Appendix

Appendix

List of papers published: Three

1) Micromorphological characters as biomarkers for some of the medicinal plants of Gujarat. *Herbal Technology: Recent Trends and Progress.*(M. Daniel *et.al.*, Eds.) Scientific Publishers, Jodhpur, (India), 77-84.

2) Foliar trichomes of some members of the family Acanthaceae and their taxonomic utility (*Int. J. Pharma and Bio* Sci. 2 (3), 2011, 231-235.)

3) Foliar micromorphological studies on some members of the family Fabaceae (*Int. J. Pharma and Bio Sci.* 2 (4), 2011, 603-611).

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MICROMORPHOLOGICAL CHARACTERS AS BIOMARKERS FOR SOME OF THE MEDICINAL PLANTS OF GUJARAT

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ABSTRACT

Twelve medicinal plants of Gujarat have been screened for their micromorphological characters such as epidermal cell complex, stomatal complex, and trichomes. Trichomes were found to be very distinctive and useful in identifying each taxon. Therefore, a key for identification of these plants have been prepared. The trichomes thus form a valid biomarker.

INTRODUCTION

Micromorphological studies of any taxa include characteristic features of epidermal cell complex, stomatal complex, trichome complex, or any other epidermal inclusions. Importance of micromorphological characters; specifically those of trichomes are widely recognized for taxonomic considerations of Angiosperms (Banerjee, 2002). A majority of the flowering plants can readily be identified with as much easy by their vegetative characters as by their floral characters (Davis and Heywood, 1973; Praveen, 2000). These characters aid as biomarkers for easy identification of plants even in vegetative state.

In the present study, the emphasis is given on trichome studies. Trichomes are epidermal appendages of diverse form, structure and functions. They may occur on all parts of the plants. Trichomes show wide

variations within families and the smaller plant groups and even in the same plant. On the other hand, one may observe considerable uniformity in trichomes within a plant group also.

Structurally, trichomes are classified as unicellular and multicellular. The unicellular may be unbranched or branched. Multicellular trichomes may consist of a single row (uniseriate) or of several layers (multiseriate). Some multicellular trichomes are branched in dendroid manner; others have the branches oriented largely in one plane.

In the present work 12 medicinal plants found in Baroda have been studied for their micromorphological characters especially trichomes and stomatal features.

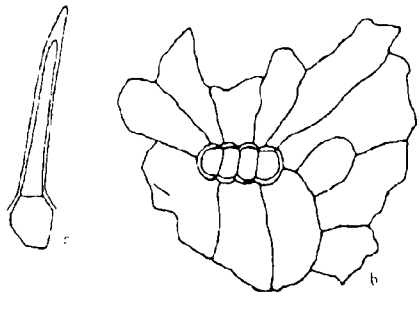
MATERIALS AND METHODS

All the plants were collected from the campus of M.S.U. The voucher specimens were deposited in the herbarium of M.S. University of Baroda. For trichome studies, the leaves were boiled in 10% KOH for 5-10 min. and after decanting the KOH, these leaf samples were washed in water. These treated leaf samples were then stained with saffranin and mounted in glycerin and observed under compound microscope.

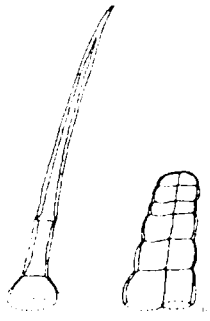
Observations

The trichomes observed in the plants studied are described below and data on trichome frequency and trichome index are provided in Table 2. The data on epidermal cells and stomata such as epidermal frequency, stomatal index and their frequency are presented in Table 1. The details of trichomes of each plant studied are presented below.

1. *Aerva lanata* Juss. Only eglandular trichome is present here. It is multicellular and uniseriate. The surface shows wartiness due to small protuberances. The walls of joints of the trichome show many finger like projections on either side. The basal cell is swollen.

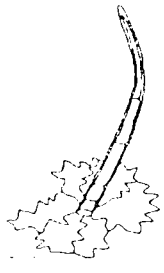


2. *Antigonon leptopus* H. & A. This plant also has both glandular and eglandular trichomes. The glandular trichome shows 3-4 celled sessile head and the polar end of the head show hemispheric cell. The eglandular trichome is unicellular and very short in length. Tip of trichome is conical.

