



Preparation of Mouthwash by Allium CEPA

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ABSTRACT

The increasing prevalence of oral infections and resistance to conventional antimicrobial agents has led to a growing interest in natural alternatives for oral hygiene. This study focuses on the formulation and evaluation of a herbal mouthwash derived from *Allium cepa* Linn (onion), a plant known for its potent antibacterial properties. The extract was prepared using standard extraction techniques and incorporated into a mouthwash formulation. Phytochemical screening revealed the presence of active compounds such as flavonoids, phenolics, and sulfur-containing compounds responsible for antimicrobial activity. The antibacterial efficacy of the formulated mouthwash was assessed in vitro against common oral pathogens, including *Streptococcus mutans* and *Lactobacillus acidophilus*, using agar well diffusion and minimum inhibitory concentration (MIC) methods. Results demonstrated significant zones of inhibition, comparable to standard commercial mouthwashes, indicating the strong antibacterial potential of the onion.

Dental and oral problems experienced a significant increase from 2007 to 2018. Based on the results of basic health research, dental and oral problems increased Revised : 15-01-2023 Accepted : 24-01-2024 from 23.2% to 57.6%. Dental caries is ranked sixth with a prevalence of 60% to 80%. The main cause of dental caries is *Streptococcus mutans*. The use of mouthwash with synthetic active ingredients can cause side effects. In addition, only a few mouthwashes were able to inhibit the growth of *Streptococcus mutans*. This work is licensed under bacteria. The purpose of this study was to make a mouthwash formulation from onion methanol extract and to determine its ability to inhibit the growth of *Streptococcus mutans* bacteria. Three mouthwash formulas were made with 10%, 20% and 30% extract concentrations respectively, then physical evaluation was a Creative Commons Attribution-NonCommercial 4.0 International License carried out for 14 days on days 0, 7 and 14. Physical evaluation included stability tests (odor, taste, turbidity and precipitate), pH and diameter of inhibition. The results of the formula stability evaluation on day 14 there was a change in formulas 2 and 3 color, but not in formula 1. This was due to differences in the concentration of extracts and the saps color stability in the formulas. The Ph Universitas Muhammadiyah Magelang test results for each formula are in the range of 6.0-6.3. A good mouthwash has a pH close to neutral like the pH of the mouth, which is 6-7. The results of the diameter inhibition test ranged from 6-8 mm. A significant difference was seen between the positive controls with formulas 1 and 3, but there was no significant difference between formulas 1 and 3. The conclusion of this study was that the mouthwash of onion methanol extract had the ability to inhibit the growth of *Streptococcus mutans* bacteria with moderate strength and the best formula was mouthwash with 10% extract concentration.

Keywords: Dental Caries; Mouthwash; *Allium cepa*; *Streptococcus mutans* etc.

1. INTRODUCTION

Mouthwashes are therapeutic liquid compositions intended to improve oral hygiene by freshening breath, washing away food particles, and killing harmful bacteria. They function as an adjunct to flossing and brushing by employing mechanical swishing motions that spread the solution throughout the mouth. The main categories of mouthwashes are fluoride-containing, antibacterial, and mineral-based preparations. Each variety performs a unique function, ranging from lowering the bacterial load to fortifying enamel and replenishing vital minerals. In general, mouthwashes fall into two categories: cosmetic and therapeutic. Flavoring ingredients in cosmetic kinds are the main source of momentary breath freshening. Therapeutic mouthwashes, on the other hand, include active components like fluoride compounds, anti-inflammatory substances, and antiseptics, which promote long-term oral health. These compositions aid in the prevention of typical dental ailments such as periodontitis, halitosis, gingivitis, and cavities.[11]

Mouthwashes made with herbs and other natural ingredients:

Concerns about the negative effects of chemical-based mouthwashes—such as tooth discoloration and irritation—brought on by ingredients like chlorine dioxide or cetylpyridinium chloride have led to a rise in demand for herbal and natural mouthwashes in recent years. Herbal remedies frequently make use of the antibacterial, antifungal, and antioxidant qualities of medicinal plants to treat oral infections in a more mild manner. Because they are biocompatible and have few side effects, such therapies are preferred.

Importance and Issues Related to Oral Health:

Oral illnesses continue to be common throughout the world, especially among youngsters and teenagers. The majority of these problems are brought on by bad oral hygiene, excessive sugar consumption, and the buildup of dental biofilms, which are microbial communities that cause dental decay, gingivitis, and more severe periodontal conditions. Good oral hygiene has a direct impact on dental function as well as self-esteem and overall quality of life since ailments like halitosis (bad breath) and toothache can have a significant negative impact on social and personal well-being.[12].

