

ANATOMICAL AND PHARMACOGNOSTIC STUDY OF *IPHIONA GRANTIOIDES* (BOISS.) ANDERB AND *PLUCHEA ARGUTA* BOISS. SUBSP. *GLABRA* QAISER

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Abstract

Anatomical and pharmacognostic study of two plants *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* of family asteraceae was carried out during 2014. *Iphiona grantioides* is a perennial herb, with soft aerial parts and woody base, covered with glandular trichomes. *Pluchea arguta* subsp. *glabra* is an erect, branched, stout shrub having pungent smell, with terete and glabrous stem with obovate, dentate, sessile, glabrous leaves varying 1.5 to 3cm in length and 0.3 to 2.0 cm in width. In *Iphiona grantioides*, stomatal number and stomatal index value were 120 to 150 (130) and 12 to 15 (13) per mm². Vein islet and vein termination number were 8 to 10 (9) and 7 to 10 (8) per mm² respectively, while the palisade ratio ranged between 5 to 6 (6.75). In *Pluchea arguta* subsp. *glabra* stomatal number and stomatal index value were in the range of 110 to 160 (130) and 10 to 12 (11) per mm² and vein islet and vein termination number were 10 to 12 (11) and 6 to 9 (8) per mm² respectively, with 6 to 7 (7.5) palisade ratio. Anatomical study of stems of both *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*, share almost same features. Higher total ash content was recorded for stem (7.06%) compared to leaf value (3.33%) for *Iphiona*. In *Pluchea arguta* subsp. *glabra* total ash values were in the range of 7.82% (leaf), 6.12% (stem) to 5.6% (root). Leaf powder of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* showed presence of different tissues. Powder drug exhibited fluorescence when treated with different reagents under UV and ordinary day light. Results of extraction with solvents revealed that highest extractive values were observed for the leaf aqueous extract of *Iphiona grantioides* (46.2%), and leaf ethyl acetate extract of *Pluchea arguta* subsp. *glabra* (30%). The present study will be helpful in the identification of these two important medicinal plants.

Key words: *Iphiona grantioides*, *Pluchea arguta* subsp. *glabra*, Extractive values, Ash contents, Fluorescence.

Introduction

Iphiona grantioides (Boiss.) Anderb. belongs to *Inuleae*, tribe of Asteraceae, growing on a wide range of soils including saline and arid plains (Abid & Qaiser, 2003). It is a perennial herb, 15-60 cm tall; the areal parts are glandular with hairy fleshy, lobed leaves covered with trichomes. *Pluchea arguta* Boiss. subsp. *glabra* Qaiser belongs to *Pluchea* tribe of the same family. It is commonly known as camphor weeds, pluchaeas, or as "fleabanes". Mostly these plants are resinous and bushy shrubs. (Qaiser & Abid, 2003).

Plants based products have been used as therapeutic alternatives in the health-care systems throughout the world. Medicinal plants are important sources of bioactive compounds, as their easy availability and low cost are the main reasons that justify their widespread application. The World Health Organization (WHO) has published several guidelines that aim at improving the quality, effectiveness and safety of the herbal medicinal preparations (Anon., 2002) and in order to make sure the safe use of these medicines, a necessary first step is the establishment of standards of quality, efficacy and safety (Bhat *et al.*, 2012). Pharmacognostic study of crude drugs gives scientific information regarding the quality and purity of plant derived drugs (Sindhu, 2012) and the valuable information thus obtained regarding morphology, macroscopic, microscopic and physical characteristics of crude drugs are found significant in the delimitation and classification of medicinal plants and can be used as tool in the identification of taxonomic problems at species and tribal levels (Rodríguez *et al.*, 2017; Teke & Binzet, 2017; Khan *et al.*, 2017). Both the

plants *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* have been studied for its medicinal evaluation (Naveed *et al.*, 2016), however, as no data was available on its anatomical characteristics so anatomical and pharmacognostic study of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* was carried out, including macroscopic description of plants, anatomy of leaf, stem and root, constant values of leaf (stomatal index, vein islet number and palisade ratio), moisture contents, ash values, fluorescence study and extractive values.

Materials and Methods

Fresh specimens of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* were collected from saline area of District Karak, in 2013. Plants samples were cleaned, washed and separated into leaf, stem roots and dried in shade for two weeks. Macroscopic characters were studied at the time of collection, while dried specimens were grinded in electric grinder, sieved, powdered and preserved in airtight bottles to keep it safe from moisture deterioration, for future use. Fresh specimens of both the plants were preserved for section cutting, stomatal and histological studies. Morphological studies of various parts (root, stem, leaf) of two plants were carried out following Trease & Evans (1985) and Evans, (2002). Thin transverse sections of leaf, stem and root of both plants were studied by following Chaffey, 2001; Dilcher 1974 and Evans 2002. Ash values (total ash, acid insoluble, water soluble ash and moisture contents of both the plants were determined by following Wallis, 1985; Anon., (2000) and Jarald & Jarald (2007). Powder drug study was carried out by following Wallis (1985). Powder drug

of each plant part was taken on clean slide, treated with various reagents and studied under visible day light and UV light (short and long wave lengths) by following Evans, (2002) for the fluorescence study. Powdered specimen of both plants were extracted with different solvents including acetone, butane, ethanol, ethyl acetate, n- Hexane, methanol and water for the determination of extractive values (Khandelwal, 2004). Arithmetic mean and standard deviation were used to analyze the data statistically (Saeed *et al.*, 2010).

Results and Discussion

Morphological studies revealed that *Iphiona grantioides* is a perennial herb, with soft aerial parts and woody base. Stem is fleshy, densely covered with glandular trichomes (Fig. 1a). Leaf length varies from 3-5cm and 0.8-1.5cm wide. Tape root is slightly fleshy, aromatic, provided with rootlets. These observed features are of taxonomic importance and could be used for proper identification of *Iphiona grantioides*. *Pluchea arguta* subsp. *glabra* is an erect, branched, stout shrub having pungent smell, with terete and glabrous stem and branches (Fig. 1b). Leaf is alternate, sessile, 1.5-3 cm long, 0.3-2.0 cm wide, attenuate at base, oblong or obovate, acute to subacute, coarsely serrate/ dentate, 5-7 toothed, glabrous on both surfaces. Root is tape root, slightly twisted, hard and woody, dark brown in colour. These observations are further supported by work of Toma *et al.* (2010) and Khan *et al.* (2010). The present observation will set additional parameters for evaluation of correct identification of these two plants.

Transverse section (T.S.) of *Iphiona grantioides* leaf (Fig. 2a) and *Pluchea arguta* subsp. *glabra*. (Fig. 3a) showed the presence of single layered upper and lower epidermis composed of rectangle shaped epidermal cells with thick cuticle on the outer side and covered with multicellular, biseriate, capitate glandular trichomes and non-glandular, uniseriate hairs with long pointed apical cell in *Iphiona* and sessile glandular trichomes, surrounded by a group of 5-9 epidermal cells, in *Pluchea*. Leaf is bifacial in both plants, mesophyll tissue is heterogeneous, with multicellular palisade tissue and spongy parenchyma tissue beneath. Palisade cells have chloroplast and oil droplets. Spongy mesophyll cells have cuboid crystals of calcium oxalate in *Iphiona* and oil globules in both plants. Meric (2009) confirms the presence of Ca Oxalate in some species of the tribe *Inuleae*. In *Iphiona*, in the midrib region, the mid-vein is very prominent, having 1-2 vascular bundles with sclerenchymatic cells on the lower side only; while in *Pluchea*, there are 3 vascular bundles in the midrib region with the central comparatively larger one, and surrounded by collenchyma on both sides. Vascular bundles are collateral and closed and Xylem vessels are provided with spiral wall thickening.

