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EVALUATION OF HERBAL MOUTHWASH TO COMBAT ORAL PATHOGENS

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Abstract: Herbal mouthwash contains natural ingredients called phytochemicals that contains desired anti-microbial effect. The aim of the present study was to evaluate herbal mouthwash against oral pathogens. Herbal mouthwash prepared from Mint (*Mentha piperita*), Neem (*Azadirachta indica*), Pomegranate (*Punica granatum*) and Tulsi (*Ocimum sanctum*) was evaluated against oral pathogens. Prepared herbal mouthwash was found to be effective against all oral pathogens. It was found that all the four extracts were effective against *S.aureus* and *E.coli*. Antibiotic resistance profile of *S.aureus* showed that all *S.aureus* were sensitive (100% each) to Erythromycin, Gentamicin, Penicillin and Tetracycline while resistant to Oxacillin (100%). Antibiotic resistance profile of *E.coli* showed that all *E.coli* were sensitive to Tobramycin followed by 80% was sensitive to Chloramphenicol, Trimethoprim and Vancomycin while all *E.coli* were resistant to Ofloxacin. Phytochemical analysis of herbal aqueous extract was performed. It was found that in Mint extract Tannins and Reducing sugars were present. In Neem extract Alkaloids, Saponins, Flavonoids were present. In Pomegranate extract Glycosides, Flavonoids were present. In Tulsi extract Alkaloids, Glycosides, Tannins, Reducing sugars, Flavonoids were present.

Keywords: **Herbal mouthwash, Oral pathogens**

INTRODUCTION

Mouthwash is a liquid oral product designed to freshen breath. It is an aqueous solution which is most often used for its deodorant, refreshing and antiseptic properties or for control of plaque (Sandhya, 2015). The need for mouthwash is a result of a condition called halitosis, or bad breath. It is estimated that over half the population occasionally has foul-smelling breath. It has been found that bad breath is mostly due to bacterial activity in an unclean mouth. Specifically, anaerobic bacteria that grow on the protein-rich food debris stuck between the teeth or on the tongue. As the bacteria breaks down the proteins, those containing sulphur give off foul odor molecules such as methylmercaptan and hydrogen sulphide which result in bad breath (Bhavna and Vidhya, 2012). One of the most common infectious disease encountered by many individuals are dental carries and periodontal diseases (Durate, 2013). Dental plaque is the material that adheres to the teeth and consists of bacterial cells, salivary polymers and bacterial extracellular products. If not taken care of, via brushing or flossing, it can lead to gingivitis or periodontal disease (Levine, 2014).

Herbal medicines are known to mankind, and medicinal plants have been used as traditional treatment for numerous human diseases for centuries in many part of the world. Many plants were reported to inhibit the growth of oral bacteria (Vargas, 2015). Mentha leaves (*Mentha piperita*) are extensively used as flavoring ingredient in breathe freshness, antiseptic mouth rinses, chewing gums and tooth paste. The antibacterial activity of peppermint oil and different extracts of *Mentha piperita* against some Gram-positive and Gram-negative bacterial strains was reported (Singh et al., 2015). Menthyl acetate and ketones are responsible for the antimicrobial activity of Mint. Its menthol component is the biologically active antioxidant. Mint leaf extract displays antimicrobial activity against plaque (Chaudhary et al., 2012).

Neem (*Azadirachta indica*) is a natural antibacterial agent. The antibacterial effect of Neem has been reported against *S.aureus* and *E.coli* (Siswomihardjo et al., 2007). Ethanolic extract of Neem leaves, sticks and bark exhibited significant antibacterial activity. Dried chewing sticks of Neem showed maximum antibacterial activity against *S. mutans* compared to other dental caries-causing organisms such as *S.aureus* and *E.coli* (Chava et al., 2012). Various parts of the Neem tree possess astringent and antiseptic activity. Leaf extracts have been widely used in both traditional and conventional times to manufacture toothpaste and mouthwash in the oral care dentistry. Its antibacterial properties due to the presence of nimbidin, azadirachtin, and nimbinin help to remove many oral aerobic and anaerobic pathogens. Neem bark and leaf extract is most effectively used in preventing cavities and gum diseases. Mouthwash containing Neem is a remedy for tooth decay, oral infection, prevents bleeding and sore gums. Twigs of Neem tree are used as chewing sticks by people all over India (Bhanwra et al., 2000).

