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Formulation Development and Evaluation of Polyherbal Mouthwash

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Abstract: ***Altered lifestyle and habits have created problems in oral hygiene and have similarly gained wide attention towards mouthwash. Overcoming the side effects of chemical-based mouthwashes, herbal mouthwashes are potentially popular for maintaining oral health. This article focuses on utilising the significant antiplaque, antibacterial, and antimicrobial properties of a few herbs in the formulation development of a polyherbal mouthwash. The aqueous extracts, mixed in an appropriate proportion, maintain an alcohol-free formulation. The various test results of physicochemical and microbial evaluation confirm the desired activity of the prepared formulation, making it effective in oral hygiene.***

Keywords: Polyherbal Mouthwash, Alcohol Free, Aqueous Extracts, Antimicrobial, Antiplaque

I. INTRODUCTION

Oral hygiene is crucial for maintaining good oral health and preventing various oral diseases. Maintaining oral hygiene involves several practices, including brushing, flossing, and using mouthwash [1]. Mouthwash is a popular oral care product that is used for freshening breath, reducing plaque and gingivitis, and promoting overall oral health. Conventional mouthwashes contain various synthetic chemicals that may have adverse effects on the oral microbiome and the environment [2]. Therefore, there is an increasing demand for herbal mouthwashes that are both safe and equally effective [3].

Polyherbal formulations have gained attention in recent years due to their multiple therapeutic benefits and minimal side effects. Polyherbal mouthwash formulations are composed of various herbal extracts that provide a synergistic effect, thereby improving overall oral health [4]. Multiple herbs, such as meswak, myrrh, clove, thyme, neem, peppermint, and tea tree, have been traditionally used for oral care due to their antibacterial, antifungal, and anti-inflammatory properties [5, 6, 7]. This article focuses on the significant use of various herbs and their potential antimicrobial effects in maintaining oral hygiene.

II. PLANT PROFILE

Table-I: Plant Profile of Selected Herbs [8, 9, 10, 11]

Sr. No.	Common name	Scientific Name	Family	Plant part used	Chemical Constituents	Uses
1.	Meswak	*Salvadora persica*	Salvadoraceae	Twig	Salvadorine, flavonoids	Antiplaque
2.	Myrrh	*Commiphora myrrha*	Burseraceae	Resin	α-, β- and γ- Commiphoric acids	Wound Healing
3.	Clove	*Syzygium aromaticum*	Myrtaceae	Flower bud	β- caryophyllene	Antibacterial
4.	Thyme	*Thymus vulgaris*	Lamiaceae	Leaf, stem	Thymol	Anti- inflammatory
5.	Stevia	*Stevia rebaudiana*	Asteraceae	Leaf	Stevioside, rebaudioside	Sweetening agent
6.	Lemon	*Citrus limon*	Rutaceae	Fruit	Phenolic acid	Antioxidant

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III. MATERIALS AND METHODS

A. Collection of Plant Powders

Roots, twigs, and stem of *Salvadora persica* (Meswak), buds of *Eugenia caryophyllus* (clove), reddish-brown dried sap of a thorny tree, Commiphora *Abyssinia* (myrrh), *Thymus vulgaris* (thyme), leaves of *Rebaudiana Bertoni* (stevia) and *Citrus limon* (lemon oil) purchased from online and local market of Nashik.

B. Formulation of Herbal Mouthwash

The selection of herbs was made, taking into account their antimicrobial efficacy, as well as the properties of the excipient. These, namely, preservative, sweetening, and flavouring effects, are required to develop an ideal mouthwash.

Table-II: Master Formula of Polyherbal Mouthwash

Sr.no	Ingredients	Botanical Names	Role	Quantity (ml)
1.	Meswak	Salvador persica	Antiplaque	30
2.	Myrrh	Commiphora myrrha	Wound Healing	20
3.	Clove	Syzgium aromaticum	Antibacterial	20
4.	Thyme	Thymus vulgaris	Anti-microbial	15
5.	Stevia	Rebaudiana Bertoni	Sweetening agent	10
	Pomegranate			
6.	Lemon oil	Citrus limon	Flavouring agent,	q.s.

