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Characterization of Chemical Constituent and Antibacterial Activity of Honje Fruit Skin (*Etlingera elatior*)

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Abstract

Characteristic of chemical constituent and antibacterial property from *Etlingera elatior* fruit have been carried out. Essential oil of plant was extracted using steam and water distillation and then analyzed by gas chromatography-mass spectrometry. Antibacterial activity of the essential oil was done through disc diffusion method against *Bacillus cereus, Staphylococcus aureus, Eschericia coli* and *Pseudomonas aeruginosa*. Percentage yield of essential oil was about 0,09% (v/w). The GC-MS profile revealed that *E. elatior* contained 35 compounds where *1-dodecanol, dodecanal* and *trans-caryophyllene* were predicted to be main components. The oil showed activity against *B. Cereus, S. Aureus, E. Coli,* and *P. aeruginosa* with zone of inhibition were about 25, 15.2, 11.67 and 12.33 mm respectively.

Keywords: Essential oil, *Etlingera elatior,* Gas chromatography-mass spectroscopy, Antibacterial activity

1. Introduction

Food generally has a relatively short shelf life where it is easily damaged (perishable). Water content in the food is the main factors to decide the shelf life of food. The higher water content of food, the greater damage of likelihood will occur, where it is as a result of internal biological activity (metabolism) and the entry of destructive microbes [1].

Most of instant foods use preservative to prevent the growing of microbial [2]. The use of synthetic food preservative has been known to have many disadvantages which cause side effects to human's body. To cope this solution, natural food preservatives have been developed which is less side effects. One of the types of compounds that can be used as a natural food preservative is a natural antimicrobial compounds [3]. Natural antimicrobial can be used as a natural food preservative which is required to replace chemical preservatives that have a risk to health such as food-borne illness. In order to reduce the number of cases of food-borne illness, applying antimicrobials into food processing is an alternative to inactivate or prevent the growth of microbial [4]. Natural food preservative containing antimicrobial substances may be derived from natural plants. Essential oil has been known to have antimicrobial property which benefits as food preservative. *Etlingera elatior* (Figure 1) is family of *Zingiberaceae* and commonly distributed in West Java and Banten. *E. elatior* has been traditionally used as a spice for cooking and odor remover [5].

Figure 1. Plant and Fruit of Honje

Previous studies on bioactivity of *E. elatior* were reported. Ethanol extract of the leaves of *E. elatior* showed activity against bacteria gram-positive (*Bacillus cereus* and *Bacillus megatrium*) and bacteria gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*). The results revealed that *E. elatior* showed antibacterial activity with minimum concentration 100-800 mg/mL and the minimum lethal concentration 400-800 mg/mL [6]. In addition, *E. elatior* had antibacterial activity against *S. aureus* with minimum inhibitory concentration values about 15% of concentrations at with the percentage of inhibition about 95.63%, *E. coli* by 50% with the percent inhibition of 92.41%. These concentrations were considered to be natural food preservative [7].

This study was aimed on characterization of chemical constituent from *E. elatior* using gas chromatography-mass spectroscopy and screening for its antibacterial activity against pathogenic bacteria.

2. Research Method

2.1 Plant material and extraction process

Honje fruit (*Etlingera elatior*) was collected from Cintaratu Village, District Parigi, District of Pangandaran, West Java. The specimen of the plant was verified at Field Botany Biology Research Center Cibinong LIPI, Bogor.

About 2 kg of *E. elatior* was placed into steam-water distillation and extracted for 6 h to obtain essential oil. After extraction, excessive water in essential oil was removed by anhydrous sodium sulphate. The essential oil was stored at 4 °C.

2.2 Gas chromatography analysis

Chemical constituent of essential oil from *E. elantior* was analyzed using gas chromatography-mass spectroscopy (GC-MS) technique. GC-MS analyzer was performed using Agilents Technologies 68890 N Network GC system, equipped with semi-polar column, type HP-5MS (30 m x 0.25 mm x 0.25 mm). Helium was performed as carrier gas and around 0.1 µL of essential oil was injected (1:100). Temperature was programmed from 80 °C to 250 °C.

2.3 Antibacterial test: Disc diffusion method

Antibacterial test of *E. elantior* against two bacteria gram positive *Bacillus cereus, Staphylococcus aureus* and gram negative *Eschericia coli, Pseudomonas aeruginosa* was evaluated using disc diffusion method. Bacteria was prepared in nutrient broth and incubated at 37 °C to standardize approximately 10^8 colony forming unit (CFU). About 0.1 ml of bacteria was subjected onto nutrient agar and swabbed using cotton swab. Sample was prepared in 20, 40 60, 80 and 100% of concentration (v/v) in DMSO. Around 10 µL of each concentration was injected into 11 mm of blank disc and allowed to dry. The discs were placed to cultured agar and incubated at 37 °C for 24 h. Chloramphenicol was used as positive control and solvent DMSO as negative control. Clear inhibition zone indicated as a presence of antibacterial property of *E. elatior*.

