

Preparation, Evaluation & Comparison of Herbal Mouthwash against Oral Micro-Organisms

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Abstract

Medicinal plants play a vital role in curing diseases due to their antimicrobial activity against human pathogens through decades. Herbal mouthwashes are in high demand, because they act on oral pathogens and relieve the pain instantly and are also less side effective. One of the most common infectious diseases encountered by many individuals is dental caries and periodontal diseases at different stages of their life time. Dental caries include the cavity formation, eruption of enamel, swollen gums, bleeding gums. Dental caries are high among children and adolescents, because they do not practice proper oral hygiene. Prepared antimicrobial Herbal Mouthwash from the solvent extract of five different herbs like Naie, Guava, Pomegranate, Dikmali and Henna that acts against the oral pathogens namely *S.aureus*, *S. pyrogen*, *E. coli* and *C. albicans* to check the antimicrobial activity by using Disc Diffusion method. The prepared Herbal Mouthwash had shown the antimicrobial activity against used test microorganisms.

Keywords: Herbal; Extraction; Micro-organisms; Mouthwash

Introduction

The mouth washes are concentrated aqueous anti-bacterial solutions that are used against oral microbes to counter oral infection, cleansing, to get rid of bad breath refreshing, antiseptic. 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 i.e. 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. Ancient Egyptians are known to be responsible for the first artistic drawings that emphasize the importance of beauty and hygiene. An unclean body was thought to be impure. Pedanius Dioscorides, a Greek physician and surgeon (40–90 AD) whose writings served as a medical textbook, suggested for treatment of bad breath a mouthwash mixture of the following: a decoct of the leaves of the olivetree, milk, the juice of pickled olives, gum myrrh with wine and oil, pomegranate peelings, nutgalls, and vinegar. The ancient Romans included teeth cleaning as part of their [1-9] religious ceremonies. The patriarchy required their slaves to clean their teeth. The Romans included a secret ingredient in their mouthwash: human urine. They imported urine from Portuguese people because they thought it had more strength. Until the 18th century, urine continued to be an active ingredient in toothpaste and mouthwash because of the ammonia's cleansing abilities.

1. Mouthwashes can be broadly classified as

a. Chemical mouthwashes

b. Herbal mouthwashes

a. Chemical mouthwashes

Usually contains antimicrobial agents, such as chlorhexidine gluconate which is very potent chemoprophylactic agent, it has a broad spectrum action especially against Mutans Streptococci group. But it has many side effects like staining of teeth, altering the taste of the mouth and desquamation of oral mucosa (Figure 1).

a. Herbal mouthwashes

The importance of herbs is highly considered as effective in contrast to chemical products. Medicinal plants play a vital role in curing diseases due to their antimicrobial and antifungal activity against human pathogens through decades. Herbs are being widely explored to discover alternatives to synthetic antibacterial agents. Herbal Mouthwash act on oral pathogens and relieves pain instantly and are also less side-effective.

a. Uses of mouthwash

1. Antiseptic/As antibacterial
2. Astringent
3. Cooling and refreshing action

b. Aim and objectives

- To prepare Herbal Mouthwash.
- To evaluate prepared Herbal Mouthwash.
- To compare the prepared Herbal Mouthwash with marketed Chemical Mouthwash.

Details of Selected Herbal Plant

Material and Method

Collection of herbal plant materials

Herbal plant materials like Guavawas collected locally from farm at Solapur city and remaining herbs like Naie, Dikmali, (Table 1) Pomegranate

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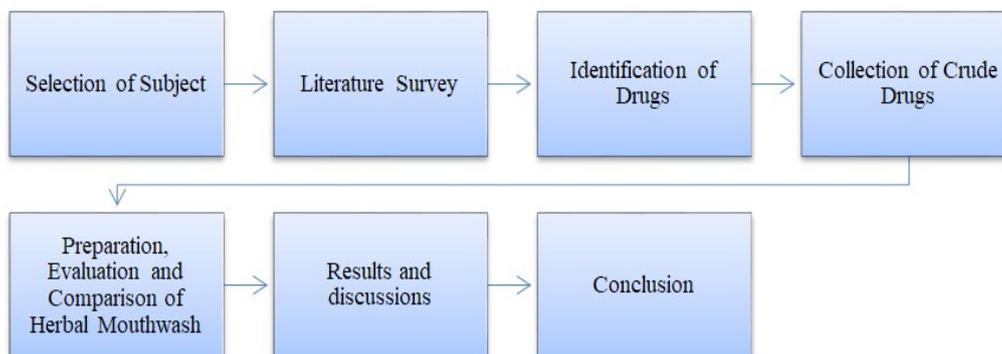


Figure 1: Plan of work.

