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Full Length Research Paper

Systematic and ethnopharmacognostic investigation of selected medicinal plants of family Asteraceae

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The present research project includes the family Asteraceae comprising the species *Calendula arvensis* L.*, Parthenium hysterophorus* L. and *Silybum marianum* (L.) Gaertn. The present study was carried out to clear up the taxonomic position of the selected taxa and their authenticity. 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. Classical approaches are morphology, anatomy, palynology, ultra violet (UV) and infra red (IR) analysis and organoleptography whereas the applied approaches include their chemical analysis. In the context of morphology, characters such as calyx and corolla shape and size, flower color, shape and size etc showed variation. Leaf epidermal anatomy was found taxonomically useful. Epidermal cells show the difference in shape of epidermal cells, subsidiary cells, guard cells, micro-hairs and macro-hairs. All the three taxa can be distinguished easily on the basis of stomata type and width found useful in the delimitation of the taxa. Pollen characters were also studied including shape and size of pollen grains in equatorial and polar view. UV, IR and organoleptography analysis also showed a lot of variations among the selected taxa. The fluorescence, solubility and chemical analysis was also done in order to delimit the taxa. All these parameters showed successful findings and can be helpful for the identification, authentication and classification of the selected plants. All the available records are listed and mapped.

Key words: Morphology, anatomy, palynology, organoleptography.

INTRODUCTION

The use of the medicinal herbs for curing disease has been documented in history of all civilizations. The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. Man in the pre-historic era was probably not aware about the health hazards associated with irrational therapy. With the onset of research in medicine, it was concluded that plants contain active principles, which are responsible, for curative action of the herbs (Yineger et al., 2007). According to the World Health Organization (WHO), over 80% of the world's population, or 4.3 billion people, rely upon traditional plant-based systems of medicine to provide them with primary health care (Bannerman et al., 1983). Of the 250,000 higher plant species on earth, more than 80,000 are medicinal. India is one of the world's 12 biodiversity centers with the presence of over 45,000 different plant species. Of these, about 15,000 to 20,000 plants have good medicinal value. However, only 7,000 to 7,500 species are used for their medicinal values by traditional communities (Qureshi and Ghufran, 2007).

Pakistan is one of the few places on earth with such a unique biodiversity, comprising of different climatic zones with a wide range of plant species. Approximately 6,000

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plant species with medicinal properties are found in Pakistan. Traditional Unani medicine is a part of our culture and Pakistan is one of those countries where traditional Unani medicine is popularly practiced among the large segment of its population. Traditional Unani medicine heavily depends on medicinal plants, apart from using animals and minerals (Ahmad et al., 2003**).** One of the most common families which have accepted medicinal value is Asteraceae or Compositae, also referred to as the aster, daisy, or sunflower family, which is the largest family of vascular plants. The family has more than 22,750 currently accepted species, spread across 1,620 genera and 12 subfamilies. In Pakistan it is represented by 650 species distributed in 15 tribes (Nasir and Ali, 1982). Many species of this family are easily obtainable and their local medicinal uses include painkiller, diuretic, febrifuges, carminative, anthelmintic, anti-inflammatory, aphrodisiac, cardio tonic, tonic, stomachache, dyspepsia, jaundice, leprosy, cough, asthma, ulcers, vomiting etc (Zafar et al., 2007).

Among the medicinally important species of Asteraceae *Parthenium hysterophourus* L. is described as a weed and is reported to be useful against dysentery, hepatic amoebiasis and skin disorders (Javaid and Anjum, 2005; Oudhia, 1998; Bremer, 1994). *Calendula arvensis* L. is a type specimen of medicinally important plants of Asteraceae and is used as antiseptic and skin stimulant in homeopathy. The plant seems to have a remedy for skin problems and is applied externally to bites and stings, sprains, wounds, sore eyes, varicose veins etc. (Herman, 2003). Another medicinally important plant of this family is *Silybum marianum* (L.) In herbalism, it is used in cases of liver diseases (cirrhosis, jaundice and hepatitis), gallbladder disease and is claimed to protect the liver against poisons (Van Breda and Barnard, 2001).

Although a lot of work has been carried out on *P. hysterophourus, C. arvensis* and *S. marianum* but still no systematic attempt has been made to carry out the work on multiple parameters including morphology, anatomy, palynology, organoleptography and pharmacognosy. This study provides the descriptions of multiple parameters and point out the important features of each of the selected taxon by combining several characters to get as much information as possible, which eventually leads to correctly identify the species. The overall objectives of the present study were to show how morphological, anatomical and pollen characters help in identification and differentiation at species level and also to investigate some medicinal plants of Asteraceae used traditionally in Pakistan.

MATERIALS AND METHODS

Anatomical analysis

Leaf samples were prepared according to the modified method of Cotton (1974), who followed Clark`s (1980) technique but with a little modification (Shaheen et al., 2010). Slides of both abaxial and adaxial surface of leaf were prepared and mounted in clean 88% lactic acid.

Palynomorph analysis

Palynomorph study was done according to the modified method of Wodehouse technique (Ronald, 2000). The pollen fertility estimation was carried out by employing the techniques used by Meo and Khan (2006).

Fluorescence and solubility analysis

The solubility and retention of original color of powdered materials was noted in various solvents in cold and hot conditions. 5 g powdered drug was mixed in 20 ml sulphuric acid, hydrochloric acid, acetic acid and water (Evers and Smith, 1955).

Organoleptic analysis

Material for organoleptic analysis was procured from herbal shops and collected from the field. All parts of herbal drugs including, wood bark, roots, rhizomes, leaves, stems, fruit, flowers and seeds of problematic medicinal plants were identified by examining macro-morphological characters. 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.

Light microscopic photographs (LM)

The light microscopic photograph (LM) of abaxial and adaxial epidermis of leaf samples and pollen was carried out. The microphotographs were taken on the 40, 60 and 100x lens

Scanning electron microscopic photographs (SEM)

The leaves and pollen samples were sent to Centralized Science Laboratory, Karachi for getting the photographs. The method was followed by the Terrel and Wergin (1979) and Hilu and Wright (1984).

