

**Original Research****Antibacterial Activity Of Combination Of n-Hexane Extract Of Buas-Buas Leaves (*Premna serratifolia* L.) And Sappan Wood (*Caesalpinia sappan* L.)**Rissa Febriyanto<sup>1</sup>, Isnindar Isnindar<sup>2\*</sup>, Sri Luliana<sup>3</sup><sup>1,2,3</sup> Department of Pharmacy, Faculty of Medical University of Tanjungpura Pontianak, Indonesia**ABSTRACT**

**Background:** The plants used in this study were buas-buas leaves (*Premna serratifolia* L.) and sappan wood (*Caesalpinia sappan* L.). The people of West Kalimantan use the buas-buas leaves and sappan wood as traditional medicine, and, based on research, they have antibacterial activity. *Staphylococcus aureus* and *Escherichia coli* are bacteria that cause nosocomial infections and have developed resistance to many antibiotics. This research was conducted to determine the antibacterial activity of the combination of n-hexane extract of buas-buas leaves and sappan wood.

**Methods:** The antibacterial activity test was carried out by the agar diffusion method using a paper disc with a diameter of 6 mm. The antibacterial activity of a combination of n-hexane extract of buas-buas leaves and sappan wood in a ratio of 1:1, 2:1, and 1:2 at concentrations of 125, 250, and 500 ppm.

**Results:** In this study, the combination of n-hexane extract of buas-buas leaves and sappan wood with various concentrations, even with increasing concentrations, did not show any inhibition zones. The factors that are thought to affect the absence of the inhibition zone are the concentration of the extract, the content of secondary metabolites, the solubility of the extract, and the length of contact time

**Conclusion:** The combination of n-hexane extract of buas-buas leaves and sappan wood did not have antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* in all comparisons and concentrations. It is recommended to test the antibacterial activity with a higher concentration, such as with a concentration of percent (%) (w/v).

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**INTRODUCTION**

Buas-buas is a plant that is widely found in West Kalimantan and its leaves are often used by the Malay people of West Kalimantan as vegetables. Buas-buas leaves are believed to have medicinal properties and have been used in traditional medicine as a cure for intestinal worms and colds, overcoming hemorrhoids, helping blood clots,

increasing appetite in children, facilitating breast milk, and as food preservatives (Kurniati, 2013). The plants belong to the Verbenaceae family. The group of compounds owned by buas-buas leaves are alkaloids, flavonoids, polyphenols, saponins, and terpenoids (Kurniati, 2013; Vadivu et al., 2009).

Based on research conducted at the level of extracts and pure compounds, buas-buas leaves have biological activities such as antioxidant, antibacterial, anti-inflammatory, cytotoxic, and hepatoprotective (Minh, 2019). The n-hexane extract of buas-buas leaves contains phytol compounds (Hadiarti, 2015). Phytol compounds are known to have antibacterial activity against *Escherichia coli* (Ghaneian, Ehrampoush, Jebali, Hekmatimoghaddam, & Mahmoudi, 2015).

*Sappan wood* is a plant that is used by some Malay people as a traditional drink. In addition, the second plant is used as a natural dye because it produces a red pigment (Nomer, Duniaji, & Nocianitri, 2019). *Sappan wood* contains chemical compounds such as alkaloids, brazilin, flavonoids, phenyl propane, saponins, tannins, and terpenoids (Sudarsono et al., 2002). Braziline is the most dominant component and gives the red color to the *Sappan wood* extract. Braziline is reported to have biological activity as antibacterial, anti-inflammatory, hypoglycemic, vasorelaxant, antiallergic, antioxidant, and anti-acne (Nirmal, Rajput, Prasad, & Ahmad, 2015).

An antibacterial is a compound that can be used to inhibit the growth of bacteria. The mechanism of antibacterial compounds is generally carried out by interfering with protein synthesis, inhibiting the work of enzymes, and damaging cell walls. Phenolic compounds, flavonoids, and alkaloids are phytochemical compounds that have the potential as natural antibacterials by damaging the cell walls of pathogenic bacteria, for example, *Staphylococcus aureus* and *Escherichia coli* (Septiani, Dewi, & Wijayanti, 2017). *Escherichia coli* and *Staphylococcus aureus* are bacteria that are generally found in the human body but can be pathogenic, causing various infectious diseases in humans (Fitri & Rahayu, 2018).

