TRADITIONAL PLANTS OF FAMILY SOLANACEAE FACING THE PROBLEM OF ADULTERATION: A CRITICAL ISSUE

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ABSTRACT: Authenticated identification of medicinal plants always had been an issue because of their local names misunderstanding and multiple morphological similarities. Hence the present study was an effort to provide some authenticated taxonomic tools for identification of three valuable selected medicinal plants of the family Solanaceae. i.e., *Datura stramonium* L., *Solanum nigrum* L. and *Withania somnifera* L. This study was based on multiple parameters (morphology, anatomy (Light Microscopy & Scanning electron microscopy), palynology (LM & Scanning Electron Microscopy), Organoleptography, UV (ultra violet) and IR (infra-red) analysis, solubility, fluorescence and chemical analysis, so as to establish some reiliable taxonomic tools for recognition of these plants. Anatomical studies were carried out to identify the species from the taxonomic point of view. A variety of characters like epidermal cells, subsidiary cells, guard cells, trichomes, macro-hairs, micro-hairs and stomata were used as a tool for the taxonomic grouping of different species. Pollen characters in all the three taxa were helpful to distinguish the taxa at species as well as at generic level. The organoleptic, ultra violet, infra red and chemical analysis of the taxa showed a lot of variation among them. The present research work provided a comprehensive detail of the systematics along with the pharmacognosy of selected taxa which can be used as an aid in the identification and exploration of the medicinal wealth of the Pakistan.

Key words: Solanaceae, Chemosystematic analysis, epidermal leaf anatomy, Pollen analysis, organoleptography, UV & IR analysis.

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INTRODUCTION

Adulteration is substitution of original plant by another morphologically similar plant which has low cost. This progression in most of the cases is dangerous due to the totally change chemical profile. Adulteration is the major issue behind the least priority of herbal drug system consequently drug efficacy will be reduced. Adulteration arises due to various reasons like non accessibility of original herbal drugs, misidentification of herbal drugs, low drug cost, variations in geographical distribution and adverse reaction of the drug (Parkash *et al*., 2013). Solanaceae is a large Family with around 3000-4000 species in 90 different genera, found in most temperate and tropical regions of Pakistan. It is a family mainly of herbs, with a few shrubs and trees, (ITIS, 1999). In Pakistan 14 genera and 52 species of Solanaceae are present of which 27 species are native, 6 naturalized and the others exclusively cultivated or found occasionally. The Solanaceae family is characteristically ethnobotanical that is extensively utilized by humans. Medicinally, members of Solanaceae have been prized and used throughout history (NHM, 2008). About 400-600 medicinal plant species are estimated to exist in Pakistan, (ITIS, 1999). Among the medicinally important species of Solanaceae, *Solanum nigrum* L. is a fairly common herb or short-lived perennial shrub. The plant has been used as a medicines from ancient time, dating back to ancient Greece. Their leaves are used for the treatment of mouth ulcers and fruit is also used for diabetes (NHM, 2008). *Datura* are potent medicinal members of the Solanaceae family, relatives of other well known strnomium plants Various species of *Datura* as traditional medicine worldwide, primary among them are *Datura innoxia, Datura metel, and Datura stramonium*. *Datura* has been used both as medicinal and ceremonial plant in many diverse cultures including Chinese, Indian, Mexican and Native Americans of the Southwest. *Datura* has played a major role in religious rites and medicine (Richard, 2002). *Withania somnifera* is also known as Ashwagandha. The herbal root extract has been traditionally used as a tonic and as a sedative. The leaves, berries and tubers of Ashwagandha have been in use for centuries as a home remedy and the extract is an important part of Indian Ayurvedic medicine (NHM, 2008). In Pakistan herbal plants are chiefly used by tibia dawakhanas. But unfortunately very little attention has been paid to the ethno-botanical and pharmacognostic aspect of plants because hakims are only interested in vegetative and floral parts of herbal plants without any concern to their botanical characteristics and their distribution in Pakistan's various ecological zones. In

Pakistan 80% of the people belonging to the rural areas still depend upon the herbal medicines (Anonymous, 2008). In the recent years, more efforts have been made to document the traditional knowledge. Although a lot of work has been carried out on *Solanum nigrum*, *Datura stramonium* and *Withania somnifera* but still there are certain gaps which need to be carried out in continuation of previous work. In this study these species were screened out for taxonomic characterization for correct identification. The pharmacognostic analysis of these medicinal plants might lead to the discovery of new herbal drugs. This study was first time done with reference to the applications of multiple parameters and helps to identify the species, which are capable of multiple uses, as well as alternative products such as medicines.

MATERIALS AND METHODS

The analytical studies were carried out by using light microscope and scanning electron microscope.

Anatomical Analysis: Leaves samples were prepared according to the method of Shaheen *et al.* (2011) and 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 slides of the leaves were prepared and observed by using light microscopy and scanning electron microscopy.

Palynological Analysis: The palynological analysis was carried out by following the Wodehouse technique by Ronald (2000) and the pollen fertility study was done by employing the techniques used by Meo and Khan (2004) through light microscopy and scanning electron microscopy.

Organoleptic Analysis: Organoleptic analysis involved the use of sight, smell, taste, touch and macroscopy of crude drugs to evaluate plant materials often comparing the properties of a known sample with that of a reference standard. Material for organoleptic analysis was procured from herbal shops and were also collected from the field.

Microphotographs (LM & SEM): Microphotographs of leaves and pollen samples were taken by Nikon (FX-35) Camera equipped with light microscopy and scanning electron microscopy.

