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Insecticidal Potential of *Ocimum basilicum* Leaves: Metabolite Distribution in Different Leaf Tissues

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ABSTRACT

Leaves serve as essential plant organs that facilitate photosynthesis and consist of several layers, such as the mesophyll and epidermis, each of which possesses unique metabolite compositions. These metabolites play a role in the plant's defensive system against insects. For instance, the leaves of *Ocimum basilicum* L. (basil) possess biocidal properties against a variety of insects. Although the insecticidal properties of these leaves have been well documented, the distribution studies on the leaf metabolites are inadequate. Thus, this study examined the metabolite profiles of the two leaf layers, epidermis and mesophylls. The separation of epidermis and mesophyll extracts was accomplished using whetstone powder, followed by gas chromatography-mass spectrometry to analyze the obtained metabolite profiles. The leaf trichomes were examined by scanning electron microscopy. Certain chemicals were only detectable within the epidermal or mesophyll tissues. For example, tricosane (16.37%) and geraniol (7.88%) were exclusively detected in the epidermis, whereas limonene oxide (1.26%) and α-humulene (1.04%) were only detected in the mesophyll. Furthermore, certain components were found in higher quantities in the epidermis and mesophyll layers, whereas others were more prevalent in the opposite layer. Our findings relevant to the trichome types, specifically glandular and non-glandular trichomes, indicated that both play a role in the initial defenses against herbivorous insects. This study offers significant insights into the chemicals that serve as plant defenses in basil leaf tissue and trichomes. Future studies on the distribution of chemical compounds in different leaf tissues can provide further

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insights into the mechanisms of plant-insect interaction and facilitate the development of strategies for identifying compounds that play a role in defense.

Keywords: Basil, epidermis, localization, gas chromatography-mass spectrometry, metabolite profile

INTRODUCTION

Plants synthesize a wide array of chemical molecules and metabolites, including both primary and secondary metabolites. Primary metabolites are crucial in fundamental biological activities, including growth, cell division, photosynthesis, and respiration. In contrast, secondary metabolites interact between plants and their surrounding environment. Secondary metabolites are crucial in plant defense against herbivores and pathogens (Aharoni & Galili, 2011; Cavalier-Smith, 2007; Chaudhary et al., 2018; Erb & Kliebenstein, 2020). However, their structural diversity and accumulation characteristics in specific plant compartments remain poorly understood (Hadacek et al., 2011). Thus, specific secondary metabolites and their accumulation may play distinct roles against diverse herbivores (Berenbaum & Feeny, 1981; Nuringtyas et al., 2012).

Herbivorous insects attack plants by eating plant parts, selecting specific plant organs such as leaves, stems and roots, or only eating specific plant tissues (War et al., 2018). It raises the question about the effect of the way an herbivorous insect eats plants on the quality and quantity of mechanical damage to plant tissues. Leaf defoliators, such as caterpillars (Lepidoptera), cause tissue damage by chewing, cutting, and tearing. Leaf miners feed on soft plant parts between epidermal cells or mesophyll tissue layers in the leaves. Piercing/sucking insects such as thrips (Thysanoptera) have tube-like structures that suck fluid from lateral cells and the epidermis. Phloem-suckers such as aphids (Hemiptera) possess stylets that penetrate cells all the way into the phloem (Chaudhary et al., 2018; Fürstenberg-Hägg et al., 2013). When chewing insects attack plants, volatile organic compounds are released, attracting herbivorous insect enemies to reduce herbivorous insect numbers (Fürstenberg-Hägg et al., 2013). Therefore, chemical compounds and their composition in different organs and cell types are expressed differently to optimize plant defenses (Martin et al., 2001; Nuringtyas et al., 2012).

