Australian Journal of Crop Science AJCS
AJCS 7(6):744-749 (2013) ISSN:1835-2707

Morphology of trichomes in *Pogostemon cablin* Benth. (Lamiaceae)

Amalia Rusydi[1, 4], Noraini Talip[1*], Jalifah Latip[2], Ruzi Abdul Rahman[1], and Idris Sharif[3]

[1]School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
[2]School of Chemical Science and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
[3]Scanning Electron Microscopy Unit, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
[4]Biology Department, Mathematics and Science Faculty, Syiah Kuala University, 23111 Darussalam, Banda Aceh, Indonesia

***Corresponding author: norainitalip@gmail.com**

Abstract

Pogostemon cablin is also known as patchouli or nilam. This species is an aromatic plant which has economic importance due to its essential oil which is widely used as raw material for the perfume industry. This study was conducted in order to determine the types, anatomy and micromorphological features of the trichomes (glandular and non-glandular) on the patchouli leaf lamina. The findings of this study showed that there were eight distinct types of trichome (two non-glandular and six glandular trichomes). The non-glandular trichomes are simple unicellular and multicellular. The glandular trichomes are short-stalked capitate, long-stalked capitate, peltate, digitiform, clavate filiform and fusiform.

Keywords: Capitate trichomes, Glandular trichomes, Lamiaceae, Non-glandular trichomes, Peltate trichomes, *Pogostemon cablin* Benth.
Abbreviations: LM-Light Microscope, SEM-Scanning Electron Microscope .

Introduction

Pogostemon cablin Benth. (Lamiaceae), commonly known as patchouli, is one of the medicinal herbal plants that produces essential oils. Patchouli is mainly distributed in Indonesia, Malaysia, China, Brazil and India (Mahanta et al., 2007). The essential oils are produced by the glandular trichomes, and may act to protect the aerial parts of the plant against herbivores and pathogens (Werker, 1993), and the biological activity is of interest to the pesticide, pharmaceutical, flavor and fragrance industries (Duke, 1994). For several decades the essential oils have been used in food industries and as fixatives in raw materials for the perfume industry. The essential oils have therapeutic properties, namely anti-depressant, anti-inflammatory, antiseptic, aphrodisiac, astringent, carminatives, diuretic, febrifuge, insecticides, fungicides, sedatives and tonic (Bunrathep et al., 2006). The production of essential oils in the plants is generally associated with the presence of specialized structures such as glandular trichomes. Generally, the aerial organs of the plant are often covered by trichomes, and the morphology of the trichome structures can vary greatly within species. Trichomes are defined as unicellular or multicellular appendages, which originate from the epidermal cells and develop outwards on the surface of various plant organs (Werker, 2000). In several genera of Lamiaceae, the leaf bears non-glandular and glandular secreting trichomes. The non-glandular trichomes are diverse in morphology, anatomy and microstructure, and serve the plants and humans in many ways. The glandular trichomes vary in the chemical composition of the substances they secrete, in their structure, location and function (Werker, 2000). The structure and function of the glandular trichomes occurring in the plants of the family Lamiaceae are well documented (Werker et al., 1985; Fahn, 1988, 2000; Werker et al., 1993; Ascensao et al., 1999; Bagherpour et al., 2010; Kahraman et al., 2010) and they are recognized as the site of essential oil biosynthesis, secretion and accumulation (Croteau and Johnson, 1984). Some variation exists among the genera in Lamiaceae as to the types of trichome (peltate, capitate, non-glandular and the different versions and combinations of these) that occur in a given species (Venkatachalam et al., 1984). The chemical composition of patchouli oil is almost entirely comprised of sesquiterpenes with patchouli alcohol (Bunrathep et al., 2006) and the secretory structures of that plant are responsible for the production of the sesquiterpenes, and many studies on the content of the essential oils have been reported (Maeda and Miyake, 1997). Although many phytochemical works have been carried out, studies on the secretory structures are scarce and only a limited number of species have been studied, concentrating on the leaves. There is only a small number of publications dealing with secretory structures, especially the types of trichome present in this species, such as previous works by Maida and Miyake (1997). Several related studies have been carried out on other Lamiaceae species, such as *Rosmarinus officinalis* (Marin et al., 2006), *Plectrantus ornatus* (Ascensao et al., 1999), *Leonotis leonurus* (Ascensao and Pais, 1998), *Teucrium capitatum* (Antunes et al., 2004), *Salvia sclarea* (Schmiderer et al., 2008), *Lavandula pinnata* (Huang et al., 2008), *Salvia*