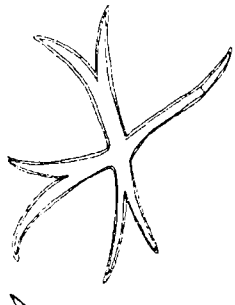


3. ***Blumea membrancea* DC.** Here also both glandular and eglandular trichomes are present. The eglandular trichome (a) shows swollen basal cell. Basal cell is somewhat hemispheric. Eglandular trichome is multicellular, uniseriate and the joints show swellings as those of *Jatropha*. Glandular trichome (b), is multicellular, biseriate and the head is not well differentiated and 3-4 celled.

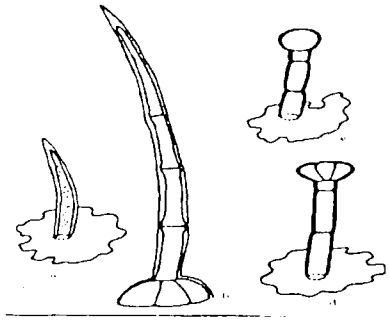
4. ***Buddleia asiatica* Lour.** Trichomes are of two types, glandular and eglandular. The eglandular trichome is two celled joined at the base and each cell is further dichotomously branched. Hence, it shows four arms and each arm is spread in the form of a cross. This trichome shows a small stalk formed of the bending of the base of two cells. The glandular trichome is 2-celled with kidney shaped cells joined at the back.



5. ***Euphorbia hirta* L.** The trichomes observed are eglandular uniseriate unbranched filaments. Apical cell is pointed.

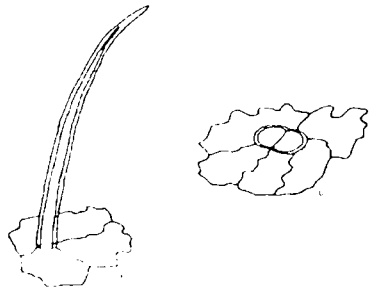


6. ***Glinus lotoides* Loefl.** This plant shows presence of 3-5 armed stellate hairs. Each arm of the stellate hair is dichotomously branched. Sometimes the arm shows presence of cross septa.

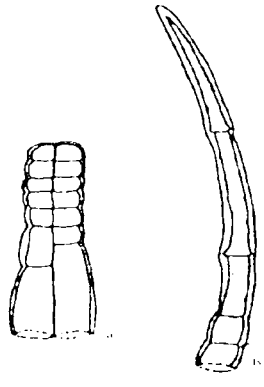


7. ***Hygrophila serpyllum* Anders.** Here also, both eglandular and glandular types of trichomes are present. Eglandular trichomes are of two types, viz. unicellular (a) and multicellular uniseriate trichome (b). Unicellular trichome show pitted surface. Multicellular uniseriate filamentous trichome show swollen base. The base is hemispheric and formed of 3 swollen cells. The glandular trichomes are of two types one is with single cell head (c) and another one with 3-celled head (d). Both trichomes are stalked (multicellular uniseriate stalk).

8. ***Jatropha gossypifolia*** **L.** Trichome observed here is multicellular, uniseriate, and eglandular. The apical cell of the trichome is pointed. Surface of the trichome is smooth.

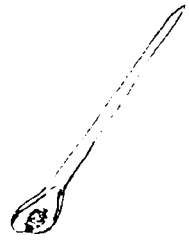


9. ***Lantana camara*** **L.** Both glandular and eglandular trichomes are present. Eglandular trichome is unicellular with slight curve at its tip. The glandular trichome is sessile and 2-celled. Each cell is hemispheric.

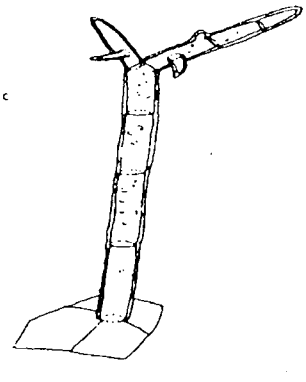


10. ***Sphaeranthus indicus*** **L.** Here two types of trichomes viz. glandular and eglandular are present. The glandular trichome is multi-cellular filamentous and biseriate. The trichome shows an undifferentiated head (4-5 celled). Stalk is absent and basal cells are swollen.

Eglandular trichome is multicellular filamentous uniseriate. The basal 2-3 cells are swollen. The joints are swollen similar to the joints of bones.



11. ***Terminalia arjuna*** **W. & A.** Here very long unicellular glandular trichomes are present. It shows a very narrow lumen at the base only.



