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Comparative study of "Commercially available mouthwashes with that of herbal mouthwashes" on pre-dominant oral cavity flora- *Streptococcus species*

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Abstract— Oral cavity is a vital part of the body and contributes to total health and wellbeing. Poor oral health affects general health and leads to systemic diseases like dental caries. The association between oral diseases and oral cavity flora is well established. There are more than 700 species of bacteria which may affect oral cavity and a number are implicated in oral diseases. Dental caries are developed by micro-organisms like *Streptococcus, Lactobacillus* and *Actinomycetes* which can be inhibited with the use of herbal and commercial mouthwashes as they show anti-microbial activity. Many researchers studied effect of anti-microbial activity of plant extracts on microbial flora of oral cavity. However, the studies on green synthesis of silver nanoparticles from the plant extracts and its effect on microbial flora of oral cavity would be a significant finding as these nanoparticles are efficient at very low concentrations and can penetrate into dental cavities easily. The silver nanoparticles play a crucial role in dental field for not only controlling the pathogens but also in other applications of nano dentistry.

The present study includes screening, isolation and identification of microbial flora of oral cavity and to determine the effect of silver nanoparticles on oral flora. 50 oral cavity samples of patients fitted with dental braces from Army College of Dental Sciences and other dental clinics were collected. *Streptococcus species* was the predominant bacteria found in all these samples and the same organism is selected for comparative study of effect of commercially available mouthwashes like Orarite, Histidine and Whisper mint with that of herbal products like Azadirachta indica (Neem), Ocimum santum (Tulsi), Mentha (Peppermint), Piper betle (Betel) and Syzigium aromaticum (Clove). The results demonstrated that herbal products have more anti-microbial activity on *Streptococcus species* than the commercially available mouthwashes. These plant products were further tested for green synthesis of silver nanoparticles. The Silver Nanoparticles produced were characterized by Spectrophotometric, and SEM analysis. The silver nanoparticles synthesized from these plant products are safe, efficient and economical to use in herbal mouthwash preparations for dental diseases.

Key words: Green synthesis, Silver nanoparticles, Plant extracts, Oral flora.

I. INTRODUCTION

The oral cavity of humans is a suitable environment for the colonization of different types of micro-organisms. These oral microbiota are some of the most complex microbial floras in the human body, consisting of more than 700 different bacterial species (1). The major microbial flora of oral cavity includes *Streptococcus sp, Lactobacillus sp, Actinomycetes sp, Fusobacterium sp.* Among these, *Streptococcus species* is the most well-established bacteria because it has the ability to form the biofilms, and appears to be the most commonly involved acidogenic microorganism in development of dental caries (2). The presence of braces or other fixed orthodontic appliances in the mouth is usually associated with the multiplication of decaying bacteria. These bacteria can cause oral health problems which may persist even after the removal of those orthodontic appliances. The use of mouthwashes reduces the incidence of microbial plaques and oral diseases and also improve oral health (3). Among the commercially available mouthwashes, products such as Listerine, Colgate plax, Orarite, Hexidine, Whisper mint mouthwashes containing chlorhexidine are known to be highly effective in preventing the multiplication of micro- organisms in the oral cavity including *Streptococcus species* and blocking the attachment of these bacteria to dental surfaces. Chlorhexidine is the gold standard to test the efficiency of mouthwashes but it causes side effects such as undesirable tooth discolouration, unpleasant taste, dryness and burning sensation in the mouth (Salehi. P, *etal.,* 2006) (4). Therefore, there is a need for efficient herbal mouthwashes to replace such chemicals so as to minimize the undesirable effects.

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Leaves of Azadirachta indica (Neem), Ocimum santum (Tulsi), Mentha (Peppermint), Syzygium aromaticum (Clove) and Piper betle (Betel) are known since ages for their antibacterial properties. More over the leaf extracts of plants are excellent bioreactors for the synthesis of nanoparticles. They do not cause any harmful effects to the oral cavity but in fact possess many medicinal uses for the maintenance of oral care. The phenols, flavonoids, polysaccharides, tannins etc., in the plant extracts help in bio-reduction of silver nitrate and synthesis of silver nanoparticles (Babu S. A., *etal*., 2011) (5). Of all the five different plant extracts selected, Syzigium aromaticum(Clove) has found to be more effective after performing the antimicrobial assays and hence was chosen for the further study.

Biological nanoparticles are non–toxic, environment-friendly and at the same time, their efficiency is greatly enhanced due to their small size (6). Incorporation of such nanoparticles into herbal plant products can eliminate the need for harmful chemicals.

