



## ***Averrhoa bilimbi* (BELIMBING WULUH) LEAVES EXTRACT EVALUATION AS ANTI-CARIOGENIC MOUTHWASH**

**Oktavia Dwi Nugraheni<sup>a</sup> ; Ayu Nala El Muna Haerussana<sup>b\*</sup>**

<sup>a</sup> Department of Pharmacy ; Poltekkes Kemenkes Bandung ; Prof. Eyckman Street Number 24 ; Bandung City 40161 ; Indonesia

<sup>b</sup> Center of Excellence on Utilization of Local Material for Health Improvement ; Poltekkes Kemenkes Bandung ; Padjajaran Street Number 56 ; Bandung City 40171 ; Indonesia

### **Abstract**

The practice of using mouthwash has become increasingly common as people have become more aware of the importance of oral hygiene in preventing dental issues. In order to mitigate any potential risks associated with alcohol-based mouthwashes, researchers are exploring natural sources of anti-cariogenic compounds. This study aimed to assess the effectivity of *Averrhoa bilimbi* leaf extract as an ingredient in mouthwash formulations, as the use of *Averrhoa bilimbi* leaves is still limited. The evaluation of the mouthwash included a thorough examination of its antibacterial activity, viscosity, density, pH, and organoleptic qualities. The results indicated that formula with 30 grams extract (F3) was the most effective, exhibiting more robust antibacterial properties, appropriate viscosity and density, and a pH level similar to that of a solution. F3 resulted in an effectiveness of 41.67%, 1.36 cPs for viscosity, 1.0785 mg/l for density, pH of 5.25±0.006, organoleptically liquid, mint odor, and blackish brown clear color. Based on these findings, it appears that *Averrhoa bilimbi* leaf extract could be a promising ingredient in mouthwash formulations.

**Keywords:** Antibacterial; *Averrhoa bilimbi* leaves extract; Mouthwash; Evaluation

### **1. Introduction**

Increased public awareness about the importance of oral health has also led to an increase in mouthwash use in Indonesia (Nurmalasari et al., 2018). Public knowledge, supported by literacy accessibility, promotes oral health awareness (Tadin et al., 2022). Mouthwash can be used preventively and therapeutically (Radzki et al., 2022). Mouthwash is used to maintain oral hygiene, eliminate bad breath, and reduce inflammation, infection, and plaque caused by microorganisms (Rajendiran et al., 2021; Renuka & Muralidharan, 2017). Mouthwash with alcohol content has several drawbacks, including dry mouth, especially in oral mucosa, a burning sensation, and the risk of mucosal pain (Werner & Seymour, 2009). The use of alcohol in mouthwash was intended as a solubilizer, stabilizer, and vehicle for delivering an active compound (Ustrell-Borràs et al., 2020).

Antibacterial acts as an active compound by inhibiting the growth of cariogenic bacteria (Ustrell-Borràs et al., 2020). Plaque development by cariogenic bacteria causes dental caries. *Streptococcus mutans*, an opportunistic pathogenic microbe, initiates plaque formation on teeth. Its growth must be inhibited so that the number of organisms is insufficient for pathogenicity (Utamaningyas et al., 2022). Inhibiting bacterial development prevents plaque, cavities, and pain caused by cavities in the teeth. Previous research has shown that medicinal herbs may inhibit the growth of these bacteria (Ravi et al., 2017). The formulation of extracts in mouthwash can impair their inhibitory effectiveness, so it is required to examine the inhibition of extracts that have been created in mouthwash. Although the production of mouthwash reduces its activity, mouthwash is considered effective due to its capacity to reach areas that are difficult

\*) Corresponding Author (Ayu Nala El Muna Haerussana)  
E-mail: ayunalael\_farmasi@staff.poltekkesbandung.ac.id

to clean with a toothbrush and can compete with the growth of plaque on teeth (Basir et al., 2023; Macfarlane et al., 2010; Yousefimanesh et al., 2015).

