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UTILIZATION OF KECOMBRANG FRUIT PEEL (*Etlingera elatior* (Jack) RMSm) AS A MOUTHWASH TO PREVENT MOUTH ULCERS AND DENTAL CARIES

Pemanfaatan Kulit Buah Kecombrang *(Etlingera elatior* (Jack) RMSm) Sebagai Obat Kumur untuk Mencegah Sariawan dan Karies Gigi

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ABSTRACT
Kecombrang fruit is used by the community as a food ingredient but actually the skin of this kecombrang fruit can be used as a mouthwash for the development of dental caries. This study aims to evaluate the preparation of mouthwash formulations with the addition of kecombrang fruit peel extract. The work procedure starts from extraction, phytochemical testing, antimicrobial testing of extracts, and evaluation of mouthwash preparations. The results contained secondary metabolites in the form of alkaloids, flavonoids, tannins, and saponins. Evaluation of mouthwash preparation with pH test is 5.0-5.6, organoleptic test which does not change significantly, viscosity test at 20% and 30% concentration is 1.0193 cP and 1.0061 cP, and antimicrobial test with inhibition diameter on Streptococcus mutans and Candida albicans is 11.3 mm and 11.1 mm. In conclusion, mouthwash with added extracts can inhibit the growth of microbes that cause mouth ulcers and tooth decay.

***Keywords**: Antimicrobial, Dental Caries, Kecombrang Peel, Mouthwash, Mouth Ulcers*

ABSTRAK
Buah kecombrang digunakan masyarakat sebagai bahan makanan namun sebenarnya kulit buah kecombrang ini dapat dijadikan sebagai obat kumur untuk penyembuhan karies gigi. Penelitian ini bertujuan untuk mengevaluasi sediaan formulasi obat kumur dengan penambahan ekstrak kulit buah kecombrang. Prosedur kerja dimulai dari ekstraksi, pengujian fitokimia, uji antimikroba ekstrak, dan evaluasi sediaan obat kumur. Hasil yang didapat terdapat metabolit sekunder berupa alkaloid, flavonoid, tanin, dan saponin. Evaluasi sediaan obat kumur dengan uji pH yaitu 5,0-5,6, uji organoleptik yang tidak berubah signifikan, uji viskositas pada konsentrasi 20% dan 30% yaitu 1,0193 cP dan 1,0061 cP, serta uji antimikroba dengan diameter zona hambat pada Streptococcus mutans dan Candida albicans yaitu 11,3 mm dan 11,1 mm. Kesimpulannya obat kumur dengan penambahan ekstrak mampu menghambat pertumbuhan mikroba penyebab sariawan dan karises gigi.

Kata kunci: *Antimikroba, Karies gigi, Kulit Kecombrang, Obat Kumur, Sariawan*

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INTRODUCTION

Mouth ulcers is an ulcerative condition in the oral cavity which is characterized by pain, recurrent ulcers, and white pseudomembranes with a white round pattern due to the growth of the fungus *Candida albicans* (Lilyawati et al., 2019). *Candida albicans* is opportunistic, namely pathogenic in individuals with low immune conditions but not pathogenic in healthy individuals. (Pranata & Sundara, 2021). Apart from canker sores, another problem is dental caries caused by the bacteria *Streptococcus mutans*. This bacteria has acidogenic properties which function in producing acidic compounds in the teeth, which results in loss of calcium or what is called decalcification and the surface of the teeth is eroded to form caries on the teeth (Gurning et al., 2019).

Efforts that can be made to prevent canker sores and dental caries include using mouthwash. One of the chemical mouthwash products is hexetidine. The way this type of drug works is by attaching it to the oral mucosa and then binding to the dental plaque which results in inhibiting the metabolism of microorganisms. However, the use of mouthwash with an alcohol content of >25% can cause human health problems because it can cause cancer of the mouth, throat and pharynx (Tampoliu et al., 2021).

Indonesia has plants that can be used as medicine, for example the kecombrang plant. The kecombrang plant or Etlingeria elatior (Jack) RMSm) has properties such as eliminating bad breath and body odour and is antimicrobial (Suryani, Nurjanah, et al., 2019). The chemical content of this kecombrang stem extract can inhibit the growth of *Streptococcus mutans* bacteria, namely flavonoids, tannins, alkaloids and saponins.

