The Journal of Animal & Plant Sciences, 23(3): 2013, Page: 872-881
ISSN: 1018-7081

SYSTEMATIC AND PHYTOCHEMICAL EVALUATION OF SELECTED HERBS OF FAMILY FABACEAE

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ABSTRACT

The present work deals with the investigation on *Cassia fistula* (Linn.), *Cassia occidentalis* (Linn.) and *Dalbergia sissoo* (Linn.) based upon multiple parameters including anatomy (LM & SEM), palynology (LM & SEM), UV and IR analysis, organoleptography, fluorescence, solubility and phytochemical analysis. The systematic attempt based upon multiple parameters of the selected medicinal plants of family Fabaceae showed a lot of variations. Such as while studying anatomical parameters different types of stomata were recognized like diacytic, paracytic, anisocytic and hypostomatal within the selected species. Also the presence or absence of trichomes, as well as their types can be useful in characterizing the species studied. Multicellular trichomes were observed in *Cassia fistula* on both abaxial and adaxial epidermis, whereas in *Cassia occidentalis* and *Dalbergia sisso* only adaxial side possessed trichomes. No silica bodies were observed in *Cassia occidentalis* and *Dalbergia sisso* where as in *Cassia fistula* both the abaxial and adaxial sides showed the presence of silica bodies. In this respect the presence of silica bodies emerged as useful taxonomic feature to delimit different taxa studied from each other. In the present investigation macro-hairs were observed in the two species i.e., *Cassia fistula* and *C. occidentalis* but no macro-hairs were found in the *Dalbergia sisso*. Studying the palynology the pollen characters obtained were generally compatible with those determined in previous studies except for pollen sizes, the number of pores and several ornamentation features. A striking fact of the evolutionary order of these selected species was that pollen characters related to *Dalbergia* species were established as porate, and reticulate; those related to *Cassia fistula* which were psilate, perfolate, oblate-spheroidal to prolate-spheroidal or sub prolate. Pollen shape was generally uniform in the polar view in all the three species. However in equatorial view it varied to spheroidal to sub-spheroidal, oblate-spheroidal, prolate-spheroidal and elleptic to cup-shaped. No colpi were found in the *Cassia fistula*, the number of colpi in the pollen of *Cassia occidentalis* may be 2 or 3 whereas in *Dalbergia sisso* they were always 3. The variation was also observed in the interspecific distance of colpi. *Dalbergia sisso* showed lowest interspecific difference 3.5 (2-5) µm whereas *Cassia occidentalis* showed highest interspecific difference 5 (3-7) µm. A distinct comparison of these species can also be seen on the basis of Ultra Voilet, infra-red, organoleptography, fluorescence, solubility and chemical analysis.

Key words: Systematic analysis, Phytochemical analysis, Fabaceae, Epidermal leaf anatomy, Palynology, Organoleptography.

INTRODUCTION

Fabaceae is the third largest family of flowering plants, which is commonly known as the legume family, pea family, bean family or pulse family. Among the medicinally important species of Fabaceae, *Cassia fistula* (Linn.) (Amaltas) is native to southern Asia and widely found in Pakistan, India and Sri Lanka. It is an important herb used in many medicinal preparations. Its fruit pulp is used as mild laxative, against fevers, arthritis, as well as cardiac conditions and stomach problems such as acid reflux. The root is considered a very strong purgative. The plants are used in folk remedies for tumors of the abdomen, glands, liver, stomach, and throat, cancer (Santos, 2008). Throughout the Far East, the uncooked pulp of the pods is a popular remedy for constipation, thought to be good for the kidneys "as those who use it much remain free of kidney stones. A decoction of the root bark is recommended for cleansing wounds. The bark and leaves are used for skin diseases: (Santos, 2008).

Cassia occidentalis (Linn.) a type specimen of medicinally important plants of Fabaceae, *Cassia occidentalis* is distributed in Pakistan (Punjab, Sind). All parts of the plant have almost similar properties. They possess purgative, tonic, febrifugal, expectorant and diuretic properties. The plant is used to cure sore eyes, typhoid, asthma and disorders of hemoglobin (Bagci and Sahin, 2004). A decoction of the plant is used in hysteria, in dysentery and other stomach troubles, and also as an application to sores, itch and inflammation of the rectum. The leaves are used in foot and mouth disease of cattle. Their extract exhibits activity against earthworms. The seed is bitter and has tonic, febrifugal and purgative properties. It is considered to be a blood tonic and

excellent diuretic. Seeds are useful in cough and whooping cough, convulsions and in heart diseases. (Bagci and Sahin, 2004).

Dalbergia sissoo (Linn.) is known as shisham or tally in Pakistan. Shisham is an important fuel wood, shade, and shelter. It is also an important source of furniture wood, which is the most precious one among all hard woods. . Many medicinal uses for its fresh leaves, dried bark, and wood raspings are reported from its native region. *Sissoo* is reported to be a stimulant used in folk medicine and remedies (Wu *et al*., 2010). Some ethnic groups in Cameroon are said to relish eating fresh young leaves of *sissoo* (Anna, 1993).

The present studies give the complete and comprehensive systematic and phytochemical description of the species studied and compare/determine the intraspecific relationship and patterns of variation associated with the anatomical and palynological features/characteristics among the studied taxa of family Fabaceae.

MATERIALS AND METHODS

Systematic Evaluation

Anatomical Investigation (LM & SEM): Leaves samples were prepared according to the method of Cotton (1974), but with a little modification. (Shaheen *et al.*, 2010). Leaves were soaked in lactic acid for a few minutes to make them soft and unfolded. Fully developed leaves were placed in test tubes containing 70% hot lactic acid and 30% ammonia solution and boiled for about 50-60 minutes to soften the leaves. The abaxial and adaxial peels were removed from the the leaves and observed under Light Microscope and Scanning Electron Microscope.

