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Effect of Leaf Extract Buasbuas (*Premna pubescens* Blume) for Against of Bacteria Growth *Staphylococcus aureus* and *Salmonella sp* in Vitro

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Abstract. Buasbuas (*Premna pubescens* Blume) is the one of the medicinal plants in Indonesia. This plant contains flavonoids potential as an antibacterial. *Staphylococcus aureus* and *Salmonella sp* is the one of the bacteria that can cause poisoning. This research aims to find out of inhibitory the extract of buasbuas leaves the growth of *Staphylococcus aureus* and *Salmonella sp*. This research was conducted in Medan Health Laboratory used hole sinks methods. Buasbuas leaves which has been condensed ethanol extract was diluted using aquadest with a concentration of 0%, 50%, 60%, 70%, 80%, 90% and dropped into MSA *(Mannitol Salt Agar)* media inoculated bacteria and wich is a hole sinks. Them incubated for 1 × 24 hours at a temperature of 37^0C. The results of this research that the buasbuas extract with the different concentrations can inhibit the growth of *Staphylococcus aureus*, then the result were analyzed used of Non Factorial Experimental Method (ANOVA) where F_{count} (337.54)> F_{table} 0,05 (2,77) ; Ft_{abel} 0.01 (4.25). While the maximum concentration buasbuas extract were more effective the growh of bacteria is 90% with average inhibition zone diameter of 11 mm.

INTRODUCTION

Indonesia is one of the mega biodiversity countries by the number of about 40,000 species of medicinal plants, but only about 2.5% have been explored and used as a traditional medicine. One of the medicinal plants in Indonesia is buasbuas (*Premna pubescens* Blume). This plant has efficacy as a drug but not many people are familiar with this plant. Plant buasbuas (*Premna pubescens* Blume) which is synonymous with the name of the plant *Premna obtitufolia, Premna integrifolia* L, *Premna corymbosa* R, and *Premna cordifolia* L is a type of plant that is often used by the people of Malay as a vegetable that is included in the slurry mixture spicy become typical food in fasting, as well as traditional medicine to cure various ailments such as colds, eliminate breath odor unpleasant, overcome the infection worms, reproduce breast milk (ASI), and can refresh the body of a woman who gave birth by mixing the decoction of the leaves, roots, bark and stem into the water Mandian women (Marbun and Restuati, 2015).

Based on preliminary studies conducted by Restuati, et al (2016) to get the result that the leaf extract buasbuas (*Premna pubescens* Blume) has antibacterial activity which can inhibit the growth of bacteria B. cereus and E. coli. With a concentration of 50% showed the greatest inhibition zone but has not been too effective in inhibiting the growth of bacteria *B. cereus* and *E. coli*. , However, future studies with a higher concentration of the extract, different methods of extraction, the nature of the flowers, fruits, bark and root extract may be useful for evaluating the antibacterial actually done for other pathogenic bacteria.

Staphylococcus aureus is a gram-positive bacterium that is common in humans and other mammals. In the amount of 105 CFU / ml *S. aureus* bacteria could potentially produce the toxin and the amount of 106 CFU / ml bacteria produce intoxication that can cause poisoning. These bacteria can be spread from the nose, mouth, skin and food, the bacteria Salmonella sp is a gram-negative bacterium that is pathogenic in humans. (Karlina et al., 2013). Food can become toxic because it has been contaminated by pathogenic bacteria which can then grow and multiply during storage, so it can produce toxins which can be harmful to humans. In addition, there are also foods that are naturally already be toxic as some fungi or plants and animals. One of the bacteria that can cause poisoning *Staphylococcus aureus* and *Salmonella sp*. (Hasanah, 2015)

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One intoxication is often the case of food intoxication caused by enterotoxin *of Staphylococcus aureus. Staphylococcus aureus* presence in food can be sourced from the skin, mouth or nasal cavity food processors. When found in high amounts is an indicator of inadequate sanitary conditions. Many foods sold at roadside made by human hands, for example pecal, hodgepodge, meatballs or snacks with other children and the food is very loved by the people. This is what is feared could be the cause of poisoning because at the time of manufacture of food are not considered cleanliness and sterility of the food and the food maker (POM, 2008). Excessive use of antibiotics and less directed may result in resistance. The emergence of resistance to certain antibiotics can lead to failure in the treatment of poisoning caused by the bacterium *Staphylococcus aureus* and *Salmonella sp*, so as to overcome it necessary to search for natural ingredients as an alternative treatment (Rastuti et al., 2013).

