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Antibacterial Effectiveness of Methanol Extract Combination Formula 3:2:1 of Tambora Leaf (*Ageratum conyzoides*), Sembalit Angin Leaf (*Mussaenda frondosa* L), Turmeric Rhizome (*Curcuma longa* L) on the growth of *Staphylococcos aureus*

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Abstract
The research was a quantitative descriptive study using laboratory experimental methods. We conducted this research to analyze the combination formula of Tambora leaves, Sembalit Angin leaves, and turmeric rhizomes using 3: 2: 1 ratio and determine its effectiveness in-inhibiting the-growth of *Staphylococcus aureus* bacteria in vitro. The width of *the inhibition zone* measured the growth of Staphylococcus aureus from the outer side of the paper disc, which has been immersed in methanol extract of Tambora leaves, Sembalit Angin leaves, and turmeric rhizomes in nutrient agar medium. Calipers did measurement, which were made during the incubation period of 24 hours, 48 hours, and 72 hours. The data obtained from the effect of a combination formula of 3:2:1 Tambora leaves, Sembalit Angin leaves, and turmeric rhizomes on the growth of *Staphylococcus aureus* were analyzed by Anava statistical test, and Duncan 1% test. The results found that the combination formula of 3:2:1 bio herbal methanol extract of Tambora leaves, Sembalit Angin leaves, and Turmeric rhizomes had a significant effect on the growth of *Staphylococcus aureus* at 1% significance, proven by the sig value. 0.00 <0.01, so the 3: 2: we can recommend 1 combination formula as the most effective combination extract formulation in inhibiting the growth of *Staphylococcus aureus*.

INTRODUCTION

Indonesia has a high level of biodiversity, proven by its abundant natural wealth, for example medicinal plants. One reason is the tropical climate and loose soil in Indonesia (Jalil, 2019). Indonesian people often use medicinal plants as clothing, food, shelter, and also use them as materials for treatment. An example of the use of medicinal plants is to treat bacterial infections. Indonesia has a lot of forest that containing many medicinal plants, including forests in Central Kalimantan.

Central Kalimantan has forest areas that are rich in medicinal plants (Ardhany & Novaryatiin, 2019). Some of these medicinal plants are Tambora, Sembalit Angin, and Turmeric. The metabolic substances of Tambora leaves include flavonoids and saponins that have a potential role as antibacterial (Saputra et al., 2019). Sembalit Angin leaves known could heal wounds and has a role as antibacteria for

Staphylococcus aureus (Garvita, 2015). Turmeric rhizome is a medicinal plant that can act as an antibacterial, because of the curcuminoids and essential oils contained in it (Jumiati et al., 2019). *Staphylococcos aureus* is one of the bacteria that causes infection in the post-partum reproductive tract (Mas'udah, 2017).

Medicinal plants such as Tambora, Sembalit Angin, and Turmeric are very easy to find, for example, on the roadside, in the yard, on the banks of the river, and can even be planted. Lack of knowledge and information about these medical plants makes people not know the efficacy of them. Nowadays, people do not know the benefits of the metabolic substances in these medicinal plants. The people also do not know how to process these medicinal plants, so that we can use them as medicines that are beneficial to health. Lack of information and documentation is one cause of this happening. The ethnobotanical experts explained that the body is more receptive to natural materials than synthetic materials (Novaryatiin & University, 2011). The combination of several medicinal plants will provide a maximum impact to treat diseases. The combination of several medicinal plant extracts was believe has effectiveness in healing diseases (Halimatussa'diah et al., 2014). Sudewi & Lolo (2016) reported that the combination of extracts of *Morinda citrifolia*, *Annona muricata*, *Piper crocatum* and *Moringa oleifera* was effective in preventing the pathogenicity of *E. coli* and *Staphylococcos aureus* . Kholifah's (2018) also states that the combination of several medicinal plants has more optimal potential as certain antibacterials.