Tulsi is the traditional herb, known as "Queen of Herbs or the Mother Medicine of nature" as it is rich in antimicrobial substances and have been used to treat a variety of illnesses. Its sun dried and powdered leaves have been used for brushing teeth. Tulsi leaves with mustard oil used as toothpaste. Tulsi leaves (*Ocimum sanctum*) are used as an antimicrobial and antifungal substance against oral microbes. It shows high microbial activity against *Streptococcus pyogenes*, *Staphylococcus aureus* and *E.coli*.

Pomegranate (*Punica granatum*) is an ancient fruit and its extracts are effective in chronic periodontal disorders. Pomegranate are anti-inflammatory in nature, hence they are used to treat against oral-ailments. Pomegranate possesses active polyphenolic flavonoids which help to prevent gingivitis (Banu et al., 2016). Pomegranate containing mouthwash may fight dental plaque and tartar formation by inhibiting the activities of the microorganisms that cause plaque. It shows that pomegranate extract suppresses the ability of these microorganisms to adhere to the surface of the tooth. Additionally, pomegranate compounds posses anti-inflammatory properties that may help soothe irritated tissues (Shukla et al., 2008). It has been reported that some of the herbal plant leaves of Mentha, Neem, Tulsi and Pomegranate inhibits the growth of oral bacteria. Therefore, aim of the present study was to evaluate herbal mouthwash against oral pathogens.

MATERIALS AND METHODS

I. Preparation of extracts:

Leaves of Mint (*Mentha piperita*), Neem (*Azadirachta indica*), Pomegranate (*Punica granatum*) and Tulsi (*Ocimum sanctum*) were collected from botanical garden. The leaves were washed with sterile water, shadow-dried and stored in air-tight bottles. The aqueous extracts were prepared by soaking 10 gm of the powdered leaves in 200ml sterile distilled water. The preparation was heat sterilized at 40^0C for 5-10 minutes and kept for incubation at 37^0C for 72hours. After incubation, the extracts were filtered with the help of a sterile Whatman's filter paper No. 1 and used further (Baron and Finegold, 1990).

II. Collection and identification of oral pathogens:

A total of ten oral pathogens were collected from pathology laboratory in Nagpur and cultures were identified on the basis of morphological, cultural and biochemical characteristics (Collee and Marr, 1996).

III. Antibiotic Sensitivity Test by Disc Diffusion Method:

The antibiotic sensitivity test was carried out by Kirby-Bauer disc diffusion method (Bauer et al., 1966) against *S. aureus* and *E.coli* (Table 1 and 2). *S. aureus* and *E.coli* strains were grown over night on nutrient agar at 37^0C for 18 hours, and the colonies were suspended in sterile saline water equivalent to a 0.5McFarland standard (1.5×108 CFU/ml). The suspension (100 µL) was spread over Mueller Hinton agar. The plates were kept at room temperature for 10 minutes for drying under strict aseptic condition. The antibiotic discs were carefully placed on to the surface of the Mueller Hinton agar plates using sterile forceps. The plates were incubated at 37^0C for 18 hrs. The agar plates were examined for zone of inhibition around the disc and the oral pathogens were classified as "resistant" or "sensitive" based on the standard interpretative chart according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2007).

