Utilization of Lime Peel (Citrus aurantiifolia (Christm.) Swingle) Ethanol Extract for Mouthwash Formulation to Prevent Dental Caries

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A B S T R A C T

Lime (Citrus aurantiifolia (Christm.) Swingle) is a plant that has medicinal properties. Lime peel contains alkaloids, flavonoids, tannins, steroids/triterpenoids and essential oils. These compounds are known to have antibacterial activity. This study aims to determine whether lime peel extract has antibacterial activity against Streptococcus mutans and antifungal against Candida albicans. So that an effective inhibition zone will be obtained for the manufacture of mouthwash preparations using the agar diffusion method. The research method used is the experimental method. The experiment consisted of 3 repetitions with a concentration of 96% ethanol extract of lime peel 25%, 30%, 35%, 40%, 45%. Lime peel samples were taken purposively from Pasar 1 Rel, Terjun, Kecamatan Medan Marelan, North Sumatra. The results showed that lime peel extract provided an effective inhibition zone diameter at a concentration of 40% and 45% against Streptococcus mutans with a diameter of 14.07 mm and 14.4 mm at a concentration of 40% and 45% against the fungus Candida albicans with a diameter of 14. mm and 14.2 mm in mouthwash preparations of lime peel extract at FI concentration of 40% FII concentration of 45%, giving satisfactory results of inhibition zone diameter which is greater than 14 mm against Streptococcus mutans and Candida albicans. Lime peel can be formulated in the form of mouthwash preparations.

Keywords:

Citrus aurantiifolia (Christm.), Antimicrobial, Streptococcus mutans, Candida albicans, Mouthwash

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1. INTRODUCTION

Dental and oral health is important. One indicator of oral health is the level of oral hygiene. Various diseases caused by poor oral hygiene such as gingivitis and periodontitis are the main factors causing bad breath [1]. Dental caries (cavities) is the most common disease in the oral cavity. The main bacteria causing dental caries (cavities) is *Streptococcus mutans*. *Streptococcus mutans* is a normal inhabitant of the oral cavity, *Streptococcus mutans* can turn into a pathogen if the bacterial environment is favorable and the population increases [2]. In addition to bacteria, there is *Candida albicans* in the oral cavity. Candida can cause mucosal candidiasis, disseminated candidiasis, and opportunistic infections [3]. Thrush, or oral thrush, is a common oral infection caused by an overgrowth of *Candida albicans* [4].

Generally, mouthwash contains antibacterial ingredients with the main component in the form of alcohol more than 20%, which can trigger oral cancer [5]. Mouthwash according to Pharmacopeia Indonesia III is a diluted solution to prevent or threat of throat infections. Mouthwash contains active formula ingredients derived from natural or synthetic ingredients [6].

One of the efforts to reduce the number of caries in the community requires an antibacterial that can kill *Streptococcus mutans*. One of the natural antibacterials that can be used as medicine is lime (*Citrus aurantiifolia* (Christm.) Swingle). Lime is an ingredient that is easily obtained and available throughout the year. Lime is a medicinal plant with many benefits and properties for preventing and treating diseases [7]. Lime fruit contains compounds of alkaloids, flavonoids, steroids, triterpenoids, saponins, tannins and phenolics that can inhibit bacterial growth. Besides that lime fruit contains elements of chemical compounds such as citric acid, fatty resins, glycosides, minerals, vitamin B1 and essential oils [8]. Lime is a plant that has antimicrobial activity that is

effective against gram-positive and gram-negative bacteria [9]. Lime peel (*Citrus aurantiifolia* (Christm.) Swingle) contains many volatile oil and flavonoid compounds. The most dominant volatile oil group compounds are hydrocarbon monoterpenes, namely limonen, α-pinen, β-pinen, γ-terpinen, β-mirsen and several sesquiterpene groups such as β-bisabolen. Meanwhile, the flavonoid compounds found in lime peel are quercetin, myristin, rutin, tangerin, narigin, and hesperidin [10]. According to Hasyim's research [11], lime peel contains high concentrations of flavonoid compounds than other parts that can be used as antibacterial and antiviral.