Phytochemical Analysis: Phytochemical screening of selected plant parts was done for the detection of alkaloids, glycosides, tannins, starch grains, Anthraquinones, saponins, fixed and volatile oils and acid hydrolysis by following the method of British Pharmacopoeia (1999).

RESULTS AND DISCUSSION

***Datura stramonium* L.**

Syn: *Datura tatula* L.

***Datura stramonium var. tatula* (Willd.) Clarke**

Anatomical Analysis (LM & SEM): In **abaxial epidermis,** the leaf epidermal cells were polygonal type, thick smooth walled, the average length of epidermal cells was 125 (90-160) µm and the average width was 110 (80-140) µm, the stomata were few in number and their type was diacytic. The number of stomata per unit area was 4, all the stomata were open and no closed stomata were present, the average length of guard cells was 27.5 (25-30) µm and average width of guard cells was 6 (5-7) µm. the average length of subsidiary cells was 120 (90-150) µm and the average width was 40 (50-90) µm. Micro-hairs were present having an average length of 12.5 (10-15) µm. Trichomes , macro-hairs and silica bodies were absent. In adaxial epidermis, the leaf epidermal cells were polygonal type, and the average length of epidermal cells was 93 (90-96) µm and the average width was 66.5 (60-73) µm, stomata were in abundance, stomatal type was diacytic or Paracytic. The number of stomata per unit area was 15. The open stomata were 11 and 4 closed stomata were present, average length of guard cells was 42.5 (40-45) µm and average width of guard cells was 9.5 (7-12) µm. The average length of subsidiary cells was 120 (90-150) µm and the average width was 40 (50-90) µm. Trichomes, macro-hairs, micro-hairs and silica bodies were absent (Fig. 1, 2, 3, 4).

Palynological Analysis: In equatorial view, the pollens were circular and semi-circular. In polar view, they were semi-angular, prolate and spheroidal (Plate 1f, 1g). Polar diameter was 172 µm (109-234 µm) and equatorial diameter was 165 µm (140-179 µm). The P/E ratio was 1.4 and exine thickness was 1 µm, entine thickness was 1.2 (0.9-1.3). The Colpi length was 22 (20-24) and the colpi width was 50.5 (48-54).inter-cellular difference was 10 (8-12). Percentage of pollen fertility in this species was 78.66% (Graph 2).

Organoleptographic Analysis: In this study **s**eeds were used. The colour was marvel brown and red has bitter oily taste. The smell was pleasant. The shape of seeds was semicircular and smooth having a length of 0.1-0.2 cm and the width was 0.1-02 cm (Fig. 9).

Fluorescence and Solubility in Different Solvents: Actual colour of the powdered material was marvel brown but colour changed in different solvents, became copper in distilled water, brown in sulphuric acid, leather brown in hydrochloric acid, beige in acetic acid and leaf green in nitric acid. While performing cold test the

powdered material was insoluble in all the solvents except sulphuric acid and hydrochloric acid but was soluble in acetic acid and remained insoluble in nitric acid during hot test.

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

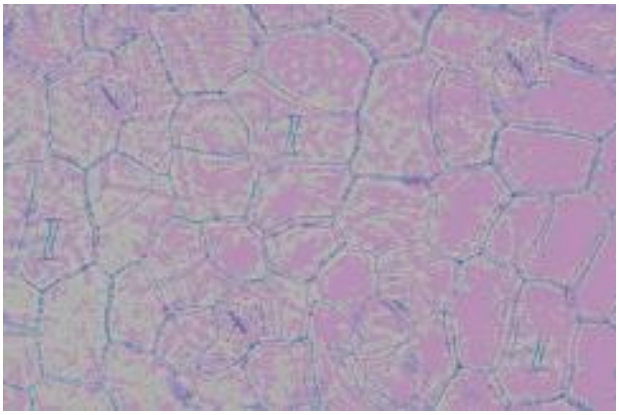


Figure 1: Abaxial side showing microhairs (LM)

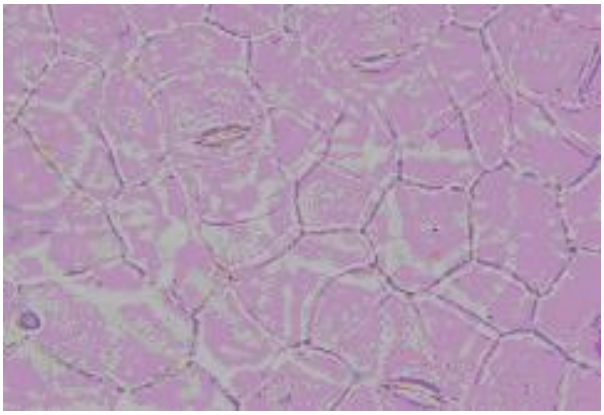


Figure 2: Adaxial side showing stomata (LM)

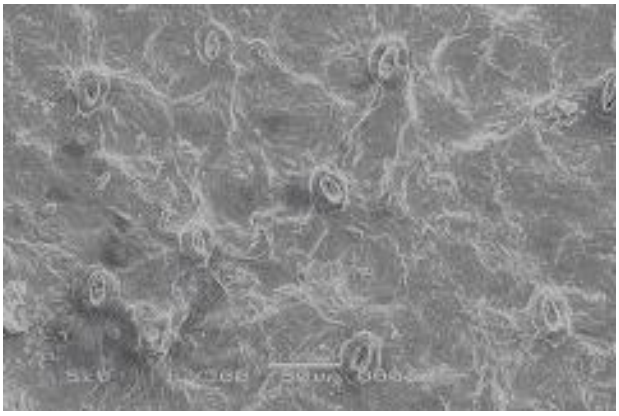


Figure 3: Abaxial side showing epidermal cells (SEM)

