

Research Article

Implication of light and scanning electron microscopy of foliar cuticular and palynological features in the correct identification of medicinal plants

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Abstract

Foliar epidermal anatomy and palynology of six medicinal plant species *Catharanthus roseus* L., *Carum carvi* L., *Trachyspermum ammi* L., *Anethum graveolens* L., *Foeniculum vulgare* Mill. and *Nigella sativa* L. are commonly used for different medicinal uses. Six medicinal species were used as an example and their foliar cuticular and pollen features were evaluated by light and scanning electron microscopy (SEM). Features allied to pollen grains (pollen type, colpi, and sculpture type) and foliar cuticular features (epidermis, stomata, and trichome) were found to be important examined characters. Pollen grain morphology of these medicinal plants revealed that the pollen is the prolate and per-prolate type. Their foliar epidermal anatomy revealed the presence of an anomocytic type of stomata in *C. roseus*, *C. carvi* and *N. sativa*, dicytic type in *F. vulgare* and anisocytic type in *A. graveolens*.

Keywords: Medicinal plants; Palynology; SEM; Taxonomic identification

Introduction

Medicinal plants are in common use in most of the developing countries [1]. Plants are morphologically similar and correct identification in crude form is very difficult. The Foliar epidermis is one of the most significant taxonomic characters from the biosystematics point of view and the taxonomic studies of a number of families are made on the basis of leaf epidermis [2, 3].

The significance of pollen morphology in the taxonomy of Apocynaceae has been investigated by many researchers including Guinet, Mareues, Schill Nilssons and Van Campo [4-8]. Apiaceae, a family of about 300 genera and 3000 species is cosmopolitan in distribution, mainly in northern regions [9, 10]. In Pakistan, it is represented by 56 genera and 167 species [11]. The family is of considerable economic importance for food,

flavoring, and ornamental plants. Apiaceae also has some poisonous plants. Seeds of Apiaceae are of importance because of their essential oils. Of this, caraway seeds (*C. carvi*) are used in bread, Dill (*A. graveolens*) is used in flavoring dill pickles. Ranunculaceae is a large family of 50 genera and 2000 species widely distributed throughout the northern hemisphere but also in southern temperate regions [10]. In Pakistan, it is represented by 22 genera and 114 species, of which genera like *Nigella*, *Anemone*, and *Aquilegia* include plants of ornamental value [12]. The level of confidence in taxonomic units supported by different kinds of data is likely to be much higher than for those supported by only one kind [13]. Thus, the main objective of the study was to characterize the taxa by using multiple and complementary perspectives to supplement the traditional morphology-based description with additional markers (foliar epidermal anatomy & palynology). The objective of the palynological studies was to provide additional knowledge about the pollen morphology of the taxa already investigated by the above-mentioned workers as well as to include additional taxa, which were not considered in the studies of previous workers. The character of spine index was used for the first time to characterize pollen of the taxa under investigation.

Materials and methods

Foliar cuticular studies

Fresh samples for the type and shape of leaf epidermal cell studies were collected from the field and prepared according to the modified method of Clark's [14], Voucher numbers are given in (Table 1). The leaves were placed in test tubes and treated with 88% lactic acid, in a water bath (Model, Memmert-91126-FRG, Germany) at 100 °C for 20-40 min. They were then removed from the test tube into a Petri dish. A drop of lactic acid was used to soften the tissue of leaf due to which it's peeling off is made easy. Slides

of both abaxial and adaxial sides of leaves were prepared and observed under light microscope. Microphotographs were taken by using a CCD digital camera (Model: DK 5000) fitted on leica light microscope (model: DM 1000). These micrographs were useful for identification and differentiation of epidermal cells on the basis of microscopic features. The leaves were prepared for scanning electron microscopy (SEM) by the standard methods described by Erdtman [15]. A leaf piece washed with acetic anhydride was directly transferred to a metallic stub and let it dry. Then it was coated with gold in a sputter chamber (Ion-sputter JFC-1100) with coating restricted to 150 °A. Scanning Electron Microscopy examination was performed by Jet microscope JSM-T 200, in Centralized Science Laboratory, University of Karachi. Foliar epidermal characters like epidermal cells, stomata and trichomes were studied.

