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Research Article

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Micromorphology studies of three important medicinal plants of Asclepiadaceae family

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Abstract

Asclepiadaceae family contains many medicinally important species of which *Hemidesmus indicus, Leptadenia reticulata* and *Tylophora india* were selected for the present micromophological studies. It was revealed that different types of stomata like anomocytic, anisocytic and paracytic were present only on abaxial surfaces of leaves. However maximum anomocytic stomata i.e. 25.50±0.43 were observed in *L. reticulata* followed by *H. indicus* (24.54±0.31) and *T. indica* (11.36±0.16). Similarly, observation for trichomes revealed that they were present on abaxial surface in *H. indicus* and *T. indica* whereas in *L. reticulata* on both of the surfaces present. They varied in their type as unicellular in *H. indicus* whereas multicellular trichomes in *L. reticulata* and *T. indica*. This different micromorphological characters will help in identification of authentic plant species.

Keywords: epidermis; foliar characters; *Hemidesmus indicus*; *Leptadenia reticulata*; stomatal index; trichome type; *Tylophora indica*

Introduction

Medicinal plants are a source of therapeutic drugs for the treatment of various ailments and diseases. However, one of the major problems in traditional medicine is the species misidentification and substitution of the plants with closely related species poses the problem of adulteration which could be fatal to the consumers (Neelam *et al*., 2014). Investigating the morphological and anatomical characters of closely related species is an indispensable tool for the identification of correct plant material (Sonibare *et al*., 2014). Micromorphological characters such as stomata, trichome and epidermal cells are useful research tools in plant taxonomy, phylogeny and their applications in the identification of species which has been well recognized (Sonibare *et al*., 2014). Stomata are a microscopic pore on the surface of land plants which are surrounded by a pair of specialized epidermal cells called guard cells, known to act as a turgor-driven valve that open and close the pores in response to environmental conditions. Stomata type, size, abundance, length and density are species specific and hence important characters in angiosperm taxonomy as well as in phylogenetic studies (El

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Sayed *et al*., 2012). Apart from stomata, the epidermis of most plants also contains epidermal appendages like trichomes, which are highly varied amongst the plants and their diversity as well as distribution has been used as important tool in delimiting plant species (Bano *et al*., 2015). Foliar trichome character has been used to resolve the taxonomic conflicts and have played an important role in plant taxonomy (Fang and Fan, 1993). They are of scientific interest due to their functional attributes and economic importance in the secretion of phytochemicals (Robles-Zepeda *et al*., 2009).

Asclepiadaceae family, commonly called as milkweed family, has around 250 genera and 2000 species. Many plants belong to this family amongst which *Hemidesmus indicus* (L.) R. Br.*, Leptadenia reticulata* (Retz.) Wight and Arn and *Tylophora indica* (Burm. F.) Merrill. are important medicinal climbers. *H. indicus*, commonly known as anantamula or Indian sarsaparilla, is a semi-erect twining shrub, found in moist part of India (Anonymous, 1959). Leaf of this plant is simple, entire, opposite decussate, short petioled, extipulated, apiculate acute or obtuse which varies in its size (Austin, 2008). The plant is reported as 'Rasayana' drug used in rejuvenation and give strength to bones and tissues (Puri, 2003). Another climber of the family, *L. reticulata* is locally known as jivanti or dodi is present in Gujarat, sub-Himalayan tracts of Punjab, Uttar Pradesh and the Deccan Peninsula (Mohanty *et al*., 2017). Leaves of the plant are opposite, ovate to cordate, long, broad, entire, acute, subacute to mucronate, base symmetrical, rounded to obtuse, almost glaberous when older (Bawra *et al*., 2010; Schmelzer *et al*., 2013). It has many medicinal properties like galactogogue/lactogenic, antioxidants, anticancerogenic, anti-inflammatory etc. (Mohanty *et al*., 2017). An important perennial climber used in traditional medicines is *T. indica*, which is known as antamul or Indian ipecac is mostly used to treat asthma and bronchitis hence it is also called as 'asthma herb' (Gupta *et al*., 1979, 2010). Its leaves are 1.5-7 cm wide, obviate-oblong to elliptic-oblong in shape and leathery cordate at base (Kirtikar and Basu, 1935).