Study includes comparison of the commercially available mouthwashes with that of herbal mouthwashes against oral cavity flora- *Streptococcus sp*. These herbal products were further tested for green synthesis of silver nanoparticles and its antibacterial effect on *Streptococcus sp*. After successful demonstration of their antibacterial property, it can be used for the synthesis of herbal mouthwashes. The nanoparticles show size-dependent variation in their properties so it is important to determine the size of the synthesized nanoparticles and for this purpose, characterization using UV-Vis spectrophotometry and SEM analysis was carried out in the present study.

The oral cavity consists of many surfaces each with a plethora of bacteria, the proverbial bacterial biofilm. Some of these bacteria may involve in oral diseases such as caries and periodontitis, which are among the most common bacterial infections in humans (7). Surprisingly, there are only fewer facts known about the microflora of the healthy oral cavity (8). There are many pathogens that cause oral cavity diseases and some of them include *Streptococcus mutans, Streptococcus salivarius, Halobacterium sps, Veilonella sps* etc. These pathogens reside in plaque, the deposit that forms on the base of the teeth and hardens to form tartar (9).

The presence of orthodontic appliances in the mouth for one to two years is usually associated with the multiplication of bacteria. The presence of such bacteria leads to oral health problems such as primary lesions and progressive enamel loss after the removal of fixed orthodontic appliances (10).

Mouthwashes are used in reduction of microbial plaques. Based on the recent studies on mouthwashes, chlorhexidine is used as a positive control to compare the efficacy of other products, since it is believed that chlorhexidine is a gold standard (11-15). However using this mouthwash there is an incidence of side effects such as undesirable tooth discoloration, unpleasant taste, dryness and burning sensation in the mouth. So, these side effects will discourage patients to use the mouthwash (16-17). Eugenol has been used to treat related dental problems to relieve pain arising from pulpits and dentinal hypersensitivity. It reduces swelling and irritation in the effective area (18). Silver nanoparticles have a range of biomedical applications, including, dental field, an antibacterial factor in dental resin composites. Dental caries are usually targeted at automatic or nonspecific control of the plaque biofilm (19). Silver nanoparticles are characterised using UV-Visible Spectrophotometry and SEM analysis (20).

II. METHODOLOGY

1) Collection of clinical sample:

Tooth scrapings were collected by gentle rubbing of the sterile cotton swabs from 50 patients wearing braces, from Army College of Dental Sciences, Balaji Nagar, Secunderabad and other dental clinics in vicinity.

2) Isolation and identification of *Streptococcus sp*:

The cotton swabs were inoculated onto Nutrient agar plates for the isolation of oral microbial flora (*Streptococcus sp*). The organism was identified based on cultural and microscopic methods. The inoculum of *Streptococcus sp* was prepared by inoculating in 5ml of Nutrient broth at 37^0C overnight.

3) Dilution of commercially available mouthwashes:

The commercially available mouthwashes like Hexidine, Orarite and Whisper mint were diluted with sterile distilled water to get 10 fold dilution. These were used for antibacterial assays.

4) Preparation of plant extracts:

Fresh leaves of Azadirachta indica (Neem) (21), Ocimum santum (Tulsi) (22), Mentha (Peppermint) (23), Syzygium aromaticum (Clove) (24) and Piper betel (Betel) (25) were collected from the campus of Bhavan`s Vivekananda Degree College, Sainikpuri.

Preparation of leaf extracts: 200gm of fresh leaves were taken from different plants and were washed twice with distilled water and these leaves were subjected to drying for 24 hours. After drying, the leaves were cut into small pieces and one litre of distilled water was added and boiled for 30 minutes. After cooling, the solution was filtered using Whatman no.1 filter paper. The filtrate of leaf extract was collected in a beaker and was stored at 4^oC for further use.

5) Green synthesis of Silver nanoparticles using silver nitrate:

Preparation of $AgNO_3$ solution:

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Different concentrations/ Molarity of silver nitrate solutions were prepared from a stock solution. Stock is prepared by dissolving 1.69g of $AgNO_3$ crystals into 10 ml of distilled water and stored in amber coloured bottle in cool and dry place.

6) **Antibacterial activity:**
Nanoparticles synthesized from the Syzigium aromaticum (Clove) extract were tested for their antibacterial efficiency against *Streptococcus sp* using agar well assay.

7) **Characterization of synthesized nanoparticles:**
Synthesized silver nanoparticles in the solution with leaves extract were characterized by UV-Vis Spectroscopy and SEM.

III. RESULTS AND DISCUSSION

Different dental samples were screened, of all the dental samples *Streptococcus sp* was found to be predominant oral cavity flora. The growth of *Streptococcus species* on nutrient agar as shown in (fig 1). *Streptococcus species* grew as yellow colored colony on nutrient agar media and the cells appeared in chains when observed microscopically (fig 2). This isolated *Streptococcus sp* was tested by various commercially available mouthwashes.

