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Controlling Oral Pathogens using Ficus Benghalensis Mediated Silver Nanoparticles

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Authors' contributions

This work was carried out in collaboration among all authors. Author KT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft manuscript. Author MJ and Author 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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Original Research Article

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ABSTRACT

Aim: To find antimicrobial property of *Ficus benghalensis* mediated silver nanoparticles (AgNPs)
Introduction: Nanoparticles have been studied in recent years because of certain properties physical, chemical, electronic, thermal, magnetic, optical, dielectric and biological. This study conducted in order to evaluate antimicrobial properties of *F. benghalensis* media AgNPs.
Materials and Methods: In this study, extract of *F. benghalensis* was used to synthesize si nanoparticles characterized using UV- visible spectrophotometer, Fresh *F. benghalensis* media AgNPs show excellent antimicrobial activity against oral pathogens, *Staphylococcus aure Streptococcus mutans, Enterococcus faecalis. and Candida albicans.*
Results: *F. benghalensis* mediated AgNPs showed antimicrobial activity against gram positive *aureus* with a zone of inhibition of 16.22 ± 0.31 mm at the concentration of 100 µl. The zone of inhibi

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against *S. mutans* was 12.14 ± 0.21 mm followed by zone of inhibition against *E. faecalis* was 12.1
0.2 mm and antimicrobial activity against *C. albicans* showed a zone of inhibition of 14.02 ± 0.24 mr
the concentration of 100 µl. The zone of inhibition of the nanoparticles was shown to be increased v
increase in concentration against all the pathogens and the maximum inhibition was shown agains
aureus.

Conclusion: Overall, antimicrobial activity was seen to be increased with increase in concentrat
Thus there was potent antimicrobial activity in *F. benghalensis* mediated AgNPs which could
beneficial when applied in treatment of infectious oral diseases in future.

Keywords: Antimicrobial activity; Ficus benghalensis; innovative technology; nanoparticles; oral pathogens.

1. INTRODUCTION

Nanoparticles size range from 10- 1000 nm so they are known as particulate dispersion or solid particles. The drugs are now dissolved, trapped and encapsulated or in other words bound to a matrix of nanoparticles. The medicinal plant from centuries used as an alternative remedy for treating human disease. This is due to various active constituents of therapeutic value. Microbial resistance development against antibiotics made the research to investigate various alternative sources to treat the resistance strain. 80% of the population of the world realised that medicine derived from the plant was severe as a first line of defense in maintenance of health and combating many diseases [1]. *F. benghalensis* is commonly known as Banyan tree or vats or vada tree in Ayurveda. 6Silver has been used for centuries as an antimicrobial. In order to fight infections, disease and stop the spoilage, it is known that silver-based ion compounds are more toxic to both Gram-negative and Gram-positive microorganisms [2]. The Barks, stems, leaves, flowers and fruits of plants, various animal tissues from which natural antimicrobials are derived. Optimum levels of total phenolic and flavonoid compounds in *F. benghalensis* aerial root are found to be present in 70 mg of extract [3]. Several metal nanoparticles, for example magnesium, gold, Iron, copper, silver, zinc have evolved. silver nanoparticles (AgNPs) have been established to be simplest because of the need sensible antimicrobial activity against various microorganisms which is in the form of nanoparticles (NPs) this can be used as more effective bactericidal materials because of their enhanced reactivity, that result from their high surface/volume ratio [4]. Particularly, AgNPs are known to show strong biocidal effects on various bacterial species that include even multidrug resistant bacteria. Silver nanoparticles has various biomedical applications such as antimicrobial agent, drug delivery agent, biodetection and labelling, antidiabetic, anticancer therapy [5]. Plant based synthesis of AgNPs found to be very simple, rapid, dependable, eco-friendly and non-toxic. The synthesis of metal nanoparticles using plant extracts produces an advantage over other types of biological synthesis methods which have difficulties maintaining microbial cultures. After the synthesis of AgNPs, characterization of the AgNPs is important for investigating their characteristic features like size, shape, surface area, morphology, solubility and aggregation etc [6]. The physical and chemical properties of nanoparticles may need a considerable influence on their biological properties [7]. Characterization of AgNPs is important before evaluating their toxicity [8]. Different analytical techniques had been employed for the characterization of the nanoparticles, like Transmission electron microscope (TEM), Scanning electron microscopy (SEM), Ultraviolet visible spectroscopy (UV-vis), X-ray diffractometry (XRD), Atomic force microscopy (AFM), Energy Dispersive Analysis (EDAX) and Fourier transform infrared spectroscopy (FT-IR) etc [9]. The antibacterial effects of AgNPs against bacterial cells are always complicated [10]. The direct morphological analysis by TEM or SEM gives structural modification of the bacterial cell [11]. This would give us useful information and good understanding on the bactericidal activity of AgNPs against bacterial cells. And the proper antibacterial mechanism of the AgNPs is still in a mysterious situation. Therefore, the antibacterial activities and its mechanisms of AgNPs against several bacteria were reported in the past. The present study is based on various plant based methods for AgNPs synthesis, characterizations, and predicted antibacterial activity against various bacteria. Before we have done various studies on biological synthesized different nanoparticles [12–24],[25–29] [30] [31]. The present study is to

