Kristu Jayanti Journal of Core and Applied Biology (KJCAB)
Volume 2, Issue 1, 2022, Pages 10-14 | Original Research

Effect of Herbal Extracts on Oral Microorganisms

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ABSTRACT

The mouth has a diverse microbial ecosystem and is yet vulnerable to infectious diseases. Dental plaque is formed when bacteria accumulate on teeth, and if left untreated, it can develop to gingivitis. Gingivitis can progress to periodontitis, which causes irreparable damage to the gums and underlying support tissues if left untreated. Hundreds of bacterial species are involved in dental caries such as streptococci species, lactobacillus species. Mouthwashes are meant to reduce oral bacteria, remove any food debris and provide a pleasant and refreshing taste in the mouth. Antiseptic chemicals including alcohol, menthol, and eucalyptol are used in mouthwash to destroy microorganisms. Plaque and gingivitis can be avoided with the use of mouthwash. Each mouthwash mixture has somewhat different chemicals, and each product serves a particular purpose. Herbal mouthwashes are considered to be an effective alternative for the commercial products. Herbal mouthwashes are in high demand, because they have less or no side effects and act on oral pathogens effectively. Herbs, in contrast to artificial products, are widely regarded as highly effective. Medicinal herbs have long been used to treat ailments because of their antibacterial and antifungal properties against human pathogens. Herbal mouth washes have the ability to deliver the therapeutic ingredients to access against the organism present on the surface of the mouth. Dental caries and periodontal diseases are among the most frequent infectious diseases experienced by many people at various phases of their lives, with a significant prevalence among children and adolescents who do not practice basic oral hygiene. Herbal mouthwash was prepared from the aqueous extracts of four different leaves namely *Ocimum tenuiflorum*, *Plectranthus amboinicus*, *Mentha* and *Foeniculum vulgare.* The antimicrobial efficacy was tested against the oral pathogens *Staphylococcus sp.*, *Streptococcus sp.,* and *Bacillus sp*. by using agar well diffusion method. The herbal mouthwash was found to be effective against the oral pathogens.

KEYWORDS: Herbal Mouth Wash, Dental Caries, Peridontal Diseases, *Ocimum tenuiflorum*, *Plectranthus amboinicus*, *Mentha* and *Foeniculum vulgare.*

Article History | Received: 13. 07. 2022; Revised: 07. 10. 2022; Accepted: 08. 10. 2022; Published Online: 23. 12. 2022

1. INTRODUCTION

Mouthwashes are mostly suggested in dentistry for prevention and treatment of a number of oral problems. Mouthwashes have the ability to supply ingredients that can fight off organisms that are present on the mouth's surface. The deterioration of ligament, cementum, gingiva, and alveolar bone can result from periodontal disorders. The primary etiological factor for gingival irritation is plaque. The role of junk foods in affecting the oral cavity of an individual is high and unavoidable. The foods like candies, chocolates, jellies and jams have high sugar content the children and adolescents are usually prone to consume this kind of sugar products but, the presence of insoluble glucan in the sugar causes it to bind to the tooth's enamel, causing a cavity to form. The carbonated drinks are other important destroyer of teeth enamel, as it erodes the enamel some may even results in depth eruption of dentine and results in tooth discolouration. The most common infectious diseases encountered by many individuals are dental carries and periodontal diseases at different stages of their life time. Dental caries includes the cavity formation, eruption of enamel, swollen gums, bleeding gums, formation of hollow black eruption on the surface of the teeth *(Bagchi et al., 2015).* In early days, dental caries is high among children and adolescents, because they do not practice proper oral hygiene.

