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Test The Activity Of A Mouthwash Preparation From The Extract Senggani Leaf Ethanol (*Melastoma Malabatricum* l.) Against The Fungi Candida Albicans

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ABSTRACT

The Candida albicans fungus is one of the factors that causes canker sores. Several researchers have proven that senggani leaves (Melastoma malabatricum L) have antifungal activity. The ethanol extract of senggani leaves is made by maceration. Formulated in mouthwash preparations with concentrations of 2%, 4% and 6%, then tested for antifungal activity against Candida albicans. The results of the antifungal activity test of the ethanol extract of senggani leaves obtained a Minimum Inhibition concentration at a concentration of 2%. The results of the antifungal activity of mouthwash preparations showed an average zone of inhibition. -average at a concentration of 2% 12.8 mm; 4% 13.4 mm and 6% 16.6 mm. Mouthwash preparations using ethanol extract of senggani leaves have been proven to have antifungal activity against Candida albicans which causes canker sores.

INTRODUCTION

Indonesia is a country that produces many plants which are rich in properties which have been passed down from generation to generation as traditional medicines and much research has been carried out on how to use plants by the community in an effort to treat disease, one of which is by using ingredients derived from plants, namely senggani leaves.(*Melastoma malabatricum*L.) (Dharma, 2001). Senggani leaves (Melastoma malabathricum L.) can be used to treat fever, canker sores, relieve pain, relieve urine, remove swelling, improve blood flow, heal wounds, and stop bleeding (Suherman, et al, 2015; Woro, 2023). In ancient times, people traditionally used senggani leaves to treat various types of diseases by boiling them and gargling them to treat toothache (Joffry, et al., 2012; Alnajar, et al., 2012). Senggani leaves contain secondary metabolite compounds including saponins, flavonoids, steroids, glycosides and tannins. This compound is able to inhibit and kill the growth of microbes, antifungal, bacteria and other types of microorganisms (Titi, et al, 2007).

Mold *Candida albicans*is one of the causes of canker sores in the oral cavity. In medical terms, canker sores are often referred to as stomatitis, which is swelling or inflammation that occurs in the mucosal lining of the oral cavity. Candida albicans is a component of the oral microflora and approximately 30-50% of people are infected with this organism. The infection that occurs can be local or systemic. Local infections can include disorders of the mouth, skin, throat and digestive tract. Systemic infections may include meningitis, endocarditis, and septicemia (Nurcahyanti, 2020).

This study aims to determine the most effective mouthwash preparation in inhibiting the growth of Candida albicans fungus from the ethanol extract of senggani leaves (Melastoma malabathricum L.) which is one of the factors causing canker sores.

METHOD

Tools used in research include laboratory glassware, blender (Turbo), water bath (mummert), analytical scales (fujitsu), Laminar Air Flow Cabinet (Astec HLF 1200L), autoclave,

oven (memmert), incubator, micrometer, pH meter , a set of spreadability test tools, vernier calipers, and antifungal test tools. The materials used in this research include, senggani leaf extract, 96% ethanol, Aquadest, Nutrient Agar, Minosep®, NaCl,Menthol, Sodium Benzoate, Saccharin, Peppermint Oil, Mg Powder, HCL,FeCl3 10%,Chloroform, Wagner's Reagent, Dragedroff, Liberman-Burchard's Reagent, 0.1 M NaOH, Mayer's Reagent and Candida albicans fungus. Sample material was collected purposively, that is, it was taken from one area only without comparing it with the same plants from other areas. The samples used in this research were senggani leaves collected from Tulumbaho Village, Lolofitu Moi District, West Nias Regency, Gunung Sitoli City, North Sumatra Province.

Simplicia powder was weighed as much as 500 g. Then maceration was carried out by adding 96% ethanol solvent until the powder was submerged. Leave it for 2x24 hours with occasional stirring until it is repeated 3 times with the aim of maximizing the process of extracting the chemical compounds found in the sample leaves. The results obtained were filtered using filter paper and extracted with water bath steam and stirred until a thick extract was obtained (Nurhayat and Marpaung, 2020).

