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RESEARCH ARTICLE
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To Evaluate the Antimicrobial Efficacy of Amla, Neem Silver Nanoparticles Mediated Dental Varnish – An In Vitro Study

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ABSTRACT

Aim: The aim of the study was to compare the antimicrobial activity of Amla, Neem incorporated with silver nanoparticles dental varnish with that of commercially available dental varnish

Objectives: The two main objectives of the study are to prepare silver nanoparticle based dental varnish and to evaluate the antimicrobial efficacy

Materials And Methods: Streptococcus mutans, Streptococcus aureus, Enterococcus faecalis and Candida albicans were grown in Mueller–Hinton agar media separately; three different concentrations of silver nanoparticle varnish ((25µl, 50µl and 100µl) were prepared and applied on the Mueller–Hinton plates. Conventional dental varnish was included as the control sample. The plates were further incubated anaerobically for 24 h at 37°C in the incubator. The antimicrobial effect of different varnishes was assessed by measuring the diameter of inhibition zones in millimetres by a ruler. Statistical analysis was performed using the descriptive data to find the mean and standard deviation for the data.

Results: The silver nanoparticle varnish showed antimicrobial effect against all test microbes, where the mean value of inhibition zone size (mm) was increased by increasing the concentration.

Conclusion: The herbal based varnish added with silver nanoparticles seems to be more effective against S. Mutans, C. Albicans, E. Faecalis, S. Aureus. Silver nanoparticle dental varnish had better antibacterial efficacy as compared to conventional dental varnish against all four microorganisms.

Keywords: *Dental varnish , children , plants , nanoparticles, Biofilm, Fluoride*

INTRODUCTION

Dental caries is a complex, dynamic, biofilm-mediated disease that causes phasic demineralization and remineralization of tooth hard tissues. Caries can occur at any age, in both primary and permanent dentitions, and can damage the tooth crown as well as exposed root surfaces in later life. The balance of pathogenic and preventive variables promotes caries start and development[1]. Fluoride varnish was created as a preventive therapy to increase the amount of time fluoride has contact with the tooth surface, making the tooth more resistant to dental caries[2]. The theorised mechanism of action involves the production of intraoral fluoride reservoirs as a result of the formation of calcium fluoride ion pairs (e.g., CaF+) that are held on enamel and in dental plaque and slowly released to assist limit mineral loss during demineralization [3,4]

Fluoride can be harmful if consumed in large doses. The American Dental Association recommends dispensing no more than 120 mg fluoride (264 mg sodium fluoride) at a time[5].In the United States, two commercially produced fluoride varnishes contain the greatest fluoride content of any fluoride vehicle (22,600 ppm F-) 80 and are intended to be administered by dental specialists. Despite the varnish's short setting time and the minimal dosage used, there is a possibility that young children will eat some of the substance during placement. Furthermore, because fluoride is released from the varnishes after treatment, some fluoride is consumed[6]. In rare cases death has occurred in infants with as little as 250 mg[7].

Due to several concerns associated with fluoride, many recent researches have taken a step towards other antimicrobial agents including plant derivatives. Silver nanoparticles are essential in the development of novel antimicrobials against a wide spectrum of harmful bacteria. Recently, the biological production of metal nanoparticles utilising plant extracts was proven to be of great effectiveness [8]. Amongst the various available metals, Silver (Ag) nanoparticles have been widely used due to their potential anti-bacterial [9], anti-fungal, and anti-proliferative action [10,11]. As a result, it is less poisonous and not a hazardous material and ecologically friendly as solvents [12], simple, quick, and cost effective[46,13], consumes less energy and performs under moderate operating circumstances and combines the potency of both silver nanoparticles and plant active components[12].

Silver nanoparticles' effect is mostly determined by their nanoscale, which modifies the level of silver ion release and interferes with surface energy[43,14]. When compared to other antimicrobial treatments, nanoparticles have good antibacterial properties due to their huge surface area, which provides high interaction with microorganisms[15]. One of the most important modes of action of AgNPs is the formation of reactive oxygen species (ROS), with hydroxyl radicals being the primary species responsible for oxidative damage[16].Terpenoids and flavanones are the two important phytochemicals present in neem which play a vital role in stabilising the nanoparticle and also act as capping and reducing agent[46,17]. Aqueous neem leaf extract reduces silver salt to silver nitrate, this capped nanoparticle with neem extract exhibits antibacterial activity[42,18]. The reduction of the metal ions and the stabilisation of the Ag nanoparticles is believed to occur by the various hydrolysable tannins present in the amla fruit.The nanoparticles are extremely stable with time and could be phase transferred into an organic medium[19].

In the present study, the antimicrobial efficacy of green synthesised silver nano-particle based dental varnish and its role in caries activity was studied by analysing the bacterial colony count using the disc diffusion method against 4 different microorganisms. The aim of the study was to compare the antimicrobial activity of Amla, Neem incorporated with silver nanoparticles dental varnish with that of commercially available dental varnish.