*Averrhoa bilimbi* (Indonesian synonym = belimbing wuluh) is a native Indonesian plant that thrives in tropical climates (Ismail et al., 2019; Rozi et al., 2022). The fruit of this plant is used as a sour spice by the Javanese and has been empirically used to be anti-acne and anti-sarial by the Indonesianese (Rahimulinsan et al., 2018; Widhianto et al., 2017). The leaves are less extensively used; therefore, articles on its activity against *Streptococcus mutans* bacteria still need to be made available. Saputera et al. (2018) reported that *Averrhoa bilimbi* leaf extract strongly inhibited *Streptococcus mutans*. *Averrhoa bilimbi* leaves contain carambolaflavone (a glycoside flavonoid) (Auw et al., 2015), also called apigenin (Lakmal et al., 2021);  $\beta$ -amyirin and  $\beta$ -sitosterol (triterpene) (Auw et al., 2015) which have antibacterial properties against Gram-positive bacteria (Abdel-Raouf et al., 2015; Lakmal et al., 2021; Wang et al., 2018). The mouthwash's effectiveness was determined to be equal to the sample's effectiveness against *Streptococcus mutans*, so the inhibitory ability has the potential to make mouthwash (Sharma et al., 2018). This investigation was carried out to assess the inhibitory ability, pH, viscosity, and organoleptic properties of *Averrhoa bilimbi* extract mouthwash formulation.

## 2. Method

### *Plants material*

The plant samples were obtained in West Java Province, Indonesia, and identified as *Averrhoa bilimbi* L. (synonym: *Averrhoa abtusangulata* Stokes) of the Oxalidaceae family at Padjajaran University on plant identification number 02/HB/01/2022. Wet-sorted *Averrhoa bilimbi* leaves were cleaned, air-dried for two weeks, then dry-sorted. A dried sample (with a 10% loss on drying) was powdered for extraction.

### *Extraction and phytochemical screening*

Grounded dry leaves were steeped for 24 hours in 96% ethanol and re-macerated twice. A rotary vacuum evaporator (Buchi at 40°C and 90 rpm) and a water bath (40°C) were utilized for ethanol removal. The crude extract was stored at 4°C until it was examined. The color and precipitate tests were used to qualitatively assess phytochemical screening for flavonoid, tannin, saponin, terpenoid, and alkaloid. Flavonoid utilized the Wilstater test to see if flavylium dark red salt formed. Tannin and FeCl<sub>3</sub> combine to create a blue-black complex, whereas saponin forms a stable foam when shaken vertically. Terpenoid oxidation results in a brownish-red complex in the Lieberman-Burchard test. Alkaloid precipitate test using Mayer, Wagner, and Dragendorff method. In the Mayer test, alkaloid forms a white precipitate from the potassium-alkaloid complex; in the Wagner test, a brown complex form; and in the Dragendorff test, nitrogen in alkaloid forms covalent bonds with potassium.

### *Mouthwash preparation*

The mouthwash was created using the formula in Table 1. *Averrhoa bilimbi* leaf extract (ABLE) was pulverized in a mortar with glycerin until homogenous. Sorbitol and sodium benzoate were then added and mashed until homogenous. Then, 100 ml of distilled water was added, filtered, and placed in a bottle. Peppermint oil was added in 3-4 drops, and the mouthwash formulation was tested for stability using organoleptic, pH, specific gravity, and viscosity tests.

**Table 1.** Mouthwash formula

Materials	Mouthwash Formula*			
	F1	F2	F3	F0
Extract	20 grams	25 grams	30 grams	-
Glycerin	4 grams	4 grams	4 grams	4 grams
Sorbitol	9 grams	9 grams	9 grams	9 grams
Sodium benzoate	0.5 grams	0.5 grams	0.5 grams	0.5 grams
Peppermint oil	0.2 grams	0.2 grams	0.2 grams	0.2 grams
Aquadest	ad 100 ml	ad 100 ml	ad 100 ml	ad 100 ml

\*F1-3: Mouthwash formula with extract variations; F0: without extract

### *Mouthwash evaluation*

#### *Organoleptic*

The mouthwash preparation was visually observed using the five senses: color, aroma, and texture.

#### *pH*

The pH of the ABLE mouthwash preparation was tested three times by dipping the pH meter electrode into the mouthwash.

#### *Density/ specific gravity*

The density was estimated using a weighed empty pycnometer. The pycnometer was filled with distilled water until full and closed, then dried with tissue before being weighed. Aquadest in the pycnometer was then discarded and dried inside and out before being filled with ABLE mouthwash mixture and weighed. The test was repeated three times, and the specific gravity was determined by weighing the mouthwash and comparing it to aquadest.

