

Antibacterial and Antioxidant Activities of Ginger Essential Oils

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Ginger is a rhizomatous perennial herb that grows abundantly in tropical areas. It has been used around the world as a spice, flavoring agent, and ingredient in traditional medicine. Ginger essential oils (GEOs) are derivatives of ginger that can be found in various products used in daily life, such as food, pharmaceutical, and cosmetics. The present study analyzed the chemical compositions, antioxidant, and antibacterial activities of three commercially available GEOs. The compositions of GEOs were identified using the gas chromatography method. The antioxidant activity was evaluated using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and 2,2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay methods. The antibacterial activity was determined using a disc diffusion assay based on the diameter of the inhibition zone (DIZ). The main compounds identified from the samples were zingiberene, α -curcumene, β -sesquiphellandrene, camphene, α -farnesene, β -bisabolene, α -pinene, and 3-carene. The IC_{50} values were found to be 5.3023 and 1.4504 mg mL⁻¹ for GEO1; 0.9249 and 0.5276 mg mL⁻¹ for GEO2; and 10.4463 and 3.3535 mg mL⁻¹ for GEO3 when evaluated using DPPH and ABTS assay methods, respectively. All samples showed antibacterial activity against *Staphylococcus aureus* ATCC 13420 and *Bacillus subtilis* (the collection of National Research and Innovation Agency), while only GEO2 and 3 displayed inhibitory effect against *Escherichia coli* ATCC 9637.

Key words: antibacterial, antioxidant, chemical composition, essential oil, ginger

Jahe adalah tumbuhan rhizoma herba perennial yang banyak ditemukan di daerah tropis. Tanaman ini banyak digunakan sebagai rempah, perisa, maupun bahan baku untuk obat tradisional. Minyak jahe, merupakan salah satu produk turunan jahe, yang telah banyak digunakan dalam produk-produk yang digunakan dalam kehidupan sehari-hari seperti makanan, produk farmasi, dan kosmetik. Artikel ini memberikan informasi mengenai komponen kimia, aktivitas antioksidan, dan antibakteri dari minyak jahe komersial. Senyawa kimia dari minyak jahe dianalisa dengan metode kromatografi gas. Aktivitas antioksidan sampel diuji dengan metode uji 2,2-diphenyl- 1-picryl-hydrazyl (DPPH) dan 2,2'-azinobis-(3-ethylbenzothiazoline-6-asam sulfonat) (ABTS). Sedangkan aktivitas antibakteri dianalisa dengan menggunakan metode difusi cakram, dengan mengukur zona hambat yang terbentuk. Senyawa kimia utama yang terdeteksi dalam sampel adalah zingiberen, α -curcumen, β -sesquifellandren, camphen, α -farnesen, β -bisabolen, α -pinena, dan 3-karena. IC_{50} sampel yang diperoleh adalah 5.3023 dan 1.4504 mg mL⁻¹ untuk GEO1; 0.9249 dan 0.5276 mg mL⁻¹ untuk GEO2; serta 10.4463 dan 3.3535 mg mL⁻¹ untuk sampel GEO3 ketika diuji dengan metode DPPH dan ABTS, secara berurutan. Semua sampel menunjukkan aktivitas antibakteri terhadap *Staphylococcus aureus* ATCC 13420 dan *Bacillus subtilis* (koleksi Badan Riset dan Inovasi Nasional), sedangkan hanya GEO2 dan GEO3 yang menunjukkan aktivitas penghambatan terhadap *Escherichia coli* ATCC 9637.

Kata kunci: antibakteri, antioksidan, jahe, komponen kimia, minyak atsiri

Zingiberaceae belongs to a large family of monocotyledonous perennial herbs. This plant is widely cultivated in tropical and subtropical regions (Kun-Hua *et al.* 2011), encompassing approximately 50 genera and more than 1,200 species (Pintatum *et al.* 2020). Ginger has been used widely as a spice, flavoring agent, and traditional remedy (Abd El-Hack *et al.* 2020). Studies revealed that ginger had various pharmacological activities, such as antioxidant, antimicrobial, gastrointestinal modulating agent,

antidiabetic, anticancer, antivomiting, neuroprotection, anti-inflammation, cardiovascular protector, anti-obesity, antinausea, anti-emetic, protective effects against respiratory disorders, and analgesic (Mao *et al.* 2019; Abd El-Hack *et al.* 2020; Anh *et al.* 2020). Ginger essential oil (GEO) is one of ginger derivatives that can be found in various products, such as food, pharmaceutical, and cosmetics. Diverse biological activities of GEO have also been reported in various studies, such as antimicrobial, antioxidant, analgesic, anti-inflammatory, bronchodilator, anti-ulcer, anticancer, and immunomodulatory (Mahboubi 2019).