C. Formulation Table

We had prepared four different formulations with varying quantities of herbs in each. The quantities of the herbs were decided based on a literature review and also the minimum concentration of herbs at which they exhibit their desired properties.

Table-III: Four Different Formulations of Polyherbal Mouthwash

Sr.no	Ingredients	F1 (ml)	F2 (ml)	F3 (ml)	F4 (ml)
1.	Meswak	25	30	25	30
2.	Myrrh	25	20	25	20
3.	Clove	20	20	20	20
4.	Thyme	10	15	10	15
5.	Stevia	10	10	10	10
6.	Pomegranate peel	10	5	-	-
7.	Lemon oil	-	-	10	5

1. A 20-gm weighed quantity of each ingredient was taken.
2. The powdered herb was taken mixed with a 200 ml quantity of water.
3. Then it is kept for incubation for 72 hours at 370 °C.
4. After 72 hours, the mixture is removed and appropriately mixed in an appropriate proportion desired for our formulation.
5. Finally, preservative added and the product will be packed in an attractive, well-closed container [14,15,16].

The development of the formulation and evaluation was a continuous process. Several characteristic issues, such as fungal growth during storage and a strong aromatic odour, were observed, and to rectify these, several combinations were tried in the formulation.

D. Preparation of Extract

The Aqueous extract of each plant material was prepared using an incubation method [12,13], where the powder sample was soaked in sterile distilled water and maintained in an Incubator at 37 °C for 72 hours. The herbal extracts were filtered using Whatman filter paper, washed with 10 ml of sterile distilled water, and pressed.

IV. EVALUATION

A. Organoleptic Evaluation

Table-IV: Organoleptic Evaluation for Herbal Mouthwash

Evaluation Parameter	Observation
Colour	Brown
Odour	Pungent, spicy, citrus odour

B. pH

With the help of a calibrated pH meter, the differences and variability in pH of the formulation were calculated. The pH of the formulation was found to be 6.7, which is suitable for use in the oral cavity.

C. Viscosity

A Brookfield viscometer was used to measure the viscosity of the formulation. Viscosity was calculated using Spindle no. 61 at 50 rpm, and it was found to be 1.92 cP.

D. Stability Testing

We have formulated the same formulation after 3 months to check its efficacy over time and have conducted all physical-chemical tests on it, which were within the desired range of parameters. Both samples showed the same pH, viscosity, and potent antimicrobial activity.

Therefore, we can conclude that our formulation is effective over a period of three months. Furthermore, additional evaluation can be conducted after 6, 9, or 12 months to assess its stability.

E. Anti-Microbial Testing

The Polyherbal Mouthwash we have developed has been evaluated to assess its effectiveness in terms of antimicrobial activity. The use of natural antimicrobials may contribute to controlling the disordered growth of oral microbiota, thus overcoming problems caused by species resistant to conventional antimicrobials.

F. Method

i. Pure strains of *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Candida sp.* were used.

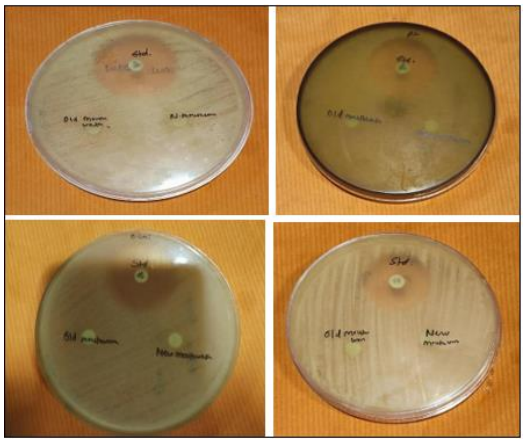
ii. The medium which was used to study was nutrient agar. The Agar well diffusion technique was used to determine the Minimum Inhibitory Concentrations (MIC).

iii. The minimum inhibitory concentration (MIC) was used to evaluate the inhibitory effects of herbal mouthwash.

iv. The test standards used were Gentamycin and Nystatin against herbal mouthwash.

v. The plates were incubated at 37°C overnight and the zone of inhibition of growth was measured in millimetres.

vi. The antimicrobial activity of the mouthwash with different test standards was screened by Agar well diffusion technique, and the zone of inhibition was measured in mm diameter. The images of the zone of inhibition are shown in Figure 1.