Examination of Flavonoid Compounds.Provide two test tubes, tubes A and B. In tubes A and B, add 0.5 g of extract. Then in tube A, NaOH reagent is given, in tube B, distilled water is given. Note the difference in color changes on each tube. A positive reaction to flavonoids is characterized by a yellow or cloudy greenish color change after being given NaOH (Simaremare, 2014). Saponin Compound Examination.0.5 g of concentrated extract into a test tube then dissolved in 5 ml of distilled water and heated over a Bunsen flame and left to cool. The extract is shaken vigorously until it produces foam. A positive reaction to saponin is characterized by the presence of foam (Syahruni and Nur 2015). Examination of Tannin Compounds.0.5 g of extract was dissolved in 5 ml of ethanol in a test tube until dissolved, then FeCl3 was added. Note the color change that occurs on the tube. A positive tannin reaction is indicated by a dark blue color change. (Directorate General of Drug and Food Control, 1995). Steroid/Terpenoid Examination: Provide 05-1 g of concentrated extract dissolved in Chloroform: Aquadest (1:1) 5 ml each into a test tube, then shake gently and leave for a moment. Separate the two layers into 2 test tubes and mark tube A for the water fraction and tube B for the chloroform fraction. The Chloroform B fraction was tested using Lieberman Burchard reagent and acetic acid reagent. The chloroform filtrate was divided into 2 holes of the drip plate and allowed to evaporate and hole 1 was added with anhydrous acetic acid reagent and hole 2 was added with Lieberman Burcahard's reagent. Pay attention to the color differences. Positive reactions to steroids are marked in blue, purple or green. Positive reactions to terpenoids are marked in red/brownish red (Syahruni and Nur 2015). Alkaloid ExaminationPrepare 0.5–1 g of concentrated extract and dissolve it with 10 ml of HCL in a beaker glass, then filter it with filter paper and divide it into 3 test tubes. Tube A filtrate was added with 1 ml of Dragendroff Reagent. Tube B filtrate was added with 1 ml of Mayer's Reagent and Tube C Filtrate was added with 1 ml of Wagner Reagent. Note the color change in the tube. Dragendroff's Positive Reaction is characterized by the presence of a brick red precipitate. Mayer's Positive Reaction is characterized by the presence of a white precipitate. Wagner's Positive Reaction is characterized by the presence of a brown precipitate (Syahruni and Nur 2015).

The mouthwash solution was made by dissolving each concentration of ethanol extract of senggani leaves by adding 2 ml of 96% ethanol. Menthol is crushed and added with 2 ml of 96% ethanol until dissolved. then mixed into the ethanol extract of senggani leaves. Next, sodium benzoate and saccharin are dissolved in 10 ml of warm water and stirred until homogeneous. Then add a mixture of ethanol extract of senggani leaves and menthol to the mixture of saccharin and sodium benzoate until homogeneous by adding*peppermint oil*4 drops. Next, 100 ml of distilled water was added to the solution, then stirred again until homogeneous, then filtered. The

mouthwash solution was put into a tightly closed bottle and stored in a cool place for further research.

Table 1. Composition of Mouthwash Formula

Material Name	Concentration (%)					Material Function
	K(-)	K(+)	F1	F2	F3	
Senggani Leaf Extract	-	Minosep® %		4%	6%	Active substance
Menthol	0.10 g	-	0.10 g	0.10 g	0.10 g	Refresher
Saccharin	0.10 g	-	0.10 g	0.10 g	0.10 g	Sweetener
Sodium benzoate	0.10 g	-	0.10 g	0.10 g	0.10 g	Preservative
Peppermint oil	4 drops	-	4 drops	4 drops	4 drops	Fragrance
Ethanol 96%	2 ml	-	4 ml	10 ml	15 ml	Material solvent
Aquadest	There's 100 ml	-	There's 100 ml	There's 100 ml	There's 100 ml	Solvent

Evaluation of Mouthwash Preparations

Organoleptic observations are carried out visually by observing color, taste, aroma and shape. A stable nanoemulsion is characterized by a color that is not cloudy and clear and does not have a rancid odor (Rachma, 2010). Test the pH (degree of acidity) in general, mouthwash has a pH of 5-7. If the pH is below 5 then the preparation is too acidic and will cause more microbial growth. A good mouthwash is close to a neutral mouth pH, namely between pH 5-7. The test is carried out by dipping pH meter paper into mouthwash for several minutes at room temperature (Yosephine, et al, 2013).