The use of leaf buasbuas as an alternative antibacterial, because the leaves contain flavonoids buasbuas. Based on research conducted by Restuati, et al. (2014) conducted phytochemical test results have been obtained the result that the secondary metabolites of positive *Premna pubescens* leaf extract contains alkaloids, steroids, flavonoids, saponins. Based on research conducted by Darmawi, et al (2013) found that the sap within China containing flavonoids can inhibit the growth of *Staphylococcus aureus* in vitro with the most optimal concentration is 100%. Then the research findings Restuati, et al (2016) to get the result that the leaf extract buasbuas *(Premna pubescens* Blume) has antibacterial activity which can inhibit the growth of bacteria *B. cereus* and *E. coli.*

This study aims to determine whether the secondary metabolites of buasbuas leaves can inhibit the growth of *Staphylococcus aureus* and *Salmonella sp* cause of poisoning so buasbuas leaf extract can be used as an antibacterial or inhibit the growth of *Staphylococcus aureus* and *Salmonella sp* cause of poisoning.

METHODS

This research was conducted in the Laboratory of Chemistry, State University of Medan and Health Laboratory at the Research Institute of Medan, Jl. Williem Iskandar V Market Barat I No. 4 Medan. In November of 2016 to January 2017. Buasbuas leaf samples obtained from the yard of a house in Jl. Lizardi 53 Silangge Putra Medan, tools and materials used is a petri dish, glas beaker, flask, knife, blender, hair drayer, paper labels, incubator, a pipette, a stirrer, pipette volume, measuring cups, analytical balance, scales, plastic, calipers, etcher, autoclave, cotton, sterile distilled water, ethanol 90%, leaves buasbuas, *Staphylococcus aureus*, *Salmonella sp* , MSA (Mannitol Salt Phenol-red Agar), NA (Nutrient Agar), HCl.

The method used is the method of pitting, by piercing the agar surface with a sterile pipette as much as 6 holes, because the concentration of the extract in this study there were 6 of 0%, 50%, 60%, 70%, 80%, 90%. The research method is non factorial completely randomized design. Leaves buasbuas which has become the ethanol extract viscous diluted using auadest with a concentration of 0%, 50%, 60%, 70%, 80%, 90%, then dropped into the hole that is already available in the agar surface that had been induced in bacteria *Staphylococcus aureus* and *Salmonella sp* then incubated for 24 hours at a temperature of 37^0C.

RESULTS AND DISCUSSION

Based on research that has been done, the result that the leaf extract buasbuas (*Premna pubescens* Blume) has antibacterial activity in inhibiting *S. aureus* but can't inhibit the growth of bacteria *Salmonella sp*. Buasbuas leaf extract with different concentrations caused the area/zone of inhibition varied to *Staphylococcus aureus* bacterium *Salmonella sp* whereas not indicate a clear zone around the wells. The higher the concentration of leaf extract buasbuas (*Premna pubescens* Blume) is given, the greater the inhibition zone produced for *Staphylococcus aureus*.

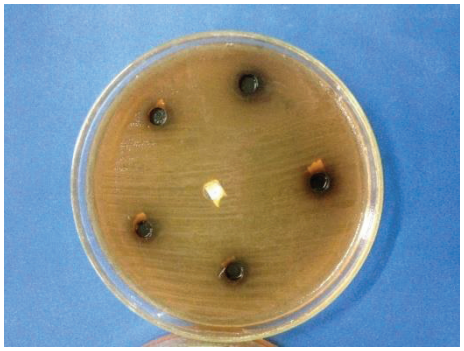

FIGURE 1. Results Buasbuas Leaf Extract on Growth bacterium Salmonella sp.

