



Preparation of Mouthwash using *Ficus benghalensis* Assisted Silver Nanoparticles and a Comparative Analysis of Its Antimicrobial Activity

M. Rangeela^a, M. Jeevitha^{b*}, S. Rajeshkumar^c and Selvaraj Jayaraman^d

^a Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.

^b Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.

^c Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.

^d Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author MR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft manuscript. Authors MJ and SR managed the analyses of the study. Author SJ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study is to prepare a mouthwash using *Ficus benghalensis* mediated silver nanoparticles (Ag NPs) and analysing its antimicrobial activity against oral pathogens

Introduction: Nanotechnology deals with the particles which are less than 100 nm and have important roles in medicines, industries, drug gene delivery. Different parts of the *F. benghalensis* show medicinal properties. Leaves are used for ulcers, aerial roots are used in gonorrhoea, seeds and fruits are used for dysentery, diarrhoea and diabetes. *F. benghalensis* have been reported to have immunomodulatory, antibacterial activity and are used to treat toothache.

Materials and methods: In the present study, the silver nanoparticles were synthesized from *Ficus benghalensis* plant extract. The preparation of plant extract was done using commercially available *F. benghalensis* leaves powder for the synthesis process. The mouthwash was prepared using 1

*Corresponding author: E-mail: jeevitham.sdc@saveetha.com;

mM of silver nitrate. The prepared nanoparticles were confirmed using a UV-vis spectrophotometer. Different concentrations of the synthesised *Ficus benghalensis* mediated silver nanoparticles were tested against *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis* and *Candida albicans* using agar well diffusion method and MIC will be determined.

Results: It has been observed that the antimicrobial effect of the prepared Ag NPs was almost similar against all the organisms used in the study with a maximum zone of inhibition against *Staphylococcus aureus*. It has also been observed that the antimicrobial activity of the AgNPs increased with increasing concentration.

Conclusion: As the synthesised *F. benghalensis* assisted silver nanoparticle mouthwash showed potent antimicrobial activity in vitro against the oral microbes: *S. aureus*, *S. mutans*, *E. faecalis*, *C. albicans*, in vivo studies are needed further to evaluate the antimicrobial effect.

Keywords: Antimicrobial activity; *Ficus benghalensis*; innovative technology; mouthwash; silver nanoparticles.

1. INTRODUCTION

Nanotechnology deals with particles which are less than 100 nm in size and have important roles in medicines, industries and drug gene delivery systems. Nanomaterials are particles having nanoscale measurement and they are estimated as small particles with upgraded synergist reactivity, warm conductivity, non-straight optical execution and compound relentlessness attributable to its enormous surface territory to its volume proportion [1,2]. Nanoparticles have begun being considered as nano antibiotics in light of their antimicrobial activities [3]. In today's world, nanoparticles have been incorporated into different modern, wellbeing, nourishment, feed, space, compound, and beauty care products which requires a green and condition cordial way to deal with their synthesis [1]. The size of the nanoparticles is similar to most of the biological molecules and structures therefore the nanoparticles can be used for both in vitro and in vivo biomedical research and applications [4,5].

The nanoparticles were made from different materials like metal oxides and the most commonly used metals are silver and gold [6]. Silver nanoparticles (AgNPs) are commonly employed as metallic nanoparticles in health care delivery systems due to their unique physicochemical and biological properties and are used as antibacterial, antifungal, antiviral and anti-cancer agents. Since the silver is known for its antimicrobial activity, the AgNPs can be used against infection [6]. The antimicrobial activity of the synthesized silver nanoparticle is due to the silver cations released from them, pertaining to the changes in the membrane structure of microbes which leads to the increased

membrane permeability of the bacteria and finally cell death [7,8]. The AgNPs were synthesized from many plant varieties. Silver salts are well known for their antimicrobial activity since antiquity. The use of Ag NPs is increasing because of their unique properties like high electrical conductivity, chemical stability, catalytic and antimicrobial activity [9]. The size, shape and surface chemistry of AgNPs have an effect on their physical, chemical, optical and electrical properties and Small sized Ag NPs are of great use because of their potential applications in emerging biomedical, antibacterial, antifungal and antiviral areas [1,10].