*Staphylococcus aureus* and *Escherichia coli* are bacteria that are commonly found as the causes of nosocomial infections. Nosocomial infections are infections that occur in patients while in the hospital, where before entering the hospital they did not show symptoms of infection (Baharutan, Rares, & Soeliongan, 2015) (Wikananda, Hendrayana, & Pinatih, 2019). *Staphylococcus aureus* and *Escherichia coli* are also known to have developed resistance to many antibiotics on the market, which causes problems in medical treatment (Fitri & Rahayu, 2018).

The method used to test the antibacterial activity in this study is the disc diffusion method. This antibacterial activity test has the advantage of being fast, easy, and inexpensive because it does not require special tools (Katrin, Idiawati, & Sitorus, 2015). Based on research by Rahman et al., (2016), essential oil from *Premna integrifolia* leaves has an inhibition zone against *Escherichia coli* bacteria in the hexane extract of  $12.2 \pm 1.1$  mm and chloroform extract of  $13.1 \pm 0.5$  mm.

Huish et al., (2014) reported that the hexane extract of buas-buas leaves had antibacterial activity against *Staphylococcus aureus* bacteria with an inhibitory concentration of 59% and 5% for *Escherichia coli*. In the study of Srinivasan et al., (2012), the petroleum ether extract of sappanwood provides antibacterial activity with a maximum value of the inhibition zone on *Escherichia coli* of  $5.0 \pm 0.3$  mm and a minimum inhibitory zone of  $2.0 \pm 1.1$  mm on *Staphylococcus aureus*. The invalid source was specified. According to Bukke, et al., (2015), the chloroform extract of sappan wood on *Escherichia coli* has an inhibition zone of  $26,166 \pm 0.170$  mm.

Several previous studies explored the potential of buas-buas leaves and *sappan wood* as antibacterial, both in the form of extracts, fractions, and isolation, but the solvents used focused on polar solvents, so research was needed to test the antibacterial potential of buas-buas leaf extract and sappan wood from nonpolar solvents. Therefore, this research was carried out to make updates on knowing the antibacterial activity of the combination of n-hexane extract of buas-buas leaves and sappan wood.

## **MATERIALS AND METHOD**

### **Tools**

The tools used in this study were glassware (Pyrex Iwaki®), autoclave (GEMMY SA-300VF), stir bar, maceration vessel, vial bottle, Buchner (Iwaki®), Bunsen, incubator (Mettler® E24899), caliper, ose wire, 6 mm paper disc, Laminar Air Flow (ROBUST), refrigerator, 100µl - 1000µl micropipette (ENDO PRO), oven (Mettler® UP400), water bath, tweezers, dropper, volume pipette, rotary evaporator (BUCHI), alcohol swab, analytical balance (Ohaus® PA 2102), and turbidimeter.

### **Materials**

The materials used were Buas-buas leaves (*Premna serratifolia* L.) and *Sappan wood* (*Caesalpinia sappan* L.), *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, Mueller Hinton Agar (MHA) (OXOID), solvent n-Hexane (technical), NaCl, DMSO, filter paper, and ciprofloxacin 5 g/disk (OXOID).

### **Extraction**

Samples of the simplicia leaves of Buas-buas and Kayu Secang, weighing respectively 1,133 grams and 3,500 grams, were put into each container, then 5 L of n-hexane solvent was added to the container containing each simplicia. Then it was allowed to stand for 3x24 hours with the same solvent change every 1x24 hours and stirring occasionally. Macerate is taken by filtering using a Buchner vacuum. The obtained macerate was evaporated using a rotary evaporator to obtain a more concentrated extract (Nuralifah, Jabbar, Parawansah, & Iko, 2018).

### **Phytochemical Screening**

The phytochemical screening in this research was qualitatively (tube test) using certain reagents with indicators of color changes that occurred (Kristianti et al, 2008; Prabawa, Khairiah, & Ihsan, 2019; Tjitda & Nitbani, 2019; Tohomi, Iswahyudi, & Wahdaningsih, 2014).

### **Antibacterial Activity Testing**

#### **Sterilization**

The equipment used for testing is sterilized first. Sterilization of non-glass tools using an autoclave at 121°C for 15 minutes. Glass utensils were sterilized using an oven at 170°C for 2 hours. Ose wire is sterilized by glowing in a Bunsen flame (Kumesan, Yamlean, & Supriati, 2013).

#### **Preparation Medium of Mueller Hinton Agar (MHA)**

Weigh 38g MHA medium (2g beef extract; 17.5 g casein hydrolysate; 1.5g starch; 17g agar) according to the composition of the ingredients on the packaging, then dissolve in 1 liter of distilled water, if necessary with the help of heating. In addition, autoclave

media sterilization at 121°C for 15 minutes. Pour the MHA medium over a sterile petri dish as much as 20 ml and place it at room temperature until it solidifies (Afnidar, 2014).

