IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Formulation And Evaluation Of Antibacterial Polyherbal Mouthwash

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Abstract

This study aims to create a polyherbal mouthwash with ethanolic extracts of leaves from Acarynthus aspera, Ocimum sanctum, and Azardicha indica and evaluate its medicinal effectiveness. After gathering A. aspera, Ocimum sanctum, and Azardicha indica, maceration was used to produce an ethanolic extract. Mouthwash formulations designated F1, F2, F3, and F4 were made with varying extract concentrations, and their stability, pH, color, and odor were assessed. Using 0.2% chlorohexidine as a control, the formulation's antibacterial and antifungal efficacy against streptococcus mutants (ATCC 21571) and candida albicans (MTCC 277) was also assessed. The in vitro antimicrobial activity of the mouthwash was tested using the agar well diffusion method, and it demonstrates good antibacterial and antifungal action against microorganism.

Keywords: Acarynthus aspera, Ocimum Sanctum, Azadirchta indica, Streptococcus mutants, Candida albicans, Maceration, Agar well diffusion Method, 0.2% Chlorohexidine.

Introduction

Mouthwash is a chemotherapy drug that patients can use as an efficient at-home dental hygiene method. The two most common diseases affecting humans are dental caries and gingivitis, both of which have multiple underlying causes. In India, dental caries affects between 60 and 70 percent of children. [2] In India, traditional medicine still employs one of the world's oldest medical systems, ayurvedic medicine. Ayurvedic medicine is mostly derived from plants, but it can also include elements of animals, metals, and minerals, in addition to dietary advice, physical activity, and lifestyle modifications. Medication therapy is prioritized in Ayurvedic medicine.

Mouth washes are concentrated aqueous anti-bacterial solutions that are used as antiseptics, cleansers, mouth fresheners, and to counteract oral infections caused by oral germs. A person's use of mouthwash is important for maintaining good oral hygiene since it relieves the signs and symptoms of swollen gums and gingivitis. Additionally, it is reliable for destroying harmful microorganisms. Most dental patients utilize mouth washes to treat sensitive teeth, sore mouths, and ulcerated throats [2, 3, 4].

Before performing oral surgery on patients, dentists always use mouthwash as an antibacterial agent to help sterilize the surface of the inflamed gums and teeth and prevent the spread of further microorganisms. Fluoride-containing mouthwash can help prevent tooth decay; however, even with fluoride mouthwash, avoid using it just after brushing your teeth, as this can remove the concentrated fluoride that was left on your teeth by the toothpaste. Pick a different time, like after, to use mouthwash. It is more effective to use mouthwash than just brushing. While brushing your teeth is a good way to remove plaque, using mouthwash can sometimes be even more beneficial. Mouthwash works well because it helps destroy bacteria and plaque.

Mouthwash comes in two varieties: chemical and herbal. Mouthwash is an aqueous solution that is mostly used for controlling plaque or for its deodorizing, refreshing, and antibacterial qualities. Alcohol, glycerine, artificial sweeteners, flavorings, colorings, surface active agents, and so forth may be present. Breath fresheners and treatments for potentially fatal secondary infections, such as oral mucositis, are examples of this [6, 7, 8].

Herbs having antimicrobial properties, such as neem, yavanisatva, nagavali, gandhapurataila, pilu, bibhitaka, ocimum, Echinacea, chameli leaves, etc., are found in many herbal mouthwashes. [10] Some of the herbs found in mouthwashes include peppermint, which has a cooling impact on the mouth, and clove, which has long been utilized for dental health due to its antiseptic, antibacterial, and antiviral qualities. Natural remedies that have been scientifically proven to be safe and effective for treating oral health issues like bleeding gums and gingivitis and preventing tooth decay include tulsi, green tea, clove oil, nagamotha, cinnamon oil, and many more [2, 10, 11].

Therefore, the majority of herbal mouthwashes are a safe substitute for mouthwash for youngsters, diabetics, and pregnant women. This study was conducted to ascertain the prevalence of mouthwash use. It was also intended to assess the safety and effectiveness of herbal mouthwash for use with human medications, as well as the type and quantity of mouthwash to be used.

