Antibacterial Activity of the Essensial Oils of Lempuyang wangi (*Zingiber aromaticum* Val.), lempuyang gajah (*Zingiber zerumbet* Sm), and lempuyang emprit (*Zingiber amaricans* Bl.) on Three Gram Negative Bacteria

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ABSTRACT--- ***The in vitro antibacterial activities of three essential oils of Lempuyang wangi (Zingiber aromaticum Val.), lempuyang gajah (Zingiber zerumbet Sm), and lempuyang emprit (Zingiber amaricans Bl.) has been evaluated against three gram negative bacteria (Pseudomonas sp., Escherichia coli and Salmonella thypii). The in vitro antibacterial activity was performed by disc diffusion method and dilution method. the three essential oils inhibited growth of the three gram-negative bacteria with zone of inhibition between 8 – 9.8 mm at 5 mg/ml . The MIC of the essential oils was in the range of 0.625 mg/ml – 1,25 mg/ml and the MBC was in range 1.25 mg/ml – 2.5 mg/ml.***
Keyword--- antibacterial, zingiber

1. INTRODUCTION

Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation or extraction but the hidrodistillation is most commonly used for commercial production. An estimated 3000 essential oils are known, of which 300 are commercially important in fragrance market (1). Essential oils are complex mixers comprising many single compounds. Chemically they are derived from terpenes and their oxygenated compounds. Each of these constituents contributes to the beneficial or adverse effects. Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties (2,3). Some oils have been used in cancer treatment (4). Some other oils have been used in food preservation , aromatherapy and fragrance industries (5,6,7).

The Zingiberaceae is among the plant families which are widely distributed throughout the tropics particularly in Indonesia (8,9). Most members of the family are easily recognised by the characteristic aromatic leaves and fleshy rhizome and several species from the genera *Zingiber* are major ingredients in traditionally medicine (10,11,12)

This study aimed at evaluating the in vitro antibacterial activity of the essential oil of rhizome from the genera *Zingiber*. The species tested were Lempuyang wangi (*Zingiber aromaticum* Val.), lempuyang gajah (*Zingiber zerumbet* Sm), and lempuyang emprit (*Zingiber amaricans* Bl.)

2. MATERIAL and METHODS

Plant materials and preparation of essential oils
All plant materials were collected from the nursery of the Balitro Bogor.

Preparation of essential oils.
Essential oils were extracted by hydrodistillation. The fresh rhizomes of Zingiberaceae were washed to remove soil, peeled and sliced. Sliced rhizomes of fresh Zingiberaceae (2 kg) were mixture with distilled water (5 L). The essential oils were extracted by hydrodistillation using a vertical hydrodistillation unit.

Micro-organisms tested. The following strains of bacteria were used: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATTC 27853, and *Salmonella thypii wild type*. The micro-organisms were grown overnight at 37°C in Nutrient Agar (Merck). Their sensitivity to the reference antibiotics (Choramphenicol)was checked for the bacteria (Table 2).

Antibacterial assay

Screening of essential oils for antibacterial activity was done by the disk diffusion method, which is normally used as a preliminary check and to select between efficient essential oils. It was performed using an 18 h culture at 37°C in 10 ml of Nutrient Broth. The cultures were adjusted to approximately 10^5CFU/ml with sterile saline solution. Five hundred microliters of the suspensions were spread over the plates containing Mueller-Hinton agar using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates.

The essential oils were dissolved in 10% aqueous dimethylsulfoxide (DMSO) with Tween 80 (0.5% v/v for easy diffusion). Empty sterilized discs (Whatman no. 5, 6 mm dia) were impregnated with 50 ul of different concentrations (2%; 1%; 0.5%; 0.25% dan 0.125%) of the respective essential oils and placed on the agar surface. Paper disc moistened with aqueous DMSO was placed on the seeded petriplate as a vehicle control. A standard disc containing Chloramphenicol (30ug/disc) was used as reference control. The plates were left for 30 min at room temperature to allow the diffusion of oil, and then they were incubated at 37°C for 18 h. After the incubation period, the zone of inhibition was measured . Studies were performed in triplicate, and mean value was calculated. The means were analysed by one way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test using SPSS software package version 13.0 for windows. The results were expressed as mean ± SD. *P* values <0.05 were considered as significant.

