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Toothpaste Formulation Ethanol Extract Mangosteen Peel (*Garcinia mangostana L.*) and Test Activity Against *Candida albicans*

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Abstract: The mangosteen peel contains phenol compounds that have antifungal activity. The study aimed to find out the antifungal activity in the dosage form of toothpaste against Candida albicans. The research methods used include the extraction of mangosteen peel (Garcinia mangostana L.) by maceration using 96% ethanol. The toothpaste formula was composed with an extract concentration of 1.25% (F1); 2.5% (F2); 5% (F3); and base (B). Antifungal testing was carried out by diffusion method using Potato Dextrose Agar (PDA) media. The results showed that the toothpaste formula with a concentration of F1; F2; F3; and base (B) has an average value of successive inhibition zone diameters of 11.86 mm; 12.03mm; 12.54mm; and 7.78 mm. The conclusion of the study showed that the toothpaste preparation of the ethanol extract of mangosteen fruit peel (Garcinia mangostana L.) concentration was 1.25%; 2.5%; 5% which performs antifungal activity.

Keywords: Antifungals; Candidiasis; Candida albicans; Mangosteen peel; Toothpastes

Introduction

Candida albicans is the most isolated fungus from the human body, one of which is present in the oral cavity as a normal flora along with other microbes. Normal conditions C. albicans are found in the range of 200 cells/mL of saliva and abnormal conditions can reach 50%. Certain normal conditions of microbe flora can become pathogenic due to predisposing factors, namely oral hygiene (Kapila, 2021).

Oral candidiasis is a disease of the oral cavity in the form of red lesions and white lesions caused by the fungus C. albicans causing opportunistic infections. Mangosteen peel contains phenol compounds that function as antifungal and antibacterial (Behiry et al., 2019). Previous studies mentioned that ethanol extract of mangosteen Peel has inhibitory activity of C. albicans with an inhibitory zone diameter of 11.59 mm at a concentration of 2.5% (Boukhennoufa et al., 2019).

Toothpaste is one of the semi-solid preparations consisting of cleaning agents, abrasives and other additives so that the active substances can work optimally on the surface of the teeth to protect against damage caused by oral microbes without damaging theteeth or oral mucous membranes dental and oral health is the well-being of the oral cavity, including the teeth and supporting tissue structures free from disease and pain. Therefore, it is necessary to keep it clean by brushing your teeth (Raison et al., 2021). The use of toothbrushes and toothpaste preparations can help prevent the occurrence of dental and oral diseases (Kossioni et al., 2018).

Based on this description, it can be formulated the problem of this study is whether the preparation of toothpaste containing ethanol extract of mangosteen Peel has antifungal activity against Candida albicans (Serra et al., 2018). This study aims to determine the antifungal activity of toothpaste preparations containing

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ethanol extract of mangosteen rind against Candida albicans (Kurniawan et al., 2020).

The benefits of this study are expected to obtain scientific data on toothpaste preparations containing ethanol extract of mangosteen Peel has antifungal activity against Candida albicans (Hsu et al., 2021).

Method

Time and Place of Research

The research was conducted in March-August 2022 at the Microbiology Laboratory of the Makassar College of Pharmaceutical Sciences and Pharmacetics of the Islamic University of Makassar (Herwin, 2022).

Tools and Materials Used

The tools used in this study are glass (Pyrex®), autoclave (Hirayama®), petri dishes, incubators (Memmert®), calipers (Mitutoyo®), micropipette (Socorex®), oven (Memmert®), introducer, rotary evaporator , and analytical scales (Electronic Balance®) (Puhalsky et al., 2023).

The ingredients used are mangosteen peel, 70% alcohol, aluminum foil, distilled water, Candida albicans culture 10231 ATCC, Calcium Carbonate (CaCO3), ethanol 96%, glycerin, physiological NaCl solution, Potato Dextrose (PDA), sodium Carboxy Methyl Cellulose (Na CMC), sodium Benzoate, pippermint oil, Sodium Lauryl Sulphate (SLS) (Kar et al., 2019).

Sample Setup

Sampling

Mangosteen peel is obtained from Barugae Village, Bulukumpa District, Bulukumba regency, South Sulawesi. The sampling location was at South Latitude (S) 5°19'03.6012" and east longitude (E) 120°07'26.6016" (Elsbury et al., 2021).