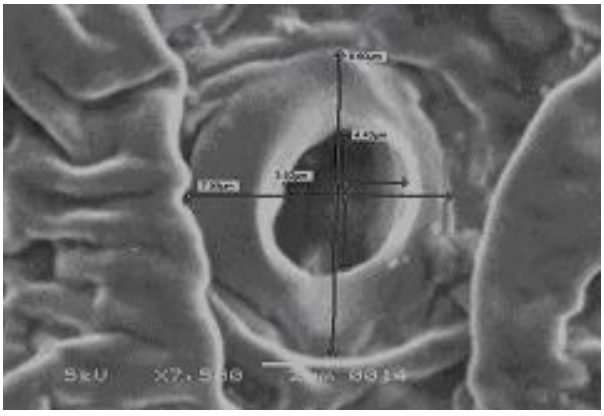


Figure 4: Open stomata (SEM)

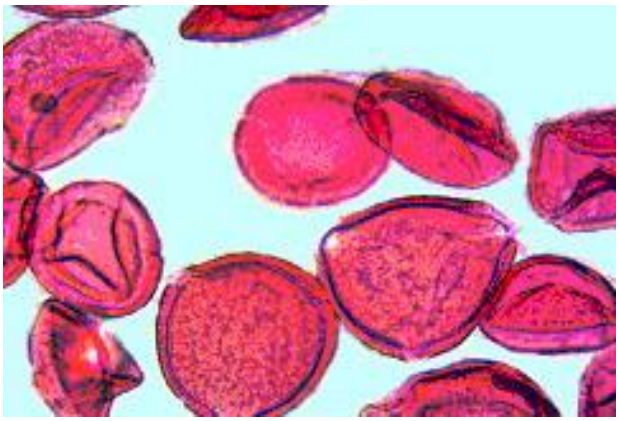


Figure 5: Pollen Fertility (LM)

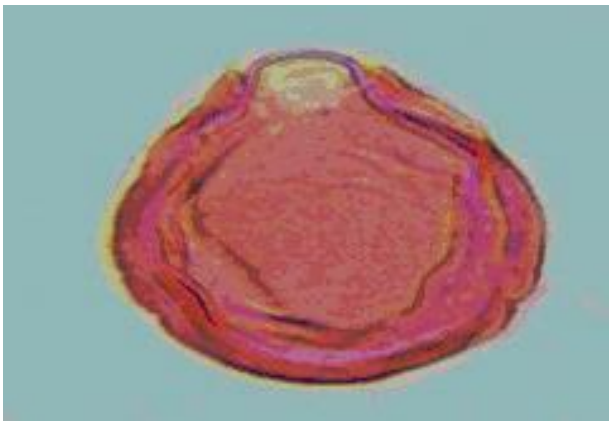


Figure 6: Polar view (LM)

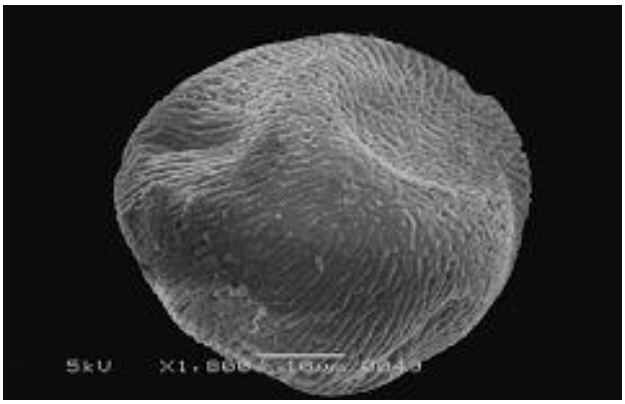


Figure 7: Patches on pollen (SEM)

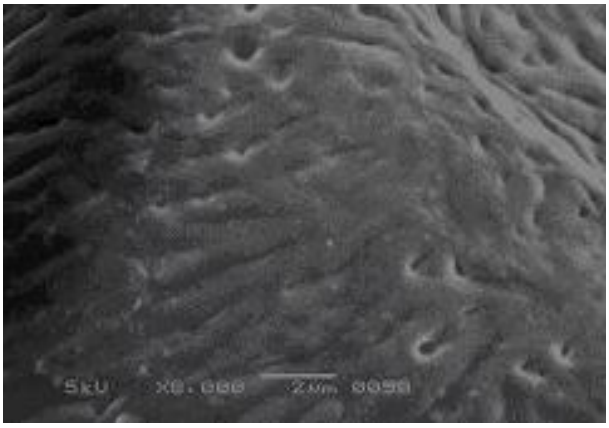


Figure 8: Pollen sculpturing (SEM)

Figure 9: Seeds under visible light

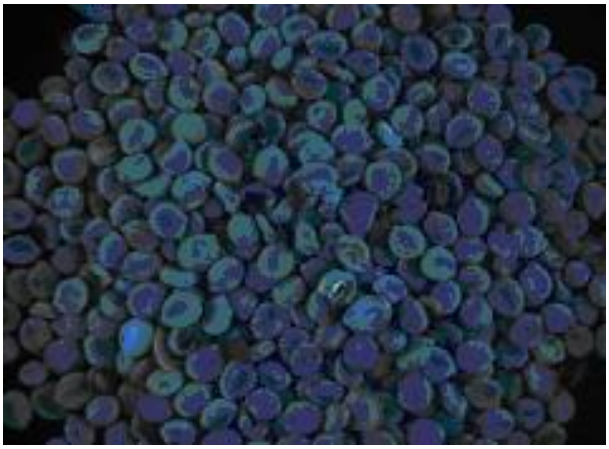


Figure 10: Seeds under UV light

Figure 11: Seeds under IR light *Solanum nigrum* (Linn) Syn: *Solanum rubrum* auct. non L.*Solanum villosum* (L.) Moench

