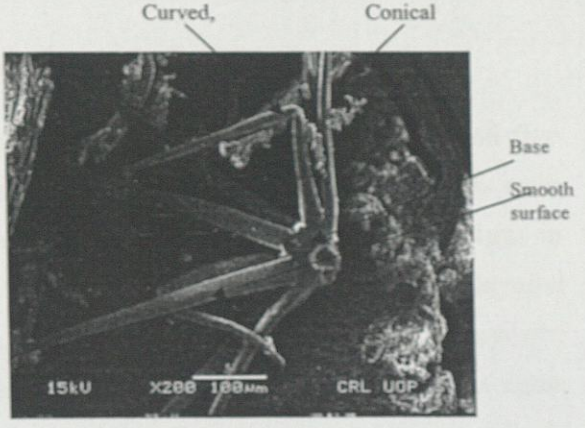



Fig. 29. SEM micrograph of stellate trichome in root of *Fagonia cretica*.

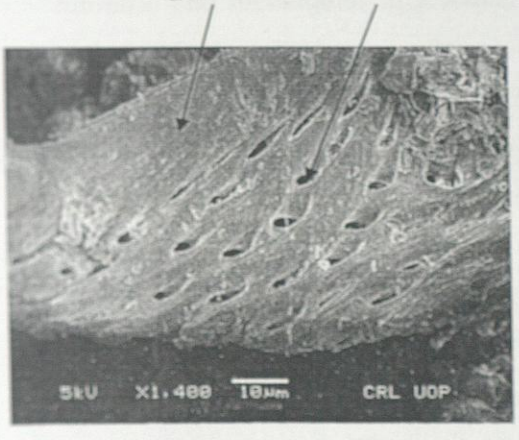



Fig. 30. SEM micrograph of vessel in root of *Ricinus communis*.

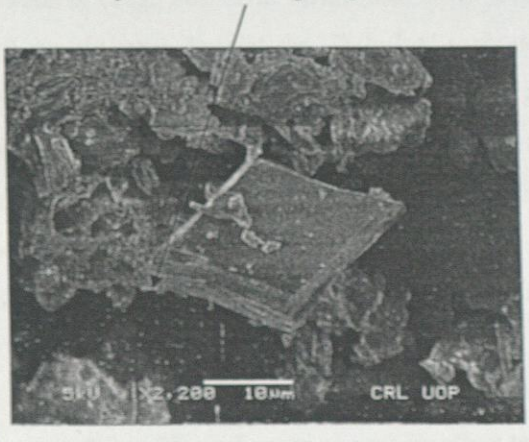



Fig. 31 SEM micrograph of crystal in root of *Ricinus communis*.

## STEM ANATOMY

### iv. Comparative anatomical features of stem

Bark was absent in all the plants except *Ricinus communis*: It showed five layers of cork cells and had cluster crystals of calcium oxalate. The cork cells were square in shape and compactly arranged (Fig. 43). The cork cells were 2.34µm in diameter. The total width of cork region was (9.54µm). Epidermis was double layered in *Peganum harmala*; single in *Fagonia cretica, Tribulus terrestris,* and *Chrozophora tinctoria* and ruptured in *Ricinus communis* (Figs. 32-33, 41). These findings agree with Patel *et al.* (2012) who reported single layer of rectangular epidermal cells in *Fagonia cretica*. These findings are in line with Baslar (2000) who reported similar findings in the stem of *Chrozophora tinctoria.* The epidermal cells shape varied from oval and beaded in *Fagonia cretica* and *Chrozophora tinctoria*; square in *Peganum harmala* and rectangular in *Tribulus terrestris* (Fig. 36). The epidermal cells length and width was highest in *Peganum harmala* and lowest in *Tribulus terrestris*. Covering trichomes were present in *Tribulus terrestris* and *Chrozophora tinctoria* and absent in *Fagonia cretica*, *Peganum harmala* and *Ricinus communis*. The length and width of covering trichomes was maximum in *Tribulus terrestris* than *Chrozophora tinctoria*. Covering trichomes were tapering, one celled with broad base and having length of (31.14µm) and width of (1.8µm). These findings agree with Benjamin & Christopher (2009), Satil *et al.* (2005) and Kumar & Sharma (2007), who also reported covering trichomes in stems of other medicinal plants. Stellate trichomes were observed only in *Chrozophora tinctoria* (Fig. 42) and were absent in other tested plants. They were not abundant and had a broad base having length of (18.72µm) and width of (1.8 µm). Kumar & Sharma (2007) also observed stellate trichomes in stems of *Turnera ulmifolia* In the present study it was observed that the number of layers of cortical cells were variable. It was three in *Fagonia cretica* and *Peganum harmala* (Figs. 32-33). This is in contrast to Koyuncu *et al.* (2008) and Patel *et al.,* (2012) who reported five to six to several layers of cortical cells in the stem of *Fagonia cretica* and *Peganum harmala.* There were six layers of cortical cells in *Tribulus terrestris*; two in *Chrozophora tinctoria* and four in *Ricinus communis* (Fig. 41, 43). Our findings are in agreement with Tüzün *et al.* (2011), Benjamin and Christopher (2009) and Zdemür *et al.* (2006), who reported variable number layers of cortical

cells in different medicinal plants. The shape of cortical cells was also different. It was square and rectangular in *Fagonia cretica*; oval in *Peganum harmala,* elongated in *Tribulus terrestris* (Figs. 32, 34, 36) and *Chrozophora tinctoria* and oblong and oval in *Ricinus communis* (Table 5). These findings agrees with Prabhu *et al.* (2011) and Sen *et al.* (2011) who reported similar cortical cells shapes in *Viburnum punctatum* and *Phyllanthus fraternus*. The length of cortical cells was highest in *Ricinus communis* and lowest in *Chrozophora tinctoriaI.* The cortical cells had maximum width in *Tribulus terrestris* and lowest in *Chrozophora tinctoria*. The total width of cortex region was highest (15.57µm) in *Tribulus terrestris* and lowest (4.23 µm) in *Ricinus communis* (Fig. 43). Excepting *Chrozophora tinctoria* the fiber cells were present in all the tested species. The presence of fiber cells in *Fagonia cretica* (Fig. 33) is supported by Patel *et al.* (2012). Fiber cells were also observed in *Peganum harmala* and these findings are supported by Koyuncu *et al.* (2008), who reported similar results for *Peganum harmala*. Space between cortical cells and endodermis was present only in *Peganum harmala* and its total width was (13.66 µm) (Fig. 34) while such space was absent in all other tested plants. The shape of endodermal cells was oval and rounded in *Fagonia cretica;* elongated in *Tribulus terrestris* (Fig. 36); square in *Chrozophora tinctoria*. These results are in agreement with Voigt *et al.* (1938) and Jain *et al.* (2010) who reported elongated endodermal cells in stems of other medicinal plants. While the endodermal cells of *Peganum harmala* and *Ricinus communis* were difficult to recognise, so neither the shapes nor the measurements were recorded for them. The endodermal cells of *Chrozophora tinctoria* had the length of (3.06µm) and width of (2.7µm) (Table 6). The shape of the xylem vessels was oval and rounded in *Tribulus terrestris* (Figs. 36-37), *Chrozophora tinctoria* and *Ricinus communis* and rounded in *Fagonia cretica* and *Peganum harmala* (Figs. 40, 43). The one-celled vessel had maximum (6.84µm) length in *Peganum harmala* and least (4.68µm) in *Tribulus terrestris*. The width was maximum (4.32µm) in *Tribulus terrestris* and *Chrozophora tinctoria* lowest (3.78µm) in *Peganum harmala*. The shape of the medullary ray cells was oval in *Tribulus terrestris*, elongated in *Chrozophora tinctoria* (Fig. 41) and square in *Ricinus communis* (Figs. 43). Baslar (2000), however, did not observe prominent medullary rays in the stem of *Chrozophora tinctoria. Tribulus terrestris*, *Chrozophora tinctoria* and *Ricinus communis* had the same length and width of (1.8µm). The total length of medullary ray was highest in *Chrozophora tinctoria* and lowest in *Ricinus communis.*

The shape of the pith cells was oval and polygonal in all the plants (Figs. 33, 39, 44) while hexagonal in *Peganum harmala* (Fig. 35). It agrees with Girish *et al.* (2010) and Patel *et al.* (2012), who reported large pith consisting of large lignified or unlignified parenchymatous cells. The length and width of pith cells was highest in *Tribulus terrestris* (Fig. 38) and lowest in *Chrozophora tinctoria*. The total length of pith cells region was same in *Peganum harmala* and *Ricinus communis* (Table 5). This is supported by Nikolova & Vassilev (2011) and Koyuncu *et al.* (2008), who also observed a wide pith region in *Peganum harmala* stem and these workers have not reported crystals of calcium oxalate in the pith cells and this is in accordance with the present findings.

**v. Statistical Evaluation of stem features**

**Comparative study based on mean value**

The average epidermal cells length and width was highest in *Peganum harmala* and lowest in *Tribulus terrestris*. The average cortical cells length was highest in *Ricinus communis* and lowest in *Chrozophora tinctoria.* The average cortical cells width was highest in *Peganum harmala* and lowest in *Chrozophora tinctoria.* These findings are in accordance with Baslar (2000) who reported similar tissues in the stem of *Chrozophora tinctoria*. The average fiber cells length was highest in *Peganum harmala* and lowest in *Ricinus communis*. The average fiber cells width was highest in *Tribulus terrestris* and lowest in *Peganum harmala* (Fig. 36). The average diameter of endodermal cells was (2.07 m) in *Fagonia cretica* and average length and width of endodermal cells was (3.06µm) and (2.7µm) in *Chrozophora tinctoria*. The average vessels length was highest in *Peganum harmala* and lowest in *Ricinus communis*. The average vessels width was highest in *Tribulus terrestris* and *Chrozophora tinctoria* and lowest in *Ricinus communis*. The diameter of vessels was (1.89 µm) in *Fagonia cretica*. The average phloem cells length was highest in *Fagonia cretica* and lowest in *Tribulus terrestris* (Fig. 36). The average phloem cells width was same for *Fagonia cretica* and *Tribulus terrestris.* This is supported by Baslar (2000) who reported similar results in *Chrozophora tinctoria*. The average pith cells length and width was highest in *Tribulus terrestris* and lowest in *Chrozophora tinctoria.* The diameter of pith cells was (7.56µm) in *Peganum harmala*. The average diameter of crystals of calcium oxalate (single) was highest in

*Ricinus communis*. The coefficient of variation of diameter of crystals of calcium oxalate (cluster) was highest in *Ricinus communis* and lowest in *Tribulus terrestris*. It is in accordance with Bhavita *et al.* (2010) who also found similar findings. In the present study the pith cells had crystals of calcium oxalate in *Chrozophora tinctoria*. This is in contrast to Baslar (2000) who found no crystals of calcium oxalate in the pith cells of stem of *Chrozophora tinctoria*. The coefficient of variation of diameter of medullary ray cells was zero in *Tribulus terrestris* and *Chrozophora tinctoria*. The coefficient of variation of total length of medullary ray cells was highest in *Chrozophora tinctoria* and lowest in *Ricinus communis* (Table 6).

**vi. Scanning Electron Microscopy (SEM) comparative study of stem**

This study was carried out to find out some further diagnostic features of stem that could not be detected with ordinary light microscopy.

A fragment of spiral vessel was observed having length and width of (40µm) and it was associated with other fragments in *Chrozophora tinctoria* (Fig. 45). In *Chrozophora tinctoria* a part of vessel showed prominent pits. The width of the rows of pit was (10µm) and each pit length was less than (10µm). Pits were of different sizes. The length of the pit varied from (5µm to12µm) and the width from (1µm to 4.5µm). The average length of the pit was (8.4 µm) and average width of the pit was (2.02µm) (Figs. 48-49). Similar results were also reported by Patel *et al*. (2012) who observed fragments of lignified pitted and spiral vessels in stem of *Fagonia arabica*.

Parenchyma cells were rectangular and elongated in shape *in Chrozophora tinctoria*. The cells length varied from (25µm to 40µm) and the width varied from (15 to 22µm). These cells were not associated with other tissues (Fig. 46).

In *Chrozophora tinctoria* a fragment of stellate trichome was consisted of five ends. Each end was variable in length and width. The length varied from (5µm to 250 µm) and the width varied from (12µm to 14µm). The average length was (145µm) and width was (13µm). The base length was (40µm) (Fig. 47). These findings agree with Voulgaridis *et al*. (2000).

*Tribulus terrestris* and lowest in *Ricinus communis*. The average diameter of crystals of calcium oxalate (cluster) was highest in *Ricinus communis* and lowest in *Tribulus terrestris*. These findings are in line with Malode and Manik (2009) who also reported calcium oxalate crystals in cortex and pith region of the stem of *Tribulus terrestris*. Saraswathy *et al.* (2009); Bhavita *et al.* (2010) and Girish *et al.* (2010) also support the present findings who reported cluster crystals of calcium oxalate in the stem of various medicinal plants. The average diameter of medullary ray cells was same. The average total length of medullary ray cells was highest in *Chrozophora tinctoria* and lowest in *Ricinus communis* (Table 6).

**Comparison of coefficient of variations**

The coefficient of variation of epidermal cells length was highest in *Chrozophora tinctoria* and lowest in *Tribulus terrestris* (Table 6). The coefficient of variation of epidermal cells width was highest in *Fagonia cretica* and lowest in *Tribulus terrestris*. The coefficient of variation of cortical cells length and width was highest in *Fagonia cretica* and lowest in *Tribulus terrestris*. The coefficient of variation of fiber cells length was highest in *Tribulus terrestris* and lowest in *Peganum harmala*. The coefficient of variation of fiber cells width was highest in *Tribulus terrestris* and lowest in *Ricinus communis*. The coefficient of variation of diameter of endodermal cells was 917.39µm) in *Fagonia cretica* and coefficient of variation of length and width of endodermal cells was (2591.2µm and 0.0µm) in *Chrozophora tinctoria*. The coefficient of variation of vessels length was highest in *Ricinus communis* and lowest in *Tribulus terrestris*. The coefficient of variation of vessels width was highest in *Peganum harmala* and lowest in *Ricinus communis*. The coefficient of variation of diameter of vessels was (9.52µm) in *Fagonia cretica*. The coefficient of variation of phloem cells length was highest in *Fagonia cretica* and lowest in *Tribulus terrestris*. The coefficient of variation of phloem cells width was lowest in *Fagonia cretica* and *Tribulus terrestris*. The coefficient of variation of pith cells length was highest in *Tribulus terrestris* and lowest in *Chrozophora tinctoria*. The coefficient of variation of pith cells width was highest in *Chrozophora tinctoria* and lowest in *Tribulus terrestris*. The coefficient of variation of diameter of pith cells was (9.52µm) in *Peganum harmala*. The coefficient of variation of diameter of crystals of calcium oxalate (single) was highest in *Tribulus terrestris* and lowest in

Table 5. Comparative anatomical features of stems of plants of family Zygophyllaceae and Euphorbiaceae.

| | | Zygophyllaceae | | Euphorbiaceae | | |
|---|---|---|---|---|---|---|
| Anatomical Features | Aspects | *Fagonia cretica* L. | *Peganum harmala* L. | *Tribulus terrestris* L. | *Chrozophora tinctoria* L. (Raf) | *Ricinus communis* L. |
| Bark | Layers | Ab | Ab | Ab | Ab | Five |
| | Shape | Ab | Ab | Ab | Ab | Square |
| | Size (µm) | Ab | Ab | Ab | Ab | L=1.8, W=1.84 |
| | T.W (µm) | Ab | Ab | Ab | Ab | 8.28 |
| Trichome | | | | | | |
| Stellate | Shape | Ab | Ab | Ab | Star | Ab |
| | Size (µm) | Ab | Ab | Ab | L=8.72, W=1.8 | Ab |
| Fiber | Shape | Ab | Ab | Fiber | Thread like | Ab |
| | Size (µm) | Ab | Ab | L=74.4, W=2.16 | L=3.14, W=1.8 | Ab |
| Epidermis | Layers | Single | Double | Single | Ab | Ab |
| | Shape | Oval, beaded | Square | Rectangular | Ab | Ab |
| | Size (µm) | L=2.54,W=2.16 | L=3.69,W= 4.05 | L=1.8, W=1.35 | Ab | Ab |
| | T.W (µm) | Nil | 4.5 | Nil | Ab | Ab |
| Cortical cells | Layers | Three | Three | Six | Ab | Four |
| | Shape | Square, Rectangular | Oval | Elongated | Ab | Oblong, Oval |
| | Size (µm) | L=3.06,W=2.34 | L=4.05, W=3.69 | L=4.43, W=4.14 | Ab | L=6.3, W=4.2 |
| | T.W (µm) | 10.12 | 6.3 | 15.57 | Ab | 4.23 |
| Fibers | Shape | Groups (17) | L=6.12,W=3.66 | Group(19) | Ab | Groups |
| | Size (µm) | 12.1 | Groups (more in no) | L=12.24, W=10.62 | Ab | 7.56 |
| Endodermis | Shape | Single, Oval | Single | Nc | Round, beaded | Beaded |
| | Size (µm) | 2.07 | Elongated | Nc | 2.7 | 2.7 |
| | T.W (µm) | 1.8 | 2.07 | Nc | Nc | |
| Space | Size (µm) | Ab | 13.66 | Ab | Ab | Ab |
| Xylem | Shape | Rounded | Rounded | Oval, rounded | Oval, rounded | Oval, rounded |
| | Dia (µm) | 1.89 | 1.89 | L=4.68, W=4.32 | 4.23 | 4.23 |
| Phloem | Shape | Rectangular | Rectangular | Rounded | Bk | Bk |
| | Diameter | L=3.24, W=2.7 | L=3.24, W=2.7 | L=2.7, W=2.7 | Bk | Bk |

| | | | | | | |
|---|---|---|---|---|---|---|
| Medullary cays | Shape | Nf | Nf | Oval | Square | Square |
| | Size (µm) | Nf | Nf | L=1.8, W=1.8 | L=1.8, W=1.8 | L=1.8, W=1.8 |
| | T.L (µm) | Nf | Nf | 24.66 | 34.56 | 22.86 |
| | Shape | Oval | Hexagonal | Rounded and polygonal | Oval and polygonal | Oval and polygonal |
| Pith | Size (µm) | L=3.6, W=3.15 | L=6.84,W=3.78 | L=7.92,W=7.74 | L=3.6, W=3.15 | 7.56 |
| | T.W (µm) | Long=45; Ver.=22.5 | T.W=7.56 | Long=99 ; Ver.=78.3 | 59.65 | 108 |
| | Shape | Ab | Ab | Stellate | Square (Cluster) | Square (Cluster) |
| | Number | Ab | Ab | 25 | 40 | 99 |
| Cry of CO | Size (µm) | Ab | Ab | L=5.04, W=3.42 | 3.6 | 6.6 |
| | Shape (Prism) | Ab | Ab | Ab | Ab | Prism |
| | Number | Ab | Ab | Ab | Ab | 15 |
| | Size (µm) | Ab | Ab | Ab | Ab | 3.5 |

Key= Ab= Absent; Bk= Break; CO= Calcium oxalate; Cry= Crystals; Nc Not clear; Nf= Not found; L=Length;W=Width; T.W=Total width; T.L=Total length

Table 6. Statistical data of stem anatomy of some plants of family Zygophyllaceae and Euphorbiaceae.

| Plant | (µm) | x | S.D | Var | Coef.V | S.E | CIM 1 | CIM 2 |
|---|---|---|---|---|---|---|---|---|
| **Zygophyllaceae** | | | | | | | | |
| 1. *Fagonia cretica* L. | | | | | | | | |
| Stellate trichome | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| Epid- cells | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | L | 2.54 | 0.44 | 0.19 | 20.41 | 0.20 | 2.61 | 1.71 |
| | W | 2.16 | 0.60 | 0.13 | 23.81 | 0.27 | 3.13 | 1.91 |
| Cortical cells | | | | | | | | |
| | L | 3.06 | 0.72 | 0.52 | 23.53 | 0.32 | 3.79 | 2.33 |
| | W | 2.34 | 0.89 | 0.78 | 37.84 | 37.84 | 87.94 | -83.26 |
| Fibers | | | | | | | | |
| | L | 11.34 | 9.96 | 99.14 | 17.56 | 4.45 | 21.41 | 1.27 |
| | W | 8.64 | 0.44 | 0.19 | 5.10 | 0.20 | 9.09 | 8.19 |
| Endo-cells | Dia | 2.07 | 0.36 | 0.13 | 17.39 | 0.16 | 2.43 | 1.71 |
| Xylem | Dia | 1.82 | 0.18 | 0.03 | 9.52 | 0.08 | 2.07 | 1.71 |
| Phloem | | | | | | | | |
| | L | 3.24 | 0.44 | 0.19 | 13.61 | 0.20 | 3.69 | 2.79 |
| | W | 2.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| Calcium oxalate crystal | | | | | | | | |
| Single | Dia | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| Cluster | Dia | Ab | Ab | Ab | Ab | Ab | Ab | Ab |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Medullary ray | | | | | | | | |
| | T. L | Nf | Nf | Nf | Nf | Nf | Nf | Nf |
| | W | Nf | Nf | Nf | Nf | Nf | Nf | Nf |
| Pith cells | L | 6.12 | 0.88 | 0.78 | 14.41 | 0.39 | 7.01 | 5.23 |
| | W | 4.32 | 0.67 | 0.45 | 15.59 | 0.30 | 5.00 | 3.64 |
| 2. *Peganum harmala* L. | | | | | | | | |
| Stellate trichome | | | | | | | | |
| | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| Epid cells | | | | | | | | |
| | L | 3.69 | 0.18 | 0.03 | 4.88 | 0.08 | 3.87 | 3.51 |
| | W | 4.05 | 0.90 | 0.81 | 22.22 | 0.40 | 4.96 | 3.14 |
| Cortical cells | | | | | | | | |
| | L | 6.03 | 2.43 | 5.88 | 40.22 | 1.08 | 8.48 | 3.58 |
| | W | 3.69 | 0.18 | 0.03 | 4.88 | 0.08 | 3.87 | 3.51 |
| Fibers | | | | | | | | |
| | L | 5.17 | 0.60 | 0.36 | 0.89 | 0.27 | 68.01 | 66.79 |
| | W | 3.6 | 0.39 | 0.15 | 7.53 | 0.17 | 5.57 | 4.78 |
| Endo-cells | Dia | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| Xylem | L | 6.84 | 0.80 | 0.64 | 11.76 | 0.36 | 7.61 | 5.99 |
| | W | 3.78 | 0.36 | 0.13 | 9.52 | 0.16 | 4.14 | 3.42 |
| Phloem | | | | | | | | |
| | L | 3.24 | 0.44 | 0.19 | 13.61 | 0.20 | 3.69 | 2.79 |
| | W | 2.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| Medullary ray | | | | | | | | |

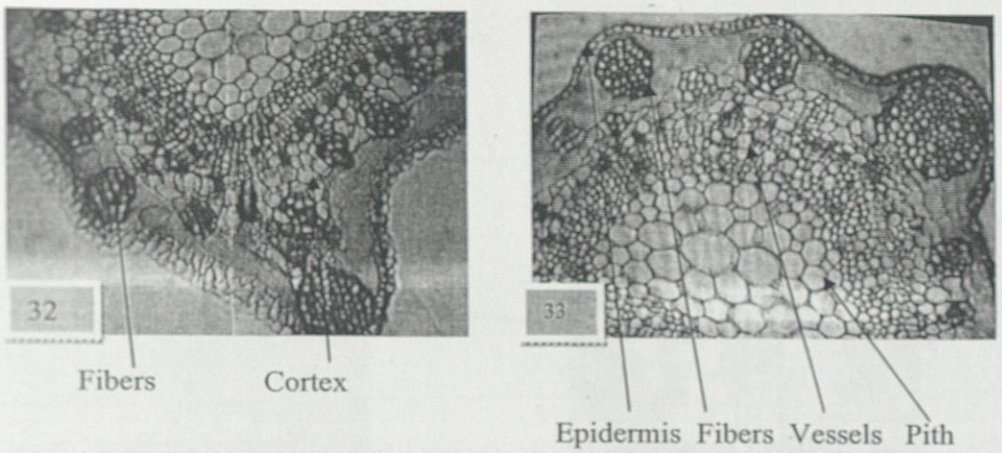
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | T. L | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | W | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| Pith cells | Dia | 7.56 | 0.72 | 0.52 | 9.52 | 0.32 | 8.29 | 6.83 |
| 3. *Tribulus terrestris* L | | | | | | | | |
| Trichome Stellate | | | | | | | | |
| | L | 77.4 | 10.00 | 99.99 | 12.98 | 4.47 | 87.16 | 66.92 |
| | W | 2.16 | 0.72 | 0.52 | 33.33 | 0.32 | 2.89 | 1.43 |
| Epid cells | | | | | | | | |
| | L | 1.8 | 0 | 0 | 0 | 0 | 0 | 0 |
| | W | 1.35 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cortical cells | | | | | | | | |
| | L | 4.43 | 0.33 | 0.11 | 5.83 | 0.15 | 5.92 | 5.26 |
| | W | 4.14 | 0.44 | 0.19 | 10.65 | 0.20 | 4.59 | 3.69 |
| Fibers | | | | | | | | |
| | L | 12.24 | 2.10 | 4.41 | 17.15 | 0.94 | 14.36 | 10.12 |
| | W | 10.62 | 1.65 | 2.72 | 15.53 | 0.74 | 12.29 | 8.95 |
| Endo-cells | | | | | | | | |
| | Dia | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| Xylem | | | | | | | | |
| | L | 4.68 | 0.36 | 0.13 | 7.69 | 0.16 | 5.04 | 4.32 |
| | W | 4.32 | 0.36 | 0.13 | 8.33 | 0.16 | 4.68 | 3.96 |
| Phloem | | | | | | | | |
| | L | 2.7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | W | 2.7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Calcium oxalate crystal | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Single | Dia | 5.04 | 0.92 | 0.84 | 18.21 | 0.41 | 5.97 | 4.11 |
| Cluster | Dia | 3.42 | 0.36 | 0.13 | 10.53 | 0.16 | 3.78 | 3.06 |
| Medullary ray | | | | | | | | |
| | T. L | 24.66 | 2.25 | 5.05 | 9.12 | 1.01 | 26.93 | 22.39 |
| | Dia | 1.802 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pith cells | | | | | | | | |
| | L | 7.92 | 0.36 | 0.13 | 4.55 | 0.16 | 8.28 | 7.56 |
| | W | 7.74 | 0.44 | 0.19 | 5.70 | 0.20 | 8.19 | 7.29 |
| **Euphorbiaceae** | | | | | | | | |
| 4. *Chrozophora tinctoria* (L.) Raf | | | | | | | | |
| Stellate trichome | | | | | | | | |
| | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| Fiber | | | | | | | | |
| | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| Epid-cells | L | 3.06 | 0.92 | 0.84 | 29.99 | 0.41 | 3.99 | 2.13 |
| | W | 1.98 | 0.22 | 0.05 | 11.14 | 0.10 | 2.20 | 1.76 |
| Cortical cells | | | | | | | | |
| | L | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | W | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Endo-cells | L | 3.06 | 79.29 | 6287.23 | 2591.24 | 35.46 | 83.27 | 0.00 |
| | W | 2.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fiber | Ab | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| Xylem | | | | | | | | |

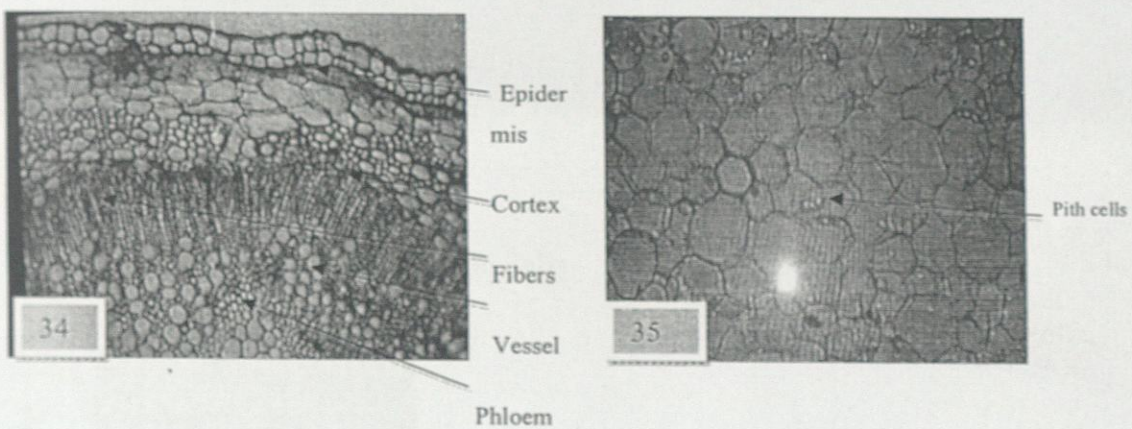
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | L | 4.95 | 0.40 | 0.16 | 8.13 | 0.18 | 5.36 | 4.54 |
| | W | 4.32 | 0.36 | 0.13 | 8.33 | 0.16 | 4.68 | 3.96 |
| Phloem | | | | | | | | |
| | L | BK | BK | BK | BK | BK | BK | BK |
| | W | BK | BK | BK | BK | BK | BK | BK |
| Calcium oxalate crystal | | | | | | | | |
| | Dia | 3.6 | 0.57 | 0.32 | 15.81 | 0.25 | 4.18 | 3.02 |
| Medullary ray | | | | | | | | |
| | T. L | 34.56 | 3.35 | 11.21 | 9.69 | 1.50 | 37.95 | 31.17 |
| | Dia | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Pith cells | L | 3.6 | 0 | 0 | 0 | 0 | 0 | 0 |
| | W | 3.15 | 3.18 | 10.08 | 100.81 | 1.42 | 6.36 | -0.06 |
| 5. *Ricinus communis* L. | | | | | | | | |
| Stellate trichome | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| Covering trichome | L | 31.14 | 5.74 | 32.92 | 18.42 | 2.57 | 36.94 | 25.34 |
| | W | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Cortical cells | | | | | | | | |
| | L | 6.3 | 0.57 | 0.32 | 9.03 | 0.25 | 6.88 | 5.72 |
| | W | 4.2 | 0.67 | 0.45 | 15.61 | 0.30 | 4.95 | 3.60 |
| Fibers | | | | | | | | |
| | L | 7.56 | 0.44 | 0.19 | 5.83 | 0.20 | 8.01 | 7.11 |
| | W | 2.7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Xylem | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | L | 4.23 | 4.25 | 18.02 | 100.36 | 1.90 | 8.52 | 0.00 |
| | W | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Phloem | | | | | | | | |
| | L | BK | BK | BK | BK | BK | BK | BK |
| | W | BK | BK | BK | BK | BK | BK | BK |
| Calcium oxalate crystal | | | | | | | | |
| Single | Dia | 3.51 | 0.60 | 0.36 | 10.15 | 0.27 | 4.11 | 2.91 |
| Cluster | Dia | 6.66 | 6.72 | 45.20 | 100.94 | 3.01 | 13.46 | 0.00 |
| Medullary ray | | | | | | | | |
| | T. L | 22.86 | 0.44 | 0.19 | 1.93 | 0.20 | 23.31 | 22.41 |
| | W | 1.8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Pith cells | Dia | 7.56 | 0.92 | 0.84 | 12.14 | 0.41 | 8.49 | 6.63 |

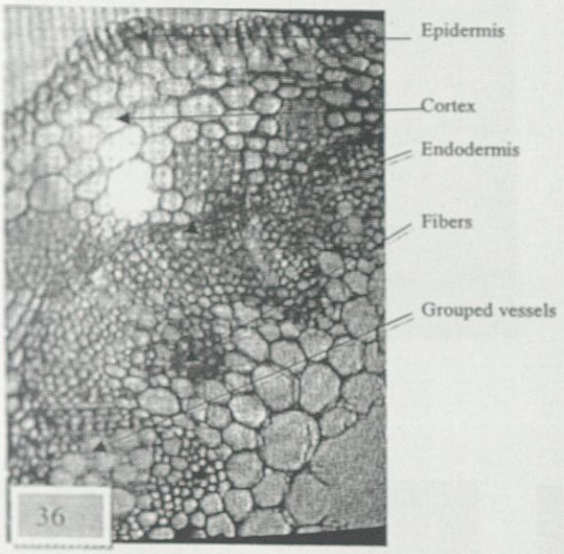
Key: Ab = Absent; BK = Break; Coef-V = Coefficient of variance; CIM = Confidence interval for mean; Epid = Epidermis; Endo = Endodermis; Nc Not clear; Nf = Not Found; S.E. = Standard Error; S.D = Standard deviation; Var = Variance; x = Mean

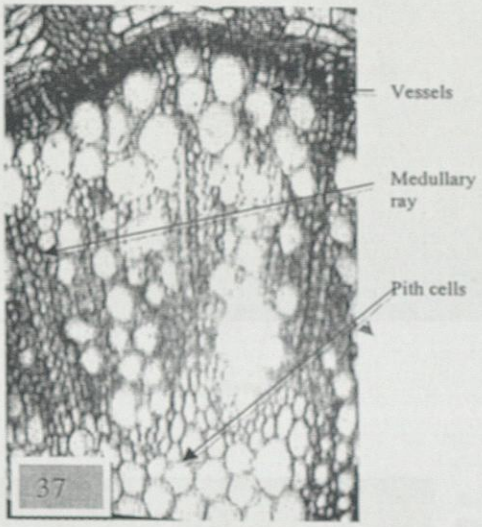


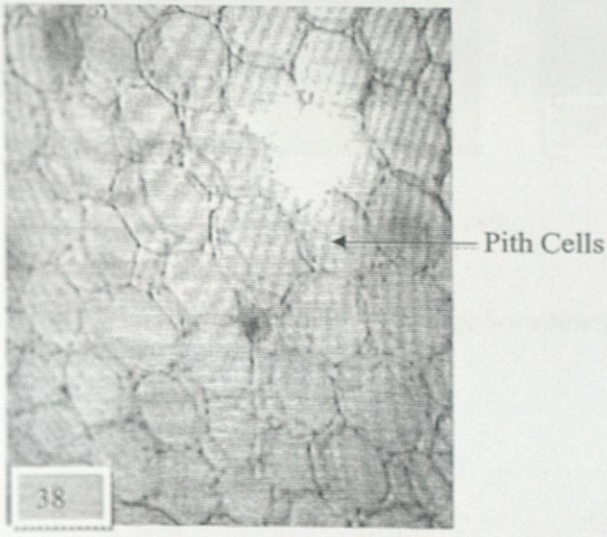

Figs. 32-33. T. S. of stem of *Fagonia cretica*.




Figs. 34-35 T. S. of stem of *Peganum harmala*.

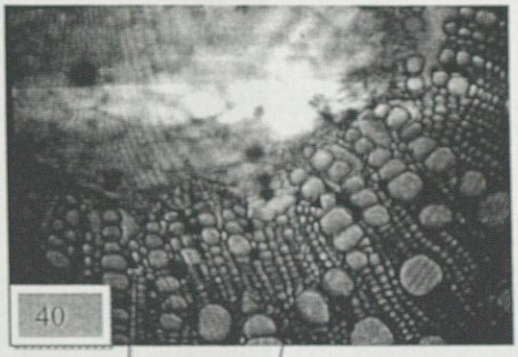


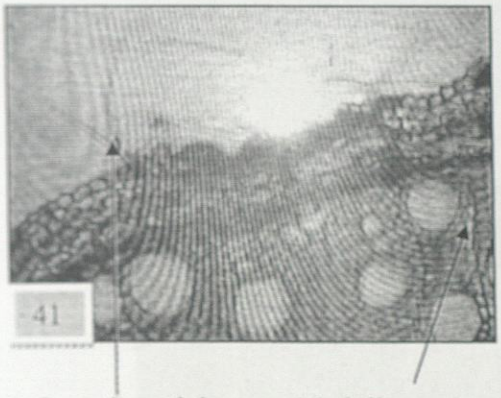



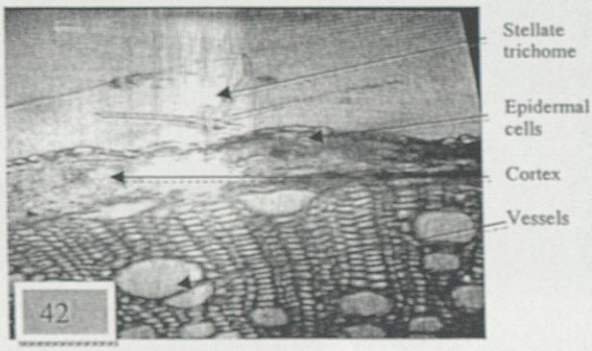




Figs. 36-38. T. S. of stem of *Tribulus terrestris* showing different anatomical features.

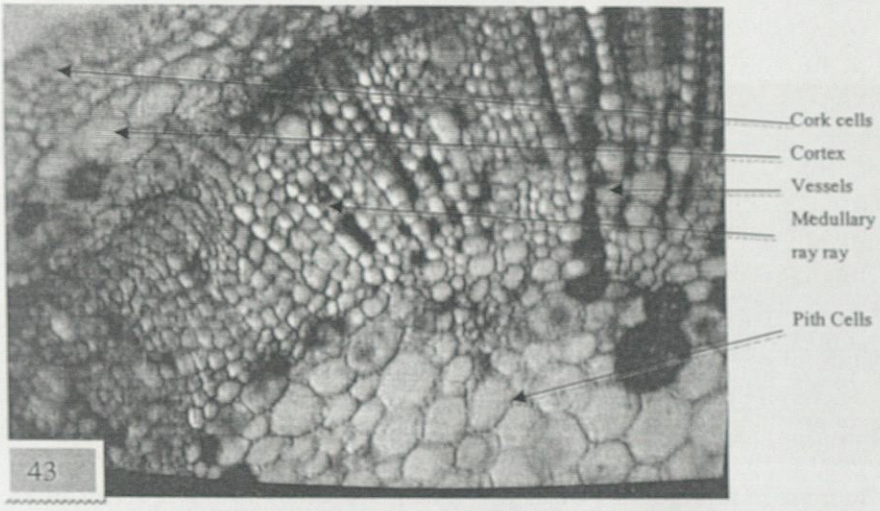


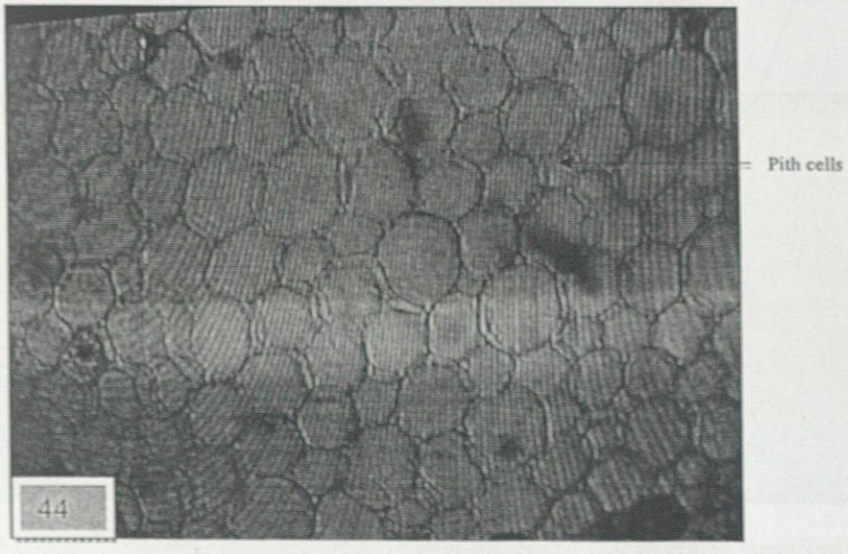




Figs. 39-42. T. S. of stem of *Chrozophora tinctoria* showing different anatomical features.




Figs. 43-44. T. S. of stem of *Ricinus communis* showing different anatomical features.

Spiral thickenings

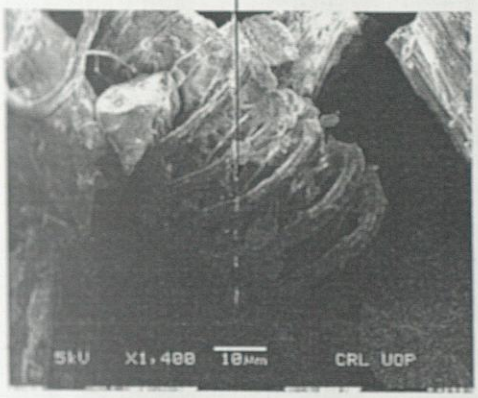



Fig. 45. SEM micrograph of vessel in stem of *Chrozophora tinctoria*.

Parenchyma

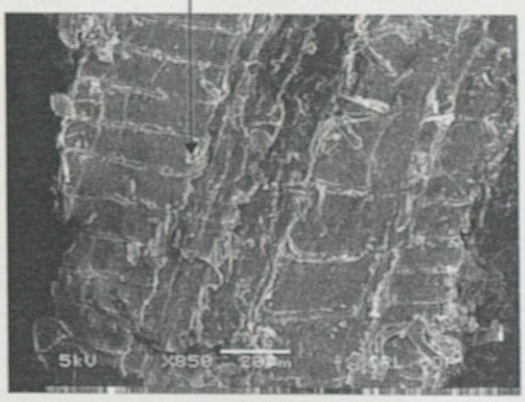



Fig. 46. SEM micrograph of stem of *Chrozophora tinctoria*. showing parenchyma

Tapering end

Group of trichomes

Broken end




Fig. 47. SEM micrograph showing stellate trichome in stem of *Chrozophora tinctoria*.

Irregular surface, Narrow Pits

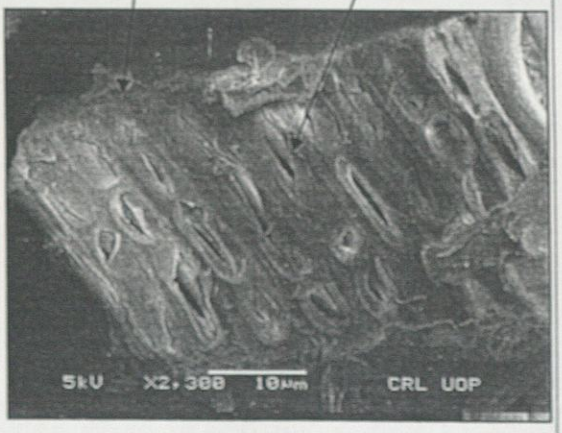



Fig. 48. SEM micrograph showing narrow pits in stem of *Chrozophora tinctoria*.

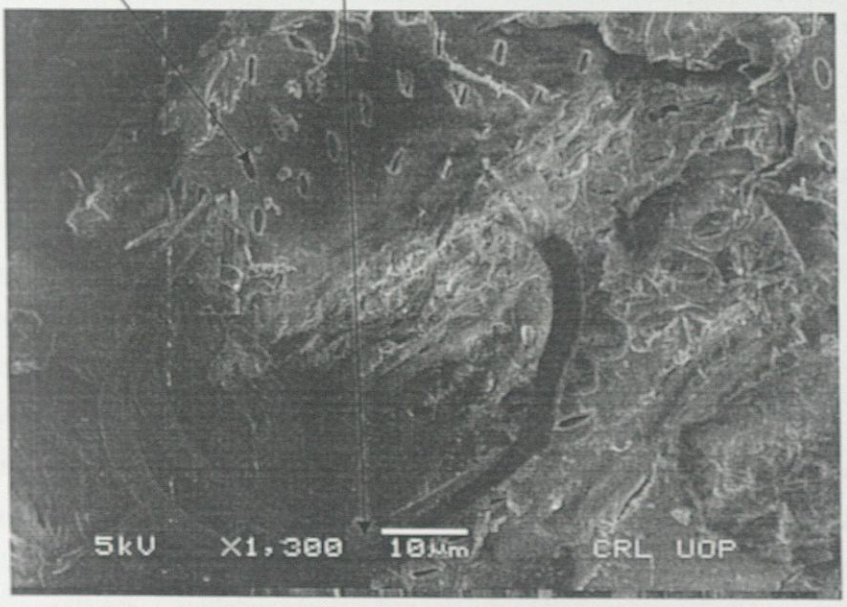



Fig. 49. SEM micrograph of stem of *Chrozophora tinctoria* showing vessels with pits and fragments of curved trichome.

## C. LEAF ANATOMY

### vii. Comparative anatomical features of leaf

The cuticle was thickest (1.35) µm in *Fagonia cretica* and thinnest (0.9) µm in *Tribulus terrestris* and *Chrozophora tinctoria* (Table 7). It agrees with Koyuncu *et al.* (2008), Fahn & Shimony (1996) and Semerdjieva (2011) who reported similar findings for *Peganum harmala*, *Fagonia arabica* and *Tribulus terrestris*. Covering trichomes were single celled and conical in shape in *Tribulus terrestris* and *Chrozophora tinctoria*. The trichomes length (L=29.43µm) and width (W=2.79µm) was maximum in *Chrozophora tinctoria* and minimum length (L=9.54 µm) and width (W=1.93µm) in *Tribulus terrestris* (Figs. 54-56; Fig. 58d). The total number of trichomes were maximum (118) in *Chrozophora tinctoria* and least (19) in *Tribulus terrestris*. *Fagonia cretica*, *Peganum harmala* and *Ricinus communis* had no trichomes. This is supported by Poornima *et al.* (2009), Shruthi *et al.* (2010), Subrata *et al.* (1993), Venkatanarayane & Lakshmi (2012) and Nataraj *et al.* (2009), who reported the absence of trichomes in *Oxystelma esculentum*, *Kirganelia reticulata*, *Gymnosporia montana* and other medicinal plants. Upper epidermis cells layers were maximum in *Fagonia cretica* and lowest in *Peganum harmala, Chrozophora tinctoria* and *Ricinus communis* (Table 7).

The shape of epidermal cells was variable. It was oval and oblong in *Fagonia cretica*; elongated in *Peganum harmala*; elongated and squarish in *Tribulus terrestris* (Figs. 50-51; Figs. 52-53); rounded and beaded in *Chrozophora tinctoria* and rectangular and elongated in *Ricinus communis* (Fig. 59b). This agrees with Behera & Raina (2011) who reported oval and rounded upper epidermal cells in *Gentiana kurroo* and this is in contradicted to the findings of Semerdjieva (2011) who observed polygonal epidermal cells in *Tribulus terrestris*. The maximum length (4.6 m) of the epidermal cells was observed in *Fagonia cretica* and least (2.87m) in *Chrozophora tinctoria*. The maximum (3.24µm) width of the epidermal cells was present in *Fagonia cretica* and lowest (1.8µm) in *Ricinus communis*. Total width of upper epidermis was highest (207µm) in *Peganum harmala* and lowest (1.8 m) in *Ricinus communis*. The maximum number (110) of epidermal cells were recorded for *Ricinus communis* and lowest (11) in *Chrozophora tinctoria* (Table 7). Palisade cells were not

clear in *Peganum harmala* and they were columnar and elongated in shape in all the tested plants. Similar columnar shaped palisade cells were also reported in leaves of other medicinal plants by (Sandeep *et al.*, 2010; Bratati & Datta, 1990; Kumar *et al.*, 2012; Madhavan *et al.* 2010; Sunita *et al.* 2011) and this strengths the present findings. The palisade was two layered in *Fagonia cretica* and *Tribulus terrestris* (Figs. 50-52). These findings agree with Vasuki *et al.* (2011), Thitikornpong *et al.* (2011), Jaiganesh & Arunachalam (2011) and Chawla *et al.* (2012), who reported double layered palisade cells in *Piper betle*, *Lagerstroemia speciosa* and *Ficus carica* leaves. These findings differ from those of Nikolova & Vassilev (2011) and Semerdjieva (2011), who reported single layer of palisade cells in *Tribulus terrestris*. *Chrozophora tinctoria* and *Ricinus communis* had single layered palisade (Fig. 59b) and this agrees with Baslar (2000) and Metcalfe & Chalk (1950). Palisade ratio was highest in *Ricinus communis* and lowest in *Tribulus terrestris* which agrees with Prajapati & Patel (2010) and Venkatesh *et al.* (2008). The palisade cells were longest (13.33µm) in *Fagonia cretica* and shortest (3.6 µm) in *Tribulus terrestris*. The palisade cells were widest (3.6µm) in *Fagonia cretica* and smallest (1.8 µm) in *Ricinus communis*. The total width of the palisade region cells was maximum (21.96 µm) in *Fagonia cretica* and least (4.24µm) in *Chrozophora tinctoria*. The spongy cells were elongated in *Fagonia cretica* and rounded in *Peganum harmala* and *Ricinus communis* (Fig. 59a). Spongy layer was not clear in *Tribulus terrestris* and *Chrozophora tinctoria*. The cells length was highest (6.93µm) in *Ricinus communis* and lowest (3.15µm) in *Peganum harmala* while the width was also highest (6.84µm) in *Ricinus communis* and lowest (2.52µm) in *Fagonia cretica* and *Peganum harmala*. The total width of the spongy layer was maximum in *Peganum harmala* and *Ricinus communis* and lowest in *Fagonia cretica*. Lower epidermis showed variation in number of layers and it was highest in *Fagonia cretica* (Fig. 51) and lowest in *Peganum harmala* and *Ricinus communis* (Fig. 60a; Table 7).

The shape of lower epidermal cells was variable. It was oval-oblong in *Fagonia cretica* (Figs. 50-51) elongated in *Peganum harmala*; elongated and oblong in *Tribulus terrestris* (Fig. 52-53) and elongated, rectangular *Ricinus communis*.The results are supported by Shantha *et al.* (2012), Dinakaran *et al.* (2011), Behera & Raina (2011) and Saravanan *et al.* (2011), who reported elongated, rectangular and

oval shaped lower epidermal cells in other medicinal plants. The lower epidermal cells were longest in *Peganum harmala* and shortest in *Ricinus communis*. The width of the lower epidermal cells was maximum (3.15µm) in *Fagonia cretica* and lowest (1.8µm) in *Ricinus communis* (Fig. 60a). Total width of lower epidermis was highest in *Peganum harmala* and lowest in *Ricinus communis*. The number of epidermal cells was maximum in *Ricinus communis* and minimum in *Peganum harmala* (Table 7). Covering trichomes were present both on upper and lower epidermis of *Tribulus terrestris* and found only on lower epidermis of *Chrozophora tinctoria* (Fig. 58b). These results are in agreement with Nikolova & Vassilev (2011), Pragapati & Patel (2010), Kandalkar *et al*. (2009), Jain *et al*. (2010) and Mathur *et al*. (2010) who reported covering trichomes on both the epidermal surfaces. However, Fahn & Shimony (1996) reported glandular trichomes in *Fagonia*. Trichomes were found singly in *Tribulus terrestris* and grouped in *Chrozophora tinctoria*. The trichomes length and width was maximum in *Chrozophora tinctoria* and least in *Tribulus terrestris*. The maximum total number of trichomes (38) were recorded in *Tribulus terrestris* (Figs. 54-57) and least in *Chrozophora tinctoria*. In *Tribulus terrestris* xylem cells were present in groups as they were individually not differentiated (Fig. 52). In *Ricinus communis* xylem was spiral in shape and cells occurred in groups (Fig. 59a-b). Xylem cells were also elongated in *Fagonia cretica*, *Peganum harmala* and *Chrozophora tinctoria*.The xylem cells length and width (group) was maximum in *Fagonia cretica* and least in *Ricinus communis*. The phloem was obscure in *Fagonia cretica*. These findings are in line with those of Robertson *et al*. (2010), who reported faint phloem in leaf of *Prosopis juliflora*. The phloem cells were oval to rounded in *Peganum harmala*, *Tribulus terrestris* and *Chrozophora tinctoria*. The phloem cells were longest (4.38µm) in *Chrozophora tinctoria* and shortest (1.8µm) in *Peganum harmala*. The width of phloem cells was greater in *Tribulus terrestris* and lessor in *Peganum harmala*. Calcium oxalate crystals were present only in *Tribulus terrestris*, *Chrozophora tinctoria* and *Ricinus communis*. The results are in agreement with (Bairagi *et al*., 2012; Monje & Baran 2002; and Kadiri *et al*. 2006) who also observed calcium oxalate crystals in other medicinal plants and cacti. The maximum length of calcium oxalate crystals was (3.53µm) in *Chrozophora tinctoria* and (3.24µm) in *Ricinus communis*. The width of these crystals of calcium oxalate was same (3.3µm) in both these plants (Table 7). Scott (1941) also reported crystals in the leaf of *Ricinus*

*communis* and this strengthened the present findings. The stellate trichomes were present in *Chrozophora tinctoria* (Fig. 58a-c).

**xiii. Statistical evaluation of leaf features**

**Comparison based on mean**

The average length of upper epidermal cells was maximum (4.6µm) in *Fagonia cretica* and lowest (2.87µm) in *Chrozophora tinctoria* (Table 8). The average width of upper epidermal cells was maximum (3.24µm) in *Fagonia cretica* and lowest (1.8µm) in *Ricinus communis*. The lower epidermal cells were longest (4.77µm) in *Peganum harmala* and shortest (3.06µm) in *Ricinus communis*. The lower epidermal cells were widest (3.15µm) in *Fagonia cretica* and narrowest (1.8µm) in *Ricinus communis*. The average palisade cells length was maximum (13.32µm) in *Fagonia cretica* and poorest in *Tribulus terrestris*. Similarly the average palisade cells were widest (3.6µm) in *Fagonia cretica* and narrowest (1.8µm) in *Ricinus communis*. The average length of spongy cells was maximum (6.93µm) in *Ricinus communis* (Fig. 59a) and least (3.14µm) in *Peganum harmala*. The average spongy cells width was maximum (6.84µm) in *Ricinus communis* and smallest (2.52µm) in *Fagonia cretica* and *Peganum harmala*. The average vascular bundles were longest (15.48µm) in *Fagonia cretica* and shortest (2.44µm) in *Tribulus terrestris*. The average vascular bundles were widest (12.15µm) in *Fagonia cretica* and smallest (1.8µm) in *Chrozophora tinctoria*. The phloem cells had a maximum (4.39µm) length in *Chrozophora tinctoria* and poorest (1.8µm) in *Peganum harmala*. The phloem cells showed maximum width (2.7µm) in *Tribulus terrestris* and narrowest (1.71µm) in *Peganum harmala*. The average trichome length (29.43 µm) and width (2.79µm) on upper epidermis was maximum in *Chrozophora tinctoria* and lowest length (10.44µm) and width (1.94µm) in *Tribulus terrestris* (Fig. 55). These results are strongly supportd by Semerdjieva (2011) who also reported simple and conical trichomes in *Tribulus terrestris*. The average trichome length (31.68µm) and width (2.39µm) on lower epidermis was highest in *Chrozophora tinctoria* and lowest length (12.6µm) and width (1.8µm) was observed in *Tribulus* terrestris (Fig. 57). The average calcium oxalate crystals length (3.42µm) from midrib region was maximum in *Chrozophora tinctoria* and least (3.24µm) in *Ricinus communis*. The average

calcium oxalate crystals width from midrib region was same (3.33µm) for both *Chrozophora tinctoria* and *Ricinus communis* (Table 8).

**Comparative study based on coefficient of variation**

The coefficient of variation of upper epidermal cells length was highest in *Peganum harmala* and lowest in *Fagonia cretica* and width was highest in *Peganum harmala* and lowest in *Chrozophora tinctoria* and *Ricinus communis* (Table 8). The coefficient of variation of lower epidermal cells length was highest in *Tribulus terrestris* and lowest in *Fagonia cretica* .The coefficient of variation of lower epidermal cells width was highest in *Fagonia cretica* and lowest in *Tribulus terrestris*. The coefficient of variation of palisade cells length was highest in *Fagonia cretica* and it was lowest *Tribulus terrestris*. The coefficient of variation of palisade cells width was highest in *Chrozophora tinctoria* and lowest in *Fagonia cretica*. The coefficient of variation of spongy cells length and width was highest in *Peganum harmala* and lowest in *Fagonia cretica*.

The coefficient of variation of vascular bundle length was highest in *Ricinus communis* and lowest in *Fagonia cretica*. The coefficient of variation of vascular bundle width was highest in *Peganum harmala* lowest in *Chrozophora tinctoria*. The coefficient of variation of phloem cells length was highest in *Chrozophora tinctoria* and lowest in *Tribulus terrestris*. The coefficient of variation of phloem cells width was highest in *Peganum harmala* and lowest in *Tribulus terrestris*. The coefficient of variation of trichome length on upper epidermis was highest in *Chrozophora tinctoria* and lowest in *Tribulus terrestris*. The coefficient of variation of trichome width on upper epidermis was highest in *Chrozophora tinctoria* and lowest in *Tribulus terrestris*. The coefficient of variation of trichome length on lower epidermis was highest in *Tribulus terrestris* and lowest in *Chrozophora tinctoria*. The coefficient of variation of trichome width on lower epidermis was highest in *Chrozophora tinctoria* and lowest in *Tribulus terrestris*. The coefficient of variation of calcium oxalate crystals length from midrib region was highest in *Ricinus communis* and lowest in *Tribulus terrestris*. The coefficient of variation of calcium oxalate crystals width from midrib region was highest in *Tribulus terrestris* and lowest in *Ricinus communis* (Table 8).

**Comparative study of stomata**

It was seen that *Fagonia cretica* had higher anomocytic stomata on lower epidermis (86%) than on the upper epidermis (81%). The guard cells had the same length (2.7µm) on upper and lower epidermis. The width of the guard cells was more on lower (1.8 µm) than on upper epidermis (1.35µm). The length of stomata central pore of stomata was maximum (2.7µm) on upper epidermis and than lower epidermis (2.52µm). The width of stomata central pore of was same (0.9µm) on both epidermises. The length and width of subsidiary cells were longest (4.14µm) and broadest (3.6µm) on upper epidermis compared to lower epidermis (3.6µm) and (2.7 µm), respectively. Similar results were also reported by Dhyani *et al.* (2009) and Poornima *et al.* (2009). In *Peganum harmala* stomata were anomocytic and were irregularly distributed. In *Tribulus terrestris* stomata were not clear on upper epidermis. These findings agree with Nikolova & Vassilev (2011) who also reported anomocytic stomata in *Tribulus terrestris*.

**ix. Scanning Electron Microscopy (SEM) comparative study of leaf**

This study was carried out to find out further diagnostic features of leaves that could not clarified by light microscopy.

**1. Trichomes**

**a. Non-glandular trichomes**

The trichomes length was maximum (260µm) in *Ricinus communis* and lowest (80 µm) in *Peganum harmala*. These trichomes were widest (25 µm) in *Chrozophora tinctoria* and narrowest (13.5µm) in *Fagonia cretica*, and some pores were seen in *Peganum harmala* (Figs. 61-62; Fig. 63, 66). The external surface of trichome showed some raised points in *Fagonia cretica* while it was irregular in *Ricinus communis* and smooth in *Peganum harmala*. The non-glandular trichomes were long and unicellular and they were more in number than stellate in *Chrozophora tinctoria*, peltate and glandular trichomes in leaves of the investigated plants in the present study (Figs. 61-63; Fig. 66). *Tribulus terristris* also showed trichomes (Fig. 72). This agrees with

Badmus & Afolayan (2010), Yasmin *et al.*, (2010), and Sitthithaworn *et al.*, (2009) who also reported non-glandular trichomes in other medicinal plants.

**b. Stellate trichomes**

Stellate trichomes were recorded in *Tribulus terrištris* and *Chrozophora tinctoria* only. They had length varying from (35 to 250µm) and width upto (125µm) in *Tribulus terristris* and *Chrozophora tinctoria.* The mean trichome length varied from 150 (*Chrozophora tinctoria*) to166.1 µm (*Tribulus terristris*) and the mean width varied from 11.6 (*Chrozophora tinctoria*) to17.95 µm (*Tribulus terristris*) (Figs. 66-67; Figs. 70-71). Similar findings were reported by Senakun and Chantaranotha (2010), Adedeji and Jewoola (2008), Chmielewska & Chernetskyy (2005) who observed stellate trichomes in *Croton, Tridax procumbens* and *Kalanchoë* and these all strength the present results.

**c. Peltate trichomes**

This type of trichome was seen only in *Chrozophora tinctoria*. The length varied from (220-250µm) and width (20-200µm). Trichomes were not abundant. Each trichome had a pointed end with a base and length was (50µm) or they were curved. External surface of the trichomes had some raised points (Fig. 66; Fig. 69). Similar findings were recorded by Shaheen *et al.* (2009) and Kahraman *et al.*, (2009) who also reported the peltate trichomes on leaves of *Sida alba*, *S. alii* S. Abedin var. alii, *S. cordata*, *S. mysorensis* and *S. ovata*, *S. spinosa* and *S. yunnanensis, Salvia chrysophylla*, *Justicia adhatoda*, *J. brandegeana,Ruellia prostrate* and *Dicliptera roxburghiana* and these all support the present findings.

The distribution and structure of trichomes on plant surfaces contribute to the control of transpiration, temperature of the organ, organ protection against UV-B radiation, pest deterrents, in plant defence or act as attractants to insects to facilitate pollination (Combrinck *et al.*, 2007).

**d. Glandular trichome**

This type of trichome was observed only in *Ricinus communis* (Figs. 74). The small trichomes were (4µm) in length and width. The average length of small trichomes was (2.2µm) and width was (2.02µm) while the average length of larger trichomes was (4.8µm) and width was (5.4µm) ). These trichomes had no prominant stalk. Similar glandular trichomes were reported by many workers (Sahreen *et al.* 2010) in *Abutilon* and *Silene* species and *Carissa carandas.*

It is interesting that SEM microscopy revealed the presence of trichomes in *Peganum harmala* and *Ricinus communis* as these trichomes were not observed in the transverse sections of leaves by light microscopy in the present case. This agrees with Kaliappan & Viswanathan (2008), who stated that SEM plays a pivotal for the identification of the microstructures as *Plectranthus amboinicus* leaf was studied under light microscope and no granules were seen but SEM microscopy revealed, lot of granules on the upper epidermis. Glandular structures are primary sites of secondary metabolite biosynthesis, secretion and storage and essential oils are also produced (Combrinck *et al.*, 2007).

