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ANTIBACTERIAL ACTIVITY OF GARGARISMA KNOB FLOWER EXTRACT PREPARATION

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Abstract

Staphylococcus aureus is a bacteria that is the main cause of abscesses in the oral cavity. One use of natural materials is to use the knob flower plant (*Balloon gomprena* L) who belongs to the family *Amaranthaceae* as a lineman. This research aimed to determine the antibacterial activity of the Konop flower extract Gargarisma preparation against Staphylococcus aureus bacteria. Antibacterial testing uses the disc diffusion method with nutrient agar media. The concentration of knob flower extract in FI, FII, FIII preparations is 25%, 50%, 75% respectively. The extraction method uses the maceration method with 80% ethanol solvent. Positive control for mouthwash on the market (Cool mint) and negative control for preparations without extract. Each formula was tested for physical quality which was presented descriptively while the data from the Staphylococcus aureus antibacterial activity test results (inhibition zone) were analyzed in the SPSS version 25 program. The results of the physical quality test of the pH test on the preparation were 5. The specific weights of the preparations FI, FII, FIII were respectively: 1.077g/ml, 1.136 g/ml, 1.206 g/ml. Meanwhile, the viscosity values for FI, FII, FIII preparations are: 16,714 cps, 39,892 cps, 69,989 cps. The antibacterial activity test of the inhibition zone produced at FI, FII, FIII has an average value respectively, namely; 8.6mm, 11.33mm and 13.33mm. Based on the test results, it can be concluded that each preparation formula has antibacterial activity against Staphylococcus aureus bacteria**.**

Keywords: Knob Flower Extract, Gargarisma, Staphylococcus Aureus.

1. INTRODUCTION

Oral health problems that often occur are bad breath, mouth infections, tooth abscesses and canker sores (Oktaviani, Rahmatullah, and Pambudi 2021). Oral health is important for society because a healthy mouth allows you to carry out daily activities such as eating, talking and socializing without experiencing pain and discomfort. *Staphylococcus* aureus is a gram-positive bacterium that can live as a commensal organism on human skin and mucous membranes. *Staphylococcus aureus* is a bacteria that is the main cause of abscesses in the oral cavity (Toy, Lampus, and Hutagalung 2015). Prevention of the emergence of microorganisms can be done by providing antibacterials packaged in the form of mouthwash preparations (Handayani, Sundu, and Sari 2018). Mouthwash is a solution or liquid that can be used to clean the oral cavity for various purposes, one of which is to remove damaging bacteria. The use of this preparation is very effective because it can reach places that are difficult to clean with a toothbrush (Kono, Yamlean, and Sudewi 2018). One of the advantages of mouthwash is that it is practical and easy to carry everywhere, apart from that it is useful for freshening the mouth, eliminating bad breath and reducing the formation of plaque or caries on the teeth (Anastasia, Yuliet, and Tandah 2017). People believe that treatment with efficacious medicinal plants can cure diseases and maintain health. Medicinal plants are also believed to have relatively fewer side effects compared to modern medicine. This has been believed by people for a long time and

has been passed down from generation to generation (Harefa 2020). One of the uses of natural materials is to use the knob flower plant (Gomphrena globosaL.) from the family *Amaranthaceae* as a mouthwash or Gargarism. According to research by Kusmiati et al 2017, positive knob flowers contain saponins and flavonoids where these two compounds can act as antibacterials and stimulate the growth of new cells in wounds. The content of flavonoid compounds in knob flowers shows bactericidal, antiviral activity and inhibits enzyme action (Kusmiati, Priadi, and Rahayu 2017). Based on research by Rilyn et al, 70% ethanol extract of knob flower leaves at a concentration of 20% can inhibit bacterial growth *Escherichia coli* although not strong (Novita, Caroline, and Yuriani n.d.2020). Based on this background, research was carried out using ethanol extract of knob flowers which was used as a mouthwash or mouthwash Gargarism. Research related to the potential of knob flower extract as a prevention of diseases that can occur in the oral cavity, for example tooth abscesses. This research aims to increase the use value of the flowers of the knob plant as a gargarisma preparation which can inhibit the growth of bacteria Staphylococcus aureus where so far no one has researched it (Andini, 2021).