Anatomical Analysis: In abaxial epidermis, the leaf epidermal cells were irregular shaped, smooth thick walled. The average length of epidermal cells was 50 (40-60) µm and the average width was 35 (30-40) µm. The stomata were present, stomatal type was anisocytic. The number of stomata per unit area was 11. The open stomata were 4 whereas 7 closed. The average length of stomatal guard cells was 13 (10-16) µm and average width of guard cells was 4.5 (2-7) µm. The average length of subsidiary cells was 115 (80-150) µm and the average width was 75 (50-100) µm. Trichomes were present. The number of trichome per unit area was 1; the

average length of trichomes was 200 (180-220) µm. The silica bodies were absent. In adaxial epidermis, the leaf epidermal cells were irregular shaped. The average length of epidermal cells was 45.25 (30-60.5) µm and the average width was 25 (20-30) µm. The stomata were present and their type was diacytic. The number of stomata per unit area was 6. The open stomata were 2 and 4 were closed. The average length of guard cells was 30.25 (20-40.5) µm and average width was 7.75 (5-10.5) µm, average length of subsidiary cells was 110 (100-120) µm and the average width was 70 (50-90) µm. Multicellular micro-hairs were present having an average length of 47.75 (35.5-60) µm and the average width was 8 (5-11) µm. Silica bodies were absent (Fig 12, 13, 14, 15).

Palynological Analysis: In equatorial view, the pollens were circular and semi-circular (Fig 16). In polar view, the pollens were semi-angular, prolate and spheroidal. Polar diameter was 103.4 µm (102.2-103µm) and equatorial diameter was 107.3 µm (101.1-107.3 µm). P/E ratio was 1.0 and exine thickness was 0.9 µm (0.9-1 µm) and entine thickness was 1.2. Colpi length was 11 and colpi width was 22.7. Pore was endoporus. Percentage of pollen fertility in this species was 78.66% (Graph 2).

Organoleptographic Analysis: in this study fruit was used. Colour of fruit was rose wood and had sweet taste and unpleasant smell, shape was round and less smooth. It was circular in shape and its diameter was about 0.02 to 0.03 cm (Fig. 20).

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

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

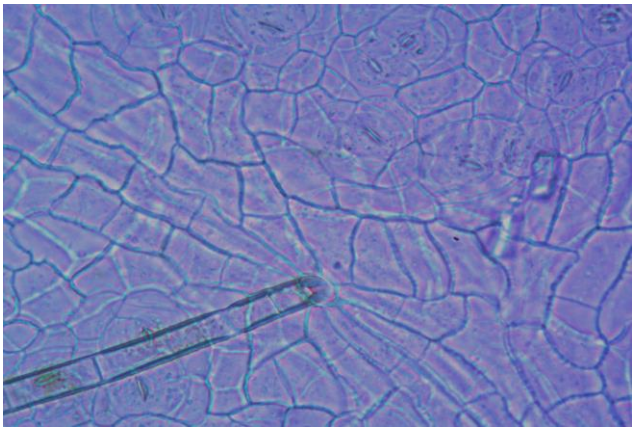


Figure 12: Abaxial side showing trichomes (LM)

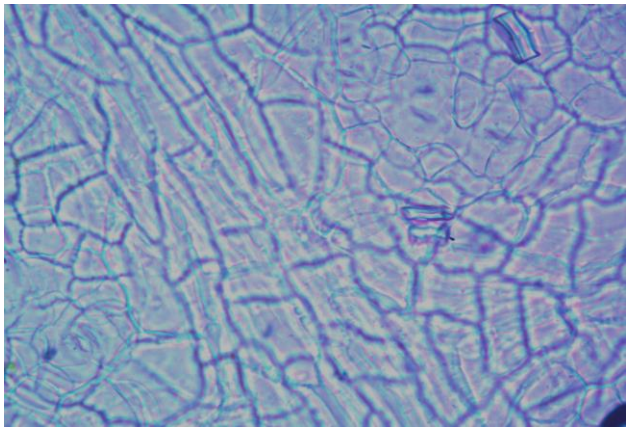


Figure 13: Adaxial surface (LM)

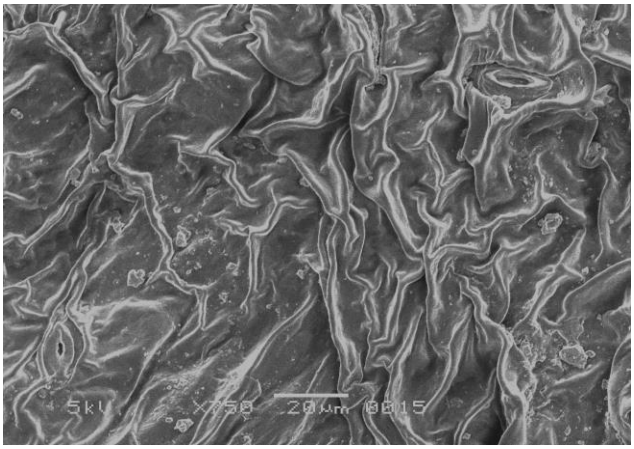


Figure 14: Epidermal cells and stomata (SEM)