**2. Stomata**

Stomata were observed in *Fagonia cretica*, *Peganum harmala* and *Tribulus terrestris*. They were not seen in other plants on screen due to imaging. The mean length and width of stomata varied from (5 and 1 µm) in *Fagonia cretica*, (30 and less than 1 µm) in *Peganum harmala* and (2.97 and 2.16 µm), respectively in *Tribulus terrestris* (Fig. 64; Fig. 73).

**3. Spongy cells**

Spongy cells were observed in *Fagonia cretica* and *Chrozophora tinctoria* only. In *Fagonia cretica* the cells were polygonal with intercellular spaces and had calcium oxalate crystals. They had length of (30µm) and width of (20µm). In *Chrozophora tinctoria* the cells were rounded to polygonal in shape and had no calcium oxalate crystals. The cells length was (2.5µm) and width was less than (1µm) (Fig. 68).

**4. Calcium oxalate crystals**

Numerous calcium oxalate crystals were observed in *Peganum harmala* (Fig. 65) and *Ricinus communis*. Their shape varied from sharply pointed to conical or star shaped or triangular. Adeshina *et al.* (2008) reported calcium oxalate crystals in *Alchornea cordifolia* (Euphobiaceae) and this supported the present findings. The results are also in agreement with Sahreen *et al.* (2010), Bairagi *et al.* (2012), Dogan *et al.* (2008), Monje & Baran (2002), Akcin & Binzet (2010), Kadiri *et al.* (2006) and Mhinana *et al.* (2010) who reported calcium oxalate crystals in different medicinal plants and cacti. Rosette and prism shaped crystals were seen in *Ricinus communis* (Fig. 75).

**5. Vessels**

They were seen only in *Tribulus terrestris* and showed small sized pits occurred in a row. Pits were crescent or rounded in shape. Spiral shaped vessels were also found. The lower surface of both the vessels was irregular. Below the spiral vessels, some narrow vessels were also seen and they were closely associated with spiral vessels (Fig. 77).

Table 7. Comparative anatomical features of leaves of some plants of family Zygophyllaceae and Euphorbiaceae.

| Anatomical features | Aspects | Zygophyllaceae | | | Euphorbiaceae | |
|---|---|---|---|---|---|---|
| | | *Fagonia cretica* L. | *Peganum harmala* L. | *Tribulus terrestris* L. | *Chrozophora tinctoria* (L.) Raf | *Ricinus communis* L. |
| Cuticle | Size (µm) | 1.35 | 1.2 | 0.9 | 0.9 | 1.8 |
| Trichome on (Upper epidermis) | No of cells | Ab | Ab | Unicellular | Unicellular | Ab |
| | Shape | Ab | Ab | Conical and Tapering | Conical , and Tapering | Ab |
| | Size (µm) | Ab | Ab | L=9.54, W=1.93 | L=29.43,W=2.79 | Ab |
| | No of trichomes | Ab | Ab | 19 | 118 | Ab |
| Upper epidermis | Layers | Five | One | Two | One | One |
| | Shape | Oval, Oblong | Elong | Square, Elong | Rond, Beaded | Rect, Elong |
| | Size (µm) | L=4.59, W=3.24 | L=4.23, W=2.7 | L=3.33, W=2.65 | L=2.86,W=2.7 | L=3.20, W=1.8 |
| | T.W (µm) | 12.67 | 207 | 4.05 | 2.81 | 1.8 |
| | No of cells | 97 | 80 | 102 | 11(others not clear) | 110 |
| Palisade cells | Layers | Two | Nc | Two | Single | Single |
| | Shape | Colum, Elong | Nc | Colum, Elong | Colum, Elong | Colum, Elong |
| | Palisade ratio | 8 | Nc | 6, 6, 4, 5 | 7(taken once) | 8, 8, 9, 9 |
| | Size (µm) | L=13.33, W=3.6 | Nc | L=3.6,W=2.44 | L=4.37,W=2.25 | L=7.56W=1.8 |
| | T.W (µm) | 21.96 | Nc | Nc | 4.24 | 7.38 |
| Spongy cells | Shape | Elong | Rond | Nc | Nc | Rond |
| | Size (µm) | L=4.32,W=2.52 | L=3.15,W=2.52 | Nc | Nc | L=6.93, W=6.840 |
| | T.W(µm) | 19.53 | 36 | Nc | Nc | 36 |
| Lower epidermis | Layers | Four | One | Two | Nc | One |
| | Shape | Oval, Oblong | Elong | Elong ,Oblong | Nc | Rect, Elong |

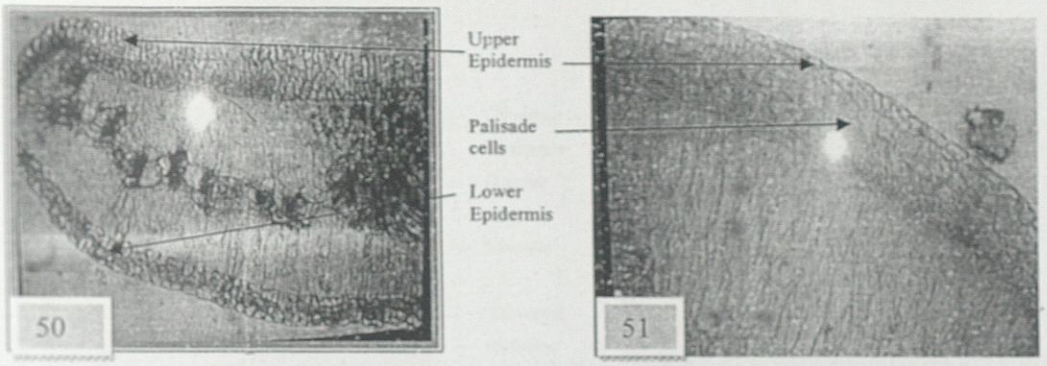
| | | | | | | |
|---|---|---|---|---|---|---|
| | Size (µm) | L=4.59,W=3.15 | L=4.77,W=2.88 | L=3.51,W=2.34 | Nc | L=3.06,W=1.8 |
| | T.W(µm) | 8.69 | 225 | 3.51 | Nc | 1.8 |
| | No of cells | 81 | 70 | 75 | Nc | 101 |
| Trichomes on (Lower epidermis) | No of cells | Ab | Ab | Unicellular | Unicellular | Ab |
| | Shape | Ab | Ab | Conical and Tapering | Conical and Tapering | Ab |
| | Size (µm) | Ab | Ab | L=12.6,W=1.8 | L=29.8,W=2.38 | Ab |
| | No of trichomes | Ab | Ab | 38 | 7-8 , M, P.L | Ab |
| Xylem | Shape | Elong, in gp | | Elong, in gp | Elong | Spiral, in gp |
| | Size (µm) | Gp=L=15.43,W=11.25 | L=2.79,W=1.89 | Gp=L=10.12,W=9.45 | L=9.56, W=1.8 | Gp=L=8.820,W=4.50 |
| Phloem | Shape | Nc | | Oval, Rond | Oval, Rond | Nc |
| | Size (µm) | | L=1.8,W=1.71 | L=3.55,W=2.7 | L=4.38,W=2.64 | |
| Calcium oxalate crystals | Shape | Ab | Ab | Rond, stellate | Rond, stellate | Rond, stellate |
| | Size (µm) | Ab | Ab | D=3.79 | M=L=3.52,W=3.3, | L=3.240, W=3.3 |
| | Occurrence | Ab | Ab | VB, PL | M, PL | M, PL |
| | Number | Ab | Ab | VB=10, PL=10 | M=160, PL=60 | M=48, P=13 |

Key: Ab= Absent; Coulm=Columnar; D= Diameter; Elong= Elongated; Gp=Group; L=Length; M=Midrib; Nc=Not clear; PL=Palisade layer; Rond=Rounded; T.W=Total width; VB=Vascular bundle; W =Width.

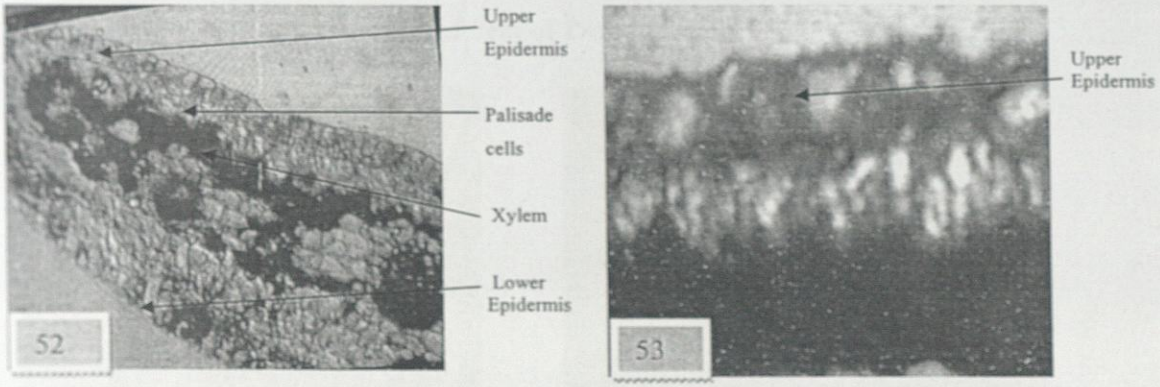
| Plants | Upper epidermis | Lower epidermis | Palisade layer | Spongy layer | Vascular bundle | Phloem | Trichomes | CO Cry (Midrib) | |
|---|---|---|---|---|---|---|---|---|---|
| **Zygophyllaceae** | | | | | | | | | |
| 1. *Fagonia cretica* L. | | | | | | | | | |
| Sta. par | L | W | L | W | L | W | L | W | L | W | L | W | L | W | L |
| Mean | 4.6 | 3.24 | 4.59 | 3.15 | 13.33 | 3.6 | 4.32 | 2.52 | 15.43 | 11.25 | Nc | Nc | TRM in U.E | | TRM in L.E | | CO Cry (Midrib) |
| dx2 | 0.73 | 1.94 | 0.73 | 20.25 | 9.4 | 0 | 1.3 | 1.3 | 5.75 | Nil | Nc | Nc | Ab | Ab | Ab | Ab | Ab |
| S.D | 0.27 | 0.44 | 0.27 | 1.42 | 3.71 | 0 | 0.36 | 0.36 | 33.02 | 2.78 | Nc | Nc | Ab | Ab | Ab | Ab | Ab |
| Var | 0.07 | 0.19 | 0.07 | 2.03 | 13.8 | 0 | 0.13 | 0.13 | 37.12 | 7.75 | Nc | Nc | Ab | Ab | Ab | Ab | Ab |
| Coef.V | 5.88 | 13.6 | 5.88 | 45.18 | 27.89 | 0 | 8.33 | 14.29 | 1.82 | 22.91 | Nc | Nc | Ab | Ab | Ab | Ab | Ab |
| S.E | 0.09 | 0.14 | 0.09 | 0.45 | 1.17 | 0 | 0.11 | 0.11 | 5.74 | 0.86 | Nc | Nc | Ab | Ab | Ab | Ab | Ab |
| CIM 1 | 4.78 | 3.56 | 4.78 | 4.17 | 15.98 | 0 | 4.58 | 2.78 | 19.59 | 14.14 | Nc | Nc | Ab | Ab | Ab | Ab | Ab |
| CIM 2 | 4.4 | 3.4 | 4.4 | 2.13 | 10.66 | 0 | 4.06 | 0.11 | 11.37 | 10.16 | Nc | Nc | Ab | Ab | Ab | Ab | Ab |
| | L | W | L | W | L | W | L | W | L | W | L | W | L | W | L | W | L |
| 2. *Peganum harmala* L. | | | | | | | | | | | | | | | | | |
| Mean | 4.23 | 2.7 | 4.77 | 2.88 | Nc | Nc | 3.14 | 2.52 | 2.79 | 1.89 | 1.8 | 1.71 | Ab | Ab | Ab | Ab | Ab |
| dx2 | 4.23 | 2.7 | 4.77 | 2.88 | Nc | Nc | 3.14 | 2.52 | 2.79 | 1.89 | 4.86 | 5.59 | Ab | Ab | Ab | Ab | Ab |
| S.D | 1.34 | 0.7 | 0.81 | 0.78 | Nc | Nc | 0.92 | 0.67 | 0.75 | 0.63 | 0.7 | 0.75 | Ab | Ab | Ab | Ab | Ab |
| Var | 1.79 | 0.49 | 0.66 | 0.62 | Nc | Nc | 0.84 | 0.45 | 0.56 | 0.4 | 0.49 | 0.56 | Ab | Ab | Ab | Ab | Ab |
| Coef.V | 31.6 | 25.8 | 17 | 27.24 | Nc | Nc | 29.2 | 26.73 | 2.68 | 33.33 | 38.73 | 43.72 | Ab | Ab | Ab | Ab | Ab |
| S.E | 0.42 | 0.22 | 0.26 | 0.25 | Nc | Nc | 0.29 | 0.21 | 0.24 | 0.2 | 0.22 | 0.24 | Ab | Ab | Ab | Ab | Ab |
| CIM 1 | 5.19 | 3.2 | 5.35 | 3.44 | Nc | Nc | 3.8 | 3 | 3.32 | 2.34 | 2.3 | 2.24 | Ab | Ab | Ab | Ab | Ab |
| CIM 2 | 3.27 | 2.2 | 4.19 | 2.32 | Nc | Nc | 2.48 | 2.04 | 2.26 | 1.44 | 1.3 | 1.18 | Ab | Ab | Ab | Ab | Ab |
| | L | W | L | W | L | W | L | W | L | W | L | W | L | W | L | W | |
| 3.*Tribulus terrestris* L. | | | | | | | | | | | | | | | | | |
| Mean | 3.87 | 2.66 | 3.51 | 2.34 | 3.6 | 2.4 | Nc | Nc | 2.44 | 10.13 | 3.56 | 2.7 | 9.54 | 1.93 | 12.6 | 1.8 | 3.65 |
| dx2 | 6.56 | 0.18 | 3.97 | 1.13 | 0 | 1.2 | Nc | Nc | 1.16 | 0.61 | 0.18 | 0 | 10.04 | 37.9 | 356.4 | 0 | 3.58 |
| S.D | 0.81 | 0.14 | 0.63 | 0.34 | 0 | 0.4 | Nc | Nc | 0.41 | 0.39 | 0.14 | 0 | 1 | 1.95 | 5.97 | 0 | 0.6 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Var | 0.66 | 0.02 | 0.4 | 0.11 | 0 | 0.2 | Nc | Nc | 0.17 | 0.15 | 0.02 | 0 | 1 | 3.79 | 35.64 | 0 | 0.36 | |
| Coef.V | 20.9 | 5.08 | 18 | 14.39 | 0 | 17 | Nc | Nc | 16.64 | 8.25 | 3.8 | 0 | 9.6 | 101 | 47.38 | 0 | 16.43 | |
| S.E | 0.26 | 0.04 | 0.2 | 0.11 | 0 | 0.2 | Nc | Nc | 0.15 | 0.39 | 0.04 | 0 | 0.32 | 0.62 | 1.89 | 0 | 0.19 | |
| CIM 1 | 4.45 | 2.75 | 3.96 | 2.58 | 0 | 2.8 | Nc | Nc | 2.79 | 10.33 | 3.65 | 0 | 11.16 | 3.33 | 16.87 | 0 | 4.07 | |
| CIM | 3.29 | 2.56 | 3.06 | 2.1 | 0 | 2.1 | Nc | Nc | 2.1 | 8.57 | 3.46 | 0 | 9.72 | 0.54 | 8.33 | 0 | 3.22 | |
| **Euphorbiaceae** | | | | | | | | | | | | | | | | | | |
| | L | W | L | W | L | W | L | W | L | W | L | W | L | W | L | W | L | W |
| *4.Chrozophora tinctoria* (L.) Raf | | | | | | | | | | | | | | | | | | |
| Mean | 2.87 | 2.7 | Nc | Nc | 4.37 | 2.3 | Nc | Nc | 8.5 | 1.8 | 4.38 | 2.64 | 29.43 | 2.79 | 31.68 | 2.39 | | 3.33 |
| dx2 | 1.59 | 0 | Nc | Nc | 0.69 | 1.2 | Nc | Nc | 5.04 | 0 | 8.57 | 0.18 | 627 | 12636 | 608.8 | 5.69 | 1.3 | 1.7 |
| S.D | 0.45 | 0 | Nc | Nc | 0.31 | 0.4 | Nc | Nc | 0.75 | 0 | 1.03 | 0.15 | 7.92 | 35.6 | 7.8 | 0.75 | 6 | 0.41 |
| Var | 0.2 | 0 | Nc | Nc | 0.1 | 0.2 | Nc | Nc | 0.56 | 0 | 1.07 | 0.02 | 62.7 | 1264 | 60.88 | 0.57 | 0.13 | 0.17 |
| Coef.V | 15.6 | 0 | Nc | Nc | 9.92 | 19 | Nc | Nc | 8.8 | 0 | 107.1 | 6.7 | 26.91 | 1274 | 24.63 | 31.63 | 10.53 | 12.38 |
| S.E | 0.16 | 0 | Nc | Nc | 0.12 | 0.2 | Nc | Nc | 0.25 | 0 | 0.36 | 0.06 | 2.5 | 11.2 | 2.47 | 0.24 | 0.11 | 0.13 |
| CIM 1 | 3.23 | 0 | Nc | Nc | 4.64 | 2.6 | Nc | Nc | 9.06 | 0 | 5.21 | 2.79 | 35.09 | 28.2 | 37.26 | 2.92 | 3.68 | 3.62 |
| CIM 2 | 2.51 | 0 | Nc | Nc | 4.1 | 1.9 | Nc | Nc | 7.94 | 0 | 3.56 | 2.5 | 23.77 | -22.6 | 26.1 | 1.85 | 3.16 | 3.04 |
| | L | W | L | W | L | W | L | W | L | W | L | W | L | W | L | W | L | W |
| *5. Ricinus communis* L . | | | | | | | | | | | | | | | | | | |
| Mean | 3.24 | 1.8 | 3.06 | 1.8 | 7.56 | 1.8 | 6.93 | 6.84 | 8.82 | 5.64 | Nc | Nc | Ab | Ab | Ab | Ab | 3.24 | 3.29 |
| dx2 | 1.94 | 0 | 1.94 | 0 | 6.8 | 0 | 29.2 | 13.28 | 8.82 | 4.5 | Nc | Nc | Nc | Ab | Ab | Ab | 1.94 | 1.64 |
| S.D | 0.44 | 0 | 0.44 | 0 | 0.82 | 0 | 1.71 | 1.15 | 197.3 | 1.62 | Nc | Nc | Nc | Ab | Ab | Ab | 0.44 | 0.41 |
| Var | 0.19 | 0 | 0.19 | 0 | 10.91 | 0 | 2.92 | 1.33 | 4.44 | 0.4 | Nc | Nc | Nc | Ab | Ab | Ab | 0.19 | 0.16 |
| Coef.V | 13.6 | 0 | 14.4 | 0 | 0.26 | 0 | 24.7 | 16.85 | 19.73 | 0.16 | Nc | Nc | Nc | Ab | Ab | Ab | 13.61 | 12.33 |
| S.E | 0.61 | 0 | 0.14 | 0 | 8.15 | 0 | 0.54 | 0.36 | 50.36 | 8.94 | Nc | Nc | Nc | Ab | Ab | Ab | 0.14 | 0.13 |
| CIM 1 | 4.63 | 0 | 3.38 | 0 | 0.26 | 0 | 8.15 | 7.66 | 1.4 | 0.13 | Nc | Nc | Nc | Ab | Ab | Ab | 3.56 | 3.57 |
| CIM 2 | 1.85 | 0 | 2.74 | 0 | 1.33 | 0 | 5.71 | 6.02 | 12 | 4.79 | Nc | Nc | Nc | Ab | Ab | Ab | 2.92 | |

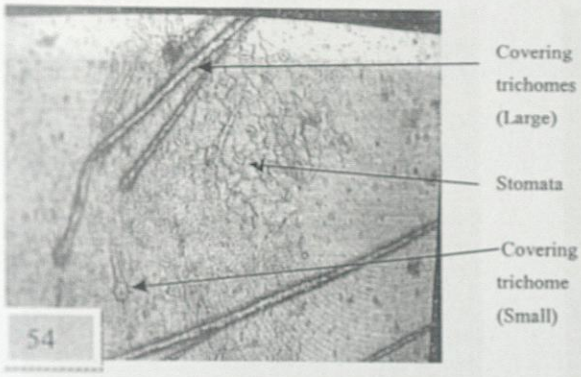
Key: Ab = Absent; CO = Calcium Oxalate; Cry = Crystals; Coef.V=Coefficient of variance; CIM =Confidence interval for mean; $dx^1$ = Difference; Nc =Not clear; L.E = Lower epidermis S.E.= Standard Error; S.D= Standard deviation; Sta. par = Statistical parameter ;TRM =Trichome; U.E = Upper epidermis; Var=Variance; L=Length; W=Width.

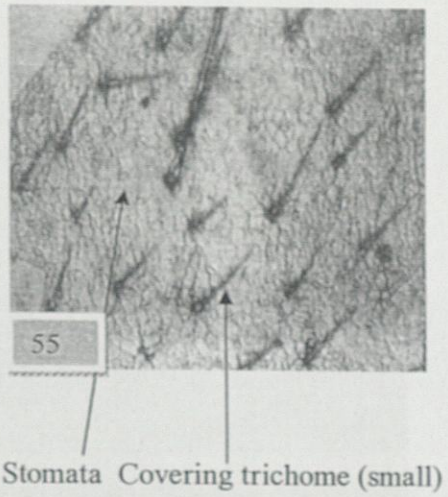



Figs. 50-51. T. S of leaf of *Fagonia cretica* showing different anatomical features.

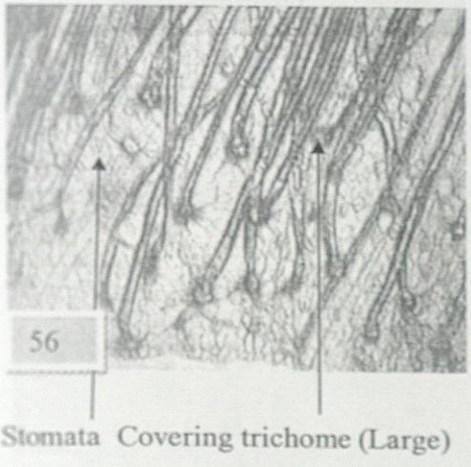


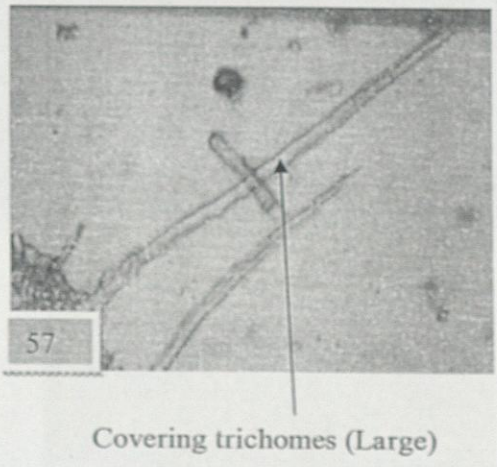

Figs. 52-53. T. S of leaf of *Tribulus terrestris* showing different anatomical features.



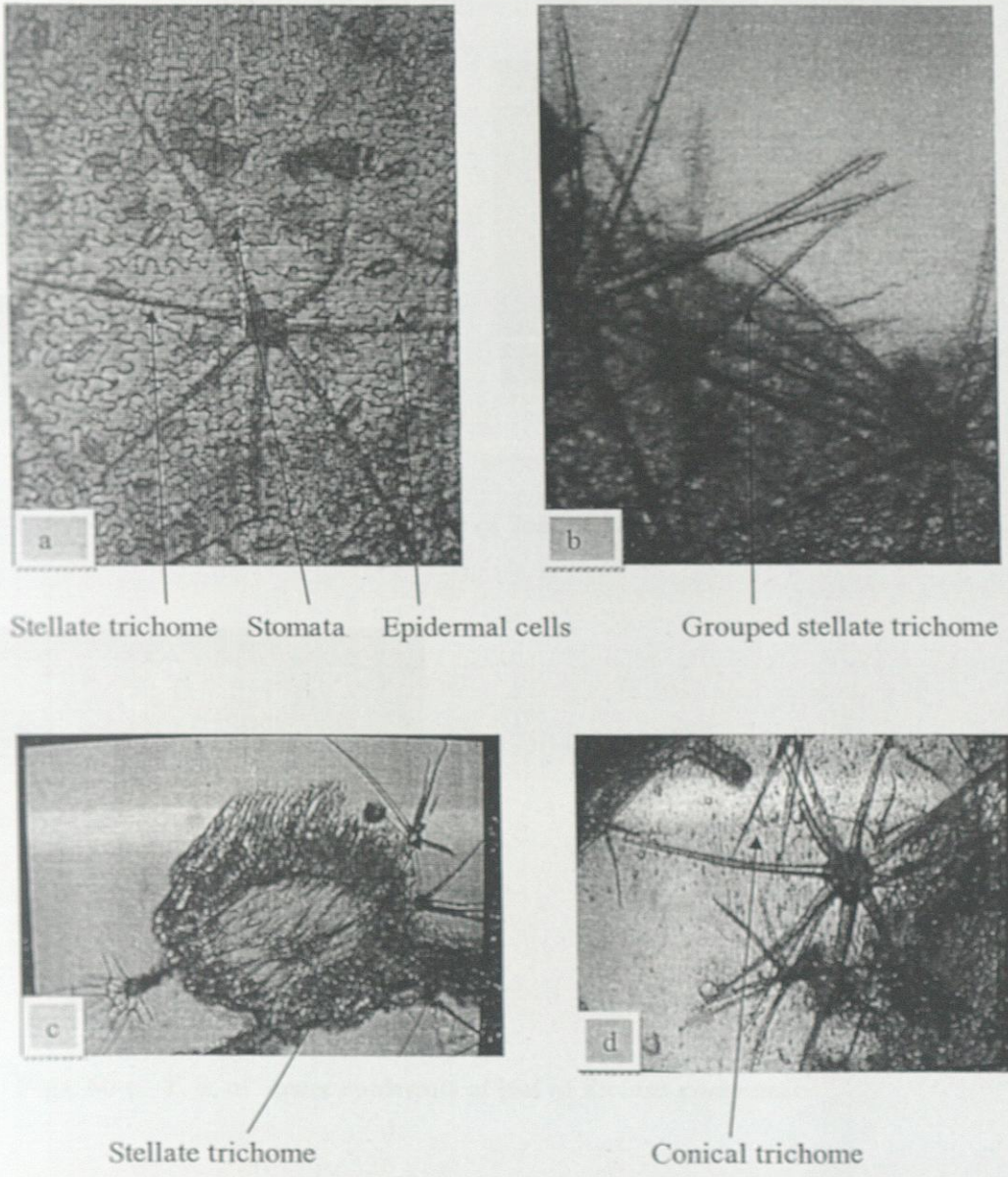




Figs. 54-55. T. S of upper surface of leaf of *Tribulus terrestris*.




Figs. 56-57. T. S of lower surface of leaf of *Tribulus terrestris*.




Figs. 58 a,b,c,d. T. S of lower surface of leaf of *Chrozophora tinctoria*.

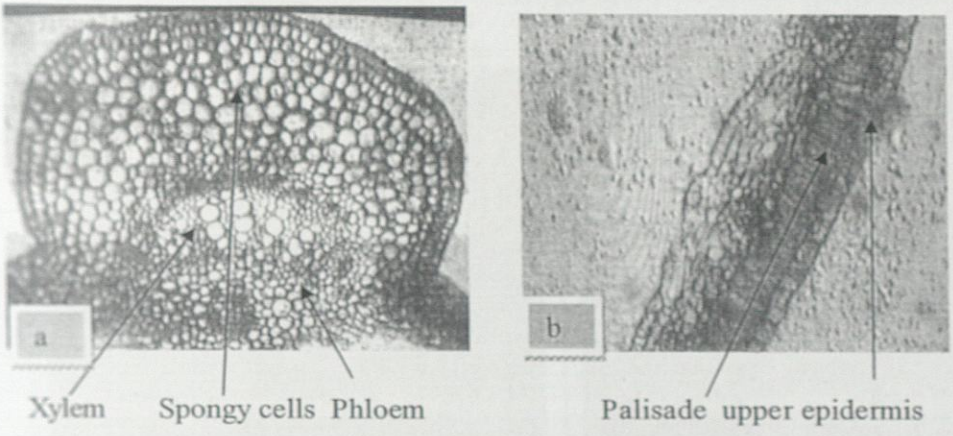
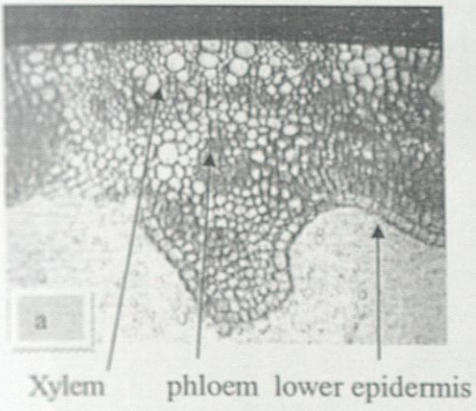



Fig. 59. a-b. T. S. of upper epidermis of leaf of *Ricinus communis*.




Figs. 60 a. T. S. of lower epidermis of leaf of *Ricinus communis*.

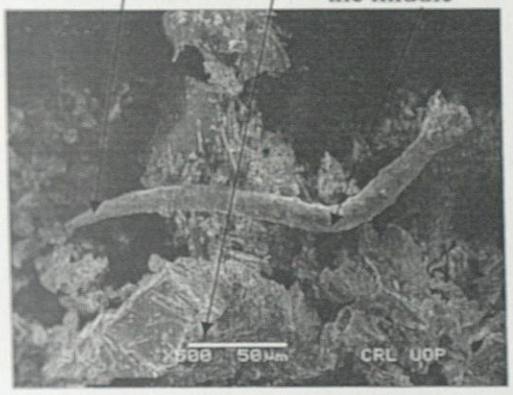



Fig. 61. SEM micrograph of curved trichome in leaf of *Fagonia cretica*.

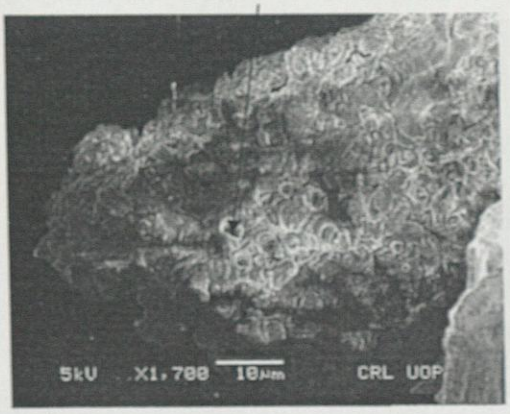



Fig. 62. SEM micrograph showing pores in leaf of *Peganum harmala* .

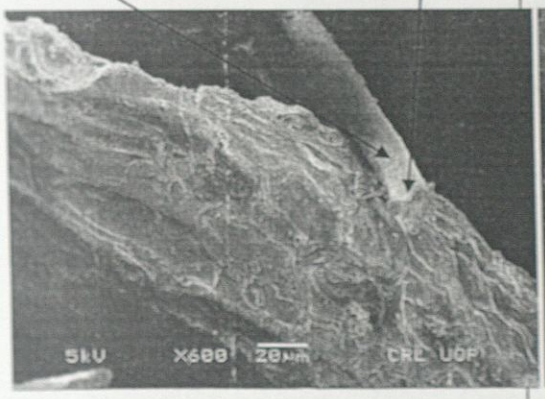



Fig. 63 SEM micrograph showing smooth surface of trichome in leaf of *Peganum harmala* .




Fig 64. SEM micrograph of stoma in leaf of *Peganum harmala*.

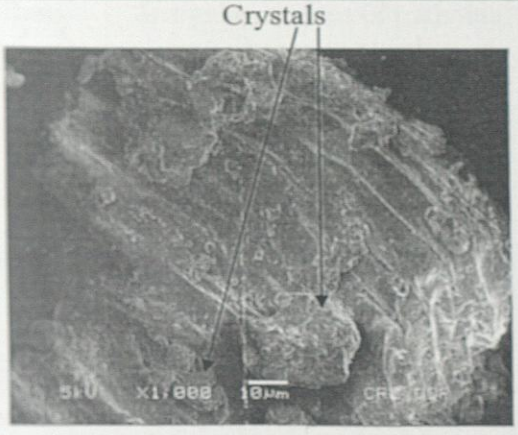



Fig. 65. SEM micrograph showing crystals in elongated cells in leaf of *P. harmala*.

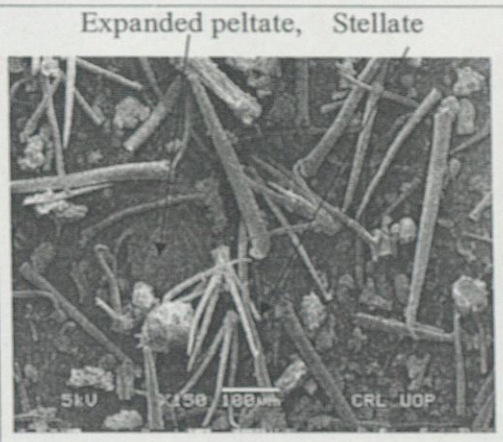



Fig. 66. SEM micrograph showing peltate, stellate & unicellular trichomes in leaf of *Chrozophora tinctoria*.

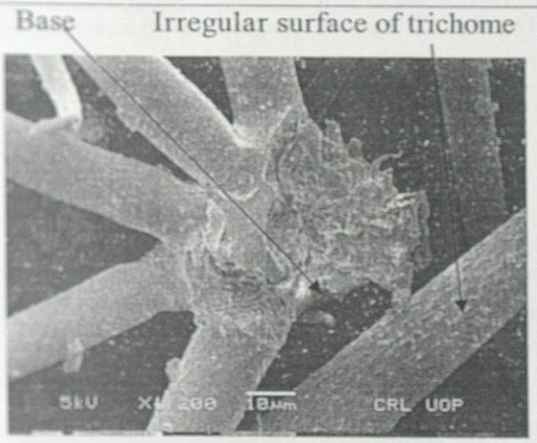



Fig. 67. SEM micrograph of stellate trichome showing irregular surface in leaf of *Chrozophora tinctoria*.

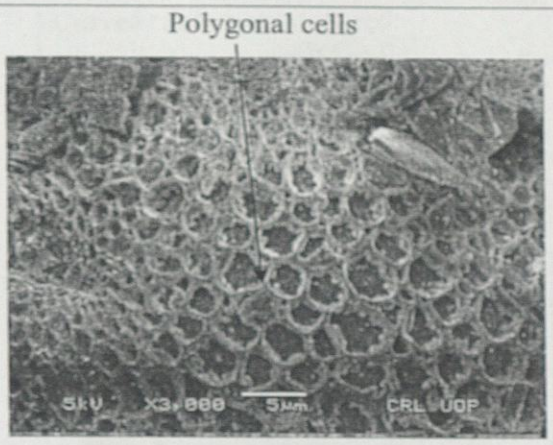



Fig. 68. SEM micrograph showing spongy cells in leaf of *Chrozophora tinctoria*.

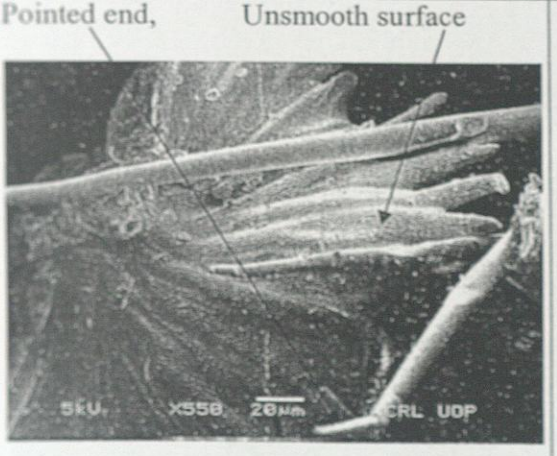



Fig. 69. SEM micrograph of peltate trichome in leaf of *Chrozophora tinctoria*.

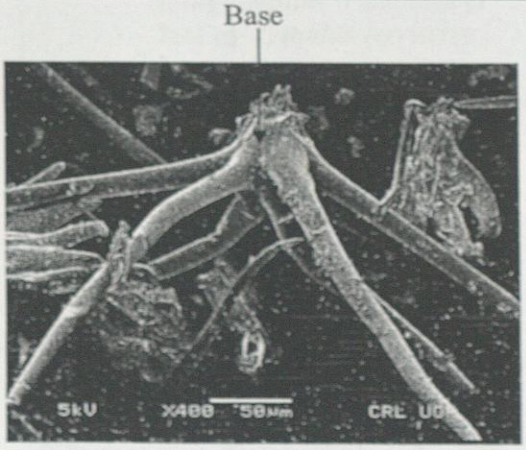



Fig. 70. SEM micrograph of stellate trichome in leaf of *Chrozophora tinctoria*.

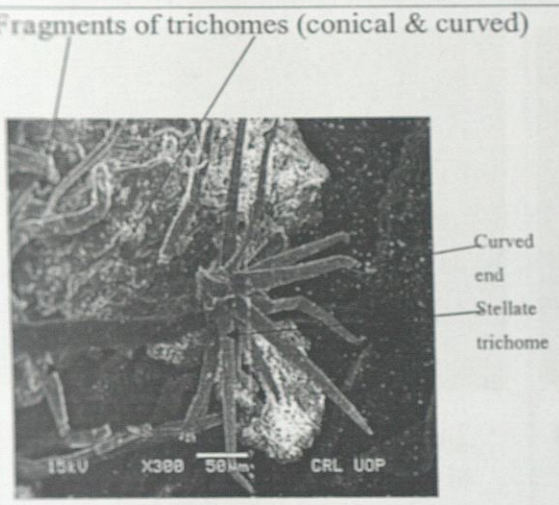



Fig. 71. SEM micrograph of stellate trichome in leaf of *Tribulus terretris*.

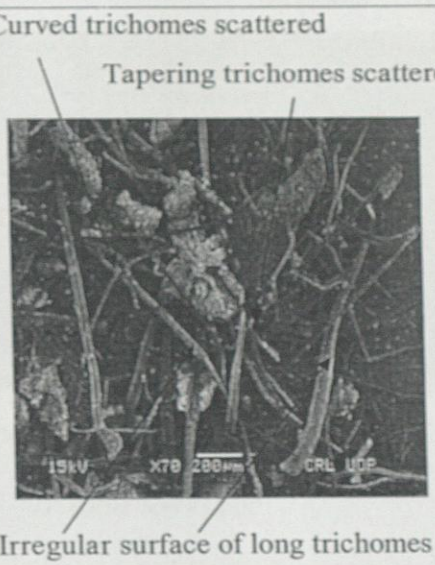



Fig. 72. SEM micrograph showing non-glandular trichomes in leaf of *Tribulus terristris*.

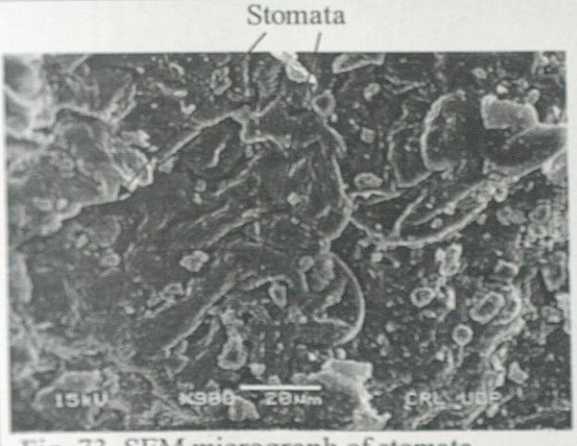



Fig. 73. SEM micrograph of stomata in leaf of *Tribulus terrestris*.

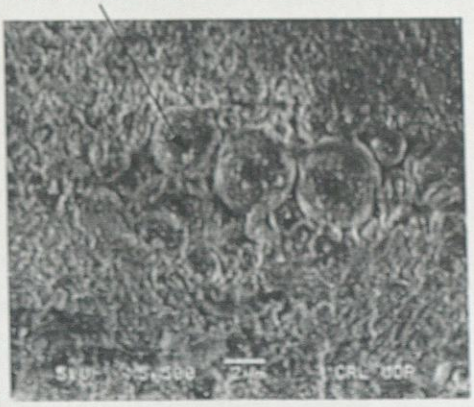



Fig. 74. SEM micrograph of glandular trichomes in leaf of *Ricinus communis*.

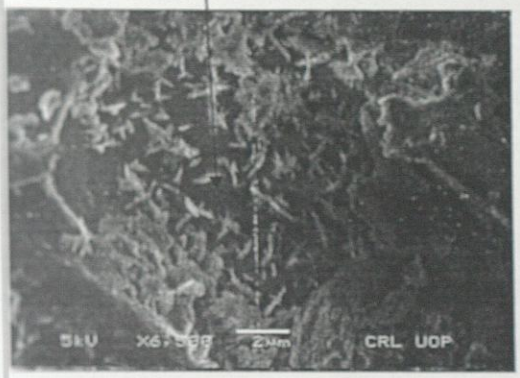



Fig. 75. SEM micrograph of rosette and prisms shaped crystals in leaf of *Ricinus communis*

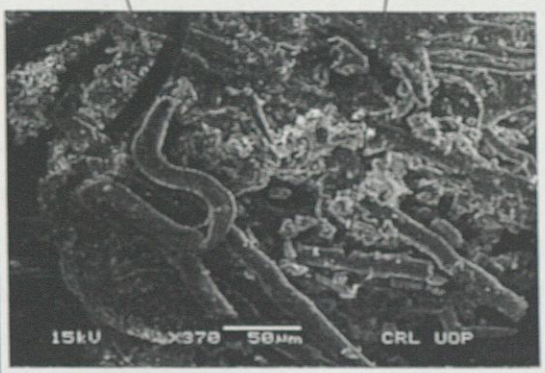



Fig. 76. SEM micrograph showing non-glandular trichomes in leaf of *Tribulus terrestris*.

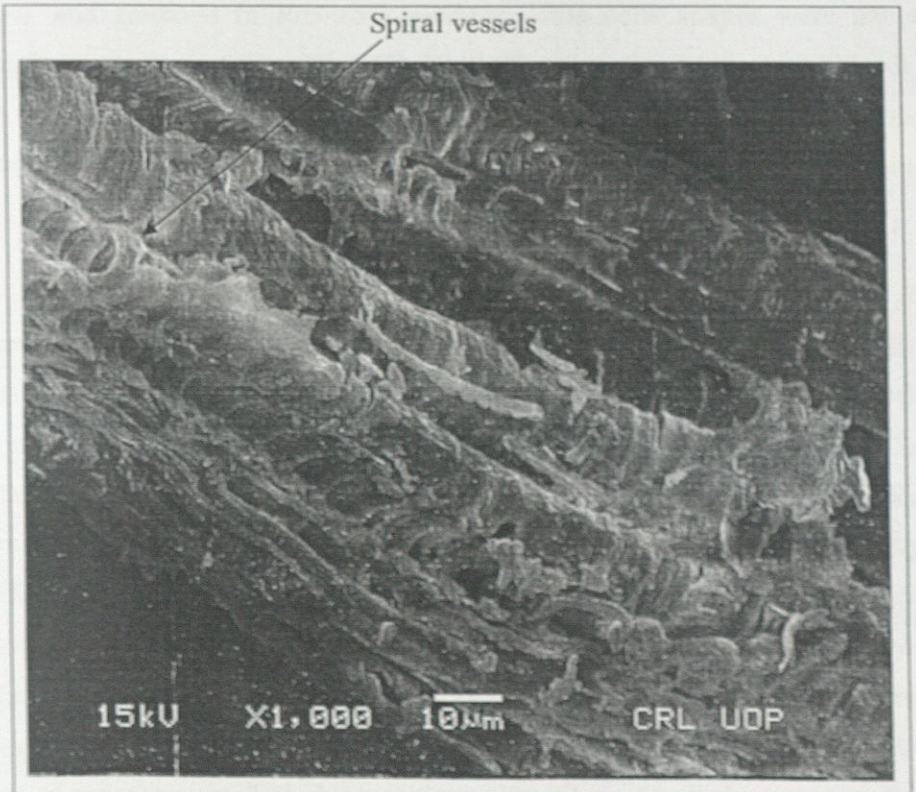



Fig. 77.Vessels in the leaf of *Tribulus terrestris*

## FRUIT ANATOMY

### x. Comparative anatomical features of fruit

The cuticle was thickest (1.5µm) in *Peganum harmala* and thinnest (0.9µm) in *Tribulus terrestris*, *Chrozophora tinctoria* and *Ricinus communis*. The trichomes were elongated and conical in *Tribulus terrestris* and *Chrozophora tinctoria*(Fig. 79b). They were absent in *Peganum harmala* and *Ricinus communis*. The number of trichomes were highest (334) in *Tribulus terrestris* than lowest (307) in *Chrozophora tinctoria* (Figs. 78c, 78b, 79a). The trichomes were longest (28.72µm) in *Tribulus terrestris* and shortest (10.16µm) in *Chrozophora tinctoria*. The trichomes were widest (2.7µm) in *Chrozophora tinctoria* (Fig. 79c) and narrowest (2.52µm) in *Tribulus terrestris* (Table 9). This agrees with Rajkala *et al.* (2011), Kumar & Sharma *et al.* (2007) who reported trichomes in the fruits of *Tribulus terrestris*, *Averrhoa carambola*, *Semecarpus anacardium* and ther medicinal plants. The shape of epicarp cells was rounded and polygonal in *Peganum harmala*; elongated in *Tribulus terrestris* and rounded in *Ricinus communis* while cells shapes were unclear in *Chrozophora tinctoria* (Figs. 78a-78b, 78d). These results agree with Trivedi *et al.* (2011), Upton *et al.* (2011) and Arif & Saeed (2010) who reported elongated, polygonal epicarp cells in *Piper nigrum*, *Vitex agnus cactus* and *Spondias mangifera* fruits.

The epicarp cells were loosely arranged in *Peganum harmala* and *Ricinus communis* and compactly arranged in *Tribulus terrestris*. The epicarp cells were longest (4.5µm) in *Peganum harmala* and smallest (2.7µm) in *Ricinus communis*. The epicarp cells were widest (3.6 µm) in *Peganum harmala* and narrowest (2.07µm) in *Tribulus terrestris*. The total width was maximum (16.25µm) in *Peganum harmala* and lowest (9.0µm) in *Tribulus terrestris*. The fibers were grouped in *Peganum harmala* and *Tribulus terrestris*. These two plants also revealed that the individual fiber cells were not clearly differentiated. They were unclear in *Chrozophora tinctoria* and *Ricinus communis* (Table 9). The fibers were found only in the epicarp region in *Peganum harmala* and *Tribulus terrestris* while fibers were also present in other regions of mesocarp in *Tribulus terrestris*. The fibers were elongated in *Peganum harmala* and *Tribulus terrestris*. These findings agree with Putiyanan *et al.* (2010) who also reported the elongated fiber cells in *Illicium verum* fruits. The fibers cells

length was maximum (123µm) in *Tribulus terrestris* and lowest (25.2µm) in *Peganum harmala* while width was highest (22.5µm) in *Tribulus terrestris* than *Peganum harmala* (10.80µm). The shape of the mesocarp cells was not clear in *Peganum harmala*. It was rounded in *Tribulus terrestris* and *Ricinus communis* (Fig. 78b, Figs. 81b). This is also supported by Rajkala *et al.* (2011), who also observed mesocarp cells, stone cells and lignified fibers in the fruits of *Tribulus terrestris*. Mesocarp cells were elongated in *Chrozophora tinctoria*. This agrees with Jain *et al.* (2007), Joseph (2001) and Arif & Saeed (2010) who reported elongated mesocarp cells in *Jatropha curcas*, *Spondias mangifera*, *Piper nigrum* and its adulterants. They were loosely arranged in all the tested plants. The mesocarp cells were longest (21.19µm) in *Chrozophora tinctoria* and smallest (4.45µm) in *Ricinus communis*. The cells had the widest range (4.4µm) in *Ricinus communis* and smallest (2.7µm) in *Chrozophora tinctoria*. The total width of mesocarp was maximum (20.16µm) in *Chrozophora tinctoria* and lowest (13.8µm) in *Ricinus communis*. The endocarp cells were spindle and hexagonal shaped in *Peganum harmala* and elongated in *Tribulus terrestris* and *Chrozophora tinctoria* (Fig. 78b, 79d). These results are supported by Joseph (2001), Rai *et al.* (2011) and Sudhakar *et al.* (2012), who reported elongated endocarp cells in *Jatropha curcas*, *Syzygium alternifolium* and *Foeniculum vulgare* fruits. It was rectangular in *Ricinus communis* (Fig. 81a) and this agrees with Selvamani *et al.* (2009) who also reported rectangular endocarp cells in *Commiphora berryi* fruits. The endocarp cells were longest (10.17µm) in *Peganum harmala* and smallest (4.45µm) in *Ricinus communis*. The width of the endocarp cells was highest (3.78µm) in *Tribulus terrestris* and lowest (1.35µm) in *Chrozophora tinctoria*. The number of cavities were highest in *Tribulus terrestris* (5) and lowest (2) in *Chrozophora tinctoria*. The calcium oxalate crystals were rounded in *Tribulus terrestris* and *Ricinus communis*, oval in *Chrozophora tinctoria*. They were absent in *Peganum harmala*. The number of calcium oxalate crystals were highest in *Chrozophora tinctoria* and lowest in *Ricinus communis*. The diameter of these crystals was highest (3.6 µm) in *Tribulus terrestris* and lowest (2.7 µm) in *Ricinus communis* (Table 9). These results agree with Singh *et al.* (2010) and Prakash *et al.* (2012) reported calcium oxalate crystals in the fruits of *Elaeocarpus ganitrus*, *Terminalia chebula* and *Foeniculum vulgare*.

### xi. Statistical evaluation of fruits features

#### Comparison based on mean values

The average trichome length was highest in *Tribulus terrestris* and lowest in *Chrozophora tinctoria* (Table 10). The average trichome width was highest in *Chrozophora tinctoria* and lowest in *Tribulus terrestris*. The average epicarp cells length was highest in *Peganum harmala* and lowest in *Ricinus communis*. The average epicarp cells width of cells was highest in *Peganum harmala* and lowest in *Tribulus terrestris*. The average mesocarp cells length was highest in *Chrozophora tinctoria* and lowest in *Ricinus communis*. The average mesocarp cells width was highest in *Tribulus terrestris* and lowest in *Chrozophora tinctoria*. The average endocarp cells length was highest in *Chrozophora tinctoria* and lowest in *Ricinus communis*. The average endocarp cells width was highest in *Ricinus communis* and lowest in *Chrozophora tinctoria*. The average diameter of crystal of calcium oxalate was highest in *Tribulus terrestris* and lowest in *Chrozophora tinctoria* (Table 10).

#### Comparison based on coefficient of variations

The coefficient of variation of trichome cells length and width was highest in *Tribulus terrestris* and lowest in *Chrozophora tinctoria*. The coefficient of variation of epicarp cells length and width was highest in *Tribulus terrestris* and lowest in *Chrozophora tinctoria*. The coefficient of variation of mesocarp cells length and width was highest in *Tribulus terrestris* and lowest in *Ricinus communis*. The coefficient of variation of endocarp cells length was highest in *Peganum harmala* and lowest in *Ricinus communis*. The coefficient of variation of endocarp cells width was highest in *Tribulus terrestris* and lowest in *Peganum harmala* and *Chrozophora tinctoria* (Table 10).

### xii. Scanning Electron Microscopy (SEM) comparative study of fruit

The SEM study clarified variation in trichomes from those observation provided by light microscopy.

**a. Non-glandular trichomes**

The SEM study showed trichome variation in fruits of *Fagonia cretica* and *Tribulus terrestris*. Some were long (*Fagonia cretica*), unicellular, tapering at both the ends (*Tribulus terrestris*). Some had long and a broad base (*Fagonia cretica* and *Tribulus terrestris*) (Fig. 85) with tapering end and they may be a fragment of multicellular trichome . They were pointed at one end and curved in the middle in (*T. terrestris*) (Fig. 87, 88-89).

The trichome length varied from 11.6 to 281µm in *Fagonia cretica* and the width varied from (9.6 to 21. 87µm) in *Fagonia cretica* (Fig. 84). The trichomes length varied from 105 (*Tribulus terrestris*) to 450µm (*Tribulus terrestris*) and the width varied from 25 to 50µm in (*Tribulus terrestris*). They were associated with other tissues in *Tribulus terrestris* (Fig. 88). Their length varied from (300 to 500µm) in *Tribulus terrestris* and the width varied from (7 to 26.66µm) in *Tribulus terrestris* (Fig. 87). The trichomes length and width was highest in *Tribulus terrestris* and lowest in *Ricinus communis*. In *Ricinus communis* a long trichome was found with a length of 200µm and width was 25µm was observed (Fig. 96). These findings are similar to that of Manisha *et al.* (2011) who also reported eglandular hairs on fruits of different medicinal plants.

The SEM study has exhibited variation in trichomes in the fruit of *Chrozophora tinctoria* and absent in other tested plants.

**Glandular trichomes**

This type was observed only in the fruit of *Chrozophora tinctoria.* It was 3 celled and without stalk. Trichome was (10µm) in length and (10µm) in width (Fig. 91).

**Peltate trichomes**

This trichome had tapering ends which were pointed and some of the ends were broken. It showed a broad base. The base length was (150µm) while the length of each pointed trichome was(50 to 60µm) and the width was (25µm) (Figs. 92). This type was found only in the fruit of *Chrozophora tinctoria* (Fig. 111). Trichome length

varied from (50-60-150μm) and the width varied from (10 to 20μm). These trichomes were slightly curved (Fig. 91). Similar findings were also reported by Bhatt *et al.* (2010), Satil & Kaya, (2007) and Machado *et al.* (2006).

**Stellate trichomes**

Stellate trichomes were recorded only in *Chrozophora tinctoria*. They had mean length of (284.2 μm) and mean width 47.14 μm (Fig. 93). A stoma was found in the fruit of *Tribulus terrestris* and *Chrozophora tinctoria* the pore length was (3μm) and the width as less than (1μm) in both the plants (Figs. 90, 94). A fragment was unknown (Fig. 95).

**Mesocarp cells**

A fragment of mesocarp cells was seen in *Tribulus terrestris* (Fig. 86), which was associated with two trichomes. One was curved while the other straight. The straight was more than (100μm) in length and about (20μm) in width Parenchyma cells and crystals were also seen in *Fagonia cretica* (Figs. 82- 83).

Table 9. Comparative anatomical features of fruits of some plants of family Zygophyllaceae and Euphorbiaceae.

| Anatomical Features | Aspects | Zygophyllaceae | | Euphorbiaceae | |
|---|---|---|---|---|---|
| | | *Peganum harmala* L. | *Tribulus terrestris* L. | *Chrozophora tinctoria* (L.) Raf. | *Ricinus communis* L. |
| Trichome | Shape | Ab | Elongated and conical | Elongated | Ab |
| | Formation | Ab | Single | Group | Ab |
| | No (Approx) | Ab | 334 | 307 | Ab |
| | Size (µm) | Ab | L=28.72, W=2.52 | L=10.16, W=2.7 | Ab |
| Cuticle | Size (µm) | 1.5 | 0.9 | 0.9 | 0.9 |
| Epicarp | Shape | Rounded, to polygonal | Elongated | Nc | Rounded |
| | Arrangement | Loose | Compact | Nc | Loose |
| | Size (µm) | L=4.5,W=3.6, T.W=16.25 | L=2.97, W=2.07, T.W=9 | Nc | L=2.7,W=2.7, T.W=9.36 |
| Fibers | Arrangement | Grouped | Grouped | Nc | Nil |
| | Location | Epicarp | Epicarp and mesocarp | Nc | Nil |
| | Shape | Elongated | Elongated | Nc | Nil |
| | Size (µm) | L=25.2, W=10.80 | L=123, W=22.5 | Nc | Nil |
| Mesocarp | Shape | Nc | Rounded | Elongated | Rounded |

| | | | | | |
|---|---|---|---|---|---|
| | Arrangement | Nc | Loose | Loose | Loose |
| | Size (µm) | Nc | L=6.12,W=4.2,T.W=18 | L=21.19,W=2.7, T.W=20.16 | L=4.45,W=4.4,T.W=13.8 |
| Endocarp | Shape | Spindle and hexagonal | Elongated | Elongated | Rectangular |
| | Size (µm) | L=10.17, W=2.7, T.W=56.50 | L=5.31, W=3.78, T.W=18 | L=6.48, W=1.35, T.W=11.70 | L=4.45,W=3.63, T.W=3.6 |
| Cavities | No | 3 | 5 | 2 | 3 |
| | Size (µm) | L=99,W=90 | L=99,W=64.8 ; L=103.50,W=35 | L=315,W=108 | L=135,W=45 |
| | | L=112.5,W=90 | L=90,W=70.2; =112.50,W=71.10 | L=270,W=184 | L=117,W=54 |
| | | L=63, W=58.50 | L=81.90, W=76.50 | | Sd |
| Calcium oxalate crystals | Shape | Ab | Rounded | Oval | Rounded |
| | Location | Ab | P, M and C | E | M |
| | No | Ab | P=105, M=130, C=20 | 440 | 176 |
| | Size (µm) | Ab | Diameter = 3.6 | L=3.96,W=1.35 | Diameter = 2.7 |

Key: Ab = Absent, L = Length, W = Width, T.W = Total Width, Sd = Solid, M = Mesocarp, Nc = Not clear; E = Epicarp, C = Cavities,P=Pericarp.

Table 10. Statistical analysis of anatomical features of fruits of plants of family Euphorbiaceae and Zygophyllaceae.

| Plants | Aspects (µm) | x | dx2 | S.D | Var | Coef.V | S.E | CIM 1 | CIM 2 |
|---|---|---|---|---|---|---|---|---|---|
| 1. *Peganum harmala* L. | | | | | | | | | |
| Trichome | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| Epicarp | L | 4.50 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | W | 3.36 | 1.70 | 0.41 | 0.17 | 12.38 | 0.13 | 3.62 | 3.04 |
| Mesocarp | L | Nc | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | W | Nc | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| Endocarp | L | 10.17 | 8608.20 | 29.34 | 860.82 | 288.49 | 9.28 | 31.16 | 0.00 |
| | W | 2.70 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Calcium-oxalate crystals | Diameter | Ab | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| 2. *Tribulus terrestris* L. | | | | | | | | | |
| Trichome | L | 28.72 | 3731.08 | 19.32 | 373.11 | 67.24 | 6.11 | 42.54 | 14.91 |
| | W | 2.52 | 2.92 | 0.54 | 0.29 | 21.43 | 0.17 | 2.91 | 2.13 |
| Epicarp | L | 2.97 | 1.70 | 0.41 | 0.17 | 13.89 | 0.13 | 3.26 | 2.68 |
| | W | 2.07 | 1.70 | 0.41 | 0.17 | 19.92 | 0.13 | 2.36 | 1.78 |
| Mesocarp | L | 6.12 | 27.22 | 1.65 | 2.72 | 26.96 | 0.52 | 7.30 | 4.20 |
| | W | 4.20 | 2.70 | 0.67 | 0.45 | 15.95 | 0.52 | 5.38 | 3.02 |
| Endocarp | L | 5.31 | 284.31 | 5.33 | 28.43 | 100.42 | 1.69 | 9.12 | 1.50 |
| | W | 3.78 | 2.92 | 0.61 | 0.38 | 16.26 | 0.19 | 4.22 | 3.34 |
| Calcium-oxalate crystals | Diameter | 3.60 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 3.*Chrozophora tinctoria* (L.) Raf | | | | | | | | | |
| Trichome | L | 10.16 | 84.56 | 2.91 | 8.46 | 28.62 | 0.92 | 12.24 | 8.08 |
| | W | 2.70 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Epicarp | L | Nc | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| | W | Nc | Nc | Nc | Nc | Nc | Nc | Nc | Nc |
| Mesocarp | L | 21.96 | 78.08 | 2.79 | 7.81 | 12.72 | 0.88 | 23.96 | 19.96 |
| | W | 2.70 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Endocarp | L | 6.48 | 7.24 | 0.85 | 0.72 | 13.13 | 0.27 | 7.09 | 5.87 |
| | W | 1.35 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Calcium-oxalate crystals | | 3.96 | 6.80 | 0.63 | 0.40 | 15.89 | 0.20 | 4.41 | 3.51 |
| | W | 1.35 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 4 .*Ricinus communis* L. | | | | | | | | | |
| Trichome | L | Ab | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| | W | Ab | Ab | Ab | Ab | Ab | Ab | Ab | Ab |
| Epicarp | L | 2.70 | 0.18 | 0.14 | 0.02 | 5.08 | 0.04 | 2.75 | 2.56 |
| | W | 2.70 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Mesocarp | L | 4.45 | 0.18 | 0.14 | 0.02 | 3.03 | 0.04 | 4.55 | 4.36 |
| | W | 4.40 | 3.34 | 0.58 | 0.33 | 14.43 | 0.18 | 4.42 | 3.59 |
| Endocarp | L | 4.45 | 0.18 | 0.14 | 0.02 | 3.03 | 0.04 | 4.55 | 4.36 |
| | W | 3.36 | 3.42 | 0.59 | 0.34 | 14.61 | 0.18 | 4.42 | 3.59 |
| Calcium-oxalate crystals | Diameter | 2.70 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Key: Ab = Absent; $Dx^2$ = Difference; Var = Variance; Coef.V = Coefficient of variance; S.E. = Standard Error; S.D = Standard deviation; CIM = Confidence interval for mean; Nc = Not clear; x=Mean.

