
CHARACTERISTICS OF ANATOMICAL STRUCTURE AND ESSENTIAL OIL GLANDS OF LEAF PEPPERMINT (*MENTHA PIPERITA*) AND SPEARMINT (*MENTHA SPICATA*)

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ABSTRACT

Mentha is one of the genera of the Lamiaceae family which can produce essential oils. *Mentha* species have antioxidant and antimicrobial activity, and the most active compound that plays a role is menthol. This study aimed to analyze the characteristics of variations in the anatomical structure and oil glands of the leaves of two *Mentha* species as essential oil producers. This type of research was descriptive qualitative in the form of leaf anatomy observations using the fresh and whole mount preparations for longitudinal incisions and the paraffin method for transverse incisions. The research results show that the anatomical structure of the leaves in *M. piperita* and *M. spicata* includes the shape, arrangement of epidermal cells and their derivatives, the structure of the tissues that make up the leaves, and variations in glandular and non-glandular trichomes including variations in type, structure, size, and location. The structure of essential oil glands in the leaves of *M. piperita* and *M. spicata* species has variations, including number, shape, size, distribution, location, and density. The results of the analysis of the anatomical characteristics of the *Mentha* species showed that *M. piperita* had more oil glands than *M. spicata*. This research has implications for better natural resource management, disaster prevention, and the development of agriculture and fisheries, and this research is the basis for better decision-making by local governments, stakeholders, and related institutions in planning regional development, environmental management, and resource allocation.

Keywords: plants, mentha, anatomy, leaves, oil glands.

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INTRODUCTION

Mentha is a genus in the Lamiaceae family with approximately 30 species. *Mentha* is widely cultivated on five continents, especially in temperate and subtropical areas, and is classified into five genera containing 25-30 species (Chen et al., 2012). Several *Mentha* species include *M. arvensis*, *M. aquatic*, *M. canadensis*, *M. pulegium*, *M. x piperita*, *M. piperita*, and *M. spicata*. Among the *Mentha* species, *M. x piperita*, *M. piperita*, and *M. spicata* are the most common in Indonesia.

Several *Mentha* species have been successfully developed in 25 species. Still, there are only four *Mentha* species that can be cultivated for essential oil production in the world, namely *M. canadensis*, *M. gracilis*, *M. piperita*, and *M. spicata* (Chen et al., 2012). Essential oils are aromatic oil liquids that can be extracted from various parts of plants, namely leaves, seeds, flowers, bark, and fruit skin (Tongkuanchan & Benjakul, 2014). *Mentha* contains essential oils, namely many chemicals in aromas such as menthol, menthone, iso menthone, and menthofuran (Hidayat & Rurini Retnowati, 2013).

M. piperita and *M. spicata* are known for their essential oil content produced from trichome glands and also for the abundance of phenolic compounds in the leaves (Dhifi et al., 2016) ; (Kalemba & Synowiec, 2019) ; (Park et al., 2019) ; (Park et al., 2019). *Mentha* species have various types of trichomes, generally glands found in the epidermis and containing essential oils. *M. piperita* can produce an essential oil commonly called peppermint, while *M. spicata* can produce an essential oil commonly called spearmint. The essential oil obtained from *Mentha* species is used extensively in the pharmaceutical industry. *Mentha* species have antioxidant and antimicrobial activity and the most active compounds that play a role are menthol (Ali et al., 2015) ; (Mahdavia & Saharkhiz, 2015).

Based on previous research on the species *M. piperita* and *M. spicata*, we examined several *Mentha* species, including *M. piperita* and *M. spicata*, which included classification of inflorescence characters, classification of leaf characters, taxonomic identification, and showing the presence of trichome types in several species. *Mentha* (Šarić-Kundalić et al., 2016) . Previous research also examined the amount of oil content or yield produced by the *M. piperita* and *M. spicata* species leaves. The results were linked to observations of the anatomy of the leaves of the oil glands of the *M. piperita* and *M. spicata* species (Puspitasari et al. , 2021).

Research can also be a research support for observing the anatomical structure of the leaves and oil glands of the *M. piperita* and *M. spicata* plants that will be studied. However, in the previous study, samples of plant species used came from different places from this study; the species to be used came from Sidomulyo Village, Kec. Batu, Malang (Šarić-Kundalić et al., 2016) ; (Puspitasari et al., 2021) .

One of the characteristic parts of the leaf is the leaf epidermis tissue and its derivatives. Research on the characteristics of the anatomical structures and oil glands in the leaves of *M. piperita* and *M. spicata* is important because there is little information about the structural characteristics of *M. piperita* and *M. spicata* in Indonesia.