Chemical analysis

A small amount of dried plant material was treated with 2 normal (2N) hydrochloric acid (HCl) and heated for one hour in a water bath at about 100°C for acid hydrolysis

Briefly, 15 g of each powdered drug were macerated with ethanol (50 ml) then acidified distilled water and dragondorff reagent was added in to this mixture (British Pharmacopoeia, 1999) for alkaloid detection.

In detecting glycosides, the species showed negative tests to alkaloidal reagent, which possibly contain glycosides and tannins were detected by briefly adding 1 ml of "ferric chloride solution to10 g of each powdered drug macerated with distilled water.

To detect starch, a 0.5 M iodine solution was briefly added to the solution (British Pharmacopoeia 1999) while anthraquinones were detected by briefly adding 20% sodium hydroxide to 2 to 3 g of each powdered drug macerated in 5 to 6 ml diethyl ether.

For saponin detection, 2 to 3 g of each powdered drug was shaken with 5 to 6 ml of distilled water while volatile and fixed oils were determined by taking 8 to 10 g of each powered drug and placed under a mechanical presser. The presence of any oily stain

showed the presence of oil (British Pharmacopoeia, 1999).

RESULTS AND DISCUSSION

The present research work included the *C. arvensis, Parthenium hysterophorus* and *S. marianum* belonging to the family Asteraceae. The present study was carried out to clear up the taxonomic position and delimitation of the taxa. Results for *C. arvensis, P. hysterophorus* and *S. marianum* are shown in Tables 1 to 3.

Anatomical variations among selected plant species

The walls of the epidermal cells of all the three species were smooth and thick walled. The average length of epidermal cells observed in *C. arvenses* was 93 (90 to 96) µm whereas the average length of epidermal cells observed in *S. marianum* was 55 (40 to 70). The epidermal characters, which had been proven to be of systematic value, were cuticular characters, epidermis, stomata, subsidiary cells and trichomes (Ellis, 1976). The present study shows diversity in the type and shape of the stomatal cells. The stomata seen in *C. arvenses* were anisocytic whereas in *P. hysterophores* were diacytic and no stomata were observed in *S. marianum* (Plates 3b and c). In the *P. hysterophores* and *S. marianum*, micro-hairs were single celled and present on both the abaxial and adaxial surface whereas in *C. arvensis* no micro-hairs were seen.

Palynological variations among selected plant species

It was observed that the pollen grain of *Parthenium hysterophores* was smaller in size 32 (28.5 to 35.5) µm and pollen grain of *S. marianum* was larger in size 60 (55 to 65) µm in equatorial diameter (Figure 1) whereas in polar view, the size ranged from 39 (32 to 39) µm to 48.5 (45 to 52) µm. *P. hysterophores* appeared to be the smallest in size whereas *C. arvensis* was the largest (Figure 1). In the present investigation, it was observed that *C. arvensis* was endoporus and pollen grains were trizonocolporate and tetrazonocolporate, which were mostly isodiametric and colpi and furrows were boat shaped. In *P. hysterophores*, pollen was tricolporate and echinate whereas, Nair (1991) reported only 3-zonocolporate pollen in *C. arvensis.* The presence of trizonocolporate and tetrazonocolporate pollen in *C. arvensis* is an evolutionary trend which would be helpful to establish a phylogenetic relationship of species within the family Asteraceae.

Praglowski and Grafstron (1980) felt that a brief palynological investigation of genus *Calendula* is helpful for taxonomic purposes. In the present studies simple and some paired spines were found in *C. arvensis*. The intine thickness ranged from 0.75 (0.5 to 1) µm to 1.25 (1 to 1.5) µm. *C. arvensis* showed the highest whereas *Parthenium hysterophores* showed the lowest value (Figure 1). *C. arvensis* showed the highest colpi length as 78.75 (77 to 82.5) µm whereas *P. hysterophores* showed the lowest value 50.25 (45 to 55.5) µm spine length which ranged from 40 (35 to 45) µm to 80 (75 to 85) µm. *S. marianum* showed the lowest value and *C. arvensis* showed the highest value. Praglowski and Grafstrom (1980) reported that numerous slender spines had a length of 100 to 130 µm of Asteraceae family. In the three species studied, the highest value of pollen fertility was found in *P. hysterophores* as 85.93% and the lowest value was in *C. arvensis* as 61.42% (Figure 2). Reitsman (2007) observed that the pollen fertility is valuable for the taxonomists in attempting to distinguish putative hybrids from the parent plants and is also useful to determine the degree of fertility/stainability in those plants that were grown under unfavorable conditions.

UV, IR and organoleptic variations among selected plant species

In the present investigation, the UV and IR analysis worked as an aid in the identification of the selected taxa (Plates 1h, 1i, 2h, 2i, 3h and 3i). Davihazy (2004) reported the use of infrared light for the blooming of house plants. The analysis of market samples of *C. arvensis* revealed the presence of smooth surface having angoori color and mild peppery and salty taste (Plate 1g). Whereas, the fresh leaves were green colored and had a length of 1.3 cm. These organoleptic characters were similar to the findings of Gilman and Howe (1999) who studied general morphological and organoleptic characters of *C. arvensis.* In case of *P. hysterophores*, market sample collaborated with actual sample collected from different localities of Lahore. The outer surface of fruit was semicircular and rough in appearance (Plate 2g). In case of *S. marianum*, leaves and seeds were used. Market samples of leaves were dried therefore they showed rough appearance and yellowish in color whereas the fresh leaves were greenish having white veins and were shiny whereas the market seed samples collaborated with the actual samples and were smooth surfaced (Plate 3g). These results are in accordance with the findings of Kemper (1999).

Fluorescence and chemical variations among selected plant species

The powdered drug of all the three species were soluble in all the solvents by cold and hot tests except *S. marianum* which was soluble in all the solvents except nitric acid and it did not retain its original dark mustard color on dry filter paper in various solvents by cold and hot tests (Table 4). Dastagir and Haq (1995) also

Table 1. Characteristics of *Calendula arvensis* L.

