ETHNOPHARMACOLOGICAL AND SYSTEMATIC STUDIES OF SELECTED MEDICINAL PLANTS OF PAKISTAN

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ABSTRACT: The present study was conducted with the aim to provide a comprehensive account on the herbal drug authentication by using classical taxonomic parameters as well as pharmacognostic approach. In the utilization of herbal medicine the key issue was their assurance of quality, purity and effectiveness. However these herbal drugs face the complication in identification, taxonomic characterization and their authentication. In the present work the taxonomic characterization was done by morphological, anatomical, palynological, organoleptic, ultraviolet and infrared analysis. A great diversity in anatomical features was perceived among these herbal species, as multi-celled trichomes were located in *Cassia angustifolia* and *Cassia occidentalis,* While absent in *Calendula officinalis.* Palynological examination revealed the highest pollen fertility in *Parthenium integrifolium* whereas lowest value was observed in *Calendula officinalis*. Moreover UV, IR, organoleptic and chemical studies proved to be vital taxonomic parameters, facilitating in authentication of various medicinal plant species found in Punjab. Remarkable achievement of this study was the addition of four new species in flora of Pakistan 1990, as these taxa were not mentioned earlier. These included *Calendula officinalis, Parthenium integrifolium, Silybum eburneum* and *Dalbergia obovata*, although all these species were common plants in Pakistan but present work gave a comprehensive account on these species with multiple parameters, which was not published earlier.

Key words: Classical taxonomy, applied taxonomy, pharmacognostic evaluation, palynology

INTRODUCTION

The medicinal use of plants has a long history worldwide. The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history. It is obvious from the literature that 80% of the world's population is dependent on herbal drugs (Sultana *et al.,* 2012). Early humans depended on nature for a healthy life and since that time humanity has depended on the diversity of plant resources for food, clothing, shelter, and medicine to cure myriads of disorder.

In Pakistan a local market system (Pansara) specifically deals with medicinal plants business. Eighty percent of people belonging to the rural areas still depend upon the herbal medicines in Pakistan. It has been reported that 600 to 700 species are used for medicinal purposes. Ethnobotanical significance of about 48 species has been recognized from Kaghan valley and Mansehra (Shinwari and Khan, 1998).The majority of medicinal plants used for the herbal drug industry and local communities come from wild collection. Excessive use of herbal medicines may lead to their unscientific collection and misidentification of adulterant plants. Adulteration of botanical medicines is an important hindrance to quality control and standardization (Shinde *et al*., 2009) and one of the major issues related to the herbal medicinal plants utilization and their trade is adulteration. Adulteration arises when morphologically similar species substitute each other to reduce their cost. Usually this adultrism is unintentional but some time it could be intentional due to various reasons. Herbal medicinal plants and their authentication is a great field and there are various contradictory theories in the use of herbal medicinal drugs in Pakistan. Hence the background knowledge of these herbal drugs is essential. More over various plants are not classified correctly, their taxonomic position is doubtful. That is why there is a need of correct taxonomic identification of medicinal plants and to overcome the problems related to the use of herbal plant drugs. The research needs to be based on the authentication of herbal medicinal plants to avoid the adulteration in use of herbal medicinal plants which is a major issue of the recent time. The present research work is conducted by employing multiple parameters. Among them some chemotaxonomic markers will be used for correct identification of problematic medicinal herbs, which are considered more acceptable and accurate for characterization and authentication of medicinal plants. While correlating these markers with the taxonomic characters results in a valuable tool for correct identification as well as authentication of adulterant medicinal herbs of Pakistan. The present study is aimed: to explore indigenous medicinal plants of Pakistan, and enlist their therapeutic effects, to highlight the

significance of classical taxonomic approaches in identification of medicinal herbs, to construct chemotaxonomy approach, a modern systematic technique, for the authentication of herbal medicines, to provide the pharmacognostic evaluation of indigenous medicinal plants and to aware the local community about adulteration problems a comprehensive study be carried out related to herbal medicines.