Palynological studies

For these studies, the glycerin jelly was prepared according to the modified method of Parveen and Qaiser [11]. 500 mL of distilled water was taken in a beaker and heated on a hot plate (model UELP Scientifica, Germany). 35 gm of gelatin was added when the temperature reached 70-80 °C. After an increase in temperature, it became a viscous liquid of glycerin jelly. Whole solution was kept on heating for one hour. 35 gm of glycerol was mixed in it with few crystals of phenol. Then 0.1% safranin was added with the 1/8th volume of glycerin jelly. It was stirred till uniform pink color appeared. Jelly was stabilized at room temperature. Specimens used for foliar epidermal analysis were also used as a source for polliniferous material. For processing of pollen for light and scanning microscopy, the slides were prepared by the modified procedure of Erdmann [16]. For light microscopy, the pollen grains were mounted in glycerin jelly stained with 1% safranin, on

a glass slide. A glass coverslip was placed on the prepared pollen glycerin jelly mixture. When cooled, the glass slide was labeled and edges of the coverslip were sealed with transparent nail varnish. The prepared slides were studied under the light microscope. Pollen shape, pollen diameter, exine thickness, exine sculpturing, the height of the spine, the width of the spine at its base, spine index, inter-spinal distance and pore diameter were examined. Details of pollen morphology were based on the measurements of 10-15 grains. For SEM studies, pollen grains suspended in a drop of 40% acetic acid were transferred to clean metallic stubs and coated with gold using a JEOL JFC 1100 E ion sputtering device. SEM observations were carried out on a JEOL microscope JSM-5910. The work was carried out in the Centralized Science Laboratory, University of Karachi Pakistan. The terminology used was that of Erdmann, Moore and Punt [16-18].

Results and discussion

The detailed medicinal uses of the six investigated plants species are shown (Table 1). The foliar epidermal anatomy of *C. roseus* showed ordinary epidermal cells irregular to cuboidal, pentagonal and hexagonal to polygonal. Cell walls thick, straight, irregular or wavy. Cells compactly arranged without intracellular spaces without glands and hairs. Stomata absent adaxially or very rare. Stomata are of anomocytic type abaxially. 36 μm to 56 μm long and 25-30 μm wide (Table 2). Foliar epidermal anatomy of *T. ammi* showed conspicuous costal and intercostals zonation. Ordinary epidermal cells irregular, longitudinally arranged and somewhat tubular, walls sinuous. Coastal zone with Ca-oxalate crystals, costal epidermal cells small, transversely flattened, compactly arranged in the form of filaments. Stomata absent coastally, prickles and glands not observed. Epidermal cells in the foliar epidermal anatomy of *A. graveolens* are irregular,

polygonal to cuboidal. Cell walls are sinuous shaped. Stomata anisocytic, common on surfaces, subsidiaries cells unequal, guard cells bean-shaped (Figure 1). 50-75 μm long and 45-55 μm wide, stomatal pore 20-35 μm long. Glands, prickles, and hairs not observed. In the foliar epidermal anatomy of *F. vulgare* costal and intercostals zones are distinct. Intercostals ordinary epidermal cells rectangular, tubular, cuboidal to polygonal, arranged regularly in vertical rows. Cell walls markedly sinuous, stomata dicytic, common on both surfaces, 22-30 μm long. Subsidiaries slightly unequal with irregular outline. While in the leaves of *C. carvi* zonation is absent. Epidermal cells irregular, walls wavy, eglandular adaxially. Stomata common, anomocytic, 42-52 μm long and 25-31 μm wide. Subsidiaries absent. Epidermal cells of *N. sativa* irregular in shape, angle somewhat stellate, stomata present, anomocytic, unicellular, glandular and club-shaped trichomes present. 150-200 μm long, narrowing into a prominent beak at the tip. Stomata are 60-90 μm abaxially and 40-60 μm adaxially, few abaxially and common adaxially.

The pollen grains of *C. roseus* is prolate with exine sculpturing elements elongated sideways and extending more or less parallel, forming grooves or gaps between them. *C. roseus* has the largest pollens (57.4 \times 37.5 μm) and *T. ammi* has the smallest pollens (Figure 2). The pollens are tri-colporate and about 57.4 μm long and 37.5 μm wide. The colpus length is about 47.4 μm . Pollen grains of *T. ammi* is per-prolate with exine sculpturing striate with sculpturing elements elongated and extending sideways and parallel (Table 3). The pollens are bi-colporate and about 13.7 μm long and 8.58 μm wide. The length of colpus is about 8.63 μm . While pollen grains of *A. graveolens* are bi-colporate with exine sculpturing psilate with the surface completely smooth. The pollens are about 20.9 μm long and 7.67 μm wide. Colpus

