

FORMULATION AND EVALUATION OF A POLYHERBAL MOUTHWASH AND ITS COMPARATIVE STUDIES WITH CHLORHEXIDINE

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ABSTRACT

Introduction: Mouthwashes are antiseptic solutions intended to reduce the microbial load in the oral cavity with other uses like analgesic, anti-inflammatory or anti-fungal action, saliva substitutes to neutralize acid and keep the mouth moist in xerostomia (dry mouth). Wide applications of mouth washes include killing of bacterial plaque in cavities, gingivitis, and bad breath, controlling supra gingival plaque, after oral surgery procedures such as tooth extraction, bed ridden and in-patients, etc. However, medicated mouthwashes have limitations like taste disturbance, tooth staining, sensation of a dry mouth and worsening of halitosis with alcohol mouth washes, soreness, ulceration and redness if the person is allergic or sensitive to mouthwash ingredients. Therefore a poly herbal mouth wash has been formulated which has successfully overcome the above limitations. **Materials and methods:** volatile oils of various crude drugs are extracted by Clavengers apparatus after careful evaluation of their morphological and organoleptic evaluation. These volatile oils have been formulated into a polyherbal mouthwash and evaluated for pH, viscosity, Isothermal stress stability and antimicrobial activity. **Results and discussion:** The pH of the given formulation was determined by using pH meter and it was found to be 6.77. The viscosity was found to be 1.67. In palatability test the degree of palatability was rated as refreshing and palatable by the volunteers. Slightly acidic pH makes the formulation potently active against microbes and aesthetic cleaning. Viscosity is optimum for swishing and long stay in oral cavity. Antimicrobial activity was done using zone of inhibition on microbial cultured agar plates. The formulation with oils ratio 2:2:1 has shown 26 mm of zone of inhibition which is greater than that of chlorhexidine which was of 20 mm. The formulation of mouth wash was isothermally stable at 2:2:1 concentration of oils. **Conclusion:** mouth washes are an essential component of prescription both medically and aesthetically. Since medicated mouthwashes have several drawbacks such as ulceration, sensitivity reactions, dry mouth etc, our polyherbal mouth wash formulation forms an excellent alternative with good palatability, viscosity, isothermal stability and anti microbial properties which would improve patient compliance in their use.

KEYWORDS: mouth wash, chlorhexidine, poly herbal, clove, cinnamon.

INTRODUCTION

Mouthwash, mouth rinse, oral rinse or mouth bath, is a liquid which is held in the mouth passively or swilled around the mouth by contraction of the perioral muscles and/or movement of the head, and may be gargled, where the head is tilted back and the liquid bubbled at the back of the mouth.^[1] Usually mouthwashes are an antiseptic solution intended to reduce the microbial load in the oral cavity, although other mouthwashes might be given for other reasons such as for their analgesic, anti-inflammatory or anti-fungal action. Additionally, some rinses act as saliva substitutes to neutralize acid and keep the mouth moist in xerostomia (dry mouth).^[2,3] Cosmetic mouthrinses temporarily control or reduce bad breath and

leave the mouth with a pleasant taste.^[4] Anti-cavity mouth rinse uses fluoride to protect against tooth decay.

The American Dental Association asserts that regular brushing and proper flossing are enough in most cases, although they approve many mouthwashes that do not contain ethanol.^[5] For many patients, however, the mechanical methods could be tedious and time-consuming and difficult. Chemotherapeutic mouth rinses have a key role as adjuncts to preventing and controlling supra gingival plaque, gingivitis and oral malodor.^[6] Another common use of mouthwash is prior to and after oral surgery procedures such as tooth extraction.

