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RESEARCH ARTICLE

Micromorphological studies of *Anisochilus scaber* Benth.

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Abstract

The diversity in trichome characters plays a major role in taxonomical and physiological studies. The present study aims to analyze the structural diversity of trichomes in *Anisochilus scaber* Benth. using scanning electron microscope and bright field microscope. In this species two basic types of trichomes were observed, glandular and non-glandular. The glandular trichomes consist of peltate and capitate types. Non-glandular trichomes were multicellular, simple or branched. These trichomes were recognized in tender leaf, mature leaf, tender stem and sub-mature stem. However, its distribution pattern varies among the plant parts. Trichomes were almost completely absent in mature stem. The stomata identified were diacytic in nature. Difference in stomatal index is observed between tender and mature leaves. Highest percentage of stomatal index is seen in the abaxial surface of the tender leaf.

Keywords: *Anisochilus scaber*; diacytic stomata; glandular and non-glandular; scanning electron microscopy; taxonomy; trichome

Introduction

Anisochilus scaber Benth. is commonly called 'Sambrani poo' in Malayalam and 'Seivenai chedi' in Tamil belongs to the family Lamiaceae of subtribe Plectranthinae and tribe Ocimeae. *Anisochilus* Wall. ex Benth. is an Asian genus with more than 20 species and most of them are reported from Western Ghats of India. *Anisochilus scaber* is a short-lived perennial herb with quadrangular stem with rooting at the node. Leaves are petiolate, fleshy, opposite-decussate, scabrous-pubescent above and densely hoary tomentose below. Head inflorescence is mostly simple, occasionally branched with white or purplish- white corolla (1). According to studies *Coleus anthoyni* Jebin Joseph & J. Mathew sp. nov. is like *A. scaber* in morphological and ecological characters (2). But there is a slight difference in growth habit, trichome and floral nature. There exists confusion between the identities of these species due to close morphological similarity.

Most of the species from Lamiaceae are medicinally important with active production of secondary metabolites. The Paliyar aboriginal community used the leaves of *A. scaber* for witchcraft practices (3). Most of the species in the genus have significant role in traditional system of curing, especially *A. carnosus* due to the presence of secondary metabolites like phenols, flavonoids, alkaloids, tannins, terpenoids etc. (4). This study aims to provide additional information about micromorphological characters of *A. scaber* for easy identification.

The velvety nature of leaf lamina and stem could be experienced and the trichomes can be observed using unaided eye. Trichomes are epidermally originated unicellular or multicellular, glandular or non-glandular, simple or branched structures seen in the aerial parts of plants like leaf, petiole, stem, calyx, seed surface etc. There exists a relation between the secondary metabolite production and nature of trichomes (5). Secondary metabolites possess different pharmacological properties such as anti-inflammatory, antibacterial, antifungal, anticancerous, antidiabetic, antioxidant, insecticidal, etc. and produced as the result of co-evolution with different kinds of organisms like pathogens. So these secretions have some specialised properties other than medicinal values such as attracting pollinators and vectors for seed dispersal (6).

Most of the species in the family Lamiaceae are medicinally important with active production of secondary metabolites. These species commonly possess peltate and capitate type of glandular trichomes, which are the source of their secretions. Several investigations of these trichomes have been carried out in different species (7–9).

Materials and Methods

Collection and identification of the plant material

The plant material was collected from Achankovil, Kollam District, Kerala, India (Longitude: 77.1252; Latitude: 9.091405; Elevation: 83.17 m / 272.86 ft, Google Earth) during the flowering season of the plant (October–November 2021). Taxonomic identification was done with the help of taxonomic experts from Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India and voucher specimens were deposited in the Madras Herbarium with accession Number MH178219. Leaf and stem of fresh material were taken for the study after proper processing.

Scanning electron microscopic studies

For the scanning electron microscopy (SEM), small pieces (1 cm^2) of leaves (tender and mature) and stems (tender, sub-mature and mature) were fixed on aluminium stubs covered by a double-sticky adhesive tape and were coated by a thin layer (ca.25 nm) of gold–palladium with a Quorum sputter- coating for 30–60 sec. Five samples of each part were taken for SEM analysis. The SEM photographs of trichomes were taken using the ZEISS EVO 18 Research model Scanning Electron Microscope. Type of trichome is identified with the help of description provided in the literature (10).

Bright field microscopic studies

Fresh peels from the clean and dirt free aerial vegetative parts were taken and mounted in glycerine and observed under microscope using different magnification. The observations were recorded with the help of stereomicroscope (ZEISS, Stemi 305) and trinocular microscope (LABOMED L X 500). The nature of trichomes and stomata were analysed using 5 replicates of each part. The number of stomata was counted in 10 random fields and the average taken as stomatal index.

$$\text{Stomatal index} = S \times 100 / E+S$$

S = No. of stomata

E = No. of epidermal cells

Results

Micromorphological studies of *A. scaber* (Fig. 1A–C) shows amphistomatic leaf with only diacytic type of stomata (Fig. 2A–C). The stomatal index of the leaf shows variation in adaxial and abaxial epidermis. Difference in stomatal index is observed between tender and mature leaves. Abaxial surface of tender leaves shows highest percentage of stomatal index (Table 1).

