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# Formulation and Evaluation of Aloe Vera Mouthwash

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***Abstract*-- The main objective of this study is to prepare and evaluate a Aloe vera mouthwash and determine physiochemical function that emphasizes on safety, efficacy, and quality of the product. Aloe vera mouthwash is a natural oral care product which is used to reduce plaque, soothe gum inflammation and mouth ulcers, promote healing, and maintain oral hygiene. Various drugs are used for the preparation of mouthwash such drugs shows various side effects. Aloe vera mouthwash works by using natural compounds like anthraquinones and saponins that kill bacteria and reduce inflammation, while its soothing gel base keeps the mouth moist unlike chemical mouthwashes that rely on alcohol or harsh antiseptics which can irritate tissues and cause dryness. Therefore, an attempt is made to formulate Aloe vera mouthwash that is free from side effects. All the ingredients were obtained and formulation was prepared and evaluated. Among the nine formulation F1, F2, F3, F4, F5, F6, F7, F8 and F9. F7 displayed better properties in comparison to other eight formulation when evaluated for their physical appearance, homogeneity, pH, density, viscosity, specific gravity, microbial activity and stability study.**

***Keywords*-- Aloe vera, soothing gel, formulation, stability study.**

## I. INTRODUCTION

*ALOE VERA:*

Aloe vera (Aloe barbadensis Miller) is a succulent plant widely recognized for its extensive medicinal and therapeutic applications. It has been traditionally used in Ayurveda, Unani, and folk medicine for centuries.

The gel obtained from the inner part of the Aloe vera leaf is rich in biologically active compounds, including vitamins (A, C, E, B12), minerals (calcium, magnesium, zinc), amino acids, polysaccharides, and enzymes, which contribute to its healing potential [1].

Among its many applications, Aloe vera has gained attention in the field of oral health care due to its antibacterial, anti-inflammatory, antioxidant, and wound-healing properties. Studies have shown its effectiveness in reducing plaque accumulation, gingival inflammation, and oral microbial load when used as a mouthwash [2][3].

Due to its biocompatibility, minimal side effects, and cost-effectiveness, Aloe vera is now considered a promising alternative to chemical-based oral hygiene products. It has shown notable efficacy against pathogens like Streptococcus mutans and Candida albicans, commonly involved in dental caries and oral infections [4].

Aloe is the solid residue obtained by evaporating the liquid which drains from the transversely cut leaves of various species of Aloe. The juice is usually concentrated by boiling and solidifies on cooling. The official (BP, EP, USP) varieties of aloes are the Cape from South Africa and Kenya, and the Barbados (Curaçao) from the West Indian Islands of Curaçao, Aruba and Bonaire. There are separate pharmacopoeial monographs for each type. Socotrine and Zanzibar varieties are no longer official. Plantsof about 180 known species of Aloe, the drug is mainly obtained from the following:

Cape variety from Aloe ferox and its hybrids; Curaçao variety from Aloe barbadensis; Socotrine and Zanzibar varieties from Aloe perryi. The genus Aloe includes herbs, shrubs and trees, bearing spikes of white, yellow or red flowers. Aloe ferox is an example of the arborescent type and A.barbadensis of the herbaceous type. Aloe leaves are fleshy, are strongly cuticularized and are usually prickly at the margins. [5]

**Fig. 1: Aloe vera (Aloe barbadensis miller)**

Aloe vera is a tender plant containing a high-water content (99–99.5%). Solid contents range from 0.5–1% and consist of a variety of active components i.e. fat and water-soluble minerals, vitamins, simple/complex polysaccharides, organic acids, enzymes and phenolic compounds.

*Gel:* Inner layer consisting of soft, clear, moist and slippery tissues having large parenchyma cells. This is a transparent mucilaginous jelly like material. It contains water (99%), glucomannans, amino acids, lipids, sterols and vitamins.

*Latex:* The middle layer containing anthraquinones, bitter yellow sap and glycosides.

*Rind:* The outer thick layer consisting of 15–20 cells which gives protection to gel matrix and helps in the synthesis of carbohydrates and proteins.