MATERIALS AND METHODS

Sr no.	Investigations	Methodology
1	**Morphological Analysis**	In the different areas of Pakistan, the fresh plant samples were collected, morphological examination was done by using the binocular light microscope.
2	**Anatomical Investigation LM & SEM**	Leaf samples were furnished by pursuing the modified methodology of (Shaheen et al., 2012).
3	**Palynomorph Investigation**	The fresh polliniferous material was utilized for palyno-morph study according to the modified method of (Zafar *et al.*, 2011).
4	**Pollen Fertility Investigation**	The pollen fertility estimation was carried out by employing the techniques used by (Meo and Khan 2004).
5	**Organoleptic Investigation**	Organoleptic analysis involved the use of sight, smell, taste, touch and microscopy of crude drugs to evaluate plant materials often comparing the properties of a known sample with those of a reference standard.
6	**Microphotographs**	Microphotographs of leaves and pollen samples were taken by Nikon (FX-35) Camera equipped light microscope and Scanning Electron Microscope.
7	**Methods for chemical authentication**	Fluorescence and Solubility Analysis, acid hydrolysis, detection of alkaloid, glycoside, tannins, starch grains, anthraquinones, saponin and detection of voltile and fixed oils were carried out by employing the method of (Sultana *et al.*,2012).

RESULTS AND DISCUSSION

The comprehensive data on morphology, leaf epidermal anatomy and palynology oganoleptography and chemical analysis of six taxa of some commonly available medicinal plants were presented.

Table 1: Morphological variations among selected medicinal plant species

Characteristics	*Cassia angustifolia*	*Cassia occidentalis*	*Calendula officinalis*	*Dalbergia obovata*	*Parthenium integrifolium*	*Silybum eburneum*
Common Name(s)	Senna,Senna India,	Coffee Senna, Coffeeweed,	Field Marigold, Pot marigold,	Climbing flat-bean.	Wild quinine, amercian feverefew,	Silver Milk Thistle, Elephant Thistle.
Habitat	Dry land	Roadsides and waste ground	Open places and wastelands	Riverine vegetation	Dry and rocky woods.	River flats, sheep camps,
Life Form	Perennial	Annual	Annual /perennial	Deciduous.	Short-lived annual.	Annual, Biennial.
Habit	Shrub	Shrub	Forb/herb	Tree	Erected, branched herb	Non grass herb
Stem Size	60-90 cm tall.	2 m tall.	80 cm tall.	5 m tall.	60 cm tall.	40-100 cm tall.
Leaves	Lanceolate to elliptic	lanceolate or ovate-lanceolate	Oblong-lanceolate,	Obovate-elleptic	Crenate-serrate	Lanceolate
Active Growth Period	Spring.	Spring.	Spring	Summer.	Spring-Summer.	Spring
Flowering Period	October-November.	October-March.	June – September	October-November.	May- August	June-August.
Flower Colour	Yellow flowers	Yellow flowers	Bright yellow to yellow-orange.	White flowers	White flowers	Pink-to-purple, rarely white flowers.
Inflorescence	Racemes, axillary	Pedunculate, axillary, corymbose raceme	A thick capitulum.	Axillary and terminal panicles.	Corymb	Panicles
Fruit	Pods	Pods glabrous	Curved achene.	Pod flat	Achenes obovate	Achene with a pappus

The morphological features can be utilized in distinguishing taxa one from another by differences in habit, habitat, inflorescence and floral morphology. In the present studies it was observed that the leaves of *Calendula officinalis* were oblong-lanceolate, 5–17 cm long, hairy on both sides, and with margins entire or occasionally waved or weakly toothed (Table 1).Whereas (Foster 2001) reported that leaves were ovate or broadly lanceolate, 2-8 cm long and 3-5 cm wide in *Calendula officinalis.* The results of the present study indicated that in the morphological examination of *Cassia angustifolia* and *Cassia occidentalis* because of the differential sensitivity of genus, it was necessary to assess the botanical and taxonomic characterization of this genus. The variation in height, branching, leaf texture, and fruits of the species studied could be used for taxonomic characterization and were of immense significance. The stem of *Dalbergia obovata* was 2-4 m in girth at base. Bark was rough with shallow broad longitudinal fissures, exfoliating in irregular woody strips and scales, light brown in colour. Whereas (Altaf and Matin 2007) concluded that *Dalbergia obovata* was a tree with crooked trunk and its bark was grey and longitudinally to somewhat reticulately furrowed having a girth of 1-2 m. The results of the morphology of studied species have proved to be of immense assistance in interpreting problems related to plant identification and classification. The results could therefore be utilized with information from other disciplines in clarifying taxonomic relationships of these taxa with other genera, species or subspecies.