Two basic types of trichomes were observed on the stem and leaf: glandular and non-glandular (Fig. 3A–C; Fig. 4A–C; Fig. 5A, B; Fig. 6A, B; Fig. 7A, B). The number of glandular trichomes is less compared to non-glandular trichomes. The types of trichomes observed and their distribution on the plant parts are summarized in Table 2.

Glandular trichomes are peltate and capitate types (Fig. 3D, 4D, 6E, F). Single type of capitate glandular trichome is present. It consists of basal cell, long stalk and unicellular secretory head. Non-glandular trichomes are multicellular, branched or unbranched (Fig. 6C, D). The branching configurations of non-glandular trichomes are biramous, triramous, tetraramous and petaramous. The length of glandular and non-glandular trichome is ranging from 1.00 mm to 2.40 mm. Number of cells per trichome ranges from 3 to 7. The leaves of *A. scaber* have single layered adaxial and abaxial epidermis with thick cuticles (Fig. 2A). Trichomes are uniformly distributed on all the portions of the leaf (petiole, lamina, margin, mid-rib regions and veins). Trichomes are present on both sides of the lamina, but their nature and density vary. The density of glandular and non-glandular trichome is higher in mature leaves than that of tender leaves. Abaxial epidermis of mature leaves has a high density and a greater number of cells per trichome (Fig. 7A, B). The surface of tender stem has high density of non-glandular trichome (Table. 2). It has similar morphological characteristics that are observed in the lamina. But the number of glandular trichome is less compared to

Table 1. Distribution of stomata on the leaf surfaces of *Anisochilus scaber*

Plant parts		Stomatal index (%)
Tender leaf	Adaxial	13.34
	Abaxial	18.57
Mature leaf	Adaxial	11.26
	Abaxial	15.72

Table 2. Distribution of different trichomes on the leaf and stem surface of *Anisochilus scaber*

Plant parts			Glandular trichome			Non-glandular trichome			
						Branched			
			Peltate	Capitate	Unbranched	Biramous	Triramous	Tetraramous	Pentaramous
Leaf	Tender	Adaxial	+	+	+	+	+	+	+
		Abaxial	+	+	++	++	++	++	+
	Mature	Adaxial	++	++	++	++	++	++	+
		Abaxial	+++	+++	+++	+++	+++	+++	++
Stem	Tender		+	+	+++	+	+	+	+
	Sub-mature		+++	++	+++	++	++	+	+
	Mature		-	-	-	-	-	-	-

(Symbols indicate the trichome density and arrangement: -, absence of trichome; +, presence of trichome; ++, higher density of trichome; +++, highest density of trichome).

Fig. 1. Habit of *Anisochilus scaber.* A: Whole plant showing the general habit; B: Stem and leaf arrangement; C: Close view of the apical portion.

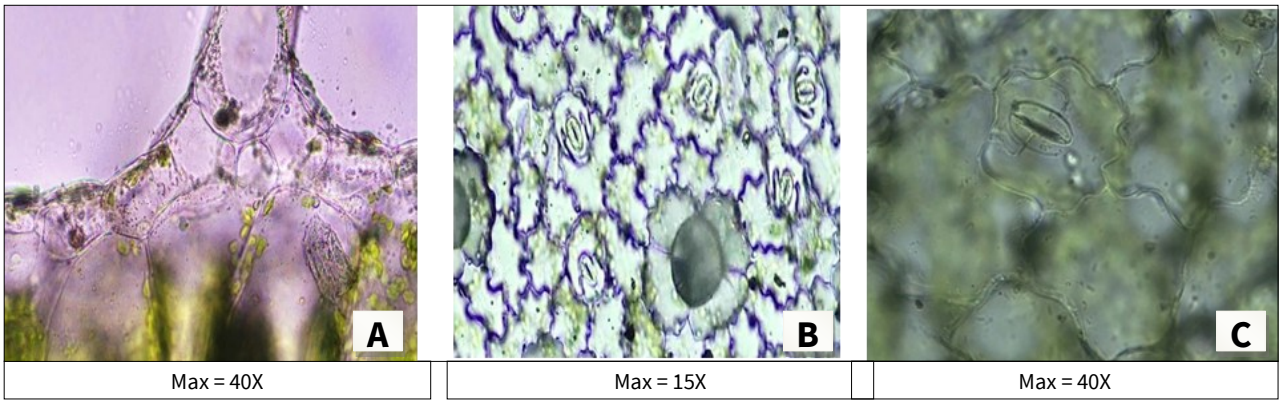

Fig. 2. Light microscopy (LM) micrographs of the epidermal tissue system of *Anisochilus scaber*. A: Epidermal cells; B: Stomata with guard cells; C: Stomatal apparatus.