12. ***Withania somnifera*** **Dun.** Only eglandular trichome is present here. It is multicellular, uniseriate and branched. Many a times these trichomes are vigorously branched. The branches appear like horns. Surface of the trichome is warty.

The general description and types of trichomes along with stomata of the investigated taxa are described below.

Epidermal cell complex

Epidermal cells were four to many sided. All the taxa investigated show epidermal cells with irregular cells with wavy and curved walls except *Withania somnifera*, Dun. and *Antigonon leptopus*, H. & A. which showed cells with straight walls. Epidermal cell frequency ranged from 18 to 61.2 per mm^2.

Stomatal complex

Stomata observed were of different types such as anisocytic, anomocytic, diacytic, tetracytic, polycytic, pentacytic and paracytic. Stomatal frequency ranged from 7 to 12 per mm^2 and stomatal index ranged from 11.56 to 26.67 per mm^2.

Trichome complex

Both glandular and eglandular types of trichomes were present. The trichome were unicellular/multicellular uniseriate or multicellular multiseriate.

All the investigated taxa studied showed unbranched trichomes except for *Withania somnifera*, Dun. where branched trichomes were observed. The data on stomata and trichomes in the investigated taxa are presented in Tables 1 & 2.

Table 1. Stomatal and Epidermal characters in some Medicinal Plants of Baroda

Taxa	Epidermal Cell Shape	Epidermal Cell Frequency (per mm^2)	Stomatal Type	Stomatal Frequency (per mm^2)	Stomatal Index (per mm^2)
Aerva lanata Juss.	Irregular	41.2	Anisocytic Anomocytic	12	22.56
Antigonon leptopus H. & A.	Irregular	61.2	Anisocytic Anomocytic Tetracytic Paracytic	8	11.56
Blumea membranacea DC.	Irregular	21.8	Anomocytic	7	24.30
Buddleia asiatica Lour.	Irregular	63.2	Anomocytic	21.4	25.29
Euphorbia hirta L.	Irregular	30	Anisocytic Anomocytic	8	21.05
Glinus lotoides Loefl.	Irregular	81.2	Anomocytic Polycytic	10.8	25.71
Hygrophila serpyllum Anders.	Irregular	25.6	Diacytic	8.6	25.14

Jatropha gossypifolia L.	Irregular	31.6	Tetracytic	9.8	23.67
Lantana camara L.	Irregular	28.6	Diacytic Anisocytic Anomocytic	10.4	26.67
Sphaeranthus indicus L.	Irregular	18	Anisocytic Anomocytic	6.2	25.61
Terminalia arjuna W. & A.	Irregular	23.4	Anomocytic Anisocytic Pentacytic	10.2	30.35
Withania somnifera Dun.	Irregular	24.4	Anisocytic	6.4	20.78

Table 2. Trichomes in some medicinal plants of Baroda.

Taxa	Trichome Type	Trichome Frequency (per mm²)		Trichome Index (per mm²)	
		Eglandular	Glandular	Eglandular	Glandular
Aerva lanata Juss.	Multicellular uniseriate eglandular	2.4	---	5.50	---
Antigonon leptopus H. & A.	Both multicellular glandular & unicellular eglandular	1	1	1.6	1.6
Blumea membranacea DC.	Both multicellular glandular and eglandular	1	1	4.38	4.38
Buddleia asiatica Lour.	Both eglandular and glandular	14.8	2	18.97	3.16
Euphorbia hirta L.	Multicellular uniseriate eglandular	1	---	3.22	---
Glinus lotoides Loef.	Stellate hair	1.2	---	3.70	---
Hygrophila serpyllum Anders.	Both eglandular and glandular	10.6 1.2	2.2 1.8	29.28 3.31	7.91 6.56
Jatropha gossypifolia L.	Multicellular uniseriate eglandular	8.5	---	9.71	---
Lantana camara L.	Both multicellular glandular and unicellular eglandular	1.2	1.2	4.02	4.02
Sphaeranthus indicus L.	Both eglandular and glandular	1	1.6	5.26	8.16
Terminalia arjuna W. & A.	Unicellular glandular	1	---	1.6	---
Withania somnifera Dun.	Multicellular uniseriate branched eglanudular	1.6	---	6.15	---

DISCUSSION

The present study of micromorphological characters of the leaf epidermis revealed that these characters are useful for the identification of these investigated taxa. These features are now considered important in taxonomic studies and they can be considered as biomarkers. In this study, the trichomes of the plants screened are very much distinct from each other. The nature, branching and surface of the trichomes are variable and hence, they appear very sound biomarkers.

