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ORIGINAL ARTICLE

Trichomes of *Coleus forskohlii*: Morphology and Volatile Compounds

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ABSTRACT

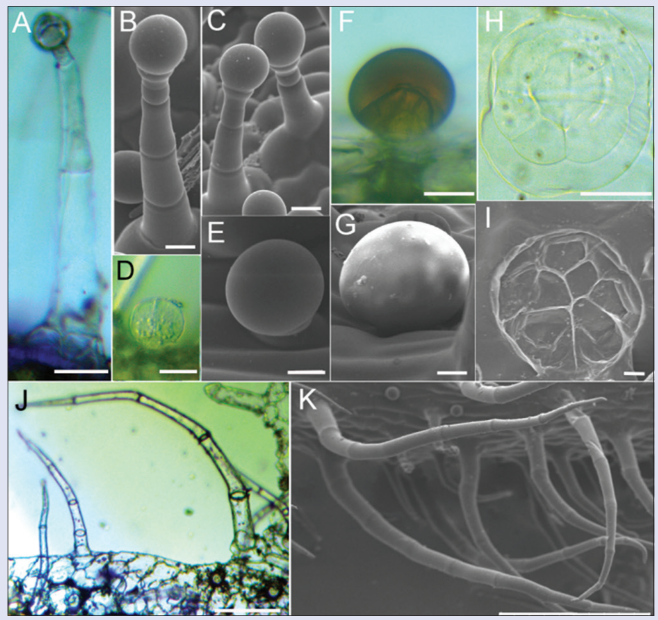
Background: *Coleus forskohlii* (*Lamiaceae*) cultivates imperishably over the tropical and subtropical regions of Asia; extracts from the roots and aerial parts of *C. forskohlii* are widely employed for the therapy of respiratory disorders, gastro-intestinal disturbance, heart diseases, and central nervous system disorders. The morphologies and structures of trichomes from *C. forskohlii*, as well as phytochemicals they secrete in China-origin plants are poorly unstated. **Objectives:** This study was intended to characterize the morphology and structure of trichomes and the profile of their secretory materials. **Materials and Methods:** To characterize the morphology and structure of trichomes, which happen on the vegetative and reproductive organs of *C. forskohlii*, it was employed light microscopy and scanning electron microscopy. The chemical qualities of volatile compounds from Glandular trichomes (GTs) of *C. forskohlii* were also studied using gas chromatography mass spectrometry (GC-MS) and histochemical reactions. **Results:** Four morphologically distinct trichomes, i.e., non GTs, peltate GTs, Type 1 and 2 capitate GTs, were categorized. Histochemical study naked the presence of polyphenols, flavonoids, lipids, and terpenes in the GTs. Essential oils, isolated from mature leaves by hydro-distillation, were investigated by GC-MS and 41 compounds were acknowledged with oxygen-containing sesquiterpenes and monoterpenes as the major compounds. **Conclusion:** Secretory materials of GTs attained by surface extraction also indicated the precise features in the China-origin samples.

Key words: *Coleus forskohlii,* essential oil, histochemistry, secretory material, trichomes

SUMMARY

- The aerial parts of *C. forskohlii* displayed 3 morphologically dissimilar GTs, i.e., short and long stalk CGT, PGT and NGT. Histochemical studies exposed the existence of polyphenols, flavonoids, lipids and terpenes in GTs secretion. Essential oils, isolated from trichomes, were investigated by GC-MS and 41 compounds were acknowledged.

Abbreviations used: CGT: Capitate glandular trichome; GC-MS: Gas chromatography mass spectrometry; GT: Glandular trichomes; LM: Light microscopy; NGT: Non-glandular trichomes; PGT: Peltate glandular trichome; SEM: Scanning electron microscopy.