Most of the plants resembled in their morphological appearance that was why reliability on morphological characters caused confusions in identification, differentiation and taxonomic delimitation of medicinal plants. This problem could be overcome by utilizing leaf anatomical features which helped in clarifying the taxonomic status of different medicinal plants (Gilani *et al*., 2002). Leaf epidermal traits i.e. epidermal cells, stomata and hairs have proved to be important tools in delimitation of taxa in many plant families (Naz *et al*., 2009; Hameed *et al.,* 2010; Riaz *et al.,* 2010). The average length of epidermal cells observed in *Calendula officinalis* was 94(90-96) µm whereas the average length of epidermal cells observed in *Silybum eburneum* was 57(34-80). The utilization of leaf epidermal features in plant systematics is gaining popularity just like the DNA makers and chemical compositions. (Nwachukwu and Mbagwu, 2006) had also supported that type and arrangement of stomata, different shapes of epidermal cells, number of vascular bundles and shape of trichomes played vital role in systematic botany. Stomata were absent in abaxial epidermis of *Dalbergia obovata* and *Silybum eburneum*. Different types of stomata were recognized like diacytic, paracytic, anisocytic and hypostomatal in all the studied species. (Adedeji *et al*., 2007) reported that the presence and absence of trichomes and their type was quite valuable in the configuration of characteristic anatomical markers, which have been proven to be of systematic value. Multicellular trichomes were observed in *Cassia angustifolia* on both abaxial and adaxial epidermis, whereas in *Cassia occidentalis* and *Dalbergia obovata* only adaxial side possessed trichomes. In the *Parthenium integrifolium* and *Silybum eburneum* micro-hairs were single celled and were present on both the abaxial and adaxial surfaces whereas in *Calendula officinalis* no micro-hairs were seen.

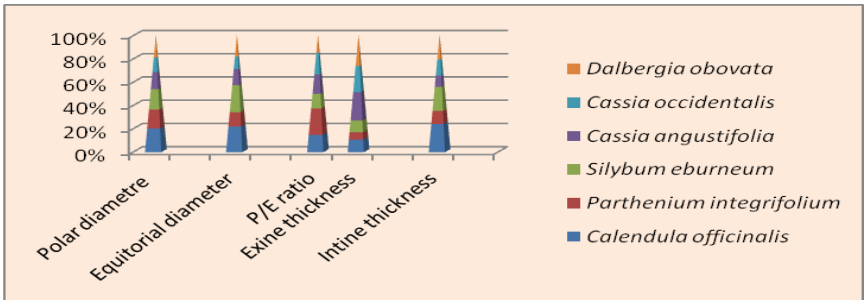
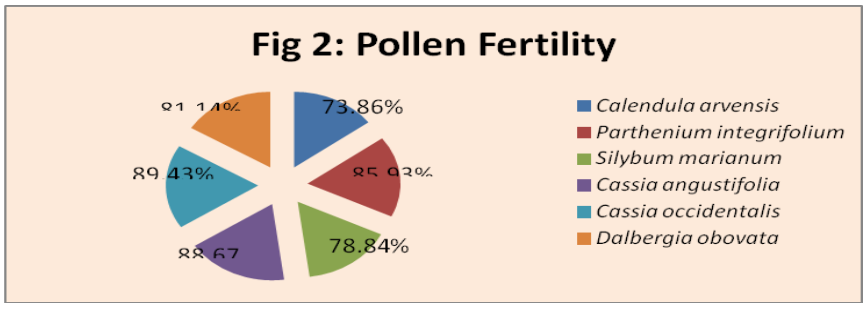