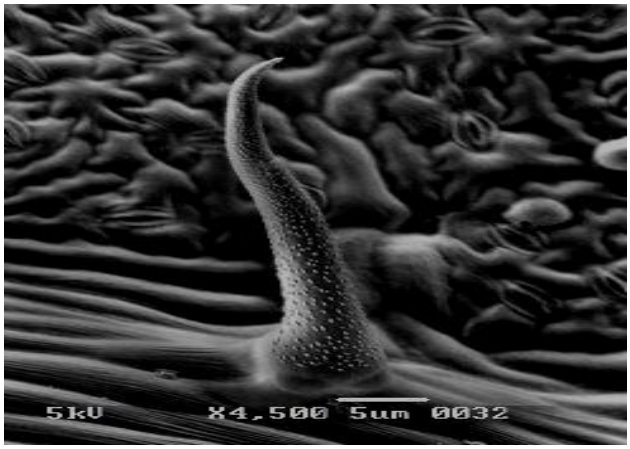


Figure 15: Trichome (SEM)

Figure 16: Circular shaped pollen (LM)

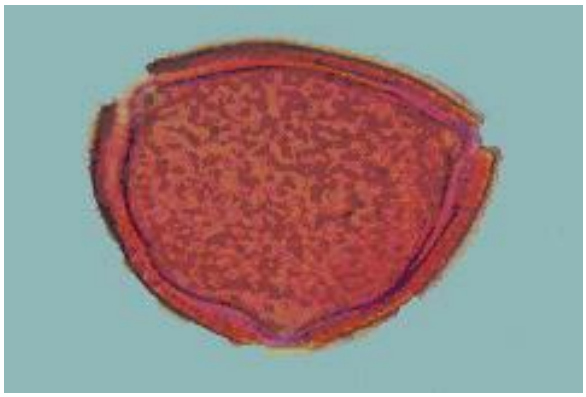


Figure 17: Pollen showing thick exine (LM)

Figure 18: Equatorial view (SEM)

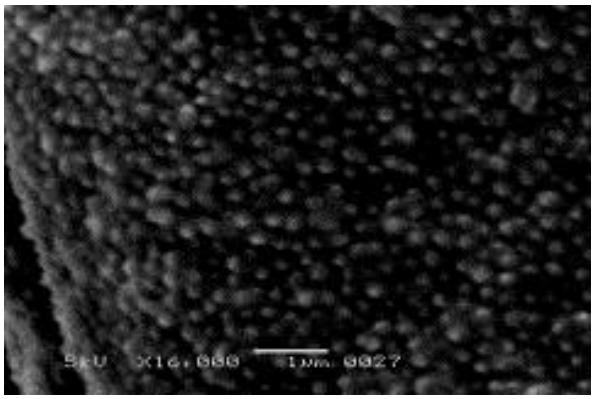


Figure 19: Granulated sculpturing (SEM)

Figure 20: Fruit under visible light

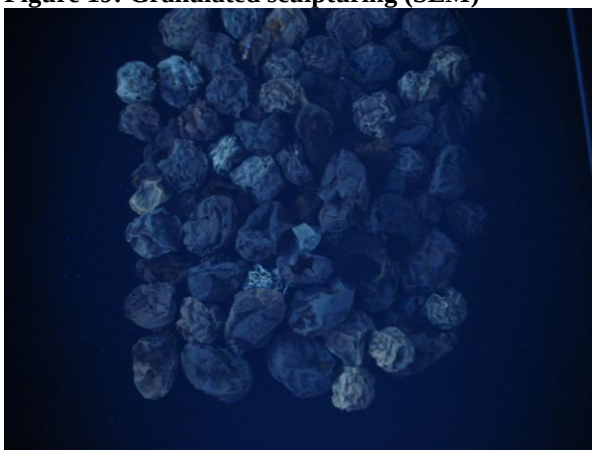


Figure 21: Fruit under UV light

Figure 22: Fruit under IR light

***Withania somnifera* (L.)**
Syn:*Physalis somnifera* L.

***Withania kansuensis* Kuang & A. M. Lu**

Anatomical Analysis (LM & SEM): In abaxial epidermis the leaf epidermal cells were irregular shaped having thick undulate walls, average length of epidermal cells was 160 (130-190) µm and the average width was 72.5 (65-80) µm. The stomata were present, stomatal type was anisocytic and diacytic. The number of stomata per unit area was 3. The open stomata were 2 and 1 closed stomata was present. The average length of guard cells was 42.5 (25-60) µm and average width of guard cells was 20 (10-30) µm, average length of subsidiary cells was 130 (100-160) µm and the average width was 85 (80-90) µm. Multicellular micro-hairs were present having an average length of 25 (20-30) µm. Silica bodies were absent. In adaxial epidermis the leaf epidermal cells were irregular shaped having thick undulate walls, average length of epidermal cells was 155 (120-190) µm and the average width was 70 (60-80) µm, stomata were present, stomatal type was anisocytic and diacytic. The number of stomata per unit area was 5. The open stomata were 4 and 1 closed stomata was present. The average length of guard cells was 40 (20-60) µm and average width of guard cells was 20 (10-30) µm. The average length of subsidiary cells was 130 (110-150) µm and the average width was 65 (60-70) µm. Multicellular micro-hairs were present having an average length of 40 (30-50) µm. Silica bodies were absent (23, 24, 25, and 26).

Palynological Analysis: In equatorial view, the pollens were circular and semi-circular. In polar view, the pollens were semi-angular, prolate and spheroidal (Fig 27, 28, 29, 30). Polar diameter was 195.9 µm (211.6-180.2 µm) and equatorial diameter was 155 µm (175.5-134.9 µm). P/E ratio was 1.3 and exine thickness was 1.1 (.9-1.1 µm). The entine thickness was 1.5. while intercellular differences was 9.9 (7.8-12) µm. The colpi length was 44.5 and the colpi width was 83.4. The percentage of pollen fertility in this species was 79.66% (Graph 2).

