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Chemical Composition and Antioxidant Activities of Citronella Essential Oil *Cymbopogon nardus* (L.) Rendle fractions

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Abstract. The human body needs antioxidants to protect the body from free radical attacks. One of the antioxidant sources is citronella oils that are mainly produced in Indonesia. This study aimed to isolate and fractionate citronella oils, to identify the compounds contained in citronella oils and its fractions, and to test their antioxidant activity. Citronella oils were afforded from C. nardus (L.) Rendle through steam distillation and the fractionation of citronella oils was performed using fractional distillation under reduced pressure. Identification of the major components from the isolated citronella oils and the fractions were carried out using gas chromatography-mass spectrometry (GC-MS). Meanwhile, the antioxidant activity test was performed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The fractionation of citronella oils successfully afforded four fractions, i.e., Fraction 1 (F1), Fraction 2 (F2), Fraction 3 (F3), and residue (R). Identification results of the dominant compound contained in citronella oil, F1, F2, F3, and R fractions were citronellal (36.63%), limonene (67.07%), citronellal (92.39%), geraniol (62.41%), and geraniol (47.03%), respectively. The antioxidant activity test showed the antioxidant activity (IC_{50}) of citronella oil, as well as fraction F1, F2, F3, and the Residues were 488, 14.254, 305, 253, and 93 µg/mL respectively.

INTRODUCTION

Degenerative chronic disease is a non-communicable disease that lasts chronically due to deterioration in the function of organs as the result of the aging process. Some chronical degenerative diseases are cancer, cardiovascular, hypertension, diabetes, and obesity. Cancer is the second-largest disease that caused death after cardiovascular. Free radicals have high reactivity, which triggers a chain reaction in cells that can cause cancer. The human body has a mechanism to inhibit free radicals by producing antioxidants, which are produced naturally or given externally through food or supplements [1].

Antioxidants can inhibit the oxidation process by donating one or more electrons to stabilize the free radicals [2,3,4]. The activity of compounds as antioxidants is determined by the presence of hydroxyl (–OH) functional groups and carbon-carbon double bonds, as it can be found in flavones, flavones, squalene, tocopherols, ß-carotene, vitamin C, and others [5,6]. Antioxidants can be obtained by isolation from natural ingredients and synthesis methods [7]. Catherine *et al*. [8] have studied some factors that influence the enhancing of resistance in some classes of polyphenols to the oxidation process [8]. Their studies supported the assumption that the antioxidant activities in the lipophilic phase were defined by the partition coefficients and reaction rate of the flavonoids with the relevant radicals.

Hussain *et al*. [9] has investigated how summer, autumn, winter and spring seasons could affect the chemical composition of the essential oils from aerial parts of basil (*Ocimum basilicum L.*), as well as tested their antioxidant and antimicrobial activities [9]. The hydro-distillation of the samples from winter seasons produced the maximum amount of essential oils, while the samples collected in summer gave the minimum result (range from 0.5 to 0.8%). Linalool was found as the main component (56.7-60.6%) in the essential oils, and epi-α-cadinol, α-bergamotene, as well as α-cadinene were identified as minor components (11.4 to 3.2%). The studies also revealed that oxygenated monoterpenes (68.9%) were richer in the samples collected in winter, while esquiterpene hydrocarbons (24.3%)

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were found in the summer samples. Statistically, different seasons significantly affected the chemical composition of the essential oils ($p < 0.05$). The antioxidant activity of the essential oils was measured by observing the bleaching of ß-carotene in the linoleic acid system, DPPH free radical-scavenging ability, and inhibition of linoleic acid oxidation, which exhibited a good antioxidant activity.

Indonesia is an agrarian country that has an abundant diversity of natural resources, include specific spices such as nutmeg, pepper, clove, and cardamom. Indonesia is also well-known as the largest producer of various kinds of essential oils worldwide, for example, nutmeg oil, clove oil, patchouli oil, vetiver oil, lemongrass oil, and citronella oil, which is become the most potential agroindustry export commodities. Essential oils are commonly used as the raw material for fragrances, flavors, pharmaceuticals, cosmetics, and aromatherapy industries. Essential oils are volatile compounds produced from secondary metabolites in plants and can be found in the roots, bark, leaves, flowers, and seeds. Citronella oil is produced by distillation of citronella leaves of *Cymbopogon nardus L.* It has been stated that essential oils, especially citronella oil, are having high economic value and it can be developed as there is a rise in the demand of essential oils in the global market [10].