The *Ocimum* genus possesses abundant and diverse secondary metabolites, including terpenoids and phenolics, which may be involved in plant defenses. However, neither the species' defense mechanisms nor secondary metabolite roles have been well characterized, although the insecticidal activity of plant leaves against several insects has been reported. One of the *Ocimum* genus species reported to have insecticidal activity is *Ocimum kilimandscharicus*. This species reportedly possesses rich secondary metabolites such as eucalyptol, camphor, limonene, germacrene D, and β-caryophyllene (Singh et al., 2014). Another investigation was conducted to determine the metabolite profile of *Ocimum gratissimum* essential

oil (EO). The findings revealed that thymol and *p*-cymene predominate EO, whereas carvacrol and thymol are predominant in the ethanolic and aqueous extracts, respectively, along with shikimic acid and rosmarinic acid (Benelli et al., 2019). In addition, the EO of *O. basilicum* possesses noteworthy biological activity against insect pests. Chromatographic analysis of the oil revealed the presence of estragole and linalool (Boulamtat et al., 2021; da Silva Moura et al., 2020), methyl cinnamate, eugenol, 1,8-cineole, α-cadinol, α-bergamotene (Chaaban et al., 2019), and methyl eugenol (Govindarajan et al., 2013). Nevertheless, there has been a lack of research on the distribution of these metabolites within leaf tissues. Investigating the metabolite profile via mass spectrometry (MS) and its distribution in leaf tissues can help identify potential biopesticides and their mechanism of protecting plants from insects.

Ocimum basilicum exerts biocide activity against different herbivorous insects, possibly caused by glandular and non-glandular trichomes in the epidermis. Trichomes are important tissues that support plant defense systems; they facilitate plant resistance against herbivores via physical and chemical deterrents (Fürstenberg-Hägg et al., 2013). In addition to its role as a biopesticide, studies on *O. basilicum* have reported its role in supporting health. For instance, *O. basilicum* possesses antioxidant properties (Srivastava et al., 2016), therapeutic potential (Bensaid et al., 2022), and anti-acetylcholinesterase activity (Farag et al., 2016).

The carborundum abrasion (CA) method has been extensively employed for abrading the epidermis and mesophyll. Murata and De Luca (2005) implemented this method to collect epidermis samples to investigate alkaloid metabolites, enzyme activity, and gene expression levels. In addition, this technique allowed mRNA extraction from the leaf epidermis to assess the spatial distribution of indole alkaloid biosynthesis (Murata et al., 2008). According to Nuringtyas et al. (2012), the differences in the metabolic profiles were investigated between the epidermal and mesophyll tissues of *Jacobea vulgaris* and *Jacobea aquatica* using CA. A similar approach was employed to investigate the accumulation of putative photoprotective group chemicals (Ilmiah et al., 2018). This study involved the optimization of CA to achieve the full removal of the leaf epidermis using whetstone powder. The fundamental idea behind this approach is to use silicon carbide powder to abrade the epidermal layer.

The current research on plant defense chemicals detected in basil leaf tissues is limited, and no studies exist that distinguish compounds in different tissue layers of basil leaves. In fact, the existing research has focused only on the content of secondary metabolites in basil. For instance, Chaaban et al. (2019), da Silva Moura et al. (2020), and Govindarajan et al. (2013) only evaluated the activity of crude basil extracts against insect pests. The present study is the first on defense chemicals found in different tissue layers of basil leaves. In this work,

the compounds in basil leaf tissues were separated using whetstone powder and the component distribution was analyzed. The current investigation serves as the first attempt to gain a deeper understanding of the underlying mechanisms of plant defense in basil leaf tissues by examining metabolite and trichrome profiles.

MATERIALS AND METHODS

Plant Material

In June 2022, fresh basil leaf samples were collected from Sengi Village, Magelang, Central Java, Indonesia (7°31'31.5''S, 110°21'47.0''E). The leaves were removed from plants that had just entered the flowering phase, which is high in EO production (Marotti et al., 1996). Basil plant species were authenticated at the Plant Systematic Laboratory, Faculty of Biology, Universitas Gadjah Mada, Indonesia.

Epidermis and Mesophyll Extraction

Epidermal extraction was performed using a modified whetstone powder approach from Ilmiah et al. (2018). Briefly, the abaxial and adaxial epidermis layers were lightly rubbed six times using whetstone powder. The abraded leaves were then placed into a 50-ml conical tube with *n*-hexane solvent and vortexed for 1 min to separate the powder from the leaves. The extract was then filtered and placed on a porcelain dish to allow solvent evaporation. The abraded leaves were defined as mesophyll, removed from the conical tube, and ground in a blender. The fine-ground leaves were then extracted by maceration in *n*-hexane solvent for 24 hr at the leaf: solvent ratio of 1:5. After 24 hr, the mesophyll extract was filtered and poured into a porcelain dish to allow solvent evaporation. The samples were then stored at 4°C until further requirement.