Based on Parama, *et al* research [12], it was found that 40% concentration of lime fruit extract was more effective than 10%, 20%, and 30% concentrations to reduce the growth of *Streptococcus mutans* in the saliva of children with early dental caries. This study was conducted to determine the inhibition of lime fruit extract on the growth of *Streptococcus mutans* in saliva samples of children with early caries.

According research from Sari, *et al* [13] states concentrations of 100%, 75%, 50%, and 25%obtained an average inhibitory zone respectively 26.69 mm, 24.76 mm, 23.37 mm, and 19.43 mm, while at concentration positive oxytarecin and negative control CMC-NA 0.1% respectively 32.59 mm and 00.00 mm. The results showed that the juice of lime skin has the potential as an antibacterial activity on E. coli growth in vitro. The 25% concentration has shown the inhibition zone in the Mueller Hinton media that has been inoculated with lime juice. The higher the concentration, the greater the inhibition zone formed.

2. RESEARCH METHOD (10 PT)

2.1. Materials

The materials were consisted of the ethanol extract of lime peel (*Citrus aurantiifolia* (Christm.) Swingle), 96% ethanol, sterile Aquadest, 0.2% Chlorhexidine gluconate, Sodium Benzoate, Sodium Sacharin, NaCl Solution, PDA Media (Potato Dextrose Agar), NA Media (Nutrient Agar), and MHA Media (Muller-Hinton Agar).

2.2. Equipment

The equipment was encompassed an autoclave, blender, incubator, gas stove, refrigerator, oven, water bath, rotary evaporator, PH meter, bunsen, glass plate, parchment paper, test tube, cotton, parchment paper, vortex, caliper, petri dish, ose needle, micropipette and analytical balance.

2.3. Methods

The research was conducted at the Microbiology Laboratory, Faculty of Pharmacy and Health Sciences, Sari Mutiara Indonesia University, Medan. The research carried out included: collection of plant materials, manufacture of extracts, which were carried out by the disc diffusion method on *Streptococcus mutans* (ATCC 25175) and *Candida albicans* (ATCC 10231) bacteria which measured their inhibition zone.

2.4. Making The Ethanol Extract of Lime Peel (*Citrus aurantiifolia* (Christm.)

A total of 500 gr crushed simplicial was put in a glass jar and soaked in 75 parts (3.75 L) of 96% ethanol solvent. It was left for 5 days protected from light while frequently stirring, filtering, squeezing, washing the dregs with a liquid filter. The 25 parts (1.25 L) of 100 parts, left for 2 days protected from light before filtered. Then, the extract was evaporated using a rotary evaporator at a temperature of 50◦C and concentrated over a water bath until a thick extract was obtained. The filter used is 96% ethanol as it tends to attract secondary metabolites from Simplicia such as alkaloids, phenols, flavonoids, saponins, tannins and terpenoids [14].

2.5. Making The Ethanol Extract of Lime Peel (*Citrus aurantiifolia* (Christm.) Concentration

Variations in the concentration of extract of lime peel (*Citrus aurantiifolia* (Christm.) were prepared by dissolving it with DMSO with concentrations of 25%, 30%, 35%, 40%, and 45%,. Then the inhibitory activity test of extract of lime peel (*Citrus aurantiifolia* (Christm.) was carried out against *Streptococcus mutans* and *Candida albicans*.

2.6. Testing the Activity of Lime (Citrus aurantiifolia (Christm.) Swingle) Peel Extract Against *Streptococcus mutans*

Sterilize all tools and materials to be used. Into a sterile petri dish, 0.1 inoculum of *Streptococcus mutans* was added, after which 15 ml of MHA was added. Furthermore, the petri dish was homogenized on a table

(Laminar Air Flow Cabinet) so that the media and bacterial suspension were evenly mixed and solidified. Make markings under a petri dish with each concentration of 25%, 30%, 35%, 40%, 45%, positive control (chloramphenicol) and negative control (DMSO 10%). Soak the disc paper into the lime peel extract with each concentration, leave for 2 minutes. Put the disc paper into the petri dish according to the concentration marking. Incubate for 18 to 24 hours at 37ºC. Observe the results by measuring the inhibition zone in the form of an area that is not overgrown with bacteria using a caliper. This test was carried out in triples (3 petri dishes at once) [15].