Therefore, based on the above background, this research was carried out, which aims to analyze the characteristics of variations in the anatomical structure and oil glands in the leaves of two *Mentha* species, which are related to the presence of essential oils. The research results will be useful in obtaining information about the diversity of anatomical structures and supporting data for *Mentha* species.

METHOD

This type of research is descriptive qualitative, namely analyzing the anatomical and oil gland structure characteristics of the leaves of *M. piperita* and *M. spicata* species. This research was conducted for 4 months, December 2022 – April 2023. Plant sampling was carried out in Sidomulyo Village, Batu, Malang. The leaves' anatomical structure was observed at the Microtechnical Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Surabaya State University.



Figure 1. Map

The plant samples used were taken fresh. Plant sample collection was carried out by randomly sampling 3 specimens for each *Mentha species*. The samples taken are leaves from nodes 3-5, which are free of disease and other materials. The preparations were made using three methods: the fresh and *wholemout incision method* for longitudinal and the paraffin method for transverse incision. Observation of the transverse anatomical structure of leaves using materials including FAA (formalin acetic acid-alcohol) alcohol graded 50%, 70%, 95%, and 100%, alcohol solution: xylol (3:1), alcohol solution: xylol (1: 1), alcohol solution: xylol (1:3, xylol absolute 1 and xylol absolute II, safranin 1%, fast green 0.5% and entel I an, while for observation of the longitudinal anatomical structure of the leaves using the appropriate *wholemout method*. Using distilled water, kloroks , safranin 0.1 %, glycerin 30%. The preparations were observed with a light microscope with a magnification of 40x10 with a scale of 0.19625/mm² (Marantika et al., 2021).

The data were analyzed descriptively and qualitatively using the two *Mentha species*' anatomical structures and oil glands. Data analysis includes qualitative and quantitative data. Qualitative data were obtained by means of a literature review and similar research results, while the application of image j processed quantitative data. The results of data analysis are then arranged in tables and figures to make it easier to characterize each species.

The anatomical characteristics observed in the longitudinal incisions were the structure of the epidermis, the shape of the epidermis, the composition of the epidermal cells and their derivatives, the number of oil glands, the shape of the oil glands, the size of the oil glands and the density of the oil glands. The anatomical characters of the leaves observed in the cross-section were leaf tissue, including adaxial and abaxial epidermal cells, palisade parenchyma and spongy parenchyma, various forms of glandular and non-glandular trichomes, shape of essential oil, size of essential oil, distribution of essential oil, location essential oils in the leaves of the *M. piperita* and *M. spicata* species respectively.

RESULTS AND DISCUSSION

The results showed that there were variations in the structure of the epidermis and its derivatives and also found the presence of essential oil glands in the leaves of *M. piperita* and *M. spicata species*. Obtained from Sidomulyo Village, Batu, Malang. Leaf anatomy was observed by

making transverse and longitudinal incisions to determine variations in leaf anatomy and characteristics that indicate the presence of oil glands.

Anatomical Structure

Mentha species, the results obtained were the presence of constituent tissues. Based on Figure 1, it is known that there are several similarities and differences in the characteristics of the two species. The anatomical characteristics of the leaves shared by both species with a magnification of 40x10 and a field of view scale of 0.19625/mm² are the presence of epidermal cells, palisade parenchyma tissue, sponge parenchyma tissue, and trichomes. Each species has one layer of leaf epidermal cells located on the adaxial and abaxial sides of the leaf. The palisade parenchyma and spongy parenchyma in both species have the same structure; namely, the palisade parenchyma looks neatly arranged under the adaxial epidermal layer, while the spongy parenchyma shows an irregular cell arrangement as well as different shapes and sizes above the abaxial epidermal layer. The transverse anatomical structure of the leaves in three *Mentha* species can be seen in Figure 1.