The preparation viscosity test was carried out to determine the viscosity of the ethanol extract mouthwash preparation of senggani leaves. The expected viscosity of mouthwash preparations is close to the viscosity of water, because water is a liquid with a viscosity that is acceptable to all consumers. Viscosity testing was carried out using an Ostwald viscometer. 10 mL of the preparation is inserted through tube B and then sucked using a pipette until the liquid passes through section A and passes the "a" boundary. The liquid is then allowed to flow from boundary "a" to boundary "b". The time required for the preparation to flow is calculated using a stopwatch. (Handayani, et al, 2017).

Testing of Mouthwash Preparations (Well Diffusion Method).

Antifungal activity test of ethanol extract of senggani leaves (*Melastoma malabathricum*L.) against the fungus Candida albicans using the well diffusion method. This method was chosen as a simple way of working, equipment that is easy to obtain. The well method involves making 3 holes using a sterile steel spatula, on solid agar media that has been incubated with fungi. Then each hole is filled with the concentration of extract to be used. After incubation at 37°C for 2x24 hours. The test was carried out by observing and measuring using a screw micrometer by looking at the inhibition zone around the well (Prayoga, 2022).

Data analysis

Data analysis was carried out using qualitative and quantitative data analysis methods. Qualitative data analysis in the form of descriptive data with tables obtained from direct

observations by researchers of organoleptic tests, pH tests and viscosity tests. Quantitative data analysis was obtained from data analysis of the antifungal activity test using the One Way Anova statistical method by measuring the diameter of the inhibitory zone of the Candida albicans fungus compared with the results of the negative control (Qhorina, et al, 2021).

RESULTS AND DISCUSSION

Plant Identification Results

Plant identification was carried out at the Medanense Hebarium Plant Systematics Laboratory (MEDA) at the University of Sumatra Urara (USU). Based on the identification results with number 074/MEDA/2022, it shows that the plant used in this research is senggani leaves (Melastoma*malabathricum* L.) family Melastomataceae.

Chemical Compound Screening Results

Based on the results of tests on the chemical compound groups in the ethanol extract of senggani leaves, it contains chemical compounds from the Flavonoid, Saponin, and Tannin groups. In this study, compounds from the Alkaloid and Steroid/Terpenoid groups were not found in the simplicia of senggani leaf extract.

Results of Making Mouthwash Preparations from Ethanol Extract of Senggani Leaves (Melastoma malabathricum L.)

The mouthwash preparation was made based on the journal Sinrang, et al, (2022) which contains 10% menthol, 10% sodium benzoate, 10% saccharin, peppermint oil, ethanol and topped up with distilled water. The ethanol extract of senggani leaves added to mouthwash preparations as an antimicrobial is in concentrations of 2%, 4% and 6%. The mouthwash preparation obtained was in the form of a yellow, dark yellow and brick colored liquid with a characteristic odor of peppermint oil at concentrations of 2%, 4% and blank while at a concentration of 6% it had a distinctive odor of ethanol extract of sengganni leaves and peppermint oil. The results of the mouthwash preparation can be seen in Figure 1

Figure 1. Senggani Leaf Ethanol Extract Mouthwash Preparation

Results of Evaluation of Mouthwash Preparations

The results of organoleptic observations showed that the mouthwash preparations produced had a distinctive smell from senggani leaves and peppermint oil and all mouthwash preparations showed good and stable color, taste, aroma and shape with a concentration of 2% yellow and 4% yellow senggani leaf ethanol extract. concentrated while 6% gives a brick color. The higher the concentration of ethanol extract of senggani leaves used, the darker the color of the mouthwash produced. Organoleptic observation results can be seen in Table 2.