Generally, humans use the kecombrang plant for its stems, leaves and flowers. Another part, namely the fruit of the kecombrang plant, is known as a cooking spice because it produces a distinctive sour taste.

However, when used, the flesh of the fruit is usually consumed while the fruit skin becomes dregs because of the hard physical characteristics of the fruit skin. The urgency of this research is to increase the utilization of kecombrang fruit peel which is formulated as a mouthwash preparation with natural ingredients to prevent canker sores and dental caries as well as developing the use of unused natural ingredients in the form of kecombrang fruit peel waste.

MATERIALS AND METHODS

Place and time of research

Implementation of this research starts from January to October 2023 in. UINSU Microbiology Laboratory and USU Laboratory.

Material

The experiment used kecombrang fruit skin extract as the primary ingredient to test against *Streptococcus mutans* bacteria, which was obtained from CV. Rudang Jaya. The materials included NA media from Himedia, MHA media from Oxoid, 96% ethanol, DMSO, Lugol's solution, safranin, methylene blue, distilled water, 0.9% NaCl, sulfuric acid, and 1% barium chloride.

The tools used are aluminum foil, plastic wrapping, sterile cotton, tissue, rotary vacuum evaporator, object glass, cover glass, hot plate, oven, incubator, petri dish, test tube, test tube rack, erlenmeyer, measuring cup, microscope, tube needle , analytical balance, spatula, dropper pipette, bunsen, disc paper, and caliper.

Method

a) Sample Identification and Preparation of Simplisia and Extract of Kecombrang Fruit Peels.

The samples were identified at the Herbarium Medanase USU, and five kilograms of kecombrang fruit peel were collected, washed with running water, and dried in a drying cabinet. The dried peels were then crushed into powder, placed into a container, and soaked in 96% ethanol. The solution was filtered using filter paper to produce the liquid extract of kecombrang fruit peel, which was evaporated using a water bath until a thick extract was obtained (Rahmayani et al. 2023). The extract was then prepared into a series of five dilutions with concentrations of 10%, 20%, 30%, 40%, and 50%.

b) Phytochemical Screening

Phytochemical screening tests include tests for the content of alkaloids, flavonoids,

saponins and tannins (Nurjannah, et al., 2022). In this study, phytochemical methods were used to identify the presence of secondary metabolites (such as alkaloids, flavonoids, tannins, and saponins) in kecombrang fruit peel extract. The following are technical steps and how to make reagents for each phytochemical test:

1. Alkaloid Test

Steps:

Take a small amount of kecombrang fruit peel extract, then add a few drops of 2% HCl. Divide the solution into three parts to be tested with Dragendorff, Mayer, and Bouchardat reagents. If precipitation occurs, the alkaloid is detected positively.

Reagent Preparation:

Dragendorff's reagent: Mix 1% potassium iodide (KI) solution with 1% bismuth nitrate ($Bi(NO_3)_3$). Mayer's Reagent: Mix 1% potassium iodide (KI) solution with 1% mercury(II) chloride ($HgCl_2$). Bouchardat reagent: Dissolve iodine in potassium iodide in a ratio of 2:1 in water.

2. Flavonoid Test

Steps:

Take kecombrang fruit peel extract and add a little magnesium (Mg) powder and concentrated HCl. Stir gently, and note the colour change of the solution. The presence of red or orange colour indicates the presence of flavonoids.

Reagent Preparation:

HCl and Magnesium Reagents: This solution does not require additional special reagent preparation; use concentrated HCl directly and magnesium powder as a catalyst.

3. Tannin test

Steps:

Take the extract and add 1% $FeCl_3$ (ferric chloride) solution. Note the colour change that occurs. The presence of tannins is indicated by a colour change to bluish green or black.

Prepare the reagent:

$FeCl_3$ 1% solution: Dissolve 1 g of ferric chloride ($FeCl_3$) in 100 ml of water.

4. Saponin test

Steps:

Take Kecombrang fruit peel extract, place it in a test tube filled with hot water, then shake the test tube vigorously. If a stable foam forms and lasts for more than 10 minutes, saponin is detected.