[Fig.1: Zone of Inhibition]

V. RESULT & DISCUSSION

A. Evaluation Parameter Results

Based on the marked characterisation, it has been observed that the Pomegranate content in formulations 1 and 2 has led to fungal growth in the formulations. In contrast, replacing the pomegranate content with lemon oil has resolved the issue. The lemon oil incorporated into the formulation has developed a strong aromatic odour, which is then rectified by adjusting the proportions of Meswak and lemon oil. Based on all evaluation tests performed, it is concluded that formulation 4 is practical, stable, and safe.

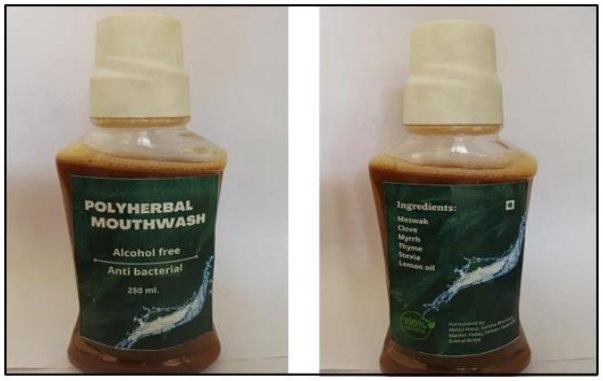
Sr No.	Parameters	Evaluation
1.	Color	Brown
2.	Odor	Pungent, spicy, citrus odour
3.	pH	6.30
4.	Viscosity	1.92cp
5.	Foam Index	250
6.	Antimicrobial activity	No Zone of inhibition

B. Antimicrobial activity report

The sample F4 was used for the antimicrobial test, where a comparative assessment was also made between the freshly prepared formulation and the formulation stored for 4 months. The formulation was assessed against standard antimicrobials, such as Gentamycin and Nystatin. The observations made are tabulated below.

Table-V: Antimicrobial Activity Report

Sample	E. coli ATCC 25922	Pseudomonas aeruginosa ATCC 27853	Staphylococcus Aureus ATCC 25923	Candida sp.
F4 stored for 4 months	No zone	No zone	No zone	No zone
Freshly prepared F4	No zone	No zone	No zone	No zone
Gentamycin	28 mm.	29 mm.	28 mm.	–
Nystatin	–	–	–	21 mm.



[Fig.2: Polyherbal Mouthwash]

VI. CONCLUSION

The test findings are sufficient to conclude that the combination of herbs is accurate and suitable for use as a mouthwash. The antiplaque, wound-healing, antibacterial, anti-inflammatory, and analgesic properties in the formulation help to address oral health issues. The aqueous extract helps sustain the formulation as an alcohol-free product.

DECLARATION STATEMENT

After aggregating input from all authors, I must verify the accuracy of the following information as the article's author.

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- **Author's Contributions:** The authorship of this article is contributed equally to all participating individuals.

REFERENCES

1. Nigam D, Verma P, Chhajed M. Formulation and Evaluation of Herbal Mouthwash against Oral Infections Disease. International Journal of Pharmacy & Life Sciences. 2020 Jul 1;11(7):6746. https://www.researchgate.net/publication/362838844_Formulation_and_Evaluation_of_Herbal_Mouthwash_against_Oral_Infections_Disease
2. Chitali SC, Dubewar A. "Formulation and Evaluation of Ayurvedic Herbal Mouth Wash Against Oral Disorders". https://www.ijnrd.org/papers/IJNRD2306176.pdf
3. Problems and Proposals for Recording Gingivitis and Plaque. International dental journal, 25, 229-235. - references - scientific research publishing [Internet]. Scirp.org. [cited 2023 Apr 12]. https://www.scirp.org/(S(lz5mqp453ed%20snp55rrgjct55))/reference/referencespapers.aspx?referenceid=296866.
4. Shambharkar SB, Thakare VM. Formulation and Evaluation of Herbal Mouthwash. World Journal