However, there is insufficient information regarding micromorphology of these important plants of Asclepiadaceae family. Thus stomata, trichomes and epidermal cells were studied for these plants which will be helpful in understanding the taxonomical and phylogenetic relationship between them. These characters will be an asset in easy identification of these species with different characters which in turn reduce chance of species misidentification for herbal medicines.

Materials and Methods

Foliar epidermal preparation

Fresh leaves of three asclepiadaceae members viz. *H. indicus*, *L. reticulata* and *T. indica* were collected from the Botanical Garden and Arboretum of The Maharaja Sayajirao University of Baroda. They were washed thoroughly followed by soaking in distilled water. The epidermal peels (upper and lower surface) of leaves were taken out manually by placing the leaf blade portion in water on clean glass slide with fine forcep and then the desired of leaf surface was slightly scrapped off with the help of sharp razor blade to remove the chlorophyll. They were then repeatedly washed with aid of soft caramel brush till the epidermal peel was transparent followed by staining with 1% safranin for 5 min. The stained tissues were carefully mounted in 50% glycerol on a clean microscopic glass slide and a cover slip was placed and the periphery sealed with nail vanish.

All the slides were studied for stomatal index under light microscope at different magnifications (10x and 40x) and observations were taken from 15 fields of view for each epidermal surface (abaxial and adaxial). The photomicrographs were obtained using Leica MC120 HD microscope (Leica Microsystems, Mumbai, India). The comparative analyses of the epidermis surface micromorphology and anatomical structure of the leaves were performed. Following formula was used to calculate stomatal index:

$$SI = \left(\frac{S + 100}{E + S}\right)$$

Where,

SI – Stomatal index
S – Number of stomata
E – Number of epidermis cells

Results

In the present experimental study three climbers of Asclepiadaceae family viz. *H. indicus*, *L. reticulata* and *T. indica* (Figure 1 A-C) were selected. Micromorphological features of both adaxial and abaxial surfaces of all the leaves (Figure 2 A-F) were studied under light microscope and the results are described below.

Foliar micromorphology of *H. indicus* epidermal cells revealed that they were irregular, large in size with sinuous or slight wavy thin anticlinal walls at abaxial surface, whereas they were polygonal, straight and single layered walls on the adaxial surface (Figure 4A, Table 1). The observations for stomata revealed that they were densely distributed on the abaxial surface only and were open, paracytic with two distinct subsidiary cells and contiguous stomata in open conditions with single distinct subsidiary cell were also observed but rarely on the abaxial surface (Figure 3A). Stomatal index 24.54±0.31 were also measured for abaxial surface of epidermis (Table 1). Apart from stomata, small sized, non-glandular, thick walled, unicellular trichomes were observed only on the abaxial surface of epidermis, but they were sparsely distributed (Figure 3B, Table 1). Non-glandular trichomes helps to protect plant from desiccation by covering the guard cells.

Leaves of *L. reticulata* when observed for its micromorphological characters, it was observed that the cells were irregular, large in size with slightly sinuous or wavy anticlinal walls on abaxial surface (Figure 3C). The characters of cells were changed to large, thick, polygonal and straight when the adaxial surface was observed (Figure 4B, Table 1). Observing the stomata revealed that it was present only on abaxial surface and were anomocytic as well as anisocytic with two distinct subsidiary cells (Figure 3C). They were densely distributed on whole surface of leaf and were 25.50±0.43 which is higher as compared to *H. indicus* (Figure 3C, Table 1). Whereas trichomes were observed on both the surfaces and were multicellular, long and non-glandular with very thick walls (Figure 3D, 4B).

Micromorphological characters of *T. indica* epidermal cells revealed that they were irregular with sinuous anticlinal walls on the abaxial surface whereas they were polygonal and straight on the adaxial surface (Figure 4C, Table 3). The paracytic stomata were sparsely distributed only on the abaxial surface (Figure 3E) and the number is less (11.36 ± 0.16) as compare to both *H. indicus* and *L. reticulata* (Table 1). Similarly, observation for trichomes also revealed that they were unicellular in nature and present only on abaxial surface (Figure 3F, Table 1).

This study revealed that in all three plants, stomata type and index, as well as trichome type and index varied. Maximum stomatal index was observed for *L. reticulata* followed by *H. indicus* and was least for *T. indica*. But the trichome index was maximum for *H. indicus* followed by *L. reticulata* and *T. indica*. However, in all the three selected species, shape of epidermal cells and pattern of anticlinal walls are same which indicate some kind of similarity between taxas.