vermifolia (Bagherpour et al., 2010) and *Salvia chrysophylla* (Kahraman et al., 2010). The objective of this study is to investigate the type, distribution and morphological features of the trichomes (secretory structures) using light microscopy (LM) and scanning electron microscopy (SEM).

Results

This paper presents the detailed morphological features of leaf trichomes (non-glandular and glandular) in *P. cablin* Benth. The results of LM and SEM analysis indicate that there are eight types of trichome on the leaves of *P. cablin* Benth. It is postulated that the presence of trichomes are positively correlated with the accumulation of essential oils in the leaf lamina of *P. cablin*. Observations under LM and SEM revealed that both the adaxial and abaxial leaf epidermis were characterized by non-glandular and glandular trichomes (Figure 1A-B). There are two types of non-glandular trichome: simple unicellular and simple multicellular. The former is composed of one large basal cell and one elongated cell (Figure 1C-E) and has a smooth surface, whereas the latter consists of two to five cells. This type of trichome has a group of four to six epidermal cells that are arranged in a circle around the basal part of the trichomes (Figure 1F-G). The surface ornamentation of the trichome is echinate or adorned with micropapillae (Figure 1H). The trichomes are found densely on the adaxial side of the epidermal surface, on the midribs and along the secondary and tertiary veins. There are six types of glandular trichome, i.e. short-stalked capitate, long-stalked capitate, peltate, digitiform, clavate filiform and fusiform. Capitate glandular trichomes can be classified into two types based on their stalk size and the morphology of their glandular head. Type I, which is the short-stalked capitate trichome, consists of one basal cell, a short stalk cell and a unicellular or bicellular glandular head (Figure 1I-J). Type II, which is the long-stalked capitate trichome, has one basal cell, a unicellular or multicellular (2-3 cells) stalk that varies in length, a short neck cell and a unicellular head (Figure 2A-C). The short-stalked capitate trichomes are more abundant and dense compared to the long-stalked capitate trichomes, which are found only on the midrib of the leaves (Figure 2C). The peltate trichomes consist of one or two basal epidermal cells, with a very short stalk and a large spherical head (Figure 2D-E). The head of a glandular trichome has either a smooth or a wrinkled surface (Fig. 2E), while the basal cell and stalk cell disappear within the epidermal cells. Peltate trichomes are more densely located on the abaxial surface (Figure 1A), and are more numerous than capitate trichomes on both the adaxial and abaxial surfaces of the leaf epidermis. The morphological structure of capitate and peltate glandular trichomes found in this present study is similar to those found in the leaf lamina of *Plectrantus ornatus* (Ascensao et al., 1999). A digitiform glandular trichome comprises of one elongated oblong basal cell attached to two or three cells in a line that are more-or-less equivalent in length and diameter, with no distinction between the stalk cell and the head cell (Figure 3A-B). A clavate filiform glandular trichome consists of one basal cell, three to five stalk cells (multicellular stalk) with distinctive length and one head cell (rounded tip). These trichomes are present along the veins, especially on the abaxial side of the leaf epidermis (Figure 3C). A fusiform glandular trichome is composed of one basal cell, one thin stalk cell and three layers of head cells. This type of trichome has a swollen part in the middle and a tapering end or spindle-like tip, and is narrowly ellipsoid (Figure 3D).

