

Research on Formulation and Evaluation of Antibacterial Herbal Mouthwash

Vedika D. Bihade¹, Rajlaxmi Deolekar², Vishakha Sahare³, Ashwini Shinde⁴

New Montfort Institute of Pharmacy, Ashti, Wardha, Maharashtra
vedikabihade31@gmail.com

Abstract: *The objective of present work is to formulate and evaluate herbal mouthwash and to evaluate its effectiveness against microbial load of oral cavity. The plant materials were collected and extracted for water soluble ingredients. Prepared mouthwash was further evaluated for its physicochemical properties and antimicrobial activity. The present mouthwash possesses a good antibacterial property. The results of stability study also confirm the effectiveness of preparation. Present mouthwash is a liquid preparation which normally contains antibacterial and antiseptic agents. Commercially available mouthwash formulations are often medicated and contain chemicals that may elicit adverse effects to the users. A mouthwash may be recommended for its efficacy in preventing dental caries other oral conditions due to its antimicrobial, anti-inflammatory properties*

Keywords: mouthwash

I. INTRODUCTION

Human beings are the creations of nature. Nature provides us everything what is needed for our survival. Although herbs had been known for their medicinal, flavoring, and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance for a while.

Mouthwash is an antiseptic aqueous solution used to clean the mouth and teeth or freshen up the breathe¹ It is most often used for control of plaque and other dental problems.² Mouthwash is a term that refers to a liquid preparation, typically antiseptic, used to clean teeth and mouths or to refresh breath. Dentistry frequently recommends mouthwash for the prevention and management of a number of oral disorders. Recently, there has been a significant increase in the usage of naturally occurring items, also known as "grandmother's remedy." This has prompted the development of more modern mouthwashes, but this study examines whether they compare favourably to the gold standard or are even superior to it. Due to their antioxidant and antibacterial properties, spices like clove, mint, neem. 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 halitosis³ Individual susceptibility to dental and periodontal disease depends on complex risk factors. Genetics, systemic factors, diet, oral hygiene, etc.⁴ Maintenance of good oral hygiene is the key to the prevention of dental diseases. The primary etiological factor for dental diseases is dental plaque. The formation of plaque on the tooth surface is characterized by the progression from a limited number of pioneer microbial species to the complex flora of mature dental plaque. This progression involves initial adherence of bacteria to the salivary pellicle and subsequent accumulation by growth and inter-bacterial adherence. Ultimately, the tooth surface gets coated with a dense, complex micro-community that ends up in the destruction of hard enamel tissue.⁽⁵⁾ In India as in other developing countries, a very significant proportion of dental problems are due to microbial infections. Dental problems are of three types, formation of dental plaques, dental caries and periodontal diseases.⁽⁶⁾ The use of plants for treating diseases is as old as the human civilization. There are many plants which have been in use as traditional medicine, so they are called as medicinal plants. The use of plants for curing diseases was inevitable as is already proven by seeing the problems associated with synthetic antibiotics.⁽⁷⁾ Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids found to have antimicrobial properties. Many of the spices and herbs used today have been valued for their antimicrobial activity in addition to their flavor and fragrance properties.⁽⁸⁾ Natural mouthwashes may offer significant advantages over the

