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Preparation of *Ocimum tenuiflorum* and *Syzygium aromaticum* of Herbal Formulation Based Mouth Wash and its Anti-Microbial Activity

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Abstract: Herbal extracts are used in dentistry for treatment of various dental disorders. The herbal remedies have an edge over conventional antibiotic treatment that suffer the limitation of low benefit to high risk as compared to herbal treatment that possess high benefit to low-risk ratio. The literature shows that several herbal formulations have the capacity to control the production of pro-inflammatory mediators, thereby managing many inflammatory processes. The use of such herbal anti-inflammatory formulation for a longer period of time was found to be safer than that of chemical anti-inflammatory drugs. The studies for assessment of safety and efficacy of herbal remedies are in demand. These herbal remedies are expected to be widely used in future. There are much more opportunities for further research in the utility of herbal remedies for oral diseases. Hence the usage of herbal mouthwash will improve the oral hygiene comparatively with routine mouthwash products.

Keywords: Tulsi, *Ocimum Tenuiflorum*, Clove, *Syzygium Aromaticum*, Anti-microbial, Oral rinse, Mouthwash

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Introduction

A mouthwash is a medicated liquid which is held in the mouth and swished by the action of pre-oral musculature to eliminate the oral pathogens. Mouth rinses have the ability to deliver the therapeutic effect all over the tooth surface including inter proximal areas in which even toothpaste is not much effective (Matthews, 2003).

The earliest reports of usage of mouth rinse are attributed to the Indian and Chinese forms of medicine. It is also well documented that Hippocrates recommended a mixture of salt, alum and vinegar (Mhaske *et al.*, 2012). Most of the medical products contain a chemical even though it plays an important role in medicinal with

unpleasant highly side-effects after long time usage. To overcome this problem plants and their extracts are demonstrated as substitutes. Greek physician Pedanius Dioscorides, formulated a mouthwash mixture of decoct extracted from the olive tree leaves, milk, wine and oil, pomegranate peelings, nutgalls and vinegar. This was how ancient mouth washes were prepared using traditional methods and herbs (Fischman, 1997). It was observed that in the 18th century urine served as a key active ingredient due to the presence of ammonia that rendered the oral cavity free from oral pathogens especially Sulphur producing organisms (Waite, 1937).

Since then a variety of herbal remedies are available such as Triphala, Tulsi Patra, Jeshthamadh, neem, clove oil, pudina, Ajwain, white oak bark, horsetail herb, plantain leaf, aloe vera, organic echinacea angustifolia root, myrrh gum, organic lobelia herb and seed, organic peppermint leaf, wildcrafted goldenseal root, clove essential oil, peppermint essential oil, tea tree essential oil (Manipal *et al.*, 2016).

Since then commercial interest in mouthwashes has been intense and several newer products claim effectiveness in reducing the build-up of dental plaque, gingivitis and halitosis. The number of mouthwash variants in the world has grown from 15 in 1970 to nearly about 113 in 2012 (Malhotra *et al.*, 2011). Chlorhexidine (CHX) is the most effective antiseptic for plaque inhibition and prevention of gingivitis when used twice daily as mouth rinse. But in oral use as a mouth rinse chlorhexidine has been reported to have a number of side effects (Aichner and Coletti, 2013).

In order to overcome such side effects, the World Health Organization (WHO) advice researchers to investigate the possible use of natural products such as herb and plant extracts. Herbs and plant extract have been used in oral hygiene products for many years (Bhat *et al.*, 2013). A number of clinical studies have shown the effects of using mouth washes extracted from herbs such as *Sanguinaria*, *Myrtus communis*,

Quercus infectoria, *Capparis spinosa* and *Cinnamon* in the prevention of dental plaque accumulation and subsequent gingival inflammation (Aichner and Coletti, 2013). *Salvadora persica* (*S. persica*) is a medicinal plant whose roots have been used by many people in Africa, South America, Middle East and Asia. It has been demonstrated that extracts of *S. persica* improved gingival health and inhibited growth of cariogenic bacteria (Mandel, 1988).