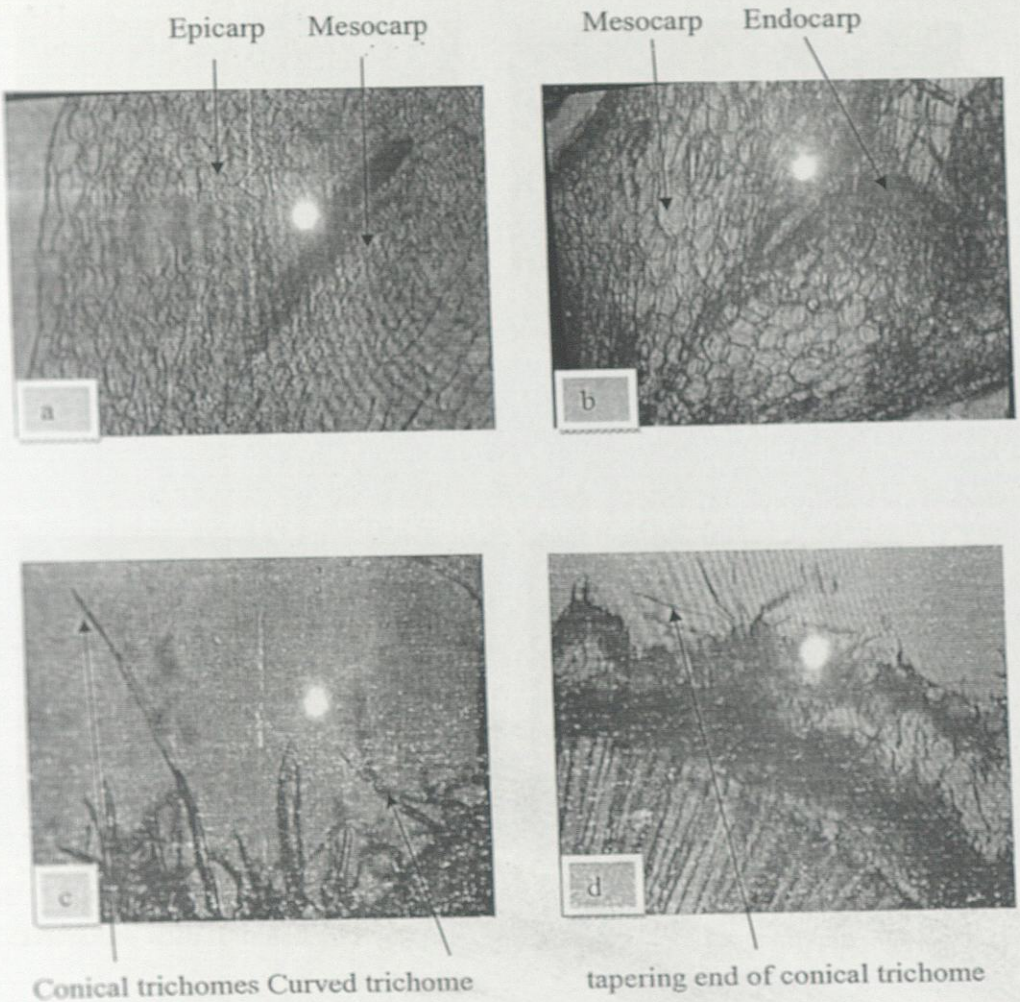
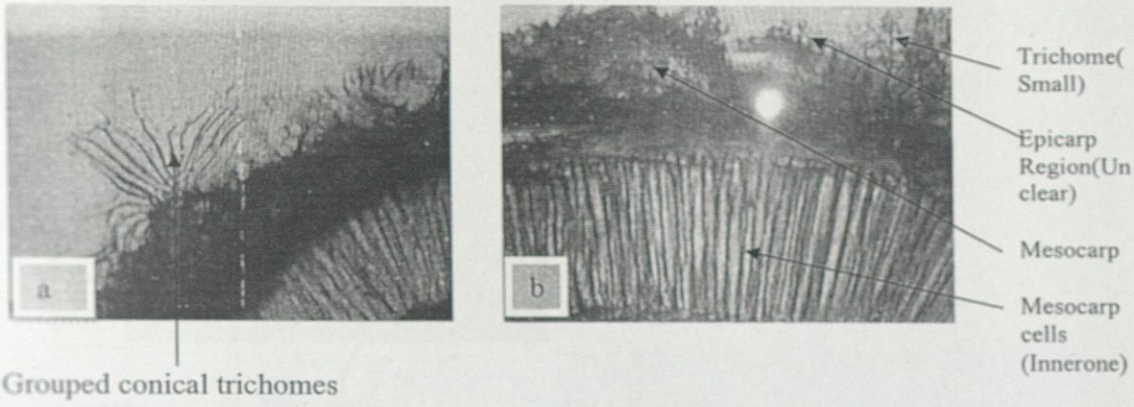
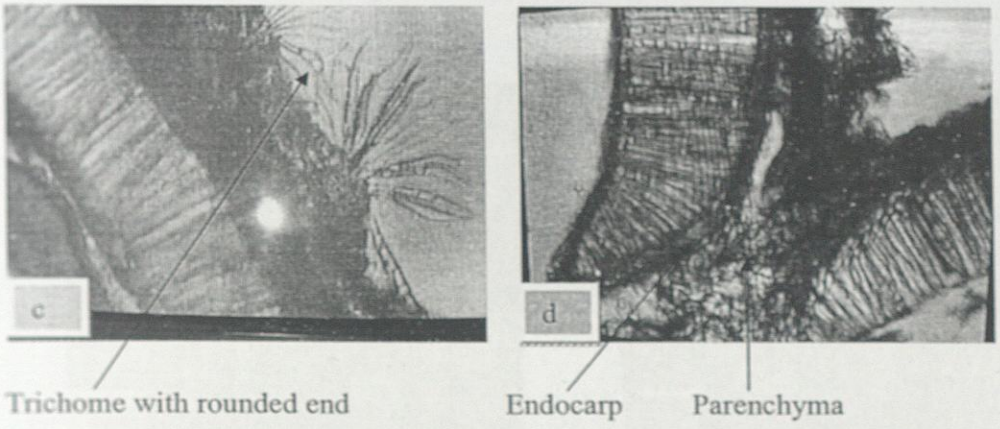



Fig. 78a. T.S of fruit of *Peganum harmala*. Fig. 78. b-c. T.S of fruit of *Tribulus terrestris*.

Fig. 78d. T.S of fruit of *Chrozophora tinctoria*.




Figs. 79 a-d. T. S of fruit of *Chrozophora tinctoria*.

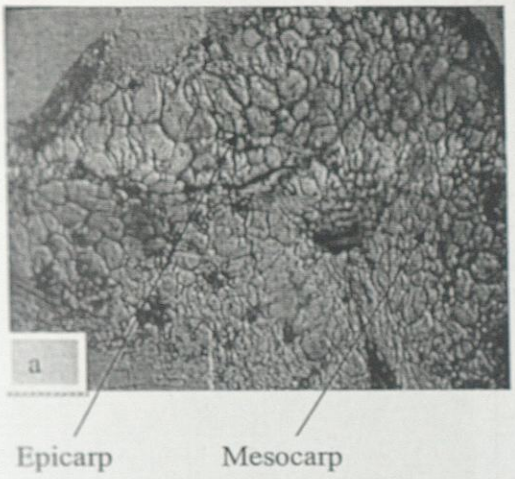
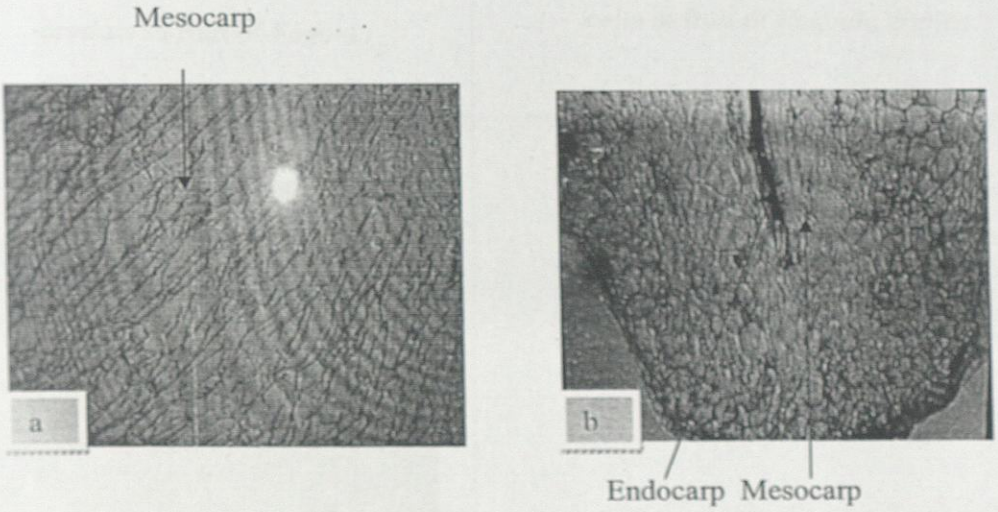



Fig. 80a. T.S of fruit of *Chrozophora tinctoria*.




Figs. 81a-b T.S of fruit of *Ricinus communis*.

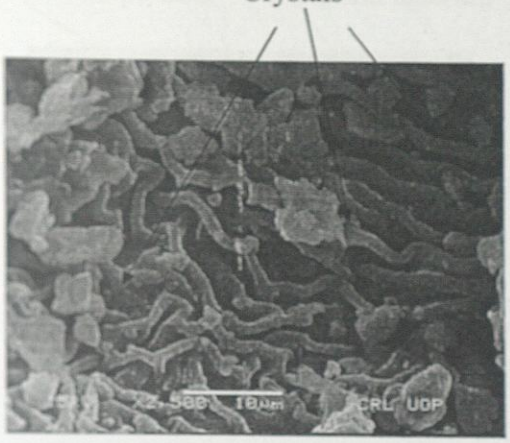



Fig. 82. SEM micrograph showing crystals in fruit of *Fagonia cretica*.

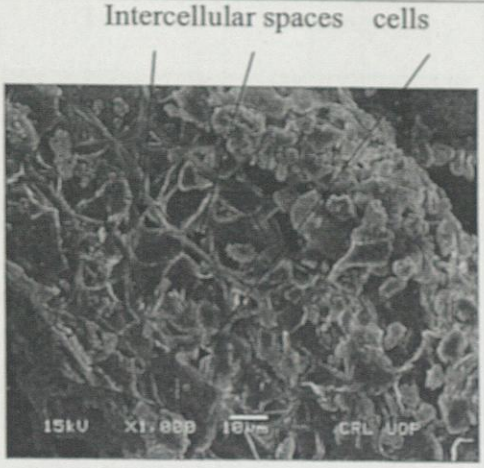



Fig. 83. SEM micrograph of parenchyma cells in fruit of *Fagonia cretica*.

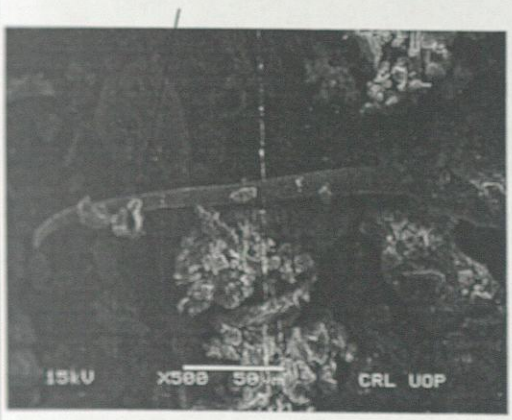



Fig. 84. SEM micrograph on non-glandular trichome in fruit of *Fagonia cretica*.

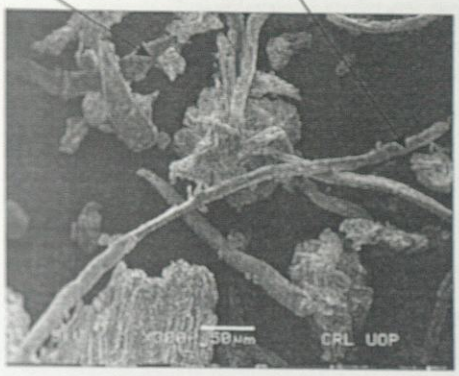



Fig. 85. SEM micrograph showing trichomes in fruit of *Tribulus terrestris*.

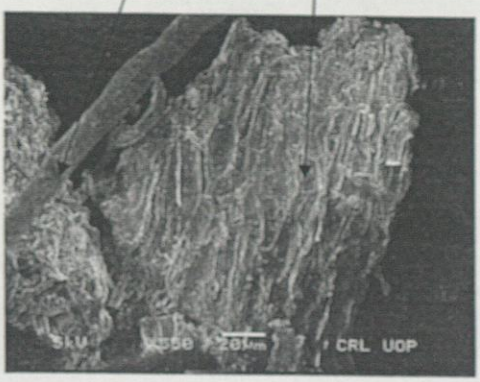



Fig.86. SEM micrograph of mesocarp cells and trichomes in fruit of *Tribulus terrestris*.

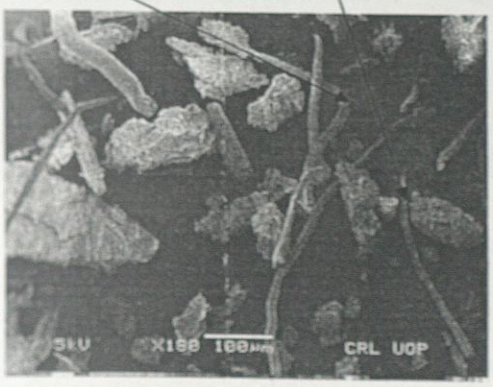



Fig. 87. SEM micrograph of tapering trichomes in fruit of *Tribulus terrestris*.

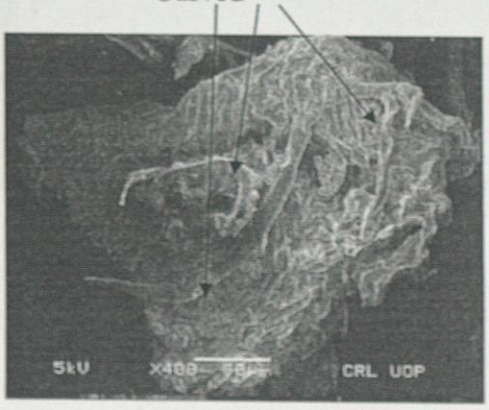



Fig. 88. SEM micrograph of tapering trichomes in fruit of *Tribulus terrestris*.

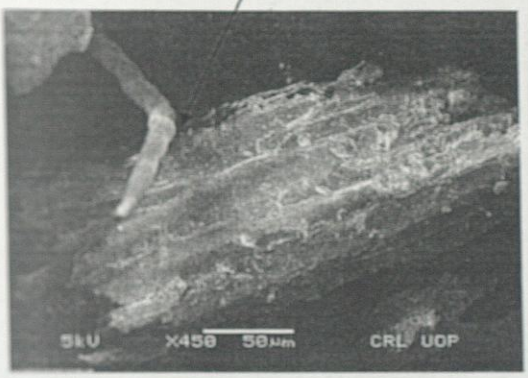



Fig. 89. SEM micrograph showing wavy trichomes in fruit of *Tribulus terrestris*..

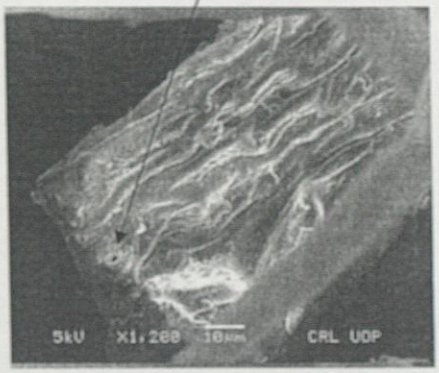



Fig. 90. SEM micrograph of stoma in fruit of *Tribulus terrestris*..

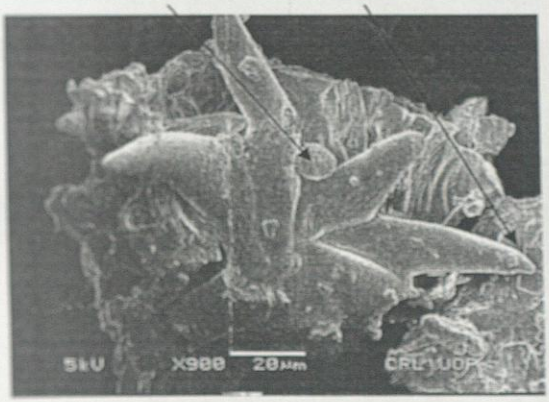



Fig. 91. SEM micrograph of glandular and peltate trichomes in fruits of *Chrozophora tinctoria*.

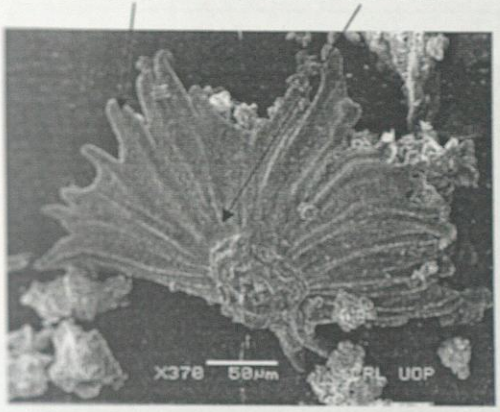



Fig. 92. SEM micrograph of peltate trichome in fruits of *Chrozophora tinctoria.*

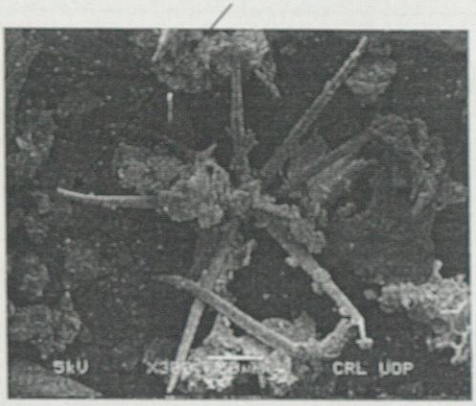



Fig. 93. SEM micrograph of stellate trichome in fruits of *Chrozophora tinctoria.*

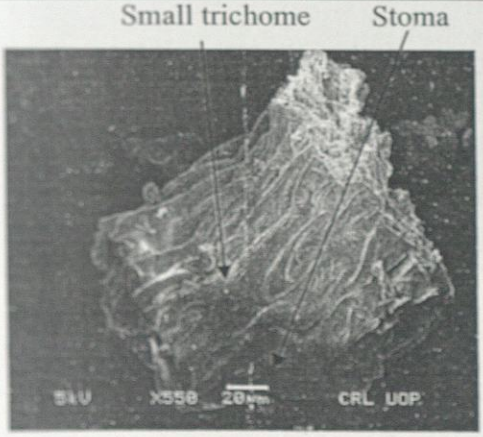



Fig. 94. SEM micrograph of stoma in fruit of *Chrozophora tinctoria.*

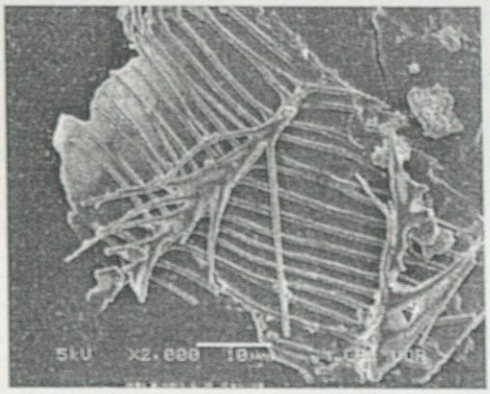



Fig. 95. SEM micrograph of unknown tissues in fruit of *Chrozophora tinctoria.*

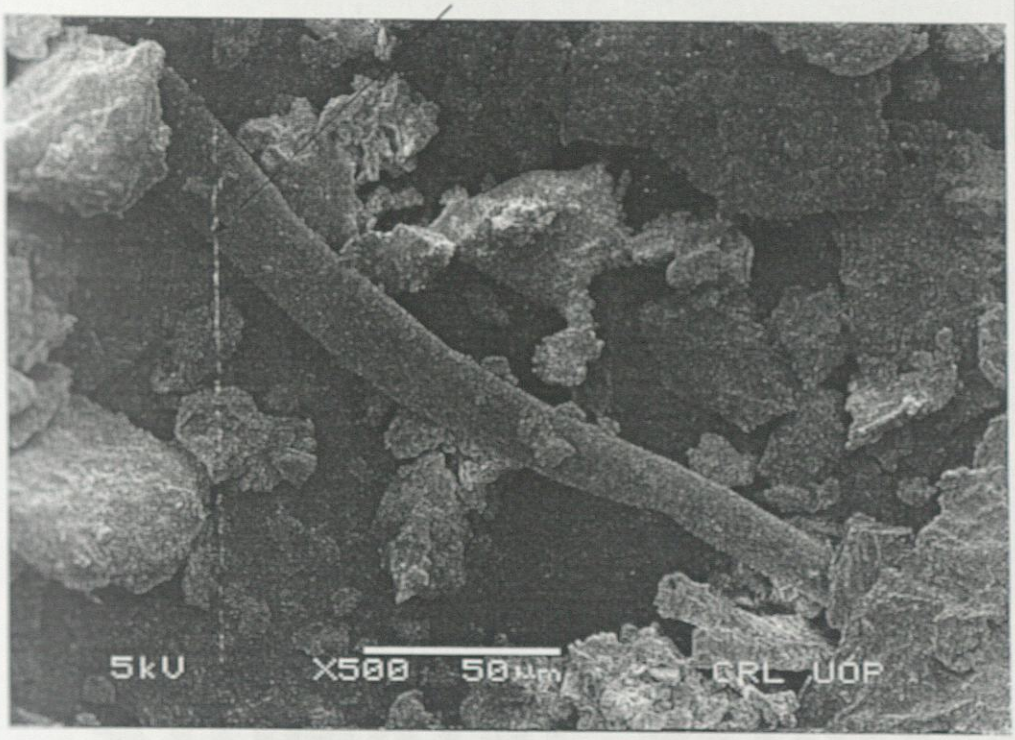



Fig. 96. SEM micrograph showing trichome in fruits of *Ricinus communis*.

# CHAPTER-6
# STOMATAL STUDY

# CHAPTER 6

# STOMATAL STUDY

The anomocytic stomata were found in the leaves of *Fagonia cretica* and *Peganum harmala* (Figs. 97a, b, c; Figs. 98-99). This agrees with the findings of Perveen *et al.* (2007) and Ahmed *et al.* (2010) who reported similar results. Ahmed *et al.* (2010) found other types of stomata in *Fagonia indica*. In the present study *Tribulus terrestris* had staurocytic stomata on both the surfaces of the leaves (Figs. 100-101). This is not in accordance with the previous findings (Vijayalakshmi, 2008; Perveen *et al.*, 2007; Ahmed *et al.*, 2010; Nikolova & Vassilev, 2011; Semerdjieva, 2011) who reported paracytic, actinocytic, anomocytic and other types of stomata in *Tribulus terrestris*. Similar type of stomatic apparatus has been reported by Metcalfe & Chalk (1950). In the present investigation *Fagonia cretica* and *Peganum harmala* had abundant anomocytic stomata on the lower surface while *Tribulus terrestris* had less stomata on the lower side (Table 11). The stauracytic stomata were found in the leaves of *Chrozophora tinctoria* and paracytic stomata in the leaves of *Ricinus communis* (Fig. 102-103).

**i. Comparative stomatal study of five plants**

The average guard cells length of stomata of upper surface was highest in *Fagonia cretica* and lowest in *Tribulus terrestris*. The average guard cells width of stomata of upper surface was highest in *Peganum harmala* and lowest in *Ricinus communis*. The average guard cells length of stomata of lower surface was highest in *Fagonia cretica* and *Ricinus communis* and lowest in *Tribulus terrestris*. The average guard cells width of stomata of lower surface was highest in *Peganum harmala* and lowest in *Tribulus terrestris*. The average central pore length of stomata of upper surface was highest in *Peganum harmala* and lowest in *Ricinus communis* and average central pore width of stomata of upper surface was highest in *Peganum harmala* and lowest in *Tribulus terrestris, Chrozophora tinctoria* and *Ricinus communis* (Table 11). The average central pore length and width of stomata of lower surface were highest in *Peganum harmala* and lowest in *Chrozophora tinctoria*. The average subsidiary cells length of stomata of upper surface was maximum in *Chrozophora tinctoria* and lowest in *Ricinus communis* .The average subsidiary cells

width of stomata of upper surface was highest in *Peganum harmala* and lowest in *Ricinus communis*. The average subsidiary cells length of stomata of lower surface was highest in *Chrozophora tinctoria* and lowest in *Peganum harmala*. The average subsidiary cells width of stomata of lower surface was highest in *Tribulus terrestris* and lowest in *Peganum harmala* .

The highest average number of stomata were found on upper epidermis in *Peganum harmala* and lowest were found on upper epidermis in *Ricinus communis*. Koyuncu *et al.* (2008) reported that *Peganum harmala* leaf was amphistomatic and plant had an amaryllis, a mesomorph and anisocytic type stomata. The highest average number of stomata were observed on lower epidermis in *Peganum harmala* and the lowest number of stomata were found on lower epidermis in *Tribulus terrestris* (Table 11).

The highest average number of epidermal cells for upper epidermis was observed in *Tribulus terrestris* and lowest in *Chrozophora tinctoria*. These findings are strongly supported by Semerdjieva (2011), who also reported maximum average number of epidermal cells on upper epidermis of *Tribulus terrestris*. The highest average number of epidermal cells for lower epidermis was found in *Ricinus communis* and lowest in *Tribulus terrestris* (Table 11). The average epidermal cells length of upper surface was highest in *Fagonia cretica* and lowest in *Chrozophora tinctoria*. The average epidermal cells width of upper surface was highest in *Fagonia cretica* and lowest in *Ricinus communis*. The average epidermal cells length of lower surface was highest in *Peganum harmala* and lowest in *Ricinus communis*. The average epidermal cells width of lower surface was highest in *Tribulus terrestris* and lowest in *Ricinus communis*. However, the findings of Ahmed *et al.* (2010) disagree with the present findings as they reported the higher values for epidermal length and width of *Fagonia cretica* and lowest in *Tribulus terrestris*. The stomatal indices have a wide range of variation. Although stomatal indices have been given considerable importance for making comparison in different taxa, it does not seem to be of any significance in the present study. This is because their values vary inconsistently in different taxa. Chaudhry & Imran (1997) compared the stomatal indices of Euphorbiaceae and Malvaceae and the ranges in these two families closely coincide with each other. The highest stomatal index of stomata was found for upper epidermis in *Tribulus terrestris* and lowest in *Ricinus communis*. The highest stomatal index of

stomata was found for lower epidermis in *Ricinus communis* and lowest in *Tribulus terrestris* (Table 11). The results are not in line with Thakur & Patil (2011), reported the highest stomatal index value in *Sapium insigne* and low value *Acalypha indica* (Euphorbiaceae). The highest stomatal index of stomata was found for lower epidermis in *Ricinus communis* in the present study (Table 11). Chaudhry & Imran (1997) reported variation in the structure and distribution of stomata in some members of Euphorbiaceae and found paracytic stomata as the dominant type in Euphorbiaceae some members and this agrees with the present study.

**ii. Statistical evaluation of stomatal study**

**Comparative study based on mean value**

The length and width of guard cells logically is the function of the activity of the meristemoid giving rise to the stomatal apparatus. Depending upon the degree of definitineness of the meristemoid together with the duration of the enlargement phase of the derivatives (Chaudhry & Imran 1997). The average guard cells length of stomata of upper surface was highest in *Fagonia cretica* and lowest in *Tribulus terrestris* and *Chrozophora tinctoria.* The average guard cells width of stomata of upper surface was highest in *Peganum harmala* and lowest in *Tribulus terrestris, Chrozophora tinctoria* and *Ricinus communis*. These findings are not in agreement with the findings of Chaudhry & Imran (1997) who reported higher values for guard cells length in *Ricinus communis*. The average guard cells length of stomata of lower surface was highest in *Fagonia cretica* and lowest in *Tribulus terrestris.* The average guard cells width of stomata of lower surface was highest in *Fagonia cretica* and *Peganum harmala* and lowest in *Chrozophora tinctoria* (Table 12). The average central pore length of stomata of upper surface was highest in *Peganum harmala* and lowest in *Ricinus communis.* The average central pore width of stomata of upper surface was highest in *Fagonia cretica* and lowest in *Tribulus terrestris*, *Chrozophora tinctoria* and *Ricinus communis*. These findings disagree with the findings of Chaudhry & Imran (1997) who reported higher values for central pore length in *Ricinus communis*. The average central pore length of stomata of lower surface was highest in *Fagonia cretica* and lowest in *Chrozophora tinctoria.* The average central pore width of stomata of lower surface was highest in *Tribulus terrestris* and lowest in *Peganum harmala* and *Chrozophora tinctoria.* The average subsidiary cells length of upper surface was highest in *Peganum harmala* and *Chrozophora tinctoria* and lowest

in *Peganum harmala*. The average subsidiary cells width of upper surface was highest in *Peganum harmala* and lowest in *Ricinus communis*. The average subsidiary cells length of lower surface was highest in *Ricinus communis* and lowest in *Peganum harmala*. The average subsidiary cells width of lower surface was highest in *Tribulus terrestris* and lowest in *Peganum harmala*. The highest average number of stomata were found on upper epidermis in *Peganum harmala* and the lowest number were found in *Chrozophora tinctoria*. The highest average number of stomata were found on lower epidermis in *Peganum harmala* and the lowest number were found in *Chrozophora tinctoria* (Table 12).

**Comparative study based on coefficient of variation**

The coefficient of variation of guard cells length of stomata of upper surface was highest in *Chrozophora tinctoria* and lowest in *Tribulus terrestris*. The coefficient of variation of guard cells width of stomata of upper surface was highest in *Peganum harmala* and lowest in *Tribulus terrestris* and *Chrozophora tinctoria*. The coefficient of variation of guard cells length of stomata of lower surface was highest in *Chrozophora tinctoria* and lowest *Peganum harmala*. The coefficient of variation of guard cells width of stomata of lower surface was highest in *Peganum harmala* and lowest *Chrozophora tinctoria* (Table 12).

The coefficient of variation of central pore length of stomata of upper surface was highest in *Tribulus terrestris* and lowest in *Fagonia cretica*. The coefficient of variation of central pore width of stomata of upper surface was highest in *Peganum harmala* and lowest in *Fagonia cretica*. The coefficient of variation of central pore length of stomata of lower surface was highest in *Ricinus communis* and lowest in *Chrozophora tinctoria*. The coefficient of variation of central pore width of stomata of lower surface was highest in *Peganum harmala* and lowest in *Fagonia cretica* (Table 12).

The coefficient of variation of subsidiary cells length of stomata of upper surface was highest in *Ricinus communis* and lowest in *Fagonia cretica*. The coefficient of variation of subsidiary cells width of stomata of upper surface was highest in *Ricinus communis* and lowest in *Chrozophora tinctoria*. The coefficient of variation of subsidiary cells length of stomata of lower surface was highest in *Ricinus communis* and lowest in *Fagonia cretica*. The coefficient of variation of subsidiary

cells width of stomata of lower surface was highest in *Peganum harmala* and lowest in *Tribulus terrestris*. The coefficient of variation of number of stomata were highest on upper epidermis in *Peganum harmala* and the lowest number were found in *Tribulus terrestris*. The coefficient of variation of number of stomata were highest on lower epidermis in *Peganum harmala* and the lowest number were found in *Fagonia cretica* (Table 12).

The family Euphorbiaceae consisted of 300 genera and 5000 species. The mature stomata in this family are rubiaceous, cruciferous and ranunculaceous which are usually confined to lower surface, more rarely on both the surfaces of the lamina. Paracytic stomata which were mesogenous in development in *Euphorbia variegata* and *Ricinus communis*. Similarly, anomocytic, paracytic, anisocytic, cyclocytic and stomata with a single subsidiary cell in the species of *Euphorbia*. Surprisingly, in such a large family only a handful workers have studied the stomatal structure and ontogeny. In the present study the staurocytic type of stomata were found in the leaves of *Chrozophora tinctoria*. These findings agree with Yasmin *et al*. (2010) who reported similar stomatal types in *Fagopyrum, Bistorta affinis* and *B. amplexicaulis*. *Ricinus communis* showed paracytic type of stomata on both the surfaces (Table 12). Chaudhry & Imran (1997) reported that the members of Euphorbiaceae had paracytic and anisocytic stomata. These findings agree with the present study.

Table 11. Comparative stomatal study of plants of family Zygophyllaceae and Euphorbiaceae (values are average of ten reading ( three fields).

| Plants | Type | Distribution | No of St | No of (E) | St. Index | Size of S P (µm) | Size of G C (µm) | Size of S C (µm) | Size of (E) | O/C St |
|---|---|---|---|---|---|---|---|---|---|---|
| **Zygophyllaceae** | | | | | | | | | | |
| *Fagonia cretica* L. | | | | | | | | | | |
| Abaxial (Upper) | Anomocytic | Irregular | 166 | 81 | 62.20 | L=2.75, W=1.52 | L=4.1, W=1.51 | L=3.7, W=2.90 | L=4.59,W=3.24 | All opened |
| Adaxial (Lower) | Anomocytic | Irregular | 230 | 97 | 70.34 | L=2.7, W=1.4 | L=3.56, W=1.55 | L=3.75, W=2.97 | L=4.59,W=3.15 | All opened |
| *Peganum harmala* L. | | | | | | | | | | |
| Abaxial(Upper) | Anomocytic | Irregular | 700 | 70 | 90.9 | L= 3.0, W=1.6 | L=2.6, W=1.6 | L=3.57 , W=3.22 | L=4.77,W=2.88 | All opened |
| Adaxial(Lower) | Anomocytic | Irregular | 850 | 70 | 92.4 | L= 2.0, W= 0.9 | L= 3.0 , W=1.54 | L=2.17, W=2.17 | L=4.77,W=2.88 | |
| *Tribulus terrestris* L. | | | | | | | | | | |
| Abaxial(Upper) | Staurocytic | Irregular | 237 | 101 | 270 | L=2.56, W=0.9 | L=2.65, W=1.35 | L=4.05, =3.193 | L=3.33,W=2.65 | All opened |
| Adaxial(Lower) | Staurocytic | Irregular | 39 | 75 | 34.21 | L=2.23,W=1.58 | L=2.65, W=0.68 | L=3.74, W=3.06 | L=4.05,W=3.51 | All opened |
| **Euphorbiaceae** | | | | | | | | | | |
| *Chrozophora tinctoria* (L.) Raf. | | | | | | | | | | |
| Abaxial(Upper) | Staurocytic | Irregular | 116 | 11 | 127 | L=2.73,W=0.9 | L=2.71, =W=1.35 | L=4.08, =2.910 | L=2.86, W=2.7 | All opened |
| Adaxial (Lower) | Staurocytic | Irregular | 92 | Nc | Nc | L=1.8,W=0.9 | L=2.95, W=1.35 | L= 3.81, W=2.50 | Nc | All opened |
| *Ricinus communis* L. | | | | | | | | | | |
| Abaxial(Upper) | Paracytic | Irregular | 43 | 110 | 28.1 | L=2.13,W=0.9 | L=2.89,W=1.34 | L=3.29,W=2.52 | L=3.240, =1.8 | All opened |
| Adaxial(Lower) | Paracytic | Irregular | 116 | 101 | 53.5 | L=2.64,W=1.27 | L=3.5,W=1.34 | L=4.39W=2.69 | L=3.06, W=1.8 | All opened |

Key: L = Length; W = Width; St = Stomata; E = Epidermal cell; St. index = Stomatal index; S P = Stomatal pore; G C = Guard cells; S C = Subsidary cells; O = Open; C = Close; Nc = Not clear.

Table 12. Statistical data of stomatal study of plants of family Zygophyllaceae and Euphorbiaceae.

| Aspects (µm) | X | | dx2 | | S.D | | Var | | Coef. V | | S. E | | CIM 1 | | CIM 2 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plants | | | | | | | | | | | | | | | | |
| | U | L | U | L | U | L | U | L | U | L | U | L | U | L | U | L |
| **Zygophyllaceae** | | | | | | | | | | | | | | | | |
| 1. *Fagonia cretica* L. | | | | | | | | | | | | | | | | |
| Guard cell. | | | | | | | | | | | | | | | | |
| Length | 4.11 | 3.55 | 0.18 | 0.05 | 0.24 | 0.13 | 0.06 | 0.02 | 5.91 | 3.69 | 0.14 | 0.07 | 4.43 | 3.66 | 3.79 | 3.33 |
| Width | 1.51 | 1.55 | 0.05 | 0.03 | 0.13 | 0.1 | 0.02 | 0.01 | 8.4 | 7 | 0.03 | 0.06 | 1.58 | 1.64 | 1.45 | 1.36 |
| Central pore of stomata | | | | | | | | | | | | | | | | |
| Length | 2.75 | 2.67 | 0.01 | 0 | 0.07 | 0.04 | 0 | 0 | 2.4 | 1.53 | 0.04 | 0.02 | 2.83 | 2.72 | 2.66 | 2.61 |
| Width | 1.52 | 1.4 | 0.13 | 0.1 | 0.21 | 0.18 | 0.04 | 0.03 | 13.7 | 13.17 | 0.12 | 0.11 | 1.79 | 1.63 | 1.25 | 1.16 |
| Subsidary cells | | | | | | | | | | | | | | | | |
| Length | 3.7 | 3.75 | 0.4 | 0.01 | 0.36 | 0.04 | 0.13 | 0 | 0.1 | 1.13 | 0.21 | 0.02 | 4.19 | 3.81 | 3.24 | 3.69 |
| Width | 2.9 | 2.97 | 0.02 | 0.07 | 0.08 | 0.15 | 0.01 | 0.02 | 2.64 | 5.08 | 0.04 | 0.09 | 2.99 | 3.17 | 2.8 | 2.78 |
| Number of Stomata | 55.3 | 78 | 370.7 | 744 | 11.12 | 15.74 | 123.6 | 248 | 20.09 | 20.17 | 6.42 | 9.09 | 69.9 | 98.6 | 40.82 | 57.44 |
| 2. *Peganum harmala* L. | | | | | | | | | | | | | | | | |
| Guard cell | | | | | | | | | | | | | | | | |
| Length | 2.6 | 3 | 9.58 | 0 | 1.79 | 0 | 3.19 | 0 | 68.73 | 0 | 1.03 | 0 | 4.93 | 0 | 0.27 | 0 |
| Width | 1.6 | 1.54 | 6.64 | 4.81 | 1.49 | 1.27 | 2.21 | 1.6 | 92.98 | 82.23 | 0.86 | 0.73 | 3.54 | 3.19 | 0 | 0 |
| Central pore of stomata | | | | | | | | | | | | | | | | |
| Length | 3 | 2 | 4.6 | 3.3 | 1.2 | 1 | 1.5 | 1.1 | 68.7 | 53 | 0.7 | 0.6 | 4.6 | 3.3 | 1.4 | 0.6 |
| Width | 1.6 | 0.9 | 6.1 | 2.4 | 1.2 | 0.9 | 2 | 0.8 | 93 | 98.8 | 0.8 | 0.5 | 3.5 | 2.1 | 0 | 0 |
| Subsidary cells | | | | | | | | | | | | | | | | |
| Length | 3.57 | 2.17 | 2.18 | 0.65 | 0.88 | 0.47 | 0.73 | 0.22 | 24.61 | 21.6 | 0.51 | 0.27 | 3.57 | 2.78 | 2.42 | 1.56 |
| Width | 3.22 | 2.17 | 31.29 | 3.52 | 3.23 | 1.08 | 10.43 | 1.18 | 100.3 | 65.57 | 1.86 | 0.63 | 7.43 | 3.07 | 0 | 0.24 |
| Number of Stomata | 617 | 350 | 11294 | 615000 | 613.6 | 452.8 | 376481 | 205000 | 99.49 | 129.4 | 354.3 | 261 | 1418 | 941 | 0 | 0 |
| 3. *Tribulus terrestris* L. | | | | | | | | | | | | | | | | |
| Guard cell | | | | | | | | | | | | | | | | |
| Length | 2.65 | 2.65 | 0 | 0.01 | 0.04 | 0.05 | 0 | 0 | 1.39 | 1.89 | 0.02 | 0.03 | 2.7 | 2.72 | 2.61 | 2.58 |
| Width | 1.35 | 0.68 | 0 | 0.25 | 0 | 0.36 | 0 | 0.13 | 0 | 52.71 | 0 | 0.25 | 0 | 1.24 | 0 | 0.11 |
| Central pore of stomata | | | | | | | | | | | | | | | | |
| Length | 2.56 | 2.23 | 26.22 | 0.05 | 2.18 | 0.16 | 8.74 | 0.02 | 84.93 | 6.97 | 1.26 | 0.11 | 5.41 | 2.47 | 0 | 1.98 |
| Width | 0.9 | 1.58 | 0 | 0.1 | 0 | 0.23 | 0 | 0.05 | 0 | 14.29 | 0 | 0.16 | 0 | 1.93 | 0 | 1.22 |
| Subsidary cells | | | | | | | | | | | | | | | | |
| Length | 4.05 | 3.74 | 0.11 | 0.04 | 0.19 | 0.14 | 0.04 | 0.02 | 4.8 | 3.61 | 0.11 | 0.1 | 4.3 | 3.95 | 3.8 | 3.52 |
| Width | 3.19 | 3.06 | 0.258 | 0 | 0.29 | 0 | 0.09 | 0 | 9.18 | 0 | 0.17 | 0 | 3.58 | 0 | 2.81 | 0 |
| Number of Stomata | 77.5 | 40.3 | 12.5 | 2617 | 2.5 | 29.5 | 6.3 | 872 | 3.2 | 73.2 | 1.8 | 17 | 81.5 | 78.9 | 73.5 | 1.8 |

| Euphorbiaceae | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4. *Chrozophora tinctoria* (L.) Raf. | | | | | | | | | | | | | | | | |
| Guard cell | | | | | | | | | | | | | | | | |
| Length | 2.71 | 2.95 | 22.03 | 0.88 | 2.71 | 0.17 | 7.34 | 0.03 | 100 | 5.8 | 1.56 | 0.1 | 6.25 | 3.18 | 0 | 2.72 |
| Width | 1.35 | 1.35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Central pore of stomata | | | | | | | | | | | | | | | | |
| Length | 2.73 | 1.8 | 0.995 | 0 | 0.06 | 0 | 0.056 | 0 | 23.77 | 0 | 0.032 | 0 | 2.44 | 0 | 2.3 | 0 |
| Width | 0.9 | 0.9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Subsidary cells | | | | | | | | | | | | | | | | |
| Length | 4.08 | 3.81 | 0.08 | 0.23 | 0.17 | 0.28 | 0.03 | 0.08 | 4.1 | 7.3 | 0.1 | 0.16 | 4.3 | 4.17 | 3.86 | 3.44 |
| Width | 2.91 | 2.59 | 0.005 | 0.24 | 0.04 | 0.15 | 0.012 | 0.01 | 1.457 | 5.92 | 0.024 | 0.09 | 2.96 | 2.79 | 2.85 | 2.39 |
| Number of Stomata | 38.7 | 30.7 | 266.7 | 133 | 9.42 | 6.65 | 88.88 | 44.2 | 24.38 | 21.68 | 5.44 | 3.83 | 38.7 | 39.4 | 26.35 | 21.98 |
| 5. *Ricinus communis* L. | | | | | | | | | | | | | | | | |
| Guard cell | | | | | | | | | | | | | | | | |
| Length | 2.89 | 3.5 | 0.18 | 0.03 | 0.25 | 0.09 | 0.06 | 0.01 | 8.49 | 0.73 | 0.14 | 0.05 | 3.21 | 3.62 | 2.57 | 3.37 |
| Width | 1.34 | 1.34 | 0.01 | 0.01 | 0.18 | 0.06 | 0 | 0 | 13.3 | 4.2 | 0.14 | 0.03 | 1.66 | 1.41 | 1.01 | 1.26 |
| Central pore of stomata | | | | | | | | | | | | | | | | |
| Length | 2.13 | 2.64 | 0.35 | 0.01 | 0.34 | 0.06 | 0.12 | 0 | 15.93 | 2.21 | 0.2 | 0.03 | 2.57 | 2.71 | 1.69 | 2.56 |
| Width | 0.9 | 1.27 | 0 | 0.04 | 0 | 0.11 | 0 | 0.01 | 0 | 8.51 | 0 | 0.06 | 0 | 1.41 | 0 | 1.13 |
| Subsidary cells | | | | | | | | | | | | | | | | |
| Length | 3.6 | 4.3 | 10.35 | 0.03 | 1.86 | 1.2 | 3.45 | 1.43 | 51.54 | 27.83 | 1.07 | 0.69 | 6.03 | 5.87 | 1.18 | 2.74 |
| Width | 2.71 | 2.67 | 28.46 | 0.04 | 3.08 | 0.11 | 9.49 | 0.01 | 113.8 | 4.2 | 1.78 | 0.06 | 6.73 | 2.82 | 0 | 2.52 |
| Number of Stomata | 47 | 50 | 1404 | 1517 | 26 | 22 | 702 | 506 | 15 | 45 | 19 | 13 | 89 | 79 | 4 | 21 |

Key: X = Mean; CIM 1 and 2 = Confidence interval for mean ; Coef.V = Coefficient of variance; $dx^2$ = Total sum of difference square; S.D = Standard deviation; S. E. Standard Error.Var = Variance.

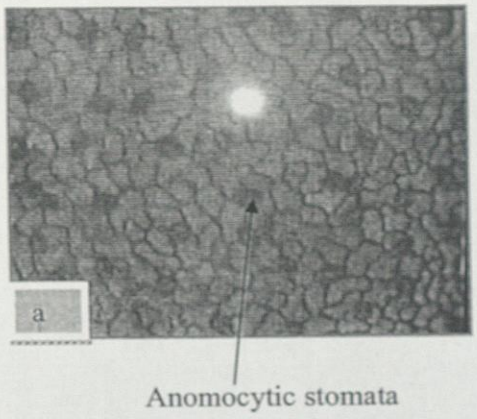



Fig. 97 a. Anomocytic stomata in *Fagonia cretica* upper epidermis.

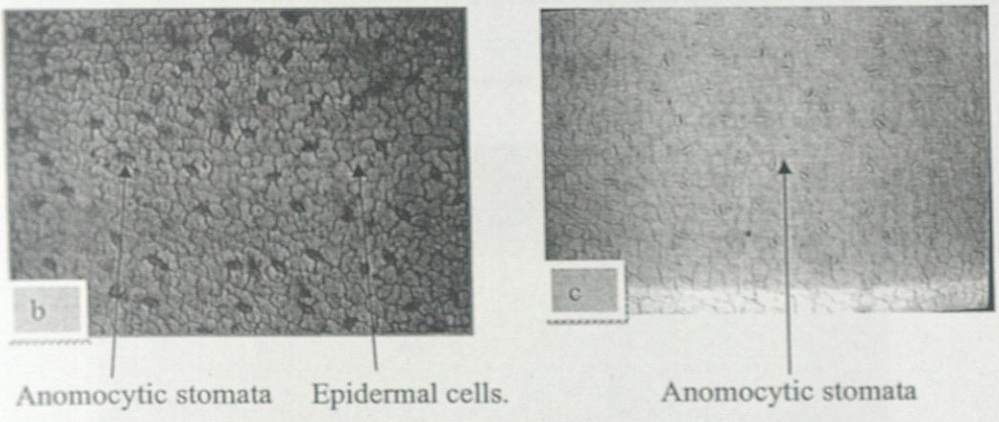



Fig. 97. b-c. Anomocytic stomata in *Fagonia cretica* lower epidermis.




Fig. 98. Anomocytic stomata in *Peganum harmala* upper epidermis.

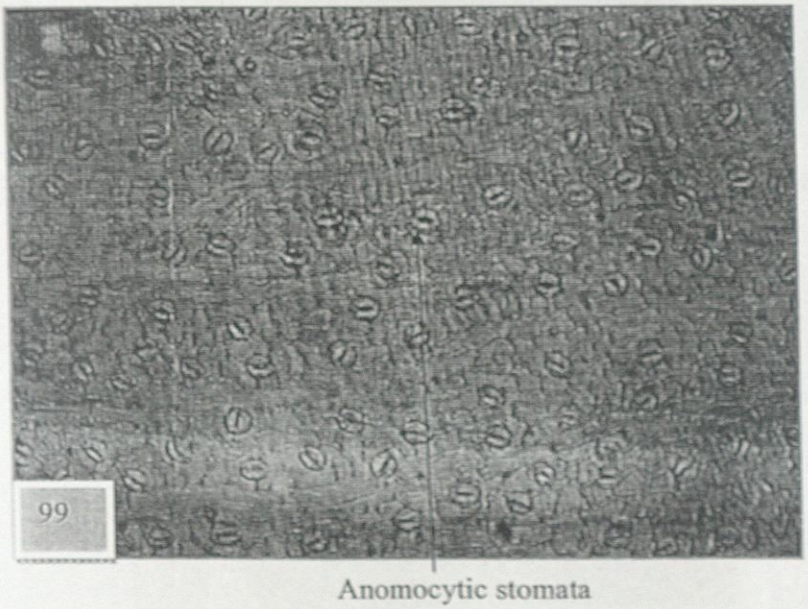



Fig. 99. Anomocytic stomata in *Peganum harmala* lower epidermis.

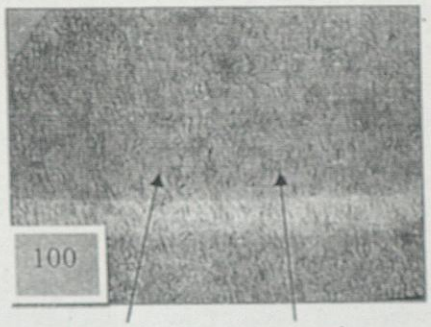



Fig. 100. Staurocytic stomata in *Tribulus terrestris* upper epidermis.

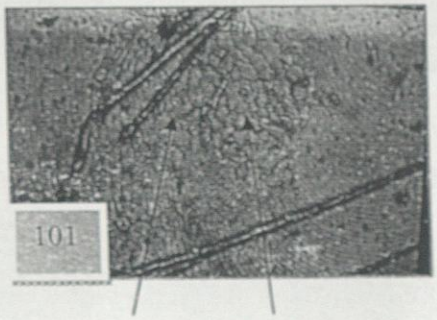



Fig. 101. Staurocytic stomata in *Terrestris terrestris* lower epidermis.

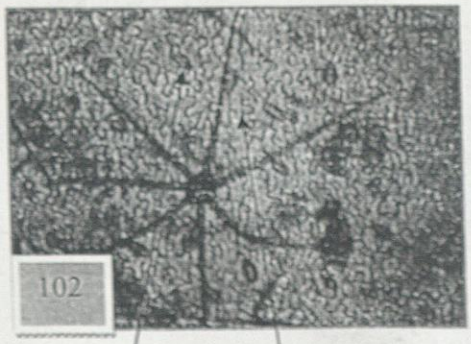



Fig. 102. Staurocytic stomata in *Chrozophora tinctoria* lower epidermis.

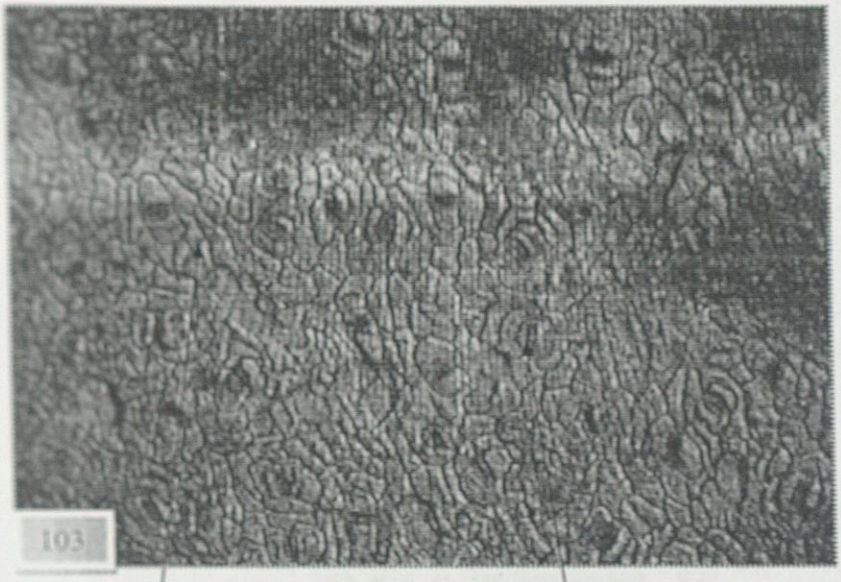



Fig. 103. Paracytic stomata in *Ricinus communis* lower epidermis

BIOLOGICAL ACTIVITIES

# CHAPTER-7
# BIOLOGICAL ACTIVITIES

# i.Antibacterial Activity

# CHAPTER 7

# ANTIBACTERIAL ACTIVITY

## . Antibacterial activity of five plants

The results of antibacterial activity of plant extracts are shown in Tables 13 nd 14. *Fagonia cretica* showed highest antibacterial activity against *Staphylococcus ureus* and lowest against *Salmonella typhi*. *Peganum harmala* showed highest hibition against *S. aureus* and *K. pneumoni*. Hayet *et al.* (2010) reported that ethanolic extract of *P. harmala* leaves showed strong antibacterial activity against $g^+$ than gram $g^-$bacteria. *Peganum harmala* showed low antibacterial activity against *E. coli* and *S. aureus* in the present study. This low activity might be due to the egligible amount of phytochemicals present in the plant. Amadi *et al.* (2010) eported that the bio-efficacy of plant extracts was affected by the extraction method nd concentration used. *Tribulus terrestris* showed highest inhibition against *E. coli*. These findings are in agreement with Mohanty *et al.* (2010). The methanolic leaf extract of *T. terrestris* had alkaloids, tannins, saponins and glycosides. This agrees with Hussain *et al.* (2011) and Kianbakht & Jahanianiian (2003). Since present study showed activity against the most prevalent $g^-$ bacteria in urinary infections namely *E. coli*, the use of the plant as a urinary anti-infective is validated. *Chrozophora tinctoria* showed highest inhibition against *P. aeruginosa* and low antibacterial activity against *S. aureus* and *K. pneumoni*. *Ricinus communis* showed highest inhibition against *S. aureus*. These findings are also supported by Kensa & Yasmin (2011). *Fagonia cretica*, *P. harmala* and *R. communis* showed highest % inhibition of *S. aureus*. This maximum inhibition of *S. aureus* may be due to the solubility of some active principles in the methanol.

The n-hexane extracts of *F. cretica* showed highest inhibition against *P. aeruginosa*. Javale and Sabnis (2010) stated that inhibition of *K. pneumoni* might be due to the presence of capsular polysaccaride surrounding *K. pneumoni* which might have interferred with the antimicrobial activity of the leaf and fruit extract of *Emblica officinalis*. *Peganum harmala* showed highest inhibition against *K. pneumoni*. Takhi *et al.* (2011) disagrees with these results.

# CHAPTER 7

# ANTIBACTERIAL ACTIVITY

**i. Antibacterial activity of five plants**

The results of antibacterial activity of plant extracts are shown in Tables 13 and 14. *Fagonia cretica* showed highest antibacterial activity against *Staphylococcus aureus* and lowest against *Salmonella typhi*. *Peganum harmala* showed highest inhibition against *S. aureus* and *K. pneumoni*. Hayet *et al.* (2010) reported that methanolic extract of *P. harmala* leaves showed strong antibacterial activity against g+ than gram g⁻bacteria. *Peganum harmala* showed low antibacterial activity against *E. coli* and *S. aureus* in the present study. This low activity might be due to the negligible amount of phytochemicals present in the plant. Amadi *et al.* (2010) reported that the bio-efficacy of plant extracts was affected by the extraction method and concentration used. *Tribulus terrestris* showed highest inhibition against *E. coli*. These findings are in agreement with Mohanty *et al.* (2010). The methanolic leaf extract of *T. terrestris* had alkaloids, tannins, saponins and glycosides. This agrees with Hussain *et al.* (2011) and Kianbakht & Jahanianiian (2003). Since present study showed activity against the most prevalent g⁻ bacteria in urinary infections namely *E. coli*, the use of the plant as a urinary anti-infective is validated. *Chrozophora tinctoria* showed highest inhibition against *P. aeruginosa* and low antibacterial activity against *S. aureus* and *K. pneumoni*. *Ricinus communis* showed highest inhibition against *S. aureus*. These findings are also supported by Kensa & Yasmin (2011). *Fagonia cretica, P. harmala* and *R. communis* showed highest % inhibition of *S. aureus*. This maximum inhibition of *S. aureus* may be due to the solubility of some active principles in the methanol.

The n-hexane extracts of *F. cretica* showed highest inhibition against *P. aeruginosa*. Javale and Sabnis (2010) stated that inhibition of *K. pneumoni* might be due to the presence of capsular polysaccaride surrounding *K. pneumoni* which might have interferred with the antimicrobial activity of the leaf and fruit extract of *Emblica officinalis*. *Peganum harmala* showed highest inhibition against *K. pneumoni*. Takhi *et al.* (2011) disagrees with these results.

*Tribulus terrestris* showed highest inhibition against *S. aureus* and these findings are supported by Hussain *et al.* (2009), Al-Bayati and Al-Mola. (2008) and Jain *et al.*(2010). *Fagonia cretica* and *P. harmala* showed maximum % inhibition of *P. aeruginosa* while *T. terrestris, C. tinctoria* and *R. communis* showed maximum % inhibition of *S. aureus*. The present results clearly show that the antibacterial activity varied with the plant species and the extracts used and it agrees with Mahesh & Satish (2008). The t-test done for comparing the antibacterial activity of methanolic and n-hexane plant extracts against various microbes revealed that there was no significant difference between the two solvents used in the present study (Table 14).

The n- hexane extract of *F. cretica* and *P. harmala* were more inhibitory than methanolic extract. Walter *et al.* (2011) reported similar results. Methanolic extract of *T. terrestris* was also more inhibitory than the n-hexane extract. This is supported by Devi *et al.* (2009) and Amadi *et al.* (2010) who found similar results. Devi *et al.* (2009) stated that methanol extraction yielded higher antimicrobial activity than n-hexane. The variation of antibacterial activity among different crude extracts of this investigation might be due to distribution of varied antimicrobial substance. Owoseni *et al.* (2010) stated that different extracts of plants show different antimicrobial activities on an organism.

Comparing the microorganisms inhibition the methanolic extract of *F. cretica, P. harmala* and *R. communis* showed highest % inhibition of *S. aureus* and this agrees with Arote *et al.* (2009). The n-hexane extract of *F. cretica* and *P. harmala* showed maximum % inhibition of *P. aeruginosa* while *T. terrestris, C. tinctoria* and *R. communis* were highly inhibitory against *S. aureus*. The factors responsible for this high susceptibility of the bacteria to the extracts are not exactly known but may be attributed to the presence of secondary plant metabolites or which is soluble in solvents tested and to the structural differences in the cell envelope compositions of the $g^-$ and the g+ bacteria (Devi *et al.* 2009). The present study showed that $g^-$ bacteria were more sensitive to the tested methanolic and n-hexane extracts as compared to the g+ bacteria. The highest sensitivity of *S. aureus* may be due to its cell wall structure and outer membrane (Shan *et al.* 2007). This is not in line with the findings of Shan *et al.* (2007). *Staphylococcus aureus* and *E. coli* were the resistant in the present study. Similar findings were also reported by Shan *et al.* (2007).