Microscopic Analysis

Microscopic observations of the leaf surfaces before and after whetstone powder extraction were performed to compare the epidermis layers. Before microscopy, longitudinal leaf sections were prepared using a sliding microtome (REICHERT Nr. 338 262, Austria). Subsequently, the specimens were observed under light microscopy (BOECO, BM-180, Germany) at 100× magnification.

Gas Chromatography-Mass Spectrometry (GC–MS) Metabolite Analysis of the Epidermis and Mesophyll

GC-MS was conducted at the Department of Organic Chemistry, Universitas Gadjah Mada, using (GCMS-QP2010S, Shimadzu Corporation, Japan) with single-quadrupole MS attached to a DB-5MS capillary column operated in the electronic ionization mode at 70 eV. The dried extract was diluted again with the solvent. Helium was used as the carrier gas, and the analyses were conducted using the splitless method with the following settings: column oven temperature = 60°C, sampling time = 1.00 min, flow control mode: pressure, injection temperature = 300°C, pressure = 16.5 kPa, total flow = 30.5 ml/min, column flow = 0.55 ml/min, linear

velocity = 27.1 cm/s, and purge flow = 3.0 ml/min. Finally, the chromatogram peaks were analyzed.

Data Analysis

GC-MS chromatogram data were cleaned by eliminating the MS values that resembled impurities. The MS splitting patterns were compared with the MS information in PubChem and KNApSAck-3D databases (http://kanaya.naist.jp/knapsack esp top.html) to validate and identify compounds. The mass spectral interpretation was based on compounds with similarity indices >86% (Dahibhate et al., 2022). The compounds were assembled into a heatmap plot using GraphPad Prism 9 for better representation. A higher value set percentage area was set for 15, and all values >15 were colored as the highest concentration.

Scanning Electron Microscopy (SEM) Analysis

SEM analysis was conducted at the Integrated Research and Testing Laboratory, Universitas Gadjah Mada. The samples were sliced into ± 5 × 7-mm sections and affixed to carbon tape above the specimen holder. The samples were then inserted into the auto coater. A vacuum coater machine applied a pressure of ±3.2 Pa, followed by coating with a layer of gold. Subsequently, the samples were placed into the SEM and subjected to vacuum, and electrons fired the sample with a certain level probe. The upper and lower epidermal surface observations were then recorded.

RESULTS

Epidermis and Mesophyll Abrasion

Epidermal abrasion using whetstone powder is depicted in Figures 1a and 1b. Observations were made using cross-sectional preparations under microscopy. This approach successfully eroded the epidermis by lightly rubbing the surface six times. Abrasion treatment with whetstone powder could successfully erode basil leaves' adaxial and abaxial epidermis layers.

Compound Identification by GC–MS

Epidermis extract GC–MS chromatograms displayed 28 peaks (Figure 2a), whereas the mesophyll extracts showed 46 peaks with varying percentages (Figure 2b). Different secondary metabolites were

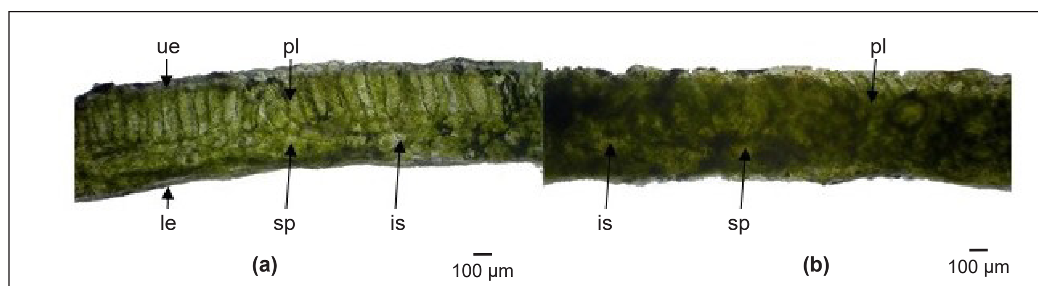

Figure 1. Microscopic comparison of *Ocimum basilicum* leaf cross-sections before (a) and after (b) whetstone powder abrasion: the upper epidermis (ue), palisade (pl), sponge (sp), intercellular space (is), and lower epidermis (le)

