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Research Article

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## Foliar micromorphology of *Pimpinella* (*Apiaceae*) species from Eastern Ghats, India

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### Abstract

*Pimpinella* L. is a potential but little-known genus of medicinal plants of Apiaceae, represented by four species from the Eastern Ghats of Andhra Pradesh. Epidermal features such as stomata and trichomes were studied. For each species, the composition of stomatal types, nature of anticlinal walls of the epidermal cells, stomatal index and frequency, and trichome morphology along with distribution were determined. Haplocytic, diacytic, amphidiacytic, paracytic, transitional, anisocytic and anomocytic types have been revealed, while trichomes were confined to non-glandular ones multicellular, unbranched type. The present study establishes the taxonomic significance of the foliar epidermis.

**Keywords** *Pimpinella*, Epidermal complex, Comparative analysis

### Introduction

*Pimpinella* L. is the third-largest genus of the family Apiaceae with 150 species, widespread in the northern hemisphere (Heywood, 1971). Of the 22 species present in India (Karthikeyan et al., 2009), eight are confined to Peninsular India, with the rest occurring in the Himalayan region (Mukherjee & Constance, 1993). Pullaiah & Ramamurthy (2007) reported five species of *Pimpinella* from Eastern Ghats, India. The diversity and distribution of stomata and trichomes can be viewed from various perspectives and used as a model system for research in developmental biology, ecology, and evolution. The leaf epidermis, which is the outermost layer of the lamina, includes stomata, trichomes, and idioblasts, varies considerably among species (Salisbury, 1927; Raju & Rao, 1977). The structural diversity and arrangement of stomata and trichomes play a significant role in the taxonomy, especially at a generic and specific level (Raju, 1981; Stace,

1984). Fruit is the essential taxonomic character for the identification of taxa in the family Apiaceae. The traditional classification and delimitation of groups within the family are primarily delineated according to fruit morphology, and the obtained results do not correspond to the most recent molecular phylogenetic analysis (Lariushin, 2012). Ostroumova (1991) worked extensively on Umbelliferous stomata, considered *Pimpinella* a complex polymorphic group, and taxonomic affinities need revision. Hence, it is necessary to explore consistent taxonomic characters other than fruit to substantiate the taxonomic diversity at generic and specific levels.

An attempt was made here to analyze the epidermal features such as stomata, trichomes, and other epidermal features to strengthen the systematics of genus *Pimpinella* in the Eastern Ghats by studying four out of five species found in the Eastern Ghats, viz., *Pimpinella bracteata* Haines, *Pimpinella heyneana* (Wall. ex DC.) Kurz, *Pimpinella tirupatiensis* Bal.

& Subr., and *Pimpinella wallichiana* (Miq. ex Hohen.) Gandhi. The present paper provides a diagnostic key to the species of *Pimpinella* using just foliar epidermal features.

## Materials and Methods

The plant material of four *Pimpinella* species was collected from various localities along the Eastern Ghats of Andhra Pradesh, India, during the fruit bearing season. The species were identified with the help of Gamble & Fisher (1969) and Flora of Eastern Ghats (Pullaiah & Ramamurthy, 2007), and the voucher specimens were deposited at SKU (Sri Krishnadevaraya University Herbarium, Ananthapuramu).

The epidermis was studied using fresh leaves. The adaxial (Ad) and abaxial (Ab) surfaces of the leaf were peeled off with the help of needles and forceps and stained with a combination of safranin-hematoxylin (differential staining) solution (Reeve, 1948; Raju & Rao, 1977). The material was mounted in glycerine jelly, observed, and measured under Nikon E-400 microscope. For scanning electron microscopy (SEM), leaf segments were anchored with carbon conductive tape on brass stubs; specimens were sputter-coated with gold, and observations were accomplished with a JSM-IT500 Scanning Electron Microscope.

The terminology adopted for the epidermal features after Ramayya (1962), Stace (1965), Patel (1979), Metcalfe and Chalk (1985) and Baranova (1987).