Therefore, mouthwashes or mouth rinses are utilised to get rid of the food particles quickly. The mouthwashes are concentrated aqueous anti-bacterial solution that is used against oral microbes to counter oral infection, for cleansing, and to get rid of bad breath *(Ritam et al., 2014).* The mouthwash plays a prominent role in the oral hygiene of an individual; it helps to relieve symptoms of gingivitis and it is reliably used to destruct the pathogenic germs *(Charles et al., 2007).* The mouthwashes are used by most of the dental patients to overcome sour mouth (xerostomia), ulcerated throat and sensitive teeth. Before performing oral surgery on patients, dentists always use mouthwash as an antibacterial agent because it helps to sterilise the surface of the inflamed gums and teeth, preventing the contamination of any other pathogens. Chlorhexidine is regarded as gold standard mouthwash but has significant side effects, apart from staining the teeth after long term use *(Manipal et al., 2016).* Herbs are considered as effective in contrast to chemical products. Due to its long-lasting antibacterial and antifungal activity against human pathogens, medicinal plants are essential in the treatment of disorders. Herbal mouthwashes are in high demand, because they act on oral pathogens and relieve the pain instantly and also have less side effects. This study includes the preparation of anti-bacterial herbal mouthwash using the aqueous extracts of leaves from four plants namely *Ocimum tenuiflorum* (Tulsi), *Plectranthus amboinicus* (Mexican mint), *Mentha* (Mint), *Foeniculum vulgare* (Fennel) that acts against the oral pathogens Staphylococcus aureus, Streptococcus pyogenes, Bacillus sp. Further the Anti-microbial activity of the leaf extracts was analysed using Agar well diffusion method *(Aldhaher ZA. 2014).* The organisms used for analysis are Staphylococcus aureus which is a gram positive, round shaped bacteria in clusters. Staphylococcus releases enterotoxins which cause food poisoning. Streptococcus pyogenes is a facultative gram-positive bacterium which is in cocci shaped grown in chains. They are usually found in the re-

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gions of upper and lower mouth which is above and below the tongue. Bacillus sp. is a gram-positive bacterium which are rod shaped. These bacteria are usually found in oro-pharynxial region of the mouth *(Harrison et al., 2014).* For the prevention and treatment of a number of oral problems, mouthwash is frequently suggested in dentistry *(Rezaei et al., 2016).* Tulsi leaves help in the clearing of the mouth, throat and help in reducing the susceptibility to mouth ulcers and teeth disorders. Mint leaves help in keeping the mouth moist and fresh. Additionally, they aid in promoting salivary and other digestive enzyme production. Mexican Mint leaves acts as an excellent remedy for oral care, it basically helps in the preventing oral cavities. They also help in checking bad odour and hinder the bacterial growth in the oral cavity. Fennel leaves act as a perfect mouth freshener and are used in reducing the digestive problems. The leaf extracts help in prevention of oral cavities and also keep the mouth fresh and healthy.

2. MATERIALS AND METHODS

2.1 Collection of the Leaf Samples

The leaf samples of Tulsi and Mexican Mint were collected from medicinal garden, Kristu Jayanti College, Bangalore. Mint and Fennel Leaves were collected from the local market, Bangalore.

2.2 Preparation of Leaf Extracts:

The leaves were washed with double distilled water and were shade dried. In case of Mexican mint leaves hot air oven was used for drying since it requires almost 10-15 days to completely dry using shade drying. Hence it was dried at 70ºC for 20 minutes. The dry leaves obtained after drying were grinded into a fine powder under sterilized conditions.

Around 10g of leaf powder was mixed with 50ml of distilled water. The mixture was thoroughly mixed and the impurities which remained in the mixture were drained out using a muslin cloth. This was done in order to retain the chemical components of the leaf extracts. The leaf extracts obtained were stored in containers for further use *(Nazreen and Gayathri, 2016).*

2. 3 Isolation of Microorganisms:

The oral microorganisms *Streptococcus sp., Staphylococcus sp., and Bacilli sp*., were selected for the study. Using a sterile cotton swab the culture swab was obtained from the upper and lower palate of five individuals. This swab was streaked onto the nutrient agar plate. The petri plates were incubated at 37°C for 24 to 48 hours. The colonies were obtained and the characteristics of the colony was observed under the microscope. To further isolate the specific species the microorganisms were sub cultured on specific media such as Mannitol Salt Agar (MSA) for *Staphylococcus sp.*, Blood Agar/ Chocolate agar for *Streptococcus sp.* and MacConkey Agar for *Bacilli sp* (*Nazreen and Gayathri, 2016*).

