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Selection of Four Lamiaceae Species (*Ocimum americanum* L., *Ocimum basilicum* L., *Leucas lavandulifolia* Sm., and *Perilla frutescens* (L.) Britton) as Antioxidant Sources and Metabolite Profile

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ABSTRACT. The Lamiaceae family which are widely used in traditional medicine in Indonesia. Plants from this family are known to have antioxidant bioactivity. Therefore, in this study, an antioxidant test was carried out first as an initial screening. This study evaluated the antioxidant capacity of four Lamiaceae species: *Ocimum americanum* L., *Ocimum basilicum* L., *Leucas lavandulifolia* Sm., and *Perilla frutescens* (L.) Britton. The four Lamiaceae species extracts were evaluated for their antioxidant capacity using 2,2-diphenyl-1-picrylhydrazyl (DPPH), cupric-reducing antioxidant capacity (CUPRAC), and ferric-reducing antioxidant power (FRAP). The highest activity extract was fractionated with *n*-hexane, ethyl acetate, and ethanol-water, and the results were tested for antioxidant capacity. The most active extract and most active fraction were analyzed for metabolites using liquid chromatography-mass spectrometry (LC-MS/MS). The results show that the ethanol 50% leaves extracts of *O. americanum* L. have the highest antioxidant capacity of about 0.439 (DPPH); 1.517 (CUPRAC); and 1.021 (FRAP) mmol ascorbic acid equivalent (AAE)/g extract. The highest antioxidant capacity from the partition results was possessed by the ethyl acetate fraction with a value of 1.109 (DPPH); 1.540 (CUPRAC); and 1.551 (FRAP) mmol AAE/g fraction. Metabolite analysis using LC-MS/MS succeeded in identifying 18 metabolites consisting of flavonoids, terpenoids, amino acids, phenolic acids, fatty acids, and other carboxylic acids.

Keywords: Antioxidant, *Leucas lavandulifolia*, *Ocimum americanum*, *Ocimum basilicum*, *Perilla frutescens*

INTRODUCTION

For generations, Indonesian people have used many medicinal plants in their daily lives. One of the plant families that is widely used as traditional medicine in Indonesia is Lamiaceae. Lamiaceae species are known to have medicinal properties, have a strong history of use since ancient times, and are famous for their high essential oil content (Handayani, 2015). Plants from this family are widely used by the community as wound healers, antibacterials, antioxidants, antifungals, anti-inflammatories, diuretics, pain relievers, and antidotes to infections (Venkateshappa and Sreenath 2013). One of the widely reported bioactivities in this family is as an antioxidant. Antioxidant activity is important to study because it can be used as an initial screening for determining other bioactivities.

Antioxidants are chemical compounds that can inhibit oxidative stress. Oxidative stress is damage to lipids, proteins, or DNA due to the presence of excessive reactive oxygen species (ROS) (Yousuf et al., 2021). Oxidative stress can cause several chronic diseases such as diabetes, chronic lung, chronic inflammation, cancer, coronary heart disease, premature aging, and neurodegenerative. These diseases can be avoided by consuming foods rich in antioxidants which can inhibit or inactivate ROS. Apart from protecting the body from the effects of excess ROS, antioxidants also play a role in protecting food and pharmaceutical products against oxidative damage (Gulcin, 2020). Therefore, in recent years, the identification of natural and safe sources of antioxidants, one of which comes from medicinal plants, has been of interest.

The types of Lamiaceae plants that are often found in Indonesia and have the potential to act as antioxidants are *Leucas lavandulifolia* Sm. (lenglengan) (Ramani et al., 2012), *Ocimum americanum* L. (kemangi) (Karau et al., 2015), *Ocimum basilicum* L. (selasih) (Teofilović et al., 2017), and *Perilla frutescens* (L.) Britton (perilla) (Shang et al., 2023). Previous research has proven the existence of antioxidant activity in these four plants. However, no one has yet selected the antioxidant activity of the four, so an antioxidant activity test is needed to determine the level of antioxidant activity of these plants.