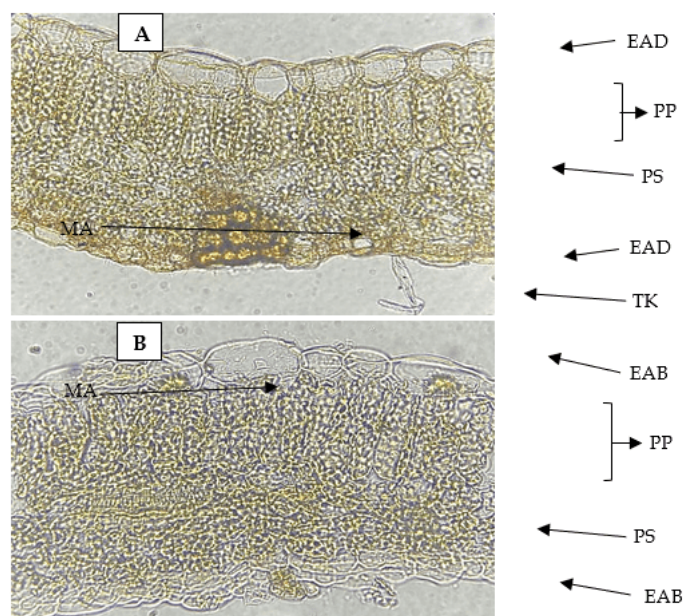


Figure 2. Structure of cross sections of two *Mentha* species with a magnification of 40x10; a) *M. piperita*, b) *M. spicata* ; (EAD): Adaxial epidermis, (PP): Palisade parenchyma, (PS): Sponge parenchyma, (MA): Essential oils, (TK): Trichomes, (EAB): Abaxial epidermis.

Observations obtained in longitudinal sections of the two *Mentha* species with a magnification of 40x10 and a field of view scale of 0.19625/mm² include The shape of the epidermal cell walls and epidermal derivatives, namely stomata and trichomes, also found the presence of essential oils, but in Figure 2 only shows the presence of oil glands in *M. spicata*. The anatomical structure of *M. piperita* and *M. spicata* leaves showed that the epidermis surface showed grooved and irregular anticlinal walls. Another difference is showing the presence of trichomes in Figure 2, which are only found in *M. spicata*. The results of observations on the type of stomata of the leaves of the two *Mentha* species have the same type, namely the anomocytic type in Figure 2. This can be seen from the sketch of the stomata in Figure 4.2. It can be seen that the number and arrangement of guard cells in the stomata consists of 1 guard cell.

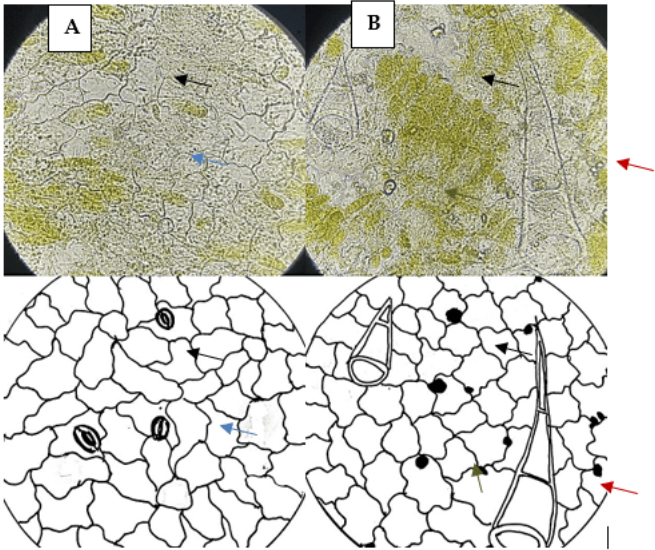


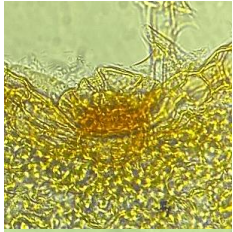
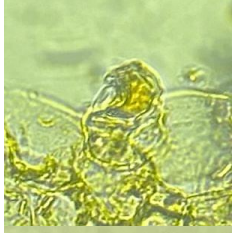
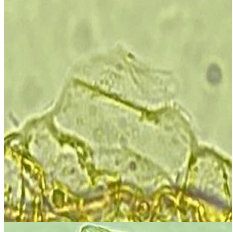
Figure 3. Leaf anatomical structure of longitudinal sections of two *Mentha species* with a magnification of 40x10; a) *M. piperita*, b) *M. spicata* ; (black arrow: cell wall, blue arrow: stomata, red arrow: trichomes, green arrow: essential oil).

Trichomes

The results of observations of other anatomical structures in the leaves of two *Mentha species* are the presence of epidermal derivatives in trichomes. Trichomes on *M. piperita* leaves are found in the abaxial epidermis, while trichomes on *M. spicata* leaves are found in both adaxial and abaxial epidermis, Table 1.

