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FORMULATION, DEVELOPMENT, EVALUATION AND OPTIMIZATION OF HERBAL ANTIBACTERIAL MOUTHWASH

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ABSTRACT

Aqueous leaf extract of a tropical variety of Psidium guajava L. (Myrtaceae), Azadirachta indica (Meliaceae) & Glycyrrhiza glabra (Leguminosae) was used to formulate fifteen batches of herbal mouthwash. The antimicrobial potentials of these herbal mouthwash formulations in oral hygiene were assessed in vitro using a modification of the conventional methods for evaluating oral antiseptics. Formulations containing Psidium guajava, Azadirachta indica & Glycyrrhiza glabra leaf extract and a standard mouthwash (Povidone iodine, Chlorhexidine & Geofresh) served as test samples and controls respectivelly. The mouthwash formulations were screened for antimicrobial activities against cultures of Staphylococcus aureus, Escherichia coli and Bacillus subtilis. The extinction time of each formulation batch was determined against each test organism. The fifteen batches containing aqueous extract showed a high level of activity against the test organisms- E. coli and S. aureus comparable to the activity shown by the standard mouthwash (Povidone iodine, Chlorhexidine & Geofresh). The study encourages further stability and Jn vivo assessment to develop P.guajava, A. indica & G. glabra leaf extract as an ingredient of commercial mouthwashes.

KEYWORDS: Antimicrobial, Herbal Mouthwash, Psidium Guajava, Azadirachta, Indica, Glycyrrhiza Glabra.

INTRODUCTION

The importance of mouth and teeth cleanliness has been recognized from the earliest days of civilization to the 21st century. Patients and oral health practitioners are faced with a multitude of mouthwash products containing many different active and inactive ingredients.

Making informed decisions as to the suitability of a particular product for a particular patient can be a complex task. 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. Various herbal products and their extracts such as Guava, Pomegranate, Neem, Liquorice, Tulsi, Green Tea, Cranberry, Grapefruit etc. have shown significant advantages over the chemical ones. 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.[1]

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.[2]

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.[3] 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, Yavanisatva, Nagavalli, Gandha purataila, Pilu, Bibhitaka, Ocimum, Echinacea, Chameli leaves, etc. Manuka honey, which is rich in flavonoids, increased the glycoprotein production in the ethanol model of gastric damage. Manuka honey was the most effective antioxidant and antibacterial honey compared to both acacia honey and wild carrot honey, possibly because of its high phenol content.[4] Guava (Psidium guajava) as a mouthwash for swollen gums and

ulceration of the mouth and also for bleeding gums.[5] Extracts from neem inhibit the growth of S. mutans and used in the treatment of periodontitis.[6] Aloe vera mouthwash, mixture of Terminalia chebula and cinnamon, mixture of Staphysagria, Chamomilla, Echinacea, Plantago, Ocimum, and cistus extracts, mouthwashes with turmeric, neem and triphala, mixture of Acacia Arabica, Punica granatum, Chameli leaves, Glycyrrhiza glabra and neem shows significant reduction of plaque indices and gingival scores comparatively to chlorhexidine mouthwash and can be a better alternative to chlorhexidine mouthwash.[7,8,9]

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.[10]

Herbs used in Mouthwash

a) GUAVA[11-16]

Table.1: Information of Guava.

Synonym	Psidium; genus Psidium; true guava; Psidium guajava; fruit tree; guava bush.
Biological Source	Guava is a common tropical fruit cultivated in many tropical and subtropical regions of Psidium guajava.
Family	Myrtaceae
Chemical Constituents	Octanol, ethyl octanoate(pink guava fruit). α-pinene, β-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β-bisabolene, caryophyllene oxide, β-copanene, farnesene, humulene, selinene, cardinene and curcumene, mallic acids, nerolidiol, β-sitosterol, ursolic, crategolic, and guayavolic acids, cineol, quercetin, 3-L-4-4-arabinofuranoside (avicularin) and its 3-L-4-pyranoside (Essential oil), resin, tannin, eugenol, caryophyllene (1a α-, 4a α-, 7 α-, 7a β-, 7b α-)]-decahydro-1H-cycloprop[e] azulene, Guajavolide (2 α-,3 β-,6 β-,23-tetrahydroxyurs-12-en-28,20 β-olide; 1) and guavenoic acid (2 α-,3 β-,6 β-,23-tetrahydroxyurs- 12,20(30)-dien-28-oic acid, triterpeneoleanolic acid, triterpenoids, flavinone-2 2'-ene, prenol, dihydrobenzophenanthridine and cryptonine.
Uses:	a)Antimicrobial activity.b)Treatment of plaque. c)Antiproliferative activity.d)Immunomodulatory activity.