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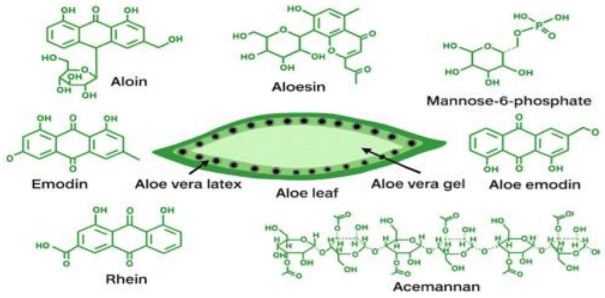


**Fig. 2: Active components**

## II. Materials And Methods

**TABLE 1:**
**List Of Materials Used In The Formulation**

| Materials Used | Manufacturer | Uses |
|---|---|---|
| Aloe vera | -- | Antimicrobial properties, Anti-inflammatory effects, Wound healing, Fresh breath. |
| EG-400 | Sisco Research Laboratories Pvt. Ltd. | Solubilizer, umectant, Thickening agent |
| Ethanol | Changshu Hong Shen Fine Chemical Co., Ltd. | Solvent, Preservative |
| Glycerin | Pallav Chemicals and Solvents Pvt. Ltd. | Humectant, Protectant, Texture modifier |
| Stevia | Organic India Pvt. Ltd | Natural sweetener |
| Peppermint oil | Loba Chemie Pvt. Ltd. | Fresh breath, Cooling sensation, Flavor enhancement |

*Collection Of Plant:*

Aloe vera plant was collected from Hosur market and subjected for authentication by Central Ayurveda research institute, Bangalore. The plant was found to be Aloe vera (L.) Burm.f.

*Chemicals:*

All chemicals PEG-400, Ethanol, Glycerine, Distilled water were used from Vaasudhara College of Pharmacy, Bangalore.

*Preparation Of Aloe Vera Extract:*

The Aloe vera leaves were removed from the plant and washed thoroughly to remove all traces of latex, which has an unpleasant bitter taste. Dry the leaves with a dry cloth or towel and cut the large Aloe vera leaves into half. By using the sharp knife, cut the side of the leaf first and then the natural Aloe vera gel is visible to scoop the gel into cup by using spoon. Wash the Aloe vera gel thoroughly with water and refrigerate them for one week [6]. The Aloe vera gel life can be extended by adding a spoon of Vitamin-C (Ascorbic acid) as a preservative and it helps to extend the life of the gel for 1 to 2 months in the refrigerator [7].

*Preparation of Base Formulation*

Different solvents were used to make base formulation. Three base formulation were made (B1 - B2) using Polyethylene glycol- 400, Glycerine, Ethanol and distilled water. Each base formulation is made upto 150 ml volume [8].

*Preparation Of Herbal Mouth Wash Formulation Using Aloevera*

Aloe vera extract of different concentrations were incorporated with base formulations (B1- B3) to come up with nine herbal mouthwash formulations (F1-F9) of 50ml.

## III. Evaluation Tests

*Physical Evaluation:*

Physical parameter such as appearance, color, taste and odour were checked visually.

*Homogeneity:*

The mouthwash formulations were placed in the container and visually inspected for uniformity. For the look and presence of any aggregate, they underwent testing.

*Measurement of Ph:*

To choose the best mouthwash, a Quality Control test was used to determine the pH of the mouthwash. The pH of the formulation was determined using a digital pH metre. Table 3 has a record of the outcomes.

*Antibacterial Activity:*

Aloe vera extract was tested for in-vitro antibacterial activity using a modified version of the standard procedure for zone inhibition assay/well-diffusion against the S. aureus bacterial strain. In essence, a sterile cotton swab was used to evenly disperse the bacterial inoculum across the surface of the agar Petri dish plate. Then, using a sterile tip, four 5 mm diameter holes were drilled. Two wells received a treatment of 10 L of drug solution (1 mg/ml), whereas the following two wells received a control treatment of 10 L of autoclaved double- distilled water. At a temperature of 37 °C and under aerobic circumstances, plates were incubated for 72 hours. The clean zone in the agar, which was assessed after the course of therapy, served as a proxy for the antibacterial effect.

*Stability Testing:*

The purpose of a stability test is to ensure that mouthwash formulations can continue to be effective and retain their original properties throughout time. The visual look, physical separation, and homogeneity of the manufactured mouthwash are all recorded during the stability test.

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An accurate pH metre was used to monitor pH stability as well. Results from a four-week experiment using a mouthwash formulation with a higher pH value were reported.[45]

*Density Test:*

Density is a fundamental physical property that can indicate changes in the mouthwash's formulation over time or due to manufacturing variations. Monitoring the density of a mouthwash is a key aspect of evaluating its stability and overall product quality.

*Viscosity Test:*

Viscosity testing is an essential evaluation parameter for mouthwash as it helps ensure the formulation has an ideal flow property that provides a pleasant mouthfeel, maintains uniform distribution of active ingredients, improves retention time in the oral cavity for better therapeutic action, and guarantees batch-to-batch stability and patient acceptability.