Stomata occurs mostly on leaf surface and are very important taxonomic character and are quite helpful in phylogeny tracing the higher level of taxonomic hierarchy (Perveen *et al.*, 2007). Leaves of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* are amphistomatic with anomocytic type of stomatal apparatus as well as with few diacytic type in *Pluchea arguta* (Dilcher, 1974).

Stomatal number and stomatal index value for *Iphiona grantioides* were 120 to 150 (130) and 12 to 15 (13) per mm² respectively, Vein islet and vein termination number were 8 to 10 (9) and 7 to 10 (8) per mm², respectively. The vein-islets were quite distinct as squares, elongated or polygonal in shape, internally provided with many forked vascular branches. The palisade ratio ranged between 5 to 6 (6.75) (Fig. 2b, c). In *Pluchea arguta* subsp. *glabra* stomatal number and stomatal index value were in the range of 110 to 160 (130) and 10 to 12 (11) per mm², respectively. Vein islet and vein termination number were 10 to 12 (11) and 6 to 9 (8) per mm² respectively, with 6 to 7 (7.5) palisade ratio (Fig. 3b, c). Many workers have reported palisade ratio, vein islet number and vein termination values for a number of plants including plants of family Solanaceae (Hameed & Hussain, 2011) *Lamium pisidicum* (Baran *et al.*, 2013) and *Pelargonium graveolens* (Boukhris *et al.*, 2013). Stomatal index values are very important and useful to distinguish between leaves of co-generic species (Evans, 2002).

Anatomical study of stems of both *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*, share some common features. Stem of both plants have single layered epidermises, covered with multi-cellular glandular and none glandular trichomes in *Iphiona* (Fig. 2d), and sessile glandular trichomes in *Pluchea* (Fig. 3d). Hypodermis is multilayered and chlorenchymatous in *Iphiona grantioides*; while 1-2 cell in thickness in *Pluchea arguta* subsp. *glabra*. General cortex is multilayered and parenchymatous. Sclerenchymatous pericycle lies inside the endodermis and is the outermost part of the stele, surrounding conjoint, collateral and open vascular bundles, arranged in a ring. Medullary rays are present between the vascular bundles, spreading from the parenchymatous pith. Cross section of the root of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* have shared some common characteristics. The outermost layer is rhizodermis (phelloderm) followed by cortex, secondary phloem with phloem fibers on the outside and secondary xylem on the inner side. Secondary xylem has large and well developed and wide lignified vessels while primary xylem occupy the center of the root. Primary and secondary xylem are separated by numerous multiseriate primary and secondary medullary rays, respectively (Figs. 2e-3e).

Many other medicinal plants have been investigated anatomically, e.g., *Zanthoxylum aramatum* (Barkatullah, 2014); *Spiraea* L. species (Rosaceae) (Omer *et al.*, 2017) and comparative study of leaf (Rodríguez *et al.*, 2017) for its anatomical characteristics. Results obtained in the present study are strongly supported by Moudi *et al.* (2017). Sultana & Zafar (2013) authenticated the herbal medicine *Lawsonia innermis* by using taxonomic and pharmacognostic techniques. Similarly Teke *et al.*, (2017) explained the anatomical and micro-morphological differentiation of the genus *Onosma* L. (Boraginaceae). Khan *et al.*, (2017) carried out the comparative foliar micro morphological studies of tribe *Arundineae*, *Aristideae* and *Chlorideae* and further supported the importance of anatomical studies in the identification, authentication and classification of medicinal plants.



Fig.1.a. *Iphiona grantioides* (Boiss)



Fig.1. b. *Pluchea arguta* subsp. *glabra*

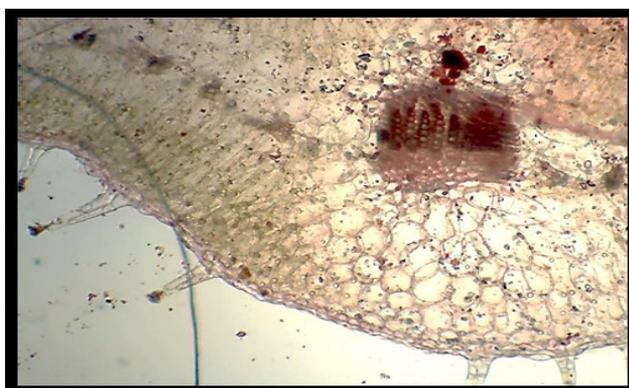


Fig. 2a. T.S. of leaf of *Iphiona grantioides*.

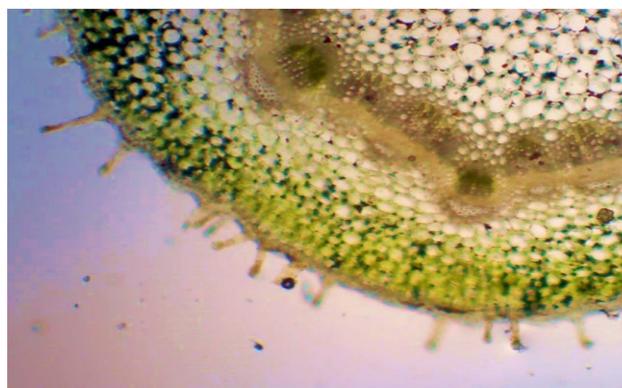


Fig. 2d. T.S of *Iphiona grantioides* stem—Glandular trichome, epidermis, hypodermis, cortex and vascular bundles arranged in a ring (10x).

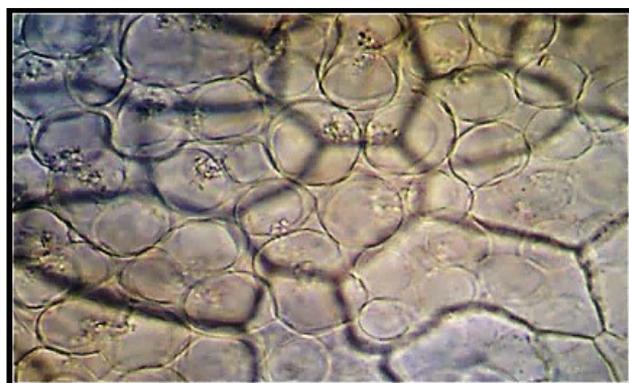


Fig. 2b. *Iphiona grantioides* leaf. Palisade cells arrangement under epidermal cells.