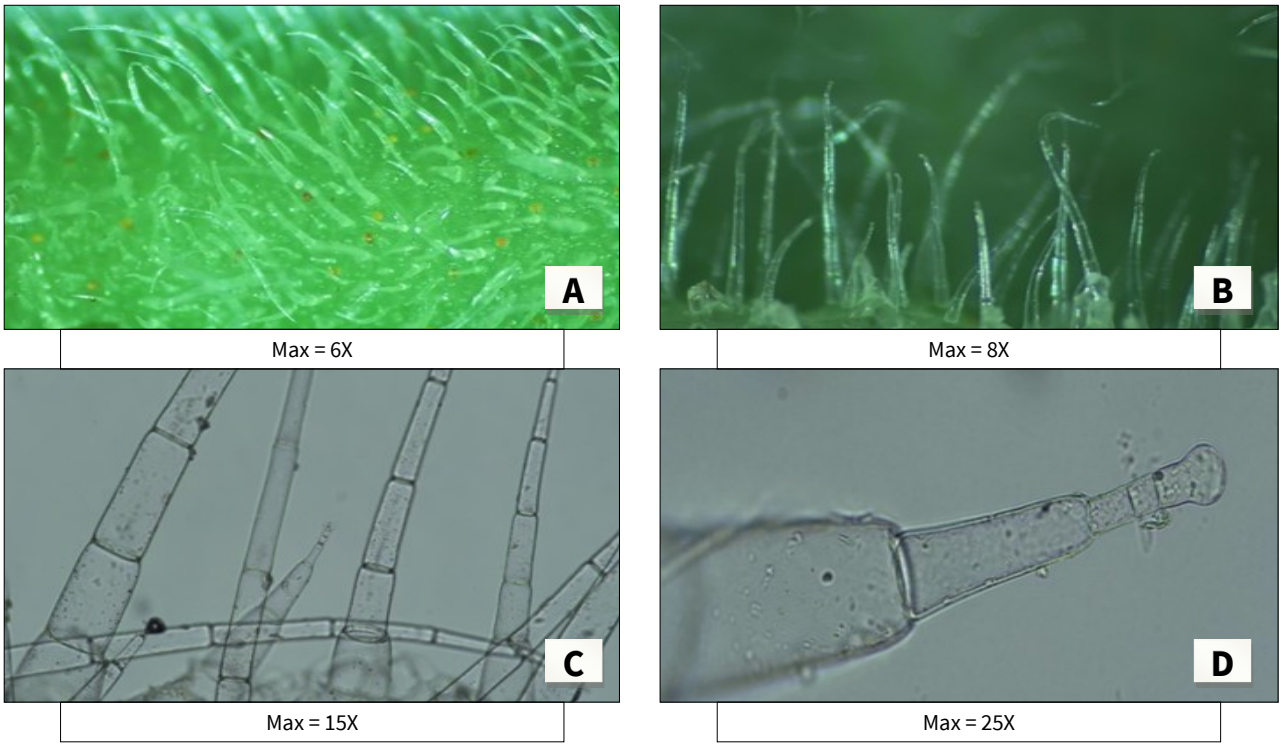

Fig. 3. Light micrographs of trichomes on the leaf of *Anisochilus scaber*. A: Non-glandular trichomes on the leaf surface; B: Enlarged view of non -glandular trichomes; C: Glandular trichomes; D: Single capitate glandular trichome.