The widespread use of mouthwashes as an aid to oral hygiene is a relatively recent phenomenon in the developing countries of the world. Hence, herbal dental products are becoming popular amongst general public (Malhotra *et al.*, 2011). One such herbal product is Hiora, an herbal mouthwash known for its antiseptic, antimicrobial, antiplaque and analgesic property. Hiora contains herbs having antimicrobial properties such as oil of *Syzygium aromaticum*, *Cinnamomum zeylanicum*, and extract of *Spinacia oleracea*, *Triphala*, *Tarikatu* and powders of Yashada Bhasma and Surya kshara. *Syzygium aromaticum* which have shown to have antifungal, antiviral, analgesic/ anesthetic, antiseptic, anticoagulant and antioxidant properties. Oil of clove has also shown antimicrobial activity (Khalessi *et al.*, 2004).

Mouthwash:

Mouthwashes are liquids which contain anti-inflammatory, antimicrobial, and analgesic action. There are two types of mouthwash - chemical and herbal.

Chlorhexidine mouthwash comes under chemical mouthwash. Chlorhexidine was developed in 1950, which is still considered the most effective anti-plaque agents in dentistry (Shreya Shetty *et al.*, 2013). Many of the plant extracts has an anti-microbial property which is effectively used in maintaining good oral hygiene. Natural herbs such as triphala, Tulsi patra, jeshthamadh, neem, clove oil, pudina, and many others are used as single or in combination have been scientifically proven to be safe and effective medicine against oral health problems such as bleeding gums, halitosis, mouth ulcers, and

preventing tooth decay without side effects (Manipal *et al.*, 2016). The main purposes of using mouthwashes are that it can be used at home as routine to maintain good oral hygiene, mouthwash provides anti-inflammatory, anti-microbial activity, it is used prior to and after oral surgery procedures such as tooth extraction as prophylaxis, the purpose of mouthwash after brushing is to clean the areas which cannot be reached during brushing, It freshens the mouth by which it helps to control bad breath, and it kills all germs that have not killed by toothpaste.

Herbal Mouthwash:

Herbal mouthwash contains a natural ingredient called phytochemical that contains desired anti-microbial and anti-inflammatory effect. Herbal mouthwash becomes more popular as they work without alcohol, artificial preservatives, flavor, or colors (Aspalli *et al.*, 2014). 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, Yavani satva, Nagavalli, Gandhapurataila, Pilu, Bibhitaka, Ocimum, Echinacea, Chameli leaves, etc. (Biswas *et al.*, 2014).

Some of the herbs that are used in mouthwashes are clove (*Syzygium aromaticum*), which is traditionally used for oral health because of their antiseptic, antibacterial, and antiviral property; peppermint which gives cooling effect to the mouth; plantain has ability for speed wound healing. Many of the herbs contain anti-microbial, anti-inflammatory, antioxidants, antiseptic properties such as neem, clove, Triphala with combination of amalaki, haritaki, vibhitaki, Tulsi, celery, licorice, oak tree, bakula, katha, spearmint, turmeric, and Aloe vera (Aspalli *et al.*, 2014).

Neem as mouthwash has been shown to have significant effects on both Gram-positive and Gram-negative organisms which include *Escherichia coli*, *Streptococcus*, and *Salmonella*. Extracts from neem inhibit the growth of *S. mutans* and used in the treatment of periodontitis. It

contains anti-microbial, anti-inflammatory, and anti-oxidant property (Prasad *et al.*, 2015).

Ocimum tenuiflorum (OT), commonly known as Tulsi is a small shrub belonging to the mint family Lamiaceae. It has small leaves with a strong smell and purple flower (Van Leeuwen *et al.*, 2011). Tulsi is a time-tested premier medicinal herb. It is a plant of Indian origin, worshipped by the Hindus and used in Ayurvedic medicine since ancient times. It is one of the holiest and most sacred herbs grown widely in India. It possesses significant antibacterial, antioxidant, and anti-inflammatory properties. It is an herb that is bestowed with enormous antimicrobial substances and is used to treat a variety of illnesses ranging from diabetes mellitus, arthritis, bronchitis, and skin diseases (Van Leeuwen *et al.*, 2011). Thus, this study was planned to evaluate the antimicrobial efficacy of *Ocimum tenuiflorum* and *Syzygium aromaticum* extracts and to assess its anti-microbial activity *in vitro*.