chemical ones. If such mouthwashes can be formulated which can be easily prepared and used safely by people at home using natural products, it may lead to improvement in the general dental health of the population.(9) Mouthwash is an aqueous solution which is most often used for its deodorant, refreshing and antiseptic properties or for control of plaque. It may contain alcohol, glycerin, synthetic sweetness, surface active agents, flavoring agents, colouring agents, etc. This can vary from breath fresheners to treatment of life threatening secondary infections such as oral mucositis in patients undergoing bone marrow transplant therapy. The use of mouthwashes requires a correct diagnosis of the oral condition and a thorough knowledge of the product to achieve effective treatment. Major side effects for some marketed brand are: Skin irritation, Allergic Skin Reaction, Redness of skin, Acneiform eruptions, Thyroid imbalances, Tooth/tongue staining, Increased tartar, Mouth/throat irritation, Dry mouth, Unusual or unpleasant taste in your mouth, Decreased taste sensation, Tongue swelling, Gingivitis.(10) To overcome all this side effects, Herbal Mouthwash can be formulated. Herbal mouthwash contains a natural ingredients called phytochemical that contains desired anti-microbial and anti-inflammatory effect. Herbal mouthwash becomes more popular they work without alcohol, artificial preservatives, flavor, or colors.(11) As it contains natural herbs that have natural cleansing and healing property to teeth and gums. Many herbal mouthwashes contain herbs with anti-microbial property such as Neem, tulsi etc. Usually, the traditional mouthwash comprises of the high alcoholic content. It can cater the harm to your teeth and gums. The lining of your mouth gets irritated using these. Even, if you have the sensitive gums, then you may encounter with the ache. While the natural mouthwash doesn't contain any alcoholic residue and is gentler on the mouth. So, it is suggested to use the natural or alcohol-free mouthwash.(12) Natural mouthwashes may offer significant advantages over the chemical ones. If such mouthwashes can be formulated which can be easily prepared and used safely by people at home using natural products, it may leads to improvement in the general dental health of the population. In this study the various natural ingredients and materials are used. Herbal mouthwash becomes more popular they work without alcohol, artificial preservatives, flavor, or colors(13) Some of the herbs that are used in mouthwashes are clove, which is traditionally used for oral health because of their antiseptic, antibacterial, and antiviral property, peppermint which gives cooling effect to the mouth, plantain has ability for speed wound healing and many of the herbs contains anti-microbial, anti-inflammatory, antioxidants, antiseptic properties such as neem, clove, tulsi, etc(14) Herbal mouthwashes are gentle enough for daily use and provide less abrasive alternative to more potent prescription formulations meant for short term use. Herbal mouthwashes do not contain any alcohol and other preservatives that cause dry mouth. Herbal mouthwashes are suitable for oral prophylaxis. As it contains herbs and its extracts, that can maintain good oral hygiene without causing any toxic effect to our body. For daily use, herbal mouthwashes are more preferable than chlorhexidine mouthwash considering its side effects on long term usage.(15) Mouth washes have the ability to deliver the therapeutic ingredients and ingredients to access against the organism present on the surface of the mouth. The role of junk foods in affecting the oral cavity of an individual is high and unavoidable. The foods like Candies, chocolates, jellies and jams have high sugar content the children and adolescents are usually prone to consume this kind of sugar products but, the sugar content possess insoluble glucan which gets attached to the enamel of the tooth resulting in the formation of cavity in tooth. The mouth washes are concentrated aqueous anti-bacterial solution that are used against oral microbes to counter oral infection, cleansing, to get rid of bad breath refreshing ,anti-septic .The mouthwash plays a prominent role in the oral hygiene of an individual ,it helps to relieve symptoms of inflamed gums gingivitis. And also it reliably used to destruct the pathogenic germs. The mouth washes are used by most of the dental patients to overcome sour mouth, ulcerated throat and sensitive teeth. Herbal mouthwash made from natural sources is used in periodontal therapy, controlling bleeding, inflammation. They are also used to control caries, reduce plaque and prevent oral infection. Daily use of mouthwash helps control dental plaque, mainly by limiting further adhesion of oral bacteria and plaque's uncontrolled development.(16) Human beings are the creations of nature. Nature provides us everything what is needed for our survival. Although herbs had been known for their medicinal, flavoring, and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance.(17) Ideally, it is required that any antimicrobial/antiseptic agent used should be able to modify the oral environment by being specifically effective against pathogens.(18)Mouthwash is an aqueous solution which is most often used for its deodorant, refreshing and antiseptic properties or for control of plaque.(19) Maintenance of oral hygiene is imperative in preventing the buildup of plaque, a sticky film of bacteria and food that accumulates on teeth. Herbal mouth wash is in very high demand. because they act on oral pathogens and relieve the pain instantly and are also less side effect. chemical mouthwash have

