Chemical Constituent, Antibacterial Activity and Mode Of action Of Elephat and Emprit Ginger

by Diki Prayugo Wibowo

CHEMICAL CONSTITUENTS, ANTIBACTERIAL ACTIVITY AND MODE OF ACTION OF ELEPHANT GINGER (*Zingiber officinale var. officinale*) AND EMPRIT GINGER RHIZOME (*Zingiber officinale var. amarum*) ESSENTIAL OILS

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ABSTRACT

Introduction: Ginger (*Zingiber officinale* Rosc) is a spice plant, which is extensively used worldwide, and morphologically classified into three types, including the red, “gajah” or “elephant”, and “emprit” ginger (common name in Indonesia). In addition, the extract and essential oils possess antibacterial pharmacological activities, due to the inherent constituents. The aim of this research, therefore, was to analyze the chemical constituents, test antibacterial activities, and observe the mode of action of elephant and emprit ginger rhizome essential oils. **Methods:** Essential oils isolation was conducted using water and steam distillation method, while microdilution method was adopted in the testing for antibacterial activities against Gram positive and negative bacteria. Furthermore, the mode of action was evaluated using *Scanning Electron Microscopy* (SEM). **Results:** The antibacterial activity demonstrated antibacterial activities in the essential oils of elephant and emprit ginger rhizome, with minimal inhibition concentrations (MIC) value of 250-1000 μg.mL^{-1} and minimal bacterial concentrations (MBC) value of 500-1000 μg.mL^{-1}, while chemical evaluation showed the presence of 45 and 38 constituents, respectively. **Conclusion**: Both essential oils possess antibacterial activities against Gram positive and negative bacteria, with different strengths, which are based on chemical composition. Conversely, SEM micrographs demonstrated the ability for elephant and emprit ginger rhizome essential oils to change the morphology of bacteria.

Keywords: antibacterial, elephant ginger, emprit ginger, chemical constituents

INTRODUCTION

Ginger (*Zingiber officinale* Rosc) is a spice plant, which is extensively used worldwide, and is morphologically classified into three types, including the red, gajah or elephant and emprit ginger[1]. These tend to differ from one another in terms of shape, color, odor, and chemical constituents. Specifically, the elephant variety possesses a huge rhizome, less odor and fiber, while the red has a relatively smaller rhizome, red skin, sharp odor and more fiber. Conversely, Emprit ginger has small rhizome, beige skin color with sharp odor and more fiber[1].

Traditionally, ginger has been used in the treatment of cold, digestion disorder, pain, fever, inflammation, and others[2]. However, the extract scientifically possesses pharmacological activities, including antidiarrheal, based on the inhibitory effect of B-FITC toxin, on the receptor binding mechanism at a concentrations of 25 and 50 μg/ml[3]. In addition, antidiabetic[4], anticonvulsion[5], antinausea[5], antibacterial [6,7], lipid decreasing[8,9], and anti-inflammatory[10] effects have also being exploited with the essential oils of ginger, e.g., *Zingiber striolatum*. Specifically, the antimicrobial activity recorded in prior studies show a minimal inhibition concentration (MIC) value of 0.78-3.12 mg/mL, anticancer effect was reported for leukemia (K562), cervical (A549), and prostate (PC-3) cancers, with IC_{50} value of 29.67; 48.87; 86.05 μg/ml, respectively[11].

Pharmacology activities of ginger essential oils depend on the inherent chemical composition. The aim of this research, therefore, was to analyze the chemical constituents, test antibacterial activities, and observe the mode of action of elephant and emprit ginger rhizome essential oils.

MATERIALS AND METHODS

Plant Material

The rhizomes of elephant and emprit ginger were collected from Balai Penelitian Tanaman Rempah dan Obat (BALITTRO), Bogor, Jawa Barat, and determined at Jatinangor Herbarium, Taxonomy laboratory, Biology faculty, Padjadjaran university, with specimen number 123/HB/03/2018. Therefore, soil and others were cleaned from the fresh specimen, using water flow, and chopped to 2 mm thickness after draining.

