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Green Synthesis and Antioxidant Activity of Silver Nanoparticles Synthesized Using *Ficus benghalensis*

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Authors' contributions

This work was carried out in collaboration among all authors. Author AKS 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

Background: Nanoparticles are materials with overall dimensions in the nanoscale, ie, under 100 nm. Silver nanoparticles (AgNPs) have shown excellent bactericidal properties against a wide range of microorganisms. *Ficus benghalensis* is a very large tree and had been considered as effective, economical and safe treatments for curing various diseases.
Aim: The present study is aimed to evaluate the antioxidant activity of AgNPs synthesized using *F. benghalensis.*
Materials and Methods: Bio-mediated synthesis of metal oxide nanoparticles using *F. benghalensis* is a promising alternative to traditional chemical synthesis. The antioxidant activity of

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AgNPs was synthesized using DPPH radical scavenging assay. 1 gm of *F. benghalensis* mixed with 100 mL of distilled water and boiled in 60-70 degree celsius in the heating mantle for 10-15 minutes. Add filtered using Whatman no. 1 filter paper. 20 milli molar (0.574g) of silver was dissolved in 60mL of distilled water. 40 mL of filtered *F. benghalensis* extract is mixed with 60 mL of silver nanoparticles.

Results: The activity of *F. benghalensis* extract was compared with standard ascorbic acid by measuring absorption intensity in the spectrophotometer at the wavelength of 517 nm. While increasing the concentration (10 µL, 20 µL, 30 µL, 40 µL, 50 µL) of *F. benghalensis* extracts, the percentage of inhibition of DPPH also increased.

Conclusion: In this study, a simple, biological and low-cost approach was done for the preparation of silver nanoparticles using *F. benghalensis*. Thus the synthesized *F. benghalensis* mediated silver nanoparticles can be subjected to the various other biological activities such as antioxidant, antibacterial, antifungal and cytotoxic evaluation to know the efficiency of these nanoparticles.

Keywords: Antioxidant; Ficus benghalensis; green synthesis; innovative technology; silver nanoparticles.

1. INTRODUCTION

Nanoparticles show novel properties which depend on their size, shape and morphology which enable them to interact with plants, animals and microbes [1]. Silver nanoparticles (AgNPs) have been proven to have bactericidal properties against a wide range of bacteria [2]. They are prepared from different perspectives, often to study their morphology or physical characteristics [3]. Biosynthesis of AgNPs involves bacteria, fungi, yeast, and plant extracts [4,5]. Recently, a number of parts of plants such as flowers, leaves and fruits, besides enzymes, have been used for the synthesis of gold and silver nanoparticles [6]. The size, morphology and stability of nanoparticles depend on the method of preparation, nature of solvent, concentration, strength of reducing agent and temperature [7]. Antioxidant compounds in food play an important role as a health protecting factor [8]. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease [9]. The potentially important derivatives of oxygen, endorsed as ROS (Reactive Oxygen Species) such as O_2, H_2O_2 and OH radicals are generated within the human body, if there is ROS overproduction the equilibrium is hindered favoring the ROS gain that leads to oxidative stress [10]. The ROS readily attack and induce oxidative damage to various biomolecules like proteins, lipids, lipoproteins and DNA [11,12]. This oxidative damage can eventually lead to diabetes mellitus, cancer, atherosclerosis, arthritis, and neurodegenerative diseases. Recently additional interest has been shown in the field of free radical biology, to avoid the causes of chronic diseases [13]. Epidemiological studies have shown that the intake of antioxidants such as Vitamin- C (ascorbic acid) can reduce the risk of coronary heart disease and cancer [14]. The use of synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, tert–butylhydroquinone and propyl gallate has been negatively perceived by consumers because of its safety and health effects [15]. Hence, there is an increased interest and demand in search of natural antioxidants from plant sources. It is well known that many botanical products possess natural antioxidants with high antioxidant activity and investigations on these were initiated based on their uses in traditional herbal medicines [16]. The genus *Ficus* includes 750 species in most tropical and subtropical forests throughout the world. The genus is remarkable for its large variation in the habits of its species. *Ficus benghalensis* is a very large tree distributed throughout India [17]. It is commonly known as 'Indian Banyan tree' and is considered the holy tree of India. Recent studies reveal that the herbal preparations of different parts of *F. benghalensis* had been considered as most effective and safe treatments for curing various types of diseases in the Indian traditional system and used as medicine. The hanging roots of *F. benghalensis* have been reported as antidiarrheal agents [18]. The fruit extract of *F. benghalensis* has been documented for its antitumor and antibacterial activities [19].The plant is used in folk medicine for respiratory disorders and certain skin diseases [20]. The bark of *F. benghalensis* has been traditionally used to cure diabetes mellitus and is also used as oral administration of bark extract showed lowering of blood glucose level and enhancement of serum insulin levels in normoglycemic as well as diabetic rats. Blood