2. MATERIALS AND METHODS

Knob flowers from the Jl. Batuyang- Batubulan Kangin Gg. Kokokan, Sukawati Village, Gianyar City, saccharin, tween 80%, peppermint oil, glycerin, distilled water. The solvent used is ethanol (*Bratachem*). Nutrient Agar (NA) media, bacteria *Staphylococcus aureus* (Labkesda Denpasar) and commercial mouthwash on the market (*Lister's*).

Making Extracts

Extraction was carried out by maceration with a solvent ratio of 80% ethanol as much as 1:10. A total of 1.7 kg of simplicia powder was extracted with 17 liters of 80% ethanol solvent. Simplicia was soaked in ethanol for 24 hours with stirring every 6 hours. After 24 hours, the macerate is filtered and then remaciated in the same way. The extraction results were then concentrated using a rotary evaporator at a temperature of 50°C. The thick extract obtained was weighed and stored in a closed glass container before being used for testing.

Phytochemical Screening

Phytochemical screening was carried out to identify secondary metabolites in knob flower extract with chemical reagents, namely alkaloids using reagents *Dragendroff* and *Meyer*, flavonoids using reagents *Willstater*, saponins use the foam test, tannins use FeCl3 and steroids or triterpenoids use reagents *Liberman Burchard*, anthraquinone with NaOH. (Handayani, Apriliana, and Natalia 2019).

Formulation

The making of the Gargarisma formula refers to the Oktaviani 2021 formula design, by replacing the extract as the active ingredient, namely using kenop flower extract (*Gomphrena globosa* L.) (Octavian*et al*. 2021)

Physical Quality Testing of Preparations

Testing the physical quality of the gargarisma kenop flower extract preparation (*Gomphrena globosa*L.) is carried out in several stages which include organoleptic tests, pH, specific gravity, and viscosity. (Widyasanti, Winaya *and* Rosalinda 2019).

Antibacterial Activity Testing Using the Agar Diffusion Method

Antibacterial testing uses the agar diffusion method with media *Nutrient Agar* (NA) 4 grams dissolved in 150 ml of distilled water. Test bacteria *Staphyllococcus aureus* used with a concentration equivalent to 0.5*Mc. Farland* has a population of 1.5 x 108 CFU/ml. The test bacteria from the bacterial suspension are taken with a sterile wire loop and then streaked onto the agar medium tightly. Next, it was incubated in an incubator at 37°C for 24 hours.

Preparation of test solution

Preparation of test samples for the preparation of gargarisma kenop flower extract (*Gomphrena globosa* L.) according to concentration, namely 25%, 50%, 75%. (FI, FII, FIII) which have been formulated and have been tested for physical quality.

3. RESULTS AND DISCUSSION

The process of making knob flower simplicia uses direct sunlight and is covered with black cloth so that the compounds contained in the knob flower are not damaged. The extraction method used in this research uses the maceration method. The maceration method is a cold extraction method so that the compounds contained in the plant are not damaged. The solvent used in the maceration process is 80% ethanol. In Kusmiati et al.'s 2017 research, the extraction process using 96% ethanol obtained a moderate antibacterial activity test, therefore in this study 80% ethanol was used. 80% ethanol is more polar, so it can attract secondary metabolite compounds which have antibacterial activity, especially flavonoid and tannin compounds which are polar in nature, which tend to dissolve more in 80% ethanol.

3.1. Phytochemical Screening Results

The phytochemical screening test aimed to determine the secondary metabolite compounds contained in the knob flower extract. Based on the results of the phytochemical screening test, the knob flower extract contains saponin, tannin, flavonoid and terpenoid compounds which can be seen in Table 1 below:

Table 1: Phytochemical Screening Results

No	Compound	Reactor	Check up result	Information
1	Alkoloid	Mayer	No precipitate is formed	Negative (-)
		Dragendrof	No precipitate is formed	Negative (-)
2	Flavonoid	Concentrated Mg + HCl powder	A yellow color forms	Positive (+)
3	Saponin	Aquadest	Forms stable foam	Positive (+)
4	Tannin	$FeCl_3$ 5%	Formed color blackish blue	Positive (+)
5	Quinones	NaOH	No happen discoloration	Negative (-)
6	Steroid &Triterpenoid	Lieberman Burchad	A green color is formed	Positive (+)