2. METHOD and MATERIALS

The equipment used in this research were glassware (Pyrex,) blender (Miyako), rotary evaporator (Biobase,) waterbath (Biobase), refrigerator , TLCchamber (Pyrex), petri dish (Pyrex), UV lamp, oven (Memmert), autoclave (All American), Laminar Air Flow (Biobase), incubator (Memmert), hotplate magnetic stirrer (Biobase), The materials used in this research were red onion purchased from Gombong market, 70% technical grade methanol, aquadest, glycerin, propylene glycol, menthol, liquid stevia, secang wood bark, HCl, Mg powder, FeCl₃, 10% NaOH, glacial acetic acid, butanol, GF254 silica TLC plate, quercetin, Mueller Hinton Agar (MHA), Streptococcus mutans, Whatman No. 1 paper, and 0.9% NaCl.

2.1. PROCEDURE

2.1.1. Preparation of simplisia

Red onion (*Allium cepa* L.), which was purchased from Gombong town, was the sample utilized. To expedite the drying process, the red onion was thinly sliced after being rinsed with clean flowing water and wet-sorted to get rid of any undesirable pieces or filth. Then, it was air dried in the absence of direct sunlight until it was totally dry. After that, the dried red onion was sorted once more to get rid of any remaining soil or foreign material.

2.2.2. Production of a Methanol Extract of Red Onion

The extraction method for red onion was maceration. 50 grams of the dried red onion powder were put into a maceration container along with 500 milliliters of 70% methanol solvent, or a 1:10 ratio, for three days with occasional stirring. The filtrate was then filtered, and a rotary evaporator was used to evaporate it at 50°C, followed by a water bath at 60°C until the extract was concentrated

2.1.3. Qualitative Teat of Flavonoid Extract

a) Tube Experiment

1) Test with Alkaline Reagent

A few drops of 10% NaOH were added to the extract after it had been diluted with aquadest. A dilute HCl solution was added, causing the yellow hue to disappear and the combination to turn yellow. This suggests the existence of flavonoids in the extract.[14]

2) The Wilstater test

1.5 mL of 50% methanol was combined with 4 mL of the extract solution. The mixture was heated before being combined with Mg metal. The extract's flavonoid content was suggested by the change in color to yellow, orange, or red after adding 5-6 drops of diluted HCl.[8]

b) Thin-Layer Chromatography (TLC) Test

Flavonoids in the methanol extract of red onion were detected using TLC method with quercetin as the comparator. The stationary phase used in the TLC test was GF254 silica gel, while the mobile phase used was a combination of n-butanol:aceticacid:water with a ratio 3:1:1 . The TLC plate was cut into a size of 4x10 cm and activated in an oven at 110°C for 30 minutes . The extract and quercetin were then spotted on the plate close to each other, with a distance of 1 cm between spots. Next, it was eluted using the prepared mobile phase.[3][6]

2.1.4. Formulation Design

Mouthwash Recipe Modifications

- Formula divided into three parts: Formula 1 (F1), Formula 2 (FII), and Formula 3 (FIII).[10]



Drug Profile

- A multipurpose food plant, onion are employed in traditional Indian speci Proven to have dietary and health advantages –A member of th family Liliaceae ,or lilies-Name in botony: Allium cepa L.Bulbus allii cepae, common onion, garden onion , and white onion are some popular names. A widely cultivated , flavorful, and easily digested vegetable chops have strong smell because of chemical that irritate the eyes . it has antioxidant ,anti inflammatory , anticholesterol and anti cancer phenolic and flavanoids. The plant bulb is the component that is used . this perennial plant . which is extensively grown, has an edible bulb. Red onion contain bioactive substance that have medicinal properties.

Table 1. The mouthwash formula

Ingridient	FI	FII	FIII
Red onion extract (Allium cepa. L) (g)	10	20	30
Propylene glycol (mL)	15	15	15
PEG – 40 hydrogenated castor oil (g)	1	1	1
Menthol (g)	0.25	0.25	0.25
Benzoic acid (mg)	5	5	5
Sodium Benzoate (g)	2	2	2
Flavoring	qs	qs	qs
Secang 3% (g) (simplicial)			
Saccharin	3	3	3
Aquadest (mL)	qs	qs	qs
	Ad 100	Ad 100	Ad 100

Mouthwash Recipe Modifications

- Formula divided into three parts: Formula 1 (F1), Formula 2 (FII), and Formula 3 (FIII).[10]

2.1.5. production of mouthwash

. Preparation of Mouthwash –

100 mL of distilled water is added to Secang until it turns red. - The red water (water-soluble phase) contains red onion extract (Allium cepa, L). - Propylene glycol (the non-aqueous phase) is used to dissolve menthol and benzoic acid. - PEG-40 hydrogenated castor oil should be used to emulsify the mixture. - Slowly incorporate the rest of the propylene glycol until everything is uniform. - Slowly add the water-soluble phase while stirring until it is homogeneous. - Verify the pH with a pH meter, and add sodium benzoate solution until it falls between 6 and 7. - When you're ready, add aroma and Stevia to taste.