sugar lowering and serum insulin raising action was also found in a dimethoxy derivative of leucocyanidin 3-O-beta-galactosyl cellobioside and a dimethoxy ether of leucopelargonidin-3-O-alpha-L-rhamnoside isolated from the bark of *F. benghalensis* [21]. Bengalenoside, a glucoside isolated from *F. benghalensis*, also showed hypoglycemic activity in normal and alloxan diabetic rabbits. The antioxidant effect of aqueous extract of the bark of *F. benghalensis* has been evaluated in hypercholesterolemic rabbits [22]. Our team has extensive knowledge and research experience that has translated into high-quality publications [12,23–34,35-41]. The aim of the present study is to evaluate the antioxidant activity of silver nanoparticles synthesized using *F. benghalensis.*

2. MATERIALS AND METHODS

2.1 Preparation of the Extract

A sample of 1 gm of *F. benghalensis* was mixed with 100 mL of distilled water and boiled in 60-70 degrees Celsius in the heating mantle for 10-15 minutes. Add filtered using Whatman number 1 filter paper. 20 millimolar (0.574 g) of silver was dissolved in 60 mL of distilled water. 40 mL of filtered *F. benghalensis* extract was mixed with 60 mL of silver nanoparticles.

2.2 Synthesis of Silver Nanoparticles (AgNPs)

Synthesis of AgNPs is done by a green synthesis method, in which the 1 mL of aqueous filtrate of the prepared extract was taken into 100 mL of Erlenmeyer flask. Then the extract was mixed into silver nitrate (AgNO3) to make the final volume concentration of 1 mm solution. The reaction mixture was exposed to sunlight irradiation conditions until the colour change arose as shown in Fig. 1. The reaction mixture of seed extracts and AgNPs was subjected to centrifugation at 8,000×g for 15 min; the resulting pellet was washed three times with deionized water and filtered.

2.3 UV Spectrometric Analysis of Synthesized Nanoparticles

The endpoint of the reaction was evaluated by UV–vis spectroscopy. The biologically reduced brown colour solution mixture was scanned by Shimazdu, Lambda UV mini-1240 instrument operated at a resolution of 1 nm. The UV–visible analysis was performed in the absorption wavelength of 200–700 nm periodically for 1 hr in order to observe rapid reduction AgNPs by the action of faeces Bengalis extracts. The distilled water was used as a blank.

2.4 Antioxidant Activity

Antioxidant activity is the ability of bioactive constituents to prevent, inhibit, and protect against oxidation of numerous substrates such as DNA and lipid components in living organisms and food products. Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method was followed for evaluating the antioxidant activity of synthesized nanoparticles. DPPH is a stable free radical in methanol that accepts an electron or hydrogen radical and transforms into a stable diamagnetic molecule. It Is commonly employed as a test subject for antioxidative activities. Five distinct extract concentrations were combined with various DPPH concentrations. By comparing the absorbance to a blank containing only DPPH and solvent, the radical scavenging activity was measured. % inhibition was calculated using the below equation:

%inhibition= Absorbance of control – Absorbance of sample X 100 / Absorbance of control.

3. RESULTS AND DISCUSSION

The synthesis of biological nanoparticles represents an alternative for the physical and chemical methods of nanoparticle formation. The majority of researchers focused on the green synthesis of nanoparticles for the formation of metal and oxide nanoparticles. The use of plants for the synthesis of nanoparticles is a rapid, low-cost, eco-friendly option and is safe for human use.

3.1 Visual Identification

In this study, the silver nanoparticles were synthesized using *F. benghalensis* extract. At various intervals of incubation time, colour changes in the reaction mixture were noticed. The silver ions are reduced to silver nanoparticles, which are detected by a change in colour (Fig. 2). The colour changed from yellow to reddish brown, indicating that silver nanoparticles had synthesized. With increasing incubation time, the brown colour intensity increased. After 24 hours, a dark brown colour appeared, followed by no colour change, indicating that the synthesis of silver

nanoparticles was complete. With respect to this current study, studies with similar results were also found. The development of intense yellowish-brown colour owing to the surface plasmon resonance confirmed the synthesis of the silver nanoparticles [42]. After 80 mins there was a significant increase in the brown colour which confirms the growth of the silver nanoparticles [43]. Colour changes of the reaction mixture 240 min after the bioreduction process, which was recorded by UV-vis spectrophotometer at 400-700 nm. In particular, absorbance in the range of 500-550 nm has been used as an indicator to confirm the reduction of Ag+ to metallic Ag [44]. In a previous study, silver nanoparticles synthesized using tulsi leaf extract, the colour change was observed from yellow to black-brown [45].