Isolation of Essential oil

The isolation of essential oils was conducted using water and steam distillation method for 3 hours, and both samples were then stored in the refrigerator prior to further analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The chemical constituent of elephant and emprit ginger essential oils were analyzed with GC (Shimadzu GCMS-QP2010 Ultra). Furthermore, the system temperature was initiated at 60°C, and subsequently raised to 280°C at the injector. The detector used helium as eluent, at a 1.31

ml/minutes flow rate, 80.2 kPa pressure, and 1.7 cm/sec linear speed. Moreover, the MS system had a source temperature of 230ºC and a 0.80 KV detector.

Antibacterial Activity

Antibacterial tests was conducted by the microdilution methods, using *Staphylococcus aureus* ATCC6538, *Bacillus subtilis* ATCC6633 *Bacillus cereus* ATCC11778, Methicillin-resistant *Staphylococcus aureus* (MRSA) as positive gram bacteria and *Pseudomonas aeruginosa* ATCC9027, *Escherichia coli* ATCC8939 as negative bacteria. Furthermore, all microorganisms were obtained from the Microbiology Laboratory, Bandung Technology Institute, and the procedures were performed according to the National Committee for Clinical Laboratory Standard.

Mode Of Action

The mode of action for both essential oils were observed using *Scanning Electrons Microscopy* (SEM) at the Mineral Faculty Laboratory, Bandung Technology Institute, using the procedure stipulated by Anam [12,13].

RESULTS

Chemicals Constituent of Elephant and Emprit Ginger Essential Oils

The chemical constituents of both samples were analyzed and the results showed the existence of differences between elephant essential oil,, which contains 45 components, including 1,8-Cineole (6.4%), ar-curcumene (2.75%), camphene (6.48%), citral (16.19%), fernesene (3.8%), linalool (2.57%), methylheptenone (2.33%), sabinene (6.19%), z-citral (11.84%), α-cedrena (4.72%), α-pinen (3.22%), β-bisabolene (2.28%), β-myrcene (2.48%) as well as β-sesquiphellandrene (3.35%), and the Emprit variety, which consist of 38 chemical constituents, encompassing 1,8-Cineole (7.95%), 1,8-p-methandiene (2.74%), ar-curcumene (6.86%), Bornyl acetate (3.21%), camphene (10.14%), citral (16.19%), geranyl acetate (2.24%), methylheptenone (2.44%), z-citral (12.23%), α-pinen (2.5%), and β-bisabolen (2.64%). Furthermore, Table 1 shows the chemical constituents of both samples.

Table 1: Chemical Constituent of Ginger Essential Oils.

No	Chemical constituent	Elephant ginger (%)	Emprit ginger (%)

1	(-)-Spathulenol		0.44
2	1,8-Cineole	6.4	7.95
3	1,8-p-Menthadiene		2.74
4	2-Heptanone	0.21	0.19
5	2-Nonanone	0.95	1.21
6	2-Undecanone	1.84	1.54
7	4-Terpineol	0.88	
8	4α-Methyl-trans-2-decalinone		0.46
9	ar-Curcumene	2.75	6.86
10	Bornyl acetate	0.63	3.21
11	Bornyl methyl ether		0.18
12	Camphene	6.48	10.14
13	Camphor	0.45	1.31
14	Caryopilen		0.42
15	Citral	16.19	16.19
16	Citronella	1.31	0.47
17	Citronellyl acetate	0.27	0.51
18	Cryptone	0.4	0.5
19	Cyclohexane		1.27
20	Cycloisolongifolene	0.31	
21	d-3-Carene	0.26	
22	d-Nerolidol		0.52
23	Farnesene	3.8	
24	ɣ-Cadinene		0.64
25	Geranic acid		1.51
26	Geraniol formate		0.46
27	Geranyl acetate	0.68	2.24
28	Germacrene	0.35	
29	ɣ-Maaliene	0.95	
30	Isoborneol	0.58	
31	Isogeraniol		0.55
32	Linalool	2.57	
33	Methylheptenone	2.33	2.44
34	Myrtenal		
35	n-Decanal	2.2	
36	Neoallocimene	0.28	
37	p-Cymene	0.29	0.56
38	Piperitenone	0.32	
39	Piperitone		0.25
40	Sabinene	6.19	
41	Squalene		0.27
42	Sylvestrene		
43	Thiogeraniol	0.31	0.36
44	Tricyclene	0.32	0.4
45	Valencene	0.32	

