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Essential Oil Profiling and Antibacterial Activity of *Curcuma xanthorrhiza* Roxb. Originated from Yogyakarta by GC-MS

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ABSTRACT

As a tropical country, Indonesia has a large diversity of flora and fauna, including numerous medicinal plants. Although herbs are widely used for medicinal purposes, their pharmacological effect often varies. This variability is frequently attributed to differences in the geographical locations where these plants are grown, leading to variations in the content of active metabolites. In this study, the profile of metabolite content in the essential oil of *C. xanthorriza* rhizomes was cultivated in three different regions in Yogyakarta, Indonesia, namely Mangunan, Ngawen, and Menoreh. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was employed to assess the metabolite content of essential oil. At the same time, antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* was evaluated using the microdilution method. Results indicated that the rhizome from Mangunan has the highest xanthorrhizol content with a percentage area of 21.07%. The Biplot on Principal Component Analysis (PCA) showed that the relationship of compound levels of the three essential oils has no similarity with one another. The Minimum Inhibitory Concentration at 50% (MIC-50) values of *C. xanthorriza* essential oils from Mangunan, Menoreh, and Ngawen regions were lower in inhibiting the growth of *E. coli* bacteria (1620.78 ppm; 1777.69 ppm; and 1688.39 ppm, respectively) compared to *S. aureus* bacteria (3080.80 ppm; 3340.14 ppm; and 2869.54 ppm, respectively).
Keywords: antibacterial; essential oil; *C. xanthorriza*; GC-MS; PCA

INTRODUCTION

As a tropical country, Indonesia has a large diversity of flora and fauna. One of Indonesia's natural resources that has been passed down from generation to generation is medicinal plants. Indonesia's tropical forests have 30,000-40,000 species of flowering plants. A total of 1,845 species of medicinal plants are scattered in various forest formations and natural ecosystems. The diversity of medicinal plants collected in various Indonesian forest formations is a national asset of high value for the welfare of mankind (Zuhud et al., 2018). The increasing level of public awareness of the side effects of chemical-based drugs has led to the increasing use of herbs as an alternative to disease prevention and treatment. The increasing use of herbs in society is the reason that research and development of herbal products are needed to encourage their utilization in the health sector (Purwono et al., 2023).

One of the medicinal plants widely used in the community is the *Zingiberaceae* family. The part of the plant that is often used is the rhizome which contains essential oils that are important for health (Washikah, 2016). The *Zingiberaceae* family consists of 47 genera and about 1.300 species. Some plants included in *Zingiberaceae* and often used in traditional medicine such as *C. xanthorriza* (Alolga et al., 2022). This herb is a plant found in various parts of Indonesia that has been designated by the Indonesian Food and Drug Authority as one of the leading medicinal plants. Moreover, *C. xanthorriza* is thought to have an antimicrobial effect due to the content of active ingredients in the form of essential oils. One of the essential oil elements is terpenoids which are thought to involve bacterial cell membrane breakdown by lipophilic components. Another content is phenol, which is thought to be toxic to bacteria through enzyme inhibition (Mashita, 2017). Phenol compounds have been reported to have inhibitory actions on microbial cell walls or membranes by altering their cell permeability, resulting in the loss of important molecules such as ATP, RNA, proteins, and DNA (Rahmat et al., 2021). Soebagio et al. (2006) stated that the minimum inhibitory concentration of the extract against *S. aureus* and *S. epidermidis* was 0.38% and 0.03%, respectively.

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However, *C. xanthorrhiza* only showed mediocre antibacterial activity against *K. pneumoniae* and *E. coli*, two Gram-negative pathogens with MIC values of 125 and 250 mg/mL, respectively (Septama et al., 2022).

Location of plant growth

Although herbs are widely used in medicine, the effects resulting from plants are often different. This phenomenon is especially caused by variations in the content of active metabolites due to variations in their growth location (Rosidi, 2020). It is known that geographical factors such as weather, altitude, soil pH, temperature, and humidity can cause changes in plant metabolite content (Thi et al., 2021). Based on this phenomenon, information on metabolite profiles is needed, which can later be used in maintaining quality, especially in their pharmacological effects (Karsinah et al., 2002). A study conducted by Li et al (2023) demonstrated that *Cinnamomum camphora* populations across 17 regions in China exhibit notable genetic diversity, moderate variation, and limited differentiation between populations. Similarly, Klau et al (2023) conducted intriguing research revealing disparities in the metabolite profiles of various Indonesian ginger varieties cultivated in Bogor, Cianjur, and Sukabumi. These differences were attributed to genetic factors, soil conditions, farming practices, and agroecological settings. Furthermore, Kharbach et al (2022) found significant variations in 36 secondary metabolites, including 33 polyphenolic compounds and 3 non-phenolic compounds, derived from *Argania spinosa* L., indicating a strong influence of geographic origin on metabolite composition. These studies collectively underscore the impact of geographical locations on metabolite content.