The goal was to create an anti-bacterial herbal mouthwash that acts against oral pathogens by combining an alcoholic extract of Ocimum sanctum (tulsi) with aqueous extracts of two different powdered drugs that act against them: Cyperus rotundus (Nagarmotha) and Camellia sinesis (green tea) [12]. The anti-microbial activity was then assessed using the paper disc method and compared to Chlorhexidine Mouthwash I.P. The annual plant Acarynthus aspera, which belongs to the Amaranthaceae family, spreads like a weed over all of India. Traditionally, it is referred to as Apamarga. Antibacterial, immunomodulatory, antioxidant, anticancer, anti-inflammatory, thyroid-stimulating, and antiperoxidative properties are just a few of the medicinal

properties of A. aspera. It has been demonstrated that an ethanolic extract of A. aspera leaves has antibacterial activity against oral pathogens. (13)

Clove:

Biological Source: The dried flower buds that make up cloves are produced by Eugenia caryophyllus, a plant belonging to the Myrtaceae family.
Chemical components: methyl amyl ketone, caryophyllene, and eugenol.
Applications 1. Clove is an analgesic for teeth. 2. Addresses foul breath 3. Prevents dental caries

Neem:

Biological Source: The leaves of the Azadirachta indica plant, which belongs to the Meliaceae family, are used. Azadirachtin, Nimbin, Nimbdin, and Nimbinin are the chemical constituents.
Applications
It stops bacteria from growing and plaque from forming. Teeth can be cleaned and bacterial diseases treated using neem leaves, twigs, and seeds. Neem extract works well to treat gingivitis and other oral infectious disorders because it stops bacteria from growing and plaque from forming. For thousands of years, people in India and South Asia have used neem leaves, twigs, and seeds to heal bacterial and fungal illnesses as well as clean teeth.

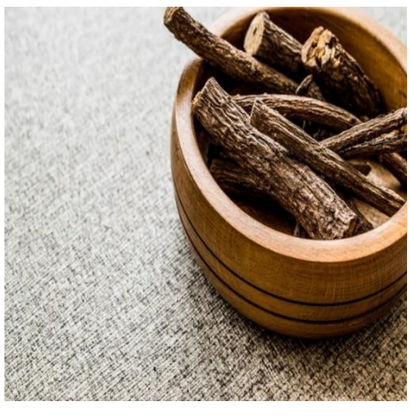
Tulsi

Biological Source: Ocimum sanctum Linn., a plant in the Lamiaceae family, is the source of fresh and dried leaves used to make tulsi (Syn. Ocimum tenuiflorum). it's active ingredients are roughly 70% eugenol, 3% carvacrol, and 20% eugenol-methyl-ether.
Uses: 1. Teeth can be cleaned with a powder made from dried leaves. 2. Oral health toothpaste with tulsi leaf preparation helps treat colds and coughs. 3. It is efficient against many bacterial strains and demonstrates bacteriostatic, immune-modulating, and antioxidant properties. 4. Moreover, it is used to treat gingivitis and periodontal diseases.

Aghada :

The plant species Achyranthes aspera Linn. belongs to the Amaranthaceae family. It is also referred to as Apamarga or Chirchita in Hindi. Its active ingredients include the triterpenoids saponins, which have oleanolic acid, and ecdysterone.
Applications:Traditional healers greatly value this plant, which they use to treat a variety of conditions, including asthma, bleeding, boils, bronchitis, colds, coughs, colics, debility, dropsy, dog bites, dysentery, ear complications, headaches, leucoderma, pneumonia, renal complications, bites from snakes and scorpions, and skin diseases.

Liquorice:



Source: biological: a blooming plant belonging to the Fabaceae family of beans. It yields a pleasant, fragrant flavor when extracted from the root. chemical components: polyphenols, polysaccharides, and tritrpenoids are present. Applications: antioxidant, antibacterial, and anti-inflammatory qualities.

Advantages :1.A clean breath. 2. Protecting teeth from decay by using sodium fluoride. 3. Reducing gum discomfort through the removal of microorganisms. 4. Using a bleaching product to whiten teeth 5. Preventing gum disease with an antiseptic or anti-plaque ingredient. 6. Mouthwash reduces gingivitis and gum disease by getting rid of microorganisms that could otherwise infect the gums and dental sockets. 7. It can help you avoid dental decay by demineralizing your teeth, strengthening the enamel, and preventing the creation of plaque.