length is about 12.6 μm . The pollen grains of *F. vulgare* is bicolporate with exine sculpturing striate with sculpturing elements much elongated and extending parallel forming gaps or grooves. Pollens are about 20.9 μm long and 9.6 μm wide. Colpus length is about 12.1 μm . The pollen grains of *C. carvi* are prolate with exine sculpturing reticulate with sculpturing elements arranged in compact networking with gaps (lumina) and muri (ridges). The pollens are bicolporate about 20.8 μm long and about 10.5 μm wide. Colpus length is about 12.0 μm . The pollen grains of *Nigella sativa* is spheroidal with exine sculpturing perforate with holes or

depression less than 1 μm . The pollens are tetra-colporate and about 41.0 μm long and 34.6 μm wide. Colpus length is about 27.9 μm . Ranunculaceae is a eurypalynous family. Pollen grains 2, 3, 4 colpate, 3 colporoidate, pantocolpate, pantoporate, inaperturate, pollen shape oblate to spheroidal, ornamentation shows variety (echinate, reticulate etc.) [15]. 5 distinct pollen types in the family on the basis of exine ornamentation and apertural types were recognized by Parveen and Qaiser [11]. The family has spinulose exine ornamentation, the most common pattern in the Ranunculaceae [19].

Table 1. Species, local names, Part used, flowering seasons and traditional medicinal uses

Species	Local names	Part used	Flowering period	Voucher No.	Traditional medicinal uses	Morphological Description
<i>C. roseus</i> (L.) G. Don	Sadabahar	Leaves & flowers	Throughout the year	126864	Antihyperglycemic, hypotensive anti-cancer and anti-diabetic	Perennial sub-shrub, woody at base, 30-60 cm tall, young branches pubescent. Leaves oblong oblanceolate, membranous, 2.5-6 x 1.5-3 cm, entire, obtuse, base obtuse or cuneate, glabrous or sparingly hairy specially on nerves beneath, shining green above, paler below, lateral nerves obscure; petiole short, 3-10 mm long, with many glands at the axil. Inflorescence of axillary 1-4 flowered cymes, Flowers pink or white, pedicel 1-3 mm long. Calyx tube short, lobes linear acute, 5-7 mm long, hairy. Corolla tube 20-25 mm long, sparsely pubescent above, throat densely hairy within below the stamens, lobes oblong-rounded, or obovate, 15-25 mm long spreading Disc scales higher than ovary, 2-3 mm long. Ovary pubescent; style 2.5 cm

						long; stigma pentagonal. Follicles 2, slender, cylindrical, striated. 15-25 mm long.
<i>T. ammi</i> L. Sprague	Ajowain	Fruits	November-March	12106	Antihyperlipidemic, digestive, antiseptic, spice, anti-oxidants.	Annual, 15-50 cm tall, branched. Stem glabrous. Leaves much divided; ultimate segments filiform. Involucre of 5-8 linear, sometimes pinnate, bracts. Rays 8-16, glabrous, unequal. Involucel of 5-8 linear, sometimes pinnate bract-lets. Pedicels twice as long as the fruit. Fruit ovoid, 2 mm long, muricate; ridges not prominent; stylopodium, conical; styles c. mm long.
<i>A. graveolens</i> L.	Sowa	Fruits	June-July	126863	Antimicrobial, antihyperlipidemic and Antihypercholesterolemic	50-150 cm tall; strongly scented. Leaves 3-4-pinnate, segments filiform, upper leaves shorter and reduced. Peduncles up to 15 cm long. Rays 8 tonumerous. Pedicels slender, c. 4 mm long. Fruit 3-4 mm long; dorsal and intermediate ridges distinct, lateral narrowly winged.
<i>F. vulgare</i> Mill.	Saunf	Fruits	July-August	12180	Carminative, used for a variety of problems like digestion, overweight, boosting metabolism and for stomach cramps, purgative	Plants perennial. Leaves much dissected, 3-4-pinnate; segments filiform to linear. Bracts and bractlets lacking. Peduncles terminal and lateral Calyx teeth obsolete. Petals yellow. Fruit elliptic to oblong, terete; stylopodium conical; styles very short; fruit ridges distinct; furrows 1-vittate; commissure 2-vittate. Inner seed face plane to slightly concave.
<i>C. carvi</i> L.	Zeera	Fruits	June-July	118455	Spice, an infusion of fruits and foliage is used as vermifuge and digestive	Perennial, up to 70 cm tall, branched, glabrous. Root fusiform, elongated. Leaves bipinnatisect; segments filiform to lanceolate or oval, lower leaves petiolate,