Table 1: Pharmacognostic scheme of Dikmali.

	Synonym	Brilliant Gardenia, Cambi resin tree, Gardenia lucida
	Botanical name	<i>Gardenia gummifera</i>
	Chemical constituents	It contains 89.9% of resins, 0.1% volatile oils and a coloring agent gardenin. Besides these it contains certain alkaloids and minerals.
	Uses	It is used as carminative, stimulant, vermin-fuge, wound healer, painkiller, insect repellent and majorly used in gums and tooth problems, anti septic and anti bacterial.
	Part used	Gum from bark part

and Henna were purchased from local Market of AyurvedicStore at Solapur, Maharashtra, India in the month of 16th January 2018 (Table 1-9).

Preparation of extract

Preparation of powder

- Dikmali, Naie and Henna: Readymade powders utilized directly (Table 2).
- Guava leaves were shadow dried at room temperature for 1 week before extraction process.
- Leaves were cut into small pieces with the help of scissor and grinded in mortar & pestle to fine texture powder form and stored in air-tight polythene bag (Figure 2).

Solvent extraction

Powdered plant material was extracted by Percolation Method (Exhaustive extraction) using ethanol and distilled water (1:4).

1. Procedure Followed For Percolation

- Drug in a suitably powdered form.
- Imbibe the powdered drug with menstruum for a period of one hour.
- Packed evenly into the percolator.
- A piece of filter paper is placed on surface followed by a layer of clean sand so that top layers of drugs are not disturbed.

- Sufficient menstruum is poured over the drug slowly and evenly to saturate it, keeping the tap at bottom open for passing of occluded gas to pass out.
- Sufficient menstruum is also added to maintain a small layer above the drug and allowed to stand for 24 hours (Figure 3).
- 75% of the volume of the finished product is collected.
- Mark is pressed and expressed liquid is added to the percolate giving 80% to 90% of the final volume.
- Volume is adjusted with calculated quantities of fresh menstruum.
- Evaporation and concentration to get finished products by applying suitable techniques and apparatus.

Evaluation Test

1. **Color:** Yellowish Brown
2. **Odour:** Aromatic
3. **Taste:** Sweet, Pleasant

Microbial test

Collection of Micro-organisms

Mouth organisms like *Staphylococcus aureus*, *Streptococcus pyrogens*, *Escherichia coli*, and *Candida albicans* were procured from Ashwini Sahakari Hospital & Research Centre, Solapur and maintained and incubated at 36°C for 2 hrs, before performing the activity.

Table 2: Pharmacognostic scheme of Naie.

Naie		
	Synonym	Indian whitehead, Bahuguni, mamejaw
	Botanical name	<i>Enicostemma axillare</i>
	Chemical constituents	A fruit mainly contains gum resin and numbers of flavonoids are also present. Whereas it includes gardenin and also woogninder along with alkaloids, tannins.
	Uses	It is mainly used in diabetes, arthritis, skindiseases, hernia, and swelling. Antibacterial, anti inflammatory and anti oxidant.
	Part used	Leaves

Table 3: Pharmacognostic scheme of Pomegranate.

Pomegranate		
	Synonym	Apple fruit
	Botanical name	<i>Punicagranatum</i>
	Chemical constituents	Ellagic acid, punicalagin, punicalin, punicalic acid, urolithin A with 85% of water and others are ascorbic acid citric acid
	Uses	It is mainly used as rich source of vitamin C, detoxifying agent, combinant tool against heart diseases and also for dental hygiene, anti oxidant, antimicrobial etc.
	Part used	Peel part

Table 4: Pharmacognostic scheme of Henna.