## Results and Discussion

The foliar epidermis in *Pimpinella* bears various stomatal types dispersed all over the leaf surface in a regular pattern. The extent of variations like the shape of epidermal cells, nature of anticlinal cell wall, types of stomata, number of stomata per unit area, size, stomatal index, stomatal frequency, and the type, distribution, density, and length of trichomes were evaluated and presented.

### Stomatal complex

The epidermal cell shapes and the nature of anticlinal walls vary among the test species. Penta- or hexagonal epidermal cells are present on both the epidermis of *P. bracteata* and only on the adaxial epidermis of *P. tirupatiensis*. The abaxial epidermal cells of *P. tirupatiensis* are of irregular cell shape, whereas *P. heyneana* and *P. wallichiana* possess irregular shape with wavy or sinuous anticlinal walls (Table 2).

All the leaves are amphistomatic. There were seven types of stomata noted: amphidiacytic, anisocytic, anomocytic, diacytic, haplocytic, paracytic and transitional type. Diacytic stomata are dominant in all four species, showing the highest percentage in *P. wallichiana* (86% in abaxial) followed by *P. bracteata* (82% in adaxial) and *P. tirupatiensis* (50% in

adaxial). However, *Pimpinella tirupatiensis* showed the highest stomatal diversity with the presence of five types of stomata. Such variation in foliar stomata in the same species was reported in *Micrococca mercurialis*, Euphorbiaceae (Raju & Rao, 1975), and the need for determining the predominant (basic) stomatal type was sought (Raju & Rao, 1987). Diacytic is the basic stomatal type in the genus, but the complex shows variation in structure within the type, Patel (1979) considered the variant as amphidiacytic type, which is present in *P. bracteata* and *P. tirupatiensis*. Guyot (1971), Ostroumova & Kljuykov (1991) concluded that all the North Eurasian *Pimpinella* species have stomata with two subsidiary cells as the predominant type. In our study, *Pimpinella heyneana* showed anomocytic type (50%) dominance over its primary type (diacytic with 39%), the same reported by Ostroumova et al., (2007) in some of (Chinese and Himalayan) *Pimpinella* species exhibit anomocytic as the dominant type.

The stomatal index and frequency (mean  $\pm$  SD) observed in *P. bracteata*, i.e.,  $34.5 \pm 1.3$  and  $251.5 \pm 26.4 \text{ mm}^2$  were highest while they (SI:  $14.4 \pm 1.5$  and SF:  $42.3 \pm 8.4 \text{ mm}^2$ ) were lowest in *P. tirupatiensis* among the four *Pimpinella* species studied. The stomata were bigger in *P. tirupatiensis* ( $42.5 \times 27.8 \text{ }\mu\text{m}$ ) whereas more or less of the same (around  $30 \times 21 \text{ }\mu\text{m}$ ) in other species (Table 1).

### Trichome complex

Although the test species possess only one type of trichome (non-glandular, multicellular, and uniseriate) in the four species *Pimpinella*, they exhibit variation in organographic distribution, number of cells per trichome, etc. Trichomes profusely occupied all parts of *P. bracteata* while restricted only to leaf in the other three species. Trichomes in *P. heyneana* are abundant on the adaxial surface than abaxial. Trichomes are associated with veins of leaf in *P. heyneana* and *P. tirupatiensis*, though they are sparingly present on intercoastal regions of the lamina. The trichome density is high in the juvenile stage, and it progressively declines and is restricted to a few parts of the *P. wallichiana* (Table 3).

The foot of the trichome (Ramayya, 1962) is simple or sometimes compound; body uniseriate, 4–12 celled, cylindrical, filiform; terminal cell pointed (*P. heyneana* and *P. wallichiana*) or blunt (*P. bracteata* and *P. tirupatiensis*); cross walls thin; lateral wall slightly convex; and surface sculpturing may be smooth (*P. heyneana*) or granulated (*P. bracteata*, *P. wallichiana* and *P. tirupatiensis*). The cells per trichome body were highest (4–12) in *P. bracteata*, and length was up to 2 mm, while the cells per trichome were 4–7 in rest of the species length was up to 0.5 mm. An interesting warty type of trichome was found and restricted only to the leaf margin, between serrations. These are two-celled, thick-walled, blunt, and bent towards the leaf apex (Fig. II).