						upper sessile; bases sheathing; sheath margin white, membranous. Involucre of 1-5 linear bracts or lacking. Rays 5-9, unequal. Involucel of minute bractlets or lacking. Flowers white or rose colored. Calyx teeth obscure. Fruit pedicels very unequal. Fruit ovoid to oblong, yellowish brown, 3-4 mm long; ridges prominent, obtuse; furrows 1-vittate; commissure 2-vittate.
<i>N. sativa</i> L.	Kalonji	Seeds	February-March	10046	Carminative, digestive, antimicrobial anti-inflammatory, anti-tumour, anti-diabetic, anti-ulcerogenic, and anti-asthmic	Rue, a hardy, evergreen, somewhat shrubby plant. The stem is woody in the lower part, the leaves are alternate, bluish-green, bi or tripinnate, emit a powerful, disagreeable odour and have an exceedingly bitter, acrid and nauseous taste. The greenish-yellow flowers are in terminal panicles. Each flower has a square of four concave yellow petals with wrinkled margins and with eight stamens. The central flower of the corymb has five concave yellow petals and ten stamens and often is placed lower on the flowering stalk than the four-petaled flowers. The first flower that opens has usually five petals and ten stamens, the others four only and eight stamens.

Table 2. Quantitative and qualitative foliar cuticular features of the medicinal plants

Species	Leaf epidermis (Ad/Ab)		Trichomes			Stomata		
	Shape	Margin/wall morphology	Length (µm)	Width (µm)	P/A (Ab/Ad)	Type	P/A (Ab/Ad)	Type (glandular/eglandular)
<i>C. roseus</i>	Irregular to cuboidal/irregular wavy, variously shape	Thick, straight	36 to 56	25 to 30	Ab	Anomocytic	Absent	Absent
<i>T. ammi</i>	Irregular	Sinuuous	13 to 16	03 to 08	Ab	Anomocytic	Absent	Absent
<i>A. graveolens</i>	Irregular Sinuuous Polygonal to cuboidal	Sinuuous	50 to 75	45 to 55	Ab	Anisocytic	Absent	Absent
<i>F. vulgare</i>	Rectangular, tubular, Sinuuous cuboidal to polygonal	Sinuuous	13 to 16	05 to 11	Ab	Dicytic	Absent	Absent
<i>C. carvi</i>	Irregular wavy	Sinuuous	42 to 52	25 to 31	Ab	Anomocytic	Absent	Absent
<i>N. sativa</i>	Irregular Somewhats tellate	Sinuuous	23 to 34	05 to 18	Ab/Ad	Anomocytic	Absent	Unicellular, glandular

Table 3. Quantitative and qualitative palyno-morphological of medicinal plants

Species	Pollen type	Pollen Size (Length × Width) µm	No. of Colpi	Colpi Length (µm)	Pollen class	Exine sculpturing
<i>C. roseus</i>	Monad	57.4 × 37.5	Tri-colporate	47.4 µm	Prolate	Striate
<i>T. ammi</i>	Monad	13.7 × 8.58	Bi-colporate	8.63 µm	Per-prolate	Striate
<i>A. graveolens</i>	Monad	20.9 × 7.67	Bi-colporate	12.6 µm	Per-prolate	Psilate
<i>F. vulgare</i>	Monad	20.9 × 9.6	Bi-colporate	12.1 µm	Per-prolate	Striate
<i>C. carvi</i>	Monad	20.8 × 10.5	Bi-colporate	12.0 µm	Prolate	Reticulate
<i>N. sativa</i>	Monad	41.0 × 34.6	Tetra-colporate	27.9 µm	Spheroidal	Perforate