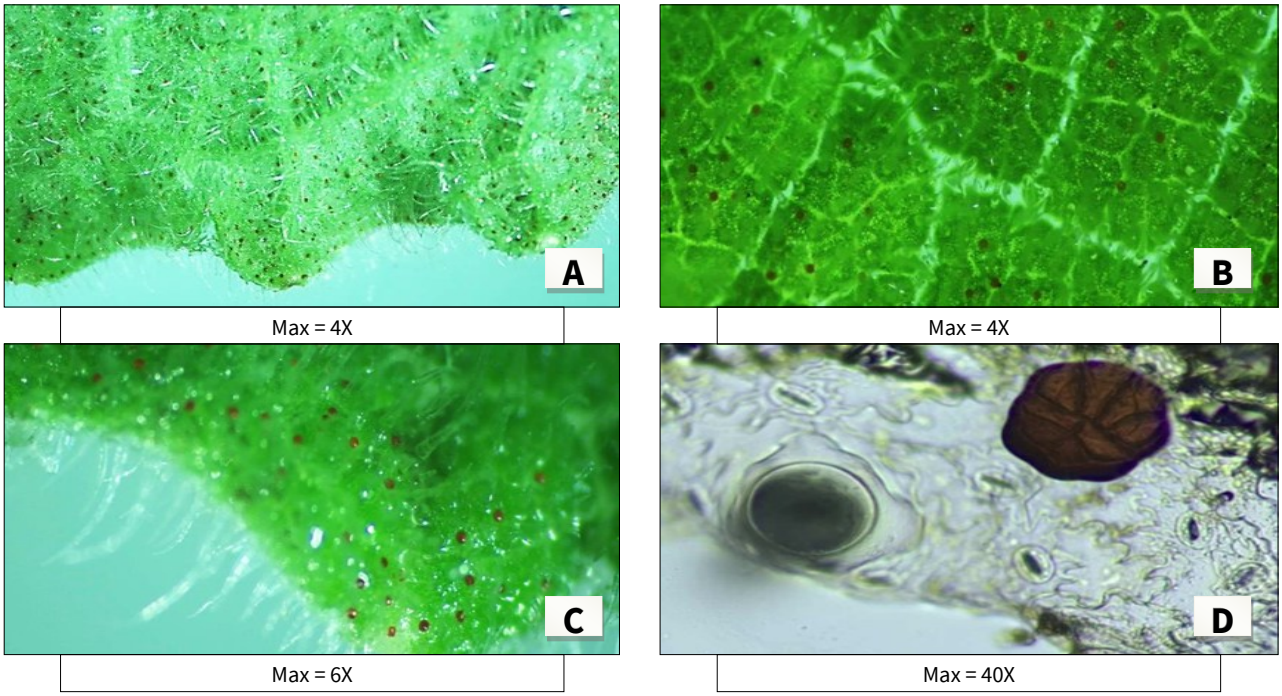

Fig. 4. LM micrograph of glandular trichomes of *Anisochilus scaber*. A, B, C: Capitate glandular trichomes; D: Peltate glandular trichome.

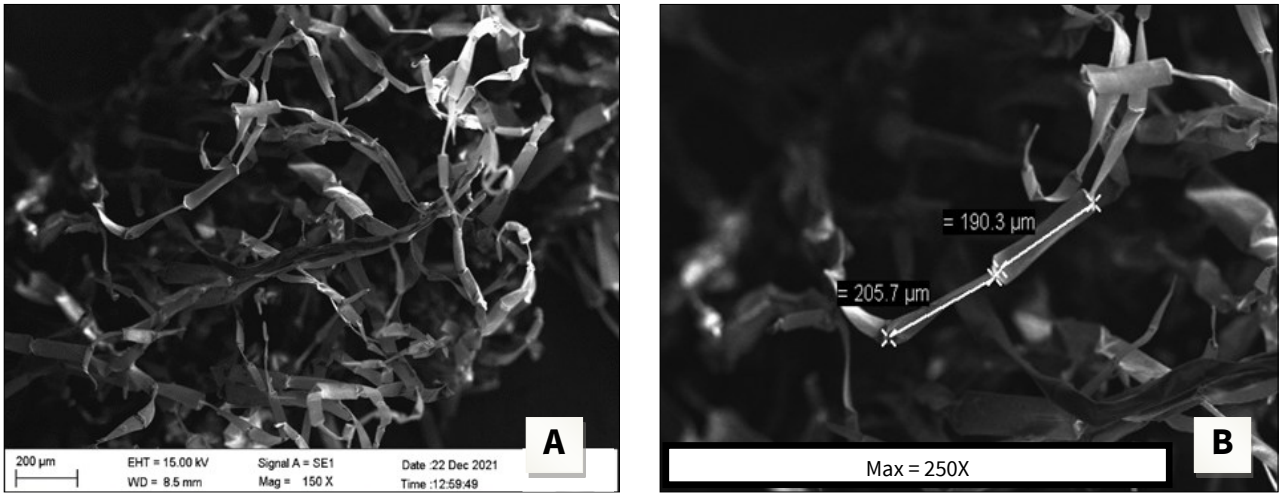

Fig. 5. SEM micrograph of the adaxial leaf surface of *Anisochilus scaber*. A: Narrow view; B: Enlarged view.