Henna		
	Synonym	Egyptian privet, Lawsonia Alba, Mehendi
	Botanical name	<i>Lawsonia inermis</i>
	Chemical constituents	The phytochemicals present in Henna are phenols, and glycosides. Lawsone is an active constituent while others are gallic acid, resin, sugars, tannins.
	Uses	It is mainly used as antibacterial, treat headache, antifungal, astringent, aromatic, cooling and analgesic
	Part used	Leaves

Table 5: Pharmacognostic scheme of Guava.

Guava		
	Synonym	Psidium, guava bush, Peru
	Botanical name	<i>Psidium guajava</i>
	Chemical constituents	Polyphenol, Flavonoids, carotenoids, essential oils, 10% tannins, saponins
	Uses	Anti-oxidant, anti-inflammatory, anti-bacterial
	Part used	Leaves

Table 6: Zone of inhibition in Dikmali extracts.

Microbes	Dilution of the extracts	Zone Of Inhibition (mm)	Sensitivity	Resistance
<i>E. coli</i>	1mg/ml	0	-	+
	2mg/ml	0	-	+
<i>S. aureus</i>	1mg/ml	0	-	+
	2mg/ml	3	+	-
<i>C. albicans</i>	1mg/ml	0	-	+
	2mg/ml	5	+	-
<i>S. pyrogen</i>	1mg/ml	5	+	-
	2mg/ml	6	+	-

Table 7: Zone of inhibition in Naie extracts.

Microbes	Dilution of the extracts	Zone Of Inhibition (mm)	Sensitivity	Resistance
<i>E.coli</i>	1mg/ml	1	+	-
	2mg/ml	1	+	-
<i>S.aureus</i>	1mg/ml	0	-	+
	2mg/ml	2	+	-
<i>C.albicans</i>	1mg/ml	0	-	+
	2mg/ml	2	+	-
<i>S.pyrogen</i>	1mg/ml	3	+	-
	2mg/ml	3	+	-

Table 8: Zone of inhibition in Henna extracts.

Microbes	Dilution of the extracts	Zone Of Inhibition (mm)	Sensitivity	Resistance
<i>E.coli</i>	1mg/ml	0	-	+
	2mg/ml	2	+	-
<i>S.aureus</i>	1mg/ml	0	-	+
	2mg/ml	0	-	+
<i>C.albicans</i>	1mg/ml	4	+	-
	2mg/ml	6	+	-
<i>S.pyrogen</i>	1mg/ml	0	-	+
	2mg/ml	2	+	-

Table 9: Zone of inhibition in Pomegranate extracts.

Microbes	Dilution of the extracts	Zone Of Inhibition (mm)	Sensitivity	Resistance
<i>E.coli</i>	1mg/ml	0	-	+
	2mg/ml	2	+	-
<i>S.aureus</i>	1mg/ml	0	-	+
	2mg/ml	2	+	-
<i>C.albicans</i>	1mg/ml	0	-	+
	2mg/ml	2	+	-
<i>S.pyrogen</i>	1mg/ml	1	+	-
	2mg/ml	4	+	-

Table 10: Zone of inhibition in Guava extracts.

Microbes	Dilution of the extracts	Zone Of Inhibition (mm)	Sensitivity	Resistance
<i>E.coli</i>	1mg/ml	0	-	+
	2mg/ml	2	+	-
<i>S.aureus</i>	1mg/ml	0	-	+
	2mg/ml	2	+	-
<i>C.albicans</i>	1mg/ml	2	+	-
	2mg/ml	3	+	-
<i>S.pyrogen</i>	1mg/ml	0	-	+
	2mg/ml	0	-	+

Collection of oral swab

The sterile cotton swab was rubbed on to the gums and periodontal region of both upper and lower jaws of the individuals (totally 6 oral swabs) were collected.

Antibacterial Activity

Disc Diffusion Method

A sterile 500 ml conical flask was used to prepare nutrient agar (HIMEDIA M001-100G) Lot 0000293649 Expiry: MAR 2022

- Prepared Media and petri dishes were autoclaved at 121°C for 15min.