Palynomorph Investigation (LM & SEM): The method was followed by Wodehouse technique Ronald (2000). Pollen were acetolized and stained by using glycerin jelly. Glycerin jelly was prepared according to modified method (Ahmad *et al*., 2003). Different parameters used for measurement of pollen were shape and size of pollen, exine thickness, polar diameter, equatorial diameter and P/E ratio.

Pollen Fertility Investigation: The numbers of stained and unstained pollen grains were tabulated. Fully stained pollen were considered fertile while the lightly stained pollen and unstained pollen were considered sterile. According to Rigamoto and Tyagi, (2002) pollen fertility is very important in fruit and seed production in flowering plants. Therefore, pollen fertility knowledge for any plant species is essential for plant breeders and commercial growers. On the basis of pollen fertility we may find compatible species in nature for getting promising hybrids.

Organoleptic Investigation: In these tests the shape and size, external color and markings, texture, internal color**,** odor, and taste of crude drugs **we**re noted to evaluate plant materials.

Microphpotographs: Micrographs of leaves and pollen samples were taken by Nikon (FX-35) Camera equipped light microscope and Scanning Electron Microscope.

Phytochemical Evaluation

Acid Hydrolysis: A small amount of dried plant material is treated with 2 normal (2N) hydrochloric acid (HCl) and heated. By this treatment normally all flavonoids-O-glycosides are converted to flavonoids aglycons, anthocyanins to anthocyanidin where as the C-glycosides remain unaffected. After cooling, the flavonoid aglycons are extracted with diethyl ether (Et_2O) from the aqueous phase.

Detection of alkaloid: In conical flasks 15 g of each powdered drug were macerated with ethanol (50 ml) covered by a cotton lid of 24 hours. The material was shaken and then filtered by filter papers.3-4 drops of this extract were taken in a test tube and 5ml of distilled water acidify with 1-2 drops of 2M HCL was mixed and 1ml of Dragendorff reagent was added in to this mixture and was shaken. Presence of orange or orange red ppt indicated the presence of alkaloid (British Pharmacopoeia, 1999).

Detection of glycoside: Those bitter species, which gave negative tests to alkaloidal reagent, were considered therefore possibly to contain glycosides.

Detection of tannins: Ten gram of each powdered drug were macerated with distilled water (40-50 ml) Then filtered and 1ml of this extract was taken in a test tube and added 1ml of "ferric chloride solution" into it and was shaken. The presence of blue, blue black or brownish color indicated the presence of tannins (British Pharmacopoeia, 1999).

Detection of starch grains: On glass slides 1-2 g of each powdered drug was taken. 1 drop of .5 M Iodine solution is added to it, the presence of blue or black color indicated the presence of starch grains.

Detection of Anthraquinones: In diethyl ether (5-6 ml) powdered drug (2-3 g) was macerated Then it was filtered. Few drops of 20% sodium hydroxide (caustic soda), (20 g of NaOH + 80 ml of distilled water) were added, and shaken. (British Pharmacopoeia 1999).

Detection of saponin: In a test tube 2-3 g of each powdered drug was first taken. 5-6ml of distilled water was added into it, and was shacked. Presence of any marked frothing indicated the presence of saponin.

Detection of voltile and fixed oils: Ten gram of each powered drug were taken on a filter paper and placed under a mechanical pressure for 5 minutes. The presence

of any oily stain on filter paper showed the presence of oil. If the stain on filter paper was still present after heating it in an oven for 2-3 hours at 90°C. It indicated the presence of fixed oil. If the stain disappeared then it indicated the presence of volatile oil.

Fluorescence and Solubility Screening of Powdered Drug: Five gram powdered drug was mixed in 20 ml sulphuric acid, hydrochloric acid, acetic acid, and water.

RESULTS AND DISCUSSION

***Cassia fistula* (Linn.)**
Syn: *Cassia rhombifolia* Roxb.
***Cathartocarpus fistulus* (L.) Pers.**

Leaf Epidermal Anatomy (LM & SEM): In **abaxial epidermis,** the leaf epidermal cells were irregular shaped and polygonal, smooth walled, average length of epidermal cells was 70 (60-80) µm and the average width was 50 (40-60) µm, Hypo-stomatic type of stomata were present, number of stomata per unit area was 2, open stomata was 1 and 1 closed stomata was present, average length of guard cells was 45.25 (20-70.5) µm and average width of guard cells was 25 (20-30) µm, average length of subsidiary cells was 32.5 (15-50) µm and the average width was 15 (10-20) µm. Multicellular trichomes were present, number of trichomes per unit area was 1, average length of trichomes was 100 (90-110) µm and the average width of trichomes was 55 (40-70) µm. In **adaxial epidermis,** the leaf epidermal cells were irregular shaped and polygonal, smooth walled, average length of epidermal cells was 67.5 (50-85) µm and the average width was 47.5 (30-65) µm, stomata were present, stomatal type was diacytic and paracytic, number of stomata per unit area were 3, open stomata were 2 and 1 closed stomata was present, average length of guard cells was 35 (30-40) µm and average width of guard cells was 20 (15-25) µm, average length of subsidiary cells was 85 (80-90) µm and the average width was 55 (30-80) µm, number of trichomes per unit area were 1-2, average length of trichomes was 50 (40-60) µm and the average width of trichomes was 30 (20-40) µm. Average length of macro-hairs was 235 (190-280) µm and average width was 140 (110-170) µm. (Plate 1a, 1b, 1c, 1d).

Palynology (LM & SEM): In equatorial view, the pollen grains were oblate-spheroidal, prolate-spheroidal or subprolate. In polar view, the pollen were semicircular, triangular and spheroidal. (Plate 1f, 1h). Polar diameter was 34 (28-39) µm and equatorial diameter was 36 (30-55) µm. P/E ratio was 0.94, exine thickness was 3.25 (2.5-5) µm and intine thickness was 0.75 (0.6-1) µm. Percentage of pollen fertility in this species was 88.67%. (Fig. 2).