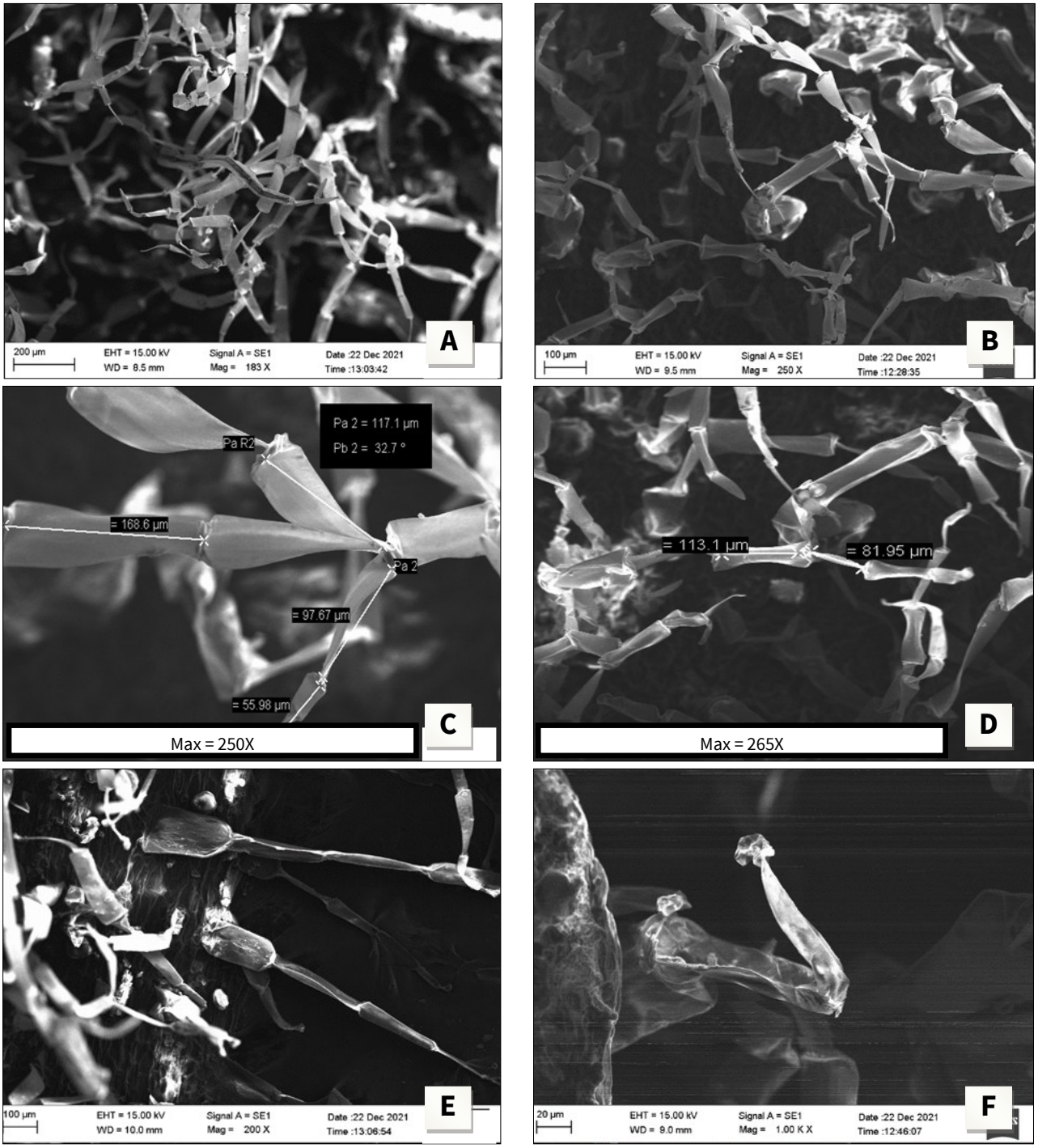

Fig. 6. SEM micrograph of the abaxial leaf surface of *Anisochilus scaber*. A, B: General view of the leaf surface; C, D: Non-glandular trichomes; E, F: Glandular trichomes.

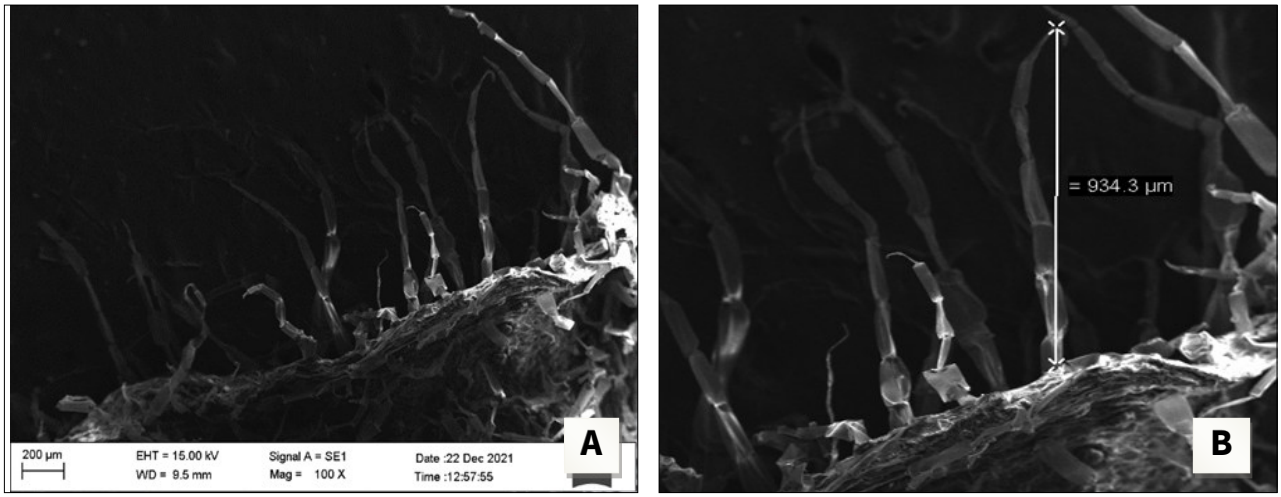

Fig. 7. SEM micrograph of the tender stem surface of *Anisochilus scaber*. A: General view; B: Close-up view.

leaf surface. The sub-mature region of stem has lower density of trichome than tender portion, but it has a greater number of glandular trichomes (Fig. 8A, B). The nature of glandular trichomes is same as that of leaf lamina. The mature stem region has only scars and remnants of trichomes (Fig. 9A, B).