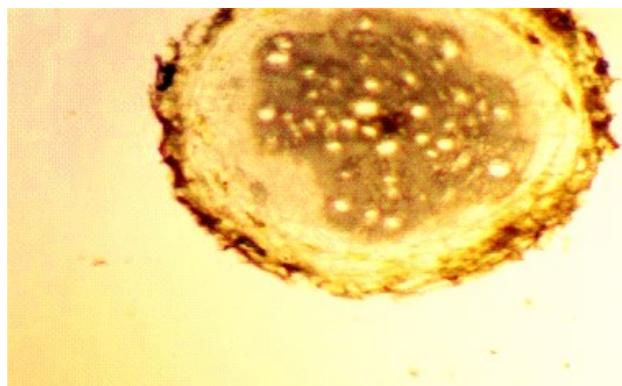


Fig. 2e. T.S. of *Iphiona grantioides* root.



Fig. 2c. *Iphiona grantioides* leaf. Veins arrangement in lamina with vein islet number and vein termination number.



Fig. 3a. T.S. of *Pluchea arguta* subsp. *glabra*, leaf.

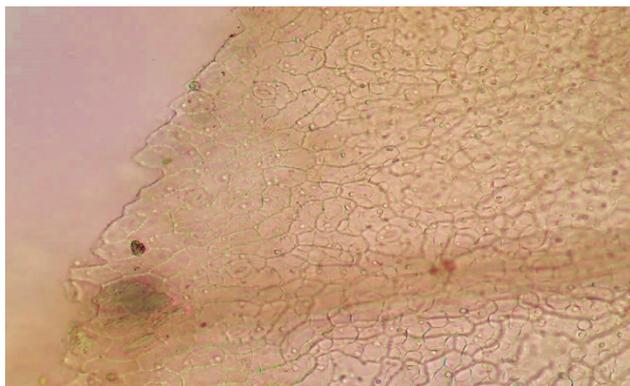


Fig. 3b. *Plucheia arguta* subsp. *glabra* leaf. Palisade cells under epidermal cell.

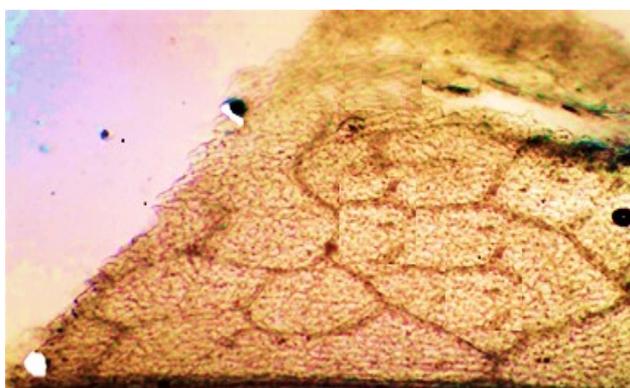


Fig. 3c. *Plucheia arguta* subsp. *glabra* leaf. Vein islet number and vein termination number.

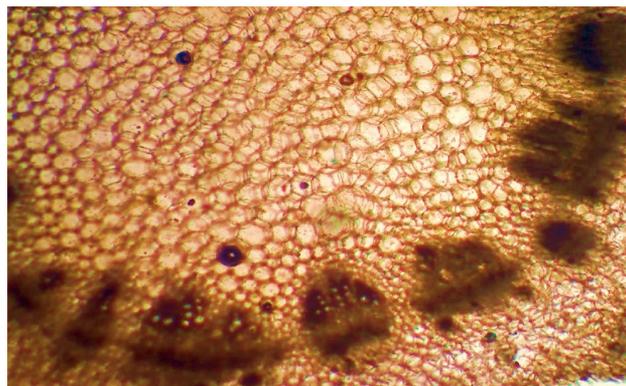


Fig. 3d. *Plucheia arguta* subsp. *glabra* stem T.S. showing vascular bundle (xylem and phloem).

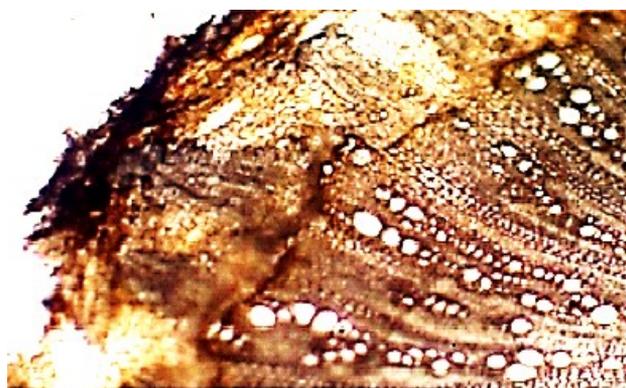


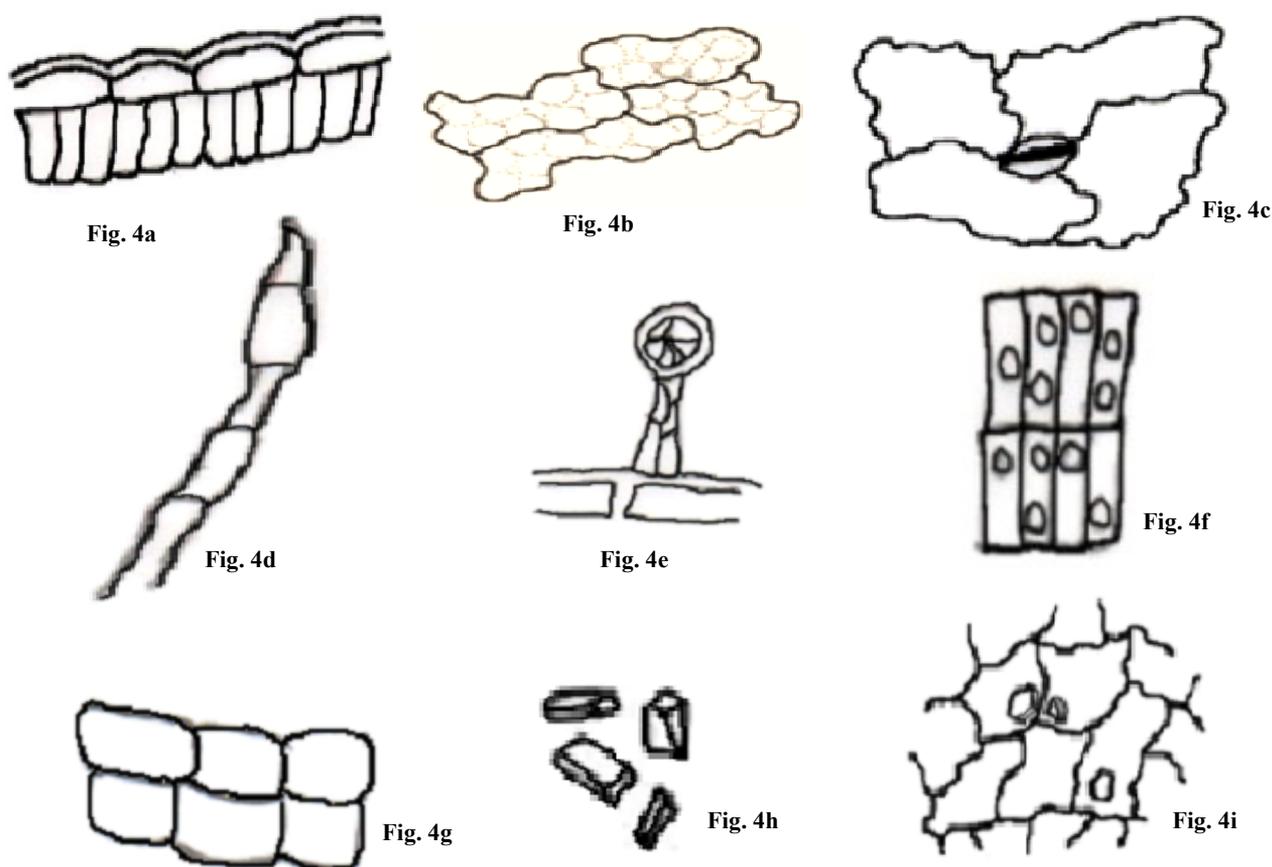
Fig. 3e. T.S. of root of *Plucheia arguta* subsp. *glabra*.