Amorati *et al.* [11] have tested the antioxidant activity of various essential oils [11]. Their studies revealed that especially thymus and oregano, as well as other essential oils such as thymol, eugenol, and carvacrol, can be applied as natural antioxidants to preserve food. Their research suggested that a standardized and rational approach is needed to design an experiment in the actual application of essential oil for food preservation and developing health-oriented products.

The study about essential oils for the pharmaceutical and food industries is very interesting due to the biological activities possessed by essential oils such as antibacterial, antioxidant, anti-inflammatory, and anticancer chemoprotective activity [12]. Essential oils are commonly used as a natural additive in culinary, pharmaceuticals, cosmetics industries because it less harmful than synthetic additives compounds [13]. Dar *et al.* [14] investigated the antioxidant and cytotoxic properties of essential oils and the main components of *Cymbopogon jawarancusa* [14]. Based on the characterization using GC-FID, GC-MS, and ^{13}C NMR, the main chemical composition from the hydro-distilled essential oil of *C. jawarancusa* was identified as piperitone (58.6%) and elemol (18.6%). The antioxidant activity of the oil and its components were evaluated by the DPPH test and showed a strong antioxidant and cytotoxic effect. The order of the scavenging activity to the DPPH radical was found to be elemol, piperitone, b-caryophyllene, and a-pinene. The research also showed that essential oil from *C. jawarancusa* could be applied to control the human disorders linked to the oxidative stress involving aging, DNA damage, and cancer.

Quercetin is a polyphenolic compound that can stop the oxidation process by inhibiting the free radicals in oxidative chain reactions [15]. Quercetin has been classified as an antioxidant compound because it could inhibit the oxidation of other molecules [16,17]. Quercetin is usually utilized as a standard compound in antioxidant activity tests.

There are many publications about the in vitro methods that measure total antioxidant capacity. The most used procedures for measuring antioxidant capacity are DPPH, ABTS, FRAP, TEAC, and ORAC [18]. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method is an independent assay for screening many samples of their free radical inhibitory activity [19,20,21]. The DPPH method is a convenient, simple, rapid, accurate, and inexpensive to measure the ability of compounds as inhibitors of free radicals or antioxidants [22]. Marinova and Batchvarov [18] have reported the determination of the radical inhibitory activity of various foods, beverages, and substrates through several methods and modifications by using DPPH according to the original method [18,23,24].

In this study, fractionation of citronella oil, as well as the identification of the compounds in each fraction, have been conducted. The antioxidant activity test of the citronella essential oil and its fraction were tested by DPPH methods with quercetin as the standard compound. This research was expected to find some compounds in citronella essential oil, which has an active role as antioxidants.

EXPERIMENTAL

In this study, citronella leaves (*Cymbopogon nardus L.*) were collected from Kebanggan-Sumbang, Banyumas, Central Java, Indonesia. In the antioxidant activity assay, methanol (Merck) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich) were used without further purification.

The equipment includes laboratory glassware, steam distillation equipment, fractional distillation set were used to obtain the essential oils and its fractions. Identification of the components in the essential oils and its fractions was carried out using Gas Chromatography-Mass Spectrophotometer (GC-MS) (Shimadzu QP 2010). The

absorbance of the essential oils and its fractions in the antioxidant activity test were recorded at the UV-Vis spectrophotometer (Shimadzu 1800).

Isolation and fractionation of essential oils from citronella leaves

Approximately 5 kg of citronella leaves of *Cymbopogon nardus L* were dried through aeration. The isolation process was carried out by putting the dried leaves in the steam distillation apparatus to isolate the essential oils of citronella leaves. The isolation process could produce 800 mL of the isolated essential oils and further fractionation by fractional distillation under reduced pressure could give three fractions (F1, F2, and F3) and residues (R). The component of the obtained essential oil and the fractions from citronella leaves were finally determined by analysis with GC-MS.