Table 2. Organoleptic Observation Results of Mouthwash Preparations

Formulation	Characteristics	Observation result
F1 (2%)	Color	Yellow

	Flavor	Sweet, fresh
	Aroma	Mint
	Form	Solution
F2 (4%)	Color	Deep yellow
	Flavor	Sweet, fresh
	Aroma	Mint
	Form	Solution
F3 (6%)	Color	Brick
	Flavor	Slightly bitter, fresh
	Aroma	Mint
	Form	Solution
Blank	Color	Clear
	Flavor	Sweet, fresh
	Aroma	Mint
	Form	Solution

Results of pH Testing of Mouthwash Preparations

The results of the pH test on the blank and sample formulation showed that the blank pH test obtained a pH of 6, while the preparation using ethanol extract of senggani leaves at concentrations of 2%, 4% and 6% had an average pH of 5. The test was carried out by dipping the pH meter paper into in mouthwash for several minutes at room temperature. The pH value of a preparation determines the type and ability of microbes to grow. According to Yosephine, et al, (2013) a good mouthwash is close to a neutral oral pH, namely between pH 5-7. So in this case it can be concluded that all mouthwash preparations made have an acceptable pH. The measurement results of the ethanol extract of senggani leaf mouthwash can be seen in Table 3.

Table 3. Results of pH Testing of Mouthwash Preparations

Formulation	pH Test Results
Blank	6
F1 (2%)	5
F2 (4%)	5
F3 (6%)	5

Viscosity Test Results for Mouthwash Preparations

The results of mouthwash viscosity testing carried out on blanks and sample formulations showed that the viscosity results of the ethanol extract of senggani leaf mouthwash preparations that had been carried out ranged from 0.81-0.98. The viscosity value of the senggani leaf extract mouthwash preparation has a smaller value than the viscosity of water, this is due to the influence of other additional ingredients which can increase the viscosity of a mouthwash preparation. The viscosity of water is around ±1 cP. The closer the viscosity level of a mouthwash formulation is to the viscosity of water, the easier and more comfortable it is to use for gargling (Handayani, et al, 2017). The viscosity test results can be seen in Table 4.

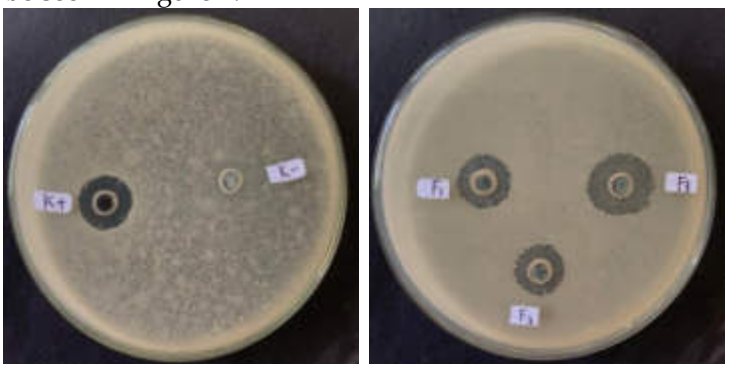
Table 4. Mouthwash Viscosity Test Results

Formulation	Repetition	Viscosity (piose)	Average
	1	0.80	
Blank	2	0.80	0.84
	3	0.94	

	1	0.82	
F1 (2%)	2	0.83	0.82
	3	0.83	
	1	0.83	
F2 (4%)	2	0.86	0.86
	3	0.91	
	1	0.97	
F3 (6%)	2	1	0.98
	3	0.97	

Antifungal Test Results of Mouthwash Preparations from Ethanol Extract of Senggani Leaves (Melastoma malabathricum L.) Against the Fungus Candida albicans