Ash analysis was carried out for *Iphiona grantioides* and *Plucheia arguta* subsp. *glabra*. Results revealed that highest total ash contents were recorded for stem (7.06%) and least value (3.33%) for leaf of *Iphiona*, while ash contents were 4.25% for root and 3.38% for flower. Acid insoluble ash was in the range of 1.54% (leaf), 1.425% (root), 1.27% (stem) and 0.35% (flower). Water soluble ash was in the range of 4.36% (leaf), 3.34% (stem), 2.23 % (root) and 1.82% (flower). Moisture contents values were in the range of 9.16% (root), 9.01% (stem), 7.80% (leaf) and 6.12% (flower) (Table 1). In *Plucheia arguta* subsp. *glabra* total ash values were in the range of 7.82% (leaf), 6.12% (stem) to 5.6% (root). Acid insoluble ash was in the range of 1.66% (root), 1.49% (leaf) and 1.09% (stem) and water soluble ash was in the range of 2.56%, 3.38% and 6.5%. Moisture contents were highest in leaf (8.87%), root (8.41%) and lowest in stem (7.5%) (Table 1). Ash value determination is an important tool for the detection of adulterants or a mixture of inorganic matter such as metallic salts, silica or clay during improper handling (Jarald & Jarald, 2007). Likewise acid insoluble ash values are carried out for drugs having calcium oxalate crystals or sand, clay or other earthy material is added (Wallis, 1985; Rangari, 2002). Water soluble ash value is important for detection of presence of water exhausted materials in genuine drugs (Jarald & Jarald, 2007).

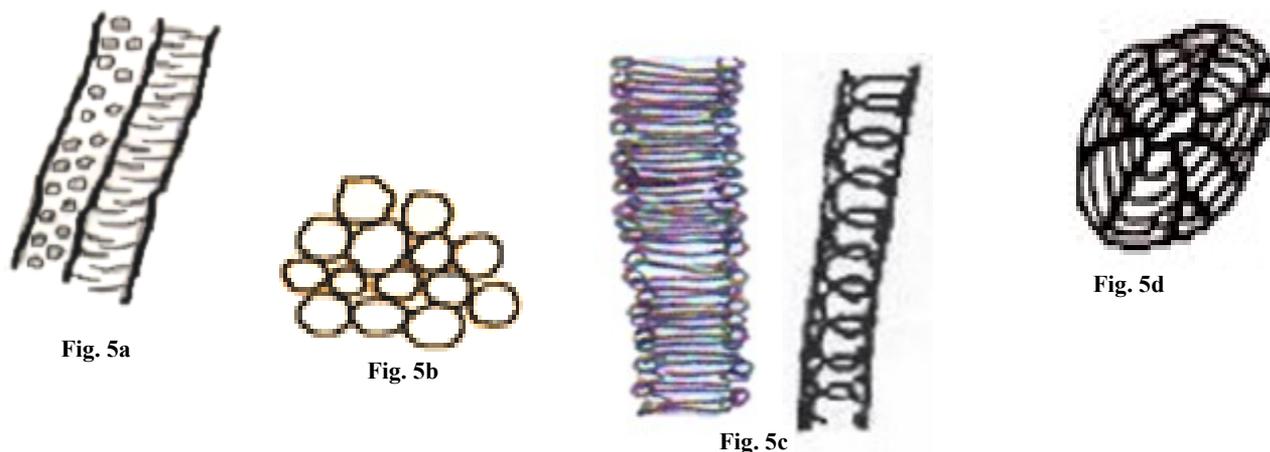
Powder drug study: *Iphiona grantioides* leaf powder appeared bright green in color with pleasant aromatic odor and acrid taste. It has fragments of both epidermises along with anomocytic type of stomata, fragments of upper epidermis with cuticle and mesophyll cells, simple multicellular and glandular trichomes, fragments of lamina with a row of palisade cells underneath, phloem fibers,

spirally/annularly thick xylem vessels and crystals of Ca oxalate and parenchyma cells with oil droplets (Fig. 4a-i). Stem powder of *Iphiona grantioides* appeared dull green in color with mild aromatic odor and slight acrid taste. The study showed rectangular epidermal and oval shaped cortical cells, vessels with pitted, reticulate, annular and spiral thickening, sclerenchymatous cells and glandular/ multicellular trichomes (Fig. 5a-d). Powder drug of the root showed the presence of cork cells, cortical cells, phloem fibers and xylem vessels and thick walled paranchymatous cells (Fig. 6a-e). The powder was light in colour with a slight aromatic taste and characteristic odour.