Parameter	Characteristic
Common name(s)	Field Marigold, Pot-marigold, Souci des champs (French).
Type	None.
Origin	It is native to Central and Southern Europe.
Habitat	Open places, wastelands, road side, graveyard and field margins, shrub-steppes and desert.
Distribution in Pakistan	Swat, Lower Hazara, Poonch, Kashmri, Balti, Ladak and Rawalpindi.
Distribution in World	South Europe, Africa, Iran, Afghanistan.
Life form	Annual
Habit	Forb/herb.
Roots and underground Structures	Tap roots, long, fleshy tubers.
Active growth period	May to November.
Flowering period	February to April.
Leaf epidermal anatomy (LM and SEM)	In abaxial epidermis, the leaf epidermal cells are irregular shaped, smooth and thick walled, average length of epidermal cells is 190 (150 to 230) µm and the average width is 110 (80 to 140) µm, stomata are present, stomatal type is anisocytic, average length of guard cells is 60 (30-90) µm, average width of guard cells is 10 (5 to 15) µm, average length of subsidiary cells is 120 (90 to 150) µm and the average width is 60 (40 to 90) µm. Trichomes, micro-hairs, macro-hairs and silica bodies are absent. In adaxial epidermis, the leaf epidermal cells are irregular shaped, smooth and thick walled, average length of epidermal cells is 93 (90 to 96) µm and the average width is 65 (60 to 70) µm, stomata are present, stomatal type is diacytic or anisocytic, average length of guard cells is 30 (20 to 4) µm and average width of guard cells is 7.5 (5 to 10) µm, average length of subsidiary cells is 120 (90 to 150) µm and the average width is 85 (70 to100) µm. Macro-hairs are present rarely. Trichomes, micro-hairs and silica bodies are absent. (Plates 1b to e).
Palynology (LM and SEM)	In equatorial view, the pollen grains are circular and semi-circular. In polar view, the pollen grains are semi-angular, prolate and spheroidal (Plates 1f and h). Polar diameter is 48.5 (45 to 52) µm and equatorial diameter is 57.75 (55.5 to 60) µm. P/E ratio is 0.83, exine thickness is 1 (0.5 to 1) µm and intine thickness is 1.25 (1 to 1.5) µm. Colpi length is 78.75 (77 to 82.5) µm and width is 20 (15 to 25) µm. Iintercelluler difference of colpi is 5.5 (3 to 8) µm. Spine length is 80 (75 to 85) µm. Percentage of pollen fertility in this species is 73.86% (Figure 2).
Organoleptography	Leaves are used for organoleptic analysis. Color of leaves is angoari and taste is salty and mild peppery. The outer surface of leaves is smooth having a length of 1.3 cm and width of 0.4 cm. The powdered drug has unpleasant smell (Plate 1j).
Fluorescence and solubility in different solvents	Actual color of the powdered material is just like the spring leaf but color changes in different solvents, becomes icy lemon in distilled water, golden in sulphuric acid, mustard in hydrochloric acid, pale yellow in acetic acid and bottle green in nitric acid. The powdered material is soluble in all the solvents.
Chemical analysis	Active chemical constituents; alkaloids, tannins, starch grains, anthraquinone and saponins are present whereas glycosides, volatile and fixed oils and ferric chlorides are absent.
Folk medicinal uses	A remedy for skin problems and applied externally to bites and stings, sprains, wounds, sore eyes, varicose veins etc. It is also a cleansing and detoxifying herb, and is taken internally in treating fevers and chronic infections. The leaves, blossoms and buds are used to make a homeopathic remedy. It is used internally in order to speed the healing of wounds.

LM, Light microscopic photograph; SEM, scanning electron microscopic photographs

reported similar results of *S. marianum* on solubility in different solvents. In the present studies active chemical constituents; alkaloids, tannins, starch grains, anthraquinone and saponins were observed whereas glycosides, volatile and fixed oils and ferric chlorides were absent in the *C. arvensis* (Table 4) and these results are in agreement with Kemper (1999). In *P. hysterophorus*, alkaloids, glycosides, starch grains, volatile and fixed

Table 2. Characteristics of *Parthenium hysterophorus* L.

Parameter	Characteristic
Common name(s)	Congress grass, parthenium weed, ragweed parthenium, whitetop weed (English) and Karottenkraut (German).
Type	None.
Origin	Native to subtropics of North and South America.
Habitat	All disturbed land including pastures, farms, agricultural areas and range/grasslands.
Distribution in Pakistan	Punjab (Sialkot, Gujranwala, Lahore, Kasur, Shekhupura, Gujrat, Jehlem, Rawalpindi/Islamabad).
Distribution in World	Australia, Taiwan, Southern China, Pacific Islands, India and has recently spread to East and South Africa.
Life form	Short-lived annual.
Habit	Erected, whitish, branched herb.
Roots and underground structures	Tap roots.
Active growth period	Summer
Flowering period	Summer To Autumn (June to November).
Leaf epidermal anatomy (LM and SEM)	In abaxial epidermis, the leaf epidermal cells are irregular shaped and polygonal, smooth thick walled, average length of epidermal cells is 70 (60 to 80) µm and the average width is 55 (50 to 60) µm, stomata are present, stomatal type is diacytic, number of stomata per unit area is 8, open stomata are two and six closed stomata are present, average length of guard cells is 60 (40 to 80) µm and average width of guard cells is 15 (10 to 20) µm, average length of subsidiary cells is 100 (80 to 120) µm and the average width is 60 (40 to 90) µm. Multicellular trichomes are present, Number of trichomes per unit area is 1, average length of trichomes is 335 (220 to 450) µm and the average width of trichomes is 135 (100 to 170) µm. Multicellular micro-hairs are present, average length of micro-hairs is 10 (6 to 14) µm and average width of micro-hairs is 2.75 (2.5 to 3) µm. Silica bodies are absent. In adaxial epidermis, the leaf epidermal cells are irregular shaped and polygonal, thick walled with wavy margins, average length of epidermal cells is 72.5 (60 to 85) µm and the average width is 52.5 (40 to 65) µm, stomata are present, stomatal type is diacytic, number of stomata per unit area is 8, open stomata are two and six closed stomata are present, average length of guard cells is 60 (40 to 80) µm and average width of guard cells is 20 (15 to 25) µm, average length of subsidiary cells is 115 (80 to 150) µm and the average width is 60 (40 to 90) µm. Trichomes are present which are of non-glandular type, Multicellular micro-hairs are present, average length of micro-hairs is 30 (20 to 40) µm and average width of micro-hairs is 6.5 (4 to 9) µm. Silica bodies are absent (Plates 1b to e).
Palynology (LM and SEM)	In equatorial view, the pollen grains are circular to semicircular. In polar view, the pollen are prolate, circular and spheroidal (Plate 1g). Polar diameter is 39 (32 to 39)µm and equatorial diameter is 32 (28.5 to 35.5) µm. P/E ratio is1.21, exine thickness is 1 (0.9 to 1.1) µm and intine thickness is 0.75 (0.5 to 1) µm. Colpi length is 50.25 (45 to 55.5) µm and width is 19 (18 to 20) µm. Intercelluler difference of colpi is 5 (4 to 6)µm. Spine length is 73 (66 to 80) µm. Percentage of pollen fertility in this species is 85.93% (Figure 2).
Organoleptography	Fruit is used. The color of fruit is whitish brown, smell is aromatic and taste is bitter. The outer surface is semicircular and rough in appearance having a length of 1.5 to 1.8 cm and a width of 0.6 to 0.8 cm (Plate 1i).
Fluorescence and solubility in different solvents	Actual color of the powdered material is dark super beige but colour changes in different solvents; becomes sonora in distilled water, brown in sulphuric acid, mustard in hydrochloric acid, zest in acetic acid and green in nitric acid. While performing cold test, the powdered material is insoluble in all the solvents except sulphuric acid but becomes soluble in all solvents during hot tests.
Chemical analysis	Alkaloids, glycosides, starch grains, volatile and fixed oils and ferric chlorides are present whereas tannins, anthraquinone and saponins are absent.
Toxicity	Toxic
Folk medicinal uses	Used as tonic and febrifuge. Fruit decoction is useful in dysentery, it is applied externally on skin disorders and decoction of the plant is often taken internally as a remedy for a wide variety of ailments.