2.4 Gram Staining

Gram staining was done to check if the cell wall of the bacteria contains peptidoglycan or not and also it is done to differentiate between the gram positive and gram-negative bacteria. Gram staining was performed by applying a primary stain (crystal violet) to a heat-fixed smear for one minute, followed by the addition of a mordant (Gram's Iodine) for one-minute, rapid decolorization with alcohol, acetone, or a mixture of alcohol and acetone for 30 seconds and lastly, counterstaining with safranin for about one minute. The slides were washed thoroughly with distilled water, air dried and observed under the microscope *(Aldhaher ZA, 2014).*

2.5 Catalase Test

A sterile loop is used to transfer a small amount of colony in the surface of a clean, dry glass slide. It is mixed with a drop of distilled water on the slide. After thorough mixing 2-3 drops of 3% hydrogen peroxide is added to it. Observe for effervescence which shows that the microorganism is catalase positive.

2.6 Anti-Microbial Activity

The oral microorganisms *Streptococcus sp., Staphylococcus sp., and Bacilli sp*., were sub cultured into Nutrient broth and incubated at 37°Cs for 24hrs. To perform antimicrobial assay. The nutrient agar media was poured into the petri plates and was allowed to solidify. The wells were made in the medium using sterile gel puncher (3mm). Six wells were punched onto the gel using a gel puncher and 20-30µl of the leaf extract was pipetted into each of the wells under sterile condition. 30µl of distilled water served as control. The plates were incubated at 37°C or 24hours. The zone of inhibition was measured. The zone of inhibition and the readings were noted down (*Suharitha et al., 2021*).

3. RESULTS & DISCUSSION

3.1. Sample collection

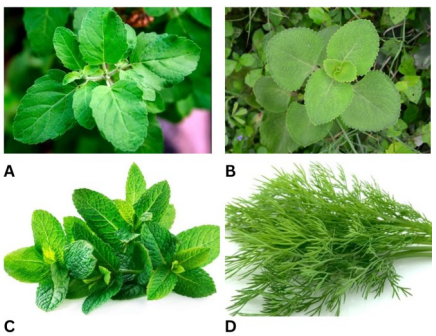

Figure 1: Leaf samples: (A) *Ocimum Tenuiflorum* (Tulsi); (B) *Plectranthus amboinicus* (Mexican Mint); (C) *Mentha* (Mint) (D) *Foeniculum vulgare* (Fennel)

The leaf samples were collected from the source. It was washed and dried for further use ***(Figure 1).***

3.2. Gram Staining

Gram-positive bacteria have cell walls that contain thick layers of peptidoglycan (90% of cell wall). These take up the purple stain. Gram-negative bacteria have walls with thin layers of peptidoglycan (10% of wall), and high lipid content. These take up the pink stain. Gram staining helped in identifying the isolated organisms. It was observed under the microscope and Gram positive cocci shaped organisms were observed and found to be Streptococcus sp., ***(Figure 2a).*** Further Gram positive round shaped bacterium was obtained which was identified as Staphylococcus aureus ***(Figure 2b).*** The Gram positive rod shaped organisms obtained were found to be Bacilli ***(Figure 2c).***

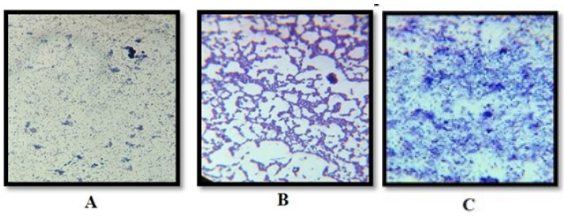

Figure 2: Gram Staining of (A) *Streptococcus sp.,* (B) *Staphylococcus sp.,* (C) *Bacillus sp.*

Streptococcus sp. is a facultative gram positive bacterium which is in cocci shaped grown in chains. They are usually found in the regions of upper and lower palate of the mouth. *Staphylococcus sp.* is a gram positive round shaped bacteria in clusters. Staphylococcus releases enterotoxins which causes food poisoning. *Bacillus sp.* is a gram positive bacteria which are rod shaped. This kind of bacteria is usually found in oropharynxial region of the mouth.

3.3. Catalase Test

This test is performed to determine the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide (H_2O_2).

The morphologically similar Streptococcus (catalase negative) and Staphylococcus (catalase positive) can be differentiated using the catalase test. It helps us to differentiate between the bacteria that produces an enzyme catalase, such as Staphylococci and Bacillus sp. (Figure 3A & B), which are positive, from non-catalase producing bacteria such as Streptococci. The lack of catalase is evident from the absence of bubble formation (Figure 3C) and are catalase negative. Catalase-positive bacteria comprise of aerobes and facultative anaerobes. Catalase-negative bacteria may be anaerobes.