The bio-activity of natural products is due to phytochemicals having therapeutic, nutritional and antibacterial properties. Phytochemical analysis of plant extracts is vital in the knowledge of their therapeutic properties. They have been found to inadvertently confer anti-microbial protections to humans due to compounds synthesized in the secondary metabolism as well as being immunomodulative (Al-Bayati and Al-Mola, 2008). Saponins have antimicrobial activity (Mandal *et al.* 2005) and this activity of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell. The methanolic extract of *F. cretica, P. harmala, C. tinctoria* revealed the presence of saponins. It disagrees with Raja & Venkataraman (2011). Alkaloids have analgesic, antimicrobial and antioxidant activity (Igbinosa *et al.* 2009 and Govindappa *et al.* 2011). They were present in all the studied plants. It agrees with Raja & Venkataraman (2011) who reported similar findings. Alkaloids interfere with cell division, hence the presence of alkaloids in the studied plants could account for the inhibition of bacterial species in this study. Mughal *et al.* (2010) and Siddiqui *et al.* (2009) also reported similar results in Euphorbiaceae members. Tannins had antibacterial activity and they reduce the bacterial proliferation by blocking key enzymes at microbial metabolism (Mungole *et al.* 2010). The methanolic extract of *C. tinctoria* and *R. communis* had tannins. Singh *et al.* (2010) also reported similar findings. Ogunwenmo *et al.* (2007) stated that water-soluble tannins are toxic to bacteria. Flavonoids have antimicrobial activity and is probably due to their ability to complex with extracellular and soluble proteins and bacteria cell wall (Njoku & Akumefula, 2007). They were found in the methanolic extract of *F. cretica, P. harmala, T. terrestris, C. tinctoria* and *R. communis*. This agrees with Abdel Khaliq *et al.* (2000), Arshad *et al.* (2008), Rajkala *et al.* (2011), Jain *et al.* (2010), Kensa & Yasmin, (2011) and Jain & Nafis (2011) who also reported similar findings. They were found in the n-hexane extract of *F. cretica, P. harmala, T. terrestris, C. tinctoria* and *R. communis*. Glycosides were present in the methanolic extract of *F. cretica, P. harmala*. They were present in the n-hexane extract of *F. cretica* and *T. terrestris*. Terponoids were found in the methanolic extract of *P. harmala*. This disagrees with Khan *et al.* (2011). They were found in the n-hexane extract of *F. cretica, T. terrestris, C. tinctoria* and *R. communis*. Steroids were present in the methanolic extract of *P. harmala, T. terrestris* and *C. tinctoria*. They were present in the n-hexane extract of *F. cretica, P. harmala*. This agrees with the results of Rajkala *et al.* (2011). Amino acids were present in the methanolic extract of *F.*

*cretica* and *P. harmala*. They were found in the n-hexane extract of *P. harmala, T. terrestris, C. tinctoria*. Reducing sugars were present in the methanolic extract of *F. cretica, P. harmala* and *T. terrestris*. This agrees with the findings of Raja & Venkataraman (2011) and Hussain *et al.* (2011). Phenolics have antiinflammatory, antioxidant and anticarcinogenic activities and they are widespread in plant-based foods, humans consume phenolic acids on a daily basis (Stalikas, 2007). Phenolics were found in the methanolic extract of *P. harmala*. Phytochemical screening of the n-hexane extract of all the plants showed the absence of saponins, alkaloids, tannins, pholobatannins and anthraquinones in the present study. The variations in the percentage of chemical constituents such as essential oils can be explained by the differences in the geographic origins, climate, age of the plant and part of the plants. These factors involve the activation or inactivation of certain enzymatic groups, which lead to the predominance of a particular biosynthetic pathway (Khadhri *et al.*, 2011; Neves & Camara, 2012).

In the present study chemical group tests showed that the methanolic extract of *F. cretica, P. harmala, T. terrestris, C. tinctoria* and *R. communis* revealed the presence of OH, carbooxylic and phenolic groups. The methanolic and n-hexane extract of *F. cretica, P. harmala, T. terrestris, C. tinctoria* and *R. communis* showed the absence of aldehyde or and ketone group. In the present study chemical group tests revealed that the n-hexane extract of *F. cretica, T. terrestris, C. tinctoria* and *R. communis* contained OH, phenolic, carbooxylic group and it was absent in *P. harmala*. Phytochemical screening Oils were found in stems and fruits of *F. cretica*, in the roots, leaves and fruits of *T. terrestris*. They were found in roots, stems and fruits. They were also present in stems and leaves of *R. communis*. Mucilage was present in the stems and fruits of *F. cretica*. It was also found in the roots, leaves and fruits of *T. terrestris*. It was present only in leaves of *C. tinctoria*. It was also present in the stems and leaves of *R. communis* in the present study. It is observed and not surprising that there are differences in the antibacterial effects of plant species in the present study. It is quite possible that some of the plants that showed ineffectiveness in this study could be due to less constituents, just not in sufficient concentrations so as to be effective or the active chemical constituents were not soluble in methanol or hexane (Parekh & Chanda, 2008). An interesting finding in the present study is that as for as solvent is concerned the n-hexane extracts exhibited the maximum

antibacterial activity as compared to methanol. This suggests that n-hexane is a best solvent to extract active principles from the plant material than the other solvent used.

The variation of the present results with the previous findings are not unexpected as phytoconstituents are known to vary with ecological factors Upadhyay *et al.* (2010). The environmental factors such as rain fall or drought enhances the quality of active compounds or diminish it (Upadhyay *et al*., 2010). Other factors like soil nature, temperature (Yang *et al.* 2011), and length of the sunny period also counts very much (Upadhyay *et al*., 2010).

The present study shows that the plants possess antibacterial activity which in the extracted form can be utilized successfully to treat infectious diseases and protect the host from microbial infections. In this regard plants used in the traditional system of medicine offer a great reservoir for the discovery of not only antimicrobial drugs but some other drugs as well.

Table 13. Antibacterial activity of some plants of family Zygophyllaceae and Euphorbiaceae.

| Treatment/Extract | Bacteria | Zone of inhibition of sample (mm) | % inhibition |
|---|---|---|---|
| Control | | 33 | |
| a. Methanol extract | | | |
| *Fagonia cretica* L. | *Salmonella typhi* | 25 | 24.2 |
| | *Pseudomonas aeruginosa* | 15 | 54.5 |
| | *Escherchia coli* | 20 | 39.3 |
| | *Staphylococcus aureus* | 12 | 63.6 |
| | *Klebsella pneumoni* | 13 | 60.6 |
| 2. *Peganum harmala* L. | *Salmonella typhi* | 15 | 54.5 |
| | *Pseudomonas aeruginosa* | 16 | 51.5 |
| | *Escherchia coli* | 24 | 27.2 |
| | *Staphylococcus aureus* | 30 | 9.09 |
| | *Klebsella pneumoni* | 15 | 54.5 |
| 3. *Tribulus terrestris* L. | *Salmonella typhi* | 12 | 63.6 |
| | *Pseudomonas aeruginosa* | 17 | 48.4 |
| | *Escherchia coli* | 10 | 69.6 |
| | *Staphylococcus aureus* | 19 | 42.4 |
| | *Klebsella pneumoni* | 23 | 30.3 |
| 4. *Chrozophora tinctoria* (L.) Raf | *Salmonella typhi* | 15 | 54.5 |
| | *Pseudomonas aeruginosa* | 10 | 69.6 |
| | *Escherchia coli* | 19 | 42.4 |
| | *Staphylococcus aureus* | 25 | 24.2 |
| | *Klebsella pneumoni* | 20 | 39.3 |
| 5. *Ricinus communis* L. | *Salmonella typhi* | 14 | 57.5 |
| | *Pseudomonas aeruginosa* | 15 | 54.5 |
| | *Escherchia coli* | 16 | 51.5 |
| | *Staphylococcus aureus* | 10 | 69.6 |
| | *Klebsella pneumoni* | 24 | 27.2 |
| | | | |

| b. n-hexane extract | | | |
|---|---|---|---|
| 6. *Fagonia. cretica* L. | *Salmonella typhi* | 18 | 45.4 |
| | *Pseudomonas aeruginosa* | 10 | 69.6 |
| | *Escherchia coli* | 23 | 30.3 |
| | *Staphylococcus aureus* | 25 | 24.2 |
| | *Klebsella pneumoni* | 20 | 39.3 |
| 7. *Peganum harmala* L. | *Salmonella typhi* | 18 | 45.4 |
| | *Pseudomonas aeruginosa* | 13 | 60.6 |
| | *Escherchia coli* | 21 | 36.3 |
| | *Staphylococcus aureus* | 29 | 12.1 |
| | *Klebsella pneumoni* | 10 | 69.6 |
| 8. *Tribulus terrestris* L. | *Salmonella typhi* | 25 | 24.2 |
| | *Pseudomonas aeruginosa* | 29 | 12.1 |
| | *Escherchia coli* | 30 | 9.09 |
| | *Staphylococcus aureus* | 12 | 63.6 |
| | *Klebsella pneumoni* | 15 | 54.5 |
| 9. *Chrozophora tinctoria* (L.) Raf | *Salmonella typhi* | 18 | 45.4 |
| | *Pseudomonas aeruginosa* | 24 | 27.2 |
| | *Escherchia coli* | 30 | 9.09 |
| | *Staphylococcus aureus* | 10 | 69.6 |
| | *Klebsella pneumoni* | 15 | 54.5 |
| 10. *Ricinus communis* L. | *Salmonella typhi* | 10 | 69.6 |
| | *Pseudomonas aeruginosa* | 16 | 51.5 |
| | *Escherchia coli* | 14 | 57.5 |
| | *Staphylococcus aureus* | 12 | 63.6 |
| | *Klebsella pneumoni* | 23 | 30.3 |

Table 14. Statistical analysis of antibacterial activity of some plants of family Zygophyllaceae and Euphorbiaceae.

| Plants | Mean | Variance | SD | Var, diff, bet means | SD of the diff | t-Value | df | Probability | Confidence limit | Result |
|---|---|---|---|---|---|---|---|---|---|---|
| *Fagonia cretica* L. | | | | | | | | | | |
| Methanol | 48.44 | 271.38 | 16.47 | 127.0694 | 11.2725 | 0.5926 | 4 | 0.9041 | (-24.618 to 37.978 | NS |
| n-Hexane | 41.76 | 308.51 | 17.56 | | | | | | | |
| *Peganum harmala* L. | | | | | | | | | | |
| Methanol | 39.36 | 417.49 | 20.43 | 16.8712 | 4.1075 | -1.3249 | 4 | 0.2558 | (-5.962 to 16.846 | NS |
| n-Hexane | 44.80 | 501.64 | 22.40 | | | | | | | |
| *Tribulus terrestris* L. | | | | | | | | | | |
| Methanol | 50.18 6 | 253.46 | 15.92 | 268.1509 | 16.3753 | 0.7427 | 4 | 0.4989 | -33.303to 57.627) | NS |
| n-Hexane | 38.70 | 673.55 | 25.95 | | | | | | | |
| *Chrozophora tinctoria* (L.) Raf | | | | | | | | | | |
| Methanol | 46.00 | 290.57 | 17.05 | 258.2550 | 16.0703 | 0.3013 | 4 | 0.778 | -39.776to 49.460 | NS |
| n-Hexane | 41.16 | 557.03 | 23.60 | | | | | | | |
| *Ricinus communis* L. | | | | | | | | | | |
| Methanol | 52.06 | 240.38 | 5.50 | 10.3626 | 3.2191 | -0.7580 | 4 | 0.4907 | -6.498 to 11.378) | NS |
| n-Hexane | 54.50 | 288.61 | 15.12 | | | | | | | |

Key: SD=Standard Deviation; df= Degree of freedom; Var=Variance

# ii.Antifungal Activity

# ANTIFUNGAL ACTIVITY

**ii. Antifungal activity of five plants**

The results of antifungal activity of plant extracts are shown in Tables 15 and 16. *Fagonia cretica* showed highest inhibition against *Candida albicans* followed by good activity against *Aspergillus niger* and *Candida glabarata* and low inhibition against *Fusarium solani*. These findings are strongly supported by Anjum *et al.* (2007) who reported that *Fagonia cretica* exhibited significant antifungal activity against *Candida albicans* and *Aspergillus flavus*. *Candida* species may cause systemic infections under immunocompromised situations due to high adaptability to different host niches (Jain & Nafis, 2011).

*Peganum harmala* has shown highest inhibition against *Fusarium solani* and lowest inhibition against *Candida albicans* and *Candida glabarata*. These findings are in line with Diba *et al.* (2010) who reported significant inhibitory effect on *Candida tropicalis* and *Candida glabarata*. The low antifungal activity against *Candida glabarata* was shown by *Peganum harmala* (Table 15) that might be due to the negligible amount of active principles at the time of collection. Takhi *et al.* (2011) and Sarpeleh *et al.* (2009) stated that the antimicrobial activity of *Peganum harmala* was due to its alkaloids present in seeds. *Tribulus terrestris* showed highest inhibition against *Aspergillus niger* and low inhibition against *Fusarium solani*. Our findings agree with Usman *et al.* (2007) and Janovska *et al.* (2003). In the present study the methanolic leaf extract of *Tribulus terrestris* had alkaloids, tannins, saponins and cardiac glycosides. Similar results were also reported by Raja & Venkataraman (2011).

*Chrozophora tinctoria* showed highest inhibition against *Aspergillus flavus* and *Candida glabarata* and low inhibition against *Fusarium solani* and *Candida albicans*. *Ricinus communis* showed highest inhibition against *Aspergillus flavus* and low inhibition against *Fusarium solani* and good antifungal activity against *Candida glabarata*. Amadi *et al.* (2010) and Verma *et al.* (2011) observed that the hexane and methanol extracts of roots of *Ricinus communis* showed good activity against *Candida albicans* and *Aspergillus niger* fungal strains. These results are contrary to our study. Panghal *et al.* (2011) reported that *Aspergillus fumigatus* was found resistant to *Ricinus communis*.

The n-hexane extracts of *Fagonia cretica* showed highest inhibition against *A. niger* and lowest inhibition against *Fusarium solani*. *Peganum harmala* showed highest inhibition against *Candida albicans* and lowest inhibition against *Fusarium solani*. These findings are in line with Nenaah (2010), who reported that *Peganum harmala* inhibited *Candida albicans*. *Tribulus terrestris* showed highest inhibition against *Aspergillus flavus* and *Candida albicans*. This agrees with the findings of Al-Bayati and Al-Mola (2008), who also reported similar findings.

*Chrozophora tinctoria* showed highest inhibition against *Candida albicans* and lowest inhibition against *Aspergillus niger*. *Ricinus communis* showed highest inhibition against *Aspergillus niger*and lowt inhibition against *Candida glabarata*. The present findings disagree with Jain &Nafis (2011). *Ricinus communis* contained tannins, steroids and flavonoids. The results of the present investigation clearly showed that the antifungal activity varied with the species of the plants and the extracts used and it agrees with Mahesh & Satish (2008). The t-test done for comparing the antifungal activity of methanolic and n-hexane plant extracts against various fungi showed that there was no significant difference between the two solvents used in the present study (Table 16).

The methanolic extracts of *Fagonia cretica, Tribulus terrestris* and *Ricinus communis* were more inhibitory than n-hexane extract. This agrees with Igbinosa *et al.* (2009) and Abo *et al.* (2011) who reported similar results. The n-hexane extract of *Peganum harmala* and *Chrozophora tinctoria* was also more inhibitory compared to the methanolic extract and these plants showed similar (77.7%) inhibition (Table 15). This agrees with Duraipandiyan *et al.* (2009) who reported that n-hexane extracts of medicinal plants showed significant antifungal activity against *Candida albicans*.

Comparing the fungi inhibition, the methanolic extract of *Chrozophora tinctoria* and *Ricinus communis* showed maximum % inhibition of *Aspergillus flavus*. This agrees with the earlier studies of Canales *et al.* (2011) who reported that methanolic extract of *Acalypha monostachya* (Euphorbiaceae) showed antifungal activity. Similar findings were also reported by Priya *et al.* (2010) who studied different extracts of *Cassis fistula*. Generally, the methanol extract was more effective than the hexane extracts on the test micro-organisms. *Candida* species are the major player in nosocomial bloodstream infections (Klotz *et al.* 2007). In the present study the n-hexane extract of *Fagonia cretica* and *Ricinus communis* showed maximum

% inhibition of *Aspergillus niger*. This is in line with Mathur *et al.* (2011) who reported that n- hexane extracts of *Berberis aristata* and *Asparagus racemosus* showed maximum inhibition of *Aspergillus niger* ut no antifungal activity against *Candida albicans*. Similarly, the n-hexane extract of *Peganum harmala* and *Chrozophora tinctoria* showed maximum % inhibition of *Candida albicans*. This study revealed that *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* were highly sensitive *Fusarium solani* and *Candida glabarata*.

The biological activity of natural products is due to phytochemicals having therapeutic and antimicrobial properties. Phytochemical screening of plant materials is thus vital in the knowledge of their therapeutic properties. They confer anti-microbial protections to humans due to compounds synthesized in the secondary metabolism as well as being immunomodulative (Al-Bayati & Al-Mola, 2008).

Saponins have antimicrobial activity (Mandal *et al.* 2005; Soetan *et al.* 2006; Avato *et al.* 2006; Sule *et al.*, 2011) and this activity is due to its ability to cause leakage of proteins and certain enzymes from the cell. The methanolic extract of *Fagonia cretica, Peganum harmala* and *Chrozophora tinctoria* had saponins. It disagrees with other workers Mohanty *et al.* (2010); Khan *et al.* (2011); Hussain *et al.* (2011); Rajkala *et al.* (2011) Abdel Khalik *et al.* (2000); Arif *et al.* (2009) who reported saponins in *Tribulus terrestris*. The present phytochemicals and also reported by earlier studies suggest that they might be responsible for the antifungal activity in the present investigation. Alkaloids are therapeutically significant plant substances and have antimicrobial properties (Igbinosa *et al.* 2009; Govindappa *et al.* 2011; Ayoola & Adeyeye, 2010; Arif *et al.* 2009). They were found in all the studied plants. Raja & Venkataraman (2011) reported similar findings. Alkaloids interfere with cell division, so presence of alkaloids in the studied plants may suggest the inhibition of fungal species in this study. Mughal *et al.* (2010) and Siddiqui *et al.* (2009) reported similar results in Euphorbiaceae members.

Tannins have antimicrobial and pharmacological activities (Mungole *et al.*, 2010 and Bobbarala and Vadlapudi, 2009). The methanolic extract of *Chrozophora tinctoria* and *Ricinus communis* had tannins. Singh *et al.* (2010) reported similar results. The previous studies and present findings suggest that the tannins could have arrested the growth of different fungi and caused maximum inhibition of them.

Flavonoids had antifungal activities (Adekunle & Ikumapayi 2006 and Njoku & Akumefula, 2007). They were found in the methanolic extract of *Fagonia cretica*, *Peganum harmala*, *Tribulus terrestris*a, *Chrozophora tinctoria* and *Ricinus communis*. They were present in the n-hexane extract of all studied plants. This agrees with Abdel Khaliq *et al.* (2000); Arshad *et al.* (2008); Rajkala *et al.* (2011); Jain *et al* .(2010); Kensa & Yasmin, (2011) and Jain & Nafis 2011). The absence of certain phytochemicals in one sample and its presence in the other can be attributed to the various physiological and biothynthetic reactions taking place inside the plant, the effect of the environment should not be neglected, as it always modify the chemistry of plants (Khan *et al.*, 2011). Several studies (Okokon & Nwafor, 2010 ; Abimbola *et al.*, 2010; Zachariah *et al.*, 2010; Racadio *et al.*, 2008; Murugesan *et al.*, 2011; Amadi *et al.*, 2010; Shihabudeen *et al.*, 2010 and Ravikumar *et al.*, 2007) reported the antifungal activities of medicinal plants and they contained phytochemicals and it was concluded that these secondary metabolites were responsible for the inhibition of various fungi.

Glycosides were present in the methanolic extract of *Fagonia cretica*, *Peganum harmala*. They were found in the n-hexane extract of *Fagonia cretica* and *Tribulus terrestris*. These results negate the findings of Jain *et al.* (2010) in this respect.

Terponoids were found in the methanolic extract of *Peganum harmala*. They were found in the n-hexane extract of *Fagonia cretica, T. terrestris, Chrozophora tinctoria* and *Ricinus communis*. Steroids were present in the methanolic extract of *Peganum harmala*, *Tribulus terrestris*and *C. tinctoria*. This agrees with the results of Rajkala *et al.* (2011). They were found in the n-hexane extract of *Fagonia cretica, Peganum harmala*. Amino acids were present in the methanolic extract of *Fagonia cretica* and *Peganum harmala*. They were found in the n-hexane extract of *Peganum harmala, T. terrestris, Chrozophora tinctoria*. Reducing sugars were present in the methanolic extract of *Fagonia cretica, Peganum harmala* and *T. terrestris*. This agrees with the findings of and Raja and Venkataraman (2011) and Hussain *et al.* (2011). Phenolics have antiinflammatory, antioxidant and anticarcinogenic activities. As they are widespread in plant-based foods, humans consume phenolic acids on a daily basis (Stalikas, 2007). Phenolics were found in the methanolic extract of *Peganum harmala*. The n-hexane extract of all the studied plants showed the absence

of saponins, alkaloids, tannins, pholobatannins and anthraquinones in the present study.

The chemical group tests showed that the methanolic extract of *Fagonia cretica, Peganum harmala, Tribulus terrestris, Chrozophora tinctoria* and *Ricinus communis* revealed the presence of OH, carbooxylic and phenolic groups. The methanolic and n-hexane extract of *Fagonia cretica, Peganum harmala, Tribulus terrestris, Chrozophora tinctoria* and *Ricinus communis* showed the absence of aldehyde or and ketone group.

In the present study chemical group tests revealed that the n-hexane extract of *Fagonia cretica, T. terrestris, Chrozophora tinctoria* and *Ricinus communis* contained OH, phenolic, carbooxylic group and it was absent in *Peganum harmala.* Oils were found in stems and fruits of *Fagonia cretica.*

The phytochemical analysis of plant parts of the studied plants revealed that oils were found in the roots, leaves and fruits of *Tribulus terrestris*. They were found in roots, stems and fruits of *Chrozophora tinctoria*. They were also present in stems and leaves of *Ricinus communis*. Mucilage was present in the stems and fruits of *Fagonia cretica.* It was found in the roots, leaves and fruits of *T. terrestris*. It was present only in leaves of *C. tinctoria*. It was also present in the stems and leaves of *Ricinus communis* in the present study.

It is observed and not surprising that there are differences in the antifungal effects of plant species in the present study. It is quite possible that some of the plants that showed ineffectiveness in this study could be due to less constituents, just not in sufficient concentrations so as to be effective or the active chemical constituents were not soluble in methanol or hexane (Parekh and Chanda, 2008). An interesting finding in the present study is that as for as the solvent was concerned the methanol extracts exhibited the maximum antifungal activity as compared to n-hexane. This suggests that n-hexane is a best solvent to extract active principles from the plant material than the other solvent used.

The variation in the present results with the previous findings are not unexpected as phytoconstituents are known to vary with ecological factors (Upadhyay *et al.*, 2010). The environmental factors such as rain fall or drought enhances the quality of active compounds or diminish it (Upadhyay *et al.* 2010). Other factors like

soil nature, temperature, (Yang *et al.* 2011), and length of the sunny period also counts very much (Upadhyay *et al.* 2010).

The present study shows that the plants possess antifungal activity which in the extracted form can be utilized successfully to treat infectious diseases and protect the host from microbial infections. In this regard plants used in the traditional system of medicine offer a great reservoir for the discovery of not only antimicrobial drugs but some other drugs as well.

Table 15. Antifungal activity of some plants of family Zygophyllaceae and Euphorbiaceae.

| Treatment/Extract | Fungus | Linear growth of sample (mm) | % inhibition |
|---|---|---|---|
| Control | | 45 | |
| a.Methanol extract | | | |
| | *Aspergillus niger* | 18 | 60 |
| | *Aspergillus flavus* | 25 | 44.4 |
| | *Fusarium solani* | 36 | 20 |
| | *Candida albicans* | 10 | 77.7 |
| | *Candida glabarata* | 16 | 64.4 |
| 2. *Peganum harmala* L. | *Aspergillus niger* | 25 | 44.4 |
| | *Aspergillus flavus* | 24 | 46.6 |
| | *Fusarium solani* | 20 | 55.5 |
| | *Candida albicans* | 39 | 13.3 |
| | *Candida glabarata* | 42 | 6.6 |
| 3. *Tribulus terrestris* L. | *Aspergillus niger* | 9 | 80 |
| | *Aspergillus flavus* | 20 | 55.5 |
| | *Fusarium solani* | 39 | 13.3 |
| | *Candida albicans* | 15 | 66.6 |
| | *Candida glabarata* | 29 | 35.5 |
| 4. *Chrozophora tinctoria* (L.) Raf | *Aspergillus niger* | 20 | 55.5 |
| | *Aspergillus flavus* | 15 | 66.6 |
| | *Fusarium solani* | 39 | 13.3 |
| | *Candida albicans* | 42 | 6.6 |
| | *Candida glabarata* | 15 | 66.6 |
| 5. *Ricinus communis* L. | *Aspergillus niger* | 11 | 75.5 |
| | *Aspergillus flavus* | 6 | 86.6 |
| | *Fusarium solani* | 40 | 11.1 |
| | *Candida albicans* | 22 | 51.1 |
| | *Candida glabarata* | 16 | 64.4 |

| b. n-hexane extract | | | |
|---|---|---|---|
| | *Aspergillus niger* | 13 | 71.1 |
| | *Aspergillus flavus* | 22 | 51.1 |
| | *Fusarium solani* | 36 | 20 |
| | *Candida albicans* | 30 | 33.3 |
| | *Candida glabarata* | 21 | 53.3 |
| 7. *Peganum harmala* L. | *Aspergillus niger* | 15 | 66.6 |
| | *Aspergillus flavus* | 20 | 55.5 |
| | *Fusarium solani* | 29 | 35.5 |
| | *Candida albicans* | 10 | 77.7 |
| | *Candida glabarata* | 34 | 24.4 |
| 8. *Tribulus terrestris* L. | *Aspergillus niger* | 25 | 44.4 |
| | *Aspergillus flavus* | 10 | 77.7 |
| | *Fusarium solani* | 37 | 17.7 |
| | *Candida albicans* | 26 | 42.2 |
| | *Candida glabarata* | 10 | 77.7 |
| 9. *Chrozophora tinctoria* (L.) Raf | *Aspergillus niger* | 36 | 20 |
| | *Aspergillus flavus* | 25 | 44.4 |
| | *Fusarium solani* | 31 | 31.1 |
| | *Candida albicans* | 10 | 77.7 |
| | *Candida glabarata* | 29 | 35.5 |
| 10. *Ricinus communis* L. | *Aspergillus niger* | 25 | 44.4 |
| | *Aspergillus flavus* | 26 | 42.2 |
| | *Fusarium solani* | 29 | 35.5 |
| | *Candida albicans* | 32 | 28.8 |
| | *Candida glabarata* | 40 | 11.1 |

Table 16. Statistical analysis of antifungal activity of some plants of family Zygophyllaceae and Euphorbiaceae.

| Plants | Mean | Var | SD | Var, diff,bet means | SD of the diff | t-Value | df | Prob | Confidence limit | Results |
|---|---|---|---|---|---|---|---|---|---|---|
| *Fagonia cretica* L. | | | | | | | | | | |
| Methanol | 53.3 | 487.8 | 22.0 | 98.920 | 9.95 | 0.75 | 4 | 0.56 | 7.540±27.614 | NS |
| n-Hexane | 45.7 | 386.5 | 19.6 | 98.93 | | | | | | |
| *Peganum harmala* L. | | | | | | | | | | |
| Methanol | 39.8 | 877.7 | 29.6 | 216.6 | 14.72 | -0.82 | 4 | 0.5 | 12.060± 40.866 | NS |
| n-Hexane | 51.9 | 479.9 | 21.9 | | | | | | | |
| *Tribulus terrestris* L. | | | | | | | | | | |
| Methanol | 50.18 | 690.7 | 26.3 | 207.02 | 14.38 | -.1223 | 4 | 0.908 | 1.760 ± 9.947 | NS |
| n-Hexane | 51.94 | 662.8 | 25.7 | | | | | | | |
| *Chrozophora tinctoria* (L.) Raf | | | | | | | | | | |
| Methanol | 41.72 | 867.2 | 29.5 | 404.62 | 20.115 | -.0010 | 4 | 0.9993 | 0.020 ± 55.849 | NS |
| n-Hexane | 41.74 | 481.24 | 21.94 | | | | | | | |
| *Ricinus communis* L. | | | | | | | | | | |
| Methanol | 57.74 | 853. | 29.2 | 183.0766 | 13.5306 | 1.8728 | 4 | 0.1344 | 25.340 plus or minus 37.567 | NS |
| n-Hexane | 32.40 | 179.0 | 13.4 | | | | | | | |

Key: SD=Standard Deviation; df= Degree of freedom; Var =Variance

# iii.Cytotoxic Activity

# CYTOTOXIC ACTIVITY

**iii. Cytotoxic activity of five plants**

The methanolic and n-hexane extracts of investigated plants in the present study showed significant lethality against brine shrimps. The methanolic extract of *Fagonia cretica* had highest LD 50117.72, while *Peganum harmala* showed low 41.70 LD50 value compared to n-hexane extract. The methanolic and n-hexane extracts of *Tribulus terrestris* showed similar LD50 values. The methanolic extract of *Chrozophora tinctoria* showed low LD50 value than the n-hexane extract. The methanolic extract of *Ricinus communis* showed highest LD50 value while the n-hexane showed lowest LD50 value (Table 23). The LD50 value < 100 was obtained from n-hexane extracts of *Fagonia cretica, Peganum harmala* and *Ricinus communis*. The n-hexane extracts of these plants also showed the highest toxicity as compare to methanolic extracts (Table 23). These findings are in accordance with Mudi & Salisu (2009), who also found highest toxicity in n- hexane soluble fraction. The present findings disagree with those of Biswas *et al.* (2011) and Akhter *et al.* (2008) who observed high LC 50 value for Euphorbiaceae members while Sharififar *et al.* (2009) and Akhter *et al.* (2008) reported less LC 50 value for test species.

Potassium dichromate was used as a standard and its 10µl, 100µl and 1000µl concentrations were used. It was observed that with increasing time from 6 to 96 hrs, the LD 50 value reduced (Table 21). The standard revealed the lowest LD50 value compared to methanolic and n-hexane extracts of all the tested plants (Table 21). Potassium dichromate LD50 value showed that it was highly toxic to the brine shrimps compared to the methanolic and n-hexane extracts of all the studied plants. Earlier workers reported different LC50 values for potassium dichromate. Sahgal *et al.* (2010) found at 6, 12, and 24 hrs as 0.76, 0.56, and 0.28 mg/ml; Moshafi *et al.* (2009) recorded 23.2893 µg/ml; Ved *et al.* (2010) reported 32 µg/mL; Rajeh *et al.* (2010) observed LC50= 0.076. Krishnaraju *et al.* (2005) reported variable LD50 values as in *Phyllanthus emblica* (58), *P. reticulatus* (60), *P. amarusi* (2,900), *Ricinus communis* (150) and *Mallotus philippensis* (32.1). The earlier findings disagree with the present findings in this regard. In the present study, dimethylsulphoxide (DMSO) was used as the solvent and as negative control. Potassium dichromate was used as

positive control. This agrees with the previous reports that brine shrimps can tolerate up to 11% of dimethylsulphoxide DMSO (Rahmatullah *et al.*, 2010).

The plants with LD50 values less than 100 were *Fagonia cretica*, *Peganum harmala, Chrozophora tinctoria* and *Ricinus communis* and these results lend further support to their traditional use (Table 23). These findings are also supported by Gupta *et al.* (2010) and Habib *et al.* (2011). The highest value was exhibited by *Peganum harmala* and the lowest by *Fagonia cretica* (Table 23). These findings agree with Hussain *et al.* (2007) who also reported less LD50 value.These findings suggest that the n-hexane extract of *Fagonia cretica* was significantly toxic. This significant lethality of n-hexane extract of *Fagonia cretica* plant to brine shrimps is indicative of the presence of potent cytotoxic components which warrants further investigation.

Several workers investigated plant extracts toxicity and reported variable LD 50 values i.e., an extract is non-toxic (LC50 > 100 µg/mL); toxic ($LC_{50}$ < 10 µg/mL) (Ramadhani *et al.*, 2011). Moshi *et al.* (2006) found LC50 >500 microg/ml non-toxic and $LC_{50}$ 30-100 microg/ml mild toxic; Montanher *et al.* (2002) found $LC_{50}$ < 1000 ppm, significant; values > 1000 ppm inactive; Krishnaraju *et al.* (2005) reported that out of the 120 plants tested, *Pistacia lentiscus* exhibited potent brine shrimp lethality with LC50 2.5 µg. The degree of lethality was found to be directly proportional to the concentration of potassium dichromate. The maximum concentration (1000µl) of the potassium dichromate showed the highest mortality of brine shrimps after 24hrs while minimum concentration (10µl) showed mortality after 120 hrs exposure. This indicated that with increasing concentration the mortality of the brine shrimps also increased as in the present study (Table 22). Similar results have been reported by earlier workers in their studies (Sumathi, *et al.*, 2011; Das *et al.*, 2011; Taha & Alsayed, 2000; Sonia *et al.*, 2009; Viji, 2011; Khade *et al.*, 2011; Gupta *et al.*, 2010 ; Habib *et al.*, 2011; Ramachandran *et al.*, 2011 and Faridur *et al.*, 2010).

**Zygophyllaceae**

The statistical analysis showed that methanolic extract of *Peganum harmala* exhibited significant differences ($p < 0.05$) in mortality (%) of brian shrimps at various doses as compared to *Tribulus terrestris* and *Fagonia cretica* (Table 17). The mortality recorded at five doses was significantly different from mortality at control. The doses 100, 300 and 1000 µl of methanolic extract of *Peganum harmala* caused

100% mortality which was the highest as compare to other two plants (Table 17). These findings agree with Nisar *et al.* (2010) and Chanda & Baravalia (2011).

Statistical analysis revealed that n-hexane extract of *Fagonia cretica* and *Tribulus terrestris* showed significant differences ($p < 0.05$) in (%) mortality of brian shrimps at various doses as compare to control; while *Peganum harmala* results were statistically insignificant. The three doses 100, 300 and 1000 µL of n-hexane extract of *Fagonia cretica* caused of 85, 93 mortality and 100% which was the highest mortality as compared to *Peganum harmala* and *Tribulus terrestris* (Tables 19).

Saponins exhibited anticancer and antineoplastic properties (Ngoci *et al.*, 2011). Saponins were present in stems, leaves and fruits of *Fagonia cretica* and *Peganum harmala,* and in roots, stems and leaves of *Tribulus terrestris*. The present study is supported by Kianbakht & Jahanianiian (2003), but this is not in line with Rajkala *et al.* (2011). Alkaloids are chemotherapeutic agents (Olaleye & Tolulope, 2007; Rizwana *et al.*, 2010) and they interfere with cell division. They were found in stems, leaves and fruits of *Fagonia cretica* and *Peganum harmala* and also in roots, stems and leaves of *Tribulus terrestris*. Mohanty *et al.* (2010), Khan *et al.* (2011) and Raja & Venkataraman (2011) reported similar findings. Alkaloids were also present in the methanolic extracts of all the plants. These active constituents could be responsible for the brine shrimps mortality recorded in this study. Anthraquinones were present in the leaves and stems of *Peganum harmala* and *Fagonia cretica.* These findings are in contrast with the findings of Hussain *et al.* (2011). Oils were found in stems and fruits of *Fagonia cretica,* and in the roots, leaves and fruits of *Tribulus terrestris*. These active principles might be responsible for the death of shrimps in the present study. Mucilage was present in the stems and fruits of *Fagonia cretica,* and in the stems and leaves of *Peganum harmala*. It was also found in the roots, leaves and fruits of *Tribulus terrestris.* Glycosides were present in the methanolic extract of *Fagonia cretica* and *Peganum harmala* and n-hexane extract of *Fagonia cretica* and *Tribulus terrestris*. Terpenoids were found in the methanolic extract of *Peganum harmala* and in the n-hexane extract of *Fagonia cretica* and *Tribulus terrestris*. Steroids were present in the methanolic extract of *Peganum harmala* and *Tribulus terrestris* and in the n-hexane extract of *Fagonia cretica* and *Peganum harmala*. Amino acids were recorded in the methanolic extract of *Fagonia cretica* and *Peganum harmala* and in the n-hexane extract of *Peganum harmala* and *Tribulus*

*terrestris*. Reducing sugars were present in the methanolic extract of *Fagonia cretica, Peganum harmala* and *Tribulus terrestris*. Phenolic compounds have insecticidal activities. Phenolics were found in the methanolic extract of *Peganum harmala*. Kensa & Yasmin (2011) strongly supported these findings.

**Euphorbiaceae**

The analysis of variance for brian shrimps mortality showed that methanolic extracts of *Ricinus communis* had highly significant differences due to various doses used as compare to *Chrozophora tinctoria*. The mortality (%) recorded at five doses significantly differed for mortality from control (Tables 17). The 50,100, 300, and 1000 µL doses of methanolic extract of *Ricinus communis* caused 100% mortality which was the highest as compared to *Chrozophora tinctoria*.

The analysis of variance for mortality of shrimps showed that n-hexane extract of *Ricinus communis* exhibited the maximum mortality of brine shrimps at five doses as compare to *Chrozophora tinctoria*. The mortality (%) recorded at five doses significantly varied from mortality recorded in the control (Table 19). The doses 100, 300, and 1000 µl of n-hexane extract of *Ricinus communis* caused mortality of 100% which was the highest as compare to *Chrozophora tinctoria*.

Saponins were found in roots and stems of *Ricinus communis*. Anthraquinones were present in the leaves of *Chrozophora tinctoria* and *Ricinus communis*. Oils occurred in roots, stems and fruits of *Chrozophora tinctoria* and also in stems and leaves of *Ricinus communis*. These active substances might be responsible for the mortality of the shrimps in the present investigation. Mucilage was present in leaves of *Chrozophora tinctoria* and also in the stems and leaves of *Ricinus communis*. Tannins were recorded in roots and leaves of *Chrozophora tinctoria;* and also in all parts of *Ricinus communis*. This also agrees with Peteros & Uy (2010), Maridass (2008), Viji (2011) and Ngoci *et al.* (2011), who detected tannins and observes cytotoxic activity of plants. The variation in BSLA results (Tables 11, 19) may be due to the difference in the amount and kind of cytotoxic substances (e.g., tannins, saponins, flavonoids, alkaloids, volatile oils etc) present in the crude extracts. It is suggested that these compounds might have caused death of brian shrimps. Terpenoids were found in the n-hexane extract of *Chrozophora tinctoria* and *Ricinus communis*. Steroids were present in the methanolic extract of *Chrozophora tinctoria*.

Amino acids were identified in the n-hexane extract of *Chrozophora tinctoria*. Phenolic compounds exhibited herbicidal and insecticidal activities. Phenolics were found in the methanolic extract of *Ricinus communis* and in the n-hexane extract of *Ricinus communis*. Phlobatannins were present in roots, leaves and fruits and absent in stems of *Chrozophora tinctoria*. The phytochemicals present in the tested plants might be responsible for the mortality of the brian shrimps in the present case.

The n-hexane extract of all the plants showed the absence of saponins, alkaloids, tannins, pholobatannins and anthraquinones. It agrees with Srinivasan *et al.* (2007) who also reported similar results. Flavonoids play role in protection against various adverse situations with biosynthetic path way (Taylor & Grotewold, 2005; Samanta *et al.,* 2011). They were identified in the methanolic and n-hexane extract of all the plants. Several workers also recorded similar results (Arshad *et al.*, 2008; Rajkala *et al.*, 2011; Delazar *et al.*, 2006; Jain *et al.*, 2010; Jain & Nafis, 2011; Siddiqui *et al.*, 2009; Daniyan *et al.*, 2011; Javale & Sabnis 2010; Mughal *et al.*, 2010) and these studies strengthened the present findings. The variation in the mortality of shrimps might be due to the change of solvents used.

In the present study chemical group tests showed that methanolic extract of *Fagonia cretica, Peganum harmala, Tribulus terrestris, Chrozophora tinctoria* and *Ricinus communis* had OH, carbooxylic and phenolic groups. The methanolic and n-hexane extract of *Fagonia cretica, Peganum harmala, Tribulus terrestris, Chrozophora tinctoria* and *Ricinus communis* showed the absence of aldehyde or and ketone group.

In *Ricinus communis* with increasing concentration of methanolic and hexane extracts, the percent mortality of brian shrimps also increased (Tables 17, 19). The methanolic and n-hexane extract of *Ricinus communis* was highly active; *Peganum harmala* methanolic and *Fagonia cretica* n-hexane was next active agent and *Chrozophora tinctoria* methanolic and n-hexane were weakly active at all the five concentrations (Tables 17, 19). Comparing the two extracts (methanolic and n-hexane), the methanolic extract of *Peganum harmala, Tribulus terrestris, Chrozophora tinctoria* and *Ricinus communis* were more inhibitory than n-hexane extract.

Several workers reported that methanolic extracts are more inhibitory than n-hexane extracts (Javidnia *et al.,* 2003; Montanher *et al.,* 2002; Sultana *et al.*, 2010) and this supports the present findings. The n-hexane extract of *Fagonia cretica* was more inhibitory than methanolic extract. Similar findings were also reported by Pisutthanan *et al.* (2004), Erdogan (2009) and Apu *et al.* (2010), who observed that n-hexane extracts were more effective in killing brine shrimps than other extracts. Reason for this differential activity might be the inadequacy or presence of additional phytochemicals in the tested plants. The statistical results showed that the tested plants had the potential to inhibit the growth or kill brine shrimps and this clearly indicates the presence of potent bioactive principles in these extractives, which might be very useful as antiproliferative, antitumor, pesticidal and other bioactive agents (Sultana *et al.*, 2010). Cytotoxic property by plant material is due to the presence of antitumor compounds (Manilal *et al.*, 2009).

Table 17. Effect of different concentration of methanolic extracts of some plants of family Zygophyllaceae and Euphorbiaceae against *Artemia salina* (expressed as % Mortality).

| Concs of Extracts (µL) | *Fagonia cretica* L. | *Peganum harmala* L. | *Tribulus terrestris* L. | *Chrozophora tinctoria* (L.) Raf | *Ricinus communis* L. | Mean |
|---|---|---|---|---|---|---|
| 10 | 19.21cde | 38.89bc | 0.000e | 28.33cd | 28.10cd | 22.91c |
| 50 | 20.56cde | 30.00bcd | 12.04cde | 0.000e | 100.0a | 32.52c |
| 100 | 56.67b | 100.0a | 100.0a | 100.0a | 100.0a | 91.33ab |
| 300 | 8.33de | 100.0a | 100.0a | 100.0a | 100.0a | 81.67b |
| 1000 | 100.0a | 100.0a | 100.0a | 100.0a | 100.0a | 100a |
| Mean | 40.95c | 73.78ab | 62.41b | 65.67b | 85.62a | ------- |

Table 18. ANOVA for effect of different concentration of methanolic extracts of some plants family Zygophyllaceae and Euphorbiaceae against *Artemia salina*.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Plants (P) | 4 | 16279.591 | 4069.898 | 14.7099 | 0.0000 |
| 4 | Conc (C) | 4 | 75313.132 | 18828.283 | 68.0513 | 0.0000 |
| 6 | PxC | 16 | 29484.933 | 1842.808 | 6.6605 | 0.0000 |
| -7 | Error | 50 | 13833.881 | 276.678 | | |
| | Total | 74 | 134911.536 | | | |

Coefficient of variation: 25.32%, LSD value for plants (12.20) and concentration (12.20), LSD value for plants and concentration interaction = 27.28, at $\alpha = 0.05$)

Table 19. Effect of different concentration of n-hexane extracts of some plants of family Zygophyllaceae and Euphorbiaceae against *Artemia salina* (expressed as % Mortality).

| Concs of Extracts (µL) | *Fagonia cretica* L | *Peganum harmala* L. | *Tribulus terrestris* L. | *Chrozophora tinctoria* (L.) Raf | *Ricinus communis* L. | Mean |
|---|---|---|---|---|---|---|
| 10 | 80.00abc | 36.67defg | 6.66g | 7.037g | 38.09defg | 33.69c |
| 50 | 17.78fg | 67.86abcd | 17.84fg | 23.06efg | 62.50bcd | 37.81c |
| 100 | 85.00ab | 51.58cde | 100.0a | 100.0a | 100.0a | 87.32a |
| 300 | 93.33ab | 20.00efg | 10.0g | 48.13cdfg | 100.0a | 54.29b |
| 1000 | 100.0a | 100.0a | 100.0a | 100.0a | 100.0a | 100.0a |
| Mean | 75.22a | 55.22b | 46.90b | 55.65b | 80.12a | ------- |

Table 20. ANOVA for effect of different concentration of n-hexane plants extracts of family Zygophyllaceae and Euphorbiaceae against *Artemia salina*.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Plants (P) | 4 | 12232.757 | 3058.189 | 7.6897 | 0.0001 |
| 4 | Conc. (C) | 4 | 52935.233 | 13233.808 | 33.2760 | 0.0000 |
| 6 | PxC | 16 | 31862.047 | 1991.378 | 5.0073 | 0.0000 |
| -7 | Error | 50 | 19884.919 | 397.698 | | |
| | Total | 74 | 116914.957 | | | |

Coefficient of variation: 31.85% LSD value for plants (14.63) and concentration (14.63), LSD value for plants and concentration interaction = 32.31, at $\alpha = 0.05$)

Table 21. LD 50 values of potassium dichromate used as a positive control.

| | Number of survived brine shrimps | | | | | | |
|---|---|---|---|---|---|---|---|
| Concentrations | 6 hrs | 12 hrs | 24 hrs | 48 hrs | 72 hrs | 96 hrs | 120 hrs |
| 10µl | 29 | 29 | 29 | 20 | 12 | 5 | 0 |
| 100µl | 29 | 29 | 29 | 10 | 1 | 0 | 0 |
| 1000µl | 20 | 20 | 0 | 0 | 0 | 0 | 0 |
| LD 50 | 10463.6 | 10463.6 | 1147.29 | 143.27 | 13.04 | 0.02 | |

Table 22. Effect of the potassium dichromate toxicity against brine shrimps (expressed as % mortality using Abbott formula).

| | Mortality (%) of brine shrimps | | | | | | |
|---|---|---|---|---|---|---|---|
| Concentration | 6 hrs | 12 hrs | 24 hrs | 48 hrs | 72 hrs | 96 hrs | 120 hrs |
| 10µl | 3.33 | 3.33 | 3.33 | 33.33 | 60 | 83.33 | 100 |
| 100µl | 3.33 | 3.33 | 3.33 | 66.67 | 96.67 | 100 | 100 |
| 1000µl | 33.3 | 33.3 | 100 | 100 | 100 | 100 | 100 |

Table 23. Cytotoxic activity of methanolic and n-hexane extracts of some plants of family Zygophyllaceae and Euphorbiaceae against *Artemia salina* with LD 50 value.

| Plants | LD 50 (µg/ml) |
|---|---|
| **Zygophyllaceae** | |
| *Fagonia cretica* L. | |
| Methanol extract | 117.72 |
| n-hexane extract | 0.32 |
| *Peganum harmala* L. | |
| Methanol extract | 41.70 |
| n-hexanev extract | 75.94 |
| *Tribulus terrestris* L. | |
| Methanol extract | 235.65 |
| n-hexane extract | 235.65 |
| **Euphorbiaceae** | |
| *Chrozophora tinctoria* (L.) Raf | |
| Methanol extract | 47.22 |
| n-hexane extract | 151.77 |
| *Ricinus communis* L. | |
| Methanol extract | 361.92 |
| n-hexane extract | 22.95 |

# iv.Phytotoxic Activity

# PHYTOTOXIC ACTIVITY

**iv. Phytotoxic activity of five plants**

The ANOVA values showed that 10g and 20g extracts of all the tested plants significantly inhibited the growth of *Lemna minor* with significant differences. The plants means were non-significant. The interaction between extracts and plants was also significant. *Fagonia cretica*, *Peganum harmala*, *Tribulus terrestris*, *Chrozophora tinctoria* and *Ricinus communis* caused significant growth inhibition of *Lemna minor* in all the dilutions. The interaction between plants and dilution was non-significant (Tables 24, 25).

Allelopathy is an important ecological factor in the plant kingdom including phytotoxic medicinal plants. Flavonoids reportedly act as allelochemicals (Samanta *et al.*, 2011). They were found in the methanolic and n-hexane extract of all the tested plants. These findings are in agreement with many workers (Alamdari, 2011; Ahmad *et al.*, 2011; Rajkala *et al.*, 2011; Hussain *et al.*. 2011; Khan *et al.*, 2011 and Raja & Venkataraman , 2011; Jain *et al.*, 2010; Jain & Nafis, 2011; Mwine & Damme, 2011; Daniyan *et al.*, 2011; Javale & Sabnis, 2010; Mughal *et al.* 2010 and Biswas *et al.*, 2011), who also reported flavonoids in the plants investigatedby them. Several reports on the allelopathic potential of some members of Euphorbiaceae are available (Sisodia & Siddiqui, 2010; Gilani *et al.* 2010; Silva *et al.*, 2006; Ma, *et al.*, 2011) and these findings support the present results. The variations in the inhibition of *Lemna* by plants might be due to solvent and plant material. Allelopathic effects are related to plants, their parts and extraction procedures.

The present study showed significant interaction between concentration and plants. The concentration and plants interaction mean value was highest in *Fagonia cretica* and lowest in *Fagonia cretica* (Table 24). The phytochemical screening of these plants showed that oils were found in stems and fruits of *Fagonia cretica*, in the stems and leaves of *Peganum harmala*, in the roots, leaves and fruits of *Tribulus terrestris*, Glycosides were present in the methanolic extract of *Fagonia cretica*, *Peganum harmala* and in the n-hexane extract of *Fagonia cretica* and *Tribulus terrestris*. Terpenoids were found in the methanolic extract of *Peganum harmala* and in the n-hexane extract of all tested plants. All these phytochemicals have the capability to exhibit phytotoxicity.This agrees with Jha *et al.* (2006), Abrosca *et al.*

(2005), Mancini *et al.* (2009), Hegazy & Farrag (2007) and Hale (2004), also observed phytotoxicity and the present findings agree with them. The phytochemicals detected in the tested plants might be responsible for the inhibition of *Lemna* plants in the present study. Steroids were present in the methanolic extract of *Peganum harmala* and *Tribulus terrestris* and in the n-hexane extract of *Fagonia cretica, Peganum harmala*. Amino acids were found in the methanolic extract of *Fagonia cretica* and *Peganum harmala* and in the n-hexane extract of *Peganum harmala* and *Tribulus terrestrisi.* Several workers reported the allelopathic effect of amino acids against plants (Uma *et al.*, 2009; Elemore 1980; Williams & Hoagland, 2007). Allelochemicals inhibit electron transport in mitochondria (Kapoor, 2011). Reducing sugars were present in the methanolic extract of *Fagonia cretica, Peganum harmala* and *Tribulus terrestris*. Phenolic compounds and their derivatives are potential inhibitors of germination and seedling growth and have allelopathic applications in agriculture and forestry as herbicides (Li *et al.*, 2010). Phenolics were found in the methanolic extract of *Peganum harmala* and in the n-hexane extract of *Tribulus terrestris*. Anthraquinones were present in the stems of *Peganum harmala, Fagonia cretica,* in leaves of *Peganum harmala*. These findings are in contrast with the findings of Hussain *et al.* (2011). The active principles detected in the above plants could be responsible for the inhibition of *Lemna* plants in the present study. Romero-Romero *et al.* (2002) stated that phytotoxins affect membrane permeability, ion uptake, electron transport in photosynthesis and respiratory chains, enzymatic activity, and cell division.

In the present study the concentration and dilution interaction was also significant. The concentration and dilution interaction mean value was highest in *Fagonia cretica* and lowest in *Ricinus communis* when 10g and 20g extracts were compared. The dilution means were highest in *Fagonia cretica* and lowest in *Ricinus communis* (Table 24).

**Zygophyllaceae**

The ANOVA values indicated that methanolic extracts of *Fagonia cretica* significantly inhibited the growth of *Lemna minor* with significant differences among concentrations. *Fagonia cretica* showed 5.57, 12.22 and 50.0 % growth inhibition of *Lemna minor* while *Peganum harmala,* and *Tribulus terrestris* did not show the significant differences and their results were statistically non-significant (Table 26).

The ANOVA values showed that n-hexane extract of *Fagonia cretica*, *Peganum harmala*, and *Tribulus terrestris* showed non-significant results (Table 27).

It was seen that methanolic extract of *Fagonia cretica* showed the maximum growth inhibition at 1000 µl as compare to the n-hexane extract of *Fagonia cretica* at 100 µl (Tables 26). The n-hexane extract of *Peganum harmala* showed the maximum growth inhibition at 1000 µl as compare to the methanolic extract at the same concentration (Table 27). These findings are in agreement with Nisar *et al.* (2010) who reported similar findings for *Impatiens bicolor*. The methanolic extract of *Tribulus terrestris* showed the maximum growth inhibition at 100 µl as compare to its n-hexane extract at 10µl. Rahman *et al.* (2011) reported similar results and observed that n-hexane extract of stem bark of *Pistacia integerrima* showed the least phytotoxic effect as compared to other solvents. Reason for this differential activity might be the solubility of phytochemicals.

Saponins have allelopathic property (Chaieb, 2010; Lee *et al.*, 2004). Saponins were found in stems, leaves and fruits of *Fagonia cretica* and *Peganum harmala* also in roots stems and leaves of *Tribulus terrestris* and in stems of *Chrozophora tinctoria*. The present findings differ from Rajkala *et al.* (2011) but agree with Kianbakht & Jahanianiian (2003). The methanolic extract of *F. cretica, P. harmala, C. tinctoria* also had saponins. Shao-Lin *et al.* (2004) stated that phytotoxins activity varies with plant tissues. The leaves, stems, roots and seeds can release allelochemicals which differ in different parts or tissues of some plants. Stems, leaves and fruits of *Fagonia cretica* and *Peganum harmala* had alkaloids in roots, stems and leaves of *Tribulus terrestris*. Khan *et al.* (2011) and Raja & Venkataraman (2011) support the present findings as they reported alkaloids in *Tribulus terrestris*. The relatively greater growth inhibition caused by *Fagonia cretica* as compared to other plants was as a result of the active constituents in the plant acting in a more synergistic manner. Mucilage was present in stems and fruits of *Fagonia cretica*. It was also found in roots, leaves and fruits of *Tribulus terrestris*. The study concludes that *Fagonia cretica* might be useful for weed control.

**Euphorbiaceae**

The ANOVA values showed that methanolic extracts of *Ricinus communis* significantly inhibited the growth of *Lemna minor* with significant differences among concentrations. The *Chrozophora tinctoria* results were statistically non-significant (Table 26). The ANOVA values showed that n-hexane extracts of *Chrozophora tinctoria* and *Ricinus communis* did not significantly inhibit *Lemna minor* (Table 27).

The methanolic extract of *Chrozophora tinctoria* showed the maximum growth inhibition at 100 µl as compare to the n-hexane extract of *Chrozophora tinctoria* at 10µl (Table 26).These results are supported by Dzoyem *et al.* (2011) who reported similar results from methanol extract of *Diospyros canaliculata*. The methanolic extract of *Ricinus communis* had the maximum growth inhibition at 1000 µl as compare to the n-hexane extract of *Ricinus communis* at 1000µl. Several researchers reported the inhibition of *Lemna* plants mostly at the high concentration (Ahmed *et al.*, 2011; Khan *et al.*, 2005; Zaidi *et al.*, 2006; Ateeq-ur-Rehman *et al.*, 2009).

Oils were detected in roots, stems and fruits of *Chrozophora tinctoria* and in stems and leaves of *Ricinus communis*. Saponins were found in stems of *Chrozophora tinctoria* and in roots and stems of *Ricinus communis*. Alkaloids were found in leaves of *Chrozophora tinctoria* and in the stems and leaves of *Ricinus communis*. This agrees with Kensa & Yasmin (2011) who detected alkaloids in *Ricinus communis*. Alkaloids interfere with cell division and presence of these substances might be responsible for the inhibition of *Lemna minor* in the present study. The toxic phytochemicals may either damage the DNA or inhibit protein synthesis, photosynthesis and plant growth of plants (Shao-Lin *et al.*, 2004). Several workers reported the inhibitory role of alkaloids in plants (Jha *et al.*, 2006; Hagazy & Farrag, 2007; Sodaeizadeh & Damme, 2009). Our findings supported them. Steroids were present in the methanolic extract of *Chrozophora tinctoria*. Amino acids were detected in the n-hexane extract of *Chrozophora tinctoria*. Phenolics were found in the methanolic and n-hexane extract of *Ricinus communis*. Anthraquinones were present in the stems of *Chrozophora tinctoria* and *Ricinus communis*. Mucilage was found in leaves of *Chrozophora tinctoria* and in the stems and leaves of *Ricinus communis*. Tannins are allelopathic and inhibitory to most test organisms. Tannins were noted in roots, leaves of *Chrozophora tinctoria* and in all parts of *Ricinus*

*communis*. Jha *et al.* (2006) and Kensa & Yasmin (2011) strongly supported these findings. The methanolic extract of *C. tinctoria* and *R. communis* had tannins. Phlobatannins were present in roots, leaves and fruits of *Chrozophora tinctoria*. The presence of these phytochemicals detected in the investigated plants suggest that the inhibition of *Lemna* plants might be due to these compounds as they are very heterogenous mixtures of a single substance acting in a synergistic way or antagonistic manner. Shao-Lin *et al.* (2004) reported that phytotoxins activity varied with solvent extraction method as several workers have reported allelopathic potentials with n-hexane, acetone and water extracts of *Evolvulus alsinoides* and *Melissa officinalis*, with water extraction having the strongest allelopathic activity.

The n-hexane extract of all the investigated plants showed the absence of saponins, alkaloids, tannins, pholobatannins and anthraquinones with the exception of flavonoids and this t agrees with Srinivasan *et al.* (2007) who reported similar behaviour. In the present study methanolic extract of *F. cretica, P. harmala, T. terrestris, C. tinctoria* and *R. communis* revealed the presence of OH, carbooxylic and phenolic groups while methanolic and n-hexane extract of *F. cretica, P. harmala, T. terrestris, C. tinctoria* and *R. communis* showed the absence of aldehyde and ketone group.

The toxicity depends upon the part assayed and solvent system. This agrees with other workers (Hussain *et al*., 2010; Hussain *et al*., 2011; Ladhari *et al*., 2011; Lopez *et al*., 2009; Shao-Lin *et al*., 2004), who reported phytotoxicity to be part and species specific and had an independent effect on various physiological processes. Toxicity is specie related and it plays important role in the plant distribution. In the present study the growth inhibition of *Lemna* plants increased with increasing concentration of extracts (Tables 26-27). These findings are in accordance with Rashid *et al.* (2009) and Nisar *et al.* (2011). In addition, release and activity of allelochemicals may be affected by soil moisture, soil pH, soil organic matter content or soil salinity (Farooq *et al.*, 2011).

Alleolochemicals, inhibiting weeds, become favorable choice for natural pesticides. Many allelochemicals can be used to produce natural pesticides and much remains to be done in order to transform the laboratory studies into commercial products. In short, diversity of allelochemicals means that they can be used in multiple purposes. Synthesizing these compounds has important ecological

significance and economic potentials. It can be predicted that allelochemicals will become an important impetus for ecoagricultural development. Allelopathy can help explain the inhibitory effects or toxicity in the processes of rotation, intercrop and mulch and such studies can help avoid wasting billions of dollars in worldwide agricultural practices (Shao-Lin *et al.*, 2004).

Table 24. ANOVA mean values for the effect of plants extracts concentration of some members of family Zygophyllaceae and Euphorbiaceae on growth inhibition of *Lemna minor*.

| Conc | Species | Dilutions (ml) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 10+90 | 30+70 | 50+50 | 10+90 | Control | CXPXD |
| 10 (g) | *Fagonia cretica* L. | 49.9 | 14.9 | 12.67 | 10.0 | 0.0 | 17.52 a |
| | *Peganum harmala* L. | 54.0 | 16.5 | 5.3 | 7.3 | 0.0 | 16.65 ab |
| | *Tribulus terrestris* L. | 31.3 | 9.6 | 9.9 | 6.6 | 0.0 | 11.53 bcd |
| | *Chrozophora tinctoria* (L.) Raf | 34.6 | 4.0 | 13.9 | 6.6 | 0.0 | 11.88 bcd |
| | *Ricinus communis* L. | 50.0 | 6.3 | 4.6 | 7.9 | 0.0 | 13.8 abc |
| 20 (g) | *Fagonia cretica* L. | 13.21 | 5.3 | 5.3 | 10.6 | 0.0 | 6.9 d |
| | *Peganum harmala* L. | 12.8 | 12.0 | 7.9 | 7.3 | 0.0 | 8.0 d |
| | *Tribulus terrestris* L. | 12.54 | 10.6 | 7.3 | 14.0 | 0.0 | 8.9 cd |
| | *Chrozophora tinctoria* (L.) Raf | 21.3 | 16.6 | 7.3 | 5.9 | 0.0 | 10.2 cd |
| | *Ricinus communis* L | 20.1 | 14.6 | 14.0 | 8.6 | 0.0 | 11.49 bcd |
| PXD | | | | | | | |
| | *Fagonia cretica* L. | 31.6 | 10.1 | 9.0 | 10.3 | 0.0 | NS |
| | *Peganum harmala* L. | 33.4 | 14.2 | 6.6 | 7.3 | 0.0 | NS |
| | *Tribulus terrestris* L. | 21.9 | 10.1 | 8.6 | 10.3 | 0.0 | NS |
| | *Chrozophora tinctoria* (L.) Raf | 27.9 | 10.3 | 10.6 | 6.3 | 0.0 | NS |
| | *Ricinus communis* L | 35 | 10.4 | 9.3 | 8.3 | 0.0 | NS |
| | 10(g) | 43.9 a | 10.3 c | 9.3c | 7.7c | 0.0d | CXD |
| | 20(g) | 16.0 b | 11.8 bc | 8.3 c | 9.3 c | 0.0 d | |
| Dilution Mean | | 30.0 a | 11.0 b | 8.8 b | 8.5 b | 0.0 c | |
| Plant Mean | | 12.2 | 12.3 | 10.2 | 11.0 | 12.6 | NS |
| LSD for Conc= 2.34 ; LSD for CXPXD= 5.24; LSD for dilution= 3.708; LSD for CXD=5.244 ; Coefficient of variation = 80.37% . | | | | | | | |

Mean values in the vertical columns followed by different letters are significantly different at 0.05 level of significance using LSD test.

