

Preparation a Novel Poly Herbal Antibacterial Formulation, Characterization and Evaluation of Mouth Wash Protecting and Tackling Oral Cavity against Oral Pathogens

Dr. Satendra Kumar^{1*}, Dr. Anil Kumar²

¹Department of Pharmaceutics, L.N Pharmacy College, Baitalpur, Deoria, U.P

²Department of Pharmacognosy, Pharmacy College, Itaura, Chandeshwar, Azamgarh, U.P

*Corresponding Author: howruskumar@gmail.com

ABSTRACT

The oral cavity is the home of various bacterial species. All through some of the oral bacteria are harmless and there are certain species that are harmful which may causes oral plaque, bad breath and mouth disease. Medicinal plant or herbs are considered to rich source of ingredients which can be used in drug development. They can prevent and cure disease because of their antimicrobial and antibacterial property against microorganisms. The importance of mouth and teeth cleanliness has been recognized from the earliest days of civilization. Patients and oral health practitioners are faced with a multitude of mouthwash products containing many different active and inactive ingredients. 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 like Leaves of *Azadiracta indica* (neem), *Mentha Piperita* (peppermint), *Ocimum sanctum* (Tulsi) bud of *Eugenia caryophyllus* (clove), and bark of *Cinnamomum verum* (cinnamon), *Glycyrrhiza glabra* (Licorice) Although many popular herbal products have helped to control dental plaque and gingivitis, they have been used for a short time and only as an adjunct to other oral hygiene measures such as brushing and flossing. By this study it was concluded that the developed herbal mouthwash possess significant, therapeutically efficacious, suitable vehicle for drug delivery in low cost but definitely with high potential. Hence there is need for increased usage of herbal preparation to avoid the adverse effects.

Keywords: Gingivitis, Antimicrobial. Herbal Mouthwash, **Plaque** maintenance

INTRODUCTION

Herbal mouthwashes are in excessive demand, because they act on oral pathogens and relieve the pain instantly and are also less side-effective. Chemical mouthwashes have hydrogen peroxide a chlorine dioxide, and cetyl pyridinium chloride, as an immediate whitener, sterilizer and pain reliever of teeth, but they tend to produce discoloration of teeth and may produce side effect, mean while they are cost effective. One of the most common infectious diseases encountered by many individuals is dental carries and periodontal diseases Periodontitis as a major oral infection may affect the Dental carries contain the

cavity formation, eruption of enamel, swelling of gums, bleeding in gums, and formation of hollow black eruption on the surface of the teeth, oral hygiene. Oral infections spread from the root of the contaminate tooth through the jaw bones and into spaces between the facial planes of surrounding soft tissue. The meaning of mouth wash is a usually antiseptic liquid preparation for cleaning the mouth and teeth or freshening the breath. Mouthwashes are often prescribed in dentistry for prevention and treatment of several oral conditions. In the recent times the use of naturally occurring products what is otherwise known as grandmothers remedy are used on a large scale. This has now called for a newer age of mouth washes but is the new age mouth washes at par with the gold standard or even better than them this study investigates. Across the world, oral health is becoming a major concern. The world oral health report 2003. highlighted oral health as an integral and essential component of general health. Most of the chemical products contain an antiseptic that plays an important role in controlling plaque accumulation. The vehicle for delivery of chemical agents with ant plaque action is tooth paste, mouth washes, spray, irrigators, chewing gums and varnishes. However, mostly accepted method of delivering the antimicrobial agents after toothpaste is mouth wash. Mouth wash are an antiseptic solution which is used to reduce the microbial load in the oral cavity.