The results of interviews with the community stated that we often used these medicinal plants for post-partum medicine. For example, a mix of Tambora leaves with turmeric can be used as herbal medicine. However, people do not yet know the right combination formula and the right way of processing plants for treatment. Based on the explanation above, it is necessary to explore the potential effect of the combination of these medicinal plants on the growth of *Staphylococcos aureus* in certain formula, so the effectiveness of the 3:2:1 combination formula as an antibacterial is the findings in this paper.

METHOD

The research stages are taking and handling medicinal plant samples, means taking or looking for as many medicinal plants in nature as samples. Next, drying and making powders to become simply, this process takes several days until the medicinal plant samples are completely dry. Then made it into a fine powder with simple extraction, means it carried the extraction process out without classifying or adjusting the chemical compounds in medicinal plants. Testing the extract with a 3:2:1 combination formula with a concentration level of 30%, 40%, 50%, 60%, 70%, and 80%, these tests are based on previous research and based on based on the habits used by the community in using the formula. We conducted this research at the Microbiology Laboratory of IAIN Palangka Raya, Central Kalimantan. We carried the measurement of the inhibitory power out between the clear zone between the *Staphylococcos aureus* colonies and the outer side of the paper disc containing the extract on the Nutrien Agar (NA) plate medium.

Tools and Materials

This study used a beaker glass, test tube, measuring cup, 500 ml and 250 ml Erlenmeyer flask, petri dish, inoculation needle (hollow), iron stirrer, glass funnel, tweezers, magnetic stirrer, micropipette, autoclave, refrigerator, oven, pipette, laf, hot plate, digital balance, micropipette tip, scissors, cutter, spirit, blender, basin, tray, pan, gas stove, thermometer, scale, and evaporator.

The materials used include: Tambora leaves, Sembalit Angin, Turmeric, pure culture of *Staphylococcos aureus*, NA medium, beef extract, becto peptone, aquadest, 70% alcohol, methanol, cotton, vaseline, filter paper, cover paper, gauze, paper labels, blotting paper, rubber bands, lysol, laundry soap, cotton buds, and aluminum foil. Preparation of extract combination formulation 3:2:1 (see Table 1).

Table 1. Preparation of Extract Combination Formulation 3:2:1.

Combination 3:2:1	Preparation
Control (+) 0,1%	2 ml *Chloramfenicol*+198 ml Aquades
*Contro*l (-)	Aquadest steril
30%	14 gram ekstrak kombinasi + 6 ml aquades
40%	12 gram ekstrak kombinasi + 8 ml aquades
50%	10 gram ekstrak kombinasi + 10 ml aquades
60%	8 gram ekstrak kombinasi + 12 ml aquades
70%	6 gram ekstrak kombinasi + 14 ml aquades
80%	4 gram ekstrak kombinasi + 16 ml aquades

Effectiveness Test of the 3:2:1 Combination Formula against *Staphylococcos aureus* .

We incubated liquid nutrients that have been planted with bacteria for 1x24 hours. After that, they planted it into NA media using a cotton bud as much as 1 ose in 10 petri dishes for 1x24 hours in order to adapt. A combination of 3:2:1 extract formula was made with a predetermined concentration level, and soaked the disc paper for 1 minute. After the incubation period of the media containing *Staphylococcos aureus* was completed, the soaked disc paper was placed in the center of the media surface. After completion, all research units were incubated. *Staphylococcos aureus* growth inhibition was observed during the incubation period.

Data analysis

We analyzed the results of the observation of the inhibition zone formed using the Anova statistical test and the Duncan 1% test.

RESULTS AND DISCUSSION

The observation data was the width diameter of the inhibition zone, measured from the outer side of the paper disc with the farthest colony. The clear zone is an indicator of inhibited growth of *Staphylococcos aureus.* Figure 1 shows the clear zone from the observations. Mean of growth inhibition zone with the combination formula of 3:2:1 (Tabel 2).