The rhizomes of *C. xanthorrhiza* cultivated in diverse geographical locations exhibit distinct metabolite variations, influenced by geographic conditions and the age of the plants at harvest (Rosidi, 2020). Research by (Rosidi, 2020) indicated that *C. xanthorrhiza* extract from the Bener region in Purworejo had a similar curcumin content compared to that from the Tembalang region in Semarang (34.06±0.10% and 34.02±0.10%, respectively). However, in terms of antioxidant activity, *C. xanthorrhiza* from the Bener region exhibited superior antioxidant efficacy with an IC_{50} value of 91.02±3.41 ppm compared to that from the Tembalang region, which had an IC_{50} value of 94.64±4.74 ppm.

In this study, we will analyze the profile of metabolite content in the essential oil of *C. xanthorriza* rhizomes grown in three different areas in Yogyakarta, Indonesia, and those are Mangunan, Ngawen, and Menoreh. Mangunan, characterized by Mediterranean and latosol soil types, maintains an average temperature of 26°C with a humidity level of 82.8% and an annual rainfall of 2288 mm (Takliviyah, 2016). Menoreh, featuring latosol soil, experiences temperatures ranging from 20°C to 32°C, humidity levels between 70% and 90%, and an annual rainfall ranging from 7849 mm to 9291 mm (Kheriawan et al., 2016). Ngawen, with Mediterranean soil, encounters temperatures varying from 23.2°C to 32.4°C, humidity levels between 80% and 85%, and an annual rainfall of 1643 mm (Miksan, 2021). These distinct environmental conditions are crucial factors that may influence the metabolite profiles and antibacterial activity of *Curcuma xanthorrhiza*, as supported by previous studies on the pharmacological activity of plant extracts against pathogens such as *E. coli* and *S. aureus*. The analysis of essential oil was carried out by GCMS for the qualitative and quantitative, while for the pharmacological activity test, and the antibacterial effect on *E. coli* as represent by gram negative bacteria and *S. aureus* as represent of gram positive bacteria were tested by microdilution method (Amare et al., 2019).

MATERIALS AND METHODS

Materials

Rhizomes of *C. xanthorriza* Roxb. were harvested around October after being planted for 8-10 months (from Mangunan, Ngawen, and Menoreh). Anhydrous Na_2SO_4, DMSO, and n-hexane were purchased from Emsure. Agar powder and Luria Bertani (LB) medium were purchased from Himedia. Ampicillin was purchased from Sigma. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were collected from the Culture Stock of Microbiology Laboratory, Faculty of Pharmacy, UGM.

Methods

Essential Oil Distillation

Five kilograms of fresh rhizomes were sorted, washed, and then sliced to a thickness of approximately 1-2 mm. The steam distillation method was employed for approximately six hours. Subsequently, the obtained essential oil underwent centrifugation at 4500 rpm for 30 minutes at 20°C to separate the oil phase from the aqueous phase. Anhydrous Na_2SO_4 was added to the oil phase to eliminate residual water content.

GC-MS Analysis of Essential Oil Profiles

The essential oil samples were diluted twice using n-hexane. The GC-MS analysis was performed under the following conditions: column oven temperature of 75°C, injection temperature of 175°C, and Split injection mode. Helium (He) was used as the carrier gas at a pressure of 45.9 kPa, with a total flow rate of 83.8 mL/minute, column flow of 0.8 mL/minute, and linear velocity of 32.9 cm/second. The purge flow was set at 3 mL/minute, and the split ratio was 100. The MS settings included an ion source temperature of 200°C, interface temperature of 225°C, and solvent cut time of 2 minutes. The total runtime for the GC-MS analysis was 40 minutes. The Wiley database was utilized for compound identification, and the separation was performed using a temperature gradient. Each sample was injected in duplicate.