The yields of essential oil from ginger rhizomes

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range between 1–5% (Bampidis *et al.* 2020). Factors that affected the yields were the source, freshness, and the treatment (drying method) of rhizomes, as well as the distillation methods. The chemical constituents of GEO primarily consist of monoterpenes, sesquiterpenes hydrocarbons, oxygenated sesquiterpenes, and phenylpropanoid compounds (Mahboubi, 2019; Pintatum *et al.* 2020). Similar to the yields, the chemical compositions of the GEOs can greatly be affected by several factors i.e., the source of rhizome, its freshness or dryness, and the extraction methods used (Mahboubi 2019). GEO is mainly obtained from *Zingiber officinale*, although other sources of Zingiberaceae family are also available, such as *Z. zerumbet* (Koga *et al.* 2016; Rana *et al.* 2017), *Z. cassumunar* (Bhuiyan *et al.* 2008), *Z. montanum* (Manochai *et al.* 2010), and *Z. kerrii* (Pintatum *et al.* 2020).

The urgent need for antibiotics to overcome resistant bacteria underlines the importance of developing new antibiotics, one of which is by sourcing the lead compounds from natural products, including essential oils. Similarly, the demands for natural food preservatives, as well as natural antioxidants for the cosmetic and food industry, are increasing. Antioxidant activity of natural products is also of interest and has been investigated for its correlation to diseases, as the production of free radicals in the body correlates with various human diseases (Lobo *et al.* 2010). As ginger has been used as traditional medicine and its medicinal properties have been widely investigated, its essential oils may be a promising source of these properties. Therefore, this study aimed to elucidate the antibacterial and antioxidant activities of three different commercial GEOs.

MATERIALS AND METHODS

Procurement of GEOs. The essential oils of ginger were obtained through online commerce.

Gas Chromatography-Mass Spectrometry Analysis. The GC-MS analysis of GEO was conducted using GC/MS apparatus (Shimadzu QP 2010 Ultra, Japan) equipped with a low polarity column (30 m x 0.25 mm x 0.25 µm; Restek-5MS, USA). Helium as the carrier gas was set at a flow rate of 0.98 mL min⁻¹. The column temperature was raised to 80 °C and kept for 3 min and steadily rose at 7.50 °C min⁻¹ to 220 °C and held for 5 min, followed by a temperature increment of 10 °C min⁻¹ rate and kept for 5 min at 270 °C. The prepared samples (10% GEO in ethanol) were injected at 8 µL with a split ratio of 1:50. The peaks were

identified by a combined search of retention time and comparison of MS fragments of the peak with mass spectra (NIST 14 library). The percentages are calculated from the peak areas given by the GC-MS.

Antibacterial Activity. The inhibitory effect of pure (100%) GEOs was tested using agar disc diffusion method against *Escherichia coli* ATCC 9637 (Supelco, Sigma Aldrich), *Bacillus subtilis* (collection of Indonesia Institute of Sciences), and *Staphylococcus aureus* ATCC 13420 (Supelco, Sigma Aldrich). The bacterial suspension was prepared using freshly grown bacteria in Nutrient Broth (NB; Merck) that was incubated at 37 °C overnight with shaking at 180 rpm. The inoculum was diluted in liquid Nutrient Agar (NA; Merck) to achieve optical density (OD) of 0.02 or 5×10⁷ CFU mL⁻¹ at 600 nm. The inoculated NA was then poured into disposable petri dishes (SPL Life Sciences) and left to set. All samples and positive controls were applied to the blank paper discs (Fioroni Filters) at 10 µL and left to dry. As positive controls, streptomycin sulphate (Merck) was used for *B. subtilis* (5 µg), whereas ampicillin (Merck) was used for *E. coli* (10 µg) and *S. aureus* (3 µg). The diameter of the inhibition zone (DIZ) was measured after 18-24 h incubation at 37 °C.

