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Activities of *Curcuma heyneana* Rhizome and *Graptophyllum pictum* Leaves Combination against *Staphylococcus aureus*

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ABSTRACT

Introduction: Infectious diseases have always been a problem, especially in the third-world countries. Due to the uncontrolled prescription of antibiotics, several bacteria developed resistance to some antimicrobial agents. One of them is *Staphylococcus aureus*. There is local wisdom in Indonesia to use natural products as potent antimicrobial agents. *Curcuma heyneana* and *Graptophyllum pictum* had been used by the locals in past centuries and seemed to be an effective agent for combatting infectious diseases. Therefore, we want to evaluate the activity of the combined extract of *Curcuma heyneana* and *Graptophyllum pictum* against *Staphylococcus aureus*..

Material and Methods: An in-vitro test by using the test tube dilution. The test tubes consisted of seven tubes; 2 control tubes and 5 experimental tubes. We used 1000mg/ml of each extract component at the highest (i.e., 1000mg/ml for *Curcuma heyneana* and *Graptophyllum pictum*, respectively). Five-times replication was conducted for each treatment. MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) were investigated after being streaked at the agar plate and incubated at 37^0C for 24 hours.

Results: We could not determine the MIC because the colour of the combination of the extract was cloudy. The bacteria grew at all concentrations from five-times replication, except for the negative control, therefore MBC could not be obtained.

Conclusion: *Staphylococcus aureus* was not inhibited effectively by combined extract of *Curcuma heyneana* and *Graptophyllum pictum*.

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INTRODUCTION

Human and animal mortality and morbidity are increasing because of infectious illnesses. This is exacerbated by the fast emergence of multidrug resistance, the narrow antibiotic spectrum, and the negative effects of currently available antimicrobial drugs. *Staphylococcus aureus* is a prominent cause of bacteraemia and is linked with increased morbidity and death when compared to other organisms [1]. The high prevalence of bacteraemia put a strain on already overburdened healthcare resources [2,3]. Because the epidemiology of *Staphylococcus aureus* has shifted and the pathogen intrinsic becomes more aggressive, improved techniques and better medications to treat and eradicate *Staphylococcus aureus* are urgently needed.

The study of antibacterial capabilities in particular indigenous plants might generate significant results. As a result, antimicrobials produced from plants have received growing attention and demand. Because of the unrivalled chemical variety of naturally generated molecules, natural products, give outstanding prospects for novel therapeutic leads [4,5]. Plants' therapeutic usefulness is linked to a group of active compounds, phytochemicals, which benefit human physiology.

Herbs with plant ingredients as traditional medicines are currently very much in demand. This follows Indonesia's condition as a country with abundant flora

and fauna biodiversity, supported by low prices and public trust in the efficacy of these plants. Temu giring rhizome (*Curcuma heyneana*) has long been used by Indonesians as a medicinal plant. It has traditionally been proven to cure several diseases such as worm infections, fever, and abdominal pain [6]. *Curcuma heyneana* was known for having its antimicrobial effects [7]. Sari and Wicaksono in 2016 reported that *Curcuma heyneana* could inhibit the growth of *Staphylococcus aureus* with MIC and MBC were 62.5 mm and 62.5 mm, respectively [8]. *Tanaman ungu* (*Graptophyllum pictum*) is also long known as a medicinal plant. People in the Maluku Islands often use the young leaves of *tanaman* ungu (*Graptophyllum pictum*) to treat fever and malaria [9]. Proboseno in 2011 reported the antibacterial activity of the ethanolic extract from *tanaman ungu* leaves against *Staphylococcus aureus* [10]. Reason why you have to combine those herbs? To date, there is no research which showsm the effectiveness of combined natural products extract. As an individual natural extract, i.e. *Curcuma heyneana* or *Graptophyllum pictum* alone showed promising results toward the growth of *Staphylococcus aureus*, it is hypothesized that the combined extracts would bring better result observed from the MIC and MBC.

We designed a study to investigate the activity of temu giring rhizome (*Curcuma heyneana*) extract solution combined with *tanaman ungu* leaf (*Graptophyllum pictum*) against *Staphylococcus aureus*.

MATERIAL AND METHODS

Study Design

This is an experimental study using test tube dilution. This research was conducted at the Medical Microbiology Laboratory, Department of Medical Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya.

The bacteria used was *Staphylococcus aureus* on a blood agar plate, which had previously been incubated at 37^0C for 18 to 24 hours and its morphology was proven in Gram staining under the microscope.

For each repetition in this dilution test, seven test tubes were provided with the labels T1 , T2 T3, T4 , T5, K1, and K2. T1 abbreviated for "test tube 1", which has meaning as the first experimental tube, and so on. Whilst the K1 was used as the negative control which only containing the extract combination only, K2 was used as the positive control containing *Staphylococcus aureus* colony in broth. A total of 1 ml of Mueller-Hinton broth was put into tubes K1, T1, T2, T3, T4, and T5. A total of 0.5 ml of extract without dilution and 0.5 ml of Mueller-Hinton broth medium were put into tube T2. 1 (one) gram of*ungu* leaf extract and 1 (one) gram of *temu giring* rhizome extract were then added and mixed into the T1 tube without being diluted. From tube T2 , 1 ml of suspension was taken and then added and mixed into tube T3 . The final extract concentration of each ingredient was0.5 volumes from the previous tube.

