



Received on 17 April 2023; received in revised form, 24 July 2023; accepted 28 July 2023; published 01 December 2023

FORMULATION AND EVALUATION OF ANTIBACTERIAL HERBAL MOUTHWASH AGAINST ORAL DISORDERS

M. Govardhan, B. Hemanth, M. Ricwin, G. K. Yuvan Shankar, K. Yuvaraj and T. P. Karunya *

Department of Biotechnology, Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College, Chennai - 600062, Tamil Nadu, India.

Keywords:

Oral bacteria, Herbal remedy, Bioactivity, Mouthwash, Healthy practice

Correspondence to Author:

T. P. Karunya

Research Scholar,
Department of Biotechnology,
Vel Tech High Tech Dr. Rangarajan
Dr. Sakunthala Engineering College,
Chennai - 600062, Tamil Nadu, India.

E-mail: karunya.tp17@gmail.com

ABSTRACT: The current study aims to develop and evaluate herbal mouthwash and ascertain its effectiveness against microbes. Conventionally herbal mouthwashes are used as a disinfectant to lessen the presence of microbes in the oral cavity. Herbal mouthwashes act as a preventative agent for bleeding gums, decoloring teeth, and killing off bad breath germs. The herbal mouthwashes also have inherent benefits of plaque control and inflammation. The formulated mouthwash was prepared using herbs and the extract was extracted by hot water extraction method. Additional analysis of the Phyto ingredients was done where the flavonoid content and phenolic content was estimated as 41.71µg/ml and 76.25µg/ml, respectively and the *in-vitro* antibacterial assay was performed against *Streptococcus mutans* and *Streptococcus salivarius* and the zone of inhibition was calculated as 18mm and 22 mm at the highest concentration. The results indicated that the mouthwash exhibits effective antibacterial qualities. These solutions can be used for various things, including reducing the growth of germs in the oral cavity and for their analgesic, anti-inflammatory, or antifungal effects.

INTRODUCTION: Around the globe, 70–100% of people have gingivitis, which is directly related to plaque. When plaque is controlled, gingival can be remedied ¹. But if oral hygiene isn't prioritized, it can advance and significantly affect the whole gingival anchoring network of the gum tissue, which can cause gingivitis, loss of teeth, and a decreased quality of life, among other adverse consequences ². Thus, controlling plaque effectively is crucial to treating and preventing gingivitis and other similar symptoms ³.

Oral diseases have a long history of being treated with natural remedies. Periodontal disease is basically caused by microorganisms contained in dental plaque. Studies have linked these microorganisms, especially those with adherent biofilm properties, to clinically specific oral diseases such as dental caries, periodontitis, and halitosis ⁴. Individual susceptibility to dental and periodontal disease depends on complex risk factors. Genetics, systemic factors, diet, oral hygiene, *etc.* ⁵.

Clinical management of these diseases is most easily achieved by reducing the oral microbial load on the plaque-biofilm ⁶. With the advent of antibiotics and their cognition that bacteria are potential causative agents of major dental diseases, caries, and periodontitis, plaque reduction has become a hallmark of preventive dentistry ⁷. Plaque

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.14(12).5725-33</p> <hr/> <p>This article can be accessed online on www.ijpsr.com</p> <hr/> <p>DOI link: https://doi.org/10.13040/IJPSR.0975-8232.14(12).5725-33</p>
---	--

reduction Chemical and mechanical oral hygiene aids are used for removal and prevention. Mechanical plaque control agents such as toothbrushes, dental floss, toothpicks, and interdental brushes are very popular and often used with chemical plaque control agents⁸. Although the toothbrush is the most widely used oral hygiene tool, a large portion of the population cannot effectively remove mechanical plaque. Therefore, chemical plaque control is required⁹. Therefore, chemical plaque reduction methods such as mouthwash are attractive as they may offer significant benefits to patients who cannot maintain adequate mechanical plaque control. They can be regarded as technically fewer demanding tools for mechanical control⁸.

Brushing is currently the most common self-administered oral hygiene method for mechanical plaque removal. However, this mechanical approach is often insufficient in most people, suggesting that chemical plaque control with mouthwash may be beneficial as an adjunct to daily oral care¹⁰. Chlorhexidine (CHX), a broad-spectrum fungicide, is considered the gold standard for chemical plaque control. Still, CHX mouthwash can cause discoloration of teeth and tongue, taste disorders, and adverse effects on the oral mucosa. These unwanted side effects limit the long-term use and patient acceptance of CHX mouthwashes. The search for alternatives continues, shifting the focus to bio-derived active ingredients¹¹.