2,2-Diphenyl-1-picryl-hydrazyl (DPPH) Assay. The DPPH assay was conducted based on a method described by Yen and Chen (1995) with slight modifications. Briefly, stock solution of GEOs was prepared by diluting the samples in methanol to get concentration of 100 µg mL⁻¹. A total of 40 µL of a solution of 1 mM DPPH in methanol was mixed with a volume of GEO stock solution adjusted for a series of concentration and then added methanol to a final volume of 200 µL to get the the desired concentration. The solution was incubated for 30 min in the dark at room temperature. The absorbance of five concentration levels of the samples were measured at 515 nm using a microplate reader (Tecan Infinite M200 Pro, Switzerland). Negative and positive controls were methanol and ascorbic acid (AA) (Merck), respectively. The inhibition percentage of the samples was calculated according to the following formula:

$$\% \text{ Inhibition} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100\%$$

2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Assay. The ABTS radical scavenging assay method was adopted from Lyu *et al.* (2020) with minor modifications. The radical mono cation of ABTS was prepared by mixing 7.4 mM ABTS with 2.6 mM potassium persulfate at 1:1 ratio, the

solution was incubated for 24 h in the dark at room temperature. The ABTS solution was diluted with phosphate-buffered saline (pH 7.4) to obtain an absorbance of 0.7 ± 0.02 at 734 nm. Aliquots of samples (10 μL) at five concentration levels were reacted with 190 μL of the ABTS solution and incubated for 6 min. Trolox was used as the positive control. The absorbance was measured at 734 nm using the Tecan Infinite M200 Pro. Antioxidant activities were calculated based on the equation below:

$$\% \text{ Inhibition} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100\%$$

The IC_{50} value shows the concentration of sample that is able to inhibit the oxidation process by 50% obtained by making a linear curve between the concentration of the test solution (x-axis) and % antioxidant activity (y-axis).

Statistical Analysis. The statistical analysis was evaluated using SPSS version 22 (SPSS Inc.). The results were expressed as mean \pm SD (standard deviation). Pearson's correlation test was used to assess correlations between means. One-way analysis of variance was performed by ANOVA and followed by Tukey's multiple comparison tests. $P < 0.05$ was regarded as significant.

RESULT

GC-MS Analysis-Based Chemical Compositions. Fig 1 represents the total ion chromatogram (TIC) of three commercial GEO samples that resume intensities of all mass spectral peaks pertaining to the same scan. Based on comparing retention time-MS fragments of the sample peaks with the mass spectra of a computer based-NIST 14 library, the chemical compounds detected in the commercial GEOs tested and are listed in Table 1. It is shown that the major compounds of GEO1 were α -zingiberene (22.89%), camphene (16.94%), α -curcumene (11.62%), β -sesquiphellandrene (11.21%), β -bisabolene (9.07%), α -pinene (5.04%), and α -farnesene (4.46%). Moreover, GEO2 had α -pinene (25.72%) as the main component, followed by α -curcumene (11.88%), α -zingiberene (8.01%), 3-carene (7.71%), β -sesquiphellandrene (6.81%), β -bisabolene (6.24%), and ar-tumerone (4.65%). While in GEO3, the major identified compounds were α -zingiberene (24.88%), β -sesquiphellandrene (13.67%), β -bisabolene (11.20%), α -curcumene (10.68%), α -farnesene (6.93%), camphene (5.08%), cis-sabinene (4.35%).

Antibacterial Activity. In this study, all samples

were tested for antibacterial activities against *E. coli* ATCC 9637, *B. subtilis* (collection of LIPI), and *S. aureus* ATCC 13420. Table 2 displays the extent of antibacterial activities of GEOs compared to the antibiotic controls when tested at a concentration of 100% with a volume of 10 μL . The antibacterial activities were measured based on the formation of DIZ and recorded in millimetres (mm). The results were categorized as follows: a DIZ of < 10 mm was taken as weak inhibition, a DIZ of 10-15 mm was taken as moderate inhibition, a DIZ of 16-20 mm was considered as strong inhibition, and above 25 mm was deemed as a very strong inhibition. GEO 2 and GEO 3 showed weak antimicrobial activity against all tested microorganisms, i.e., *S. aureus*, *E. coli*, and *B. subtilis*. On the other hand, GEO 1 exhibited a weak activity only against the Gram-positive bacteria, namely *S. aureus* and *B. subtilis*.