Materials and Methods

Collection of plant:

Tulsi (*Osmium tenuiflorum*) and Clove (*Syzygium aromaticum*) were collected from Avalurpet, Tamil Nadu, India. After collection, the plants were washed thoroughly and shade dried for 15 days.

Preparation of extract:

The *Ocimum tenuiflorum* and *Syzygium aromaticum* were dried and powdered into uniform particle size. 1 g of each powder was dissolved in 100 ml of distilled water. Then the mixed solution was boiled for 30 min. The boiled solution was filtered by using Whatman filter paper. The filtered solution was boiled again continuously until 100 ml reduced to 50 ml solution quantity. Then the prepared solution was maintained under shaker with few hours.

Preparation of mouth wash:

The mouth wash was prepared by adding 2 ml of plant extract, 8 ml of water, 0.3 g of sucrose, 0.001g of sodium benzoide, 0.01 g of Sodium Lauryl sulfate and 100 ml pepper mint oil.

Collection of organisms:

Oral swab: The sterile absorbent cotton was rolled against the tip of the wooden stick besides the flame to avoid spores and were sterilized by autoclaving at 121°C for 15 min. The swab was rubbed on to the gums and periodontal region of both upper and lower jaws of the individuals. Totally 25 oral swabs were collected from the individuals under sterile condition inside the laboratory.

Peptone water: 1.5 g of peptone water was weighed and mixed with 100 ml of distilled water and autoclaved at 121°C for 15 min. Cooled to 50-55°C and transferred to sterile test tubes. The oral swabs were inoculated and incubated at 37°C for 1 h and results were observed.

Maintaining the culture:

The culture was allowed to store in Refrigerator and frequently sub cultured by inoculating it in Nutrient slant or Peptone water for long period of usage.

The collected samples were cultivated and the colony morphology on the culture media-nutrient agar were examined. A colony was picked, inoculated in peptone water and incubated at 37°C for 1 h. The isolates were streaked on specific medium- Blood agar, MacConkey agar, Mannitol salt agar, Eosin methylene blue and *Lactobacillus* Mann rogosa agar. Meanwhile, the isolates were identified with Gram staining techniques, motility test and biochemical tests.

Agar Well Diffusion Technique:

A sterile conical flask was taken and filled with 500 ml of distilled water. 19 g 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 min, cooled to 55°C. 5-7 ml of agar was poured in the 25 petri plates, to form a thick agar plate and kept at room temperature to get settled. The plates were then air dried and UV radiated in the laminar air flow for 5-10 min. The agar wells were punctured by using sterile micropipette tips with equal intervals

and the dilutions were marked as 50 µl, 100 µl, 150 µl for each extract. The isolated bacterial colonies were picked from the test organism culture media and inoculated in peptone water or saline and incubated at 37°C for 2 h. After incubation the bacterial cultures were compared with 0.5 Mac Farland's standard, for the standardization of the bacterial cultures. With the help of oral swab, the isolates were swabbed on the upper surface of the agar gently, under sterile condition. The micro pipette is used to transfer the aqueous extracts in each well and incubated at 37°C for 24 h. After 24h, the plates show agar diffusion around the wells and the results were identified by measuring the zone of inhibition which was identified for the resistance of the organisms.

Antimicrobial Activity:

The antimicrobial activity of *Ocimum tenuiflorum* leaves and *Syzygium aromaticum* flower buds essential was investigated using agar gel diffusion. The result obtained showed that the *Ocimum tenuiflorum* and *Syzygium aromaticum* exhibited more antibacterial activity on *S. mutans*, *Candida albicans*, *Lactobacillus* and *S. aureus*.