Antioxidant activity assay with the DPPH method

The antioxidant activity was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method involving some steps below:

Preparation of DPPH solution [25]

A DPPH solution with a concentration of 0.05 mM was prepared by dissolving 1.97 mg of DPPH crystals in methanol in the 100 mL volumetric flask.

Determination of the maximum wavelength of DPPH

The maximum wavelength of DPPH was determined by putting a 4 mL DPPH solution (0.05 mM) in the cuvette, followed by the addition of 1 mL methanol. The solution was then let to stand in the dark condition for 30 minutes. The absorption of the solution was measured with a UV-Vis spectrophotometer at a wavelength of 400-600 nm to determine the maximum absorption wavelength.

Determination of the operating time of the test solution

The operating time was determined by employing 4 mL of DPPH solution (0.05 mM) with the addition of 1 mL of 100 ppm test solution. The absorbance of the solution was measured at the maximum wavelength at intervals of 5 minutes until a stable absorbance was obtained.

Determination of antioxidant activity

Determination of antioxidant activity was carried out by measuring the absorbance of 4 mL DPPH solution (0.05 mM) with 1 mL of the sample solution in various concentrations of 0; 12.5; 25; 50; and 100 ppm (in DMSO). The mixture was allowed to stand for the obtained operating time, and the absorbance was measured at the maximum wavelength.

Determination of the percentage inhibition (IC_{50})

The IC_{50} value shows the concentration of the test sample that gives 50% inhibition (able to inhibit or reduce the oxidation process by 50%). The IC_{50} value was determined by making a linear curve between the concentration of the test solution and the % inhibition. The value of the concentration of the test solution was entered as abscissa (X-axis) and the value of percent inhibition/activity as ordinate (Y-axis) into the linear regression curve equation.

$$\text{Percentage of inhibition} = \frac{(A_1 - A_2)}{A_1} \times 100\% \qquad \text{(Eq. 1)}$$

A_1 = absorbance of control, A_2 = absorbance of sample

The IC_{50} value is inversely related to the antioxidant activity. The smaller the IC_{50} value, the better the antioxidant activity.

RESULTS AND DISCUSSION

Isolation and fractionation of essential oils from citronella leaves

The type of plant used in this study was *Cymbopogon nardus L.* Rendle. Identification of the plants taxonomy was carried out at the Faculty of Biology, Environmental Laboratory, Jenderal Soedirman University. Isolation of citronella oil was carried out by steam distillation with a specific gravity of 0.8699. As a result, based on the analysis by GC-MS, the dominant component of citronella oil was identified as citronellal (36.63%) and geraniol (25.715%) (Fig. 1).

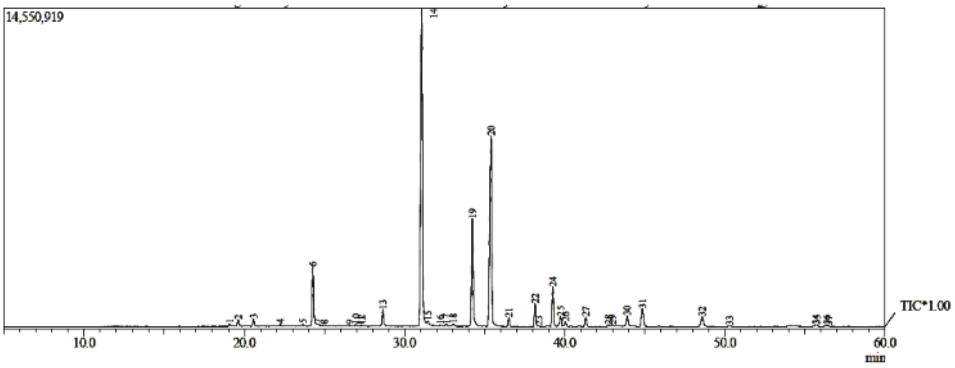

FIGURE 1. GC Chromatogram of citronella oil

Fractional distillation was carried out to get the fractions from citronella oil in a vacuum condition to reduce vapor pressure from 60 to 40 mmHg. From the distillation process, three fractions (F1-F3) were obtained along with residue (R). Table 1 showed the temperature condition in fractional distillation from each fraction.