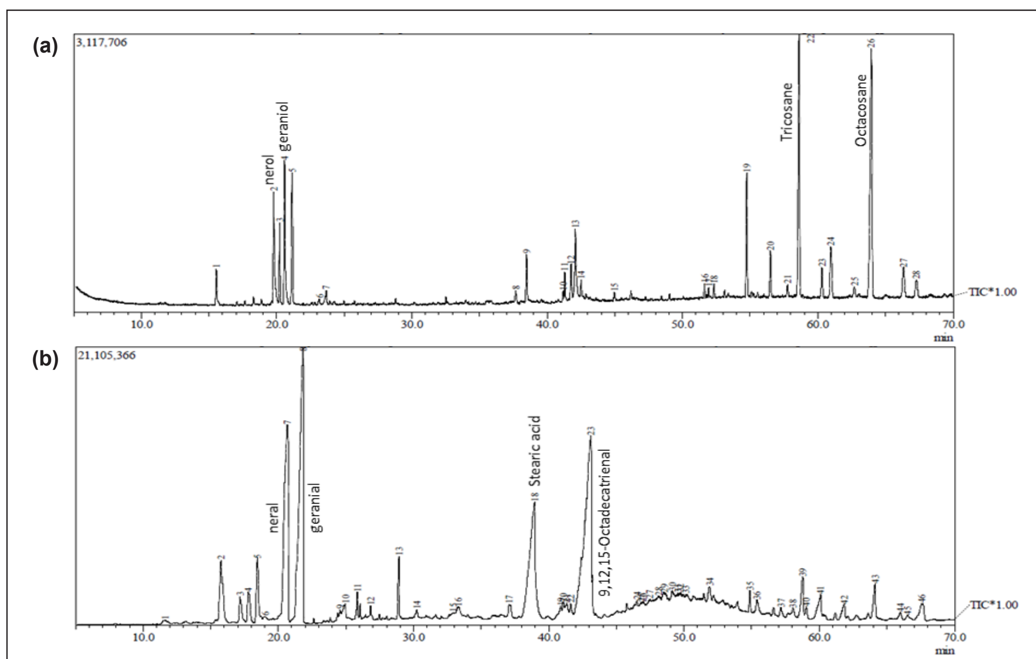

Figure 2. Gas chromatography-mass spectrometry chromatograms of bioactive components from basil (*Ocimum basilicum*) leaf: (a) epidermis and (b) mesophyll extracts, respectively

identified using chromatograms' mass spectra and compound detection (Table 1). A typically different chromatogram of the different tissues was observed immediately, wherein the epidermis showed significant signals at retention time (RT) of 58.62 and 65.95 min, corresponding to tricosane and octacosane. The mesophyll metabolite profiles had significant signals at RT 20.23, 21.15, 42.49, and 43.10 min, which were identified as neral, geranial, stearic acid, and 9,12,15-octadecatrienal, respectively.

A heatmap of the basil leaf epidermis and mesophyll compounds was assembled to visualize metabolite distribution (based on the compound percentage areas; Figure 3). Dark blue indicates a high compound concentration, while white indicates low concentrations. Several compounds, including tricosane, octacosane, nerol, and geraniol, were detected at high concentrations in the epidermal extracts. In contrast, the mesophyll extracts detected neral, geranial, stearic acid, and 9,12,15 octadecatrienal at high concentrations.

Trichome Analysis Using SEM

Trichome morphology and distribution were compared on the adaxial and abaxial leaf surfaces (Figure 4). SEM revealed that the basil leaves had two trichome types, glandular and non-glandular (Figures 4a and b), which were nonuniformly distributed on the adaxial and abaxial epidermis surfaces. The adaxial sections showed more non-glandular trichomes, whereas the abaxial sections showed more glandular trichomes (Figure 4d).