Lamiaceae plants contain many phenolic compounds, polyphenols, and essential oils (Frezza et al., 2019), but not all of them play an active role as antioxidants. Therefore, it is important to know the active metabolites that act as antioxidants. Different plants will have different activities, because the metabolites they contain are also different. Several factors also affect the content of extracted plant metabolites, such as differences in extraction methods and differences in extraction solvents (Sayuti, 2023). One extraction method that is rarely used in research but is widely used in industry is the autoclave extraction (AE) method. The AE method has the advantages of higher extraction yields, increasing the bioactivity of bioactive compounds, saving time and solvents, being efficient in extracting macromolecules, and is considered a promising method for extracting active ingredients (Kim et al., 2016; Ko et al., 2015). Previous studies showing active compounds that act as antioxidants among the four plants extracted using the AE method are still few. Therefore, it is necessary to identify metabolite profiles that act as antioxidants in these plants. This research carried out a selection of antioxidant activity from the plant extracts of *O. americanum* L., *O. basilicum* L.*, L. lavandulifolia* Sm., and *P. frutescens* (L.) Britton. as well as searching for active metabolites that have potential as antioxidants through compound identification using liquid chromatography-mass spectrometry (LC-MS /MS).

EXPERIMENTAL SECTION

Material

The sample used in this study was *O. americanum* L. (determination result letter No. 7767/IT1.C11.2/ TA.00/2022) and *O. basilicum* L. (determination result letter No. 668/IT1.C11.2/TA.00/2022) taken in Bogor Regency at coordinates -6.54714 LS, 106.71637 BT, *P. frutescens* (L.) Britton (determination result letter No. 193/1T1.C11.2/TA.00/2022) taken in Sukabumi at coordinates -6.85250 LS, 106.95306 BT, and *L. lavandulifolia* Sm. (determination result letter No. 668/IT1.C11.2/TA.00/2022) taken in Padang at coordinates -0.82061 LS, 100.32421 BT. The materials used in this research were ethanol, ethyl acetate, *n*-hexane, chloroform, methanol, copper(II) chloride, iron(III) chloride, ammonium acetate, dimethyl sulfoxide (DMSO) (Merck, Darmstadt, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, neocuproine, 2,4,6-tripiridyl-s-triazine (TPTZ) (Sigma-Aldrich, Steinheim, Germany).

Sample Preparation and Determination of Water Content

The four samples were determined at the School of Biological Sciences and Engineering, Bandung Institute of Technology. The leaves were taken from each sample, then cleaned and washed with water. Next, the samples were dried in an oven at 45 °C until dry. After drying, the samples were ground to a size of ±80 mesh. Determination of water content refers to AOAC (2000) with modification. The empty porcelain cup was dried in an oven at 105 °C for up to 30 minutes, then cooled for 30 minutes in a desiccator, and the empty weight was weighed. A sample of 3 g was added to the cup, then dried in an oven at 105 °C for 5 hours. After cooling in a desiccator for 30 minutes, the cup containing the sample was weighed. The same thing was done with a drying time of 1 hour until a constant weight was obtained. Water content is expressed as the percentage of sample weight lost due to evaporation.

Sample Extraction and Partitioning

The samples were extracted with hot water and 50% ethanol using an autoclave process. 20 g of dry powder was put into a closed container, then 200 mL of solvent was added and put into an autoclave with a temperature setting of 100 °C for 10 minutes and an environmental pressure of 1 atm. After that, the container containing the sample was kept in a water bath at 60 °C for 1 hour and left overnight at room temperature to cool naturally. The extract was filtered using filter paper to remove the supernatan which was then concentrated using a rotary evaporator and lyophilized. The yield is shown as a percentage of the weight of the extract obtained per initial weight of the simplicia. The obtained extracts were tested for activity. The most active extract was partitioned with the solvent *n*-hexane (nonpolar) until the organic phase obtained was colorless, followed by the solvent ethyl acetate (semipolar), and finally ethanol-water (polar).

Phytochemical Test

The concentrated extracts were tested for phytochemistry to determine the class of compounds contained in each extract, including tests for flavonoids, alkaloids, saponins, triterpenoids, steroids, and tannins. This test uses standard procedures and procedures from previous research by Shaikh & Patil (2020) and Mlozi et al. (2022). (1) Flavonoids: 0.1 g of extract was dissolved in 1 mL of distilled water, then 2 mL of 2% NaOH solution was added to the solution. The mixture was

shaken, then a few drops of dilute HCl were added, and the color change was observed. Extracts are positive for containing flavonoids if they change color from dark yellow after adding NaOH solution to colorless after adding a few drops of dilute HCl. (2) Alkaloid: 0.1 g of extract was dissolved in 1 mL of 1% HCl, then the mixture was stirred and filtered, then 2 mL of Dragendorff/Mayer reagent was added to the filtrate. The presence of alkaloids is indicated by the formation of a reddish-brown precipitate after adding Dragendorff's reagent or a white/creamy yellow precipitate after adding Mayer's reagent. (3) Saponin: 0.5 g of extract is shaken using 2 mL of distilled water. The foam formed is observed. The presence of saponin is indicated by the formation of foam which can last for 10 minutes. (4) Triterpenoids and Steroids: To a solution of 0.1 g of extract in 1 mL of chloroform, 1 mL of acetic anhydride was added, followed by 2 drops of concentrated H_2SO_4 added slowly, then the color change was observed. The presence of triterpenoids is indicated by a color change from purple to pink to red, while the presence of steroids is indicated by a color change from purple to blue or green. (5) Tannin: 0.1 g of extract is dissolved in 1 ml of distilled water, then the mixture is stirred and filtered. 10% FeCl solution was added 3 drops into the filtrate. The formation of a blue-black precipitate indicates the presence of tannins.