These chemicals have potential toxic effects on the environment and health. Hence, green methods for synthesis of nanoparticles is preferred. Use of plants is still being explored and is considered to be a promising treatment option for various diseases. In the present study the AgNPs were synthesized from *Ficus benghalensis*. *F. benghalensis* belongs to the Moraceae family which is a very large tree with wide-spreading branches bearing aerial roots. The root extract has been used in medicine since ages to boost the immune system and used extensively in folk medicines as a vermicide, astringent, hypotensive, and anti dysentery drug. Different parts of the *F. benghalensis* have medicinal properties [8,11]. Leaves are used for ulcers, aerial roots are used in gonorrhoea, seeds and fruits are used for dysentery, diarrhoea and diabetes [12]. The extract of *F. benghalensis* has been reported to have more immunomodulatory, antibacterial and hair growth promoting activity [13,14]. There are many secondary metabolites present in *F. benghalensis* such as alkaloids, phenols, saponins, proteins, tannins, flavonoids. These

phytochemicals can be used for many treatments such as ulcers, diarrhoea, dysentery, skin diseases, piles, gonorrhoea [15].

Dental caries is a major problem mainly consisting of bacterial plaque and oral microbes, which is caused by the bacteria such as *Streptococcus mutans*, *Lactococcus*, *Staphylococcus*, *Enterobacter* species. Many Plants are able to produce varieties of compounds against pathogens [16,17]. Such that the roots of *Ficus* species can be used to treat the toothache. The herbal products used in dentistry are effective to treat dental infection. The herbal mouthwashes have lesser side effects than the conventional mouth washes. Plants products can be used as mouthwashes because they are eco-friendly, economical and effective in maintaining oral hygiene. Nano scale compound is used for the preparation materials which are used in dentistry. This method is simple and less expensive [18]. Our team has extensive knowledge and research experience that has translated into high quality publications [19–38]. The aim of this study is to prepare a mouthwash using *F. benghalensis* mediated AgNPs and analysing its antimicrobial activity against oral pathogens.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract

The preparation of plant extract was done using commercially available *F.benghalensis* leaves powder for the synthesis process. 1 gm of *F. benghalensis* dried powder was mixed with 100 mL of distilled water and boiled at 60-70 degree celsius in the heating mantle for 10-15 minutes. The heated solution was filtered using Whatman no.1 filter paper. 20 mM (0.574g) of Silver nitrate was dissolved in 60 mL of distilled water. 40 mL of filtered *F. benghalensis* extract is mixed with 60 mL of metal solution and was made into 100 mL solution.

2.2 Synthesis of Nanoparticles

The solution was kept in a magnetic stirrer for nanoparticle synthesis. The colour change was observed visually and photographs were recorded in particular intervals. The solution was centrifuged using a lark refrigerated centrifuge. The solution was centrifuged at 8000 rpm for 10 minutes and the pellet was collected and washed with distilled water twice. The final purified pellet

was collected and dried at 60°C for 24 hours. The final product was stored in an airtight eppendorf tube.

2.3 Conformation of AgNPs

The AgNPs synthesis was confirmed by using UV-vis-spectroscopy. 3mL of the solution was taken in a cuvette and scanned in a double beam UV-vis-spectrophotometer from 300 nm to 700 nm wavelength. The results were recorded for confirmation of AgNPs synthesis.

2.4 Preparation of Mouthwash

The mouthwash was prepared using silver nanoparticles, ethanol, distilled water, sucrose, sodium benzoate, clove oil, Sodium dodecyl sulphate. Silver nanoparticles are the main constituent and ethanol acts as a solvent to solubilize the ingredients. Sodium benzoate acts as a preservative and clove oil acts as a flavoring agent.