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INTRODUCTION

Plant trichomes are epidermal appendages of the aerial parts with diverse morphologies and functions, which can be unicellular or multicellular and glandular trichomes (GT) or non-glandular trichomes (NGT).[1] NGTs are classically not able to secrete phytochemicals but distinction in shape, size, and structure, and they form defensive physical structures against herbivores, function as sinks for xenobiotics and control leaf temperature, water loss, and solar radiation.[2,3] GTs synthesize, store and occasionally secrete a

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varied set of plant secondary metabolites (e.g., terpenes, alkaloids, tannins, and fatty acid derivatives), which encompass in the plant variation to abiotic and biotic factors and serve as high-value natural products of attention as pharmaceuticals, flavors, fragrances, and pesticides.[4,5] In plants of *Lamiaceae* family, two main types of GT, i.e., peltate GT (PGTs) and capitate GT (CGTs), are considered morphologically by their head size and stalk length.[6]

Coleus forskohlii (*Lamiaceae*) cultivates perennially over the tropical and subtropical regions of Asia (such as India, Pakistan, South of Arabian Peninsula, and China), East Africa and Brazil with varied medicinal uses. Extracts from the roots and aerial parts of *C. forskohlii* are widely employed for the treatment of respiratory disorders, gastrointestinal disturbance, heart diseases, and central nervous system disorders.[7,8] The roots of the plant are the alone source of forskolin, a heterocyclic labdane-type diterpenes with exact adenylyl-cyclase activation properties resulting in a dramatic surge of the intracellular level of cAMP.[9] Root aqueous extracts of *C. forskohlii* are often employed to treat cough, asthma, hypertension, and common cold in the Indian, Chinese, and African folk medicine.[7] Hydroalcoholic extracts gained from the aerial parts of the plants were found to have hypotensive, hypoglycemic, and antispasmodic activity.[10] The leaves of *C. forskohlii* are used as an herbal tea for the treatment of digestive and nervous system illnesses in South America, Africa, and Asia.[11] The volatile component was shown to be the chief constituents in leaves.[12]

Essential oils from the leaves of *C. forskohlii* were formerly studied, and their chemical composition and biological activity were assessed.[10,13-15] The surface of *C. forskohlii* vegetative and reproductive organs is enclosed by non-glandular and GTs, as in utmost *Lamiaceae* species.[16-20] GTs are the chief structures to be accountable for synthesizing, accumulating and storing essential oils.[21]

Despite the morphologies of *Lamiaceae* trichomes have been widely considered, little is known about the morphologies and structures of trichomes from *C. forskohlii*, as well as phytochemicals they secrete in China-origin plants. This study intended to describe the morphology and structure of trichomes that happen on the vegetative and reproductive organs of *C. forskohlii* with light microscopy (LM) and scanning electron microscopy (SEM). The chemical qualities of volatile compounds from GTs of *C. forskohlii* were also examined using the gas chromatography mass spectrometry (GC-MS) and histochemical reactions.

MATERIALS AND METHODS

Plant materials

Mature seeds of *C. forskohlii* Briq. (Syn. *Plectranthus barbatus* Andr.) were collected from Huize, Yunnan Province of China, were propagated in pots and watered frequently. Fresh material (leaves, stems, and flowers) was assembled from seedling to full flowering stage (specimen number: 20160923019).

Morphological and anatomical investigation

For SEM, the samples were frozen in the liquid nitrogen and directly surface scanned in an adjustable pressure scanning electron microscope (Hitachi S-3400 N, Tokyo, Japan) with accelerated voltage of 15 kV.

Histochemical analyses were achieved on transverse, hand-cut sections of fresh material to perceive the presence of the main classes of compounds secreted by trichomes, using the following different histochemical tests: (a) Sudan III for total lipids,[22] (b) ferric trichloride for polyphenols,[23] (c) concentrated sulfuric acid for sesquiterpene,[24] (d) 2,4-dinitrophen ylhydrazine for terpenes,[25] and aluminum trichloride for flavonoids.[26-27] All observations were made with an Olympus microscope (BHS-RFK, Olympus Optical Co. Ltd., Tokyo, Japan).

Extraction and analysis of secretory materials from glandular trichomes

Secretory materials from GTs were extracted by a surface extraction method as described by Bisio *et al*.[28] and Robles-Zepeda *et al*.[29] with slight alterations. Briefly, fresh leaves (15 g) of *C. forskohlii* were submerged in 500 mL of dichloromethane for 5 s. Then, the residual moisture in the extracts was detached using anhydrous sodium sulfate. The solvent was then concentrated to 10 mL and kept in the dark at 4°C for further analysis.