Table 1. Micromorphological characteristics of few members of Asclepiadaceae family

Characters	*H. indicus*		*L. reticulata*		*T. indica*	
	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
Shape of epidermal cells	Irregular	Polyglonal	Irregular	Polyglonal	Irregular	Polyglonal
Pattern of anticlinal walls	Sinuous	Straight	Sinuous	Straight	Sinuous	Straight
Stomata type	Paracytic/ contiguous	-	Anomocytic/ anisocytic	-	Paracytic	-
Stomatal index	24.54 ± 0.31	-	25.50 ± 0.43	-	11.36 ± 0.16	-
Trichome type	Unicellular	-	Multicellular	Multicellular	Multicellular	-

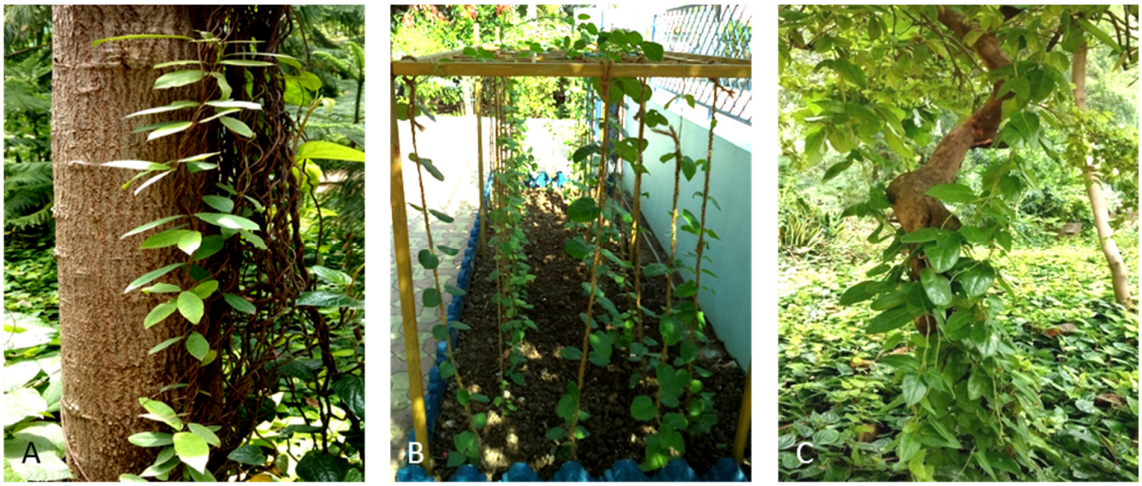

Figure 1. Plant species selected for micromorphology studies: (A) *H. indicus*, (B) *L. reticulata* and (C) *T. indica*