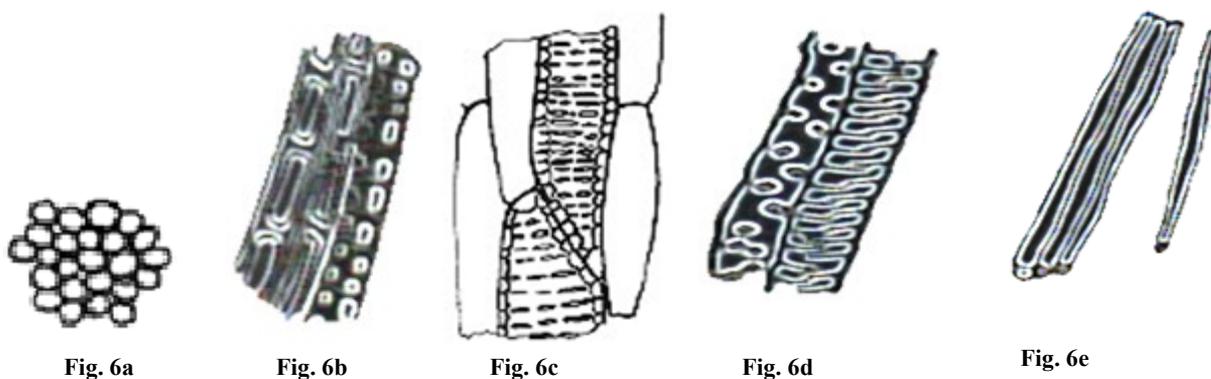
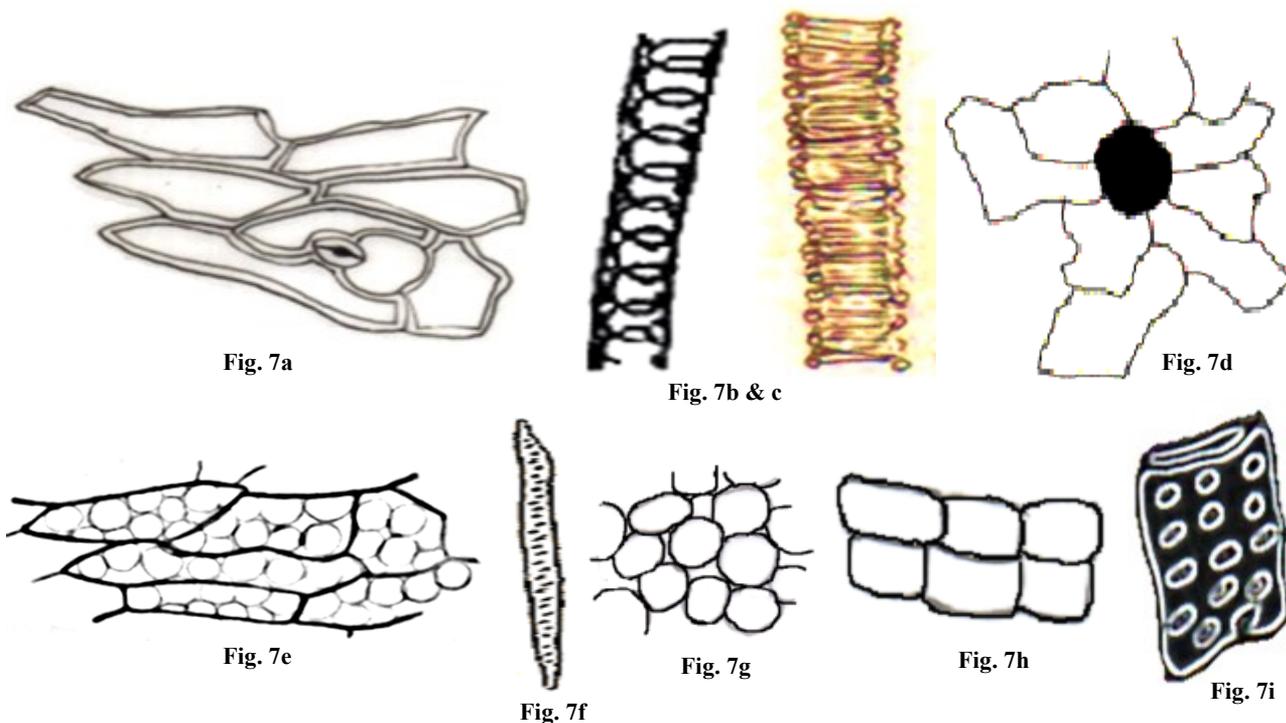
Dark green leaf powder of *Plucheia arguta* subsp. *glabra* with pungent odour and unpleasant taste showed pieces of upper and lower epidermises in surface view with anomocytic type of stomata, numerous multi-cellular glandular and non-glandular hairs. Fragments of lamina, with a row of palisade cells underneath the upper epidermis, fragments of phloem fibres and xylem vessels with spiral/annular wall thickenings (Fig. 7a-i). Dull green stem powder of *Plucheia arguta* subsp. *glabra* was slightly pungent in taste and bitter odour, showing pieces of epidermal cells, paranchymatous pith cells, xylem vessels with annular/spiral wall thickening and sclerenchymatous cells (Fig. 8a-f). Light brown root powder of *Plucheia arguta* subsp. *glabra* was found odour less and unpleasant, having the presence of some cork cells, fragments of cortex, phloem fibers, xylem vessels and paranchymatous cells (Fig. 9a-i). The present study is well in accordance with those of Sultana & Zafer, 2013 (*Lawsonia innermis* L.), who also conducted pharmacognostic studies for standardization of various crude drugs of plants origin.

Powder drug study of *Iphiona grantioides*.

- Fig. 4a. *Iphiona grantioides* leaf. Epidermal cells with cuticle and mesophyll cells.
 Fig. 4b. *Iphiona grantioides* leaf. Epidermal cells with palisade cells attached below.
 Fig. 4c. *Iphiona grantioides* leaf. Epidermal cells with anisocytic type of stomata.
 Fig. 4d. *Iphiona grantioides* leaf. Non-glandular multicellular trichome.
 Fig. 4e. *Iphiona grantioides* leaf. Glandular trichome.
 Fig. 4f. *Iphiona grantioides* leaf. Parenchyma cells with oil droplets.
 Fig. 4g. *Iphiona grantioides* leaf. Epidermal and palisade cells.
 Fig. 4h. *Iphiona grantioides* leaf. Isolated crystals of calcium oxalates.
 Fig. 4i. *Iphiona grantioides* leaf. Spongy mesophyll cells with crystals of calcium oxalates.

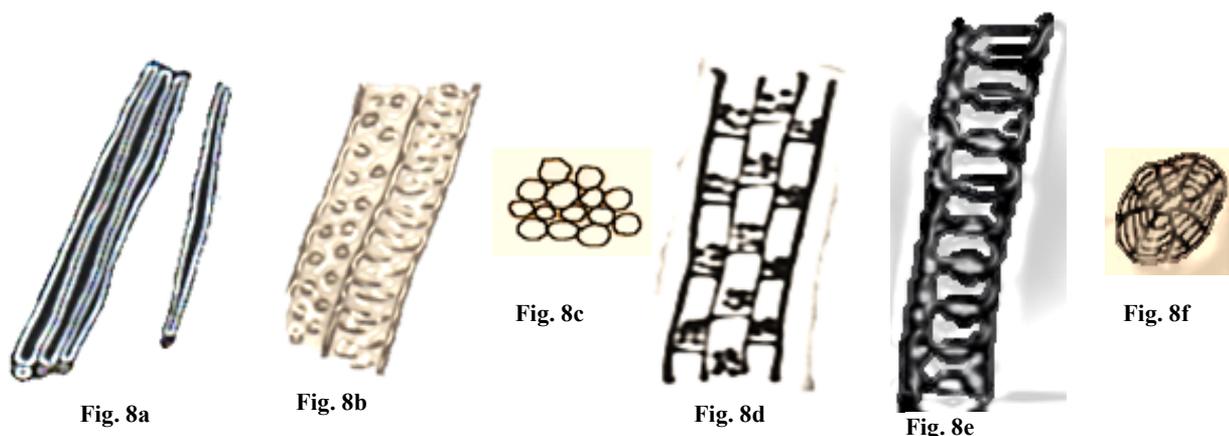
Powder drug of stem of *Iphiona grantioides*.

- Fig. 5a. *Iphiona grantioides* stem. Vessels with pitted and reticulate thickening.
 Fig. 5b. *Iphiona grantioides* stem. Parenchyma cells in cortex.
 Fig. 5c. *Iphiona grantioides* stem. Vessels with annular and spiral thickenings.
 Fig. 5d. *Iphiona grantioides* stem. Sclerenchymatous cells.