46	Z-Citral	11.84	12.23
47	Zingiberene	0.07	0.22
48	α-Bergamotene	0.28	
49	α-Cedrene	4.72	
50	α-Cubebene	0.21	
51	α-Guaiene	0.24	
52	α-Phellandrene	0.53	
53	α-pinene	3.22	2.5
54	α-Terpineol	2.24	
55	α-Terpinolene	0.64	
56	β-Bisabolene	2.28	2.64
57	β-Elemene	0.5	0.21
58	β-Myrcene	2.48	1.09
59	β-Pinene	0.87	0.49
60	β-Sesquiphellandrene	3.35	0.4

Antibacterial Activities

The test for antibacterial activity in both samples provided positive results, as shown by the MIC and MBC values, which was more potent in the elephant essential oil. This variety inhibited the growth of 5 bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus, Escherichia coli,* and MRSA, while the emprit ginger prevented the growth of 3 species, encompassing *Staphylococcus aureus, Escherichia coli,* and MRSA. Table 2 shows MIC and MBC of both samples.

Table 2 : MIC and MBC value of ginger essential oil against test bacteria

Essential oils	Inhibition ($\mu g.mL^{-1}$)	*E. coli*	*P.aeruginosa*	*B.cereus*	*B.subtilis*	*S.aureus*	MRSA
Elephant ginger	MIC	500	-	1000	1000	250	>1000
	MBC	500	-	>1000	-	>1000	-
Emprit ginger	MIC	1000	-	-	-	250	1000
	MBC	>1000	-	-	-	>1000	>1000

Table 2 demonstrates that the strongest inhibition of growth by both essential oils occurred with *Staphylococcus aureus* at 250 $\mu g.mL^{-1}$ MIC. Therefore, the mode of bacteria damage action was observed using *Scanning Electron Microscopy* (SEM). Figure 1 and 2 show SEM results.