#### *Viscosity*

The mouthwash's viscosity was measured with an Ostwald viscometer. The tube was filled with a specific amount of sample (temperature set to  $20.0^{\circ}\text{C}\pm 0.1^{\circ}\text{C}$ ). The meniscus of the liquid in the capillary tube was raised to the top graduation line using pressure or suction. Allow the liquid to flow freely into the container by opening the feeding tube and the capillary tube against air pressure. The time it took the liquid in the capillary tube to flow from the upper limit to the lower limit was recorded.

#### *Antibacterial activity*

Tests were performed under aseptic conditions. Petri dishes were sterilized in an oven at  $170^{\circ}\text{C}$  for 1 hour. NA medium for reculture and MHA for testing were sterilized for 15 minutes in a  $121^{\circ}\text{C}$  autoclave. *Streptococcus mutans* ATCC 31987 (*S. mutans*) bacteria from reculture were suspended in physiological NaCl and measured for equivalence with McFarland 0.5 (density of  $1.5\times 10^8$  CFU/ml). The well diffusion method was used to experiment. A petri dish was packed with sterile MHA media and then inoculated with bacterial suspension. The swabs were applied to the agar surface three times. Wells were made with a 6 mm cork-borer. Each well received up to 50  $\mu\text{l}$  of prepared mouthwash, negative control, and positive control for three replications. Testing was done three times. The diameter of the inhibition zone (clear zone minus the diameter of the well) was measured using a caliper, and the efficiency against chlorhexidine was determined in percent.

#### *Statistical analysis*

The data was statistically examined using SPSS, and the normalcy test was performed using Shapiro-Wilk test data. Homogeneity test with one-way ANOVA to detect the difference in inhibition zone formation from mouthwash.

### **3. Result and Discussion**

Before extraction, the simplisia is sorted, washed, and dried to ensure the best possible condition (Sulasmi et al., 2016). During the extraction procedure, the solvent will penetrate the cell wall of Averrhoa bilimbi leaves and draw the active compounds out. Stirring every few hours is required to balance the concentration of active chemicals in the liquid faster and dissolve the active substances in the simplicia so that they do not precipitate. The concentrated extract yielded 127.48 grams from 500 grams of simplicia.

The extract contains flavonoids, tannins, saponins, terpenoids, and alkaloids, according to the test results in this study. Averrhoa bilimbi leaves contain the main compound squalene, a flavonoid (Gunawan et al., 2013) isolated from methanol extract,  $\beta$ -amyirin, a triterpene isolated from 70% ethanol extract (Leliqia & Safitri, 2021), while the main compounds of the leaves extracted using 96% ethanol solvent are unpublished.  $\beta$ -amyirin, on the other hand, dissolves well in 96% ethanol (Ferraz-Filha et al., 2016). Due to the similar solubility properties, it is conceivable that its compound is close to that in the 70% ethanol extract.

ABLE formulations were evaluated in weights of 20, 25, and 30 grams. Because the extract is prepared in water, glycerin is required to enhance its solubility in water. Sorbitol serves as a sweetener, and sodium

benzoate acts as a preservative. Sodium benzoate is a way to stabilize the pH of this acidic preparation. Peppermint oil provides a distinct aroma and taste that masks the mouthwash's unpleasant taste and odor (Radzki et al., 2022). So, the finished product tastes cooler and smells fresh and slightly spicy. The mouthwash's color intensifies as the ABLE concentration rises, but this has no effect on the mouthwash's form or odor (Table 2). The pH of the mouthwash is within an acceptable range; a pH greater than seven may promote thrush-causing fungus growth, while a pH less than five may irritate (Rahman & Ariastuti, 2021). Due to the compound of the ABLE, the pH of this mouthwash formulation tends to be near 5 (acidic).

**Table 2.** *Averrhoa bilimbi* leaves extract mouthwash evaluation

Sample	Color	Odor	Texture	pH	Density (mg/l)	Viscosity (cPs)
F0 (negative control)	Clear	Mint	Liquid	4.090±0.006	1.0310	1.07
F1	Brown clear	Mint	Liquid	5.250±0.006	1.0577	2.26
F2	Dark brown clear	Mint	Liquid	5.310±0.006	1.0687	1.39
F3	Blackiesh brown clear	Mint	Liquid	5.250±0.006	1.0785	1.36
Chlorhexidine (positive control)	Clear	Mint	Liquid	6.200±0.002	0.9985	0.97