3.4. Antimicrobial Activity of Leaf Extracts

The leaf extracts of the plants exhibited difference of inhibition activity against the bacterial species (Figure 4); and the zone of inhibition results were expressed in terms of the diameter (mm) (Table 1), distilled water was used as control. The results clearly showed that tested bacteria were vulnerable to the four-leaf extracts. *Foeniculum vulgare* (Fennel leaves) shows sensitivity towards *Streptococcus sp.* but is less effective against *Staphylococcus sp.* and *Bacillus sp*. It acts as a perfect mouth freshener to remove the bad breath. *Mentha* (Mint) shows sensitivity towards all the three bacterial species. Mint is known for its aroma hence it helps to get rid of morning-bad breath. It helps to nourishes the salivary activity in mouth hence suppress the acidic bacteria in oral cavity. *Plectranthus amboinicus* (Mexican Mint) shows sensitivity towards *Staphylococcus sp*. but is less sensitive towards *Streptococcus* sp. and *Bacillus* sp. It helps in preventing oral cavities. *Ocimum tenuiflorum* (Tulsi) shows sensitivity towards *Bacillus* sp. but is less effective against *Staphylococcus sp*. and *Streptococcus sp*. It helps in reducing the susceptibility to mouth ulcers and teeth disorders (Figure 5). The mixture of all these leaf extracts (200µl of each sample), shows higher sensitivity towards *Bacillus* sp. and *Staphylococcus sp*. when compared to *Streptococcus* sp.

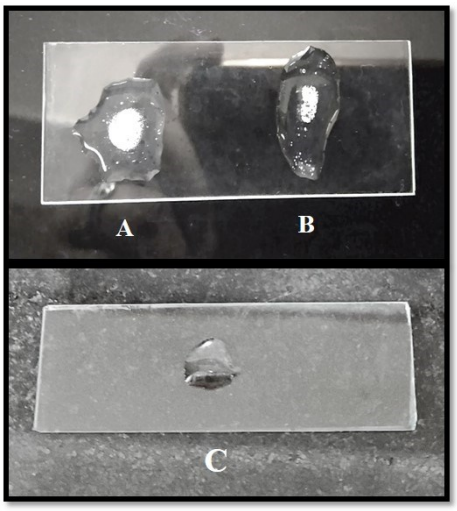

Figure 3: Catalase positive- (A) *Staphylococcus aureus*, (B) *Bacillus sp,* and Catalase negative- (C) *Streptococcus sp.*

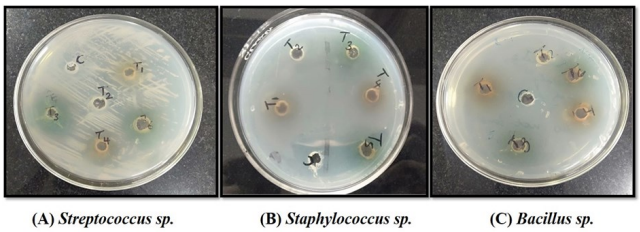

Figure 4: Antimicrobial activity of leaf extracts- Zone of inhibition in millimetres

4. Conclusion

The mouthwashes are concentrated aqueous anti-bacterial solution that are used against oral microbes which might lead to oral infection, bad breath. The mouthwash provides a prominent role in the oral hygiene of an individual, it helps to relieve symptoms of inflamed gums due to gingivitis. It is reliably used to destruct the pathogenic germs (*Van der Weijden et al., 1998*). The mouthwashes are used by most of the dental patients to overcome sour mouth (xerostomia), ulcerated throat and sensitive teeth. This study helps us to analyse the effect of herbal mouthwash on oral microorganisms. The herbal mouthwash was prepared using leaf extracts of different beneficial plants such as the *Foeniculum vulgare* (Fennel leaves), *Mentha* (Mint Leaves), *Plectranthus amboinicus* (Mexican mint leaves), *Ocimum tenuiflorum* (Tulsi Leaves).

