Mouthwash Formulation and Antimicrobial Activity of Aqueous Infusion of Tanjung Fruit Flesh (Mimusops Elengi L.)

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Abstract

One way that is considered effective in maintaining oral hygiene apart from brushing your teeth is to use mouthwash. Mouthwash is a liquid preparation most often used for refreshing or antiseptic effect and to control plaque, has a temporary antibacterial effect. Tanjung fruit flesh (*Mimusops elengi* L.) contains flavonoid compounds, tannins, and saponins that act as antibacterial, and it has a potential efficacy in inhibit the growth of some microorganisms including oral microorganims. The purpose of this study was to formulate the mouthwash solution from Tanjung fruit with a good physical quality test, then evaluate its antimicrobial activity. The type of research used is Laboratory Experimental research with a post-test only control group design. The formula with 30% concentration of fruit infusion treatment showerd greater activity as antimicrobial agent, with the inhibition zone of >18mm. The inhibition zone showed the ability to inhibit *Candida albicans* with the interpretation of the sensitive inhibition zone measurement (p=0.000). Additionally the parameters that observed in the physical evaluation of mouthwash preparation include changes in color, aroma and taste showed that each formula had different physical characteristics. The most efficient concentration of tanjung fruit pulp mouthwash that can inhibit *Candida albicans* is at a concentration of 30% with an inhibition zone of > 18 mm.

Keywords: Fruit flesh, *Mimusops elengi* L mouthwash, *Candida albican*

1. Introduction

Dental and oral health is important for a person's general health because a healthy mouth allows a person to eat, talk, and socialize, without experiencing pain and discomfort [1]. Oral health is a reflection of the overall health of the body. Dental and oral health is important for general health and well-being and greatly affects the quality of life, including speech, mastication, and self-confidence. Dental and oral health problems will have an impact on a person's performance. In Indonesia, dental and oral diseases, especially caries and periodontal disease, are still suffered by many, both children and adults [2]. Most dental and oral health problems can actually be prevented. There are many ways to reduce and prevent dental and oral diseases with various approaches that include community-started prevention, self-care and professional care [3]. The use of natural ingredient has its own advantages compared to chemicals because natural materials are more acceptable to humans and are widely available in nature [4]. The reason why natural product is so beneficial, it is because of the phytochemicals contain [5]. The fruit of *Mimusops elengi* L. contains a number of biologically active compounds with beneficial effects on human health. It is contains polyphenolic phytochemicals such as resveratrol, tannins, flavonoids (cathecin), roanthocyanidins and fatty acids (oleanolic acid, oleanolic aldehyde) which function as glucosylation inhibitors in the plaque formation process. Moreover *Mimusops elengi* L also can be used as an alternative antibacterial in caries prevention [6].

Research using fruit extract *Mimusops elengi* L. has been done previously, fruit (*Mimusops elengi* L has been proven to be effective in inhibiting the growth of oral microflora because it contains tannins, saponins, alkaloids, flavonoids and steroids [7]. One way to get rid of bad breath is to use mouthwash. Mouthwash is a solution that is used as a cleanser to improve oral health, aesthetics, and breath freshness. Mouthwash can be used to kill bacteria, remove bad odors and prevent dental caries. Another effectiveness of mouthwash is its ability to reach the most difficult places to clean with a toothbrush and can damage

plaque formation, its use can be as a substitute for a toothbrush [8]. So that in the study, a mouthwash formulation of fruit flesh *Mimusops elengi* L. was carried out including evaluation of physical quality test and a test for the inhibition of *Candida albicans*.

2. Materials and methods

Materials

The tools used in this study were autoclave (GEA®), climatic chamber (MMM®), homogenazer (WiseStir®) freeze dryer, aluminum voil, stir bar, petri dish, funnel, measuring cup (IWAKI), incubator, ose, pH meter, LAF (Laminar Air Flow), bunsen, micropipette (Eppendrof®), oven (Falc®), tweezers, rotary evaporator (THOMAS®), analytical balance (Mettler Toledo®), pipette scale, horn spoon, syringe,, tube reaction (Pyrex), refrigerator, and digital caliper. The materials used in this study were fruit flesh of *Mimusops elengi*. L, distilled water, glycerin, menthol, 70% alcohol and other materials for testing.