Antioxidant Capacity Test

The antioxidant capacity test for each extract and fraction was performed using three methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity, Cupric Ion Reducing Antioxidant Capacity (CUPRAC), and Ferric Ion Reducing Antioxidant Power (FRAP). Radical scavenging activity was measured with the DPPH method refer to Rafi et al. (2018). Each extract sample is dissolved in ethanol, then 40 μL of the sample solution is added to 250 μL of 125 μmol/L DPPH solution in a 96-well plate. Ascorbic acid solutions were prepared with six different concentrations as positive controls. The ethanol was prepared as a negative control (blank). Then, 40 μL of each control is added to 250 μL of 125 μmol/L DPPH solution in a 96-well plate. Each sample and control was prepared in triplicate. The mixture was incubated for 30 minutes in the dark at room temperature, then the absorbance was measured at a wavelength of 517 nm using a microplate reader. The radical scavenging activity was determined using the following equation: $y = 2.6151x - 0.0097$, with $R^2 = 0.9979$, and it is expressed in mmol AAE/g sample.

The CUPRAC method of antioxidant capacity test refers to Badrunanto et al. (2024). Each 40 μL sample solution was added with 50 μL of 10 mM $CuCl_2$ solution, 50 μL of 7.5 mM neucuproin solution, and 60 μL of 1 M NH_4CH_3COO solution (pH 7) into a 96-well plate. The mixture was incubated for 1 hour at room temperature and the absorbance was measured at a wavelength of 450 nm using a microplate reader. Ascorbic acid solutions were prepared with six different concentrations as positive controls. The antioxidant capacity was determined using the following equation: $y = 1.2808x + 0.0012$, with $R^2 = 0.9971$. Each sample and control was prepared in triplicate. Antioxidant capacity is expressed in mmol ascorbic acid equivalent (AAE)/g sample.

The FRAP method refers to Rafi et al. (2018), and the FRAP reagent was prepared following the procedure of Benzie and Devaki (2017). 30 μL of sample solution is taken, then 270 μL of FRAP reagent is added to a 96-well plate. The mixture was incubated for 30 minutes at room temperature. Absorbance was measured at a wavelength of 593 nm using a microplate reader. Ascorbic acid solutions were prepared with six different concentrations as positive controls. The antioxidant capacity was determined using the following equation: $y = 1.7419x - 0.0448$, with $R^2 = 0.9979$. Each sample and control was prepared in triplicate. Antioxidant power is expressed in mmol AAE/g sample.

Analysis of Metabolite Profiles using LC-MS/MS

LC-MS/MS analysis for metabolite identification refers to the method of Farag et al. (2016) with mobile phase modification. The LC-MS/MS used was a Vanquish Flex ultra-high-performance liquid chromatography (UHPLC) system coupled to an Orbitrap high-resolution mass spectrometer (HRMS) (Q Exactive Plus LC-MS/MS System, Thermo Scientific, Germany). LC-MS/MS analysis was performed at The Advanced Research Laboratory of IPB. The column used was Accucore C18 100 × 2.1 mm, 1.5 μm (ThermoScientific) with a temperature of 30 °C. The mass spectrometer was operated with an electrospray ionization (ESI) source with a Q-Orbitrap mass analyzer. The scanning range was 100–1500 m/z and the ionization mode used was negative ion. The sample was separated using 2 mobile phases, namely 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), with a flow rate of 0.2 mL/minute and an injection volume of 2 μL. The elution system used is a gradient with a mobile phase composition of 0–1 minute (5% B), 1–25 minutes (5–95% B), 25–28 minutes (95% B), 28–33 minutes (5% B). The resulting data was processed using Compound Discoverer 3.2 software (Thermo Scientific, Germany) with an online database and an in-house database. Next, MS-MS was confirmed to predict the compounds contained in the sample.