Discussion

The epidermal tissue system, especially trichome and stomata has a significant role in systematics in species and subspecies level of classification and useful in phylogeny reconstruction (11). Stomata assist movement of water via transpiration in addition to the gas exchange. The type of stomata varies among species to species like anomocytic, anisocytic, diacytic and paracytic based on the nature of subsidiary cells and their distribution on leaves may be amphistomatic, epistomatic and hypostomatic depends on the habitat. Most of the species in Lamiaceae have amphistomatic leaves as that of *A. scaber*. However certain species rarely show epistomatic or hypostomatic nature (12). In amphistomatic leaves stomata were distributed on both the adaxial and abaxial epidermis. But more numbers are seen in the lower epidermis. When compared to other species of Lamiaceae, *A. scaber* shows comparatively a smaller number of stomata. It may be due to the

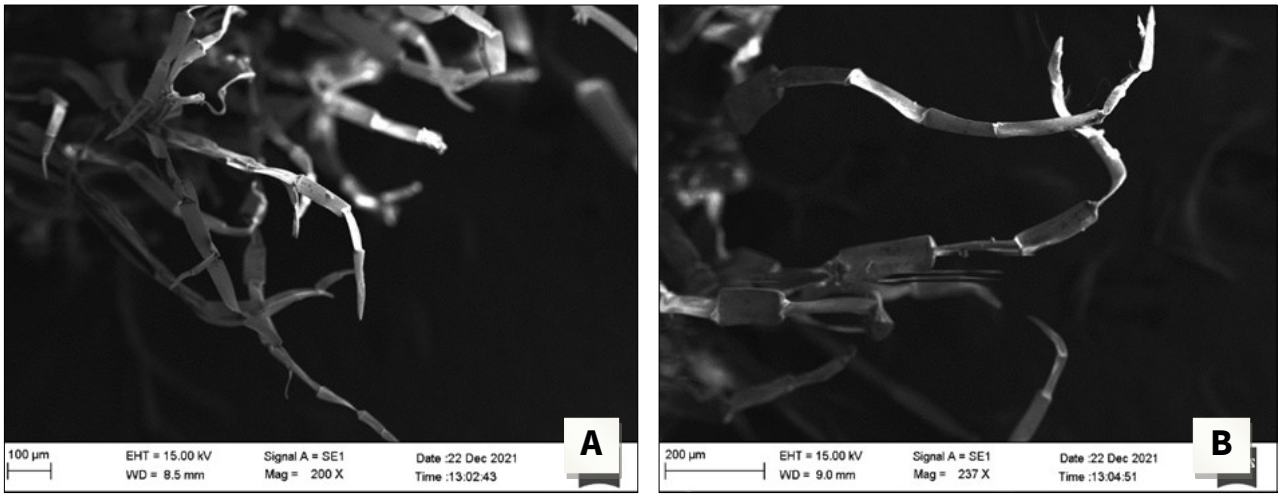

Fig. 8. SEM micrograph of the sub-mature stem surface of *Anisochilus scaber*. A: General view; B: Close-up view.

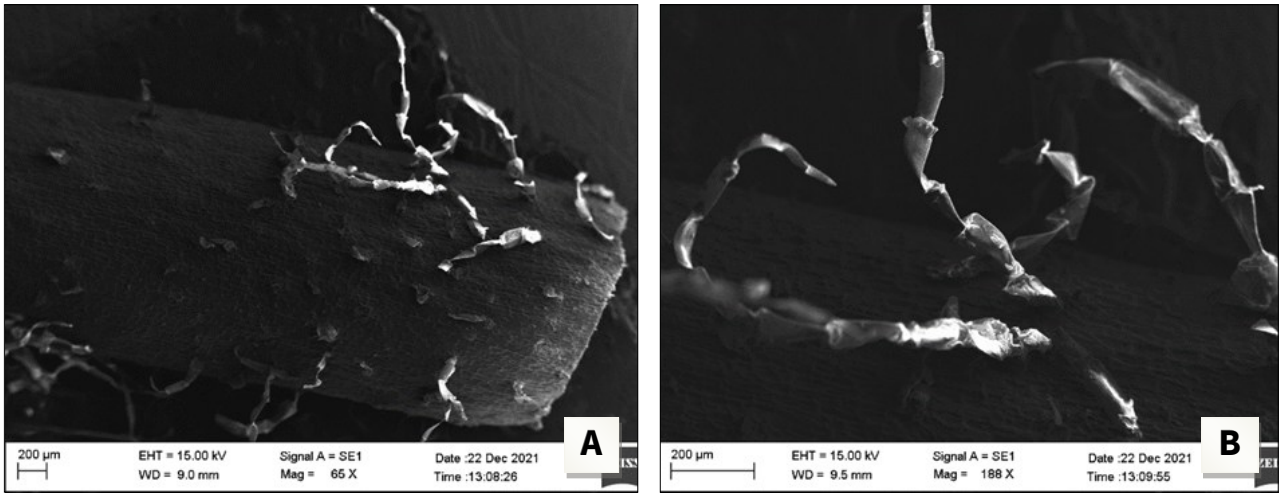

Fig. 9. SEM micrograph of the mature stem surface of *Anisochilus scaber*. A: General view; B: Close-up view.