Plant collection and Preparation

Samples were taken from cape plants that are fruiting and ripe in Medan City. The dried sample were obtained by drying process using drying cabinet, the dried samples is ground using grinder [9].

Preparation of Infusion by Decoction Method

A 1000 grams of powder dissolved in 1000 ml of water heated over a water bath for 30 minutes starting from the temperature reaching 90^{o}C while stirring occasionally. Sprinkle while hot through or by using a flannel, and add enough hot water through the dregs to obtain the volume of the infusion [10].

Formulation of Mouthwash

The design of mouthwash formula is showed in the table 1.

Table 1 Formulation of Mouthwash

Composition	Amount (% b/v)			
	F1 (10 %)	F2 (30 %)	F3 (50 %)	F4 (70 %)
infusion solution	10	10	10	10
glycerin	10	10	10	10
Menthol	1.5	1.5	1.5	1.5
Ethanol 70%	10	10	10	10
Distilled water	Add to 100 ml	Add to 100 ml	Add to 100 ml	Add to 100 ml

The formulation that has been made, then measures the characteristics of the preparation including organoleptic test, pH test, density test, viscosity test and refractive Index test using standard procedure [11], and also antimicrobial testing against the fungus *Candida albican*.

Antimicrobial test procedure

Antimicrobial tests were carried out against the fungus Candida albicans in various concentrations in the previously prepared test group. The antimicrobial test was carried out by disc diffusion method with the following test groups: K1: 10% mounthwash solution, K2: 30% mounthwash solution, K3: mounthwash solution, K4: 50% mounthwash solution k4: 70% mounthwash solution, K5 is positive namely Nystatin and K6: negative control without sample, In Laminar Air Flow Work Station, the results of incubation of mushrooms for 24 hours in liquid medium are taken 0.1 ml using a volume pipette and dripped into a petri dish containing SDA that has been frozen and leveled using a spreader. Then let it sit for about 15 minutes, then it is divided into five parts and each part is given a paper disk [12]. Each paper disk on the inoculated media was dripped with 10 µl of the test solution. The media that had been dripped with infusion and control were incubated for 24 hours at 37°C. The results are read with the area of inhibition [13]. Then measured using a caliper.

3. Results and Discussion

Physical Evaluation of Mouthwash Preparations

Organoleptic examination, refractive index test, viscosity, and pH can be seen in tables 1 and 2. Organoptic testing is done by looking at the color, smell, and taste.

Table 2 Result of Organoleptic Evaluation

No	Group	Color	Smell	Taste
1	K1	white	Menthol	tasteless
2	K2	brown	Menthol	bitter
3	K3	white	Menthol	bitter
4	K4	Beige	odorless	bitter
5	K5	Transparant	Menthol	tasteless
6	K6	Transparant	Menthol	Less bitter

Table 3 Result of Physical Evaluation

No	Group	Refractive index (n)	Density	Viscosity (π)	pH
		MEAN ± SD			
1	K1	1,634± 0,004	1,082± 0,001	0,916± 0,01	4,23±0,15
2	K2	1,64±0,001	1,092±0,002	1,003±0,001	5,36±0,15
3	K3	1,744±0,168	1,103±0,001	1,014±0,002	6,16±0,2
4	K4	1,670±0,01	1,109±0,005	1,018±0,001	7,1±0,1
5	K5	1,6393	1,079	0,824	5,3
6	K6	1,6421	1,093	0,832	7,3