MarcoAntonioBotelho *et al.*, (2009) (26) have done similar studies and bacterium was identified as *Streptococcus sp*.
The commercially available mouthwashes i.e., Orarite, Hexidine and Whisper mint were tested using agar well diffusion assay against gram positive oral cavity flora-*Streptococcus sp.* Among the commercially available mouthwashes tested, all the three mouthwashes showed good zone of inhibition. The highest was for Chlorhexidine followed by Orarite (fig 4).

Among the plant extracts tested, Clove extract showed the highest zone followed by Neem, Mint etc (fig 6) Comparatively the plant extracts showed a high zone of inhibition than the commercially available mouthwashes.

Gradual colour change of the plant extract, Syzigium aromaticum (Clove) after addition of $AgNO_3$ is considered as an indication of synthesis of silver nanoparticles. The colour is referred to the surface plasmon resonance of silver nanoparticles. These colour intensities recorded using UV-Vis spectrophotometry between 400nm to 430nm. An extreme absorption was detected at 420nm after 24hrs incubation.
On mixing plant extract with silver nitrate solution, a colour change was observed in the plant extract (Figure 7) which clearly indicates the reduction of silver ions and formation of silver nanoparticles.

Antibacterial activity of the synthesized silver nanoparticles:
To determine the antibacterial activity of the synthesized nanoparticles, the samples were collected from patients fitted with dental braces which were used in the experiment. By performing cultural and microscopic tests, the predominant oral cavity flora isolated was *Streptococcus sp*. The antibacterial activity of the nanoparticles was tested against these *Streptococcus sp* using Agar well assay. The silver nanoparticles showed good zone of inhibitions against oral cavity flora *Streptococcus sp*. (Figure 8).

Characterization of silver nanoparticles by UV-Vis Spectrophotometry:
The synthesized nanoparticles from clove extract were characterized using UV-Vis Spectrophotometry and the absorption maxima was found to be 420nm. (Figure 10).
The concentration of silver nitrate solution used also has an effect on Silver nanoparticle formation. The optimum silver ion concentration at most cases is reported as 1mM(H.R. *et al*., 2016) (12). While nanoparticles were found be produced with all the concentrations of $AgNO_3$, i.e., 1mM, 2.5mM, 5mM, 10mM; more nanoparticles were produced at a molarity of 5mM (Figure 11). Silver nitrate solution of molarity 5mM supported rapid formation compared to other concentrations on the basis of UV-Vis studies.

Characterization of Silver nanoparticles by SEM analysis:
The silver nanoparticles were characterized by scanning electron microscopy (SEM). In this micrograph observed spherical nanoparticles in the size range of 70.5nm-120nm respectively (Figure 12).
Song *et al.*, (2009) (27) have performed similar methods of synthesis and characterisation of silver nanoparticles and observed similar results.

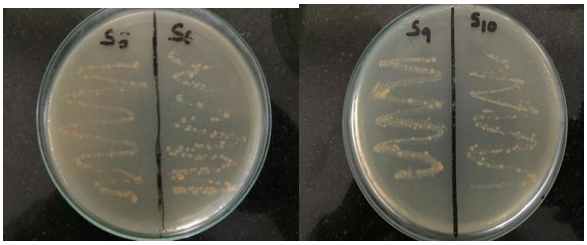

Fig 1: Growth of *Streptococcus species* on nutrient agar

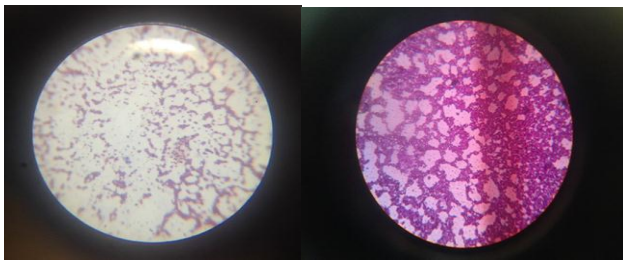

Fig 2: Microscopic observation of *Streptococcus species*

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O- Orarite H- Hexidine W- Whisper mint

Fig 3: The zone of inhibition of differ commercially available mouthwashes indicated significant anti-bacterial activity on *Streptococcus species.*

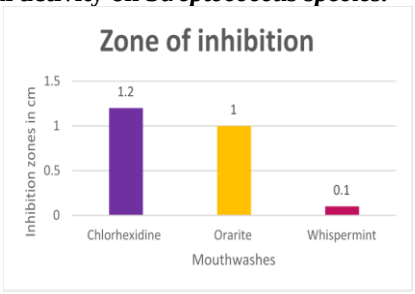
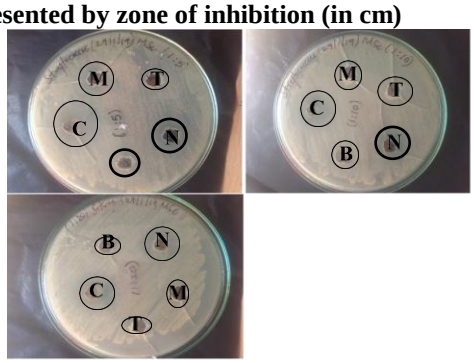


