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Preparation and Evaluation of Herbal Mouthwash

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ABSTRACT: Medicinal plant or herbs are considered to have 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 main objective of this literature work is to prepare and evaluate a herbal mouthwash and check its effectiveness against microorganisms of oral cavity. To prepare Antibacterial herbal Mouthwash from aqueous extract of 7 different Herbs namely Azadirachtaindica (Neem), Ocimum bacilicum(Tulsi), Menthalogifolia(Mint), Curcuma longa(Turmeric), Syzygiumaromaticum(Clove), Glycyrrhizaglabra(Liquorice), Cinnamomumverum(Cinnamon). That act against the oral Staphylococcus aureus,E.coli and to check the antimicrobial activity by using Agar well diffusion method. The medicinal plants which were suitable were collected and their water soluble extracts was prepared. The prepared mouthwash was evaluated against pathogens and was found effective for controlling and demolishing microbial growth in mouth. Further physiochemical properties were tested for prepared mouthwash and itpossesses a good antibacterial activity. The current mouthwash is liquid formulation with potent action.

KEYWORDS: Herbal mouthwash, Natural extracts, Plaque maintenance, Antimicrobial, Staphylococcus aureus, and E.coli.

I. 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. Usually mouthwashes are antiseptic solutions intended to reduce the microbial load in the mouth, 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).Cosmetic mouth rinses temporarily control or reduce bad breath and leave the mouth with a pleasant taste.

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 plats an 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.

The uses of Neem are known from ancient dates back since 45,000 years against oral diseases. The neem leaves Azadirachtaindica belongs to the family Lamiaceae. The neem solutions are used in decreasing the inflammation of gums, to remove plaque and against dental cavities. Nowadays the neem extracts are used as antiseptic substance against inflammation of mouth. The neem extract shows significant effects on both Gram positive

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and Gram negative bacteria such as Staphylococcus aureus, E.coli. The inhibition is against the production of insoluble glucan,which is the main cause for plaque formation. Theneem sticks has proven to show markedly inhibition against Streptococci ,whichcolonize the surface of the tooth.

Turmeric (Curcuma longa) a rhizomatous, herbaceous plant of the ginger family zingiberaceae. It is native to south west India requiring temperatures between 20º and 30º C and the plants are gathered annually for their rhizomes. Turmeric was first used as a dye by ancients, then later for its medicinal properties. The important chemical components of turmeric are a group of compounds called curcuminoids that includes demethoxycurcumin and bisdemethoxycurcumin. The other volatile compounds include turmerone, altantone and zingiberine. According to the National centre for complementary and Integrative health, some researches shows compound in turmeric to have antifungal and antibacterial properties.

The Mentha leaves (Menthalongifolia) are aromatic, perennial herbs. The menthe belongs to the family Lamiaceae. Peppermint was first described in 1753 by Carl Linnaeus. Mentha are extensively used as flavouring ingredient in breath freshness, antiseptic mouth rinses, chewing gums and tooth paste. The pulegone primarily responsible for the aroma and flavour of spearmint is L-Carvone. Menthol oil contains menthone, menthyl esters, menthyl acetate and menthofuran. Mint also consists of small amounts of many additional compounds including Limonene, Pulegone, Caryophyllene and Pinene.

Tulsi(Ocimum bacilicum) is a legendary herb which has been used for ages due to its religious and medicinal values. Several pharmacological studies have established a scientific basis for therapeutic uses of this plant belongs to family Lamiaceae. . It can prove beneficial in treating oral diseases also because of its antibacterial, anti-inflammatory, ulcer healing, antioxidant, immunomodulatory properties. Future studies should be directed to explore and evaluate therapeutic significance of this miraculous plant in periodontal diseases.Tulsi leaves contain bright, yellow coloured and pleasant volatile (0.1 - 0.9 per cent). The oil content of the drug varies depending up the type, the place of cultivation and season of its collection. The oil is collected by steam distillation method from the leaves and flowering tops. It contains approximately 70 per cent eugenol, carvacrol (3 percent) and eugenol-methyl-ether (20 percent). It also contains caryophyllin in seeds which contain fixed oil with good dental properties. The plant is also reported to contain alkaloids, glycosides, saponin, tannins, an appreciable amount of vitamin C and traces of maleic, citric and tartaric acid.