Mouth washes are liquids which contains anti-inflammatory, Anti-microbial and analgesic action. There are two types of mouthwashes-chemical and herbals Herbal mouthwash contains natural ingredients called phytochemicals that contains desired anti-microbial and anti-inflammatory effects. Herbal mouthwash becomes more popular they work without alcohol, artificial preservatives, flavors and colors. As it contains natural herbs that have natural cleansing and healing property to teeth and gum. Herbal Mouthwashes are in high demand, because they act on oral pathogens and relieve the pain instantly and are also less side-effective. Chemical mouthwashes have hydrogen peroxide and chlorhexidine as an immediate whitener, sterilizer and pain reliever of teeth, but they tend to produce discoloration of teeth and may produce side effect, meanwhile they are cost effective. One of the most common infectious diseases encountered by many individuals is Dental carries and Periodontal diseases at different stages of their life time. 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 (xerostomia), ulcerated throat and sensitive teeth. Dentists always use mouthwash as an antimicrobial agent before oral surgery of the patients, because they help to sterilize the surface of the inflamed gums and teeth, thereby the contamination of any other microorganisms can be avoided. Almost all chemical mouthwashes contain alcohol and fluoride which is toxic to our body in overdoses. Hence, most herbal mouthwashes are safe alternative to pregnant women, peoples with dry mouth, diabetic and to children. The purpose of this study was to determine the prevalence of mouthwash use and not only the type of mouthwash but quantity of mouthwash to be taken for use is also important and also this study was performed to evaluate the efficacy and safety of herbal mouthwash for human medicines.

Herbal Mouthwash

Herbal mouth wash is prepared from natural plant extracts. The natural extract present in the herbal mouth washes are obtained from various plant leaves, fruits, seeds and various tree oils etc.

Why should we prefer Herbal Mouthwash?

Herbal mouthwashes are highly prefer because they have following properties

- Herbal mouth wash are demanded because they instantly relives the pain.
- Herbal mouthwash can help to prevent and various infectious disease.
- From gingivitis Mouthwash act on oral pathogen and also producing less stable effects as compared to synthetic herbal products.

Aim of the Herbal Mouthwash

1. It can reduce the plaque growth in your mouth, decrease your chances of developing gum disease, and prevent tooth decay.
2. Determine whether there has been an increased risk observed with alcohol-based mouthwash.
3. To determine if commercially available mouthwashes are capable of performing the advertised elimination of oral bacteria.
4. To determine the antimicrobial activity.
5. To evaluate mouthwash for its consistency.
6. To develop formulation of herbal mouthwash
7. Role of alcohol in the mouthwash after the advent of more effective agents.

Advantages of Herbal Mouthwash

- A. Get a fresh breath. This one is the most common.
- B. Herbal mouthwash is gentle for even the most sensitive mouth.
- C. Say goodbye to particles. It is very common nowadays that most of the people use mouthwash only after brushing their teeth.
- D. Avoids plaque.
- E. Fights cavities from growing.
- F. Use of herbal mouthwash is to improve oral hygiene
- G. Cures canker sores.
- H. Herbal mouthwash has naturally antibacterial property.
- I. It contains no harsh additives.
- J. Herbal mouthwash does not cause dry mouth.
- K. It is highly in demand.
- L. It keeps your mouth healthy.
- M. 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.
- N. They have very minimal or no side effect and they are less harmful.
- O. All herbal mouthwashes do not contain alcohol and/or sugar.
- P. Herbal mouth wash do not contain.

Stannous fluoride
Artificial sweeteners
Artificial colors
Cetylpyridinium chloride (CPC)
Harsh chemical preservative
Sodium Lauryl Sulphate (SLS)
Dye

Application of Herbal mouthwash

1. It is use to clean septic sockets.
2. It relieves pain and inflammation.

3. Many conditions within the oral cavity require the use of a mouthwash.
4. This can vary from breath freshener to treatment of life-threatening secondary infections such as oral mucositis in patient undergoing bone marrow transplant therapy
5. It helps to control dental plaque
6. The use of mouthwashes requires a correct diagnosis of the oral condition and through knowledge of the product to achieve an effective treatment.
7. Use of herbal mouthwash is to improve oral hygiene.
8. It can be used in gum diseases.
9. Used for killing germs in oral cavity.
10. It freshens breath and covers bad breath.
11. Using a mouthwash for gum disease prevention is very important.