Organoleptographic Analysis: The dried part of roots was used. Colour of root was yellowish brown or light brown, its outer surface was bugg to grey yellow with longitudinal wrinkles and in the center soft and solid mass with scattered pores were also present. The smell was unpleasant like horse's smell and taste was bitter and acrid. Length ranged from 0.9-1.5 cm and diameter was 0.3 cm (Fig. 31)

Fluorescence and Solubility in Different Solvents: Actual colour of the powdered material was Camel colour but colour changes in different solvents, become copper in distilled water, brown in sulphuric acid, mustard in hydrochloric acid, pale cream in acetic acid and honey-dew in nitric acid. While performing cold test the powdered material was soluble in all the solvents except sulphuric acid but became soluble in all solvents during hot tests.

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

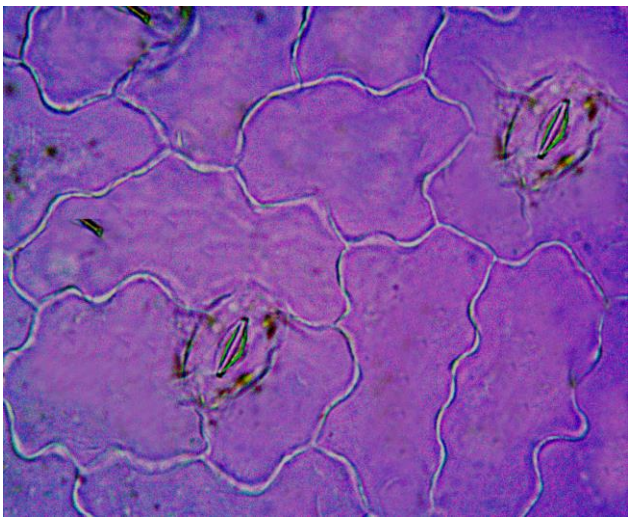


Figure 23: Abaxial side (LM)

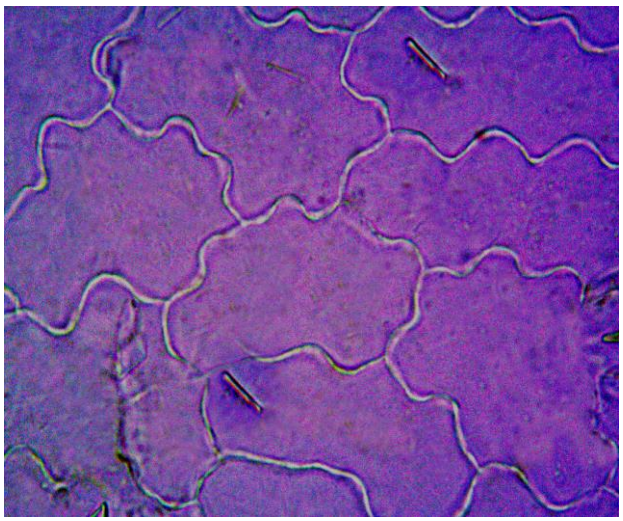


Figure 24: microhairs (LM)

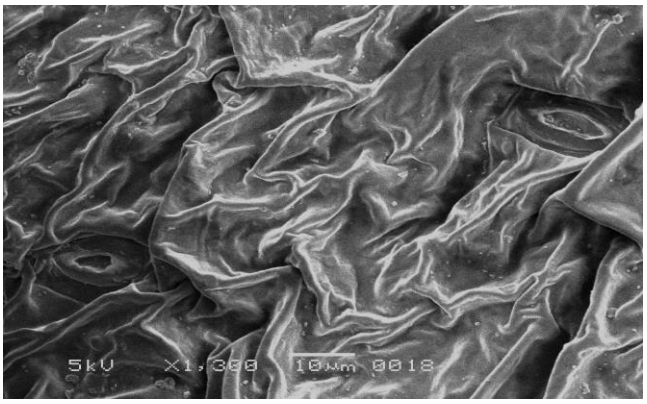


Figure 25: Stomata (SEM)

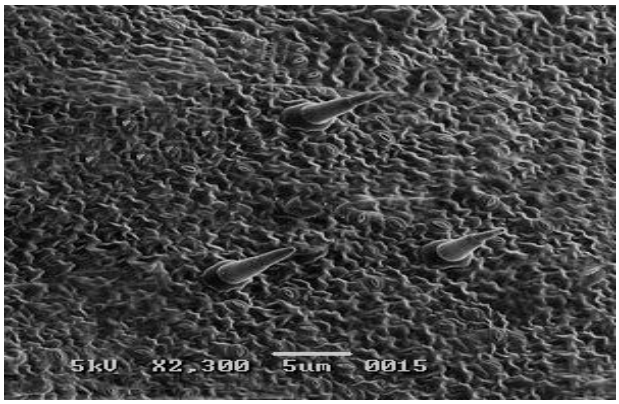


Figure 26: Microhairs (SEM)

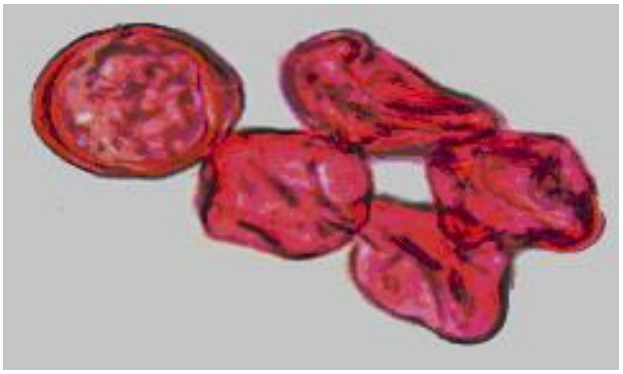


Figure 27: Equatorial view (LM)