Over the counter, various types of mouthwash are available, but chlorhexidine is the most popular. It is recognized as the leading chemical plaque control agent, and its clinical efficacy is well known in the art. In recent years immense use of CHX in the perioperative period has had adverse side effects on oral cavities¹². Chlorhexidine has also reported an ancillary effect on mouth gums, impairment of taste, tooth staining, and mucous¹³. One more contemporary problem is the chemical-based Chlorhexidine mouthwashes which consist of chemicals such as ethyl alcohol or ethanol¹⁴. The latest studies have illustrated that the high usage of alcohol in mouthwash can lead to oral cancer. Additional side effects have been associated with the use of ethanol in CHX, such as dry mouth feeling, burning mouth syndrome, and dysgeusia¹⁵.

In the Indian traditional form of medicine in Ayurveda and Siddha, several species and herbs have been reported to exhibit medicinal properties such as antithrombotic, antiatherosclerotic, hypolipidemic, hypoglycemic, anti-inflammatory, antiarthritic, *etc.*¹⁶.

Indian spices have grown popular as a biotherapy in both the developed and developing worlds, with individual spices like cinnamon, clove, peppermint, and curcumin being used in immune system management and antimicrobial therapy. Though spices have remarkable bioactivity, research on them is limited¹⁷.

From time immemorial, plants have provided food, cosmetics, antiseptic ointments, and many readily available remedies for human ailments. It has been used as a source of medicine by humans for centuries due to the presence of active compounds¹⁸. As the popularity of these herbal products continues to grow, dentists are becoming increasingly concerned about the safety and effectiveness of these products. It is expected to provide patients with information about their sexuality¹⁹. However, this can be difficult as no expert consensus exists on the subject. To date, Asia, especially India and other Southeast Asian countries where these products are most popular and widely used, have reported insufficient clinical studies on herbal mouthwashes and dentifrices²⁰.

Herbal medicines derived from plant sources have long been used in dentistry to inhibit microorganisms, reduce inflammation, soothe irritation, and relieve pain. A number of herbal mouthwashes have recently been reported to have promising results in controlling plaque and gingivitis²¹. Herbal mouthwashes are designed and manufactured using extracts and essential oils of Phytotherapeutic plants containing a blend of active ingredients such as catechins, tannins and sterols²². Mixing substances usually has a mild therapeutic effect. Compared to the antimicrobial mechanisms provided by synthetic chemicals, herbal mouthwashes may have additional anti-inflammatory and antioxidant properties that may further benefit gum health²³. Many herbal mouthwashes have been introduced and tested. However, results in the existing literature are conflicting regarding the clinical efficacy of herbal

mouthwashes on both plaque and gingivitis compared with placebo or CHX, suggesting that the overall effect of emphasizing herbal mouthwashes is consistent²⁴. Little meta-analytical evidence supports its efficacy as a supplement to daily self-administered oral hygiene in patients with gingivitis. Without this information, it is not possible to provide comprehensive evidence-based advice to patients and practitioners²⁵.

MATERIALS AND METHODS:

Collection of Leaves: The leaves of betel (*Piper betle*) and Peppermint (*Mentha piperita*) were aimlessly gathered from a well-grown plant and authenticated by Siddha Central Research Institute,

and the plant authentication number is P04072301B.

Extraction Process: Shade drying was done for 15 days to avoid chemical degradation due to sunlight. The leaves were grinded with a grinder and converted into coarse powder. The leaf powder was boiled in sterilized water for 15 minutes by keeping it in the water bath (10g/100ml). The liquid thus obtained was filtered using Whatman paper²⁶.

Formulation of Herbal Mouthwash: The herbal mouthwash was formulated using **Table 1** and then all the other extracts were blended in different ratios.