Material and method

Collection of Plants

Leaves *Azadiracta indica* (neem), *Mentha Piperita* (peppermint), *Ocimum sanctum* (tulsi) flower bud of *Eugenia caryophyllus* (clove), bark of *Cinnamomum verum* (cinnamon), and *Glycyrrhiza glabra* (Licorice) were randomly collected from plants. Clove oil, peppermint oil, Patent v, purchased from local market of Deoria.

1-Liquorice (*Glycyrrhiza glabra*) belongs to family Fabaceae. The root has long been used in Chinese and alternative medicine to enhance the efficacy of other herbal remedies. More recently, studies have discovered that two other compounds, licoricidin and licorisoflavan-A, found in dried licorice root, act as an effective antibacterial agent that can prevent or reduce the growth of bacteria connected with tooth decay and gum disease. The major constituent of the roots of *G. glabra* and *G. uralensis* is a sweet triterpene saponin glycyrrhizin, which is a potassium and calcium salt of glycyrrhizic acid in the range of 6-14 per cent. It is a triterpene of oleanan skeleton, which after hydrolysis, affords two molecules of glucuronic acid and an aglycone, glycyrrhetic acid.

2- Tulsi (*Ocimum sanctum*) Tulsi is belonging to family Lamiaceae has also proven to be very effective in preventing halitosis. Its anti-inflammatory property makes it a suitable treatment for gingivitis and periodontitis, and it can be used for massaging the gingiva in these conditions. The constituent of tulsi eugenol acts as an analgesic. Tulsi leaves contain 0.7% volatile oil consisting about 71% eugenol and 20% methyl eugenol. Oral infections tulsi leaves are quite effective in treating common oral infections. When chewed, tulsi leaves help in maintaining oral hygiene. Antibacterial agents namely carvacrol and terpineol are present in this plant. Menthol gives a cooling sensation when applied to the skin or other tissues (such as the tongue, gums, or inside the cheeks). Menthol topical oral mucous membrane is used to treat minor sore throat pain, or mouth irritation caused by a canker sore.

3 Cloves (*Eugenia caryophyllus*) belongs to family Myrtaceae have been shown to have antimicrobial properties, meaning they can help stop the growth of microorganisms like bacteria. One test-tube study showed that clove essential oil killed three common types of bacteria, including *E. coli*, which is a strain of bacteria that can cause food poisoning. What's more, the antibacterial properties of cloves could even help promote oral health. In one test-tube study, the compounds extracted from cloves were found to stop the growth of two types of bacteria that contribute to gum disease. Another study in 40 people tested the effects of an herbal mouthwash consisting of tea tree oil, cloves, and basil. After using the herbal mouthwash for 21 days, they showed improvements in gum health, as well as the amount of plaque and bacteria in the mouth. In combination with regular brushing and proper oral hygiene, the

antibacterial effects of cloves may benefit your oral health. Clove contains volatile oil, about 14-20 per cent and gallic acid, 10-13 per cent.

4-Peppermint (*Mentha piperita*) Peppermint is a hybrid mint, a cross between water mint and spearmint. Indigenous to Europe and the Middle East, the plant is now widely spread and cultivated in many regions of the world. It is occasionally found in the wild with its parent species.

Peppermint also known as *Mentha balsamea* Wild is a hybrid mint, a cross between water mint and spearmint. Indigenous to the plant is now widely spread and cultivated in many regions of the world. It is occasionally found in the wild with its parent species.