Organoleptography: Dried fruits were used, fruit shape was elongated, 30-60 cm long and 4-9 cm wide, straight or slightly curved and smooth but finely striated transversely, the striations appearing as fine fissures having rounded distal ends. Internally the pod was divided by thin, buff coloured, transverse compartments at intervals of about 0.3-0.5cm. Each compartment contained one seed which is flat, oval, reddish brown with a well marked raphe. Colour of fruit was chocolate-brown to almost black. Smell was pungent. (Plate 1i, 1j).

Fluorescence and Solubility in Different Solvents: Actual colour of the powdered material was rose wood but colour changed in different solvents, becomes chocolate in distilled water, conker in sulphuric acid, chocolate in acetic acid and sonora in nitric acid. While performing cold test the powdered material was insoluble in all the solvents except sulphuric acid and nitric acid but became soluble during hot test.

Chemical Analysis: Alkaloids, glycosides, starch grains, tannins, anthraquinone, saponins and ferric chlorides whereas fixed and volatile oils were absent.

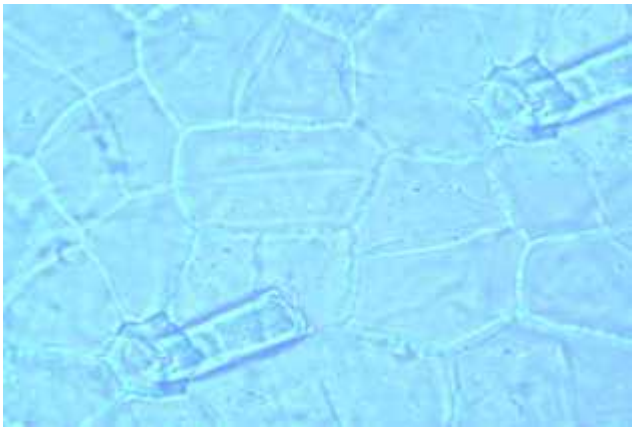
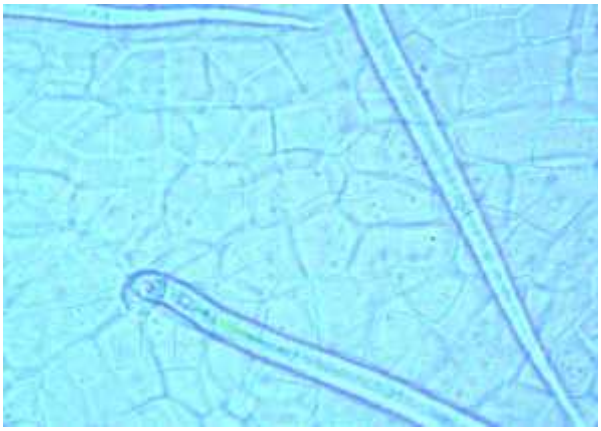
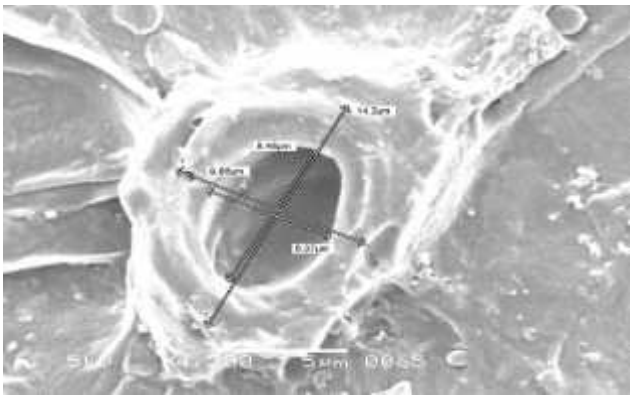


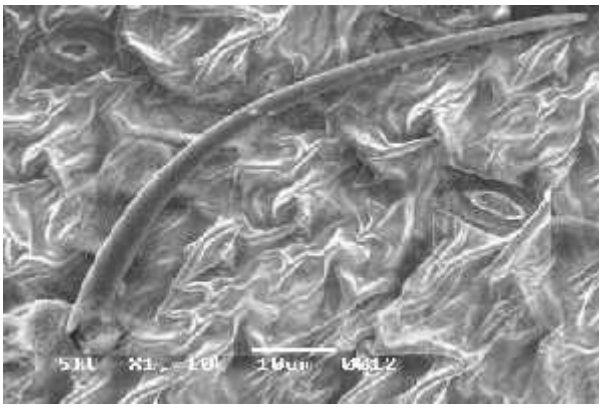
Plate:(1a): Abaxial side showing trichomes (LM)



b). Adaxial side showing macrohairs (LM)

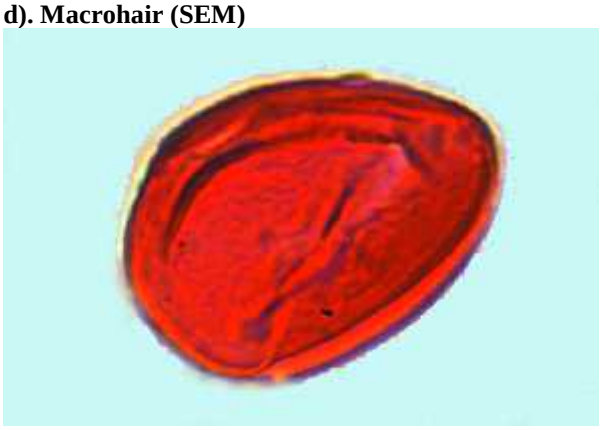


c). Stomata (SEM)

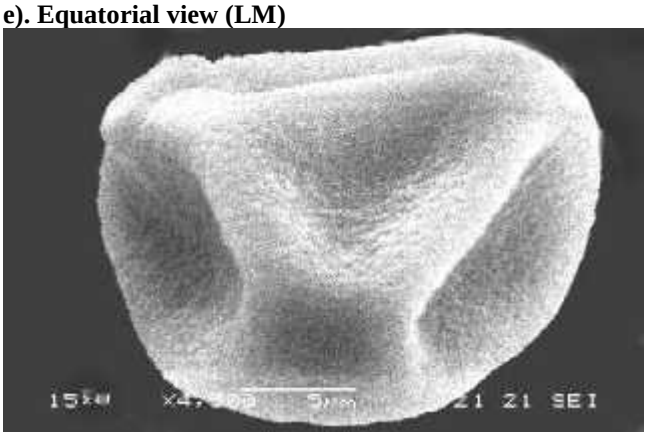


d). Macrohair (SEM)

e). Equatorial view (LM)

