

The Secretary Structure of Essential Oils In Some Species Of Lamiaceae For Traditional Cosmetics of Besemah Tribes Lahat's District

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ABSTRACT

Besemah tribe uses plants in addition to the treatment is also used for beauty treatments traditionally. Based on Agustina's (2015) study, 109 species of plants were used for traditional medicine by the Tribe of Besemah. Some species of Lamiaceae for traditional medicine such as patchouli (*Pogostemon cablin* (Blanco) Benth.), wild mint (*Mentha arvensis* L.), hoary basil (*Ocimum americanum* L.), mexican mint (*Plectranthus amboinicus* (Lour.) Spreng.), and sweet basil (*Ocimum basilicum* L.) contains essential oils that can be used for traditional cosmetic ingredients. Essential oils are stored in the secretory structure of plants in leaf and stem organs. This study aims to determine the type and location of the secretory structure of essential oils in some species of lamiaceae used for traditional cosmetics of Besemah Tribes Lahat's District. The research was conducted in November 2016 until February 2017. The results showed that patchouli plants was found in the presence of glandular trichomes, oil cells, and idioblast cells. The wild mint plants have glandular trichomes. The hoary basil plants have glandular trichomes and oil cells. The mexican mint plants have glandular trichomes and idioblast cells. The sweet basil plants have glandular trichomes.

Keywords: essential oils, lamiaceae, traditional cosmetics, Besemah tribes, glandular trichomes, oil cells, idioblast cells

INTRODUCTION

The world's biodiversity besides Brazil and Tanzania. From Sabang to Merauke scattered about 40,000 species of plants (Agusta, 2000). Essential oil-producing plant covers about 200 species, 40 species of which are found in Indonesia. The types of essential oils that have been produced and circulated in the world market currently reaches 70 - 80 kinds, 15 of which are from Indonesia. Essential oils can be used as fragrance or soap and perfume or perfume products (Wibowo, 2012).

The Encyclopedia of Chemical Technology states that essential oils are compounds, which are generally fluid, obtained from parts of plants, roots, skins, stems, leaves, fruits and seeds or from flowers by steam distillation. Not all types of plants produce essential oils, only plants that have glanula cells alone can produce essential oils (Bangkit et al., 2012). One of the people who have local wisdom in the form of utilization of plants in the activity of life is the Tribe Besemah.

Besemah tribe is a tribe that occupies the area Pagaralam Lahat regency of South Sumatra. The tribe is known to use many herbs for traditional medicine. Based on research that has been done by Agustina (2015), obtained 109 types of plants used as medicine by Besemah tribe and some of them including Lamiaceae species that produce essential oils that can also be used for beauty treatments such as patchouli, wild mint, hoary basil, mexican mint, and sweet basil.

This study examines the type and distribution of secretory structures contains essential oils on several types of Lamiaceae which are used as traditional cosmetics tribe Besemah Lahat regency. The result of the research is expected to explain about secretory structures containing essential oils in plant organs, so as to provide insightful knowledge of the essential oil-producing plants of Lamiaceae as well as the basis for further research.

MATERIALS AND METHODOLOGY

This research was conducted from November to February 2017. The sample was taken at Bantunan and Sukabumi Village, Pajar Bulan Sub-district, Lahat District, South Sumatera. Preparation of vegetative preparations of plant vegetative organ is done in Mikroteknik Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Inderalaya.

The ingredients used are alcohols with different concentrations (100% alcohol, 96% alcohol, 70% alcohol, 50% alcohol, 30% alcohol), aquades, balsam canada, 4% formalin, Haupt adhesive (gelatin, phenol, glycerin), solution (paraxin, Na-hypochlorite, leaf and stem organs from patchouli, wild mint, hoary basil, mexican mint, and sweet basil, paraffin 48, paraffin 58, safranin-fastgreen dye solution, TBA, and xylol.

Field observations and interviews

Field observation was conducted by looking directly at the research location. The collection of information is done by interviewing local community or traditional healers expert on some kind of Lamiaceae are useful addition to the treatment can also be used for beauty treatments. From the interviews that have been conducted, showed that 5 kind used Lamiaceae Besemah Tribe communities for traditional cosmetics (Table 3.1).

Table 3.1. Types of Lamiaceae essential oil for traditional cosmetics used by the Tribe of Besemah people

Num	The name of the plant		Usability	Parts used
	Local name / common name	Scientific name		
1.	Patchouli	<i>Pogostemon cablin</i>	As perfume and deodorant	Leaf
2.	Taghok mim/wild mint	<i>Mentha arvensis</i>	Smooth skin and remove acne scars	Leaf
3.	Hoary basil	<i>Ocimum americanum</i>	Deodorant and bad breath (halitosis)	Leaf
4.	Mexicant mint	<i>Plectranthus amboinicus</i>	Breastfeeding	Leaf
5.	Sweet basil	<i>Ocimum basilicum</i>	Deodorant	Leaf

Preparation of research samples

Plant patchouli, wild mint, hoary basil, mexican mint, and sweet basil collected parts of the organ leaves and stems. Leaves taken are still young while the stem and petiole taken only at the shoot. Leaf organs and plants are soaked in FAA solution (*Formaldehyde Acetic-acid Alcohol*) to avoid damage and can be used for further processing.

The making of preparations by paraffin method (Sass, 1958)

Patchouli, wild mint, hoary basil, mexican mint, and sweet basil (leaves and stems) are collected and cut 3 x 3 mm, then fixed with a minimum of 24 hours FAA (*Formaldehyde Acetic-acid Alcohol*) solution. Next in aspiration for 1 hour with 3-5 repetitions for 5 days. Samples were included in 50% alcohol solution for 1 hour with 2 repetitions, Johansen I for 2 hours, Johansen II + safranin 24 hours, Johansen III, IV, and V for 2 hours, TBA 3 x for 2 hours, and TBA: oil paraffin (1: 1) for 1 hour. Continue with infiltration with paraffin 48 at oven temperature 48⁰C for 2 hours 3 x repetition, and paraffin 58 at 58⁰C for 2 hours 3 x repetition.

Embedding in paraffin 58 on paper box then cut and pasted on wooden block. Then in rotate with rotary microtom with a thickness of 10 - 15 µm and in the paste on the glass object that has been in tethesion of haupt adhesive and 4% formalin solution. Preparations in color with safranin – fastgreen staining method, spilled canada balsam, and covered with cover glass.

The making of preparations by whole mount method (Sass, 1958)

Samples were collected and slashed with a razor as thin as possible, after which the sample was fixed with a FAA fixative solution (Formaldehyde Acetic-acid Alcohol). Then washed by fixative solution was removed and replaced with aquades, and was done by soaking in Na-hypochlorite for 3 minutes then washed with aquades and then stained by the incision colored with safranin 1% for 3 minutes. Samples are placed on the glass object. The final closure was performed by means of an incision given 10% glycerol, then covered with a cover glass and observed under a microscope.

RESULTS AND DISCUSSION

Observations showed the secretory structure of essential oils in 5 species of Lamiaceae in the form of glandular trichomes, oil cells, and idioblast cells (Table 2). Glandular trichomes are found in 5 plant species (patchouli, wild mint, hoary basil, mexican mint, and sweet basil). Oil cells are found in *Pogostemon cablin* (patchouli) and *Ocimum americanum* (hoary basil), and idioblast cells are found in *Pogostemon cablin* and *Plectranthus amboinicus* (mexicant mint).