2.5 Antimicrobial Activity of *F. benghalensis* Mediated AgNPs Mouthwash against Oral Pathogens

The agar well diffusion method was used to determine the antimicrobial activity of the mouthwash. The 25 µl, 50 µl, and 100 µl concentration prepared by mouthwash and they were added to the wells and was tested against *Staphylococcus aureus*, *Streptococcus mutans* (gram positive), *Enterococcus faecalis* and *Candida albicans*. The fresh bacterial suspension was dispersed on the surface of Muller Hinton agar plates. Different concentrations of nanoparticles (25 µl, 50 µl, and 100 µl) were incorporated into the wells and the plates were incubated at 37°C for 24 hours. The antibiotics were used as positive control and a zone of inhibition was recorded in each plate.

3. RESULTS AND DISCUSSION

3.1 Visual Observation

It is known that Ag NPs exhibit dark brown colour depending on the intensity and size of nanoparticles. This colour arises due to the excitation of the surface plasmon resonance (SPR) of the silver nanoparticles. When *F. benghalensis* was added to the Silver nitrate solution, the colour of the solution transformed from colorless to dark brown. This colour change

has been shown in Fig. 2. This colour change indicates the formation of AgNPs which is further confirmed by UV - vis spectrophotometry.



Fig. 1. *F. benghalensis* plant extract



Fig. 2. Colour change exhibited by the prepared *F. benghalensis* assisted silver nanoparticles

3.2 UV-Vis Spectroscopy

UV-vis spectroscopy is a significant technique to show the formation and stability of Ag NPs in an aqueous solution. The UV-vis spectra were recorded for the prepared *F. benghalensis* silver nitrate solution (Fig. 3). It has been observed from the spectra that the AgNPs SPR peak occurs at 450 nm with high absorbance, which is specific for silver nanoparticles. The peak represents the purity of silver nanoparticles. This confirms the formation of *F. benghalensis* mediated silver nanoparticles.

3.3 Antimicrobial Activity of *F. benghalensis* Mediated AgNPs Mouthwash

The prepared *F. benghalensis* mediated AgNPs mouthwash were studied for antimicrobial activity against the following oral pathogens: *S. aureus*,

S. mutans, *E. faecalis* and *C. albicans*. The diameter of the zone of inhibition (ZOI) in millimeters around each well in different concentrations levels of *F. benghalensis* mediated AgNPs against the bacterial species were determined (Fig. 4). The results have been tabulated (Table 1) and Graph has been plotted against the antimicrobial activity of *F. benghalensis* mediated AgNPs mouthwash and antimicrobial drug (Fig. 5).

The nanoparticles obtained in the present study are prominent and possess potential antagonism against the oral pathogens. It has been observed that the mean zone of inhibition against bacterial pathogens was found to increase as the concentration of mouthwash increased, however the maximum was found for the standard antibiotic.

The zone of inhibition of the prepared AgNPs mouthwash against *Candida albicans* at 25 μ l, 50 μ l and 100 μ l was found to be 9 mm, 14 mm and 21 mm respectively indicating excellent antifungal activity against *C. albicans* at 100 μ l concentration which was better than the standard which exhibited zone of inhibition of only 12 mm. Zone of inhibition for *S. aureus* was found to be 9 mm, 14 mm and 20 mm. For *S. mutans*, the zones of inhibition were 10 mm, 11 mm and 17 mm respectively. For *E. faecalis*, it was 9 mm, 12 mm, 21 mm (Table 1). The antimicrobial activity of the synthesized AgNPs is due to the silver cations released from them, pertaining to the changes in the membrane structure of microbes, which lead to the increased membrane permeability of the bacteria and finally cell death [39].

Here in this report the mouthwash was prepared using silver nanoparticles. The chlorhexidine gluconate has a bitter taste, unpleasant and it makes microorganisms resistant to drugs by repeated use. The mouthwash prepared using nanoparticles was tested against oral pathogens. It showed a good inhibition zone. So it indicates that the herbal based nano mouthwash was better, safer, less costly and eco friendly compared to chemically prepared mouthwash.