LM, Light microscopic photograph; SEM, scanning electron microscopic photographs.

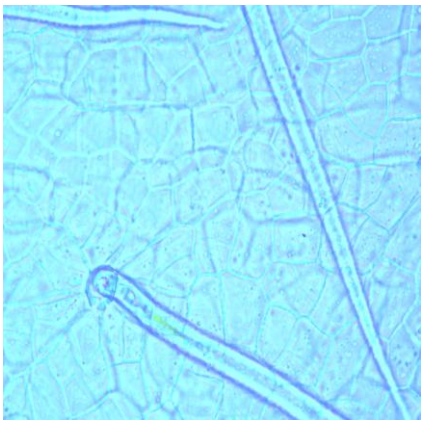
Table 3. Characteristics of *Silybum marianum* (L.) Gaertn.

Parameter	Characteristic
Common name(s)	Blessed milk thistle, marian thistle, mary thistle, spotted thistle and variegated thistle.
Type	None
Origin	Originally a native of Southern Europe through to Asia, and now found throughout the world.
Habitat	River flats, sheep camps, around stock yards and any other area of higher than normal soil nitrogen levels, especially if the area has been disturbed.
Distribution in Pakistan	Punjab and NWFP.
Distribution in World	England, North America, Australia, New Zealand, Germany, Hungary, Poland, China and Argentina.
Life Form	Annual, Biennial
Habit	Forb/herb
Roots and underground structures	Long, creeping roots.
Active growth period	March to May.
Flowering period	June to August.
Leaf epidermal anatomy (LM and SEM)	In abaxial epidermis, the leaf epidermal cells are irregular shaped and polygonal, smooth and thick walled, average length of epidermal cells is 55 (40 to 70) µm and the average width is 25 (20 to 30) µm, stomata are absent. Multicellular micro-hairs are present, average length of micro-hairs is 40 (25 to 55) µm and average width of micro-hairs is 11 (10 to 12) µm. Trichomes and silica bodies are absent. In adaxial epidermis, the leaf epidermal cells are irregular shaped and polygonal, smooth and thick walled, average length of epidermal cells is 55 (30 to 80) µm and the average width is 45 (30 to 60) µm, stomata are present, stomatal type is diacytic, number of stomata per unit area is 14, open stomata are 11 and 3 closed stomata is present, average length of guard cells is 30 (20 to 40) µm and average width of guard cells is 10 (5 to 15) µm, average length of subsidiary cells is 40 (20-60) µm and the average width is 35 (20-50) µm. Trichomes are present, average length of trichomes is 200 (110 to 290) µm and average width is 75 (60 to 90) µm. Multicellular micro-hairs are present, average length of micro-hairs is 22.5 (15 to 30) µm and average width of micro-hairs is 17 (15 to 20) µm. Silica bodies are absent (Plates 1d to g).
Palynology (LM and SEM)	In equatorial view, the pollen grains are circular and semi-circular. In polar view, the pollen are circular, prolate and spheroidal (Plate 1h). Polar diameter is 41.75 (38 to 45.5) µm and equatorial diameter is 60 (55 to 65) µm. P/E ratio is 0.69, exine thickness is 1.5 (1 to 1.5)µm and intine thickness is 1 (0.5 to 1.5) µm. Colpi length is 70 (60 to 80) µm and width is 25 (22 to 28)µm. Iintercelluler difference of colpi is 5 (3 to 7) µm. Spine length is 40 (35 to 45) µm (Table 3). Percentage of pollen fertility in this species is 78.84% (Figure 2).
Organoleptography	Leaves and seeds are used. Leaves are either wavy loved or pinnated and have spiny edges. They are hairless, shiny, with milk white veins having a length of 1.2 to1.5 cm and 0.4 to 0.7 cm width. Color of leaves is yellowish green having squash taste and smell is pungent. Seeds are smooth and shiny, brownish to black in color and have a tuft of minutely barbed bristles. (Plate 1i and m).
Fluorescence and solubility in different solvents	Actual color of the powdered material is dark mustard but color changes in different solvents, becomes copper in distilled water, golden brown in sulphuric acid, leather brown in hydrochloric acid, light brown in acetic acid and leafy green in nitric acid. While performing cold test the powdered material is insoluble in distilled water, acetic acid and nitric acid but becomes soluble in all solvents during hot tests.
Chemical analysis	Alkaloids, tannins, starch grains, saponins and volatile and fixed oils are present whereas glycosides, anthraquinone and ferric chlorides are absent.
Toxicity	Non-toxic
Folk medicinal uses	One of the herbs that believed to be beneficial in toning liver and increase its resistance to infections.