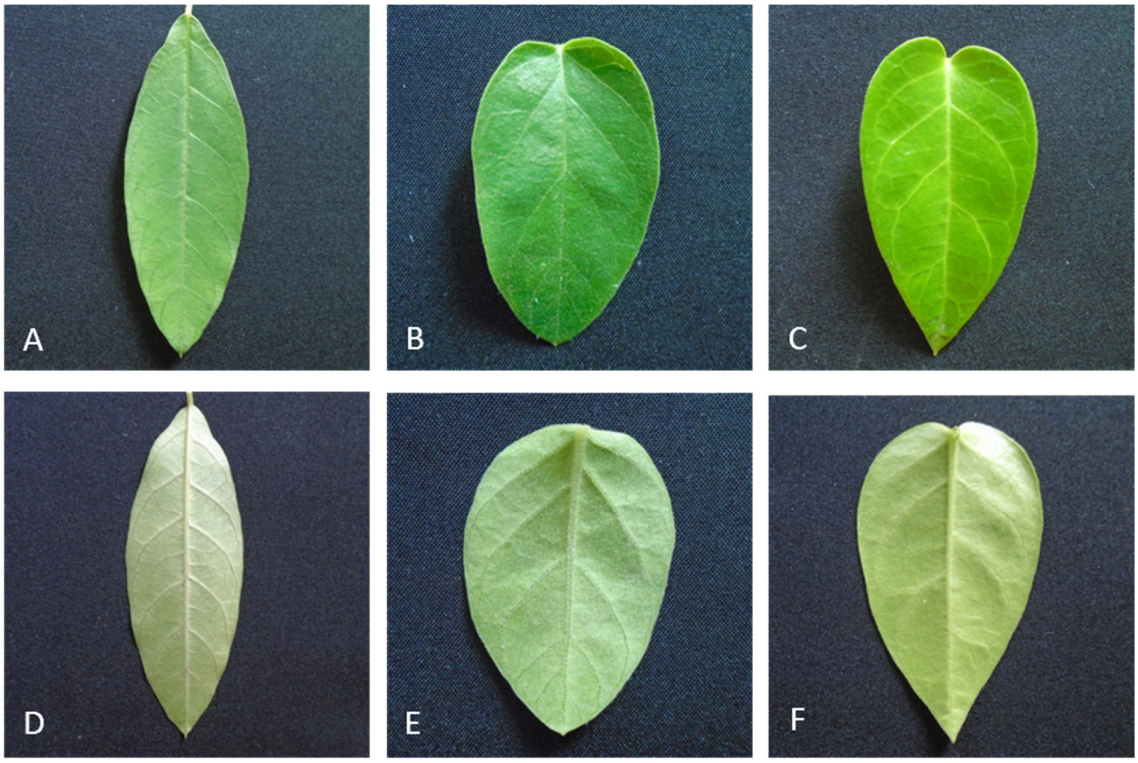

Figure 2. Adaxial (A-C) and abaxial (D-F) surfaces of leaves: (A, D) *H. indicus*, (B, E) *L. reticulata* and (C, F) *T. indica*

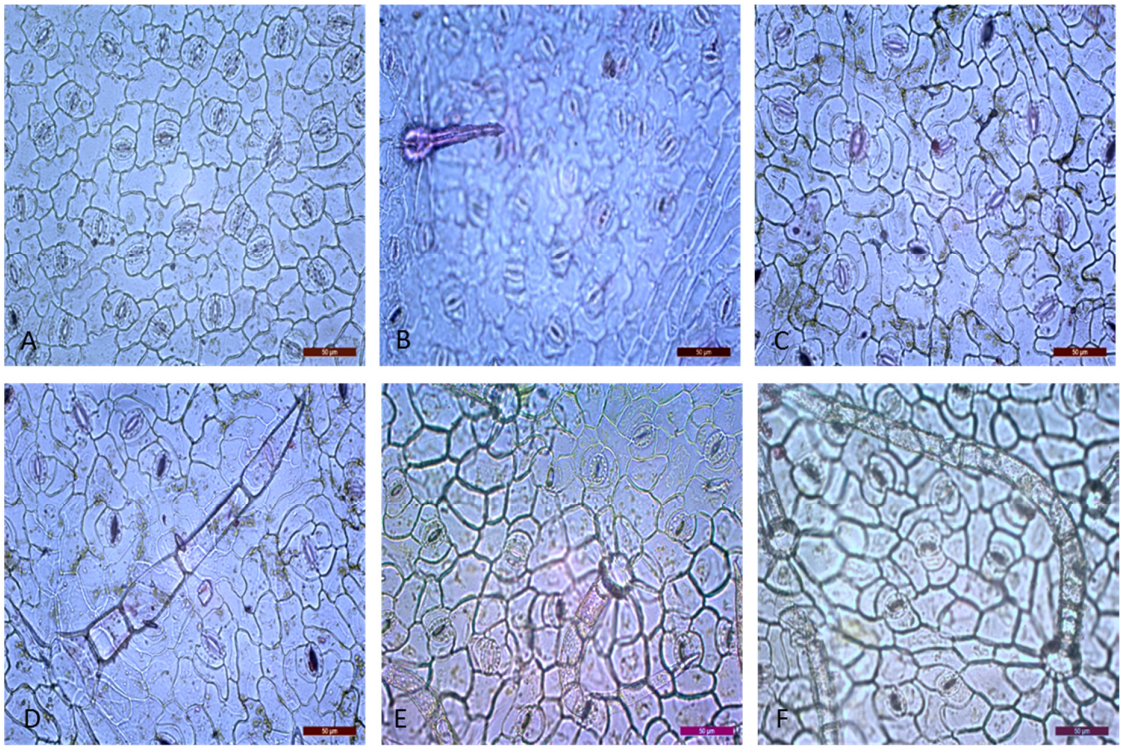

Figure 3. Type of stomata and trichome from the light micrograph of abaxial surfaces at 40x magnification-(A) paracytic stomata and (B) unicellular trichome in *H. indicus*, (C) anomocytic/anisocytic stomata and (D) multicellular trichome in *L. reticulata*, (E) paracytic stomata and (F) multicellular trichome in *T. indica*. Bar = 50 µm