Table 1: Antibiotic discs used against *S. aureus*

Antibiotics	Concentration
Erythromycin	15 mcg
Gentamicin	10 mcg
Oxacillin	1mcg
Penicillin	10 units
Tetracycline	30 mcg

Table 2: Antibiotic discs used against *E. coli*

Antibiotics	Concentration
Chloramphenicol	30mcg
Ofloxacin	5mcg
Tobramycin	10mcg
Trimethoprim	5mcg
Vancomycin	30 mcg

IV. Antimicrobial Activity by Agar Well Diffusion Method:

S.aureus and *E.coli* isolates were grown overnight on nutrient agar at 37^0C for 18 hours, and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5X 108 CFU/ml). The suspension (100µl) was spread on the Mueller-Hinton agar plate. The wells of 6 mm diameter were cut into agar medium with a sterilized cork borer. A 20µl of each herbal extracts were added separately into the separate wells. The plates were incubated at 37^0C for 18 hours. The diameter of the zone of inhibition around each well was measured and recorded (Bauer et al., 1966).

VII. Phytochemical Analysis of Extracts:

The extracts were analyzed by the following procedures for the presence of alkaloids, flavonoids, saponins, tannins, glycosides and reducing sugars.

- **Test for Alkaloids:** To 2 ml of extract, 2 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green colour or white precipitate indicates the presence of alkaloids.
- **Test for Flavonoids:** To 2 ml of extract, 1 ml of 2N sodium hydroxide was added. Presence of yellow colour indicates the presence of flavonoids.
- **Test for Saponins:** To 2 ml of extract, 2 ml of distilled water was added and shaken in a graduated cylinder for 15 minutes. It resulted in the formation of 1 cm layer of foam that indicated the presence of saponins.
- **Test for Tannins:** To 1 ml of extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black colour indicates the presence of tannins.
- **Test for Glycosides:** To 2 ml of extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink colour indicates presence of glycosides.
- **Test for Reducing sugars:** To 2 ml of extract, 5ml mixture of equal volumes of Fehling's solution A and B was added and heated in a water bath for 2 minutes. Brick red precipitate indicates the presence of reducing sugars.

RESULTS AND DISCUSSION

Antimicrobial activity of Mint extract, Neem extract, Pomegranate extracts, and Tulsi extract was evaluated against 10 oral pathogens (*S.aureus* (n=5) and *E.coli* (n=5)). It was found that all four extracts were effective against *S.aureus* and *E.coli*. Prepared herbal mouthwash was found to be effective against all oral pathogens (Table 3, 4). Newman and Lansky (2007) found that plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development. Leaf extract of Mentha showed broad spectrum antimicrobial activity since various water, ethanol and methanol extracts of leaves have exhibited antibacterial activities. The study of Saharkhiz et al., (2012) showed *M. longifolia* shows strong antibacterial and antifungal activity against various Gram positive and Gram negative bacteria as well as several strains of fungi. Peppermint is an excellent breath freshener. Mint is also a good remedy for gingivitis. In the study of Vijayanand and Hemapriya (2011) showed that pomegranate extract have strong antibacterial activity against gram positive and gram negative bacteria. Jalaluddin et al., (2017) exhibited that extracts from neem inhibit the growth of *S. mutans* and used in the treatment of periodontitis. The leaves, twigs, and seeds of neem have been used in India and South Asia for thousands of years to clean the teeth and fight bacterial infections (Sandhya, 2015). Tulsi is useful in teeth disorders. Its leaves, dried in the sun and powdered, can be used for brushing teeth. This is very good for maintaining dental health counteracting bad breath and for massaging the gums. It is also useful in pyorrhea and other gum disorders. The anti-inflammatory and anti-infectious properties of tulsi make it a powerful treatment for gum disease (Biswas and Biswas, 2005). Rajalakshmi Rakshanna and Lakshmi (2017) reported that natural herbs such as tulsi patra, neem, clove oil, pudina, ajwain and many more used either as whole single herb or in combination have been scientifically proven to be safe and effective medicine against various oral health problems such as bleeding gums, halitosis, mouth ulcers, and preventing tooth decay.