MIC assay

The dilution methods were used to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The Strains were cultured overnight at 37°C in Brain heart Infusion. Essential oils were emulsified in sterile tween 2% and diluted with BHI to a concentration of 2%. Test tubes filled with liquid BHI medium of 2.5 ml and added emulsi oil 2% 0f 2.5 ml. Further 1:2 serial dilutions were performed by addition of culture broth to reach concentrations ranging from 5 to 0.0625 mg/mL;). Each test and growth control tube was inoculated with 5 µL of a bacterial suspension (10^8 CFU/mL) then incubated at 37 ° C for 24 hours.

3. RESULTS and DISCUSSION

The anti-bacterial activity of zingiberaceae essential oils against three gram negative bacterial species is summarized in Table 1 and 2. The zone of inhibition above 7 mm in diameter was taken as positive result.

Table 1. Diameter of Zone of inhibition of zingiberaceae essential oils against *E.coli, P. aeruginosa* and *S. thypii*

Concentration % Oils	*E.Coli*			*P. aeruginosa*			*S. thypi*		
	LW	LE	LG	LW	LE	LG	LW	LE	LG
1	11,1	10.9	10,93	11,8	12,1	11,9	11,9	12,43	12,33
0,5	8,97	8,97	8,90	9,3	9,8	9,67	9,7	9,80	9,83
0,25	7,5	7,27	7,23	8,3	8,6	8,5	8,43	8,83	8,7
0,125	6,5	6,53	6,37	7,1	7,37	7,3	7,33	7,60	7,47

LW: Lempuyang Wangi (*Z.aromaticum*); LE: Lempuyang Emprit (*Z.amaricans*); LG: Lempuyang Gajah (*Z.zerumbet*)

The mean of diameter zone inhibition of zingiberaceae essential oils *Z. aromaticum, Z. amaricans and Z. zerumbet* were analyzed using ANOVA showed that there was no significant difference between the average diameter of the zingiberaceae essential oils at all concentrations against *E. coli* with p > 0 , 05. Whereas in bacteria P.aeruginosa, essential oil of Z. amaricans was significantly different with essential oil of Z.aromaticum and Z. zerumbet at concentration of 1% and the bacterium S. thypi, essential oil of Z. aromaticum was significantly different with essential oil of Z. zerumebt and Z. amaricans at concentration of 1%.

Table 2. **Minimum Inhibitory concentration of zingiberaceae essential oils against *E.coli, P. aeruginosa* and *S. thypii***

Microorganism	Minimum Inhibitory concentration mg/ml		
	Z.aromaticum	*Z. amaricans*	*Z. zerumbet*
Escherichia coli	1.25	0.625	1.25
P. aeruginosa	0.625	0.625	1.25
Salmonella thypii	0.625	0.625	0.625

Table 3. **Minimum Bactericidal concentration of zingiberaceae essential oils against *E.coli, P. aeruginosa* and *S. thypii***

	Minimum Bactericidal Concentration mg/ml		
Microorganism	*Z.aromaticum*	*Z. amaricans*	*Z. zerumbet*
Escherichia coli	2.5	2.5	2.5
P. aeruginosa	1.25	2.5	2.5
Salmonella thypii	1.25	1,25	1.25

Zingiberaceae family has been used for many thousand of years in food preservative, alternative medicine and natural therapy (13). MIC and MBC test results showed that the three types lempuyang have antibacterial activity of relatively similar to *Escherichia coli*, *Salmonella thypii* and *Pseudomonas.aeruginosa.*The varying degrees of sensitivity of the bacterial test organismsmay be due to both the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds present in the essential oil. In one genus or family, plants often contain chemical compounds that resemble both the type and amount quantitatively, so there are also similarities pharmacological effects. The main components of the essential oil of family Zingibereceae include terpenes (zingiberene, geraniol, methyl chavicol, terpinene, tumerone). At present, however, the mode of action of terpenic constituents on microorganisms is not fully understood (14,15). Nevertheless, in view of their hydrophobicity, it is generally considered that they are involved in such mechanism as cytoplasmic membrane, coagulation of cell contents (16)

4. CONCLUSION

The essential oilof Lempuyang wangi ,lempuyang gajah and lempuyang emprit have a potential to inhibit three gram negative bacteria. The diameter zone inhibition of essential oils showed that there was nosignificant difference at all concentrations.The MIC of the essential oils was in the range of 0.625 mg/ml – 1,25 mg/ml and the MBC was in range 1.25 mg/ml – 2.5 mg/ml.

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