Next, 1 ml of *Staphylococcus aureus* was prepared from a 0.5 Mc Farland liquid culture tube and then put into tubes K1, T1, T2, T3, T4, and T5. After tubes K1, T1, T2, T3, T4, and T5 were mixed with bacteria, the tubes were incubated for 24 hours at 37^0C. After incubation, the MIC was determined by observing the colour changes of the tube. If the colour of the tube is not clear, the MBC was obtained after the test tube was streaked onto the nutrient agar plate and observed 24 hours later. The growth of *Staphylococcus aureus* was observed after being incubated again for 24 hours at 37^0C.

Curcuma heyneana Rhizome and *Graptophyllum pictum* Leaves Extract

Curcuma heyneana rhizome and *Graptophyllum pictum* leaves used in this study were obtained from Balai Materia Medica Batu. The rhizome and leaves obtained were dried using a drying machine so that those were obtained in powder form. Extracts were made using the maceration method. Each powder was weighed as much

Table 1. Minimum Inhibitory Concentration from the Combined Extract

Test Tube	Extract Concentration	Observation of Sterile Liquid Medium				
		Replication 1	Replication 2	Replication 3	Replication 4	Replication 5
K1	Extract combination	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
K2	*Staphylococcus aureus* colony	Turbid white	Turbid white	Turbid white	Turbid white	Turbid white
T1	1000 mg/ml for each	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
T2	500 mg/ml for each	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
T3	250 mg/ml for each	Brown	Brown	Brown	Brown	Brown
T4	125 mg/ml for each	Light brown	Light brown	Light brown	Light brown	Light brown
T5	62.5 mg/ml for each	Turbid yellow	Turbid yellow	Turbid yellow	Turbid yellow	Turbid yellow

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Table 2. Minimum Bacterial Concentration from the Combined Extract

Test Tube	Extract Concentration	Observation of Sterile Liquid Medium				
		Replication 1	Replication 2	Replication 3	Replication 4	Replication 5
K1	Extract combination	-	-	-	-	-
K2	*Staphylococcus aureus* colony	+	+	+	+	+
T1	1000 mg/ml for each	+	+	+	+	+
T2	500 mg/ml for each	+	+	+	+	+
T3	250 mg/ml for each	+	+	+	+	+
T4	125 mg/ml for each	+	+	+	+	+
T5	62.5 mg/ml for each	+	+	+	+	+

Note: "-" means that there was no growth of *Staphylococcus aureus* on the nutrient agar plate. "+" means that there was a presence of *Staphylococcus aureus* observed on the nutrient agar plate after being inoculated for 24 hours at 37^0C incubator.

as 600 grams, which was then wetted using 600 ml of 70% ethanol as solvent. After that, the wetted powders were then put into a levelled jar and added with 70% ethanol solvent until submerged (the solvent used was at least 2 times the weight or more). The solvent added to the jar amounted to 1.9 litres. The jars were left for incubation for 24 hours and centrifuged at 50 rpm.

The centrifuged extract was filtered through a cloth filter and collected to an Erlenmeyer tube. The extract was then put on a 25^0C rotary evaporator for two and half an hours and re-evaporated in a water bath for 2 hours. The extracts that have been obtained are then put into a glass bottle and stored in a refrigerator at 4^0C.

The next step was testing the contamination of the extracts. The contamination test was carried out by taking a small portion of the extracts in a test tube, then taking 2 (two) ml of the extract with a loop and streaking it on the nutrient agar plate. The plate that had been streaked with the extract was then incubated at 37^0C for 24 hours and observed. From the observations, there was no contamination in the extracts.

Ethics

This study obtained ethical clearance from The Health Research Ethics Committee, Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia with number 158/EC/KEPK/FKUA/2017. All procedures used in this study were carried out in accordance with the required instructions and regulations.

Data Analysis

Measurement of the results of the study was carried out qualitatively by observing the MIC and MBC of each extract combination at each replication. Replication was carried out 5 (five) times using the Federer formula. The data were then analysed qualitatively. The data obtained are data on the growth of *Staphylococcus aureus*. The data collected will be separated between the control group and the group with certain treatments. After being separated, the data will be analysed using Microsoft Excel spreadsheets and compared between each replication for obtaining the MIC and MBC. If needed, IBM SPSS Statistics 25 would be used in the analysis.