Silver nanoparticles, due to their size, surface area and low toxicity have a significant role in nanotechnology [40,41]. The important factors in regard to the antimicrobial activity of silver are stability, adhesion and good dispersion of silver particles in organic matrix [42,43]. Silver in the form of nanoparticles has found acceptance in the field of nanotechnology, as their

efficacy is primarily related to the fact that they reduce bacterial resistance. The current study was conducted to assess the antimicrobial activity of mouthwash incorporated with AgNPs against oral pathogens and showed good potential as an antimicrobial agent that maintains control of biofilm, preventing initial colonization of bacteria. They had potential antimicrobial activity

of the synthesized silver nano-particles in mouthwash against gram positive bacteria, similar to various other studies [44–46] and in this current study has certain limitations, it was not tested against gram negative bacteria. Hence, further research should be conducted to gather evidence regarding [47] the potential of AgNPs and its antimicrobial activities.

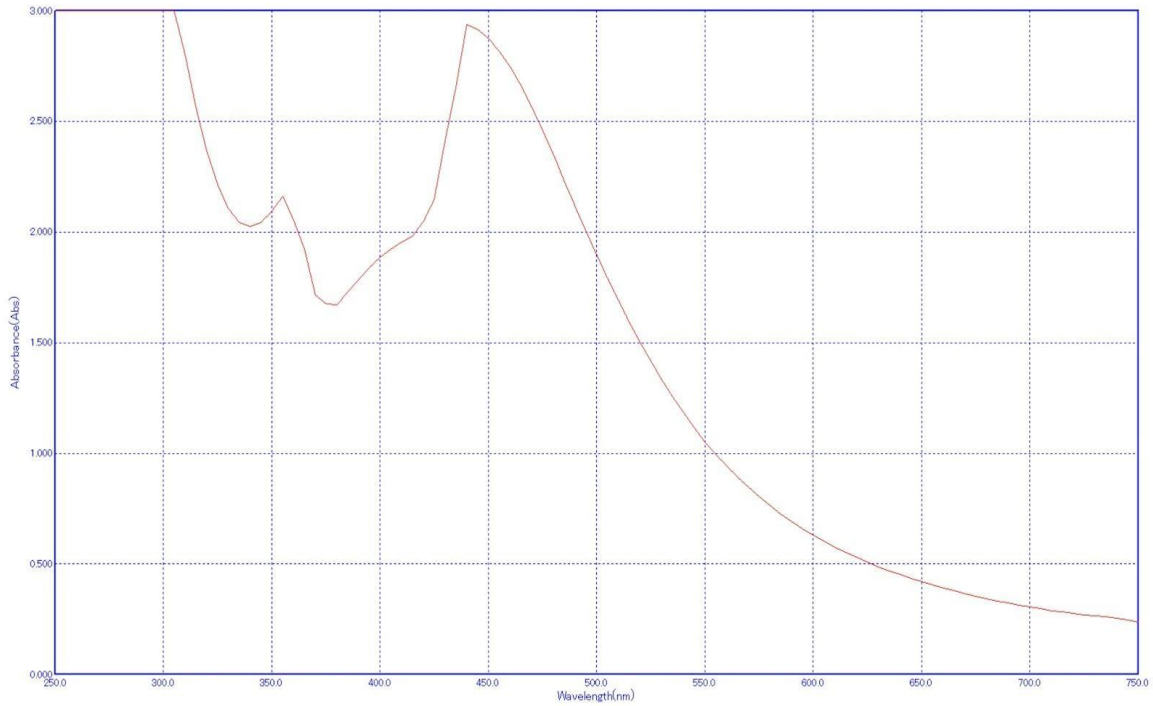


Fig. 3. Spectroscopic analyses of *F. benghalensis* mediated AgNPs mouthwash recorded as function of time