TABLE 1: FORMULATION OF HERBAL MOUTHWASH

S. no.	Ingredients	Botanical Name	Role	Quantity
1.	Peppermint	<i>Mentha piperita</i>	Anti-bacterial and anti-microbial	40 ml
2	Betel	<i>Piper beetle</i>	Anti-septic and Breath freshener	40 ml
3	Clove oil	<i>Syzygium aromaticum</i>	Anti-septic and analgesic	2 ml
4	Peppermint oil	--	Anti-inflammatory	2 ml

Preparation: 40 ml of each extract (peppermint extract and betel leaf extract) were mixed with 2 ml of clove oil and pepper mint oil the sample thus prepared was now kept in two volumetric flasks at two varied temperatures, one at ambient temperature and the other at low temperature. Thus, the prepared herbal mouth wash was subjected to further evaluation.

Phytochemical Analysis: The aqueous extracts of the plant sample were evaluated for the presence of Alkaloids, Steroids, Tannins, Flavonoids, Terpenoids, Saponins, Cardiac Glycosides and Phenols by the phytochemical analysis techniques²⁷.

Alkaloids: About 2ml of extract was taken and 2 drops of Mayer's reagent were added. The formation of a creamy or white precipitate indicates the positive result.

Steroids: 2ml of the extract was taken to which 2ml of chloroform and 2ml of concentrated H₂SO₄ was added. The appearance of red in the upper layer and a yellowish green fluorescence in the lower layer indicates positive results for steroids.

Tannins: For Tannins analysis 5ml of extract was taken and few drops of neutral 5% ferric chloride solution was added. Appearance of dark green colour indicates positive result.

Flavonoids: For Flavonoids analysis, drops of 20% NaOH were added to 2ml of the extract. The yellow color disappears on the addition of concentrated hydrochloric acid, indicating a positive result.

Terpenoids: To a 3ml of extract, 1ml of chloroform and 1.5ml of concentrated sulphuric acid was added to the sides of the test tube. Positive result is denoted by the presence of reddish-brown colour in the interface.

Saponins: 5ml of the extract was take ninatest-tube and 1ml of distilled water was added and shaken vigorously, the formation of forth indicates positive result for Saponins.

Cardiac Glycosides: To 5ml of the extract, 2ml of glacialacetic acid was added and a dropwise ferric chloride solution was added to it. Then 1ml of concentrated sulphuric acid was added. The formation of brown ring indicated a positive test for the presence of deoxy sugar of cardenolides. Formation of violet ring beneath the brown layer and in the acetic layer green ring might appear.

Phenols: To a 2ml of extract, 5% alcoholic ferric chloride was added. Appearance of blue colour indicates positive result for phenols.

Quantitative Analysis:

Total Phenolic Content: The total Phenolic compound of extract was evaluated by Folin-Ciocalteu method. The standard Gallic Acid curve was built by preparing dilution in methanol from the standard solution of gallic acid. 100 µl of each dilution were mixed with 500µl of distilled water and the Folin-Ciocalteu reagent of 100µl was added and allowed to stand still for about 6 minutes. After that, 1ml of 7% Na₂CO₃ and 500µl of distilled water was added to the mixture. The reaction mixture was incubated for 90 min, and the absorbance was determined at 760nm. The total phenolic content of the extract was calculated as gallic acid equivalents (mgGAE/g). The standard gallic acid curve and regression equation is used to measure the total phenolic content in the extracts²⁸.

Total Flavonoid Content: The Aluminum chloride complex forming procedure was used to determine the total flavonoid content. Flavonoid content was measured as quercetin equivalent, where Quercetin was employed as standard. A calibration curve was constructed for evaluation. The solution with varying dilution was prepared in methanol. Where 100 µl of each dilution was mixed with 500µl of water, followed by 100µl of 5 % NaNO₃ and the reaction mixture was allowed to stand still for 6 minutes. After 6 minutes 150 µl of 10% Aluminium chloride solution was admitted to sit for 5 minutes after that 200 µl of 1 MNaOH solution was added slowly. The absorbance was measured at 510 nm using spectrophotometer and the total flavonoid content was expressed as quercetin equivalent (mgQE/g)²⁹.

Examination of Herbal Mouth Wash Odour and Color: The physical parameter of the extract such as odour and color were visually examined.

pH: The pH of the prepared herbal mouth rinses was measured using a Digital pH meter. A standard buffer was used to calibrate the pH meter. About 1ml of mouth wash was weighed, dissolved in 50ml of distilled water, and its pH was measured.