3. Research Result

3.1 Characteristic of Essential Oil

Essential oil of *Etlingera elatior* was produced about 15.5 mL (0.009%) of 16.8 kg of dry weight sample. Physical properties of *E. elatior* were compared with Indonesian National Standard (SNI) ginger oil (SNI 06-1312-1998) and International Standard Oil of Ginger (ISO 7355:1995) as shown in Table 1.

Table 1. Characteristic of the physical properties of fruit skin honje essential oil

No	Characteristic	*E. elatior* Fruit essential oil	SNI 06-1312-1998	ISO 7355:1995
1	Specific gravity (g/mL) $25^0C/25^0C$	0,8814	0,8720 – 0,8890	0,870 – 0,890
2	Refractive index 25^0C	1,451	1,4853 – 1,4920	1,480 – 1,490
3	Solubility in alcohol 90%	1 : 1	–	1 : 4

3.2 Chemical Composition of the Essential Oil

Bioactive compounds of *E. elatior* were identified through GC-MS analysis. Thirty-five compounds were observed in fruit plants. Based on GC analysis, there are five main components in the essential oil of *E. elatior*, 1-dodecanol (RT= 20.70 min; 25.59%), dodecanol (RT= 19.05 min; 18.11%), trans-cryophyllene (RT= 19.94 min; 12.22%), cyclododecane (RT= 25.38 min, 6.47%) and dodecyl ester (RT= 23.72 min; 5.99%) (Library Wiley 7). Alpha pinene appeared as the first compound to come out with retention time 8.76 min (0.41%) and cyclotetradecane was the last compound with RT 29.72 min (0.16%). Several compounds were also detected such as alpha copaene (RT 18.62 min, 0.16%), 2-methoxy-4-(2-propenyl) (RT= 21.88 min, 0.15%), alpha farnesene (RT= 21.46 min, 0.19), 2-pentadecanone (RT= 25.85 min, 0.17%) and cycloundecane (RT= 20.30 min, 0.19%). Chemical constituent of *E. elatior* with molecular weight, peak area and retention time was shown in Table 2.

Table 2. The chemical components of *E. elatior* fruit essential oil

RT	Compound	molecular formula	MW	% Peak Area	% Similarity
8.76	Alpha pinene	$C_{10}H_{16}$	136	0.41	97
13.85	Decanal	$C_{10}H_{20}O$	156	1.38	91
15.06	Geraniol	$C_{10}H_{18}O$	154	1.06	94
15.41	1-Decanol	$C_{10}H_{22}O$	158	1.51	91
15.99	2-Undecanone	$C_{11}H_{22}O$	170	0.35	94
16.36	Undecanal	$C_{11}H_{22}O$	170	0.7	91
17.94	2-methoxy-4-(2-propenyl) phenol	$C_{10}H_{12}O_2$	164	3.26	98
18.21	Geranyl acetate	$C_{12}H_{20}O_2$	196	0.26	91
18.62	Alpha copaene	$C_{15}H_{24}$	204	0.16	99
19.05	**Dodecanal**	$\mathbf{C_{12}H_{24}O}$	**184**	**18.11**	**91**
19.94	**Trans caryophyllene**	$\mathbf{C_{15}H_{24}}$	**204**	**12.22**	**99**
20.15	Beta bisabolene	$C_{15}H_{24}$	204	0.22	86
20.30	Cycloundecene, 1-methyl	$C_{12}H_{22}$	166	0.19	95
20.70	**1-Dodecanol**	$\mathbf{C_{12}H_{26}O}$	**186**	**29.59**	**91**
21.11	2-Tridecanone	$C_{13}H_{26}O$	198	1.14	94
21.38	Germacrene D	$C_{15}H_{24}$	204	0.19	98
21.46	Alpha farnesene	$C_{15}H_{24}$	204	0.19	97
21.88	Azulene	$C_{15}H_{24}$	204	0.15	98
21.98	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	$C_{12}H_{14}O_3$	206	0.16	98
22.10	Naphthalene	$C_{15}H_{24}$	204	0.54	98
22.94	Dodecanoic acid	$C_{12}H_{24}O_2$	200	8.4	99
23.45	Cyclododecene	$C_{12}H_{22}$	166	0.11	93
23.72	Acetic acid, dodecyl ester	$C_{14}H_{28}O_2$	228	5.99	94
23.91	Hexadecanal	$C_{16}H_{32}O$	240	1.33	95
23.98	Caryophyllene oxide	$C_{15}H_{24}O$	220	0.58	94
25.02	Cis-9-tetradecen-1-ol	$C_{14}H_{28}O$	212	0.42	91
25.13	1,13-tetradecadiene	$C_{14}H_{26}$	194	1.59	91
25.38	Cyclododecane	$C_{12}H_{24}$	168	6.47	98
25.53	Ar-tumerone	$C_{15}H_{20}O$	216	0.29	49
25.85	2-Pentadecanone	$C_{15}H_{30}O$	226	0.17	50
26.00	Patchouli alcohol	$C_{15}H_{26}O$	222	0.53	99
27.22	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.11	99
27.95	E-7-dodecen-1-ol acetate	$C_{16}H_{30}O_2$	254	0.21	91
28.15	Cyclotetradecane	$C_{14}H_{28}$	196	0.85	98
29.72	Cyclotetradecane	$C_{14}H_{28}$	196	0.16	95