b) NEEM[17-21]

Table 2: Information of Neem.

Synonym:	Hin.-Nira, nimb; Mal. – Veppa; Mar. – Limba, Oriya- Nimba; Tam- Vembu.
Biological Source:	Neem consists of the fresh or dried leaves and seed oil of Azadirachta indica J. Juss (Melia Indica or M. azadirachta Linn.)
Family:	Meliaceae.
Chemical Constituents:	Nimbin, 6- desacetylnimbinene, Nimbinene, Nimbandiol, nimbolide. Quercetin, β-sitosterol. Ascorbic acid, n-hexacosanol, nonacosane and amino acid, Nimbin &Nimbidinin.
Uses:	a) Poultice, applied to boils. b) In worm, jaundice and in skin disease. c) Ulceration of cow-pox. d) Insect-repellent. e) Antiviral and antifungal.

c) LIQUORICE[21,22]

Table 3: Information of Liquorice.

Synonym:	Mulethi, Radix glycyrrhizae, Licorice, JethiMadh, Yashtimadhu
Biological Source:	It consists of subterranean peeled and unpeeled stolons, roots, and subteraean stems of Glycyrrhiza globraLinn., and Glycyrrhiza glabra L.
Family:	Leguminosae., and Fabaceae.
Chemical Constituents:	Saponin glycosides: Glycyrrhizin and glycyrrhetic acid. Flavonoids: Liquiritin, liquiritigenin, isoliquiritin and isoliquiritigenin. Coumarin derivatives: Herniarin, umbelliferone. Bitter principle: glyceramarin. Asparase, β-sitosterol, starch, resin and malic acid.
Uses:	a) Demulcent and expectorant. b) Tonic. c) Laxative. d) Diuretic and emollient property. e) Anti-inflammatory agent. f) Chewing gums, chocolate candy, cigarette and chewing tobacco.

MATERIALS AND METHODS

Test organisms: Standard culture of Staphylococcus aureus, Escherichia coli and Bacillus subtilis were used in the assay. They were cultured and preserved at 4°C.

Reagents/Chemicals: Culture media used were nutrient- agar, (LobaChemie, Mumbai), Chemicals used were sodium lauryl Sulphate (LobaChemie, Mumbai), Sodium chloride (LobaChemie, Mumbai), Methyl paraben (LobaChemie, Mumbai), peppermint emulsion (Prepared by own laboratory), Sorbitol (LobaChemie, Mumbai), Beet powder (Prepared by own laboratory), Mint oil(Purchased from local market) chloroform-water (double strength).

Collection of plant material: Leaves of Psidium guajava (Guava), Azadirachta indica (Neem), Glycyrrhiza glabra (Liquorice) were randomly collected from mature plants. The

leaves are kept for sundried for 5 days. After 5 days leaves were taken and powdered by using mixer. The pulverized leaves are transferred to air tight container.