TABLE 1. Temperature and pressure conditions for fractional distillation of citronella oils

Compounds	Temperature Condition of Fractination		
	Heater (°C)	Batch (°C)	Distillate (°C)
F1	130	65	40-82
F2	160	110	82
F3	175	116	82-86

Perry [26] has distilled citronella oil at a pressure of 60 mmHg, where the citronella oil sample boiled at 125-150 °C [26]. Decreasing in vapor pressure causes a decrease in boiling point, so in this study, Fraction 1 (F1) came out at a temperature range of 40-82 °C, Fraction 2 (F2) at 82 °C, and Fraction 3 (F3) collected at 82-86 °C. The fractional distillation was started with 822 mL of citronella oil, and the volume of each fraction and the residue obtained can be seen in Table 2.

TABLE 2. Percentage of fractional distillation results with pressure reduction

Citronella oil's fraction	Volume (mL)	Percentage (%)
F1	75	9.12
F2	262	31.85
F3	320	38.85
Residue	165	20.18

Kadarohman *et al*. [27] have carried out a quantitative approach based on the GC-MS analysis, where the citronella oil was distilled at a lower boiling point [27]. Their work reported Fraction I contained around 4.52%, while citronellal or Fraction II about 32.15% and the last fraction (residue) whose boiling point is above the citronellal was 63.33%. Their research aimed to isolate citronellal from citronella oils with only collected three

fractions of oils. However, in this study, the last fraction was still separated into Fraction 3 (F3) and residues (R). The percentage of each fraction F1, F2, F3, and the residue (R) were 9.12, 31.85, 38.85, and 20.18, respectively.

Further characterization to identify the composition of each fraction was carried out using GC-MS. Figure 2, Figure 3, Figure 4, and Figure 5 presented the chromatogram GC of Fraction 1 (F1), Fraction 2 (F2), Fraction 3 (F3), and residue (R), respectively.

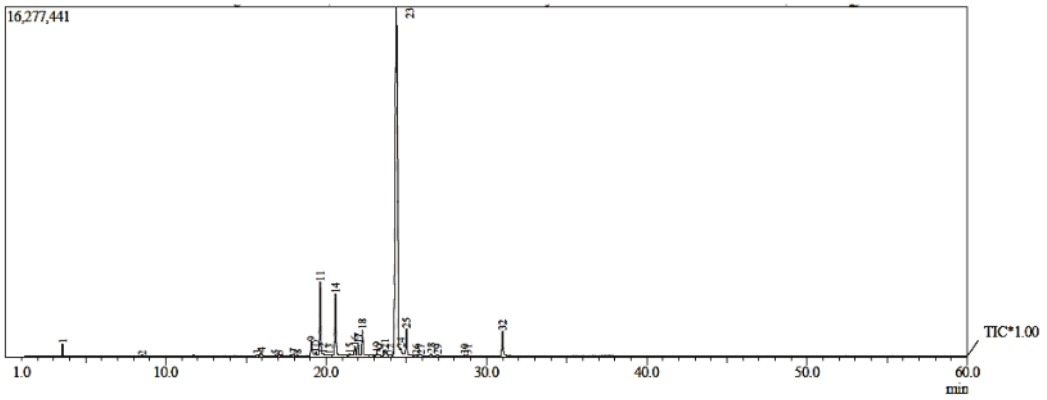

FIGURE 2. Chromatogram GC of Fraction 1 (F1)