evaluate the antimicrobial activity of *F. benghalensis* mediated AgNPs against oral pathogens.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract

1 mg of *F. benghalensis* was collected from the local field and was dried in the shade region. After getting dried they were crushed & powdered and mixed with 100 mL of distilled water in a conical flask. The solution was labelled and boiled in 60-70 degree Celsius in the heating mantle for 10-15 minutes. The heated solution was taken out where there was an appearance of small bubbles. After the heating process, the solution was filtered using Whatman no.1 filter paper (Fig. 1).

Fig. 1. Synthesis of AgNPs from F. benghalensis extract

2.2 Synthesis of Nanoparticles

20 milli molar (0.574g) of Ag was dissolved in 100 mL of distilled water. 40 mL of filtered *F. benghalensis* extract was mixed with 60 mL of Silver nitrate. The flask containing the mixture was incubated in an orbital shaker and observed for colour change at various periods of incubation time. The synthesized nanoparticles were optically measured using a double beam UV-vis spectrometer. AgNPs synthesized from *F. benghalensis* was tested for antimicrobial activity by agar well diffusion method against *S.aureus, S.mutans, E.faecalis, C. albicans.* 1 millimolar of silver mixed with double distilled water. The plant extract of *F. benghalensis* was added with the metal solution and was made into a 100 mL solution. The colour change has been observed visually and photographs were recorded (Fig. 2). The solution is kept in a magnetic stirrer/orbital shaker for nanoparticle synthesis.

2.3 Antimicrobial Activity

The agar well diffusion method was used to determine the antimicrobial activity of silver nanoparticles. Different concentrations of silver were tested against *Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis. and Candida albicans.* The freshly prepared bacterial suspension was made to disperse on the Muller Hinton agar plates. Different concentrations of nanoparticles like 25 µl, 50 µl, 100 µl and standard (Ab) were incorporated into each of the well and plates were incubated at 37°C for 24 h. The Zone of inhibition for different concentrations of *F. benghalensis* mediated AgNPs were measured.

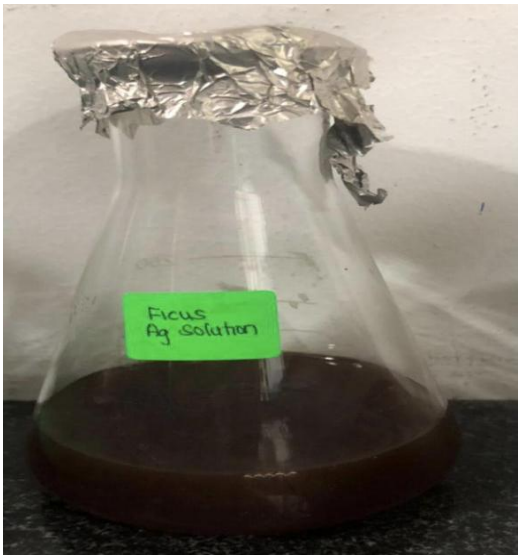

Fig. 2. Synthesis of AgNps visually identified by color change

3. RESULTS AND DISCUSSION

As observed visually at various periods of incubation, the colour of the solution transformed from colorless to dark brown which indicated formation of AgNPs (Fig. 2). UV- vis spectrophotometer revealed surface plasmon resonance peak positioned at 450 nm (Fig. 3). The antimicrobial activity of *F. benghalensis* mediated AgNPs was assayed by well diffusion method (Fig. 4). Table 1 shows the inhibition of bacterial growth in various concentrations of *F. benghalensis* mediated AgNPs against *S. aureus, S. mutans, E. faecalis, C. albicans.* As the concentration increased gradually the

antimicrobial activity of *F. benghalensis* assisted AgNPs also increased (Fig. 5). The obtained results were comparable with that of the standard antimicrobial agents. The *F. benghalensis* mediated AgNPs showed antimicrobial activity against gram positive *S. aureus* with maximum zone of inhibition of 16.22 ± 0.31 mm at the concentration of 100 µl. The zone of inhibition against *S. mutans* was 12.14 ± 0.21 mm followed by zone of inhibition against *E. faecalis* was 12.12 ± 0.2 mm and antimicrobial activity against *C. albicans* showed a zone of inhibition of 14.02 ± 0.24 mm at the concentration of 100 µl. The zone of inhibition of the nanoparticles was shown to be increased with increase in concentration against all the pathogens and the maximum inhibition was shown against *S. aureus.*