MATERIALS AND METHODS

It was an in vitro study conducted in the month of September 2022 – December 2022 in the city of Chennai, Tamil Nadu.

Ethical approval

The study was registered with the Institutional Review Board of the Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India. Ethical approval was obtained from the Institutional Review Board of the SIMATS.

Plant material and characterization of the silver particles

Leaves of Neem were collected from University Campus in the month of November from chennai, Tamil Nadu, India. Leaves were thoroughly washed in running water to remove the dirt and dust on the surface of the leaves. They were air dried for 10 days and kept in the hot air oven at 600C for 24-48 hours. These leaves were then ground to a fine powder. Indian Gooseberry was crushed and the pure liquid was extracted. 1 mM silver nitrate was added to the plant extracts separately to make up a final solution of 200 ml and centrifuged at 18,000 rpm for 25 min. The supernatants were heated at 50 to 950C. A change in the colour of the solution was observed during the heating process within 10-15 minutes. The colour changes indicate the formation of silver nanoparticles

FIGURE 1: Showing microbial zone of inhibition after 24 hours plates swabbed with Streptococcus aureus.

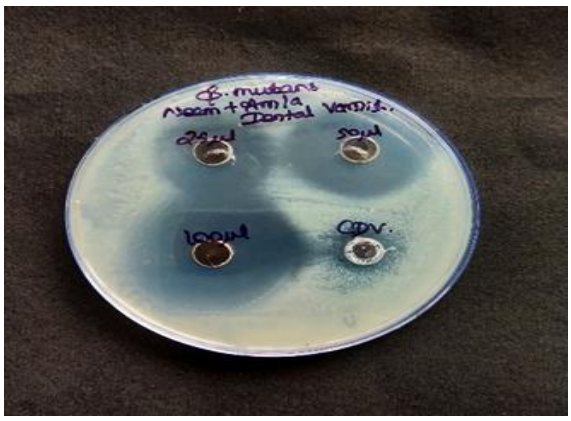

FIGURE 2: Showing microbial zone of inhibition after 24 hours plates swabbed with Streptococcus Mutans.

FIGURE 3: Showing microbial zone of inhibition after 24 hours plates swabbed with E.Faecalis.

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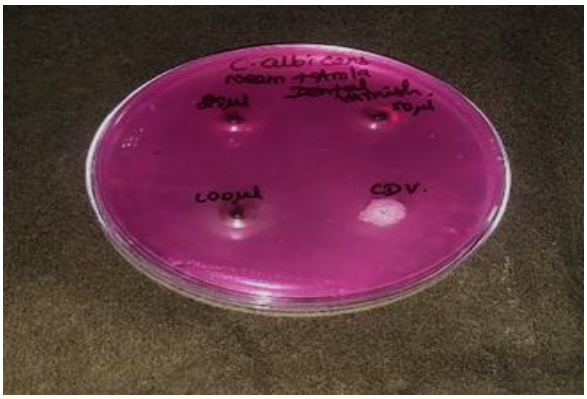


FIGURE 4: Showing microbial zone of inhibition after 24 hours plates swabbed with E.Faecalis.

Antimicrobial analysis

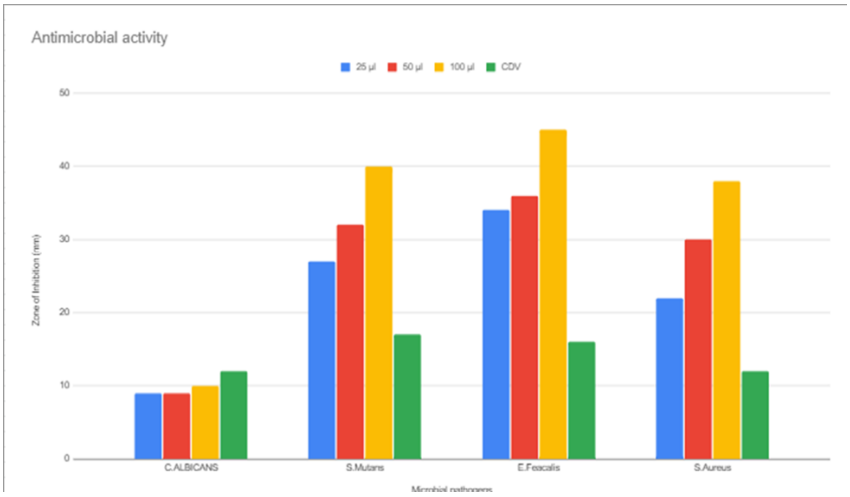
The antibacterial activities were carried out by disc diffusion method. Mueller Hinton agar plates were prepared, sterilized for 15 min and solidified. After solidification wells were cut using a 9mm sterile polystyrene tip and bacterial cultures(C.Albicans, S.Mutans, E.Faecalis, S.Aureus) were swabbed on these plates. Silver nanoparticles solution of various concentrations (25µl, 50µl and 100µl) and the conventional dental varnish solution were placed on four different wells on the agar plate and kept for incubation at 37 degrees Celsius for 24 hours. Zones of inhibition were measured. The experiments were repeated thrice and mean values of zone diameter were presented.