Discussion

The aerial surfaces of almost all of the aromatic plants belonging to the Lamiaceae family examined are covered with trichomes, including non-glandular trichomes and glandular or secretory trichomes (Werker et al., 1985; Werker, 1993). Basically, the non-glandular trichomes are classified according to their morphology. They may be unicellular or multicellular, and both types can be unbranched and branched (Werker, 2000). In this present study, two types of non-glandular trichome were identified, namely as simple unicellular and simple multicellular. The simple unicellular non-glandular trichomes, all called prinkle hairs (Metcalfe, 1960), have smooth cell wall surfaces. These trichomes develop from single protoderm initials without any divisions (Ramayya, 1972). In contrast the wall surfaces of the simple multicellular non-glandular trichomes are covered with cuticular micro-papillae. In non-glandular trichomes, the cuticle may acquire variable thickness. The outer surface of the trichomes can be smooth or may exhibit micro-ornamentation, such as micro-papillae, warty, reticulate, seriate, etc (Werker, 2000). According to Bathlott (1981), the cuticular micro-papillae are a continuation of the cuticular folding, present on the surface of the surrounding epidermal cells. Within the Lamiaceae family, different species can have different types, distribution, morphology, and density of glandular trichomes, which could be of important taxonomic value (El-Gazzar and Watson, 1970), such as having both peltate and capitate trichomes, or with only either peltate or capitate trichomes, or, more rarely, having neither (Huang et al., 2008). In this study, six distinct glandular trichomes, i.e. short-stalked capitate, long-stalked capitate, peltate, digitiform, clavate filiform and fusiform glandular trichomes were found. According to Fahn (1988), high diversity exists in the morphology of glandular trichomes in the organ, and at the cellular and subcellular levels, which can be unicellular or multicellular, uniseriate or multiseriate, and variously shaped (Werker, 2000). However, to date, there has been no comprehensive report on the diversity (types) of trichomes in *P. cablin*. Maeda and Miyake (1997) noted that external glandular trichomes were present in large numbers on both the adaxial and abaxial epidermis of patchouli leaves and the trichomes project above the leaf surface, but no specific type of trichomes were reported. The glandular trichomes are known to be the primary sites of secondary metabolite biosynthesis, secretion and storage, and generally consist of either simple subcutaneous glands or of trichomes (Weiss, 1997). Much evidence has revealed that glandular trichomes are the storage site of terpenoids in some species of Lamiaceae (Kelsey et al., 1984). Henderson et al. (1970) also reported on the correlations between the number of external glandular trichomes and the sesquiterpenoid content of patchouli (*P. cablin*) leaf sections. Two types of capitate trichomes are found based on their morphological features, namely short-stalked and long-stalked capitate trichomes. The differences between these trichomes are in the stalk length, the neck cell and the shape of the glandular head. The result is consistent with the findings of Werker et al. (1985) who noted that capitate trichomes are very variable in stalk length, glandular head shape and secretions, and can be classified into various types. The short-stalked capitate trichomes found on the patchouli leaf in this study are similar to those reported by Maeda and Miyake (1997). The glandular trichomes of *P. cablin* found in both studies are project above the leaf surface and each consists of a secretory head, a stalk and basal cells. The capitate trichomes with those features are also similar to those observed in several