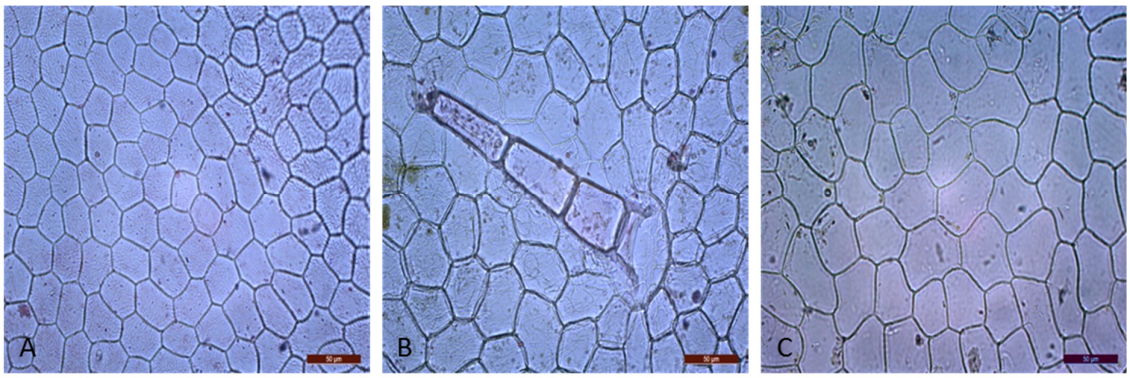

Figure 4. Light micrograph of adaxial surfaces at 40x magnification-(A) polygonal epidermal cells in *H. indicus*, (B) polygonal epidermal cells with muticellular trichome in *L. reticulata* and (C) polygonal epidermal cells in *T. indica*. Bar = 50 µm

Discussion

In the present work three important medicinal plants belonging to family Asclepiadaceae have been investigated with regards to micromorphology of the leaf studied under light microscope and identified according to Esau (2002). Micromorphological features like epidermal cells, stomata and trichome types of both the surfaces of all the species were recorded and variation between the species and the surfaces of the leaf was observed.

Observations revealed that in all the three species stomata were absent on adaxial surface which may help in preventing water loss as it is highly exposed to sunlight (Martin and Glover, 2007). In *H. indicus* and *T. indica* only paracytic stomata were observed on abaxial surface with polygonal epidermal cells, similar observation was documented in *T. asthmatica* (Santhan, 2014). However contiguous stomata were observed on abaxial surface in *H. indicus* and is also reported earlier in the same plant (Shekhawat and Manokari, 2016). Similar findings are also reported in another Asclepiadaceae members like *Pentatropis capensis* (Saralla *et al*., 2012), *Gymnema* sps. (Satheesh *et al*., 2013) and *Calotropis gigantean* (Maiti *et al*., 2018). In *L. reticulata* both anisocytic and anomocytic stomata were observed on the abaxial surface which was also reported by Mammen *et al*. (2012) however they have recorded less stomatal index (11.67±0.68) as compared to present study.

Trichomes study revealed that they were non-glandular in all the species. Unicellular trichomes were observed in *H. indicus* which is in line with the earlier report on same plant (Shekhawat and Manokari, 2016). Whereas multicellular non-glandular trichome was observed in *L. reticulata* and *T. indica* which is documented in *Secamone afzelii* (Abere and Onwukaeme, 2012) and *Gymnema* sps. (Satheesh *et al*., 2013). The anticlinal walls of epidermal cells were straight on the adaxial surface while on abaxial surface they were slightly wavy in all three plants. Whereas the epidermal cells in *Araujia* and *Morrenia* sps. are slightly curved, wavy with thick anticlinal walls (Carvalho *et al*., 2017).

Conclusions

This study revealed that there is a variation in micromorphology of the species and these can be considered as identifying characters of the same. In *H. indicus* and *T. indica* the adaxial surface was glabrous and epidermis was without stomata whereas stomata and trichomes were observed on abaxial surface of both the plants. However, in *L. reticulata* stomata was observed only on abaxial surface and trichomes were on both surfaces.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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