Fig 1: Palynological variations among studied medicinal plants



Palynological studies not only provided additional information but also provided taxonomic characters, which proved to be helpful to improve the systematic position of taxa within their respective classification. (Meo and Khan 2003, Nasreen and Khan 1998 and Anozie 2002) have sporadically reported palynology of different families in Pakistan. According to Perveen (2006) pollen shape is one of the strikingly significant character which could help in delimiting the taxa. There were remarkable variations observed in pollen shapes of these selected medicinal plants, ranging from semicircular (*Calendula officinalis*) to oblate-spheroidal (*Cassia angustifolia*). Pollen size was quite variable among the species indicating that pollen morphological characters are helpful at specific level also. The size of pollen grains in the polar view ranged from 29 µm to 47.5 µm. *Cassia occidentalis* possessed the largest size while *Calendula officinalis* had the smallest one. In equatorial view the size ranged from the 28 µm to 58.5 µm. *Cassia occidentalis* appeared to be smallest whereas *Calendula officinalis* was the largest. Meo and Khan (2003) also reported the lower values of equatorial diameter in *Calendula officinalis*. P/E ratio i.e., polar and equatorial relationship appeared to be variable. It varied from 0.71 to 1.28. The highest value belonged to *Parthenium integrifolium* while the lowest value was exhibited by *Silybum eburneum*. These results indicated that P/E ratio was an important diagnostic character in identification of species. This ratio also helped at specific level. The lowest value of exine thickness was characterized by *Parthenium integrifolium* i.e., 0.9 µm whereas *Cassia angustifolia* was observed to be high i.e., 3.55 µm. The values of intine thickness also showed remarkable variations and ranged from 0.7 µm to 1.75 µm. The lowest value showed by *Cassia angustifolia* while the highest limits were observed in *Calendula officinalis.* These variations among exine and intine thickness suggested that this character could be helpful in segregation and identification of species. Pollen fertility was a useful tool to determine genetic variations. In the present study, the highest value of pollen fertility was found in *Cassia occidentalis* i.e., 89.43% and the lowest value was in *Calendula officinalis* i.e., 73.86%. Pollen fertility may indicate the adaptability of pollen grains to the environment and it was also suggested that their ploidy level may exist due to higher level of pollen fertility (Awan *et al*., 2001).This test could cooperate significantly in solving taxonomic troubles in the cases when taxonomic characters were interrelated with the morphological characters to become a quantitative character.

The present research was also confined to the chemotaxonomic markers including the macro and microscopic features of the powdered drug and their solubility and fluorescence analysis which were used as powerful tool in the identification. According to World Health Organization (WHO) these chemotaxonomic techniques were of utmost importance to resolve the problems like adulteration, nomenclatural confusion and morphological similarities. The powdered drugs of all studied species were soluble in all the solvents by cold and hot tests except *Silybum eburneum* which was soluble in all the solvents except nitric acid and distilled water. Moreover *Silybum eburneum* original mustard colour disappeared on dry filter paper during cold and hot tests. Similar findings about *Silybum eburneum* solubility were also reported by (Dastagir and Haq 1995). The chemical nature of plants also played a key role in resolving taxonomic confusions. In the present studies active chemical constituents like alkaloids, tannins, starch grains, anthraquinone and saponins were observed in all plant species. In *Calendula officinalis* glycosides, volatile and fixed oils and ferric chlorides were absent and these results ressembled with (Kemper 1999 and Dweck 1997) who reported the presence of tannins and saponins in the *Parthenium integrifolium.* While in this present work they were found to be absent. This study showed that fixed and volatile oils were present in *Parthenium integrifolium* and *Silybum eburneum* whereas absent in all other studied species.