A dichotomous key for identification of these investigated taxa is given below

1. Trichomes eglandular only:
   2. Trichomes branched:
      3. Trichomes dichotomously branched like Stellate hairs ..... ***Glinus lotoides*** Loefl.
      3. Trichome multicellular uniseriate randomly branched ..... ***Withania somnifera*** Dun.
   2. Trichome unbranched:
      4. Trichome multicellular uniseriate with Smooth surface:
         5. Stomata only tetracytic ..... ***Jatropha gossypifolia*** L.
         5. Stomata both anisocytic and anomocytic ..... ***Euphorbia hirta*** L.
      4. Trichome multicellular uniseriate with spiny surface ..... ***Aerva lanata*** Juss.
1. Trichomes both eglandular and glandular or only glandular:
   6. Eglandular trichome absent, glandular unicellular trichome ..... ***Terminalia arjuna*** W. & A.
   6. Eglandular trichome present with glandular trichomes.
      7. Eglandular unicellular trichomes:
         8. Multicellular glandular trichome with two celled head..... ***Lantana camara*** L.
         8. Multicellular glandular trichome with 3-4 celled head ..... ***Antigonon leptopus*** H. & A.
      7. Eglandular multicellular trichomes:
         9. Eglandular trichome with dichotomous branching ..... ***Buddleia asiatica*** Lour.
         9. Eglandular trichome unbranched:
            10. Eglandular both unicellular and multicellular uniseriate ..... ***Hygrophila serpyllum*** Anders.
            10. Eglandular but only multicellular:
               11. Stomata only anomocytic... ..... ***Blumea membranacea*** DC.
            10. Stomata both anisocytic and anomocytic..... ***Sphaeranthus indicus*** L.

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RESEARCH ARTICLE

PHARMACOGNOSY

FOLIAR TRICHOMES OF SOME MEMBERS OF THE FAMILY ACANTHACEAE AND THEIR TAXONOMIC UTILITY

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ABSTRACT

Looking into the importance of micromorphological characters in classification of taxa at various levels and for identifying the important plants even in the absence of floral characters, a preliminary study is conducted in 13 members of the family Acanthaceae. Trichomes were a common feature in the plants screened having located in 11 out of the 13 plants studied. Both glandular and non-glandular trichomes were present. A key based on the trichome characters was prepared to identify the plants bearing them even in the vegetative conditions. These characters can serve as pharmacognostic biomarkers in cases of medicinally important plants.

KEY WORDS

Acanthaceae, micromorphological characters, biomarkers, trichomes.