Test for Microbial Growth in Formulated Mouth Wash: A control was prepared by inoculating an agar medium plate with a mouthwash containing by the streak plate method.

The plate was placed in an incubator and incubated at 37 degree for 24 hours. After the incubation period, the plates were removed and checked for microbial growth by comparing it with control²².

In-vitro Antibacterial Activity: *In-vitro* antibacterial activity was carried out for both formulated herbal mouth wash and commercially available mouthwash in the market. The activity was performed on isolated strain of streptococcus mutans. The agar well diffusion method was used to determine zones of inhibition and minimum inhibitory concentrations (MIC). Readymade blood agar plates were used to inoculated streptococcus strains. 5mm agar well cutters were used to cut five wells in the dried plates³⁰. In order to allow the inert diffusion of the formulated herbal mouthwash, the agar plates were stored properly at 37° for 24 hours. The zones of inhibition were calculated inmm, the same procedure was carried out for commercially available mouthwash, and zones of inhibition (ZOI) were calculated in mm³¹.

RESULTS:

Collection of Leaves and Extraction Process: The betel leaves (*Piper beetle*) were collected from freshly from the garden and were shaded dried for 15 days and the dried leaves were powdered finely and the extract was obtained by hot water extraction.

**FIG. 1: BETEL LEAF****FIG. 2: SHADED DRIED LEAF**



FIG. 3: COARSE POWDER



FIG. 4: BEETLE LEAF EXTRACT

Formulation of Herbal Mouth Wash: Herbal mouthwash was formulated by using **Table 1** and the prepared mouthwash was thus subjected to phytochemical analysis.

steroids showed a negative result, and the extract tested positive result for Flavonoids, saponins, tannins, phenols and glycosides where the flavonoid³² **Table 2.**



FIG. 5: FORMULATED MOUTH WASH

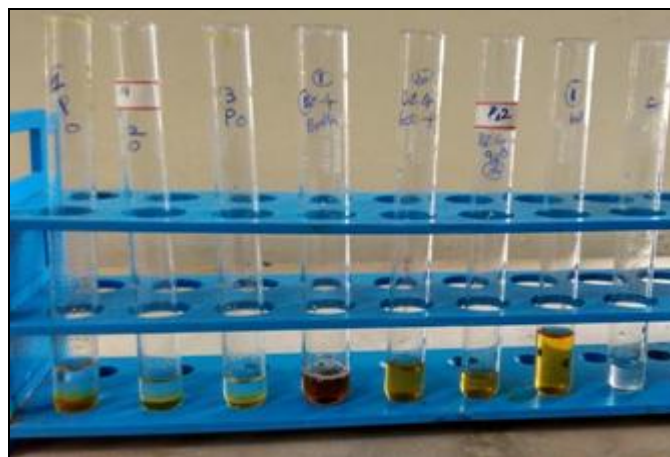


FIG. 6: PHYTOCHEMICAL ANALYSIS OF BEETLE LEAF EXTRACT

Phytochemical Analysis: The phytochemical assay was carried out for the formulated herbal mouthwash where alkaloids, terpenoids, and

TABLE 2: PHYTOCHEMICAL ANALYSIS OF BEETLE LEAF EXTRACT

Sample	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Terpenoids	Glycosides	Steroids
Extract	-	+++	+++	+++	+++	-	++	-

Quantitative Analysis:

Total Flavonoid Content: The Total flavonoid content is expressed in mg of Quercetin equivalent per gram of extract³³. The color change in the sample indicates the presence of flavonoids. From the standard graph Fig. 7, we can calculate the value of the concentration of total flavonoids present in the sample was 41.71µg/ml.

