

Leaf Epidermal Anatomy as an Aid to the Identification of Genera *Lantana*, *Verbena* and *Vitex* of Family Verbenaceae from Pakistan

SHAKIRA MUNSIF, MIR AJAB KHAN¹, MUSHTAQ AHMAD, MUHAMMAD ZAFAR, GHULAM MUJTABA SHAH AND NIGHAT SHAHEEN

Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan

¹Corresponding author's e-mail: mirajab@qau.edu.pk

ABSTRACT

Abaxial and adaxial leaf epidermal anatomy of eight species of Verbenaceae was studied based on leaf epidermal characters of *Lantana camara*, *L. indica*, *Verbena bonariensis*, *Ver. officinalis*, *Ver. bipinnatifida*, *Ver. hybrida*, *Vitex negundo* and *V. trifolia*. These species showed variations in stomata types and size, epidermal cell shape size and trichomes shape. Most of the characters especially trichomes were diagnostic and used for distinguishing taxa. Trichomes were mostly macrohair but on adaxial surface of *Ver. bonariensis* and both the surfaces of *V. negundo* and abaxial surface of *V. trifolia* microhair were also observed. Macrohair (197 x 24 µm) were observed in *Ver. hybrida* and glandular hairs were also seen. Microhairs (12 x 12 µm) were noted in *V. negundo*. Glandular hairs were observed in *L. camara*, *Ver. bonariensis*, *V. negundo* and *V. trifolia*. Trichomes were well segmented in *Vitex* spp. Trichomes of *L. camara* on abaxial surface had aggregates of 5 basal-cells, in *L. indica* there was just one basal, while in *L. indica* they were different from that of *L. camara* being broader at the base and swollen little bit before tip. Leaf epidermal anatomy was found taxonomically useful in the identification at the generic and species level.

Key Words: Epidermis; Trichomes; Glands; Verbenaceae; Pakistan

INTRODUCTION

Great part of Pakistan, except the districts of the Himalayan foot, is arid (Kitamura, 1964). Practically, whole the area lies in the watershed of the river Indus. Flora of Pakistan is varied and diverse. Of the nearly six thousand species of flowering plants reported to be occurring in Pakistan and Kashmir, a large number is found in the Northern and North-Western parts of Pakistan. Ten percent of the flora is Sino-Himalayan. The phytogeographical regions were adapted after (Ali & Qaiser, 1986; Nasir & Rafiq, 1995). There is no clear demarcation between each region and thus flora intermingles.

Major floristic zones include Saharo-Sindian, Irano-Turanian, Sino-Japanese and Indian. The family Verbenaceae is mainly distributed in the Tropics and Subtropics of the southern hemisphere. Genera *Lantana*, *Verbena* and *Vitex* are commonly found in Pakistan in the sub-Himalayan tract, plains of Baluchistan, North Waziristan, Sulaiman mountains, Kurram, NWFP, Kohat, Jehlum, Mirpur in Kashmir, lower Sawt, Rawalpindi etc., (Jafri & Ghafoor, 1972). In Pakistan the common species are *Lantana camara*, *L. indica*, *Verbena bonariensis*, *Ver. officinalis*, *Ver. bipinnatifida*, *Ver. hybrida*, *Vitex negundo* and *V. trifolia*. Verbenaceae includes many ornamental and medicinal plants in addition to teak, which is highly prized for its wood (Jafri & Ghafoor, 2005). *L. camara* is cultivated as garden ornamental. Decoction of this plant is given in

tetanus, rheumatism and malaria. It is also used in atoxy of abdominal viscera. A strong decoction of the leaves is taken internally and a poultice of crushed leaves is applied to the wound. The secretion of leaves have remarkable ecological functions due to their action against herbivores and pathogens, in the attraction of pollinators as well as fruit and seed dispersal (Fahn, 1979; Gottlieb & Salatino, 1987; Gershenzon & Croteau, 1991; Pare & Tumlinson, 1999). The leaves of *L. alba* are used as a cure for snake bite (Vashista, 1972). *Vitex* yields good timber and many species are cultivated as ornamentals such as *V. trifolia* and *V. negundo*. The dried leaves of *V. negundo* are smoked like cigarette for relieving headache the leaves are chewed to reduce toothache and to relieve labor pain in women (Farrukh, 2006). *V. hybrida* is commonly cultivated as garden ornamentals. The *V. officinalis* is useful in nerve complaints the root of this species is considered as a remedy for Scrofula. Family Verbenaceae has been least studied for leaf epidermal anatomy in Pakistan. This study was conducted to investigate some of the leaf epidermal anatomical features in view of its great role in establishing the taxonomic position of eight species of the family Verbenaceae.