Although the genus *Mentha* comprises more than 25 species, the one in most common use is peppermint. While Western peppermint is derived from *Mentha piperita*,

5-Neem (*Azadirachta indica*) Neem is belongs to family Meliaceae which have Antimicrobial, fungicidal, anti-inflammatory property. Neem bark and leaf are used to treat and prevent the onset of many dental disorders since ancient time. It is temporary used for treatment of tooth ache. It proved to reduce local inflammation in oral cavity. Neem is also a bitter taste, and various active chemical constituents present such as, Antiviral action Antibacterial, antifungal properties nematicide and antimicrobial properties.

6-Cinnamon (*Cinnamomum verum*) Cinnamon is belong to Lauraceae. Cinnamon has antioxidant property, bactericidal and anti-inflammatory property. It also gives a pleasant flavor to many oral formulations. It is reported to protect dental health and fresh breath naturally. The main reservoir for *Candida* in the oral cavity has found to be the buccal mucosa and tongue. It has also been found to co-aggregate with bacteria in the sub gingival biofilm and adhere to the epithelial cells and invade the gingival connective tissue. It is also found in the periodontal pocket of chronic periodontitis patients. Cinnamon being an ancient spice in the kitchen has been found to have multiple medicinal values too. *Cinnamomum zeylanicum* and Cinnamon cassia are the only two approved medicinal herbs of the genus *Cinnamomum*. It has been found to have anti-cholesterol, anti-bacterial as well as anti-fungal property. Cinnamon in addition to its popularity as a spice has been found to have medicinal properties such as anti-bacterial, anti-fungal, anti-oxidant.

Drug profile

Sr. No.	Herbal Ingredients	Botanical Name	Active constituents	Medicinal use
1	Liquorice	Glycyrrhiza glabra	Glycyrrhizin	Sweetening agent
2	Tulsi	Ocimum sanctum	Eugenol	Dental care
3	Clove	Eugenia caryophyllus	Eugenol	Dental Analgesic, Fight bad breath, stimulate circulation
4	Peppermint	Mentha piperita	Menthol	It gives fragrance, Anti-viral
5	Neem	Azadirachta indica	Nimbin, Nimdin	inhibit plaque formation, Antiseptic
6	Cinnamon	Cinnamomum verum	cinnamate, cinnamic acid	Flavoring agent, Bactericidal
7	Patent v	-	-	Colouring agent
8	Coco glucoside	-	-	Surfactant

List of Herbal Material

Sr. No.	Materials Name (Herbal)
1	Liquorice
2	Tulsi
3	Clove
4	Peppermint
5	Neem
6	Cinnamon
7	Patent v
8	Coco glucoside

List of Equipment

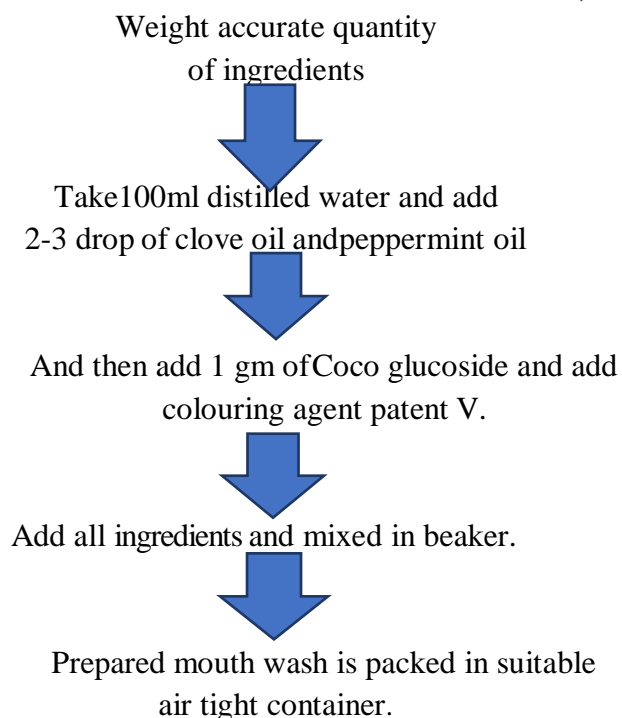
Sr. No.	Equipment Name
1	Measuring cylinder
2	Beaker, Autoclave
3	Hot air-oven
4	volumetric flasks, Conical flask
5	Funnel
6	Water bath, mortar pestle
7	Burner
8	Sterile Petri plates
9	pH meter
10	Incubator