Key: P=Plants; C=Concentration; D=Dilution;NS=Non-significant

Table 25. Mean squares values for the effect of various concentration of plants extracts of some members of family Zygophyllaceae and Euphorbiaceae on growth inhibition of *Lemna minor* L.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob | |
|---|---|---|---|---|---|---|---|
| 2 | Weight (C) | 1 | 1659.202 | 1659.202 | 18.7674 | 0.0000 | S |
| 4 | Plants (P) | 4 | 208.325 | 52.081 | 0.5891 | - | NS |
| 6 | WxP | 4 | 860.138 | 215.035 | 2.4323 | 0.0488 | S |
| 8 | Dilution (D) | 4 | 24522.300 | 6130.575 | 69.3436 | 0 .0000 | S |
| 10 | CxD | 4 | 8204.223 | 2051.056 | 23.1997 | 0.0000 | S |
| 12 | PxD | 16 | 1222.116 | 76.382 | 0.8640 | - | NS |
| 14 | CxPxD | 16 | 1942.172 | 121.386 | 1.3730 | 0.1579 | S |
| -15 | Error | 200 | 17681.725 | 88.409 | | | |
| | Total | 249 | 56300.201 | | | | |

Key: S = Significant; NS = Non-significant

Table 26. Effect of different concentrations of methanolic plant extracts of some members of family Zygophyllaceae and Euphorbiaceae on growth inhibition of *Lemna minor*.

| | **Zygophyllaceae** | | | **Euphorbiaceae** | |
|---|---|---|---|---|---|
| Conc (µL) | *Fagonia cretica* L. | *Peganum harmala* L. | *Tribulus terrestris* L. | *Chrozophora tinctoria* (L.) Raf | *Ricinus communis* L |
| | Mean ± SE | Mean ± SE | Mean ± SE | Mean ± SE | Mean ± SE |
| Control | 10.0 b ± 4.68 | 10.0 ± 5.18 | 10.0 ± 9.63 | 10.0± 5.47 | 10.0b± 8.74 |
| 10 | 5.57 b ± 4.68 | 13.3c ± 5.18 | 26.5 ± 9.63 | 13.3± 5.47 | 25.55b± 8.74 |
| 100 | 12.2 b ± 4.68 | 6.6 ± 5.18 | 34.4 ± 9.63 | 23.33± 5.47 | 33.33b± 8.74 |
| 1000 | 50.0 a ± 4.68 | 20.0 ± 5.18 | 26.6 ± 9.63 | 17.77± 5.47 | 61.09b± 8.74 |
| Conc Means | 19.44 | 12.5 | 24.41 | 16.11 | 32.49 |
| COV | 41.70% | 71.89% | 68.28% | 58.83% | 46.59% |
| LSD at $\alpha$=0.05 | 15.27 | Nil | Nil | Nil | 28.50 |

Key: SE = Standard Error; COV = Coefficient of variation, LSD = Least significant difference.

Table 27. Effect of different concentrations of n-hexane plant extracts of some members of family Zygophyllaceae and Euphorbiaceae on growth inhibition of *Lemna minor*.

| | **Zygophyllaceae** | | | **Euphorbiaceae** | |
|---|---|---|---|---|---|
| Conc (µL) | *Fagonia cretica* L. | *Peganum harmala* L. | *Tribulus terrestris* L. | *Chrozophora tinctoria* (L.) Raf | *Ricinus communis* L |
| | Mean ± SE | Mean ± SE | Mean ± SE | Mean ± SE | Mean ± SE |
| Control | 10.0 ± 12.07 | 10.0 ± 10.86 | 10.0 ± 5.3 | 10.0± 3.09 | 10.0± 11.95 |
| 10 | 0.0 ± 12.07 | 16.6 ± 10.86 | 14.0 ± 5.3 | 7.7± 3.09 | 25.55± 11.9 |
| 100 | 43.0 ± 12.07 | 10.0± 10.86 | 3.3 ± 5.3 | 0.0± 3.09 | 33.33± 11.9 |
| 1000 | 1.0 ± 12.07 | 51.0 ± 10.86 | 4.4 ± 5.3 | 0.0± 3.09 | 61.09± 11.9 |
| Conc Means | 13.61 | 21.95 | 8.05 | 4.44 | 22.78 |
| COV | 153.61% | 85.70% | 114.57% | 120.56% | 90.9% |
| LSD at $\alpha$=0.05 | Nil | Nil | Nil | Nil | Nil |

Key: SE= Standard Error; COV=Coefficient of variation, LSD=Least significant difference

# v.Insecticidal Activity

# INSECTICIDAL ACTIVITY

**v. Insecticidal activity of *Tribolium castaneum***

**Effect on mortality of *Tribolium castaneum***

**Zygophyllaceae**

Among the three plants extracts, *Peganum harmala* showed the highest insects mortality (21.0%), followed by *Fagonia cretica* (9.0%) at the same dose (Table 28). *Tribulus terrestris* showed the lowest (2.6%) mortality. All the doses (5 to 20%) of the plant extracts showed significant differences of mortality of *Tribolium castaneum* as compared to control. The highest dose mean (12.8%) was shown by *Peganum harmala*, while the lowest dose mean was shown by *Tribulus terrestris* (Tables 28). This is in agreement with the findings of Jbilou & Sayah (2006). Several workers reported the insecticidal activities of *Peganum harmala* (Jbilou & Sayah, 2006; Nateghpour, *et al.*, 2006; Khattak, *et al.*, 2006; Shonouda, *et al.*, 2008 ; Haq, *et al.*, 2005; Ramya, *et al.*, 2009) and these findings strengthened the present results. Many workers have reported the biological activities of *Peganum harmala* (Edziri *et al.*, 2010; Sarpeleh, *et al.*, 2009 and Takhi, *et al.*, 2011) and suggested that active principles present in *Peganum harmala* might be responsible for the mortality of *Tribolium castaneum* as in the present study. ANOVA for the effect of aqueous extract of *Fagonia cretica*, *Peganum harmala* and *Tribulus terrestris* on the moetality of insects revealed highly significant differences (Tables 29-31).

Saponins are harmful to insects (Chaieb, 2010). They have been detected in stems, leaves and fruits of *Fagonia cretica* and *Peganum harmala* and in the roots of, stems and leaves of *Tribulus terrestris*. Glycosides were identified in the methanolic extract of *Fagonia cretica*, *Peganum harmala* and in the n-hexane extract of *Fagonia cretica* and *Tribulus terrestris*. Terpenoids were recorded in the methanolic extract of *Peganum harmala* and in the n-hexane extract of all the plants, except *Peganum harmala*. The insecticidal activity of terpenoides has been reported by early workers (Afshar, *et al.*, 2011; EL-Kamali, 2009), which agree with the present study. The methanolic extract of *Peganum harmala* and *Tribulus terrestris* and n-hexane extract of *Fagonia cretica, Peganum harmala* had steroids. The phytochemicals detected in the above plants might be responsible for the mortality of *Tribolium castaneum*. The variation in the mortality of *Tribolium castaneum* might be due to the change in the

solvents used. The stems, leaves and fruits of *Fagonia cretica* and *Peganum harmala*, and roots, stems and leaves of *Tribulus terrestris* contained alkaloids. Anthraquinones were recorded in the stems of *Peganum harmala, Fagonia cretica* and in the leaves of *Peganum harmala*. Oils were identified in stems and fruits of *Fagonia cretica* and in the roots, leaves and fruits of *Tribulus terrestris*. Amino acids occurred in the methanolic extract of *Fagonia cretica* and *Peganum harmala* and in the n-hexane extract of *Peganum harmala, Tribulus terrestris, Chrozophora tinctoria*. The methanolic extract of *Fagonia cretica, Peganum harmala* and *Tribulus terrestris* had reducing sugars. Phenolic compounds have insecticidal activities and these were found in the methanolic extract of *Peganum harmala, Ricinus communis* and in the n-hexane extract of *Tribulus terrestris,* and *Ricinus communis*. The secondary metabolites presently investigated might be responsible for causing the death of *Tribolium castaneum*. The mortality caused by the present plants might be attributed to several mechanisms. Abdullahi *et al.* (2010) reported that the chemicals present in the plant extracts block the spiracles of insects which impairs the respiration leading to the death of the insects.

The mortality of *Tribolium castaneum* increased significantly with the increasing concentration of extracts from 5 to 20% of extracts of all the tested plants (Table 28). These findings are supported by Khanna *et al.* (2003); Hameed *et al.* (2012); Abbasipour *et al.* (2010) and Hussain *et al.* (2010).

**Euphorbiaceae**

Among the two plants, *Chrozophora tinctoria* showed the maximum insects mortality (40.0%) while *Ricinus communis* showed the lowest (5.0%) mortality at the same dose (Table 28). In the case of *Chrozophora tinctoria* the 5% and 10 % doses were ineffective showing insignificant mortality compared to the higher doses. In the case of *Ricinus communis* all the doses (5% to 20%) showed significant ($p< 0.05$) differences in the mortality of *Tribolium castaneum* compared to control (Table 33). The highest dose mean (15.75%) was revealed by *Chrozophora tinctoria* followed by (3.5%) by *Ricinus communis* (Table 28). ANOVA for the effect of aqueous extract of *Chrozophora tinctoria* and *Ricinus communis* on the mortality of *Tribolium castaneum* showed that doses were significant (Tables 32-33).

Saponins were detected in stems of *Chrozophora tinctoria* and in roots and stems of *Ricinus communis*. Tannins were present in roots and leaves of *Chrozophora tinctoria* and in all parts of *Ricinus communis*. Phlobatannins were detected in roots, leaves and fruits of *Chrozophora tinctoria*. Eearlier workers reported various phytochemicals members of Family Euphorbiaceae (Nwokocha *et al*., 2011; Daniyan *et al*., 2011; Edeoga *et al*., 2005; James & Friday, 2010; Reuben and Abdulrahman, 2008; Sule and Sani 2008; Mughal *et al*., 2010; Singh *et al*., 2010; Kensa & Yasmin, 2011 and Verma *et al*., 2011). The presence of these phytochemicals may explain the toxic effects on *Tribolium castaneum*. Flavonoids were found in the methanolic and n-hexane extract of all the analysed plants. Similar findings (Rajkala *et al*., 2011; Hussain *et al*., 2011; Khan *et al*., 2011; Raja & Venkatarama, 2011; Jain *et al*., 2010; Jain & Nafis 2011; Siddiqui *et al*., 2009; Mwine & Damme 2011; Daniyan *et al*., 2011; Javale & Sabnis 2010). have been reported that strengthened the present findings. Alkaloids were found in roots, stems, fruit and leaves of *Chrozophora tinctoria,* and in the stems and leaves of *Ricinus communis*. Mucilage was present in leaves of *Chrozophora tinctoria* and in the stems and leaves of *Ricinus communis* that might have caused the mortality of *Tribolium castaneum* in the present case. *Chrozophora tinctoria* had steroids. Phenolic compounds with insecticidal activities were found in the methanolic extract of *Ricinus communis* and n-hexane extract of and *Ricinus communis*. The secondary metabolites found in the above plants could be responsible for the death of *Tribolium castaneum*.

Table 28. Effect of extracts of some plants on mortality (%) of *Tribolium castaneum*.

| Mortality of *Tribolium castaneum* after 72 Hrs. | | | | | |
|---|---|---|---|---|---|
| | **Zygophyllaceae** | | | **Euphorbiaceae** | |
| Doses | *Fagonia cretica* L. | *Peganum harmala* L. | *Tribulus terrestris* L. | *Chrozophora tinctoria* (L.) Raf | *Ricinus communis* L |
| | Mean ± SE | Mean ± SE | Mean ± SE | Mean ± SE | Mean ± SE |
| Control | 0.8 c ± 0.87 | 0.6d ± 1.22 | 1.0 c ± 0.45 | 0.75 a ± 0.38 | 1.58 c ± 0.83 |
| 5% | 4.0 b ± 0.87 | 7.3 c ± 1.22 | 1.0 c ± 0.45 | 0.0 c ± 0.38 | 2.33 c ± 0.83 |
| 10% | 6.6 ab ± 0.87 | 16.6b ± 1.22 | 7.7 a ± 0.45 | 0.0 c ± 0.38 | 7.6 a ± 0.83 |
| 15% | 7.0 a ± 0.87 | 18.3a b ± 1.22 | 2.6b ± 0.45 | 38.0b ± 0.38 | 1.0c ± 0.83 |
| 20% | 4.0 b ± 0.87 | 21.0a ± 1.22 | 2.6b ± 0.45 | 40.0a ± 0.38 | 5.0b ± 0.83 |
| Plant Means | 4.5 | 12.8 | 3.01 | 15.75 | 3.5 |
| COV | 33.58% | 16.48% | 25.77% | 4.20% | 40.71% |
| LSD | 2.75 | 3.8 | 1.4 | 1.2 | 2.6 |

Key: SE= Standard Error; COV=Coefficient of variation, LSD=Least significant Difference.

Table 29. ANOVA for effect of aqueous extract of *Fagonia cretica* on the mortality of *Tribolium castaneum*.

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F-value | Prob |
|---|---|---|---|---|---|
| Timings | 2 | 16.30 | 8.150 | 9.98 | 0.0067 |
| Conc. | 4 | 74.67 | 18.667 | 22.86 | 0.0002 |
| Error | 8 | 6.53 | 0.817 | | |
| Total | 14 | 97.50 | | | |

Table 30. ANOVA for effect of aqueous extract of *Peganum harmala* on the mortality of *Tribolium castaneum*.

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F-value | Prob |
|---|---|---|---|---|---|
| Timings | 2 | 35.76 | 17.879 | 15.55 | 0.0018 |
| Conc. | 4 | 886.40 | 221.600 | 192.70 | 0.0000 |
| Error | 8 | 9.20 | 1.150 | | |
| Total | 14 | 931.36 | | | |

Table 31. ANOVA for effect of aqueous extract of *Tribulus terrestris* on the mortality of *Tribolium castaneum*.

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F-value | Prob |
|---|---|---|---|---|---|
| Timings | 2 | 2.61 | 1.304 | 3.04 | 0.1043 |
| Conc. | 4 | 89.02 | 22.254 | 51.85 | 0.0000 |
| Error | 8 | 3.43 | 0.429 | | |
| Total | 14 | 931.36 | | | |

Table 32. ANOVA for effect of aqueous extract of *Chrozophora tinctoria* on the mortality of *Tribolium castaneum*.

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F-value | Prob |
|---|---|---|---|---|---|
| Timings | 2 | 0.18 | 0.088 | 0.17 | 0.8493 |
| Conc. | 4 | 5412.75 | 1353.188 | 2577.50 | 0.0000 |
| Error | 8 | 4.20 | 0.525 | | |
| Total | 14 | 5417.13 | | | |

Table 33. ANOVA for effect of aqueous extract of *Ricinus communis* on the mortality of *Tribolium castaneum*.

| Source | Degrees of Freedom | Sum of Squares | Mean Square | F-value | Prob |
|---|---|---|---|---|---|
| Timings | 2 | 13.63 | 6.817 | 7.94 | 0.0126 |
| Conc. | 4 | 92.68 | 23.171 | 27.00 | 0.0001 |
| Error | 8 | 6.87 | 0.858 | | |
| Total | 14 | 113.18 | | | |

# vi. Termicidal Activity

Methanolic extract of *Peganum harmala* and *Tribulus terrestris* and n-hexane extract of *Fagonia cretica* and *Peganum harmala* had steroids (Table 43). These phytoconstituents detected in the above plants might be responsible for the mortality of *Heterotermes indicola*. Alkaloid are reportededly insecticidal (EL-Kamali, 2009). The presence of alkaloids in stems, leaves and fruits of *Fagonia cretica* and *Peganum harmala*, and in roots, stems and leaves of *Tribulus terrestris* could be the cause of inhibition of termites. Verma *et al.* (2009) reported that anthraquinones have termicidal activity. Anthraquinones were present in the stems of *Peganum harmala, Fagonia cretica* and in the leaves of *Peganum harmala*. Ayvaz *et al.* (2010) stated that oils from different plant species possessed larvicidal and repellent properties against various insect species. Aboua and Koua (2010) stated that oils may be toxic by penetrating the insect body via the respiratory system. Oils were found in stems and fruits of *Fagonia cretica* and in the roots, leaves and fruits of *Tribulus terrestris*. Amino acids were found in the methanolic extract of *Fagonia cretica* and *Peganum harmala* and in the n-hexane extract of *Peganum harmala* and *Tribulus terrestris*. Reducing sugars were present in the methanolic extract of *Fagonia cretica, Peganum harmala* and *Tribulus terrestris*. Phenolic compounds have insecticidal activities. Phenolics occurred in the methanolic extract of *Peganum harmala* and in the n-hexane extract of *Tribulus terrestris*. This agrees with Takhi *et al.* (2011) who also reported phenolic compounds in *Peganum harmala*. Rahman *et al.* (2005) stated that phenolic compounds of *Juniperus procera* showed 100% mortality of tea termites, *Odontotermes assamensis*. The active principles present in these plants might be responsible for the death of *Heterotermes indicola* under experimental condition.

**Effect of Concentrations**

**Zygophyllaceae**

The ANOVA results (Table 35) showed that all the concentrations (120,160,180 and 240 ml) of *Peganum harmala* and *Tribulus terrestris* exhibited significant ($P<0.05$) mortality of *Heterotermes indicola* with significant differences among themselves. These results are supported by Badshah *et al.* (2004) who recorded that all the concentrations of *Calotropis procera* extracts caused significantly higher mortality of *Heterotermes indicola* as compared to control. Mortality is concentration and time dependent, it increased with concentration and the exposure time (24 to 72Hrs) in the present case. These findings agree with Seo *et al.*

(2009) and Park and Shin (2005) and Boussaada *et al.* (2008) who reported that different medicinal plants, *Melaleuca dissitiflora*, *Eucalyptus citriodora*, *Eucalyptus globulus*, *Allium cepa*, *Mantisalca duriaei*, *Rhaponticum acaule*, and *Scorzonera undulata* showed time and concentration dependent mortality of termites and against other insects. Exposure time was highly significant ($P<0.05$) in *Tribulus terrestris* as compared to *Peganum harmala* in the present investigation. On the contrary, concentration and exposure time interaction was statistically significant ($P<0.05$) in *Tribulus terrestris* while it was insignificant in *Peganum harmala*. Concentrations and exposure time means significantly ($P<0.05$) differed in both the plants. *Tribulus terrestris* caused maximum mortality of termites at 48 and after 72 hrs as compared to *Peganum harmala* (Tables 35) at the same exposure time. These findings agree with Park and Shin (2005) who reported that *Allium cepa* and *Eugenia caryophyllata* bud caused 100% mortality of termites after 72 hrs. ANOVA for the effect of aqueous extract of different concentrations of *Peganum harmala* and *Tribulus terrestris* on the mortality of termites showed that concentrations were highly significant (Tables 38-39).

**Effect of Concentrations**

**Euphorbiaceae**

The ANOVA values showed that aqueous extracts (20g) of *Chrozophora tinctoria* and *Ricinus communis* showed significant ($P<0.05$) mortality of *Heterotermes indicola* after 72 hrs. Plants and time were highly significant while their interactions were non- significant. Plant mean value was highest for *Chrozophora tinctoria* and lowest for *Ricinus communis*. The exposure time means were not significantly different (Tables 34, 37). These results are supported by Sharma *et al.* (1990) who reported the termicidal activity of *Ricinus communis* that damage the wood of *Mangifera indica* and *Pinus longifolia*. All doses significantly reduced weight loss in wood pieces exposed to termites. These findings are in agreement with Vasant & Narasimhacharya (2008) who reported that *Ricinus communis* showed strong termicidal activity as compared to other medicinal plants. The present study also agrees with Upadhyay *et al.* (2012) who reported that various extracts of *Capparis decidua* showed termicidal activity and mortality was concentration and time dependent.

**Euphorbiaceae**

The ANOVA results (Table 35) indicated that all the concentrations (120, 160, 180 and 240 ml) of *Chrozophora tinctoria* showed significant ($P<0.05$) mortality of *Heterotermes indicola* with significant differences among themselves. The mortality of termites increased with the increase of concentration and time (24 to 72Hrs). The exposure time was highly significant at ($P<0.05$). Similarly concentration and exposure time interaction was statistically significant (Tables 40).

Saponins were found in stems of *Chrozophora tinctoria* and in roots and stems of *Ricinus communis*. Tannins were present in roots, leaves of *Chrozophora tinctoria* and in all parts of *Ricinus communis*. Flavonoids exhibit insecticidal activities and are important phenolics in terms of repellent and feeding deterrent functions (Ajayi *et al.*, 2012). Verma *et al.* (2009) and Elsayed (2011) stated that *Larix leptolepis* had large quantities of flavonoids and caused the mortality of termites. Flavonoids were found in the methanolic and n-hexane extract of all the analysed plants. Several workers reported similar findings (Raja & Venkataraman, 2011; Jain *et al.*, 2010; Jain & Nafis 2011; Rajkala *et al.*, 2011; Hussain *et al.*, 2011; Khan *et al.*, 2011; Siddiqui *et al.*, 2009; Mwine & Damme, 2011) that support the present results. Badshah *et al.* (2004) stated that roots, stems, leaves, and seeds containing alkaloids showed insecticidal (termicidal) activity. Alkaloids were found in leaves of *Chrozophora tinctoria* and in the stems and leaves of *Ricinus communis*. This agrees with Takhi *et al.* (2011) and Ajayi *et al.* (2012) who reported that *Morinda lucida* had alkaloids, tannins, flavonoids, anthraquinones and saponins and they caused the mortality of termites. Mucilage was present in leaves of *Chrozophora tinctoria* and in the stems and leaves of *Ricinus communis*. The presence of these phytochemicals might be responsible for the mortality of termites in the present study. Steroids were detected in *Chrozophora tinctoria*. Phenolic compounds had insecticidal activities (Seo *et al.*, 2009) and were present in the methanolic and n-hexane extract of *Ricinus communis*. This agrees with Ajayi *et al.* (2012) who identified flavonoids in *Ricinus communis* causing mortality of *Callosobruchus chinensis*. These compounds affect insect development, as antijuvenile hormones, resulting in sterile adults. The secondary metabolites occurring in the tested plants might be responsible for the mortality of *Heterotermes indicola*.

The present study showed that the methanolic extract of *F. cretica, P. harmala, T. terrestris, C. tinctoria* and *R. communis* had OH, carbooxylic and

phenolic groups. *Fagonia cretica, Peganum harmala, Tribulus terrestris,* and *Chrozophora tinctoria* have not been previously investigated for their insecticidal activity (against termites) in Pakistan. The previous studies revealed that *Fagonia cretica, Peganum harmala, Tribulus terrestris, Chrozophora tinctoria* and *Ricinus communis* had toxic phytochemicals and showing biological activities (Anjum *et al.* 2007; Abdel Khalik *et al.*, 2000; Hayet *et al.*, 2010; Jain *et al.*, 2010; Raghu & Ravindra, 2010 and Krishnaiah *et al.*, 2009). These compounds might be responsible of termitidal activity in the present case.

**Effect of methanolic extract after 24 hrs**

The total highest plant mean 50.0 was shown by *Fagonia cretica*, *Peganum harmala, Tribulus terrestris* and *Chrozophora tinctoria*. The total mortality mean of methanolic extract was 100.0 as compared to control (Table 41). Manzoor *et al.* (2011) reported 100% mortality of *Heterotermes indicola* from methanolic extracts of *Ocimum sanctum* and this agrees with the present case. Rahman *et al.* (2007) worked on different solvent extracts of *Polygonum hydropiper* against tea mound building termite, *Odontotermes assamensis* and showed that chloroform extract showed better insecticidal activity than methanol extract. The direct exposure to 2.0% chloroform extract caused 100%. Termite mortality occurred within 24h of treatment. This agrees with the present findings. The above results show that methanolic extracts of tested plants took less time as compared to aqueous extracts to cause death of termites.

Table 34. Effect of aqueous extracts (20g) of some selected plants of family Zygophyllaceae and Euphorbiaceae on the mortality of *Heterotermus indicola*.

| Plants | Exposure Time (hrs) | | | Plants Mean |
|---|---|---|---|---|
| Control | 24 | 48 | 72 | |
| **Zygophyllaceae** | | | | |
| | 10.3c | 11.3c | 25.0a | 15.56b |
| *Fagonia cretica* L. | 19.67b | 21.67ab | 25.0a | 22.11a |
| *Peganum harmala* L. | 20.0b | 21.0b | 25.0a | 22.0a |
| *Tribulus terrestris* L. | 20.0b | 21.67ab | 25.0a | 22.4a |
| **Time Mean** | 17.67b | 18.91b | 25.0a | |
| Significance at $\alpha$=0.05 | S | S | S | S |
| **Euphorbiaceae** | | | | |
| Control | 10.3 | 11.3 | 25.0 | 21.11 |
| *Chrozophora tinctoria* (L.) Raf | 18.3 | 11.3 | 25.0 | 19.88 |
| *Ricinus communis* L. | 16.67 | 18.0 | 25.0 | 15.55 |
| **Time Mean** | 15.58 | 16.91 | 25.0 | |
| Significance at $\alpha$=0.05 | NS | NS | NS | NS |

Mean values in rows followed by different letters are significantly different at the 0.05 level of significance using LSD test.

Table 35. The effect of different concentrations of some plants of family Zygophyllaceae and Euphorbiaceae on the mortality (%) of *Heterotermus indicola*.

| **Zygophyllaceae** | Exposure Time (hrs) | | | Concentration Mean |
|---|---|---|---|---|
| *Peganum harmala* L. | 24 | 48 | 72 | |
| Concentration (%) | | | | |
| Control | 10.33 | 11.33 | 12.0 | 11.33c |
| 120 | 16.67 | 23.0 | 25.0 | 21.56ab |
| 160 | 15.0 | 19.0 | 25.0 | 19.67ab |
| 180 | 18.0 | 20.67 | 25.0 | 21.22ab |
| 240 | 21.0 | 24.33 | 25.0 | 23.44a |
| Exposure Time Mean | 16.2c | 19.67b | 22.47a | |
| *Tribulus terrestris* L. | | | | |
| Control | 10.33c | 11.33c | 12.33c | 11.33c |
| 120 | 12.67c | 21.0b | 25.0a | 19.56b |
| 160 | 21.0b | 25.0a | 23.67a | 23.67a |
| 180 | 13.33c | 17.67b | 25.0a | 18.67b |
| 240 | 19.67b | 25.0a | 25.0a | 23.11a |
| Exposure Time Mean | 15.4c | 19.93b | 22.4a | |
| **Euphorbiaceae** | | | | |
| *Chrozophora tinctoria* | | | | |
| Control | 10.33c | 11.33c | 12.33c | 11.33c |
| 120 | 14.33e | 17.33b | 25.0a | 18.89b |
| 160 | 21.67a | 22.67a | 25.0a | 23.11a |
| 180 | 13.67cde | 15.33bc | 25.0a | 18.0b |
| 240 | 22.67a | 23.33cde | 25.0a | 23.67a |
| Exposure Time Mean | 16.53b | 18.0b | 22.5a | |

Mean values in rows followed by different letters are significantly different at the 0.05 level of significance using LSD test.

Table 36. ANOVA for the effect of aqueous extract (20g) of Fagonia *cretica*, *Peganum harmala* and *Tribulus terrestris* on mortality of *Heterotermus indicola* after 72 hrs.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Plants (P) | 2 | 297.639 | 99.213 | 24.8032 | 0.0000 |
| 4 | Time(t) | 2 | 369.389 | 184.694 | 46.1736 | 0.0000 |
| 6 | Pxt | 6 | 149.944 | 24.991 | 6.2477 | 0.0005 |
| -7 | Error | 24 | 96.000 | 4.000 | | |
| Total | | 34 | 912.972 | | | |

COV = 9.74 % LSD for plants = 1.95; LSD for time LSD for plants and time interaction=3.37

Table 37. ANOVA for effect of aqueous extract (20g) of *Chrozophora tinctoria* and *Ricinus communis* on mortality of *Heterotermus indicola* after 72 hrs.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Plants(P) | 1 | 164.111 | 54.704 | 9.4226 | 0.0003 |
| 4 | Time(t) | 2 | 623.167 | 311.583 | 53.6699 | 0.0000 |
| 6 | Pxt | 6 | 82.389 | 13.731 | 2.3652 | 0.0616 |
| -7 | Error | 24 | 139.333 | 5.806 | | |
| Total | | 33 | 1009.000 | | | |

COV=12.57

Table 38. ANOVA for effect of different concentrations of *Peganum harmala* on mortality of *Heterotermus indicola* after 72 hrs.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| | | 2 | 295.644 | 147.822 | 12.2505 | 0.0001 |
| 4 | Conc.(C) | 4 | 805.111 | 201.278 | 16.6805 | 0.0000 |
| 6 | HXC | 8 | 78.356 | 9.794 | 0.8117 | |
| -7 | Error | 30 | 362.000 | 12.067 | | |
| Total | | 44 | 1541.111 | | | |

COV= 17.86% LSD for Time = 2.59; LSD for Conc =3.34

Table 39. ANOVA for effect of different concentrations of *Tribulus terrestris* on mortality of *Heterotermus indicola* after 72 hrs.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Hours (H) | 2 | 384.533 | 192.267 | 40.2419 | 0.0000 |
| 4 | Conc. (C) | 4 | 877.689 | 219.422 | 45.9256 | 0.0000 |
| 6 | HXC | 8 | 153.244 | 19.156 | 4.0093 | 0.0025 |
| -7 | Error | 30 | 143.333 | 4.778 | | |
| Total | | 44 | 1558.800 | | | |

COV= 11.35%; LSD for Time = 2.59; LSD for Conc = 3.34; LSD for time and conc = 3.65

Table 40. ANOVA for effect of different concentrations of *Chrozophora tinctoria* on mortality of *Heterotermus indicola* after 72 hrs.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Hours(H) | 2 | 286.533 | 143.267 | 35.4231 | 0.0000 |
| 4 | Conc.(C) | 4 | 886.222 | 221.556 | 54.7802 | 0.0000 |
| 6 | HXC | 8 | 151.911 | 18.989 | 4.6951 | 0.0008 |
| -7 | Error | 30 | 121.333 | 4.044 | | |
| Total | | 44 | 1446.000 | | | |

COV= 10.58%; %; LSD for Time = 1.5; LSD for Conc = 1.94; LSD for time and conc = 3.35

Table 41. Effect of methanolic extracts of plants of family Zygophyllaceae and Euphorbiaceae on mortality (%) of *Heterotermes indicola* after 24 hrs.

| Plants | *Fagonia cretica* L. | *Paganum harmala* | *Tribulus terristris* | *Chrozophora tinctoria* | Means |
|---|---|---|---|---|---|
| Control | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Methanol | 100.000 | 100.000 | 100.000 | 100.000 | 100.000 |
| Means | 50.000 | 50.000 | 50.000 | 50.000 | ---------- |

# CHAPTER-8
# CHEMICAL EVALUATION

# a.Phytochemical Screening

# CHAPTER 8

# PHYTOCHEMICAL SCREENING

## a. Phytochemical screening of plant parts

Saponins were present in stems, leaves and fruits of *Fagonia cretica* and *Peganum harmala*. The roots, stems and leaves of *Tribulus terrestris* also had saponin. These findings agree with those of Dinchev *et al*. (2008) and Rajkala *et al*. (2011) who reported saponin in the fruits of *Tribulus terrestris*. These findings agree with EL-Kamali (2009) who reported saponins in *Ageratum conyzoides, Glycyrrhiza glabra* and *Ipomoea carnea*. Alkaloids were detected in stems, leaves and fruits of *F. cretica* and *P. harmala* and in roots, stems and leaves of *T. terrestris* (Table 42). Anthraquinones were present in the stems of *P. harmala, F. cretica* and in the leaves of *P. harmala* and were absent from the stems. Oils were found in stems and fruits *F. cretica* and in the roots, leaves and fruits of *T. terrestris*. Mucilage was present in the stems and fruits of *F. cretica*, and in the roots, leaves and fruits of *T. terrestris* (Table 42).

Saponins were found in the stems of *Chrozophora tinctoria* and in the roots of *Ricinus communis*. This agrees with the findings of Okoli *et al*., (2009) who reported saponins in various species of Euphorbiaceae. Alkaloids were present only in leaves of *C. tinctoria* and stems and leaves of *R. communis*. These findings are supported by Mughal et *al*. (2010) who reported that *R. communis* leaves contained alkaloids.

Anthraquinones were found in the leaves of *C. tinctoria* and *R. communis*. Oils were present in roots, stems and fruits of *C. tinctoria* while stems and leaves of *R. communis* also had oils. Mucilage was present only in leaves of *C. tinctoria*, and stems and leaves of *R. communis* (Table 42).

**Screening of plant extracts**

Saponins were found in the methanolic extract of *F. cretica, P. harmala, C. tinctoria*. The n-hexane extract of all the studied plants showed the absence of saponins, alkaloids, tannins, pholobatannins and anthraquinones in the present study.Alkaloids were present in the methanolic extract of all the tested plants. Raja & Venkataraman (2011) reported similar findings.

Tannins were found in the methanolic extract of *C. tinctoria* and *R. communis*. Singh *et al.* (2010) also reported similar results in various extracts of *R. communis* stem and found tannins in the benzene, petroleum ether and chloroform extracts.

Terpenoids were detected in methanolic extract of *P. harmala* while terpenoids were found in the n-hexane extract of *F. cretica, T. terrestris, C. tinctoria* and *R. communis*. This agrees with Raja & Venkataraman (2011), Khan *et al.* (2011) and Abdel Khalik *et al.* (2000) who also reported triterpenoids in *F. cretica* (Table 43).

Steroids were spotted in the methanolic extract of *Peganum harmala*, *Tribulus terrestris* and *Chrozophora tinctoria*. Similarly Rajkala *et al.* (2011) also reported steroids in the fruits of *Tribulus terrestris*.

The methanolic extract of *F. cretica*, *P. harmala* had cardiac glycosides. These findings are in line with those of Jain *et al.* (2010) who also reported similar findings in methanolic and hexane leaf extracts of *R. communis*. Cardiac glycosides were found in the n-hexane extract of *F. cretica* and *T. terrestris* (Table 43). Likewise Usman *et al.* (2007) also reported cardiac glycosides in *T. terrestris*. These findings also are in line with Khan *et al.* (2011) and Hussain *et al.* (2011) who also reported glycosides in different parts of *T. terrestris*.

Methanolic extract of all the analysed plants; and n-hexane extract of *F. cretica, T. terrestris, C. tinctoria* and *R. communis* had flavonoids (Table 43). This is in accordance with Jain & Nafis (2011) who reported similar findings in *R. communis*. Abdel Khalik *et al.* (2000) and Arshad *et al.* (2008) and Sharaf *et al.* (1997) also reported flavonoids in *F. cretica* and *P. harmala*. Similarly, Raja & Venkataraman (2011),

Rajkala *et al.* (2011), Khan *et al.* (2011) and Hussain *et al.* (2011) reported flavonoids in *T. terrestris*.

Amino acids were detected in the methanolic extract of *F. cretica* and *P. harmala* and n-hexane extract of *P. harmala, T. terrestris, C. tinctoria* (Table 43). Uma *et al.* (2009) and Sivasankari *et al.* (2010) reported amino acids in solvents other the present one.

The methanolic extract of *F. cretica, P. harmala* had reducing sugars (Table 43). This agrees with Hussain *et al.* (2011) and Raja & Venkataraman (2011) who reported reducing sugar in *T. terrestris*. Reuben and Abdulrahman (2008) also reported reducing sugar in some members of family Euphorbiaceae that also supports the present findings. Reducing sugars were absent in n-hexane extract of *F. cretica, P. harmala, T. terrestris, C. tinctoria*.

Phenolics were detected in the methanolic extract of *P. harmala, R. communis* while the n-hexane extract of *T. terrestris* and *R. communis* had phenolics (Table 43).

**Functional groups**

The methanolic extract of *Fagonia cretica, Peganum harmala, Tribulus terrestris, C. tinctoria* and *R. communis* revealed the presence of OH group, carbooxylic group and phenolic group.

The n-hexane extract of *F. cretica, T. terrestris, C. tinctoria* and *R. communis* revealed the presence of OH group. The n-hexane extract of *F. cretica, P. harmala, T. terrestris, C. tinctoria* and *R. communis* revealed the presence of carbooxylic group. The n-hexane extract of *F. cretica, T. terrestris, C. tinctoria* and *R. communis* revealed the presence of phenolic group.

Table 42. Phytochemical screening of plants of family Zygophyllaceae and Euphorbiaceae

| Plant name | Part used | Saponin | | | Alkaloid | | Anthraquinone | | Oil | | Mucilage | Tannin | | Phlobatannin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | D.C | Froth Yes/No | +ve/-ve | D.C | +ve/-ve | D.C | +ve/-ve | D.C | +ve/-ve | +ve/-ve | D.C | | D.C | |
| *Fagonia cretica* L. | Rt | Y | Ys | +++ | Gr | - | Pnk | + | Y | - | - | L Br | - | L Br | - |
| | St | Y | Ys | +++ | Pnk | + | Pnk | + | L Y | ++ | + | YBr | - | YBr | - |
| | Lvs | Y | Ys | ++ | Pnk | + | Gr | - | Y Gr | - | + | YBr | - | YBr | - |
| | Frt | Y | Ys | +++ | YGr | + | L Gr | - | Y Gr | + | + | W | - | W | - |
| *Peganum harmala* L. | Rt | Y | N | - | YBr | - | YBr | - | YBr | - | - | Gr | - | Gr | - |
| | St | Y | N | - | Pnk | + | Y | - | L Y | - | + | DBr | - | DBr | - |
| | Lvs | L Br | N | - | Y | + | Pnk | + | L Br | - | + | Gr | - | Gr | - |
| | Frt | Y | N | - | Rd | + | Y | - | Y | - | + | LY | - | LY | - |
| *Tribulus terrestris* L. | Rt | Y | Ys | +++ | Y | + | H W | - | Du Gr | ++ | + | LY | - | LY | - |
| | St | Y | Ys | + | Y | + | LGr | - | L Gr | - | + | Ow | - | Ow | - |
| | Lvs | Y | Ys | +++ | Y | + | LGr | - | L Y | ++ | + | GBr | - | GBr | - |
| | Frt | Y | N | - | Pnk | - | LGr | - | Gr | + | - | LBr | - | LBr | - |
| *Chrozophora tinctoria.* | Rt | Y | N | - | Pnk | - | P | - | Y | + | - | BluB | + | BluB | + |
| | St | Y | Ys | ++ | Y | - | L Y | - | Y Gr | + | - | YG | - | YG | - |
| | Lvs | R | N | - | Pnk | + | Pnk | + | D Gr | - | + | BB | + | BB | + |
| | Frt | R | N | - | Pnk | - | P | - | Gr | + | - | P | - | P | + |
| *Ricinus cmmunis L.* | Rt | Br | Ys | + | Y | - | Y | - | Y | - | - | LBluB | + | LBluB | - |
| | St | Y Br | Ys | + | Pnk | + | Y Br | - | L Gr | ++ | + | DBluB | + | DBluB | - |
| | Lvs | Rd Br | N | - | Pnk | + | Y | - | D Gr | + | + | DBluB | + | DBluB | - |
| | Frt | Br | N | - | Br | - | Br | - | Br | + | + | DBluB | + | DBluB | - |

Key: Rt = Root; St = Stem; Lvs = Leaf; Frt = Fruit; Blu = Blue; Br = Brown; B = Black; D = Dark; Du = Dull; Gr = Green; = Ow = Offwhite; H.W = Half white; L = Light; Pnk = Pink; P = Purple; Rd = Red; W = white Y = Yellow; Ys = Yes; N = No; + = (+) Presence; (-) = Absence; DC = Drug color.

Table 43. Phytochemical analysis of plants of family Zygophyllaceae and Euphorbiaceae.

| Phytochemicals | Sap | Alk | Anth | Tan | P-ta | Gly | Fla | Terp | Ste | Ami | Red | Phe |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Zygophyllaceae** | | | | | | | | | | | | |
| **Methanolic Extract** | | | | | | | | | | | | |
| *Fagonia cretica* L. | + | + | - | - | - | + | + | - | - | - | + | - |
| *Peganum harmala* L. | + | + | - | - | - | + | + | - | + | + | + | + |
| *Tribulus terrestris* L. | - | + | - | - | - | - | + | - | - | + | + | - |
| **Euphorbiaceae** | | | | | | | | | | | | |
| *Chrozophora tinctoria* (L.) Raf. | + | + | - | + | - | - | + | - | - | - | - | - |
| *Ricinus communis* L. | - | + | - | - | - | - | + | - | - | - | - | + |
| **N-hexane Extract** | | | | | | | | | | | | |
| **Zygophyllaceae** | | | | | | | | | | | | |
| *Fagonia cretica* L. | - | - | - | - | - | + | - | - | + | + | - | - |
| *Peganum harmala* L. | - | - | - | - | - | | - | - | - | + | - | - |
| *Tribulus terrestris* L. | - | - | - | - | - | + | - | - | + | - | - | - |
| **Euphorbiaceae** | | | | | | | | | | | | |
| *Chrozophora tinctoria* (L.) Raf. | - | - | - | - | - | - | - | - | + | - | - | + |
| *Ricinus communis* L. | - | - | - | - | - | - | - | - | + | - | - | + |

Key : Alk=Alkaloid, Anth=Anthraquinone, Ami=Amino acid, Fla=Flavonoid, Gly=Glycoside P-ta=Phlobatannin,Phe=Phenol, Red=Reducing sugar, Sap=Saponin, Ste=Steroids, Terp=Terpenoid.

# b.Proximate Composition

## NUTRITIONAL EVALUATION

### b. Proximate composition

#### i. Moisture contents

The presence of moisture in a crude drug can lead to its deterioration due to either activation of certain enzymes or growth of microbes. Thus moisture contents should be maintained to a reasonable level during storage. In the present case it was seen that the moisture contents was high (9.1%) in the stems and lowest (7.4%) in fruits (7.4%) and in roots of *Fagonia cretica* (Table 44). Hameed *et al.* (2008); Otunola *et al.* (2010) and Hussain *et al.* (2009) observed similar moisture contents in *Rumex hastatus, Allium sativum, Zingiber officinale Capsicum frutescens* and *Vitis venifera*. However, Shad *et al.* (2002) reported high (58.51%) moisture contents in the leaves and seeds of *Fagonia arabica* thus contradicting the present results. This could be due to variation in drying, storage or time. The moisture contents varied from 7.7% in *Chrozophora tinctoria* in summer to 9.5% in *Ricinus communis* in winter. Moisture contents increased in winter than summer in *Fagonia cretica*, *Tribulus terrestris* and *Chrozophora tinctoria*; while it decreased during winter in *Peganum harmala* and *Ricinus communis* (Fig. 104). Kochhar *et al.* (2006) reported that the moisture contents of bittergourd fruit, fenugreek seeds and Jumbo seeds differed significantly. ANOVA showed that means were insignificant while seasonal differences between winter and summer were significant ($P < 0.05$). The plants and seasons and plant interaction were insignificant (Tables 47, 46).

#### ii. Ash contents

The total ash contents is also a measure of the presence of inorganic compounds in a drug. High ash contents indicate high inorganic compounds in the plant material (Mammen *et al.*, 2010). The ash contents are also index of the mineral contents in biota (Hassan & Umar, 2006), which consists of oxides, salts and minerals. Ash contents were high (14.1%) in leaves and low (5.3%) in roots of *Tribulus terrestris* (Table 44). Like the present study, Hassan & Umar (2006) and Hussain *et al.* (2009) also reported high ash contents in the leaves of *Momordica balsamina* and *Fumaria officinalis*. The ash contents varied from 10.4% in *Tribulus terrestris* in summer to 13.8% *Peganum harmala* in winter in the present study. Ash contents decreased in winter in *Fagonia cretica* and *Ricinus communis*. Mammen *et*

*al.* (2010) reported higher total ash contents for *Leptadenia reticulata* when collected in summer, compared to monsoon and winter. Similar findings were also reported by Parlak *et al.* (2011) who found least ash contents in *Thymus longicaulis* in February (winter) which also supports the present findings. Ash contents increased in winter in *Peganum harmala, Tribulus terrestris* and *Chrozophora tinctoria* (Fig. 105). This also agrees with Parlak *et al.* (2011) who reported increased ash contents in *Phillyrea latifolia* and *Cistus creticus* in winter. This increase or decrease of ash contents might be due to variation of misture in soil or atmospheric humidity. Plants means and seasonal means and interactions were insignificant (Table 46) as ANOVA showed insignificant differences between the two seasons, among the plants and seasons (Table 48). Parlak *et al.* (2011) stated that ash contents increasesd significantly in kermes oak, and halophyte shrubs with maturation while in *Hippocrepis balearica* ash contents elevated in the spring as compared to summer. The observed variation in ash might be due to the difference in anatomical structures of these plants.

**iii. Protein contents**

Proteins have structural role in tissues, vital in cell division, growth, reproduction, transport nutrients, maintain fluid balance, blood pressure, regulate enzymes, hormones, blood clotting substances and immune system. The present study revealed that the protein contents varied from 6.8% in *Chrozophora tinctoria* in winter to 16. 1% *Peganum harmala* in winter. The protein (13.3%) was highest in fruits and lowest (8.0%) in roots in winter while it was (20.3%) in roots and (11.2%) in stems of *Peganum harmala* in summer (Tables 44, 45). Bukhsh *et al.* (2007) reported that crude proteins varied with plant parts thus supporting the present results. Protein contents increased in winter than summer in *Fagonia cretica*, *Peganum harmala* and *Ricinus communis* (Fig. 106). Chinnasamy *et al.* (2003) stated that two reasons for increase in protein contents during adaptation to stress depending on the plant species: increased *de novo* synthesis and decreased utilization of protein as a consequence of low growth rate. Protein contents decreased in winter in *Tribulus terrestris*, and *Chrozophora tinctoria*. The results agree with Parlak *et al.* (2011), who reported also decreased crude protein in *Sarcopoterium spinosum* in winter as compared to summer. Similar results however were reported by Haddi *et al.* (2009) who observed decreased crude protein in *Sueada mollis* in winter. Haddi *et al.* (2009) also stated that crude protein of arid plants decreased in warm and dry seasons and

increased in cold and humid sessons. Hussain *et al.* (2009) also reported high and significant levels of protein in *Terminalia chebula, Pistacia vera*, and *Coriandrun sativum*. There is always variability in the amount and increase/decrease of crude protein in different species and between growth stages. Zavodnik *et al.* (1998) reported the lowest proteins contents both in leaves and roots of *Cymodocea nodosa* during August and September. Protein contents in roots (10%) showed pronounced seasonal changes and lower values than in leaves (14%) during all vegetative periods, except in February-March when they attained their maximum (17%) level. The ANOVA revealed that plants and easons means and seasons and plant interaction were insignificant (Table 49).

**iv. Fat contents**

Fats and oils are the most abundant lipids found in nature and important constituents of plant and animal tissues (Oladele & Oshodi, 2007). The fat contents in the present case varied from 5.9% in *Fagonia cretica* in summer to 17.1% in *Ricinus communis* in winter (Tables 44, 45). Fat contents increased in winter than summer in all the plants (Fig. 107). Otunola *et al.* (2010) reported that pepper had a significantly high crude fat contents compared to garlic and ginger. Similarly, Hussain *et al.* (2009) also reported higher fat contents in *Fumaria officinalis*. Kochhar *et al.* (2006) reported the fat contents of bittergourd fruit, fenugreek seeds differed significantly from each other. This agrees with the present findings. ANOVA showed significant seasonal differences ($P < 0.05$) while plants and seasons and plant interaction were insignificant (Table 50).

**v. Fibre contents**

The crude fibre is the organic residue remaining after digesting with acids. Crude fibre in diet consists mostly of plant polysaccharides that cannot be digested by human dietary enzymes. The importance of fiber in diet cannot be neglected as it decreases serum cholesterol levels, risk of coronary heart diseases, hypertension, diabetes, colon and breast cancer (Ishida *et al.*, 2000). It is commonly used as an index of value in poultry and feeding stocks feeds (Oladele & Oshodi, 2007). The fibre was highest (62.3 %) in roots and lowest (41.3%) in leaves of *Fagonia cretica* like the present study (Table 44). The fiber contents varied from 17.1% in *Peganum harmala* in winter to 53.27% in *Fagonia cretica* in summer. Fiber contents

decreased in winter than summer in all the plants (Fig. 108). This agrees with Parlak *et al.* (2011) who reported decreased fibre contents in *Cistus creticus* in winter as compared to summer. Similarly Ahmed *et al.* (2011) reported that fiber contents in *Mentha longifolia* were influenced by summer and fibers were maximum in summer than other seasons. This variation in biochemical ingredients might be due to soil moisture contents, plant maturity and change of temperature during different seasons. Plants means were insignificant while seasons means were significant (Table 46) as ANOVA showed significant differences ($P < 0.05$) of fat contents between two seasons. The plants and seasons and plant interaction were insignificant (Table 51). Kochhar *et al.* (2006) reported the fibre contents of bittergourd fruit, fenugreek seeds and Jumbo seeds and differed significantly from each other and this what this study also reports.

**vi. Carbohydrate contents**

Carbohydrate are essential for the maintenance of plant and animal life and also provide raw materials for many industries (Oladele & Oshodi, 2007). High level of carbohydrates 63.8 % were recorded in *Chrozophora tinctoria* (Table 44). This agrees with Iniaghe *et al.* (2009) who also reported high carbohydrate contents in leaves of *Acalypha*. Hussain *et al.* (2009) reported pronounced levels of carbohydrates in *Terminalia belerica* and *Ziziphus vulgaris*. Similarly, Rajkala *et al.* (2011) also detected carbohydrates in fruit of *Tribulus terrestris* and agrees with the present study. Carbohydrate contents varied from 11.2% in *Tribulus terrestris* in summer to 36.225% in *Fagonia cretica* in winter suggesting increase in winter than summer in all the analysed plants (Fig. 109). ANOVA showed that means for plants, seasons and plant interactions were also insignificant (Tables 52, 46). Kochhar *et al.* (2006) also reported insignificant differences in the total carbohydrate contents of bittergourd fruit, fenugreek seeds and Jumbo seeds. Variations in plant metabolites occur due to factors such as genetic component, biotic and abiotic features, seasonal changes in biochemical characteristics that are caused by shifting patterns of resource allocation that reflect different physiological demands associated with growth, defense and reproduction. Simultaneouly, a diversity of environmental stresses contributes to spatial variation within and among populations (Niknam & Lisar, 2004).

**vii. Gross Energy contents**

All carbon containing compounds have a certain amount of energy which are a primary source of medicines, fibre, food, and other items in daily use by humans (Hemingway, 2004). Gross energy (GE) represents the total combustible energy in a feed stuff. The amount of energy needed are measured as digestible energy (DE), metabolized energy (ME) and total digestible nutrients (TDN). These values always differ among the species and phenological stages of plants. Gross energy is important in the evaluation of drugs. The GE contents varied from 395.07% in *Chrozophora tinctoria* to 446.925% in *Ricinus communis* in winter. Gross energy increased in winter in *Peganum harmala, Tribulus terrestris* and *Ricinus communis* and it decreased in *Fagonia cretica* and *Chrozophora tinctoria* (Fig. 110). Dongmeza *et al.* (2009) reported significant changes in gross energy in *Musa nana* and *Manihot esculenta* leaves collected in summer and winter and this variation agrees with the present results. ANOVA revealed that plants means, seasons means (Table 46) and seasons and plant interaction were insignificant (Table 53).

Considering the overall comparative, it was seen that the average values revealed that *Peganum harmala* excelled in high fat, carbohydrate, protein and moisture contents than other two species, therefore it can be considered a good nutritive plant followed by *Fagonia cretica* that contained the highest fibre. The *Tribulus terrestris* had the maximum protein and GE (Tables 44-45). The differences found in the proportion of proximate parameters of these medicinal plants might be attributed to the habitat, environment and time of harvest (Hussain *et al.* 2009) (Tables 44-45).

Among the tested Euphorbiaceae plant *Chrozophora tinctoria* and *Ricinus communis* also had variation in various analysed biochemicals (Table 44). The average values indicated that *Chrozophora tinctoria* had high the moisture contents, ash contents, protein, fats, fibre, carbohydrate and gross energy than its counterpart *Ricinus communis* (Table 44).

The present study suggests that the various analysed parameters such as ash values, extractive values and moisture contents showed variation due to parts analysed, variation in season and region of collection. These values however, can help in judging the best time and place of collection for a particular plant. It is suggested

that similar studies need to be carried out for other medicinal plants for these and other chemical parameters. Thus a parallel study of a number of samples can help in deciding the best time and place of collection for a particular medicinal plant.

It is then concluded that the investigated plants can significantly add to the nutritional requirements of people and should be used as a source of nutrients to supplement other major sources. The analysed plants also have high biological activities and therefore are of great medicinal value.

Table 44. Proximate composition of some selected species of Family Zygophyllaceae and Euphorbiaceae showing seasonal differences by parts during summer.

| Plants | Parts | Percent on dry matter basis | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Moisture contents (%) | Ash content %) | Protein contents (%) | Fat contents (%) | Fibre contents (%) | Carbohydrate (%) | Gross Energy (Kcal/g) |
| *Fagonia cretica* L. | Roots | 7.4 | 6.7 | 9.0 | 3.7 | 62.3 | 10.9 | 428.9 |
| | Stems | 9.1 | 11.9 | 8.6 | 4.9 | 54.3 | 11.2 | 400.8 |
| | Leaves | 8.4 | 16.5 | 10.0 | 8.9 | 41.3 | 14.9 | 399.5 |
| | Fruits | 7.4 | 10.7 | 10.2 | 6.2 | 55.2 | 10.3 | 423.1 |
| | **Mean** | **8.07** | **11.45** | **9.45** | **5.9** | **53.27** | **11.8** | **413.0** |
| *Peganum harmala* L. | Roots | 7.3 | 6.5 | 8.0 | 3.2 | 60.2 | 14.8 | 424.1 |
| | Stems | 10.7 | 10.4 | 11.6 | 8.7 | 33.4 | 25.2 | 410.5 |
| | Leaves | 9.6 | 15.1 | 11.5 | 7.8 | 22.3 | 33.7 | 382.4 |
| | Fruits | 9.3 | 10.0 | 13.3 | 11.6 | 34.0 | 21.8 | 438.1 |
| | **Mean** | **9.22** | **10.5** | **11.1** | **7.8** | **37.5** | **23.8** | **413.7** |
| *Tribulus terrestris* L. | Roots | 7.5 | 5.3 | 9.5 | 2.1 | 52.1 | 23.5 | 418.2 |
| | Stems | 7.7 | 11.3 | 11.1 | 8.4 | 57.4 | 4.1 | 434.2 |
| | Leaves | 8.8 | 14.1 | 15.3 | 8.9 | 42.3 | 10.6 | 423.0 |
| | Fruits | 6.4 | 10.9 | 14.2 | 9.3 | 52.6 | 6.6 | 448.1 |
| | **Mean** | **7.6** | **10.4** | **12.5** | **7.1** | **51.1** | **11.2** | **430.8** |
| *Chrozophora tinctoria* (L.) Raf | Roots | 5.7 | 8.1 | 5.2 | 6.9 | 10.3 | 63.8 | 401.6 |
| | Stems | 7.8 | 15.6 | 9.7 | 8.0 | 51.1 | 7.8 | 407.1 |
| | Leaves | 8.9 | 15.1 | 20.0 | 9.8 | 56.3 | 0.0 | 436.4 |
| | Fruits | 8.4 | 15.5 | 13.8 | 11.0 | 53.7 | 0.0 | 431.0 |
| | **Mean** | **7.7** | **13.5** | **12.1** | **8.9** | **42.9** | **35.8** | **419.0** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Ricinus communis* L. | Roots | 7.9 | 19.3 | 9.6 | 5.9 | 57.0 | 0.3 | 385.0 |
| | Stems | 7.1 | 12.1 | 7.7 | 6.8 | 52.6 | 13.7 | 416.1 |
| | Leaves | 7.2 | 11.8 | 24.9 | 11.8 | 11.3 | 33.0 | 441.5 |
| | Fruits | 7.2 | 6.2 | 2.6 | 6.2 | 22.5 | 55.3 | 404.2 |
| | **Mean** | **7.35** | **12.35** | **11.2** | **7.67** | **35.9** | **25.57** | **411.7** |

Table 45. Proximate composition of some selected species of Family Zygophyllaceae and Euphorbiaceae showing seasonal differences by parts during winter.

| Plants | Parts | Percent on dry matter basis | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Moisture contents (%) | Ash content (%) | Protein contents (%) | Fat contents (%) | Fibre contents (%) | Carbohydrate (%) | Gross Energy (Kcal/g) |
| *Fagonia cretica* L. | Roots | 8.5 | 7.5 | 8.4 | 7.6 | 56.8 | 11.2 | 437.3 |
| | Stems | 10.3 | 12.0 | 9.8 | 9.9 | 50.8 | 7.2 | 422.8 |
| | Leaves | 9.3 | 16.0 | 7.7 | 10.4 | 25.1 | 31.5 | 389.9 |
| | Fruits | 8.3 | 10.0 | 13.2 | 12.5 | 28.6 | 27.4 | 341.5 |
| | **Mean** | **9.1** | **11.4** | **9.8** | **10.1** | **40.3** | **19.32** | **397.8** |
| *Peganum harmala* L. | Roots | 9.3 | 8.1 | 20.3 | 8.9 | 26.6 | 26.8 | 436.0 |
| | Stems | 9.7 | 11.2 | 11.2 | 11.8 | 21.1 | 35.0 | 418.1 |
| | Leaves | 8.6 | 15.3 | 19.8 | 14.4 | 11.0 | 30.9 | 427.2 |
| | Fruits | 8.9 | 11.7 | 13.2 | 12.3 | 9.7 | 44.2 | 416.8 |
| | **Mean** | **9.1** | **11.5** | **16.1** | **11.85** | **17.1** | **34.22** | **424.4** |
| *Tribulus terrestris* L. | Roots | 7.8 | 6.7 | 8.0 | 8.9 | 56.0 | 12.5 | 448.7 |
| | Stems | 9.2 | 12.1 | 5.2 | 13.3 | 55.2 | 5.0 | 440.7 |
| | Leaves | 8.7 | 15.7 | 13.1 | 9.9 | 15.5 | 37.2 | 393.1 |
| | Fruits | 8.0 | 11.0 | 11.2 | 13.4 | 37.1 | 19.3 | 446.7 |
| | **Mean** | **8.42** | **11.4** | **9.4** | **11.3** | **40.95** | **18.5** | **432.3** |
| *Chrozophoratinctoria* (L.) Raf | Roots | 6.1 | 8.3 | 3.0 | 8.5 | 56.3 | 17.8 | 439.1 |
| | Stems | 10.1 | 15.7 | 6.9 | 8.5 | 30.9 | 27.9 | 380.6 |
| | Leaves | 9.7 | 16.0 | 10.5 | 13.0 | 6.7 | 44.1 | 393.2 |
| | Fruits | 8.0 | 15.3 | 6.8 | 7.6 | 7.2 | 55.1 | 367.4 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Mean** | **8.45** | **13.8** | **6.8** | **9.4** | **25.27** | **36.2** | **395.0** |
| *Ricinus* | Roots | 8.1 | 9.7 | 8.0 | 8.2 | 34.0 | 23.8 | 382.3 |
| *Communis* L. | Stems | 10.3 | 10.1 | 8.4 | 9.2 | 46.2 | 15.8 | 420.2 |
| | Leaves | 9.8 | 16.2 | 16.2 | 12.9 | 23.8 | 21.1 | 414.1 |
| | Fruits | 10.1 | 11.7 | 17.2 | 38.1 | 24.4 | 0.0 | 571.1 |
| | **Mean** | **9.5** | **11.9** | **12.45** | **17.1** | **32.1** | **20.2** | **447.0** |

Table 46. Statistical comparison of proximate composition of plants of family Zygophyllaceae and Euphorbiaceae for summer and winter.

| Plants | Moisture Contents (%) | | Means | Ash Contents (%) | | Means | Protein Contents (%) | | Means |
|---|---|---|---|---|---|---|---|---|---|
| **Zygophyllaceae** | Summer | Winter | | Summer | Winter | | Summer | Winter | |
| *Fagonia cretica* L. | 8.075 | 9.10 | 8.588 | 11.450 | 11.375 | 11.412 | 9.450 | 9.775 | 9.612 |
| *Peganum harmala* L. | 9.225 | 9.125 | 9.175 | 10.500 | 11.575 | 11.038 | 11.100 | 16.125 | 13.612 |
| *Tribulus terrestris* L. | 7.600 | 8.425 | 8.012 | 10.400 | 11.375 | 10.888 | 12.525 | 9.375 | 10.950 |
| **Euphorbiaceae** | | | | | | | | | |
| *Chrozophora tinctoria* (L.) Raf | 7.70 | 8.475 | 8.087 | 13.575 | 13.825 | 13.700 | 12.175 | 6.800 | 9.488 |
| *Ricinus communis* L. | 7.350 | 9.575 | 8.463 | 12.350 | 11.925 | 12.138 | 11.200 | 12.450 | 11.825 |
| **Mean** | 7.990b | 8.940c | | 11.655 | 12.015 | | 11.290 | 10.905 | |
| **Zygophyllaceae** | **Fat Contents (%)** | | | **Fiber Contents (%)** | | **Carbohydrates (%)** | | | |
| *Fagonia cretica* L. | 5.925 | 10.100 | 8.012 | 53.275 | 40.325 | 46.800 | 11.825 | 19.325 | 15.575 |
| *Peganum harmala* L. | 7.825 | 11.850 | 9.838 | 37.475 | 17.100 | 27.288 | 23.875 | 34.225 | 29.050 |
| *Tribulus terrestris* L. | 7.175 | 11.375 | 9.275 | 51.100 | 40.950 | 46.025 | 11.200 | 18.500 | 14.850 |
| **Euphorbiaceae** | | | | | | | | | |
| *Chrozophora tinctoria* (L.) Raf | 8.925 | 9.400 | 9.163 | 42.850 | 25.275 | 34.062 | 14.800 | 36.225 | 25.512 |
| *Ricinus communis* L. | 7.675 | 17.100 | 12.387 | 35.850 | 32.100 | 33.975 | 25.575 | 14.800 | 20.187 |
| **Mean** | 7.505b | 11.965a | | 44.110a | 31.15 b | | 17.455 | 24.615 | |

| Gross Energy (Kcal/g) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Zygophyllaceae** | | | | **Euphorbiaceae** | | | |
| *Fagonia cretica* L. | 413.075 | 397.875 | 405.475 | *Chrozophora tinctoria* (L.) Raf | 419.025 | 395.075 | 407.050 |
| *Peganum harmala* L. | 413.775 | 424.525 | 4129.150 | *Ricinus communis* L. | 411.700 | 446.925 | 429.313 |
| *Tribulus terrestris* L. | 430.875 | 432.300 | 431.588 | | 417.690 | 419.340 | |

Means followed by the same letter are not significantly different at 0.05%

Table 47. ANOVA for the comparison of seasonal variation of moisture contents and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 9.025 | 9.025 | 7.7813 | 0.0091 |
| 4 | Factor B | 4 | 6.931 | 1.733 | 1.4940 | 0.2289 |
| 6 | AB | 4 | 5.560 | 1.390 | 1.1984 | 0.3319 |
| -7 | Error | 30 | 34.795 | 1.160 | | |
| | Total | 39 | 56.311 | | | |

COV=12.72% LSD=0.6956 for seasons at 0.05

Table 48. ANOVA for the comparison of seasonal variation of ash contents and seasons-plants of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 1.296 | 1.296 | 0.0910 | |
| 4 | Factor B | 4 | 42.256 | 10.564 | 0.7419 | |
| 6 | AB | 4 | 3.414 | 0.853 | 0.0599 | |
| -7 | Error | 30 | 427.185 | 14.239 | | |
| | Total | 39 | 474.151, | COV=31.88% | | |

Table 49. ANOVA for the comparison of seasonal variation of protein contents and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 1.482 | 1.482 | 0.0681 | |
| 4 | Factor B | 4 | 93.388 | 23.347 | 1.0732 | 0.3871 |
| 6 | AB | 4 | 129.981 | 32.495 | 1.4938 | 0.2290 |
| -7 | Error | 30 | 652.618 | 21.754 | | |
| | Total | 39 | 877.470, | COV=42.03% | | |

Table 50. ANOVA for the comparison of seasonal variation of fat contents and seasons-plantsinteraction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 198.916 | 198.916 | 7.6413 | 0.0097 |
| 4 | Factor B | 4 | 84.421 | 21.105 | 0.8107 | |
| 6 | AB | 4 | 81.739 | 20.435 | 0.7850 | |
| -7 | Error | 30 | 780.955 | 26.032 | | |
| | Total | 39 | 1146.031 | | | |

COV=52.41% LSD=3.295 for seasons at 0.05

Table 51. ANOVA for the comparison of seasonal variation of fibre contents and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 1679.616 | 1679.616 | 6.2226 | 0.0183 |
| 4 | Factor B | 4 | 2300.946 | 575.237 | 2.1311 | 0.1014 |
| 6 | AB | 4 | 338.002 | 84.500 | 0.3131 | |
| -7 | Error | 30 | 8097.640 | 269.921 | | |
| | Total | 39 | 12416.204 | | | |

COV=43.66% LSD=10.61 for seasons at 0.05

Table 52. ANOVA for the comparison of seasonal variation of carbohydrate contents and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 512.656 | 512.656 | 1.9526 | 0.1726 |
| 4 | Factor B | 4 | 1224.578 | 306.145 | 1.1661 | 0.3454 |
| 6 | AB | 4 | 1070.931 | 267.733 | 0.0198 | 0.4130 |
| -7 | Error | 30 | 7876.405 | 262.547 | | |
| | Total | 39 | 10684.571 | COV=77.03% | | |

Table 53. ANOVA for the comparison of seasonal variation of gross energy and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value Prob | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value |
|---|---|---|---|---|---|
| 2 | Factor A | 1 | 27.225 | 27.225 | 0.0218 |
| 4 | Factor B | 4 | 4714.941 | 1178.735 | 0.9424 |
| 6 | AB | 4 | 4298.846 | 1074.712 | 0.8592 |
| -7 | Error | 30 | 37522.715 | 1250.757 | |
| | Total | 39 | 46563.727 | COV=8.45% | |

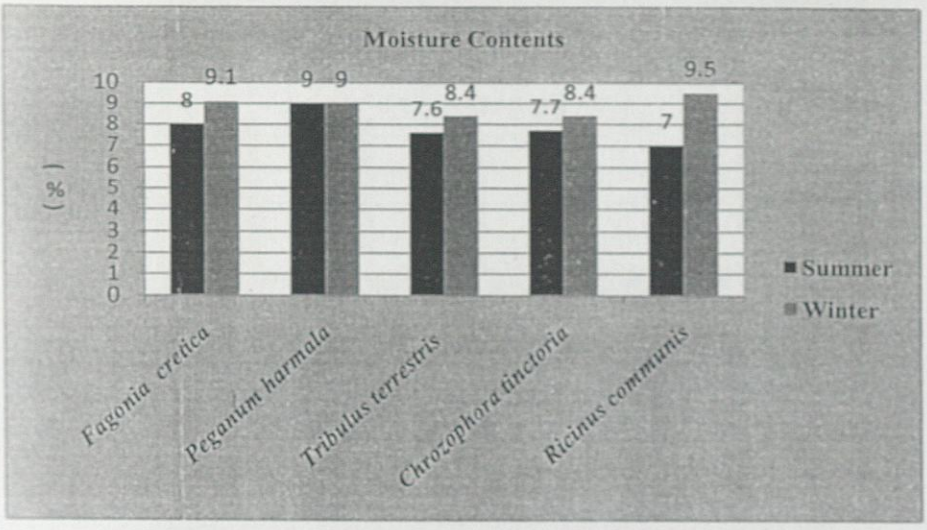



Fig. 104. Seasonal differences in moisture contents (%) of plants of family Zygophyllaceae and Euphorbiaceae.