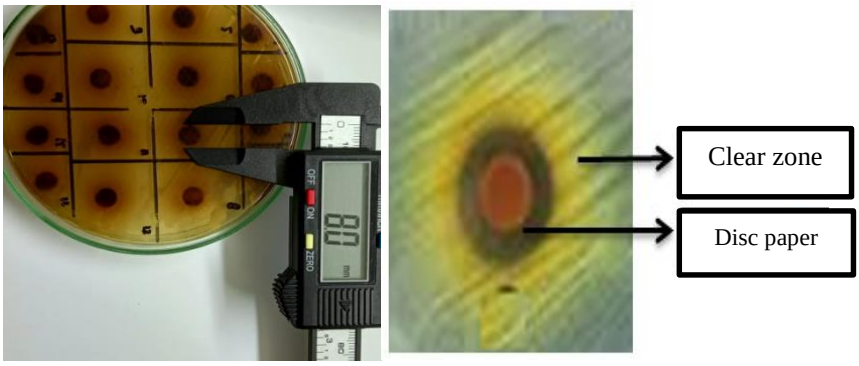

Figure 1. Zone Inhibition Growth of *Staphylococcus aureus*

Table 2. Recapitulation of DMRT 15 of the Diameter of *Staphylococcos aureus* Growth Inhibition 24 h, 48 h, and 72 h

Combination Formula of 3:2:1	Mean of Growth Inhibition Zone (mm)		
	24 hours	48 hours	72 hours
Chloramfenicol (+) 0,1%	0,96 b	1,11 b	0,62 ab
Aquades (-)	0 a	0 a	0 a
30% 14 gram combination exctract + 6 ml aquades	2,56 d	2,59 c	3,26 c
40% 12 gram combination exctract + 8 ml aquades	1,72 c	2,58 c	3,09 c
50% 10 gram combination exctract + 10 ml aquades	0,70 b	1,03 b	1,01 ab
60% 8 gram combination exctract + 12 ml aquades	0,71 b	1,15 b	0,99 ab
70% 6 gram combination exctract + 14 ml aquades	0,88 b	1,32 b	1,58 b
80% 4 gram combination exctract + 16 ml aquades	0,81 b	1,01 b	1,04 ab

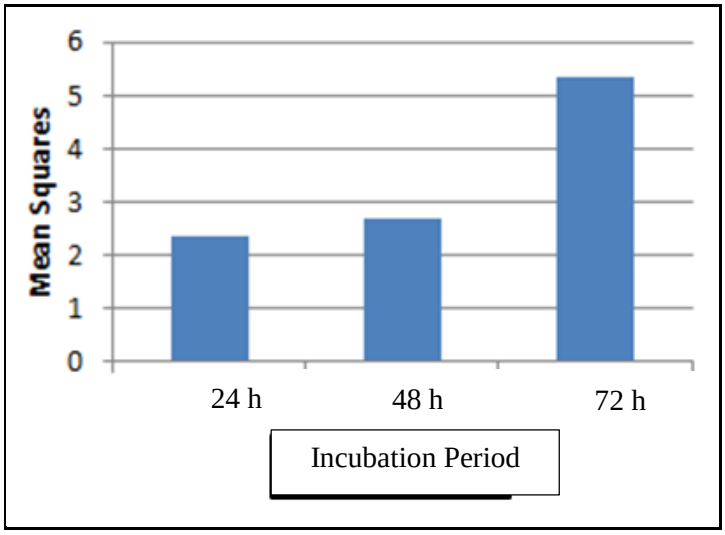

Figure 2. Mean Square 3:2:1 Combination Formula of *Staphylococcos aureus*

All data on the 3:2:1 combination were analyzed using ANOVA and followed by Duncan's 1% test to determine the optimal and effective concentration in inhibiting the growth of *Staphylococcos aureus* .

Based on the data recapitulation, it can be seen that the research treatment had a significant effect on the growth of *Staphylococcos aureus* with a significance of 1% during the entire incubation period. Furthermore, the optimal and effective extract concentration were displayed in Table 2..

Based on the results of Duncan's 1% test, the combination formula of P5, P6, P7, and P8 were not significantly different from P1. It was proven that the concentration had the same ability as 0.1% Chloramphenicol as the positive control of the study. However, these concentrations were significantly different when compared to P4 or concentrations of 40%, and P3 (30%). This indicates that the concentration of 30% is effective and optimum in inhibiting the growth of *Staphylococcos aureus* in the 3:2:1 combination formula for 24 hours incubation. Observations were continued for an incubation period of 48 hours. (Table 2).