Extraction process: The leaves were sun-dried, pulverized and stored in air tight bottles. About 50g of pulverized leaves were cold macerated by soaking in 200ml of distilled water for 72 h. The aqueous extract was later filtered using Whattmann filter paper and stored in air tight bottles.[22]

Formulation of Herbal Mouthwash: A batches of Mouthwash tested was prepared by adding varying propertions of Guava extract, Chloroform water(Double strength), Sodium lauryl sulphate, Methyl parabean, Sorbitol, Peppermint emulsion, Mint oil, Beet powder and Distilled water.[23]

EVALUATION OF PREPARED MOUTHWASH

1. Preliminary antimicrobical activity of the pure leave extract[22]: The preliminary sensitivity of the aqueous leave extract against the test-organisms was assessed by the agar-well diffusion method. Stock solution of the aqueous extract can be prepared. Holes of diameter 4mm were aseptically bored with a sterile cork-borer on plates previously seeded with 0.1ml standardized inocula of the respective test organisms and then 0.2 mL of the graded concentration of the aqueous extract of psidium guajava, Azadirachta indica, Glycyrrhiza glabra, was introduced into them using separate plates for each organism. The assay plates were held at 4°C for 24 h for rapid diffusion of extract into the assay medium and suppression of immediate microbial growth and subsequently incubated at standard growth conditions for each test microorganism. After incubation, plates were observed for the presence or absence of growth inhibition zones.

2. Antimicrobial Study: Agar Diffusion Method[10]

A sterile conical flask was taken and filled with 100 ml of distilled water 2g of agar was weighed and dissolved in the water and a pinch of agar- agar was added for solidifying the media. The media was autoclaved at 121°C for 15 mins, cool to 55°C. Using a marker, each petridish is divided into three halves. 0.05 ml of Staphylococcus aureas is added to one petridish again 0.05 ml of E. coli is added to the second petridish and 0.05 ml of Bacillus subtalis is added to the other. After adding the inoculum, the inoculum is spread by the spreader. Using the borer, wells are created in the inoculum-applied agar media on each of the halves of the petridishes. This method is known as the agar diffusion method or bore well

method. The petridishes are now kept in the incubator at 37 °C for 24 h. After 24 hr, petridishes are looked for inhibition zones around the wells.

3. **pH determination:**[24] pH of mouthwashes was measured using digital pH mater.
4. **Palatability determination:**[25] Palatability is the property of being acceptable to the mouth. The mouthwashes were tested separately for that Criterion by three research members in a blind-style. The test was done on scale of 5 levels:

   5 = Really Good; 4 = Good; 3 = Not Sure; 2 = Bad; And 1 = Really Bad
5. **Stability study:**[24] Stability studies were done with open and close container. Here, by subjecting the product to room temperature for 1 month.

RESULTS AND DISCUSSION

1. Organoleptic Properties, pH and Palatability and Stability determination

Various organoleptic properties, pH and palatability and Stability study was perform and result was found as follows:

Guava

Table 4: Organoleptic Properties of Guava preparation.

Parameters	Colour	Odour	Taste	pH	Palatability	Stability
Herbal Powder	Faint Greenish	Aromatic	Bitter Sweet	-	-	-
Herbal Extract	Brown	Aromatic	Bitter	-	-	-
Herbal Mouthwash						
G1	Red	Mild Aromatic	Agreeable	6.51	Good	Not stable
G2	Cherry Red	Pleasant	Slightly Sweet	7.01	Good	Stable
G3	Dark Red	Pleasant	Sweet	6.65	Good	Not stable
G4	Brownish Red	Pleasant	Sweet	6.79	Good	Not stable
G5	Brownish Red	Strong Aromatic	Sweet	6.87	Good	Not stable

Neem

Table 5: Organoleptic Properties of Neem preparations.

Parameters	Colour	Odour	Taste	pH	Palatability	Stability
Herbal Powder	Dark green.	Typical	Bitter	-	-	-
Herbal Extract	Brown	Garlic	Bitter.	-	-	-
Herbal Mouthwash						
N1	Cheery Red	Pleasant	Sweet	6.06	Not sure	Not stable
N2	Cheery Red	Pleasant	Sweet	6.19	Good	Stable
N3	Cheery Red	Pleasant	Sweet	6.31	Good	Not stable
N4	Cheery Red	Pleasant	Sweet	6.25	Not sure	Not stable
N5	Cheery Red	Pleasant	Sweet	6.40	Good	Not stable

Liquorice

Table 6: Organoleptic Properties of Liquorice preparations.