Materials and Methods

Materials: **Collection of leaves and roots** :

Leaves of A.aspera ,ocimum sanctum ,Azardicha indica , Glycerrhiza gabra were collected from outskirts of Solapur . Maharashtra and roots of Glycyrrhiza glabra were obtained from Vasundhara agrimart Solapur. Maharashtra . They were authenticated by the Department of Botany DBF Dayanand College of Arts and science , Solapur as Acarynthus aspera Glycerrhiza glabra , Azardicha indica , Ocimum sanctum.

Test organisms: strains of streptococcus mutants (ATCC 25175) were obtained from National Collection of Industrial Laboratories Pune Maharashtra, India and strains of candida albicans (MTCC 277) was obtained from Depatment of Biotechnology V.G. Shivdare Coleege of Arts and Science, Solapur, Maharashtra.

Standard: Chlorhexidine mouthwash – 0.2 % Chlorhexidine Gluconate was used as standard antimicrobial agent.

Method of Extraction:

The A. aspera plant roots of Glycyrrhiza gglabra were gathered and then cleaned with sterile water, let to dry naturally, and ground into a coarse powder using a mixer grinder band. The powder was then stored individually in airtight containers. The powdered leaves of Acarynthus aspera, Ocimum sanctum, and Azardicha indica were soaked in ethanol for seven days to prepare the ethanolic extract of the leaves. Similarly, the powdered roots were soaked in a solution of 30:70 ethanol and water for seven days to prepare the root extract. The herbal extracts were filtered through Whatman filter paper; the leftover material was then rinsed with 20 milliliters of ethanol and compressed. The obtained filterates were put on petri plates, and the solvents were left to evaporate. 3.9 grams of Ocimum sanctum, 4.1 grams of Azadarachita indica, and 4.8 grams of Acarynthus aspera extract and 5,6 gm of Glycyrrhiza glabra root extract was obtained. The extract was collected and stored at 4-5 c in air tight container .14

Extract of neem

Extract of Liquorice

Extract of Acarynthus aspera

Phytochemical analysis: The phytochemical analysis of extracts of Ocimum sanctum , Glycerrhiza glabra , A. aspera ,Azardicha indica were investigated and summarized in below table . the presence of tannins, saponins ,flavonoids ,alkaloids , and steroids were determined .

Composition of formulation

Ingredients	Scientific name	Chemical constituents	Uses
clove	Eugenia caryophyllus	Eugenol	Dental analgesic antimicrobial agent
Neem	Azardirachta indica	NImbin, Nimidin , Azardichinitin	Antiseptic , Inhibit plaque formation.
Tulsi	Ocimum sanctum	Eugenol Carvecol and eugenol –methyl-ether	Antimicrobial activity
Liquorice	Glycyrrhiza glabra	Glycyrrhizin	Antibacterial
Propylene glycol	-	-	Humectant
Aghada	Acarynthus aspera	Triterpenoids , Saponins, ecdysterone	Antimicrobial activity
Water	-	-	Vehicle

Method of preparation of Mouthwash :

Acarynthus aspera leaf extract, Azadirachta indica, Ocimum sanctum, and Glycyrrhiza glabra root extract were all precisely weighed and then diluted in a small amount of water in a single beaker. After adequately dissolving saccharin in a small amount of water in a separate beaker, clove oil was added drop by drop. A measured amount of glycerol was added and thoroughly stirred. The second beaker, which contained a mixture of the remaining ingredients, was then filled with the extract solution gradually. The volume was then adjusted with water. Finally, preservative was added and thoroughly mixed, and the formulation was placed in a well-sealed container for storage.