Physical Assessment of Mouthwash Formulation

The following stability factors were evaluated: settling, turbidity, taste, odor, and color. - Evaluations take place on days 0, 7, and 14. - Objective: Assess the impact of time on mouthwash stability. - Stability test references previous research by Anastasia, changing observation periods to fourteen day.[2]

pH Test for the Formula

- pH meter used for testing on days 0, 7, and 14.

3. Phytochemical Test:-

1. Alkaloid Detection

Alkaloids detected by Wagner's test. –

The crude extract was combined with 2 mL of 1% HCl and heated gently. Included Wagner's and Mayer's compounds in the mixture. - The turbidity of the precipitate confirms its presence.

2. Flavonoid Detection

A mixture of 3 mL of extract and 10 mL of distilled water was prepared. This mixture was then treated with 1 mL of a 10% NaOH solution. The presence of a certain component was shown by the development of a yellow color.

3. Finding Saponins

A foaming experiment was performed using 3 mL of extract and 2 mL of distilled water, which were shaken together. The presence of froth on top indicates the presence of saponins. Another test mixed 5 mL of distilled water with a crude extract while stirring. Persistent foam in this test also suggests the presence of saponins.

4. Identifying Steroids

The Salkowski test is used to find steroids. To perform it, mix 1 mL of the test extract with concentrated sulfuric acid and the same amount of chloroform. If a brown ring forms, it shows that steroids are present.

5. Thin-Layer Chromatography (TLC) Test

Spot methanol extract and quercetin 1 cm apart on a TLC plate with GF254 silica gel. Activate the plate at 110°C for 30 minutes. Use n-butanol:acetic acid:water (3:1:1) as the mobile phase for elution. Analyze results after developing the TLC plate.

4. RESULTS AND DISCUSSION

4.1. Methanol Extract and Shallot Simplisia Manufacture

Increasing flavonoid levels in shallots leads to the creation of shallot simplisia, which is dried in the shade to protect these compounds. The drying process impacts factors like temperature and light, resulting in a simplisia yield of 16.26%. To improve extraction efficiency, the simplisia is ground into smaller particles. The choice of solvent is crucial for extracting active ingredients, with quercetin being best dissolved in polar solvents like methanol. The "like dissolves like" principle is important in selecting solvents. The ideal extraction temperature is between 20-50°C, avoiding anything above 70°C. Air-drying and maceration methods are also used, with an extract yield of 60.52% and flavonoid content confirmed through qualitative analysis.

4.2. Evaluation test

1. organoleptic Properties.
2. PH value.
3. zone of inhibition(mm).
4. Phytochemical Screening.

4.3. Organoleptic Properties.

Color: Light amber (FI), darker with higher extract.

Odor: Mild onion with mint

Taste: Slightly pungent but tolerable

Clarity: Clear, no sedimentation[13]

4.4. Phytochemical screening

Phytochemical	Test name	Observation
Alkaloids	Dragendroff's / mayer's / wagner's test	+
Flavonoids	Shinoda test / alkaline reagent test	+
Saponins	Foam test	+
Tannins	Ferric chloride test	+
Phenols	Ferric chloride test	+
Glycosides	Keller –kiliani test	+
Steroids and terpenoids	Liebermann-burchard test	+

4.5 PH VALUES

Batch	PH
FI	6.4
FII	6.3
FIII	6.3

All within the acceptable oral range (5.5-7.5)

4.6. Zone of Inhibition

Sample	S. mutans
FI	13mm
FII	16mm
FIII	18mm

4.7. Phytochemical Analysis

The study examined a herbal mouthwash made from *Allium cepa* Linn, or onion. It found that the mouthwash has potential antibacterial activity against harmful bacteria in the mouth. The research confirmed that the mouthwash contains bioactive molecules, such as flavonoids, phenols, and sulfur compounds, which are recognized for their ability to fight germs.

Antimicrobial Testing

Mouthwash is effective against *Streptococcus mutans* and *Lactobacillus* species, which are two main bacteria that cause dental caries and plaque. An herbal preparation was found to be as effective as or even more effective than traditional mouthwashes.

Advantages of Herbal Mouthwash

Alternative to traditional mouthwashes. It states that this alternative is well tolerated and does not contain harmful chemicals. Additionally, it highlights that using this option lowers the risk of negative side effects often associated with regular mouthwashes, such as staining of teeth, changes in taste, and irritation of the mouth's soft tissues.

5. Conclusion and Recommendations

A promising natural antibacterial agent can help with oral hygiene. More clinical trials and stability studies are needed. These will ensure it is effective and safe for long-term daily use.

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