Data Analysis

The research data were analyzed descriptively and presented in the form of pictures, graphs, and tables. Results are expressed as the mean ± standard deviation of three replicates. Statistical significance was

determined using analysis of variance (ANOVA) followed by Tukey's test where applicable, using Minitab software version 20 (Minitab, LLC, New York). Analysis of significant differences was carried out on the yield and activity of each sample using a confidence level of 95% ($p < 0.05$).

RESULTS AND DISCUSSION

Water Content and Yield

The four samples have a water content of <10%, meaning the samples are in good condition and can be stored for a long time (BPOM, 2019). The variance test showed significantly different results ($p < 0.05$), and the follow-up test (Tukey) also showed significantly different perilla samples, with the highest water content. The water content in a sample is influenced by several factors, including drying factors, temperature, size, and thickness of the sample (Gómez-de la Cruz et al., 2015; Morakinyo & Taiwo, 2016).

The extraction yield of the four samples ranged from 5.98 ± 0.54 to 20.17 ± 1.44%. Analysis of variance showed that the yields of these extracts were significantly different with $p < 0.05$, with the results of further tests shown in **Table 1.** The highest yield was possessed by selasih extract with hot water, namely 20.17 ± 1.44%, which shows that the metabolites in selasih plants are more extracted with hot water. Compared with other extracts, the yield of selasih extract was the highest in both solvents (hot water and 50% ethanol) (**Table 1**), meaning that selasih contained more compounds that had the same polarity as the two solvents. In addition, the yield of lenglengan, perilla, and kemangi extracts with 50% ethanol was higher than with hot water (**Table 1**). This shows that the three samples contained more compounds with the same polarity as 50% ethanol. The four samples were extracted using the autoclave method because this method has been reported to produce more extract, increase the bioactivity of compounds, and save time and solvents, making it more efficient (Kim et al., 2016; Ko et al., 2015).

The kemangi 50% ethanol extract was further separated using the liquid-liquid extraction (partition) method with three solvents of different polarity, namely n-hexane (nonpolar), ethyl acetate (semipolar), and 50% ethanol (polar), respectively. Partition is carried out to obtain more specific compounds based on their polarity. The yield of the fractions obtained is shown in **Table 1**, expressed as a percentage of the weight of the fraction obtained per weight of extract used. The highest yield was obtained by the 50% ethanol fraction, which shows that the metabolites in the kemangi 50% ethanol extract are mostly polar in nature, so they are more distributed in t he 50% ethanol solvent. These results are by Vinnata et al. (2018) who also partitioned the ethanol extract of kemangi using three solvents (*n*-hexane, ethyl acetate, and ethanol-water) and obtained the highest yield in the ethanol-water fraction (41.33%).

Phytochemical Content

Extracts were tested for phytochemistry as an initial qualitative screening of the compound content in each extract. The test results in **Table 2** show differences in the phytochemical content in the extracts of the four plants with the two solvents. Flavonoid compounds were contained in each extract, while steroid compounds were only positive in three extracts (lenglengan, perilla, and selasih with 50% ethanol). This is because most of the flavonoid group has the same polarity as the solvent used, that is, it is polar, while the steroid group is mostly nonpolar, so it is less extracted. Previous studies have shown different results with tables such as Ali et al. (2022) did not showing triterpenoid content in water and ethanol-water extracts of kemangi. Islam et al. (2017) also showed different results in methanol extracts of lenglengan from Bangladesh containing steroid, alkaloid, tannin, and flavonoid compounds. Nadeem et al. (2022) also showed different results in water extracts of selasih containing alkaloids, flavonoids, steroids, tannins, and terpenoids, while the ethanol extract contained flavonoids, steroids, saponins, and tannins. Differences in compound content screening results with literature can be caused by several factors such as differences in environmental (water, drought, salinity, temperature, radiation, chemical, seasonal variation, and region/location) (Verma & Shukla, 2015), plant parts, extraction methods, and extraction solvents (Sayuti, 2017).

The partition results of the 50% ethanol extract of kemangi were also tested for phytochemistry. The results (**Table 3**) are different from the research results of Ali et al. (2022) which showed that the ethyl acetate fraction resulting from the partition of the ethanol-water extract of kemangi positively contained flavonoids, saponins, tannins, terpenoids, and phytosterols. Based on **Table 3**, the 50% ethanol fraction contains the largest group of compounds; These results are in line with the results yield.

Antioxidant Capacity

The antioxidant test on each extract and fraction is intended to determine the potential of each as an antioxidant by looking at its antioxidant capacity. The antioxidant capacity is expressed as comparable or equivalent to the antioxidant capacity of ascorbic acid in the form of mmol of ascorbic acid equivalents (AAE) per g of extract or fraction. So the higher the antioxidant capacity value, the higher its potential as an antioxidant. The results of the antioxidant test for each extract using three methods (DPPH, CUPRAC, and FRAP) are shown in **Figure 1**.