Based on the results of the phytochemical screening test, knob flowers are positive for containing flavonoids, saponins, tannins and steroids. In contrast to research conducted by Kusmiati 2017, the results of the phytochemical screening were positive for containing flavonoids, saponins, steroids. Factors that can influence differences in the content of secondary metabolite compounds from this research are differences in growing place, harvest time and ethanol solvent used in extraction. So it can affect the metabolite compounds contained in the knob flower plant.

3.2 Physical Quality Test Results of Gargarisma Flower Knob Preparations

Testing the physical quality of the Gargarisma dosage formulation includes organoleptic tests, pH tests and viscosity tests. The results of the organoleptic test showed that Gargarisma preparations FI and FII had a liquid consistency, while FIII had a slightly thick liquid consistency, Gargarisma was purple-brown in color, with a distinctive smell of knob flower extract and peppermint. Based on the results obtained, it shows that the higher the extract concentration has an effect on the consistency of the preparation. Organoleptically, the clarity of the Gargarisma preparation is classified as cloudy, this is caused by the greater the concentration of the extract used, the more intense the color produced. The cloudy color produced from Gargarisma preparations is caused by the use of active ingredients in the form of high concentration extracts (Handayani, Warnida, *and* Nur 2016). The pH test in this study aims to measure the acidity level of the preparation produced and must match the pH range of the mouth so as not to irritate the oral mucosa. The pH of a good mouthwash or gargarisma preparation is in the range of 4.5-7.5 (Riani, Darusman, *and* Suparman 2020). The pH test results for Gargarisma preparations, both FI, FII, and FIII, have the same value, namely 5 things, indicating that the pH value of Gargarisma preparations meets the requirements. The specific gravity test in this study used a pycnometer and analytical scales. Gargarisma knob flower extract preparations in formulas I, II and II have different specific gravities. Formula I has a specific gravity of 1.077 g/ml. Formulation II is 1.136 g/ml, and formulation III has a specific gravity of 1.206 g/ml. The difference in specific gravity could be caused by an increase in the concentration of knob flower extract used. The specific gravity results in the three formulas do not meet the requirements because they have a specific gravity of the preparation greater than the specific gravity of water. In this study, viscosity testing was carried out using an Ostwald viscometer. The viscosity value in each formula is different. In formula I the viscosity value is 16,714 cps, formula II is 39,892 cps and formulation III has a value of 69,989 cps. Formulation III is the formulation that has the highest viscosity value, this is because the higher the concentration used, the higher the viscosity value. This is influenced by the amount of extract used in the formula (Tampoliu, Ratu,*and* Rustiyaningsih 2021).

3.3 Antibacterial Activity Test Results

The results of the antibacterial activity test are shown by the presence of a clear colored area which is then measured by the diameter of the antibacterial activity inhibition zone. The results of the antibacterial activity test for knob flowers can be seen in Table 2 below

Table 2: Results of Antibacterial Activity of Gargarisma Knop Flower Extract Preparations

Replication					
	F1 25%	F2 50%	F3 75%	Control -	Control +
1	10	12	14	0	0
2	9	11	12	0	0
3	7	12	14	0	0
Rate rate	8,6	11,66	13,33	0	0