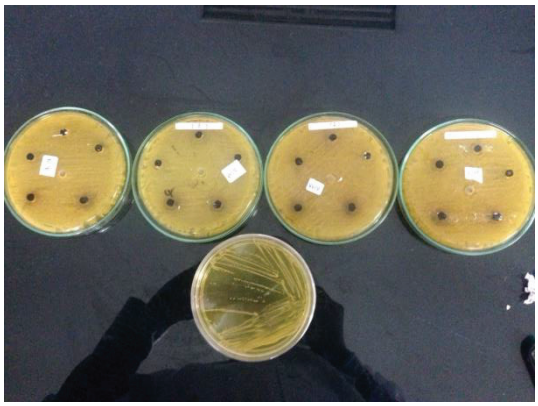

FIGURE 2. Results Buasbuas Leaf Extract on Growth bacterium *Staphylococcus aureus*.

Inhibition produced by leaf extract buasbuas marked by discoloration of the surface to be around sinks into a translucent color. Media surrounding area is overgrown by bacteria *Staphylococcus aureus* look yellowish and visible colonies of the bacterium *Staphylococcus aureus*. Inhibition zone that occurs is stretched around the place of planting leaf extract buasbuas performed using the method of pitting holes. The results of the research can be seen in Table 1 below.

TABLE 1. Results Effect of Leaf Extract Inhibits the Growth of Bacteria Buasbuas in *Staphylococcus aureus*

Treatment	repeat				total treatment	mean
	1	2	3	4		
0%	7 mm	5 mm	6 mm	4 mm	22 mm	5,5 mm
50%	6 mm	5 mm	7 mm	6 mm	24 mm	6 mm
60%	8 mm	6 mm	8 mm	7 mm	29 mm	7,25 mm
70%	9 mm	6 mm	8 mm	7 mm	30 mm	7,5 mm
80%	11 mm	8 mm	10 mm	10 mm	39 mm	9,75 mm
90%	10 mm	12 mm	11 mm	11mm	44 mm	11 mm
		amount			188 mm	7,83 mm

Can be seen from the table above that the greatest inhibition zone formed is 11 mm which is the treatment of the extract concentration of 90%. It showed that the leaf extract buasbuas less effective in inhibiting the growth of bacteria *Straphylococcus aureus* cause of poisoning. *Premna pubescens* Blume is a family-owned plants lamiales. *Premna pubescens* Blume is native to Southeast Asia and is one shrub widespread in the forests of Sumatra and the Malay Peninsula. Growing up as an ornamental plant. Most parts of the plant *P. pubescens* Blume has been used in traditional medicine systems in the Malays for treating various infectious diseases. Other uses of *P. pubescens* is an antioxidant, anticancer, antiviral, antifungal and antibacterial. As with other Premna, *P. pubescens* Blume contain secondary metabolites such as alkaloids, flavonoids and steroids. Phenolic compounds such as flavonoids contained in plants is usually beneficial as an antioxidant, anticancer, antiviral, antifungal and antibacterial (Restuati et al., 2016).

Congenital intoxication caused by toxic food from *Staphylococcus aureus*. This intoxication is common throughout the world. Enterotoxin produced by *Staphylococcus aureus* strains associated with food poisoning. *Staphylococcus aureus* bacteria can be found in the nose, throat, skin, hair and fur animals including birds and humans. These bacteria can also be found infecting skin wounds or swelling in humans and livestock. Bacterial contamination is common Seara from such sources (Wardah, 2013).

Flavonoids are phenolic compounds largest. Mechanism of action of flavonoids work as an antibacterial compound by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cell membranes. The mechanism of action in a way denature bacterial cell protein and cell membrane damage without irreparably (Haryati et al., 2015).

Flavonoids are water-soluble compounds. Can be extracted with ethanol 70% and remain in the water layer after this extract is shaken with petroleum ether. Flavonoids are phenolic compounds form. Flavonoids are found in all vascular plant but some classes more spread out than others. Flavonoid compounds have the ability to form a complex with bacterial cell protein through hydrogen bonds. The structure of the cell wall and cytoplasmic membrane of bacteria containing proteins become unstable because of the protein structure of the bacterial cell becomes damaged because of their hydrogen bonds with flavonoids, sehngga protein bacterial cells become lose activity, consequently the function of the permeability of the bacterial cell is disrupted and the bacterial cell will undergo lysis will result in bacterial cell death (Harbone, 1987).