2.7. Testing the Activity of Lime (*Citrus aurantiifolia* (Christm.) Swingle) Peel Extract against the Fungi *Candida albicans*

Sterilize all tools and materials to be used. Into a sterile petri dish, 0.1 inoculum *Candida albicans* was added, after which 15 ml of PDA was added. Furthermore, the petri dish was homogenized on a table (Laminar Air Flow Cabinet) so that the media and bacterial suspension were evenly mixed and solidified. Make markings under a petri dish with each concentration of 25%, 30%, 35%, 40%, 45%, positive control (ketoconazole) and negative control (DMSO 10%). Soak the disc paper into the lime peel extract with each concentration, leave for 2 minutes. Put the disc paper into the petri dish according to the concentration marking. Incubate for 48 hours at 27ºC. Observe the results by measuring the inhibition zone in the form of an area that is not overgrown with fungus using a caliper. This test was carried out in triples (3 petri dishes) [15].

2.8. Making a Mouthwash Preparation Formula

Making mouthwash formulations with active ingredients of Extract of Lime Peel (*Citrus aurantiifolia* (Christm.) solution and substances added as corigensia, namely saccharin, menthol, nipagine and aquadest are used as solvents. The formulation of mouthwash was prepared with Extract of Lime Peel and additional substances were 0.2% saccharin as a sweetener, 0.2% menthol as a freshener, 0.1% nipagin as a preservative and a solvent, 100 ml aquadest ad.

2.9. Evaluation of Mouthwash Formulations

Organoleptic Examination

The physical and chemical characteristics evaluation of mouthwash preparation was observed to the shape, color, smell, and taste of Extract of Lime Peel.

Formula Evaluation

Physical evaluation was included to an examination of the preparation's stability and pH of the preparation. Biological evaluation was encompassed the determination of the antibacterial activity of Extract of Lime Peel mouthwash against *Streptococcus mutans* and *Candida albicans* by the disc diffusion method.

Stock Stability Check

Evaluation of formulation stability consisted of visually observed shape, color, odor and taste [15]. A mouthwash is declared stable if it does not change color, odor, taste or appearance during storage.

pH Determination of Preparation

pH measurements were performed using a pH meter as follows. Rinse with distilled water before drying with tissue paper. The electrodes were immersed in a mouthwash solution. The device was left to show a constant pH. The reading on the pH meter was the pH of the preparation.

Viscosity Measurement

The viscosity of the formulation was measured using an Oswald viscometer. Preparations were measured as much as 5 ml in which the viscometer was reinforced with static poles. The sample was poured into the Oswald tube before being drawn through the Kugelrohr to the limit mark. Allow the samples to flow upwards from the n to m marks and use a stopwatch to calculate the time.

Microbiological Test of Preparations

Antibacterial activity testing of a mouthwash made from lime peel extract (*Citrus aurantiifolia* (Christm) was performed by the dish diffusion method. Media were MHA for *Streptococcus mutans* and PDA for Candida albicans. 0.1 ml of *Streptococcus mutans* suspension was inoculated into MHA medium, homogenized before solidification, impregnated with various formulations (FI, FII) for preparing mouthwash, and applied to the surface of the solidified medium. Place in Petri dish before incubating at 37°C for 18-24 hours. His 0.1 ml suspension of *Candida albicans* was inoculated into PDA medium and then homogenized and allowed to solidify. The discs

were impregnated with various formulations (FI, FII) for the preparation of mouthwashes from water containing fragrant lemongrass leaf extract and placed in Petri dishes before incubation at 25 °C for 48 h. placed on the surface of the solidified medium.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Compound Identification

The determination of the phytochemical screening test was carried out to identify the compounds of lime peel extract (*Citrus aurantiifolia* (Christm). Based on the research, the lime peel extract contained alkaloids, flavonoids, saponins, tannins and triterpenes/steroids.