Bacterial Culture Preparation

LB agar and liquid media were prepared according to the protocols outlined by (Jira et al., 2018) that solid media preparation was carried out by mixing 2.5 g of LB powder with 1 g of agar powder and then adding 100 mL of distilled water, while liquid media was made by mixing 1.5 g of LB powder with 50 mL of distilled water. *Escherichia coli* and *Staphylococcus aureus* cultures were inoculated onto solid LB media and incubated for 14-18 hours at 37°C (Tuttle et al., 2021). Bacterial starters were prepared by inoculating *E. coli* and *S. aureus* cultures into 10 mL of liquid LB medium in Erlenmeyer flasks. The flasks were then incubated at 37°C with agitation at 180 rpm until an OD_{600} value of 0.3 was reached (approximately 3 hours) which is equal to 2.3 x 10^8 CFU/mL for *S. aureus* and 1.2 x 10^8 CFU/mL for *E. coli* (Hall et al., 2014).

Antibacterial Activity Analysis

The antibacterial activity of essential oils of *C. xanthorrhiza* was evaluated using the microdilution method in a 96-well plate. Each well contained 70 µL of sample (dissolved in 1% DMSO solution), 105 µL of liquid LB medium, and 25 µL of bacterial suspension. Ampicillin at 0.01% concentration served as the positive control. The Minimum Inhibitory Concentration (MIC) was determined based on the ELISA-reader readings at a wavelength of 600 nm (Stoimir et al., 2016).

Data Analysis

Principal Component Analysis (PCA) was employed to analyze the chromatogram and mass spectral results of the essential oil samples using Minitab 17. The PCA was exploratory and descriptive, aiming to reduce dataset dimensionality and explore underlying patterns.

RESULTS

Curcuma xanthorriza rhizomes in this research were taken from 3 different areas. The geographical conditions of each area can be observed in Table I.

Distillation of rhizome

Visually, the essential oil from *Curcuma xanthorriza* showed that the oil from the Menoreh area is purplish, while that from the Ngawen and Mangunan areas is slightly blackish and has a distinctive odor (Figure 1) and similar yield

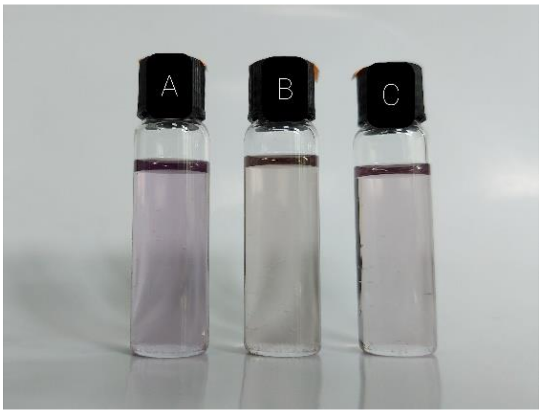

Figure 1. The essential oil of *Curcuma xanthorriza* from the Menoreh (A), Ngawen (B), and Mangunan (C) regions

(Table II). According to the Indonesian National Standard, the color of *C. xanthorriza* essential oil is generally light yellow to brownish yellow.

GC-MS Analysis

The GC-MS method was used to analyze the essential oil content. Each essential oil content from the three regions produces similar contents but with different levels (Table III).

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a statistical technique applied to a collection of variables. In this research, the compound content of *C. xanthorriza* essential oil from three regions is found to have relationships or similarities between one test sample and another. The PCA method which was analyzed by Minitab 17 software produces many data such as score plots and biplots. The score plot is used to present a plot of the relationship or similarity between the essential oils of a rhizome based on PC1 and PC2 which are marked by points that have a certain

Table I. Geographical condition of area of *C. xanthorriza* growth

No	Area	Geographical condition	Citation
1.	Mangunan	Soil : Mediterranean and Latosol Temperature : 26°C Humidity : 82.8% Rainfall : 2,288 mm/year	(Takliviyah, 2016)
2.	Menoreh	Soil : Latosol Temperature : 20-32°C Humidity : 70-90% Rainfall : 7,849-9,291 mm/year	(Kheriawan et al., 2016)
3.	Ngawen	Soil : Mediteran Temperature : 23,2-32,4°C Humidity : 80-85% Rainfall : 1,643 mm/year	(Miksan, 2021)

Table II. *Curcuma xanthorriza* essential oil yield

No.	Area	Wet weight (Kg)	Distillation time (Hr)	Volume of Essential Oil (mL)	Yield (% v/w)
1.	Ngawen	5	5.5	9	0.180
2.	Mangunan	7.7	5.5	12	0.156
3.	Menoreh	5	5.5	8	0.160

Table III. Essential Oil Content of *C. xanthorriza* (% Area)