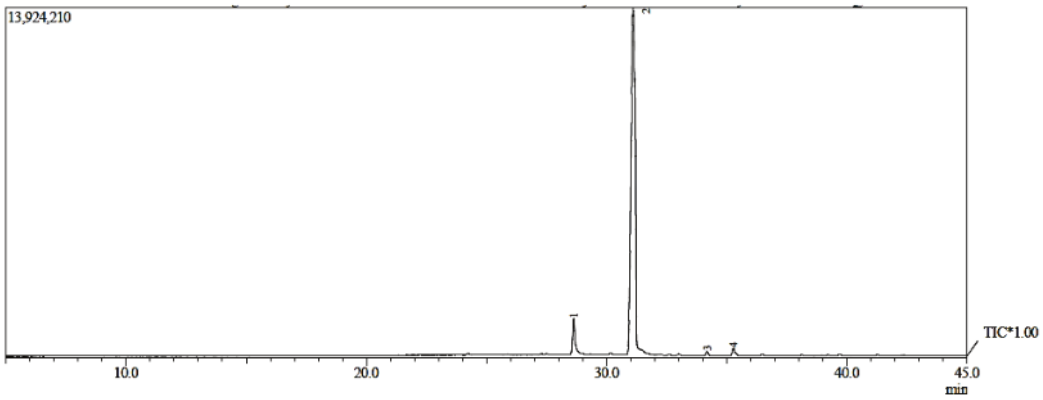

FIGURE 3. Chromatogram GCMS of Fraction 2 (F2)

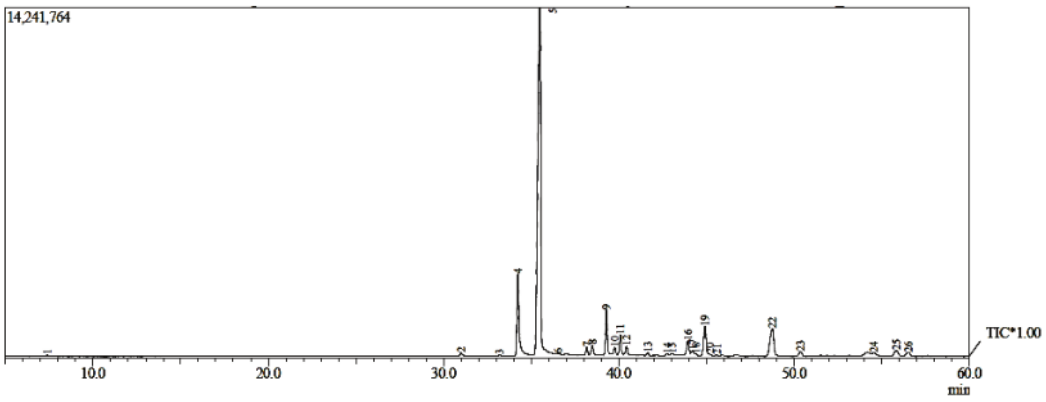

FIGURE 4. Chromatogram GC of Fraction 3 (F3)

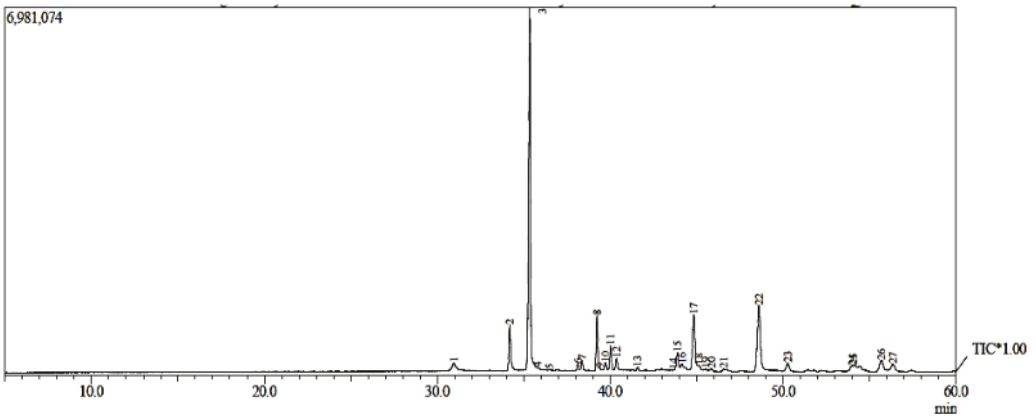

FIGURE 5. Chromatogram GC of Residue (R)

Based on the analysis result using GC-MS, the component in each fraction can be determined according to the retention time and mass spectra. Table 3 showed that the most component in Fraction I (F1) is limonene (67.07%), whereas in Fraction II (F2) is citronella (92.39%), and Fraction III (F3), as well as a residual fraction (R), are geraniol (62.41 and 47.03%). Further studies were conducted to examine the antioxidant activity of the three fractions and residues compared with un-fractionated citronella oil and quercetin.