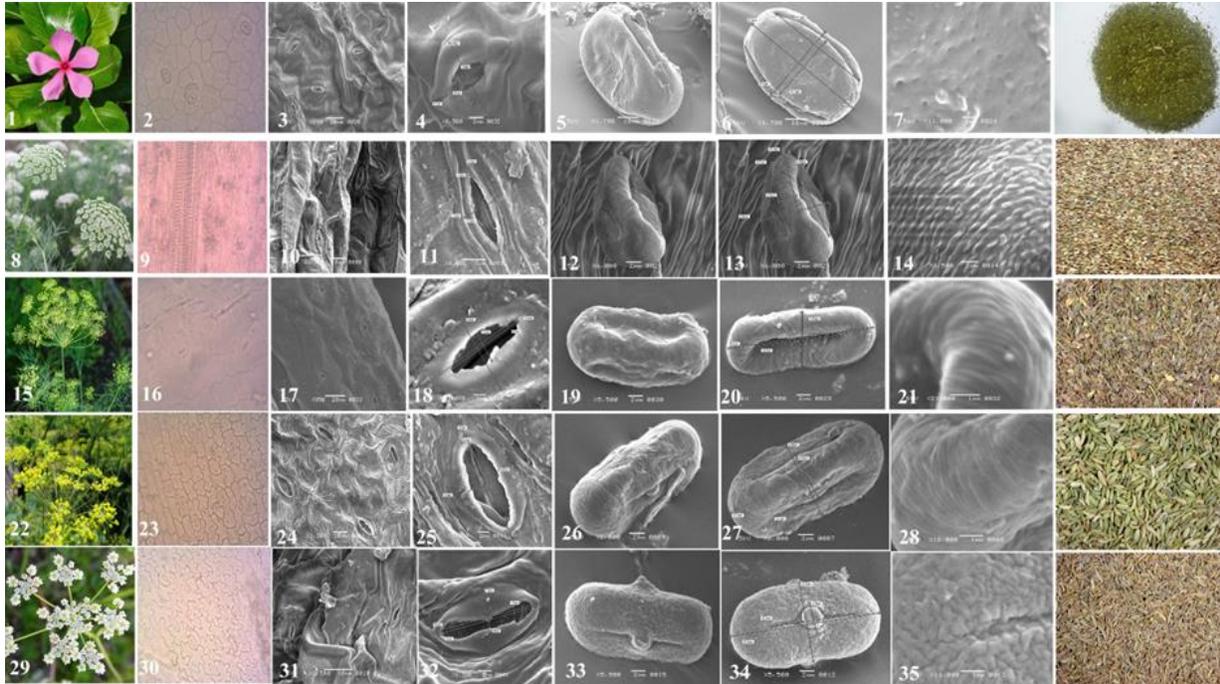


Figure 1. SEM images of stomata and pollen of different medicinal plants

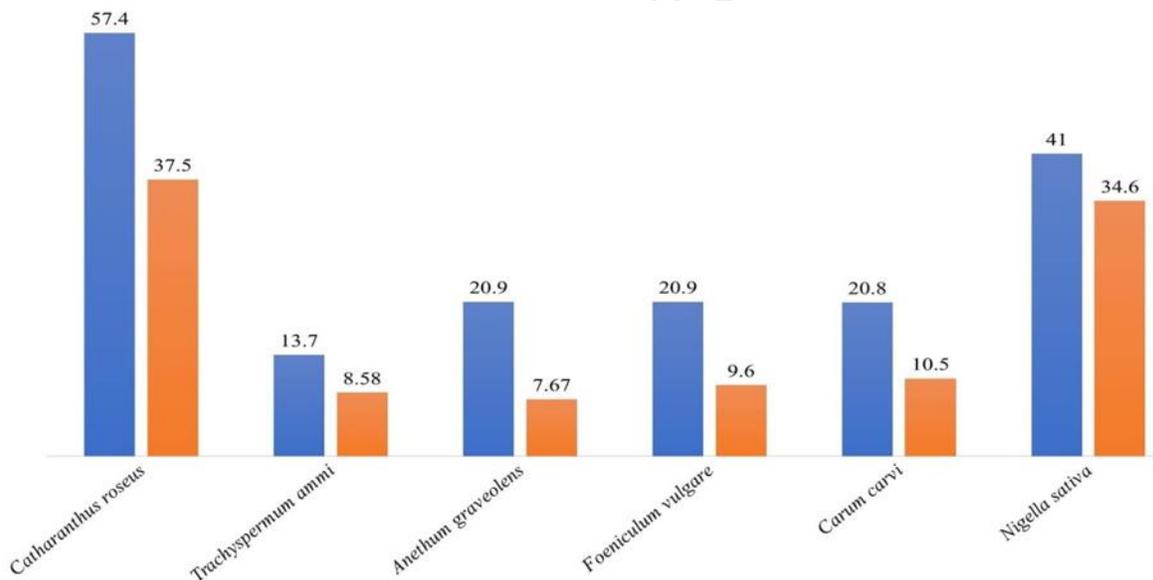


Figure 2. Percentage comparison in medicinal plants

Conclusion

Both leaf cuticular and Palynological characters were found useful in identification of medicinal plants. Different foliar cuticular and palynological characters in the taxonomic identification for

pharmacognostic, physicochemical and ethnobotanical uses.

Authors' contributions

Designed & idea of the work: N Seema, Supervision of the idea: M Hamayun & A Ullah, Literature survey and graphs setting:

M Zakaria, Manuscript writing and graphs setting: R Khan & Omerkilic.

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