INTRODUCTION

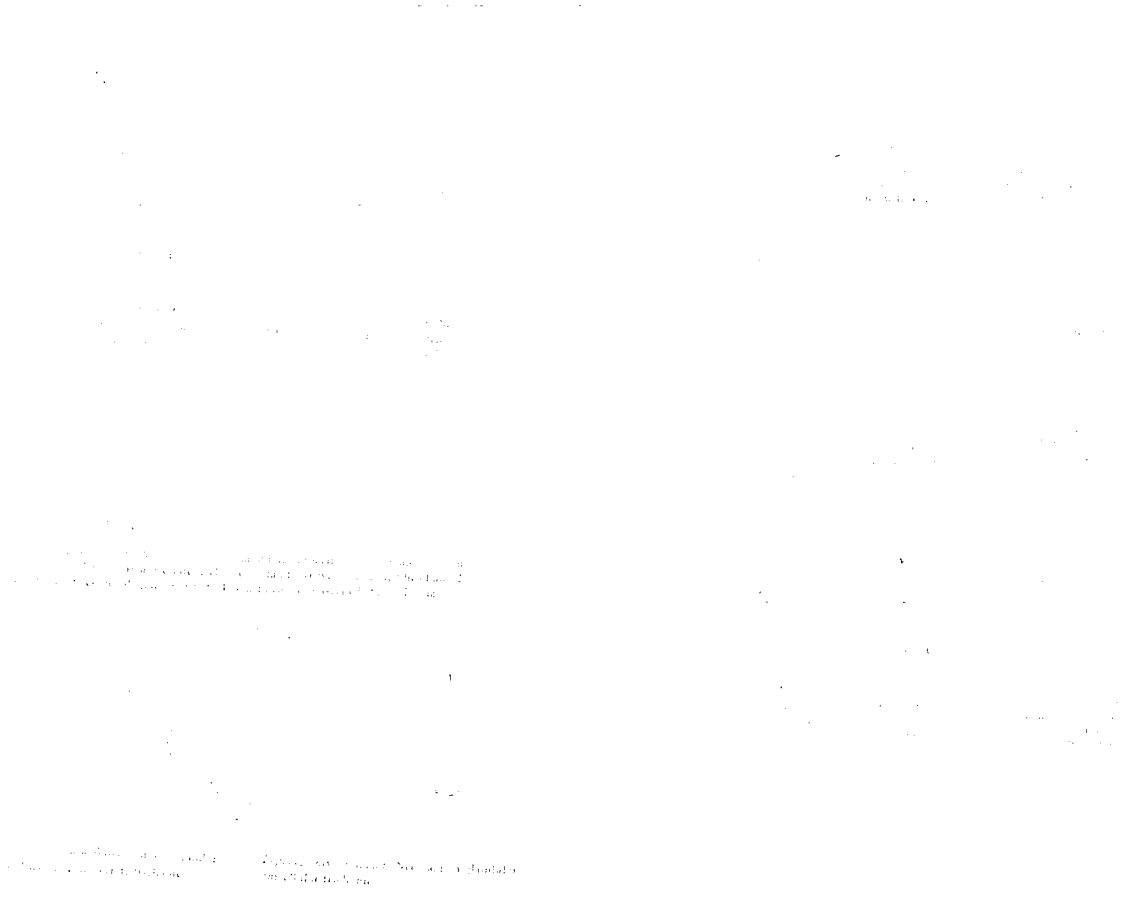
Micromorphological characters including the trichomes of plants have assumed great taxonomical significance recently as viable taxonomic markers. These characters which would be of help in identifying plants even in vegetative state will be of great use for field taxonomists in identifying plants in the absence of flowers and fruits which are available only in certain seasons of the year. It was Solereder[1] and Metcalfe and Chalk[2] who made some significant contributions to the micromorpological characters of plants. Plant morphologists had used many micromorphological characteristics as foliar trichomes to resolve the taxonomic conflicts and thus these characters have played an important role in plant taxonomy[3-4]. Recently, foliar trichomes are used for the discrimination of different taxa within the genus *Artemesia*. In case of medicinal plants, trichome characters act as biomarkers to identify the plant even in the raw material or powder form[5]. The presence of glandular trichomes in many of the medicinal plants is considered indicative on the concentration of secondary metabolites with pesticidal, pharmacological, and fragrant properties[6]. The family Acanthaceae and Asteraceae are the families which are particularly rich in different types of trichomes which are used as an aid for identification[7-8]. Therefore, in the present work, 13 members of the family Acanthaceae were studied for their foliar trichomes, to examine their utility in identification.

MATERIALS AND METHODS

The epidermis was peeled or was cut into suitable sizes. One end of leaf was held firmly with a thumb and the other end scraped gently with a razor blade, until a thin clear peel was cut off. The pieces were washed off in water and was stained with in safranin, and mounted in glycerin on a clean slide.

RESULTS AND DISCUSSION

The trichomes of the plants of Acanthaceae screened is presented in Fig. 1.Out of the 13 plants studied, two plants, *Daedalacanthus roseus* Anders. and *Rungia parviflora* var. *pectinata* Nees. were found to be glabrous. Of the remaining 11 plants, 6 possessed glandular trichomes and each of these trichomes was found to be different from others. Of the plants containing glandular trichomes, both *Ecbolium linneanum* Kurz. *Asteracantha longifolia* Nees. contained sessile, disc shaped trichomes with 4-8 cells. Both contained non-glandular uniseriate trichomes also. But the uniseriate trichomes of *Ecbolium linneanum* had the apical cell which is long and swollen at the base. All the remaining four plants contained stalked glandular trichomes. The glandular trichomes of *Adhatoda vasica* Nees. and *Neuracanthus sphaerostachys* Dalz. were having a single celled stalk and 4 celled head but the latter plant possessed unicellular trichomes also. In *Lepidagathis cuspidata* Nees. and *Hygrophila serpyllum* Anders. there were two types of glandular trichomes. The large trichome of *Lepidagathis cuspidata* Nees. had 2 celled stalk (with thick walls) and 4 celled head. While, that of *Hygrophila serpyllum* Anders. the stalk was 4-celled. The small trichome of the former possessed a four celled head while that of latter had a single celled head.