Table 1. Yield of extracts and fractions

Extracts	Yield (%)
Lenglengan hot water extract	5.98 ± 0.54[e]
Lenglengan 50% ethanol extract	13.30 ± 0.70[d]
Kemangi hot water extract	14.32 ± 0.83[cd]
Kemangi 50% ethanol extract	16.7 ± 0.29[bc]
Perilla hot water extract	16.06 ± 1.67[bc]
Perilla 50% ethanol extract	17.48 ± 0.88[ab]
Selasih hot water extract	20.17 ± 1.44[a]
Selasih 50% ethanol extract	18.57 ± 0.27[ab]
Fractions	Yield (%)
N-hexane fraction	4.39
Ethyl acetate fraction	12.48
50% Ethanol fraction	76.92

Note: Different letters indicate significantly different results ($p<0.05$) in the Tukey test.

Table 2. Phytochemical content of extracts

	LA	LE	KA	KE	PA	PE	SA	SE
Flavonoid	+	+	+	+	+	+	+	+
Saponin	+	+	+	+	+	+	-	+
Tannin	-	+	+	+	+	+	+	+
Alkaloid (Dragendorff)	+	+	+	+	-	+	-	-
Alkaloid (Meyer)	+	+	+	+	-	+	+	-
Triterpenoid	+	+	+	+	+	+	+	+
Steroid	-	+	-	-	-	+	-	+

Note: + (detected), - (not detected LA (lenglengan hot water extract), LE (lenglengan 50% ethanol extract), KA (kemangi hot water extract), KE (kemangi 50% ethanol extract), PA (perilla hot water extract), PE (perilla 50% ethanol extract), SA (selasih hot water extract), SE (selasih 50% ethanol extract).

Table 3. Phytochemical content of the partitioned fractions

	n-hexane fraction	Ethyl acetate fraction	50% Ethanol fraction
Flavonoid	-	+	+
Saponin	-	+	+
Tannin	-	+	+
Alkaloid (Dragendorff)	-	-	+
Alkaloid (Meyer)	-	-	-
Triterpenoid	+	-	+
Steroid	+	-	-

Note: + (detected), - (not detected)

The highest average antioxidant capacity for the DPPH and CUPRAC methods is owned by kemangi extract with 50% ethanol solvent, while the FRAP method is owned by selasih 50% ethanol extract, but based on the results of the Tukey test, it was not significantly different ($p > 0.05$) with 50% ethanol extract of kemangi. Therefore, kemangi extract with 50% ethanol was concluded to have the highest antioxidant capacity.

Tests with several methods (DPPH, CUPRAC, and FRAP) are used to determine the antioxidant ability of samples in each method based on the mechanism, hydrogen atom transfer (HAT) or single electron transfer (SET) (Munteanu and Apetrei, 2021). The DPPH method has a HAT and SET mechanism for hydrophilic antioxidants, the CUPRAC method has a SET mechanism for lipophilic and hydrophilic antioxidants under neutral

conditions, and the FRAP has a SET mechanism for hydrophilic antioxidants under acidic conditions (Munteanu and Apetrei, 2021; Shahidi and Zhong, 2015). Based on the mechanism of action of antioxidants according to Munteanu and Apetrei (2021), these results show that the 50% ethanol extract of kemangi contains more lipophilic and hydrophilic antioxidant compounds which play a synergistic role than other extracts, with HAT and SET mechanisms. **Figure 1** also shows that the average antioxidant capacity of the 50% ethanol extract is generally higher than that of the hot water extract. The results of the variance test showed that the antioxidant capacity of the samples using the DPPH method was not significantly different ($p > 0.05$), meaning that the antioxidants contained in the samples played a less active role in the HAT mechanism in neutralizing DPPH radicals.

Antioxidant tests using three methods (DPPH, CUPRAC, and FRAP) were also carried out on the fractions and the results are shown in **Figure 2**. The ethyl acetate fraction has the highest antioxidant capacity in the three methods, meaning that the fraction contains more compounds that play an active and synergistic role as antioxidants, in inactivating reactive species by either SET or HAT mechanisms. Dibala et al. (2016) who fractionated the ethanol-water extract of kemangi also showed the highest antioxidant activity of the FRAP method in the ethyl acetate fraction compared to the ether, dichloromethane, and *n*-butanol fractions.