Reagent preparation:

Hot water: No special reagents are required; hot water is sufficient for the saponin test.

c) Gram Staining

A bacterial culture was placed on a glass slide and fixed over a Bunsen burner. Crystal violet was applied to the culture and left for 1–2 minutes before being rinsed with running water. Lugol's solution was applied to the preparation and left for 30 seconds, followed by rinsing with running water. Finally, safranin was applied, left for 2 minutes, and then rinsed with running water before observation under a microscope.

d) Testing the Activity of Kecombrang Fruit Peel Extract

The activity of kecombrang fruit peel extract was tested against *Candida albicans* and *Streptococcus mutans*. A total of 0.1 ml inoculum for each isolate was streaked onto the surface of solidified MHA media in each petri dish. Disk paper was then soaked in the extract at various concentrations and placed on the MHA media surface at designated points. Incubation was carried out for 24 hours at 37ºC. The inhibition zones formed were observed and measured with a caliper. Positive controls were used, with chloramphenicol 250 mg for bacteria and ketoconazole 200 mg for for fungi, while sterile distilled water served as the negative control.

e) Preparation of Mouthwash Formulation

The mouthwash formulation was prepared using the active ingredient of kecombrang fruit peel extract solution, along with additional ingredients as corrigents, including saccharin (0.2%), peppermint oil (0.2%), Nipagin (0.1%), and 100 ml of distilled water (Widya, 2011).

f) Evaluation of Mouthwash Formulation

Evaluation of physicochemical properties of mouthwash using organoleptic test by observing colour, odour, and taste by 30 participants. Physical evaluation includes

checking the stability of the preparation and the pH of the preparation. Biological evaluation by testing the antimicrobial activity of kecombrang shell extract mouthwash preparations against two test microbes.

The kecombrang fruit peel extract mouthwash formulation was tested against *Candida albicans* and *Streptococcus mutans*. A volume of 0.1 ml inoculum from each isolate was streaked onto the surface of solidified MHA media in each petri dish. Disk paper was soaked in the mouthwash formulation and placed at designated points on the media surface. The petri dishes were incubated at 37ºC for 24 hours, and the inhibition zones were observed and measured using a caliper.

Storage The condensed extract is stored under conditions protected from light at 22 C using tightly sealed containers to prevent exposure to air and moisture. The container is lined with aluminium foil to protect the extract from light-induced degradation.

RESULTS AND DISCUSSION

Kecombrang Fruit Skin Extraction

The extraction process of kecombrang fruit peel is carried out using the maceration method, which is one of the simple extraction methods. In this method, the material is extracted by soaking in a solvent at room temperature for several days, usually under conditions protected from light to maintain the stability of the bioactive compounds contained therein. This technique is often chosen because it is relatively easy to do and effective for producing extracts without the need for sophisticated equipment, provided that parameters such as the type of solvent, soaking time, and protection against light are well managed (Kurniawati, 2019).

Phytochemical Screening of Combrang Fruit Peel Extract

Phytochemical screening functions to obtain information on the class of secondary metabolite compounds contained therein.

Table 1. Results of Phytochemical Screening of kecombrang fruit skin

Secondary Metabolites	Reactor	Results
Alkaloids	Dragendorf	+
	Bouchardat	+
	Meyer	+
	Wagner	-
Flavonoids	FeCl3 5%	-
	Mg++ HCl powder	+
	NaOH 10%	+
	H2SO4	-
Glycosides	Molish	-
Saponin	Hot water/shaken	+
Tannin	FeCl3	+
Triterpenoids/Steroids	Lieberman-Bourchat	-

Description: (+) Detected; (-) Not detected

Based on Table 1, the results of the phytochemical screening test of kecombrang fruit peel extract contain alkaloids, flavonoids, tannins and also saponins. The alkaloid test results were positive for Dragendroff, Mayer, and Bouchardat reagents with the formation of a brown precipitate. The flavonoid test was carried out with Mg and HCl reagents to produce a brick red color. Likewise, tannins provide a positive response by changing the color to bluish green because they react with Fe (III) to form complex compounds. Furthermore, the foam formed in the saponin test also showed positive results.