The antimicrobial property of materials was assessed at which it appeared to form circular zones of bacterial inhibition (halo) around each hole. The diameter of these zones of bacterial inhibition was measured in millimeters using a ruler (from the edge of the zone from one end to the next edge).

Statistical Analyses

The collected data was tabulated into Microsoft office Excel 2013 transferred to SPSS version 26.0 software (SPSS, Chicago, IL, USA) for statistical analysis. Descriptive data analysis was done to find the mean and standard deviation for the data. The confidence interval was set at 95%. Data are represented as the average values with standard error of at least three values of each independent experiment.

RESULTS



GRAPH 1: Zone of inhibition of Silver nanoparticles mediated dental varnish against various pathogens

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From the above graph it can be seen that on the whole the dental varnish at the 100µl appeared to be the most effective. As compared fungal to bacterial colonies all the varnishes were more effective against bacterial colonies. The conventional dental varnish appeared to function better with respect to C.Albicans. Silver nanoparticle based dental varnish at all concentrations performed better as compared to conventional dental varnish. Furthermore, with increasing concentration silver nanoparticle solution performed better. Maximum zone of inhibition was seen for E. Faecalis.

DISCUSSION

In recent years, various ways of managing dental caries have been studied, such as topical administration of antimicrobials on the surface of teeth that are at risk of or impacted by dental caries. The silver ions displayed strong bactericidal action against oral streptococci[20]. Many investigations have shown that silver nanoparticle-containing materials have high antibacterial properties against microbes, biofilms, and oral pathogenic streptococci[21,22].

AgNPs produced biologically have been found to be promising medicinal compounds with substantial antibacterial and antiviral properties[10]. These are environment conscious approaches that prevent chemical toxicity. Plant extracts contain useful compounds, such as cyclic peptides, sorbic acid, citric acid, euphol, polyhydroxy limonoids, ascorbic acid, retinoic acid, tannins, ellagic acid, and gallic acid, which are thought to play an important role in the bioreduction and stability of nanoparticles[23]. Overall, this study reported green-synthesis AgNPs mediated by Azadirachta indica and Phyllanthus emblica plant extracts and displayed good antibacterial efficacy.

Most of the studies that compared AgNPs in formation of significant zone of inhibition as compared to conventional dental varnish could be due to the ability of the nano silver particles to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell[24,,41 ,25]. There was a direct association between the diameter of the inhibitory zones and the quantities of nano silver particles in dental varnish against S.Mutans, S.Aureus and E.faecalis[26].

The zone of inhibition of the prepared nanoparticles against C.Albicans at 25, 50,100 µL and commercially available Copal Varnish was found to be 8mm , 9mm , 10mm and 12mm respectively .Similarly for S.Mutans was found to be 28mm , 32mm 40mm , 18mm . For E.faecalis was found to be 32mm , 35mm ,45mm , 15mm . For S.Aureus was found to be 23mm , 30mm , 38mm , 17mm . Silver ions can infiltrate and precipitate in carious lesions, causing enamel hardening. Hence when dental varnish is used they can reduce microbial load and also inhibit caries progression. Dental practitioners employ sodium fluoride varnish in the therapeutic setting to remineralize incipient lesions. When 5% nanosilver is added to sodium fluoride varnish, the growth of caries lesions in remaining teeth is inhibited by 77%, without the presence of a metallic taste or severe ulcerations[27].

It has been demonstrated in Candida species that the destructive activity of AgNP is related to both the ROS-mediated pathway, which induces aberrant mitochondrial apoptosis, and the ROS-independent mechanism, which results in the same cell death conclusion[28]. AgNP functions similarly to antibacterial agents in Candida species by interfering with membrane potential, integrity, and fluidity, as well as proliferation and cell cycle[29]. But in the present study the effect of the silver nanoparticles on C. Albicans appeared to be less as compared to the other microbes.

Our team has extensive knowledge and research experience that has translate into high quality publications (Neelakantan et al. 2013; Aldhuwayhi et al. 2021; Sheriff et al. 2018; Markov et al. 2021; Jayaraj et al. 2015; Paramasivam et al. 2020; Li et al. 2020; Gan et al. 2019; Dua et al. 2019; Mohan and Jagannathan 2014)

CONCLUSION

Within the limitations of this study, the following could be concluded:

(1) The three concentrations of silver nanoparticle varnish had better antibacterial effects than conventional dental varnish.
(2) The antibacterial effect against S. mutans organisms gradually increases with the increase of the nanosilver particle concentration.

(3) The synthesised AgNPs showed better antibacterial activity than antifungal activity when compared against different oral pathogens
(4) The effectiveness of silver nanoparticle varnish was better against bacteria as compared to fungal colonies.

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Nil.

CONFLICT OF INTEREST

There are no conflicts of interest.

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