Mouthwash should be liquid, like a solution, and applied by gargling. The closer the density and viscosity are to water, the more favorable it is (Ardini & Mulatasih, 2020). The density of the samples revealed that all formulations had a density similar to water. Table 2 shows that the density of mouthwash is less than or close to the density of water. The sample's density rises as the quantity of extract added to the formula increases. The preparation's viscosity can be stated in terms of viscosity, which is assessed by calculating the time it takes the liquid to flow from the upper to the lower limit point on the Ostwald viscometer. As shown in Table 2, the viscosity of F3 and F2 is closer to water than that of F1. F1 had the greatest viscosity, and F3, with 30 grams of extract, had the least viscosity. The viscosity of the mouthwash is qualified if it is around water viscosity, which is close to 1 cPs (Yelfi et al., 2022).

**Table 3.** Antibacterial activity of *Averrhoa bilimbi* leaves extract mouthwash

Sample	Inhibition zone (mm)										Average	Efectivity (%)
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10		
F0	0	0	0	0	0	0	0	0	0	0	0.00±0.00	-
F1	5.24	5.99	4.30	4.43	4.73	5.67	4.97	5.67	5.63	4.77	5.14±0.58 <sup>ab</sup>	35.52
F2	6.67	6.23	6.70	6.24	6.13	5.10	5.19	5.50	5.77	4.67	5.82±0.69 <sup>ab</sup>	40.22
F3	7.15	7.63	5.32	6.17	5.27	6.63	6.07	5.17	5.30	5.57	6.03±0.87 <sup>ab</sup>	41.67
Chlorhexidine	14.7	14.68	14.50	14.37	14.00	14.48	14.25	14.47	14.50	14.79	14.47±0.23 <sup>ab</sup>	-

<sup>a</sup>significantly different at p<0.05 compared to control (DMSO)

<sup>b</sup>significantly different at p<0.05 compared to standard mouthwash (chlorhexidine)

Table 3 demonstrates that increased extract concentrations can boost the antibacterial properties of mouthwash (Chismirina et al., 2020). According to research conducted by Imran et al. (2022) on mouthwashes containing active ingredients derived from a mixture of *Averrhoa bilimbi* fruit juice and rosella, mouthwashes can inhibit bacterial growth and plaque formation on the teeth. The results of this study outperform the inhibition zone in mouthwash formulations derived from *Amaranthus hybridus* leaves conducted by Yelfi et al. (2022) but are inferior to *Elephantopus scaber* leaves conducted by (Ifmaily & Fitriani, 2020); research on mouthwash-derived from *Averrhoa bilimbi* leaves using the same testing method has not yet been located. The mouthwash preparation has a smaller inhibition zone than the *Averrhoa bilimbi* leaf extract that Saputera et al. (2018) tested. In the study by Saputera et al. (2018) a 25% concentration of the extract was categorized as moderate, whereas in the formulation (F2) with the same concentration, inhibition decreased to a weak category (Ponce et al., 2003). Because the extract in the mouthwash dosage form is attenuated to a maximum of 100 ml in the preparation, the inhibitory capacity is reduced in comparison to that of the extract form. However, this is not a limitation. Increasing the quantity of extract in the product, altering the extraction method, and substituting a different solvent can enhance its antibacterial properties. It's crucial to understand that the extraction method and solvent are key factors. The extraction method influences the compound's stability and the interaction of dissolved compounds in the solvent, allowing for variations in the types and concentrations of extracted compounds. The secondary metabolite compounds are attracted to differ according to their solubility properties when the solvent is altered. These differences in the types and concentrations of active compounds in extracts

can result in variations in their inhibitory actions against microorganisms. Therefore, the influence of the extraction method and solvent cannot be overstated (Zhang et al., 2018; Yuvanatemiya et al., 2022).

#### 4. Conclusion and Suggestion

The antibacterial properties of mouthwash are present in all formulations, with F3 exhibiting the most effective antimicrobial properties. The most effective anti-cariogenic formula, obtained in F3 and containing 30 grams of extract, has an effectiveness of 41.67% and an inhibition zone of  $6.03 \pm 0.87$  mm. Furthermore, F3 is closer to water pH, density, specific gravity, and viscosity than the other two formulations. On the basis of combining or utilising various extraction methods and solvents, it is necessary to conduct research in an effort to enhance its inhibitory capacity.

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