Streptococcus mutans Gram stain

Gram staining is one of the important and common techniques used to identify bacteria. *Streptococcus mutans* bacteria are gram-positive, appearing purple due to their ability to retain methylene blue dye. They

have a characteristic streptococcus shape, forming round chains. This happens because the cell walls of gram-positive bacteria contain a lot of peptidoglycan. Giving alcohol will not lyse the bacterial cell walls, causing the pores to shrink and the permeability of the cell walls and membranes to decrease so that the Lugol solution given can be trapped in the protoplasmic membrane and safranin cannot enter.

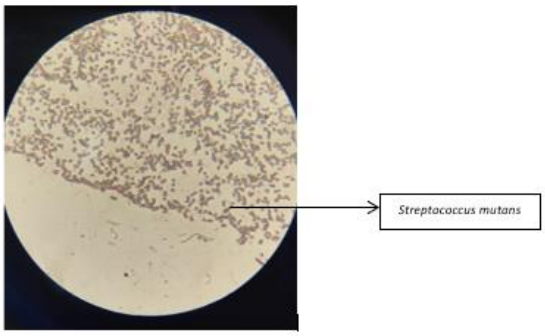

Figure 1. Gram staining of *Streptococcus mutans*

Anti-Bacterial Test Results of Kecombrang Fruit Peel Extract against *Streptococcus mutans*

Table 2. Observation Results of the Antibacterial Test of Kecombrang Fruit Peel Extract on the Growth of *Streptococcus mutans*

Treatment	Test (mm)				Average Zone of Inhibition (mm)	Category
	U1	U2	U3	U4		
10%	7.5	8.2	7.4	8.1	7.8	Currently
20%	9	8.9	8.7	8.3	8.7	Currently
30%	11.3	11.1	10.5	9.8	10.6	Strong
40%	12.2	12.7	11.8	12.9	12.4	Strong
50%	15	13.3	14	13.2	13.8	Strong
C+	28.5	29.5	30	30.4	29.6	Very strong
C-	-	-	-	-	-	No activity

The average results of the inhibition zone carried out on kecombrang fruit peel extract against *Streptococcus mutans* bacteria, namely at concentrations of 10%, 20%, 30%, 40%, and 50% were 7.8 mm, 8.7 mm, 10.6 mm, 12.4mm, and 13.8mm. This shows that the higher the concentration of kecombrang fruit peel extract, the higher the inhibition zone produced. The inhibition zone produced by the chloromphenicol positive control was also very strong, namely 29.6 mm, while the DMSO negative control did not produce an inhibition zone. Based on the research of Apriani *et al* (2022), the most effective inhibition zone was produced with a diameter of 14 mm to 16 mm. So it can be seen from the treatment that the most effective concentration of kecombrang fruit peel extract is a concentration of 50% with an inhibitory zone diameter of almost 14 mm.

The existence of an inhibition zone resulting from this antibacterial test is due to the content of secondary metabolites. This plant secondary metabolite has an antibacterial function with a synergistic mechanism of action. The secondary metabolites found in kecombrang fruit peel extract are flavonoids, alkaloids, saponins and tannins.

Flavonoids provide antimicrobial effects through their ability to form complexes with extracellular and soluble proteins as well as with bacterial cell walls. The mechanism of action of flavonoids as antibacterial compounds is divided into 3, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting metabolism (Yan et al, 2023).

The mechanism of action of tannin as an antibacterial is by causing bacterial cells to lyse. This happens because tannins have

target cells in the cell wall polypeptides become less than perfect and then the bacterial cells will die (Wati et al, 2022). Alkaloids also have the ability to act as antibacterials and their inhibitory mechanism is by interfering with the components that make up the peptidoglycan in bacterial cells so that the cell wall layer does not form completely and causes cell death. Apart from that, alkaloids also inhibit the formation of protein synthesis so that they can disrupt bacterial metabolism (Anggraini et al, 2019).