(Autoclave LabHosp size 12X12)

- Then 15-20 ml of agar was poured in each sterile Petri dish.
- The plates were air dried under UV radiated in the laminar air flow (Klenzaid's Bioclean devices Ltd.) for 15 min.
- The bacterial colonies were inoculated into nutrient broth and incubated for 24hrs.
- In broth turbid solution indicated the viable microorganisms ready to use for testing the antibacterial activity.
- From this turbid solution having micro-organism inoculated into prepared agar plate.
- The discs were diffused in testing samples.
- Then these diffused discs were placed on agar plate and kept it in incubator for 48 hrs. at 37°C.

Table 11: Zone of Inhibition in Formulated Herbal Mouthwash.

Microbes	Dilution of the extracts	Zone Of Inhibition (mm)	Sensitivity	Resistance
<i>E.coli</i>	Formula 1	3	+	-
	Formula 2	4	+	-
	Formula 3	6	+	-
<i>S.aureus</i>	Formula 1	3	+	-
	Formula 2	4	+	-
	Formula 3	6	+	-
<i>C.albicans</i>	Formula 1	3	+	-
	Formula 2	6	+	-
	Formula 3	6	+	-
<i>S.pyrogen</i>	Formula 1	3	+	-
	Formula 2	6.3	+	-
	Formula 3	7	+	-
Mouth Saliva (Random)	Formula 1	6	+	-
	Formula 2	6	+	-
	Formula 3	7	+	-



Figure 2: Set up for the Extraction.



Figure 3: Collection of extraction after 24 hours.

- After 48 hrs the results were identified and recoded the zone of inhibition (Figure 4).
- His zone of inhibition was identified for the resistance of the micro-organisms too (Figure 5-12).

Antimicrobial testing by Disc Diffusion method

- **Extracts:-**(Naie, Dikmali, Henna, Guava & Pomegranate)
- **Formulated Herbal Mouthwash:-** (Formula-1, Formula-2, Formula-3)
- **Standard Mouthwash:** (Standard-1 & Standard-2)

Result & Discussion

Solvent extraction of herbal plants

The herbs used in the prepared herbal mouthwash like Dikmali, Naie, Pomegranate, (Table 3) Henna and Guava can be used in oral care formulations like mouthwash. The solvent extracts were prepared as it shows very less side-effects than commercially available chemical mouthwashes.

Collection of Micro-organisms

The test organisms were identified & the results were recorded based on morphological & cultural characters.

Antimicrobial activity

The extracts were taken in different dilutions. The sensitivity & resistance of the organisms against formulated herbal mouthwash were analyzed. The result showed that all the herbslike Dikmali, Naie, Pomegranate, Henna (Table 4) and Guava used in this study have antimicrobial activity (Table 5, Table 9).

Dikmali: shows sensitivity towards *Staphylococcus aureus*, *Streptococcus pyrogens*, *Candida albicans* and shows resistance (Table 6) towards *Escherichia coli*.

Naie: shows sensitivity (Table 7) towards *Staphylococcus aureus*, *Streptococcus pyrogens*, *Candida albicans* & *Escherichia coli*.

Guava: shows sensitivity towards *Staphylococcus aureus*, *Candida albicans* & *Escherichia coli* & shows resistance towards *Streptococcus pyrogens* (Table 10).

Pomegranate: shows sensitivity towards *Staphylococcus aureus*, *Streptococcus pyrogens*, *Candida albicans* & *Escherichia coli*.

Henna: shows sensitivity (Table 8) towards *Streptococcus pyrogens*, *Candida albicans* & *Escherichia coli* & shows resistance towards *Staphylococcus aureus*.