Parameters	Colour	Odour	Taste	pH	Palatability	Stability
Herbal Powder	Yellowish brown	Charact-eristics	Sweet	-	-	-
Herbal Extract	Yellowish brown	Charact-eristics	Bitter sweet	-	-	-
Herbal Mouthwash						
L1	Cheery red	Pleasant	Sweet	6.47	Good	Not stable
L2	Cheery red	Pleasant	Sweet	6.35	Good	Stable
L3	Cheery red	Pleasant	Sweet	6.29	Good	Not stable
L4	Cheery red	Pleasant	Sweet	6.45	Good	Not stable
L5	Cheery red	Pleasant	Sweet	6.59	Good	Not stable

B) Antimicrobial Study

The result of the preliminary antimicrobial sensitivity showed that the aqueous extract of the leaves was highly active against Staphylococus aureus, Escherichia coli and Bacillus subitilis.

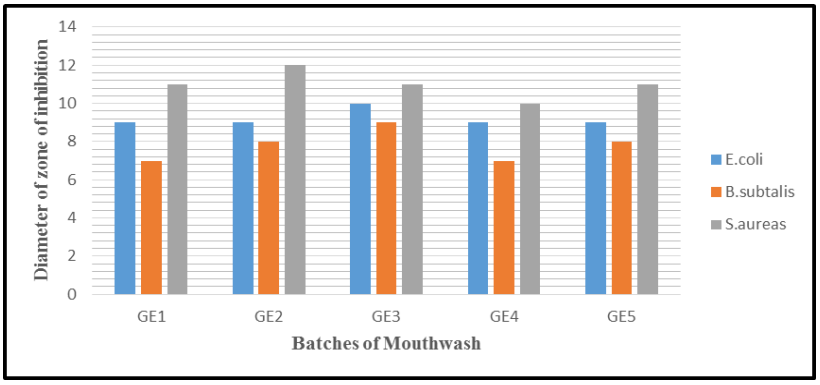

Fig. 1: Zone of inhibition of Pure Guava Extracts.

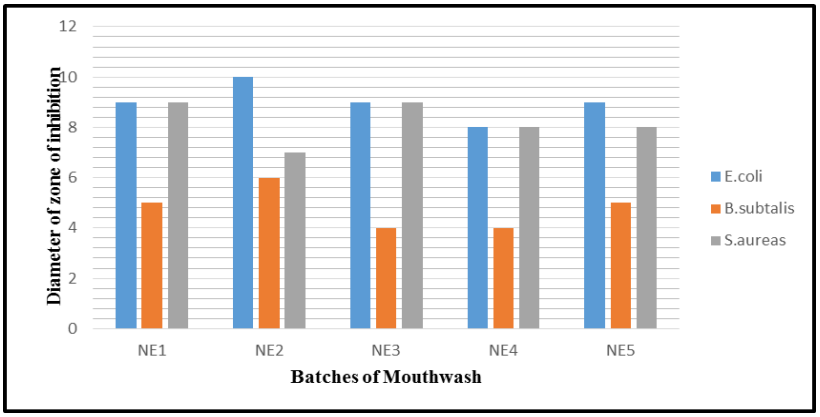

Fig. 2: Zone of inhibition of Pure Neem Extracts.