The antioxidant capacity of the ethyl acetate fraction (**Figure 2**) was higher than the kemangi 50% ethanol extract (**Figure 1**). Based on these results, further separation to obtain more specific and pure compounds such as partitioning can increase the antioxidant capacity. This can be caused by the mixture of compounds in the extract having a greater antagonistic effect than the synergistic effect on antioxidant capacity. The synergistic effect means that the combined effect of bioactive compounds is higher than the sum of the effects of each compound. On the other hand, the antagonistic effect means that the combined effect of bioactive compounds is lower than the additive effect (Chen et al., 2022).

The antioxidants in the DPPH method reduce DPPH radicals (purple) to DPPH-H (yellow). Absorption was measured at 517 nm which is the maximum absorption of DPPH radicals (Gulcin, 2020). Therefore, the higher the antioxidant activity in the sample, the fainter the purple color, so the measured absorbance is lower. In the CUPRAC method, the light blue Cu^{2+}-neocuproin complex is reduced to a yellow-orange Cu^{+}-neocuproine complex with a maximum absorption peak at 450 nm (Gulcin, 2020). The higher the antioxidant activity of the sample, the more orange the final color, and the higher the measured absorbance. Meanwhile, In the FRAP method, antioxidants reduce the colorless Fe^{3+}-TPTZ complex to a dark blue Fe^{2+}-TPTZ complex with a maximum absorption peak at 593 nm. In contrast to other methods, the FRAP test is carried out under acidic conditions (pH = 3.6) to maintain iron solubility (Munteanu and Apetrei, 2021). If the antioxidant activity of the sample is higher, the color is bluer, and the measured absorbance is higher.

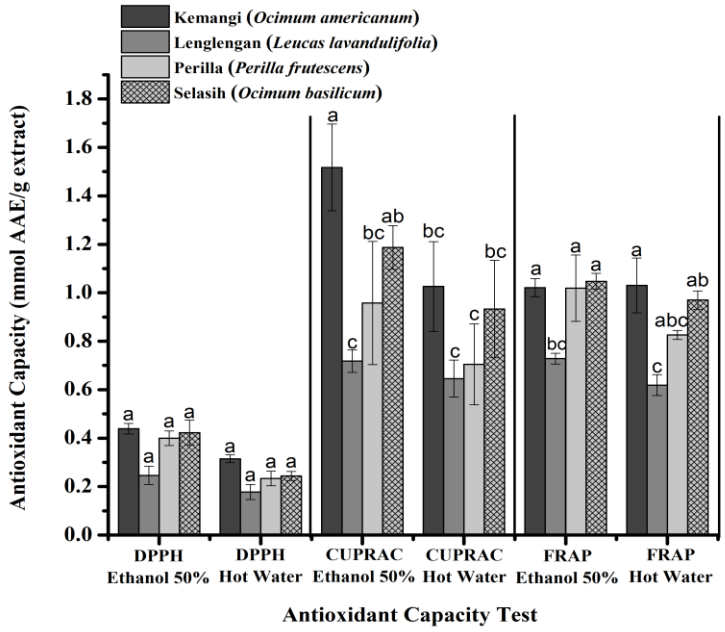

Figure 1. Average antioxidant capacity based on test methods (DPPH, CUPRAC, FRAP) and solvents from four plants (Note: different letters indicate significantly different results ($p < 0.05$) in the Tukey test)

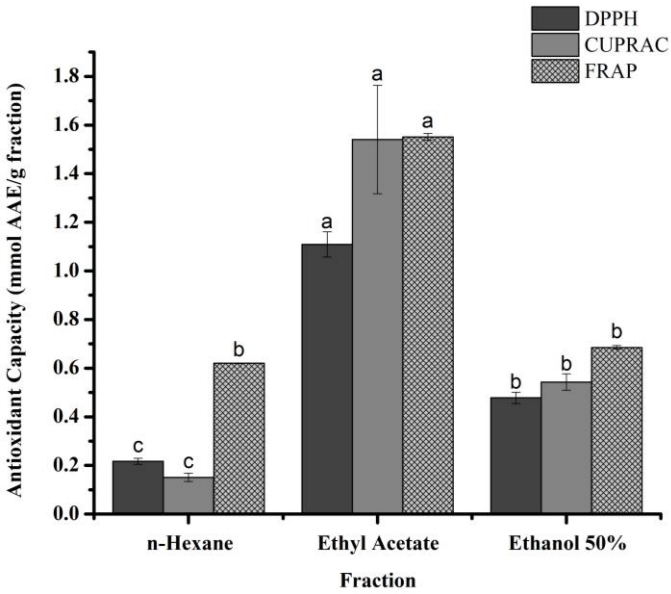

Figure 2. Average antioxidant capacity of the partitioned fraction of kemangi 50% ethanol extract based on the DPPH, CUPRAC, and FRAP test methods (Note: different letters indicate significantly different results ($p < 0.05$) in the Tukey test)

Metabolite Profile

The kemangi 50% ethanol extract and ethyl acetate fraction as the highest antioxidant capacity were analyzed for the metabolite profile using LC-MS/MS. The chromatogram of the analysis results is displayed in the form of a base peak in negative ionization mode (**Figure 3**). Based on **Figure 3**, the differences in each sample can be seen, some showing similar peaks with different intensities. The similarity of the chromatogram peaks of each sample indicates that there are similarities in the metabolites contained. The difference in intensity indicates a difference in the concentration of metabolites in the sample.