MATERIALS AND METHODS

Leaf epidermal anatomy of the genera *Lantana*, *Verbena* and *Vitex* was studied. Specimens were obtained

from Quaid-i-Azam University herbarium Pakistan. For epidermal studies Shultze's method of maceration with improved technique was followed (Subrahmanyam, 1996). Leaves were taken in a test tube, covered with 4 mL of concentrated nitric acid to which 2 g of potassium chloride and one mL of distilled water were added. The mixture was carefully boiled. Shortly epidermal peels were separated, which were washed with water and placed on glass slide then poured one to two drops of bleach for 30 sec to remove the chloroplast. After bleaching the strips were again washed with water, than poured one to two drops of lactic acid and covered with cover slip. The epidermis was peeled by hand because it is the most effective method for peeling large number of specimens. To prepare the abaxial surface, the leaf blade was placed on a tile or a cutting plate with its adaxial surface facing upwards. The adaxial surface was carefully scrapped with sharp razor until only the abaxial surface was left behind and all the above materials were removed with the help of camel hairbrush. The peels of the epidermis were kept in one to two drops of bleach to remove extra traces of chloroplast and again washed with water, placed on a clean glass slide in a drop of lactic acid and followed by a cover slip over it.

RESULTS

Leaf epidermal studies of genera *Lantana*, *Verbena* and *Vitex* were carried out to find the anatomical characters of taxonomic value. Anatomical features of genera *Lantana*, *Verbena* and *Vitex* are presented in Table I. In *L. camara*, on the adaxial surface, epidermal cells were thick walled, rectangular, square and hexagonal. Macrohair very prominent, much broad 5-celled base and somewhat acute apex, glandular hair found. Stomata diacytic. On the abaxial surface, epidermal cells square and rectangular shape. Macrohair broader at the base and somewhat tapering at the apex. Stomata prominent (Fig. 1A). In *L. indica* on adaxial surface, the epidermal cells rectangular. Macrohair bit swollen above the base but tapering at the apex. Stomata amphianisocytic. On the abaxial surface, the epidermal cells were square, rectangular and somewhat polygonal in shape. Glandular and macrohair present. Stomata were prominent (Fig. 1B).

In *Ver. bonariensis* on the adaxial surface, the epidermal cells were thick walled, rectangular, polygonal

and square shape. Macrohair larger in size. Multicellular glandular hair present. Microhair present only on adaxial surface. Stomata anisocytic. On the abaxial surface, Epidermal cells thick walled rectangular polygonal and square in shape. Macrohair as well as multicellular glandular hair present (Fig. 1C). On adaxial surface in *Ver. officinalis* epidermal cells were found irregular and polygonal. Macrohair were more in number with spheroidal base and tapering ends. Stomata anisocytic. On the abaxial surface, epidermal cells were irregular and somewhat polygonal. Macrohair spheroidal at the base and tapering at the end. Stomata were smaller in size (Fig. 1D). In *Ver. bipinnatifida* on the adaxial surface, epidermal cells were thick walled, oblong rectangular, square and polygonal. Macrohair with broader cells at the base and not much tapering at the apex. Stomata anisocytic and staurocytic, while on the abaxial surface epidermal cells oblong rectangular and polygonal. Macrohair broader at the base even to the end, which seemed to be somewhat round (Fig. 1E). In *Ver. hybrida* on adaxial surface, epidermal cells smaller square shaped. Macrohair very much tapering. Glandular hair present. Stomata anisocytic, whereas on abaxial surface epidermal cells rectangular to oblong shape. Stomata very prominent; Macrohair sharp and very long in size. Glandular hair, were present (Fig. 1F).