Cloves((Syzygiumaromaticum)belongs to family Myrtaceaehave 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 gallotannic acid, 10-13 per cent. Clove oil contains about 84-95 per cent of eugenol, about 3 per cent of eugenol acetate and 5-8 per cent ß-caryophyllene. Other constituents which are present in trace quantities include esters, ketones and alcohols. Clove oil has been reported to contain some 28 constituents and is devoid of monoterpenoids.

Liquorice(Glycyrrhizaglabra) belongs to family FabaceaeThe 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 triterpenesaponin 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, glycyrrhetinic acid.

Cinnamon(Cinnamomumverum) belongs to family Lauraceae.The main reservoir for

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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. Cinnamomumzeylanicum and Cinnamom 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.Cinnamon oil contains 60 - 70 per cent of cinnamaldehyde, 5 - 10 per cent eugenol, benzaldehyde, cuminaldehyde and other terpenes like phellandrene, pinene, cymene, caryophyllene, etc.

The aim to prepare Anti-bacterial Herbal Mouthwash from the aqueous extracts of 7 different herbs namelyAzadirachtaindica (Neem), Ocimum bacilicum(Tulsi), Menthalogifolia(Mint), Curcuma longa(Turmeric), Syzygiumaromaticum(Clove), Glycyrrhizaglabra(Liquorice), Cinnamomumverum(Cinnamon)that acts against the oral pathogens- Staphylococcus aureus and Escherchia coli to check the Anti-microbial activity by using Agar well diffusion method.

AIM: To perform the agar well diffusion technique in the Muller Hinton agar and to identify the anti-bacterial activity of leaf extracts against test organisms, by measuring the zone of inhibition.

OBJECTIVE: To compare the efficacy of extracts of 7 different herbs on the basis of their resistance and sensitivity towards the oral microbes and to formulate a herbalmouthwash.

II. MATERIALS AND METHODS:

Collection of Plant Leaves: Leaves of Azadirachtaindica(Neem), Ocimumteniflorum(Tulsi), Mentha (Mint), bark of Cinnamomumverum (cinnamon), flower bud of syzygiumaromaticum (clove), stem of Glycyrrhizaglabara (Liquorice) and rhizomes of Curcuma longa (Turmeric) were randomly collected from mature plants.

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±2°C for 72 hours and were filtered using Whattmann filter paper.

Equipments: Sterile Petri plates, volumetric flasks, Test tubes, Conicalflask, Whattmann filterpaper, mortar pestle, Incubator, Autoclave, Pippetting device, Hot air-oven.

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 mouthwash.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 (alcohol 70%) will be added and the product will be packed in an ambered coloured, well closed container. The herbal Mouthwash was prepared by the formula given in table.

TABLE 1:COMPOSITION OF HERBAL MOUTHWASH

Sr.no	Ingredients	Botanicalname	Role	Quantity
1	Turmeric	Curcuma longa	Antibacterial	1ml
2	Neem	Azadirachta Indica	Antimicrobial	2ml
3	Tulsi	Ocimumtenuiflorum	Dental care	4ml

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4	Mint	Mentha	Anti-microbial, Flavoring agent	2ml
5	Clove	Syzygiumaromaticum	Anti-inflammatory	1.5 ml
6	Cinnamon	Cinnamomumverum	Sweetening agent	2.5ml
7	Liquorice	Glycyrrhizaglabara	Anti-bacterial	2 ml

Aqueous extracts of Leaves by Shadow drying technique:

1. The leaves of mature plants were collected and washed 3 to 5 times with tap water to remove dust and dirt.
2. The leaves were allowed to soak in already boiled water bath at 30-40°C for 10 to 15 minutes to kill microbes present in the surface of the leaves.
3. The leaves undergoes Shadow drying technique were the leaves were spread in sterile container trays and kept at ambient temperature for 5 days.
4. After 5 days, the dried leaves were taken and powdered by using sterile mixer under aseptic condition.
5. The pulverized leaves are transferred to air-tight sterile container jars.
6. 100ml of sterile distilled water was taken in conical flasks (250 ml), the pulverized leaves were weighed and suspended in distilled water under sterile condition.
7. The preparation was heat sterilized at 40°C for 5-10 minutes and was kept for incubation at 37±2°C for 72 hours.
8. After incubation, the extracts were filtered with the help of a sterile Whattmann filter paper no: 1 and a funnel under lab condition.
9. The filtered extracts are boiled vigorously again to kill the bacterial spores, which will prevent from contamination.
10. The extracts after heating is ready to use for the formulation of Mouth wash and also can be tested against the oral pathogens by using Agar well diffusion techniques.

Evaluation Of Prepared Mouthwash

Color and Odour:Physical parameters like odour and colour were tested by visual examination.

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 pH meter.

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.

Stability Studies: The formulation and preparation of any product is incomplete without proper stability studies of the prepared 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 1 month for the prepared formulation. The samples were stored under the following conditions of temperature as 3-50° C, 250 C RH-60%, 400 C±2% RH- 75%. Finally the samples were kept under accelerated studies and were withdrawn on weekly intervals and were analyzed.

In-vitro antibacterial activity: In-vitro antibacterial activity was performed on isolated colonies of Streptococcus aureus. The Agar well diffusion technique was used for determining the zone of inhibition. The strains of S. aureuswere inoculated in prefabricated agar plate. Plates were dried and 4 wells were made with the help of 6 mm agar well cutter. 20µl, 40µl, 60µl, 80µl of prepared mouthwash was loaded in all the respective wells. The agar plates were kept undisturbed to allow the passive diffusion of herbal mouth wash into the agar culture medium. Then the plates were incubated at 37±2°C for 24 hours. The zone of inhibition was calculated in mm.

TABLE 2: CHEMICAL TEST

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Sr No	Name of herbs	Tests	Observation
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
4	Turmeric	Take turmeric powder in transparent glass + few drop of water and conc.HCl + shake it vigorously	Pink Colour
5	Cinnamon	Aq. Extract + potassium permanganate	Decolourisation
6	Liquorice	Aq. Extract + lead acetate reagent	White precipitate
7	Mint	5 mg extract of Ocimum sanctum in test tube + 1% HCl	Red colour

III. RESULTS AND DISCUSSION:

The pH of the formulation was found to be 6.1. As the skin is having an acidic pH around 5.5 this pH range of the formulation is suitable for oral disorders. The formulation was found to be free from heavy metals. The formulation was free from microbes as they have not produced any microbial growth when they got inoculated in the agar medium. This mouthwash is a purely herbal prepared without the addition of any kind of additives as other products found in the market. The formulation was undertaken stability studies for physical and chemical change. No considerable variations in properties of the formulation were observed. The results of stability studies are shown in the given table.When used in mouthwashes antimicrobial ingredient neem, tulsi, liquorice, cinnamon, mint turmeric and clove extracts have been found to reduce plaque and gingivitis when combined with daily brushing and flossing. Volatile sulfur compounds are the major contributing factor to bad oral odour. They arise from a variety of sources that is breakdown of food, dental plaque and bacteria associated with oral disease.

The antibacterial activity was evaluated by agar diffusion method for different concentrations of mouthwash. The result of one of inhibition for S.aureus was found to be 18 mm for 80µl, 15 mm for 60µl, 12 mm for 40µl and 7 mm for 20µl respectively. These results showed that the herbal mouthwash has significant antibacterial activity and the present preparation is able to inhibit bacterial growth in oral cavity. The association of oral microbial load on oral diseases in well established, thus this herbal mouthwash helps in motivating good oral health.