hydrogen peroxide and chlorhexidine as an immediate whitener, sterilizer and pain reliver of teeth but they tend to produce discoloration of teeth and may produce side effect, meanwhile they are cost effective. The antibacterial and antimicrobial properties of the mouthwashes can prevent the growth of cavity causing bacteria, reduce plaque, fight bad breath and keep the teeth and gums strong and healthy. Salt heals mouth sores because of producing exosmosis is improves swollen gum conditions of many periodontal disorders. Saline has a mechanical cleansing action and an antiseptic action as it is a hypertonic solution in relation to bacteria, which is undergo lysis. The heat of solution produces therapeutic increase in blood flow (hyperemia) to the surgical site, promoting heating.(20)

It also encourages drainage of pulse from dental abscesses.(21) Mouthwash is an aqueous solution which is most often used for control of plaque and is a medicated liquid which is held in the mouth and swished by the action of perioral musculature to eliminate the oral pathogens. It 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. They are also known as oral rinse or mouth rinse or mouth bath.(22) Usually, mouthwash is an antiseptic solution which is supposed to reduce the microbial load in the cavity, although there are other reasons such as for their analgesic, anti-inflammatory or anti-fungal action. Mouthwash is most commonly used at home as part of an oral hygiene anti cavity mouth rinse, which contain fluoride, which protects teeth from decay. Some natural products are effective as an adjunct in improving the oral health.(23) Herbal mouthwash usually do not contain alcohol or any added sugars or preservatives.(24) Oral infections are one of the most common chronic infections caused by colonization of healthy oral cavity by fungi, bacteria and viruses.(25)

Herbal mouthwash is a non-sterile solution which consists of natural ingredients known as phytochemicals that have anti-inflammatory and anti-microbial effects. The demand for natural mouthwash is high because they offer significant advantages over chemical mouthwashes.(26)

The antibacterial and antimicrobial properties of the mouthwashes can prevent the growth of cavity causing bacteria, reduce plaque, fight bad breath and keep the teeth and gums strong and healthy. The heat of solution produces therapeutic increase in blood flow (hyperemia) to the surgical site, promoting heating. It also encourages drainage of pulse from dental abscesses.(27)

Benefits of Herbal Mouthwash

For the following reasons, herbal mouthwash has become more advantageous than chemical mouthwashes:

- * Herbal mouthwashes are non-irritant and they have non-staining properties.
- * They are less harmful and have very few or no side effects.
- * Herbal mouthwashes are the better option for even the most sensitive mouth.
- * Herbal mouthwashes have naturally antibacterial property as they have polyphenols.
- * It doesn't contain any abrasive additives.
- * Unlike chemical mouthwashes, herbal mouthwash does not cause dry mouth.(28)

Uses :

Herbal mouthwashes are used:

1. To enhance oral hygiene.
2. To control dental plaque.
3. For eradicating bacterias present in oral cavity.
4. To cover bad breath and refresh the breath.
5. For gum disease prevention.
6. To relieve pain and inflammation.
7. To treat Mucositis (swelling and irritation in the mouth) and Halitosis (bad breathe).(29)

Advantages:

1. Fresh breathe
2. Reducing tooth decay using sodium fluoride.
3. Reducing gum inflammation by killing bacteria

4. Whitening teeth using a bleaching agent
5. Preventing gum disease using an antiseptic or anti-plaque ingredient.
6. Mouthwash prevents gingivitis and gum disease by killing the bacteria that would otherwise infect the dental sockets and gums.
7. It can prevent the buildup of plaque, strengthen the enamel, and demineralize your teeth, allowing you to prevent tooth decay.
8. The use of herbal mouthwash has grown advantage over chemical mouthwashes due to their non-irritant and non-staining properties and it does not contain alcohol.
9. Herbal mouthwashes is gentle for even the most sensitive mouth
10. Herbal mouthwash doesn't cause dry mouth and It is highly in demand.

Disadvantages .