GC-MS analysis was performed on Thermo ISQ QD-TRACE 1300 using a VF-5MS capillary column (5% phenyl/95% dimethyl polysiloxane, 30 m mm × 0.25 mm, 0.1 µm film thickness). The following oven temperature programs were employed: 1 min at 50°C, followed by a temperature increment rate of 20°C/min up to 100°C, held for 1 min, then at a rate of 15°C/min up to 125°C, a rate of 6°C/min up to 135°C, a rate of 4°C/min up to 150°C, a rate of 3°C/min up to 160°C, a rate of 1.5°C/min up to 175°C, a rate of 4.5°C/min up to 190°C, a rate of 1.6°C/min up to 200°C, increasing at 5.5°C/min up to 240°C, a rate of 2.5°C/min up to 260°C and a rate of 5.0°C/min up to 280°C, sustained for 2 min. The injection temperature was 260°C, respectively. Helium was employed as the carrier gas with a persistent flow rate of 1.5 mL/min. The split ratio was 1:100, and the acquisition mass range was 40–650 *m/z*. The mass spectra were developed in electron-impact mode with an ionization voltage of 70 eV. The volatile components were enumerated by the integration of total ion chromatograms and recognized by comparison with the mass spectra obtained from NIST library. Diesel oil (a mixture of C4-C30 n-alkanes corresponding to 400–3000 KI) used as a typical for the determination of retention indices.

RESULTS AND DISCUSSION

Distribution and morphology of trichomes

The aerial parts of *C. forskohlii* present an indumentum of abundant NGTs and GTs and four morphologically separate trichomes, i.e., NGT, PGT, Type 1 and 2 CGT, could be illustrious on the surface of different parts of seedling and mature plants [Figure 1]. PGT and type 2 CGT with saffron yellow exudates are understandable on the cotyledon and young stem [Figure 1a-c]. Young stem also keep prevailing NGTs [Figure 1c]. The trichomes on the adaxial and abaxial surfaces of euphylla were detected using SEM which clearly viewing mature [Figure 1d and e] and lots of developing trichomes [Figure 1f]. Young leaves were compactly covered with a large proportion of NGT either on the surface of the upper and lower epidermis [Figure 1g and h] and that lessened as the leaf matured [Figure 1j and k], as well as that on the stem [Figure 1i]. A large number of GTs also happens on the bracts, sepals, and petals [Figure 1l-n]. Commonly established trichomes on the surface of *Lamiaceae* plants are ordinarily eminent as glandular and non-glandular ones.[30] Certainly, several studies have discovered different trichome types in the representatives of genus *Coleus* or *Plectranthus* and species exhibit a variety of GTs and NGTs on different plant organs.[16-18,20,31] However, the glandular hairs of C. *forskohlii* still need to be studied systematically.

Two different types of CGT were detected using LM and SEM Type 1 CGT contains of one basal cell, 3–5 stalk cells and a unicellular secretory head with thin cuticle and a small subcuticular chamber [Figure 2a-c]. Type 2 CGT is collected by a basal cell, a short unicellular stalk, a round head of multicellular and a small subcuticular chamber could also be perceived [Figure 2d and e]. PGTs existing a basal cell, a short