Flavonoids are a group of secondary metabolites and is one of the biggest phenols which are naturally present in plants. Flavonoids present in all land plants, but not found in marine plants (algae), microorganisms, bacteria, fungi, and algae, flavonoids have several benefits such as drug infection of wounds, reduce blood clotting in the body, antioxidant, antitumor and anticancer properties. Flavonoids (the active chemical compounds are suspected of having a hypoglycemic effect) are secondary

metabolites found in green plants, except algae. Flavonoid commonly found in higher plants (Angiospermae) is flavones and flavonols C- and O-glycoside, khalkon with C- and O-glycoside, and dihidrokhalkon, proantosianidindan anthocyanin, Auron O-glycoside and dihidroflavonol O-glycoside. Group flavones, flavonols, flavanones, isoflavones, and khalkon also often found in the form of aglikonnya. Flavonoids can be catchers of free radicals that can cause cancer, heart disease, cataracts, premature aging, and other regenerative diseases. Among other benefits of flavonoids protect the cell structure, improve the effectiveness of vitamins, anti-inflammatory, preventing bone loss and as an antibiotic. The existence of flavonoids in the leaves is affected by the process of photosynthesis so that not too many young leaves contain flavonoids. Flavonoids are antioxidants that can capture free radicals. Flavonoids stop the early stages of the reaction to liberate the hydrogen atoms of the hydroxyl groups later bonded to one free radicals (Wahyuni et al., 2014).

In inhibiting the growth of *Staphylococus aureus* by leaf extract buasbuas in this study showed that the leaf extract buasbuas which have active substances such as flavonoids and tannins that have properties such as phenol, a statement supported by Darmawi, et al (2013) that the phenolic compounds capable of severing ties peptidoglycan in an attempt to break through the cell walls. After breaking through the cell wall, phenolic compounds will cause leakage of nutrients the cells by damaging the bonds of hydrophobic components of cell membranes (such as proteins and fospolipida) so that damage to the bacterial cell membrane resulting in inhibition of the activity and biosynthesis enzymes are specifically needed in reaction to bacterial metabolism.

Buasbuas leaf extract is unable to inhibit the growth of bacteria *Salmonella sp*. It is anticipated by *Salmonella sp* are gram-negative bacteria. The structure of the cell wall between the bacterium *Salmonella sp* with different *Staphylococcus aureus* bacteria. Secondary metabolites, flavonoids contained in extracts of leaves buasbuas polar so easy to penetrate the layers of peptidoglycan in bacteria *S. aureus* also is polar so that the bacteria *S. aureus* is more sensitive than the *Salmonella sp*. Gram-positive bacterial layer structure of peptidoglycan, a little lipid and teikoat acid. Teikoat acid is a water-soluble polymer and polar. Flavonoid compounds are compounds that are polar making it easier to penetrate the peptidoglycan layer polar than nonpolar lipid layer as that of the cell wall of the bacterium *Salmonella sp*. Special components of bacterial cell walls *Salmonella sp* which is a Gram-negative bacteria is a protein, phospholipids and lipopolysaccharide (Volk and Wheeler, 1988). The outside walls are owned by the bacterium *Salmonella sp* have a high permeability so that the active substances contained in the leaf extract buasbuas can not enter into bacterial cells, as a result the bacteria are not damaged or stunted.

CONCLUSION

Based on the research that has been done, it can be concluded that: The extract of leaves buasbuas with different concentrations of various types: the concentration of, 50%, 60%, 70%, 80%, 90% can inhibit the growth of *Staphylococcus aureus* and is not able to inhibit *Salmonella sp* bacterial growth in vitro. The higher level of concentration is given the greater the diameter / inhibition zone produced on the surface to be planted with the bacterium *Staphylococcus aureus*. Buasbuas leaf extract has an antibacterial barrier responses are less effective against *Staphylococcus aureus*. Where the clear zone/inhibition zone resulting from the administration of the extracts from buasbuas (*Premna pubescens* Blume) on the growth of *Staphylococcus aureus* bacteria that is the most optimal is 11 mm on leaf extract concentration buasbuas 90% so that the response obstacle leaf extract buasbuas as antibacterial less effective, due to the concentration reaching nearly 100%, but has yet to show a larger zone diameters compared with previous studies and are unwilling buasbuas leaf extract inhibits the growth of bacteria Salmonella sp which is a gram-negative bacteria.

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