1. Short Shelf Life:
2. Herbal mouthwash made with natural ingredients may have a shorter shelf life than synthetic mouthwash , which can contain preservatives that extend their shelf life. This means that herbal mouthwash may need to be used up more quickly to avoid spoilage.
3. It doe snot stay in the mouth for long period
4. Herbal Mouthwash can stain and darken teeth
5. Poor absorption
6. Bad breath

II. INGREDIANTS AND THEIR USES

1. NEEM



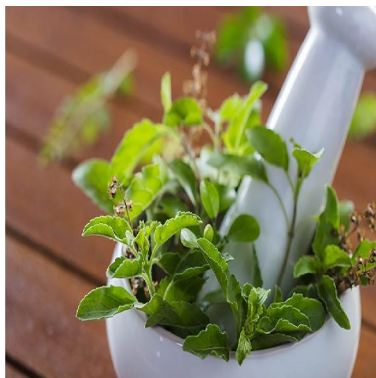
Biological sources -

Neem consists of the fresh or dried leaves and seed oil of *Azadirachta indica* J. Juss (*Melia Indica* or *M. azadirachta* Linn.).

Synonyms - margosa, nimtree or Indian lilac, family tree in the mahogany family Meliaceae.

The history of the neem tree is closely related to the history of the Native American way of life. Today, neem extract is used as an antiseptic, against internal and external parasites, or simply as an herbal mouthwash to treat a variety of skin ailments. Neem extract is also highly effective as a non-toxic repellent, insecticide, and insecticide .Most of this work has been done in the laboratory, as bacteria are relatively easy to treat (unlike viruses and cancer)

2. TULSI



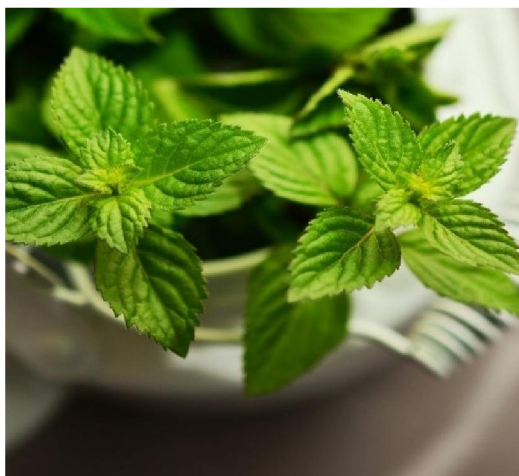
Biological source:

Tulsi consists of the fresh and dried leaves of *Ocimum* species like *Ocimum sanctum* L. and *Ocimum basilicum* L. etc. family Lamiaceae

Ocimum sanctum (family Lamiaceae), commonly known as Holy Basil or Tulsi, Synonyms *Ocimum sanctum*, *Ocimum tomentosum*

Use tulsi (*Ocimum sanctum*) as a mouthwash. Tulsi is a small plant and subshrub with multiple uses. Ayurveda mentions the importance of using it medicinally. The leaves are very effective for mouth ulcers and infections. Chewing a few leaves cures these conditions. Herbs help with tooth disease. Its leaves are dried in the sun and ground into a powder that can be used to brush your teeth. It can also be mixed with patterned oils to form a paste and used as a toothpaste.

3. Peppermint



Biological source It obtained from fresh leaves of *Mentha piperita*. belonging to family Labiatae.

Synonyms: *mentha piperita*

Chemical constituent: Peppermint oil mainly contains menthol (about 70%). It also contains menthone, menthyl acetate, and other terpene derivatives like cineole

Uses:

The dried flowering tops are used to prepare beverages like peppermint tea and in the formulation of liqueurs and bitter.

The current use of peppermint is mainly for colic and irritable bowel syndrome.

Peppermint oil is used as carminatives, aromatic, stimulant and flavouring agent.

It is widely used as an antiseptic in various preparations and in mouth fresheners.

Copyright to IJARSCT

DOI: 10.48175/IJARSCT-18396

www.ijarsct.co.in



It is also used as antipruritic and counter irritant over skin and mucous membrane.
Helps in removing the bad breath

4. Clove



The plant product used in the preparation are dried flower buds.