HISTORY

The first known references to mouth rinsing are in Ayurveda and Chinese medicine, about 2700 BC, for treatment of gingivitis.^[7] Later, in the Greek and Roman periods, mouth rinsing following mechanical cleansing became common among the upper classes, and Hippocrates recommended a mixture of salt, alum, and vinegar.^[8] The Jewish Talmud, dating back about 1,800 years, suggests a cure for gum ailments containing "dough water" and olive oil.^[9] Anton van Leeuwenhoek, (17th century) discovered living organisms in teeth deposits and experimented with alcoholic beverages but concluded that the mouth wash failed either because it didn't reach all corners of mouth or did not have a long stay to affect killing of microbes.^[10]



Figure 1: dental plaque.

The effectiveness of chlorhexidine was discovered in 1960s.^[11] Since then commercial interest in mouthwashes has been intense and several newer products claim effectiveness in reducing the build-up in dental plaque and the associated severity of gingivitis, in addition to fighting bad breath. For example, the number of mouthwash variants in the United States of America has grown from 15 (1970) to 66 (1998) to 113 (2012).^[12]

COMMON INGREDIENTS OF MOUTH WASH

Each commercial brand of mouthwash has different ingredients. The active ingredients are usually as seen in the following table:

Table 1: Types of mouthwashes and their respective ingredients.

| Type of mouthwash | Ingredients |
|----------------------|---|
| Antiseptic | chlorhexidine gluconate, hexetidine, triclosan, benzalkonium chloride domiphen bromide, Betadine. |
| Antimalodor | cetylpyridinium chloride, Sanguinarine. |
| Antiplaque/oxidizing | Zinc ,Hydrogen peroxide, Calcium. |
| Anticavity | Fluoride. |
| Antiinflammatory | Betamethasone. |
| Analgesics | Benzydamine. |
| Saliva substitute | enzymes and proteins such as Lactoperoxidase, Lysozyme, Lactoferrin. |
| Astringents | zinc chloride |
| Topical antibleeding | tranexamic acid |

Some of the common formulation ingredients of mouth washes and their uses are given in table 2.

Table 2: ingredients used in the formulation of mouth washes and their respective uses.

| Ingredient | Use |
|--|---|
| phenolic compounds like essential oils | Antibacterial properties. |
| zinc chloride | Astringent. |
| Alcohol | Carrier, antibacteria. |
| sodium benzoate or methylparaben | Preservatives. |
| zinc ion | Odor-preventing agent. |
| sorbitol, sucralose, sodium saccharin, and xylitol | Flavoring agents. |
| benzoic acid | Buffer. |
| methyl salicylate | Anti-septic, Anti-inflammatory, Analgesic, Flavoring and Fragrance. |
| Sodium lauryl sulfate | Foaming agent. |
| Water | Bulking agent, solvent |
| Salt | Antibacterial. |

Of all the above, alcohol is an essential ingredient and added in significant quantity (27%).

Alcohol is a drying agent, which encourages bacterial activity in the mouth, releasing more malodorous volatile sulfur compounds.^[13] Therefore, alcohol-containing mouthwash may temporarily worsen halitosis in those who already have it, or indeed be the sole cause of halitosis in other individuals.^[13] It is hypothesized that alcohol mouthwashes acts as a carcinogen.^[14,15,16] The

use of mouthwashes that do not contain alcohol may be equally effective.

Moreover some of the authors highlighted the side effects from several mainstream mouthwashes that included dental erosion and accidental poisoning of children.^x

Drawbacks of some of the ingredients are as follows:

Table 3: common ingredients in mouth washes and their side effects.

| Ingredient | Drawback |
|--------------------------|--|
| Chlorhexidine | Not suited for acute necrotizing ulcerative gingivitis, ^[18] extrinsic staining of teeth in coffee and tea users, overgrowth of entero bacteria in persons with leukemia, desquamation and irritation of oral mucosa, salivary gland pain and swelling, and hypersensitivity reactions including anaphylaxis. ^[19] |
| Fluoride | Staining of teeth in long term. |
| Sodium lauryl sulfate | aphthous stomatitis, deactivates cationic agents present in the mouthrinse. ^[20] |
| Triclosan | Safety questioned ^[21] |
| Cetylpyridinium chloride | has less anti-plaque effect, staining of teeth, or sometimes an oral burning sensation or ulceration. ^[19] |
| Hydrogen peroxide | hypertrophy of the lingual papillae. ^[18] |
| Sanguinarine | leukoplakia in buccal sulcus("sanguinaria-associated keratosis"). ^[19, 22] |
| Sodium chloride(salt) | Not suitable for BP patients |
| Tetracycline | Reddish teeth staining. |

Therefore a polyherbal formulation free from alcohol and having good stability and antimicrobial activity has been prepared.