It was found that the antimicrobial activity of 10% w/v of extract was found to be substantial and at the same time it is tolerable as regards the palatability due to bitterness. So, the formulation prepared by the 10%

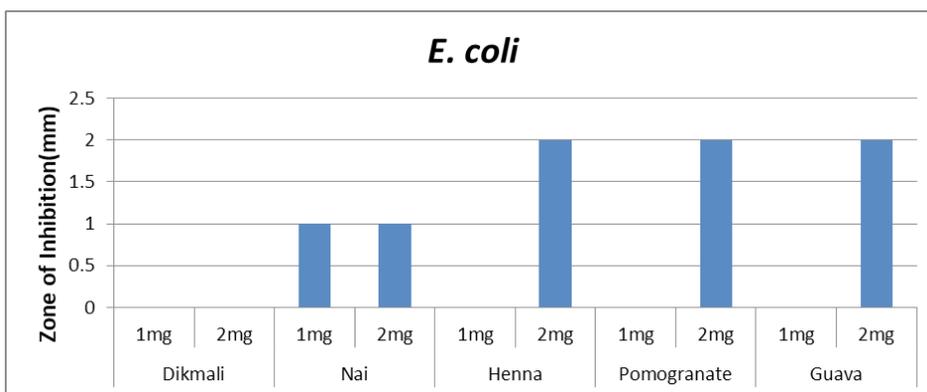


Figure 4: Zone of inhibition for *E. coli*.

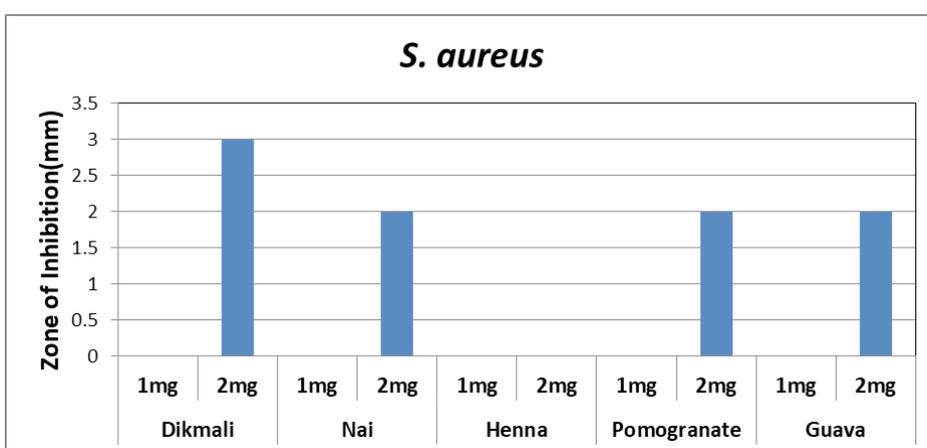


Figure 5: Zone of inhibition for *S. aureus*.

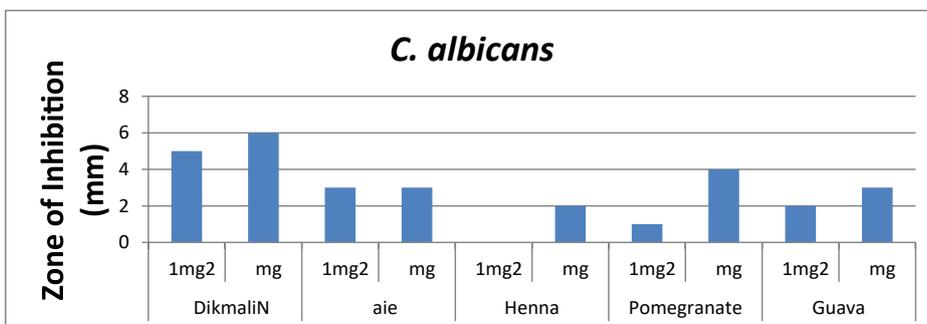


Figure 6: Zone of inhibition for *C. albicans*.