RESULTS

After being replicated five times, the MIC could be determined in the Table 1. To obtain more accurate data, further research was conducted to determine the MBC by streaking each tube on Nutrient Agar Plate media and then incubating for 24 hours at 37^0C. Furthermore, the presence of *Staphylococcus aureus* growth in the growing media was observed with the following results. After being inoculated, it was found that *Staphylococcus aureus* could not be killed nor inhibited from the greatest to the lowest concentration. At each replication, uniform results were obtained. This result was proved from the table 2, which clearly showed that the growth of *Staphylococcus aureus* could be observed at the K2, T1, T2, T3, T4, and T5 from all of the replications (Table 2).

DISCUSSION

Temu giring (*Curcuma heyneana*) is one of the herbs that is often used in Indonesia. *Temu giring* is believed to be able to overcome skin diseases and skin abrasions. Some of the active ingredients that can be found in *temu giring* include zerumbon, furanodienon, zederon, sigmasterol, curcumenol, and other ingredients [9,10]. The crude extract of *Curcuma heyneana* did not cause any cytotoxicity effect on CEM-SS cell cultures. In this research, it is desired to have the most effective active ingredient to kill bacteria and not harmful to living cells, namely curcumenol. The best extraction to get the yield containing the most curcumenol is to use acetone [11].

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In the study of the antimicrobial effect of *temu giring* extract that has been carried out by several researchers, the following results were obtained: Disc diffusion method using n-hexane solvent obtained a diameter of less than 10 mm, which indicates a weak inhibition against *Staphylococcus aureus* [12]. In the same study with acetone solvent, the diameter of the inhibition zone was 13 mm, which means it had a moderate inhibition zone. Another research conducted showed the results of the diffusion test using a disc, the extract of *temu giring* rhizome against *Staphylococcus aureus* had an inhibition zone diameter of 9.26 mm, which means the slow power is weak [13]. The same researcher also conducted a diffusion test on the *temu giring* rhizome extract, in this study, the MIC and MBC could not be obtained. Fresh extract of *temu giring* rhizome could not kill *Staphylococcus aureus*. A multilevel filtering method of *temu giring* extract through fractionation and chromatography resulted from the MIC and MBC on the microdilution test of 250 g/ml extract. This shows a weak antimicrobial effect to the activity of *Pseudomonas aeruginosa* [14].

The leaves of *tanaman ungu* (*Graptophyllum pictum*) are one of the plants that are spread throughout Southeast Asia and have been widely used by local residents to treat wounds, such as wounds on hemorrhoids and other open wounds [15,16]. There is less research about the active content of *tanaman ungu* leaves on its antimicrobial activity, but rather on its anti-cancer, anti-inflammatory, and antioxidant effects. Some of the active substances that can be found in *tanaman ungu* leaves include flavonoids, tannins, formic acid, and alcohol/ethanol [17]. In a study using 96% ethanol as a solvent and aquadest, the results of the inhibitory power of purple plant leaf extract through the Duncan Multiple Range Test method of diffusion obtained an average inhibition zone of 1.093 cm2 with 96% ethanol solvent and 0.505 cm2 with aquadest solvent [18]. The same researcher also examined the MIC from the obtained extract, which was 50 mg/ml. In another study on 70% ethanol extract of purple plant leaves, it was found that at a concentration of 100 mg/ml, the extract had no antibacterial effect against *Staphylococcus aureus* and several other bacterial species but had antifungal effects on several fungal species.

From some of the results of the research above, in general, the results of the research in the form of a dilution test method against *Staphylococcus aureus* using an independent *temu giring* extract or the leaves of the purple plant itself did not give the results of being able to kill bacteria strongly, if antibacterial activity could be obtained. carried out using the microdilution method or using the Kirby-Bauer well and disc diffusion method, with the results having a weak to moderate antimicrobial effect, not showing a strong result. These results are also in accordance with the research that has been carried out on the combined extract of the rhizome of *Curcuma heyneana* and *Graptophyllum pictum* leaves against *Staphylococcus aureus* bacteria through the dilution test, it was found that there was no antibacterial activity since it was carried out in the first tube with a concentration of 1000 mg/ml each for the rhizome of *temu giring* and *tanaman ungu* leaves.

There are several predictable factors for further research in order to obtain better results, including the need for further testing of *Staphylococcus aureus* isolates, whether the available isolates have formed a biofilm or not, because when a biofilm is formed, the active ingredients used are present in the extract cannot reach its working point, including the bacterial cell wall [19,20]. Then, for extraction, it can be done through stratified filtering (fractionation) and chromatography so that the material obtained is the desired active substance in accordance with studies that have been carried out by other researchers. The last thing that can be done is to conduct research with combined extracts through a diffusion test to see the minimal inhibition zone so that more accurate results are obtained.

CONCLUSION

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the combined extract between *temu giring* rhizome and *tanaman ungu* leaves at the exact concentration could not be determined as the *Staphylococcus aureus* still growth after being incubated. Therefore, a combination extract of *Curcuma heyneana* rhizome and *Graptophyllum pictum* leaf at the same level did not have an antimicrobial activity effect or was not effective against *Staphylococcus aureus* bacteria.

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CONFLICT OF INTEREST

The authors declared that there was no conflict of interest regarding the publication of this article.

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