adaptation for survival in xerophytic conditions, where water is the limiting factor. The distribution of the plant is concentrated in rocky areas of moist deciduous forests and its histology also shows certain xeric adaptations. Lower values of stomatal index were observed in xerophytic plants for maximum conservation of water and larger values observed in plants grown in mesophytic conditions with surplus water (13). Stomatal index decreases from tender to mature leaves. Lowest value of stomatal index observed in adaxial surface of the mature leaf. Anisocytic and diacytic stomata were commonly seen in members of Lamiaceae such as *Clinopodium vulgare* L., *C. umbrosum* (M.Bieb.) K.Koch, *Hyptis suaveolens* (L.) Poit., *Lallemantia royleana* (Benth.) Benth. and *Lamium amplexicaule* L. Most of the species with more than one type of stomata like *Salvia aegyptiaca* L. (12). *Anisochilus scaber* has only single type of stomata, diacytic.

The presence of glandular and non-glandular trichomes on the aerial vegetative and reproductive parts can be considered as the general character of the family Lamiaceae. The classification of glandular and non-glandular trichomes was done in different ways. The structure and function of trichomes in the family were documented in several studies (14–16). Trichome studies of several genus of the family such as *Stachys* (17, 18), *Chelonopsis* (19) and *Teucrium* (20, 21) have already been done. The SEM analysis showed the morphology and characters of the trichomes in the aerial vegetative parts of *A. scaber*. The epidermis of leaf and stem of the species carries both glandular and non-glandular trichomes.

Several types of trichomes were observed in Lamiaceae including glandular, non-glandular, unicellular, multicellular, branched and unbranched with diverse functions including synthesis, secretion and storage of secondary metabolites like essential oils, protection of aerial parts against herbivores and pathogens, etc (11, 22). Morphological, steriological and histochemical studies of trichomes present in *Rosmarinus officinalis* L. of Lamiaceae were carried out using light and fluorescence microscopy (22). The leaf surfaces possess non-glandular and glandular types of trichomes as in *A. scaber*. But volume density of trichomes in the adaxial epidermis (upper) was higher than abaxial epidermis (lower) and peltate trichomes were higher than that of capitate trichomes. In the case of *A. scaber*, density of trichomes in the abaxial epidermis is higher than adaxial epidermis and has a similar proportion of capitate and peltate glandular trichome. The calyx of *R. officinalis* also has capitate and peltate glandular trichomes (23). Another species of Lamiaceae, *Pogostemon cablin* (Blanco) Benth. commonly called Patchouli or Nilam with distribution concentrated in Indonesia, Malaysia, China, Brazil and India is rich in essential oil possess eight distinct types of trichomes (two non-glandular and six glandular) on leaf epidermis (24). Simple non-glandular trichomes were unicellular and multicellular in nature. The glandular trichomes include long stalked (capitate, peltate, digitiform, clavate, filifom and fusiform) and short stalked (capitate) (25). In *A. scaber*, non-glandular trichomes are multicellular types. Unicellular non-glandular trichomes are completely absent. Among glandular trichomes, short-capitate trichomes were more common in Lamiaceae (26). These kinds of trichomes were observed in certain species such as *Salvia aurea* L. (27), *Leonotis leonurus* (L.) R.Br. (28), *Plectranthus ornatus* Codd (26) and *Salvia blepharophylla* Brandegee ex Epling (29). But *A. scaber* possess capitate trichome with long stalk and unicellular secretory head. Peltate and capitate trichomes were also reported in *Teucrium* species (*Teucrium arduini* L., *Teucrium chamaedrys* L., *Teucrium flavum* L., *Teucrium montanum* L., *Teucrium polium* L. and *Teucrium scordium* L. subsp. *scordioides* Schreb.) from Croatia (30).

Studies of micromorphology of trichomes in the genus *Colquhounia* (Lamiaceae) also recognised glandular and non-glandular trichome. Glandular trichomes were capitate and peltate type. Non-gladular trichomes were simple (unicellular, two - celled, three- celled and more than three celled) or branched (biramous, stellate and dendroid) (11). The multicellular non-glandular trichomes are simple or branched in *A. scaber* also. The branching pattern is biramous to pentaramous. The length of the trichomes of *A. scaber* is almost like recorded *Epibolium* species mentioned in the reports (31), but its nature varies.