MATERIALS AND METHODS

Cinnamon, clove and ginger oils.

Objectives

- Extraction of volatile oils by clavenger apparatus.
- Evaluation of herbal mouth of different concentrations.
- To compare the effect of antimicrobial activity against Streptococcus mutants of poly herbal mouth wash with the chlorhexidine.

METHODOLOGY

- Collection and identification of crude sources of essential oils: all the 3 crude drugs i.e ginger rhizomes, cinnamon bark and clove flower buds were purchased from a local market in Hyderabad, India after careful studying of their morphological and organoleptic properties. The organoleptic characters studied were colour odour taste and solubility. The morphological characters studied were appearance, shape and size.

Extraction of volatile oil

The respective volatile oils were obtained by distillation of the plant parts which with the help of clevengers apparatus. Clove flower buds (100g), cinnamon bark and fresh ginger rhizomes(150g), distilled water (800ml) were kept in clavenger's apparatus.

Extraction of clove oil

To 100g of clove flower buds, 200ml of distilled water was added and clavenger's apparatus was setup at a temperature of 30° c for 4hrs. Clove oil being denser than water got collected as lower in graduated tube.

Extraction of cinnamon oil: To 150g of fresh cinnamon bark, 300ml of distilled water was added and clavenger's apparatus was adjusted at a temperature of 30°c for 3hrs. cinnamon oil being less denser than water got collected as upper layer in the graduated tube.

Extraction of ginger oil: To 150g of fresh ginger rhizomes, 250ml of distilled water was added and clavenger's apparatus was adjusted at a temperature of 30°c for 30hrs. clove oil being less denser than water got collected as upper layer in the graduated tube.



Figure 2: Clavenger apparatus with clove, cinnamon and ginger.

Collecting the Essential Oil

The extracted oil sits on top of the water, allowing it to be easily collected by skimming it off the top. The essential oil can then be used for its intended purpose. The leftover water has some soothing properties because it retains some of the aroma of the oil.

Oil Separation

The final step in the distillation of essential oils is the separation from the water flowing from the condenser using a special flask called a Florentine. This is a very important stage as small quantities of oils of very high

value are being handled and maximum efficiency is the key to profitability. Most essential oils are lighter than water and float to the surface of the Florentine. Some oils, however, are denser than water and sink to the bottom. For this reason two types of Florentine are used.

FORMULATION

Preparation of Mouthwash

The mouthwash was prepared by making an emulsion of cinnamon, clove, ginger oils by the dry gum method and various proportions of oils, water and gums were taken and evaluated for the stability of emulsion.

Table 4: commonly used ratios of oils, gums and water in the mouth wash and their stability.

| Oils | Gums | Water | Stability |
|------|------|-------|-----------|
| 0.1% | 0.1% | 0.1% | - |
| 0.5% | 0.5% | 0.5% | - |
| 1% | 1% | 1% | - |
| 2% | 2% | 2% | - |
| 2% | 1% | 2% | STABLE |

Based on the stability of the ratios of oil: water: gums the formulation with ratios 2:2:1 was most stable and based on which the final formulation was prepared as follows:



Figure 3: Poly herbal volatile oil solution.