#### **Preparation of Standards for Turbidity Solution Mc. Farland 0.5**

In an Erlenmeyer, 9.95 ml of 1% H<sub>2</sub>SO<sub>4</sub> solution was mixed with 0.05 ml of 1% BaCl<sub>2</sub> solution in an Erlenmeyer. The mixture is then shaken or vortexed until a cloudy solution forms. This turbidity is used as a standard for the turbidity of the test bacterial suspension (Kumesan et al., 2013).

#### **Manufacturing of Bacterial Suspension**

*Staphylococcus aureus* and *Escherichia coli* bacteria were obtained from the stock culture results at the Health Office of the Health Laboratory Unit of West Kalimantan Province. *Staphylococcus aureus* and *Escherichia coli* were each taken with sterile wire loops from the culture stock that had grown and then suspended in each tube containing 10 ml of 0.9% NaCl until the turbidity of the bacterial suspension was the same as the standard Mc. Farland 0.5 (Ilhani, 2018).

#### **Preparation of Test and Control Solutions**

Preparation of test solution for the combination of n-hexane extract of buas-buas leaves and sappan wood in various concentration series at a ratio of 1:1, 2:1, and 1:2. The first test solution was prepared in a 3,000 ppm main solution using 10% DMSO. The main solution was diluted into various concentrations of 125, 250, and 500 ppm (Huish et al., 2014). The second test solution was prepared at 66,000 ppm with 20% DMSO and was diluted into various concentrations, 2,500, 5,000, and 10,000 ppm. The positive control used ciprofloxacin 5 g/disk and the negative control was DMSO 10% and DMSO 20%.

#### **Antibacterial Activity Test with Disc Diffusion**

The antibacterial activity test was carried out by the agar diffusion method using a paper disc with a diameter of 6 mm. The test bacteria used were *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. The sterilized MHA media was poured into a petri dish until solidified, and then on the surface of the solidified MHA media, the bacterial suspension was streaked using a sterile cotton swab. Paper discs of 6 mm were soaked for 3 minutes in the sample combination of n-hexane extract of buas-buas leaves and sappan wood in a ratio of 1:1, 2:1, and 1:2, at concentrations of 125, 250, and 500 ppm. Then they are placed on MHA media that has been inoculated with the test bacteria. The antibacterial activity test was carried out with three repetitions (Agustin et al., 2018). The same treatment was also carried out on the test samples with concentrations of 2500, 5000, and 10000 ppm.

#### **Bacterial Incubation**

The bacteria-inoculated MHA media were then incubated for 1 hour at 37°C in an incubator. Observations were made on the formation of an inhibition zone around the paper disc in the form of a clear zone using a caliper (Agustin, Puspawaty, & Rukmana, 2018; Ilhani, 2018).

## RESULTS

The results of phytochemical screening were found to contain only terpenoid compounds in the n-hexane extract of buas-buas leaf and sappan wood, respectively. The negative results of flavonoid compounds, phenols, alkaloids, saponins, and tannins on the phytochemical screening of the n-hexane extract of buas-buas leaves and sappan wood were thought to be due to several influencing factors, namely environmental conditions, plant age, extraction method, and solvent used (Astuti, Sunarminingsih, Jenie, Mubarika, & Sismindari, 2014) (Tiwari, Kumar, Mandeep, Kaur, & Kaur, 2011). The location of the main plant material such as altitude, temperature, pH, and nutrient content in the soil can affect the metabolite compounds possessed (Katuuk, Wanget, & Tumewu, 2019).

**Table 1.** Result of Phytochemical Screening

Compound Name	Extract n-Hexane	
	Buas-buas Leaves	Sappan wood
FlavonoidS	-	-
Phenol	-	-
Alkaloids	-	-
Saponins	-	-
Tannins	-	-
Terpenoids	+	+

ket: (+) = contain compounds (-) = does not contain compounds

**Table 2.** Antibacterial Test Results on *Staphylococcus aureus*

Sample Test	Concentrati on (ppm)	Inhibition zone (mm)			Mean	Ket.
		Repeat to-				
		I	II	III		
1:1	125	0	0	0	0	No inhibition zone
	250	0	0	0		
	500	0	0	0		
1:2	125	0	0	0	0	No inhibition zone
	250	0	0	0		
	500	0	0	0		
2:1	125	0	0	0	0	No inhibition zone
	250	0	0	0		
	500	0	0	0		
Ciprofloxacin 5µg/disc		26,5	26	26	26,167	Sensitive
DMSO 10%		0	0	0	0	No inhibition zone