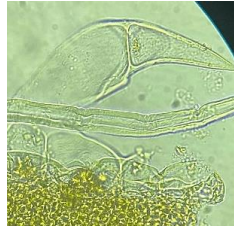
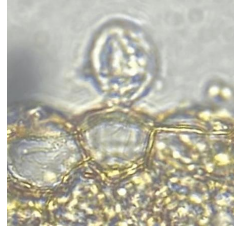
Table 1. Characteristics of trichomes on two types of leaf species: *M. piperita* and *M. spicata*

<i>A. M. piperita species</i>						Picture
Type	Structure	Basal Cells	location	Size (µm)		
Glandular (Unicellular)	Round blunt stalk tip	4	Leaf adaxial	36.362 µm		
Glandular (Unicellular)	The pointed end of the stalk	4	Leaf adaxial	12.105 µm		

Peltate glandular (Unicellular)	1 blunt stalk cell	6	Leaf adaxial	32.146 μm	
Capitate glandular (Multicellular)	1 stalk cell, 1 round head cell	2	Abaxial leaves	27.296 μm	
Non-Glandular (Multicellular)	1 stalk cell, 1 blunt head cell	6	Leaf adaxial	19,999 μm	
Non-Glandular (Unicellular)	1 cell is flat	2	Leaf adaxial	54.677 μm	

B. Species *M. spicata*

Type	Structure	Basal Cells	location	Size (μm)	Picture
Glandular (Unicellular)	1 blunt stalk cell	2	Abaxial leaves	37.332 μm	
Non-Glandular (Multicellular)	2 stalk cells, 1 tapered head cell	6	Leaf adaxial	53.778 μm	
Non-Glandular (Unicellular)	The tip of the stem is tapered	2	Abaxial leaves	87.566 μm	

Non-Glandular (Multicellular)	1 stalk cell, 1 elongated pointed head cell	4	Abaxial leaves	203.812 µm	
Non-Glandular (Unicellular)	1 round stalk cell	4	Abaxial leaves	39.992 µm	
Non-Glandular (Unicellular)	1 blunt stalk cell	4	Abaxial leaves	125.479 µm	
Dendroid	1 long stalk cell	4	Abaxial leaves	∞	

Of trichomes found on the abaxial epidermis of *M. piperita* leaves, namely glandular trichomes (secretions) and non-glandular trichomes (non-secretions) as presented in Table 1. An abaxial transverse section of *M. piperita* found: 1) glandular trichomes unicellular, blunt rounded stalk tip structure, 4 basal cells, located at the adaxial side of the leaf, size 36.362 µm; 2) unicellular glandular trichomes, rounded structures, and pointed stalk ends, 4 basal cells, located at the adaxial side of the leaf, 12.105 µm in size; 3) unicellular plate glandular trichomes, the structure of 1 blunt stem cell, 6 basal cells, located in adaxial leaf, size 32.146 µm; 4) multicellular capitate glandular trichome, structure of 1 stalk cell, 1 round head cell, 2 basal cells, located abaxially on the leaf, size 27.296 µm; 5) multicellular non-glandular trichomes, structure of 1 blunt stalk cell, 6 basal cells, located in adaxial leaf, size 19.999 µm; 6) unicellular non-glandular trichomes, the structure of 1 flat stalk cell, 2 basal cells, located in adaxial leaves, size 54.677 µm.

Types of trichomes found in the abaxial and adaxial epidermis of *M. spicata* plants, namely glandular trichomes and non-glandular trichomes as presented in (Table 4.1). An abaxial transverse section of *M. spicata* found: 1) unicellular glandular trichomes, the structure of 1 blunt stalk cell, 2 basal cells, located abaxially on the leaf, size 37.332 µm; 2) unicellular non-glandular trichomes, tapered stalk tip structure, 2 basal cells, located abaxially on the leaf, size 87.566 µm; 3) multicellular non-glandular trichomes, the structure of 1 stalk cell, 1 elongated pointed head cell, 4 basal cells, located abaxially on the leaf, 203.812 µm; 4) unicellular non-glandular trichomes, the structure of 1

round stalk cell, 4 basal cells, located abaxial in the leaf, size 39.992µm; 5) unicellular non-glandular trichomes, structure of 1 blunt stalk cell, 4 basal cells, located abaxially on the leaf, size 125.479 µm; 6) unicellular non-glandular trichomes, structure of 1 round stalk cell, 4 basal cells, located abaxially on the leaf, size 39.992 µm; 7) dendroid trichomes with a structure of 1 cell on a long stalk, 4 basal cells, located abaxially on the leaf. An adaxial longitudinal section of *M. spicata* found 1) multicellular non-glandular trichomes, the structure of 2 stalk cells, 1 tapered head cell, and 6 basal cells, located abaxially on the leaf, size 53.778 µm (Table 1).

Oil Glands

Based on observations of the structure of oil gland cells in the leaves of two *Mentha species* with a magnification of 40x10 and a field of view scale of 0.19625/mm², which has similarities and differences in the number, shape, distribution, and location of cells. The oil glands in *M. piperita* are located in the lower epidermis. They are also spread across the palisade parenchyma cells and sponge parenchyma, while the oil gland cells in *M. spicata* are located in the upper epidermis (Figure 3).