Metabolites	Mangunan	Menoreh	Ngawen
(Z)-.beta.-Farnesene	1.36	1.23	2.12
.beta.-Bisabolol	1.94	1.965	2.645
Ar-curcumene	8.66	12.165	15.84
Camphor	5.07	3.985	7.075
Curzerene	2.57	3.11	2.51
Germacrene B	5.57	6.15	5.34
Germacrone	13.29	15.37	9.34
Xanthorrhizol	21.07	18.64	20.91
Zerumbone	0.86	0.51	1.79
Zingiberene	0.85	0.83	1.14

distance between one point and another. The closer the three points are, the more similar the properties of the essential oil are. On the other hand, the further the distance between these points, the less similar the essential oils are based on the value of the compound content in them (Widyastuti et al., 2021). A biplot is a combination of a score plot and a loading plot. The loading plot shows how strongly each variable influences the PC by depicting it as a vector. If two vectors have an angle of less than 90°, then the two variables have a positive correlation. If it forms a right angle or around 90°, the two variables can't correlate. If they form an angle greater than 90° to 180°, then the two variables show a negative correlation.

Antibacterial activity

Based on the microdilution results, the three essential oils had similar patterns against *E. coli* and *S. aureus.* with *E. coli*, essential oil from Menoreh had the highest activity, followed by Ngawen and Mangunan, with MIC-50 values were 1,777.7 ppm with a standard deviation of 30 ppm; 1,688.5 ppm with a standard deviation of 105.6 ppm; and 1,620.8 ppm with a standard deviation of 159 ppm, respectively, as shown in Figure 3, while against *S. aureus*, the highest activity also come from Menoreh, followed by Mangunan and Ngawen, with MIC-50 value were 3,340 ppm with a standard deviation of 451.2 ppm; 3,080 ppm with a standard deviation of 340 ppm; and 2,869 ppm with a standard deviation of 16 ppm, respectively, as shown in Figure 4.

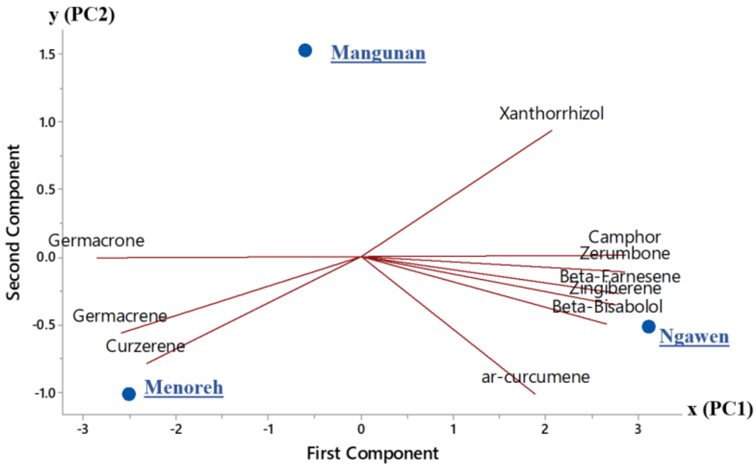

Figure 2. Biplot analysis of components from essential oils of *Curcuma xanthorrhiza* rhizomes sourced from the Mangunan, Menoreh, and Ngawen regions. The analysis, conducted using Minitab software, illustrates the relationship between compounds and their antibacterial potential. Xanthorrhizol, marked as a key compound, exhibits a positive correlation with zingiberene and ar-curcumene, suggesting potential antibacterial properties. Previous studies by Wang et al. (2020), Al-Dhahli et al. (2020), and Rialita et al. (2018) support these findings.

