ANTIMIKROBIAL PROCEEDING

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ANTIMICROBIAL TEST OF "*TUTUP*" FLOWERS (*Macaranga tanarius* (L.) Mull.Arg.)

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Abstract

Preservation "*siwalan*" sap in Lamongan done in two ways, namely by the addition of lime solution and by addition of liquid of "*tutup*" flowers. "*Tutup*" flowers ((*Macaranga tanarius* (L.) Mull.Arg.) (name of Lamongan district) is included in the family *Euphorbiaceae*. Sugar is produced from the sap with liquid of "*tutup*" flowers preservative have better quality than the other sap preservative. The results also showed that the sap with liquid of "*tutup*" flowers preservative have a longer shelf life, with a fewer the number of total microbes. Therefore, it needs to be studied a potential "*tutup*" flowers extract as an anti-microbial material. The purpose of this study is as follows: (1) to know and effectiveness test of "*tutup*" flowers preservative against pathogenic microorganisms and food destroyer, (2) to determine part of "*tutup*" plants which potentially as a preservative, and (3) to determine the minimum concentration of preservative with Contact Method.

The results showed the following: (1) Growth of *Saccharomyces cerevisiae* and *Staphylococcus aureus* can be inhibited by liquid of "*tutup*" flowers, but the growth of *Pseudomonas fluorecens* are not inhibited by liquid of "*tutup*" flowers; (2) *Pseudomonas fluorecens* bacterial growth can be inhibited by a solution of lime, but the growth of *Staphylococcus aureus* and the *Saccharomyces cereviseae* not inhibited by a solution of lime; (3) Inhibited the growth of *Staphylococcus aureus* by addition of "*tutup*" plant leaves extract with concentration 30 % and "*tutup*" plant flowers extract with 20% and 30% concentration, inhibited the growth of *Pseudomonas fluorecens* by addition of "*tutup*" plant leaves and flowers with 20% and 30% concentration, whereas inhibited the growth of *Saccharomyces cereviceae* by the addition of leaf extract of "*tutup*" plant with a concentration of 20% and 30% and a flower extract of "*tutup*" plant with a minimum concentration of 10%; and (4) furthermore used flower extract of "*tutup*" plant with concentration of at least 20% can inhibited the growth of *Staphylococcus aureus, Pseudomonas fluorecens,* and *Saccharomyces cereviceae*.

Key words: "*tutup*" flowers (*Macaranga tanarius*); antimicrobial; natural preservatives

1. Introduction

Siwalan (*Borassus flabellifer* Linn) farmers in Lamongan Indonesia using "*tutup*" flowers ((Macaranga tanarius (L.) Mull. Arg)) and lime solution as palm sap preservative at the time of harvesting. Photo of "*tutup*" flowers can be seen at Figure 1.

Figure 1. "*Tutup*" flowers plants

Used "*tutup*" flowers extract as a palm sap preservative produce solid sugar with higher yield, shorter boiling time, color and hardness as well as better quality in comparison with used of lime solution as a preservative (Rejeki, F.S., et al., 2009). This is because the use of "*tutup*" flower plants as palm sap preservative will produce palm sap with better quality and the number of microorganisms that are less than with the use of lime solution as a preservative (Rejeki, F.S., et al., 2013).

Formaldehyde, benzoic acid and the antioxidant BHT, BHA, TBHQ and sourced from petroleum or synthetic materials are widely used as a preservative (Deiana M, 2003; Freidon Shahidi, 2003 and Anonymous, 2008). The use of synthetic preservatives and antioxidants are not currently recommended by the Ministry of Health for allegedly can cause cancer (carcinogenic Agent) (Hernani; Mono Rahardjo, 2005), so it is necessary to look for alternative natural preservative. For that needs to be further investigated as a potential

of "*tutup*" flowers as a natural preservative.