Glandular trichomes are found to consist of 2 types, namely trichomes of capitate gland and peltat spread all over the surface of leaf blade, petiole, and stem of plant. Both types of trichomes are distinguished by their structure and secretory cells. Glandular trichomes are generally formed by basal cells, stem cells, and head cells. The head cells of the capitate gland tricoma consist of several sizes and shapes, including short, long, unicellular, or multicellular. However, a capitate gland trichome composed of basal cells, short stem cells with 1-2 head cells is the most commonly encountered trichome in Lamiaceae plants.

Trichomes of the peltate gland consist of basal cells, 1 stalk cells, and head cells consisting of 4-18 cells. This type of trichome has a head cell sheathing that develops over the head cells in its mature phase (Aprilia, 2016). Glandular trichomes are external secretory structures whereas oil cells and idioblast cells are part of the internal secretory structure.

Table 4.1. The type and location of the secretory structure of essential oils in some species of Lamiaceae

Num	The name of the plant	Part of observation	Secretory structure
1.	<i>Pogostemon cablin</i>	Leaf	Glandular trichomes, oil cells, and idioblast cells
		Stem	Glandular trichomes and oil cells
2.	<i>Mentha arvensis</i>	Leaf	Glandular trichomes
		Stem	Glandular trichomes
3.	<i>Ocimum americanum</i>	Leaf	Glandular trichomes and oil cells
		Stem	Glandular trichomes
4.	<i>Plectranthus amboinicus</i>	Leaf	Glandular trichomes and idioblast cells
		Stem	Glandular trichomes and idioblast cells
5.	<i>Ocimum basilicum</i>	Leaf	Glandular trichomes
		Stem	Glandular trichomes

The secretory structure of essential oils on patchouli plants

Leaf

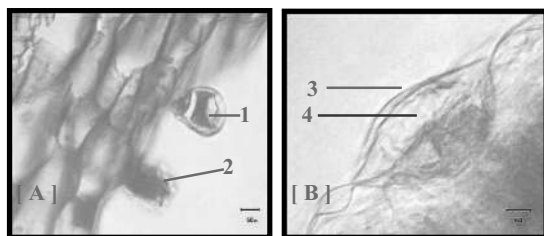


Figure 1. Glandular trichomes in cross section of patchouli leaves with paraffin (A: 40 x 10 magnification) and whole mount method (C: magnification 40 x 10)

Description:

- 1 – 2 : Capitata glandular trichomes
- 3 : Peltate glandular trichomes
- 4 : SCS (*Sub-cuticular space*)

Based on figure 1. Glandular trichomes found in patchouli leaves there are 2 types of capitata trichomes and peltate trichomes. The tricoma of the capitata gland has a basal cell, stalk cell, and 1 round head cell with a smooth surface (Fig. A (1)), or irregular wrinkles (Fig. A (2)). Trichomes of the peltate gland have basal cells, stalk cells, and a large head composed by several secretory cells (Fig. B).

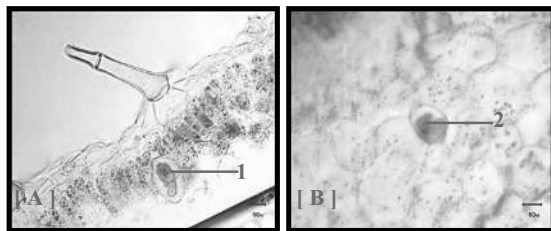


Figure 2. Oil cell and idioblast cells on cross section of patchouli leaves with whole mount method (A: magnification 10 x 10; B: magnification 40 x 10)

Description:

- 1 : Oil cells in palisade tissue
- 2 : Idioblast cells in spongy tissue

Based on figure 2. In addition to trichoma, essential oil of patchouli leaves is also produced by oil cells that can be found on parenchyma palisade (Fig. A) and idioblast cells in the sponge parenchyma (Fig. B). This idioblast cell is brownish yellow and contains oil. According to Guo *et al.* (2013), the internal glands of patchouli leaves with a length of 40 μm (± 9) spread among the palisade cells. A mature internal gland with basal cells, narrow stalk cells, and a large cytoplasmic cell head that is often filled with oil secretions.

Stem

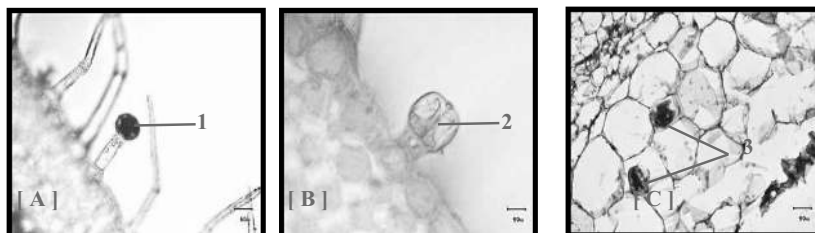


Figure 3. Glandular trichomes and oil cells in cross section patchouli stem with whole mount (A - B: magnification 40 x 10) and paraffin method (C: magnification 40 x 10)

Description:

- 1 - 2 : Capitate glandular trichomes
- 3 : Oil cells in cortical tissue

Based on figure 3. Trichomes of the glands in the stem are the type of capitate. Trichomes of the capitate gland have basal cells, longer stalk cells, and 1 round head cell (Fig. A) and tricoma of the capitate gland with basal cells, shorter stem cells, and 1 round head cell (Fig. B). Tricoma of the peltate gland is not found in the stem and the number of trichomes of the larger capitic gland in the leaves. In the area of the stem cortex also found an oil cell suspected to contain essential oils (Fig. C).

The secretory structure of essential oils on wild mint plants

Leaf

Based on figure 4. Capitate and peltate glandular trichomes are found in the upper epidermis and under epidermis (Fig.A). Trichomes of the peltate gland are made up of basal cells, do not have stalk cells, with rounded head cells (Fig. B). While capitate glandular trichomes has basal cell, stalk cell, and 1 round cell head (Fig. C). According to Turner *et al.* (2000), the biosynthetic pathway of monoterpene compounds in *Mentha piperita* has been

located specifically on the secretory cells of glandular trichoma located on the outer surface of the leaf. Three types of trichomes are present in the leaves: non glandular, multicellular, simple trichomes; small, trichoma capitate gland with 1 secretory head cells, and pelvic gland trichoma with secretory cells having 8 apical cells.

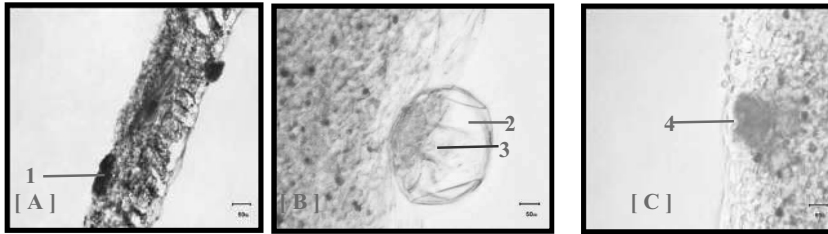


Figure 4. The location and type of glandular trichomes in a cross section of wild mint leaves with paraffin method (A: magnification 10 x 10) and whole mount (B-C: magnification 40 x 10)

Description:

- 1 : Glandular trichomes
- 2 : Peltate glandular trichome
- 3 : *SCS (Sub-cuticular space)*
- 4 : Capitate glandular trichomes

Stem

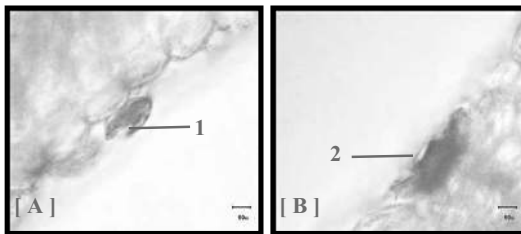


Figure 5. Glandular trichomes in cross section patchouli stem with whole mount method (A - B: magnification 40 x 10)

Description:

- 1 – 2 : Capitate glandular trichomes

Based on Figure 5. Trichomes of the capitate gland in the stem have basal cells, stem cells, and 1 head cells of different shapes (Fig. A-B).