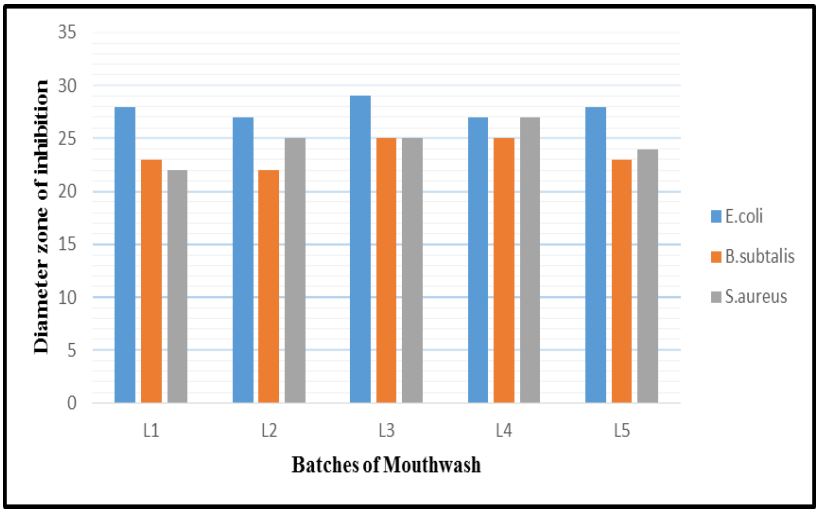

Fig. 3: Zone of inhibition of Pure Liquorice Extracts.

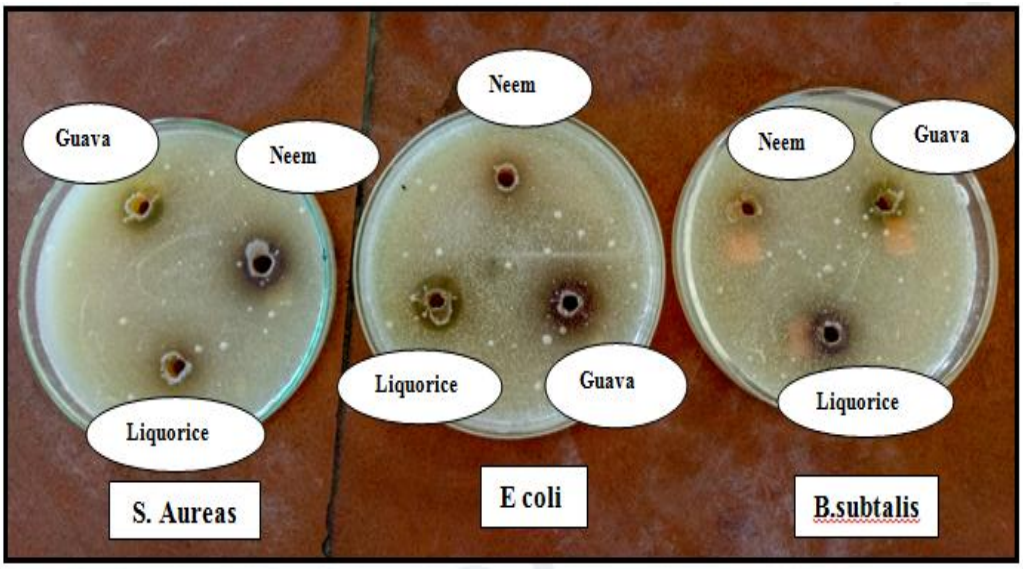

Fig. 4: Zone of inhibition of pure extract.

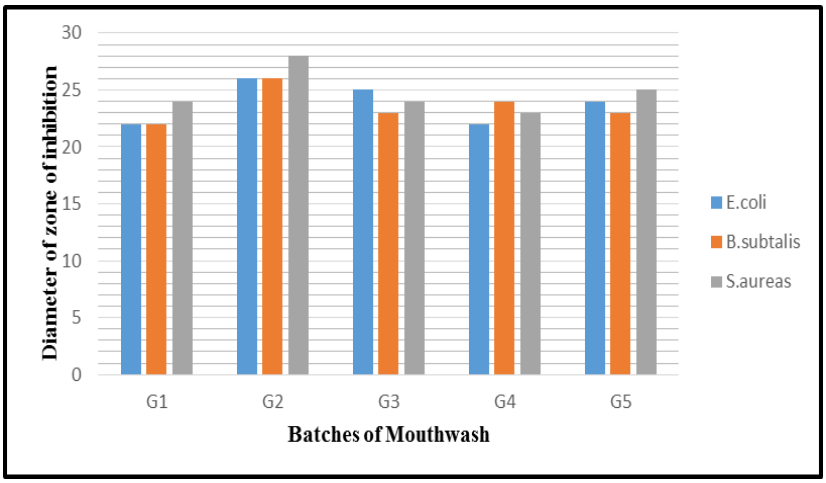

Fig. 5: Zone of inhibition of formulated Guava mouthwash.