Antimicrobial Test Results of Kecombrang Fruit Peel Extract against *Candida albicans*

Table 3. Observation Results of Antimicrobial Test of Kecombrang Fruit Peel Extract on the Growth of Candida albicans

Treatment	Test (mm)				Average Zone of Inhibition (mm)	Category
	U1	U2	U3	U4		
10%	10.9	11.2	11.5	11.9	11.3	Strong
20%	12.1	11.8	11.7	12	11.9	Strong
30%	12.8	13.4	13.6	12.5	13	Strong
40%	15.2	15.8	17.1	16.5	16.1	Strong
50%	19.6	18.5	18.7	19.2	19	Strong
C+	27.5	26.8	25.9	25.7	26.4	Very strong
C-	-	-	-	-	-	No activity

The average inhibition zones produced by concentrations of 10%, 20%, 30%, 40%, and 50% were 11.3 mm, 11.9 mm, 13 mm, 16.1 mm, and 19 mm. Likewise, with the *Streptococcus mutans* antibacterial test, the inhibition zone produced is also higher in line with the higher concentration. Effectiveness decision making is also seen from the Pharmacopoeia book IV edition with the provisions of 14 mm to 16 mm. So it can be seen that the most effective inhibition zone is at a concentration of 40% with a diameter of 16.1 mm.

The positive control for antibacterial testing against *Streptococcus mutans* used was chloramphenicol. For antifungal testing against *Candida albicans* ketoconazole was used. The Negative Control used was sterile distilled water to ensure that there was no microbial inhibitory effect caused by solvents or other additives that were not part of the active compounds in the extracts.

The ability of the extract to inhibit the growth of *Candida albicans* fungus is due to the content of secondary metabolites in the form of alkaloids, flavonoids, tannins and saponins. Alkaloids are able to disrupt the components that make up the peptidoglycan in the cell membrane, so that the cell membrane layer does not form completely and causes death of the fungus. Likewise, tannins are able to inhibit fungi by making their cell walls shrink so that cell permeability is disrupted and causes the fungus to die. Flavonoids can form complex compounds with extracellular proteins which can cause cell denaturation, resulting in damage to fungal cell membranes (Pitopang etal, 2022).

However, it can be seen from the inhibition zone that is formed that unlike the *Streptococcus mutans* bacteria, the inhibition zone produced is not very clear so that the streaks of Candida albicans fungus that are formed can still be seen. This is because the large amount of flavonoid content does not kill microbial cells but instead induces the formation of bacterial aggregates and thus is only able to reduce the number of bacterial colonies in large numbers (Fahruddin et al, 2016).

Evaluation Results of Mouthwash Preparation Formulations

A. Organoleptic Test

Table 4. Organoleptic test results

Preparation	Observation	Storage Time (week)				
		0	1	2	3	4
Formulation 1	Aroma	Kecombrang spices, a little mint	A little combrang, strong mint	A little combrang, strong mint	Mint is strong	Mint weakens
	Texture	A little thick	Dilute	Dilute	Dilute	Dilute
	Color	Dark brown, cloudy	Bright dark brown, slightly cloudy	Bright brown, clear	Bright brown, clear	Bright brown, clear
Formulation 2	Aroma	Combrang spices, a little mint	A little combrang, strong mint	A little combrang, strong mint	A little combrang, strong mint	Mint weakens
	Texture	A little thick	Dilute	Dilute	Dilute	Dilute `
	Color	Dark brown, cloudy	Bright dark brown, slightly cloudy	Bright dark brown, clear	Bright brown, clear	Bright brown, clear

Based on the organoleptic test table, it can be seen that during the 4 week storage period there were no significant differences. The 4-week storage was used to observe if there were any significant changes in pH, viscosity, aroma, colour and antimicrobial effectiveness. This ensures that the formulation remains of good quality until it is ready to be used as mouthwash. The aroma of formulation 2 with the addition of 30% combrang shell extract produces a more pungent aroma. This is because the greater the kecombrang fruit peel extract, the sharper the aroma produced by the extract. Apart from that, the color produced by formula 2 is more concentrated because it contains more kecombrang extract. The brownish color found in mouthwash preparations is produced by the tannin compound content in plants (Pardeny et al, 2022).