nicellular trichome was the only type in *epidagathis fasciculata* Nees. The remaining contained uniseriate 3-4 celled long ichomes. These trichomes were istinguished by the thickening of the trichome alls. Both *Ruellia patula* Jacq. and *Rungia epens* Nees. had broader cells in trichomes. ut the former plant contained a number of cell nclusions, while such type of inclusions were ot observed in the later plant. Of the remaining 2 plants, *Tubiflora acaulis* Kuntz. and *Justicia diffusa* Willd. the trichome of the former plant had very thick walls and very narrow lumen whereas that of *Justicia diffusa* had a swollen basal cell.

An artificial key using trichome character for the identification of some species belonging to the Acanthaceae is prepared and presented below.

RTIFICIAL KEY USING TRICHOME CHARACTERS FOR THE IDENTIFICATION OF SOME PECIES BELONGING TO THE ACANTHACEAE:

Plants glabrous.....*Daedalacanthus roseus* Anders and
Rungia parviflora var. Nees.

Plants with trichomes:

2. Glandular trichome present, non-glandular trichome present or absent:
   3. Glandular trichome sessile;
      4. Non-glandular trichome 3-celled with upper cell long and swollen at the base.....*Ecbolium linneanum* Kurz.
      4. Non-glandular trichome 3-celled without any cell being swollen.....*Asteracantha longifolia* Nees.
   3. Glandular trichome stalked:
      5. Stalk of glandular trichome unicellular
         6. Unicellular non-glandular trichome present .....
            Neuracanthus sphaer stachys Dalz.
         6. Unicellular trichome absent.....*Adhatoda zeylanica* Medic.
      5. Stalk multicellular:
         7. Stalk 2-celled.....*Lepidagathis cuspidata* Nees.
         7. Stalk more than 2-celled.....*Hygrophila serpyllum* Anders.

2. Glandular trichome absent:
   8.Non-glandular trichome unicellular.....*Lepidagathis fasciculata* Nees.
   8. Non-glandular trichome multicellular:
      9. Cells broad:
         10. Cells with brown contents.....*Ruellia patula* Jacq.
         10. Cells empty.....*Rungia repens* Nees.
      9. Cells narrow:
         11. Cell wall thick, lumen very narrow.....*Tubiflora acaulis* Kuntz.
         11. Cell wall thin, with swollen basal cell.....*Justicia diffusa* Willd.

ONCLUSION

he significance of micromorphological atures is proved beyond doubt in the present udy. The utility of the artificial key in entification of plants in the absence of floral characters is a proof to this. In case of medicinal plants the trichomes aid as biomarkers useful in identifying the plants in the powder form.

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RESEARCH ARTICLE

PHARMACOGNOSY

FOLIAR MICROMORPHOLOGICAL STUDIES ON SOME MEMBERS OF THE FAMILY FABACEAE

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ABSTRACT

Twenty-nine plants belonging to the family of Fabaceae were investigated for their foliar trichome characters. Five plants were found glabrous, and the rest showed both glandular and non-glandular trichomes, which ranged from unicellular to multicellular structures. Three plants contained both glandular and non-glandular trichomes whereas four contained only the former and the rest non-glandular trichomes. These trichome characters being specific to the plants containing them are diagnostic features which can also be utilized as biomarkers to recognize the drug plants and to identify the plants in vegetative state.

KEY WORDS

abaceae, micromorphological characters, glandular trichomes, non-glandular trichomes, biomarkers, axonomy.

NTRODUCTION

he family Fabaceae is one of the largest amilies among Angiosperms which is xtensively studied for anatomical as well as pidermal characters[1-6]. Carlquist[7], Metcalfe[8] nd Stace[9] had stated that the leaf characters re the most varied anatomical features in ngiosperms. These features can be employed s useful taxonomic characters. Leaf or foliar nd floral micromorphological characters are onsidered as some of the primary diagnostic eatures in segregating the major groups of lants. In fact, the leaf characters are considered s second to those of flowers and fruits in axonomic studies. Foliar and floral epidermal haracters are used successfully in the elimitation of a number of taxa[10-13]. Cutler[14] had tated that apart from the usefulness of those haracters in taxonomy, they are also used fficiently in the identification of fossil specimens, pecifically the leaf impressions in Paleobotany. n addition to this, the epidermal icromorphological features help in authentification of foliar drugs in pharmacognosy and thus serve as biomarkers[15]. Chandra *et al*[16] had emphasized of the significance of epidermal morphology and arrangement of phylogenetic considerations. Looking into the importance of these characters, in the present work, 29 plants, belonging to Fabaceae, are subjected to an analysis of micromorphological features in their leaves with a view to find out, the relevance of these characters, in identifying the taxa and if possible, defining the taxonomic affinities.