*Specific Gravity:*

Specific gravity as an evaluation parameter for mouthwash is important because it measures the ratio of the formulation's density to that of water, helping to confirm uniformity, detect any errors in formulation or mixing, ensure proper dosage accuracy, and maintain consistency and quality across different batches

## IV. Results & Discussion

The different solvent mixtures that added upto 150ml were prepared as mouthwash base formulation (B1-B3) using PEG-400, Glycerin, Ethanol, Distilled water as mentioned in table.

**TABLE 2:**
**Composition Of Various Base Formulations**

| Base Formulations | PEG-400 (Ml) | Glycerin (Ml) | Ethanol (Ml) | Water (Ml) | Stevia (Mg) | Peppermint Oil (Ml) |
|---|---|---|---|---|---|---|
| B1 | 50 | 50 | 10 | 40 | 150 | Qs |
| B2 | 60 | 40 | 5 | 45 | 300 | Qs |
| B3 | 70 | 75 | 0 | 5 | 350 | Qs |

*Formulation Using Aloe Vera*

Aloe vera extract of different concentrations were incorporated with base formulations (B1- B3) to come up with nine herbal mouthwash formulations (F1-F9) of 50ml as shown in table.

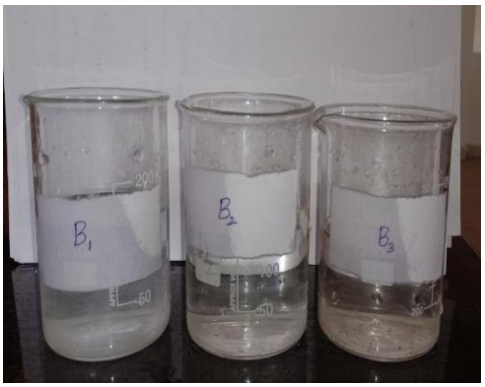


**Fig. 3: Base formulations (B1-B3)**

*Preparation Of Herbal Mouthwash*


**Fig 4. Various mouthwash formulations (F1-F9)**

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**TABLE 3:**
**PREPARATION OF HERBAL MOUTHWASH OF DIFFERENT FORMULATIONS USING ALOE VERA EXTRACT**

| Mouthwash Formulation | Base Formulatio | Volume (Ml) | |
|---|---|---|---|
| | | Aloe Vera Extract | Base Formulatio (50ml) |
| F1 | B1 | 1 | 49 |
| F2 | B1 | 3 | 47 |
| F3 | B1 | 5 | 45 |
| F4 | B2 | 1 | 49 |
| F5 | B2 | 3 | 47 |
| F6 | B2 | 5 | 45 |
| F7 | B3 | 1 | 49 |
| F8 | B3 | 3 | 47 |
| F9 | B3 | 5 | 45 |

*Physical Evaluation*

Physical parameter such as appearance, color, taste and odour were found to be clear, colorless, characteristic taste and characteristic odour.

*Homogeneity*

The mouthwash formulations were packed in the container and tested for homogeneity by visual inspection. It shows that there is no aggregate present in it.

**Fig. 5: Homogeneity test**

*Measurement Of Ph*

The pH of the formulation was determined using a digital pH metre. Table 3 has a record of the outcomes. The herbal mouthwash formula F7 was chosen for its antibacterial activity and had a decent pH value.

**TABLE 4:**
**The Ph Of The Different Herbal Mouthwash Formulation (F1 – F9)**

| Mouthwash formulation (f) | pH |
|---|---|
| F1 | 5.96 |
| F2 | 5.70 |
| F3 | 5.56 |
| F4 | 5.84 |
| F5 | 5.79 |
| F6 | 5.61 |
| F7 | 7.27 |
| F8 | 6.62 |
| F9 | 5.70 |

**TABLE 5:**
**Optimized Mouthwash Formulation F7 Was Observed For The Following Parameter**

| Parameters observed | Inference |
|---|---|
| Appearance | Clear |
| Taste | Sweet |
| Odour | Characteristic odour |
| Homogeneity | Good |
| pH | 7.27 |

*DENSITY:*

*Procedure to determine density :*

1. Clean thoroughly the specific gravity bottle with chromic acid or nitric acid.
2. Rinse the bottle at least two to three times with distilled water.

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3. If required, rinse the bottle with an organic solvent like acetone and dry.
4. Take the weight of empty dry bottle with capillary tube stopper (w1).
5. Fill the bottle with unknown liquid and place the stopper, wipe out excess liquid from outside the tube using tissue paper.
6. Weight bottle with unknown liquid on analytical balance (w2).
7. Calculate weight in grams of unknown liquid (w3).