The results of Duncan's 1% test showed that the treatments P5 to P8 were not significantly different from the positive control (P1). This result proves that the concentration still has the same ability as 0.1% Chloramphenicol as a positive control of the study. The effective and optimum concentration in inhibiting the growth of *Staphylococcos aureus* during the 48-hour incubation period was 30% concentration. This shows that the effective and optimum concentration at 48 hours of incubation is almost the same as 24 hours of

incubation in the 3:2:1 combination formula. Furthermore, to confirm the data on the incubation period of 24 hours and 48 hours, observations were made at the incubation period of 72 hours (Table 2).

The treatments at P5, P6, and P8 were not significantly different from the positive control (P1). The Duncan 1% test results in the Table 4 show that those concentration still has the same ability. Based on the data from each incubation period, it proves that the 30% concentration was the most effective and optimum in inhibiting the growth of *Staphylococcos aureus* using 3:2:1 combination formula.

Based on the data in Table 1, it can be seen that the concentration of 30% (P3) in the 3:2:1 combination formula is effective as an antibacterial agent for *Staphylococcos aureus* . This is because the main composition in the 3:2:1 combination formula is 50% Tambora leaves. Tambora leaves are known containing secondary metabolites, namely flavonoids which are very effective in suppressing the growth of *Staphylococcos aureus* .

The treatment level of 30% was the most effective concentration against the growth of Staphylococcos aureus , supported by Duncan's test 1% which was interpreted as the effective concentration of P3 (30%). This is because P3 is significantly different from other concentrations. Tambora leaves have the ability to inhibit the growth of *S.aureus* which is stronger than Sembalit Angin leaves and Turmeric rhizome. This was proven because only in 30% concentration, it could be effective in inhibiting the growth of *Staphylococcos aureus*. In addition, the content of saponins and flavonoids in tambora leaves has antibacterial activity. This fact is in accordance with study by Hidayati & Harjon (2017) which explained that tambora leaf extract has the potential as an antibacterial, both gram-positive and gram-negative bacteria. Research by Safani et al. (2019) also mentions that the main compounds contained in Tambora leaves are flavonoids and alkaloids that have the potential effect to inhibit the growth of *Staphylococcos aureus*.

The level of effectiveness and the optimum concentration of the 3:2:1 combination formula at 48 hours of observation was a concentration of 30% (P3). The test results showed that the concentration of 30% was still able to survive the incubation period of 48 h as the most effective and optimum concentration. The content of tambora leaves as the main component in the 3:2:1 combination formula is still able to survive and still active at 48 h. Some of the content of tambora leaves are flavonoids, alkaloids, essential oils, coumarins, and others. The content of these chemical compounds makes tambora leaves effective to use as an antibacterial. This is in accordance with two previous studies (Sugara et al., 2016; Hayati et al., 2020) that the content of tambora leaves has the potential role as an antibacterial.

The Duncan 1% test results showed that the concentration of 30% (P3) was effective and optimum in inhibiting the growth of Staphylococcos aureus during the 72-hour incubation period. This proves that the concentration of 30% (P3) is still able to survive until the third day. This result is also supported by the larger mean square value at the 72 h incubation period. Flavonoids in tambora leaves can damage bacterial cell walls to coagulate protoplasm, so the most effective concentration of 30% (P3) is still able to last up to an incubation period of 72 hours (Iqlima et al., 2017).

The concentration of 30% was concluded as the effective concentration in inhibiting the growth of *Staphylococcos aureus* in the 3:2:1 combination formula, the lowest concentration was able to maximally inhibit the growth of *Staphylococcos aureus* . The effectiveness of the growth inhibition of this 3:2:1 combination formula as an antibacteria of *Staphylococcos aureus* becomes the finding of this study..

CONCLUSION

The results of the analysis showed that the 3:2:1 combination formula was able to inhibit the growth of *Staphylococcos aureus.* In addition, The 3:2:1 combination formulation with a concentration of 30% is effective and can be used to inhibit the growth of *Staphylococcos aureus.*

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