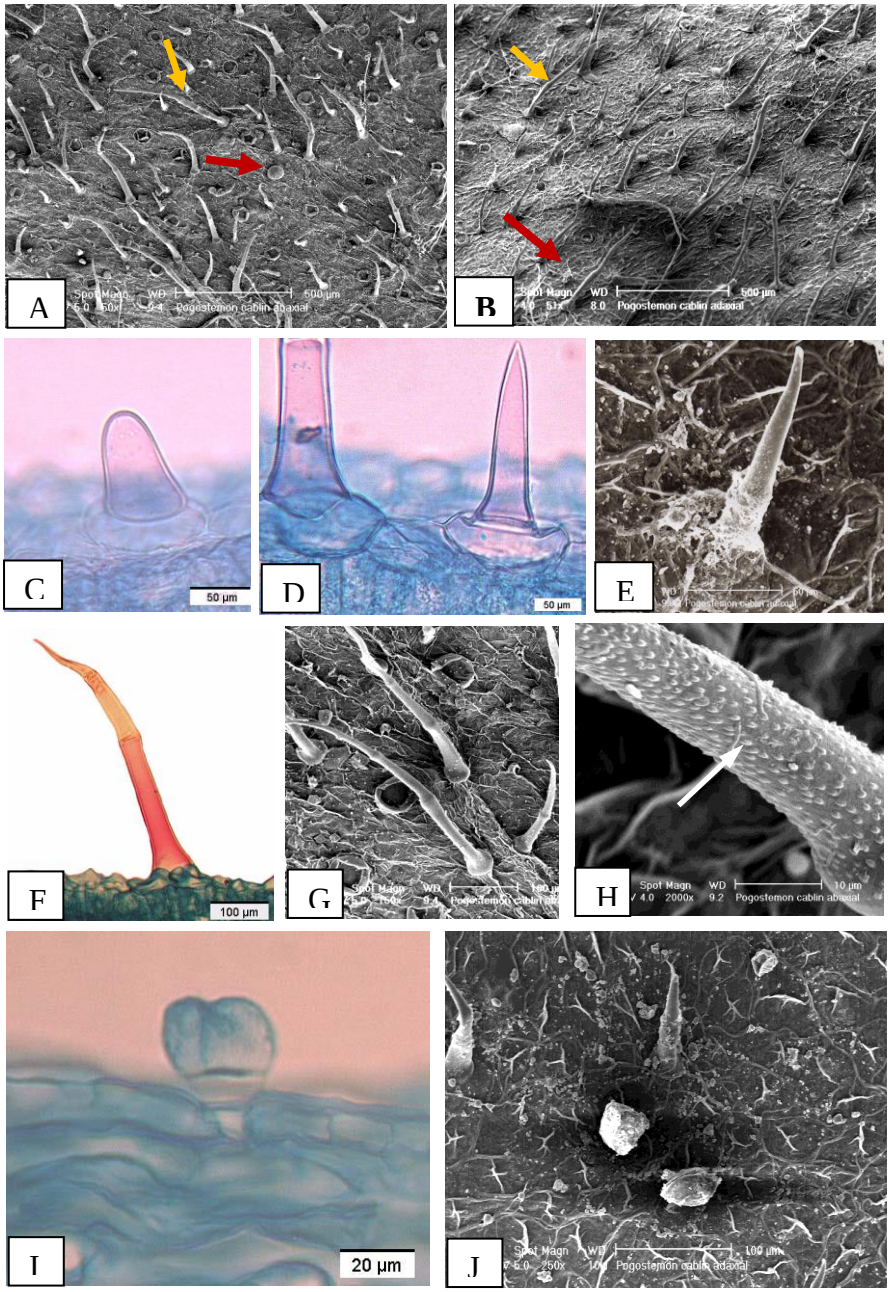

Fig 1. SEM micrographs of non-glandular trichome (yellow arrow) and glandular trichome (red arrow) in abaxial (A) and adaxial (B) leaf surface. LM and SEM micrographs of simple unicellular non-glandular trichomes (C-E), simple multicellular non-glandular trichomes (F-G), SEM micrograph of echinate ornamentation or micropapillae (white arrow) on the surface of a simple multicellular non-glandular trichome (H), short-stalked capitate trichomes (I-J).

several studies on *Plectranthus ornatus* (Ascensao et al., 1999), *Leonotis leonorus* (Ascensao and Pais, 1998), *Salvia blepharophylla* (Bisio et al., 1999) and *Salvia aurea* L. (Serrato-Valenti et al., 1997). The short-capitate trichomes are the commonest type of capitate trichome found in Lamiaceae, and these types have globoid to obovoid uni- or bicellular glandular heads (Ascensao et al., 1999). In the present study, we also found differences in the basal cells of the short-stalked capitate trichomes between the adaxial and abaxial leaf epidermal surfaces. On the adaxial surface, the basal cell is bigger than the stalk cell, while on the abaxial surface the basal cell is smaller than the stalk cell. The long-stalked capitate trichomes consist of one basal cell, one or two elongated stalk cells, a neck cell and a single pear-shaped glandular head cell. These trichomes are sparsely distributed compared to the short-stalked capitate trichomes which are normally found more densely on the midrib of the abaxial surface of the leaf. These trichomes can be classified into two types based on the number of stalk cells where the unicellular long-stalked capitate trichome has one elongated stalk cell (Figure 2A) while the multicellular long-stalked capitate trichome has two or three elongated stalk cells (Figure 2B-C), the former being more abundant than the latter. According to Ascensao et al. (1999), capitate trichomes may differ in terms of their morphological characteristics, reflecting the different secretory processes, and would probably have distinctive functions. Peltate trichomes are very frequently found on both the abaxial and adaxial leaf surfaces of *P. cablin*. These trichomes consist of a basal cell embedded in between the epidermal cells, one short unicellular stalk cell and a large