Results

Herbal medicine is both promotive and preventive in its approach. The major strength of these natural herbs is that their use has not been reported with any side-effects. Mouth rinses may be as effective as chlorhexidine as chemical anti-plaque agents with fewer side effects. However, alternative study designs using larger sample sizes and longer duration are needed to further reiterate its benefits.

The effect with the mixture of *Osmium tenuiflorum* and *Syzygium aromaticum* on antimicrobial activity is shown in Figure 1. The minimum inhibitory concentration was examined in *Candida albicans* (Fig. 2).

Discussion

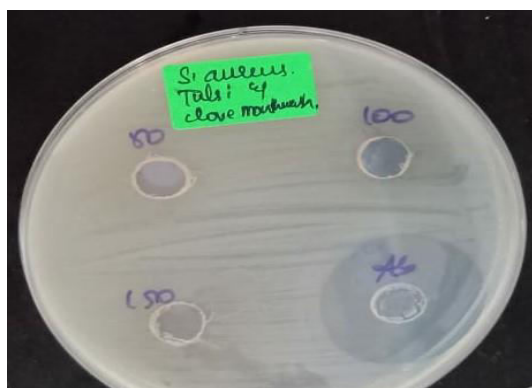
Although chlorhexidine mouthwash has some side effects it is effective in maintaining good oral



Candida albicans



Lactobacillus Sp



Staphylococcus aureus



Streptococcus mutans

Fig. 1: Anti-microbial activity of Mouth Wash.

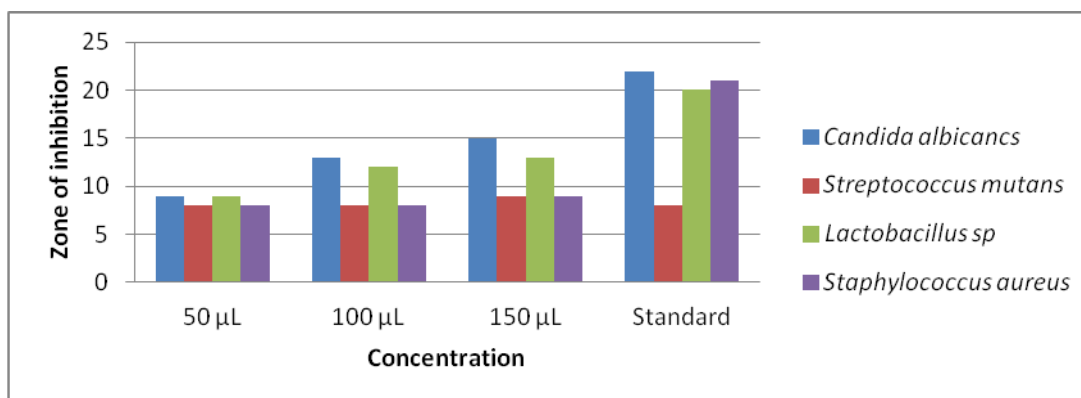


Fig. 2: Anti-microbial activity of mouth wash.

hygiene during gingivitis, periodontitis, traumas, oral cyst and after wisdom tooth extraction. It lasts longer in the mouth than other mouthwashes. It is required for healing and regeneration of oral tissues. However, continuous use of products containing chlorhexidine for long periods can cause stains on teeth, tongue and gingiva also on silicate and resin restorations, alter taste sensation, sweeping, xerostomia, ulcers, etc. Hence, it cannot be used for daily prophylactic measures (Prakash and Gupta, 2005).

Other chemical mouthwashes are made of potassium nitrate, hydrogen peroxide, iodide, etc. Some of the harmful chemicals in mouthwashes are thymol, hexetidine, methyl salicylate, eucalyptol, benzalkonium chloride, cetyl pyridinium, chloride, methyl paraben, alcohol, and hydrogen peroxide which cause harmful effects (Brookes *et al.*, 2020).