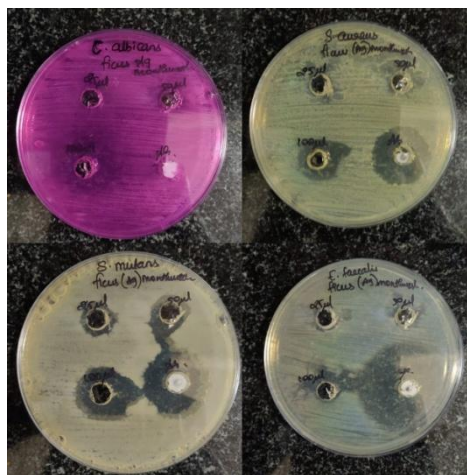


Fig. 4. Antimicrobial activity of *F. benghalensis* mediated AgNPs mouthwash against oral pathogens, *S. mutans*, *C. albicans*, *E. faecalis*, *S. aureus*

Table 1. Zone of inhibition of *F. benghalensis* mediated AgNPs mouthwash against oral pathogens, *S. mutans*, *C. albicans*, *E. faecalis*, *S. aureus* at different concentrations compared to standard antimicrobial agent

Oral pathogens	25 µl	50 µl	100 µl	AB
<i>S. mutans</i>	9 mm	12 mm	21 mm	29 mm
<i>E. faecalis</i>	12 mm	14 mm	17 mm	33 mm
<i>S. aureus</i>	9 mm	14 mm	20 mm	27 mm
<i>C. albicans</i>	9 mm	14 mm	21 mm	12 mm

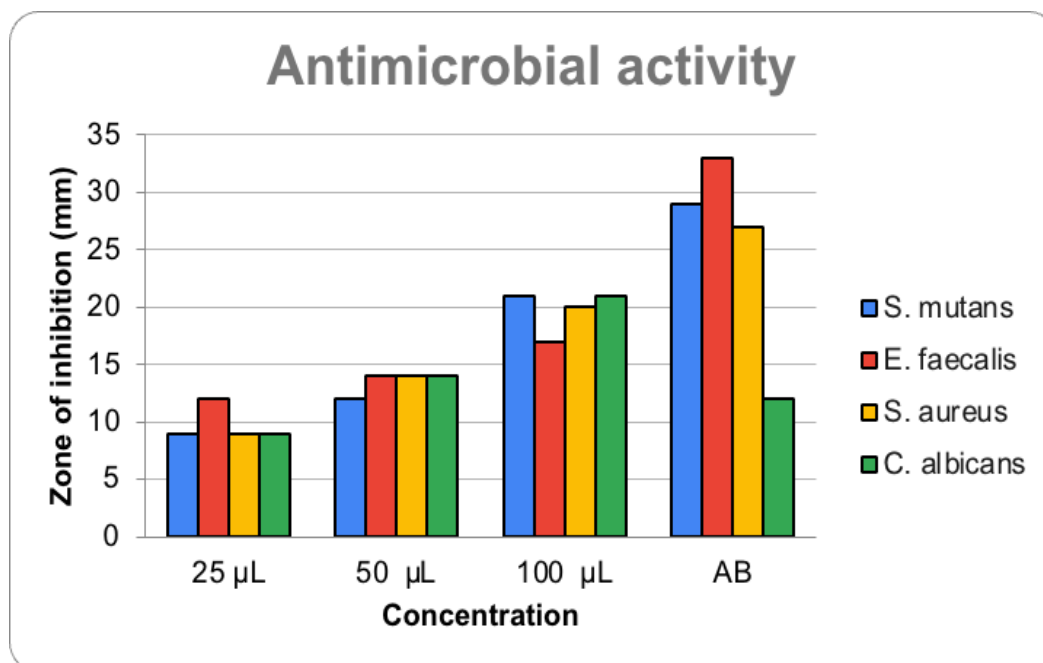


Fig. 5. Antimicrobial activity of *F. benghalensis* mediated AgNPs mouthwash compared to standard antimicrobial drug (AB)

4. CONCLUSION

From this study we conclude that the formation of AgNPs was observed by the color change from colourless to dark brown and it was confirmed by UV-Vis spectroscopy. The synthesized *F. benghalensis* assisted silver nanoparticle mouthwash showed positive antimicrobial activity against the oral microbes and can be used to prevent the formation of microbial biofilm.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by

the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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