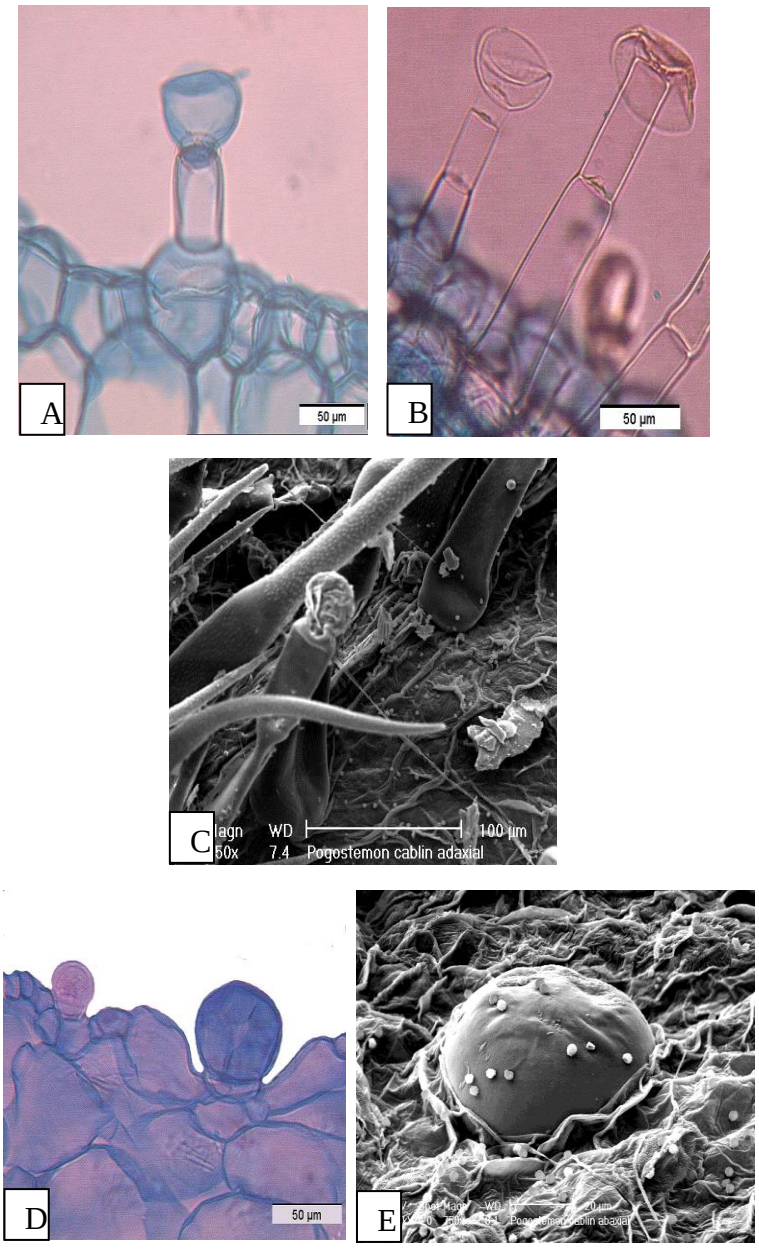

Fig 2. LM and SEM micrographs of long-stalked capitate trichomes with unicellular stalked cell (A) and multicellular stalked cell (B-C), and peltate trichomes (D-E).