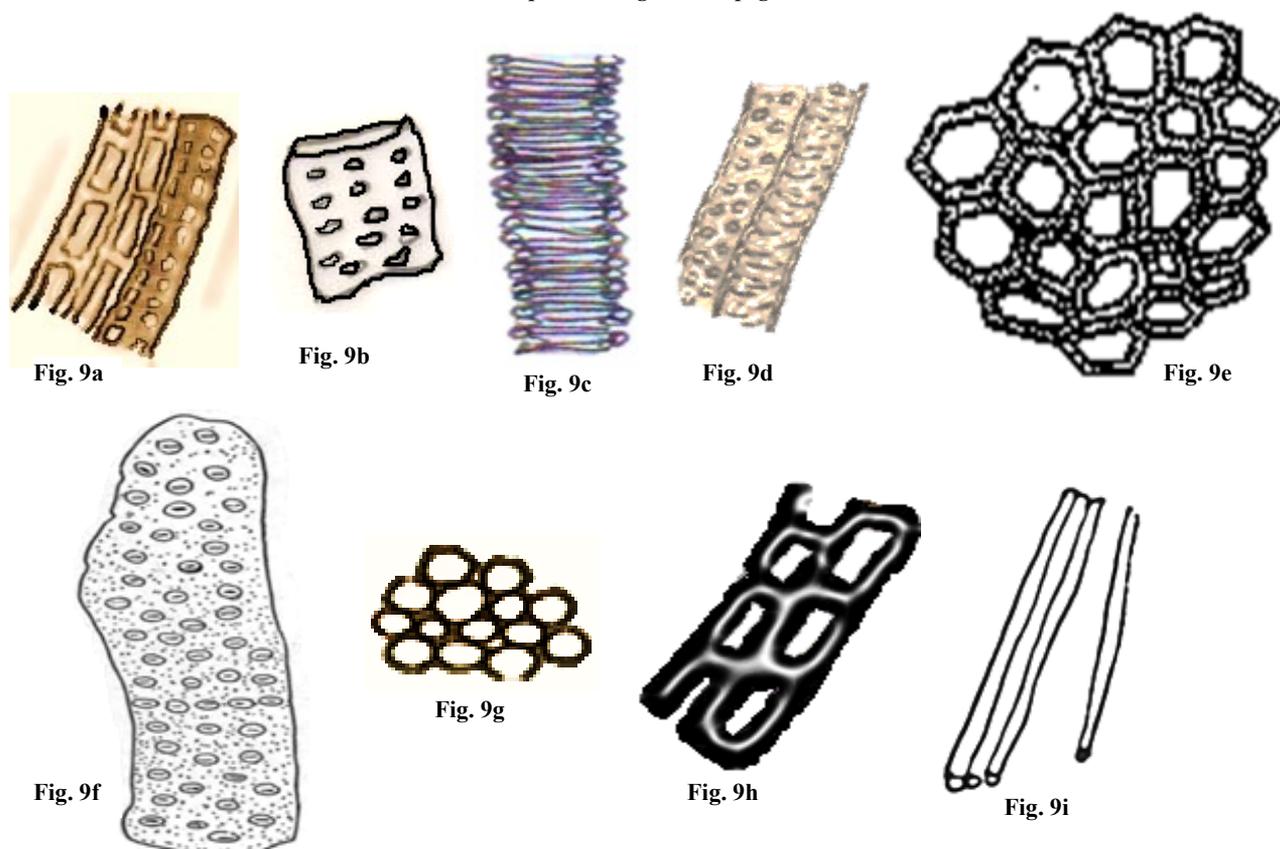
Powder drug of root of *Iphiona grantioides*.Fig. 6a. *Iphiona grantioides* root. Fragments of cortex.Fig. 6b. *Iphiona grantioides* root. Cork cells.Fig. 6c. *Iphiona grantioides* root. Xylem vessels with pits and lignified paranchymatous cells, attached.Fig. 6d. *Iphiona grantioides* root. Vessels with pitted and reticulate thickenings.Fig. 6e. *Iphionagrantioides* root. Fibers.Fig. 7a. Powder drug study of *Pluchea arguta* subsp. *glabra* leaf. Fragments of epidermal cells with anomocytic stomata.Fig. 7b & c. Powder drug study of *Pluchea arguta* subsp. *glabra* leaf. Vessels with annular and spiral thickening.Fig. 7d. Powder drug study of *Pluchea arguta* subsp. *glabra* leaf. Sessile glands attached to epidermal cells.Fig. 7e. Powder drug study of *Pluchea arguta* subsp. *glabra* leaf. Epidermal cells with mesophyl cell attached beneath.Fig. 7f. Powder drug study of *Pluchea arguta* subsp. *glabra* leaf. Tracheids.Fig. 7g. Powder drug study of *Pluchea arguta* subsp. *glabra* leaf. Cortical cells.Fig. 7h. Powder drug study of *Pluchea arguta* subsp. *glabra* leaf. Epidermal cells and mesophylls attached.Fig. 7i. Powder drug study of *Pluchea arguta* subsp. *glabra* leaf. Pitteted vessels.Table 1. Ash contents of different parts of *Iphiona grantioides* and *pluchea arguta* subsp. *glabra*.

Sample	Total ash %		Water soluble %		Acid insoluble %		Moisture content %	
	I	P	I	P	I	P	I	P
Flower	3.38%	-	1.82%	-	0.35%	-	6.12%	-
Roots	4.25%	5.6%	2.23%	3.38%	1.42%	1.66%	9.16%	8.41%
Leaves	3.33%	7.82%	4.36%	6.5%	1.54%	1.49%	7.80%	8.87%
Stem	7.06%	6.12%	3.34%	2.56%	1.27%	1.09%	9.01%	7.5

I = *Iphiona grantioides*, P = *Pluchea arguta*

Powder drug study of *Pluchea arguta* subsp. *glabra* stem

- Fig. 8a. Powder drug study of *Pluchea arguta* subsp. *glabra* stem. Bundles of fiber.
 Fig. 8b. Powder drug study of *Pluchea arguta* subsp. *glabra*. stem. Vessels with reticulate and pitted wall thickening.
 Fig. 8c. Powder drug study of *Pluchea arguta* subsp. *glabra* stem. Parenchyma cells.
 Fig. 8d. Powder drug study of *Pluchea arguta* subsp. *glabra* stem. Cortical cells with starch grains.
 Fig. 8e. Powder drug study of *Pluchea arguta* subsp. *Glabra* stem. Vessels with spiral wall thickening.
 Fig. 8f. Powder drug study of *Pluchea arguta* subsp. *glabra* stem. Sclerenchyma cells.

Root *pluchea arguta* subsp. *glabra*

- Fig. 9a. Powder drug study of *Pluchea arguta* subsp. *glabra* root. Patches of cork cells.
 Fig. 9b. Powder drug study of *Pluchea arguta* subsp. *glabra* root. Vessels with pits.
 Fig. 9c. Powder drug study of *Pluchea arguta* subsp. *glabra*. Root. Reticulate wall thickening.
 Fig. 9d. Powder drug study of *Pluchea arguta* subsp. *glabra* root. Vessels with different wall thickening.
 Fig. 9e. Powder drug study of *Pluchea arguta* subsp. *glabra* root. Sclerenchymatous cells.
 Fig. 9f. Powder drug study of *Pluchea arguta* subsp. *glabra* root. Pitted vessels.
 Fig. 9g. Powder drug study of *Pluchea arguta* subsp. *glabra* root. Parenchymatous cells.
 Fig. 9h. Powder drug study of *Pluchea arguta* subsp. *glabra* root. Fragments of cork cells.
 Fig. 9i. Powder drug study of *Pluchea arguta* subsp. *glabra* root. Bundles of fibers.