**Tabel 3.** Antibacterial Test Results on *Escherichia coli*

Sample Test	Concentrati on (ppm)	Inhibition zone (mm)			Mean	Ket.
		Repeat to-				
		I	II	III		
1:1	125	0	0	0	0	No inhibition zone
	250	0	0	0		
	500	0	0	0		
1:2	125	0	0	0	0	No inhibition zone
	250	0	0	0		

Sample Test	Concentration (ppm)	Inhibition zone (mm)			Mean	Ket.
		Repeat to-				
		I	II	III		
	500	0	0	0	0	
	125	0	0	0	0	
2:1	250	0	0	0	0	No inhibition zone
	500	0	0	0	0	
Ciprofloxacin 5µg/disc		26,5	26	26	26,167	Sensitive
DMSO 10%		0	0	0	0	No inhibition zone

The results of the observations contained in the table can be seen that the concentration used did not produce the diameter of the inhibition zone on the test bacteria. Antibacterial testing was also carried out at higher concentrations.

**Table 4.** Optimization Results of Antibacterial Activity Concentration Test.) against *Staphylococcus aureus*

Sample Test	Concentration (ppm)	Inhibition zone (mm)			Mean	Ket.
		Repeat to-				
		I	II	III		
	2500	0	0	0	0	
1:1	5000	0	0	0	0	No inhibition zone
	10000	0	0	0	0	
1:2	2500	0	0	0	0	No inhibition zone
	5000	0	0	0	0	
	10000	0	0	0	0	
2:1	2500	0	0	0	0	No inhibition zone
	5000	0	0	0	0	
	10000	0	0	0	0	
Ciprofloxacin 5µg/disc		26,5	26	26	26,167	Sensitive
DMSO 20%		0	0	0	0	No inhibition zone

**Table 5.** Optimization Results of Antibacterial Activity Concentration Test against *Escherichia coli*

Sample Test	Concentration (ppm)	Inhibition zone (mm)			Mean	Ket.
		Repeat to-				
		I	II	III		
	2500	0	0	0	0	
1:1	5000	0	0	0	0	No inhibition zone
	10000	0	0	0	0	
1:2	2500	0	0	0	0	No inhibition zone
	5000	0	0	0	0	
	10000	0	0	0	0	
2:1	2500	0	0	0	0	No inhibition zone
	5000	0	0	0	0	
	10000	0	0	0	0	
Ciprofloxacin 5µg/disc		26,5	26	26	26,167	Sensitive
DMSO 20%		0	0	0	0	No inhibition zone

## DISCUSSION

The combination of n-hexane extract from buas-buas leaves and sappan wood at all concentrations did not provide an inhibition zone against *Staphylococcus aureus* and *Escherichia coli* bacteria. Based on the results obtained, it can be seen that only the positive control gave the inhibition zone. The positive control used was the antibiotic Ciprofloxacin 5 g/disk. Ciprofloxacin is a fluoroquinolone antibiotic.

The mechanism of action of Ciprofloxacin is by inhibiting topoisomerase II (DNA gyrase) and topoisomerase IV, which are required by bacteria for DNA replication. Ciprofloxacin is a broad-spectrum antibiotic that can kill both gram-positive and gram-negative bacteria (Raini, 2017; Rieuwpassa, Yunus, & Arsana, 2011). Ciprofloxacin gave an inhibition zone of 26.167 mm for *Staphylococcus aureus* and 27.167 mm for *Escherichia coli* bacteria. The inhibition zone formed on *Staphylococcus aureus* and *Escherichia coli* bacteria indicated that the Ciprofloxacin 5 g/disk antibiotic used was sensitive. This is following the ciprofloxacin antibiotic sensitivity standard of 5 g/disk according to CLSI (Primadianti, Retnaningsih, & Ningrum, 2019).

The results of the observations contained in the table can be seen that the concentration used did not produce the diameter of the inhibition zone on the test bacteria. Antibacterial testing was also carried out at higher concentrations, namely at concentrations of 2500, 5000, and 10000 ppm. The increase in the concentration series of the combination of n-hexane extract of buas-buas leaf and sappan wood is expected to produce inhibition zones for *Staphylococcus aureus* and *Escherichia coli* bacteria. Observations of increasing the concentration series can be seen in (Table 4) and (Table 5).

The results of antibacterial testing on the combination of n-hexane extract of buas-buas leaves and sappan wood with various concentrations, even with increasing concentrations in this study, did not show any inhibition zones. The factors that are thought to affect the absence of the inhibition zone are the concentration of the extract. The content of secondary metabolites, the solubility of the extract, and the length of contact time (Lingga, Pato, & Rossi, 2015) (Suryani, Nurjanah, & Indriatmoko, 2019).