MATERIALS AND METHODS

All the 29 plants screened were collected from in and around Vadodara, Gujarat, India. For studying the trichomes, epidermal peels were taken out manually with the help of a blade and stained with safranin. Stained peel was mounted in glycerine and then observed under the microscope.

RESULTS AND DISCUSSION

No.	Plant name	Glandular trichome						Non-glandular trichome				
		unicellular	multicellular trichome differentiated into head and stalk				multicellular sessile trichome	unicellular		two-celled	three-celled	medifixed
			head two-celled and stalk one celled	four-eight celled head and one celled stalk	12-celled and one celled stalk	16-celled head and one celled stalk		base flat	base round			
1.	*Indigofera cordifolia* Heyne ex Roth.							+				+
2.	*Indigofera tinctoria* L.											+
3.	*Indigofera enneaphylla* L.											+
4.	*Indigofera trita* L.											+
5.	*Crotalaria retusa* L.	+										
6.	*Alhagi camelorum* Fisch.	+										
7.	*Clitoria ternatea* L.	+										
8.	*Teramnus labialis* (L.f.) Spreng.						+					
9.	*Pongamia pinnata* (L.) Pierre.									+		
10.	*Tephrosia villosa* (L.) Pers.							+		+		
11.	*Desmodium triflorum* (L.) DC.		+					+		+		
12.	*Desmodium velutinum* (Willd.) DC.		+		+					+		
13.	*Dolichos lablab* L.			+		+		+			+	
14.	*Stylosanthes hamatus* (Linn.)Taub.										+	
15.	*Crotalaria medicagenia* Lamk.								+			
16.	*Alysicarpus vaginalis* (L.) DC.								+			
17.	*Melilotus indica* (L.) All.								+			
18.	*Tephrosia purpurea* (L.) Pers.								+			
19.	*Crotalaria sericea* Retz.							+				
20.	*Tephrosia tenuis* Wall. ex Dalz.							+				

21.	*Crotalaria leptostachya* Benth.	+
22.	*Sesbania grandiflora* (L.) Poir.	+
23.	*Crotalaria hirsuta* Willd.	+
24.	*Mucuna pruriens* (L.) DC.	+

The distribution and characteristics of trichomes in 24 members of the Fabaceae is presented in Table I. Out of the total 29 plants studied, five plants viz. *Canavalia ensiformis, Aeschynomene indica, Desmodium gangeticum, Rhynchosia bracteata* and *Sesbania aculeata* are found glabrous. Of the remaining 24 plants with trichomes, seven possessed various unicellular and multicellular glandular trichomes. Unicellular glandular trichomes were seen in three plants, i.e., *Alhagi camelorum, Crotalaria retusa* and *Clitoria ternatea. Alhagi camelorum* possessed oblong thin walled trichome whereas *Crotalaria retusa* had spindle shaped (pointed at the ends) trichome (Fig I and II). In *Clitoria ternatea* elongated obovate trichome was present with slightly warty walls. Glandular trichomes of the remaining four plants were multicellular differentiated into a head and a stalk or sessile. The trichome of *Dolichos lablab* had a biseriate two or four tiered head and a single celled stalk. In addition, this plant contained two types of non-glandular trichomes; one unicellular and the other linear and three-celled. The unicellular trichome here was curved at the tip while the three-celled trichome had a very long apical cell and two-square shaped lower cells. The glandular trichomes of *Desmodium velutinum* had one or three tiered heads. This plant also possessed characteristic non-glandular trichomes having single basal cells and very long narrow pointed apical cells, which was smooth or warty. The glandular trichomes of *Desmodium triflorum* had two-celled heads. The non-glandular trichomes of this plant were of two types; one two-celled with a spreading small basal cell and a narrow pointed apical cell. The second type of trichome was unicellular and curved. Trichomes of the fourth plant *Teramnus labialis* were sessile, flat, linear and four-celled.