TABLE 3: RESULT OF STABILITY STUDY OF HERBAL MOUTHWASH

Temperature	Evaluationparameters	Observation(weeks)			
		1	2	3	4
3-5°C	VisualAppearance	Lightbrown	Lightbrown	Lightbrown	Lightbrown
	PhaseSeparation	Nil	Nil	Nil	Nil

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	Homogeneity	Good	Good	Good	Good
Room Temperature(25° CRH=60%)	VisualAppearance	Lightbrown	Lightbrown	Lightbrown	Lightbrown
	PhaseSeparation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
40°C±2°C RH=75%	VisualAppearance	Lightbrown	Lightbrown	Lightbrown	Lightbrown
	PhaseSeparation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good

TABLE 4: RESULT OF AGAR WELL DIFFUSION ANTIBACTERIAL STUDY

Organism	**Zoneofinhibition(mm)**			
	20 µl	**40 µl**	**60 µl**	**80 µl**
S.aureus	7	12	15	18
E.coli	12	17	19	21
Standard Penicillin G	**Zoneofinhibition(mm)**			
	20 µl	**40 µl**	**60 µl**	**80 µl**
S.aureus	9	15	18	20
E.coli	13	18	20	22

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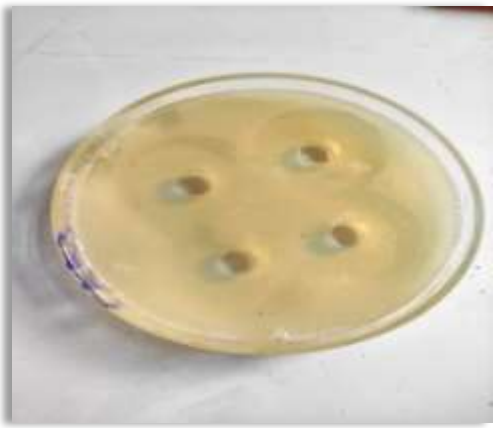

Fig. 1 Antimicrobial activity of standard Penicillin G against S. aureus

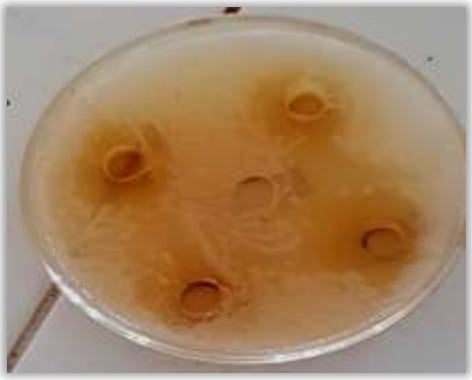

Fig. 2 Antimicrobial activity of standard Penicillin G against S. E. coli

Fig. 3Antimicrobial activity of prepared mouthwash against S. aureus

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Fig. 4 Antimicrobial activity of prepared mouthwash against E.coli

IV. CONCLUSION:

A study effort has been made to combine the active constituents of different extracts to make an effective polyherbal mouthwash formulation which is free from alcohol. For the present study neem, tulsi, Liquorice, cinnamon, mint turmeric and clove extracts have been used for their reported activities including anti-bacterial activity.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. Thus, these can be used as an adjunct to mechanical therapy for treating plaque induced gingivitis. Present study has an important impact in order to create an effective and inexpensive herbal oral health intervention for low social economic communities. However this study was short-term study so long term studies are required with larger. The natural herbs used in present formulation have been medicinally proven to prevent the problem of oral hygiene and bad breath. Since years and decades, these herbs have been known for working wonders as reflected in many research findings. Person can easily rinse his mouth using this herbal mouthwash and stay clear of wide variety of oral health issues.

REFERENCES:

[1]. Khan A. Ahmad A Manzoor N. Khan Lt Antifungal activities of Ocimum sanctum essential oil and its lead molecules Nat Prod Commun.2010; 5: 345-349.