Table 1
Bioactive compounds in the basil leaf epidermis and mesophyll extracts

No.	RT	Compounds	Groups	MF	MW	Area (%)	
						Epidermis	Mesophyll
1.	15.56	1,6-Octadien-3-ol, 3,7-dimethyl- (linalool)	Monoterpene	$C_{10}H_{18}O$	154	1.82	3.27
2.	17.79	Limonene oxide	Monoterpene	$C_{10}H_{16}O$	152	-	1.26
3.	18.45	Limonene epoxide	Monoterpene	$C_{10}H_{16}O$	152	-	2.56
4.	19.79	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)- (nerol)	Monoterpene	$C_{10}H_{18}O$	154	**7.25**	1.08
5.	20.23	2,6-Octadienal, 3,7-dimethyl-, (Z)- (neral)	Monoterpene	$C_{10}H_{16}O$	152	3.38	**14.64**
6.	20.59	2,6-Octadien-1-ol, 3,7-dimethyl-, (E)- (geraniol)	Monoterpene	$C_{10}H_{18}O$	154	**7.88**	-
7.	21.15	2,6-Octadienal, 3,7-dimethyl-, (E)- (geranial)	Monoterpene	$C_{10}H_{16}O$	152	4.96	**18.13**
8.	24.96	4-Methyl-3-penten-1-ol	Homoallylic alcohol	$C_6H_{12}O$	100	-	1.00
9.	28.90	alpha.-Humulene	Sesquiterpene	$C_{15}H_{24}$	204	-	1.04
10.	38.49	Hexadecanoic acid (palmitic acid)	Fatty acid	$C_{16}H_{32}O_2$	256	2.26	-
11.	41.29	11-Octadecenoic acid, methyl ester	Fatty acid	$C_{19}H_{36}O_2$	296	1.18	-
12.	42.10	9-Octadecenal	Fatty aldehyde	$C_{18}H_{34}O$	266	5.03	-
13.	42.49	Stearic acid	Fatty acid	$C_{18}H_{36}O_2$	284	1.23	**12.00**
14.	43.10	9,12,15-Octadecatrienal	Linolenyl aldehyde	$C_{18}H_{30}O$	262	-	**21.52**
15.	48.55	1-Octanol, 2-butyl-	Fatty alcohol	$C_{12}H_{26}O$	186	-	1.14
16.	54.74	Dotriacontane	Alkanes	$C_{32}H_{66}$	450	5.72	-
17.	56.52	Pentacosane	Alkanes	$C_{22}H_{52}$	352	2.29	-
18.	58.62	Tricosane	Alkanes	$C_{23}H_{48}$	324	**16.37**	-
19.	58.78	Tetradecane	Alkanes	$C_{14}H_{30}$	198	-	1.53
20.	60.31	Eicosane	Alkanes	$C_{20}H_{42}$	282	1.96	-
21.	60.98	Nonacosane	Alkanes	$C_{29}H_{60}$	408	3.60	-
22.	63.95	Octacosane	Alkanes	$C_{28}H_{58}$	394	**22.43**	1.35
23.	66.34	Hexatriacontane	Alkanes	$C_{36}H_{74}$	507	2.87	-
24.	67.28	Eicosane, 2-methyl-	Alkanes	$C_{21}H_{44}$	296	1.74	-

Note. Bold numbers indicate the major compounds in the extracts; RT = Retention time; MF = Molecular formula; MW = Molecular weight

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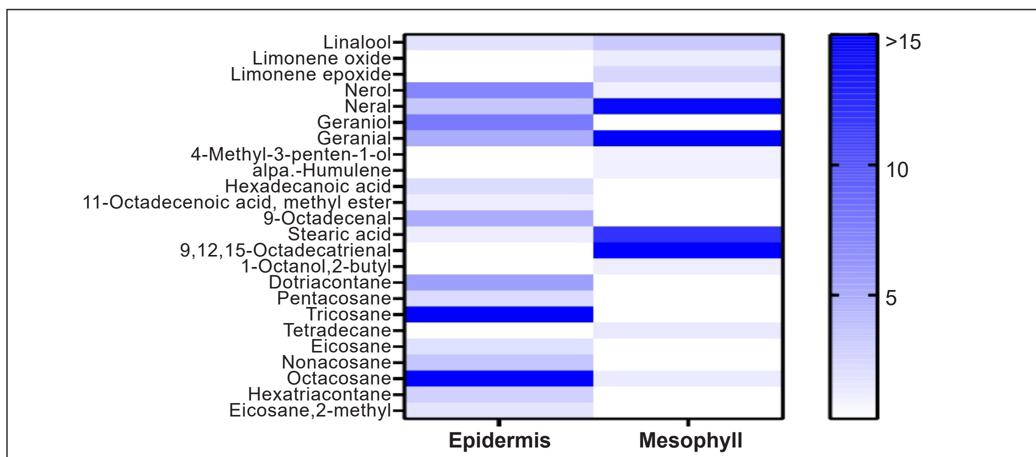