BIOLOGICAL SOURCE: Plant *Eugenia caryophyllus* dried flower buds are used Family –Myrtaceae synonym: *Eugenia caryophyllata*

uses: Helps to remove plaque, bacteria and inflammation. Clove not only relieve bad breath About also effective at fighting cavities. It stimulate circulation as well as help to reduce gum disease. It is good for dental health so for that it is used as a mouthwash.

5. HONEY



Synonym: Madhu,

Biological source: Honey is a sugary substance/secretion deposited in the honey comb by the hive bee *Apis mellifera* and other species of *Apis* belonging to the family Apidae.

Biological source: Honey is produced in India and major produce comes from the state of Himachal pradesh, Other chief countries are australia, newzealand, west India

Honey is a sweet liquid that bees produce using nectar from flowers. People throughout the world have hailed the health benefits of honey for thousands of years.

Copyright to IJARSCT

DOI: 10.48175/IJARSCT-18396

www.ijarsct.co.in

METHYL PARABENS:

Methylparabens are a type of chemical that manufacturers often use as a preservative. People can add them to food, cosmetics, and pharmaceuticals to increase shelf life and avoid bacterial and fungal growth. Methylparabens belong to a group of chemicals known as parabens.

III. FORMULATION OF HERBAL MOUTHWASH

Formulation table:

Sr.No.	Ingredients	F1	F2	Uses
1.	neem	10ml	7ml	Antibacterial
2.	tulsi	15ml	12ml	Anti-inflammatory
3.	clove	10ml	15ml	Anti-inflammatory
4.	Peppermint	10ml	10ml	Analgesic
5.	Honey	3ml	4ml	Antibacterial, Antiseptic
6.	Methyl paraben	2gm	2gm	Preservative

METHOD OF PREPARATION:

Four different extracts of mouthwash were developed.

The mouthwash formula made and the four main herbal ingredients are used: Clove, Tulsi, Neem, Peppermint. one minor ingredient honey added. And The minor components methyl paraben were used for the preservation and for improving the action.

In order to test the anti-bacterial activity of the mouthwash herbs, different percentage of herbal extract were prepared.

For the formulation, the mouthwash herbal components were grind to obtain their bark form.

10 gm of each Neem, Clove, Peppermint, Tulsi were separately soaked into 100mL of distilled water and incubated at 37 degree C for 48 hours.

After incubation, the herbal extracts were filtered.

Then the extracts were boiled separately and let it cool.

10gm of each solid minor ingredients were added separately into 100 mL of distilled water.

After the ingredients extracts cool down

IV. EVALUATION OF MOUTHWASH

Physical evaluation

Physical parameter such as colour, odour taste and consistency was examined by visual examination.

pH -The pH of prepared herbal mouthwash was measured by using digital pH meter.

Viscosity- Viscosity is measured with the help of digital viscometer.

Microbial Assay – The antibacterial activities were evaluated by measuring the zones of inhibition (in mm).

Stability studies – Physical parameters like colour, odor , consistency and PH was determined at room temperature and 400c.

Taste – The taste is strong and remain almost same over the week except for the ambient temperature sample.

Flavor – The flavor is almost unchanged and has an excellent fragrance of clove and peppermint. Only a week after, the fragrance is somewhat lost when kept at ambient temperature.

Following evaluation parameters were perform to ensure superiority of prepared mouthwash-

Physical Evolution

Mouthwash was evaluated for Morphological parameters shown in the table. The color of Formulation is green . The odour is unpleasant and bitter clove phenolic woody . The texture is liquid

pH

The pH meter was calibrated with the help of standard buffer solutions weight 1 ml of mouthwash and 50 ml of distilled water and its pH was measured with the help of digital pH meter. Ph should be 6.5

Viscosity

Viscosity of the mouthwash was determined with the help of digital viscometer at 100 rpm with the spindle

Microbial evalution

Agar media was prepared then the formulated mouthwash was inoculated on the plates agar media by steak plate method and controlled is prepared by mouthwash. The plates were placed in the incubator and are incubated 370c for 24 hours. After the incubation period the plates were taken out and the Microbial growth were checked and compared with the control.