Table 2. Results of observations on the amount, shape, distribution, and location of oil in *Mentha species*

Species Name	Amount	Form	Distribution	Size (µm)	location
<i>M. piperita</i>	25-35	Round	Spread	3,519 µm-8,642 µm	Located in palisade parenchyma and spongy parenchyma
	20-25	Round	Huddle	13,604 µm-24,012 µm	Located in the abaxial epidermis
<i>M. spicata</i>	10-15	Round	Huddle	38,986 µm-11,418 µm	Located in the adaxial epidermis

The characteristics of the oil glands with transverse sections on the 2 leaf species of *M. piperita* and *M. spicata* showed that in *M. piperita*, 1) oil glands number 25-35, have a small round shape, are spread out and are located in the palisade parenchyma cells and spongy parenchyma, have a size of 3.519 µm-8.642 µm; 2) oil glands number 20-25, have a large round shape, are clustered in distribution and are located in the lower epidermis, have a size of 13.604 µm-24.012 µm; whereas in *M. spicata*; 1) Oil glands number 10-15, have a large round shape, are clustered in distribution and are located in the upper epidermis, have a size of 38.986 µm-11.418 µm (figure 3).

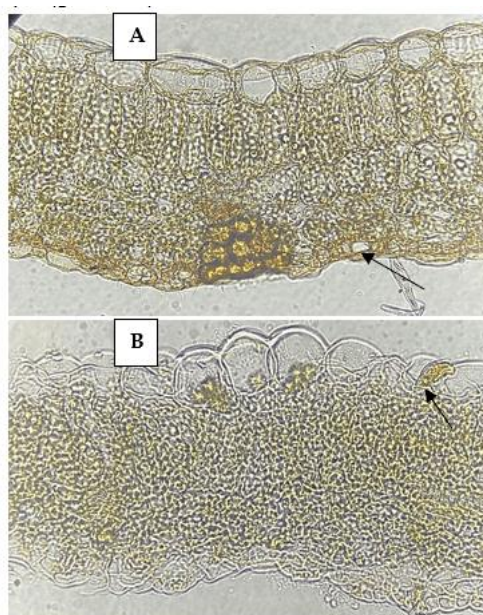


Figure 4. Images of the oil glands of two *Mentha* species: a) *M. piperita*, *M. spicata*; black arrow: location of oil glands

The results of anatomical observations of longitudinal incisions in the leaf epidermis of the two *Mentha* species with a magnification of 40x10 and a field of view scale of 0.19625/mm² show that the two species have the same epidermal cell shape Figure 4.1. Epidermal cells of *Mentha* leaves are curved, irregular, and have different cell sizes. There are variations in the characteristics of the oil glands, namely in the size and number of the epidermis, Table 3.

Table 3. Number, shape, and size of oil gland cells in leaves of two species *M. piperita* and *M. spicata*

Species Name	Amount	Form	Size
<i>M. piperita</i>	698	Round	3.124 μ m - 6.719 μ m
<i>M. spicata</i>	482	Round	2.112 μ m - 3.734 μ m

Mentha species in a longitudinal section with a magnification of 40x10 and a field of view scale of 0.19625/mm² can be seen in Figure 4.4. It can be seen in the picture that there are round characters that are inside the epidermal cells and have a round shape and also spread. Some of these oil glands are yellow and clear (Figure 4.4). The number of oil glands in Figure 4.4 is that in the species *M. Piperita* has a total of 698 and has a size of 3.124 μ m - 6.719 μ m while in the species *M. spicata*, there are 482 and a size of 2.112 μ m-3.734 μ m (table 3)

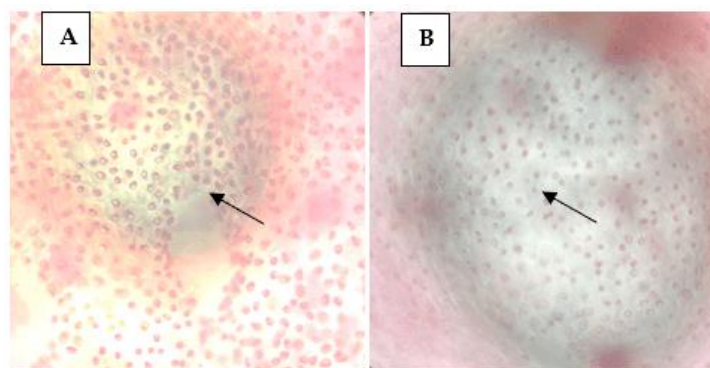


Figure 5. Image of sebaceous gland cell density in incision longitudinal in two *Mentha* species ; a) *M. piperita* , b) *M. spicata*

Plants from the *Mentha* clan, namely *M. piperita* and *M. spicata*, can produce essential oils. Essential oils or essential oils are liquid oils extracted from plant parts, namely leaves, seeds, flowers, bark, and fruit peels (Tongkuanchan & Benjakul, 2014). Several food and pharmaceutical industries widely use the essential oil of the *Mentha* clan because the plant has antioxidant and antimicrobial activity (Ali et al., 2015) ; (Mahdavia & Saharkhiz, 2015).