Among *Vitex* species, in *V. negundo*, on adaxial surface epidermal cells thickwalled and rectangular to hexagonal. Only macrohair found well-segmented, on adaxial surface. On the abaxial surface, epidermal cells were covered with tomentose hair, so stomata could not be conserved. But trichomes e.g., macrohair, microhair and glandular hair were segmented (Fig. 1G). *V. trifolia* showed hexagonal or square in shape epidermal cells on adaxial surface. Trichomes one-three segmented macrohair. Glandular hair, were present. On the abaxial surface, epidermal cells square or oval in shape, while stomata were not observed. Trichomes were microhair and glandular hair in large numbers (Fig. 1H).

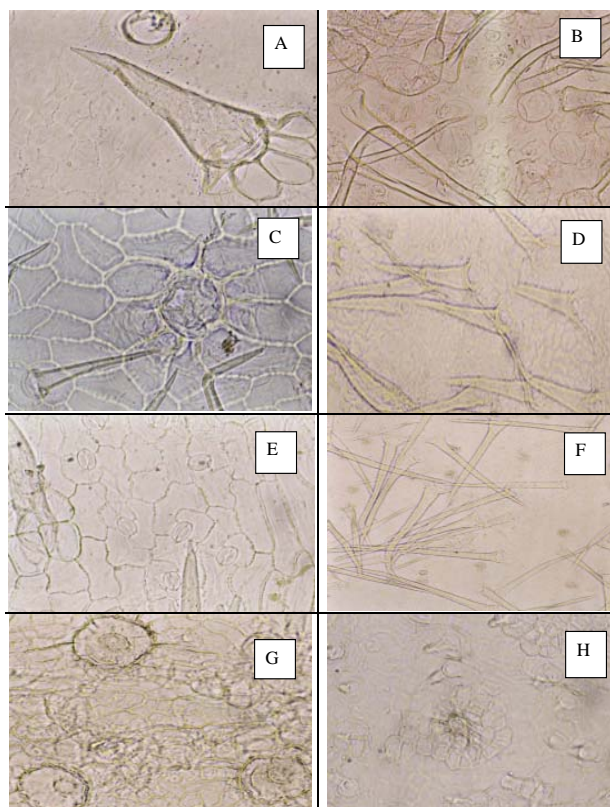
DISCUSSION

Anatomical studies revealed clear cut differences in size and shapes of stomata, epidermal cells, presence of macro- and micro-hair, their size and type etc. The size and shapes of organelles were different on both sides. Presence

Table I. Comparative Anatomy of genera *Lantana*, *Verbena* and *Vitex* (Verbenaceae) from Pakistan

Species	Stomata		L Stomata		Epidermal		Epidermal		Trichomes		Trochomes		Trichomes (microhair) L x B	
	x B	Adaxial	L	x B	cells L x B	cell L x B	Abaxial	L x B	(macrohair)	L x B	(macrohair)	L x B	Abaxial	
<i>L. camara</i>	17.5x10µm		15x20µm		14x6µm	13x12µm			192x47µm		135x27µm			-
<i>L. indica</i>	15x5µm		10x5µm		24x12µm	10x10µm			145x15µm		132x12µm			-
<i>Ver. bonariensis</i>	33x23µm		20x12µm		52x60µm	48x35µm			305x32µm		185x24µm			50 x 23µm at Adaxial
<i>Ver. officinalis</i>	10x10µm		25x20µm		35x18µm	44x23µm			112x25µm		170x35µm			-
<i>Ver. bipinnatifida</i>	27x17µm		28x16µm		45x40µm	50x38µm			170x37µm		108x18µm			-
<i>Ver. hybrida</i>	18x13µm		21x13µm		33x30µm	20x18µm			275x55µm		197x24µm			-
<i>V. negundo</i>	-		-		27x18µm	-			-		190x10µm			37 x 10 µm on Adaxial, 12 x 12 µm on Abaxial
<i>V. trifolia</i>	-		-		25x12µm	-			108x18µm		95x9µm			17x20µm on Abxial