B. Test pH

Table 5. pH test results on mouthwash

Preparation	Length of Observation				
	Week 0	1st week	2nd week	3rd week	4th week
Formulation 1	5.1	5.4	5,6	5.5	5.3
Formulation 2	5.0	5.2	5.3	5.5	5.4

pH testing was carried out using a digital pH. The results obtained for the pH of mouthwash preparations in formulation 1 with the addition of 20% extract and formulation 2 with the addition of 30% extract ranged from 5.0-5.6. The addition of extracts to the mouthwash preparation as much as 20% or 30% is an effort to achieve a balance between antimicrobial effectiveness, physicochemical stability, and convenience of use. This concentration was chosen based on the results of laboratory tests to provide maximum protection against microbes that cause canker sores and dental caries. So the results are still said to be in accordance with the pH of the mouth, which is around 5-7. This appropriate pH means that the mouthwash formula preparation can still react optimally without interfering with the mechanism of action of the drug and without affecting the skin mucosa. pH is also useful for maintaining comfort in the mouth and pre-

venting irritation. The pH value greatly influences the growth of bacteria in the preparation. It can also be seen that the pH produced by formula preparations 1 and 2 still meets the standards for good mouthwash between 5-6, a pH of less than 5 indicates that a strong acid preparation can cause more bacterial growth and if the pH is more than 6 it can increase fungal growth (Febrianti S et al, 2022).

C. Viscosity Test

Table 6. Viscosity Test

Treatment	Viscosity (cP)
20%	1.07
30%	0.85

This viscostatic test is carried out with the aim of determining the viscosity value of a substance. The viscostatic level of a formulation greatly influences the viscosity level of the mouthwash preparation when used. The closer the viscostatic level of a formulated product is to the viscosity level of water, the easier and more comfortable the product is to use for gargling. The viscosity level of pure water is 1 mPa.S or approximately 1cP, while the standard viscosity of mouth-wash on the market is 7.25 (Noval et al, 2020).

Based on viscosity test data on the first day of mouthwash with a concentration of 20%, the viscosity value was 1.07 cP, while at a concentration of 30% it was 0.85 cP. It can be seen that the viscosity value of the 20% concentration mouthwash preparation is closer to the viscosity value of pure water because the extract content is less than 30%.

D. Antimicrobial Test

Table 7. Antimicrobial Test Results on Mouthwash

Mouthwash Preparations	Inhibition Zone Diameter (mm)											
	Streptococcus mutans (week)						*Candida albicans* (week)					
	0	1	2	3	4	Average	0	1	2	3	4	Average
Formulation 1	7.7	8.5	9.4	10.6	11.3	9.5	7.3	8.1	8.9	9.4	10	8.7
Formulation 2	8.4	10.3	11.5	12.8	13.4	11.3	8.5	8.6	9.0	10.2	11.1	9.4
C+			24.3			24.3			20.6			20.6
C-	-	-	-	-	-	-	-	-	-	-	-	-

The antibacterial test results of the kecombrang fruit peel extract mouthwash preparation against *Streptococcus mutans* bacteria increased in both mouthwash formulations. Then it can be seen that formulation-1 with the addition of kecombrang fruit peel extract has an average of 9.5 mm while formulation 2 with the addition of 30% kecombrang fruit peel extract has an average inhibition zone of 11.3 which is higher when compared to formulation 1. Likewise with the antimicrobial test on *Candida albicans* which has an increase in the diameter of the inhibition zone every week.

When compared to formulation 1 with an average inhibition zone of 8.7 mm, it is lower than formulation 2 with an average of 9.4 mm. This is because the greater the concentration of the extract contained in the mouthwash preparation, the more secondary metabolite compounds there will be (Noval et al, 2020). Based on the Pharmacopoeia, formulations 1 and 2 do not meet the strong inhibition zone standards. However, mouthwash preparations can be declared successful because they can inhibit the growth of *Streptococcus mutans* bacteria and *Candida albicans* fungi, which if used continuously can prevent canker sores and dental caries.

CONCLUSION

Combrang fruit peel extract can inhibit the growth of the bacteria Streptococcus mutans which causes dental caries and Candida albicans which causes canker sores. As a result of testing the mouthwash formulation with the addition of combrang fruit peel extract in concentrations of 30% and 20%, the results of the pH test, viscosity test, organoleptic and antimicrobial tests were quite good.

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