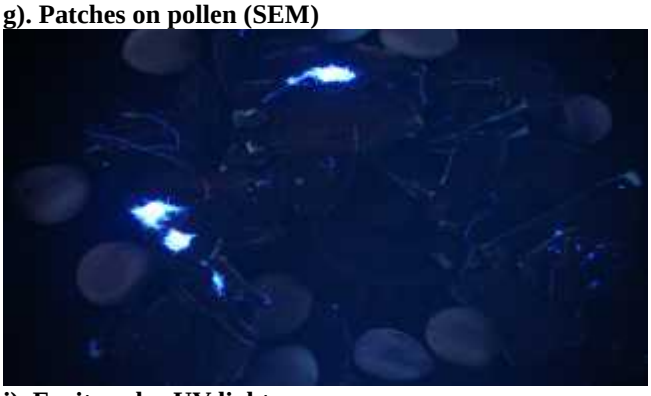


f). Semi-circular pollen showing thick exine (LM



g). Patches on pollen (SEM)

h). Semi-circular shaped pollen (SEM)



i). Fruit under UV light
Syn: ***Cassia obtusifolia*** **(L.).**

j). Fruit under IR light *Cassia occidentalis* (Linn.)
Senna occidentalis **(L.).**

Leaf Epidermal Anatomy(LM & SEM): In **abaxial epidermis,** the leaf epidermal cells were irregular shaped, smooth and thick walled, average length of epidermal cells was 100 (40-160) µm and the average width was 90 (50-130) µm, stomatal type was diacytic, paracytic and anisocytic, number of stomata per unit area was 11, open stomata were 5 and 6 closed stomata were present, average length of guard cells was 40 (30-50) µm and average width of guard cells was 30 (20-40) µm, average length of subsidiary cells was 65 (50-90) µm and the average width was 50 (30-70) µm. Silica bodies, trichomes, macro-hairs and micro-hairs were absent. In **adaxial epidermis,** the leaf epidermal cells were irregular shaped, smooth and thick walled, average length of epidermal cells was 70 (60-80) µm and the average width was 50 (40-60) µm, stomatal type was diacytic, paracytic and anisocytic, number of stomata per unit area was 15, open stomata were 4 and 11 closed stomata were present, average length of guard cells was 60 (40-80) µm and average width of guard cells was 20 (15-25) µm, average length of subsidiary cells was 100 (80-120) µm and the average width was 50 (40-60) µm, number of trichomes per unit area was 1, average length of trichomes was 75 (60-90) µm and the average width of trichomes was 55 (40-70) µm, average length of macro-hairs was 205 (130-280) µm and average width was 170 (140-200) µm. (Plate 2a, 2b, 2c, 2d).

Palynology (LM & SEM): In equatorial view, the pollen grains were elliptic and speroidal. In polar view, the pollen were trilobed and circular. (Plate 2f). Polar diameter was 28 (23-30) µm and equatorial diameter was 26.5 (21-32)µm. P/E ratio is 1.05, exine thickness was 3.0 (2.5-6)µm and intine thickness was 1 (0.5-1.5)µm. Colpi length was 57.5 (55-60)µm and width was 20.5 (18-23)µm. Iintercelluler difference of colpi was 5 (3-7)µm. Percentage of pollen fertility in this species was 89.43%. (Fig 2).

Organoleptography: Leaves were used. Colour of leaves was dark grayish green and had spicy taste and foul smell. Leaves were pubescent, leaflets pale green to bluish green, 3 to 9 pairs, lanceolate or elliptic, varying on the same plant, 1.5 to 5 cm. (Plate 2i, 2j).

Fluorescence and Solubility in Different Solvents: Actual colour of the powdered material was dark grayish green but colour changed in different solvents, became pink or magenta-red in distilled water, straw-yellow in sulphuric acid, yellow in hydrochloric acid and acetic acid and bright red colour in nitric acid. While performing cold test the powdered material was insoluble in all the solvents except sulphuric acid and nitric acid but became soluble during hot test.

Chemical Analysis: Alkaloids, glycosides, starch grains, tannins, anthraquinone, saponins and ferric chlorides whereas fixed and volatile oils were absent.

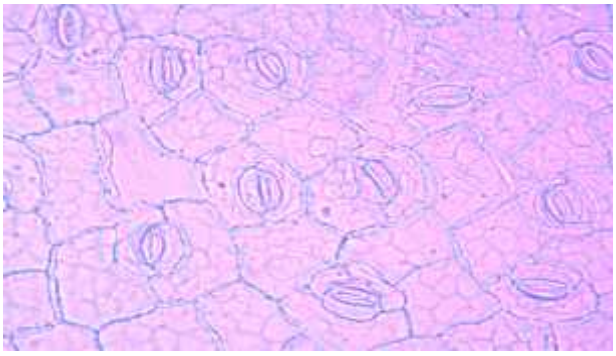
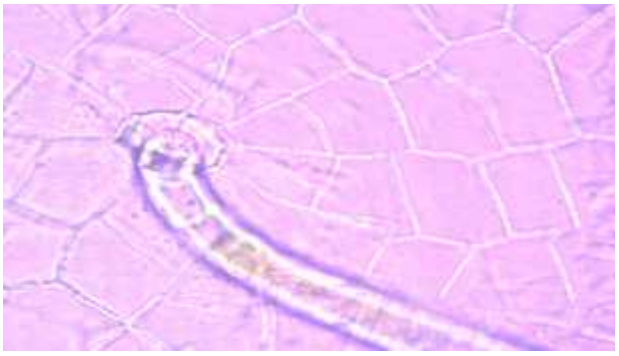
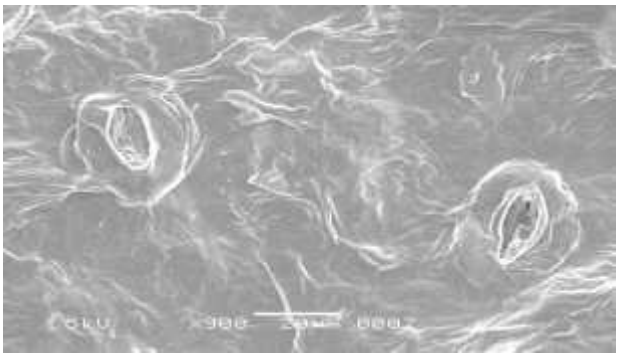