The secretory structure of essential oils on hoary basil plants

Leaf

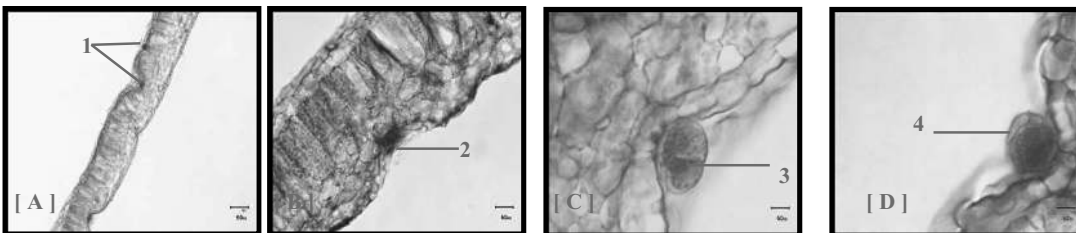


Figure 6. The location and type of glandular trichomes on cross-section of hoary basil leaves by paraffin method (A: magnification 4 x 10; B: magnification 10 x 10; C-D: magnification 40 x 10)

Description:

- 1 : Glandular trichomes
- 2 : Peltate glandular trichome
- 3 - 4 : Capitate glandular trichomes

Based on figure 6. Capitate glandular trichomes are present in the upper and lower epidermis of the leaves (Fig. A) whereas the peltate glandular trichomes is found only in the

lower epidermis of the leaf (Fig. B). Trichomes of the peltate gland have basal cells, stem cells, and a large head composed of several secretory cells (Fig. B). The tricoma of the capitate gland has a basal cell, a stem cell, with 2 heads (Fig. C) and 1 round head (Fig. D).

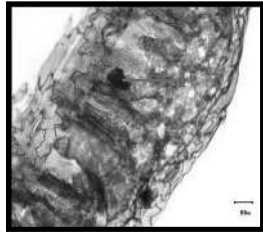


Figure 7. Oil cells on cross section of hoary basil leaves by paraffin method (magnification 10 x 10); 1: Oil cells, 2: Palisade tissue

Based on figure 7. Hoary basil leaf besides having glandular trichomes also have oil cells that can produce essential oils. The oil cell is present in the palisade tissue cells with a blackish brown.

Stem

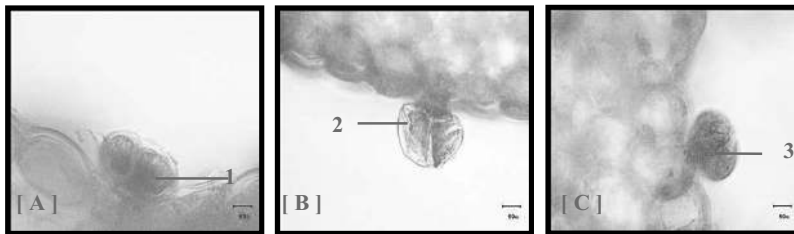


Figure 8. Glandular trichomes of cross section of hoary basil stem with whole mount method (magnification 40 x 10)

Description:

1 - 3 : Capitate glandular trichomes

Based on figure 8. In stem there is a capitate gland trichomes composed of basal cells and stalk cells with 2 round cells (Fig. A), or slightly oval (Fig. B), and 1 round head cell (Fig. C). These glandular trichomes are commonly found in the 4 corners of the stem epidermal layer. Basil stems also produce essential oils due to the trichomes of this gland, but the number is less than in the leaf.

The secretory structure of essential oils on mexicant mint plants

Leaf

Based on figure 9. Glandular trichomes found on the surface of mexicant mint leaves is a type of capitate. Trichomes of the capitate gland may be found in the upper and lower epidermis of the leaves. The capitate trichomes have basal cells, longer stalk cells, with 1 oval-shaped head cells (Fig. A), round (Fig. B), and oval (Fig. C). Capitate trichomes having basal cell, short stalk cell with 1 round head cell (Fig. D-E), or narrow head neck with blunt end (Fig. F). According to Sharma *et al.* (2014), this plant is covered by glandular and non-glandular trichomes. Glandular trichomes are type of capitate trichomes consisting of an epidermal basal cell, a unicellular or biselular stalk of varying length, having a neck cell, and a large, unicellular round head secretion.

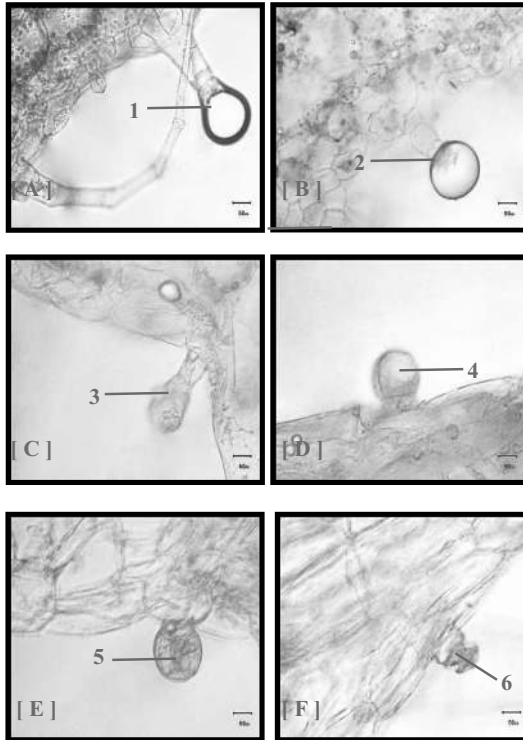


Figure 9. Glandular trichomes in cross section of mexicant mint leaves with whole mount method (magnification 40 x 10)

Description:

1 - 6 : Capitata glandular trichomes

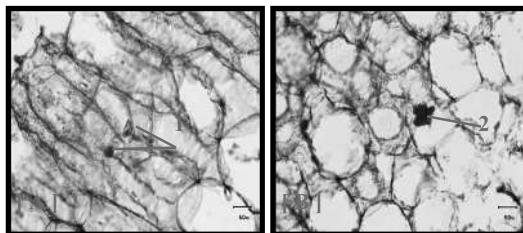


Figure 10. Idioblast cells on cross section of mexicant mint leaves with paraffin method (A - B: magnification 40 x 10)

Description:

1 : Idioblast cells in palisade tissue

2 : Idioblast cells in spongy tissue

Based on figure 10. on the cross section of mexicant mint leaves found the idioblast cell. This idioblast cell is found in palisade tissue cells (Fig. A) and spongy tissue (Fig. B). This idioblast cell is thought to contain essential oils. According to Novitasari (2015), idioblast cells are a cell of different sizes, shapes, and contents of the contents of other cells within a network. The idioblast cell size varies in the same organ, as well as between the different organs, but the size variation is low.