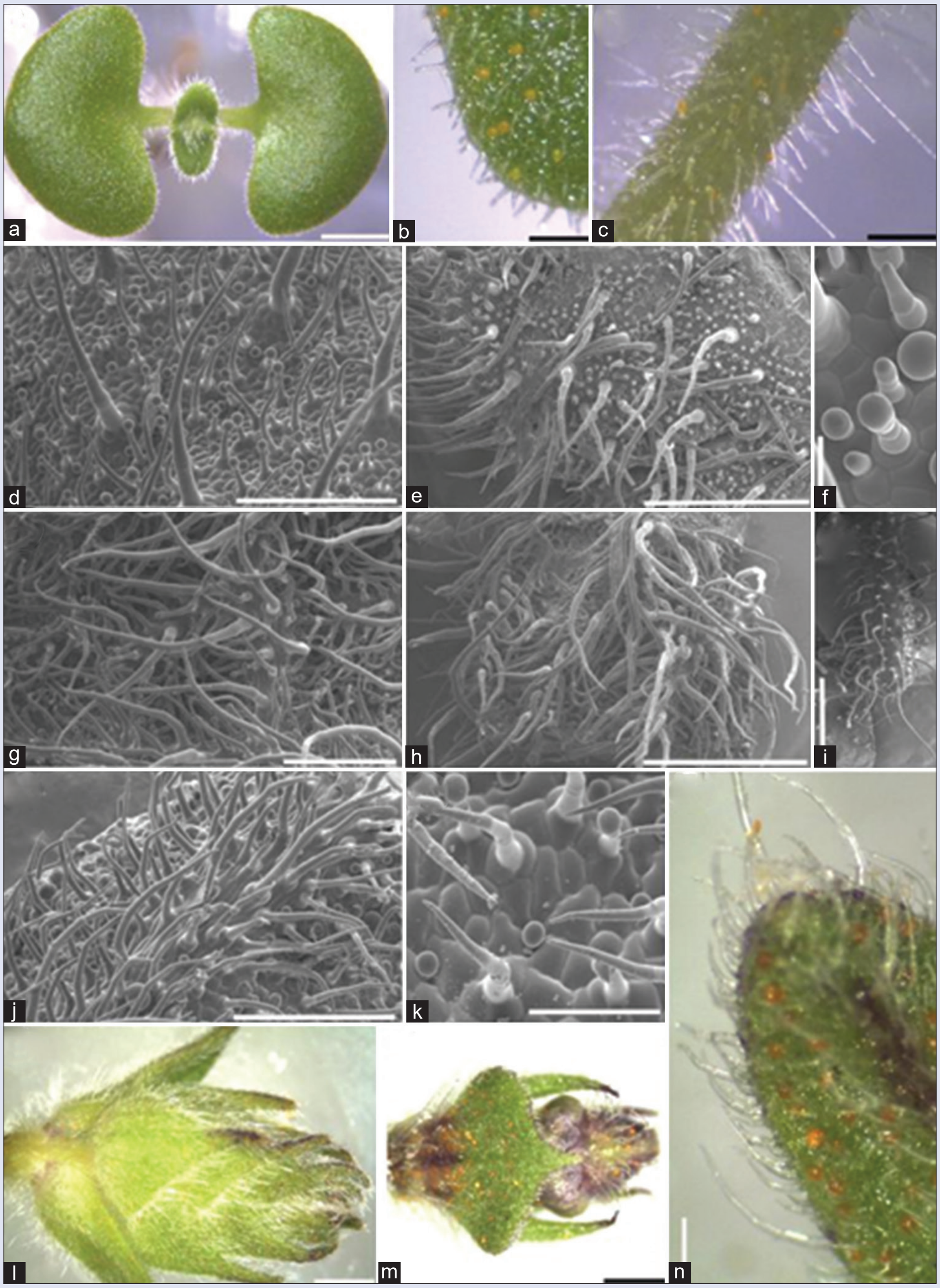

Figure 1: Trichomes distribution in *Coleus for skohlii*. Stereo-microscopic images (a-c and l, m) and scanning electron micrographs (d-k). (a and b) Cotyledon. (c) stem. (d-f) Upper (d) and lower (e) surface of tender leaf with developing trichomes (f). (g and h) Upper (g) and lower (h) surface of young leaf. (i) Stem surface. (j and k) Upper (j) and lower (k) surface of mature leaf. (l-n) Flower about to bloom (l), perianth (m), and bracts (n). Scale bars = 20µm (b), 30µm (c), 100µm (a, n and f), 200µm (k and l), 500µm (d, g, j and m), 2mm (e), 3mm (h), 4mm (i)