In this study, observations were made of the anatomical structure of the leaves of two *Mentha* species in Sidomulyo Village, Batu, Malang, namely *M. piperita* and *M. spicata*, by making transverse and longitudinal incision preparations with a microscope magnification of 40x10 magnification and a field of view scale of 0.19625/mm² which was carried out based on preliminary tests and previous research references which are useful for knowing variations in leaf anatomy between the *Mentha* species studied and the anatomical characteristics of leaves that have oil glands (Šarić-Kundalić et al., 2016).

Based on the results of observations of cross-sections on the leaves of the two *Mentha* species, they have one thing in common, one of which is a thick, unstained epidermal layer, because this tissue does not absorb *fast green dye* during the staining process. Neatly arranged palisade parenchyma beneath the adaxial epidermis and irregularly shaped spongy parenchyma. The parenchyma in both *Mentha* species is included in the assimilation parenchyma because there are two types of assimilation parenchyma: palisade and spongy (Palennari et al., 2016). Palisade parenchyma and spongy parenchyma belong to the mesophyll structure of the leaves of two *Mentha* species, which function in photosynthesis. This is supported by other research stating that the palisade parenchyma consists of several layers of columnar cells with many chloroplasts. At the same time, the sponge parenchyma consists of 4-6 layers of cells with chloroplasts and irregular shapes and air spaces between cells where these chloroplasts function in the process. Photosynthesis (Rita & Animesh, 2011).

Based on the results of observations of longitudinal incisions that were carried out on the leaves of two *Mentha* species, namely *M. piperita* and *M. spicata*, there were results that the epidermal cell walls were in the form of polygonal anticlinals, namely grooved and curved. The cell wall can be curved if indicated without the formation of corners and has inward and outward curves (Febriyani et al., 2022). There are differences in the shape of the epidermal cells, but the epidermis is a uniform tissue (Anu et al., 2017). The similarity in the shape of the epidermal cell walls in the

two *Mentha species* is because both belong to the same genus, namely *Mentha*. The *Mentha* plant is a genus of the Lamiaceae family, which has approximately 30 species and various *hybrids* and generally grows in sub-tropical areas (Chen et al., 2012). Among the several species of the *Mentha plant*, *Mentha x piperita*, *M. piperita*, and *M. spicata* are the most common in Indonesia.

The results of observations on the type of leaf stomata of both *Mentha species* have the same type, namely the anomocytic type. The type of stomata is anomocytic; the guard cells are surrounded by several cells that are the same shape and size as other epidermal cells (Fauziah & Izzah, 2019). These two *Mentha species* have little or no stomata, and this is following research (Rita & Animesh, 2011). The similarity in the number of stomata in the two *Mentha species* is caused by data collection carried out in the same area so that more or less environmental factors in light intensity, temperature, and humidity do not have much difference. Previous research stated that stomata density can also be influenced by environmental factors, namely temperature, light intensity, and humidity (Sundari & Atmaja, 2017). The number of stomata on the leaves of *M. piperita* and *M. spicata* is small because it is influenced by the environment, where it is known that the habitat of *M. piperita* and *M. spicata* is in cool to cold places, one of which is located in Sidomulyo Village, Batu, Malang. is located at the foot of a mountain to the south of Mount Arjuna and has an ^{altitude} of 800–850 meters above sea level, with an air temperature of 17oC– 25oC and fertile soil conditions (Ningtias, 2022). The number of stomata tends to decrease in an environment with low temperature and high humidity (Paluvi & Mukarlina, 2015).

Trichomes are found on both sides of the leaf, both on the adaxial and abaxial sides in *M. spicata*, while in *M. piperita*, the trichomes are only found on the abaxial side. This aligns with previous research that *M. spicata* has trichomes on both sides of its leaves (Šarić-Kundalić et al., 2016). The types of trichomes found in both species consist of glandular trichomes and non-glandular trichomes. Glandular trichomes are trichomes capable of producing secretions, while non-glandular trichomes are trichomes that do not. The types and forms of non-glandular trichomes are scale hairs, branched (multi-celled) hairs, and single hairs, while the types and forms of glandular trichomes are trichomes, salt glands, oil glands, and itchy hairs. The structure and morphology of trichomes are diverse and can be used as a key to identifying genera, species, subspecies, and varieties from various families (Harisha & Jani, 2013).