Stem

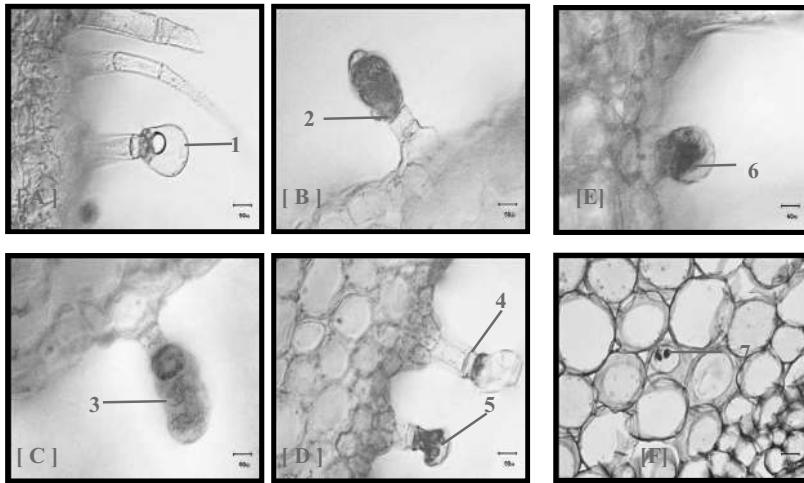


Figure 11. Glandular trichomes and idioblast cells of cross section of mexicant mint stem with whole mount (A – E: magnification 40 x 10) and paraffin method (F: magnification 40 x 10)

Description:

- 1 - 6 : Capitate glandular trichomes
- 7 : Idioblast cells

Based on figure 11. Trichomes of the capitate glands found on the stem are similar to those in the leaves. The trichomes have basal cells, longer stem cells, with oval head shape cells (Fig. A), oval (Fig. B), very oval (Fig. C), or slightly oval (Fig. D (4)). Trichomes with basal cells, shorter stem cells, and narrow head cells with a dull end (Fig. D (5)) or 1 round head cell (Fig. E). The idioblast cells are also found in stems that can be found among the cortical cells (Fig. F). This idioblast cell is thought to contain essential oils.

The secretory structure of essential oils on sweet basil plants

Leaf

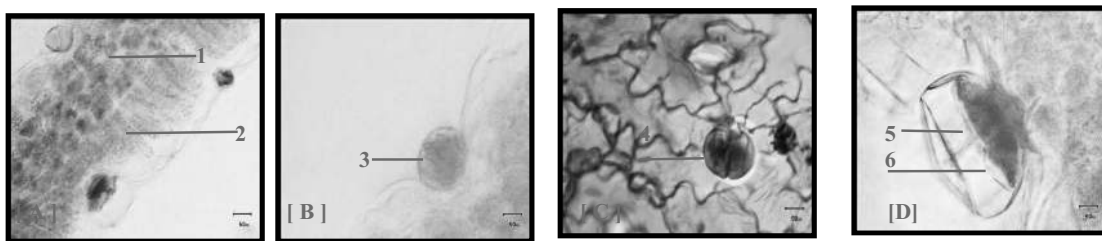


Figure 12. The location and type of glandular trichomes at cross section (A, B, D) and paradermal (C) sweet basil leaves by whole mount (A: magnification: 10 x 10; B, D: magnification 40 x 10) and paraffin method (C: 40 x 10 magnification)

Description:

- 1 : Spongy tissue
- 2 : Palisade tissue
- 3-4 : Capitate glandular trichomes
- 5 : Peltate glandular trichomes
- 6 : SCS (*sub-cuticular space*)

Based on figure 12. Capitate glandular trichomes may be found in the upper epidermis and lower epidermis whereas peltate glandular trichomes is only found in the upper leaf

epidermis (Fig. A). Capitulate glandular trichomes have basal cells, stem cells, with 1 head cell (Fig. B) or 2 round head cells (Fig. C), and peltate glandular trichomes with basal cells, short stem cells, and large heads with the presence of SCS (Fig. D). Trichomes of these peltate glands and capitates produce essential oils. According to Werker *et al.* (1993), glandular trichomes of the *O. basilicum* consists of short capitulate and peltate gland trichome. Capitulate trichomes consists of 1 basal cell, stem cell, and head cell. The peltate trichomes have basal cells, stem cells but large head cells which contain 4 cells. Very rare cell head contains more than 4 cells. Added by Iijima *et al.* (2004), glandular trichomes in sweet basil plant (*Ocimum basilicum*), rich in phenyl propanoid compounds such as monoterpenes and sesquiterpenes.

The peltate trichomes store larger volumes of essential oils than the capitulate trichomes. This is because the peltate tricomal head cells have SCS (*sub-cuticular space*) which can store oil secreted material, whereas in SCS tritomy the skit is too narrow. According to Werker *et al.* (1993), the compound is secreted through the accumulation of the peltate gland trichome, as well as the trichomes of other species of Lamiaceae studied, between the outer wall of the head cell and the cuticle attached to this layer, breaking out and forming a dome or space above the head cell. When the sub-cuticle space is fullwith secreting materials and head cells stop secreting, then they are destroyed. Only a slight loss of the cuticle is observed in a capitulate trichome with 2 head cells and none in the capitulate trichomes whose head is unicellular.

Stem

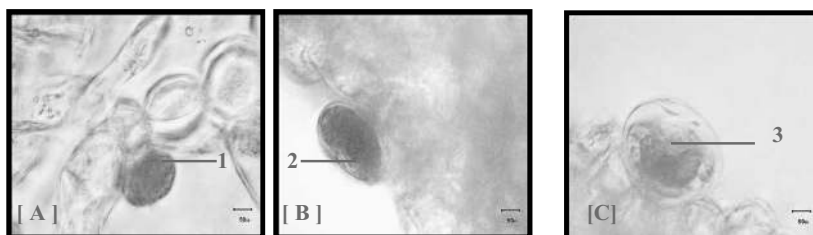


Figure 13. Glandular trichomes on the cross section of sweet basil stem with whole mount method (magnification 40 x 10)

Description:

- 1 - 2 : Capitulate glandular trichomes
3 : Peltate glandular trichomes

Based on Figure 13. Glandular trichomes can be found in the epidermal layer of the stem with a limited amount. Glandular trichomes are a type of peltate and capitulate. Capitulate gland trichome with 1 head cell (Fig. A) or with 2 round head cells (Fig. B), and peltate gland trichome with 1 round head cell with some secretory cells (Fig. C). Peltate trichomes in the stem with the leaves have different head shape. In the leaf, the peltate trichomes have bowl-shaped cells with large SCS (*subcuticular space*) space, whereas on the stem, peltate trichomes have round heads and the SCS spaces are not so large. Compared to the leaves, the density of trichomes is greater in the leaves so that the amount of essential oil produced in the leaves is much greater than that of the stems.

CONCLUSIONS

1. The patchouli plants (*Pogostemon cablin* (Blanco) Benth.) have a type of secretory structure of essential oils in the form of glandular trichomes and oil cells found in leaves and stems, and idioblast cells found in leaves.

2. The wild mint plants (*Mentha arvensis* L.) have a type of secretory structure of essential oil in the form of glandular trichomes found in leaves and stems.
3. The hoary basil plants (*Ocimum americanum* L.) have a type of secretory structure of essential oils of glandular trichomes found in leaves and stems, and oil cells found in leaves.
4. The mexican mint plants (*Plectranthus amboinicus* (Lour.) Spreng.) have a type of secretory structure of essential oils of glandular trichomes and idioblast cells found in leaves and stems.
5. The sweet basil plants (*Ocimum basilicum* L.) have a type of secretory structure of essential oil in the form of glandular trichomes found in leaves and stems.