Method

Extraction process: The leaves, stem and bark were washed with sterile water, shadow dried, pulverized and stored in air-tight bottles. The Aqueous extracts were prepared by soaking the powdered leaves, stem and bark in sterile distilled water and maintained in Incubator at $37\pm 2^{\circ}\text{C}$ for 72hours and were filtered using Whatmann filter paper.

Formulation of Herbal Mouthwash: Different mouthwashes containing various herbs were prepared using established standard procedures. The selection of the herbs were made, keeping in mind the anti-microbial efficacy along with their excipients properties namely, preservative, sweetening and flavoring effects, which are required to develop an ideal mouth wash. Weighed quantity of each ingredient will be taken Extract were taken mixed thoroughly in mortar and pestle property with small quantity of water. All other remaining ingredient will be gradually added with good mixing. Finally, water added to make volume and preservative will be added and the product will be packed in an amber colored, well closed container. The herbal Mouthwash was prepared by the formula given in table.

(Flow chart of Method of Herbal formulation)



Herbal mouth wash Formulation:

Herbal Ingredient	Function	Formulation		
		A (mg)	B (mg)	C (mg)
Neem	Active drug	250	500	1000
Tulsi	Active drug	250	500	1000
Clove oil	Active drug	0.1ml	0.15ml	0.20ml
Peppermint oil	Flavor	0.1ml	0.1ml	0.1ml
Liquorice	Sweetener	0.1mg	0.1mg	0.1mg
Coco glucoside	Surfactant	6 g	6 g	6 g
Cinnamomum	Active drug	0.1ml	0.1ml	0.1ml
Patent v	Preservative	2 ml	2 ml	2 ml
Purified water	Up to 100ml	Up to 100ml	Up to 100ml	Up to 100ml

(Prepared mouth wash formulation)



Evaluation of Herbal mouth wash formulation:

- 1 **Colour and Odour:** Physical parameters like odour and colour were test by visual examination.
- 2 **pH:** PH of prepared herbal mouthwash was measured by using digital PH meter the Ph meter was calibrated using standard buffer about 1 ml of mouthwash was weighed and dissolved in 50 ml of distilled water and its PH was measured by pHmeter
- 3 **Stability Studies-** The formulation and preparation of any pharmaceutical product is incomplete without proper stability studies of the prepared product. This is done in order to determine the physical and chemical stability of the prepared product and thus determine the safety of the product. A general method for predicting the stability of any product is accelerated stability studies, where the product is subjected to elevated temperatures as per the ICH guidelines. A short term accelerated stability study was carried out for the period of 3 months for the prepared formulation. The samples were stored at under the following conditions of temperature as 3-5⁰ C, 25⁰ C RH=60%, 40⁰ C ±2% RH= 75%. Finally, the samples kept under accelerated study were withdrawn on monthly intervals and were analyzed.
- 4 **Test for microbial growth in formulated mouthwash:** The formulated mouthwash was inoculated in the plates of agar media by streak plate method and a control was prepared. The plates were placed in the incubator and are incubated at 37±2°C for 24 hours. After the incubation period, the plates were taken out and checked for the microbial growth by comparing it with the control or standard.
- 5 **Viscosity:** Viscosity of the mouthwash was determined with the help of digital viscometer at 100 rpm with the spindle 6
- 6 **Microbial evaluation:** 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 37⁰ c for 24 hours. After the incubation period the plates were taken out and the Microbial growth were checked and compared with the control.
- 7 **Stability studies:** The result of stability was shown in table no change in color, order, texture was observed. The Stability studies showed a slight change in PH formulation at 40⁰ c.
- 8 **Taste:** Clove oil contains a chemical called eugenol, which acts as an anesthetic and antibacterial agent. Clove oil 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.
- 9 **Flavor:** It has a tingy, spicy flavor similar to cinnamon