Types of trichomes in *M. spicata* are glandular and non-glandular in the abaxial and adaxial epidermis; they consist of unicellular glandular trichomes as well as unicellular and multicellular non-glandular trichomes. *M. Piperita* has a glandular, glandular planet, glandular capitate, and non-glandular trichomes, which are found only in the abaxial epidermis; consists of unicellular glandular trichomes, unicellular plate glandular trichomes, unicellular capitate glandular trichomes and unicellular and multicellular non-glandular trichomes (table 4.1). Unicellular trichomes consist of one constituent cell, while multicellular trichomes consist of more than one constituent cell (Yuliani & Ratnawati, 2018). Variations in the cells make up the trichomes in *M. piperita* and *M. spicata*, including the shape and number of basal cells, stalk cells, and head cells.

M. piperita shows the presence of glandular peltate and glandular capitate trichomes. In the Lamiaceae family, two types of glandular trichomes are found: peltate and capitate. In contrast to *M. spicata*, where no glandular peltate and glandular capitate trichomes were found, this was

because the leaf samples used were not young leaves but leaves at nodes 3-5. The density of glandular trichomes in Lamiaceae will decrease as the organ ages, and the peltate and capitate trichomes will experience damage to the head cell sheath when entering the aging phase of the secretory structure along with the aging of the leaf organs. The trichomes found in plants of the *Coleus* genus, which are members of the Lamiaceae, show morphologically, the head cells of pelvic and capitate glandular trichomes in the three species from the visible presence of essential oils (essential oils) and other secondary metabolites which experience an increase in production at the 1st node and decrease at the nodes 3 and 5. Plant adaptation mechanisms because leaves in the early growth phase require higher protection to protect themselves from predators. Secretory structures are generally capable of secreting substances that are toxic to herbivores. Peltate glandular trichomes that accumulate monoterpenes. These trichomes contain secretory cells responsible for oil synthesis (Rios-Esteva et al., 2010). The difference that the *M. spicata* species has and that is not found in *M. piperita* is the presence of dendroid trichomes (branched trichomes). This follows previous research that branched trichomes (dendroid trichomes) are only found in a few *Mentha* genus species and not in all types (Šarić-Kundalić et al., 2016).

Previous research stated that the formation of trichomes begins with a bulge in the epidermal cells. The bulge can enlarge and grow into multicells, and then various shape modifications occur following growth (Kurrataa'yun, 2013). *Mentha* species, especially parenchyma cells, will undergo a process of proliferation and elongation so that stalk cells develop and glandular trichomes can form. This is in accordance with the statement that essential oils are produced in secretory cells initially originating from parenchymal cells undergoing a differentiation process (Dorly et al., 2015). The phase of trichome formation can be called the pre-secretory phase. The glandular trichomes develop and become complete structures consisting of basal, stalk, and secretory cells. The development phase can continue in the secretory phase; namely, the glandular trichomes can accumulate secondary metabolites optimally, which is characterized by a gradual increase in size.

Secretory cells are special secretory structures that can secrete certain compounds where these compounds are not excreted by plants (Nindyawati & Indriyani, 2017). The secretion results through the secretory are in the form of oil, resin, latex, mineral salts, and various other chemical compounds. Secretory structures can take the form of idioblast cells, secretory cavities, glandular trichomes, non-glandular trichomes, laticifers, resin ducts, and oil glands (Kuster & Vale, 2016) (de Almeida et al., 2020). Secretory cells have a location that can be divided into two, namely internal and external secretory. The internal secretaries are in the form of idioblasts, secretory cavities, secretory ducts, and laticifers, while the external secretaries are in the form of trichomes, nectaries, stigmas, and hydathodes (Dorly et al., 2015). *Mentha essential oil* species found were in two secretory places, namely the internal and external secretories. The internal secretory of essential oils is in idioblast cells. Idioblasts can be found in the mesophyll area, which can be in the form of cell spaces or oil glands, mucus cells, or crystal cells. The external secretory of essential oils is in glandular trichomes. Trichomes in the genus *Mentha* function as storage and as synthesis in the oil glands, following Lange's statement that the essential oils of *Mentha* plants are synthesized and stored in trichomes, namely in the modified epidermis, and are called glandular trichomes. *Mentha* plant trichomes contain secretory cells which are responsible for oil synthesis. Gershenzon and

McCaskill supported that *Mentha*'s biosynthetic processes and accumulation of monoterpenes were specifically localized to the glandular trichomes.