*Formula for density:* Density of liquid under test (syrup) = weight of liquid under test/volume of liquid under test = w3/v

**Fig. 6: Density test**

*SPECIFIC GRAVITY:*

*Procedure to determine specific gravity*

1. Clean thoroughly the specific gravity bottle with chromic or nitric acid.
2. Rinse the bottle at least two to three times with purified water.
3. If required, rinse the bottle with an organic solvent like acetone and dry.
4. Take weight of empty dry bottle with capillary tube stopper.
5. Fill the bottle with distilled water and place stopper; wipe out excess liquid from side tube using tissue paper (w2).
6. Weight bottle with stopper and water on analytical balance (w2).
7. Repeat the procedure for liquid under test by replacing the water after emptying and drying as mentioned in step 4 to 6.
8. Weight bottle with stopper and liquid under test on analytical balance (w3).

**Fig. 7: Specific gravity test**

*VISCOCITY:*

*Procedure to determine viscosity*

1. Thoroughly clean the Ostwald viscometer with warm chromic acid and if necessary, use an organic solvent such as acetone.
2. Mount viscometer in vertical position on a suitable stand.
3. Fill water in dry viscometer up to mark G.
4. Count time required, in second for water to flow from mark A to mark B.
5. Repeat step 3 at least 3 times to obtained accurate reading.
6. Rinse viscometer with test liquid and then fill it up to mark A, find out the time required for liquid to flow to mark B.
7. Determination of densities of liquid as mentioned in density determination experiment

**Fig. 8: Viscosity test**

*ANTI-BACTERIAL ACTIVITY:*

In-vitro antibacterial activity of the optimized formulation was performed through well-diffusion assay or cup plate assay against S. aureus, and E. coli bacterial strain using the standard secondary strain culture (prepared from 100 µl of primary suspension containing approx. 1 x 108 CFU/mL pathological tested bacteria) was evenly spread over the surface of the agar Petri dishes plate using a sterile cotton swab.

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Thereafter, 5 mm diameter holes was made using a sterile tip followed by addition of 10ul of drug solution (5mg/ml) was added into the wells and the controls well was treated with 10 µl of autoclaved double distilled water as the control treatment. Each treated plate was incubated under aerobic conditions at 37°C temperature for 72 h. The antimicrobial effect was determined by the clear zone in the agar and measured as the efficacy of optimized ointment formulation.

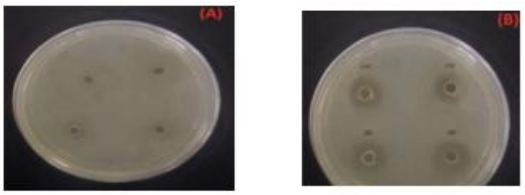

**Fig. 9: Anti-bacterial activity of Aloe vera extract Figure (A) represents control plate while figure (B) represents zone of inhibition of Aloe vera**

**TABLE 6:**
**Anti-Bacterial Activities Of Various Concentrations Of The Optimized Aloe Vera Mouthwash**

| Concentration(µg/ml) | 1000 | 500 | 250 | 125 | 62.5 |
|---|---|---|---|---|---|
| Reading 1 | 2.1265 | 2.1542 | 1.6888 | 1.1452 | 0.4224 |
| Reading 2 | 2.1255 | 2.0245 | 1.4245 | 1.2345 | 0.7365 |
| SD | 0.0046 | 0.1201 | 0.2123 | 0.2813 | 0.3876 |

*STABILITY TEST:*

**TABLE 7:**
**Stability Study Observations**

| Mouthwash formulation | Evaluation parameters | Observations |
|---|---|---|
| F7 | Visual appearance | No color change |
| F7 | Phase separation | NIL |
| F7 | Homogeneity | Good |

## V. CONCLUSION

This study successfully developed and evaluated an herbal mouthwash using Aloe vera extract. Aloe vera gel was extracted from the leaves of Aloe barbadensis Miller, a member of the Xanthorrhoeaceae family. Different base formulations were developed using PEG-400, glycerin, ethanol, and distilled water, and Aloe vera extract was incorporated into the base formulation to create an herbal mouthwash. The mouthwash underwent evaluation tests, including homogeneity, stability, and pH, which showed satisfactory results within acceptable limits. The study demonstrated that the Aloe vera mouthwash has significant antibacterial activity, indicating its potential in controlling oral infections. The preparation of herbal mouthwash is an efficient and eco-friendly method, aligning with the growing demand for natural and sustainable products. The findings suggest that Aloe vera mouthwash could be a valuable addition to oral hygiene products, providing a natural alternative to chemical-based mouthwashes.

The study concludes that the developed Aloe vera mouthwash is a promising product with potential applications in oral care, offering benefits such as natural and herbal composition, antimicrobial properties, potential to control oral infections eco-friendly production process. Overall, this study highlights the potential of Aloe vera as a natural ingredient in oral care products, warranting further research and development.

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