The density of trichomes in tender leaves and mature leaves varies in *A. scaber*. Because of the presence of fully developed trichomes, mature leaves are most suitable for trichome studies (31). According to taxonomic reference, the leaves are scabrous-pubescent above and densely hoary tomentose below (1). It is because the nature and density of trichomes varies on both sides of lamina. Lower epidermis is with high density and a greater number of cells per trichome. Mature stem or old stem of the species is glabrous. Active trichomes are completely absent in this portion. Numerous scars of trichomes are observed in this region. The trichomes observed on the vegetative surface of *A. scaber* are in accordance with the analysis of angiosperm trichomes (5).

Scanning electron microscopic studies of the epidermis of 5 species of *Ballota* genus (*Ballota damascena* Boiss., *Ballota kaiseri* Täckh., *Ballota pseudodictamnus* (L.) Benth., *Ballota saxatilis* (Raf.) Guss. and *Ballota undulate* (Sieber ex Fresen.) Benth.) in the flora of Egypt revealed the presence of glandular and non-glandular trichomes. The characteristics of trichome morphology were helpful for the separation of *Ballota* species (6). As the same way, trichome characteristics can be considered as a tool for the distinction of confusing *Epilobium* species (31). The proportion of distribution may vary between species, different parts of a species and different positions of a plant part. The trichome characters show certain variations among the members of the family such as peltate glandular, capitate glandular, non-glandular and different versions and combinations of these (18).

The trichome nature has an important role in modern taxonomy, so it is used for the systematic analysis and phylogenetic revision of species in Lamiaceae (12, 17, 32, 33). Especially the type, distribution, morphology and density of glandular trichomes present (34). Stellate and dendritic trichomes were significant for *Ballota* species (6). So trichome analysis of several species were done like *P. ornatus* (23), *T. capitatum* (35), *Lavandula pinnata* Moench (36), *Orthosiphon labiatus* N.E.Br. (37), *Isodon rubescens* (Hemsl.) H.Hara (38), *Salvia sclarea* L. (39) *Salvia chrysophylla* Stapf (16), *S. vermifolia* Hedge & Hub.-Mor. (15), *S. aegyptiaca* L. (40) and *Ocimum Gratissimum* L. (41). This particular result of *A. scaber* can be used for systematics after obtaining a broad idea about the trichome micromorphology of other species in the genus such as *Anisochilus carnosus* (L.f.) Wall. ex Benth., *Anisochilus pallidus* Wall. ex Benth., *Anisochilus robustus* Hook.f., *Anisochilus wightti* Hook.f., etc.

The histological analysis of leaves of *R. officinalis* shows the presence of proteins and polysaccharide in capitate and peltate

types of glandular trichomes. But phenolic compounds only in peltate trichome (22). The glandular trichomes in the young leaves of rosemary are the site of synthesis of phenolic diterpenes (42). This is important group of secondary metabolites with significant pharmacological activity especially, antimicrobial and antioxidant properties. The essential oil produced from the secretory structures occurs in the plant parts of *P. cablin* comprised sesquiterpenes (43). Thus the glandular trichome of *A. scaber* may also help in the production, secretion and synthesis of secondary metabolites.

Trichome has a significant role in plant- environmental interaction (44). Non-glandular trichomes were considered as part of physiological adaptation against drought resistance, UV irradiation, high or low temperature stress etc (45). Trichomes makes physical resistance by making disturbances in locomotions (46), chemical resistance to phytophagous insects by the secretion of secondary metabolites and resist natural enemies of the plants (6). So, these structures help to reduce the destruction due to herbivory (47). But it is not true for all the trichome bearing plants. Trichomes of soyabean plants did not inhibit the foraging of fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (48). The trichomes present on the abaxial surface of the leaves were able to regulate the water quantity of plants in the coastal Mediterranean macchia ecosystem (49). In xerophytic plants of Lamiaceae trichomes help to reduce transpiration in different ways and the trichomes may have some role in xerophytic adaptations of *A. scaber* (50).

Conclusion

The leaf of *Anisochilus scaber* has diacytic stomata with comparatively lower stomatal index. The leaf and stem of the plant are covered with glandular and non-glandular trichomes, like several members of Lamiaceae. Its distribution pattern varies among different parts of the plant, different region of a part and the maturity of the plant part. The *Anisochilus* genus has close superficial similarity with *Plectranthus*, *Leocus* and *Coleus* (1). At the same time, the species within the genus are also difficult to distinguish because of the closeness in external morphology. So, the comparative trichome analysis of *Anisochilus* species will make the identification easy like *Epibolium*. In this study only the types of trichomes present in the vegetative parts of the species were done. Its floral parts also carry certain kinds of hairy structures. This includes only a preliminary morphological study. Based on these findings more detailed analysis of the trichomes can be done. The role of trichomes on the production of secondary metabolites can also be studied.

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Authors' contributions

FSS, VV and GGP carried out the identification of the plant species and microscopic studies. GMD, HJB and JCSK provided support in manuscript writing. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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