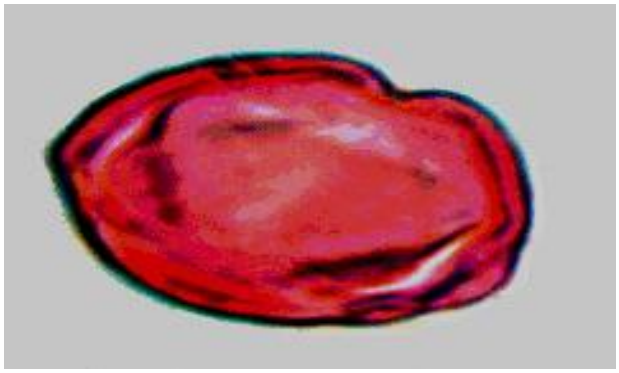


Figure 28: Porate pollen (LM)

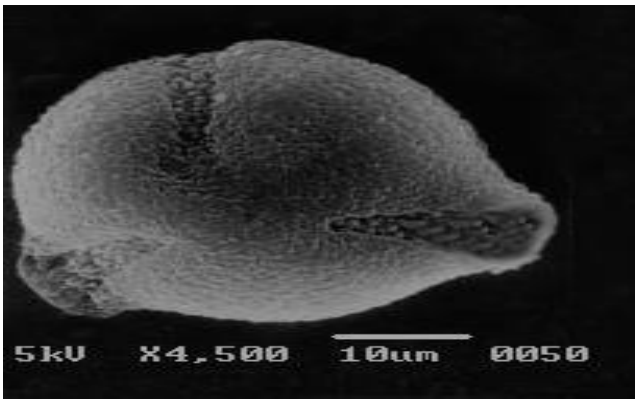


Figure 29: Tricolporate pollen

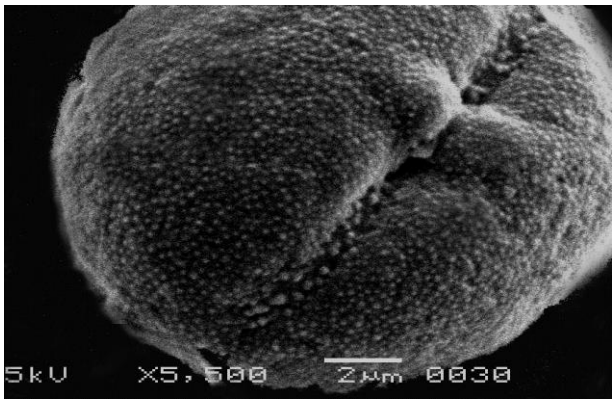
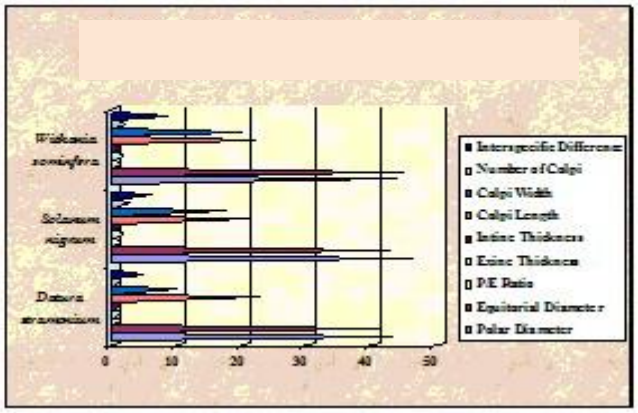


Figure 30: Pollen Cleave (SEM)

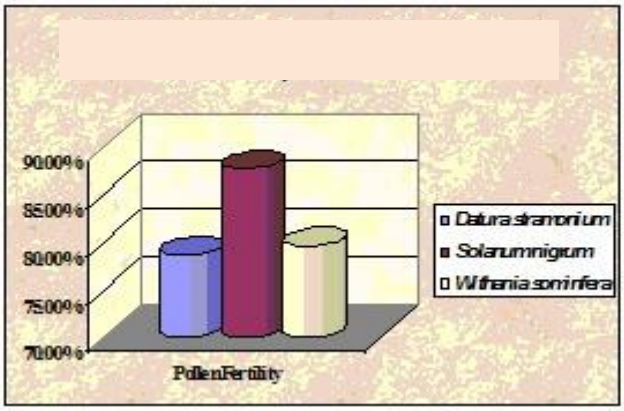
Figure 31: Roots under visible light

Figure 32: Roots under UV

Figure 33: Roots under IR light



Graph 1: Palynological distinction among selected members of family solanaceae



Graph 2: Pollen fertility of selected members of family solanaceae

DISCUSSIONS

Epidermal Leaf Anatomy: The present study described the biological significance and implications of leaf anatomical characteristics of the three species. The walls of the epidermal cells of the two species (*Datura stramonium* and *Solanum nigrum*) were smooth and thick whereas the *Withania somnifera* showed the thick undulate walls. The distribution of stomata varied in the taxa studied. In *Datura stramonium* the number of stomata per unit area was 4, in *Solanum nigrum* 11 and in *Withania somnifera* 3. Rogers and Ogg (2001) reported that stomata number were two to three-fold higher on abaxial than on adaxial epidermis of the Solanum species (*S. nigrum, S. sarrachoides, S. americanum* and *S. ptycanthum*). The stomatal type showed variation in the species under study. In *Datura stramonium* stomata were diacytic, in *Solanum nigrum* stomata were anisocytic and in *Withania somnifera* stomata were diacytic and anisocytic. In a study Lana *et al.* (2002) reported anomocytic to anisocytic stomata in *S. nigrum.* Microhairs were seen in almost all the taxa studied. These hairs differed in form, length and width. Glandular hairs were not numerous. Mostly hairs seen were multicellular as in *S. nigrum* and *W. somnifera.* Seithe and Anderson *et al.* (2002) investigated hair morphology of some species from the genus *Solanum*, but not in *S. nigrum*. Silica bodies were absent in all the taxa studied. The present study provided the anatomical structure of leaves with the aim of determining their structural adaptations.