A total of 18 compounds in **Table 4** were successfully identified putatively based on confirmation of precursor ion values, best match values, references, and MS-MS fragmentation patterns from the library (ChemSpider and mzCloud). These metabolites consist of 9 flavonoids, 1 terpenoid, 1 amino acid, 2 phenolic acids, 3 fatty acids, and 2 other carboxylic acids. Several previous studies also reported the presence of these metabolites in kemangi extract. Zengin et al. (2019) carried out LC-MS/MS analysis on ethyl acetate, methanol, and water extracts from kemangi leaves and flowers and reported the presence of flavonoids, carboxylic acids, fatty acids, and other phenolic metabolites. Karau et al. (2015) also confirmed the presence of flavonoids, terpenoids, alkaloids, phytosterols, fatty acids, and phenolic metabolites in kemangi ethyl acetate extract.

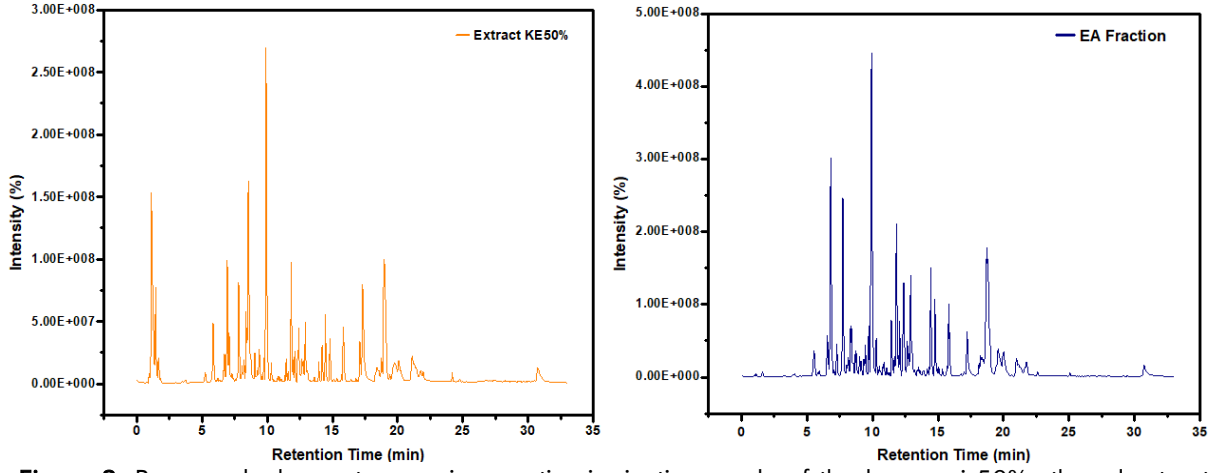

Figure 3. Base peak chromatogram in negative ionization mode of the kemangi 50% ethanol extract and the ethyl acetate fraction.