Antioxidant Activity. The antioxidant activity of GEOs expressed as IC_{50} values is illustrated in Figure 2. The IC_{50} values of GEO1, GEO2, and GEO3 when evaluated using DPPH assay (Figure 2a) were $5.3023 \pm 0.1486 \text{ mg mL}^{-1}$, $0.9249 \pm 0.0138 \text{ mg mL}^{-1}$, $10.4463 \pm 0.4662 \text{ mg mL}^{-1}$, respectively. While the IC_{50} values when assessed using ABTS assay (Figure 2b) were $1.4504 \pm 0.0551 \text{ mg mL}^{-1}$, $0.5276 \pm 0.0107 \text{ mg mL}^{-1}$, and $3.3535 \pm 0.1601 \text{ mg mL}^{-1}$ for GEO1, GEO2, and GEO 3, respectively. A similar pattern was observed with both methods as GEO2 displayed the highest overall antioxidant activity, while GEO3 exhibited the least activity. Overall, all the GEOs tested had significantly lower anti-radical scavenging activity ($p < 0.05$) compared to the positive control used in the tests, ascorbic acid ($\text{IC}_{50} = 0.0059 \pm 0.0002 \text{ mg mL}^{-1}$) in the DPPH assay and trolox ($\text{IC}_{50} = 0.0054 \pm 0.0001 \text{ mg mL}^{-1}$) in the ABTS assay.

DISCUSSION

The complex and various constituents of essential oils, including GEOs, possess a wide array of biological properties (Miguel, 2010), including antimicrobial and antioxidant properties of GEOs (Bellik 2014; Mahboubi 2019; Wang *et al.* 2020). The urgent need to find new sources of antimicrobial compounds and the demands for naturally-sourced preservatives are a few reasons why GEO and other essential oils are often investigated for their antimicrobial and antioxidant properties. This present study aims to characterise the chemical constituents of three commercially-available GEOs and elaborate on

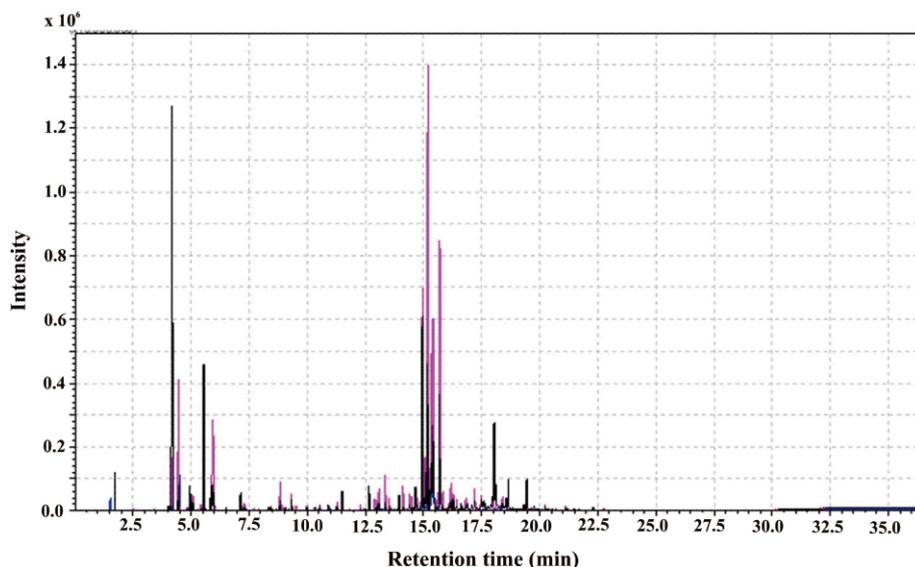


Fig 1 Total ion chromatogram of GEOs. (Blue = GEO1; Black = GEO2; Pink = GEO3).