LM, Light microscopic photograph; SEM, scanning electron microscopic photographs.

Table 4. Solubility and fluorescence analysis of powdered drug of selected medicinal plants in various solvents by cold and hot method.

Plant name	Parameter					
	Solvent	**Distilled water**	**Sulphuric acid**	**Hydrochloric acid**	**Acetic acid**	**Nitric acid**
Calendula arvensis	Actual color of powdered drug	Spring leaf	Spring leaf	Spring leaf	Spring leaf	Spring leaf
	Cold test	Insoluble	Soluble	Soluble	Soluble	Soluble
	Hot test	Soluble	Soluble	Soluble	Soluble	Soluble
	Color in solvents	Icey lemon	Golden	Mustard	Pale yellow	Bottle green
	Color on filter paper	Lemon	Brown	Creamy color	Basanti	Dark green
Parthenium hysterophorus	Actual color of powdered drug	D-super beige	D-super beige	D-super beige	D-super beige	D-super beige
	Cold test	Insoluble	Soluble	Insoluble	Insoluble	Insoluble
	Hot test	Soluble	Soluble	Soluble	Soluble	Soluble
	Color in solvents	Sonora	Brown	Mustard	Zest	Green
	Color on filter paper	Chocolate	Brown	Brown	Brown	Angori
Silybum marianum	Actual color of powdered drug	D-mustard	D-mustard	D-mustard	D-mustard	D-mustard
	Cold test	Insoluble	Soluble	Soluble	Insoluble	Insoluble
	Hot test	Soluble	Soluble	Soluble	Soluble	Insoluble
	Color in solvents	Copper	Golden brown	Leather brown	Light brown	Leafy green
	Color on filter paper	Antique gold	brown	brown	Brown	Dark green



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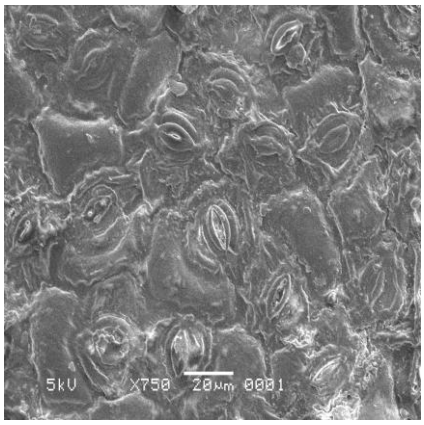

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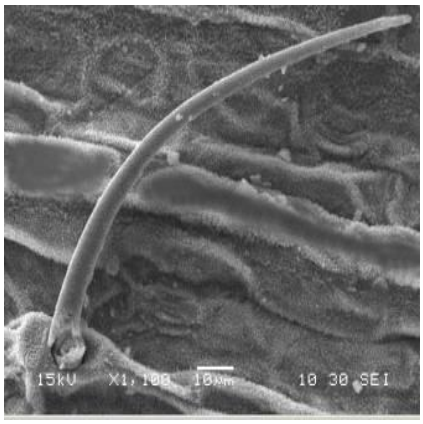

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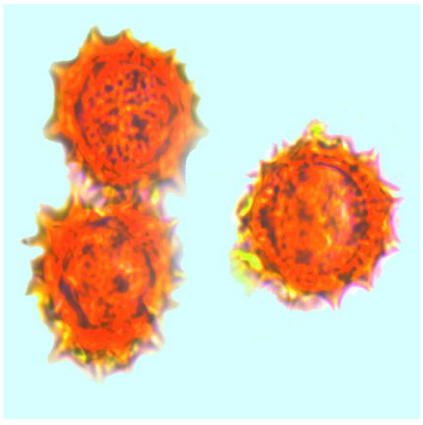

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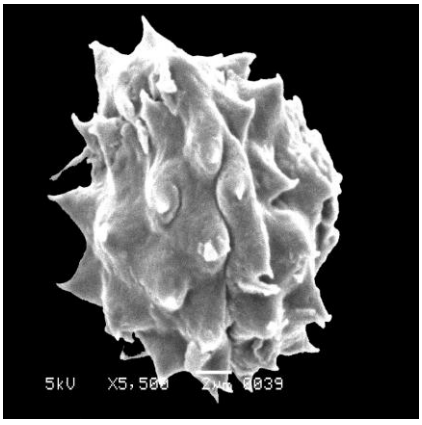

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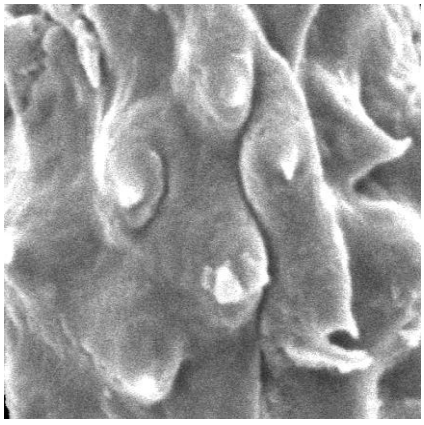
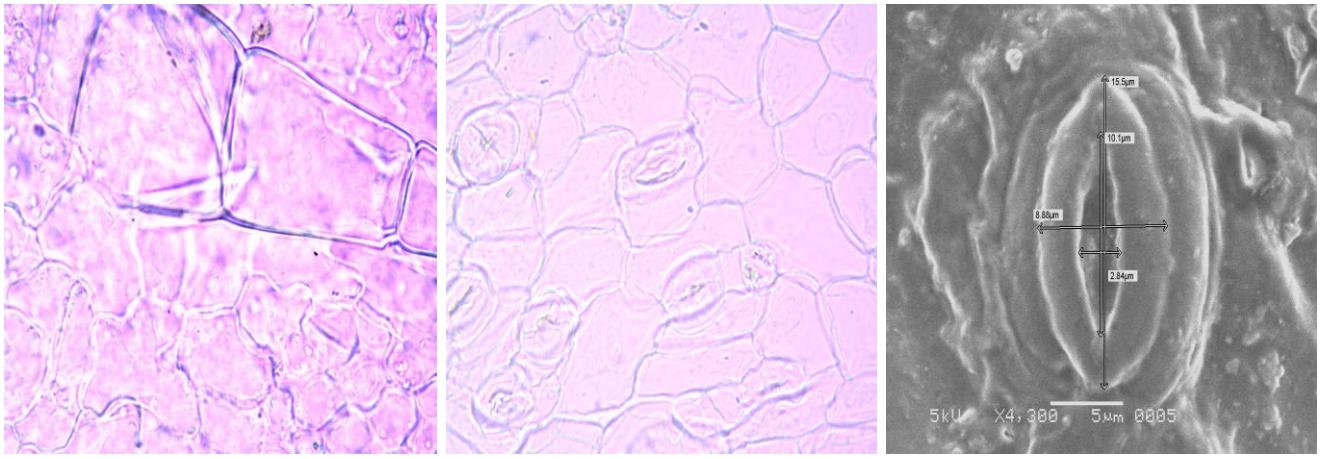