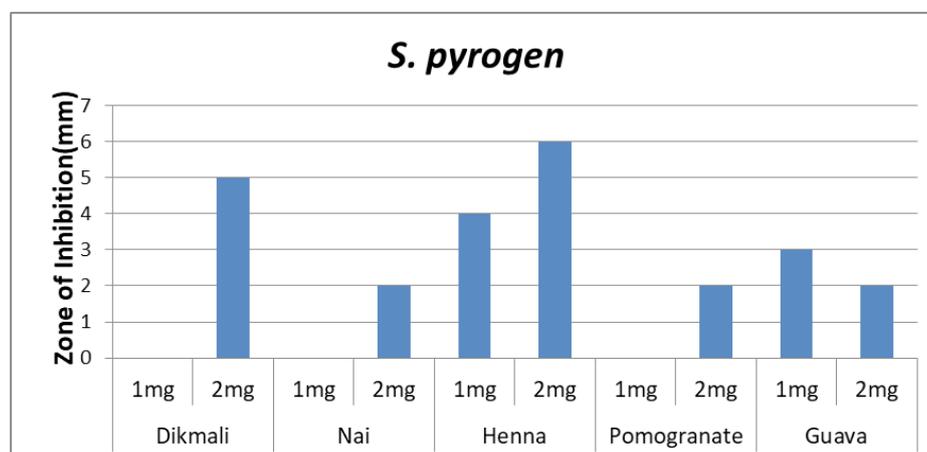


Figure 7: Zone of inhibition for *S. pyrogen*.

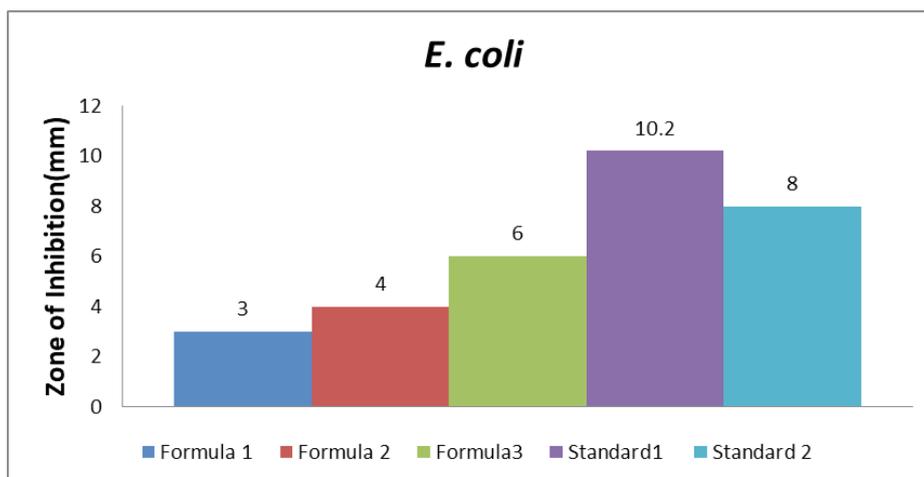


Figure 8: Zone of inhibition of formulations for *E. coli*.

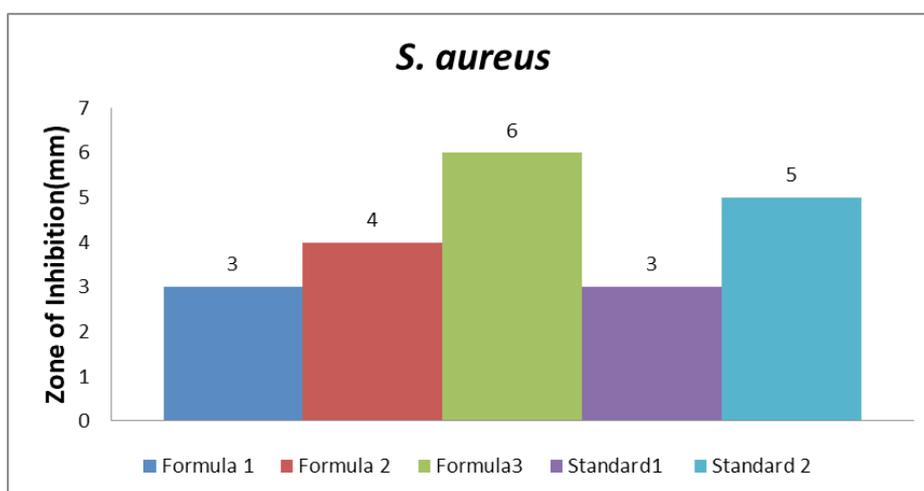


Figure 9: Zone of inhibition of formulations for *S. aureus*.