Chemical test perform on herbal ingredients

Sr No	Name of Herbal (Ingr.)	Chemical Tests	Result
1	Neem	Aq. Extract + ferric chloride 5% solution	Dark colourisation
2	Tulsi	5 mg extract of Ocimum sanctum in test tube + 1% HCl	Red colour
3	Clove	Aq. Extract + ferric chloride 5% solution	Dark colourisation
5	Cinnamon	Aq. Extract+ potassium permanganate	Decolourisation
6	Liquorice	Aq. Extract + lead acetate reagent	White precipitate

7	Peppermint	5 mg extract of <i>Ocimum sanctum</i> in test tube + 1% HCl	Red colour
---	------------	---	------------

RESULT AND DISCUSSION.

The color of Formulation is blue. The older is sweet spicy clove phenolic woody nutmeg powder. The texture is veryhard wood texture that may pose a physical hazard if biting it to it unnoticed.

(Visual inspection)

Sr. No	Observation	Result
1.	Colour	Blue wish
2.	Odour	Deep spicy and wood clove
3.	Appearance	Visual Appearance
4.	Texture	Liquid

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

(pH of Herbal mouth wash)

Sr.no	Day of Measurement	(pH) Prepared herbal mouth wash
1	1st day	5.3 to 5.5

Viscosity-Viscosity of the mouthwash was determined with the help of digital viscometer at 100 rpm with the spindle 6.

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

(Stability test)

Sr. No	Observation	Room Temp	On 40 ⁰ c
1.	Color	Not Change	Not Change
2.	Odour	Not Change	Not Change
3.	Texture	Liquid	Liquid
4.	PH	5.57	5.34

CONCLUSION

The present liquid herbal mouthwash can work in long way to help people to get rid of bad breath and many oral disorders. Besides we can be rest assured and take comfort in the fact that there aren't any unhealthy ingredients present in this preparation. The physicochemical evaluation results confirm that the colour and odour of present herbal formulation is acceptable with a pleasant odour and a better after effect used appropriately. Herbs contain active ingredients that may interact negatively with prescribed medications or other remedies. It is wise, therefore, to consult a doctor and health expert in situations in which you question the appropriateness of the herb or its interaction with other remedies. The use of herbs in dentistry should be based on evidence of effectiveness and safety. The anti-bacterial activities could be removed infectious agent in mouth. The present results therefore offer a greater use for traditional use of herbal mouth wash