Fig. 1. Light micrographs of leaves: (A)- macroshaiars and glands of *L. camara* (adaxial surface 100 x), (B)- Stomata, macrohairs and glands of *L. indica* (adaxial surface 200 x), (C)- cacrohairs and glands of *Ver. bonariensis* (abaxial surface 200 x), (D)- Stomata and trichome of *Ver. officinalis* (adaxial surface 100 x), (E)- macrohairs, stomata and epidermal cells of *Ver. bipinatifida* (adaxial surface 200 x), (F)- macrohairs, stomata and glands of *Ver. hybrida* (adaxial surface 100 x), (G)- Glands of *V. negundo* (abaxial surface 200 x), (H)- microhairs and glands of *V. trifolia* (adaxial surface 200 x)



of trichomes is the characteristic feature of family Verbenaceae. The most comprehensive description of types of glandular and non-glandular hair and their distribution play an important role in correct identification of the specimen and its classification even to the specific level. All these three genera are quite variable with respect to the modifications of epidermal cells. The epidermal wall whether thick or thin is also helpful to some extent for identification. The epidermal morphology and stomatal ontogeny are distinguishing characters between primitive and advance types and are helpful in morphological and ontogenetical classification (Ramassamy, 1995).

The ontogenesis of internal secretory cells in the leaves of *L. camara*, was observed by Moura *et al.* (2005). Solereder (1908), Raghavan and Arora (1960), Inamdar (1969), Theobald *et al.* (1979), Sinha and Sharma (1984),

Gottlieb and Salatino, (1987), Rueda (1993), Judd *et al.* (1999) have reported the presence of glandular trichomes secreting essential oil in Verbenaceae. Such a precocious synthesis provides chemical defense against herbivores acting as deterrents. The sites of accumulation have an important influence on the effectiveness of anti-herbivory substance (Gershenson & Croteau, 1991).

The micromorphological characters of the leaf provided information on the fossil's phylogeny and showed taxonomic relationships (Thomson *et al.*, 1984). The stomata larger in size on adaxial surface were observed in *Ver. bonariensis* with 33 x 23 μm and smaller were in *L. indica* with 15 x 5 μm size (Table I). On abaxial surface large sized stomata were observed in *Ver. bipinnatifida* with 45 x 40 μm and smaller in *L. indica* with 10 x 5 μm . The epidermal cells larger in size were seen on adaxial surface in *Ver. bonariensis* with 52 x 16 μm and smaller ones were in *L. camara* with 14 x 6 μm . On abaxial surface larger cells were seen in *Ver. bipinnatifida* with 50 x 38 μm and smaller ones were in *L. indica* with 10 x 10 μm . Trichomes mostly observed were macrohair but in Adaxial surface of *Ver. bonariensis* and both the surfaces of *V. negundo* and abaxial surface of *V. trifolia* microhair were also observed (Table I; Fig. 1G - H).

The larger Macrohair observed were in *Ver. hybrida* with 197 x 24 μm along with macrohair, the glandular hair was also seen. The smaller microhair were observed in *V. negundo* with size 12 x 12 μm (Table I). The presence of trichomes is a characteristic feature of family Verbenaceae. Glandular hair was observed in *L. camara*, *Ver. bonariensis*, *V. negundo* and *V. trifolia*. Trichomes were well segmented in *Vitex* spp. The trichomes of *L. camara* on abaxial surface were present as aggregate of five cells at the base (Fig. 1A). Trichomes of *L. indica* were just one celled at the base. Trichomes of *L. indica* were somewhat different from that of *L. camara* as it was broader at the base but not tapering like others. It is swollen a little bit before tip. Stomata were diacytic in *L. camara*, amphianisocytic in *L. indica*, anisocytic in *Ver. bonariensis*, *V. officinalis*, *V. hybrida*, mixture of staurocytic and anisocytic in *V. bipinnatifida*, the stomata and epidermal cells could not be seen in *Vitex* due to thick trichomes. Epidermal cells were rectangular, polygonal, square, sometimes spheroid and hexagonal.

From these studies it is concluded that it not only widens spectrum and scope of taxonomy but also provides an efficient approach to identify the plant scraps, which otherwise would have required the complete information on the specimen plant for its correct identification.

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