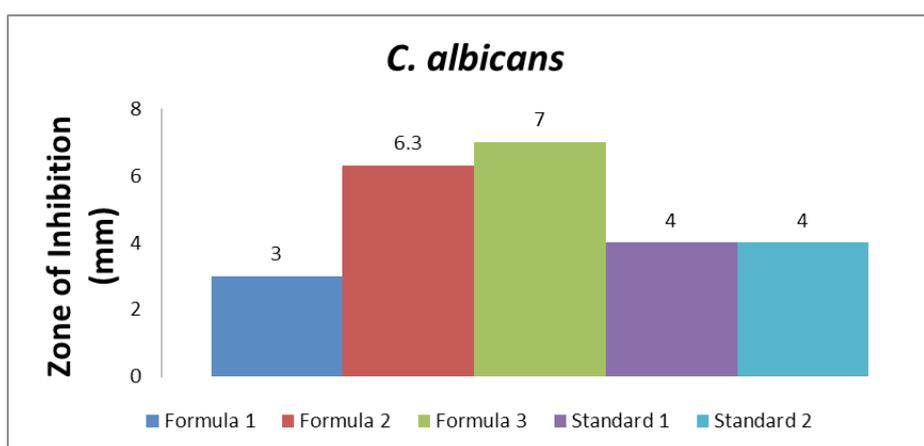


Figure 10: Zone of inhibition of formulations for *C. albicans*.

w/v of extract provides optimal antimicrobial activity and palatability to the formulation. The prepared formulations shows positive results against the randomly taken mouth saliva sample. The formulation of 10% w/v of extract shows significant effect in terms of antimicrobial activity compare with the marketed synthetic drug like chlorhexidine (Table 11-17).

Conclusion

Inclusion of Naie in mouthwash showed sensitivity and significantly effective against oral micro-organisms. Pomegranate in mouthwash helps in destruction of microbes. Hence the prepared herbal mouthwash shows effectiveness against oral micro-organisms namely

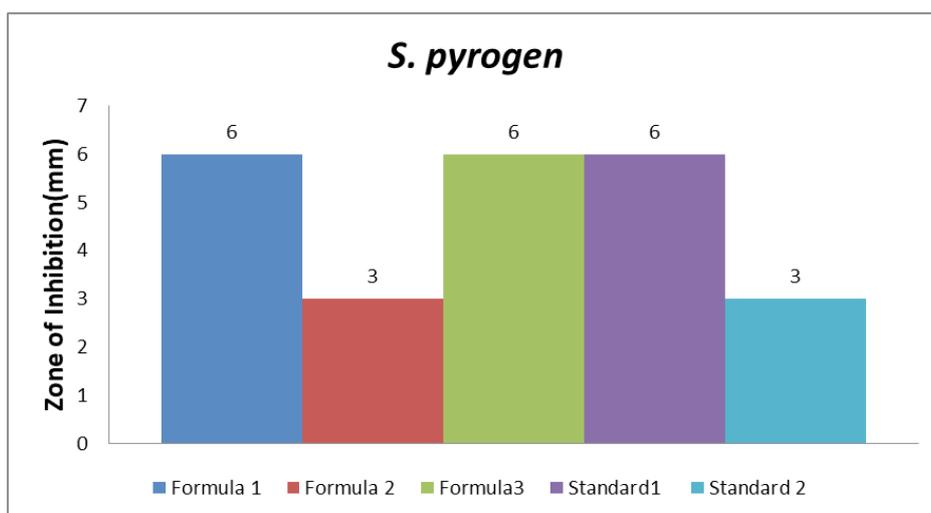


Figure 11: Zone of inhibition of formulations for *S. pyrogen*.