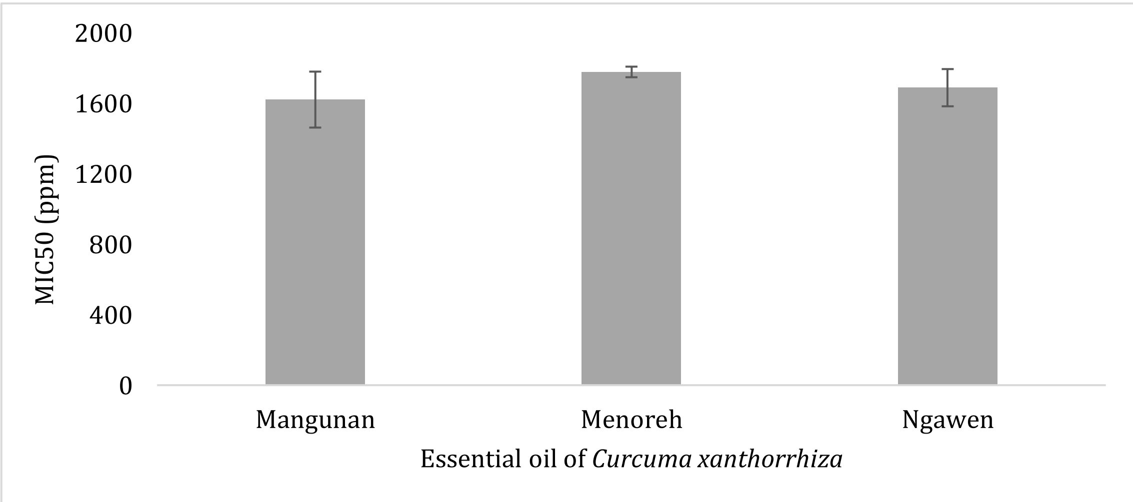

Figure 3. MIC-50 values of *Curcuma xanthorrhiza* essential oils from the Mangunan, Menoreh, and Ngawen regions, were analyzed against *E. coli* using the microdilution method. Triplicate measurements were conducted for each data point.

DISCUSSION

Curcuma xanthorriza is a plant that can grow well in many types of soil, including latosol, andosol, podzolic, and regosol soil types, with rainfall of around 1,500 mm/year, and altitude between 100–600 meters above sea level (Rahman et al., 2022). The Mangunan area which has a loose soil structure has a large absorption-desorption capacity. Absorption-desorption involves ion exchange which will determine the availability of nutrients for plants. The greater the absorption-desorption capacity, the better the nutrients needed by plants. Loose soil can convert water into soil moisture which is useful for plants. That is why rhizomes in this area can grow to a large size on average, they typically exceed 10 cm in length and have a diameter greater than 4 cm. Meanwhile, the Menoreh area has a clay or loam structure with a high clay content, causing the soil to be more efficient in binding and releasing water and nutrients for plants. This soil condition is more favorable conditions to produce larger and harder rhizomes (Yusron & Bogor, 2009). Whereas the Ngawen area has a calcareous soil

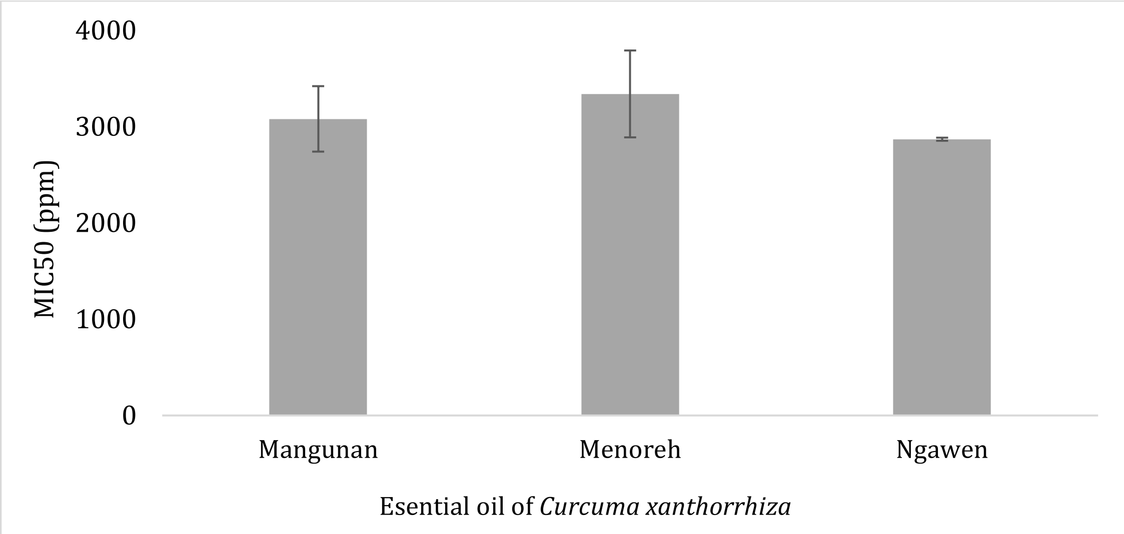

Figure 4. MIC-50 values of *Curcuma xanthorrhiza* essential oils from the Mangunan, Menoreh, and Ngawen regions, were analyzed against *S.aureus* using the microdilution method. Triplicate measurements were conducted for each data point.

structure where chalky soil has low nutrient elements. Chalky soil conditions still allow rhizomes to grow normally (Hudiyani et al., 2017).