of Pharmacy Research. 2021 May 28;10(9):775-91. DOI: http://dx.doi.org/10.20959/wjpps20226-22344
5. Lone ZA, Jain NK. Phytochemical Analysis of Clove (Syzygium aromaticum) Dried Flower Buds Extract and its Therapeutic Importance. Journal of Drug Delivery and Therapeutics. 2022 Aug 15;12(4-S):87-92. https://www.researchgate.net/publication/362888775_Phytochemical_Analysis_of_Clove_Syzygium_aromaticum_Dried_Flower_Buds_Extract_and_its_Therapeutic_importance
6. Da Silva NB, Alexandria AKF, De Lima AL, Claudino LV, De Oliveira Carneiro TF, Da Costa AC, et al. In vitro antimicrobial activity of mouth washes and herbal products against dental biofilm-forming bacteria. Contemp Clin Dent [Internet]. 2012 [cited 2023 May 31];3(3):302–5. Available from: DOI: http://dx.doi.org/10.4103/0976-237X.103623
7. Shivani B. Shambharkar and Vinod M. Thakare. 2021. Formulation and Evaluation of Herbal: Mouthwash Research Article, World Journal of Pharmaceutical Research; 10(9): 775-791. https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/bb6553398fd8dbf6711c84491d8ee866.pdf
8. Haque MM, Alsareii SA. A review of the therapeutic effects of using miswak (Salvadora Persica) on oral health. Saudi medical journal. 2015 May;36(5):530. DOI: https://doi.org/10.15537/smj.2015.5.10785
9. Batiha GE, Wasef L, Teibo JO, Shaheen HM, Zakariya AM, Akinfe OA, Teibo TK, Al-Kuraishy HM, Al-Garbee AI, Alexiou A, Papadakis M. Commiphora myrrh: a phytochemical and pharmacological update. Naunyn-Schmiedeberg's archives of pharmacology. 2023 Mar;396(3):405-20. DOI: https://doi.org/10.1007/s00210-022-02325-0
10. Kong M, Hwang DS, Yoon SW, Kim J. The effect of clove-based herbal mouthwash on radiation-induced oral mucositis in patients with head and neck cancer: a single-blind randomized preliminary study. Onco Targets and therapy. 2016 Jul 22:4533-8. DOI: https://doi.org/10.2147/ott.s108769
11. Sharma, S., Mali, A. M., & Dixit, S. (2017). Formulation and evaluation of a polyherbal mouthwash for oral health maintenance. Indian Journal of Dental Research, 28(2), 140-145. DOI: http://doi.org/10.4103/ijdr.IJDR_162_15
12. Lohar, D. R., Mali, R. G., & Vhyawahare, N. S. (2016). Development and Evaluation of a Polyherbal Mouthwash for the Management of Oral Diseases. Journal of Ayurveda and Integrative Medicine, 7(2), 85-90. DOI: http://doi.org/10.1016/j.jaim.2016.05.005
13. Ghogre, P. V., Kakade, S., & Deshmukh, V. (2018). Formulation and Evaluation of an Herbal Mouthwash Containing Triphala for Improving Oral Health. Journal of Oral Research and Review, 10(1), 25-30. DOI: http://doi.org/10.4103/jorr.jorr_19_17
14. Mahajan, R., Bansal, K., & Mahajan, A. (2017). Comparative evaluation of a polyherbal mouthwash with chlorhexidine on gingival health and microbial plaque. Journal of Clinical and Diagnostic Research, 11(9), ZC17-ZC21. DOI: http://doi.org/10.7860/JCDR/2017/27623.10677
15. Jeddy N, Ravi S, Radhika T, Lakshmi LS. Comparison of the efficacy of herbal mouth rinse with commercially available mouth rinses: A clinical trial. Journal of Oral and Maxillofacial Pathology. 2018 Sep;22(3):332. DOI: https://doi.org/10.4103/jomfp.jomfp_303_18
16. Khan I, Chauhan N, Khan Ma. Evaluation Of Phytoconstituents, And Antimicrobial and Antioxidant Activity of Juglans Regia from Kashmir Region. Asian Journal of Pharmaceutical and Clinical Research. 2019 Feb 7:89-92. https://journals.innovareacademics.in/index.php/ajpcr/article/view/28800