3.2. Results of Antimicrobial Testing of Lime Peel Extract Against *Streptococcus mutans* and *Candida albicans*

The results of measuring the diameter of the inhibition zone of the ethanol extract of lime peel against *Streptococcus mutans* can be seen in the clear area around the paper disk, the measurement results can be seen in table 1 below:

Table 1. Measurement Results of Growth Inhibition Area of *Streptococcus mutans*

Concentration (%)	Bacterial Growth Inhibition Zone Diameter (mm)			Zone of Inhibition and SD	Inhibition Response
	P1	P2	P3		
25	8,7	8,3	9	8,6 ± 0,35	Moderate
30	11,8	12,3	12	9,5 ± 0,35	Moderate
35	14,1	14,6	14,8	11 ± 0,47	Strong
40	15,2	15,3	15,9	14,07 ± 1,01	Strong
45	16	16,6	17,1	16,56 ± 0,55	Strong
Kloramfenikol	27,4			27,4 ± 0	Very strong
Aquadest	0			0 ± 0	Weak

The antibacterial effectiveness test (Table 1) was observed in 24 hours indicated that there was inhibition zones at concentrations of 25%, 30%, 35%, 40%, 45%. The smallest inhibition zone was at a concentration of 25% with an average inhibition zone diameter of 8.6 mm and the largest inhibition zone was a concentration of 45% with an average inhibition zone of 14.4 mm. This shows that the level of inhibition of the growth of *Streptococcus mutans* by lime peel increased from 25% to 45% concentration treatment. From the table the results of measuring the diameter of the inhibition zone for the growth of *Streptococcus mutans* by lime peel extract above show that at a concentration of 25%, the average inhibition zone is 8.6 mm. These results indicate that the minimum inhibitory concentration is in the medium category, the average concentration is 30%. The average inhibition zone of 9.5 mm was categorized as moderate, the average concentration of 35% of the inhibition zone was 11 mm which was categorized as strong, 40% concentration averaged 14.07 mm was categorized as strong, and the concentration of 45% average zone The inhibition of 14.4 mm is categorized as strong. Hal ini sesuai dengan penelitian yang dilakukan

The fungal effectiveness test showed the lime peel extract (*Citrus aurantiifolia* (Christm) inhibit the growth of *Candida albicans*. Similarly to antibacterial effect, the higher the extract concentration resulted the larger the diameter of the inhibition area.

Table 2. Measurement Results of Growth Inhibition Area of *Candida albicans*

Concentration (%)	Fungi Growth Inhibition Zone Diameter (mm)			Zone of Inhibition & SD	Inhibition Response
	P1	P2	P3		
25	10,3	9,6	10,1	9,5 ± 0,80	Moderate
30	10,4	13,2	10,4	10,8 ± 2,18	Strong
35	12	14,2	13,34	11,8 ± 2,51	Strong

40	13,7	14,4	15,9	14 ± 2,54	Strong
45	14,9	14,6	16,3	14,2 ± 2,69	Strong
Ketokonazole		25,6		25,6 ± 0	Very Strong
Aquadest		0		0 ± 0	Weak

The antifungal activity test (Table 2) which were observed in 48 hours showed The smallest inhibition zone is at a concentration of 25% with an average diameter of the inhibition zone of 9.5 mm. These results indicate that the minimum inhibitory concentration is in the medium category, the concentration of 30% with an average diameter of the inhibition zone of 10.8 mm is categorized as strong. 35% with an average inhibition zone diameter of 11.8 mm is categorized as strong, a concentration of 40% with an average inhibition zone diameter of 14 mm is categorized as strong, and a concentration of 45% with an average inhibition zone diameter of 14.2 mm is categorized as strong. From the data above, it can be seen that the ethanolic extract of lime peel provides an effective limit of inhibition area at a concentration of 45 mg/ml against *Streptococcus mutans* with a diameter of 14.1 mm, while *Candida albicans* does not provide an effective limit of the zone of inhibition. The boundary of the inhibition area was considered effective if it had a diameter of inhibition of approximately 14 mm to 16 mm Ditjen POM [15].