unicellular stalk and a secretory head of 6–8 cells arranging in a circle [Figure 2f-i]. The secretory head of PGT has a thick cuticle which lifts to form a large subcuticular chamber for accumulating secretory materials [Figure 2f and g]. The NGTs of *C. forskohlii* are multicellular, unbranched and uniseriate, with dissimilar articulation between cells [Figure 2j and k]. CGTs and PGTs are viewed as the most mutual glandular trichome of *Lamiaceae* family.[17,30,32] Performed histochemical localization of forskolin in the leaves and glandular trichome of *C. forskohlii,* but other substances were not emphasis on the trichome. Three types of GT, i.e., short and long stalk CGT and PGT and one type of NGT were defined in *C. blumei* and *Plectranthus madagascariensis*, which is comparable to our results in *C. forskohlii*[16,20,31] presented CGT and NGT of *C. amboinicus*[18] and stated 5 types of GT (PGT, short-and long-stalked CGTs, digitiform trichomes and conoidal trichomes), as well as 2 types of NGT in *Plectranthus ornatus*.[18] Therefore, the hairy structure in this study appears to be a communal hairy body in plants, whereas further studies are still desirable to assess their physiological functions and likely roles of systematic phylogeny at genus or sub-familial level.

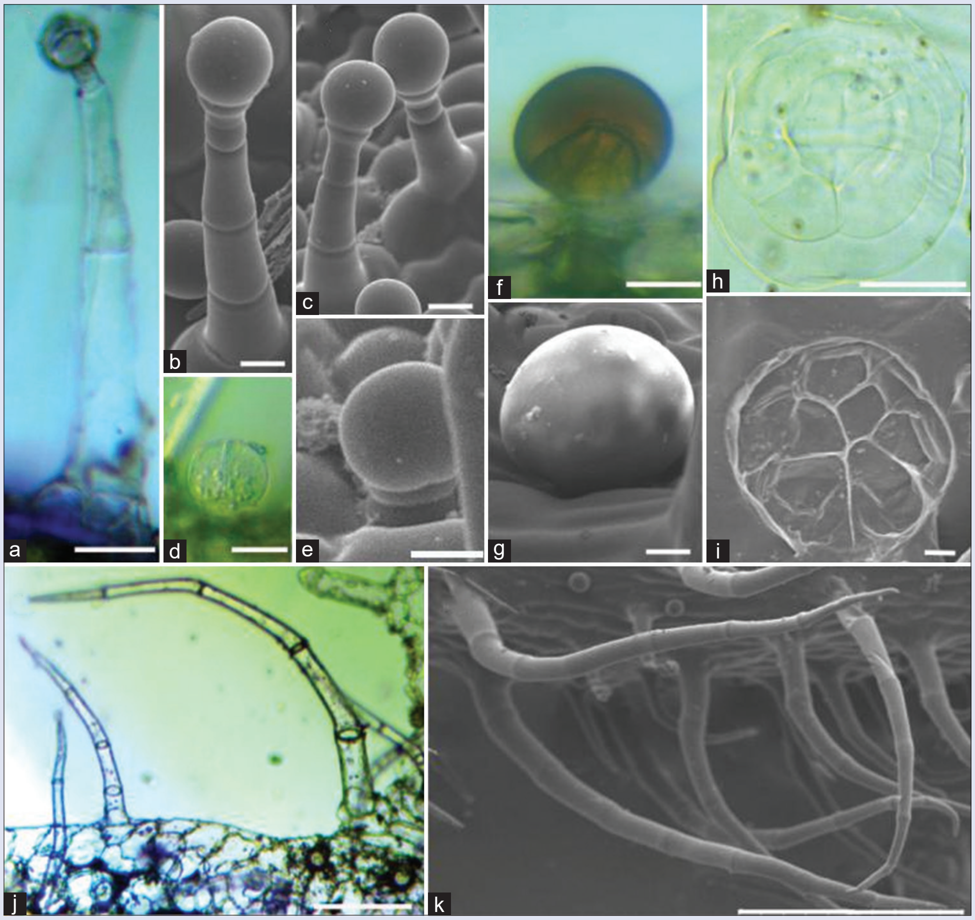

Figure 2: Types of trichomes of *Coleus forskohlii*. Light micrographs (a, d, f, h and j) and scanning electron micrographs (b, c, e, g, i and k). Type 1 Capitate glandular trichomes (a-c). Type 2 Capitate glandular trichomes (d and e). Peltate glandular trichomes (f-i). non-glandular trichomes (j and k). Scale bars = 15µm (a, d, e, i), 10µm (b, c), 50µm (f, h), 30µm (g), 400µm (k), 200µm (j)