Fig 4: Anti-bacterial activity of different mouthwashes, represented by zone of inhibition (in cm)



C- Clove N- Neem B- Betel M- Mint T- Tulsi

Fig 5: The zone of inhibition of different plant extracts indicated significant anti-bacterial activity on *Streptococcus species.*

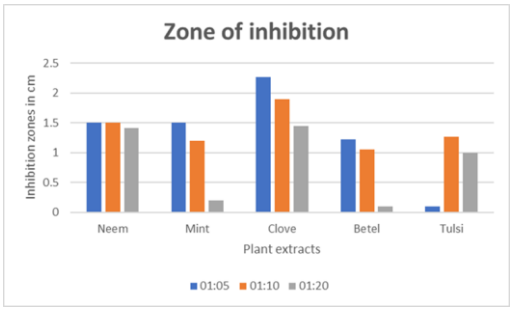


Fig 6: Anti-bacterial activity of different plant extracts, represented by zone of inhibition (in cm) on *Streptococcus species*.

Fig 7: Green synthesis of silver nanoparticles using the aqueous extract of Syzygium aromaticum (Clove)

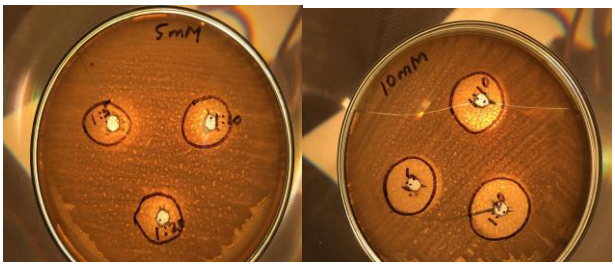


Fig 8: Zone of inhibition of silver nanoparticles indicated significant anti-bacterial activity on *Streptococcus species.*

Antibacterial activity of the synthesized silver nanoparticles:

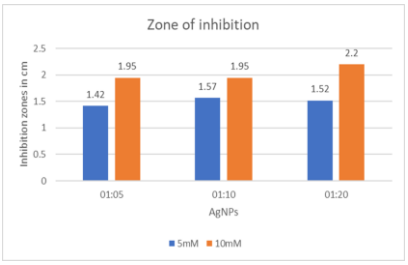


Fig 9: Anti-bacterial activity of AgNPs, represented by zone of inhibition (in cm) *Streptococcus species*.

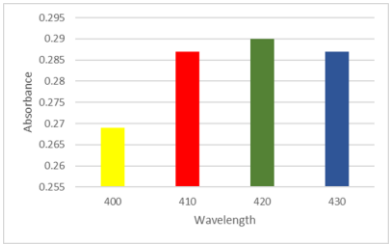


Fig 10: Absorption maxima for AgNP formation.

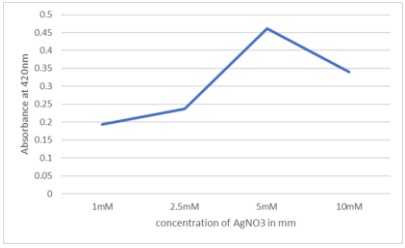


Fig 11: Effect of AgNO3 concentration on AgNP formation

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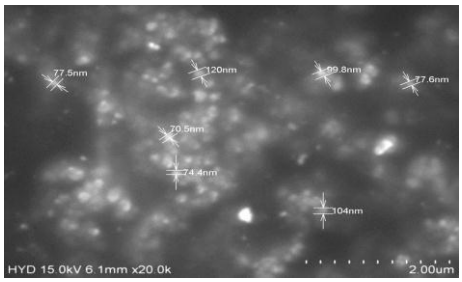

Fig 12: SEM image of the synthesized AgNPs

IV. CONCLUSION AND FUTURE SCOPE

In the present study, *Streptococcus sp* was found to be the predominant oral cavity flora. Of all the plant extracts tested, Syzigium aromaticum (Clove) was found to be most potent agent for the green synthesis of silver nanoparticles. Maximum nanoparticles were produced with 5mM AgNO3 solution and 1ml of plant product. The comparative study was done between commercially available mouthwashes with that of herbal mouthwashes of which herbal mouthwashes have more anti-microbial activity on *Streptococcus species* than the commercially available mouthwashes. The characterization of nanoparticles using SEM demonstrated that the size of the synthesized nanoparticles ranged from 70.5-120nm. Further, the silver nanoparticles synthesized by Syzigium aromaticum (Clove) in an aqueous solution can be used as an appropriate formulation for oral care.

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