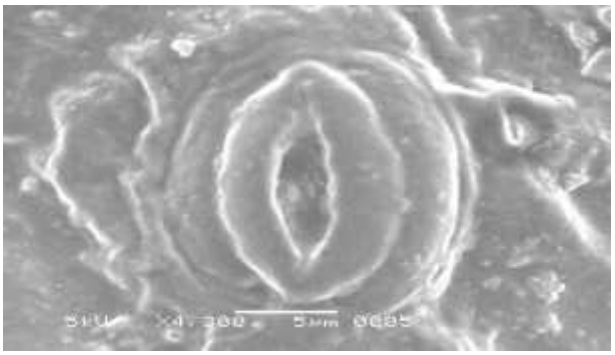
Plate 2: a). Abaxial side showing stomata (LM)



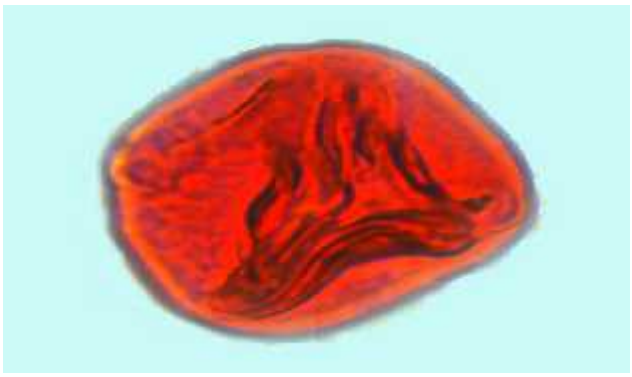
b). Adaxial side showing trichome (LM)



c). Stomata with guard cells (SEM)

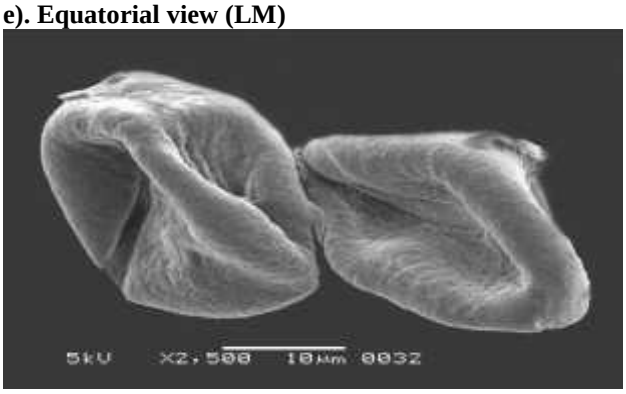


d). Open stomata (SEM)

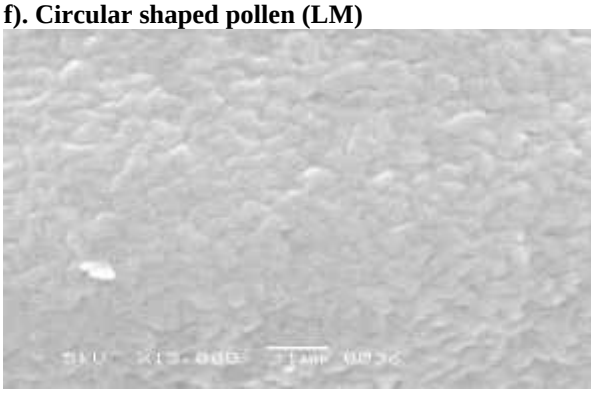


e). Equatorial view (LM)

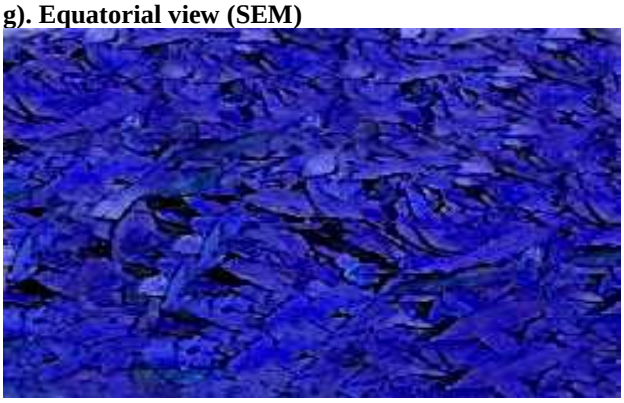
f). Circular shaped pollen (LM)