**Table 1. Epidermal cell characteristics of *Pimpinella* species in the present investigation  
i. Stomatal characteristics**

S. No.	Name of the species	Stomatal type		Stomatal Index		Stomatal Frequency (in mm <sup>2</sup> )		Size (in µm)	
		Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
1	<i>Pimpinella bracteata</i> Haines	Diacytic, Anisocytic, Transitional	Diacytic, Anisocytic, Paracytic	34.5 ± 0.7	32 ± 1.0	251.5 ± 15.2	213.6 ± 11.7	30×21	27×18
2	<i>Pimpinella heyneana</i> (Wall. ex DC.) Kurz	Diacytic, Anomocytic	Diacytic, Anomocytic	31.4 ± 1.6	29.8 ± 0.1	145.4 ± 12.8	187.5 ± 6.3	30×21	29×17
3	<i>Pimpinella tirupatiensis</i> Bal. & Subr.	Haplocytic, Diacytic, Hemidiacytic, Anomocytic	Diacytic, Hemidiacytic, Anisocytic	14.4 ± 0.8	21.1 ± 0.4	42.3 ± 4.8	99.4 ± 3.1	35×27	42×28
4	<i>Pimpinella wallichiana</i> (Miq. ex Hohen.) Gandhi	Diacytic, Transitional	Diacytic, Hemidiacytic, Anisocytic	28.6 ± 2.8	28.2 ± 2.8	144.3 ± 2.6	174.9 ± 14.3	30×21	27×19

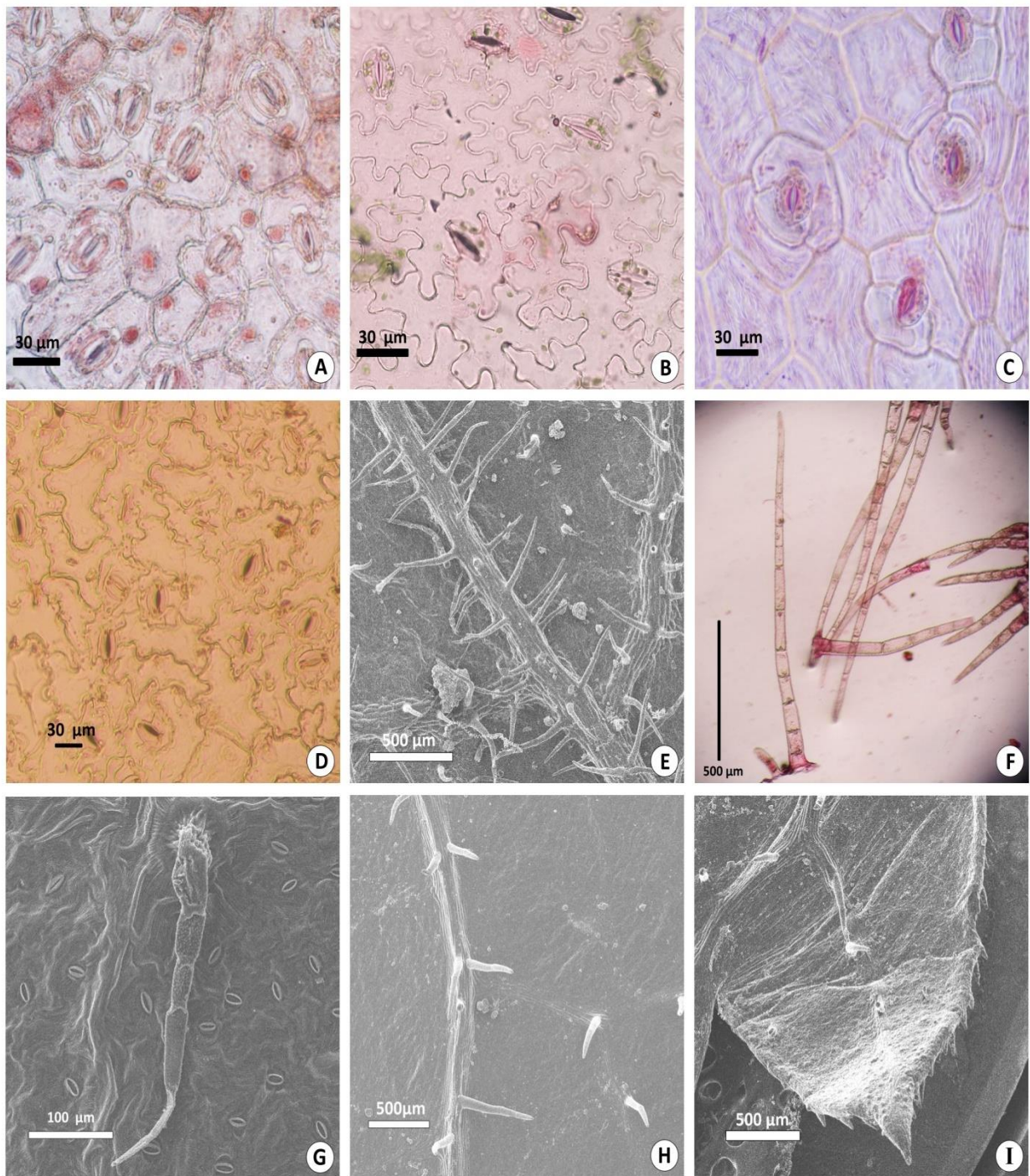
**Table 2. Epidermal cell characteristics of *Pimpinella* species in the present investigation  
ii. Epidermal cells**