Table 4. Putatively identified metabolites using LC-MS/MS

Name	Formula	RT [min]	Error [ppm]	Molecular weight	MS^2	Reference	KE	FEA
Flavonoids								
Kaemferol-3-rutinoside	$C_{27}H_{30}O_{15}$	9.097	-0.32	594.15828	68, 245, 255, 285, 593	Čulina et al. 2021		✓
Astragalin	$C_{21}H_{20}O_{11}$	9.424	-0.46	448.10036	67, 227, 255, 285, 447	Abdelaty et al. 2021		✓
Quercetin-3β-D-glucoside	$C_{21}H_{20}O_{12}$	8.806	-0.34	464.09532	67, 151, 463	Farag et al. 2016	✓	✓
Rutin	$C_{27}H_{30}O_{16}$	8.477	-0.38	610.15315	151, 179, 255, 300, 609	Beltrán-Noboa et al. 2022		✓
Trifolin	$C_{21}H_{20}O_{11}$	9.164	-0.46	448.10036	67, 227, 255, 284, 447	Database MzCloud		✓
Apigenin	$C_{15}H_{10}O_5$	12.708	-1.64	270.05238	68, 117, 151, 225, 269	Beltrán-Noboa et al. 2022	✓	✓
Glycitein	$C_{16}H_{12}O_5$	15.861	-0.78	284.06825	68, 268, 283	Martínez-Cruz dan Paredes-López 2014	✓	✓
Luteolin	$C_{15}H_{10}O_6$	11.528	-0.94	286.04747	153, 241, 285	Zengin et al. 2019	✓	✓
Skrofulein/Cirsimaritin	$C_{17}H_{14}O_6$	14.477	-1.31	314.07863	67, 133, 151, 161, 313	Dharsono et al. 2022		✓
Terpenoid								
Asiatic acid	$C_{30}H_{48}O_5$	17.132	-1.13	488.34962	69, 296, 407, 487	Velamuri et al. 2020	✓	
Amino Acid								
2-(acetylamino)-3-(1H-indol-3-yl)propanoic acid	$C_{13}H_{14}N_2O_3$	9.084	-1.72	246.10002	74, 203, 245	Database MzCloud		✓
Phenolic Acids								
Caffeic acid	$C_9H_8O_4$	6.929	-4.71	180.04141	135, 179	Beltrán-Noboa et al. 2022	✓	
(R)-(+)-rosmarinic acid	$C_{18}H_{16}O_8$	9.981	-1.44	360.08400	59, 133, 161, 359	Beltrán-Noboa et al. 2022		✓
Fatty Acids								
(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	$C_{18}H_{34}O_5$	13.105	-1.14	330.24025	68, 171, 329	Database MzCloud	✓	✓
Azelaic acid	$C_9H_{16}O_4$	9.717	-4.92	188.10393	57, 125, 143, 187	Database MzCloud		✓
16-Hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$	24.197	-0.42	272.23503	68, 225, 271	Database MzCloud	✓	
Other Carboxylic Acids								

Indole-3-acetic acid	$C_{10}H_9NO_2$	8.545	-4.83	175.06248	67, 130, 174	Database MzCloud		✓
Citric acid	$C_6H_8O_7$	1.454	-3.87	192.02626	87, 111, 191	Shanaida et al. 2017	✓	

Note: RT (retention time), error (molecular weight tolerance), MS2 (MS-MS fragmentation), KE (kemangi 50% ethanol extract), FEA (ethyl acetate fraction)

The most abundant metabolite profile in **Table 4** is the flavonoid group. This is possible because flavonoids are one of the main groups of natural plant products with more than 9000 known structures (Pilon et al., 2019). Flavonoids have many bioactivities, one of the best known being an antioxidant. The profile is dominated by the flavonoid group, so the metabolites that play an active role in increasing antioxidant capacity are thought to be the flavonoid group. The group of flavonoids that have been detected and have been proven to have antioxidant activity includes astragalin (Choi et al., 2013), rutin (Choi et al., 2021), trifolin, quercetin-3β-D-glucoside (Zhang et al., 2014), kaemferol-3-rutinoside (Chen et al., 2013), apigenin, and luteolin (Tian et al., 2021). In addition, identified phenolic acid compounds such as rosmarinic acid (Elansary et al., 2020) as well as fatty acids such as azelaic acid (Jones, 2009) have also been shown to have antioxidant activity. The combination of these compounds is thought to produce a higher synergistic effect on antioxidant activity compared to their antagonists. Previous research by Zengin et al. (2019) also showed the presence of flavonoid compounds in kemangi leaf and flower extracts such as visenin-2, eriodictyol-7-O-glucoside, viteksin, luteolin, luteolin-7-O-glucoside, isovitexin, quercetin-O-glucuronide, isoquercitrin, kosmosin, eriodictyol, quercetin, sirsiliol, apigenin, pilosin, sirsimaritin, sirsilineol, xantomirol, nevadensin, genkwanin, salvigenin, and gardenin B.

CONCLUSIONS

The highest antioxidant capacity was possessed by kemangi plants (*O. americanum* L.) with 50% ethanol solvent with a value of 0.439 (DPPH); 1.517 (CUPRAC); and 1.021 (FRAP) mmol AAE/g extract. Further separation showed that the ethyl acetate fraction had the highest antioxidant capacity in all three methods with a value of 1.109 (DPPH); 1.540 (CUPRAC); and 1551 (FRAP) mmol AAE/g fraction. Metabolite analysis using LC-MS/MS succeeded in identifying 18 metabolites consisting of flavonoids, terpenoids, amino acids, phenolic acids, fatty acids, and other carboxylic acids.

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