The parameters observed in the physical evaluation of mouthwash preparation include changes in color, aroma and taste. The results of the organoleptic test showed that each formula had different organoleptic characteristics. Meanwhile, the determination of pH showed that the preparations made from infusion fruit flesh of *Mimusops elengi* L in test groups K2, K3 and K4 met the required pH range. The pH value of the mouthwash produced must be in the pH range of the oral cavity which ranges from 5.5 to 7.9 [14], so that when the preparation is consumed it does not cause irritation to the oral mucosa. Viscosity is an index of resistance to flow of liquids, viscosity can be measured by measuring the flow rate through a cylindrical tube. Viscosity is also known as the viscosity of a substance. The more viscous a liquid substance, the greater the force required to make it flow at a certain viscosity [15]. Measurement of the viscosity value of the citronella leaf infusion mouthwash was carried out using an Oswaltd viscometer [16]. The results of the measurement of the viscosity of the mouthwash showed the unit of viscosity of the dispersing medium of a solution, the measurement of the viscosity of the four formulas showed that the mouthwash preparations had various viscosities. The difference in the viscosity value obtained is due to the greater the specific gravity value of a liquid, the higher the viscosity.

Results of the inhibition test of Candida Albicans

The results of testing the antifungal potential of the mouthwash formulation of *Mimusops elengi* can be seen in table 3.

Table 4 Antimicrobial test results

Group	Inhibition (mm)
	Mean ± SD
K1	11,66± 1,52

K2	20,66± 3,21*
K3	12,33± 1,52
K4	12,66± 1,52
K5	20
K6	0

Data expressed as mean ± SD (n = 3), *significant difference versus all group at P <0.01

Table 3 shows that various concentration of fruit flesh *Mimusops elengi* L. can be made to mouthwash solution which has the ability to inhibit the growth of *Candida albicans*, the obtained inhibition zone at a concentration of 30% has highest inhibition zone by more than >18mm. Additionally it can be seen that the inhibition zone was formed on the pulp formula of *Mimusops elengi* L. infusion and positive control nystatitne drop had similar efficacy. The activity of this product is affected by pythochemical substances containing in *Mimusops elengi* L. The fruit part of *Mimusops elengi* L is known to contain triterpenoid compounds (ursolic acid, -sitosterol, -spinasterol) and flavonoids (quercitol, dihydro quercetin, quercetin) [17]. Based on the structure, all of these compounds contain hydroxyl groups that can act as antioxidants, but only compounds from the flavonoid group in *Mimusops elengi* L have phenolic groups [18]. Meanwhile, the antifungal mechanism of *Mimusops elengi* L. is by inhibiting lipid synthesis, especially ergosterol which will eventually lead to death of fungal cells. Furthermore the concentration of the formula with fruit (Mimusops elengi L.) at concentrations of 10%, 50%, and 70%, respectively, was resistant with an inhibition zone of <12mm. However, at a concentration of 30% the resulting inhibition zone was sensitive with an inhibition zone >18mm and close to a positive control in the form of nystatin drop.

Various phytochemical compound has been found in *Mimusops elengi* L including essential oil. Essential oils can interfere with the formation of fungal cell membranes. and fungal cell walls, so that the fungal cell membranes and walls are not completely formed. Flavonoids are also antioxidants [19]. Moreover, flavonoids also can inhibit nucleic acid synthesis, inhibit cytoplasmic membrane function, and inhibit cell energy metabolism. Flavonoids are compounds of the phenol group. Phenol can inhibit fungal activity by inhibiting the process of forming fungal cell walls or by lysing cell walls that have already been formed [20]. This can result in permanent damage and death of fungal cells. The activity of tannins in is able to cause shrinkage of the fungal cell wall, so that as a result the activity of cell life is disrupted, its growth is inhibited, even at certain doses it can cause fungal death. One of the phenol derivatives, eugenol, interacts with bacterial cells through an absorption process involving hydrogen bonds. At low levels, phenol protein complexes are formed with weak bonds and are immediately decomposed, followed by penetration into cells and causing precipitation and protein denaturation [21].

4. Conclusions

The mouthwash formulation from *Mimusops elengi* L has the potential effect as an herbal mouthwash. The most efficient concentration of mouthwash (Mimusops elengi L) in inhibiting Candida Albicans is at a concentration of 30% with an inhibition zone of > 18 mm.

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