2. Methodology

2.1 Research place

This research was conducted at Microbiology Laboratory and Analysis of Industrial Products Laboratory, Study Program of Agriculture Industrial Technology, Department of Engineering, Wijaya Kusuma Surabaya University

2.2 Materials and Tools

The materials used are leaves and flowers of the "*tutup*" plant, *Staphylococcus aureus* FNCC 0047, *Pseudomonas fluorescens* FNCC 0070, and *Saccharomyces cereviceae* FNCC 3012, Agar Nutrient medium, Nutrient Broth medium, peptone, Yeast Extract, Glucose. The tools used are Laminair Air Flow, autoclave, vortex, test tubes, Erlenmeyer, and measuring tools

2.3 Research Methods

This study consists of two phase, namely:

First Stage of Research: Optimization of part of the "*tutup*" plants that has the potential as preservatives. This study used a randomized block design (RBD) with 2 factors pattern that was repeated three times. The first factor is part of the plant (S1) with two levels, namely: S1: flowers of "*tutup*" plant and S2: leaves of of "*tutup*" plant. The second factor is solution concentration (K) with three levels, namely: K1: 10%, K2: 20%. And K3: 30%

Optimization of part of the "*tutup*" plants that has the potential as preservatives will be determined by testing a preservative against microbial activity test, namely: *Staphylococcus aureus, Pseudomonas fluorescens* and *Saccharomyces cereviceae*. Analysis is conducted by descriptive analysis

Second stage of Research: Determination of antimicrobial minimum concentration with Contact Method

10 ml of Nutrient Broth (NB) sterile in erlenmeyer addition the sample extract to obtain the concentration (% w/v) as desired. Furthermore, this medium was inoculated with 0.1 ml of bacterial suspension test 24 hours old. 1 ml of this mixture were taken on plates (0 hour) with a dilution rate of 10^{-5}, 10^{-6}, and 10^{-7}. The rest of the mixture of sample and bacteria shaking in a shaker at 150 rpm, 37°C for 24 hours. At 24th hour, 1 ml of the mixture was taken and made the level of dilution 10^{-5}, 10^{-6}, and 10^{-7}. 10 NB sterile without the addition of extract used as control. Each dish was incubated for 48 hours at a temperature of 37°C. The minimum inhibitory concentration causing bacteria do not grow on plates after 24 hours of contact time with the sample.

3. Results and Discussion

Optimization of part of the "*tutup*" plants that has the potential as preservatives

This study was conducted to determine which parts of the "*tutup*" plants are leaves and flowers has a potential as *Staphylococcus aureus. Pseudomonas fluorescens* and *Saccharomyces cereviceae* antimicrobial.

Pure microbial cultures obtained from the Inter-University Center for Food and Nutrition, University of Gadjah Mada (PAU Food and Nutrition UGM) are *Staphylococcus aureus* FNCC 0047, *Pseudomonas fluorescens* FNCC 0070, and *Saccharomyces cereviceae* FNCC 3012. Pure microbial isolate can be seen at Figure 2.

Figure 2. Pure microbial isolate

The results of antimicrobial activity test with two factors, namely the part of the plant (S) and concentration (K) can be seen at Figure 3 and Table 1.

Figure 3. Inhibition zone of "*tutup*" plant leaves and flowers

Antimicrobial test results showed that the leaves and flowers of "*tutup*" plants has antimicrobial effects with varying concentrations. *Staphylococcus aureus* growth inhibited by the addition of "*tutup*" plants leaves extracts at concentration of

30% and "*tutup*" plants flowers extract at concentration of 20% and 30%. *Pseudomonas fluorescens* growth inhibited with the addition of "*tutup*" plants leaves extracts at concentration of 20% and 30%; and "*tutup*" plants flowers extract at concentration of 20% and 30%. While *Saccharomyces cereviceae* inhibited growth with the addition of "*tutup*" plants leaves extracts at concentration of 20% and 30% and "*tutup*" plants flowers extract with concentration of 10%, 20% and 30%. Based the test results shown that the inhibition zone diameter of "*tutup*" plant leaves relatively smaller than the zone of inhibition of "*tutup*" plants flowers. This is probably caused by the concentration of the antimicrobial at the "*tutup*" plants flowers is higher than the concentration of the antimicrobial at the "*tutup*" plants leaves. "*Tutup*" plants contains diterpenoids, flavonoids, and tannins. Phommart, S., et al. (2005) have isolated tanarifuranonol, tanariflavonona C, and tanariflavonona D of "*tutup*" plant leaves. Furthermore, Kawakami, A., et al. (2008) have isolated seven prenylated flavonones compounds are macaflavonones A - G. Compounds alkaloids, saponins, phenolics, flavonoids, triterpenoids are compounds that can inhibit microbial activity