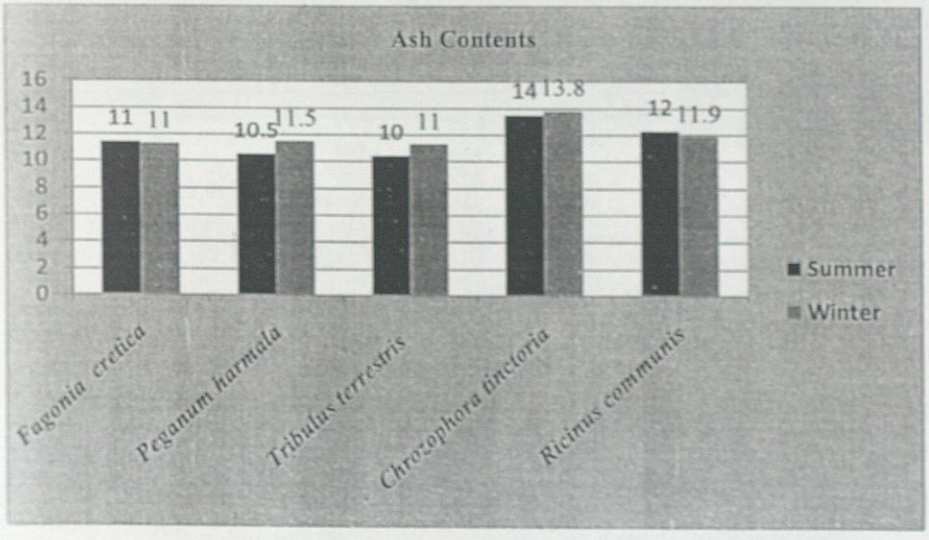



Fig. 105. Seasonal differences in ash contents (%) of plants of family Zygophyllaceae and Euphorbiaceae .

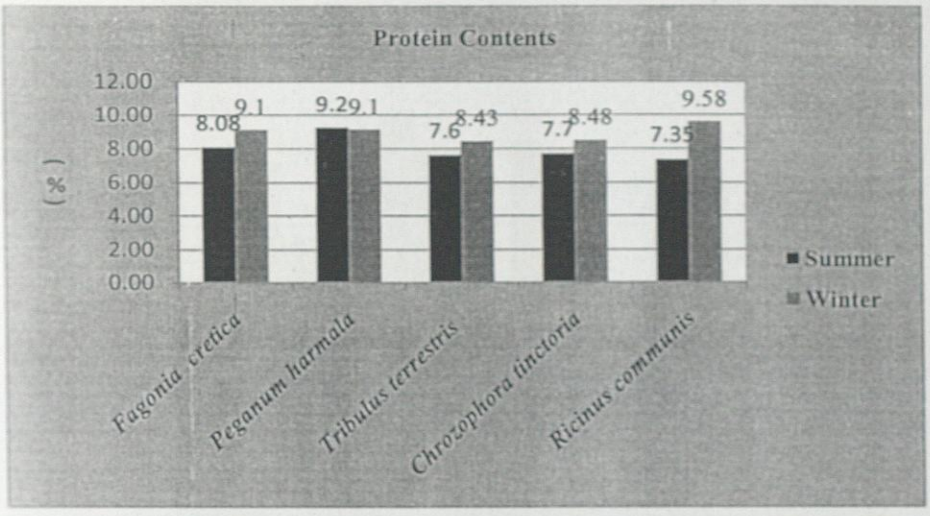



Fig. 106. Seasonal differences in protein contents (%) in plants of family Zygophyllaceae and Euphorbiaceae.

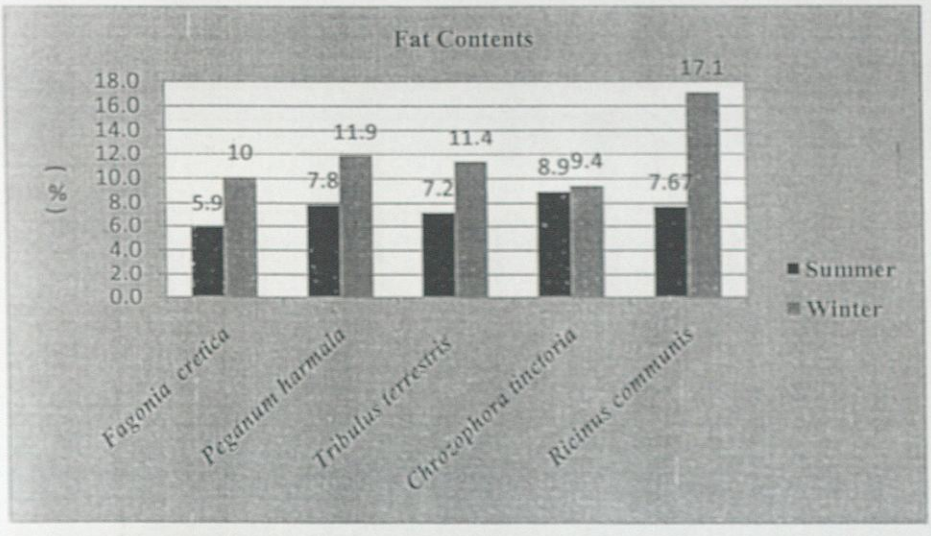



Fig. 107. Seasonal differences in fat contents (%) of plants of family Zygophyllaceae and Euphorbiaceae

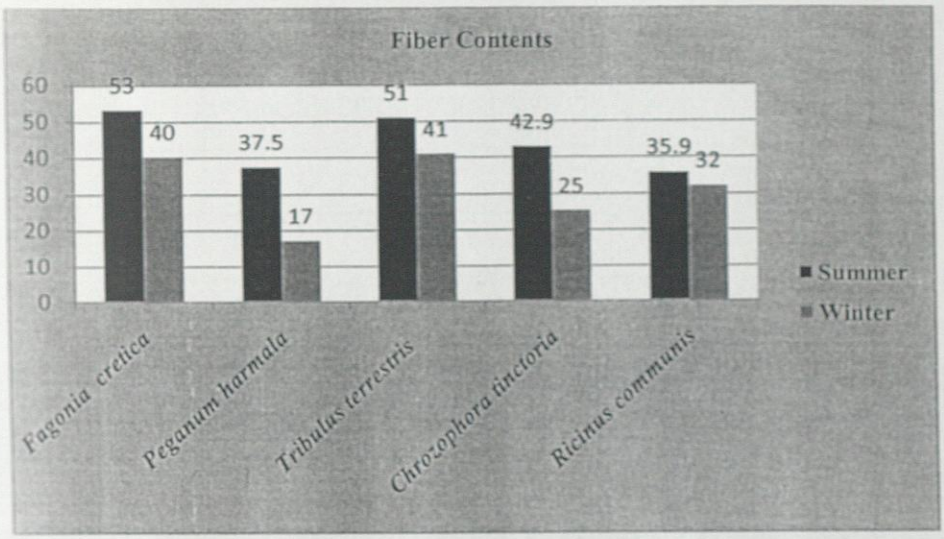



Fig. 108. Seasonal differences in fiber contents in plants of family Zygophyllaceae and Euphorbiaceae.

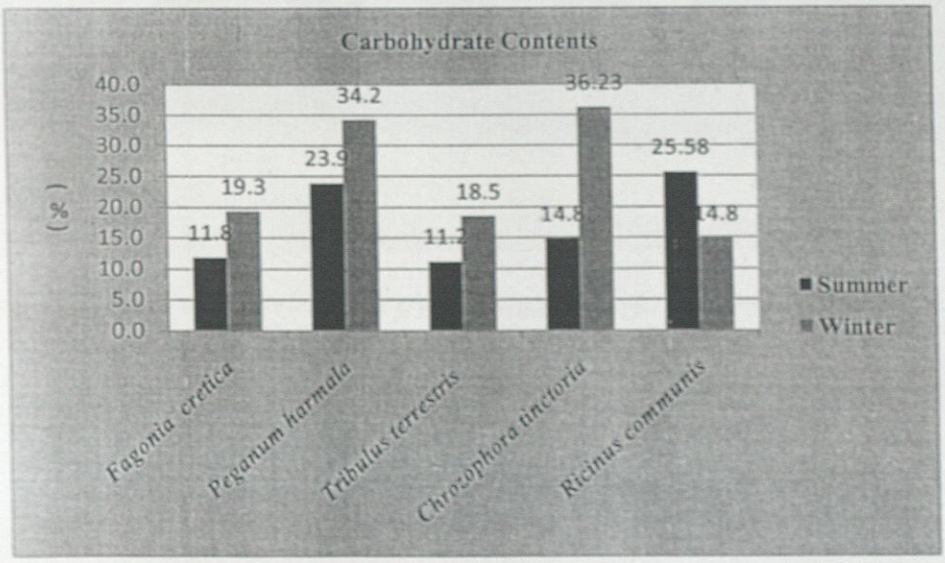



Fig. 109. Seasonal differences in carbohydrate contents (%) in plants of family Zygophyllaceae and Euphorbiaceae.

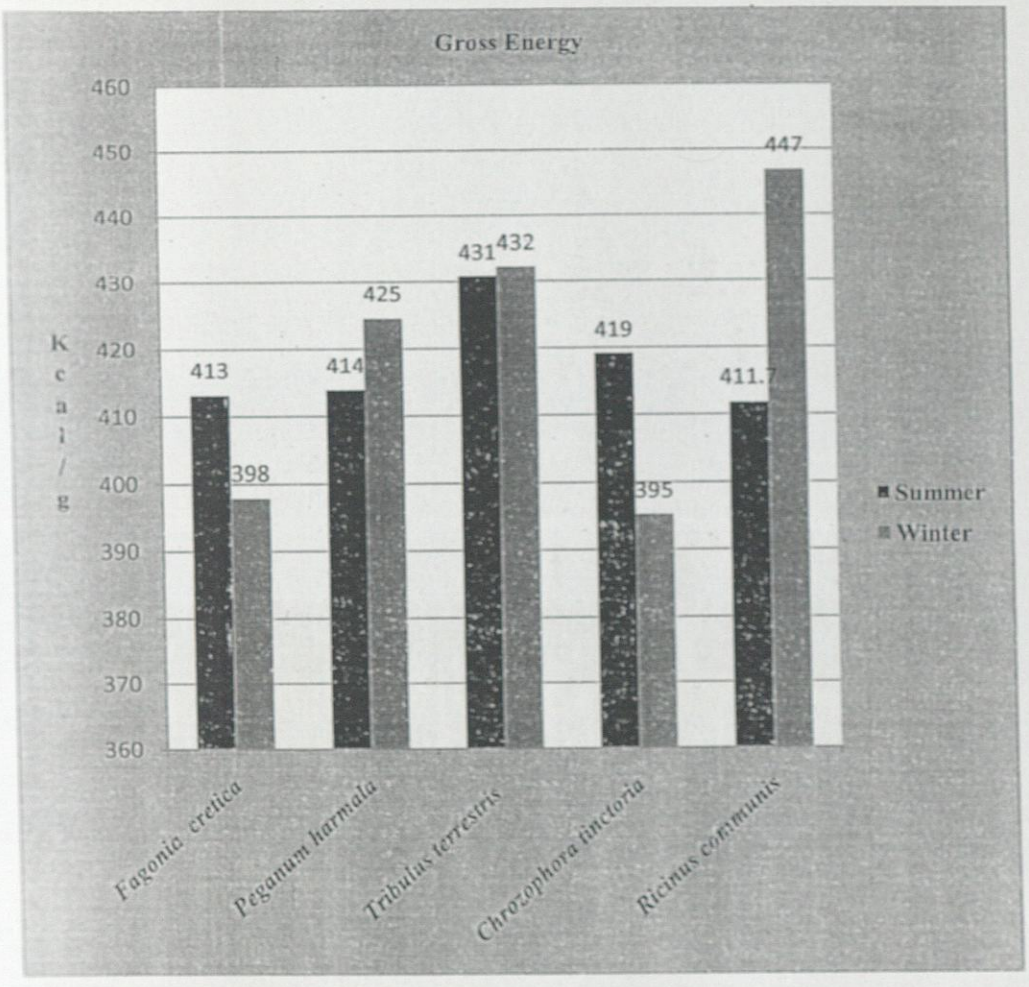



Fig. 110. Seasonal differences in gross energy (Kcal/g) in plants of family Zygophyllaceae and Euphorbiaceae.

# c.Macro and Micronutrients Composition

**c. Macro and micronutrients composition**

**i. Calcium (Ca)**

Calcium is the main constituent of bones, teeth and component of cell membranes and regulates permeability, blood coagulation, neuromuscular transmission and muscle contraction (Yamashita *et al.*, 2005; Sanjay *et al.*, 2010). Clinical symptoms linked with Ca deficiency are paralysis, hypertension, increased cholesterol levels, tooth decay and brittle nails (Chowdhary & Rasool, 2010). The macro and micro nutrients of plant parts roots, stems, leaves and fruit during summer and winter are summarised in (Tables 54-59).

The mean values varied from 116.38ppm (*Fagonia cretica*) to 196.25ppm in (*Tribulus terrestris*) (Table 61) which disagrees with Zafar *et al.* (2010) who reported low (28ppm) Ca contents in the aerial parts of *Fagonia indica*. This plant has been traditionally used in skin diseases, stomach, cough and in some parts to treat diabetes (Hussain *et al.*, 2011). Hussain *et al.* (2009), Adnan *et al.* (2010) and Ashraf *et al.* (2010) also reported similar results in *Hypericum perforatum*, *Allium sativum*, *Zingiber officinalis* and *Valeriana officinalis* and in other medicinal plants of Khyber Paktunkhwa. Khanzada *et al.* (2008) reported high Ca contents in *Calotropis procera* while Chowdhary & Rasool (2010) observed high Ca contents in members of Boraginaceae than Umbelliferae and Leguminosae. The Ca contents of *Onosma bracteatum* leaves were higher than *Cordia latifolia* fruits. *Ferula foetida* resin contained maximum Ca contents followed by *Carum bulbocastanum* and *Foeniculum vulgare*. The roots of *Glycyrrhiza glabra* yielded the maximum while *Tamarindus indica* fruits had the lowest Ca contents. The values of Ca found in present study were significantly higher than those reported in previous studies. The geochemical environment strongly influences the trace element contents of plants, which explain the wide difference of Ca concentrations observed in the previous and present studies. In the present case, Ca contents significantly increased ($P < 0.05$) in winter than summer in *Fagonia cretica*, *Peganum harmala* and *Chrozophora tinctoria* while it significantly decreased in winter in *Tribulus terrestris* and *Ricinus communis* (Fig. 111). Plants and season means were significant (Table 60). These results vary from those of Al- Rumaih *et al.* (2002) who observed the high mineral levels in each part

of *Rumex vesicarius* during spring compared to autumn and winter. ANOVA showed highly significant differences ($P < 0.05$) between two seasons in all studied plants and interaction between seasons and seasons and plant was also significant (Tables 62, 60). However Shad *et al.* (2002) reported extremely less Ca contents in *Fagonia arabica*. Due to high Ca and Mg contents, herbal preparations from these medicinal plants can be used to neutralize the excess of stomach acidity, avoiding lesions in the stomach inner walls. Chowdhary & Rasool (2010) stated that Ca is protective against hypertension, osteoporosis and colon cancer. The Recommended Dietary Allowance (RDA) demands 800 mg of Ca++ as daily dose for adults, while 1200 mg for teenagers and pregnant women. The intake of Ca reduces absorption of Na+ and assists in reducing blood pressure. Its deficiency causes rickets, osteomalacia and osteoporosis. The intake of aqueous extracts could provide about 200 mg of Ca from 100 g of the studied plants. This will certainly mitigate the deficiency of Ca content in the needy individuals.

**ii. Potassium (K)**

The K maintains body water balance, controls nerve functions, blood pressure and influences muscle activities and participates in the maintenance of the cardiac rhythm and in constipation (Singh *et al.*, 2010; Ahmed & Chaudhary, 2009; Yamashita *et al.*, 2005).

The mean value varied from 27.1ppm (*Ricinus communis*) to 27.07ppm (*Tribulus terrestris* and 27.07ppm (*Chrozophora tinctoria*) (Table 61). However, Jabeen *et al.* (2010) reported less K content in *Ricinus communis*, *Convolvulus arvensis* and *Hordeum vulgare*. Ghazanfar *et al.* (2011) and Hussain *et al.* (2011) found less K content in fodder medicinal plants compared to reported value. K contents significantly increased ($P < 0.05$) in winter compared to summer in all the analysed plants (Fig. 112). The present results agree with those of Roca-P´erez *et al.* (2006) who also reported that K contents decreased in summer in *Digitalis obscura* leaves. ANOVA (Table 63) showed highly significant differences ($P < 0.05$) of K contents in all the analysed plants between two seasons and also showed significant differences in seasons and plant interaction (Tables 63, 61). Zafar *et al.* (2010) also reported that K contents were high in the aerial parts of *Fagonia indica* while Shad *et al.* (2002) who found low K contents in *Fagonia arabica*. Individual plant species differ markedly in their elemental composition due to genetic make-up, even under

the same climatic conditions (Chinnasamy *et al.*, 2003). AI- Rumaih *et al.* (2002) reported that K and other elements increased significantly in *Rumex vesicarius* with age of the plant from juvenile throughout to flowering stage which then decreased with further development in the fruiting stage. Deficiencies in K can result in reduced intake, weight loss and stiff joints while high concentration of K leads to the dilation of arteries and normalize the blood pressure.

**iii. Magnesium (Mg)**

Magnesium is an important electrolyte and mineral element responsible for proper nerve and muscle function that also works as co-factor for more than 300 metabolic reactions (Yamashita *et al.*, 2005). It is related to the carbohydrate and fat metabolism. Diabetes mellitus is most frequently associated with Mg deficiency as it plays a role in the release of insulin. It is a component of bones and many enzymes and controls blood pressure, and its deficiency causes neurological disturbances including semi coma, tremor and susceptibility to auditory (Rajendran *et al.*, 2007; Ahmed & Chaudhary, 2009).

The mean value varied from 9.67ppm (*Peganum harmala*) to 11.15ppm (*Tribulus terrestris*) (Table 61). Ashraf *et al.* (2010), Khanzada *et al.* (2008) and Hussain *et al.* (2011) reported poor Mg contents in *Artemesia* spp. and *Calotropis procera* as compared to the present findings. Mg contents were significantly high ($P < 0.05$) in winter than in summer in the case of *Fagonia cretica* and while it significantly decreased in winter in *Tribulus terrestris, Chrozophora tinctoria* and *Ricinus communis* (Fig. 113). Similar to the present results Jabeen *et al.* (2010) also observed poor Mg contents in *Ricinus communis*. The differences in the concentration of the various elements within the different plants is attributed to the preferential absorbability of a particular plant for the corresponding element and the mineral composition of the soil in which the plant grows as well as its surrounding climatological conditions (Rajurkar & Damame, 1998). ANOVA (Table 64) showed highly significant differences ($P < 0.05$) of Mg contents in all analysed plants and it also showed highly significant differences of seasons and plant interaction (Tables 64, 60). Zafar *et al.* (2010) reported low Mg contents in the aerial parts of *Fagonia indica* which differ from the present findings. Mg is the fourth most abundant element in the human body and is essential to good health (WHO, 2009). The Daily Requirement Allowance (RDA) of $Mg^{++}$ has not been fully established, but its Estimated Safe

Intake Requirement falls between 200-300 mg/day. Luckily we can get about 300 mg of Mg++ from 100 g of the studied plants, which can be utilized to play its pharmacological function to sustain a healthy body.

**iv. Sodium (Na)**

Sodium is necessary for regulation of blood volume, blood pressure, body fluids, heart activity, for transmission of nerve and muscular impulses, regulates the acid/base balance in the body (Ahmed & Chaudhary, 2009).

The mean value varied from 3.55ppm (*Fagonia cretica*) to 5.63ppm (*Tribulus terrestris*) (Table 61) which differ from the findings of other workers as (Zafar *et al.*, 2010; Shad *et al.*, 2002; Hussain *et al.*, 2011) who reported low Na (0.015ppm) in the aerial parts of medicinal plants. Jabeen *et al.* (2010) also recorded similar level of Na in *Ricinus communis*, and *Hordeum vulgare*. On the contrary Adnan *et al.* (2010) reported high Na contents in sub-humid regions species compared to humid regions in North-west Pakistan. It has been reported that the stage of growth and environmental conditions are important factors affecting mineral composition of plants. AI- Rumaih *et al.* (2002) reported that growth stage of species significantly effects mineral composition of range grasses and also found that Na contents of *Panicum turgidum* decreased with advance in stage of growth. Na contents significantly increased ($P < 0.05$) in winter than summer in all the analysed plants in the present study (Fig. 114). During summer Na contents differences were significant in *Fagonia cretica*, *Peganum harmala*, *Chrozophora tinctoria* and *Ricinus communis*; Na contents in winter significantly differed in all the tested plants. The plant and season means were significant ($P < 0.05$). There was high significant differences between seasons and plant interaction (Tables 65, 60). These results vary from those of AI- Rumaih *et al.* (2002) who reported that Na increased significantly in *Rumex vesicarius* with advancing age of the plant. Similar to our present study. YinPing *et al.* (2009) also reported seasonal variation of Na content in the leaves of *Sabina przewalskii* and *S. chinensis* and that Na content increased in winter than summer. These results agree with the present investigation. Differences in Na contents observed in the present study could be due to the genotypic differences, stage of maturity, levels of available Na in the soil and soil pH (Khan *et al.*, 2006).

**Micronutrients**

**i. Chromium (Cr)**

The biological effects of the trace elements including Cr in living system strongly depend upon their concentration in human (Bukhsh *et al.*, 2007). Chromium regulates development of lean muscle mass, in diabetes and for weight loss, metabolism of proteins, fats and carbohydrates. Its deficiency leads to fatigue, high cholesterol level, anxiety and slow healing. Thus, it is essential to add chromium to patients fed with insulin intravenously, to avoid the onset of diabetes. There is considerable possibility that supplemental Cr may help to treat impaired glucose tolerance and type- 2 diabetes (Ahmed & Chaudhary, 2009).

Chromium contents were absent in all the analysed plants in summer and winter and it varied from 0.004ppm in (*Peganum harmala*) to 0.185ppm in (*Fagonia cretica*) in summer. *Ricinus communis* had 0.114 ppm in winter. (Table 58) Many workers (Hussain *et al.*, 2011; Ashraf *et al.*, 2010; Adnan *et al.*, 2010; Hussain *et al.*, 2010 reported high Cr contents in *Nepeta suavis, Calotropis procera, Aerva javanica, Artemesia* species and many other medicinal plants of humid and sub-humid regions of Pakistan as compared to the analysed plants. It has been reported that Cr at 5ppm proved to be toxic. In this regard, present reported low Cr contents both in summer and winter seasons. Chronic exposure to Cr may result in liver, kidney and lung damage. The permissible limit set by FAO/WHO(1984) in edible plants was 0.02 ppm. However, for medicinal plants the WHO (2005) limits have not yet been established for Cr. Vegetables/fruits provide very little Cr and its absorption in intestinal tract is low. The body's Cr content may be reduced due to pregnancy, lactation and stressful states etc.

**ii. Copper (Cu)**

Copper is contained in human and animal ceruloplasmin and is cofactor of many enzymes. An excess of Cu causes chronic inflammatory processes (myocardial infarction, bronchial asthma, and leads to Zn and Mn deficiency (Sokolova *et al.*, 2008). Copper regulates cardiovascular system, immune functions, anemia, brings infirmity of bones, haemoglobin formation, and iron absoption (Ahmed & Chaudhary, 2009; Singh *et al.*, 2010; Shirin *et al.*, 2008).

The mean value varied from 0.105ppm (*Fagonia cretica*) to 0.09ppm (*Chrozophora tinctoria*) (Table 61) which are higher than those reported by Zafar *et al.* (2010) which is 0.00018ppm in *Fagonia indica*. Similar results were also reported by Ashraf *et al.* (2010) in *Artemesia japonica* and *A. persica*. Cu contents increased insignificantly in winter than summer in all the tested plants. Chinnasamy *et al.* (2003) reported that Cu showed significant variation among nodule tissues of *Lathyrus maritimus* in summer and winter. According to Khan *et al.* (2006) $Cu^{++}$ interacts strongly with trace minerals and macro minerals for absorption by the plants. Calcium in the form of carbonate precipitates $Cu^{++}$, making it unavailable for the plants.

The plants and seasons means were significant (Table 71). ANOVA showed highly significant differences ($P < 0.05$) between plants (Fig. 115) and between seasons but plant interactions were insignificant (Tables 66, 60). The present results disagree with Shad *et al.* (2002) who reported low Cu contents in *Fagonia arabica*. Similarly, Hashem & Alfarhan (1993) also reported low Cu contents in four desert plant species including *Peganum harmala* and that Cu contents not only varied between root and shoots *Peganum harmala* but also from different localities of Saudi Arabia.

**iii. Iron (Fe)**

Iron is a component of many enzymes, hemoglobin and myoglobin and maintains a healthy immune system and digestive action (Ahmed & Chaudhary, 2009). Its deficiency causes fatigue, impairs immune functions and anemia. Iron deficiency anemia is probably the most common nutritional disease in the world, affecting at least five hundred million people. Many foods and medicinal plants contain iron and using a wide range of foods and medicinal plants can help most people meet their needs for this important element. However excessive intake of Fe may cause tissue damage, especially in liver. Pharmacological doses of Fe adversely affect Zn utilization (Rajendran *et al.*, 2007).

The mean value varied from 2.7ppm (*Peganum harmala*) to 4.7ppm (*Ricinus communis*) (Table 61). Hussain *et al.* (2011) and Shad *et al.* (2002) reported lower Fe contents in *Nepeta suavis, Calotropis procera, Aerva javanica, Fagonia arabica* and selected medicinal plants from Pakistan compared to the present findings. Likewise,

Hussain *et al.* (2010) also found low Fe contents in *Sonchus eruca, Melia azadirachta*, *Withania coagulans* and *Fagonia indica* with statistically significant variation among different metals. *Melia azadirachta* had the highest Fe contents than other plants.

Iron contents decreased in winter in *Fagonia cretica* and *Tribulus terrestris* but increased in *Peganum harmala* while Fe contents increased in winter in *Chrozophora tinctoria* and decreased in winter in *Ricinus communis* (Fig. 116). The results were statistically insignificant. Hussain & Khan (2010) also reported insignificant variations in Fe contents in *Eclipta alba*. ANOVA showed no significant differences between seasons, plants and plants and seasons interactions (Tables 67, 60). Zafar *et al.* (2010) reported low Fe in *Fagonia indica* which differ from the present case. These results are in line with AI- Rumaih *et al.* (2002).

**iv. Manganese (Mn)**

Manganese is needed in digestion, immune system, regulates blood sugar levels, production of energy, cell reproduction and bone growth. It works with vitamin K to support blood clotting, with B-complex vitamins to control the stress and also reduces nervous irritability. It has antioxidant property and might help in aging. Its deficiency might cause skin damage, hypercholesterolemia, fertility, birth defects and cancer (Shirin *et al.*, 2008; Djama *et al.*, 2011).

The mean values varied from 0.2ppm for (*Fagonia cretica, Peganum harmala* and *Chrozophora tinctoria*) to 0.3ppm (*Ricinus communis*) (Table 61). Unlike the present study Zafar *et al.* (2010) and Gul & Safdar (2009) reported high Mn contents (32.2) ppm in the aerial parts of *Fagonia indica* and in cinnamon. On the contrary, Hussain *et al.* (2011) and Shad *et al.* (2002) recorded extremely low Mn contents in *Nepeta suavis, Calotropis procera*, *Aerva javanica* and *Fagonia arabica* while Ashraf *et al.* (2010) and Jabeen *et al.* (2010) recorded poor Mn contents in *Artemesia* spp., *Justacia adhatoda* and *Achyranthes asper*. The present findings disagree with Roca-Perez (2006) who observed that Mn contents in *Digitalis obscura* showed no substantial seasonal changes. *Peganum harmala* had 0.2 ppm Mn content that differed from Hashem & Alfarhan (1993) who reported low Mn contents in *Peganum harmala*. Mn contents did not vary between root and shoots of the *Peganum harmala* but varied in *Peganum harmala* collected from different localities in Saudi Arabia.

The permissible limit set by FAO/WHO (1984) for Mn in edible plants is 2 ppm which suggests that all plants had Mn contents below this limit. However, WHO (2005) limits for medicinal plants has not yet been established. Mn contents increased in winter than summer in all the plants (Fig. 117) without any statistical significance. The differences between plant means were insignificant while those between the seaons means were significant ($P < 0.05$) (Table 60). ANOVA showed that seasons were significant ($P < 0.05$) while plants and seasons and plant interaction were not significant (Tables 68, 60).

**v. Molybdenum (Mo)**

Molybdenum is necessary in extremely small amounts for cells growth, promoting healthy teeth and reducing the risk of asthma attacks, improving mouth, gum disorders, cancer, anemia, loss of appetite, loss of weight and stunted growth in animals. The amount of Mo in foods varies significantly which is dependent upon the mineral contents of the soil and plants grown in it. Molybdenum is relatively safe for humans and animals. The mean value varied from 0.0075ppm (*Fagonia cretica*) to 0.09ppm (*Peganum harmala* and *Tribulus terrestris*) (Table 61). The Mo contents were less in winter in *Fagonia cretica*, *Tribulus terrestris* while it increased in *Peganum harmala*, *Chrozophora tinctoria* and *Ricinus communis* during winter (Fig. 118). All plants means showed that they were significantly different from each other (Table 60). ANOVA (Table 69) showed that plants were significant ($P < 0.05$) while seasons and plants seasons interaction were insignificant (Tables 69, 60). Shamat *et al.* (2009) reported that seasonal differences were insignificant ($P < 0.05$) in tested plants. Reddy *et al.* (1981) reported seasonal changes in Mo contents in *Trifolium subterraneum, Vulpia* sp., *Lolium rigidum,* and *Arctotheca calendula* growing on lateritic podzolic soils. The Mo content tended to remain constant throughout the season, *T. subterraneum* had lower Mo than *Vulpia* sp while *A. calendula* had more Mo. The concentration of Mo was similar in *A. calendula* and the grasses. Kostova *et al.* (2008) reported Mo contents in selected vegetables of family Fabaceae, Malvaceae, Brassicaceae, and Cruciferae and found variation of Mo contents in every species of Brassicaceae. The differences between the mean values were statistically proven. This agrees with the present investigation.

**vi. Zinc (Zn)**

Zinc has an essential role in metabolism, diabetes, antioxidant capacity and has low toxicity compared with other trace elements (Rajendran *et al.*, 2007). Zn promotes growth and sexual maturity (Yamashita *et al.*, 2005). It improves appetite, immune functions, healing of wounds, coronary artery disease and ulcer problems (Ahmed & Chaudhary, 2009; Singh *et al.*, 2010; Yamashita *et al.*, 2005). Chronic Zn deficiency causes dwarfism and hypogonadism (Shirin, 2008; Djama *et al.*, 2011).

This study shows that the mean value varied from 0.02ppm (*Ricinus communis*) to 1.77ppm (*Fagonia cretica*) (Table 61). Jabeen *et al.* (2010), Zafar *et al.* (2010) and Shad *et al.* (2002) recorded poor Zn contents in *Ricinus communis*, *Convolvulus arvensis*, *Hordeum vulgare* and *Fagonia indica.* Hussain *et al.* (2009) found similar results from fourteen medicinal plants. Bukhsh *et al.* (2007) reported significantly high Zn contents in seeds and leaves of *Carthamus oxycantha* and *Plantago ovata*. In the present study Zn contents were poor in winter than summer in *Fagonia cretica*, *Peganum harmala* and *Tribulus terrestris,* and Zn increased in *Chrozophora tinctoria* and *Ricinus communis* (Fig. 119). On the other hand ANOVA (Table 70) showed results were statistically insignificant for plants and seasonal (Tables 70, 60). The present findings agree with Roca-Perez (2006) who reported that Zn contents in the leaves of *Digitalis obscura* did not show a clear seasonal trend. Hussain & Durrani (2008) reported significant differences in the Zn contents of herbaceous plants of Balochistan. The considerable amount of Zn was present in all the studied plants and highest in *Fagonia cretica* in the current study which may be directly or indirectly helpful in the management of diseases.

**vii. Aluminum (Al)**

Aluminum is required in the action of enzymes such a succinic dehydrogenase and d-aminolevulinate dehydrase (involved in porphyrin synthesis).

The mean value (Table 61) varied from 0.25ppm (*Peganum harmala*) to 3.74 (*Chrozophora tinctoria*). Higher Al contents in *Polygonum plebejum*, *Alstonia scholaris* and *Viola odorata* compared to the present case were recorded by Hameed & Dastagir (2004), Dastagir & Pervaiz (2004) and Bibi *et al.* (2006). Similar results were also reported by Khan *et al.* (2006) in *Paeonia emodi* and *Aconitm laeve.* Al contents were less in winter in *Fagonia cretica*, *Peganum harmala* and *Ricinus*

*communis* which increased in *Tribulus terrestris* and *Chrozophora tinctoria* winter (Fig. 120). The summer data for *Ricinus communis* showed that Al contents were significantly different than other four plants, while the other plants among themselves were insignificant. In winter, *Tribulus terrestris*, *Chrozophora tinctoria* and *Ricinus communis* showed that the Al contents were significantly varied among themselves and these plants were significantly different than *Fagonia cretica* and *Peganum harmala* (Fig. 120). Chinnasamy *et al.* (2003) reported that Al showed significant variation among nodule tissues of *Lathyrus maritimus* in summer and winter seasons. The plants and seasons means were significantly different from each other (Table 60). ANOVA showed significant differences ($P < 0.05$) of Al content in all plants in both the seasons, and it also showed highly significant seasons and plant interaction (Tables 71, 60).

**Trend of accumulation of macro and micronutrients**

Trend of accumulation of macro nutrients in plants of family Zygophyllaceae and Euphorbiaceae remained same and showed that Ca > K > Mg > Na while the trend of accumulation of micro nutrients in the same plants of family Zygophyllaceae and Euphorbiaceae showed variation as given below :

Fe > Zn > Mn > Al > Cu > Mo > Cr in *Fagonia cretica* , Fe > Zn and Mn > Mo > Cu > Al > Cr in *Peganum harmala*, Fe > Al > Zn > Mn > Cu > Mo > Cr in *Tribulus terrestris,* Al > Fe > Mn > Zn > Cu > Mo > Cr in *Chrozophora tinctoria*, Fe > Al > Mn > Zn > Cu > Mo > Cr in *Ricinus communis*. Mean values obtained from summer and winter seasons were considered in the trend of accumulation of nutrients (Table 61). The differences in the concentration of the various elements within the different plants is attributed to the preferential absorbability of a particular plant for the corresponding element and the mineral composition of the soil in which the plant grows as well as its surrounding climatological conditions (Rajurkar & Damame, 1998).

Table 54. Macro-nutrients status of plants of some family Zygophyllaceae and Euphorbiaceae during summer.

| Plants | Parts | Ca (ppm) | | | K (ppm) | | | Mg (ppm) | | | Na (ppm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M | SD | % RSD | M | S | % RSD | M | SD | % RSD | M | SD | % RSD |
| **Zygophyllaceae** | | | | | | | | | | | | | |
| *Fagonia cretica* L. | Root | 24.46 | 0.334 | 1.36 | 27.02 | 0.001 | 0.00 | 9.263 | 0.0882 | 0.95 | 1.969 | 0.0048 | 0.24 |
| | Stem | 25.50 | 0.298 | 1.17 | 26.96 | 0.000 | 0.00 | 8.777 | 0.0590 | 0.67 | 1.787 | 0.0041 | 0.23 |
| | Leaf | 29.99 | 0.245 | 0.82 | 27.03 | 0.002 | 0.01 | 9.243 | 0.0316 | 0.34 | 1.618 | 0.0124 | 0.76 |
| | Fruit | 29.25 | 0.150 | 0.51 | 26.99 | 0.000 | 0.00 | 9.527 | 0.0524 | 0.55 | 1.445 | 0.0019 | 0.13 |
| | **Mean** | **27.36** | | | **27.0** | | | **9.20** | | | **1.70** | | |
| *Peganum harmala* L. | Root | 31.14 | 0.219 | 0.70 | 26.89 | 0.003 | 0.01 | 9.181 | 0.0709 | 0.77 | 2.156 | 0.0211 | 0.98 |
| | Stem | 29.85 | 0.054 | 0.18 | 26.86 | 0.000 | 0.00 | 9.450 | 0.1687 | 1.79 | 2.293 | 0.0220 | 0.96 |
| | Leaf | 35.24 | 0.123 | 0.35 | 27.00 | 0.001 | 0.00 | 9.251 | 0.0554 | 0.60 | 1.827 | 0.0150 | 0.82 |
| | Fruit | 23.50 | 0.055 | 0.23 | 26.92 | 0.001 | 0.00 | 8.665 | 0.1213 | 1.40 | 1.969 | 0.0034 | 0.17 |
| *Tribulus terrestris* L. | **Mean** | **29.9** | | | **27.0** | | | **9.13** | | | **1.81** | | |
| | Root | 202.0 | 12.81 | 6.34 | 26.90 | 0.001 | 0.00 | 12.48 | 0.100 | 0.80 | 4.463 | 0.0165 | 0.37 |
| | Stem | 252.7 | 3.95 | 1.56 | 26.90 | 0.001 | 0.00 | 13.08 | 0.113 | 0.86 | 6.444 | 1.6670 | 25.87 |
| | Leaf | 240.3 | 7.22 | 3.00 | 26.89 | 0.005 | 0.02 | 12.62 | 0.195 | 1.55 | 5.716 | 0.0309 | 0.54 |
| | Fruit | 252.4 | 6.41 | 2.54 | 26.91 | 0.001 | 0.00 | 11.16 | 0.074 | 0.67 | 2.254 | 0.0501 | 2.22 |
| | **Mean** | **236.9** | | | **27.0** | | | **12.33** | | | **4.71** | | |
| **Euphorbiaceae** | | | | | | | | | | | | | |
| *Chrozophora tinctoria*(L.) Raf | Root | 251.5 | 9.41 | 3.74 | 26.97 | 0.001 | 0.00 | 10.91 | 0.052 | 0.48 | 1.994 | 0.0165 | 0.83 |
| | Stem | 250.7 | 10.99 | 10.99 | 26.96 | 0.001 | 0.00 | 11.29 | 0.166 | 1.47 | 1.952 | 0.0065 | 0.33 |
| | Leaf | 91.88 | 0.700 | 0.76 | 27.07 | 0.002 | 0.00 | 9.083 | 0.0636 | 0.70 | 1.555 | 0.0108 | 0.69 |
| | Fruit | 82.06 | 0.588 | 0.72 | 27.10 | 0.002 | 0.01 | 8.243 | 0.0446 | 0.54 | 1.568 | 0.0063 | 0.40 |
| | **Mean** | **169.0** | | | **27.0** | | | **9.9** | | | **1.76** | | |
| *Ricinus communis* L. | Root | 144.2 | 0.99 | 0.69 | 27.01 | 0.003 | 0.01 | 10.42 | 0.086 | 0.83 | 3.713 | 2.6333 | 70.92 |
| | Stem | 147.6 | 23.39 | 15.84 | 27.08 | 0.001 | 0.00 | 10.00 | 0.088 | 0.88 | 2.235 | 0.0100 | 0.45 |
| | Leaf | 236.9 | 6.70 | 2.83 | 27.10 | 0.000 | 0.00 | 9.992 | 0.2226 | 2.23 | 2.235 | 0.0100 | 0.41 |
| | Fruit | 51.50 | 0.218 | 0.42 | 27.11 | 0.001 | 0.00 | 10.26 | 0.035 | 0.34 | 2.837 | 0.0087 | 0.31 |
| | **Mean** | **145.0** | | | **27.0** | | | **10.16** | | | **2.75** | | |

Table 33 Cr, Cu, Fe and Mn concentration of some plants of family Zygophyllaceae and Euphorbiaceae during summer

| Plants | Parts | Cr (ppm) | | | Cu (ppm) | | | Fe (ppm) | | | Mn (ppm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Zygophyllaceae** | | M | SD | %RSD | M | SD | %RSD | M | SD | %RSD | M | SD | %RSD |
| *Fagonia cretica* L. | Root | 0.0 | 0.0301 | 296.5 | 0.064 | 0.0009 | 1.36 | 2.197 | 0.0236 | 1.07 | 0.192 | 0.0029 | 1.53 |
| | Stem | 0.0 | 0.0137 | 6.27 | 0.030 | 0.0009 | 2.89 | 1.965 | 0.0072 | 0.37 | 0.100 | 0.0014 | 1.38 |
| | Leaf | 0.269 | 0.0079 | 2.94 | 0.039 | 0.0009 | 2.18 | 11.30 | 0.133 | 1.17 | 0.184 | 0.0045 | 2.45 |
| | Fruit | 0.101 | 0.0179 | 17.77 | 0.033 | 0.0012 | 3.58 | 2.165 | 0.0051 | 0.24 | 0.102 | 0.0033 | 3.25 |
| | **Mean** | **0.185** | | | **0.04** | | | **4.40** | | | **0.15** | | |
| *Peganum harmala* L. | Root | 0.0 | 0.0081 | 18.33 | 0.038 | 0.0012 | 3.05 | 2.156 | 0.0211 | 0.98 | 0.098 | 0.0069 | 7.04 |
| | Stem | 0.0 | 0.0188 | 24.56 | 0.047 | 0.0014 | 2.97 | 2.293 | 0.0220 | 0.96 | 0.249 | 0.0020 | 0.82 |
| | Leaf | 0.0 | 0.0127 | 8.99 | 0.034 | 0.0012 | 3.46 | 1.827 | 0.0150 | 0.82 | 0.098 | 0.0019 | 1.91 |
| | Fruit | 0.0 | 0.0088 | 2.50 | 0.037 | 0.0020 | 5.55 | 1.969 | 0.0034 | 0.17 | 0.094 | 0.0020 | 2.16 |
| | **Mean** | **0.0** | | | **0.04** | | | **2.06** | | | **0.14** | | |
| *Tribulus terrestris* L. | Root | 0.0 | 0.0213 | 0.0213 | 0.067 | 0.0039 | 5.77 | 2.503 | 0.0020 | 0.08 | 0.099 | 0.0012 | 1.24 |
| | Stem | 0.0 | 0.0021 | 0.74 | 0.079 | 0.0035 | 4.44 | 1.893 | 0.0428 | 2.26 | 0.141 | 0.0018 | 1.30 |
| | Leaf | 0.0 | 0.0296 | 8.03 | 0.069 | 0.0007 | 0.97 | 5.408 | 0.0094 | 0.17 | 0.148 | 0.0048 | 3.27 |
| | Fruit | 0.0 | 0.0031 | 0.61 | 0.058 | 0.0016 | 2.72 | 5.444 | 0.0199 | 0.37 | 0.361 | 0.0020 | 0.57 |
| | **Mean** | **0.0** | | | **0.06** | | | **3.81** | | | **0.14** | | |
| **Euphorbiaceae** | | | | | | | | | | | | | |
| *Chrozophora tinctoria* (L.) Raf | Root | 0.0 | 0.0039 | 0.59 | 0.059 | 0.0022 | 3.78 | 3.549 | 0.0106 | 0.30 | 0.265 | 0.0037 | 1.40 |
| | Stem | 0.0 | 0.0047 | 0.76 | 0.073 | 0.0036 | 4.90 | 3.852 | 0.0094 | 0.24 | 0.432 | 0.0038 | 0.88 |
| | Leaf | 0.0 | 0.0241 | 3.32 | 0.060 | 0.0023 | 3.79 | 2.989 | 0.0258 | 0.86 | 0.130 | 0.0017 | 1.27 |
| | Fruit | 0.0 | 0.0058 | 0.64 | 0.031 | 0.0023 | 7.54 | 1.819 | 0.0096 | 0.53 | 0.122 | 0.0014 | 1.17 |
| | **Mean** | **0.0** | | | **0.05** | | | **3.05** | | | **0.24** | | |
| *Ricinus Communis* L. | Root | 0.135 | 0.0195 | 14.46 | 0.074 | 0.0013 | 1.72 | 6.339 | 0.214 | 2.06 | 0.247 | 0.0039 | 1.59 |
| | Stem | 0.135 | 0.0106 | 7.80 | 0.055 | 0.0017 | 3.06 | 2.148 | 0.0113 | 0.16 | 0.170 | 0.0031 | 1.85 |
| | Leaf | 0.0 | 0.0257 | 252.22 | 0.050 | 0.0059 | 11.74 | 2.735 | 0.0113 | 0.45 | 0.216 | 0.0066 | 3.05 |
| | Fruit | 0.006 | 0.0235 | 367.42 | 0.076 | 0.0012 | 1.64 | 10.41 | 0.044 | 0.72 | 0.233 | 0.0029 | 1.25 |
| | **Mean** | **0.092** | | | **0.076** | | | **5.4** | | | **0.22** | | |

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Table 56. Mo, Zn and Al status of plants of family Zygophyllaceae and Euphorbiaceae during summer.

| Plants | Parts | Mo (ppm) | | | Zn (ppm) | | | Al (ppm) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Zygophyllaceae** | | M | SD | %RSD | M | SD | %RSD | M | SD | %RSD |
| *Fagonia cretica* L. | Root | 0.014 | 0.0051 | 36.38 | 9.590 | 0.0815 | 0.85 | 0.634 | 0.0713 | 11.25 |
| | Stem | 0.004 | 0.0237 | 630.55 | 0.0 | 0.0021 | 3.18 | 0.361 | 0.1195 | 33.06 |
| | Leaf | 0.002 | 0.0141 | 647.49 | 0.385 | 0.0008 | 0.20 | 0.802 | 0.1162 | 14.49 |
| | Fruit | 0.011 | 0.0302 | 279.04 | 0.297 | 0.0015 | 0.52 | 0.0 | 0.0880 | 27.86 |
| | **Mean** | **0.0075** | | | **3.4** | | | **0.6** | | |
| *Peganum harmala* L. | Root | 0.012 | 0.0336 | 288.75 | 0.221 | 0.0018 | 0.81 | 0.0 | 0.0349 | 6.64 |
| | Stem | 0.032 | 0.0115 | 35.74 | 0.231 | 0.0030 | 1.31 | 0.097 | 0.0538 | 55.30 |
| | Leaf | 0.063 | 0.0166 | 26.30 | 0.255 | 0.0041 | 1.59 | 0.034 | 0.1229 | 363.98 |
| | Fruit | 0.111 | 0.0011 | 1.01 | 0.192 | 0.0010 | 0.54 | 0.080 | 0.1981 | 246.77 |
| | **Mean** | **0.05** | | | **0.22** | | | **0.07** | | |
| *Tribulus terrestris* L. | Root | 0.165 | 0.0293 | 17.76 | 0.313 | 0.0022 | 0.72 | 0.0 | 0.0718 | 33.63 |
| | Stem | 0.0 | 0.0286 | 133.43 | 0.316 | 0.0044 | 1.38 | 0.826 | 0.1236 | 14.97 |
| | Leaf | 0.0 | 0.0172 | 23.17 | 0.371 | 0.0007 | 0.18 | 0.527 | 0.1041 | 19.76 |
| | Fruit | 0.0 | 0.0170 | 37.03 | 0.240 | 0.0027 | 1.11 | 0.384 | 0.0252 | 6.56 |
| | **Mean** | **0.165** | | | **0.31** | | | **0.6** | | |
| **Euphorbiaceae** | | | | | | | | | | |
| *Chrozophora tinctoria*(L.) Raf | Root | 0.00 | 0.0118 | 35.08 | 0.211 | 0.0022 | 1.03 | 0.0 | 0.1198 | 5.354.5 |
| | Stem | 0.00 | 0.0149 | 77.71 | 0.232 | 0.0016 | 0.71 | 0.0 | 0.0867 | 94.12 |
| | Leaf | 0.01 | 0.0048 | 12.32 | 0.232 | 0.0021 | 0.91 | 0.0 | 0.0679 | 63.05 |
| | Fruit | 0.00 | 0.0161 | 31.57. | 0.231 | 0.0019 | 0.81 | 0.9 | 0.0580 | 6.78 |
| | **Mean** | **0.00** | | | **0.22** | | | **0.9** | | |
| *Ricinus Communis* L. | Root | 0.00 | 0.0101 | 18.97 | 0.225 | 0.0009 | 0.41 | 10.10 | 2.117 | 20.96 |
| | Stem | 0.00 | 0.0030 | 4.34 | 0.159 | 0.0020 | 1.29 | 0.0 | 0.2818 | 64.56 |
| | Leaf | 0.01 | 0.0155 | 21.96 | 0.170 | 0.0025 | 1.44 | 0.0 | 0.0782 | 10.23 |
| | Fruit | 0.00 | 0.0164 | 19.85 | 0.180 | 0.0028 | 1.57 | 2.847 | 0.2391 | 8.40 |
| | **Mean** | **0.00** | | | **0.18** | | | **6.5** | | |

Key : M=Mean; SD=Standard Deviation; %RSD= Relative Standard Deviation

Table 37. Macro-nutrients of some plants of family Zygophyllaceae and Euphorbiaceae during winter.

| Plants | Parts | Ca (ppm) | | | K (ppm) | | | Mg (ppm) | | | Na (ppm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Zygophyllaceae** | | M | SD | %RSD | M | SD | %RSD | M | SD | %RSD | M | SD | %RSD |
| *Fagonia cretica* L. | Root | 100.8 | 0.33 | 0.33 | 27.08 | 0.001 | 0.00 | 10.37 | 0.127 | 1.23 | 6.764 | 0.0599 | 0.89 |
| | Stem | 234.2 | 7.31 | 3.12 | 27.12 | 0.001 | 0.01 | 11.04 | 0.205 | 1.86 | 4.423 | 0.0083 | 0.19 |
| | Leaf | 230.6 | 4.90 | 2.12 | 27.07 | 0.003 | 0.01 | 10.62 | 0.059 | 0.56 | 5.254 | 0.0137 | 0.26 |
| | Fruit | 255.9 | 1.88 | 0.74 | 27.06 | 0.003 | 0.01 | 11.12 | 0.031 | 0.28 | 5.210 | 0.0290 | 0.56 |
| | **Mean** | **205.4** | | | **27.08** | | | **10.8** | | | **5.41** | | |
| *Peganum harmala* L. | Root | 261.0 | 5.01 | 1.92 | 27.12 | 0.001 | 0.00 | 9.677 | 0.0951 | 0.98 | 6.132 | 0.2378 | 3.88 |
| | Stem | 254.6 | 5.94 | 2.33 | 27.07 | 0.000 | 0.00 | 10.19 | 0.104 | 1.02 | 6.229 | 0.0361 | 0.58 |
| | Leaf | 163.7 | 12.85 | 7.85 | 27.09 | 0.001 | 0.00 | 10.44 | 0.068 | 0.65 | 6.671 | 1.3255 | 19.87 |
| | Fruit | 164.4 | 32.02 | 19.48 | 27.16 | 0.000 | 0.01 | 10.57 | 0.086 | 0.81 | 6.163 | 0.0354 | 0.57 |
| *Tribulus terrestris* L. | **Mean** | **210.0** | | | **27.11** | | | **10.21** | | | | | |
| | Root | 251.1 | 8.18 | 3.26 | 27.21 | 0.000 | 0.01 | 9.973 | 0.1370 | 1.37 | 7.422 | 2.1833 | 29.42 |
| | Stem | 68.15 | 0.584 | 0.86 | 27.09 | 0.000 | 0.01 | 10.18 | 0.013 | 0.13 | 6.621 | 0.050 | 0.76 |
| | Leaf | 105.3 | 1.36 | 1.29 | 27.11 | 0.000 | 0.00 | 9.895 | 0.1042 | 1.05 | 5.394 | 0.0255 | 0.47 |
| | Fruit | 197.8 | 5.55 | 2.81 | 27.17 | 0.000 | 0.00 | 9.800 | 0.1147 | 1.17 | 6.792 | 1.2553 | 18.48 |
| | **Mean** | **155.6** | | | **27.15** | | | **9.96** | | | **6.55** | | |
| **Euphorbiaceae** | | | | | | | | | | | | | |
| *Chrozophora tinctoria* (L.) Raf | Root | 91.27 | 0.617 | 0.68 | 27.15 | 0.001 | 0.00 | 9.780 | 0.0987 | 1.01 | 8.740 | 0.1823 | 2.09 |
| | Stem | 201.7 | 15.08 | 7.47 | 27.17 | 0.001 | 0.01 | 9.463 | 0.1177 | 1.24 | 8.375 | 0.1560 | 1.86 |
| | Leaf | 221.8 | 3.23 | 1.45 | 27.14 | 0.001 | 0.01 | 9.101 | 0.0410 | 0.45 | 9.255 | 0.9327 | 10.08 |
| | Fruit | 251.6 | 8.42 | 3.34 | 27.09 | 0.000 | 0.01 | 10.90 | 0.248 | 2.28 | 8.148 | 0.0282 | 0.35 |
| | **Mean** | **191.6** | | | **27.14** | | | **9.81** | | | **8.63** | | |
| *Ricinus communis* L. | Root | 65.85 | 0.309 | 3.26 | 27.25 | 0.000 | 0.00 | 8.395 | 0.0887 | 1.06 | 7.913 | 1.9739 | 24.95 |
| | Stem | 48.48 | 0.259 | 2.94 | 27.11 | 0.001 | 0.00 | 9.315 | 0.1393 | 1.50 | 7.163 | 0.0709 | 0.99 |
| | Leaf | 88.72 | 0.617 | 0.24 | 27.15 | 0.001 | 0.00 | 9.790 | 0.0934 | 0.95 | 9.061 | 1.5972 | 17.63 |
| | Fruit | 151.5 | 9.05 | 0.28 | 27.21 | 0.000 | 0.00 | 9.903 | 0.1021 | 1.03 | 6.287 | 0.0136 | 0.22 |
| | **Mean** | **88.6** | | | **27.2** | | | **9.35** | | | **7.6** | | |