TABLE 3. Composition of the citronella oil fractions

Retention Time (min)	Compounds	Citronella Oil (%)	F1 (%)	F2 (%)	F3 (%)	Residue (%)
19.084	Trycelene	0.17	1.85			
19.626	Alpha-pinene	0.63	8.24			
20.585	Champene	0.71	7.05			
21.792	Sabinene	0.18	1.07			
21.989	Beta-pinene	0.18	1.39			
22.989	Beta-myrene	0.18	2.69			
24.278	Limonene	5.92	67.07			
28.646	Linalool	1.65		5.65		
31.076	Citronellal	36.63	3.00	92.39		1.26
34.237	Citronellol	11.22		0.55	9.78	5.58
35.408	Geraniol	25.715		1.41	62.41	47.03
38.159	Citronellyl acetate	1.95				
38.373	Methyl citronellate					1.02
9.264	Geranyl acetate	3.51			4.78	5.91
39.749	Elemen	1.14				
40.049	Eugenol				2.13	2.74
41.316	Caryofilene	1.04				
43.828	Germacrene/ gamma cadinene					2.39
43.913	Naphtalena	1.32				
43.936	Copaene				1.88	
44.854	Cadinene	2.43			4.39	9.18
48.76	Elemol				5.94	13.84
55.836	Alpha-cadinol				1.36	2.35

Antioxidant activity assay with the DPPH method

According to [22], the principle of the DPPH method is that bioactive compounds as antioxidants could reduce DPPH free radicals to 2,2-Diphenyl-1-picrylhydrazyl. DPPH that reacts with antioxidants produces a reduced form of 2,2-Diphenyl-1-picrylhydrazyl and antioxidant radicals. The DPPH antioxidant activity test method was carried out by making a solution of DPPH crystals at a concentration of 0.05 mM [25].

The absorbance of DPPH was measured first to set the maximum wavelength using a UV-Vis spectrophotometer. A 4 mL DPPH 0.05 mM solution was added to the cuvette and added with 1 mL methanol. After being left for 30 minutes in the dark, the absorption of the solution was measured at a wavelength of 400-600 nm. Figure 6 showed that the maximum wavelength obtained was 515.1 nm.

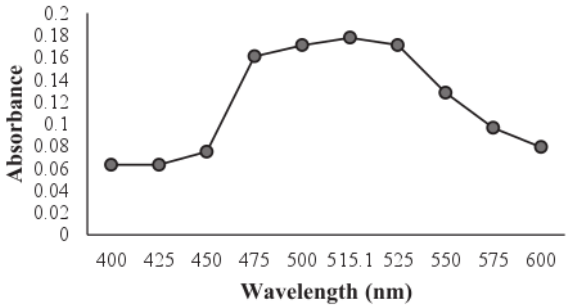

FIGURE 6. Maximum Wavelength of DPPH

Determination of the operating time in the antioxidant activity test aims to determine the optimum time for reading an absorbance with a spectrophotometer that described the stable conditions of the compound. The absorbance of the solution was measured at the maximum wavelength, at intervals of 5 minutes until a stable absorbance was obtained. Figure 7 displayed the time course of the DPPH solution with the optimum time was observed after 55 min, which indicated by the relatively constant absorbance.

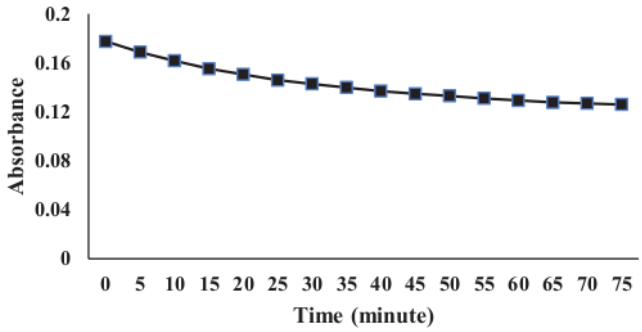

FIGURE 7. Operating Time of DPPH

The antioxidant activity was conducted to test solutions of citronella oil and fractions F1, F2, F3, and Residue in varied concentrations. In this study, 4 mL of 0.05 mM DPPH solution was added with 1 mL of the test solution. After the mixture was allowed to stand for the optimum operating time (55 min), the absorbance of the solution was then measured at the maximum wavelength (515.1 nm).