Table 1: Antimicrobial activity of leaf extracts

Leaf Extracts	Zone of inhibition in mm Streptococcus sp.	Zone of inhibition in mm Staphylococcus sp.	Zone of inhibition in mm Bacillus sp.
Control	0	0	0
T-1 (Fennel Leaves extract)	90	80	60
T-2 (Mint Leaves extract)	170	140	130
T-3 (Mexican Mint leaves extract)	40	70	50
T-4 (Tulsi leaves extract)	60	70	80
T-5 (Mixture of all extracts)	90	100	120

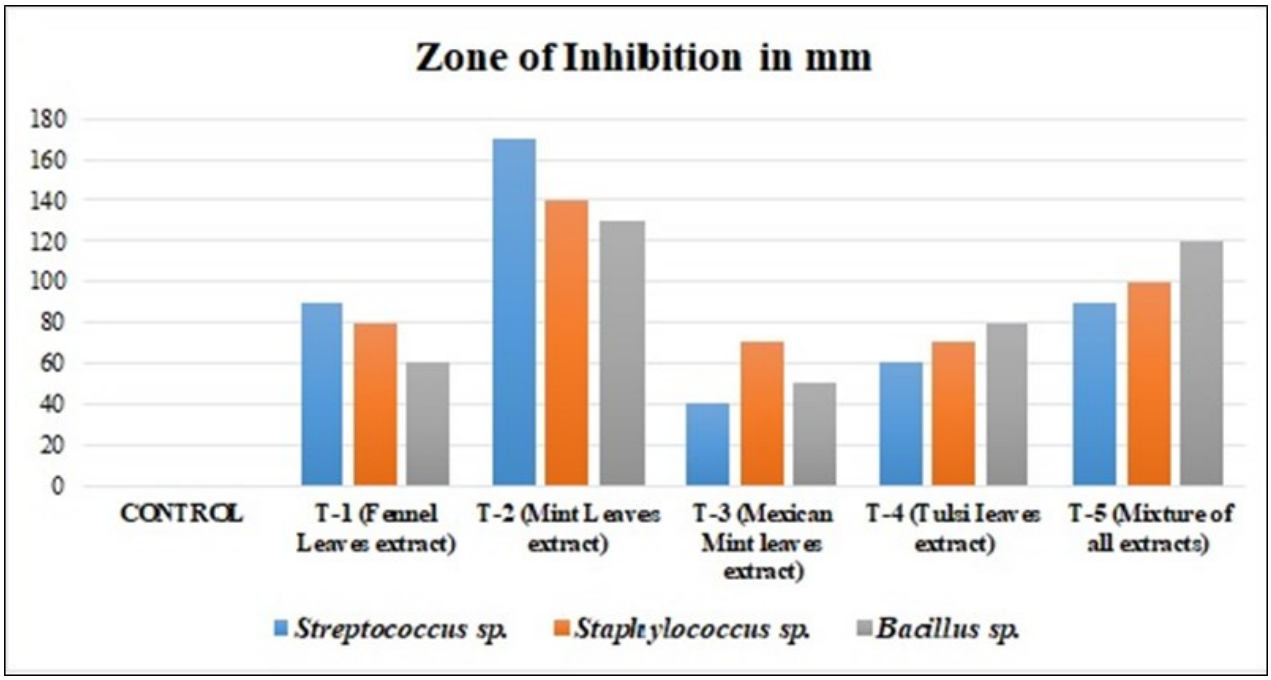

Figure 5: Antimicrobial activity of leaf extracts

The anti- microbial activity was performed for the oral micro-organisms such as *Staphylococcus sp., Streptococcus sp.,* and *Bacillus sp* (*Nam – Hui et al., 2013*). The mouthwash consists of purely aqueous extracts hence can be preferred over the commercial mouthwashes which consists of hydrogen peroxide and mercury chloride (*Pathan et al., 2017*), which acts an immediate pain reliever, sterilizer of gums and whitener of teeth, but long-term use of such products may lead to discoloration of teeth by eroding the tooth enamel which can be harmful to the teeth (*Netuschil et al., 2003*). The use of herbal mouthwash is effective as it does not produce any side effect and also economically affordable (*Sandhya, 2017*). *Ocimum tenuiflorum, Plectranthus amboinicus, Mentha and Foeniculum vulgare* will be good choice of herbs for the preparation of herbal mouthwash.

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