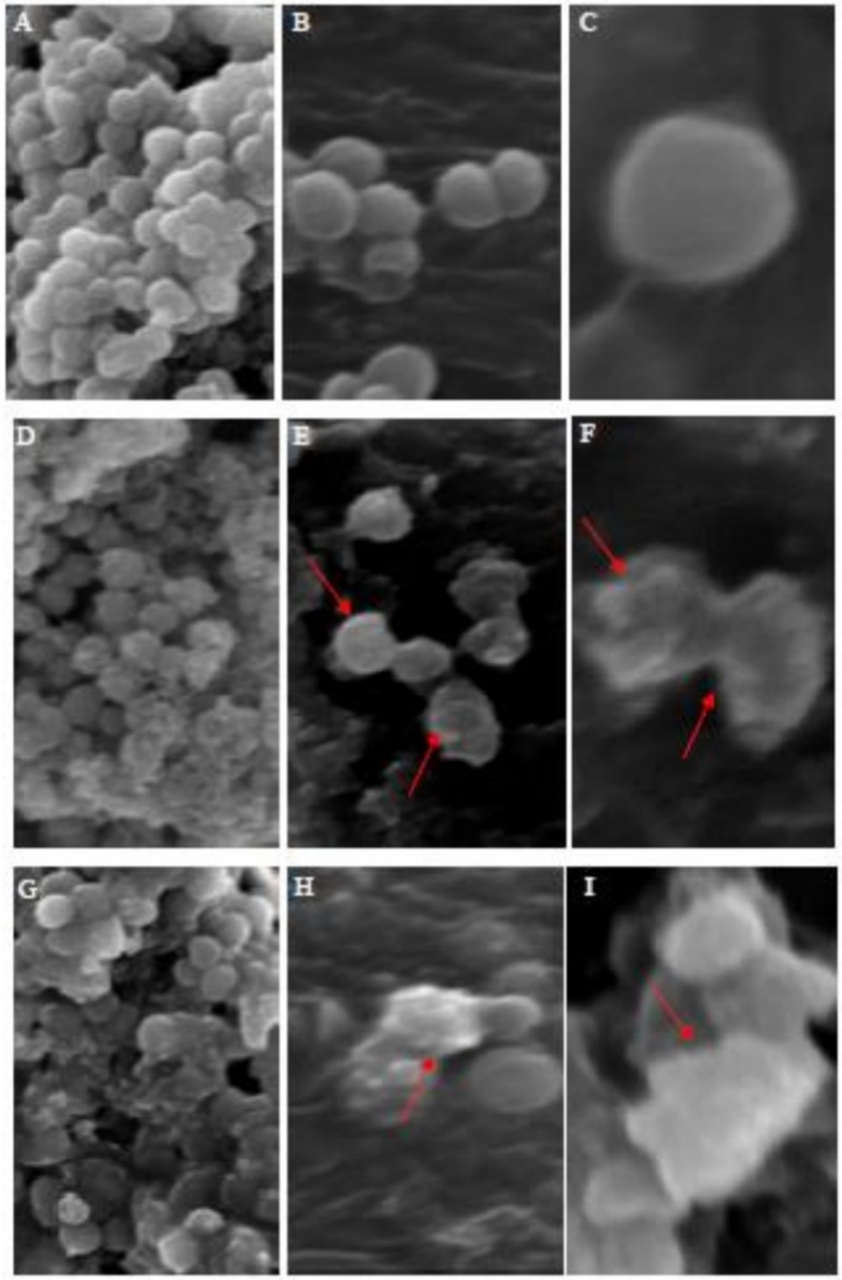

Figure 1: SEM micrograph of *S. aureus* (A-C) Control, (D-F) after growth in the presence of 1000 μg.mL^{-1} emprit ginger essential oils, (G-I) after growth in the presence of 20 μg.mL^{-1} Amoxicilin. Red arrows show morphology changes.