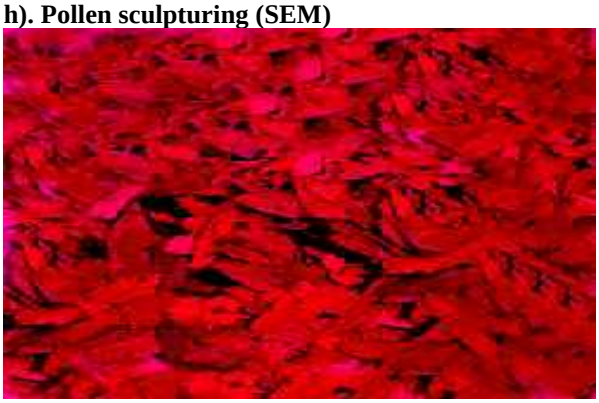
g). Equatorial view (SEM)



h). Pollen sculpturing (SEM)



i). Leaves under UV light



j). Leaves under IR light

***Dalbergia sissoo* (Linn.)**
Syn: *Amerimnon sissoo* (Roxb. ex DC.)

Leaf Epidermal Anatomy (LM & SEM): In **abaxial epidermis,** the leaf epidermal cells were irregular shaped and polygonal, smooth and thick walled, average length of epidermal cells was 65 (40-90) µm and the average width was 45 (40-50) µm, stomata were absent. Trichomes, macro-hairs, micro-hairs and silica bodies were absent. In **adaxial epidermis,** the leaf epidermal cells were irregular shaped and polygonal, smooth and thick walled, average length of epidermal cells was 60 (40-80) µm and the average width was 45 (30-60) µm, stomatal type was diacytic, number of stomata per unit area was 5, open stomata are 3 and 2 closed stomata were present, average length of guard cells was 30 (20-40) µm and average width of guard cells was 25 (20-30) µm, average length of subsidiary cells was 70 (60-80) µm and the average width was 55 (30-80) µm. Multicellular trichomes were present, number of trichomes per unit area was 6-10, average length of trichomes was 150 (140-160) µm and the average width of trichomes was 60 (40-80) µm. (Plate 3a, 3b, 3c, 3d).

Palynology (LM & SEM): In equatorial view, the pollen grains were elliptic and cup shaped. In polar view, the pollen were rounded, tri-lobed, elongated and spheroidal. (Plate 3e, 3f, 3h). Polar diameter was 47.5 (45-50)µm and equatorial diameter was 52.75 (50-55.5)µm. P/E ratio was 0.89, exine thickness is 3 (2-4)µm and intine thickness was 1.25 (1-1.5)µm. Colpi length was 87.5 (75-100)µm and width was 22.5 (20-25)µm. Intercellular

difference of colpi was 3.5 (2-5)µm. Percentage of pollen fertility in this species was 81.14%. (Fig 2).

Organoleptography: Leaves were used. Colour was yellowish to dark green having a rough surface. Leaves were 10-15 cm long and 4-6 cm wide. Taste was sweetish-sour. Smell was unpleasant. (Plate 3i, 3j).

Fluorescence and Solubility in Different Solvents: Actual colour of the powdered material was champagne but colour changed in different solvents, became rose wood in distilled water, conker in sulphuric acid, leaf green in hydrochloric acid and nitric acid. The powdered material was soluble in all the solvents during the cold test.

Chemical Analysis: Alkaloids, glycosides, starch grains, tannins, anthraquinone, saponins and ferric chlorides were present whereas fixed and volatile oils were absent.

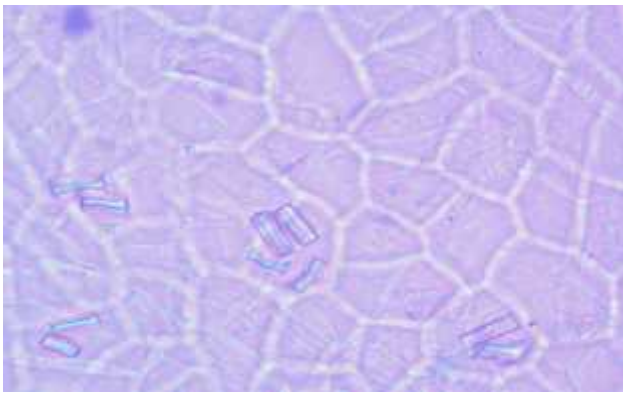
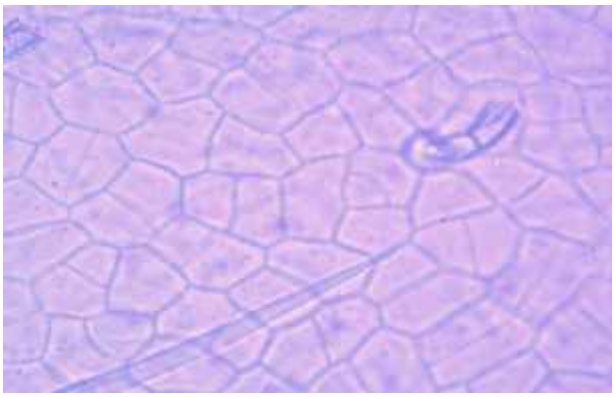
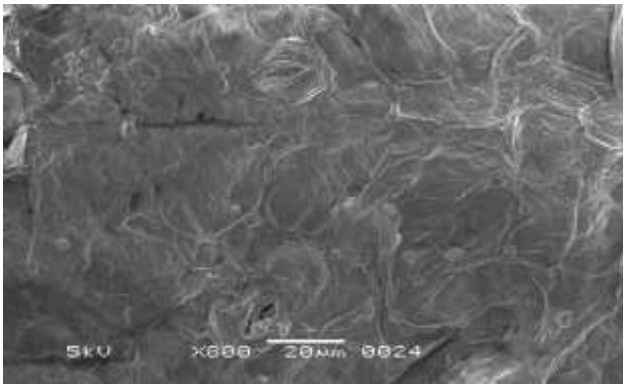


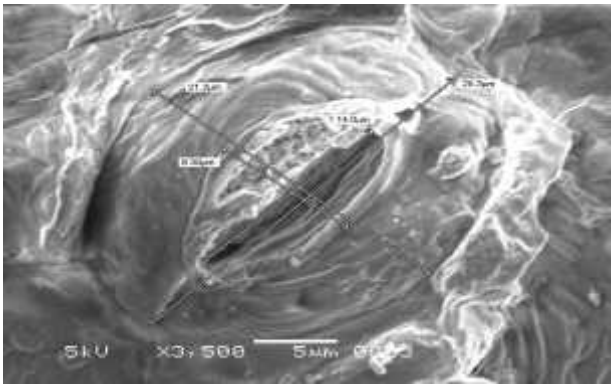
Plate 3:a). Abaxial side showing microhairs (LM)