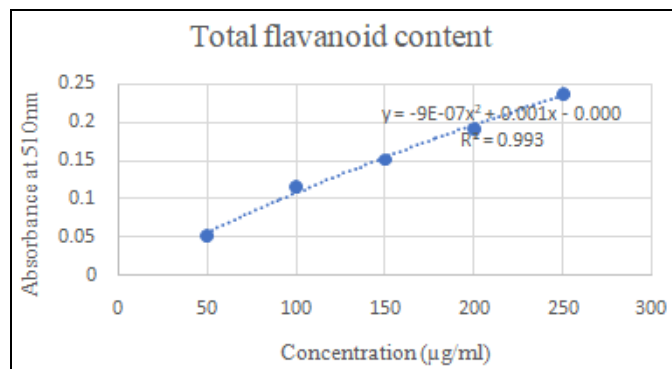


FIG. 7: STANDARD QUERCETIN GRAPH

TABLE 3: TOTAL FLAVONOID CONTENT IN EXTRACT

S. no.	Sample	OD of Extract	Total Flavonoid Content (µg/ml)
1	Formulated Mouth Wash	0.052	41.71

TABLE 4: TOTAL PHENOL CONTENT IN EXTRACT

S. no.	Sample	OD of Extract	Total Phenolic Content (µg/ml)
1	Formulated Mouth Wash	0.052	76.25

Examination of Herbal Mouth Wash: The formulated mouth was found to be 6.1 pH. Since, the skin has an acidic pH of roughly 5.5, this formulation's pH range is suited for mouth conditions.

Heavy metals were discovered to be absent from the formulation. This mouthwash is made entirely from herbs and contains no alcohol or other additives like other brands on the market³⁵.

Test for Microbial Growth in Formulated Mouth Wash: Beetle Leaf, Peppermint leaves, clove oil, and other vital plant extracts have been reported to reduce plaque and gingivitis when used as antibacterial ingredients in mouthwashes. Volatile sulfur compounds are the main cause of

Total Phenol Content: The above procedure examines the amount of phenolic content in the sample. The Phenolic Content in the mouthwash have reducing oxygen properties, and these activities provide them to act as an antioxidant³⁴.

The result is expressed in mg GAE/g. The maximum phenolic content in the extract is accountable for intro vitro studies; therefore, this extract is expected to exhibit acceptable results in bioactivities. The total phenolic content was 76.25µg/ml.

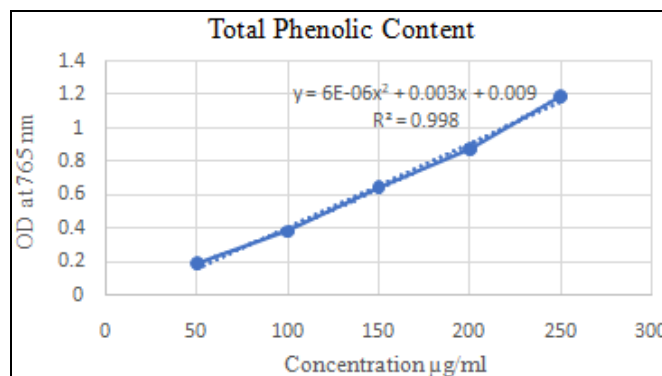


FIG. 8: STANDARD GALLIC ACID GRAPH

foul breath in humans. They come from a number of sources, including food breakdown, tooth plaque, and microorganisms linked to oral diseases³⁶.

In-vitro Antibacterial Activity: To examine the zone of inhibition and minimum inhibitory concentration well diffusion method was used with various mouthwash concentrations. The resultant ZOI for streptococcus strains was 18mm for 50µl and 9mm for 10µl and for *Streptococcus salivarius* the zone of inhibition was 22mm for 50µl and 12mm for 10 µl. These results indicated the plant mouthwash's significant antibacterial activity and the current product's capability to prevent bacterial growth in the oral cavity³⁰.

TABLE 5: ANTIBACTERIAL ASSAY RESULT

S. no.	Concentration	ZOI (Zone of Inhibition)	
		<i>Streptococcus mutans</i>	<i>Streptococcus salivarius</i>
1.	10 µl	9mm	12 mm
2.	20 µl	11mm	17 mm