Stability studies

The result of stability were shown in table no change in color, odour, texture was observed. The Stability studies showed a slight change in PH formulation at 400c.

Taste

Clove extract contains a chemical called eugenol, which acts as an anesthetic and antibacterial agent. Clove is anti-inflammatory and antifungal. It is available from many supermarkets, drug stores, and health food shops, or can be bought online. It has a strong, warm, and spicy taste

Flavor

It has a spicy and flavor similar to clove.

V. RESULT

Morphological Evolution:

Mouthwash was evaluated for Morphological parameters shown in the table.

The colour of Formulation is yellow brown. The odour is pleasant wood clove The texture is liquid that may pose a physical hazard if biting it to it unnoticed.

Table 2: Morphological Evolution

Sr.No.	Parameter	F1	F2
1.	Color	Greenish yellow	Yellowish brown
2.	Odour	unpleasant	Pleasant
3.	Texture	liquid	liquid
4.	Appearance	Visual appearance	Visual appearance
5.	Taste	bitter	Spicy

pH

The pH meter was calibrated with the help of standard buffer solutions weight 1 ml of mouthwash and 50 ml of distilled water and its pH was measured with the help of digital pH meter.

Ph of F1	Ph of F2
6.3	6.4

VI. CONCLUSION

Safe and effective herbal formulation for dental problem patients was successfully formulated. Prepared formulation was selected for the further studies. When we taken it for herbal mouthwash analysis, it shows best results. Also, it has high Antibacterial activity. No microbial growth was found in the respective formulation. The therapeutic effects of

these herbal products and their role in improving oral health. The results of zone of inhibition also confirmed that this herbal mouth rinses was found to be a potent plaque inhibitor, and were preferred by the patients for its taste, convenience of use and test duration in their mouth after rinsing. In these study herbal mouthwash had better antibacterial activity in comparison with Marketed mouthwash.