head cell. These features are also found in many species of Lamiaceae such as in *Ocimum basilicum* (Werker et al., 1993), *Salvia aurea* (Serrato-Valenti et al., 1997), *Salvia officinalis* (Corsi and Bottega, 1999), *Salvia blepharophylla* (Bisio et al., 1999), and *Lavandula pinnata* (Huang et al., 2008), *Salvia vermifolia* (Bagherpour et al., 2010) and *Salvia chrysophylla* (Kahraman et al., 2010). In many of the species of the Lamiaceae examined, the broad head of the peltate trichomes usually consisted of four to twelve cells (Werker et al., 1993; Serrato-valenti et al., 1997; Bisio et al., 1999; Corsi and Bottega, 1999; Turner et al., 2000; Huang et al., 2008; Bagherpour et al., 2010; Kahraman et al., 2010), or many secretory cells arranged in one layer in a broad head (Fahn, 1988). The peltate trichomes produce most of the essential oils, i.e. terpenes (Clark et al., 1997; Turner et al., 2000), and patchouli alcohol, which is the main component found in this plant. In the trichomes, secreted oils are accumulated in a large subcuticular space formed by distension of the cuticle and part of the cell wall surmounting the secretory cells (Werker et al., 1985; Werker, 1993). The peltate trichomes are the most abundant type of trichomes present in *P. cablin*. The digitiform glandular trichomes, each of which consists of a big basal cell, one or two stalk cells and an apical secretory cell, are fewer than the capitate and peltate glandular trichomes, and can be found on both the abaxial and adaxial leaf surfaces. Likewise, the scarcely distributed clavate filiform (rounded tip/multicellular stalk) glandular trichomes occur only on the midribs of the leaves. All these trichomes are similar to those reported by Ramayya (1972). The clavate filiform trichomes have a uni- or multicellular

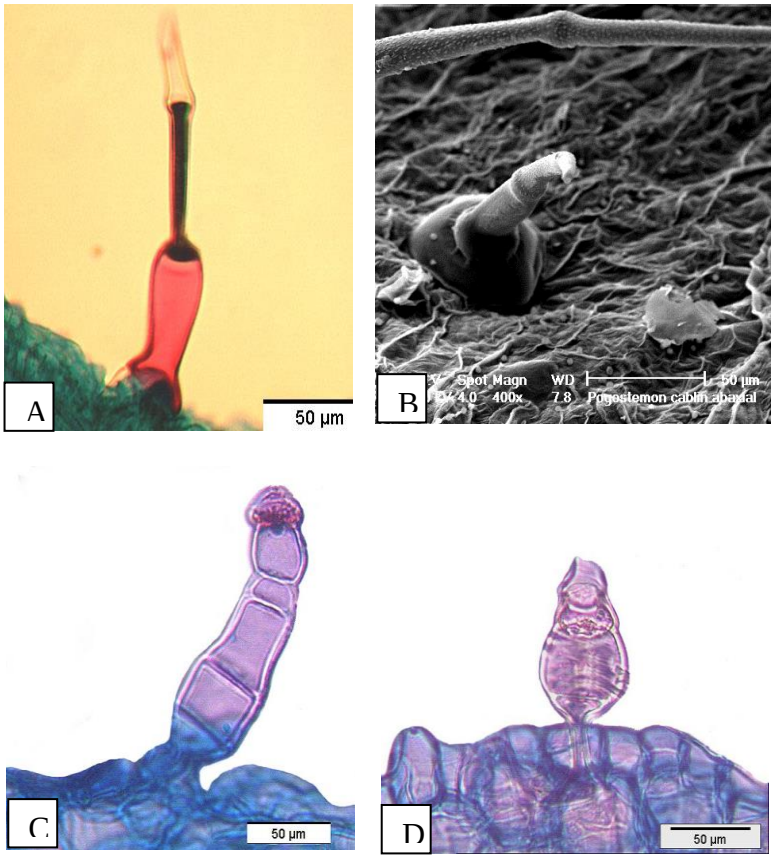

Fig 3. LM and SEM micrographs of digitiform glandular trichomes (A-B). LM of clavate filiform glandular trichomes (C), and fusiform glandular trichomes (D).

basal cell, usually somewhat projecting above the epidermis, stalk cells that are uniseriate, cylindrical, broad to elongated, with a unicellular rounded head, while the fusiform glandular trichomes are sparsely distributed on the midrib. Previous studies on Lamiaceae did not report these types of trichome which have swollen parts in the middle, a tapering or spindle-like end or tip, are narrowly ellipsoid, and have no clear secretory head cell.