Table 5: formulation of polyherbal mouth wash.

| Ingredients | Amount Required for 100ML |
|------------------------------|---------------------------|
| CINNAMON OIL | 2ml |
| CLOVE OIL | 2ml |
| GINGER OIL | 1ml |
| ACACIA GUM | 1g |
| TRAGACANTH GUM | 0.2g |
| HONEY | 5ml |
| TWEEN 80 | 1ml |
| BENZALKONIUM CHLORIDE | 0.1% |
| SUNSET YELLOW FCF C.I- 15985 | 0.1% |
| WATER | Q.S |

Cinnamon, clove, ginger oils shows antimicrobial activity (against plague), gums are used as primary emulsifiers, tween80 as a secondary emulsifier, honey has antimicrobial activity, it is a soothing agent & sweetener, benzalkonium chloride is preservative, sunset yellow is a coloring agent and water is a vehicle.

Evaluation of polyherbal mouthwash

(i) pH and viscosity

pH of mouthwashes was measured using pH meter. Viscosity of mouthwashes was measured at 25°C using Ostwald- U tube viscometer and the results was compared to that of an equal volume of distilled water as a reference.

(ii) Palatability

Palatability is the property of being acceptable to the mouth. The mouthwashes were tested separately for that Criteria by three research members in a blind-style. The

test was done on scale of 5 levels: 5 = really good; 4 = good; 3 = not sure; 2 = bad; and 1 = really bad.

(iii) Isothermal stress stability

The selected formulation were stored in 3 storage condition 8 °C, 35 °C and 70 °C . 3 (60-ml). Samples of each tincture were stored in tightly closed amber glass bottles at each of those conditions. Periodical evaluation of those tinctures were carried out at 1, 2 and 4 weeks intervals. 1 ml of the stored emulsion was diluted up to 10ml with water to prepare a corresponding mouthwash. The prepared mouthwash was then evaluated in terms of its antimicrobial activity against Streptococcus mutans, physical change such as turbidity, sedimentation and color change as well as in terms of its pH and palatability.

(iv) Antimicrobial activity

Requisite quantity of the liquid agar media was poured in the sterile petridish to give a depth of 3 to 4 mm under

the sterile condition. After a solidify the liquid media test organism was spread over the solidified agar media and incubate the petridish at 37°C for 24 hours to grow the microorganism. With the help of the sterile steel rod make a hole on the media and poured the know concentration (30-80ml) test solution in that hole. Repeat the same procedure for the standard drug Chlorhexidine. Measured the diameter of zone of inhibition of the both of the test solution as well as the standard antibiotics solution up to 24 hours.

RESULTS AND DISCUSSION

CLOVE OIL

Organoleptic characteristics

- Colour: crimson to dark brown
- Odour: aromatic
- Taste : pungent and aromatic, numbness.

Morphological characteristics

- Appearance: solid dried flower buds
- Shape: hypanthium with divergent sepals surrounded by dome shaped corolla.

GINGER OIL

Organoleptic characteristics

- Colour: buff coloured.
 - Odour: agreeable and aromatic
 - Taste: pungent and agreeable
- Morphological characteristics
- Appearance: tuberous rhizome.

- Shape: laterally compressed bearing short flat, ovate and oblique branches on the upper side with bud at the apex.

CINNAMON OIL

Organoleptic characteristics

- Colour: the outer surface is dull yellowish-brown while the inner surface is dark yellowish-brown.
- Odour: fragrant
- Shape: found in the form of compound quills
- Size: about 1m in length and 1cm in diameter. The thickness of the bark is approximately 0.5mm.
- Taste: aromatic and sweet followed by warm sensation
- Fracture: splintery.

Physico-chemical properties and palatability

- The pH of the given formulation was determined by using pH meter and it was found to be 6.77
- The viscosity was found to be 1.67
- The palatability test was performed by the blind fold test by three volunteers and it was rated as good, very good and good respectively by the volunteers.

Isothermal stress stability

The formulation of mouth wash was stable at 2:2:1 concentration(oils).