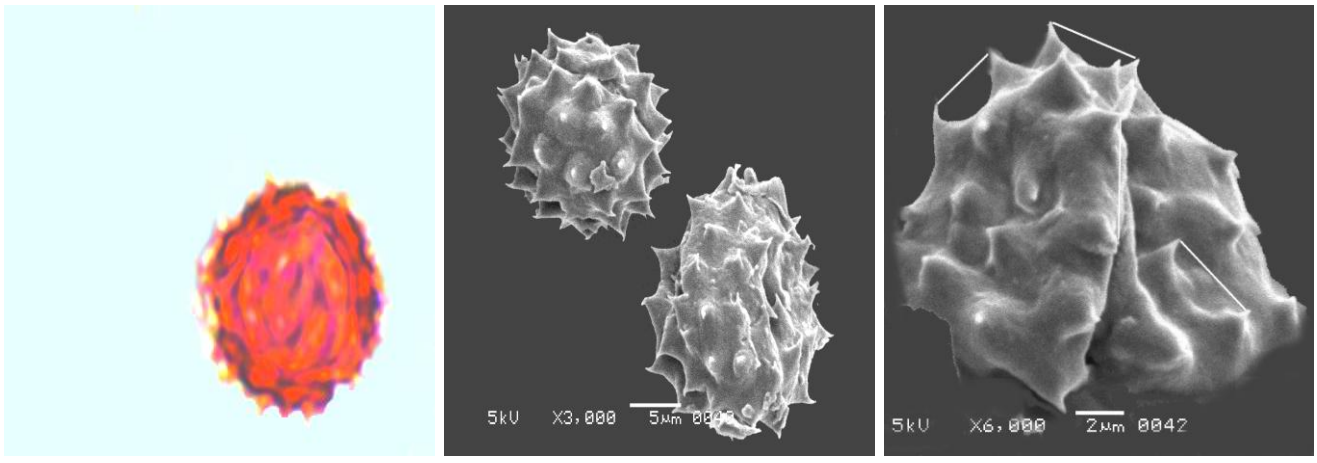
(f)

(g) (h) (i)

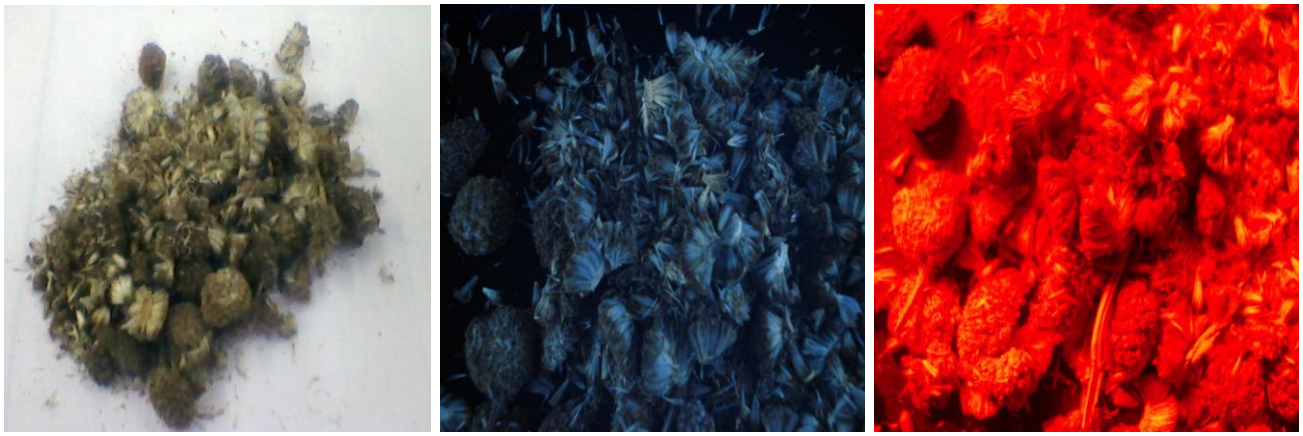
Plate 1. *Calendula arvensis* L. (a) Adaxial side showing macrohairs (LM). (b) Stomata (SEM). (c) Macrohair (SEM). (d) Circular to semi-circular pollen (LM). (e) Semi-circular pollen (SEM). (f) Echinate surface of pollen. (g) Leaves under visible light; (h) Leaves under UV light; (i) Leaves under IR light.