Histochemistry of the secretory materials

The secreting material of type 1 CGTs showed positive to all the histochemical dyes employed in this study, representing the presence of polyphenols, flavonoids, lipids, and terpenes [Figure 3a, c, d, f, h, j]. The secretion of type 2 CGTs stained positive with ferric trichloride, aluminum trichloride, Sudan III, concentrated sulfuric acid, and 2,4-dinitrophenylhydrazine demonstrating the production of polyphenols, flavonoids, lipids, and terpenes [Figure 3b, c, e, g, j]. In PGTs, Sudan III and 2,4-dinitrophenylhydrazine staining gave positive reactions, demonstrating the secretion of lipids and terpenes [Figure 3e, i]. The staining intensity of the histochemistry is recorded in Table 1.

The secreting material of GTs is a complex mixture collected of terpenoids, lipids, polyphenols, etc.[32] It has been stated that PGTs is the key source of the essential oil produced in *Lamiaceae*.[17,32] Our results propose that terpenoids are produced not only by PGTs but also by CGTs, as other reports in *Plectranthus* genus.[18,33] Also terpenoids, CGTs also secret lipids, flavonoids, and phenols, as conveyed in other *Lamiaceae* plants, such as *Plectranthus grandidentatus*, *Stachys tymphaea,* and *Hyssopus officinalis*.[18,34,35]

Analysis of secretory materials from glandular trichomes

The surface extraction was engaged to get the secretory materials of GTs using dichloromethane as solvent. In dichloromethane extraction sample, 41 compounds were recognized using GC-MS with aliphatic compounds viewing dominance [Table 2]. The major contributors of GTs were sesquiterpenoids (58.76%), diterpenes (6.66%), monoterpenoids (1.22%), and others (18.83%), with 6-isocedrol (28.91%), Bornyl acetate (10.56%), and Caryophyllene (7.59%) being the most illustrative constituents of these fractions, respectively. The substances in the glandular hairs have diverse functions, as a functional bicyclic monoterpene, endo-borneol has been mightily employed as the chemical and active ingredients in skin creams, wound care, and biologic preservatives.[36] In addition, bornyl acetate has revealed antioxidant and anti-inflammatory effects in different types of cells and tissues.[37] In fact, Caryophyllene, extracted from *Aquilaria crassna*, disclosed anticancer, antioxidant and antimicrobial properties,

Table 1: Histochemical test performed on glandular trichomes of *Coleus forskohlii*

Staining procedure	Target compounds	Type I CGTs	Type II CGTs	PGTs
Ferric chloride	Polyphenols	+	+	−
Aluminum trichloride	Flavonoids	++	+	−
Sudan III	Total lipids	+	++	++
Concentrated sulfuric acid	Sesquiterpenes	++	−	++
2, 4-dinitrophenylhydrazine	Terpenes	±	++	+

The symbols −, ±, +, and++indicate negative, weak, intense, and very intense results, respectively. PGTs: Peltate glandular trichomes; CGTs: Capitate glandular trichomes