The results obtained were comparable to similar biosynthesized nanoparticles. From the dried ginger copper nanoparticles were synthesised and these were characterised by UV-vis spectroscopy and exhibited potent antimicrobial activity against common oral pathogens [32]. In their study, the approach on green synthesis of iron nanoparticles by using dried ginger can provide pharmacological evidence of antioxidant activity [33]. Previous studies have shown that adding metal nanoparticles to various materials will improve the antimicrobial activity [11,34]. Initially the iron nanoparticles were identified by stable dark brown colour and the surface plasmon resonance was at the peak positioned at 370 nm. Their study supported that dried ginger Zingiber are important sources for potent biologic activities and thus these plant- based nanoparticles may be essential in the treatment of various pathologic conditions [35,36]. The morphology of copper nanoparticles synthesized from the extract of *Eclipta prostrata* leaves as analysed by HRTEM shows spherical and agglomerated particles ranging from 28 to 45 nm [37]. Silver nanoparticles has shown to have higher antifungal activity against *C. albican*s and *Candida tropicalis* which will represent an alternative for fungal infection treatment [38]. Further research on antimicrobial studies in vivo assigns possible applications in the dental field which will be efficient to treat oral infectious diseases. With further studies, the *F. benghalensis* mediated AgNPs can be clinically applied for its antimicrobial, anti-inflammatory, antioxidant effects, wound healing and bone regeneration.

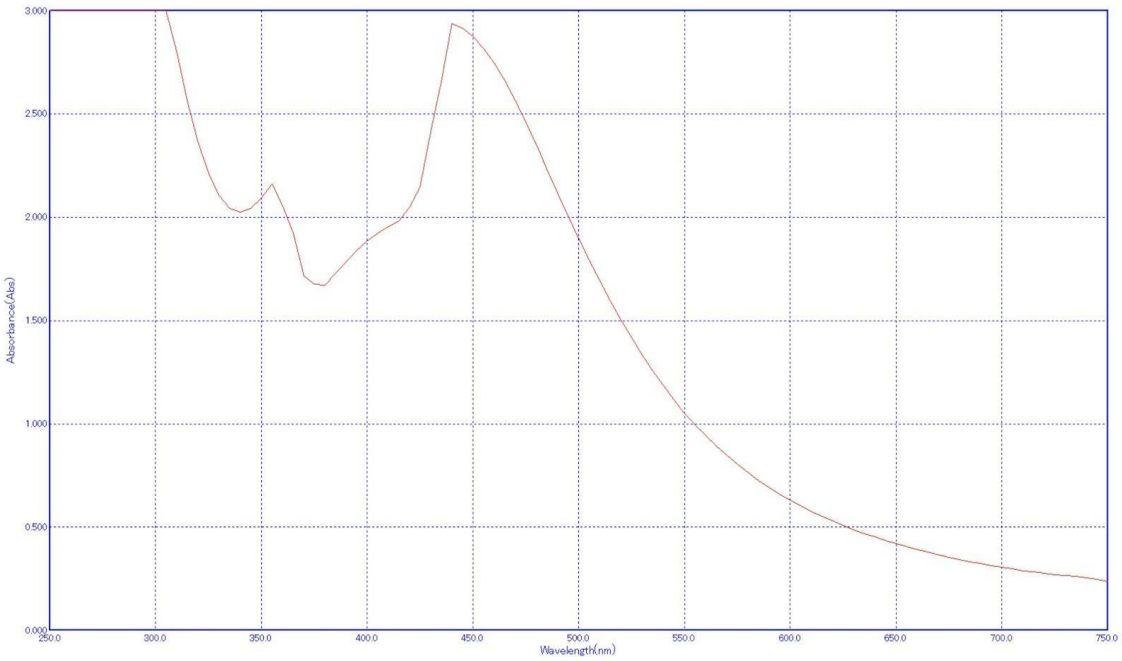

Fig. 3. Spectroscopic analyses of F. benghalensis mediated AgNPs mouthwash recorded as function of time