Table 58: Cr, Cu, Fe and Mn contents of some plants of family Zygophyllaceae and Euphorbiaceae during winter

| Plants | Parts used | Cr (ppm) | | | Cu (ppm) | | | Fe (ppm) | | | Mn (ppm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Zygophyllaceae** | | M | SD | %RSD | M | SD | %RSD | M | SD | %RSD | M | SD | %RSD |
| *Fagonia cretica* L. | Root | 0.0 | 0.0171 | 2.46 | 0.060 | 0.0040 | 6.62 | 4.634 | 0.0083 | 0.18 | 0.247 | 0.0015 | 0.59 |
| | Stem | 0.0 | 0.0097 | 1.19 | 0.045 | 0.0017 | 3.70 | 2.520 | 0.0115 | 0.46 | 0.367 | 0.0014 | 0.38 |
| | Leaf | 0.0 | 0.0186 | 2.27 | 0.050 | 0.0030 | 5.95 | 2.824 | 0.0252 | 0.89 | 0.297 | 0.0039 | 1.33 |
| | Fruit | 0.0 | 0.0083 | 0.87 | 0.087 | 0.0005 | 0.61 | 3.845 | 0.0181 | 0.47 | 0.264 | 0.0052 | 1.98 |
| | **Mean** | **0.0** | | | **0.17** | | | **3.45** | | | **0.29** | | |
| *Peganum harmala* L. | Root | 0.0 | 0.0095 | 1.03 | 0.086 | 0.0030 | 3.49 | 4.799 | 0.0233 | 0.49 | 0.198 | 0.0033 | 1.68 |
| | Stem | 0.0 | 0.0212 | 2.17 | 0.064 | 0.0030 | 4.62 | 2.384 | 0.0042 | 0.18 | 0.247 | 0.0022 | 0.89 |
| | Leaf | 0.0 | 0.0210 | 2.02 | 0.082 | 0.0027 | 3.36 | 3.497 | 0.0011 | 0.03 | 0.231 | 0.0010 | 0.42 |
| | Fruit | **0.0** | 0.0056 | 0.52 | 0.070 | 0.0013 | 1.83 | 2.813 | 0.0183 | 0.65 | 0.179 | 0.0044 | 2.43 |
| | **Mean** | **0.0** | | | **0.07** | | | **3.37** | | | **0.21** | | |
| *Tribulus terrestris* L. | Root | 0.0 | 0.0128 | 1.13 | 0.065 | 0.0006 | 0.96 | 6.848 | 0.0218 | 0.32 | 0.232 | 0.0030 | 1.30 |
| | Stem | 0.0 | 0.0170 | 1.47 | 0.097 | 0.0026 | 2.68 | 3.308 | 0.0119 | 0.36 | 0.213 | 0.0017 | 0.78 |
| | Leaf | 0.0 | 0.0072 | 0.58 | 0.067 | 0.0008 | 1.14 | 1.859 | 0.0249 | 1.34 | 0.163 | 0.0027 | 1.65 |
| | Fruit | 0.0 | 0.0036 | 0.29 | 0.068 | 0.0019 | 2.76 | 3.108 | 0.0101 | 0.32 | 0.222 | 0.0058 | 2.60 |
| | **Mean** | **0.0** | | | **0.07** | | | **3.78** | | | **0.2** | | |
| **Euphorbiaceae** | | | | | | | | | | | | | |
| *Chrozophora tinctoria*(L.) Raf | Root | 0.0 | 0.0091 | 0.71 | 0.100 | 0.0019 | 1.87 | 4.866 | 0.0243 | 0.50 | 0.226 | 0.0088 | 3.90 |
| | Stem | 0.0 | 0.0004 | 0.03 | 0.085 | 0.0038 | 4.55 | 2.643 | 0.0124 | 0.47 | 0.177 | 0.0023 | 1.28 |
| | Leaf | 0.0 | 0.0158 | 1.15 | 0.082 | 0.0024 | 2.94 | 4.167 | 0.0100 | 0.24 | 0.196 | 0.0054 | 2.78 |
| | Fruit | 0.0 | 0.0125 | 0.93 | 0.109 | 0.0025 | 2.33 | 6.027 | 0.0215 | 0.36 | 0.411 | 0.0004 | 0.10 |
| | **Mean** | **0.0** | | | **0.09** | | | **4.425** | | | **0.25** | | |
| *Ricinus communis* L. | Root | 0.114 | 0.0386 | 1.10 | 0.061 | 0.0037 | 5.97 | 2.716 | 0.0109 | 0.40 | 0.762 | 0.0089 | 1.17 |
| | Stem | 0.0 | 0.0404 | 0.91 | 0.085 | 0.0053 | 6.24 | 6.789 | 0.0073 | 0.11 | 0.248 | 0.0033 | 1.32 |
| | Leaf | 0.0 | 0.0163 | 0.64 | 0.076 | 0.0022 | 2.95 | 2.652 | 0.0094 | 0.35 | 0.280 | 0.0059 | 2.11 |
| | Fruit | 0.0 | 0.0187 | 0.45 | 0.067 | 0.0044 | 6.55 | 3.894 | 0.0108 | 0.28 | 0.283 | 0.0050 | 1.77 |
| | **Mean** | **0.114** | | | **0.07** | | | **4.01** | | | **0.39** | | |

Table 39. Mo, Zn and Al contents of some plants of family Zygophyllaceae and Euphorbiaceae during winter.

| Plants | Parts | Mo (ppm) | | | Zn (ppm) | | | Al (ppm) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Zygophyllaceae** | | M | SD | %RSD | M | SD | %RSD | | | |
| *Fagonia cretica* L. | Root | 0.0 | 0.0112 | 14.08 | 0.123 | 0.0018 | 1.42 | 0.0 | 0.0201 | 15.33 |
| | Stem | 0.0 | 0.0059 | 11.53 | 0.117 | 0.0015 | 1.31 | 0.0 | 0.0728 | 39.60 |
| | Leaf | 0.0 | 0.0438 | 1397.19 | 0.132 | 0.0030 | 2.27 | 0.0 | 0.1055 | 48.55 |
| | Fruit | 0.001 | 0.0163 | 1398.43 | 0.189 | 0.0008 | 0.43 | 0.0 | 0.0932 | 179.19 |
| | **Mean** | **0.001** | | | **0.14** | | | **0.0** | | |
| *Peganum harmala* L. | Root | 0.067 | 0.0134 | 19.99 | 0.150 | 0.0011 | 0.70 | 0.023 | 0.0373 | 159.87 |
| | Stem | 0.149 | 0.0220 | 14.69 | 0.123 | 0.0002 | 0.14 | 0.0 | 0.1987 | 72.71 |
| | Leaf | 0.208 | 0.0063 | 3.05 | 0.215 | 0.0021 | 0.97 | 0.0 | 0.1095 | 30.33 |
| | Fruit | 0.0 | 0.0081 | 22.98 | 0.132 | 0.0009 | 0.69 | 0.832 | 0.0348 | 4.18 |
| | **Mean** | **0.14** | | | **0.16** | | | **0.42** | | |
| *Tribulus terrestris* L. | Root | 0.0 | 0.0169 | 45.15 | 0.186 | 0.0012 | 0.62 | 2.228 | 0.1921 | 8.62 |
| | Stem | 0.0 | 0.0087 | 63.49 | 0.216 | 0.0060 | 2.77 | 2.856 | 0.1916 | 6.71 |
| | Leaf | 0.0 | 0.0162 | 405.17 | 0.142 | 0.0012 | 0.87 | 4.307 | 0.1224 | 2.84 |
| | Fruit | 0.029 | 0.0041 | 14.06 | 0.180 | 0.0009 | 0.49 | 5.958 | 0.3545 | 5.95 |
| | **Mean** | **0.029** | | | **0.18** | | | **3.83** | | |
| **Euphorbiaceae** | | | | | | | | | | |
| *Chrozophora Tinctoria* (L.) Raf | Root | 0.038 | 0.0026 | 6.76 | 0.160 | 0.0045 | 2.79 | 7.674 | 0.4596 | 5.99 |
| | Stem | 0.063 | 0.0076 | 12.22 | 0.120 | 0.0012 | 0.96 | 8.968 | 0.4311 | 4.81 |
| | Leaf | 0.022 | 0.0019 | 8.57 | 0.188 | 0.0037 | 1.97 | 11.83 | 0.375 | 3.17 |
| | Fruit | 0.022 | 0.0060 | 26.95 | 0.485 | 0.0065 | 1.34 | 0.812 | 0.5840 | 71.93 |
| | **Mean** | **0.022** | | | **0.264** | | | **7.321** | | |
| *Ricinus Communis* L. | Root | 0.060 | 0.0129 | 21.58 | 0.189 | 0.0021 | 1.13 | 0.765 | 0.0708 | 9.25 |
| | Stem | 0.049 | 0.0162 | 32.74 | 0.256 | 0.0020 | 0.79 | 0.953 | 0.0216 | 2.27 |
| | Leaf | 0.027 | 0.0045 | 16.62 | 0.159 | 0.0022 | 1.37 | 0.330 | 0.0558 | 16.88 |
| | Fruit | 0.055 | 0.0072 | 13.19 | 0.275 | 0.0014 | 0.50 | 0.407 | 0.1752 | 43.04 |
| | **Mean** | **0.04** | | | **0.23** | | | **0.407** | | |

Key : M=Mean; SD=Standard Deviation; % RSD= Relative Standard Deviation

Table 60: Statistical analysis of seasonal variation of macro and micronutrients in some plants of family Zygophyllaceae and Euphorbiaceae.

| PLANTS | Ca (ppm) | | | K (ppm) | | | Mg (ppm) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Summer | Winter | Mean | Summer | Winter | Mean | Summer | Winter | Mean |
| **Zygophyllaceae** | | | | | | | | | |
| *Fagonia cretica* L. | 27.300 d | 205.2ab | 116.26c | 27.0e | 27.08bcd | 27.04bc | 9.203d | 10.79b | 9.995b |
| *Peganum harmala* L. | 29.933 d | 210.9ab | 120.43bc | 26.918f | 27.11bc | 27.01c | 9.137d | 10.22bc | 9.678b |
| *Tribulus terrestris* L. | 236.850a | 155.6abc | 196.22a | 26.90f | 27.15ab | 27.02c | 12.34a | 9.962bcd | 11.15a |
| **Euphorbiaceae** | | | | | | | | | |
| *Chrozophora tinctoria* (L.) Raf | 169.035abc | 191.6ab | 180.31ab | 27.025de | 27.14abc | 27.08b | 9.882bcd | 9.811cd | 9.846b |
| *Ricinus communis* L. | 145.050 bc | 88.64cd | 116.84c | 27.08cd | 27.18a | 27.13a | 10.17bc | 9.351cd | 9.759b |
| **Mean** | 121.63b | 170.39a | | 26.98b | 27.13a | | 10.145 | 10.026 | |
| **Zygophyllaceae** | | | | | | | | | |
| | Na (ppm) | | | Cr (ppm) | | | Cu (ppm) | | |
| *Fagonia cretica* L. | 1.705e | 5.413cd | 3.559b | 0.0 | 0.0 | 0.0 | 0.042 | 0.060 | 0.051c |
| *Peganum harmala* L. | 1.817e | 6.299c | 4.058b | 0.0 | 0.0 | 0.0 | 0.039 | 0.076 | 0.057bc |
| *Tribulus terrestris* L. | 4.719d | 6.557bc | 5.638a | 0 .0 | 0 .0 | 0 .0 | 0.068 | 0.074 | 0.071a |
| **Euphorbiaceae** | | | | | | | | | |
| *Chrozophora tinctoria* L.) Raf. | 1.767e | 8.629a | 5.198a | 0.0 | 0.0 | 0.0 | 0.056 | 0.094 | 0.075a |
| *Ricinus communis* L. | 2.755e | 7.606ab | 5.181a | 0.0 | 0 .0 | 0.0 | 0.064 | 0.072 | 0.068ab |
| **Mean** | 2.553b | 6.901a | | 0.0 | 0.0 | 0 .0 | 0.054b | 0.075a | |
| **Zygophyllaceae** | | | | | | | | | |
| | Fe (ppm) | | | Mn (ppm) | | | Mo (ppm) | | |
| *Fagonia cretica* L. | 4.407 | 3.456 | 3.931 | 0.145 | 0.294 | 0.219 | 0.008 | 0.00 | 0.004b |
| *Peganum harmala* L. | 2.061 | 3.373 | 2.717 | 0.135 | 0.214 | 0.174 | 0.055 | 0.106 | 0.08a |
| *Tribulus terrestris* L. | 3.812 | 3.781 | 3.796 | 0.187 | 0.207 | 0.197 | 0.041 | 0.007 | 0.02b |
| **Euphorbiaceae** | | | | | | | | | |
| *Chrozophora tinctoria* L.) Raf | 3.052 | 4.426 | 3.739 | 0.237 | 0.253 | 0.245 | 0.002 | 0.036 | 0.01b |
| *Ricinus communis* L. | 5.408 | 4.013 | 4.710 | 0.216 | 0.393 | 0.305 | 0.002 | 0.048 | 0.02b |
| **Mean** | 3.748 | 3.810 | | 0.184b | 0.272a | | 0.022 | 0.04 | |
| **Zygophyllaceae** | Zn (ppm) | | | Al (ppm) | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Fagonia cretica* L. | 2.55 | 0.14 | 1.34 | 0.44cd | 0.00d | 0.22b |
| *Peganum harmala* L. | 0.22 | 0.15 | 0.19 | 0.05d | 0.21cd | 0.13b |
| *Tribulus terrestris* L. | 0.31 | 0.18 | 0.24 | 0.43cd | 3.83b | 2.13ab |
| **Euphorbiaceae** | | | | | | |
| *Chrozophora tinctoria* L.) Raf | 0.22 | 0.23 | 0.23 | 0.22cd | 7.3a | 3.77a |
| *Ricinus communis* L. | 0.18 | 0.22 | 0.2 | 3.23bc | 0.61cd | 1.92ab |
| **Mean** | 0.69 | 0.18 | | 0.87b | 2.39a | |

Means followed by the same letter are not significantly different at 0.05%

Table 61. Variation in macro and micronutrients in two seasons in some plants of family Zygophyllaceae and Euphorbiaceae

| | | Macronutrient (ppm) | | | | Micronutrient (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plants | Season | Ca | K | Mg | Na | Al | Cr | Cu | Fe | Mn | Mo | Zn |
| **Zygophyllaceae** | | | | | | | | | | | | |
| *Fagonia cretica* L. | Summer | 27.36 | 27 | 9.20 | 1.70 | 0.37 | 0.0 | 0.04 | 4.4 | 0.14 | 0.014 | 2.55 |
| | Winter | 205.4 | 27.08 | 10.78 | 5.41 | 0.0 | 0.0 | 0.17 | 3.45 | 0.29 | 0.001 | 0.14 |
| | **Mean** | **116.38** | **27.04** | 9.99 | **3.55** | **0.37** | **0.0** | **0.105** | **3.9** | **0.2** | **0.023** | **1.35** |
| *Peganum harmala* L. | Summer | 29.9 | 27.0 | 9.13 | 1.81 | 0.07 | 0.0 | 0.04 | 2.06 | 0.14 | 0.05 | 0.22 |
| | Winter | 210.9 | 27.11 | 10.21 | 6.3 | 0.42 | 0.0 | 0.07 | 3.37 | 0.21 | 0.14 | 0.162 |
| | **Mean** | **120.4** | **27.05** | **9.67** | **4.05** | **0.25** | **0.0** | **0.05** | **2.7** | **0.2** | **0.09** | **0.2** |
| *Tribulus terrestris* L. | Summer | 236.9 | 27.0 | 12.33 | 4.71 | 0.38 | 0.0 | 0.06 | 3.81 | 0.18 | 0.165 | 0.31 |
| | Winter | 155.6 | 27.15 | 9.96 | 6.55 | 3.83 | 0.0 | 0.07 | 3.78 | 0.20 | 0.029 | 0.18 |
| | **Mean** | **196.25** | **27.07** | **11.15** | **5.63** | **2.1** | **0.0** | **0.06** | **3.8** | **0.2** | **0.09** | **0.25** |
| **Euphorbiaceae** | | | | | | | | | | | | |
| *Chrozophora tinctoria* L.) Raf | Summer | 169.0 | 27.0 | 9.88 | 1.76 | 0.16 | 0.0 | 0.05 | 3.05 | 0.24 | 0.0 | 0.22 |
| | Winter | 191.6 | 27.14 | 9.81 | 8.63 | 7.321 | 0.0 | 0.09 | 4.425 | 0.25 | 0.02 | 0.264 |
| | **Mean** | **180.3** | **27.07** | **9.85** | **5.2** | **3.74** | **0.0** | **0.07** | **3.7** | **0.25** | **0.02** | **0.24** |
| *Ricinus communis* L. | Summer | 145.0 | 27.0 | 10.16 | 2.75 | 2.93 | **0.0** | 0.06 | 5.4 | 0.21 | 0.0 | 0.18 |
| | Winter | 88.6 | 27.2 | 9.35 | 7.6 | 0.407 | 0.0 | 0.07 | 4.01 | 0.39 | 0.04 | 0.23 |
| | **Mean** | **116.8** | **27.1** | **9.8** | **5.2** | **1.67** | 0.0 | **0.06** | **4.7** | **0.3** | 0.04 | **0.20** |

Table 62. ANOVA values for seasonal variation of Ca contents and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 23775.377 | 23775.377 | 6.3928 | 0.0170 |
| 4 | Factor B | 4 | 48701.201 | 12175.300 | 3.2737 | 0.0243 |
| 6 | AB | 4 | 125645.411 | 31411.353 | 8.4459 | 0.0001 |
| -7 | Error | 30 | 111573.361 | 19.112 | | |
| | Total | 39 | 309695.351 | | | |

COV: 41.77%: LSD=39.39 (Seasons), LSD=62.27 (plants), LSD=88.07 (seasons and plants interaction)

Table 63. ANOVA values for seasonal variation of K contents and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 0.218 | 0.218 | 99.0044 | 0.0000 |
| 4 | Factor B | 4 | 0.071 | 0.018 | 8.0699 | 0.0002 |
| 6 | AB | 4 | 0.038 | 0.009 | 4.2748 | 0.0074 |
| -7 | Error | 30 | 0.066 | 0.002 | | |
| | Total | 39 | 0.392 | | | |

COV: 0.17%; LSD= 0.028 (Seasons), LSD=0.045 (plants), LSD=0.064 (seasons and plants interaction)

Table 64. ANOVA values for seasonal variation of Mg contents and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 0.141 | 0.141 | 0.3174 | |
| 4 | Factor B | 4 | 11.743 | 2.936 | 6.6183 | 0.0006 |
| 6 | AB | 4 | 19.835 | 4.959 | 11.1796 | 0.0000 |
| -7 | Error | 30 | 13.307 | 0.444 | | |
| | Total | 39 | 45.025 | | | |

COV=6.60% ; LSD= 0.68 (Seasons), LSD=0.96 (plants)

Table 65. ANOVA values for seasonal variation of Na contents and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 189.064 | 189.064 | 256.8355 | 0.0000 |
| 4 | Factor B | 4 | 24.565 | 6.141 | 8.3426 | 0.0001 |
| 6 | AB | 4 | 26.604 | 6.651 | 9.0351 | 0.0001 |
| -7 | | 30 | 22.084 | 0.736 | | |
| Total | | 39 | 262.317 | COV: 18.15% | | |

LSD=0.55 (Seasons), LSD=0.87 (plants), LSD=1.239 (seasons and plants interaction)

Table 66. ANOVA values for seasonal variation of Cu contents and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 0.005 | 0.005 | 26.1722 | 0.0000 |
| 4 | Factor B | 4 | 0.003 | 0.001 | 4.4699 | 0.0059 |
| 6 | AB | 4 | 0.002 | 0.000 | .5712 | 0.0580 |
| -7 | Error | 30 | 0.005 | 0.000 | | |
| Total | | 39 | | | | |

COV: 20.76% LSD=0.0081 (Seasons), LSD=0.012 (plants)

Table 67. ANOVA values for seasonal variation of Fe contents and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 0.038 | 0.038 | 0.0072 | |
| 4 | Factor B | 4 | 16.159 | 4.040 | 0.7701 | |
| 6 | AB | 4 | 12.882 | .220 | 0.6139 | |
| 7 | Error | 30 | 157.381 | .246 | | |
| Total | | 39 | 186.460 | COV: 60.61% | | |

Table 68. ANOVA values for seasonal variation of Mn contents and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 0.078 | 0.078 | 6.3895 | 0.0170 |
| 4 | Factor B | 4 | 0.081 | 0.020 | 1.6629 | 0.1846 |
| 6 | AB | 4 | 0.043 | 0.011 | 0.8887 | |
| -7 | Error | 30 | 0.364 | 0.012 | | |
| | Total | 39 | 0.566 | | | |

COV: 48.32% LSD = 0.07 (Seasons), LSD=0.035 (plants), LSD=0.055 (seasons and plants interaction)

Table 69. ANOVA values for seasonal variation of Mo contents and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 0.003 | 0.003 | 1.7719 | 0.1932 |
| 4 | Factor B | 4 | 0.027 | 0.007 | 3.7682 | 0.0134 |
| 6 | AB | 4 | 0.011 | 0.003 | 1.5287 | 0.2190 |
| -7 | Error | 30 | 0.054 | 0.002 | | |
| | Total | 39 | 0.095 | | | |

COV: 138.19% ; LSD=0.05(plants)

Table 70. ANOVA values for seasonal variation of Zn contents and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 2.625 | 2.625 | 1.1880 | 0.2844 |
| 4 | Factor B | 4 | 8.165 | 2.041 | 0.9238 | |
| 6 | AB | 4 | 9.047 | 2.262 | 1.0235 | 0.4111 |
| -7 | Error | 30 | 66.291 | 2.210 | | |
| | Total | 39 | 86.128 | COV: 335.53% | | |

Table 71. ANOVA values for seasonal variation of Al contents and seasons-plants interaction of family Zygophyllaceae and Euphorbiaceae.

| K Value | Source | Degrees of Freedom | Sum of Squares | Mean Square | F Value | Prob |
|---|---|---|---|---|---|---|
| 2 | Factor A | 1 | 23.030 | 23.030 | 4.7938 | 0.0365 |
| 4 | Factor B | 4 | 73.203 | 18.301 | 3.8095 | 0.0128 |
| 6 | AB | 4 | 115.053 | 28.763 | 5.9873 | 0.0012 |
| -7 | Error | 30 | 144.121 | 4.804 | | |
| | Total | 39 | 355.407 | | | |

COV: 133.407%; LSD=1.416 (Seasons) LSD=2.238 (plants); LSD=3.165 (seasons and plants interaction

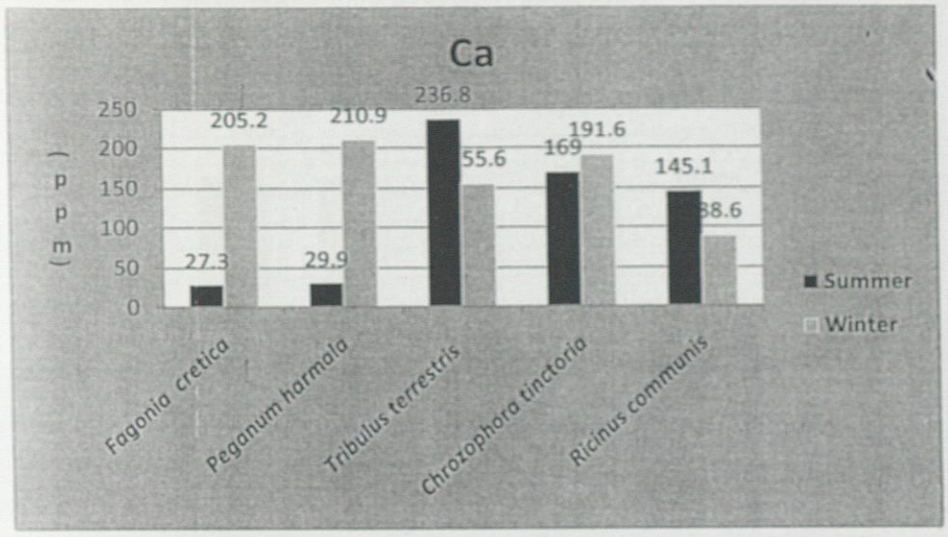



Fig. 111. Seasonal differences of calcium contents in plants of family Zygophyllaceae and Euphorbiaceae

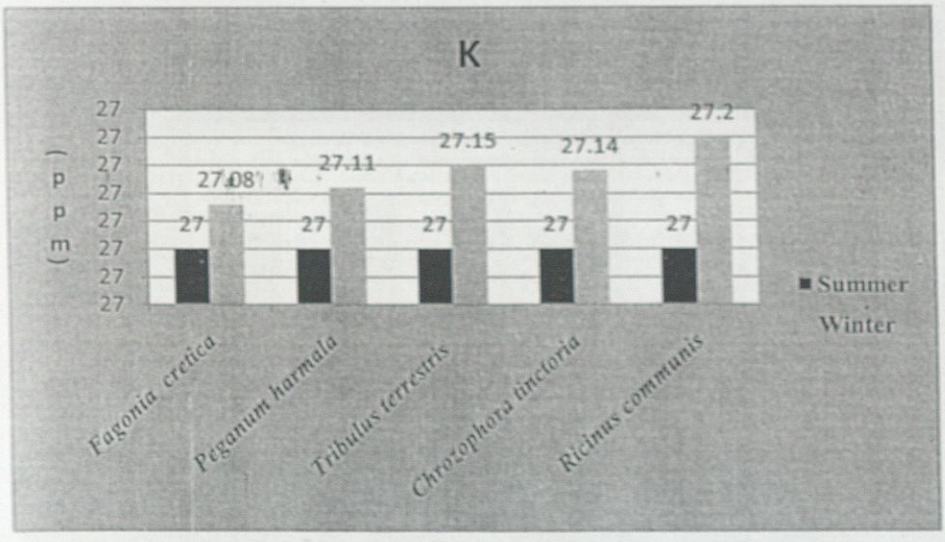



Fig. 112. Seasonal differences of potassium contents in plants of family Zygophyllaceae and Euphorbiaceae

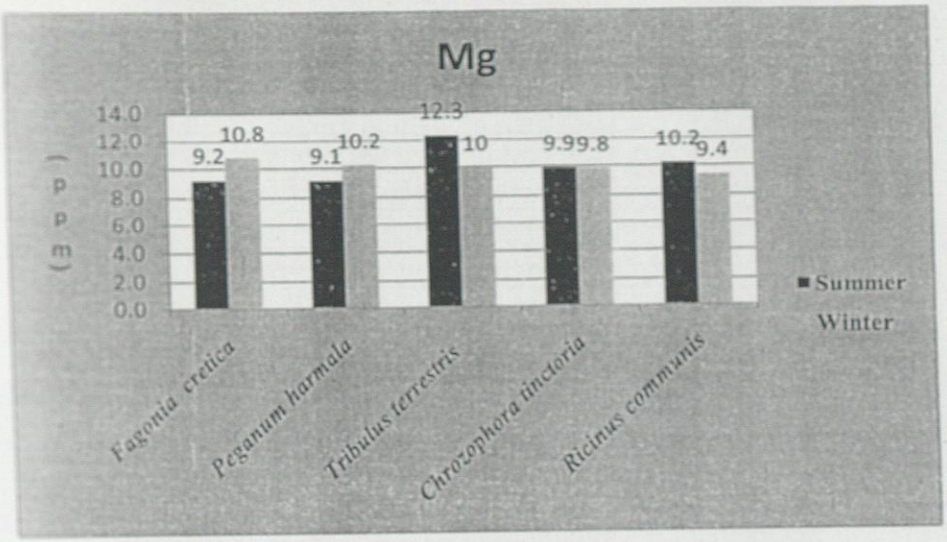



Fig. 113. Seasonal differences of magnesium contents in plants of family Zygophyllaceae and Euphorbiaceae

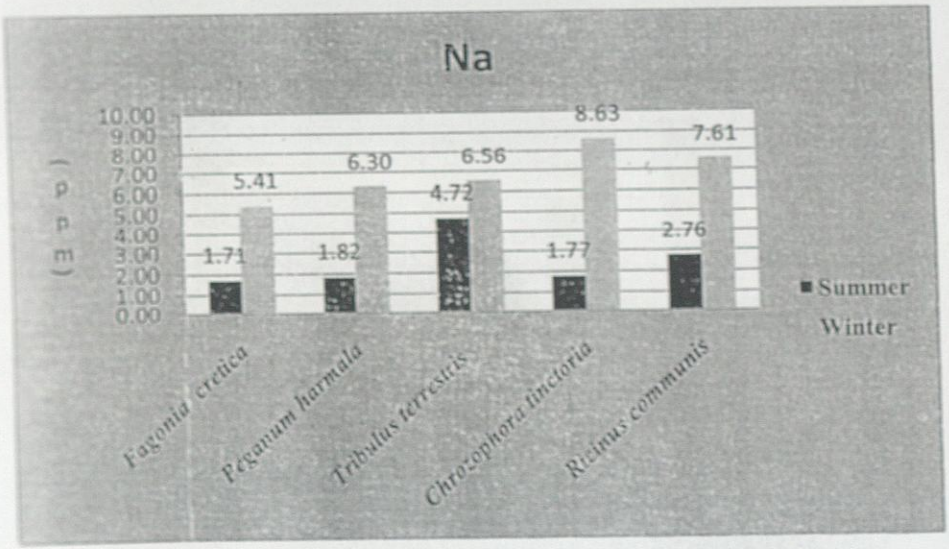



Fig. 114. Seasonal differences of sodium contents in plants of family Zygophyllaceae and Euphorbiaceae

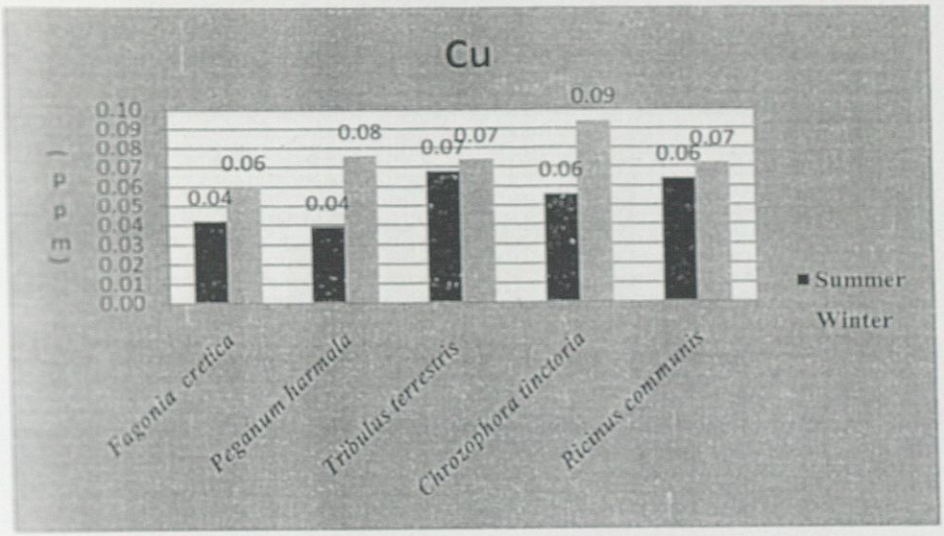



Fig. 115. Seasonal differences of copper contents in plants of family Zygophyllaceae and Euphorbiaceae

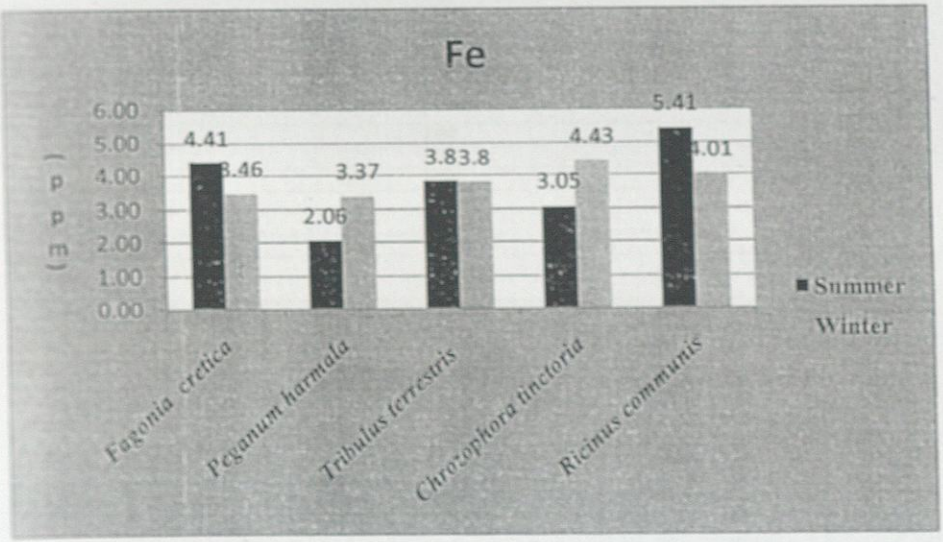



Fig. 116. Seasonal differences of iron contents in plants of family Zygophyllaceae and Euphorbiaceae

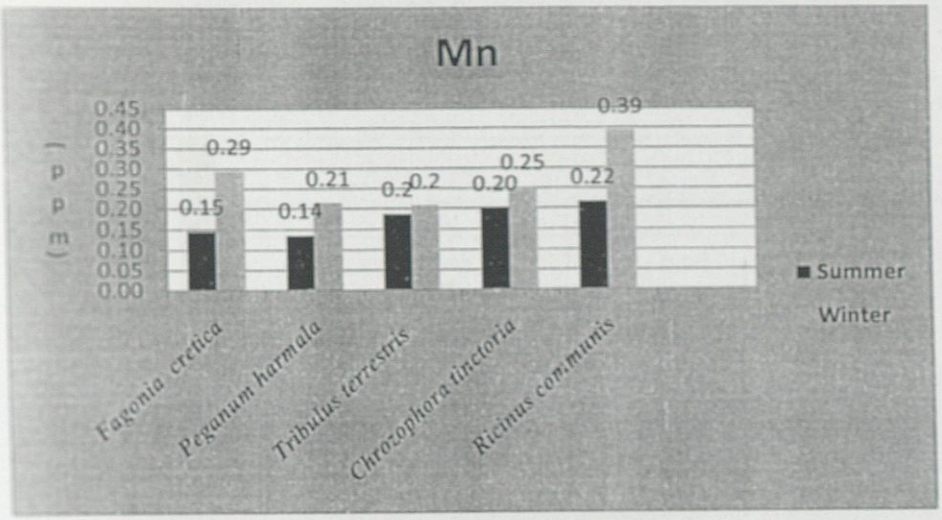



Fig. 117. Seasonal differences of manganese contents in plants of family Zygophyllaceae and Euphorbiaceae

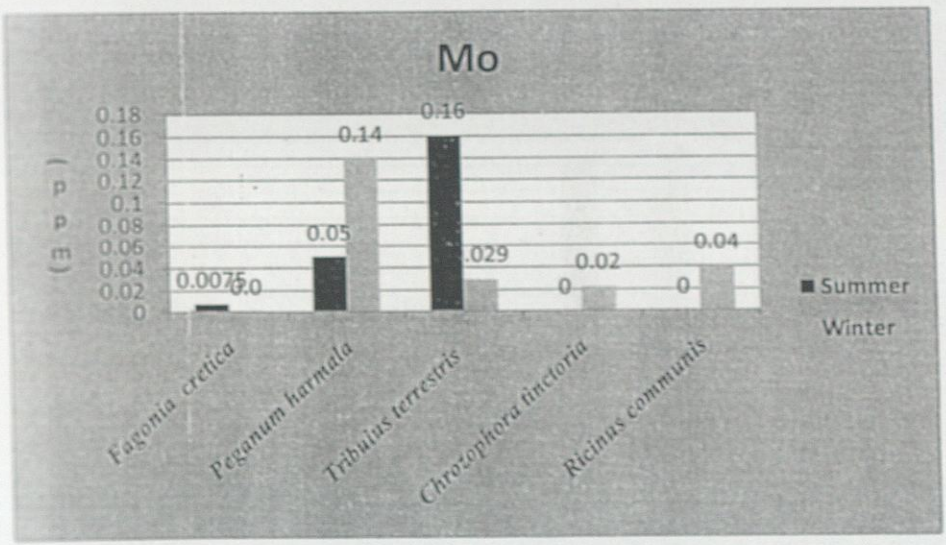



Fig. 118. Seasonal differences of molybdenum contents in plants of family Zygophyllaceae and Euphorbiaceae

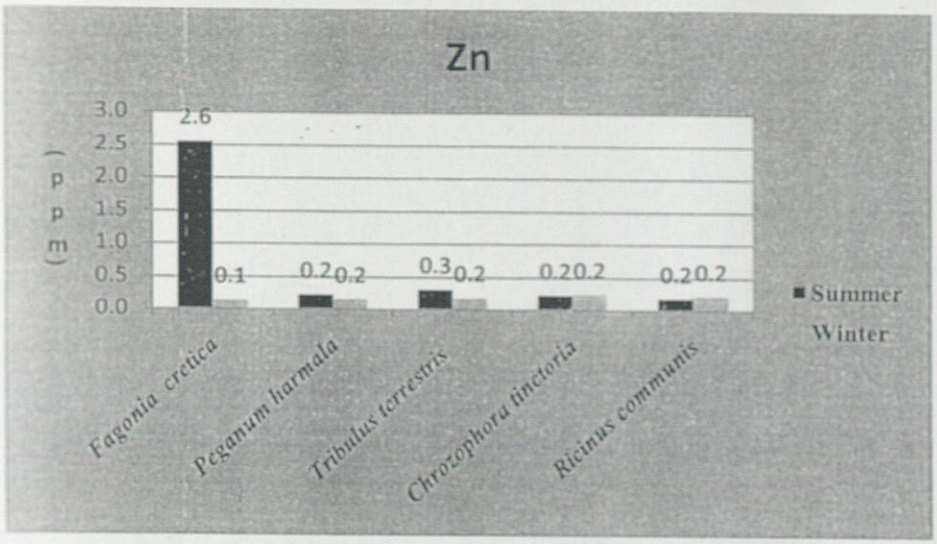



Fig. 119. Seasonal differences of zinc contents in plants of family Zygophyllaceae and Euphorbiaceae

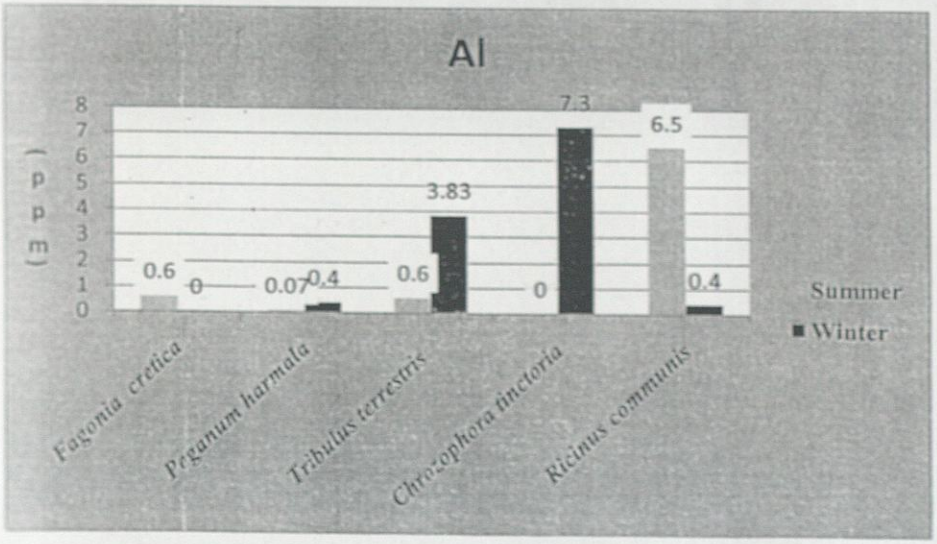



Fig. 120. Seasonal differences of aluminum contents in plants of family Zygophyllaceae and Euphorbiaceae

# CHAPTER-9

# ANALYSIS OF ESSENTIAL OIL

# CHAPTER 9

# ANALYSIS OF ESSENTIAL OIL

**i. Composition of essential oils of five plants**

The identified oil components in the aerial parts of investigated plants are summarised in Table 72. and oil compounds chromatograms are given in Figs. 121-125.

The three analysied plants of family Zygophyllaceae revealed that oxygenated monoterpenes were highest (90.99%) in *Tribulus terrestris,* followed by in *Fagonia cretica* (89.94%) and the lowest (36.01%) occurred in *Peganum harmala*(Tables 73). *Peganum harmala* had maximum esters 11.58% followed by *Tribulus terrestris* 5.8% and *Fagonia cretica* 5.5%. Monoterpenes hydrocarbons were maximum (1.22%) in *Fagonia cretica* followed by *Peganum harmala* and absent in *Tribulus terrestris*. Sesquiterpenes hydrocarbons were highest (11.01%) in *Peganum harmala* and absent in *Tribulus terrestris* (Table 73). However, the chemical groups in two tested plants of family Euphorbiaceae, showed that oxygenated monoterpenes were highest (88.63%) in *Ricinus communis,* followed 89.94% in *Chrozophora tinctoria*. Monoterpenes hydrocarbons were maximum (3.5%) in *Chrozophora tinctoria* followed by 2.86 % in *Ricinus communis*. Sesquiterpenes hydrocarbons were highest (9.85%) in *Chrozophora* and absent in *Ricinus communis*. Oxygenated sesquiterpenes were highest (3.5%) in *Chrozophora tinctoria* followed 2.4 % in *Ricinus communis*. Esters were absent in both the plants (Tables 73).

**A. Zygophyllaceae**

**ii. % of major groups of constituents**

**Oxygenated monoterpene**

Oxygenated monoterpenes have antispasmodic, sedative and tranquilizing action and beneficial to various systems and human metabolic processes (Gyawali & Kyong-Su Kim, 2012). They have antimicrobial properties (Kordali *et al*. 2009; Kotan *et al*. 2008; Gundidza *et al*. 2009, and Abu-Darwish *et al*., 2012), act as food preservatives and natural antioxidants. They have antitumoral activity and prevented mammary, lung, skin, liver and forestomach cancers in rat models (Fayed, 2009). They may also act as

radical scavenging agents (Judpentiene and Mockute (2004) stated 1,8-cineole helps in cold and other hardships of the Himalayan region and higher amounts are toxic. They widely used in the flavoring agents (Chang & Kim, 2008).

In *Fagonia cretica* oils, 17 compounds were identified constituting 100% of the oil composition (Table 72; Fig. 121). Oxygenated monoterpenes (89.94%), was a major group of compounds and it consisted of 2(4H)-Benzofuranone,5,6,7,7a tetrahydro-4,4,7a-trimethy (77.645% ), alcanfor (63.925%), O-cymene (6.166%), cineole (5.088%), trans-geraniol (1.884%), eugenol methy (1.748%) and α-citral (1.084%), and esters had lower amounts (5.5%), respectively. Other chemical groups had less than 1.22% components (Tables 73). Several workers found low oxygenated monoterpenes in different medicinal plants (Moradalizadeh *et al*., 2012; Bozin *et al*., 2008; Sukari *et al*., 2008) while *Fagonia cretica* showed increseased percentage of oxygenated monoterpenes. Several researchers (Sonibare & Effiong, 2008; Kanjilal & Kotoky 2010) reported less alcanfor in different medicinal plants. Ebadollahi *et al*. (2010) also reported poor alcanfor in *Lavendula stoechas*, *Eucalyptus globulus* and *Dittrichia graveolens* as compared to *Fagonia cretica* and *Peganum harmala*. Earlier workers (Zhang *et al*., 2011, Fayed, 2009; and Chun-Di Hu *et al*., 2010) found high amount of trans-geraniol in *Pelargonium graveolens*, *Mentha arvensis*, and *Osmanthus fragrans*. These results are not in line with the present study.

*Peganum harmala* oils had 18 compounds constituting 99.8% of the total oil composition (Table 72; Fig. 122). These oils consisted of oxygenated monoterpenes 36.01%, with major components as alcanfor 28.244% and capillin 13.176%. Esters were 11.58% (Tables 73). Other chemical groups were lower than 11.01% in the oil.

There were 10 compounds in *Tribulus terrestris* oils that consisted 100% of the total oil composition (Table 72; Fig. 123). It consisted of oxygenated monoterpenes (90.99%), having 2(4H)-Benzofuranone, 5, 6, 7, 7a tetrahydro-4, 4, 7a-trimethy 38.194% and capallin 23.556% and cyclohexyl ketone 14.768%. Esters were 5.8%. (Tables 73). Other chemical groups shared less than 5.8% components in the oil. Several workers have reported variation of 2(4H)- Benzofuranone, 5, 6, 7, 7a tetrahydro-4, 4, 7a-trimethy(volatile terpenes) in different medicinal plants (Akhbari *et al*., 2011; Akkhol *et*

*al.*, 2011; Chalannava *et al.*, 2011; Wang *et al.*, 2006; Ramasubramaniaraja, 2011). That agree with the present findings. All these studies found less 2(4H) - Benzofuranone, 5, 6, 7, 7a tetrahydro-4, 4, 7a-trimethy as compared to *Fagonia cretica, Peganum harmala, Tribulus terrestris*. Abirami & Rajendran (2011) found α-Amyrin (63.73%) in the methanolic extract of *Tribulus terrestris* and found long chain hydrocarbons and eight minor components. This disagrees with the present findings.

Essential oils rich in alcohols, aldehydes, phenols and esters, sesquiterpenes and ketones had antimicrobial and spasmolytic effect this is also correlated with the relaxant effect on man (Lis-Balchin & Hart, 1999; Derwich *et al.*, 2004; Hammer & Carson 2011). It has been proposed that an aldehyde group conjugated to a carbon to carbon double bond is a highly electronegative arrangement, which may explain their activity suggesting an increase in electronegativity increases the antibacterial activity (Chikhi *et al.*, 2012). Aldehydes are intermediates between the alcohols and the acids having lower molecular weight and characterized by their unpleasant and pungent odors and irritant effect in the nose. As the molecular weight increases, the odor profile gradually tends to be a more pleasant fruity (Chang & Kim, 2008).

Comparing the chemical groups in three tested plants of family Zygophyllaceae, it was found that oxygenated monoterpenes were highest (90.99%) in *Tribulus terrestris,* followed by in *Fagonia cretica* (89.94%) and the lowest (36.01%) occurred in *Peganum harmala* (Tables 73). These findings agree with Al-Qudah *et al.* (2010) and Judpentiene & Mockute (2004) who reported less amount of oxygenated monoterpenes and sesquiterpenes hydrocarbons in oils of *Dittricha viscosa* and *Artemisia absinthium*. *Peganum harmala* had maximum esters 11.58% followed by *Tribulus terrestris* 5.8% and *Fagonia cretica* 5.5%. Monoterpenes hydrocarbons were maximum (1.22%) in *Fagonia cretica* followed by *Peganum harmala* and absent in *Tribulus terrestris*. Sesquiterpenes hydrocarbons were highest (11.01%) in *Peganum harmala* and absent in *Tribulus terrestris* (Table 73). Njorage *et al.* (2005) reported high amount of monoterpenes hydrocarbons and sesquiterpenes hydrocarbons in *Citrus sinensis*. The variations in occurrence of these sesquiterpenes might be used to differentiate the respective oils.

**Euphorbiaceae**

Eight compounds were identified in *Chrozophora tinctoria* oils giving complete oil composition (Table 72; Fig. 124). It had oxygenated monoterpenes 86.93%, constituting of 2(4H)- Benzofuranone, 5, 6, 7, 7a tetrahydro-4, 4, 7a-trimethy 50.718%, cyclohexyl ketone 13.742%, capallin 9.948%. Esters were absent (Tables 73). The remaining chemical groups had less than 5.8% oil components.

*Ricinus communis* oil had 8 compounds with 100% of the oil composition. (Table 72; Fig. 125). The oxygenated monoterpenes were 88.63%, and it consisted of 2(4H)-Benzofuranone, 5, 6, 7, 7a tetrahydro-4, 4, 7a-trimethy 77.645%, this value was the highest individual component in the present study. Esters were absent (Tables 73). Other chemical groups had less than 2.4% components in the oil.

Several scientists observed variation of 2(4H) - Benzofuranone, 5, 6, 7, 7a tetrahydro-4, 4, 7a-trimethy in different plants (Akhbari, 2011; Ramasubramaniaraja, 2011). That agrees with the present results. These findings reported less 2(4H)-Benzofuranone, 5, 6, 7, 7a tetrahydro-4, 4, 7a-trimethy as compared to *Chrozophora tinctoria* and *Ricinus communis*.

Comparing the chemical groups in two plants of family Euphorbiaceae it was observed that oil was composed of oxygenated monoterpenes were highest (88.63%) in *Ricinus communis* and lowest in (86.93%) (Table 73). Monoterpenes hydrocarbons were highest (3.5%) in *Chrozophora tinctoria* than in *Ricinus communis* 2.86% (Table 73). These findings agree with Kadri *et al.* (2011) who also reported increased monoterpenes hydrocarbons and oxygenated monoterpenes from essential oil of *Ricinus communis*. *Chrozophora tinctoria* had 9.85% sesquiterpenes hydrocarbons and were absent in *Ricinus communis*. *Chrozophora tinctoria* had more (3.5%) oxygenated sesquiterpenes than in *Ricinus communis* (2.4%) (Table 73). Similar results were also reported by Lima *et al.* (2009) and Patricia *et al.* (2011) who found similar volatile constituents in the oils of *Acalypha ornata* and *Julocroton triqueter*.

The variations in the percentage of chemical composition of essential oils can be explained by the differences in the geographic origins, climate, nature of soil, solar radiation, age of the plant and part of the plants. All these factors involve the activation or

inactivation of certain enzymatic groups, leading to the predominance of a particular biosynthetic pathway (Khadhri *et al*., 2011; Neves & Camara, 2012).

The knowledge of the chemical composition of an essential oil is of key importance for determining its potential industrial or medicinal applications. The literaure shows that there are no reports on the detailed physicochemical features of the oils of the studied plants from Pakistan.

In the present study all the plants were found to be a rich source of oxygenated monoterpenes representing 2(4H)-Benzofuranone, 5, 6, 7,7a tetrahydro-4, 4,7a-trimethy as the major constituent. This study revealed that phytochemical attributes and chemical composition of the studied plants have potential uses for food, pharmaceutical and cosmetic applications. Detailed research work on the antioxidant principles and biological bioactivities of the studied plants is further recommended.

Table 72. Comparative percentage of various constituents in some plants of family Zygophyllaceae and Euphorbiaceae.

| | Family | Zygophyllaceae | | | Euphorbiaceae | |
|---|---|---|---|---|---|---|
| S No | Compounds | *Fagonia cretica* | *Peganum harmala* | *Tribulus terrestris* | *Chrozophora tinctoria* | *Ricinus communis* |
| 1 | Beta Phellandrene | 0.337 | 0.0 | 0.0 | 0.0 | 0.0 |
| 2 | o-Cymene | 6.166 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | Cineole | 5.088 | 0.0 | 0.0 | 0.0 | 0.0 |
| 4 | Alcanfor | 63.925 | 28.24 | 0.0 | 0.0 | 0.0 |
| 5 | n-Octyl acetate | 5.541 | 6.671 | 5.811 | 0.0 | 0.0 |
| 6 | Trans-Geraniol | 1.884 | 2.753 | 0.0 | 0.0 | 0.0 |
| 7 | Alpha -Citral | 1.638 | 2.602 | 0.0 | 0.0 | 0.0 |
| 8 | Geraniol-acetate | 1.229 | 2.635 | 0.0 | 0.0 | 0.0 |
| 9 | Eugenyl methy | 1.748 | 9.674 | 5.473 | 3.692 | 0.0 |
| 10 | Alpha Curcumene | 1.084 | 2.135 | 0.0 | 0.0 | 0.0 |
| 11 | Alpha-Limonene diepoxide | 0.801 | 0.0 | 0.0 | 0.0 | 0.0 |
| 12 | Cyclohexyl Ketone | 3.464 | 6.663 | 14.768 | 13.742 | 3.745 |
| 13 | 2(4H)-Benzofuranone,5,6,7,7a tetrahydro-4,4,7a-trimethy | 2.19 | 7.175 | 38.194 | 50.718 | 77.645 |
| 14 | Citronellyl propionate | 1.142 | 2.259 | 0.0 | 0.0 | 0.0 |
| 15 | Alpha Cedrol | 0.082 | 1.433 | 0.979 | 3.497 | 2.384 |
| 16 | Elemicin | 0.636 | 3.669 | 7.162 | 8.558 | 2.947 |

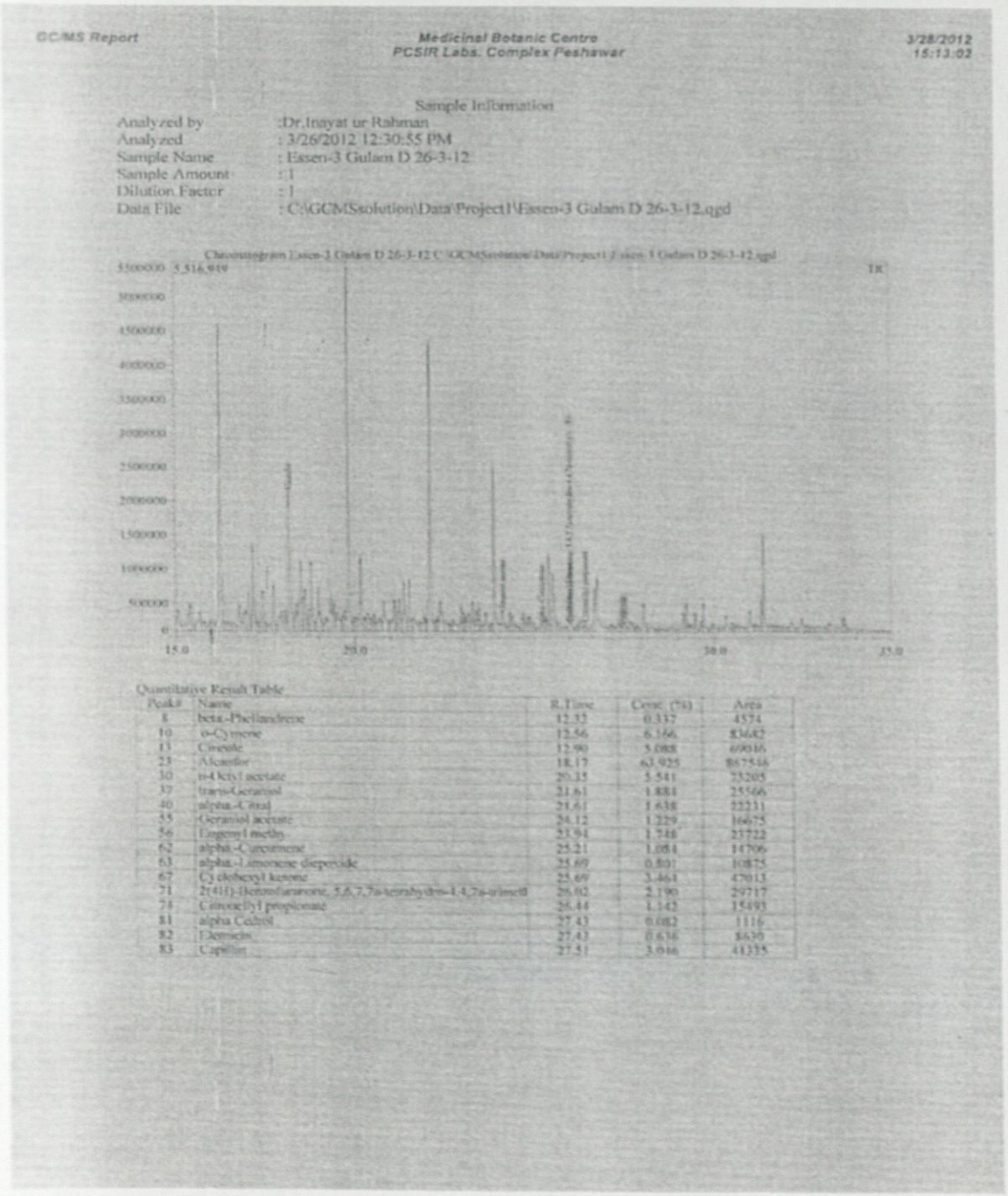
| | | | | | | |
|---|---|---|---|---|---|---|
| 17 | Capillin | 3.046 | 13.176 | 23.556 | 9.948 | 3.156 |
| 18 | p-Anisaldehyde | 0.0 | 0.380 | 0.0 | 0.0 | 0.0 |
| 19 | 6-Isopropyl-3,6-dimethyl-2-cyclohexane-1-one | 0.0 | 0.545 | 0.0 | 0.0 | 0.0 |
| 20 | Germacrene D | 0.0 | 0.208 | 0.0 | 0.0 | 0.0 |
| 21 | 1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl | 0.0 | 8.671 | 0.0 | 9.845 | 8.504 |
| 22 | Caryophyllene oxide | 0.0 | 0.581 | 0.0 | 0.0 | 0.0 |
| 23 | Z,E)-Farnesol | 0.0 | 0.523 | 0.0 | 0.0 | 0.0 |
| 24 | Phenol,2-methyl-5-(1-methylethyl)- | 0.0 | 0.0 | 1.839 | 0.0 | 0.0 |
| 25 | Beta Farnesene | 0.0 | 0.0 | 1.698 | 0.0 | 0.0 |
| 26 | Delta-cadinol | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 27 | Beta Iraldeine | 0.0 | 0.0 | 0.0 | 0.0 | 1.136 |
| 28 | P-cymene | 0.0 | 0.0 | 0.0 | 0.0 | 0.481 |

Table 73. Composition of essential oil of some selected plants of family Zygophyllaceae and Euphorbiaceae.

| Constituents | Zygophyllaceae | | | Euphorbiaceae | |
|---|---|---|---|---|---|
| Major Groups | *Fagonia cretica* | *Peganum harmala* | *Tribulus terrestris* | *Chrozophora tinctoria* | *Ricinus communis* |
| a. Oxygenated Monoterpenes (%) | 89.94 | 36.01 | 90.99 | 86.93 | 88.63 |
| b. Monoterpenes Hydrocarbons (%) | 1.22 | 1.01 | 0.0 | 3.5 | 2.86 |
| c. Sesquiterpenes Hydrocarbons (%) | 1.08 | 11.01 | 1.7 | 9.85 | 0.0 |
| d. Oxygenated Sesquiterpenes (%) | 0.082 | 2.54 | 1.5 | 3.5 | 2.4 |
| e. Esters (%) | 5.5 | 11.58 | 5.8 | 0.0 | 0.0 |
| Total Oil (%) | 100 | 99.8 | 100 | 100 | 100 |
| Number of Compounds | 17 | 18 | 10 | 7 | 8 |

## FAMILY ZYGOPHYLLACEAE

Fig. 121. GC Chromatogram of different compounds in oil of *Fagonia cretica* L.




| Peak# | Name | R.Time | Conc. (%) | Area |
|---|---|---|---|---|
| 8 | beta.-Phellandrene | 12.33 | 0.337 | 4574 |
| 10 | o-Cymene | 12.56 | 6.166 | 83682 |
| 13 | Cineole | 12.90 | 5.088 | 69046 |
| 25 | Alcanfor | 18.17 | 63.925 | 867516 |
| 30 | n-Hexyl acetate | 20.35 | 1.541 | 21205 |
| 37 | trans-Geraniol | 21.61 | 1.884 | 25566 |
| 40 | alpha.-Citral | 21.61 | 1.638 | 22231 |
| 55 | Geraniol acetate | 24.12 | 1.229 | 16675 |
| 56 | Eugenyl methy | 23.94 | 1.748 | 23722 |
| 62 | alpha.-Curcumene | 25.21 | 1.084 | 14706 |
| 63 | alpha.-Limonene diepoxide | 25.69 | 0.891 | 10875 |
| 67 | Cyclohexyl ketone | 25.69 | 3.464 | 47013 |
| 71 | 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimeth | 26.02 | 2.190 | 29717 |
| 74 | Citronellyl propionate | 26.44 | 1.142 | 15493 |
| 81 | alpha Cedrol | 27.43 | 0.082 | 1116 |
| 82 | Dermacin | 27.43 | 0.636 | 8630 |
| 83 | Capillin | 27.51 | 3.046 | 41335 |

Fig. 122. GC Chromatogram of different compounds in oil of *Peganum harmala* L.