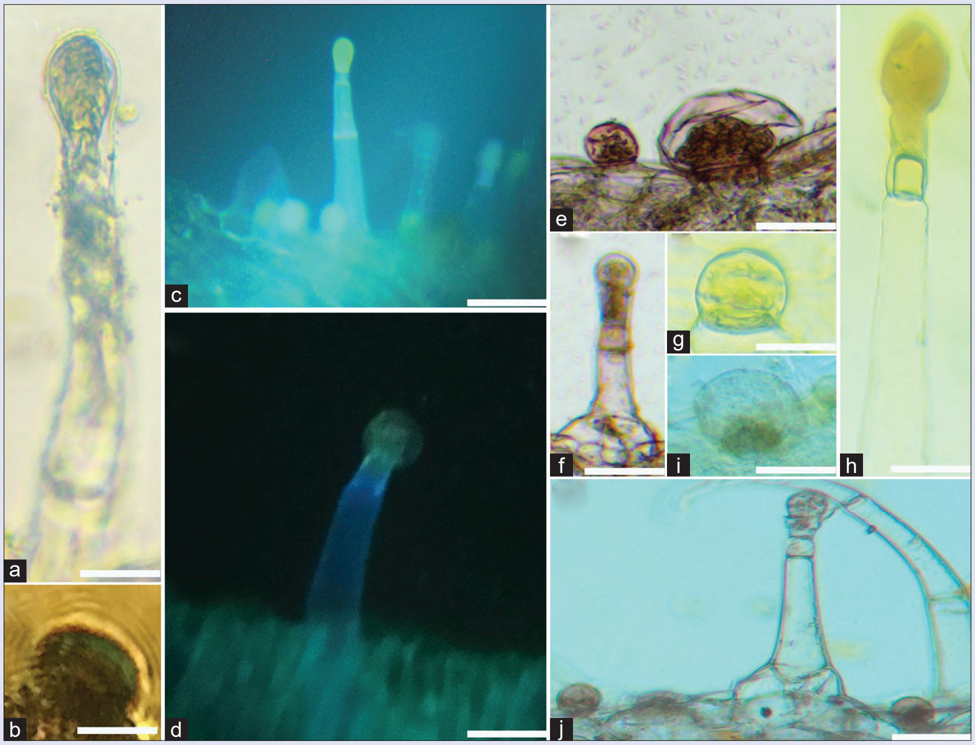

Figure 3: Histochemistry of trichomes of *Coleus forskohlii*. Ferric chloride (a and b), aluminum trichloride (c and d), Sudan III (e and f), concentrated sulfuric acid (g and h), and 2.4-dinitrophenylhydrazine (i and j) staining showed secreted material stained bright green, week green, pink-red, yellow, and light black, respectively. Scale bars = 25µm (a, g and h), 10µm (b), 100µm (c and i), 50µm (d, e and j)

Table 2: Composition of secretory materials in glandular trichomes of *Coleus forskohlii* isolated by dichloromethane surface extraction

Compound name	Retention index	Molecular formula	Relative content (%)
Monoterpenoids			
Camphene	942	$C_{10}H_{16}$	0.39
β-Pinene	961	$C_{10}H_{16}$	0.25
2-Decen-1-ol	1220	$C_{10}H_{20}O$	0.58
Sesquiterpenoids			
(+)-a-Longipinene	1296	$C_{15}H_{24}$	0.53
Naphthalene, 1,2,3,4,4a, 5,6,7-octahydro-1,4a-dimethyl-7-(1-methylethenyl)-, (1S,4aR,7R)-	1344	$C_{15}H_{24}$	3.57
(-)-α-Santalene	1351	$C_{15}H_{24}$	0.34
α-Cedrene	1367	$C_{15}H_{24}$	2.96
Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-penten-1-yl)-	1382	$C_{15}H_{24}$	0.52
Caryophyllene	1398	$C_{15}H_{24}$	7.59
(E)-β-Famesene	1412	$C_{15}H_{24}$	0.31
Prezizaene	1452	$C_{15}H_{24}$	0.77
1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z, Z, Z-	1486	$C_{15}H_{24}$	0.34
2-Hepten-1-ol, 2-methyl-6-(4-methyl-1,4-cyclohexadien-1-yl)-, (2Z,6R)-	1501	$C_{15}H_{24}O$	1.49
β-Bisabolene	1508	$C_{15}H_{24}$	1.42
Germacrene B	1519	$C_{15}H_{24}$	1.28
3,9-Guaiadiene	1523	$C_{15}H_{24}$	0.42
Sesquisabinen	1527	$C_{15}H_{24}$	3.15
β-Maaliene	1532	$C_{15}H_{24}$	1.45
γ-Maaliene	1535	$C_{15}H_{24}$	0.27
Caryophyllene oxide	1590	$C_{15}H_{24}O$	0.48
6-Isocedrol	1617	$C_{15}H_{26}O$	28.91
Humulene oxide II	1615	$C_{15}H_{24}O$	0.54
δ-acorenol	1623	$C_{15}H_{26}O$	0.26
Juniper camphor	1632	$C_{15}H_{26}O$	0.50
8,9-Epoxyacorenon-B	1607	$C_{15}H_{24}O_2$	1.66