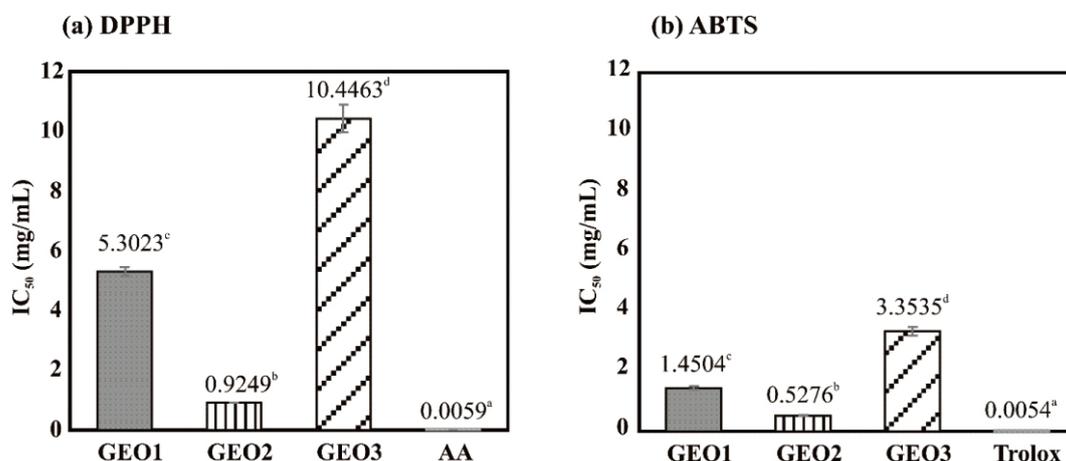


Fig 2 Antioxidant activity of GEOs based on the (a) DPPH and (b) ABTS methods. The numbers in each bar followed by the same letter are not significantly different at the 0.05 level of Tukey's multiple comparison tests.

their antibacterial and antioxidant properties.

It was known from the label and/or website of the e-commerce that GEO1 and GEO3 were produced from the root part of *Z. officinale* R., while the GEO3 source was unknown or not mentioned. Previous studies revealed that the major component of *Z. officinale* is zingiberene (Kamaliroostaa *et al.* 2013; Ribeiro *et al.* 2015; Sharma *et al.* 2016; Mahboubi, 2019). Those were different from GEO2, which contained α -pinene as the major component (25.72%). A study by Pintatum *et al.* (2020) also identified α -pinene as the main component of *Z. kerrii* essential oil, suggesting the presence of α -pinene can be used as the basis of identifying the source of GEO within Zingiberaceae family members with a high content of α -pinene.

GEO has been reported to have various

pharmacological and biological activities, including antimicrobial and antioxidant activities (Mahboubi, 2019). GEO has shown antimicrobial activity against *S. aureus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *B. subtilis*, *E. coli*, *Acinetobacter baumannii*, *S. pyogenes*, *S. typhi*, and multidrug-resistant (MDR) *A. baumannii* (Stoyanova *et al.* 2006; Mesomo *et al.* 2013; Meliani *et al.* 2014; Wang *et al.* 2020). In this study, weak inhibitory effects against *E. coli* ATCC 9637, *B. subtilis*, and *S. aureus* ATCC 13420 were observed in all samples (Table 2). This finding was similar to earlier studies conducted by Bag and Chattopadhyay (2015) and Mostofa *et al.* (2018). On the other hand, a number of studies reported good antimicrobial activity (Sivasothy *et al.* 2011; Silva *et al.* 2018; Mahboubi, 2019), which suggested that the