Table 1. Test results of antimicrobial

Microbes	Parts of the plant	Concentration	Antimicrobial activity
Staphylococcus aureus	Leaves	10 %	-
		20 %	-
		30 %	+
	flowers	10 %	-
		20 %	+
		30 %	+++
Pseudomonas fluorescens	Leaves	10 %	-
		20 %	+
		30 %	++
	flowers	10 %	-
		20 %	++
		30 %	+++
Saccharomyces cereviceae	Leaves	10 %	-
		20 %	+
		30 %	++
	flowers	10 %	+
		20 %	++
		30 %	+++

Description: -) no inhibition of growth
+) There is inhibition of growth

3.2. Determination of Antimicrobial Minimum Concentration with Contact Method

Antimicrobial Minimum Concentration Test with Contact Method conducted to determine the minimum concentration "*tutup*" flower extract that can inhibit microbial growth of *Staphylococcus aureus*, *Pseudomonas fluorescens* and *Saccharomyces cereviceae* after contact with preservative for 24 hours. The results of antimicrobial minimum inhibitory concentration test can be seen at Table 2 and Figure 4.

Table 2. Antimicrobial minimum inhibition concentration

Microbes	Parts of the plant	Consentration (%)								
		10	12,5	15	17,5	20	22,5	25	27,5	30
Staphylococcus aureus	Leaves	+	+	+	+	+	+	+	+	-
	Flowers	+	+	+	+	-	-	-	-	-
Pseudomonas fluorescens	Leaves	+	+	+	+	+	-	-	-	-
	Flowers	+	+	+	-	-	-	-	-	-
Saccharomyces cereviceae	Leaves	+	+	+	+	-	-	-	-	-
	Flowers	-	-	-	-	-	-	-	-	-

Description: -) no growth ; +) There is growth

Table 2 shows that the growth of *Staphylococcus aureus* inhibited by the addition of "*tutup*" plant leaves extracts at concentration of 30%, and inhibited started with the addition of "*tutup*" plants flowers extract at concentration of 20%. *Pseudomonas fluorescens* inhibited by the addition of "*tutup*" plant leaves extracts at concentrations of 22.5%, and inhibited started with the addition of "*tutup*" plants flowers extract at concentration of 17.5%. While *Saccharomyces cereviceae* began inhibited by the addition of "*tutup*" plant leaves extracts at concentrations of 20%, and inhibited started with the addition of "*tutup*" plants flowers extract at concentration of 10%. This is likely due to "*tutup*" plants flowers contains more inhibitors than "*tutup*" plant leaves. Based on the results of antimicrobial minimum inhibitory concentrations test, it can be concluded that the use of "*tutup*" plants flowers extract with concentration of at least 20% can inhibit the growth of *Staphylococcus aureus, Pseudomonas fluorescens,* and *Saccharomyces cereviceae*.

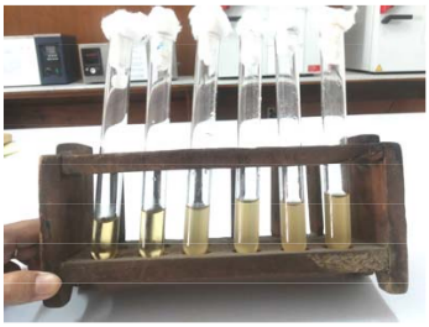

Figure 4. Result of minimum inhibition concentration test

4. Conclusions

Staphylococcus aureus inhibited by the addition of leaves of "*tutup*" plant extracts with concentration of 30% and flower of "*tutup*" plant extracts with concentration of 20% and 30%; *Pseudomonas fluorescens* inhibited by the addition of leaves of "*tutup*" plant extracts with concentration of 20% and 30% and flower of "*tutup*" plant extracts with concentration of 20% and 30%. While *Saccharomyces cereviceae* inhibited by the addition of leaves of "*tutup*" plant extracts with concentration of 20% and 30% and flower of "*tutup*" plant extracts with concentration of 10%, 20% and 30%.

Used flower of "*tutup*" plant extracts with a concentration of at least 20% can inhibit the growth of *Staphylococcus aureus, Pseudomonas fluorescens,* and *Saccharomyces cereviceae*.

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