From the results of the inhibition zone measurements, the diameter of the clear zone for each formulation increased, the greater the extract concentration, the greater the diameter of the clear zone obtained. In formulation I with a 2% concentration, the clear zone diameter obtained was 12.8 mm, formulation II with a 4% concentration was 13.4 mm, formulation III with a 6% concentration was 16.6 mm. The Minosep® positive control had an inhibition zone with an average diameter of 13.6 mm and the blank negative control showed no effectiveness or there was no clear zone around the sample. The results of this test show that the ethanol extract mouthwash of senggani leaves has antifungal activity against Candida albicans with MIC at a concentration of 2% with an inhibition zone diameter of 12.8 mm due to the greater concentration of the extract contained in the preparation, The greater the active compound it has, the greater the diameter of the clear zone produced. This is in accordance with the statement that the higher the concentration of an antimicrobial ingredient, the greater the antimicrobial activity (Ningsih, 2017). Classes of compounds such as flavonoids, saponins, tannins are thought to have antifungal activity by binding to microtubule proteins in cells and disrupting the function of mitotic spindles, thereby inhibiting fungal growth (Bhaskara, 2012). The antifungal activity test of the mouthwash preparation of ethanol extract of senggani leaves can be seen in Figure 2. Classes of compounds such as flavonoids, saponins, tannins are thought to have antifungal activity by binding to microtubule proteins in cells and disrupting the function of mitotic spindles, thereby inhibiting fungal growth (Bhaskara, 2012). The antifungal activity test of the mouthwash preparation of ethanol extract of senggani leaves can be seen in Figure 2. Classes of compounds such as flavonoids, saponins, tannins are thought to have antifungal activity by binding to microtubule proteins in cells and disrupting the function of mitotic spindles, thereby inhibiting fungal growth (Bhaskara, 2012). The antifungal activity test of the mouthwash preparation of ethanol extract of senggani leaves can be seen in Figure 2.



(a) (b)

Figure 2. MIC Test of Senggani Leaf Ethanol Extract Mouthwash

Information :

(a) K+ (Minosep®) and K- (blank)

(b) F1 = 2% concentration, F2 = 4% concentration, F3 = 6% concentration

Inhibitory power according to Handayani, et al, (2017) is divided into: very strong (clear zone > 20 mm), strong (clear zone 10-20 mm), moderate (clear zone 5-10 mm) and weak (clear zone < 5 mm) so it can be stated that formulation I, formulation II and formulation III have strong inhibitory power against the Candida albicans fungus. The results of the antifungal activity test of the ethanol extract of senggani leaf mouthwash can be seen in Table 5.

Table 5. Antifungal Activity Test Results of Senggani Leaf Ethanol Extract Mouthwash Preparations

	Average Diameter of Fungal Growth Inhibition Zone Measurement (mm)				
Repetition	F1	F2	F3	K(+)	K(-)
I	12.2	12.8	16.8	13.7	0
II	13.3	13.9	17.1	13.6	0
III	13.1	13.6	16.0	13.5	0
Average	12.8	13.4	16.6	13.6	0

Results of Data Analysis of Antifungal Activity Test of Mouthwash Preparations

The statistical analysis test was used to compare the results of the antifungal activity test of the ethanol extract of senggani leaves, the blank negative control and the red Minosep® positive control. Based on the results of the normality test, it was found that each variable had a significance above 0.05, which means the data was normally distributed. Based on the homogeneity test, a significance value of 0.08 > 0.05 was obtained, so it can be concluded that the homogeneity data showed homogeneous data. Furthermore, One Way ANOVA obtained a significance value of 0.000 < 0.05, meaning that there was a significant difference in the variation of senggani leaf extract concentration in inhibiting growth. Candida albicans fungus and showed that there was a significant average difference between the inhibitory zone concentrations of 2%, 4%, 6%, positive controlMinosep®and blank.

CONCLUSION

Mouthwash preparations from ethanol extract of senggani leaves can inhibit the growth of Candida albicans. The mouthwash preparation of ethanol extract of senggani leaves has effective antifungal activity against Candida albicans at a concentration of 6% with an inhibitory zone diameter of 16.6 mm.

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