3.	30 μ l	14 mm	20 mm
4.	40 μ l	16 mm	22 mm
5.	50 μ l	18 mm	22mm

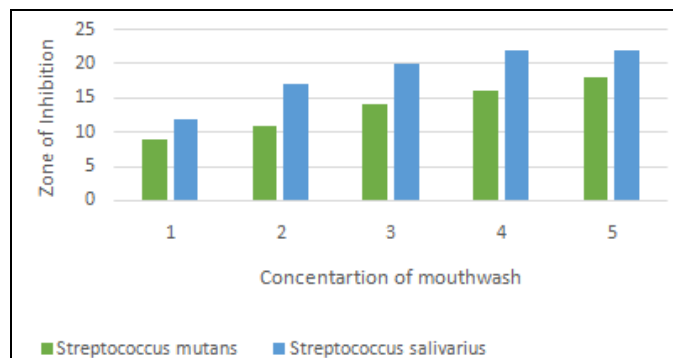


FIG. 9: IN-VITRO ANTIBACTERIAL ASSAY RESULT

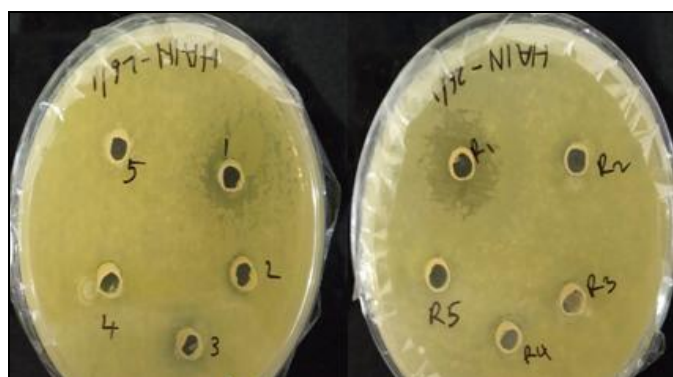


FIG. 10: ANTIBACTERIAL ASSAY I) *STREPTOCOCCUS MUTANS* II) *STREPTOCOCCUS SALIVARIUS*

DISCUSSION: Herbal mouthwashes can cover up unpleasant odours and offer a distinctive flavour. Mouthwashes with herbs may have therapeutic substances like antimicrobials efficient for long-term odour control³⁷.

The burbling of the liquid which is caused due to the wind down in the exhalation of air. Gargling with herbal formulations is safe because they have no negative side effects when ingested in trace amounts through the system³⁸. The phytochemical analysis **Table 2** confirmed the presence of Phyto ingredients responsible for the antibacterial activity³⁹. The amount of phenolic content was determined as 76.25 μ g/ml and flavonoid content was determined as 41.71 μ g/ml, which were accountable for the bioactivity of the formulated mouthwash⁴⁰. The *in-vitro* antibacterial test in **Fig. 10** confirmed that the formulated herbal mouthwash possessed good antibacterial activity.

CONCLUSION: The current aqueous herbal mouthwash may be effective in assisting

individuals to get rid of foul breath and other oral health issues. Furthermore, this preparation's absence of dangerous components gives us peace of mind and relaxation. The findings of the physicochemical examination show that the current herbal formulation's colour and aroma are acceptable, with a pleasant odour and better lasting effects.

The zone of inhibition analyses further supported the findings that this herbal mouthwash was a powerful plaque regulator. Patients favoured it for its taste, ease of use, and test duration in the mouth after washing. They can be employed as a supplement to physical treatment to treat enamel gingivitis. The current study has a massive effect on efforts to develop an herbal oral health intervention for low social and economic populations that is both efficient and affordable. However, since this research was brief, more studies with bigger sample sizes are necessary.

ACKNOWLEDGMENTS: The authors would like to thank the Department of Biotechnology and the college for their immense support during the entire Laboratory studies

CONFLICTS OF INTEREST: The authors have no conflicts of interest regarding this investigation.

REFERENCES:

1. Cai H, Chen J, Panagodage Perera NK and Liang X: Effects of herbal mouthwashes on plaque and inflammation control for patients with gingivitis: A systematic review and meta-analysis of randomised controlled trials. Evidence-Based Complement Altern Med 2020; 2020.
2. Peres MA, Macpherson LMD and Weyant RJ: Oral diseases: a global public health challenge. Lancet 2019; 394(10194): 249-260.
3. Hugoson A, Sjödin B and Norderyd O: Trends over 30 years, 1973--2003, in the prevalence and severity of periodontal disease. J Clin Periodontol 2008; 35(5): 405-414.
4. Li Y, Jiang X, Hao J, Zhang Y and Huang R: Tea polyphenols: application in the control of oral microorganism infectious diseases. Arch Oral Biol 2019; 102: 74-82.
5. Sedghi L, DiMassa V, Harrington A, Lynch SV and Kapila YL: The oral microbiome: Role of key organisms and complex networks in oral health and disease. Periodontol 2000. 2021; 87(1): 107-131.