Sample Processing

The skin of the mangosteen fruit is thoroughly washed under running water. After that drained and weighed the wet weight of the sample. Further dried-aerated without direct sunlight. Then it is pollinated with 40 degree fine mesh (Salzman et al., 2020).

Sample Extraction Process

A total of 500 g of samples were wetted first with 1 L of 96% ethanol for 15 minutes, then added to 5 L of 96% ethanol until the sample was submerged with a height of 3 cm (Tamiji & Nezamzadeh-Ejhieh, 2019). Furthermore, the sample was extracted using maceration method for 3 days while stirring occasionally (Adugna et al., 2022). After that, it is filtered with filter paper to get the macerated, the pulp is macerated again until it is clear. The macerate is combined and then evaporated with a rotary evaporator to obtain a dry extract (Sulastri et al., 2018).

Result and Discussion

Results should be clear and concise.The discussion should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Table 1. Toothpaste Formula Plan

Composition	Function	Formula Concentration (% w/v)			
		B	F1	F2	F3
Mangosteen peel extract	Active substance	-	1.25	2.5	5
Glycerin	Moisturizer	25	25	25	25
Calcium carbonate	abrasive	50	50	50	50
Na-CMC	binder	1	1	1	1
Na-Benzoate	Preservative	0.5	0.5	0.5	0.5
Sodium Lauryl Sulfate	foaming	1	1	1	1
Peppermint oil	Aroma	1	1	1	1
Aquadest ad (mL)	Solvent	10	10	10	10

Toothpaste Making

Making the formula first dissolved Na-benzoate in distilled water which has been heated to a temperature of 80°C then stirred and added Na-CMC until homogeneous. Next, calcium carbonate is added little by little until it forms a paste (Cao et al., 2019).

Ethanol extract of mangosteen fruit peel is dissolved with glycerin, then mixed into the first mixture until homogeneous. Sodium lauryl sulfate is added by stirring slowly to prevent frothing. Pippermint oil is added to the paste then adjusted to the required weight by adding distilled water. How to work for each subsequent treatment is done the same as the previous work (He et al., 2019).

Sterilization of Tools

The tools used are washed thoroughly with flowing distilled water, then the glass tools are dried and then wrapped in paper and sterilized in an oven at 180°C for 2 hours. Scaly glassware made of plastic and not heat-resistant is sterilized in an autoclave at a temperature of 121°C at a pressure of 2 atmospheres for 15 minutes. Ose is sterilized by exposure to a spirit lamp (Lestari et al., 2020).

Preparation of Potato Dextrose Agar (PDA)Media

A total of 7.8 g of PDA media (pH 5.5) was added 3% sterile tartaric acid and dissolved into 200 mL of distilled water, then heated on a hot plate until the media is cooked and homogeneous. Next, the media is sterilized in the autoclave at a temperature of 121^{o}C for

15 minutes pressure of 15 lbs and leave to a temperature of 45-50°C (Pensantes-Sangay et al., 2020).

Rejuvenation of the Test Fungus

The test fungus is Candida albicans, taken one ose from pure culture then inoculated on oblique PDA medium. After that, the test tubes are placed in the incubator for 3 times 24 hours at a temperature of 37 µC to grow the tested mushrooms (El Nahrawy et al., 2021).

Preparation of the Test Mushroom Suspension

The preparation of the mushroom test suspension was carried out by taking one Ose Candida albicans fungus and inserted into a test tube containing 0.9% NaCl as much as 4 ml, then homogenized to obtain a mushroom suspension with turbidity levels according to MC Farland 0.5 turbidity standards (Khusnul et al., 2022).

Antifungal Activity Testing of Toothpaste

A total of 5 mL of PDA media is inserted into the petri dish as a base layer and then flattened and allowed to solidify. Next, the introducer is placed using sterile tweezers on the surface of the previous medium with a distance of 2 cm from the lip of the petri Cup. Then pour as much as 10 mL of PDA media into a petri dish as the top layer (seed layer) of the brown bottle that has been mixed as much as 20 µL of test mushroom suspension, then flattened and allowed to solidify (Carr et al., 2023). After the PDA media has solidified, the introducer is taken back. Toothpaste Formula is taken as much as 0.05 mL per concentration and placed into the well and then incubated at a temperature of 37°C for 3 times 24 hours (Killilea & Schultz, 2023).