Formulation of Polyherbal Mouthwash

Formulation of Mouthwash

Ingredients	Function	Formulations			
		F1	F2	F3	F4
Acarynthus aspera	Active drug	250mg	500mg	1000mg	1500mg
Liquorice roots (Glycerrhiza glabra)	Active drug	200	400	600	800
Azadirachta indica	Active drug	200	200	200	200
Ocimum sanctum	Active drug	200	200	200	200
Clove oil	Active drug	0.1ml	0.1ml	0.1ml	0.1ml
Saccharin	Sweetener	0.1mg	0.1mg	0.1mg	0.1mg
Glycerol	Cosurfactant	3.9ml	3.9ml	3.9ml	3.9ml
Alcohol	Preservative	1.2ml	1.2ml	1.2ml	1.2ml
Purified water	Upto 60ml	60ml	60ml	60ml	60ml

Evaluation

Colour and odour: Visual examining was used to asesss physical characteristics like colour and odour .

pH :A digital meter used to check the ph of formulation . Caliberation of pH meter done by using standard buffer solution. The pH of mouthwash was determined by dissolving in 1ml of mouthwash in 50 ml of distilled water .

Stability studies : Stability studies are carried out to evaluate the formulation's chemical and physical stability, as well as its overall safety. When a formulation is workable and retains its properties over time, it is said to be stable. For prepared mouthwash, accelerated stability investigations are conducted over a brief period of time. While the PH of the mouthwash was tested with a digital pH meter, the physical stability of the product was assessed based on its appearance, phase separation, and homogeneity.Monthly analyses were performed on the samples.

Test for microbial growth in formulated mouthwash :

Agar (3 grams) and Sabouraud dextrose agar (6.5 grams) were precisely weighed, then transferred to two conical flasks, each holding 1000 milliliters of distilled water, and heated over a water bath.Agar solutions in conical flasks containing petri plates are autoclaved for 45 minutes at 121 °C to disinfect them. Following room-temperature cooling, the solutions were moved to a sterile petri plate and given time to harden. Using the streak plate approach, these petri plates were inoculated with moutheashes that had been created, and a control was set up. For 24 hours, these petri plates were incubated at 37 degrees Celsius in an incubator. Following the incubation period, the plates were taken out and compared to the control to see if any microbiological growth had occurred.

In vitro Antimicrobial activity :

S. mutants and C. albicans bacteria were used to test the mouthwash's in vitro antibiotic properties. To solidify in aseptic circumstances, the right amount of agar and Sabouraud dextrose agar medium were transferred. Using a micropipette, samples of S. mutants and C. albicans were moved to petri plates, and they were equally distributed around the petri plate using a sterile spreader. Following the solidification of the medium, 5.5-mm cup borer wells were created. Next, mouthwashes with a 100-microliter concentration were added to the wells, and 0.2% chlorhexidine mouthwash was used as the control. After removing the plates, the growth inhibition zone was noted and measured in millimeters. These were contrasted with the control group's zone of growth inhibition.

Result and Discussions

Results

Preliminary phytochemical analysis of alcoholic extract revealed the presence of the tannins, saponins ,glycosides , Alkaloids, triterpenoids

. shown in Table No 1

Phytochemicals	Acarynthus aspera	Ocimum sanctum	Azardicha indica	Glycerrhiza glabra
Tannins	+	+	+	+
saponins	+	+	+ +	+
Triterpenoids	-	-	+ +	-
Glycosides	-	-	+ +	-
Alkaloids	+	+	+ +	-
Carbohydrates	-	-	- -	+
Proteins	-	-	- -	-
Flavonoids	+	+	+ +	+
Steroids	-	-	+	-

Stability studies

A temperature change was applied to the produced mouthwash, and its appearance, phase separation, homogeneity, and pH were assessed. It was discovered that there was no phase separation, no color change, and that it was homogenous. The formulation's pH ranged from 6.8 to 7.2, making it appropriate for treating oral diseases. The results are displayed in the table 2 below.