REFERENCES

- [1]. Blot S. Antiseptic mouthwash, the nitrate-nitrite-nitric oxide pathway, and hospital mortality: a hypothesis generating review. *Intensive Care Med.* 2021;47(1):28-38. doi: 10.1007/s00134-020-06276-z, PMID 33067640. PMCID PMC7567004.
- [2]. Vranić E, Lacević A, Mehmedagić A, Uzunović A. Formulation ingredients for toothpastes and mouthwashes. *Bosnian J Basic Med Sci.* 2004;4(4):51-8. doi: 10.17305/bjbm.2004.3362, PMID 15628997.
- [3]. 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.
- [4]. 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,
- [5]. Gamboa F, Estupinan M, Galindo A. Presence of *Streptococcus mutans* in saliva and its relationship with dental caries: Antimicrobial susceptibility of the isolates. *Univ Sci*, 2004; 9: 23-7.
- [6]. Clarke JK. On the bacterial factor in the etiology of dental caries. *Brit J Exp Pathol*, 1924; 5: 141-7.
- [7]. Jahir Alam Khan, Sonali Hane. Antibacterial Properties of *Punica Granatum* Peels. *IJABPT*, 2011; 2: 23-7.
- [8]. Sundaram Ravikumar, Gopi Palani Selvan, Anitha Anandha Gracelin A. Antimicrobial Activity of Medicinal Plants along Kanyakumari Coast, Tamil Nadu, India. *AJBAS*, 2010; 2: 153-7.
- [9]. Jha B., Dodwad V., "Herbal Mouthwashes – A Gift of Nature" *Int. J. Sci.*, 2012; 3(2): 48-53.
- [10]. Sandhya R., "Herbal Products As Mouthwash – A Review", *Int. J. Sci. Res.*, 2017; 6(7): 1134-1136.
- [11]. Mini Priya R., "Review on Nutritional, Medicinal And Pharmacological Properties of Guava (*Psidium Guajava* Linn.)" *Int. Res. J.*, 2011; 2(1): 53-69.
- [12]. T. Anjali., K. Krishnakumar., K. Dinesh., J. Anish., "Herbal Remedies For Mouth Ulcer: A Review" *J. Bio.*, 2017; 6(4): 521-527.
- [13]. Mini Priya R., "Review on Nutritional, Medicinal And Pharmacological Properties of Guava (*Psidium Guajava* Linn.)" *Int. Res. J.*, 2011; 2(1): 53-69
- [14]. Biswas G, Anup N, Acharya S, Kumawat H, Vishnani P, Tambi S. Evaluation of the efficacy of 0.2% chlorhexidine versus herbal oral rinse on plaque induced gingivitis - A randomized clinical trail. *IOSR J Nurs Health Sci* 2014;3(2):58-63.
- [15]. Gupta D, Nayan S, Tippanawar HK, Patil GI, Jain A, Momin RK, et al. Are herbal mouthwash efficacious over chlorhexidine on the dental plaque? *Pharmacognosy Res* 2015;7(3):277-81.
- [16]. Takenaka, S.; Sotozono, M.; Ohkura, N.; Noiri, Y. Evidence on the Use of Mouthwash for the Control of Supragingival Biofilm and Its Potential Adverse Effects. *Antibiotics* 2022, 11, 727.
- [17]. Nasry B, Choong C, Flamiatos E, Chai J, Kim N, et al. Diversity of the oral microbiome and dental health and disease-review. *Int J Clin Med Microbiol* 2016;1:108
- [18]. Siddeshappa ST, Bhatnagar S, Yeltiwar RK, Parvez H, Singh A, Banchhor S. Comparative evaluation of antiplaque and antigingivitis effects of an herbal and chlorine dioxide mouthwashes: A clinicomicrobiological study. *Indian J Dent Res* 2018;29:34-40
- [19]. Bennett JE. Antimicrobial agents. In: Goodman and Gilman's, the Pharmacological Basis of Therapeutics. 10th ed. New York: McGraw-Hill. 2001; 7(2): 1295-302.
- [20]. Matthews R W, Hot salt water mouth baths, *British Dental Journal*. 2003, 195 (1); 3-3.
- [21]. ICH Harmonized Tripartite Guidelines, Stability Testing of New Drug Substances and Products, ICH Committee. Federal register 2003:68
- [22]. Gandini S, Negri E, Boffetta P, La Vecchia C, Boyle P (2012). "Mouthwash and oral cancer risk quantitative meta analysis of epidemiologic studies". *Annals of Agricultural and Environmental Medicine*. 19 (2): 173–80

- [23]. Ashok. 2020. Awareness on Herbal Mouthwash among Dental Students. Journal of Pharmaceutical Research International, 32(17): 48-56.
- [24]. Ahmad, Shafi & Shina, Saloni & Ojha, Smriti & Chadha, Hina & Aggarwal, Babita, Meenu. 2018. Formulation and Evaluation of Antibacterial Herbal Mouthwash Against Oral Disorders. Indo Global Journal of Pharmaceutical Science, 8(2): 37-40
- [25]. Pedesern, A. L. M. (2015). Oral infections and general health: from molecule to chairside. Springer international publishing, Switzerland, Page 3-4.
- [26]. Dodward, V., and Kurkreja, K. J. (2012). Herbal mouthwashes-gift of nature. International journal of pharmacology and biology sciences, 2(3), 47-52.
- [27]. ICH Harmonized Tripartite Guidelines, Stability Testing of New Drug Substances and Products, ICH Committee. Federal register, 2003; 68
- [28]. Renuka S, Muralidharan NP. Comparison in benefits of herbal mouthwashes with chlorhexidine mouthwashes: a review. Asian J Pharm Clin Res. 2017;10(2):3-7.
- [29]. Reddy HT, Preethi. Herbal mouthwashes. Eur J Mol Clin Med. 2020;7(2):6655-