ANTI MICROBIAL ACTIVITY

The antimicrobial activity was tested by using different concentrations of oils which includes:

Table 6: zone of inhibition of 1:1:1 polyherbal formulation: plate1.

| TEST SAMPLE | ZONE OF INHIBITION(against <i>Streptococcus mutans</i>) |
|--|--|
| Ratio of clove, cinnamon, ginger oils. (1:1:1) | 16 mm |
| Chlorhexidine | 20 mm |
| Excipients | No zone of Inhibition |

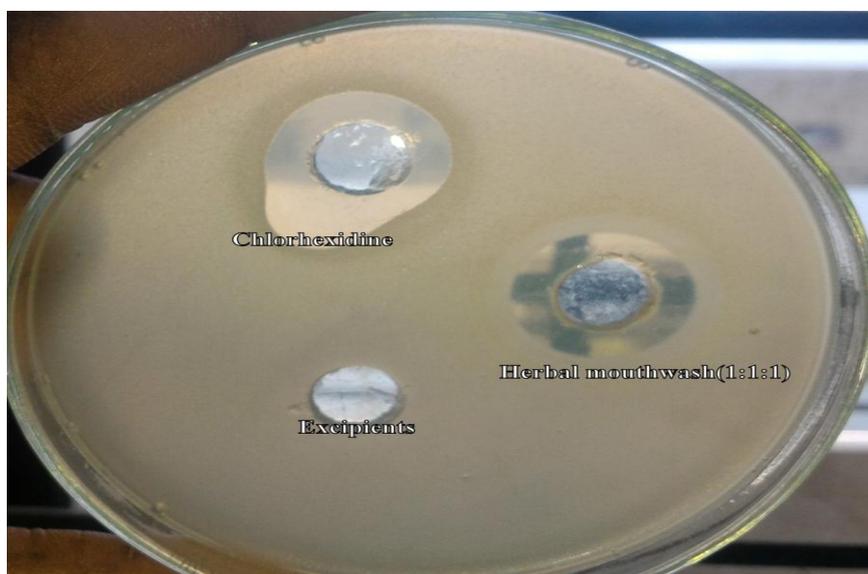


Figure 4: Plate 1: zone of inhibition of 1:1:1 polyherbal formulation.

Table 7: Zones of inhibition of 2:1:1 polyherbal formulation: plate2.

| TEST SAMPLE | ZONE OF INHIBITION (against <i>Streptococcus mutants</i>) |
|--|--|
| Ratio of clove, cinnamon, ginger oils. (2:1:1) | 19mm |
| Chlorhexidine | 20 mm |
| Excipients | No zone of Inhibition |



Figure 5: Plate 2: zone of inhibition of 2:1:1 polyherbal formulation.

Table 8: zones of inhibitions of 2:2:1 polyherbal formulation: plate3.

| TEST SAMPLE | ZONE OF INHIBITION (against <i>Streptococcus mutants</i>) |
|--|--|
| Ratio of clove, cinnamon, ginger oils. (2:2:1) | 26 mm |
| Chlorhexidine | 20 mm |
| Excipients | No zone of Inhibition |



Figure 6: Plate 3: zone of inhibition of 2:2:1 polyherbal formulation.

The formulation with oils ratio of 1:1:1 has shown a zone of inhibition of 16 mm when compared with that of standard chlorhexidine that has shown 20 mm of zone of inhibition, however the sample concentration of 2:1:1 has shown a 19 mm of zone of inhibition, whereas our third concentration of ratio 2:2:1 has shown 26 mm of zone of inhibition which is greater than that of chlorhexidine which was of 20 mm.

CONCLUSION

Commercial formulations of mouthwashes have alcohol and chlorhexidine as the essential ingredients. Alcohol has disadvantages of having questionable carcinogenic property, cause dry mouth and worsen halitosis, whereas chlorhexidine cannot be used in situations of ulcerative gingivitis, also staining the teeth and has other disadvantages like causing overgrowth of entero bacteria in persons with leukemia, desquamation and irritation of oral mucosa, salivary gland pain and swelling, and hypersensitivity reactions. Our herbal mouthwash of concentration (oils) ratio 2:2:1 has greater stability and has shown greater antimicrobial activity when compared with that of chlorhexidine. We were also successful in preparing 100% alcohol free mouthwash that has a potent antibacterial action against *Streptococcus mutants*.