REFERENCES

- 1 Sunayanamanipal, SajjidHussain, UmeshWadgave, prabhuDuraismwamy and K. Ravi, The mouthwash warchlorhexidine Herbal mouth Rinses: A meta- Analysis J clinDiagn Res. 2016 may; 10(s): ZC81-ZC83 .
- 2 Diego Francisco Cortes - Rojas, Claudia Regina fernandes de Souza, and wanderley Pereira Oliveria clove(*Syzygiumaromaticum*): a precio -les Spice Asian Pac J Trop Biomed 2014 Feb; 4(2) 90-96.
- 3 RozaHaghgoo and faridAbbasi, evaluation of the use of a peppermint mouth rinse for halitosis by J intsocprev community pent. 2013 Jan-Jun; 3(1) : 29-31.
- 4 NilimaThorar, silpiBasak, Rakesh N. Bahadure and monaliRajurkar, antimicrobial efficacy of five essential oils against oral pathogens: An in vitro study, Eur J Dent. 2013 Sep 7 (Suppl 1) 571-577.
- 5 Shivani B. Shambharkar, and Vinod m. Thakare, fomulation and evaluation of Herbal mouthwash, Article Recived on 28 may 2021.
- 6 Banani Ray chowdhury, Souptik Bhattacharya, Madhupama deb, ArnavGarai, Development of Alcohol-free Herbal mouthwash Having Anticancer property, Department of BiotechnologyBengal Institute of Technology, West Bengal university of Technology india
- 7 Matthews R W. Hot salt water mouth baths, British Dental Journal. 2003, 195 (1); 3-3.
- 8 F.M Viera, M.C Macial, F.R Nascimento and V.P Rodrigues,Plant species used in dental disease: activity evaluation, Journal of ethanopharmacology. Vol. 155 (3), 1441-1449, 2014.
- 9 Kumar P. Ansari S.H.Ali J. Herbal remedies for the treatment of pridental disease: A patient review. Recent pat drug deliver formula 2009; 3:221-8.
- 10 Rao N.J. Subhas K.R, Kumar K.S. Role of phytotherapy in gingivitis, a review. J pharmacol 2012; 8:1-5.
- 11 Clive E, Paul S. Minor illness or major disease?.The clinical pharmacist in the community (4th edition).Pharmaceutical press, 223.
- 12 Khan A. Ahmad A Manzoor N. Khan Lt Antifungal activity of *Ocimum sanctum* essential oil and its lead molecules Nat Prod Commun.2010; 5: 345-349.
- 13 Bhavna, J.K. Vidhya D. 2012 Herbal Mouthwash-A gift of Nature. Int J Pharma and Bio. Sci. 2014 278-280
- 14 Pathan M, Bhat K, Joshi V. Comparative evaluation of the efficacy of a herbal mouthwash and chlorhexidine mouthwash on select periodontal pathogens: An in vitro and ex vitro study. J ind S per. 2017: 270- 275.
- 15 C. K. Kokate, Pharmacognosy, vol. 12, NiraliPrakashan, 1999.
- 16 J. G. Hardman, L. E. Limbird, L. S. Goodman, and A. G. Gilman, Goodman and Gilman's the Pharmacological Basis of Therapeutics, McGraw Hill, New York, NY, USA, 10th edition, 2001.
- 17 Baron, J. E. and \$. M. Finegold, Methods for Testing Antimicrobial Effectiveness. In: Barley Scotts: Diagnostic Microbiology Mosby, C.V. (Ed.). Missouri, USA, 1990;171-174.
- 18 Kumar P. Ansari S.H.Ali J. Herbal remedies for the treatment of pridental disease: A patient review Recent pat drug deliver formula 2009 3:221-8.
- 19 Dhar R. Zhang K, Talwar GP. Garg S, Kumar NJ.Inhibition of the growth and development of asexual and sexual stages of drug-sensitive and resistant strains of the human malaria parasite *Plasmodium falciparum* by Neem (*Azadirachta indica*)nfractions.JEthnopharmacol.1998; 61: 31-39

- 20 AmitParashar, Mouthwashes and their use in different oral condition., Sch. J. Dent. Sci.2015; 2(2): 186-191.
- 21 Asadoorian Joanna. Cetylpyridinium Chloride Mouth Rinse on Gingivitis And Plaque. Journal of Dental Hygiene 2008.
- 22 Naveen Pattnaik. The Tree of Life. PDR for Herbal Medicines, 4thEdn. Thomson Healthcare: 640p. ISBN 978-1-56363678-3.
- 23 R. Eccles. Menthol and Related Cooling Compounds. J. Pharm.Pharmacol 1994; 46(8):618-630p. PMID 7529306.
- 24 Balch James. Prescription for Nutritional Healing,3rdedn. Avery Publishing: 2000; 94p. USA (2012-05-24). The Effect of Clove and Benzocaine versus Placebo as... J Dent 2006; PubMed-NCBI. Ncbi.nlm.nih.gov. Retrieved 2012- 09-07