Table 2. Fluorescence analysis of powder drug of flower and leaf of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra*,s leaf with different reagents.

S. No.	Reagents	<i>Iphiona grantioides</i>						<i>Pluchea arguta</i> subsp. <i>glabra</i>		
		Visible light		UV256		UV 310		Visible light	UV256	UV 310
		L	F	L	F	L	F	L	L	L
1.	Powder as such	DkG	LY	OIG	BY	LG	LB	DkG	LG	BG
2.	Powder + 50% HNO ₃	LY	O	LY	YO	O	RB	O	Y	OY
3.	Powder + Acetic acid	W	IY	LY	LB	CW	LB	W	C	CY
4.	Powder + Picric acid	G	Y	Y	LG	LY	DG	IY	D Y	Y
5.	Powder + NH ₃	Y	Bk	LY	YB	Y	GY	O	O	R
6.	Powder + 50% H ₂ SO ₄	LG	O	D G	R B	G Bk	YR	BkG	Bk	Bk
7.	Powder + NaOH	Y G	Bf W	LY	BY	C	LB	Y	I Y	Y
8.	Powder + 50%HCl	Y G	LY	B	C Y	L B	Y G	DkG	Bk G	Bk G
9.	Powder + NaOH + Ethaol	Y G	Y O	LY	YO	DY	BR	Y	Y	Y B
10.	Powder + Iodine	GB	C	B	B	C Y	LBr	W	W	C
11.	Powder + FeCl ₃	Bk B	Bk	Bk	DB	Bk	BkB	BkG	Bk	BK G

Key: Bf. Buff, B. Brown, C. Cream, D. Dull, Dark. Dk, I. Intense, L. Light, O. Orange, Y. Yellow, W. White, G. Green, Bk. Black, L. Leaf, F. Flower

Table 3. Fluorescence analysis of stem and root of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* powder with different reagents.

S.No.	Reagents	<i>Iphiona grantioides</i>						<i>Pluchea arguta</i> subsp. <i>glabra</i>					
		Visible light		UV256		UV 310		Visible light		UV256		UV 310	
		St	Rt	St	Rt	St	Rt	St	Rt	St	Rt	St	Rt
1.	Powder as such	DLG	LB	LB	B	D G	LB	DG	LB	L G	B	G	LB
2.	Powder + Picric acid	IY	C	B	C	Y	W	IY	C	Y	LB	Y	Bf
3.	Powder + NH ₃	Y	LY	LY	C	C	LY	Y	Y	Y	Y	Y	Y
4.	Powder + H ₂ SO ₄	G B	C B	Bk G	LB	DB	B	Bk B	B	B	B	Bk G	DB
5.	Powder + NaOH	IY	DB	Y	B	LY	LB	LY	BY	LY	LY	DY	B
6.	Powder + Acetic acid	BC	LB	C	LY	C	DB	W	LY	C	C	C	C
7.	Powder + HCl	LC	LY	LY	C	W	Y	Bk G	LY	Bk	C	Bk	C
8.	Powder + NaOH + Ethanol	Y	C	LY	Bf	Y	W	S Y	W	Y	W	LY	B W
9.	Powder + Iodine	C	B	LB	B	C B	LB	Bf	B	W	LB	C	LB
10.	Powder + FeCl ₃	Bk	YO	B	LY	B Bk	C	G Bk	LY	Bk	LY	Bk	LY
11.	Powder + 50% HNO ₃	LY	Y	Y	Y	L O	GY	Y	LY	Y	LY	Y	C

Key: Bf. Buff, B. Brown, C. Cream, D. Dull, Dark. Dk, I. Intense, L. Light, O. Orange, Y. Yellow, W. White, G. Green, Bk. Black, St. Stem, Rt. Root

Table 4. Extractive values of different parts of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* plants in different solvents.

S. No.	Solvents	Extractive Values (%)						
		IF	IL	IS	IR	PL	PS	PR
1.	Aqueous	5.25	46.2	31.8	16.6	27.2	22.8	4.2
2.	Acetone	2.6	7.5	4.25	20	1.75	5	35.5
3.	Butane	8.8	36	2.4	7.6	3.6	13.2	2.5
4.	Chloroform	2.75	8.25	0.75	20	5	3.5	5.5
5.	Ethanol	22.25	37	11.25	19	22.2	13.4	8.75
6.	Ethyl acetate	13.75	10	2.75	13.75	30.25	22.4	2.5
7.	Hexane	2.75	22.8	1.27	2.5	20.75	22.75	0.25

Key = I = *Iphiona grantioides*, P = *Pluchea arguta* subsp. *glabra*, F= Flower, L = Leaf, S = Stem, R = Root

Fluorescence study: Powder drugs of *Iphiona grantioides* (flower, leaf, stem, and root) and *Pluchea arguta* subsp. *glabra* (leaf, stem, and root) were studied for fluorescence analysis. Observations were made under ordinary visible day light light and under UV light of short wave length and long wave length (Tables 2-3). Iqbal *et al.*, (2011) conducted fluorescence Studies on *Tribulus teresstris*. Fluorescence study is very useful, quick, easy and unfailing method for the detection of adulteration.

Extractive values: In the present study seven different solvents including ethanol, methanol, chloroform, acetone, distilled water, butanol and n-hexane were used for extractive values determination of different parts (leaf, flower, root and shoot f *Iphiona grantioides* and leaf, shoot and root of *Pluchea arguta* subsp. *glabra*). Results

obtained are presented in the Table 4. Highest extractive values were given by *Iphiona grantioides* leaf i.e., (Aqueous extract (46.2%), Ethanol (37%), Butane (36%), Hexane (22.8%), Ethyl acetate (13.75) followed by root extracts i.e., Acetone (20%), Chloroform (20%), Ethanol (19 %) and Aqueous extract (16.6%). Highest extractive values of *Pluchea arguta* subsp. *glabra* are Ethyl acetate (30%), Aqueous (27.2%), Ethanol (22.2%), Chloroform (20%) and Hexane (20.75%). In other solvent both the parts showed variable values (Table 4). Many workers have carried out studies for the determination of extractive values of different medicinal plants including Quan *et al.*, 2013 (*Carica*, papaya) and Gaikwad *et al.*, 2016, *Xanthium strumarium* L.). So it is suggested that determination of extractive values is an important tool for evaluation of crude drugs, detection of adulterants and for selection of suitable solvent for extraction.