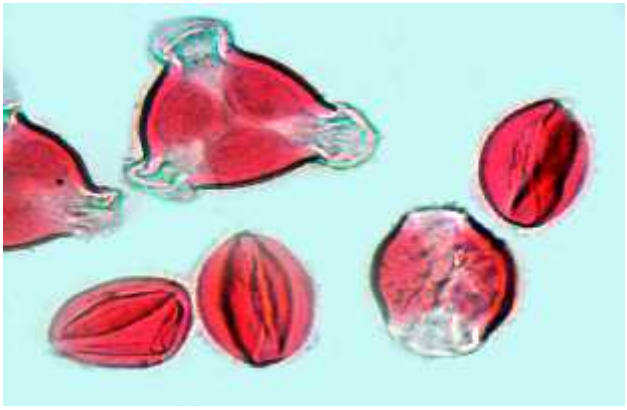
b). Adaxial side showing micro and macro hairs



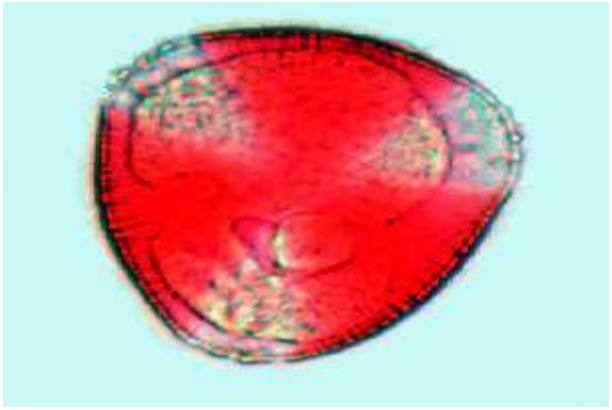
c). Abaxial side (SEM)



d). Stomata (SEM)

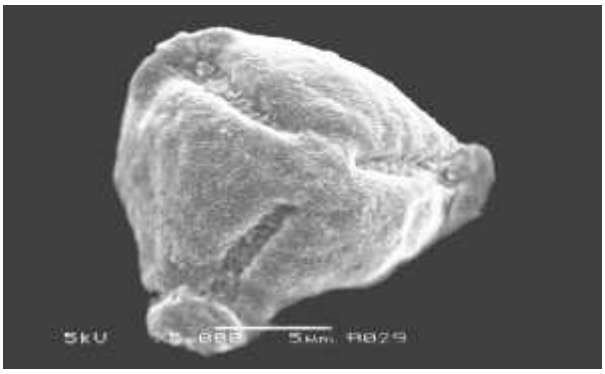


e). Different shapes of pollen (LM)



f). Pollar view (LM)

g). Elongated shaped pollen (SEM)



h). Tri-lobed shaped pollen (SEM)

i). Leaves under UV light

j). Leaves under IR light

Epidermal Leaf Anatomy: The present study provided a variety of anatomical features that can be employed as useful taxonomic characters. Stomata were absent in abaxial epidermis of *Dalbergia sisso* and different types of stomata were recognized like diacytic, paracytic, anisocytic and hypostomatal in all the three species studied. Ahmed *et al*., (2003) observed seven types of stomata (anisocytic, amphianisocytic, axillocytic, anomotetracytic, actinocytic, diacytic and staurocytic) in the members of family Fabaceae. Santos *et al*.,(2008) recognized four broad categories of stomata based on the presence and arrangement of accessory cells, as well as their mode of development in dicots. Adedeji *et al*., (2007) reported that the presence and absence of trichomes and their type is quite valuable in the configuration of characteristic anatomical markers, which have been proven to be of systematic value. In this study, the presence or absence of trichomes, as well as their types can be useful in characterizing the species studied. Multicellular trichomes were observed in *Cassia fistula* on both abaxial and adaxial epidermis, whereas in *Cassia occidentalis* and *Dalbergia sisso* only adaxial side possessed trichomes. (Plate 2b, 3a, 3b). Silica bodies play an important role in the identification and classification of different taxa. No silica bodies were observed in *Cassia occidentalis* and *Dalbergia sisso* where as in *Cassia fistula* both the abaxial and adaxial sides showed the presence of silica bodies. In this respect the presence of silica bodies emerged as useful taxonomic feature to delimit different taxa studied from each other. Shouling *et al.* (1996) discussed that the presence of macro-hairs and their average length is also an important character in identification. In the present investigation macro-hairs were observed in the two species i.e., *Cassia fistula* and *C. occidentalis*. (Plate 1b). No macro-hairs were found in the *Dalbergia sisso.* On the basis of the anatomical features we may distinguish different taxa and the present studies provided the taxonomic value of anatomical characters which along with other characters are of great taxonomic value in the classification and identification of the taxa studied.

Palynology: In the present study pollen morphology of selected three species had been investigated in the family Leguminosae. Pollen grains in all species were tricolporate and trizonoporate. Pollen size was variable among the species. It was observed that the pollen grain of *Cassia occidentalis* was smaller in size 26.5 (21-32) µm and pollen grain of *Dalbergia sissoo* was larger in size 52.75 (50-55.5) µm in equatorial diameter where as in polar view the size ranged from 28 (23-30) µm to 47.5 (45-50) µm. *Cassia occidentalis* appeared to be the smallest in size whereas *Dalbergia sissoo* was the largest. (Fig 1). Dahlgren (2004) found great variation in pollen characteristics among 34 species belong to Leguminosae in Taiwan.