Distillation of rhizome

The increasing number of extracted components causes the color of essential oil to become more intense due to the influence of the length of extraction time. The highest yield of essential oil was found in Ngawen area and achieved 0,18% v/w. According to the Herbal Pharmacopoeia 2nd edition of 2017, the essential oil in *C. xanthorriza* contains no less than 1.2% v/w. *C. xanthorriza* essential oil from three different regions had essential oil levels below 1.2% v/w, as seen in Table II. It was probably caused by the inappropriate harvest time where the age of the ginger samples harvested was between 8-10 months, whereas according to Aziz (2019), the recommended harvest age for *C. xanthorriza* to produce good levels of essential oil is 10-12 months.

GC-MS Analysis

As described before, differences in the levels of essential oil content of the three samples are influenced by the geographical conditions where it grows, such as weather, soil height, soil pH, temperature, and humidity, thus affecting the levels of plant metabolite content (Thi et al., 2021). The marker compound for *C. xanthorriza* is xanthorrhizol, where this compound has been reported to show various biological activities such as antibacterial.

Principal Component Analysis (PCA)

Figure 2 shows the score plot and biplot where the score plot shows the three essential oil points from the three regions which are far away from each other. It means that the relationship between the three essential oils is not similar to one another. Soil type, temperature and weather factors where the rhizomes grow are causing the dissimilarity of the three rhizomes. The observed PCA results in Figure 2 in the biplot section showed that the xanthorrhizol compound has a positive correlation as an antibacterial with the zingiberene and ar-curcumene or α-curcumene compounds which have an angle of less than 90^o. It can be interpreted that the content of the essential oil compounds namely xanthorrhizol, zingiberen and ar-curcumene can act as an antibacterial. Several studies on zingiberene and ar-curcumene compounds in their role as antibacterials including research on zingiberene and ar-curcumene compounds had been studied by Wang et al (2020). Research conducted by Al-Dhahli et al (2020), showed that zingiberene and ar-curcumene which have a higher content have the potential to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria. The activity of those chemical compounds as an antibacterial agent was also reported by Rialita et al (2018).

Antibacterial Activity

This antibacterial activity is similar to *C. xanthorriza* essential oil which grows in another place. *C. xanthorriza* essential oil in Padang, West Sumatra, Indonesia has an MIC-50 value of 6.250 ppm, while *C. xanthorriza* essential oil in Sukabumi, West Java, Indonesia has an MIC-50 value of 31.2 ppm against *S. aureus* bacteria and 125 µg/mL against *E. coli* (Septama et al., 2022).

Xanthorrhizol which was the active chemical compound in *C. xanthorriza* was reported to have activity as an antibacterial agent. Based on Figures 3 and 4, it can be seen that the MIC-50 value of *C. xanthorriza* essential oil is smaller in inhibiting the growth of *E. coli* bacteria than *S. aureus*. *E. coli* bacteria are Gram-negative bacteria whose cell walls have a thin peptidoglycan layer, while *S. aureus* bacteria are Gram-positive bacteria whose cell walls have thick peptidoglycan. Peptidoglycan is the main component of bacterial cell walls, while xanthorrhizol can disrupt the peptidoglycan layer (Khalid et al., 2021).

The mechanism of xanthorrhizol against bacteria is determined by the substances that make up xanthorrhizol, which is a phenolic compound. This compound contains hydroxyl (-OH) functional groups which can interact with bacterial cells through an adsorption process in the form of hydrogen bonds and can change the permeability of cell membranes. High concentrations of phenol in cells can cause protein coagulation and lysis of cell membranes. Furthermore, the formation of hydrogen bonds between the hydroxyl groups of phenolic compounds and cell membrane proteins will disrupt membrane permeability, causing vital cell components to leave the cell and induce a cell to die (Khalid et al., 2021). *S. aureus* bacteria, whose cell walls have thicker peptidoglycan than the cell walls of *E. coli* bacteria, will require a higher concentration to inhibit their growth.

CONCLUSION

GC-MS results showed that Mangunan, Menoreh, and Ngawen have different profiles of essential oil compounds although those differences were not significantly different. This essential oil profile is related to their antibacterial activity. This data shows that for therapeutic purposes, we can choose any location from the three locations, because of the similarity of their activities.

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CONFLICT OF INTEREST

All authors declare there is no conflict of interest.

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