3.3 Antibacterial activity

Essential oil of *E. elatior* was evaluated for antibacterial activity against *E. coli, P. aeruginosa, B. cereus* and *S. aureus* by observing clear inhibition zone of discs. All bacteria were successfully inhibited by essential oil at various concentrations as shown in Table 3. The largest zone inhibitions were observed such as 41.67; 19.33; 14; 13.33 mm for *B. cereus*, *S. aureus*, *E. coli* and *P. aeruginosa*, respectively at 100% of concentration. At 20% of concentration, essential oil demonstrated the highest inhibition against *B. cereus* and *S. aureus* with zone of inhibition 25 and 15.2 mm, respectively but showed no activity against *E. coli* and *P. aeruginosa*. The oil started to inhibit *B. cereus* with a moderate activity at lowest concentration and strongly inhibited at 100% of concentration.

Table 3. Disc diffusion result

	Zone of inhibition (mm)			
Concentrations (%)	*B. cereus*	*S. aureus*	*E.coli*	*P. aeruginosa*
20%	25 ± 2.00	15.2 ± 0.80	-	-
40%	30 ± 3.00	16.8 ± 1.80	-	12.33 ± 0.58
60%	34.33 ± 6.80	18.27 ± 0.30	11.67 ±1.15	13 ± 0.00
80%	39.33 ± 3.21	18.57 ± 1.10	13.67 ± 1.53	13 ± 0.00
100%	41.67 ± 6.03	19.33 ± 1.58	14 ± 1.00	13.33 ± 0.58

4. Discussion

Several researches about bioactivity of various parts of *E. elatior* such as fruit, leave and steam have been reported. Inhibitory activity of essential oil from *E. elatior* fruit increased as concentration of sample increased based on increasing of inhibition zone of treated discs [8]. Among of bacteria, *B. cereus* was the most sensitive to essential oil because it had the greatest inhibition zone at 20% of concentration. *S. aureus* was inhibited by the essential oil better than *E. coli* and *P. aeruginosa*. Gram-negative bacteria (*E. coli* and *P. aeruginosa*) were sensitive to polar compounds because the cell walls of gram-negative bacteria had similar polarity which easier passed by. Meanwhile sensitivity of gram-positive bacteria to non-polar compounds occurred because of peptidoglycan which one of the constituent amino acid alanine is hydrophobic (nonpolar). Antimicrobial compounds could react with the phospholipid component of cell membranes causing lysis in cell [9]. Terpenoids are majority compounds in essential oil which commonly exhibit antibacterial activity. It can bind proteins and lipids in cell membranes which cause cell cell lysing [10]. Membranes contain of proteins and lipids that are highly susceptible to chemical substances which are able to lower the surface tension. Damage on cell membrane causes disruption of the nutrient transport (such as compounds and ions) through the cell membrane which impacted on nutrient deficiency of growth cell [11]. Alpha-pinene, patchouli alcohol, 1-dodecanol [12], Ar-tumeron and caryophyllene oxide [13], were predicted as terpenoids compounds in the essential oil which showed antibacterial activity against *S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginose*.

Essential oils have been known to have antibacterial property due to phenolic compounds. Phenol compounds bind to the protein through hydrogen bonds, resulting on protein structures damage. Most of the structure of the cell wall and cytoplasmic membrane of bacteria contains proteins and fats. Instability in the cell wall and cytoplasmic membrane of bacteria causing the function on selective permeability, active transport, and control of protein composition of bacteria cells become impaired which will result in the losing of macromolecules and ions from the cell. This condition effects on loss of bacterial cell shape and lysing process [14].

5. Conclusion

This study revealed that *E. elatior* fruit produced a low yield of essential oil. The oil showed s strongly antibacterial activity which has potential as antibacterial agent. Further studies about isolation of bioactive compounds and mode of action on *E. elatior* should be conducted.

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