Materials and methods

Plant material

The leaf specimens used in this study were collected from the natural population in two locations, namely Nicah and Blang Malau in the Pidie district of Aceh, Indonesia. Fresh mature leaves were harvested at five or six nodes from the plant apex. Three individuals from each location were examined for the anatomical study. The voucher specimens were then deposited in the Herbarium of Universiti Kebangsaan Malaysia (UKMB), Bangi, Malaysia.

Light microscopy

Leaf specimens were fixed in Acetic acid solution (1:3, Acetic acid: 70% alcohol). Fixation, embedding and sectioning were performed following the methods described by Johansen (1940) and Sass (1958) with suitable modifications. The sectioning was done using a sliding microtome, and sections were stained in Safranin and Alcian Blue and then dehydrated in an alcohol series of 50 %, 70 %, 95 %, and 100 %. Slides were mounted in Euparal, then sections of the specimen were photographed using a video camera (JVC) attached to a Leica Diaphlan microscope and images were processed using Analysis Docu Software.

Scanning Electron Microscopy

The 1 cm^2 dried leaf sections were mounted on stubs using double sided adhesive tapes. All stubs were coated for 2 to 3 minutes with gold palladium. The specimens were then examined in the field emission of a scanning electron microscope model Phillips XL 30.

Acknowledgements

We would like to thank Prof. Dr. Maimon Abdullah for her constructive comments of this manuscript, and we would also like to express our gratitude to Prof. Dr. Idris Ghani, the head of the OUP- biodiversity of terrestrial research grant for supporting this research (Grant: UKM-OUP-2012-043).

References

Antunes T, Sevinato-Pinto I, Barroso JG, Cavaleiro C, Salgueiro LR (2004)
Micromorphology of trichomes and composition of essential oil of *Teicrium capitatum*. Flavour Fragr J 19: 336-340.
Ascensao L, Pais MS (1998) The leaf capitates trichomes of *Leonotis leonorus*: histochemistry, ultrastructure and secretion. Ann Bot-London 81: 263-271.
Ascensao L, Mota L, Castro MD (1999) Glandular trichomes on the leaves and flowers of *Plectranthus ornatus*:

morphology, distribution and histochemistry. Ann Bot-London 84: 437-447.
Bagherpour S, Kahraman A, Doğan M, Celep F, Başer B, Pehlivan S (2010) The anatomical and micromorphological characteristics of *Salvia vermifolia* (Sect. *Aethiopis* Bentham, Lamiaceae) from central Anatolia, Turkey. Cent Eur J Biol 5(6): 872-879.
Barthlott, W (1981) Epidermal and seed surface characters in plants: systematic applicability and some evolutionary aspects. Nord J Bot 1: 345-355.
Bisio A, Corallo A, Gastaldo P, Romussi G, Ciarallo G, Fontana N, De Tommasi N, Profumo P (1999) Glandular hairs and secreted material in *Salvia blepharophylla* Brandegee ex Epling grown in Italy. Ann Bot-London 83: 441-452.
Bunrathep S, Lockwood GB, Songsak T, Ruangrungsi N (2006) Chemical constituents from leaves and cell cultures of *Pogostemon cablin* and use of precursor feeding to improve patchouli alcohol level. J Sci Asia 32:293-296.
Clark LJ, Hamilton JGC, Chapman JV, Rhodes MJC, Hallahan DL (1997) Analysis of monoterpenoids in glandular trichomes of catmint *Nepeta racemosa*. Plant J 11(6):1387-1393.
Corsi G, Bottega S (1999) Glandular hairs of *Salvia officinalis*: New data on morphology, localization and histochemistry in relation to function. Ann Bot-London 84: 657-664.
Croteau R, Johnson MA (1984) Biosynthesis of terpenoid in glandular trichomes. In: Rodriguez E, Healey PL, Mehta I (eds) Biology and Chemistry of Plant Trichomes. Plenum Press, New York.
Duke SO (1994) Glandular trichomes – A focal point of chemical and structural interactions. Int J Plant Sci 155(6): 617-620.
El-Gazzar A, Watson IA (1970) Taxonomic study of Labiatae and related genera. New Phytol 69: 451-486.
Fahn A (1988) Secretory tissues in vascular plants. New Phytol 108: 229-257.
Fahn A (2000) Structure and function of secretory cells. In: Hallahan DL and Gray JC (eds), Advances in Botanical Research. Academic Press, London. Vol 31: 37-75.
Henderson W, Hart JW, How P, Judge J (1970) Chemical and morphological studies on sites of sesquiterpene accumulation in *Pogostemon cablin* (Patchouli). Phytochemistry 9(6): 1219-1223.
Huang SS, Kirchoff BK, Liao JP (2008) The capitate and peltate glandular trichomes of *Lavandula pinnata* L. (Lamiaceae): histochemistry, ultrastructure, and secretion. J Torrey Bot Soc 135: 155-167.
Johansen DA (1940) Plant Microtechnique. McGraw-Hill Book Company, New York.
Kahraman A, Celep F, Dogan M (2010) Anatomy, trichome morphology and palynology of *Salvia chrysophylla* Stapf (Lamiaceae). S Afr J Bot 76: 187-195.
Kelsey RG, Reynolds GW, Rodriguez E (1984) The chemistry of biologically active constituents stored in plant glandular trichomes. In: Rodriguez E, Healey PL, Mehta I (eds) Biology and Chemistry of Plant Trichomes. Plenum Press, New York.
Maeda E, Miyake H (1997) Leaf anatomy of patchouli (*Pogostemon patchouli*) with reference to the disposition of mesophyll glands. Japan J Crop Sci 66(2): 307-317.
Mahanta JJ, Chutia M, Sarma TC (2007) Study on weed flora and their influence on patchouli (*Pogostemon cablin* Benth.) oil and patchouli. J Plant Sci 2(1): 96-101.
Marin M, Koko V, Duletic-Lausevic S, Marin PD, Rancic D, Dajic-Stevanovic Z (2006) Glandular trichomes on the leaves of *Rosmarinus officinalis*: morphology, stereology and histochemistry. S Afr J Bot 72: 378-382.
Metcalfe CR (1960) Anatomy of the Monocotyledons: 1. Gramineae. Clarendon Press, Oxford. UK.
Ramayya N (1972) Classification and phylogeny of the trichomes of angiosperms. In: Ghouse AKM and Yunus M (eds) Research Trends in Plant Anatomy. Tata McGraw-Hill Publishing Company Ltd, New Delhi.
Sass JE (1958) Botanical Microtechnique. 3rd ed. Iowa State University Press, Iowa USA.
Schmiderer C, Grassi P, Novak J, Weber M, Franz (2008) Diversity of essential oil glands of clary sage (*Salvia sclarea* L., Lamiaceae). Plant Biol 10: 433-440.
Serrato-Valenti G, Bisio A, Cornara L, Ciarallo G (1997) Structural and histochemical investigation of the glandular trichomes of *Salvia aurea* L. leaves, and chemical analysis of the essential oil. Ann Bot-London 79: 329-336.
Turner GW, Gershenzon J, Croteau R (2000) Development of peltate glandular trichomes of peppermint. Plant Physiol 124:665-680.
Venkatachalam KV, Kjonaas R, Croteau R (1984) Development and essential oil content of secretory glands of sage (*Salvia officinalis*). Plant Physiol 76: 148-150.
Weiss EA (1997) Essential Oil Crops. CAB International. New York.
Werker E, Ravid U, Putievsky E (1985) Structure of glandular hairs and identification of main components of their secreted material in some species of the Labiatae. Israel J Bot 34: 31-45.
Werker E (1993) Function of essential oil-secreting glandular hairs in aromatic plants of the Lamiaceae - a Review. Flavour Frag J 8: 249-255.
Werker E, Putievsky E, Ravid U, Dudai N, Katzir I (1993) Glandular hairs and essential oil in developing leaves of *Ocimum basilicum* L. (Lamiaceae). Ann Bot-London 71: 43-50.
Werker E (2000) Trichome diversity and development. In: Hallahan DL and Gray JC (eds), Advances in Botanical Research. Academic Press, London. Vol 31: 1-35.