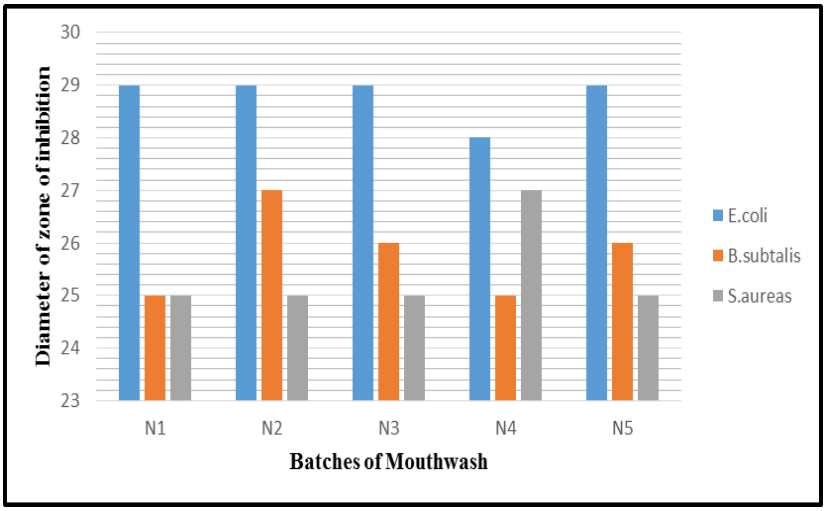

Fig. 6: Zone of inhibition of formulated Neem mouthwash.

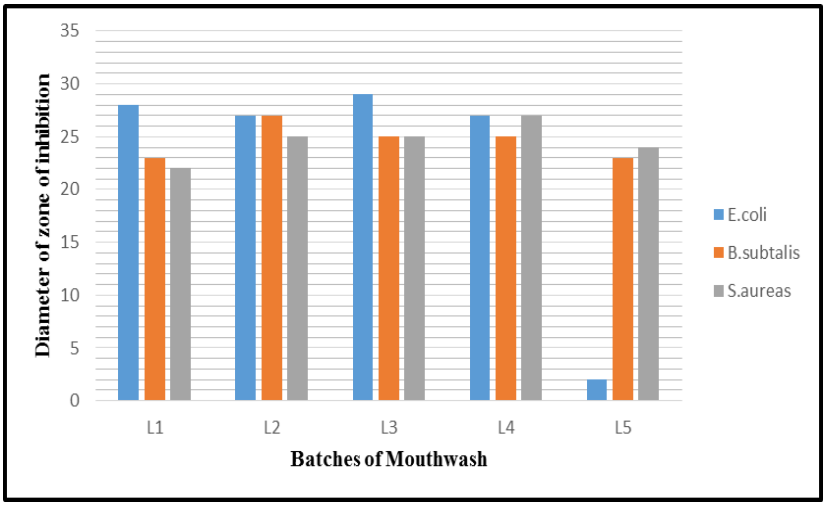

Fig. 7: Zone of inhibition of formulated Liquorice mouthwash.

