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EVALUATION OF SYNERGISTIC EFFECT OF KAEMPFERIA GALANGA L. RHIZOME EXTRACTS WITH ANTIBIOTICS AGAINST BACTERIAL PATHOGENS

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ABSTRACT

The aromatic ginger rhizome (*Kaempferia galanga* L.) is a member of Zingiberaceae family, has been known to have an antibacterial effect is used for everyday cooking spices, but the synergistic effect on broad spectrum antibiotics was unknown. This research was conducted to find out the synergistic effect is on the antibiotics or the synergistic effect of the combination of the extract and antibiotic against gram positive and negative bacterial pathogens in vitro. KGR was taken from farmers in Samarinda City, East Kalimantan Province, Indonesia. Extract was obtained by maceration with ethanol solvent. Antibacterial activity test of KGR ethanol extract and antibiotic using Mueller-Hinton agar, Kirby-Bauer disc diffusion method against *Staphylococcus aureus* and *Escherichia coli*. Results were expressed in percentage increase of the inhibition zone. Statistical test with t-test, significantly different if $p < 0.05$. The combination of each antibiotics and KGR ethanol extract showed synergistic effect by increasing the percentage of inhibition zone. Conclusion: KGR ethanol extract showed synergistic effect on antibiotic ampicillin (*E. coli*), meropenem and cefuroxime in *S. aureus* and *E. coli*.

Keywords: *Kaempferia galanga*, synergistic, antibiotic, *Escherichia coli*, *Staphylococcus aureus*

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INTRODUCTION

Antibiotic resistance in the world has been very threatening and has become a worldwide concern [1,2]. The main causes of pathogenic bacteria to be

resistant are overuse of antibiotics, improper administration of antibiotics, overuse in livestock, lack of new antibiotic preparations, and difficulty in obtaining regulatory approval [3-5]. In

Indonesia, research found antimicrobial resistance in the year 2000-2004 in dr. Kariadi Hospital Semarang and Dr. Soetomo hospital Surabaya proved that there were already multi-resistant bacteria such as ESBL-producing bacteria (Extended Spectrum Beta Lactamase) and MRSA (Methicillin Resistant *Staphylococcus aureus*) [6]. By 2014, deaths from AMR (Antimicrobial Resistance) have reached 700,000 lives per year. It is estimated that AMR deaths in 2050 will reach 10 million, greater than cancer deaths [7].

It is urgent to research in order to discover new antibiotics that is still sensitive to various pathogenic bacteria. Research can be done by studying the synergistic effect of an antimicrobial with natural materials. One potential plant to be studied was Aromatic Ginger Rhizome by the Latin name *Kaempferia galanga* L. Rhizoma (KGR) from the Zingiberaceae family. The content of KGR is essential oils, alkaloids, proteins, amino acids, fats, starches, and minerals [8]. The antibacterial activity of KGR is a combination of various components, not just one active compound [9]. The contributing components are *ethyl-p-methoxycinnamate* (EPMC) and flavonoids [10,11]. KGR has been studied to have antibacterial activity in *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) in vitro [12]. Other Zingiberaceae family members such as *Zingiber officinale* (*Z. officinale*) and *Curcuma longa* (*C. longa*) have been investigated to have a synergistic effect with antibiotics [13]. KGR is one family with *Z. officinale* and *C. longa*, it was suspected that KGR can increase antibacterial effectiveness of conventional antibiotic drugs. The objective of this study was to evaluate the synergistic effect of KGR extract with broad-

spectrum antibiotic activity against pathogenic bacteria.

MATERIALS AND METHOD

Material

The materials used were *Kaempferia galanga* L. Rhizome, obtained from farmers in Samarinda City, East Kalimantan Province, Indonesia. Identification of types assisted by botanists from the Laboratory of Dendrology and Forest Ecology Faculty of Forestry Mulawarman University. Herbarium is stored in Pharmacology Laboratory of Faculty of Medicine with Code Voucher No. KG 01/V/2017. *E. coli* (ATTC® 35218), *S. aureus* (ATCC® 33591); DMSO and ethanol GR from Merck; *Muller-Hinton* agar, blank discs, standard antibiotics ciprofloxacin (5 µg), chloramphenicol (30 µg), ampicillin (10 µg), cefuroxime (30 µg), and meropenem (10 µg) from Oxoid.

Method

KGR was sorted, washed with clean running water, re-sorted, then cut into small pieces, then inserted in a 50°C temperature dryer closet and turned back and forth daily. Once dry the KGR was ground into a fine powder. The 50 g of KGR simplified was macerated with 300 ml ethanol solvent, then stood for 72 hours at room temperature, and stirred every 24 hours for 10 min. The extract was filtered using Whatman filter paper no. 1, then concentrated using rotary evaporator temperature 50°C. The concentrated extract was further dried in with a desiccator at a temperature of 60°C to a water content of <10. The extract was stored in a lid-covered container at 4°C.

Microorganisms

Bacteria *S. aureus* and *E. coli* were each cultured in nutrient agar, then

incubated at 37°C for 24 hours. Then the bacteria were taken using a swab stick then made in the form of bacterial suspension. The required amount of bacteria was measured using a spectrophotometer with OD₆₂₅ 0.08-0.1 which was estimated to contain 1-2×10⁸ CFU/ml [14].