Figure 3. Heatmap showing epidermis and mesophyll basil leaf extracts based on gas chromatography-mass spectrometry analyses

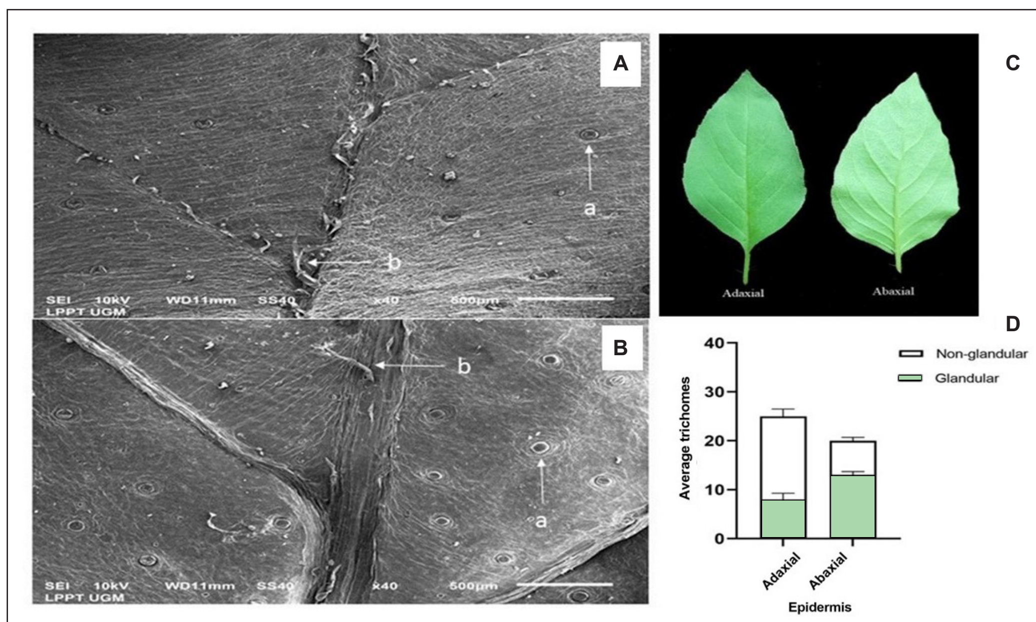

Figure 4. *Ocimum basilicum* leaves. Trichomes on adaxial (A) and abaxial (B) leaves (scanning electron microscopy): glandular trichomes (a) and non-glandular trichomes (b). The physical appearance of basil leaves (C) and the average numbers of adaxial and abaxial trichomes (D), respectively

DISCUSSION

Until recently, leaf metabolite profiling was largely conducted without considering the secondary metabolite distribution across different tissues. Different insect types must be considered when examining secondary metabolites as defense chemicals. For instance, sucking insects are exposed to different chemicals when compared with chewing insects, which consume the entire

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plant's leaves (Nuringtyas et al., 2012). In our study, the epidermis and mesophyll layers were used to determine the location of chemical compounds or chemical collections. A comparative investigation of metabolites in these layers may provide insights into the metabolite accumulation in different tissues and their roles as defense chemicals against herbivore insects and pathogens.

The CA method is the most common and straightforward method of isolating the epidermis (Ilmiah et al., 2018; Murata et al., 2008; Nuringtyas et al., 2012). Ilmiah et al. (2018) used whetstone powder to replace CA because it could not erode the *Sonneratia caseolaris* (L.) leaf epidermis. Several factors can affect tissue separation, including the leaf anatomy, cell wall thickness, and the pressure applied to leaves (Ilmiah et al., 2018). The basil leaves epidermis (upper and lower) and mesophyll tissues were effectively separated using whetstone powder, as shown in Figure 1. When compared with CA, whetstone powder offers several advantages, such as its ample availability and lesser cost. Therefore, whetstone powder was used to separate and isolate plant tissues in this study.