In the present study we dealed with the taxonomic positions of the species under the light of the palynological data obtained. The pollen characters

obtained were generally compatible with those determined in previous studies except for pollen sizes, the number of pores and several ornamentation features. A striking fact of the evolutionary order of these selected genera was that pollen characters related to *Dalbergia* species were established as porate, and reticulate; those related to *Cassia fistula* which were psilate, perfolate, oblate-spheroidal to prolate-spheroidal or sub prolate. Pollen shape was generally uniform in the polar view in all the three species. However in equatorial view it varied to spheroidal to sub-spheroidal, oblate-spheroidal, prolate-spheroidal and elleptic to cup-shaped in all the species studied. (Plate 1f, 2f, 3e). Rickett (2007) utilized pollen character as additional information for systematic purposes. In the present investigation variation was seen in the number of colpi. No colpi were found in the *Cassia fistula*, number of colpi in the pollen of *Cassia occidentalis* may be 2 or 3 whereas in *Dalbergia sisso* they were always 3. The variation was also observed in the interspecific distance of colpi among the species studied. *Dalbergia sisso* showed lowest interspecific difference 3.5 (2-5) µm whereas *Cassia occidentalis* showed highest interspecific difference 5 (3-7) µm. On the basis of pollen fertility we may find compatible species in nature for getting promising hybrids. This information may be important to a plant breeder and can be used as diagnostic characteristics in the identification of species (Meo *et al.*, 1988). In the three species studied, the highest value of pollen fertility was found in *Cassia occidentalis* as 89.43% and the lowest value was in *Dalbergia sisso* as 81.14% (Fig 2).

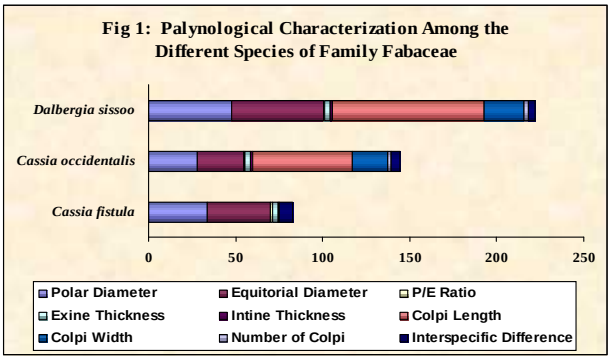


Fig 1: Palynological Characterization Among the Different Species of Family Fabaceae

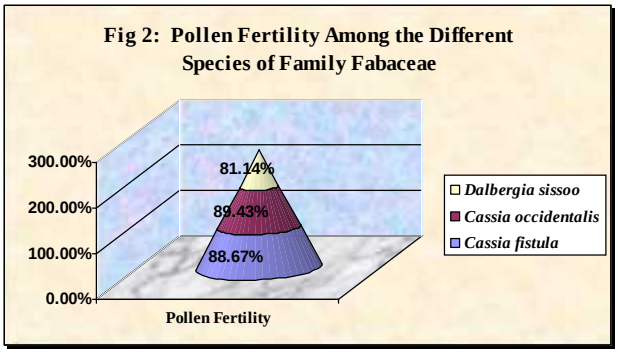


Fig 2: Pollen Fertility Among the Different Species of Family Fabaceae

UV, IR and Organoleptic Distinctions: The use of UV and Infra-Red photography is a unique and reliable method for identification and authentication of plant species. In the present work the UV and IR analysis showed assistance in the identification of the selected taxa. (Plates 1i, 1j, 2i, 2j, 3i, 3j). Organoleptic evaluation was used for the identification of sensory characteristics like colour, odour, taste, shape, size, texture and fracture. The market samples of *Cassia fistula* collaborated with actual sample collected from different localities of Lahore. Fruit was straight or slightly curved, smooth having chocolate-brown to almost black colour. These organoleptic characters were similar to the finding of Barthakur *et al.,* (1995). The analysis of market samples of *Dalbergia sisso* revealed the presence of rough surface of leaves having sweetish-sour 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).

Fluorescence and Chemical Distinctions: The present research work was confined to the macro, microscopic features of the powdered drug and their solubility and fluorescence analysis. The powdered drug of all the three species were soluble in all the solvents except nitric acid by cold test but became soluble by hot test. *C. occidentalis* did not retain its original dark grayish green colour on dry filter paper and became pink on filter paper in various solvents by cold and hot tests. Zaman, and Khan. (1997) also reported similar results of *C. occidentalis* on solubility in various solvents. Fabaceae is well suited with respect to chemical components. Lipids from some more common Fabaceae have been investigated to some extent, and some species of the family Fabaceae (Leguminosae) are also sources of cheap protein for both humans and animals. The fatty acids, alkaloids, flavonoids, glycosoids and volatile oils can provide characteristic information in order to confirm taxonomical and phylogenetic relationships in the plant kingdom (Bagci *et al.*, 2004). The results in the present investigation showed the presence of alkaloids, glycosides, starch grains, tannins, anthraquinone, saponins and ferric chlorides whereas fixed and volatile oils were absent in all the three selected plant species. The results corroborate with the findings of Palmer, 2003 according to which members of Fabaceae produce variety of compounds such as alkaloids, glycosides, starch grains, tannins, anthraquinone, triterpenoid, flavonoid and arylpropanoid.

Conclusion: The present project included a comprehensive study of multiple parameters (Anatomy (LM & SEM), palynology (LM & SEM), Organoleptography, UV and IR analysis, solubility, fluorescence and chemical analysis of the selected

medicinal plants of family Fabaceae. i.e. *Cassia fistula, cassia occidentalis* and *Dalbergia sisso*. The present research had been carried out to update the inventory of existing natural drug plants of the country. Leaf epidermal anatomy was found taxonomically useful which helps at the species level. Epidermal cells showed the difference in shape of epidermal cells, subsidiary cells, guard cells, micro-hairs and macro-hairs. Pollen characters in the all the three taxa had been helpful to distinguish the taxa at species as well as at generic level. The organoleptic, UV, IR and chemical analysis of the taxa showed a lot of variation among them, and can be used as a taxonomic tool for the identification and authentication of the taxa. It is concluded that Pakistan is quite rich in medicinal plants but no systematic attempt has been made to work on these natural resources properly. Many of the plans which are used for various ailments have either not been properly investigated or the findings have not been correlated with the phytochemical and pharmacological studies. The present research work gave a comprehensive detail of the systematics along with the pharmacognosy of the selected taxa which can be used as an aid in the identification and exploration of the medicinal wealth of the Pakistan.

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