Palynology: Pollen morphology of the family Solanaceae was quite heterogenous. Most striking variation was found in the shape class and apertural types. The greatest variation was observed in pollen morphology of the Solanaceae species. Exine thickness ranged from 1.4(1.3-1.5) µm -1.8(1.7-1.9) µm among the species studied. *Withania somnifera* showed the highest value whereas *Solanum nigrum* showed the lowest value. *Datura stramonium* showed the highest intine thickness i.e. 1.5(1-2) µm whereas *Solanum nigrum* showed the lowest value i.e. 1(0.5-1.5) µm. Exine and intine thickness were prominent features in this study. Nwachukwu and Okeke (2001) reported that pollen grains were very helpful in assigning the status of plant since pollen grain wall had specific characters. Pollen size was variable among the species. It was observed that the pollen grain of *Solanum .nigrum* was smaller in size 43.25(42.5-44) µm and pollen grain of the *Withania somnifera* was larger in size 46.5(45-48) µm in polar diameter where as in equatorial view the size ranged from 41.5 (40.5-42.5) µm to 45(44-

46) µm. *Solanum nigrum* appeared to be the smallest in size whereas *Datura stramonium* was the largest. The colpi length ranged from 21.5(21-22) µm to 23(22-24) µm. *Solanum nigrum* showed the highest where as *Withania somnifera* showed the lowest value. This variation in size may be due to indiscriminate mating, leading to hybridization. This is not surprising since previous workers have made similar observations in other groups of angiosperm. The pollen grains of the species studied showed similarities in their pollens attributes of wall sculpture, aperture, and symmetry. The pollen grain of each specie was radially symmetrical, isodiametric and isopolar. These results corroborated with the findings of Nwachukwu and Okeke (2001) according to which the pollens of Solanaceae were grain polar and radially symmetrical. The pollen shape was found to be more or less circular to semi-circular and angular in polar view and angular, semi angular spheroidal, elliptic and oval in equatorial view in the species studied. Further more, similarities were found in aperture type (tricolporate), and wall structure (scabrate) of the pollen grain of species studied. P/E ratio ranged from 1 to 1.4 among the species. The pollen grains were monoporate and psilate (smooth). The pore position was endoporus in *Solanum nigrum* and *Withania somnifera* where as exoporus in *Datura stramonium*. The taxonomic significance of pollen morphology in Solanaceae was more or less obscure. Sometimes different tribes or sub tribes had similar type of pollen or vice versa and sometimes species referred to the same genera and tribe had different type of pollens.

UV, IR and Organoleptic Analysis: The Solanaceae family was characteristically ethno-botanical that is, extensively utilized by humans. It was an important source of food, spice and medicine. Organoleptic evaluation with the advanced microscopic equipments provided more accuracy for botanical authentication (Jackson and Snowdown, 2000). The analysis of market samples of *Datura stramonium* when compared with the samples collected from different localities of Lahore showed that seeds were semi-circular and smooth surfaced. But the colour of seeds of market samples i.e., marvel brown differed from fresh having dark brown to black colour. These results were similar to the findings of (Davihazy, 2004). In case of *solanum nigrum* the market samples revealed the presence of non smooth surface and semi circular fruit. Market fruit was dried therefore its shape become semicircular while the fresh fruit was circular, shiny and red with smooth surface. In case of *Withania somnifera* market sample collaborated with actual sample. The outer surface of roots was bugg to grey yellow with longitudinal wrinkles and in the center soft, solid mass with scattered pores whereas fresh roots were brownish grey with long fleshy tubers. These results were in accordance with the finding of Seithe and Anderson (2009) who reported the similar results in their study.

Fluorescence and Chemical Analysis: The present research work was confined to detailed analysis of the powdered drug and their solubility and fluorescence analysis. The powdered drug of all the three species was soluble in all the solvents by cold and hot tests except *Datura stramonium* which was soluble in all the solvents except nitric acid and on dry filter paper it did not hold its original marvel brown colour. Dastagir and Haq (2005) also reported similar results of *Datura stramonium* on solubility in different solvents. In all the three studied species alkaloids, glycosides, starch grains, tannins, anthraquinone, saponins were present whereas absence of fixed and volatile oils were reported in current study. These findings were similar to the results of Dweck (2007) research.

Conclusion: The present study was a step towards preparing a systematic inventory of selected medicinal plants of family Solanaceae. Medicinal plants face the problems in their identification due to confusion in nomenclature, taxonomic ranking, and differentiation of various species at specific level sometimes at generic level also, but these problems can be overcome by using classical and applied approaches of taxonomy. The present account includes the comprehensive study of these approaches. Classical approaches are morphology, anatomy, palynology, UV and IR analysis and organoleptography whereas the applied approaches includes their chemical analysis. All these parameters showed successful findings and can be helpful for the identification, authentication and classification of the selected plants.

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