(a) (b) (c)

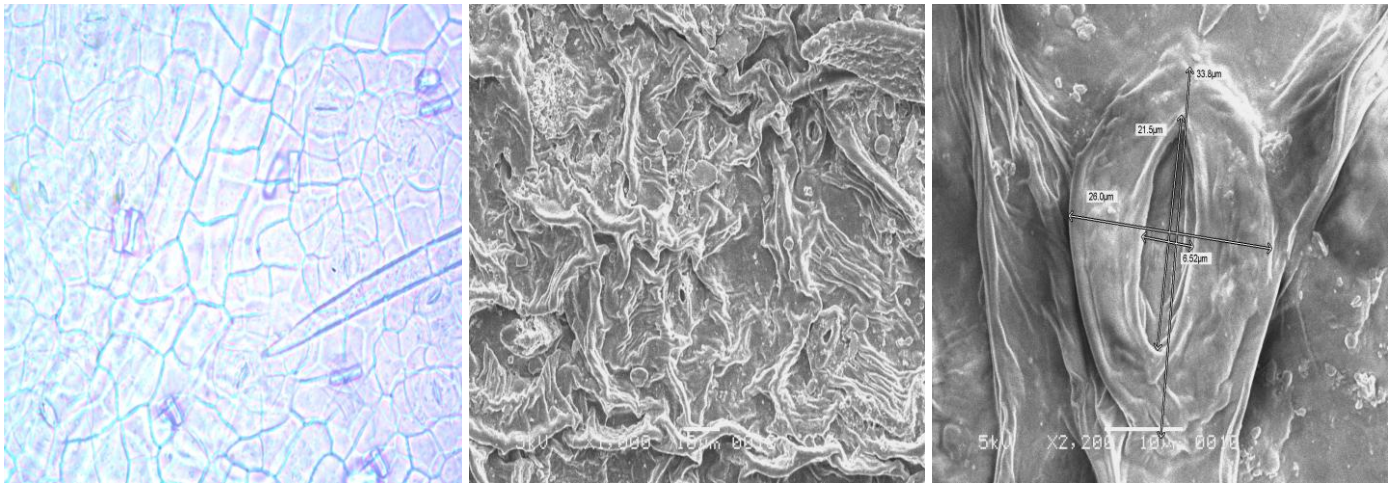


(d) (e) (f)

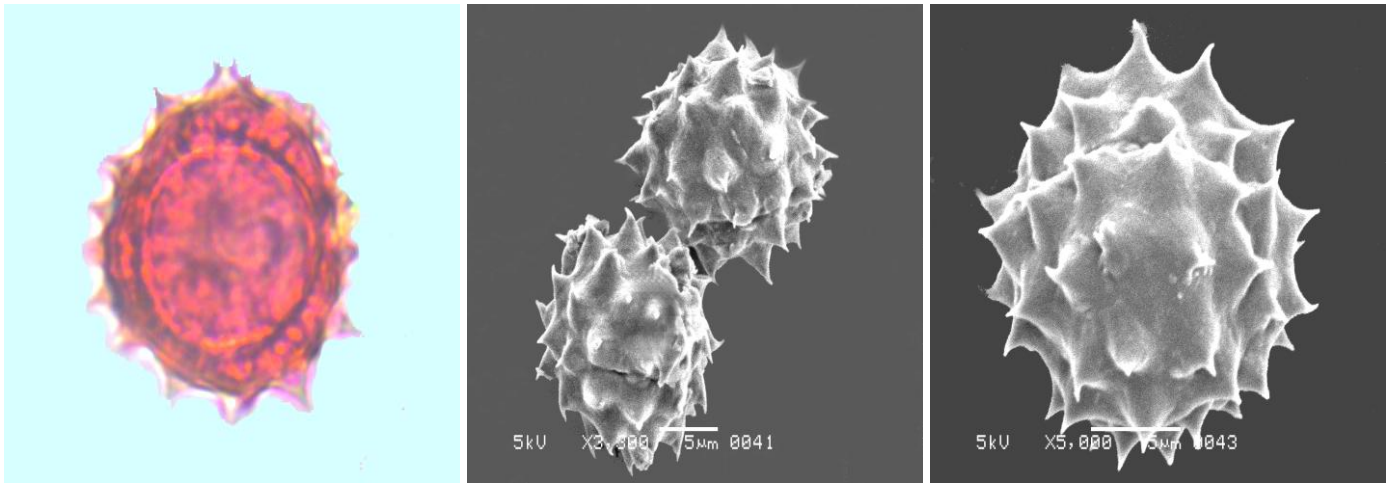


(g) **(h)** **(i)**

Plate 2. *Parthenium hysterophorus* L. (a) Abaxial side showing multicellular hair. (b) Adaxial side showing stomata (LM). (c) Stomata and guard cells. (d) Circular pollen (LM). (e) Pollen showing spines. (f) Pollen showing interspinal distance. (g) Fruit under visible light. (h) Fruit under UV light. (i) Fruit under IR light.



(a) **(b)** **(c)**



(d) **(e)** **(f)**

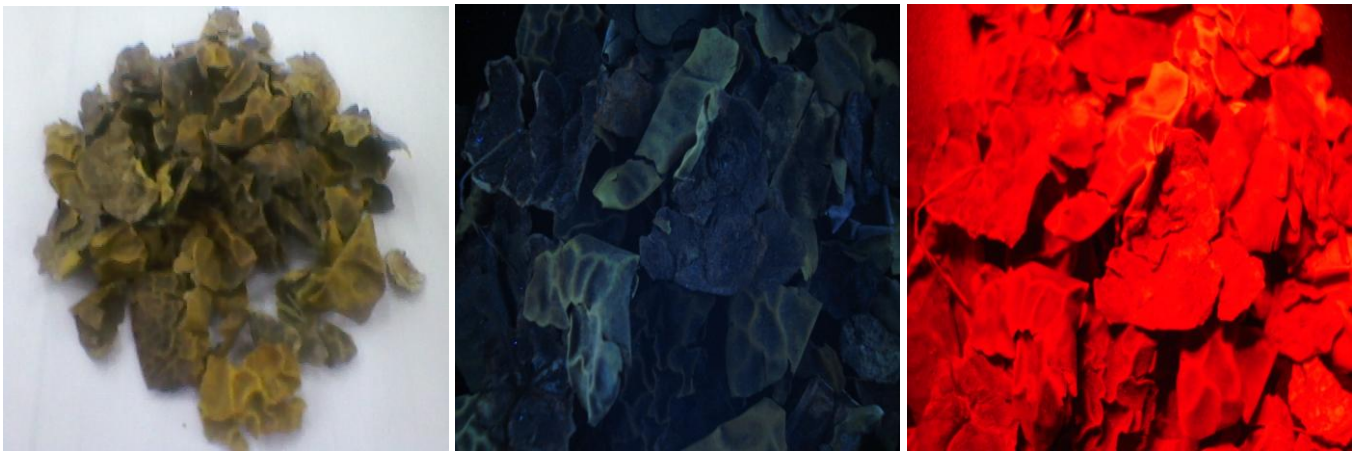


Plate 3. *Silybum marianum* (L.) Gaertn. (a) Micro and macro hairs. (b) Irregular thick walled epidermal cells. (c) Stomata. (d) Pollen having thick exine. (e) Circular to semi-circular pollen. (f) Pollen having spines on surface. (g) Leaves under visible light. (h) Leaves under UV light. (i) Leaves under IR light.