S. No.	Name of the species	Cuticle thickness (in µm)	Cell shape		Anticlinal cell wall pattern		Size (in µm)	
			Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
1	<i>Pimpinella bracteata</i> Haines	6.9	Hexagonal	Hexa and Heptagonal	Straight to slightly curved	Straight to slightly curved	53.4×40.5	39.6×24.8
2	<i>Pimpinella heyneana</i> Wall. ex DC.) Kurz	3.2	Irregular	Irregular	Sinuous to wavy with U or V sinuses	Sinuous to wavy with U or V sinuses	65.9×32.5	68.7×33.1
3	<i>Pimpinella tirupatiensis</i> Bal. & Subr.	6.5	Penta and Hexagonal	Irregular	Straight	Undulate U-shaped	49.2×30.4	42.6×32.8
4	<i>Pimpinella wallichiana</i> (Miq. ex Hohen.) Gandhi	4	Irregular	Irregular	Sinuous U-shaped	Sinuous U-shaped	55.6×33.3	48.3×21.9

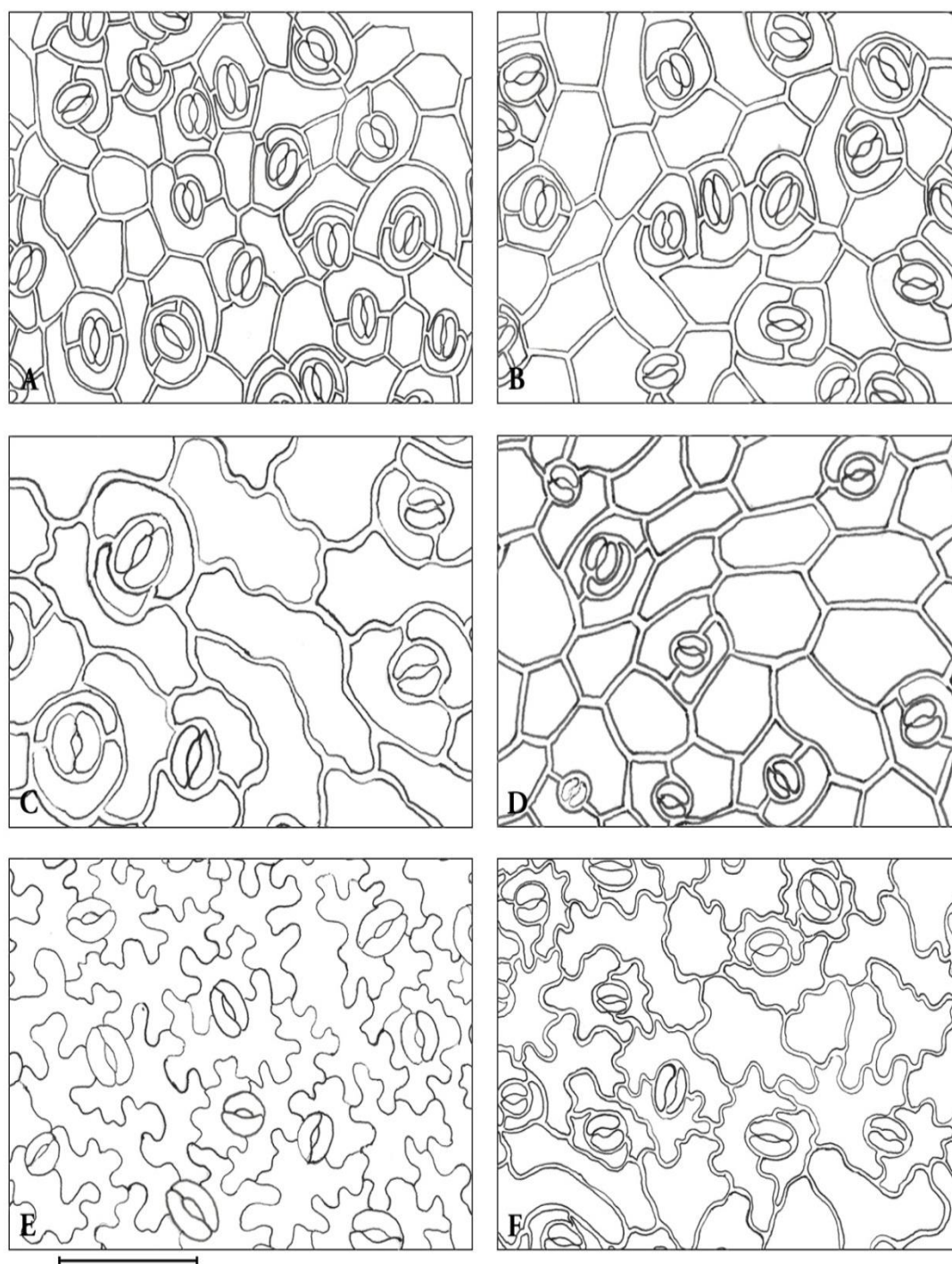
**Table 3. Epidermal cell characteristics of *Pimpinella* species in the present investigation  
iii. Trichome complex**

S. No.	Name of the species	Type	Distribution	Density		Maximum number of cells	Length up to (in mm)
				Adaxial	Abaxial		
1	<i>Pimpinella bracteata</i> Haines	Non-glandular, Multicellular, Uniseriate, conical (4 to 12 celled)	Throughout the upper and lower lower surfaces more on veins	201.8±11.9	236.8±10.3	12	20
2	<i>Pimpinella heyneana</i> (Wall. ex DC.) Kurz		More frequent on the upper surface than lower, more on veins	32.1±4	17.4± 3.3	7	5
3	<i>Pimpinella tirupatiensis</i> Bal. & Subr.		Sparsely distributed on veins, rare on lamina	15.6±2.6	10.1±1.3	6	2.5
4	<i>Pimpinella wallichiana</i> (Miq. ex Hohen.) Gandhi		Sparsely distributed on veins, rare on lamina	70.6±9.6	82.3±11.3	7	3