Table 1 Chemical compositions of the commercial GEOs

No.	Rt (min)	Compounds	% Area		
			GEO1	GEO2	GEO3
1	4.021	Tricyclene	0.53	-	-
2	4.191	α -Pinene	5.04	25.72	1.95
3	4.469	Camphene	16.94	1.28	5.08
4	4.956	β -Pinene	0.37	1.24	-
5	5.090	β -Myrcene	1.57	0.41	0.51
6	5.552	3-Carene	-	7.71	-
7	5.813	o-Cymene	-	0.55	-
8	5.918	D-Limonene	1.82	1.80	-
9	5.937	<i>cis</i> -Sabinene	-	-	4.35
10	5.940	α -Phellandrene	1.76	-	-
11	5.998	Eucalyptol	1.31	0.83	2.60
12	7.156	α -Terpinolene	0.46	0.86	0.26
13	7.317	Linalool	0.51	-	0.32
14	8.867	endo-Borneol	0.38	0.45	1.04
15	9.319	α -Terpineol	-	0.53	0.57
16	10.343	Neral	0.74	-	-
17	10.928	Citral	1.03	0.23	-
18	11.306	Bornyl acetate	0.69	-	-
19	11.502	Carvacrol	-	1.01	-
20	12.658	3-Allylguaiacol	-	1.28	-
21	13.114	Copaene	0.58	0.39	0.82
22	13.381	β -Elemene	0.58	0.41	1.34
23	13.957	Caryophyllene	-	0.82	-
24	14.116	γ -Elemene	-	-	0.98
25	14.402	(E)- β -Farnesene	-	-	0.55
26	14.655	Ethyl cinnamate	-	1.22	-
27	14.956	α -Curcumene	11.62	11.88	10.68
28	15.091	Pentadecane	-	2.19	-
29	15.065	Germacrene D	-	-	2.36
30	15.200	α -Zingiberene	22.89	8.01	24.88
31	15.348	α -Farnesene	4.46	2.70	6.93
32	15.435	β -Bisabolene	9.07	6.24	11.20
33	15.455	β -curcumene	-	0.51	-
34	15.608	<i>cis</i> -Muuroloa4(15),5-diene	-	-	0.88
35	15.705	β -sesquiphellandrene	11.21	6.81	13.67
36	15.834	<i>trans</i> - γ -bisabolene	-	-	0.69
37	16.186	Elemol	-	0.42	0.95
38	16.283	<i>trans</i> -Nerolidol	-	0.56	0.59
39	17.203	Zingiberenol	-	0.40	0.79

Table 1 Chemical compositions of the commercial GEOs, - Continued-

No.	Rt (min)	Compounds	% Area		
			GEO1	GEO2	GEO3
40	17.480	β -Acorenol	-	0.98	-
41	17.482	7- <i>epi-cis</i> -Sesquisabinene hydrate	0.33	-	0.69
42	17.620	Aromandendrene	-	0.55	-
43	17.974	β -Eudesmol	-	0.52	-
44	18.044	aR-Turmerone	-	4.65	-
45	18.116	Tumerone	-	1.08	-
46	18.646	Curlone	-	1.55	-
47	19.450	Ethyl <i>p</i> -methoxycinnamate	-	1.82	-

Table 2 Antibacterial activity of GEOs

Sample	Diameter of Inhibition Zone (mm)*					
	<i>S. aureus</i> ATCC 13420		<i>E. coli</i> ATCC 9637		<i>B. subtilis</i>	
GEO1	7.60	± 0.24	0.00	± 0.00	7.97	± 0.33
GEO2	7.17	± 0.05	7.85	± 0.31	7.42	± 0.02
GEO3	7.30	± 0.08	7.20	± 0.08	8.37	± 0.33
Antibiotic control#	21.52	± 0.22	17.63	± 0.13	14.32	± 0.30

differences in the results may be attributed to the difference in assay methods employed in these studies. The physicochemical properties may affect the diffusion of the oil component into the agar, which interferes with the result. Dilution of the GEO to a surfactant may help to increase the diffusion ability and lower the density of the oil, which in turn may improve the antibacterial activity (Li *et al.* 2015).

The antimicrobial activity of ginger GEOs has been attributed to these chemical constituents, namely β -sesquiphellandrene, caryophyllene, α -zingiberene, α -farnesene, ar-curcumene, citral, β -bisabolene, geranyl acetate, and geraniol (Mahboubi 2019). These compounds were identified in the GEOs tested; thus, it is likely that the diffusion of the oils to the agar media was not optimal, which resulted in the lower antibacterial activity than expected. In addition, it is also likely that the lower abundance of phenolic compounds contributed to the weak antibacterial activity. This finding is in accordance with a previous study by Pintatum *et al.* (2020) where *Z. kerrii* extracts with fewer phenolic compounds did not display good antibacterial activity.