Temperature	Evaluation	Observation (months)			
		0	1	2	3
3- 5^0 C	Visual appearance	Dark Green	Dark green	Dark Green	Dark Green
	Phase separation	Nil	Nil	Nil	Nil
	Homogenicity	Good	Good	Good	Good
	pH	6.8	6.8	6.8	6.8
Room Temperature (25^0 C RH= 60%)	Visual appearance	Dark Green	Dark Green	Dark Green	Dark Green
	Phase separation	Nil	Nil	Nil	Nil
	Homogenicity	good	Good	Good	Good

	pH	6.8	6.8	6.8	6.8
40 ^{0}c ±2^0 RH = 75%	Visual Appearance	Dark Green	Dark Green	Dark Green	Dark Green
	Phase separation	Nil	Nil	Nil	Nil
	Homogenicity	Good	Good	Good	Good
	pH	6.8	6.8	6.8	6.8

Table No 2

Test for microbial growth in formulated mouthwash :

A temperature change was applied to the produced mouthwash, and its appearance, phase separation, homogeneity, and pH were assessed. It was discovered that there was no phase separation, no color change, and that it was homogenous. The formulation's pH ranged from 6.8 to 7.2, making it appropriate for treating oral diseases. The results are displayed in the table below.

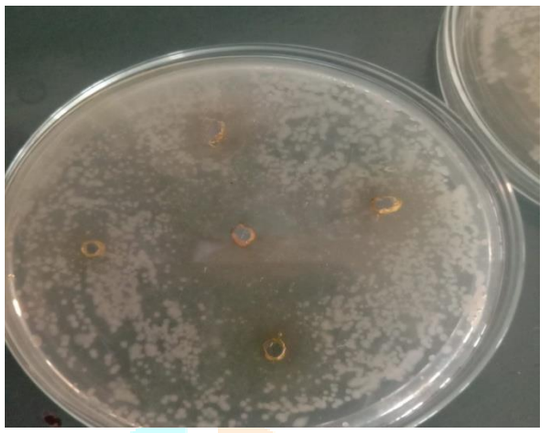


Fig. 1

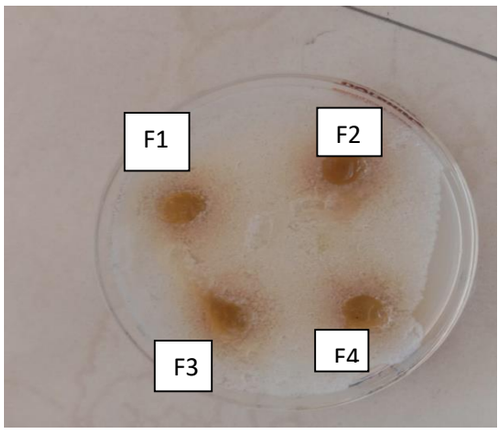
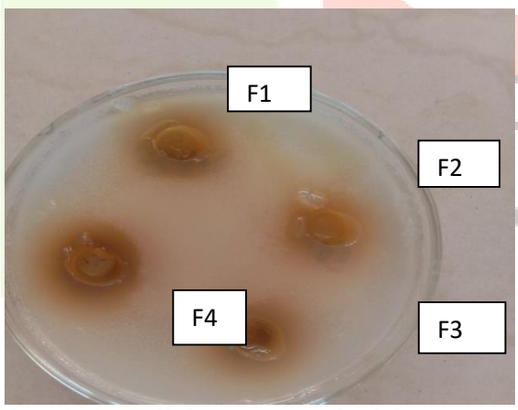