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INTRODUCTION

The world's biodiversity besides Brazil and Tanzania. From Sabang to Merauke scattered about 40,000 species of plants (Agusta, 2000). Essential oil-producing plant covers about 200 species, 40 species of which are found in Indonesia. The types of essential oils that have been produced and circulated in the world market currently reaches 70 - 80 kinds, 15 of which are from Indonesia. Essential oils can be used as fragrance or soap and perfume or perfume products (Wibowo, 2012).

The Encyclopedia of Chemical Technology states that essential oils are compounds, which are generally fluid, obtained from parts of plants, roots, skins, stems, leaves, fruits and seeds or from flowers by steam distillation. Not all types of plants produce essential oils, only plants that have glanula cells alone can produce essential oils (Bangkit et al., 2012). One of the people who have local wisdom in the form of utilization of plants in the activity of life is the Tribe Besemah.

Besemah tribe is a tribe that occupies the area Pagaralam Lahat reGENCY of South Sumatra. The tribe is known to use many herbs for traditional medicine. Based on research that has been done by Agustina (2015), obtained 109 types of plants used as medicine by Besemah tribe and some of them including Lamiaceae species that produce essential oils that can also be used for beauty treatments such as patchouli, wild mint, hoary basil, mexican mint, and sweet basil.

This study examines the type and distribution of secretory structures contains essential oils on several types of Lamiaceae which are used as traditional cosmetics tribe Besemah Lahat reGENCY. The result of the research is expected to explain about secretory structures containing essential oils in plant organs, so as to provide insightful knowledge of the essential oil-producing plants of Lamiaceae as well as the basis for further research.

MATERIALS AND METHODOLOGY

This research was conducted from November to February 2017. The sample was taken at Bantunan and Sukabumi Village, Pajar Bulan Sub-district, Lahat District, South Sumatera. Preparation of vegetative preparations of plant vegetative organ is done in Mikroteknik Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Inderalaya.

The ingredients used are alcohols with different concentrations (100% alcohol, 96% alcohol, 70% alcohol, 50% alcohol, 30% alcohol), aquades, balsam canada, 4% formalin, Haupt adhesive (gelatin, phenol, glycerin), solution (paraxin, Na-hypochlorite, leaf and stem organs from patchouli, wild mint, hoary basil, mexican mint, and sweet basil, paraffin 48, paraffin 58, safranin-fastgreen dye solution, TBA, and xylol.

Field observations and interviews

Field observation was conducted by looking directly at the research location. The collection of information is done by interviewing local community or traditional healers expert on some kind of Lamiaceae are useful addition to the treatment can also be used for beauty treatments. From the interviews that have been conducted, showed that 5 kind used Lamiaceae Besemah Tribe communities for traditional cosmetics (Table 3.1).

Table 3.1. Types of Lamiaceae essential oil for traditional cosmetics used by the Tribe of Besemah people

Num	The name of the plant		Usability	Parts used
	Local name / common name	Scientific name		
1.	Patchouli	<i>Pogostemon cablin</i>	As perfume and deodorant	Leaf
2.	Taghok mim/wild mint	<i>Mentha arvensis</i>	Smooth skin and remove acne scars	Leaf
3.	Hoary basil	<i>Ocimum americanum</i>	Deodorant and bad breath (halitosis)	Leaf
4.	Mexicant mint	<i>Plectranthus amboinicus</i>	Breastfeeding	Leaf
5.	Sweet basil	<i>Ocimum basilicum</i>	Deodorant	Leaf

Preparation of research samples

Plant patchouli, wild mint, hoary basil, mexican mint, and sweet basil collected parts of the organ leaves and stems. Leaves taken are still young while the stem and petiole taken only at the shoot. Leaf organs and plants are soaked in FAA solution (*Formaldehyde Acetic-acid Alcohol*) to avoid damage and can be used for further processing.

The making of preparations by paraffin method (Sass, 1958)

Patchouli, wild mint, hoary basil, mexican mint, and sweet basil (leaves and stems) are collected and cut 3 x 3 mm, then fixed with a minimum of 24 hours FAA (*Formaldehyde Acetic-acid Alcohol*) solution. Next in aspiration for 1 hour with 3-5 repetitions for 5 days. Samples were included in 50% alcohol solution for 1 hour with 2 repetitions, Johansen I for 2 hours, Johansen II + safranin 24 hours, Johansen III, IV, and V for 2 hours, TBA 3 x for 2 hours, and TBA: oil paraffin (1: 1) for 1 hour. Continue with infiltration with paraffin 48 at oven temperature 48⁰C for 2 hours 3 x repetition, and paraffin 58 at 58⁰C for 2 hours 3 x repetition.

Embedding in paraffin 58 on paper box then cut and pasted on wooden block. Then in rotate with rotary microtom with a thickness of 10 - 15 µm and in the paste on the glass object that has been in tethesion of haupt adhesive and 4% formalin solution. Preparations in color with safranin – fastgreen staining method, spilled canada balsam, and covered with cover glass.

The making of preparations by whole mount method (Sass, 1958)

Samples were collected and slashed with a razor as thin as possible, after which the sample was fixed with a FAA fixative solution (*Formaldehyde Acetic-acid Alcohol*). Then washed by fixative solution was removed and replaced with aquades, and was done by soaking in Na-hypochlorite for 3 minutes then washed with aquades and then stained by the incision colored with safranin 1% for 3 minutes. Samples are placed on the glass object. The final closure was performed by means of an incision given 10% glycerol, then covered with a cover glass and observed under a microscope.

RESULTS AND DISCUSSION

Observations showed the secretory structure of essential oils in 5 species of Lamiaceae in the form of glandular trichomes, oil cells, and idioblast cells (Table 2). Glandular trichomes are found in 5 plant species (patchouli, wild mint, hoary basil, mexican mint, and sweet basil). Oil cells are found in *Pogostemon cablin* (patchouli) and *Ocimum americanum* (hoary basil), and idioblast cells are found in *Pogostemon cablin* and *Plectranthus amboinicus* (mexicant mint).

Glandular trichomes are found to consist of 2 types, namely trichomes of capitate gland and peltat spread all over the surface of leaf blade, petiole, and stem of plant. Both types of trichomes are distinguished by their structure and secretory cells. Glandular trichomes are generally formed by basal cells, stem cells, and head cells. The head cells of the capitate gland tricoma consist of several sizes and shapes, including short, long, unicellular, or multicellular. However, a capitate gland trichome composed of basal cells, short stem cells with 1-2 head cells is the most commonly encountered trichome in Lamiaceae plants.

Trichomes of the peltate gland consist of basal cells, 1 stalk cells, and head cells consisting of 4-18 cells. This type of trichome has a head cell sheathing that develops over the head cells in its mature phase (Aprilia, 2016). Glandular trichomes are external secretory structures whereas oil cells and idioblast cells are part of the internal secretory structure.