Fungal growth was observed and measured the diameter of the inhibition zone marked by a clear area around the well. The test was conducted on toothpaste base (B) and ethanol extract of mangosteen peel with concentrations of 1.25 % (F1), 2.5 % (F2) and 5% (F3).

Observation and Measurement of Drag Zone Diameter

Observation and measurement of the diameter of the inhibition zone was carried out using a caliper after being incubated for 3 times 24 hours at a temperature of 37°C. The fungal inhibition zone test is determined by measuring the diameter of the inhibition zone in which the test is performed three times (triple) repetition (Wang et al., 2018).

Table 2. The Yield of Ethanol Extract of Mangosteen Peel (Garcinia mangostana L.)

Simplisia	Simplicia (g)	Filter liquid volume (mL)	Dry extracts (g)	Percent yield (%)
Mangosteen Peel	500	5000	53.08	10.6

Table 3. Measurement Results of Inhibition Zone Diameter Formula Toothpaste Extract Ethanol Mangosteen Peel (Garcinia mangostana L.)

Replication	Inhibition Zone Diameter(mm)			
	FI	FII	FIII	B
I	11.84	12.02	12.78	7.75
II	11.52	11.67	12.23	7.81
III	12.22	12.41	12.62	7.79
Total	35.58	36.1	37.63	23.35
Average	11.86±0.35	12.03±0.37	12.54±0.38	7.78±0.03

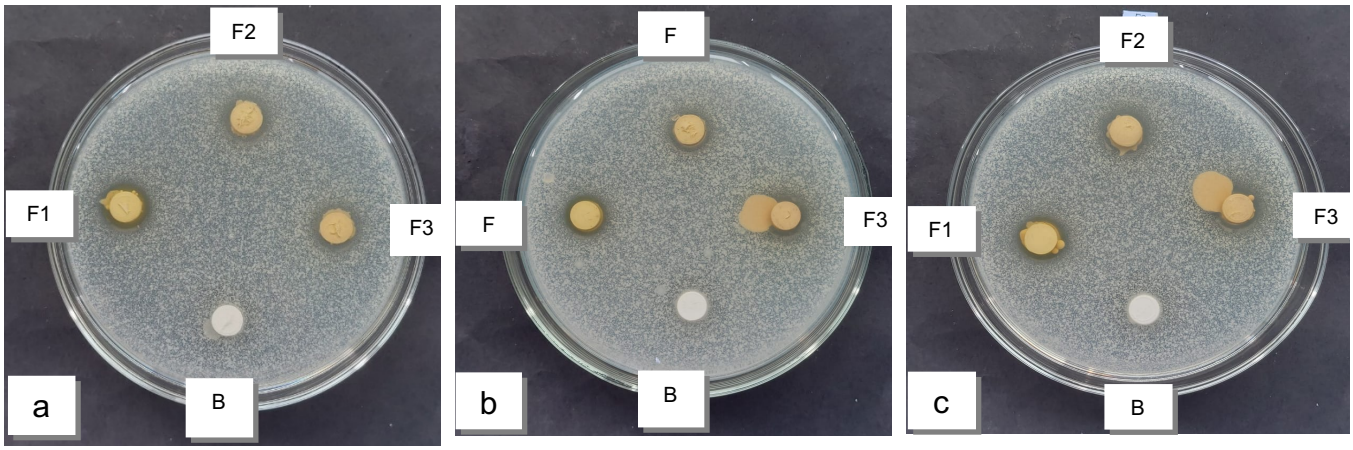

Figure 1. Antifungal activity test of a toothpaste preparation. a. Replication I, b. Replication II, c. Replication III

This study used samples of mangosteen peel (Garcinia mangostana L.). The extraction method used is maceration or immersion method. Maceration is a method of extraction that is done cold or at room temperature without any increase in temperature or heating. This is useful so that Simplicia or natural materials that are not heat resistant so as to avoid decomposition or damage to some active chemical components.

Toothpaste is a semi-solid product consisting of a mixture of scrubbing agents, cleaning agents, and additives that are used to help clean the teeth without damaging the teeth or oral mucous membranes.

The ingredients used in making the paste are ethanol extract of mangosteen peel as the active substance, glycerin as a moisturizer, calcium carbonate as an abrasive, Na-CMC as a binder, Na-benzoate as a preservative, sodium lauryl sulfate as a foamer and peppermint oil as a preservative.