The concentration of the extract used affects the results of antibacterial inhibition. Poeloengan & Praptiwi (2010) stated that the higher the concentration, the higher the inhibition zone that will be formed. Based on the results of the literature study, the researchers showed that the concentration used in this study was in the low category compared to other studies. Even though the concentration has been increased to see the antibacterial inhibition of the combination of n-hexane extract of buas-buas leaf and sappan wood, it is not effective in forming an inhibitory zone on *Staphylococcus aureus* and *Escherichia coli* bacteria.

The metabolite content of the n-hexane extract of buas-buas leaves and sappan wood in (Table 5) only contained terpenoid compounds. Terpenoid compounds are known to have antibacterial activity. The results of the antibacterial test observations did not give an inhibition zone result, presumably because the terpenoid levels possessed by the two extracts were low. The low antibacterial compound possessed by the extract makes the compound unable to damage and disrupt the physiological processes of bacterial cells (Cowan, 1999).

The content of metabolites contained in this extract is influenced by the solubility properties of these compounds. This study used n-hexane as a solvent in the extraction process and DMSO as a solvent in the process of making variations in concentration. The n-hexane solvent used during the extraction process is non-polar and will only

attract non-polar metabolites (Tiwari et al., 2011). These nonpolar metabolites affect the penetration of these compounds against *Staphylococcus aureus* and *Escherichia coli* bacteria.

DMSO, which was used as a solvent for the manufacture of concentration variations, did not affect the results of antibacterial inhibition. However, DMSO is a solvent that can dissolve polar and nonpolar compounds (Setyowati & Damayanti, 2014) (Utomo, Fujiyanti, Lestari, & Mulyani, 2018) (Verdiana, Widarta, & Permana, 2018). The use of DMSO when making mother liquor when hot has good solubility.

Meanwhile, when cold, the extracted sample has poor solubility. During the antibacterial testing process, it was suspected that there were metabolites that did not dissolve completely, which caused at least the active compounds to diffuse on the disc paper. The contact time of the disc paper at the time of immersion with the extracted sample of this study was  $\pm 3$  minutes.

It is suspected that the contact time of 3 minutes is not long enough for the active compound in the extracted sample to diffuse on the paper disc. The length of time the active compound diffuses on the disc paper is also influenced by the solubility of the extracted sample. As a result of the less than optimal diffusion of active compounds on the disc paper, there is no clear zone around the disc paper for *Staphylococcus aureus* and *Escherichia coli* bacteria (Suryati, Bahar, & Ilmiawati, 2017).

In addition to some of the factors that have been described above, It is suspected that other factors influence the non-formation of the inhibition zone, namely the interaction between the active substances possessed by the n-hexane extract of buas-buas leaves and sappan wood. It is known that the combination of the two samples can provide a synergistic effect, an additive effect, or an antagonistic effect. The synergistic effect is an advantageous effect where combining two samples can give better antibacterial results than a single sample. An additive effect occurs if the resulting antimicrobial effect is the same as that of a single sample.

Meanwhile, the antagonistic effect is a detrimental effect that arises as a result of the results of the combination between samples characterized by smaller results than the single sample (Baljeet, Simmy, Ritika, & Roshanlal, 2015) (Hilal A. Syahrir, Mochamad Afendi, & Susetyo, 2016). In this study, it is suspected that the effect that appears is the antagonistic effect of the combination of n-hexane extract of buas-buas leaf and sappan wood, which is characterized by the absence of an inhibitory zone. To be clear about the effects that arise from this combination, research is needed that specifically discusses this.

According to Schlegel and Schmidt (1994), in Siregar et al., (2012), the size of the inhibition zone formed can be influenced by the rate of agar diffusion. The concentration of microorganisms is a factor that affects the rate of agar diffusion. It is suspected that when the bacterial inoculum was taken using a wire loop and suspended with 0.9% NaCl, it was suspected that it was not suspended completely, so it affected the results of the study. The scratching factor of bacteria onto the MHA Agar media, which is not evenly distributed, can also cause the antibacterial effect to not occur around the disc (Suryati et al., 2017).

## CONCLUSION

The combination of n-hexane extract of the buas-buas leaf (*Premna serratifolia* L) and sappan wood (*Caesalpinia sappan* L) did not have antibacterial activity against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 in all



comparisons and concentrations. It is recommended to test the antibacterial activity with a higher concentration, such as with a concentration of percent (%) (w/v), and to test the antibacterial activity using different methods, such as the dilution method, as well as to test to determine the value of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

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