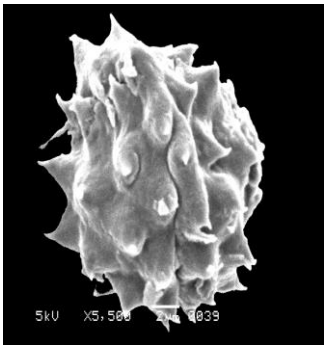


Plate 1: ***Calendula officinalis***

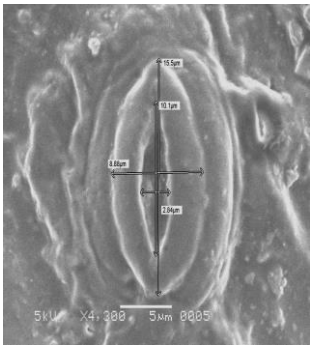


Plate 2: ***Parthenium integrifolium***

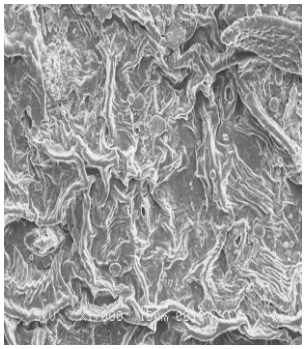


Plate 3: ***Silybum eburneum***

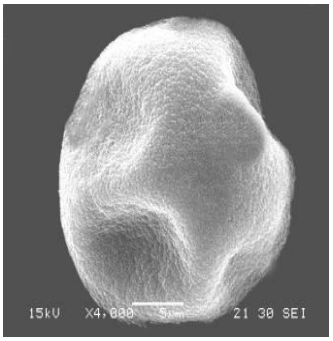


Plate 4: ***Cassia angustifolia***

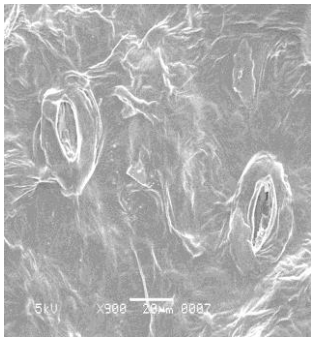


Plate 5: ***Cassia occidentalis***

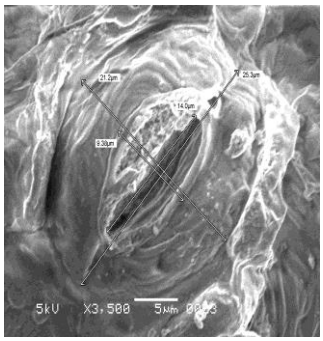


Plate 6: (a) ***Dalbergia obovata***

Ultraviolet and Infra-red spectroscopy was powerful tool for identifying the presence or absence of particular types of organic and inorganic compounds as well as pigments and these played a vital role in systematics in identification, authentication and delimitation of various plant taxa. Generally, both spectroscopy techniques were found to be useful in identifying simultaneously characteristic key bands of individual plant components e.g. alkaloids, carotenoids, polyacetylenes, fatty acids, terpenoids, amino acids. (Davihazy 2004). In the present investigation the UV and IR analysis proved to be helpful in the identification of the selected taxa. The market sample analysis of *Calendula officinalis* revealed the presence of smooth surface having grape's color and salty taste. The results of organoleptic characters were similar to those of (Gilman and Howe 1999) who studied organoleptic characters of *C. officinalis.* In case of *Parthenium integrifolium* market sample compared with actual samples collected from different localities of Lahore, the outer surface of fruit was rough and semicircular in appearance. In case of *Silybum eburneum* both leaves and seeds were used for the identification. Market samples of dried leaves showed rough appearance and yellowish color whereas the fresh leaves were greenish in color having white veins. The results of the present study were in line to the findings of (Kemper 1999).

The ultraviolet and infra-red analysis was of great interest for the authentication of herbal drugs along with the chemotaxonomic markers. (Mattivi and Reneiro 1996). The samples of *Cassia angustifolia* procured from market were collaborated with actual samples collected from various localities of Lahore. Fruit shape was straight smooth or slightly curved, having greenish-brown to almost brown in color. These organoleptic characters were similar to the finding of (Barthakur *et al.,* (1995). The analysis of market samples of *Dalbergia obovata* revealed the presence of rough surface of leaves having spicy taste. The market samples were similar to those of fresh samples. In case of *Cassia occidentalis* market sample collaborated with actual samples. Leaves were dark green and lanceolate or elliptic. These results corroborate with the findings of (Nina *et al.*, 1990).

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