Table 4.1. The type and location of the secretory structure of essential oils in some species of Lamiaceae

Num	The name of the plant	Part of observation	Secretory structure
1.	<i>Pogostemon cablin</i>	Leaf	Glandular trichomes, oil cells, and idioblast cells
		Stem	Glandular trichomes and oil cells
2.	<i>Mentha arvensis</i>	Leaf	Glandular trichomes
		Stem	Glandular trichomes
3.	<i>Ocimum americanum</i>	Leaf	Glandular trichomes and oil cells
		Stem	Glandular trichomes
4.	<i>Plectranthus amboinicus</i>	Leaf	Glandular trichomes and idioblast cells
		Stem	Glandular trichomes and idioblast cells
5.	<i>Ocimum basilicum</i>	Leaf	Glandular trichomes
		Stem	Glandular trichomes

The secretory structure of essential oils on patchouli plants

Leaf

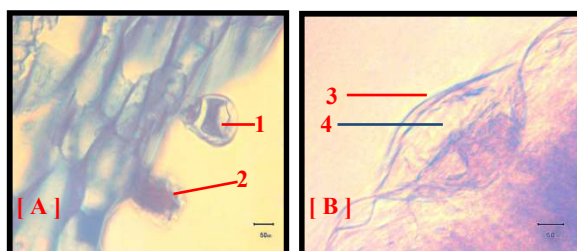


Figure 1. Glandular trichomes in cross section of patchouli leaves with paraffin (A: 40 x 10 magnification) and whole mount method (C: magnification 40 x 10)

Description:

- 1 – 2 : Capitulate glandular trichomes
- 3 : Peltate glandular trichomes
- 4 : SCS (*Sub-cuticular space*)

Based on figure 1. Glandular trichomes found in patchouli leaves there are 2 types of capitulate trichomes and peltate trichomes. The tricoma of the capitulate gland has a basal cell, stalk cell, and 1 round head cell with a smooth surface (Fig. A (1)), or irregular wrinkles (Fig. A (2)). Trichomes of the peltate gland have basal cells, stalk cells, and a large head composed by several secretory cells (Fig. B).

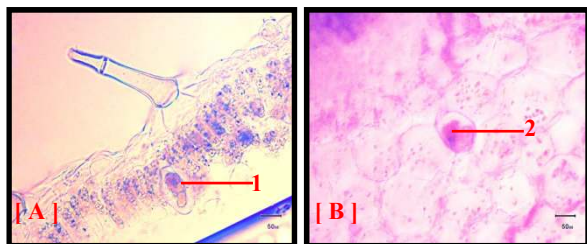


Figure 2. Oil cell and idioblast cells on cross section of patchouli leaves with whole mount method (A: magnification 10 x 10; B: magnification 40 x 10)

Description:

- 1 : Oil cells in palisade tissue
- 2 : Idioblast cells in spongy tissue

Based on figure 2. In addition to trichoma, essential oil of patchouli leaves is also produced by oil cells that can be found on parenchyma palisade (Fig. A) and idioblast cells in the sponge parenchyma (Fig. B). This idioblast cell is brownish yellow and contains oil. According to Guo *et al.* (2013), the internal glands of patchouli leaves with a length of 40 μm (± 9) spread among the palisade cells. A mature internal gland with basal cells, narrow stalk cells, and a large cytoplasmic cell head that is often filled with oil secretions.

Stem

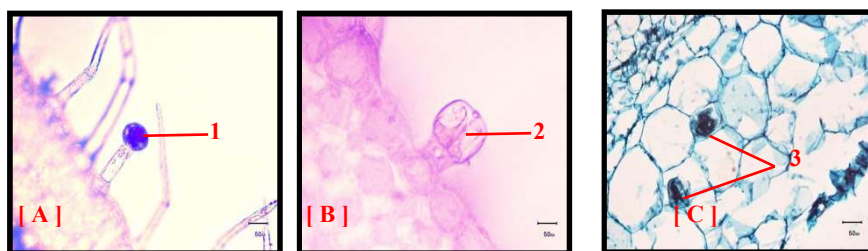


Figure 3. Glandular trichomes and oil cells in cross section patchouli stem with whole mount (A - B: magnification 40 x 10) and paraffin method (C: magnification 40 x 10)

Description:

- 1 – 2 : Capitate glandular trichomes
- 3 : Oil cells in cortical tissue

Based on figure 3. Trichomes of the glands in the stem are the type of capitate. Trichomes of the capitate gland have basal cells, longer stalk cells, and 1 round head cell (Fig. A) and tricoma of the capitate gland with basal cells, shorter stem cells, and 1 round head cell (Fig. B). Tricoma of the peltate gland is not found in the stem and the number of trichomes of the larger capitic gland in the leaves. In the area of the stem cortex also found an oil cell suspected to contain essential oils (Fig. C).

The secretory structure of essential oils on wild mint plants

Leaf

Based on figure 4. Capitate and peltate glandular trichomes are found in the upper epidermis and under epidermis (Fig.A). Trichomes of the peltate gland are made up of basal cells, do not have stalk cells, with rounded head cells (Fig. B). While capitate glandular trichomes has basal cell, stalk cell, and 1 round cell head (Fig. C). According to Turner *et al.* (2000), the biosynthetic pathway of monoterpene compounds in *Mentha piperita* has been

located specifically on the secretory cells of glandular trichoma located on the outer surface of the leaf. Three types of trichomes are present in the leaves: non glandular, multicellular, simple trichomes; small, trichoma capitate gland with 1 secretory head cells, and pelvic gland trichoma with secretory cells having 8 apical cells.

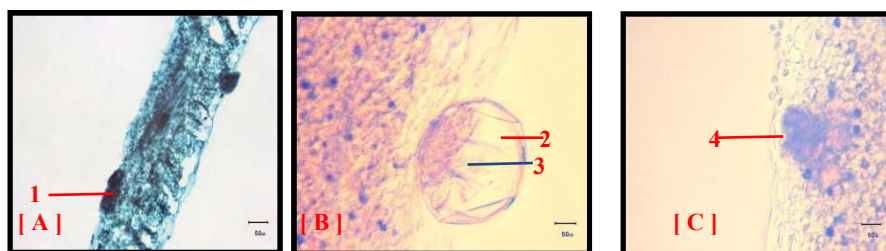


Figure 4. The location and type of glandular trichomes in a cross section of wild mint leaves with paraffin method (A: magnification 10 x 10) and whole mount (B-C: magnification 40 x 10)

Description:

- 1 : Glandular trichomes
- 2 : Peltate glandular trichome
- 3 : *SCS* (*Sub-cuticular space*)
- 4 : Capitate glandular trichomes

Stem

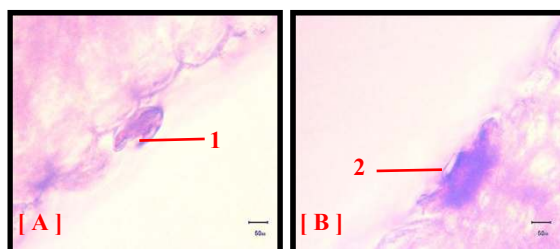


Figure 5. Glandular trichomes in cross section patchouli stem with whole mount method (A - B: magnification 40 x 10)

Description:

- 1 - 2 : Capitate glandular trichomes

Based on Figure 5. Trichomes of the capitate gland in the stem have basal cells, stem cells, and 1 head cells of different shapes (Fig. A-B).