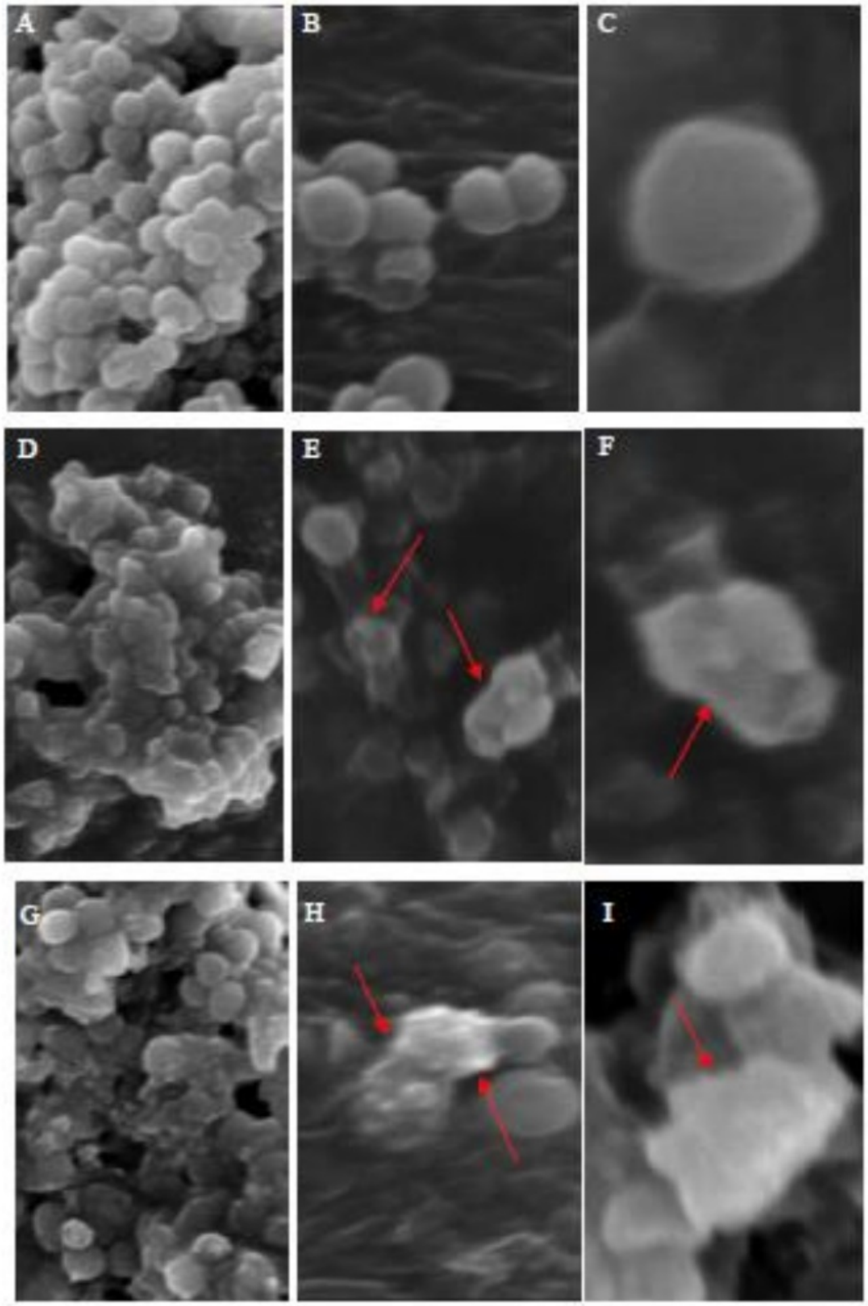

Figure 2: SEM micrograph of *S. aureus* (A-C) Control, (D-F) after growth in the presence 1000 μg.mL^{-1}of elephant ginger essential oils, (G-I) after growth in the presence of 20 μg.mL^{-} Amoxicilin. Red arrows show morphology changes.

DISCUSSION

There was a difference in the components and concentrations between elephant and emprit ginger essential oil. The main component also varied between the samples evaluated and as seen in study by Noori (2018)[14] and Sharma (2016)[15], the main component of ginger essential oils was α-zingiberene, while the main component in the sample is Citral. These differences are possibly influenced by the location of the growing plant, time of harvest, plant genetics and other factors. Furthermore, the statement is supported by the research of Bhattarai[16], which stipulated the existence of variation in components amongst three types of ginger grown in different location.

The chemical component is the determinant of antibacterial activity, exhibited by the sabinen, α-pinen, β-pinen, β-phellandrene, γ-elemene, δ-elemene, β-caryophyllene, γ-caryophyllene, and germacrene B content[17,18,19,20,21]. These were all identified in the elephant ginger variety, making it a comparably better antibacterial agent, due to the ability to inhibit the growth of all test bacteria, including the Gram positive. Conversely, emprit ginger only restrained the growth of 2 microorganisms (*E. coli* and *S. aureus*), while a research by El-baky (2010)[22] showed the specific inhibitory activity of β-sesquiphellandrene, caryophyllene and zingiberene against the growth of Gram positive bacteria. These components, however, tend to be more available in the elephant ginger essential oil.

SEM micrograph (Figure 1. and figure 2: A-C) showed the normal *Staphylococcus aureus* cell as circular in shape with smooth surface, which were modified after growth in the presence of elephant and emprit ginger essential oils, as well as Amoxicillin. This drug is known to act by binding to penicillin-binding proteins, which inhibit a transpeptidation process, therefore leading to the activation of autolytic enzymes in the bacterial cell wall, followed by lysis[23]. Based on previous research, damage of bacteria has been affiliated with interactions between essential oil and enzyme, as well as the protein membrane[24,25,26], as the chemical constituents penetrate the cellular bilayer membrane. This leads to increased permeability, and the subsequent leakage [24,25,26].

CONCLUSION

Based on the results and discussion, elephant and emprit ginger essential oils possess antibacterial activity against Gram positive and negative bacteria, at a strength that is determined by the chemical constituents. In addition, SEM micrograph showed modification in bacteria morphology, following a treatment with both samples.

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