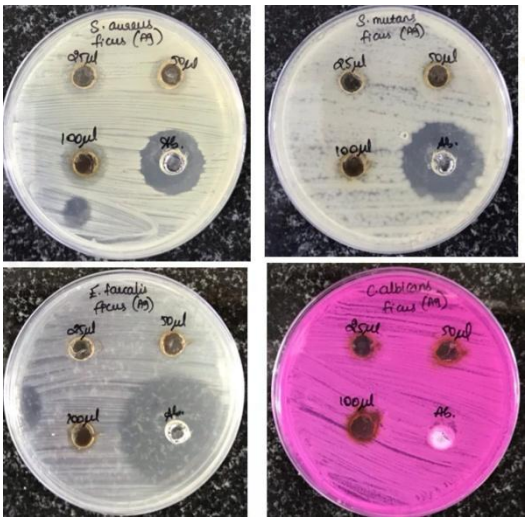

Fig. 4. Antimicrobial activity of F. benghalensis mediated AgNPs against oral pathogens

Table 1. Antimicrobial activity of AgNPs against oral pathogens

	Concentrations of Nanoparticles			
Pathogens	**25 µl**	**50 µl**	**100 µl**	**Ab**
S. aureus	13.02 ± 0.34	15.41 ± 0.33	16.22 ± 0.31	21.02 ± 0.23
S. mutans	9.24 ± 0.12	9.62 ± 0.24	12.14 ± 0.21	27.14 ± 0.31
E.faecalis	9.42 ± 0.22	9.02 ± 0.22	12.12 ± 0.21	14.02 ± 0.24
C.albicans	9.06 ± 0.14	9.04 ± 0.42	14.02 ± 0.24	12.04 ± 0.21

The above figure shows that AgNPs synthesized from *F. benghalensis* was tested for antimicrobial activity by agar well diffusion method against *S. aureus, S. mutans , E. faecalis, C. albicans.*

Table 1 shows the zone of Inhibition of AgNps against various oral pathogens.

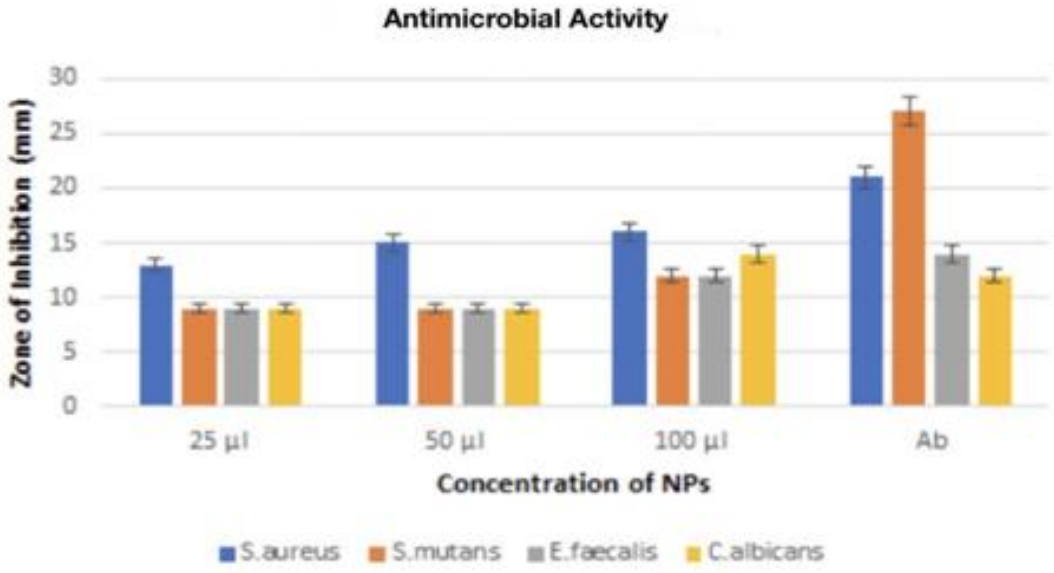

Fig. 5. Analysis of Antimicrobial activity of F. benghalensis mediated AgNPs against oral pathogens

4. CONCLUSION

The present study reported that silver nanoparticles can be synthesized in a simple and easy method using *F. benghalensis* extract. *F. benghalensis* mediated AgNPs showed excellent antimicrobial activity against oral pathogens. This study showed an increase in the zone of inhibition was seen while increasing the dosage*. The F. benghalensis* mediated AgNPs are potential candidates in biomedical applications with various benefits such cost-effectiveness, less side effects and large scale commercial production.

DISCLAIMER

The products used for this research are commonly and predominantly used 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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