Lamiaceae plants are distinguished by their ability to synthesize the most volatile terpenes in glandular trichomes. These organs possess a high concentration of volatile compounds, enhancing their botanical attributes as aromatic plants (de Sena Filho et al., 2023). EO derived from *Ocimum*, a member of the Lamiaceae family, is commonly obtained from hydrodistillation. It has been documented to possess various properties, such as antibacterial and biopesticide activities (da Silva Moura et al., 2020; Sneha et al., 2022). The selection of maceration with n-hexane as the solvent in this study was based on a previous study conducted by Kayesth et al. (2018). Their study demonstrated the discovery of various chemical compounds with insecticidal properties. This finding aligns with the objective of the present study, which identifies a wider range of chemicals hypothesized to contribute to plant defense mechanisms.

In a comparison of compounds between the epidermis and mesophyll tissue extracts by GC–MS, several compounds were observed only in the epidermis or mesophyll tissues. In addition, other compounds were more abundant in the epidermis or mesophyll layers and *vice versa* (Table 1; Figure 3). Geraniol is a monoterpenoid alcohol that contains a mixture of two cis-trans isomers-geraniol and nerol (Chen & Viljoen, 2010). Both were sevenfold higher in the epidermis than in the mesophyll tissues (Table 1). Research has indicated the presence of geraniol as a rich component in EOs of plants such as *Cymbopogon* spp. and *Pelargonium graveolens* (Dangol et al., 2023; Džamić et al., 2014).

As common components of some EOs, geraniol and nerol have promising biological properties. Both possess strong antifungal activities against *Fusarium* species, such as *Fusarium verticillioides* (Brito et al., 2019) and *Fusarium graminearum* (Krzysko-Łupicka et al., 2019). Geraniol demonstrated

fumigant potency against common pests in stored products, *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Lipocelis bostrychophila* (Psocodea: Liposcelididae) (Quan et al., 2018), along with *Tuta absoluta* (Lepidoptera: Gelechiidae), a major tomato plant pest (Rahmani & Azimi, 2020). Thus, geraniol and nerol in epidermis layers may act as first-line defense mechanisms against pathogens or pests.

Citral is a mixture of monoterpenoid aldehydes, predominantly neral and geranial, and has higher area percentages in the mesophyll tissues (Table 1). The percentage area of neral in mesophyll was fivefold higher than in the epidermis tissues. In addition, the percentage area for geranial cells increased fourfold in the mesophyll when compared with other compounds. High citral accumulation in the mesophyll was also reported in mesophyll lemongrass (*Cymbopogon flexuasus*), determined using a histochemical approach (Lewinsohn et al., 1998). Furthermore, Dancewicz et al. (2020) showed that citral is a strong repellent and functioned as a pre-and ingestive probing deterrent to *Myzus persicae* (Hemiptera: Aphididae). Citral was also found responsible for the insecticidal activity of lemongrass extracts against *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (de Oliveira et al., 2018).

Citral and geraniol are often investigated with respect to their biopesticide and pharmaceutical characteristics. In this study, the accumulation in different tissues observed for geraniol and nerol when compared with the citral component, that is, geranial and neral, is interesting to observe. In terms of biosynthesis, these monoterpenes are derived from the same pathways, either through the cytoplasmic mevalonate pathway or the plastid 2-C-methyl-d-erythritol 4-phosphate pathway (Lewinsohn et al., 1998). However, specific metabolite accumulation has been associated with specialized secretory structures inside leaves, such as glandular trichomes, resin ducts, and glandular epidermis (Luthra et al., 2017). Neral and geranial citral components are obtained as nerols and geraniol oxidation products (Kanehisa & Goto, 2000).

Distribution differences were observed in comparison of GC–MS-identified compounds in the epidermis and mesophyll tissues (Table 1). Compounds identified only in the epidermis tissues with significant area percentages include tricosane and octacosane. Concurrently, compounds identified only in mesophyll tissues with significant area percentages include stearic acid and 9,12,15-octadecatrienal. The higher stearic acid levels observed in this study are consistent with the report of Lessire and Stumpf (1983), who reported that palmitic and stearic acid distributions were localized. Palmitic acid is a major product of epidermis cells, whereas stearic and palmitic acids are in parenchymal cells.