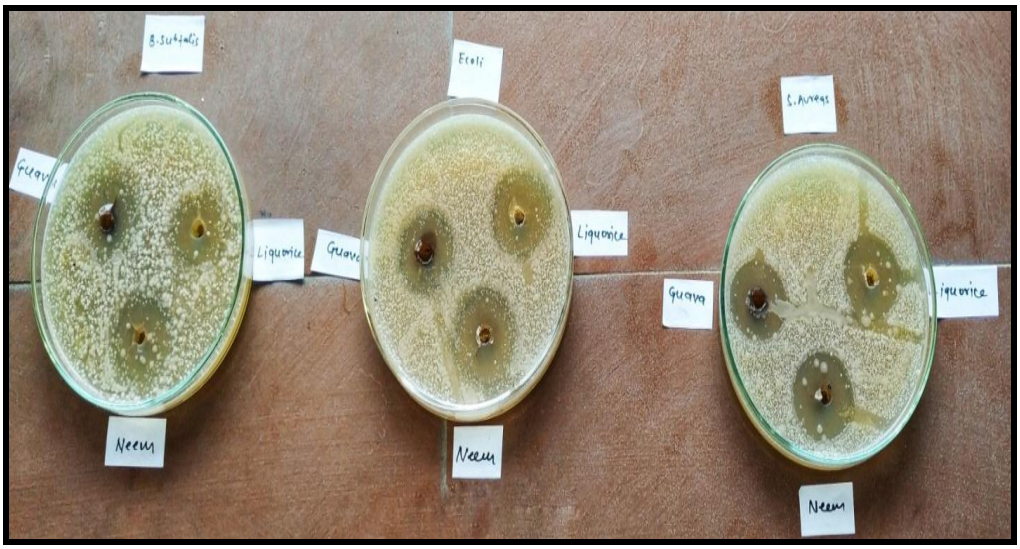

Fig. 8: Zone of inhibition of Guava, Neem & Liquorice prepared mouthwash

Comparision of Zone of inhibition of prepared herbal mouthwash and Marketed brands

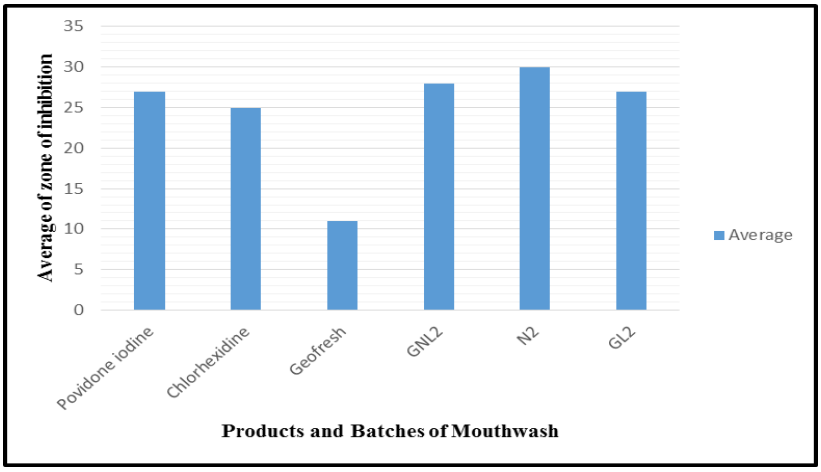

Fig. 9: Comparision of Zone of inhibition of prepared herbal mouthwash and Marketed brands against E. coli.

Fig. 9: Marketed products.

Fig. 10: Zone of inhibition of highest (Diameter) selected concentration.

The antimicrobial potency of the formulations is in the order G2>N2>L2. The formulation N2 which contains only the aqueous extract of Azadirachta indica, and G2 contains a Psidium guajava, and L2 contains a Glycyrrhiza glabra showed the highest level of antimicrobial activities and aqueous extract of showed the level of antimicrobial activities could be attributed to the presence of potent bioactive compounds with antimicrobial activities. Thus, the use of aqueous extract of P. guajava as mouthwash and gargles against oral infections could be effective.

The potency of formulations G4, N3, and L3 appears to be potentiated by the incorporation of sodium lauryl sulphate, an anionic surfactant.

Antimicrobial properties of mouthwashes is desired as it helps to reduce bacterial flora of the mouth, especially the anaerobic flora which produce the volatile sulfur compounds which are responsible for bad breath. The biocidal activity of the formulations could be related to the nature and concentration of exicipients as well as to dilution effect. Hence, formulations N2, containing mainly the aqueous neem extract being the most lethal; followed by formulation NL4 and GL4 which has high concentration of preservative and surfactant. Surfactants have been shown to increase the antimicrobial activity of mouthwash formulations.

From the results of this study, formulations G2, N2 & L2 are effective and could be explored further for possible commercialization and use as mouthwashes. Thus, there is a need for further stability, safety and In vivo efficacy studies to validate and corroborate the result of this studies.

CONCLUSION

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

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