3.2 UV-Vis Absorption Spectrophotometer Activity

The UV-vis spectroscopy showed the excitation of the surface plasmon resonance band peak positioned at the wavelength from 250 nm to 650 nm and was observed at various time intervals. The spectrum clearly demonstrates that the absorbance steadily increases as the incubation time is increased. The UV- vis spectroscopic analysis of silver nanoparticles synthesized from the *F. benghalensis* showed absorbance peak at 450 nm (Fig. 3). Similarly, a study showed that UV absorption spectra of the silver nanoparticles from methanolic stem extract of *Gymnema sylvestre* was reported to be 443 nm [46].

3.3 Antioxidant Activity of Silver Nanoparticles using *F. benghalensis*

Antioxidants are an integral part of plants as secondary metabolites which play an important role as free radical scavengers and by converting highly reactive free radicals into less reactive species [47]. Different studies verify the significant role of antioxidants in the reduction of oxidative stress, which provoke us to determine the antioxidant potential of various extracts of *F. benghalensis* [47,48]. The antioxidant capability of samples cannot be assessed by a single assay [48].

Therefore, DPPH free radical scavenging, total reduction power and TAC assays were performed to verify the antioxidant potential. DPPH is the stable free radical and antioxidant potential of crude extract was determined on the basis of scavenging of free radical i.e. DPPH. The principle of the assay is based on the conversion of the purple color of the free radical to the yellow color molecule by accepting a hydrogen electron from donor antioxidants present in samples. In the current study, high percentage free radical scavenging activity was shown by *F. benghalensis*. The activity of *F. benghalensis* extract was compared with standard ascorbic acid by measuring absorption intensity in the spectrophotometer at the wavelength of 517 nm. While increasing the concentration (10 µL, 20 µL, 30 µL, 40 µL, 50 µL) of *F. benghalensis* extracts, the percentage of inhibition of DPPH also increased. However, the inhibition percentage is directly proportional to the concentration of extract as shown in Fig. 4. Previous research works have reported on the various activities exhibited by the nanoparticles synthesized from natural sources such as antibacterial, antioxidant and cytotoxic activity [49-51]. In a study, silver nanoparticles were synthesized from aqueous leaf extract of *Cestrum nocturnum* and its antioxidant and antibacterial activities were tested against bacteria and the results confirmed that the silver nanoparticles have more antioxidant activity as compared to vitamin C [52]. In other study, Antioxidant and antibacterial activity of silver nanoparticles is due to the presence of bioactive molecules on the surface [53].