References

- Abid, A. and M. Qaiser. 2003. Chemotaxonomic study of *Inula* L. (S. Str.) and its allied genera (*Inuleae - Compositae*) from Pakistan and Kashmir. *Pak. J. Bot.*, 35(2): 127-140.
- Anonymous. 2000. A.O.A.C. (Association of Official Analytical Chemists). Official methods of analysis. Gaithersburg, Washington, USA.
- Anonymous. 2002-2005. WHO. Traditional Medicine strategy. World Health Organization, http://whqlibdoc.who.int/hq/2002/who_edm_tm_2002.1.pdf. Accessed 5 Jul 2011.
- Baran, P. and C. Özdemir. 2013. Morphological, anatomical and cytological studies on endemic *Lamium pisidicum*. *Pak. J. Bot.*, 45(1): 73-85.
- Barkatullah, M. Ibrar, G. Jilani and I. Ahmad. 2014. Leaf, stem bark and fruit anatomy of *Zanthoxylum aramatum* DC. (Rutaceae). *Pak. J. Bot.*, 46(4): 1343-1349.
- Bhat, J.U., S.Q. Nizami, M. Parry, N. Aslam, A. Fahamiya, M. Siddiqui, R. Mujeeb, M. Khanam and M.A. Khan. 2012. Pharmacognostical and phytochemical evaluation of *Melissa parviflora* and HPTLC finger printing of its extracts. *J. Nat. Prod. Plant Resour.*, 2(1): 198-208.
- Boukhris, M., C.B. Ahmed, I. Mezghani, M. Bouaziz and S. Sayadi. 2013. Biological and anatomical characteristics of the rose-scented Geranium (*Pelargonium graveolens*, L'HER.) grown in the south of Tunisia. *Pak. J. Bot.*, 45(6): 1945-1954.
- Chaffey, N.J. 2001. Putting plant anatomy in its place. *Trends in Pl. Sci.*, 6: 439-440.
- Dilcher, D.L. 1974. Approaches to the identification of angiosperm leaf remains. *Bot. Rev.*, 40(1): 1-157.
- Evans, W.C. 2002. Pharmacognosy. 15th ed. English Language Book, Society Bailliere Tindall, Oxford University Press.
- Gaikwad, S., R. Torane and K. Mundhe. 2016. Preliminary screening and comparative evaluation of antioxidant potential of medicinally important plant *Xanthium strumarium* L. *J. Pharmacog. & Phytochem.*, 5(2): 141-144.
- Hameed, I. and F. Hussain. 2011. Stomatal studies of some selected medicinal plants of family Solanaceae. *J. Med. Plant Res.*, 5(18): 4525-4529.
- Iqbal, H., F.A. Khan, H. Khan, S.U. Rehman and Badrullah. 2011. Antimicrobial, phytochemical and fluorescence Studies on *Tribulusteresstris*. *J. Pharm. Res.*, 4(5): 1556.
- Jarald, E.E. and S.E. Jarald. 2007. A text book of pharmacognosy and phytochemistry (1st Ed.) CBS Publisher and distributors, New Delhi, India. pp. 6.
- Khan, R., Asadullah, B. Khan, S.M. Khan and A. Rashid. 2017. Comparative foliar micro morphological studies of tribe *Arundineae*, *Aristideae* and *Chlorideae* from Malakand Agency, Khyber Pakhtunkhwa, Pakistan. *Pak. J. Bot.*, 49(SI): 33-42.
- Khan, S., R. Rawat, A.K.S. Rawat and A. Shirwaiker. 2010. A report on the quality control parameters of aerial parts of *Pluchea lanceolata* (DC.) Oliv. & Hiern, Asteraceae. *Brazilian J. Pharmacog.*, 20(4): 563-567.
- Khandelwal, K.R. 2004. Practical Pharmacognosy, Techniques and experiments (12th Edition).
- Meric, C. 2009. Calcium oxalate crystals in some species of the tribe Inuleae (Asteraceae). *Acta Biologica Cracoviensia Series Botanica.*, 51(1): 105-110.
- Moudi, M. and R. Go. 2017. Morphological study of four sections of genus *Dendrobium* SW. (Orchidaceae) in Peninsular Malaysia. *Pak. J. Bot.*, 49(2): 569-577.
- Naveed, S., M. Ibrar and I. Khan. 2016. *In vitro* evaluation of medicinal, antioxidant activities and phytochemical screening of *Iphiona grantioides* and *Pluchea arguta* subsp. *glabra* Qaiser. *Pak. J. Bot.*, 48(6): 2505-2511.
- Omer, S.A., M.T.M. Rajput and S.S. Tahir. 2017. Micromorphological studies on petals of *Spiraea* L. species (Rosaceae) from Pakistan. *Pak. J. Bot.*, 49(1): 283-287.
- Perveen, A., R. Abid and R. Fatima. 2007. Stomatal types of some Dicots. within flora of Karachi, Pakistan. *Pak. J. Bot.*, 39(4): 1017-1023.
- Qaiser, M. and R. Abid. 2003. Flora of Pakistan. Asteraceae (II) Inuleae, Plucheeae and Gnaphalieae, (Eds.): S.I. Ali & M. Qaiser. Department of Botany, University of Karachi and Missouri Botanical Press. Missouri Botanical Garden St. Louis, Missouri, U.S.A. 210: 1-215.
- Quan, V. Vuong, S. Hirun, P.D. Roach, M.C. Bowyer, P.A. Phillips and C.J. Scarlett. 2013. Effect of extraction conditions on total phenolic compounds and antioxidant activities of *Carica papaya* leaf aqueous extracts. *J. Herbal Med.*, 3(3): 104-111.
- Rangari, V.D. 2002. Pharmacognosy and phytochemistry. 1st Edn., New Delhi: Carrier Publication. p. 100-101.
- Rodríguez, H.G., R. Maiti and A.K. Ch. 2017. Comparative anatomy of leaf lamina of twenty six woody species of Tamaulipan thorn scrub from north eastern Mexico and its significance in taxonomic delimitation and adaptation of the species to xeric environments. *Pak. J. Bot.*, 49(2): 589-596.
- Saeed, M., H. Khan, M.A. Khan, F.U. Khan, S.A. Khan and N. Muhammad. 2010. Quantification of various metals accumulation and cytotoxic profile of aerial parts of *Polygonatum verticillatum*. *Pak. J. Bot.*, 42(6): 3995-4002.
- Sindhu, R.K. and A. Sandeep. 2012. Phytochemical and Pharmacognostical Studies on *Murraya koenigii* (L) spreng. Roots. *Drug Invention Today*, 4(1): 325-333.
- Sultana, S. and M. Zafar. 2013. Authentication of herbal medicine Henna (*Lawsonia inermis* L.) by using taxonomic and pharmacognostic techniques. *Pak. J. Bot.*, 45(SI): 165-176.
- Teke, H.I. and R. Binzet. 2017. Anatomical, morphological and palynological studies of some *Onosma* L. (Boraginaceae) taxa endemic to Anatolia. *Pak. J. Bot.*, 49(2): 579-588.
- Toma, C., M.N. Grigore, M. Afemei and I.E. Stănescu. 2010. Histo-anatomical considerations on some Romanian *Inula* L. species, with pharmacological action. *Analele științifice ale Universității "Al. I. Cuza" Iași Tomul LVI, fasc. 1, s. II a. Biologie vegetală*, pp. 5-13.
- Trease, G.E. and W.C. Evans. 1985. Pharmacognosy; Bailliere Tindall: London, 12.
- Wallis, T.E. 1985. Text book of pharmacognosy; 5th ed. CBS Publisher and Distributors, Darya Ganj New Delhi.

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