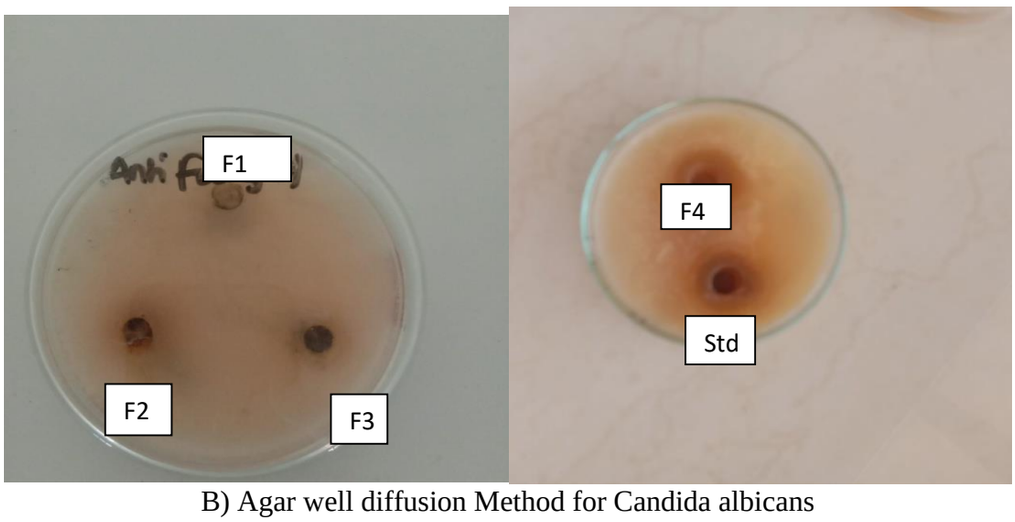
Fig. 2

In vitro Antimicrobial activity

The agar-well diffusion method was utilized to estimate the zone of inhibition in order to evaluate the antibacterial and antifungal activity. For S. mutants, the zone of inhibition was measured as follows: 19 mm, 21 mm, 22 mm, and 24 mm for F1, F2, F3, and F4; for C. albicans, it was 22 mm, 24 mm, 25 mm, and 26 mm for F1, F2, F3, and F4 accordingly. For S. mutants and C. albicans, the 0.2% chlorohexidine zone of inhibition was 21 mm and 24 mm, respectively. All mouthwash preparations have antibacterial and antifungal activity, according to the data. The largest zones of inhibition against S. mutants and C. albicans, respectively, are found in F3 and F4.



A) Agar well diffusion method S. mutants



B) Agar well diffusion Method for Candida albicans

Table 3. Zone of inhibition (mm) at 100 microliter concentration of polyherbal formulation and standard against Streptococcus mutants and Candida albicans

Formulation	Zone of inhibition (mm)	
	Streptococcus mutants	Candida albicans
F1	19	22
F2	21	24
F3	22	25
F4	24	26
Standard (0.2% Chlorhexidine)	21	24

Discussion:

Aspera, Ocimum sanctum, and Azadirachta indica leaf ethanolic extracts have shown antibacterial and antifungal efficacy against S. mutants and C. albicans. Mouthwash that contains polyherbal extracts such as neem, aghada, tulsi, clove oil, and liquorice is favored because it has fewer adverse effects. Neem, clove oil, and A. aspera work well against oral bacteria that can lead to tooth caries and other oral health problems. The mouthwash formulations (F1-F4) exhibited stability under varying conditions and were devoid of microorganisms due to the absence of microbial growth upon inoculation in nutritional agar and sabouraud dextrose agar mediums.calculating the zone of inhibition using the agar-well diffusion method in order to evaluate the antifungal and antibacterial effectiveness.

It was discovered that the zone of inhibition for S. mutants was 19 mm, 21 mm, 22 mm, and 24 mm for F1, F2, F3, and F4, respectively, and 22 mm, 24 mm, 25 mm, and 26 mm for F1, F2, F3, and F4 for C. albicans. The 0.2% chlorhexidine zone of inhibition measured 21 mm for S. mutants and 24 mm for C. albicans, respectively. The strongest zone of inhibition against S. mutants and C. albicans is found in F3 and F4.

Conclusion :

Mouthwash with polyherbal extracts from plants such as Azadirachta indica, Glycyrrhiza glabra, Ocimum sanctum, and Aspera possessing medicinal properties, including antibacterial, anti-inflammatory, and dental analgesic properties, in clove oil. Mouthwash containing polyherbal extracts is intended to treat bad breath and provide a pleasant, revitalizing feeling. Herbal remedies with antibacterial action, like A. aspera, can be used to prevent synthetic medications and the negative effects associated with them. There are numerous medicinal uses for Acarynthus aspera. ethanolic extract from A. aspera leaves. The antibacterial and antifungal properties of clove oil, Glyc. gabra, Ocimum sanctum, and Azadirachta indica have been demonstrated against oral microorganisms. A well-formulated mouthwash with herbal ingredients has strong antimicrobial efficacy against oral microorganisms like Candida albicans and S. mutants, which are mostly responsible for fungal infections and dental caries. It also promotes overall health.

Acknowledgements

The authors like to thank national collection of Laboratories Pune. Department of Botany DBF Dayanand College of Arts and Science Solapur and Depatment of biotechnology V.G. Shivdare college of arts and Science Solapur for their kind support.

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