6. Priyal G, Jose M, Nayak S, Pai V and Prabhu S: Evaluation of efficacy of different tooth paste formulations in reducing the oral microbial load-An *in-vivo* study. *Biomedicine* 2021; 41(2): 465-471.
7. Stájer A, Kajári S, Gajdács M, Musah-Eroje A, Baráth Z. Utility of photodynamic therapy in dentistry: Current concepts. *Dent J* 2020; 8(2): 43.
8. Vyas T, Bhatt G, Gaur A, Sharma C, Sharma A and Nagi R: Chemical plaque control-A brief review. *J Fam Med Prim Care* 2021; 10(4): 1562.
9. Sälzer S, Graetz C, Dörfer CE, Slot DE and der Weijden FA: Contemporary practices for mechanical oral hygiene to prevent periodontal disease. *Periodontol 2000* 2020; 84(1): 35-44.
10. Bunk D, Eisenburger M, Häckl S, Eberhard J, Stiesch M and Grischke J: The effect of adjuvant oral irrigation on self-administered oral care in the management of peri-implant mucositis: A randomized controlled clinical trial. *Clin Oral Implants Res* 2020; 31(10): 946-958.
11. Alshahrani S, Alshuaibi A, Alkhaldi M and Koppolu P: Perception and Knowledge of Patients from Different Regions in the Kingdom of Saudi Arabia towards Oral Hygiene and Oral Hygiene Aids. In: *Healthcare* 2021; 9: 592.
12. Al-Maweri SA, Nassani MZ and Alaizari N: Efficacy of aloe vera mouthwash versus chlorhexidine on plaque and gingivitis: A systematic review. *Int J Dent Hyg* 2020; 18(1): 44-51.
13. Leiva-Cala C, Lorenzo-Pouso AI and Centenera-Centenera B: Clinical efficacy of an Aloe Vera gel versus a 0.12% chlorhexidine gel in preventing traumatic ulcers in patients with fixed orthodontic appliances: a double-blind randomized clinical trial. *Odontology* 2020; 108: 470-478.
14. Felix-Cuencas L, Delis-Hechavarria E and Jarro A: Bioactivity characterization of herbal molecules. In: *Herbal Biomolecules in Healthcare Applications*. Elsevier 2022; 145-183.
15. Stornetta A, Guidolin V and Balbo S: Alcohol-derived acetaldehyde exposure in the oral cavity. *Cancers (Basel)* 2018; 10(1): 20.
16. Fazil M and Nikhat S: Therapeutic and palliative role of a unani herbal decoction in COVID-19 and similar respiratory viral illnesses: Phytochemical & pharmacological perspective. *J Ethnopharmacol* Published online 2022; 115526.
17. Kumar V: Retracted Article: Seven spices of India from kitchen to clinic. *J Ethn Foods* 2020; 7(1): 1-16.
18. Chauhan L and Gupta S: Creams: A review on classification, preparation methods, evaluation and its applications. *J drug Deliv Ther* 2020; 10(5): 281-289.
19. Mukherjee PK: Quality Control and Evaluation of Herbal Drugs: Evaluating Natural Products and Traditional Medicine. Elsevier 2019.
20. Biswas D, Nandy S, Mukherjee A, Pandey DK and Dey A: *Moringa oleifera* Lam. and derived phytochemicals as promising antiviral agents: A review. *South African J Bot* 2020; 129: 272-282.
21. Jethawa S, Gopale O and Shelke S: Herbal mouthwash: A Review. *Res J Pharm Dos Forms Technol.* 2022; 14(3): 217-223.
22. Yadav AR, Mohite SK and Magdum CS: Preparation and evaluation of antibacterial herbal mouthwash against oral pathogens. *Asian J Res Pharm Sci* 2020; 10(3).
23. Jena S, Kumar G, Singh DK and Sethi AK: others. Herbs used in dentistry: Need of the new era. *J Prim Care Dent Oral Heal* 2021; 2(1): 11.
24. Moghadam ET, Yazdani M and Tahmasebi E: Current herbal medicine as an alternative treatment in dentistry: *In-vitro*, *in-vivo* and clinical studies. *Eur J Pharmacol* 2020; 889: 173665.
25. Dubar M, Delatre V, Moutier C, Sy K and Agossa K: Awareness and practices of general practitioners towards the oral-systemic disease relationship: A regionwide survey in France. *J Eval Clin Pract* 2020; 26(6): 1722-1730.