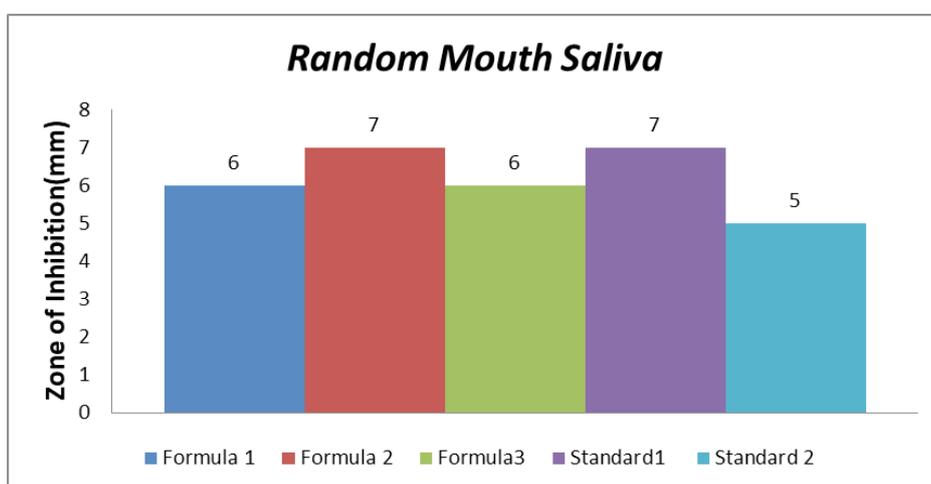


Figure 12: Zone of inhibition of random mouth saliva.

Table 12: Zone of Inhibition in Standard Mouthwash.

Microbes	Dilution of the extracts	Zone Of Inhibition (mm)	Sensitivity	Resistance
<i>E. coli</i>	Standard 1	10.2	+	-
	Standard 2	8	+	-
<i>S. aureus</i>	Standard 1	3	+	-
	Standard 2	5	+	-
<i>C. albicans</i>	Standard 1	6	+	-
	Standard 2	3	+	-
<i>S. pyrogen</i>	Standard 1	4	+	-
	Standard 2	4	+	-
Mouth Saliva (Random)	Standard 1	7	+	-
	Standard 2	5	+	-

Table 13: Following table shows total yield obtained after percolation.

Crude drugs	Weight of drug before Extraction	Weight of drug after extraction
<i>Naie</i>	40 gm	2.39 gm
Guava	40 gm	3.8 gm
<i>Dikmali</i>	15 gm	0.94 gm
Pomegranate	50 gm	5.63 gm
<i>Henna</i>	20 gm	0.51 gm

Table 14: Formulation: After percolation all the extracts were dried for a week to get the residual mass. Using this dried mass prepared the herbal mouthwash by using following different formulae for 100 ml.

Sr No.	Ingredients	Formula 1	Formula 2	Formula 3
1	Naie Extract	10mg	20mg	30mg
2	Dikmali Extract	10mg	20mg	30mg
3	Guava Extract	10mg	20mg	30mg
4	Pomegranate Extract	10mg	20mg	30mg
5	Henna Extract	10mg	20mg	30mg
6	Menthol	0.2g	0.2g	0.2g
7	Sodium Saccharine	0.15g	0.15g	0.15g
8	Methyl paraben	0.11g	0.11g	0.11g
9	Propyl Paraben	0.014g	0.014g	0.014g
10	PEG 400	6.59ml	6.59ml	6.59ml
11	Distilled Water	Q.S	Q.S	Q.S

Table 15: Standard Mouthwash Used for comparison study.

Sr. No.	Brand Detail	Ingredients	Mfg Date	Exp Date
1.	(Standard 1) 'Hexidine' ICPA Health Products Limited, Mumbai, Maharashtra, India	Chlorhexidinegluconate BP 0.2%W/V	Jul.17	Jun.20
2.	(Standard 2) 'CMW' Radius agencies, Mumbai, Maharashtra, India	Chlorhexidinegluconate IP 0.2%W/V	Oct.16	Sep.19

Table 16: pH Test :(Standard Range: 5.1 to 7.3).

Mouthwash	pH
Formula 1	6.47
Formula 2	5.85
Formula 3	5.77

Table 17: Viscosity: (Standard Range: 1 to 4 cps).

Mouthwash	Viscosity
Formula 1	0.9528 cps
Formula 2	0.9135 cps
Formula 3	0.9403 cps

Staphylococcus aureus, *Streptococcus pyrogens*, *Candida albicans* & *Escherichia coli*. An attempt has been made to outline some of the commonly available herbs which are readily available can be used as effective mouthwashes. People can use and promote such cost effective measures of maintaining the oral health which are also devoid of many unwanted side effects. Thus the prepared herbal mouthwashes can also be routinely used for improving oral hygiene of children and adults as well as in patients with dental caries and gingivitis.

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