Contd...

Table 2: Contd...

Compound name	Retention index	Molecular formula	Relative content (%)
Diterpenes			
Phenanthrene, 1,2,3,4,4a, 9,10,10a-octahydro-1,1,4a-trimethyl-7-(1-methylethyl)-	2187	$C_{20}H_{30}$	4.14
(+)-Manoyl oxide	2227	$C_{20}H_{34}O$	0.90
Ferruginol	2218	$C_{20}H_{30}O$	1.62
Others			
3,6-Heptanedione	892	$C_7H_{12}O_2$	0.25
2-Butenedioic acid	749	$C_5H_6O_4$	0.33
Bornyl acetate	1286	$C_{12}H_{20}O_2$	10.56
Tetrahydroionone	1307	$C_{13}H_{24}O$	0.53
4,8,13-Cyclotetradecatriene-1,3-diol	2302	$C_{20}H_{34}O_2$	2.66
Eicosane, 2-methyl-	2305	$C_{21}H_{44}$	0.63
Nonadecane, 2-methyl-	3015	$C_{21}H_{44}$	0.80
Pregnanetriol	3052	$C_{21}H_{36}O_3$	0.41
7-hexylicosane	3064	$C_{26}H_{54}$	0.42
Benz[e]azulene-3,8-dione, 5-[(acetyloxy) methyl]-3a, 4,6a, 7,9,10,10a, 10b-octahydro-3a, 10a-dihydroxy-2,10-dimethyl-,(3aà,6aà,10á,10aá,10bá)-(+)-	3160	$C_{19}H_{24}O_6$	0.42
Tricyclo[20.8.0.0(7,16)]triacontane	3232	$C_{30}H_{52}O_2$	0.70
Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	3315	$C_{27}H_{42}O_4$	0.56
Tetratriacontane	3431	$C_{34}H_{70}$	0.56

against human pathogenic bacterial and fungal strains.[38] Furthermore, α-cedrene, a material with natural insecticidal and antibacterial biological activity, used to deliver a combination drug with the effects of promoting wound healing, astringency, and antipruritic and for treating trauma.[39]

Surface extraction is commonly used for the studies of the chemical composition of GTs and leaf surface to diminish the levels of interfering substances.[28,29,40] Little reports were found in surface extraction and consequently analysis of their components in GTs of *C. forskohlii*. Our results recommended a small amount of aliphatic compounds secreted by GTs of *C. forskohlii,* including Tetratriacontane and 2-Methyleicosane which are hardly detected before.[41]

CONCLUSION

The aerial parts of *C. forskohlii* displayed 3 morphologically dissimilar GTs, i.e., short and long stalk CGT, PGT and one type of uniseriate multicellular unbranched NGT, which may be viewed as collective and universal trichome morphology of plants in genus *Coleus*. Histochemical studies exposed the existence of polyphenols, flavonoids, lipids and terpenes in GTs secretion. Oxygen-containing sesquiterpenes and monoterpenes and aliphatic compounds presented dominance in essential oil from leaves of *C. forskohlii*. Sesquiterpenoids are the key components of GTs secreted substances, which may contribute to the biological and pharmacological activities of *C. forskohlii*. The data attained in this study may deliver a morphological and chemo taxonomical tactic for taxa delimitation of genus *Coleus*, respectively, and the discrete compounds also shown precise features of the China-origin samples.[41]

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Conflicts of interest

There are no conflicts of interest.

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