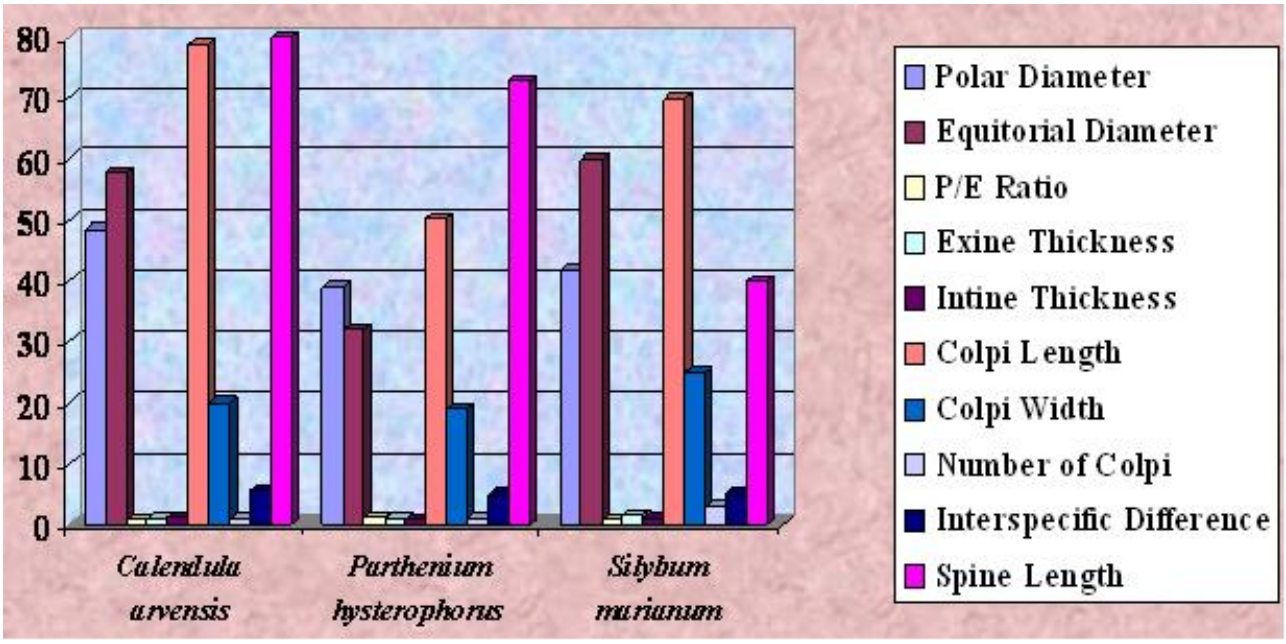


Figure 1. Palynomorph variations among the selected species of family Asteraceae.

oils and ferric chlorides were present whereas tannins, anthraquinone and saponins were absent (Table 4). These findings are different from Dweck (1997) who reported the presence of tannins and saponins in the *P. hysterophorus.* Alkaloids, tannins, starch grains, saponins and volatile and fixed oils were observed in *S. marianum* whereas glycosides, anthraquinone and ferric chlorides were absent (Table 4). These results corroborate with the findings of Foster (2001).

Conclusion

It is concluded that for the recognition and circumscription of the tribes, genera and species, very limited characters were used. While this present research suggest the anatomical studies, palynomorph features, UV, infra-red analysis, organoleptography, fluorescence, solubility and chemical analysis should be used as a tool in taxonomic characterization. Moreover the present study included

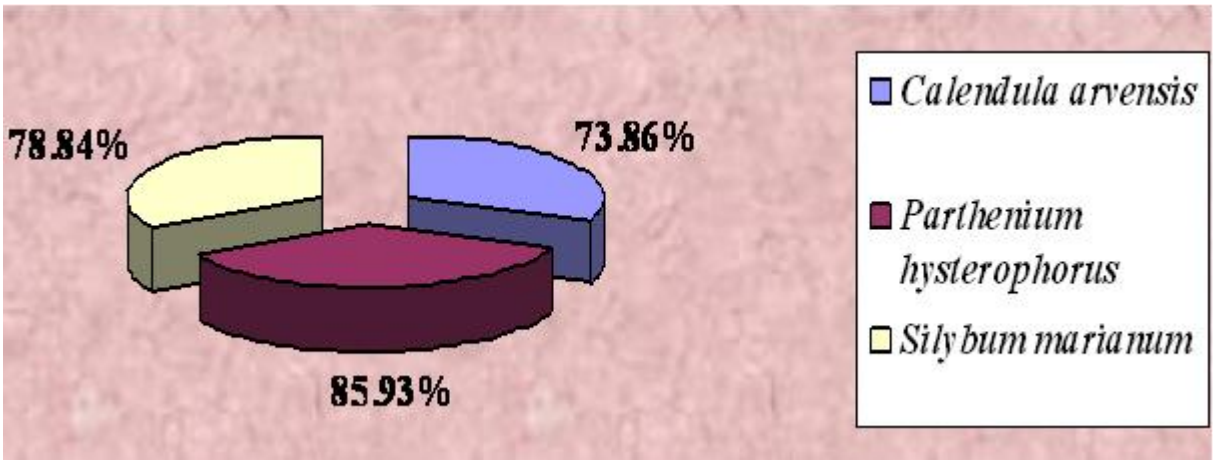


Figure 2. Fertility range among the selected species of family Asteraceae.

some addition in flora of Pakistan also, although all the three selected genera, *C. arvensis, P. hysterophorus* and *S. marianum* are common plants in Pakistan and a lot of work has been done on them with many aspects but still in the flora of Pakistan (1990), no description of all the three taxa was given and the present study gives a comprehensive account on these taxa with multiple parameters.

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