The antifungal testing method used is the diffusion method pitting. This method has the advantage that it is easier to measure the diameter of the inhibition zone not only on the upper surface of the nutrient but also to the bottom.

Based on the results of observations in Table 3 shows that testing toothpaste ethanol extract mangosteen Peel has antifungal activity. The average values of the inhibition zone diameter of ethanol extract toothpaste mangosteen peel with concentrations of 1.25% (F1), 2.5% (F2) and 5% (F3) were 11.86 mm, 12.03 mm, and 12.54 mm, respectively. The diameter values of the drag zones F1, F2 and F3 belong to the strong category. While the base (B) has an average value of the diameter of the inhibition zone is 7.78 mm. The value belongs to the medium category.

The activity of the antimicrobial inhibition zone is classified into four categories, namely: weak activity (⑧ 5 mm), moderate (6-10 mm), strong (11-20 mm), very strong (>20 mm). The results of this study are also consistent with previous research that mangosteen peel extract effectively inhibits C. albicans in vitro with an inhibitory zone diameter of 11.59 mm at a concentration of 2.5%.

Toothpaste containing mangosteen peel extract has antifungal activity against Candida albicans. This is because the skin of the mangosteen fruit contains phenol compounds that function as antifungals. This statement is in line with Chusnul's research et.al. (2018) that phenol compounds contained in the skin of mangosteen have antifungal activity. In addition, the ethanol extract of mangosteen peel also contains flavonoid compounds, saponins and tannins. This class of compounds also has antifungal activity.

Compounds of the phenol group, flavonoids and tannins exert antifungal effects by disrupting membrane permeability, inhibiting cell wall formation and disrupting activity in mitochondria (Christoper et al., 2018). Phenol compounds make the cell cycle stop in the replication phase and disrupt the cell division process. In addition, phenols damage the mitochondria which will lead to the accumulation of reactive oxygen species (ROS), working by inhibiting the synscription of chitin which is essential for the formation of the cell wall. Flavonoid compounds have antifungal mechanisms by interfering with mitochondrial homeostasis and the integrity of cell membranes. Tannins have antifungal activity by inhibiting the synthesis of chitin which is used for the formation of cell walls and damaging cell membranes so that fungal growth is inhibited. Saponins, including terpenes, have a mechanism of action such as detergents.

The measurement data were analyzed by complete randomized design (RAL) method, this can be seen in ANAVA table where F count is greater than F table at the level of 5% and 1%, which indicates that there is an effect of treatment differences on the diameter of the fungal inhibition Zone C. albicans, to see the effect of which treatment gives a difference in the effect of each group then performed a follow-up test with the BNT method. The determination of the advanced test is based on the value of the coefficient of diversity (KK) obtained, because the condition of the value of the KK for the BNT test is if the KK value is smaller or equal to 5%.

The BNT test is performed to show a comparison of the antifungal activity of toothpaste against C. albicans and between treatments of each concentration. Further test results obtained data comparison of all treatments against the base showed very significant results, meaning F1, F2 and F3 have better antifungal activity than the base.

The base has the smallest inhibitory zone diameter of all treatments. Furthermore, data comparison of F1 treatment against F2 showed no significant results, meaning antifungal activity is not different. While the treatment of F1 against F3 and treatment of F2 against F3 showed very significant results, meaning that there is a significant difference in antifungal activity. Then the formula that has the greatest antifungal activity is F3 with a concentration of 5%.

Conclusion

Based on the results of research, data analysis and discussion, it can be concluded that ethanol extract of mangosteen peel (Garcinia mangistana L) with concentrations of 1.25%, 2.5%, and 5% formulated in toothpaste dosage form has antifungal activity with average values of inhibition zone diameter of 11.86 mm (F1), 12.03 mm (F2), and 12.54 mm (F3) against Candida albicans.

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Author Contributions

In this research, the authors made different contributions. Nur Khairun Nisa, as the corresponding author, was responsible for planning the study, processing samples, and drafting the manuscript. Ermina Pakki and Nurida played a role in assisting with the interpretation of the research findings.

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Conflicts of Interests
Throughout the process of research preparation and report writing, there were no conflicts of interest among the author that could have influenced the research outcomes. All author received the result of this study based on their respective contribution, without any financial connections, personal interests, or affiliations that could introduce bias in the interpretation or reporting of the research findings.

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