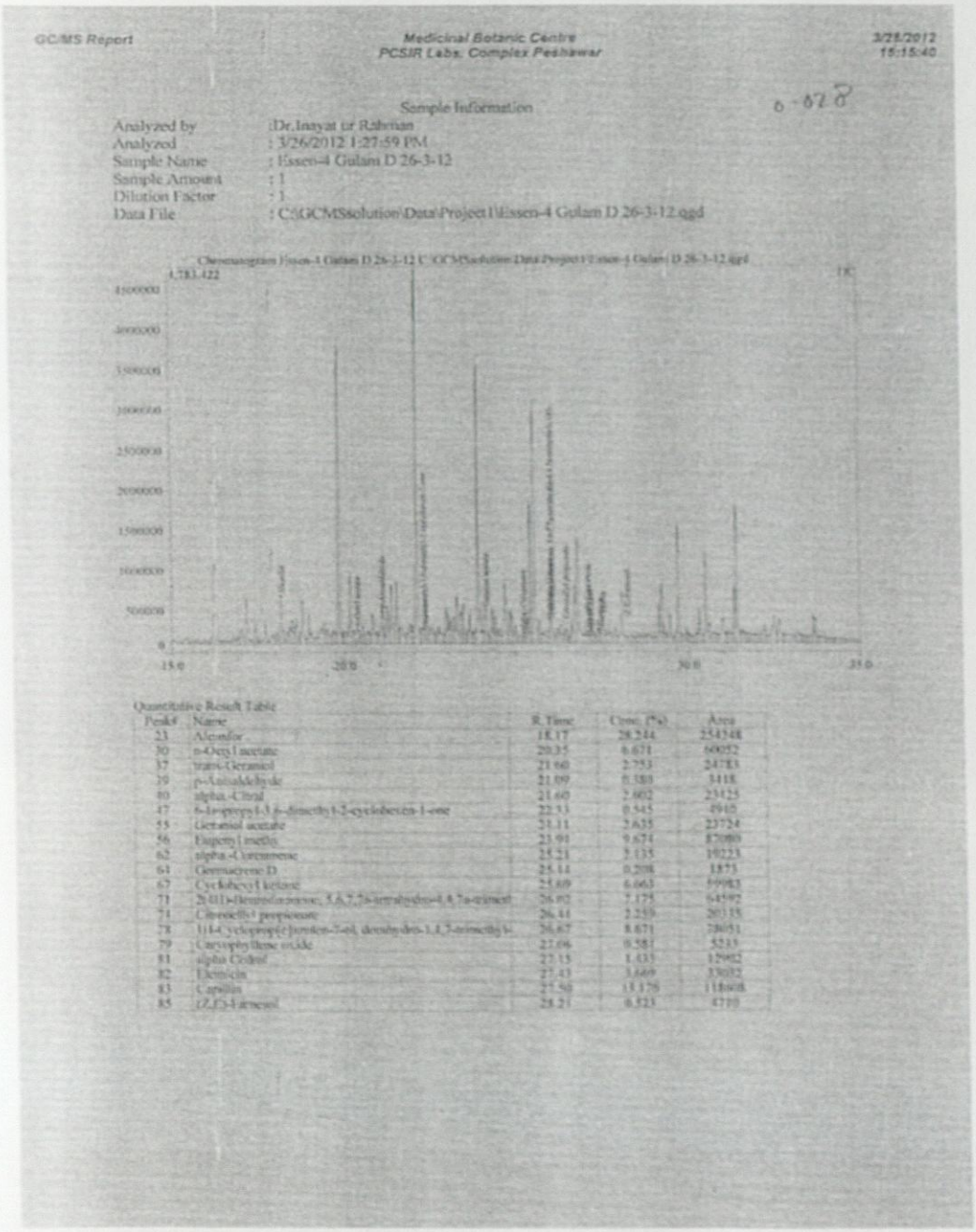
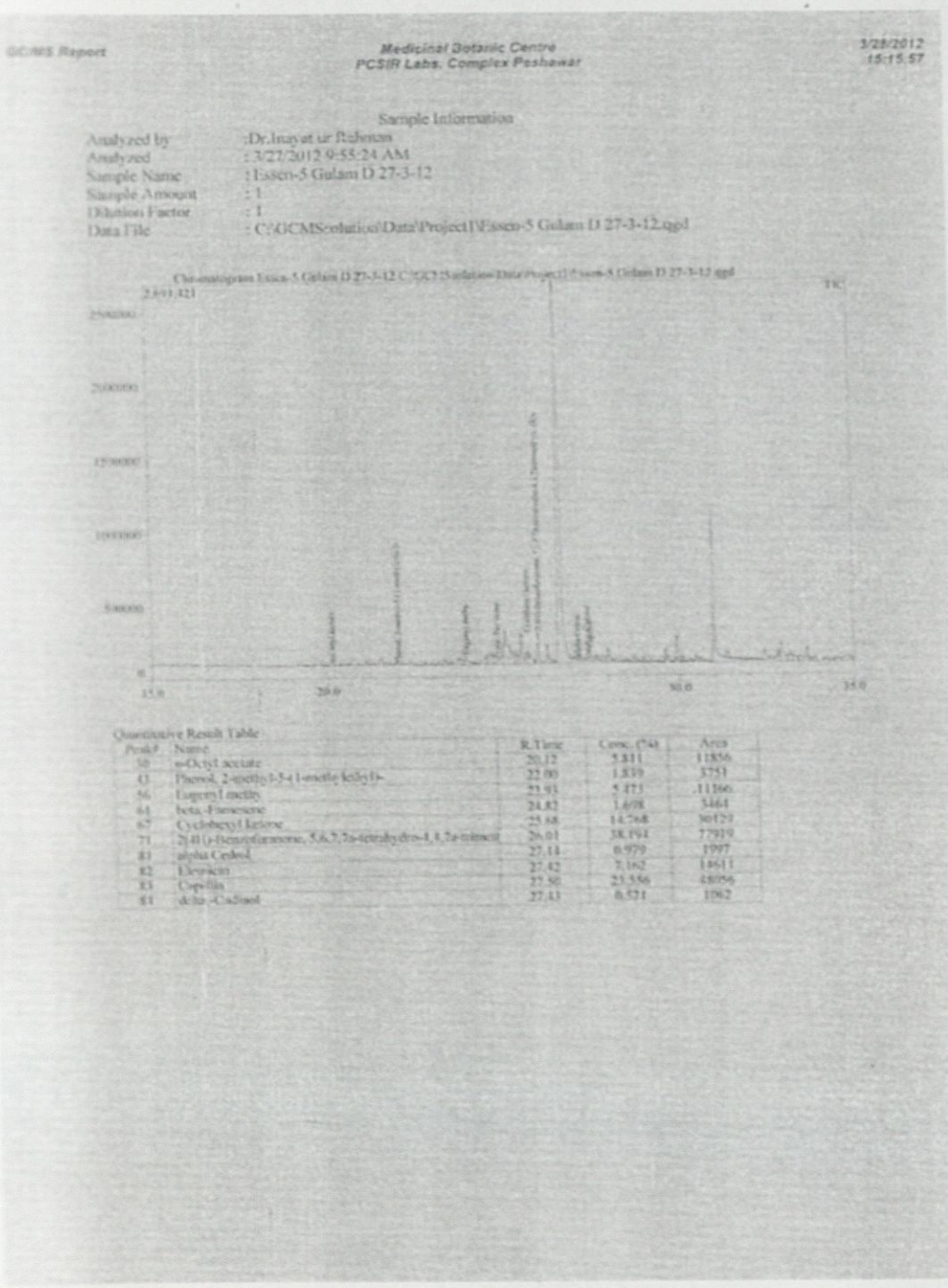


Fig. 123. GC Chromatogram of different compounds in oil of *Tribulus terrestris* L.



GC/MS Report

Medicinal Botanic Centre
PCSIR Labs. Complex Peshawar

3/28/2012
15:15:57

Sample Information

| | |
|---|---|
| Analyzed by | :Dr.Inayat ur Rahman |
| Analyzed | : 3/27/2012 9:55:24 AM |
| Sample Name | : Essen-5 Gulam D 27-3-12 |
| Sample Amount | : 1 |
| Dilution Factor | : 1 |
| Data File | : C:\GCMSolution\Data\Project1\Essen-5 Gulam D 27-3-12.qgd |

Chromatogram Essen-5 Gulam D 27-3-12 C:\GCMSolution\Data\Project1\Essen-5 Gulam D 27-3-12.qgd

TIC

2,891,421

15.0 20.0 30.0 35.0

Quantitative Result Table

| Peak# | Name | R.Time | Conc. (%) | Area |
|---|---|---|---|---|
| 50 | n-Octyl acetate | 20.12 | 5.811 | 11856 |
| 63 | Phenol, 2-methyl-5-(1-methylethyl)- | 22.00 | 1.839 | 3753 |
| 66 | Eugenyl methyl | 23.93 | 5.475 | 11366 |
| 68 | beta.-Farnesene | 24.82 | 1.698 | 3464 |
| 69 | Cyclohexyl ketone | 25.68 | 14.768 | 30129 |
| 71 | 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimeth | 26.01 | 38.194 | 77939 |
| 81 | alpha Cedrol | 27.14 | 0.979 | 1997 |
| 82 | Elemicin | 27.42 | 7.162 | 14611 |
| 83 | Copaiba | 27.50 | 23.556 | 48056 |
| 84 | delta.-Cadinol | 27.43 | 0.571 | 1062 |

## FAMILY EUPHORBIACEAE

Fig. 124. GC Chromatogram of different compounds in oil of *Chrozophora tinctoria* (L.) Raf.

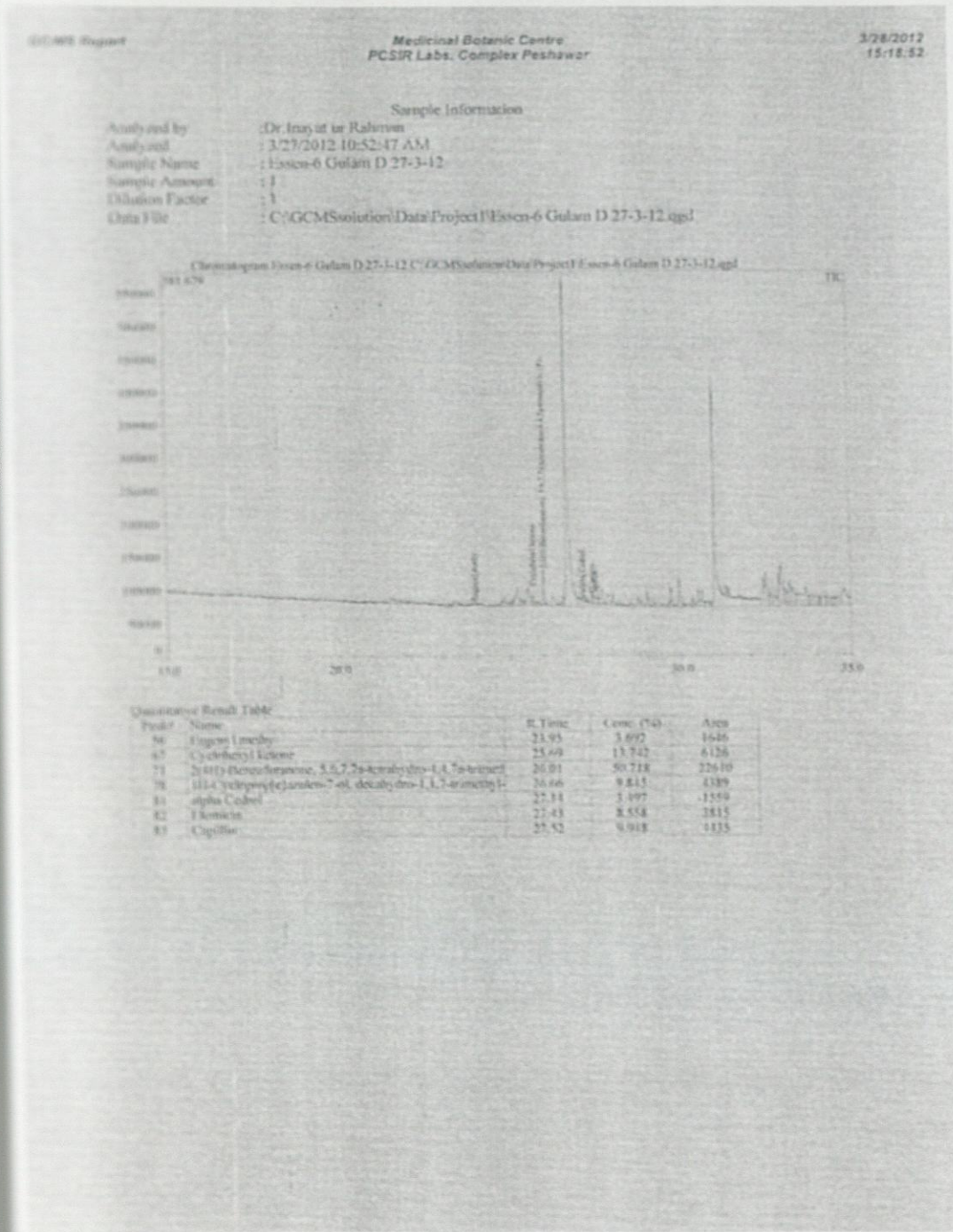
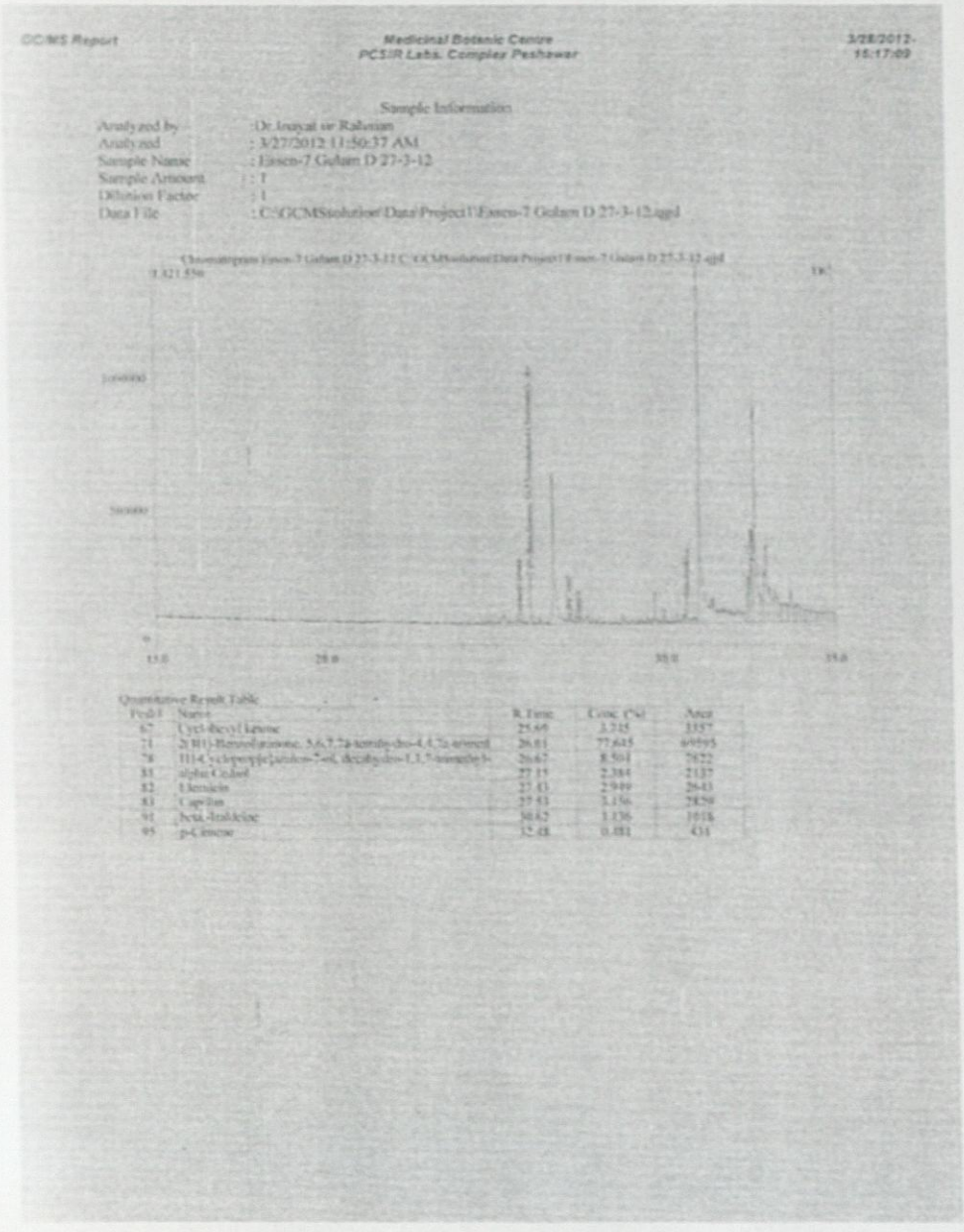


Fig. 125. GC Chromatogram of different compounds in oil of *Ricinus communis* L.



# CHAPTER-10

# FLUORESCENCE CHARACTER

# CHAPTER 10

# FLUORESCENCE CHARACTER

## i. Comparative fluorescence character of various parts in different chemicals

### A. Family Zygophyllaceae

#### a. ROOT

The fluorescence characteristic of various parts of analyzed plants is given in table 74.

1. Ammonia. It was light yellow in (DL), dark yellow in (SWL) and gray in (LWL) in *Fagonia cretica*, while it was cream colored in (DL), light yellow in (SWL) and brown in (LWL) in *Peganum harmala*. The color was dark brown in (DL), cream in (SWL) and brown in (LWL) in *Tribulus terrestris*. 2. Picric acid. The color was dark yellow in (DL), yellow in (SWL) and dark brown in (LWL) in *F. cretica*. It became yellow in (DL), in (SWL) and dark brown in (LWL) in *P. harmala*. Brown color was seen in (DL), (LWL) and light yellow in (SWL) in *T. terrestris* 3. Chloroform. It revealed that root color was light yellow in (DL), (SWL) and yellow in (LWL) in *F. cretica*. It became cream in (DL), light blue in (SWL) and cream in (LWL) in *P. harmala*. It was dark brown in (DL), cream in (SWL) brown in (LWL) in *T. terrestris*. 4. Ferric chloride. The color was light yellow in (DL), (SWL) and yellow in (LWL) in *F. cretica* while it was cream in (DL), (SWL) and brown in (LWL) in *P. harmala*. It was dark brown in (DL), cream in (SWL) and brown in (LWL) in *T. terrestris*. 5. Ether. The root color was light yellow in (DL), yellow in (SWL) and (LWL) in *F. cretica* while it became cream in (DL), light cream in (SWL) and cream in (LWL) in *P. harmala*. It was light brown in (DL), cream in (SWL) and light black in (LWL) in *T. terrestris*. 6. Ethanol. When treated with roots gave light yellow color in (DL), (SWL) and (LWL) in *F. cretica* while it was yellow in (DL), dark brown in (SWL) and black in (LWL) in *P. harmala*. It was dark brown in (DL), light black in (SWL) and black in (LWL) in *T. terrestris*. 7. Hydrochloric acid. It showed that root color was brown in (DL), (SWL) and (LWL) in *F. cretica* while it was yellow in (DL), brown in (SWL) and light brown in (LWL) in *P. harmala*. It was dark brown (DL), black in (SWL) and (LWL) in *T. terrestris*. 8. Distilled water. It showed that the root color was light yellow in (DL), yellow in (SWL) and (LWL) in *F. cretica*

while it was cream in (DL), light cream in (SWL) and (LWL) in *P. harmala*. It was light brown in (DL), (SWL) and black in (LWL) in *T. terrestris*. 9. Iodine. It indicated that root color was dark purple in (DL), light yellow in (SWL) and brown in (LWL) in *F. cretica* while it was purple in (DL), cream in (SWL) and dark brown in (LWL) in *P. harmala*. It was dark brown in (DL), black brown in (SWL) and black in (LWL) in *T. terrestris*. 10. Acetic acid. When subjected to roots showed that it was black in (DL), dark yellow in (SWL) and brown in (LWL) in *F. cretica* while it was purple in (DL), brown in (SWL) and (LWL) in *P. harmala*. It was purple in (DL), light cream in (SWL) and black in (LWL) in *T. terrestris*. 11 Sulphuric acid. On treatment with roots gave light pink color in (DL), dark pink in (SWL) and yellow in (LWL) in *F. cretica* while it was brown in (DL), red in (SWL) and brown in (LWL) in *P. harmala*. It was black in (DL), dark black in (SWL) and black in (LWL) in *T. terrestris*. 12. Conc nitric acid. When added to roots exhibited yellow color in (DL), (SWL) and (LWL) in *F. cretica* while it was light red in (DL), dark brown in (SWL) and brown in (LWL) in *P. harmala*. It was dark brown in (DL), red brown in (SWL) and dark black in (LWL) in *T. terrestris*. 13. Butanol. When tested with roots produce yellow color in (DL), (SWL) and (LWL) in *F. cretica* while it was cream in (DL), orange in (SWL) and dark cream in (LWL) in *P. harmala*. It was brown in (DL), cream in (SWL) and black in (LWL) in *T. terrestris* (Table 74).

**B. Family Euphorbiaceae**

Table 74. summarises the fluorescent characteristic of tested plants.

1. Ammonia. When treated with roots gave purple color in (DL), cream in (SWL) and light yellow in (LWL) in *Chrozophora tinctoria* while it was brown in (DL), brown in (SWL) and black in (LWL) in *Ricinus communis*. 2. Picric acid. It was yellow in (DL), light brown in (SWL) and black in (LWL) in *C. tinctoria*. It was light yellow in (DL), yellow in (SWL) and black yellow in (LWL) in *R. communis*. 3. Chloroform. It exhibited the purple color in (DL), brown in (SWL) and (LWL) in *C. tinctoria*. It became brown in (DL), in (SWL) and black in (LWL) in *R. communis*. 4. Ferric chloride. When subjected to roots gave purple color in (DL), brown in (SWL) and (LWL) in *C. tinctoria* while it was brown in (DL), (SWL) and light black in (LWL) in *R. communis*. 5. Ether. It revealed purple in (DL), cream in (SWL) and (LWL) in *C. tinctoria*. It was light brown in (DL), (SWL) and (LWL) in *R. communis*. 6. Ethanol. It produced purple color in (DL), brown in (SWL) and yellow brown in

(LWL) in *C. tinctoria*. It was light yellow in (DL), (SWL) and cream in (LWL) in *R. communis*. 7. Hydrochloric acid when treated with roots gave yellow purple in (DL), cream in (SWL) and yellow brown in (LWL) in *C. tinctoria*. It was dark brown in (DL), (SWL) and brown in (LWL) in *R. communis* (Table 74). 8. Distilled water. When added to roots exhibited purple color in (DL), cream in (SWL) and yellow black in (LWL) in *C. tinctoria*. It was cream in (DL), brown in (SWL) and black in (LWL) in *R. communis*. 9. Iodine. it was black in (DL), brown in (SWL) and black in (LWL) in *C. tinctoria*. It was yellow in (DL), reddish black in (SWL) and black in (LWL) in *R. communis*. 10. Acetic acid. It gave red in (DL), brown in (SWL) and black in (LWL) in *C. tinctoria*. It was dark brown in (DL), black in (SWL) and (LWL) in *R. communis*. 11 Sulphuric acid. The color was dark black in (DL), black in (SWL) dark black in (LWL) in *C. tinctoria* while it was light yellow in (DL), yellow in (SWL) and black in (LWL) in *R. communis*. 12. Conc nitric acid. On addition it produced yellow purple in (DL), cream in (SWL) and black in (LWL) in *C. tinctoria* while it was light yellow in (DL), yellow in (SWL) and black in (LWL) in *R. communis*. 13. Butanol. It also produced purple in (DL), light cream in (SWL) and cream in (LWL) in *C. tinctoria*. It was brown in (DL), cream in (SWL) and black in (LWL) in *R. communis* (Table 74).

The fluorescence colour is specific for each compound. A non-fluorescent compound might fluoresce if mixed with impurities that are fluorescent. Some constituents show fluorescence in visible range in day light. The UV light produces fluorescence in many natural products. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation (Kumar *et al.*, 2012).

**A. Family Zygophyllaceae,**

**STEM**

Table 74. reveals the fluorescent character of stem of tested plants.

1. Ammonia. It was light green in (DL), light yellow in (SWL) and cream in (LWL) in *F. cretica* while it was yellow brown in (DL), light brown in (SWL) and (LWL) in *P. harmala*. It was dull green in (DL), gray in (SWL) and dull green in

(LWL) in *T. terrestris*. 2. Picric acid. The color was yellow in (DL), light green in (SWL) and light brown in (LWL) in *F. cretica*. It was dark yellow in (DL), light brown in (SWL) and dark brown in (LWL) in *P. harmala*. It was yellow in (DL), (SWL) and yellowish brown in (LWL) in *T. terrestris*. 3. Chloroform. It was confirmed that it was light yellow in (DL), light green in (SWL) and cream in (LWL) in *F. cretica* while it was half white in (DL), light brown in (SWL) and (LWL) in *P. harmala*. It was half white in (DL) and (SWL) and light green in (LWL) in *T. terrestris*.4. Ferric chloride. It exhibited dark green in (DL), light green in (SWL) and light brown in (LWL) in *F. cretica* while it was light brown in (DL), (SWL) and (LWL) in *P. harmala*. It was half white in (DL), (SWL) light green in (LWL) in *T. terrestris*. It was dark green in (DL), dark purple in (SWL) and light brown in (LWL) in *R. communis*. 5. Ether. On treatment with stem gave light green in (DL), (SWL) and half white in (LWL) in *F. cretica*. It was cream in (DL), light brown in (SWL) and half white in (LWL) in *P. harmala*. It was light yellow in (DL) and (SWL) and light brown in (LWL) in *T. terrestris*. 6. Ethanol. When subjected to stems gave light green in (DL), (SWL) and (LWL) in *F. cretica*. It was light brown in (DL) and (SWL) and light green in (LWL) in *P. harmala*. It was light green in (DL), (SWL) and (LWL) in *T. terrestris*. 7. Hydrochloric acid. When added to stems showed light brown in (DL) and (SWL) and dark brown in (LWL) in *F. cretica*. It was light brown in (DL), (SWL) and dark brown in (LWL) in *P. harmala*. It was dark green in (DL), (SWL) and light brown in (LWL) in *T. terrestris*. (Table74). 8. Distilled water. Half white color was seen in (DL), (SWL) and (LWL) in *F. cretica*. It became yellow in (DL), half white in (SWL) and light brown in (LWL) in *P. harmala* while it was yellow in (DL) and (SWL) and half white in (LWL) in *T. terrestris*. 9. Iodine. It was yellow in (DL), light brown in (SWL) and light green in (LWL) in *F. cretica*. It was yellow in (DL), light brown in (SWL) and (LWL) in *P. harmala*. It was yellow in (DL), light brown in (SWL) and (LWL) in *T. terrestris*. 10. Acetic acid. It was light green in (DL), (SWL) and light brown in (LWL) in *F. cretica* while it was yellow in (DL), half white in (SWL) and in (LWL) in *P. harmala*. It was dull green in (DL), half white in (SWL) and (LWL) in *T. terrestris*. 11 Sulphuric acid. The color was black in (DL), dark green in (SWL) and sea green in (LWL) in *F. cretica*. It became black in (DL), dark blue in (SWL) and (LWL) in *P. harmala*. It was black in (DL), dark green in (SWL) and (LWL) in *T. terrestris*. 12. Conc nitric acid. Golden brown color was seen in (DL), (SWL) and (LWL) in *F. cretica*. It was also golden brown in

(DL), light brown in (SWL) and dark brown in (LWL) in *P. harmala.* Golden brown color was seen in (DL), (SWL) and (LWL) in *T. terrestris.* 13. Butanol. It yielded light green in (DL), (SWL) and (LWL) in *F. cretica*. It was yellow in (DL), half white in (SWL) and (LWL) in *P. harmala*. It was light green in (DL), half white in (SWL) and (LWL) in *T. terrestris.* (Table 74).

**Euphorbiaceae**

Table 74. characterizes the fluorescent behavior of stems.

1. Ammonia. The color was yellow green in (DL), (SWL) and half white in (LWL) in *C. tinctoria* while it was light brown in (DL), dark brown in (SWL) and (LWL) in *R. communis*. 2. Picric acid. It produced light green in (DL), (SWL) and cream in (LWL) in *C. tinctoria*. It became yellow in (DL), light brown in (SWL) and dark brown in (LWL) in *R. communis.* 3. Chloroform. It exhibited dark green in (DL), light green in (SWL) light brown in (LWL) in *C. tinctoria* while it was light brown in (DL), (SWL) and (LWL) in *R. communis*. 4. Ferric chloride. It revealed dark green in (DL), (SWL) and (LWL) in *C. tinctoria*. It was light brown in (DL) and (SWL) and cream in (LWL) in *R. communis*.

5. Ether. It revealed light yellow in (DL), light brown in (SWL) and cream in (LWL) in *C. tinctoria*. It was light brown in (DL) and (SWL) and cream in (LWL) in *R. communis*. 6. Ethanol. It has given light green in (DL), dull green in (SWL) and light green in (LWL) in *C. tinctoria*. It was cream in (DL), dark brown in (SWL) and light brown in (LWL) in *R. communis.* 7. Hydrochloric acid .It showed light brown in (DL) and (SWL) and light green in (LWL) in *C. tinctoria*. It was dark brown in (DL), (SWL) and (LWL) in *R. communis* (Table 74). 8. Distilled water. It was light green in (DL), (SWL) and half white in (LWL) in *C. tinctoria*. It was yellow colored in (DL), light brown in (SWL) and (LWL) in *R. communis.* 9. Iodine. It produced light green in (DL), (SWL) and light yellow in (LWL) in *C. tinctoria*. It was dull green in (DL), light brown in (SWL) and dull brown in (LWL) in *R. communis*. 10. Acetic acid. After treatment with stem it showed light green in (DL) and (SWL) and half white in (LWL) in *C. tinctoria*. It was dull green in (DL), light brown in (SWL) and yellow in (LWL) in *R. communis*. 11. Sulphuric acid, it was black in (DL) and (SWL) and black brown in (LWL) in *C. tinctoria*. It was black in (DL) and (SWL) and dark purple in (LWL) in *R. communis*. 12. Conc nitric acid it was light brown in (DL), (SWL) and

(LWL) in *C. tinctoria*. It was golden brown in (DL), (SWL) and (LWL) in *R. communis*. 13. Butanol. It also gave light green in (DL) and (SWL) and half white in (LWL) in *C. tinctoria*. It was dull brown in (DL), (LWL) and light brown in (SWL) in *R. communis* (Table 74). These results disagree with Mughal *et al*. (2010) who reported fluorescence characters of *R. communis* in different solvents.

**A. Family Zygophyllaceae**

**LEAF**

Table 74. indicates the fluorescent characteristic of leaves.

1. Ammonia. The color was light green in (DL), (SWL) and (LWL) in *F. cretica* while it was light brown in (DL), (SWL) and (LWL) in *P. harmala*. It was light green in (DL), (SWL) and (LWL) in *T. terrestris*. 2. Picric acid. It was yellow in (DL), yellow green in (SWL) and light brown in (LWL) in *F. cretica*. It was light brown in (DL), yellow brown in (SWL) and dark brown in (LWL) in *P. harmala*. It was yellow in (DL), light yellow in (SWL) and light brown in (LWL) in *T. terrestris*. 3. Chloroform. Light green color was seen in (DL), (SWL) and (LWL) in *F. cretica*. It changed to half white in (DL), light brown in (SWL) and (LWL) in *P. harmala*. It became light green in (DL), (SWL) and (LWL) in *Tribulus terristris*. 4. Ferric chloride. When added to leaves showed light brown in (DL), (SWL) and dark brown in (LWL) in *F. cretica*. It turned to black in (DL), dark brown in (SWL) and (LWL) in *P. harmala*. It was dull green in (DL) and (SWL) and dull brown in (LWL) in *T. terrestris*. 5. Ether. It was yellowish green in (DL), light brown in (SWL) and yellowish green in (LWL) in *F. cretica*. It became light brown in (DL), dull brown in (SWL) and light brown in (LWL) in *P. harmala*. Its color was light green in (DL) and (SWL) and light brown in (LWL) in *T. terrestris*. 6. Ethanol. The color was light green in (DL), (SWL) and (LWL) in *F. cretica*. It changed to light brown in (DL), dark brown in (SWL) and sea green in (LWL) in *P. harmala*. It became dark green in (DL), dull green in (SWL) and dark brown in (LWL) in *T. terrestris*. 7. Hydrochloric acid. When treated with leaves it was light brown in (DL) and (SWL) and dark green in (LWL) in *F. cretica*. It exhibited dull brown in (DL), light brown in (SWL) and (LWL) in *P. harmala*. It was dull green in (DL) and (SWL) dark green in (LWL) in *T. terrestris* (Table 74). 8. Distilled water. It was light green in (DL), light brown in (SWL) (LWL) in *F. cretica*. It became yellow in (DL), light brown in (SWL) and

(LWL) in *P. harmala*. It was light green in (DL), half white in (SWL) and (LWL) in *T. terrestris*. 9. Iodine. The color was light green in (DL), half white in (SWL) and light green in (LWL) in *F. cretica*. It changed to yellowish brown in (DL), light brown in (SWL) and (LWL) in *P. harmala*. It was light green in (DL) and (SWL) and dark green in (LWL) in *T. terrestris*. 10. Acetic acid. It was light green in (DL), dark green in (SWL) and light brown in (LWL) in *F. cretica*. It became yellowish green in (DL), light green in (SWL) and sea green in (LWL) in *P. harmala*. It exhibited light green in (DL), dark green in (SWL) and light brown in (LWL) in *T. terrestris*. 11. Sulphuric acid. The color was dark brown in (DL), sea green in (SWL) and (LWL) in *F. cretica*. It changed to dark brown in (DL), light blue in (SWL) and sea green in (LWL) in *P. harmala*. It was black in (DL), sea green in (SWL) and dark green in (LWL) in *T. terrestris*. 12. Con nitric acid. Golden brown color occurred in (DL), dark brown in (SWL) and (LWL) in *F. cretica*. It became light brown in (DL) and (SWL) dark brown in (LWL) in *P. harmala*. It was also sea green in (DL), golden brown in (SWL) dark brown in (LWL) in *T. terrestris*. 13. Butanol. it was light green in (DL) and (SWL) and dark yellow in (LWL) in *F. cretica*. It was light brown in (DL), (SWL) and (LWL) in *P. harmala*. It was light green in (DL), (SWL) and (LWL) in *T. terrestris* (Table 74).

**b. Euphorbiaceae**

Table 74. exhibits the fluorescent characteristic of leaves.

1. Ammonia. It showed that it was light pink in (DL), light brown in (SWL) and dark brown in (LWL) in *C. tinctoria*. It was dark green in (DL), dull green in (SWL) and light brown in (LWL) in *R. communis*. 2. Picric acid. When exposed to leaves gave light green color in (DL), (SWL) and (LWL) in *C. tinctoria*. It was dark green in (DL), dull green in (SWL) and dark brown in (LWL) in *R. communis*. 3. Chloroform. When treated with leaves exhibited light brown in (DL) and (SWL) and dark brown in (LWL) in *C. tinctoria*. It was black green in (DL), black in (SWL) and dark brown in (LWL) in *R. communis*. 4. Ferric chloride. When added to leaves produced light green in (DL), (SWL) and dark brown in (LWL) in *C. tinctoria*. It became black in (DL), black purple in (SWL) and dark green in (LWL) in *R. communis*. 5. Ether. It exhibited light green in (DL), (SWL) and dark brown in (LWL) in *C. tinctoria*. It was dull green in (DL), dark brown in (SWL) and dull green in (LWL) in *R. communis*. 6. Ethanol. It exhibited light green in (DL), (SWL) and light

brown in (LWL) in *C. tinctoria*. It was black brown in (DL), (SWL) and dark gray in (LWL) in *R. communis*. 7. Hydrochloric acid. It yielded light green in (DL), (SWL) and light brown in (LWL) in *C. tinctoria*. The leaves show dark gray in (DL), (SWL) and black brown in (LWL) in *R. communis*. 8. Distilled water. When added to leaves exhibited half white in (DL), (SWL) and light brown in (LWL) in *C. tinctoria*. It was dark green in (DL), gray in (SWL) and (LWL) in *R. communis*. 9. Iodine. It was light brown in (DL), (SWL) and (LWL) in *C. tinctoria*. It was dark green in (DL), gray in (SWL) and light brown in (LWL) in *R. communis*. 10. Acetic acid. It was dark green in (DL), (SWL) and dark brown in (LWL) in *C. tinctoria*. It was dull gray in (DL), black in (SWL) and dark gray in (LWL) in *R. communis*. 11. Sulphuric acid. It gave dark brown in (DL), dark green in (SWL) and sea green in (LWL) in *C. tinctoria*. It was black in (DL), dark blue in (SWL) and (LWL) in *R. communis*. 12. Con nitric acid. On treatment with leaves it gave light green color in (DL), (SWL) and (LWL) in *C. tinctoria*. It became reddish brown in (DL), dark brown in (SWL) and light brown in (LWL) in *R. communis*. 13. Butanol. It was light green in (DL), light brown in (SWL) and (LWL) in *C. tinctoria*. It was dark green in (DL), black in (SWL) and light brown in (LWL) in *R. communis* (Table 74). These results disagree with Dhiraj *et al.* (2009), Rajagopal *et al.* (2010), Krishnaveni *et al.* (2008), Mughal *et al.* (2010) and Joseph *et al.* (2001) who reported the fluorescence features of *Jatropha curcas, R. communis* (Euphorbiaceae) and leaves of *Acacia modesta*.

**A. Family Zygophyllaceae**

**FRUIT**

Table 74. summarises the fluorescent characteristic of fruits.

1. Ammonia. It exhibited light green in (DL), light brown in (SWL) and (LWL) in *F. cretica*. It became light brown in (DL), light blue in (SWL) and sea green in (LWL) in *P. harmala*. It was half white in (DL), yellow green in (SWL) and light brown in (LWL) in *T. terrestris*. 2. Picric acid. It was yellow in (DL), light green in (SWL) and light brown in (LWL) in *F. cretica*. The color was light brown in (DL), light green in (SWL) and (LWL) in *P. harmala*. It was yellow in (DL), light brown in (SWL) and (LWL) in *T. terrestris*. 3. Chloroform. Light green was seen in (DL), (SWL) and (LWL) in *F. cretica*. It was black in (DL), light blue in (SWL) and sea green in (LWL) in *P. harmala*. It was cream colored in (DL), light brown in (SWL)

and (LWL) in *T. terrestris*. 4. Ferric chloride. When treated with fruits showed dark green color in (DL), dark brown in (SWL) and dark purple in (LWL) in *F. cretica*. It became reddish black in (DL), dark green in (SWL) and blue green in (LWL) in *P. harmala*. It also changed to dark green in (DL) and (SWL) and dark brown in (LWL) in *T. terrestris*. 5. Ether. When tested with fruits gave light green in (DL), light brown in (SWL) and dull green in (LWL) in *F. cretica*. It was light brown in (DL), dark green in (SWL) and light green in (LWL) in *P. harmala*. It exhibited dark green in (DL), (SWL) and dark brown in (LWL) in *T. terrestris*. 6. Ethanol it showed the color as yellow green in (DL) and (SWL) and light brown in (LWL) in *F. cretica*. It was light brown in (DL), light green in (SWL) and (LWL) in *P. harmala*. It was light green in (DL), dark green in (SWL) and light brown in (LWL) in *T. terrestris*. 7. Hydrochloric acid has revealed light brown in (DL) and (SWL) and dark brown in (LWL) in *F. cretica*. It was light brown in (DL), light green in (SWL) and (LWL) in *P. harmala*. It was light green in (DL), dark green in (SWL) and light brown in (LWL) in *T. terrestris* (Table 74). 8. Distilled water. It was yellow green in (DL), half white in (SWL) and (LWL) in *F. cretica*. It became dark brown in (DL), light brown in (SWL) and dark green in (LWL) in *P. harmala*. It exhibited dark green in (DL), dark brown in (SWL) and dark green in (LWL) in *T. terrestris*. 9. Iodine. It confirmed light green color in (DL), light brown in (SWL) and (LWL) in *F. cretica*. It was light brown in (DL), (SWL) and (LWL) in *P. harmala*. It was yellow in (DL), half white in (SWL) and (LWL) in *T. terrestris*. 10. Acetic acid. The color was yellowish green in (DL), light brown in (SWL) and (LWL) in *F. cretica*. It was light brown in (DL), light green in (SWL) and sea green in (LWL) in *P. harmala*. Finally it became yellow in (DL), half white in (SWL) and light green in (LWL) in *T. terrestris*. 11. Sulphuric acid. It exhibited black color in (DL), dark green in (SWL) and sea green in (LWL) in *F. cretica*. It was black in (DL), green in (SWL) and sea green in (LWL) in *P. harmala*. It was black in (DL), dark green in (SWL) and light green in (LWL) in *T. terrestris*. 12. Con nitric acid. Golden brown color was observed in (DL), and (SWL) and dark brown in (LWL). It was light brown in (DL) and (SWL) and dark brown in (LWL) in *P. harmala*. It was light brown in (DL), golden brown in (SWL) and (LWL) in *T. terrestris*. 13. Butanol. It has revealed light green color in (DL), light brown in (SWL) and (LWL) in *F. cretica*. It was green yellow in (DL), sea green in (SWL) and light green in (LWL) in *P. harmala*. It was yellow in (DL), half white in (SWL) and light green in (LWL) in *T. terrestris*. (Table74). These findings agree with Mohanty *et*

*al.* (2010) who reported the fluorescence features of *T. terrestris*. These findings disagree with Raja and Venkataraman (2011) and Hussain *et al.* (2011) who reported the phytochemical analysis (fluorescence) features of fruits of *T. terrestris*. These findings disagree with the results of Rajkala *et al.* (2011) who worked on the phytochemical analysis of fruit of *T. terrestris*.

**b. Euphorbiaceae**

Table 74. indicates the fluorescent characteristic of fruit.

1. Ammonia. It produced brown color in (DL) and (SWL) light black in (LWL) in *C. tinctoria*. It was brown in (DL) and (SWL) and black in (LWL) in *R. communis*. 2. Picric acid .It became brown in (DL), dark brown in (SWL) and black in (LWL) in *C. tinctoria*. It was light brown in (DL), yellow in (SWL) and brown in (LWL) in *R. communis*. 3. Chloroform. When added to fruits gave brown in (DL), cream colored in (SWL) and black in (LWL) in *C. tinctoria*. It was brown in (DL), cream colored in (SWL) and brown in (LWL) in *R. communis*. 4. Ferric chloride. When exposed to fruits yielded purple in (DL) Black in (SWL) and (LWL) in *C. tinctoria*. It was dark brown color in (DL) and (SWL) and black in (LWL) in *R. communis*. 5. Ether. It gave the brown color in (DL), cream colored in (SWL) and (LWL) in *C. tinctoria*. It was dark brown in (DL), cream in (SWL) and black in (LWL) in *R. communis*. 6. Ethanol. It exhibited purple color in (DL), dark cream in (SWL) and light black in (LWL) in *C. tinctoria*. It was brown in (DL), cream in (SWL) and black in (LWL) in *R. communis*. 7. Hydrochloric acid. On treatment with fruits produced pink brown in (DL), black in (SWL) and black in (LWL) in *C. tinctoria*. It was brown in (DL), yellow in (SWL) and brown in (LWL) in *R. communis* (Table 74). 8. Distilled water. It was black in (DL), light brown in (SWL) and (LWL) in *C. tinctoria*. It was dark brown in (DL), light brown in (SWL) and black in (LWL) in *R. communis*. 9. Iodine. When added to fruits it produced light brown in (DL), (SWL) and gray in (LWL) in *C. tinctoria*. It was dark brown in (DL), brown in (SWL) and black in (LWL) in *R. communis*. 10. Acetic acid. It has revealed gray color in (DL), light brown in (SWL) and yellow in (LWL) in *C. tinctoria*. It was light purple in (DL), brown in (SWL) and black in (LWL) in *R. communis*. 11. Sulphuric acid. When exposed to fruits gave black color in (DL), dark green in (SWL) and light green in (LWL) in *C. tinctoria*. It was black in (DL) and (SWL) and dark green in (LWL) in *R. communis*. 12. Con nitric acid. Golden brown in (DL) and

(SWL) and dull brown in (LWL) in *C. tinctoria*. It was orange in (DL), orange in (SWL) and brown in (LWL) in *R. communis*. 13. Butanol. It was black in (DL), light brown in (SWL) and (LWL) in *C. tinctoria*. It was orange in (DL), light black in (SWL) and black in (LWL) in *R. communis* (Table 74). These findings are in agreement with Joseph *et al*. (2001) who reported the fluorescence analysis of *Jatropha curcas* (Euphorbiaceae). These findings disagree with Shruthi *et al*. (2010) who reported the fluorescence analysis of *Kirganelia reticulata* (Euphorbiaceae). Mughal *et al*. (2010) worked on *Euphorbia prostrata, Euphorbia hirta, Euphorbia splendens, R. communis* and *Jatropha integearrima* (Euphorbiaceae) for fluorescence analysis and their results disagree with the present case.

The fluorescence analysis represent the behavioral changes of the powder plant parts with different chemicals which can dentify the plants (Sunita *et al*. 2011). This can show the presence of secondary metabolites. This is as a result of conjugation between the double bonds from the five oxygen atoms with the single bonds present in the structure allowing absorption of the UV light. The different colours of the fluorescence rings are due to different atoms present in the compound having different wavelengths. When atoms are excited to a higher energy level, they may fall back to their original position using the same or a different wavelength resulting to emission of different colours (Pascaline *et al*. 2011).

Table 58. Fluorescence character of some plants of family Zygophyllaceae and Euphorbiaceae.

| FAMILY | | ZYGOPHYLLACEAE | | | | | | | | | | | | EUPHORBIACEAE | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Fagonia cretica* L. | | | | *Peganum harmala* L. | | | | *Tribulus terrestris* L. | | | | *Chrozophora tinctoria* (L.) Raf | | | | *Ricinus communis* L. | | | |
| Chemicals | Part | RT | ST | Lvs | Fr | Rt | ST | Lvs | Fr | RT | ST | Lvs | Fr | RT | ST | Lvs | Fr | RT | ST | Lvs | Fr |
| Ammonia | DL | LY | LGr | LGr | LGr | Cr | YBr | LBr | LBr | Br | DuGr | LGr | Hw | P | YGr | LP | Br | Br | LBr | DGr | Br |
| | SWL | DY | LY | LGr | LBr | Ly | LBr | LBr | LBlue | Cr | Gy | LGr | YGr | Cr | YGr | LBr | Br | Br | DBr | Du Gr | Br |
| | LWL | Gy | Cr | LGr | LBr | Br | LBr | LBr | SeaGr | Br | DuGr | LGr | LBr | Ly | Hw | DBr | LBlk | Blk | DBr | LBr | Blk |
| Picric acid | DL | DY | Y | Y | Y | Y | DY | LBr | LBr | Br | Y | Y | Y | Y | LGr | LGr | Br | LY | Y | DGr | LBr |
| | SWL | Y | LGr | YGr | LGr | Y | LBr | YBr | LGr | Ly | Y | Ly | LBr | LBr | YGr | LGr | DBr | Y | LBr | DuGr | Y |
| | LWL | DBr | LBr | LBr | LBr | DBr | DBr | DBr | LGr | Br | YBr | LBr | LBr | BLK | Cr | LGr | Blk | BlkY | DBr | DBr | Br |
| Chloroform | DL | Ly | LY | LGr | LGr | Cr | HW | Hw | BLK | DBr | Hw | LGr | Cr | P | DGr | LBr | Br | Br | LBr | BlkGr | Br |
| | SWL | Ly | LGr | LGr | LGr | LB | LBr | LBr | LBlu | Cr | Hw | LGr | LBr | Br | LGr | LBr | Cr | Br | LBr | Blk | Cr |
| | LWL | Y | Cr | LGr | LGr | Cr | LBr | LBr | SeaGr | Br | LGr | LGr | LBr | Br | DGr | DBr | Blk | Blk | LBr | DBr | Br |
| Ferric chloride | DL | Ly | DGr | LBr | DGr | Cr | LBr | BLk | RBlk | DBr | Hw | DuGr | DGr | P | DGr | DBr | P | Br | LBr | Blk | DBr |
| | SWL | Ly | LGr | LBr | DBr | Cr | LBr | DBr | DGr | Cr | Hw | DuGr | DGr | Br | DGr | LGr | Blk | Br | LBr | BlkP | DBr |
| | LWL | Y | LBr | DBr | DP | Br | LBr | DBr | BluGr | Br | LGr | DuBr | DBr | Br | DGr | DGr | Blk | LBlk | Crm | DGr | Blk |
| Ether | DL | LY | LGr | YGr | LGr | Cr | Crm | LBr | LBr | LBr | Ly | LGr | DGr | P | LY | LGr | Br | LBr | LBr | Du Gr | DBr |
| | SWL | Y | LGr | LBr | LBr | LCr | LBr | DuBr | DGr | Cr | Ly | LGr | DGr | Cr | LBr | LGr | Cr | LBr | LBr | DBr | Cr |
| | LWL | Y | HW | YGr | DuGr | Cr | Hw | LBr | LGr | LB | LBr | LBr | DBr | Cr | Cr | DBr | Cr | LBr | Crm | Du Gr | Blk |
| Ethanol | DL | Ly | LGr | LGr | YGr | Y | LBr | LBr | LBr | DBr | LGr | DGr | LGr | P | LGr | LGr | P | LY | Crm | BlkBr | Br |
| | SWL | Ly | LGr | LGr | YGr | DBr | LBr | DBr | LGr | LB | LGr | DuGr | DGr | Br | DuGr | LGr | DCr | LY | DBr | Blk Br | Cr |
| | LWL | Ly | LGr | LGr | LBr | B | LGr | SeaGr | LGr | B | LGr | DBr | LBr | YBr | LGr | LBr | LBlk | Cr | LBr | DGy | Blk |
| Hydrochlor ic acid | DL | Br | LBr | LBr | LBr | Y | LBr | DuBr | LBr | DBr | DGr | DuGr | LGr | YP | LBr | LGr | PiBr | DBr | DBr | DGy | Br |
| | SWL | Br | LBr | LBr | LBr | Br | LBr | LBr | LGr | B | DGr | DuGr | DGr | Cr | LBr | LBr | Blk | DBr | DBr | DGy | Y |
| | LWL | Br | DBr | DGr | DBr | LBr | DBr | LBr | DGr | B | LBr | DGr | LBr | YBr | LGr | LBr | Blk | Br | DBr | BlkBr | Br |
| Distilled. water | DL | Ly | HW | LGr | YGr | Cr | Y | Y | DBr | LBr | Y | LGr | DGr | P | LGr | Hw | Blk | Cr | Y | DGr | DBr |
| | SWL | Y | HW | LBr | Hw | LCr | Hw | LBr | LBr | LBr | Y | Hw | DBr | Cr | LGr | Hw | LBr | Br | LBr | Gy | LBr |
| | LWL | Y | HW | LBr | Hw | LCr | LBr | LBr | DGr | B | Hw | Hw | DGr | YBlk | Hw | LBr | LBr | Blk | LBr | Gy | Blk |
| Iodine | DL | Dp | Y | LGr | LGr | P | Y | YBr | LBr | DBr | Y | LGr | Y | BLK | LGr | LBr | LBr | Y | DuGr | DGr | DBr |
| | SWL | LY | LBr | Hw | LBr | Cr | LBr | LBr | LBr | Br | LBr | LGr | Hw | Br | LGr | LBr | LBr | RBlk | LBr | Gy | Br |
| | LWL | Br | LGr | LGr | LBr | DBr | LBr | LBr | LBr | B | LBr | DGr | Hw | Blk | LY | LBr | Gy | Blk | DuBr | LBr | Blk |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acetic acid | DL | B | LGr | LGr | YGr | P | Y | YGr | LBr | P | DuGr | LGr | Y | R | LGr | DGr | Gy | DBr | DuGr | DuGy | LP |
| | SWL | DY | LGr | DGr | LBr | Br | Hw | LGr | LGr | LGr | Hw | DGr | Hw | Br | LGr | DGr | LBr | Blk | LBr | Blk | Br |
| | LWL | Br | LBr | LBr | LBr | Br | Hw | SeaGr | SeaGr | B | Hw | LBr | LGr | Blk | Hw | DBr | Y | Blk | Y | DGy | Blk |
| Sulphuric acid | DL | LPi | BLk | DBr | Br | Br | B | DBr | BLk | B | BLk | Blk | Blk | DB | Blk | DBr | Blk | LY | Blk | Blk | Blk |
| | SWL | Dpi | DGr | SeaGr | DGr | R | D Blu | LBl | Gr | DB | DGr | SeaGr | DGr | Blk | Blk | DGr | DGr | Y | Blk | DBlu | Blk |
| | LWL | Y | SeaGr | SeaGr | SeaGr | Br | D Blu | SeaGr | SeaGr | B | DGr | DGr | LGr | DB | BlkBr | SeaGr | LGr | Blk | DP | DBlu | DGr |
| Nitric acid | DL | Y | GBr | GBr | GBr | LR | GBr | LBr | LBr | DBr | GBr | SeaGr | LBr | OR | YBr | LGr | GBr | LY | GBr | RBr | Or |
| | SWL | Y | GBr | DBr | GBr | DBr | LBr | LBr | LBr | RBr | GBr | GBr | GBr | OR | YBr | LGr | GBr | Y | GBr | DBr | Or |
| | LWL | Y | GBr | DBr | DBr | Br | DBr | DBr | DBr | DR | GBr | DBr | GBr | OBr | YBr | LGr | DuBr | Blk | GBr | LBr | Br |
| Butanol | DL | Y | LGr | LGr | LGr | Cr | Y | LBr | GrY | Br | LGr | LGr | Y | P | LGr | LGr | Blk | Br | DuBr | DGr | Or |
| | SWL | Y | LGr | LGr | LBr | OR | HW | LBr | SeaGr | Cr | Hw | LGr | Hw | Lcr | LGr | LBr | LBr | Cr | DuBr | Blk | LBlk |
| | LWL | Y | LGr | Dy | LBr | OR | HW | LBr | LGr | B | Hw | LGr | LGr | Cr | Hw | LBr | LBr | Blk | LBr | LBr | Blk |

Key: P=Part ; Rt=Root; St=Stem; Lvs =Leaf; Fr= Fruit; DL= Day light, S.W= Short wavelength ; L.W= Long wavelength ;Bl=Blue, Br=Brown; Blk=Black; Cr= Cream; D=Dark; Du=Dul; G=Golden; Gr=Green; Gy=Gray; H.W=Half white; L=Light; R=Red; Sea= Sea Green; OR=Orange; P=Purple; Pi=Pink; Y=Yellow; W=White.

# CHAPTER-11

# GENERAL CONCLUSIONS AND RECOMMENDATIONS

# CHAPTER 11

# GENERAL CONCLUSIONS

## 1. Conclusions

Following conclusions could be drawn from the present study.

1. The macroscopic, microscopic features, preliminary phytochemical screenings and fluorescence analysis have been used as a diagnostic tool in the identification of the investigated plants and also to establish the quality and purity of these valuable drugs.
2. The epidermis that shows variation including stomatal size, shape, frequency, epidermal cells shape, stomata type, distribution and stomata index are of taxonomic importance in both the families.
3. Leaf epidermal anatomy can be used to solve taxonomic problem in the identification of species.
4. Differences in epidermal structure and stomata arrangements are also prominent features for separating related species.
5. The present results show that the selected plants possessed antimicrobial activity which justifies its use in traditional medicine in the treatment of microbial infection. Plants can be used as an alternative for chemical compounds. The activities observed could be due to the presence of some of the phytochemicals detected which might have been associated with antimicrobial activity. The study initiated on the effectiveness of these medicinal plants enable to bring these plants to the business front. This will definitely help in the economic development of the country by preventing the purchase of foreign drugs and consequently the foreign exchange as well. This study has yielded the hope that these drugs may be used as substitute for antibiotics provided that drugs quality and quantity is standardized.
6. These plants could be a cheaper substitute for conventional drugs since the plants are easily available and the extract can easily be made.
7. The cytotoxicity exhibited by the present plants clearly indicates the presence of potent bioactive compounds and they might have antitumor or pesticidal activity. These cytotoxic samples may have clinical and therapeutic

proposition in the most life threaten disease like tumor or cancer and further bioactivity guided investigation can be done to find out potent antitumor and pesticidal agents. In addition, *in vivo* and *in vitro* with different human cell lines study is needed to demonstrate the antitumor activity of these plants and isolated the lead compound responsible for cytotoxic activity, which might be utilized for the development of novel anticancer drug.

8. In insecticidal activity all the plants showed significant mortality of *Tribolium castaneum* as compared to control and these plants can be used in the control of *Tribolium castaneum* population with integrated pest management which seems to be economically feasible and ecologically sound. Similarly, plants were also effective against *Heterotermes indicola* (termites) with significant differences among plants and control and these plants possessed toxic principles with significant insecticidal effect and could be a potential grain protectant against *Tribolium castaneum*. These plants could be used singly as potential bioactive agents for the management of termites particularly by our poor farmers who cannot afford to buy expensive insecticides from markets. The plants extracts could be used in the infected houses. These plants could be used for the isolation of active constituents as plants are promising source of pest control compounds that have generated extraordinary interest in the recent years as a potential source of natural insect pest control agents. Botanical pesticides tend to have broad-spectrum activity, are relatively specific in their mode of action, easy to process, produce, use and safe for higher animals and the environment. They are cost effective and may abate the environmental pollution and health hazards.

9. Considering the overall comparative, it was seen that the average values revealed that *Peganum harmala* excelled in high fat, carbohydrate, protein and moisture contents than other two species; therefore it can be considered a good nutritive plant followed by *Fagonia cretica* that contained the highest fibre. The *Tribulus terrestris* had the maximum protein and GE. Among the tested Euphorbiaceae plant *Chrozophora tinctoria* and *Ricinus communis* also had variation in various analysed biochemicals. The average values indicated that *Chrozophora tinctoria* had high the moisture contents, ash contents, protein, fats, fibre, carbohydrate and gross energy than its counterpart *Ricinus communis*

10. This study could be a beneficial source to the dieticians/nutritionist to consider these plants as best nutraceutical plants for manufacturing commercially viable food products.
11. The investigated plants can significantly add to the nutritional requirements of people and should be used as a source of nutrients to supplement other major sources. The analysed plants also have high biological activities and therefore are of great medicinal value.
12. These nutritional parameters Proteins, fats, fibers and carbohydrates have shown medicinal value as adequate intake of dietary fiber can decrease serum cholesterol levels, risk of coronary heart diseases, hypertension, diabetes, colon and breast cancer. Fibers increase digestibility and restore bowel action. Proteins have antifungal, antibiotic properties and effective against tapeworm infrstation. Carbohydrates stimulate the immune system and have anti-peptic, anti-inflammatory and anti-microbial properties.
13. The present study of elements with respect to indigenous medicinal plant shows that macro and micro-nutrients have significant roles in combating a variety of diseases and human ailments. This study could be helpful to determine the dosage to be administered to patients considerind elemental contents and concentrations. This could also help to understand the pharmacological action of these plant.
14. Phytochemical screening of these plants revealed the presence of tannins, saponins, phenols, terpenoids, steroid, alkaloids and flavonoids. The presence of these bioactive components may be responsible for antibacterial, antifungal, cytotoxic, phytotoxic, insecticidal activities. These plants could be potential sources of new antimicrobial, cytotoxic, phytotoxic, insecticidal agents. The presence of the chemical constituents shows the potential of these plants as a source of therapeutic agents and may provide leads in the ongoing search for antimicrobial agent from plants.
15. In recent decades, essential oil of plants have drawn great importance as sources of natural products in both academia and the food, cosmetic and pharmaceutical industries, since their possible use as natural additives emerged from a growing tendency to replace synthetic preservatives by natural ones.

Essential oil study showed that in *Fagonia cretica* oils, 17 compounds were identified constituting 100% of the oil composition. Oxygenated monoterpenes (89.94%) was a major group of compounds. *Peganum harmala* oils had 18 phytochemicals. There were 10 compounds in *Tribulus terrestris* oils that consisted 100% of the total oil composition. Eight compounds were identified in *Chrozophora tinctoria* oils giving complete oil composition. It had oxygenated monoterpenes 86.93%, constituting of 2(4H) - Benzofuranone, 5, 6, 7, 7a tetrahydro-4, 4, 7a-trimethy 50.718%. *Ricinus communis* oil had 8 compounds with 100% of the oil composition. Some oils showed to have applications in food preservation, aromatherapy, and pharmacological properties as antibacterial, antifungal, antioxidant, spasmolytic, antiplasmodial, anti-inflammatory, and anticancer activities. Camphor and 1, 8- cineole as well as borneol were well-known chemical with their pronounced antimicrobial properties.

The present results also indicate the possibility of exploitation of the essential oils of five plants as an effective antibacterial, antifungal, cytotoxic, phytotoxic, insecticidal agents. These oils can be further tested against the same bacteria, fungi, brine shrimps, *Lemna minor, Tribolium* and termites to find out oils efficacy and potential and further isolation can be done to find out the bioactive oils constituents. Our findings suggest that the essential oil from five plants have potential as antibacterial, antifungal and antioxidant agent and could be useful in the pharmaceutical and cosmetics industries. Research is needed to prove this.

*Fagonia cretica, Tribulus terristris* and *Chrozophora tinctoria* are used ethnobotanically and for the treatment of different diseases in various parts of the country. *Fagonia cretica* is sold by the local people in the crude form in KPK, but also available in selected markets. Similarly, *Tribulus terristris* and *Chrozophora tinctoria* are neither sold in any form in the country nor available commercially in the drug markets. Still these plants have medicinal and commercial value as *Fagonia cretica* leaves are used for skin lesions and applied over snake and scorpion bite to reduce pain. *Tribulus terristris* leaves aqueous extract is used to treat cough. Its seeds are used to stop bleeding fron nose. Economically, *Tribulus terristris*is antiseptic, tonic and demulcent. Leaves are used as pot herb. Plant ash is used in rheumatism. Paste of herb is effective for stones in bladder.

# RECOMMENDATIONS

**iii. Recommendations**

On the basis of this study and conclusion following recommendations are forwarded for future workers and pharmaceutical industry.

1. These findings will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of the plants, which is gaining relevance in plant drug research.
2. Other pharmacological activities e.g., anticancer, antidiabetic, antilashmanial, antiinflammatory and analgesic etc can be carried out in future to assess these plants therapeutic potential. There are numerous opportunities for those in academic instititions and in research areas particularly to perform highly socially relevant research on these botanical products in the field of pharmaceutical industries and health sciences as a whole.
3. It is desirable to make in-depth pharmacolgical, biological and clinical studies to adjudicate the veracity of the claim
4. As the plants have shown significant cytotoxic activity, therefore activity guided isolation and characterization to uncover the active phytochemicals can be carried out.
5. As effective insecticidal activities were observed, more research should be directed towards isolation of insecticidal bioactive compounds as well as further field trials can be carried out to confirm the present findings.
6. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent drugs of natural origin.
7. Due to the prevalence of various diseases, further research on macro and micronutrients can be carried out, whose functions are still not known. To encourage the curative effects of medicinal plants, it is needed to investigate the elemental composition of all the medicinal plants.
8. The cultivation of *Ricinus communis* should be encourged on large scale for the development of biodiesel that will help people. More oil can be obtained that will not only help in the treatment of different diseases but can also be exported to other countries. As oil is inexpensive and environmental friendly.

It will boost up the economic condition of the local communities who would be involved in different cultural practices to get the maximum yield of this oilseed crop. More seeds can be obtained that will be helpful for pharmaceutical, cosmetic, food and insecticidal industries. Plant reportedly has shown antioxidant activity. The oil can be used for industrail purposes e.g., coating fabrics, protective coverings, manufacture of high-grade lubricants, textile dyeing, leather preservation and fiber production. All such activities will strength the economy of the country.

# CHAPTER-12
# REFERENCES

# CHAPTER 12

## REFERENCES

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