The secretory structure of essential oils on hoary basil plants

Leaf

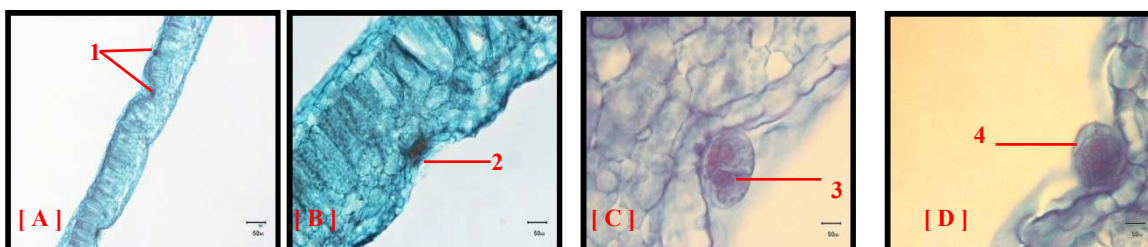


Figure 6. The location and type of glandular trichomes on cross-section of hoary basil leaves by paraffin method (A: magnification 4 x 10; B: magnification 10 x 10; C-D: magnification 40 x 10)

Description:

- 1 : Glandular trichomes
- 2 : Peltate glandular trichome
- 3 - 4 : Capitate glandular trichomes

Based on figure 6. Capitate glandular trichomes are present in the upper and lower epidermis of the leaves (Fig. A) whereas the peltate glandular trichomes is found only in the

lower epidermis of the leaf (Fig. B). Trichomes of the peltate gland have basal cells, stem cells, and a large head composed of several secretory cells (Fig. B). The tricoma of the capitate gland has a basal cell, a stem cell, with 2 heads (Fig. C) and 1 round head (Fig. D).

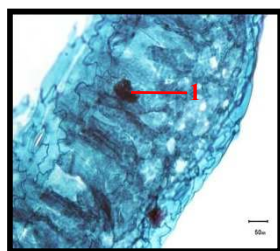


Figure 7. Oil cells on cross section of hoary basil leaves by paraffin method (magnification 10 x 10); 1: Oil cells, 2: Palisade tissue

Based on figure 7. Hoary basil leaf besides having glandular trichomes also have oil cells that can produce essential oils. The oil cell is present in the palisade tissue cells with a blackish brown.

Stem

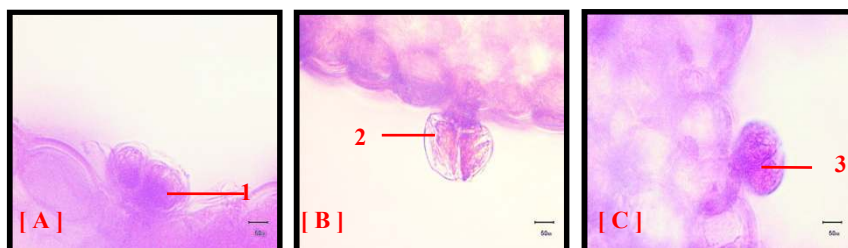


Figure 8. Glandular trichomes of cross section of hoary basil stem with whole mount method (magnification 40 x 10)

Description:

1 - 3 : Capitate glandular trichomes

Based on figure 8. In stem there is a capitate gland trichomes composed of basal cells and stalk cells with 2 round cells (Fig. A), or slightly oval (Fig. B), and 1 round head cell (Fig. C). These glandular trichomes are commonly found in the 4 corners of the stem epidermal layer. Basil stems also produce essential oils due to the trichomes of this gland, but the number is less than in the leaf.

The secretory structure of essential oils on mexicant mint plants

Leaf

Based on figure 9. Glandular trichomes found on the surface of mexicant mint leaves is a type of capitate. Trichomes of the capitate gland may be found in the upper and lower epidermis of the leaves. The capitate trichomes have basal cells, longer stalk cells, with 1 oval-shaped head cells (Fig. A), round (Fig. B), and oval (Fig. C). Capitate trichomes having basal cell, short stalk cell with 1 round head cell (Fig. D-E), or narrow head neck with blunt end (Fig. F). According to Sharma *et al.* (2014), this plant is covered by glandular and non-glandular trichomes. Glandular trichomes are type of capitate trichomes consisting of an epidermal basal cell, a unicellular or biselular stalk of varying length, having a neck cell, and a large, unicellular round head secretion.

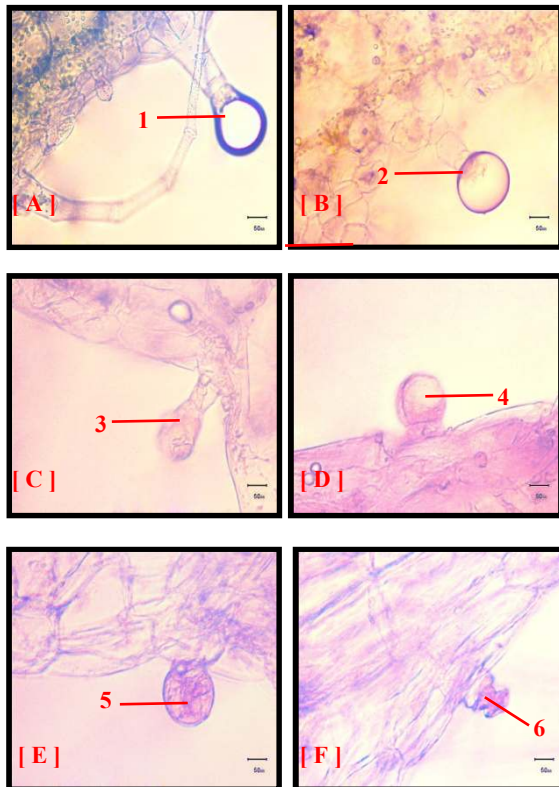


Figure 9. Glandular trichomes in cross section of mexicant mint leaves with whole mount method (magnification 40 x 10)

Description:

1 - 6 : Capitate glandular trichomes

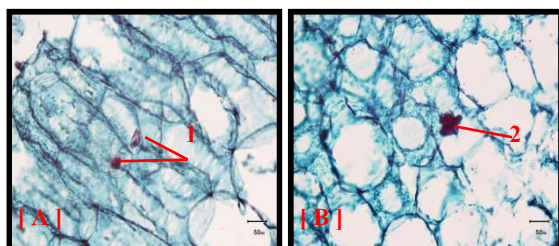


Figure 10. Idioblast cells in cross section of mexicant mint leaves with paraffin method (A - B: magnification 40 x 10)

Description:

1 : Idioblast cells in palisade tissue

2 : Idioblast cells in spongy tissue

Based on figure 10. on the cross section of mexicant mint leaves found the idioblast cell. This idioblast cell is found in palisade tissue cells (Fig. A) and spongy tissue (Fig. B). This idioblast cell is thought to contain essential oils. According to Novitasari (2015), idioblast cells are a cell of different sizes, shapes, and contents of the contents of other cells within a network. The idioblast cell size varies in the same organ, as well as between the different organs, but the size variation is low.