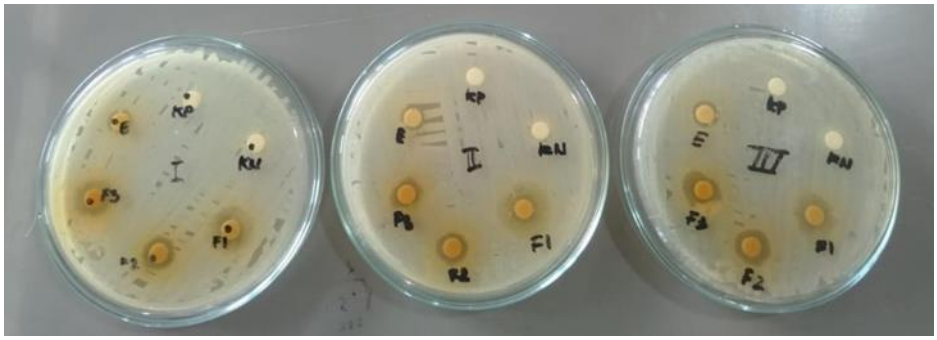

Figure 1: Inhibitory activity *Staphylococcus aureus*

In this study, the antibacterial activity of the Knob flower gargarsma preparation was tested against bacterial growth *Staphylococcus aureus* carried out using the agar diffusion method with media *Nutrient agar* (NA). Media use *Nutrient Agar* (NA) as an antibacterial test medium, namely because the nutrient agar medium is a selective medium and the composition contained in it is in accordance with the growth needs of the test bacteria, which are gram-positive and gram-negative bacteria (Azizah, Lingga, and Rikmasari 2020). The classification of bacterial growth inhibition responses based on the diameter of the clear zone consists of four groups, namely weak response (diameter ≤5 mm), medium (diameter 5-10 mm), strong (diameter 10-20 mm), and very strong (diameter ≥20 mm). mm) (Mahmudah and Atun 2017). In this study, the negative control used was the Gargarisma formula without extract, where the negative control showed that there was no inhibitory effect on bacteria because it did not contain active substances that act as antibacterials. Meanwhile, the positive control used in this study used a preparation on the market, namely listerin mouthwash (Cool mint). Listerin in this study did not show any inhibitory effect on bacteria *Staphylococcus aureus*. Zone of inhibition in the preparation *Gargarism* knob flower extract shows that FI, FII and FIII have different inhibitory activities, including formula I with an active ingredient concentration of 25% showing an average inhibitory zone diameter of 8.6 mm which is included in the medium category. Formula II with an active ingredient concentration of 50% shows an average inhibitory zone diameter of 11.66 mm, which is included in the strong category. Meanwhile, formula III with an active ingredient concentration of 75% shows that the average inhibition zone produced is 13.33 mm, which is included in the strong category. This is because the higher the concentration of the active ingredient in the meal knob flower extract, the greater the inhibitory power produced.

In contrast to previous research, in research conducted by Kusmiati et al, 2017, the results of the antibacterial activity test of the ethanol extract of kenop flowers against bacteria *Staphylococcus aureus* has a medium category inhibition zone. This can occur due to differences in the solvents used in the research, differences in the amount of extract concentration used and the content of secondary metabolite compounds contained in different ethanol extracts of knob flowers.

The presence of bacterial inhibitory activity is caused by the knob flower containing secondary metabolite compounds, namely flavonoids and tannins. The mechanism of flavonoids in inhibiting bacterial growth, among others, is that flavonoids cause damage to the permeability of bacterial cell walls, bacterial microsomes and bacterial lysosomes. Apart from that, flavonoids can also inhibit bacterial motility. The hydroxyl groups contained in the structure of flavonoid compounds cause changes in organic

components and nutrient transport which will ultimately result in toxic effects on bacteria. Meanwhile, the mechanism of action of tannins has antibacterial activity which is related to its ability to inactivate bacterial cell adhesins, through enzymes and disrupt protein transport in the inner layer of bacterial cells. Tannins also target bacterial cell wall polypeptides so that bacterial cell wall formation is less than perfect (Egra et al. 2019). Based on the results of antibacterial tests against bacteria *Staphylococcus aureus* shows that the knob flower gargarsma preparation has the opportunity to act as an antibacterial so it is necessary to carry out further testing on other bacteria both in vitro and in vivo on experimental animals so as to enrich the antibacterial source.

4. CONCLUSION

Based on the research results, it can be concluded that the preparation of gargarisma kenop flower extract (*Gomprhena globosa* L.) has antibacterial activity *Staphylococcus aureus* which increases towards strength with increasing concentration.

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Conflict of Interest Declaration

"All authors declare that they have no conflict of interest regarding this manuscript."

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