Tricosane is an oil component in plants and insects. It is extensively distributed and reportedly occurs in more than 50 plant species. However, the role of this compound in plant resistance remains unclear. Al-Maawali et al. (2021) reported that tricosane is an antifungal compound that acted against

tomato rot caused by *Alternaria alternata*. In insects, tricosane is a common female pheromone. In addition, some insects may exploit these compounds to assist hormone formation. Interestingly, this compound was also found in insect frass and functioned as an oviposition deterrent for the potato tubeworm moth *Phthorimaea operculella* (Lepidoptera: Gelechiidae) (Zhang et al., 2019).

Other specific compounds in tissue extracts were also identified, and their biological activities have been previously reported. In mesophyll tissue extracts, limonene oxide and α-humulene were identified. Limonene oxide was reported as a repellent for the tomato leaf miner *Tuta absoluta* (Miano et al., 2022). The α-humulene bioavailability in plants may indicate plant defense responses. Critically, *Spodoptera litura* (Lepidoptera: Noctuidae) reacted to this compound and may have signaling roles when locating hosts (Jönsson & Anderson, 1999).

In our study, 9,12,15-octadecatrienal was the most abundant compound in the mesophyll and reportedly regulated *Plutella xylostella* L. (diamond moth) (Lepidoptera: Plutellidae) behavior on pakchoi leaves (Zhandi et al., 2021). Thus, 9,12,15-octadecatrienal could be used to develop lead compounds for the green control of *P. xylostella* (Zhandi et al., 2021). *Plutella xylostella* attacks many vegetables, especially the Brassicaceae family, and targets young and old leaves during the larval stages.

The presence of other phenylpropenes was not detected because of several factors. Research conducted by Ahmed et al. (2019) explained that the chemical composition of *O. basilicum* can change according to the geographical location. In addition, research conducted by Dmitruk et al. (2019) also mentioned that the composition of EOs and their quantitative content depend on the part of the plant used and the plant's growth stage during the extraction.

Two major glandular and non-glandular trichome types were observed on basil leaves. Physical and chemical deterrents showcase first-line plant defense roles against herbivorous insects (Peiffer et al., 2009; Tian et al., 2012; Wang et al., 2021). Kariyat et al. (2017) demonstrated that non-glandular *Solanum* spp. trichomes functioned as mechanical post-ingestive defense mechanisms by harming the peritrophic matrix in caterpillars. More non-glandular trichomes were observed on basil adaxial leaf surfaces than on glandular trichomes, but the opposite was observed on abaxial leaf surfaces. Reportedly, jasmonic acid may account for this variation in trichrome synthesis (Tian et al., 2012; Traw & Bergelson, 2003; van Schie et al., 2007). Moreover, several studies demonstrated that glandular and non-glandular trichome density levels on leaves were possibly influenced by environmental factors, such as water availability and herbivore presence (Gonzáles et al., 2008; Rautio et al., 2002; Sá et al., 2016; Sletvold et al., 2010).

When defending against herbivorous insects, glandular trichomes also contain chemicals such as volatile terpenes (Iijima et al., 2004; Peiffer et al., 2009), which

release alkaloids, repellents, and poisonous compounds (Wang et al., 2021). Gang et al. (2002) reported that enzymes involved in phenylpropene production (such as chavicol, eugenol, and derivatives) were localized in the trichomes of basil leaves. Xie et al. (2008) also characterized the terpenoid metabolism processes in basil leaf trichomes.

CONCLUSION

In this study, the epidermal and mesophyll tissue metabolite profiles in basil leaves were successfully distinguished using the CA technique. This technique allows the identification of several chemicals with varying concentrations in distinct layers. The localization of promising compounds, geraniol and citral, respectively, in the epidermis and mesophyll layer of *O. basilicum*, leaves implies that different tissues play different roles in plant defense against pests. Moreover, distinct trichrome distribution patterns were observed on basil leaves' upper and lower epidermal surfaces. The results of our study offer more knowledge regarding the mechanisms of plant defense *in O. basilicum* leaf tissues.

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