The higher the extract concentration resulting in a larger area diameter resistor. This research is the same as the research conducted by Sapitri, *et al* [16] The results showed that fragrant lemongrass leaf extract provided an effective inhibition zone for *Streptococcus mutans* at concentrations of 30% (14.3 mm) and 40% (15.46 mm). In *Candida albicans* with a concentration of 30% (14.28 mm) and 40% (15.46 mm). The mouthwash preparation of fragrant lemongrass leaf extract at 30% F1 concentration and 40% FII concentration gave satisfactory inhibition zone diameter results which was greater than 14 mm.

3.3. Mouthwash Formulations

According to Indonesian Pharmacopoeia IV edition, antibiotic potential determination by microbiology revealed a significant effective limit of inhibition of about 14 mm to 16 mm. Based on the above results, the production of mouthwash from ethanol extract lime peel was 40% and 45%. Preparation of mouthwash administration formulations according to Yuliana, *et al* [17] in Table 3 below:

Table 3. Formulation of Mouthwash

Material	Formula I	Formula II
Citrus aurantiifolia (Christm)	40%	45%
Saccharin (g)	0,2%	0,2%
Peppermint oil	0,2%	0,2%
Nipagin	0,1 %	0,1 %
Aquadest ad (ml)	100 ml	100 ml

The mouthwash formulation with the active ingredient of ethanol lime peel extract was added to 0.2% saccharin as a sweetener, 0.2% Peppermint oil as a refresher, 0.1%. Nipagin as a preservative and 100 ml of aquadest ad as a solvent.

3.4. Evaluation Result of Mouthwash Formulations

Stock Stability Check

Sensory testing of lime peel extract (*Citrus aurantiifolia* (Christm) mouthwash revealed changes in shape, color, odor and taste, indicating that all formulations prepared were stable with respect to change. it was done. Liquid state stable - characteristic lime odor (*Citrus aurantiifolia* (Christm), preparation taste sweet. Color observations showed that the mouthwash formulation of Formulation I (FI) remained stable with a light brown color and Formulation II (FII). Increasing concentrations of lime extract (*Citrus aurantiifolia* (Christm) increased the color of the resulting mouthwashes. All formulations were sweet in taste and stable during storage. The scent produced by all mouthwash formulations was the distinctive scent of lime and flavored peppermint oil. Sensory tests were available in Table 4.

Table 4. Organoleptic Test of Mouthwash Preparation of Lime Peel Extract

Formulation	**Observation**			
	Shape	**Color**	**Smell**	**Taste**
FI	Liquid	Light brown	Typical aroma of Lime	Sweet
FII	Liquid	Dark brown	Typical aroma of Lime	Sweet

Information:
FI : Formulation I (40 %)

FII : Formulation II (45%)

Results of Determination of Preparation pH

The results of the pH examination showed that the preparations made from ethanol extract of lime peel at a concentration of 40% and a concentration of 45% were not much different, having a pH ranging from 5.0-5.2. The pH test results for mouthwash preparations were classified as acidic because the pH was below 7. Common mouthwashes have a pH ranging from 5-6. If the pH is below 5, the preparation is too acidic, it will cause more bacterial growth and if it is above 6 the preparation is too alkaline and will cause fungal growth, resulting in canker sores [18]. From the results of the pH examination above, it shows that the mouthwash preparations made have met the pH requirements of the preparation. The resulting pH indicates that all formulation mouthwash formulations are within the commercial standard pH range as seen from the herbal mouthwash quality standard, pH 5-7 [19].