Stem

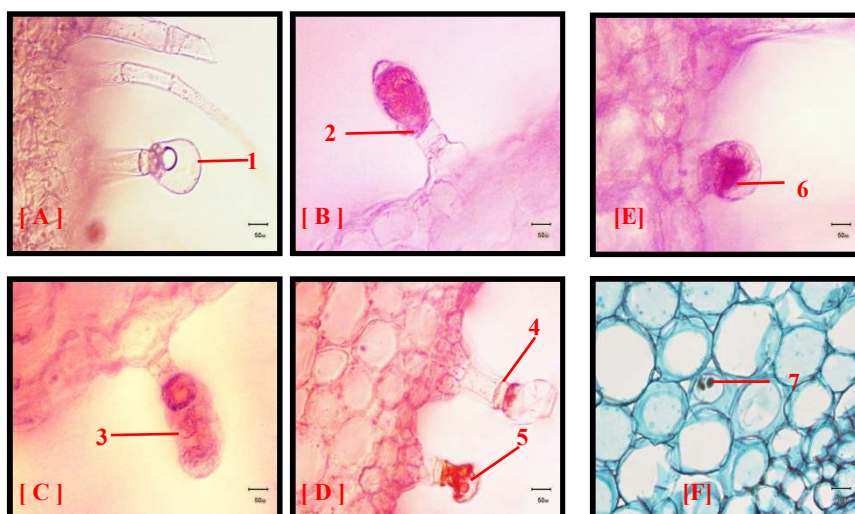


Figure 11. Glandular trichomes and idioblast cells of cross section of mexicant mint stem with whole mount (A – E: magnification 40 x 10) and paraffin method (F: magnification 40 x 10)

Description:

- 1 - 6 : Capitata glandular trichomes
 7 : Idioblast cells

Based on figure 11. Trichomes of the capitata glands found on the stem are similar to those in the leaves. The trichomes have basal cells, longer stem cells, with oval head shape cells (Fig. A), oval (Fig. B), very oval (Fig. C), or slightly oval (Fig. D (4)). Trichomes with basal cells, shorter stem cells, and narrow head cells with a dull end (Fig. D (5)) or 1 round head cell (Fig. E). The idioblast cells are also found in stems that can be found among the cortical cells (Fig. F). This idioblast cell is thought to contain essential oils.

The secretory structure of essential oils on sweet basil plants

Leaf

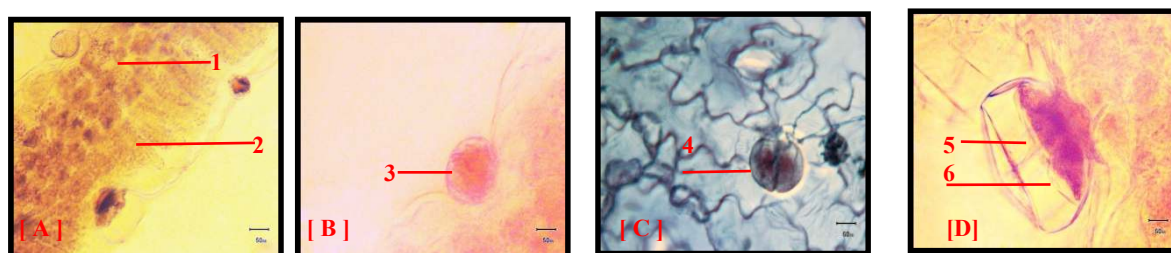


Figure 12. The location and type of glandular trichomes at cross section (A, B, D) and paradermal (C) sweet basil leaves by whole mount (A: magnification: 10 x 10; B, D: magnification 40 x 10) and paraffin method (C: 40 x 10 magnification)

Description:

- 1 : Spongy tissue
 2 : Palisade tissue
 3- 4 : Capitata glandular trichomes
 5 : Peltate glandular trichomes
 6 : SCS (*sub-cuticular space*)

Based on figure 12. Capitata glandular trichomes may be found in the upper epidermis and lower epidermis whereas peltate glandular trichomes is only found in the upper leaf

epidermis (Fig. A). Capitulate glandular trichomes have basal cells, stem cells, with 1 head cell (Fig. B) or 2 round head cells (Fig. C), and peltate glandular trichomes with basal cells, short stem cells, and large heads with the presence of SCS (Fig. D). Trichomes of these peltate glands and capitates produce essential oils. According to Werker *et al.* (1993), glandular trichomes of the *O. basilicum* consists of short capitulate and peltate gland trichome. Capitulate trichomes consists of 1 basal cell, stem cell, and head cell. The peltate trichomes have basal cells, stem cells but large head cells which contain 4 cells. Very rare cell head contains more than 4 cells. Added by Iijima *et al.* (2004), glandular trichomes in sweet basil plant (*Ocimum basilicum*), rich in phenyl propanoid compounds such as monoterpenes and sesquiterpenes.

The peltate trichomes store larger volumes of essential oils than the capitulate trichomes. This is because the peltate tricomal head cells have SCS (*sub-cuticular space*) which can store oil secreted material, whereas in SCS tritomy the skit is too narrow. According to Werker *et al.* (1993), the compound is secreted through the accumulation of the peltate gland trichome, as well as the trichomes of other species of Lamiaceae studied, between the outer wall of the head cell and the cuticle attached to this layer, breaking out and forming a dome or space above the head cell. When the sub-cuticle space is fullwith secreting materials and head cells stop secreting, then they are destroyed. Only a slight loss of the cuticle is observed in a capitulate trichome with 2 head cells and none in the capitulate trichomes whose head is unicellular.

Stem

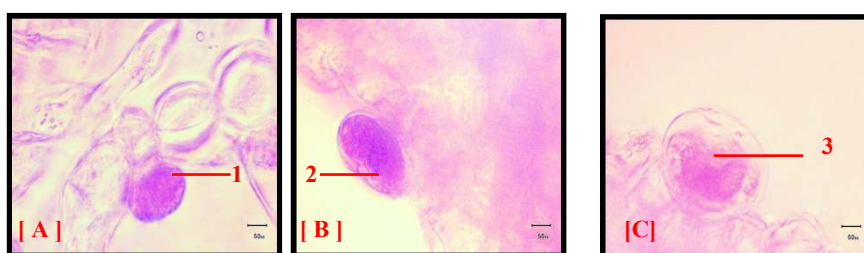


Figure 13. Glandular trichomes on the cross section of sweet basil stem with whole mount method (magnification 40 x 10)

Description:

- 1 - 2 : Capitulate glandular trichomes
3 : Peltate glandular trichomes

Based on Figure 13. Glandular trichomes can be found in the epidermal layer of the stem with a limited amount. Glandular trichomes are a type of peltate and capitulate. Capitulate gland trichome with 1 head cell (Fig. A) or with 2 round head cells (Fig. B), and peltate gland trichome with 1 round head cell with some secretory cells (Fig. C). Peltate trichomes in the stem with the leaves have different head shape. In the leaf, the peltate trichomes have bowl-shaped cells with large SCS (*subcuticular space*) space, whereas on the stem, peltate trichomes have round heads and the SCS spaces are not so large. Compared to the leaves, the density of trichomes is greater in the leaves so that the amount of essential oil produced in the leaves is much greater than that of the stems.

CONCLUSIONS

1. The patchouli plants (*Pogostemon cablin* (Blanco) Benth.) have a type of secretory structure of essential oils in the form of glandular trichomes and oil cells found in leaves and stems, and idioblast cells found in leaves.

2. The wild mint plants (*Mentha arvensis* L.) have a type of secretory structure of essential oil in the form of glandular trichomes found in leaves and stems.
3. The hoary basil plants (*Ocimum americanum* L.) have a type of secretory structure of essential oils of glandular trichomes found in leaves and stems, and oil cells found in leaves.
4. The mexican mint plants (*Plectranthus amboinicus* (Lour.) Spreng.) have a type of secretory structure of essential oils of glandular trichomes and idioblast cells found in leaves and stems.
5. The sweet basil plants (*Ocimum basilicum* L.) have a type of secretory structure of essential oil in the form of glandular trichomes found in leaves and stems.

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