It is also interesting to note that for GEO1, *S. aureus* and *B. subtilis* were more susceptible to GEO1 compared to *E. coli* as no DIZ was observed against *E.*

coli. This finding is similar to a previous study where extracted ginger GEO exhibited a better antibacterial activity against *S. aureus* than *E. coli*, potentially attributed to the structure of Gram-negative bacteria that provides an additional protective barrier against antimicrobial compounds (Wang *et al.* 2020). Other studies also revealed that GEO had a better antibacterial activity against *S. aureus* compared to *E. coli* (Bellik 2014; Mahboubi 2019; Mesomo *et al.* 2013). The variation in the antibacterial activities of the three GEOs is likely to be influenced by the differences in the overall chemical compositions detected (Mahboubi 2019), which may play different roles in exerting their activities against the tested bacterial strains. Afterall, ginger GEOs can affect bacterial viability via multiple mechanisms, namely by disrupting the cell membrane permeability, interfering with the respiratory metabolism, and inhibiting DNA metabolism activities (Wang *et al.* 2020).

The antioxidant activity of GEOs in the present study was tested using DPPH and ABTS methods. The higher IC₅₀ values obtained using DPPH compared to ABTS method (Fig 2) is also similar to the results obtained in a previous study by Höfer *et al.* (2015) where a number of methods measuring the antioxidant activity of GEO were employed. GEO was found to be

a weak DPPH reducer compared to BHT (Höferl *et al.* 2015), thus the use of other methods may elaborate further on the antioxidant activity of GEOs tested in this study. Different plant essential oils may exhibit varying antioxidant activity when different tests are employed, as the chemical compositions of the essential oils are also different and may exert antioxidant activity by various mechanisms (Miguel 2010).

Plants secondary metabolites that contributed to the antioxidant activities are from the phenolic groups (Minatel *et al.* 2017), which play an important role in neutralizing the free radicals and inhibiting oxidative damage (Pintatum *et al.* 2020). Phenolic compounds are the main components that play a role in the antioxidant activity of ginger (Yeh *et al.* 2014). As such, phenolic compounds correlate with the antioxidant activity, as exemplified in a study by Pintatum *et al.* (2020), where a lower abundance of phenolic compounds compared to terpenoid compounds in *Z. kerrii* extract was suggested to be associated with the weak antioxidant activity observed.

The present study showed intriguing result that the antioxidant activity of GEO2 was the highest, considering that the major component found was α -pinene, a non-phenolic compound. Despite weak antioxidant activity as tested with DPPH and ABTS methods observed in a range of plant essential oils when low levels or absence of phenolic compounds are detected (Miguel 2010), a study on essential oils from different plant parts of *Myrtus communis* var *italica* showed the lack of phenolic compounds and the presence of α -pinene still translated into antioxidant activity (Wannes *et al.* 2010). Furthermore, it is also likely that the presence of some oxygenated monoterpenes such as carvacrol and α -terpineol (albeit in small amounts) may also contribute to the antioxidant activity of GEO2, as such compounds have been attributed to antioxidant activity of essential oils (Bayala *et al.* 2014).

The chemical constituents of GEOs primarily consist of monoterpene and sesquiterpene compounds. The most abundant compound present in GEOs from *Zingiber officinale* was α -zingiberene at 22.89% and 24.88% concentrations for GEO1 and GEO3, respectively. In comparison, GEO2 constituted α -pinene (25.72%) as the main component.

The GEOs showed low antibacterial and antioxidant activities compared to the respective positive controls. The low antibacterial activity observed in this study was likely caused by the dense

characteristic of the oil that caused difficulty to diffuse into the agar media. Therefore, it is recommended to mix the GEOs with surfactant and use a dilution method to evaluate the antibacterial activity. Meanwhile, the low antioxidant activity of the GEOs may be due to the few phenolic compounds present. As different combinations of compounds may contribute to antioxidant activity, it would be desired to perform additional methods to assess antioxidant activity to obtain a better insight into which compounds are likely to play a role in antioxidant activity in the GEOs tested.

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