Iodine-based mouth rinse was clearly effective in reducing the number of harmful bacteria in the mouth when used daily. However, the most interesting part is that it significantly lowers cholesterol levels in the patients. In a recent study, the effect of povidone-iodine and chlorhexidine mouthwash is compared on plaque *Streptococcus mutans* count after restoration (Renuka and Muralidharan, 2017). After using mouth rinse, there was a significant reduction in *S. mutans* count in the population. After 3 months interval, the count started to gradually increase with iodine mouth rinse.

Hydrogen peroxide is a liquid substance which is also commonly used as a mouthwash. It works to destroy bacteria via oxidation damage. This oxidation creates free-radicals which can ultimately destabilize the molecular structure and cellular strength of the bacteria cells. Hydrogen peroxide is also used in teeth bleaching agents to whiten your teeth. However, this does not mean that hydrogen peroxide will be effective at whitening your teeth (Brookes *et al.*, 2020). The amount of hydrogen peroxide you use determines the safety of the mouthwash. The problem with hydrogen peroxide is that it has a cytotoxic effect

on the dental pulp cells, which means that it literally kills them (Neeraja *et al.*, 2008).

Alkaline mouthwash is predominantly used as mouthwash. It helps in eliminating bad breath, soothes mouth ulcers, restores pH balance, reduces acidity, low alcohol content, and reduces stinging and burning (Malhotra *et al.*, 2011).

The use of sodium bicarbonate as a mouthwash helps in patients with suffering from xerostomia or erosion due to its ability to increase salivary pH and suppress the growth of acid producing microorganisms such as *S. mutans* and also helps in treatment for mouth ulcers. This medication is used to prevent cavities and to reduce pain from sensitive teeth (dentinal hypersensitivity). Sodium fluoride works by making the teeth stronger and more resistant to decay caused by acid and bacteria. Potassium nitrate works by calming the nerves in the teeth. Mouth/gum irritation may rarely occur (Malhotra *et al.*, 2011; Aspalli *et al.*, 2014).

Herbal mouthwash contains a natural ingredient called phytochemical that contains desired anti-microbial and anti-inflammatory effect. Herbal mouthwash becomes more popular as they work without alcohol, artificial preservatives, flavor, or colors. As it contains natural herbs that have natural cleansing and healing property to teeth and gums (Mhaske *et al.*, 2012). Many herbal mouthwashes contain herbs with anti-microbial property such as Neem, *Yavani satva*, *Nagavalli*, *Gandhapurataila*, *Pilu*, *Bibhitaka*, *Ocimum*, *Echinacea*, Chameli leaves, etc (Agarwal *et al.*, 2010; Parwani *et al.*, 2013). Some of the herbs that are used in mouthwashes are clove, which is traditionally used for oral health because of their antiseptic, antibacterial, and antiviral property, peppermint which gives cooling effect to the mouth, plantain has ability for speed wound healing and many of the herbs contains anti-microbial, anti-inflammatory, antioxidants, antiseptic properties such as neem, clove, triphala with combination of amalaki, haritaki, vibhitaki, Tulsi, celery, licorice, oak tree, bakula, katha, spearmint, turmeric, and *Aloe vera* (Pourabbas *et*

al., 2005; Agarwal *et al.*, 2010; Vyas *et al.*, 2018).

Conclusion

Many studies have been conducted to compare chlorhexidine with many herbal extracts. Although herbal mouthwashes have the ability to maintain good oral hygiene on daily basis, still it is less effective than chlorhexidine mouthwash during treatments such as gingivitis, periodontitis, and trauma. Many herbal extracts contain similar antiplaque, anti-bacterial property with chlorhexidine mouthwash.

Hence, it is most preferable than chlorhexidine for post-treatment prophylaxis because chlorhexidine has some adverse effects on long term use. Besides all herbal mouthwashes fruit extract containing mouthwash will be more effective against the microorganism present in the oral cavity without any side effect on over usage. The aim of this study was to give an overview of mouthwashes and to compare the Chlorhexidine mouthwash with herbal mouthwash to aware people about the uses of herbal mouthwashes. Besides the disadvantages, chlorhexidine mouthwash plays effective role during dental treatments on short term usage. Herbal mouthwashes are suitable for maintaining good oral prophylaxis.

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