[2]. Bhavna, J.K. Vidhya D. 2012 Herbal Mouthwash-A gift of Nature. Int J Pharma and Bio. Sci. 2014 278-280.

[3]. 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.

[4]. C. K. Kokate, Pharmacognosy, vol. 12, NiraliPrakashan, 1999.

[5]. 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.

[6]. 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.

[7]. 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.

[8]. 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 (Azadirachtaindica) fractions.JEthnopharmacol.1998; 61: 31-39.

[9]. Sagbo 1J, AfolayanAJ, Bradley G. Antioxidant, antibacterial and phytochemical properties of two medicinal plants against the wound infecting bacteria. A Pac J Tropi Biomed 2017; 7(9): 817-825.

[10]. Chitsazi M, Shirmohammadi A, Balayi E. Effect of herbal and chemical mouth-rinses on periodontal indices; comparison of

matrica, persica and chlorhexidine. J Dent Shiraz Univ Med Sci. 8(4), 2008; 54-60.
[11]. GeethaR.V, Anitha Roy. In Vitro Evaluation of Anti bacterial Activity of Ethanolic root extract of Glycyrrhizaglabra on Oral microbes. Int. J. Drug Dev. &Res., October-December 4(4), 2012, 161-165.
[12]. Weaver A, Fleming SM, Smith DB. Mouthwash and oral cancer; Carcinogen or coincidence? J Oral Surg 1979;37(4):250-3
[13]. Jha B., Dodwad V., "Herbal Mouthwashes - A Gift of Nature" Int. J. Sci. 2012; 3(2): 48-53.
[14]. Renuka s, Muralidharannp. Comparison in benefits of herbal mouthwashes with chlorhexidine Mouthwash: a review. Asian J Pharm Clin Res, Vol 10, Issue 2, 2017, 1-5.
[15]. Anushri M and Yashoda R: Herbs: A good alternatives to current treatments for oral health problems. International Journal of Advanced Health Sciences 2015; 1(12): 26-9.
[16]. Kukreja BJ and Dodwad V: Herbal mouthwashes- a gift of nature. International Journal of Pharma and Bio Sciences 2012; 3(2): 46-52.
[17]. Weaver A, Fleming SM, Smith DB. Mouthwash and oral cancer; Carcinogen or coincidence? J Oral Surg, 1979; 37(4): 250-3.
[18]. Blom T, Slot DE, Quiryen M, Van der Weijden GA. The effect of mouthrinses on oral malodour; a systemic review. Int. J Dent Hyg, 2012;10(3): 209-22
[19]. Vinoth B and Manivasagaperumal R: Phytochemical analysis and antibacterial activity of Azadirachtaindica. Juss.Int J of Res in Plant Sci. 2012; 2(3): 50-55.
[20]. Nasreen BJ and Gayathri V: Preparation of antibacterial herbal mouthwash against oral pathogens. Int. J. Curr. Microbiol. App. Sci 2016; 5(11): 205-21.
[21]. Rao N.J. SubhasK.R, Kumar K.S. Role of phytotherapy in gingivitis, a review, J pharmacol 2012; 8:1-5.
[22]. Charles, O.E., Chukwuemeka, S.N., Ubong, S.E., Ifeanyichukwu. R.I., &Chidimma, S.O. 2007. A Case for the use of Hebal Extracts in oral Hygiene: the efficacy of Psidiumguajava- based mouthwash formulations, Res. J. Appl. Sci., 2(11): 1143 - 1147.
[23]. Firas, H.Q., Abbas, S.A. 2009. The antimicrobial effect of aqueous & alcoholic extracts of eucalptus leaves on oral Mutans streptococci, Lactobacilli & Candida albicans (an in vitro study), J. Bagh. Col. Dent. 21(4).
[24]. https://www.ebooknetworking.net/ebooks/pharmacognosy-c-k-kokate.html
[25]. https://content.kopykitab.com/ebooks/2018/07/19884/sample/sample_19884.pdf