26. de Alba SL, Garcí\`ia-González C, Coronado Ortega MA, Ayala Bautista JR, Alp\`irez GM and Montes Núñez DGL: Extraction Methods and Applications of Bioactive Compounds from Neem (*Azadirachta indica*): A Mini-Review. *Mini Rev Org Chem* 2023; 20(7): 644-654.
27. Tambe BD, Pedhekar P and Harshali P: Phytochemical screening and antibacterial activity of *Syzygium cumini* (L.) (Myrtaceae) leaves extracts. *Asian J Pharm Res Dev* 2021; 9(5): 50-54.
28. Molole GJ, Gure A and Abdissa N: Determination of total phenolic content and antioxidant activity of *Commiphora mollis* (Oliv.) Engl. resin. *BMC Chem* 2022; 16(1): 48.
29. Phuyal N, Jha PK, Raturi PP and Rajbhandary S: Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. *Sci World J* 2020; 2020.
30. Patil SS, Yadav AR, Chopade A and Mohite S: Design, development and evaluation of herbal mouthwash for antibacterial potency against oral bacteria. *J Univ Shanghai Sci Technol* 2020; 22(11): 881-898.
31. Samal HB, Boyeena L, Patra NC, Sriram S and Das IJ: Curcumin *in-situ* gel for local treatment of periodontitis: preparation, *in-vitro* evaluation and clinical assessment. *Indian Drugs* 2022; 59(11).
32. Nigam D, Verma P and Chhajer M: Formulation and Evaluation of Herbal Mouthwash against Oral Infections Disease. *Int J Pharm & Life Sci* 2020; 11(7).
33. Sharma H, Maheshwari P, Khantal A and Sethiya P: Formulation and Evaluation of *Orchis laxiflora* L. Antibacterial Mouthwash. *Int J Pharm & Life Sci.* 2021; 12(11).
34. Keshavarz Mirzamohammadi H, Modarres-Sanavy SAM, Sefidkon F, Mokhtassi-Bidgoli A and Mirjalili MH: Irrigation and fertilizer treatments affecting rosmarinic acid accumulation, total phenolic content, antioxidant potential and correlation between them in peppermint (*Mentha piperita* L.). *Irrig Sci* 2021; 39: 671-683.
35. Kerdar T, Rabienejad N, Alikhani Y, Moradkhani S and Dastan D: Clinical, *in-vitro* and phytochemical, studies of *Scrophularia striata* mouthwash on chronic periodontitis disease. *J Ethnopharmacol* 2019; 239: 111872.
36. Arumugam B, Subramaniam A and Alagaraj P: A Review on Impact of Medicinal Plants on the Treatment of Oral and Dental Diseases. *Cardiovasc & Hematol Agents Med Chem (Formerly Curr Med Chem & Hematol Agents)* 2020; 18(2): 79-93.
37. Sharmeen JB, Mahomoodally FM, Zengin G and Maggi F: Essential oils as natural sources of fragrance compounds for cosmetics and cosmeceuticals. *Molecules.* 2021; 26(3): 666.
38. Sharad S and Kapur S: Indian herb-derived phytoconstituent-based antiviral, antimicrobial and antifungal formulation: an oral rinse candidate for oral hygiene and the potential prevention of COVID-19 outbreaks. *Pathogens* 2021; 10(9): 1130.
39. Abdulrasheed M, Ibrahim IH and Luka A: Antibacterial effect of Cinnamon (*Cinnamomum zeylanicum*) bark

extract on different bacterial isolates. J Environ Microbiol Toxicol 2019; 7(1): 16-20.

40. Goud NS and Prasad G: Antioxidant, antimicrobial activity and total phenol and flavonoids analysis of *Sambucus*

nigra (elderberry). Int J Curr Pharm Res 2020; 12(1): 35-37.

How to cite this article:

Govardhan M, Hemanth B, Ricwin M, Shankar GK, Yuvaraj K and Karunya TP: Formulation and evaluation of antibacterial herbal mouthwash against oral disorders. Int J Pharm Sci & Res 2023; 14(12): 5725-33. doi: 10.13040/IJPSR.0975-8232.14(12).5725-33.

All © 2023 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)