The results of anatomical observations of cross-sectional sections of *M. piperita* and *M. spicata* leaves showed the structure of essential oil glands, which had a round and brownish-yellow color. The structure of the oil glands in both species varies in number, shape, distribution, and location of cells. Oil gland cells in *M. piperita* are distributed in the palisade and spongy parenchyma and cluster in the abaxial epidermis. In contrast, the oil gland cells in *M. spicata* are distributed in the adaxial epidermis (Figure 4.3). This is also supported by the results of longitudinal incisions in the adaxial epidermis of the two *Mentha* species, showing the presence of oil glands that appear only in *M. spicata* species (Figure 4.2).

The number of oil gland cells in the *M. piperita* leaf species shows that it has 20-35 oil gland cells with details of 25-35 oil gland cells in the abaxial epidermis, which has a size of 13.604 μm -24.012 μm and 20-25 oil gland cells are spread out in the palisade parenchyma and spongy parenchyma which have a size of 3.519 μm -8.642 μm while *M. spicata* has several oil glands of 10-15 cells which are on the abaxial side which has a size of 38.986 μm -11.418 μm . This certainly affects the oil content results in each *Mentha* species, where the more oil gland cells, the more oil content will be produced. According to Atmono's statement, the increase in secretory cells aligns with cell division. This is also in line with other studies on the maceration test using 96% pa ethanol solvent with each species using 50g of simplicia powder; the results of the study stated that the *M. piperita* species obtained more oil yield, namely 70.4%, whereas in *M. spicata* obtained less oil yield, namely 69% (Puspitasari et al., 2021).

The shape of the oil gland cells in the leaves of *M. piperita* and *M. spicata*, as seen in longitudinal sections, is round and spread out (Figure 4.4). There are variations in the characteristics of oil glands, namely in size and number in one field of view. *M. Piperita* has more oil gland cells than *M. spicata*, namely 698, while *M. spicata* has several oil gland cells, 482. There is a relationship between the density of gland trichomes and the results of essential oil content (Gupta et al., 2017), (Mishra et al., 2018). The diameter of the *M. piperita* oil gland cells is larger than that of *M. spicata*, namely 3.124 μm - 6.719 μm , while *M. spicata* has an oil gland cell diameter of 2.112 μm - 3.734 μm . The increase in size of secretory cells containing essential oil glands can be in line with growth, which includes the process of cell and tissue expansion. The greater the density of the oil glands and the larger the size of the oil glands, the more essential oil can be produced.

Species from the *Mentha* genus are known to have medicinal and commercial importance (Chen et al., 2012) ; (Jeyakumar et al., 2011). Previous research stated that the *M. piperita* species had the highest total phenolic content, followed by *M. spicata* (Zaidi & Dahiya, 2015). This is also supported by (Gharib & da Silva, 2013) ; (Naidu et al., 2012). Furthermore, in other research, phytochemical screening tests were obtained and the results showed that two *Mentha* species contained flavonoids, steroids, saponins, and tannins (Puspitasari et al., 2021). This is also supported by previous research on phytochemical screening tests showing the presence of various bioactive substances such as phenols and tannins, flavonoids, glycosides, and alkaloids in methanol (Khanal, 2020). *Mentha*'s antioxidant properties come from the content of active components such as menthone, menthol, rosmarinic acid, and carvone (Lawrence, 2013).

Other previous studies stated that the essential oil content of *M. piperita* and *M. spicata* species found antimicrobial activity against clinical isolates, phytochemicals, phenolic content, and bioactive compounds, which are responsible for antibacterial potential by TLC (*Thin-Layer Chromatography*) bioautographic analysis (Zaidi & Dahiya, 2015). Several previous studies also revealed that there was antibacterial activity in *M. piperita* and *M. spicata* species, namely *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp* and also had antifungal activity against *Aspergillus spp* . and *Candida albicans* (Sujana et al., 2013) ; (Jeyakumar et al., 2011) ; (CHAUHAN & AGARWAL, 2013). This correlates with studies where the essential oil content of *M. piperita* and *M. spicata* species showed antibacterial activity (Singh et al., 2015). Thus, the species *M. piperita* and *M. spicata* can be said to contain essential oils, which are a good source of natural antimicrobial agents.

CONCLUSION

Based on the results of research on the anatomical structure of the leaves of two *Mentha* species, it shows that *M. piperita* has a greater number of essential oil glands compared to *M. spicata*. *M. piperita* in longitudinal section has 698 oil gland cells, while *M. spicata* has 482 oil gland cells. *M. piperita* in transverse section has 20-35 oil gland cells, while *M. spicata* has 10-15 oil gland cells. The diameter of the oil gland cells of *M. piperita* is larger than that of *M. spicata*, namely 3.124µm - 6.719µm, while *M. spicata* has an oil gland cell diameter of 2.112µm - 3.734µm. These results certainly affect the results of the essential oil produced.

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