Preparations Viscosity

The results of the measurement of the viscosity of the mouthwash showed the unit of the viscosity of the dispersing medium of a solution, the viscosity measurement of the two formulas showed that the mouthwash preparation had an FI viscosity of 0.63 cp and an FII of 0.67 cp close to 0.89 cp water [20].

Microbiological Test Preparations

Microbiological tests for mouthwash preparations were carried out on two formulas: formula I and formula II with the agar diffusion method against *Streptococcus mutans* and *Candida albicans*. The results is in table 5 below:

Table 5. The Results of Lime Peel Extract Mouthwash Activity

Mouthwash	**Inhibition Zone Diameter (mm)**							
	Streptococcus mutans				***Candida albicans***			
	P1	**P2**	**P3**	**Average & SD**	**P1**	**P2**	**P3**	**Average & SD**
Formulation I	15,2	15,5	15,9	15,5 ± 0,35	14,9	13,7	14,5	14,37 ± 0,85
Formulation II	17,3	16,9	17,8	15,6 ± 0,45	15,4	14,8	15,8	15,33 ± 0,42
Control (+)		12,5		12,5 ± 0		18,4		18,4 ± 0

The table of antibacterial activity test results for mouthwash ethanol extract of lime peel against *Streptococcus mutans*, that formula I (FI) mouthwash preparation with a concentration of 40% had an average inhibition zone diameter of 15.5 mm and against *Candida albicans* 14, 37mm. Formula II (FII) mouthwash preparation with a concentration of 45% against *Streptococcus mutans* had an average inhibition zone diameter of 15.6 mm and against *Candida albicans* 15,33 mm. *Streptococcus mutans* have a diameter of the inhibition zone greater than *Candida albicans*. This is because the chemical content in the ethanol extract of lime peel is more sensitive in inhibiting the growth of *Streptococcus mutans* compared to *Candida albicans*. Ladytama, *et al* [21] Results showed that lime extract was effective in reducing plaque index at many concentrations.

Streptococcus mutans have a diameter of the inhibition zone greater than *Candida albicans*. This is because the chemical content in the ethanol extract of lime peel is more sensitive in inhibiting the growth of *Streptococcus mutans* than *Candida albicans*, so that *Candida albicans* has an inhibitory diameter. the smaller one. While the positive control (Total Care mouthwash) provided a zone of inhibition that was not too far away, namely for *Streptococcus mutans* 14.37 mm and for *Candida albicans* 18.4 mm. *Streptococcus mutans* have diameter of the inhibition zone is smaller than the inhibition zone for the *Candida albicans* fungus. This is because the chemical content contained in the positive control (Total Care mouthwash) is more sensitive in inhibiting the growth of *Candida albicans* than *Streptococcus mutans*, so that the *Candida albicans* has a larger inhibitory diameter. This research is in accordance with evaluation of mouthwash includes examination of organoleptic, pH, stability, and viscosity. The evaluation results showed that the physical properties of the mouthwash preparation gave good results and met the requirements for the mouthwash preparation [22]. Since mouthwashes are believed to be effective in preventing microbial plaque formation, natural ingredients are required to formulate mouthwashes with antimicrobial properties [23]. Jannata., *et al* [24] explained that *Streptococcus mutans* has a relatively simple cell envelope structure and a thick peptidoglycan layer makes Gram-positive bacteria more sensitive to antibiotics. *Streptococcus mutans* most often found in the oral cavity that have an important role in dental caries disease [25].

4. CONCLUSION

Based on the results of this study, ethanol extract of lime peel has potential as an antimicrobial agent in mouthwash formulations. The mouthwash formulations tested produced potent inhibition against *Streptococcus*

mutans with Formulation I (40%) having an inhibition zone of 15.5 mm and Formulation II (45%) having an inhibition zone of 14.37 mm. Similarly, its efficacy against *Candida albicans* was similar in vitro to Formulation I (40%) with a zone of inhibition of 12.32 mm and Formulation II (45%) with a zone of inhibition of 15.33 mm.

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