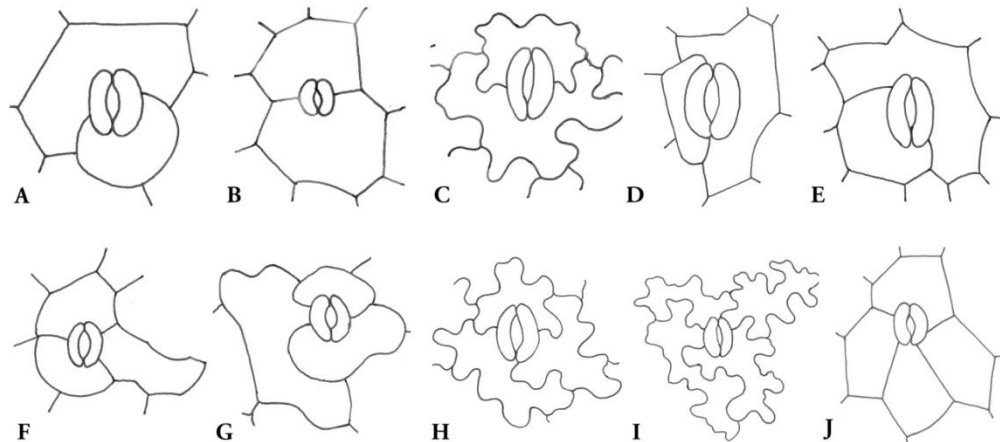


**Fig. 1 Organography of epidermal structures. A-D: Stomata - A) *P. bracteata*; B) *P. heyneana*; C) *P. tirupatiensis*; D) *P. wallichiana*. E-I: Trichomes - E&F) *P. bracteata*; G) *P. heyneana*; H) *P. wallichiana* and I) SEM image showing Warty type of trichomes at segments of leaf margin.**



**Fig. 2** Leaf epidermis. A&B: *Pimpinella bracteata*; C&D: *Pimpinella tirupatiensis*; E. *Pimpinella heyneana*; F. *Pimpinella wallichiana*. A, C&F: abaxial surface. B, D&E: adaxial surface. Scale bar = 0.1 mm.





**Fig. 3 Stomatal types: A. Haplocytic stomata; B. Diacytic; C. Amphidiacytic; D. Paracytic; E. Transitional between diacytic and paracytic; F&G. Anisocytic; H. Anomocytic with 3 neighboring cells; I&J. Anomocytic with 4 neighboring cells. A,B,G & J: *Pimpinella tirupatiensis*; C,H&I: *Pimpinella heyneana*; E&F: *Pimpinella bracteata*.**

Based on the features observed above, the following dichotomous key has been developed to allow for separation of the four species of *Pimpinella* studied in this work:

1. Epidermal cells regular, penta to heptagonal in shape; anticlinal cell wall straight or undulate:

2. Epidermal cell pattern distinctive in two surfaces; stomatal types more than four; trichome pilose, 3-6 celled.....*P. tirupatiensis*

2. Epidermal cell pattern similar in both surfaces; stomatal types diacytic, anisocytic, paracytic and transitional; trichome hirsute, densely covered, 5-12 celled.....*P. bracteate*

1. Epidermal cells isodiametric to irregular in shape; anticlinal cell wall sinuous:

3. Diacytic and anomocytic in equal proportion, transitional type is absent; trichome surface smooth, length up to 500  $\mu\text{m}$ .....*P. heyneana*

3. Diacytic with highest frequency percentage than anomocytic; transitional type on both surfaces; trichome surface granulated, length up to 300  $\mu\text{m}$ ..... *P. wallichiana*

## Conclusions

Morphological types of stomata in the family Apiaceae have certain taxonomic values (Guyot, 1966, 1984; Ostroumova & Kljuykov, 1991). For the first time, the foliar epidermal

studies of the genus *Pimpinella* of Eastern Ghats, Andhra Pradesh, India were studied. It demonstrates that leaf epidermal micromorphological characteristics are of *Pimpinella* as a genus in Apiaceae and distinguish its species. It is to conclude that the dominant diacytic (stoma surrounded with two subsidiary cells) type is to be allied to the family, whereas anomocytic and anisocytic types are allied as taxonomic characters.

## Conflict of interest

The author hereby declares no conflict of interest.

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