Evaluation of Antibacterial Activity of Extract

Evaluation of antibacterial activity of extract was evaluated by using Kirby-Bauer disc diffusion method. A culture smear on the Mueller-Hinton surface for a straight Petri dish with sterile swab sticks until dry for 3-5 minutes. Concentrations of extract used were 1, 2.5, 5, 7.5 and 10 mg/disc dripped as much as 20 µl on empty disc then affixed to media that have been swabbed with bacteria. Negative controls of solvents were dripped onto empty discs. Incubate at 37°C for 24 hours. The zone of inhibition was measured in millimeters. Repetition was done three times

Antibiotic Sensitivity Test

Using sterile tweezers, 5 antibiotic discs were placed over the *Mueller-Hinton* agar, sufficiently separated from each other to avoid overlapping. Incubation at 37°C for 24 hours. The zone of inhibition was measured in millimeters. Repetition was done three times.

Evaluation of Synergistic Effects of Extract and Antibiotic

The procedure was similar to the antibiotic sensitivity test with an additional 20 µl extract solution which has the largest inhibitory zone. The extract was dropped on an antibiotic disc. Incubation at 37°C for 24 hours. The zone

of inhibition was measured in millimeters. Repetition was done three times

Statistical Analysis

Data was presented in the form of mean±SD, statistical test with t-test, significantly different if p <0.05.

RESULT AND DISCUSSION

Table 1 shows that the largest inhibitory zone was found at a concentration of 5 mg / disc, reaching (9.6 ± 0.28) mm in *S. aureus* and (9.8 ± 1.60) mm in *E. coli*. In Table 2 shows that the combination of KGR extract and the five antibiotics has increased zone of inhibition in both bacteria. The highest increased of zone of inhibition against *S. Aureus* was showed by meropenem and the weakest were ciprofloxacin. The highest increased of zone of inhibition against *E. coli* were ampicillin and the weakest were ciprofloxacin.

The secondary metabolite compounds in the ethanol extract of KGR that are responsible as antibacterial agents are flavonoids and EPMC [15,16]. Flavonoids themselves have multi-cellular targets, not just one mechanism. In addition, some studies suggest that flavonoids may be bacteriostatic and bactericide [17]. Flavonoids itself is a metabolite that is often found in plants. Flavonoids contained in the KGR extract are apigenin, luteolin, kaempferol [18].

Secondary metabolites suspected to play a role in the synergistic effect of KGR extract are flavonoid compounds apigenin, luteoin, and kaempferol by the mechanism of action changing the permeability of the outer membrane and cytoplasmic cells, inhibiting ATPase activity, inhibition of peptidoglycan synthesis, and inhibition of β-lactamase enzyme activity [19-20].

Table 1. Zone of Inhibition of the KGR Ethanol Extract and Controls

| Concentration (disc) | Zone of Inhibition (mm) | |
|-------------------------|-------------------------|----------------|
| | <i>S. aureus</i> | <i>E. coli</i> |
| Control (-) | 6.0 ± 0.0 | 6.0 ± 0.0 |
| 1 mg | 7.3 ± 1.5 | 7.0 ± 0.0 |
| 2 mg | 9.0 ± 0.0 | 8.6 ± 1.4 |
| 5 mg | 9.6 ± 0.3 | 9.8 ± 1.6 |
| 7.5 mg | 8.6 ± 0.6 | 9.0 ± 1.3 |
| 10 mg | 8.0 ± 1.0 | 8.5 ± 1.0 |

Description: repetition three times, value are represented as Mean ± SD

Table 2. Zone of Inhibition on Antibiotic and Antibiotic-Extract combination

| Treatment | Zone of Inhibition on Antibiotic and Antibiotic-Extract combination | | | | | |
|-------------|---|---------|--------|-------------------------|---------|--------|
| | <i>S. aureus</i> | | | <i>E. coli</i> | | |
| | Zone of Inhibition (mm) | % | Sig. | Zone of Inhibition (mm) | % | Sig. |
| CIP (5 µg) | 29.0 ± 4.58 | | | 29.5 ± 4.09 | | |
| CIP + EEKg | 30.0 ± 0.50 | + 3.4% | 0.726 | 33.3 ± 1.52 | + 12.8% | 0.203 |
| C (30 µg) | 23.6 ± 2.08 | | | 24.3 ± 5.50 | | |
| C + EEKg | 27.3 ± 1.60 | + 15.6% | 0.073 | 27.6 ± 3.05 | + 13.5% | 0.411 |
| AMP (10 µg) | 12.8 ± 5.29 | | | 9.3 ± 2.51 | | |
| AMP + EEKg | 14.0 ± 0.50 | + 9.3% | 0.724 | 17.1 ± 2.51 | + 83.8% | 0.019* |
| CFX (30 µg) | 7.6 ± 0.76 | | | 7.3 ± 0.76 | | |
| CFX + EEKg | 9.8 ± 0.76 | + 28.9% | 0.025* | 12.8 ± 1.25 | + 75.3% | 0.003* |
| MEM (10 µg) | 7.6 ± 0.76 | | | 7.0 ± 0.50 | | |
| MEM + EEKg | 10.5 ± 0.50 | + 38.1% | 0.006* | 11.8 ± 1.75 | + 68.5% | 0.010* |

Description: repetition three times, value are represented as Mean ± SD, concentration extract 5mg/disc; *t-test is significantly different if $p < 0.05$, CIP: Ciprofloxacin, C: Chloramphenicol, AMP: Ampicillin, CFX: Cefuroxime, MEM: Meropenem, EEKg: Ethanol Extract *Kaempferia galanga L.*

CONCLUSION

The combination of ethanol extract KGR and some antibiotic has a synergistic effect on against *S. aureus*, and *E. coli* in vitro.

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