Table 4 showed the antioxidant activity that was expressed as IC_{50}, based on the calculation using Eq.1. According to the result, F1 has the highest IC_{50} value, which means Fraction 1 has no potential as an antioxidant. Based on the analysis, the dominant compound in F1 is limonene, which can be proposed to has no potential as an antioxidant. Fraction 3 and residue R have a low IC_{50} value with 253.10 and 92.62 µg/mL, respectively. This result showed that F3 and residue R have the potential as an antioxidant, which can be proposed by the fact that it contains geraniol and elemol. Geraniol and elemol are the alcohol group that has been reported for its potential as an antioxidant. A compound that can be expressed as an antioxidant potential if it has an IC_{50} value of less than 100 µg/mL. Based on this study, quercetin as a standard antioxidant compound has high antioxidant activity (IC_{50} of 5.58 µg/mL), almost 17 times better than the residue fraction (92.62 µg/mL)

TABLE 4. Antioxidant activity

Compounds	IC_{50} (µg/mL)
Citronella oil	487.79
F1	14.254.00
F2	305.00
F3	253.10
Residue (R)	92.62
Quercetin	5.58

Stobiecka [29] has investigated the mechanism of scavenging free radicals geraniol experimentally and theoretically [28]. The fragrant acyclic terpenoids were treated with the ABTS and DPPH tests. The results revealed that the preferred mechanism in geraniol was explained in non-polar and polar media. The computational studies proposed that the presence of an allylic H-atom close to the OH-group (at position 1C) in geraniol appears to be essential to determine the anti-radical activity (Fig. 8).

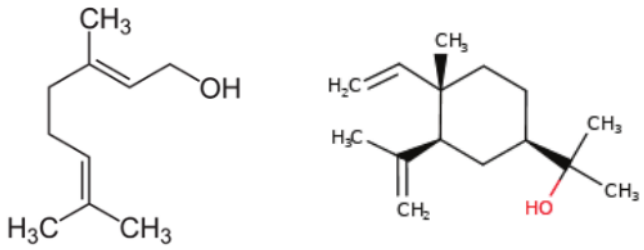

FIGURE 8. Geraniol and elemol structures

Elemol is commonly used as an additive in cosmetics, shampoos, fine fragrances, bath soaps, and other toiletries, as well as non-cosmetic products such as household cleaners and detergents. Its use worldwide on a scale of 1–4 metric tons per year. Elemol is identified as the main component of the two species of Dioscorea, which occupy 41 and 22% of *D. floribunda* and *D. composita* essential oils. Amyris oil and elemol is a flea medicine. Elemol not significantly show any different effectiveness against *A. americanum* compared with the widely used deet repellent. After 2 and 4 hours since the application to filter paper, 827 µg/cm^2 Amyris oil could repel up to 80 and 55% of the nymph *A. americanum*, respectively. Amyris and elemol oils showed a repellent effect in lower concentrations to *Ixodes scapularis* than *A. americanum* [29,30].

CONCLUSION

Based on the GC-MS analysis, the dominant compound contained in citronella oil was citronellal (36.63%). Fractionation of the citronella oil afforded three fractions (F1-F3) and a residue. Fraction F1 was identified to contain limonene (67.07%), citronellal in F2 (92.39%), geraniol (62.41%) in F3, and geraniol (47.03%) in the residue. Antioxidant test results showed that the antioxidant activity (IC_{50}) of citronella oil, F1, F2, F3, and the residues were 488, 14.254, 305, 253, and 93 µg/mL respectively. Fraction F3 and residues contain geraniol and elemol that is expected to be very potent as an antioxidant.

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