Antibiotic resistance profile of *S.aureus* showed that all *S.aureus* were sensitive (100% each) to Erythromycin, Gentamicin, Penicillin and Tetracycline while resistant to Oxacillin (100%). Antibiotic resistance profile of *E.coli* showed that all *E.coli* were sensitive to Tobramycin followed by 80% was sensitive to Chloramphenicol, Trimethoprim and Vancomycin while all *E.coli* were resistant to Ofloxacin (Table 6).

Phytochemical analysis of herbal aqueous extract was performed. It was found that in Neem extract Alkaloids, Saponins, Flavonoids were present. In Tulsi extract Alkaloids, Glycosides, Tannins, Reducing sugars, Flavonoids were present. In Pomegranate extract Glycosides, Flavonoids were present. In Mint extract Tannins and Reducing sugars were present (Table 7).

CONCLUSION

Herbs, which are powerful healing agents, must be used appropriately. The use of herbs in dentistry should be based on evidence of effectiveness and safety. The anti-bacterial activities could be enhanced if active components are purified and adequate dosage determined for proper administration. The present results therefore offer a scientific basis for traditional use of herbal mouthwash. The present study concluded that herbal mouthwash could be used as an alternative to commercially available mouthwash on a daily basis to maintain oral hygiene.

Table 3: Antimicrobial Activity of Herbal Extracts and Herbal mouthwash against *Staphylococcus aureus*

Bacterial Pathogen	Mint	Neem	Pomegranate	Tulsi	Herbal Mouthwash
S.aureus 1	16mm	14mm	23mm	14mm	11mm
S.aureus 2	20mm	13mm	25mm	18mm	16mm
S.aureus 3	17mm	15mm	26mm	20mm	14mm
S.aureus 4	22mm	15mm	28mm	23mm	14mm
S.aureus 5	20mm	17mm	26mm	21mm	13mm

Table 4: Antimicrobial Activity of Herbal Extracts and Herbal mouthwash against *Escherichia coli*

Bacterial Pathogen	Mint	Neem	Pomegranate	Tulsi	Herbal Mouthwash
E.coli 1	18mm	17mm	20mm	19mm	16mm
E.coli 2	14mm	21mm	15mm	15mm	14mm
E.coli 3	15mm	22mm	24mm	25mm	16mm
E.coli 4	18mm	21mm	25mm	24mm	17mm
E.coli 5	20mm	25mm	22mm	18mm	14mm

Table 5: Antibiotic Resistance Profile of *S.aureus*

Bacterial Pathogen	Erythromycin	Gentamicin	Oxacillin	Penicillin	Tetracycline
S.aureus 1	17mm	18mm	R	12mm	23mm
S.aureus 2	31mm	29mm	R	29mm	26mm
S.aureus 3	21mm	22mm	R	26mm	23mm
S.aureus 4	26mm	22mm	R	21mm	23mm
S.aureus 5	26mm	26mm	R	18mm	21mm

Table 6: Antibiotic Resistance Profile of *E.coli*

Bacterial Pathogen	Chloramphenicol	Ofloxacin	Tobramycin	Trimethoprim	Vancomycin
E.coli 1	26mm	R	14mm	24mm	R
E.coli 2	R	R	14mm	R	23mm
E.coli 3	24mm	R	18mm	22mm	20mm
E.coli 4	21mm	R	17mm	21mm	16mm
E.coli 5	19mm	R	12mm	18mm	17mm

Table 7: Phytochemical Analysis of Herbal Aqueous Extracts

Tests	Neem	Tulsi	Pomegranate	Mint
Alkaloids	+	+	-	-
Flavonoids	+	+	+	-
Saponins	+	-	-	-
Tannins	-	+	-	+
Glycosides	-	+	+	-
Reducing sugars	-	+	-	+

Where (+ = Present; - = Absent)

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