Herbal Mouthwash preparations have potent action and minimal side effects when compared with that of the other marketed mouthwashes, hence there is need for increased usage of herbal preparations to avoid the adverse effects. Thus there is a need to create awareness among prescribers and public about the use of herbal mouth washes and many other such studies should be encouraged.

REFERENCES

1. Matthews, R W, 2003. "Hot salt water mouth baths". British Dent J. 195(1): 3–3.
2. <https://www.wsj.com/articles/there-is-more-to-your-mouthwash-than-a-minty-taste-142109637.htm>.
3. <https://m.medicalxpress.com/news/2015-03-mouthwash.htm>.
4. <http://www.ada.org/en/member-centre/oral-health-topics/mouthrinse.htm>.
5. <http://brushflossandmouthwash.com/should-i-be-using-mouthwash-when-should-i-use-mouthwash/htm>.
6. Cortelli, José Roberto; Thénoux, Raul Emilio de La Sotta, 2007. "The effect of mouth rinses on oral microorganisms" Brazilian Oral Research. 21: 23–28.
7. Singh, Abhinav; Purohit, Bharathi, 2011. "Tooth brushing, oil pulling and tissue regeneration: A review of holistic approaches to oral health". Journal of Ayurveda and Integrative Medicine. 2: 64–68.
8. Fischman, Stuart L, 1997. "The history of oral hygiene products: How far have we come in 6000 years?" Periodontology 2000; 15(1): 7–14.
9. Shifman, A; Orenbuch, S; Rosenberg. M, 2002. "Bad breath—a major disability according to the Talmud". The Israel Medical Association journal. 4(10): 843–5.
10. <https://www.readings.com.au/products/6133341/toxin-the-cunning-of-bacterial-poisons.htm>.
11. Budtz-Jørgensen, E; Løe, H, 1972. "Chlorhexidine as a denture disinfectant in the treatment of denture stomatitis". Scandinavian journal of dental research. 80(6): 457–64.
12. Aichner T., Coletti P, 2013. "Customers' online shopping preferences in mass customization". Journal of Direct, Data and Digital Marketing Practice. 15(1): 20–35.
13. <http://www.thecologist.org/green-green-living/behind-the-label/269558/behind-the-label-listerine-teeth-and-gum-defence.html>.
14. Cole, P; Rodu, B; Mathisen, A (2003). "Alcohol-containing mouthwash and oropharyngeal cancer: A review of the epidemiology". Journal of the American Dental Association. 134(8): 1079–87.
15. Carretero Peláez, MA; Esparza Gómez, GC; Figuero Ruiz, E; Cerero Lapiedra, R, 2004. "Alcohol-containing mouthwashes and oral cancer. Critical analysis of literature". Medicina oral. 9(2): 120–3 116–20.
16. Lachenmeier, Dirk W, 2008. "Safety evaluation of topical applications of ethanol on the skin and inside the oral cavity". Journal of Occupational Medicine and Toxicology. 3(1): 26.
17. <http://www.aapd.org/assets/1/25/Shulman-19-06.pdf>.
18. British National Formulary March 2014 "Mouthwashes, gargles, and dentifrices". MJ Group and the Royal Pharmaceutical Society of Great Britain 2014.
19. Scully C 2013. Oral and maxillofacial medicine: the basis of diagnosis and treatment (3rd ed.). Edinburgh: Churchill Livingstone. pp. 39, 41.
20. Rosenberg, Mel (2002). "The Science of Bad Breath". Scientific American. 286(4): 72–9.
21. <http://www.newyorkorthodontist.net/triclosan-dangers-side-effects-in-tooth-pastes-mouthwashes.htm>.
22. <http://www.aaomp.org/public/docs/leukoplacia.pdf>.