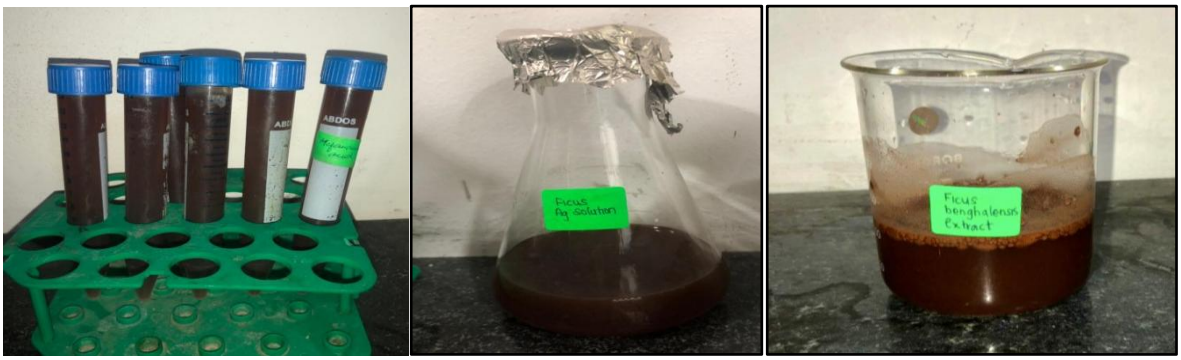

Fig. 1. Synthesis process of silver nanoparticles

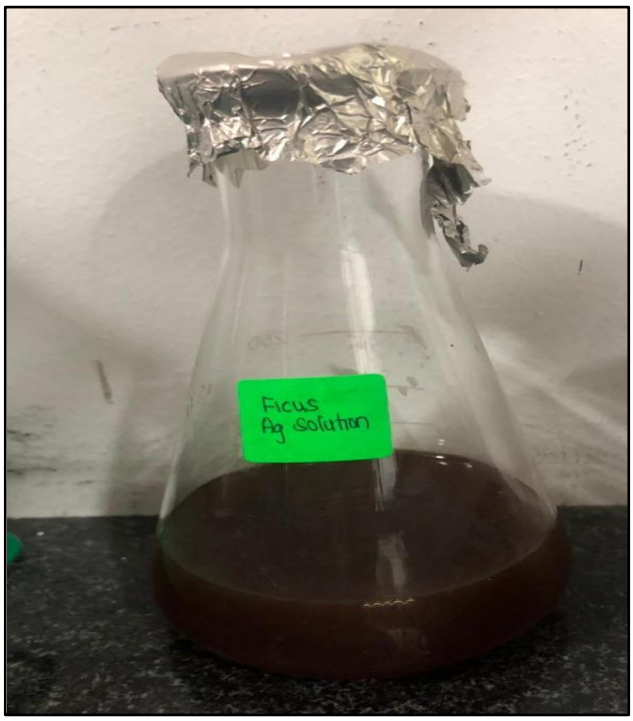

Fig. 2. Reduction of silver ions to silver nanoparticles visually identified by colour changed at various periods of incubation time

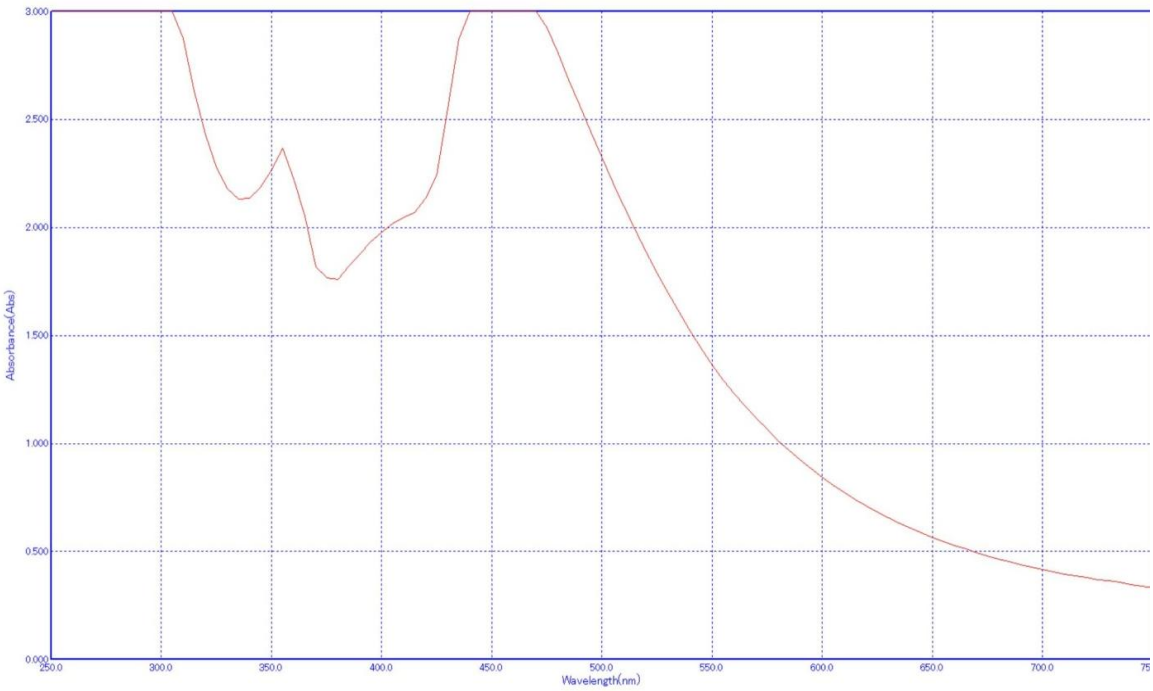

Fig. 3. UV- Vis Spectroscopic analyses of silver nanoparticles synthesized from *F. benghalensis* recorded as function of time

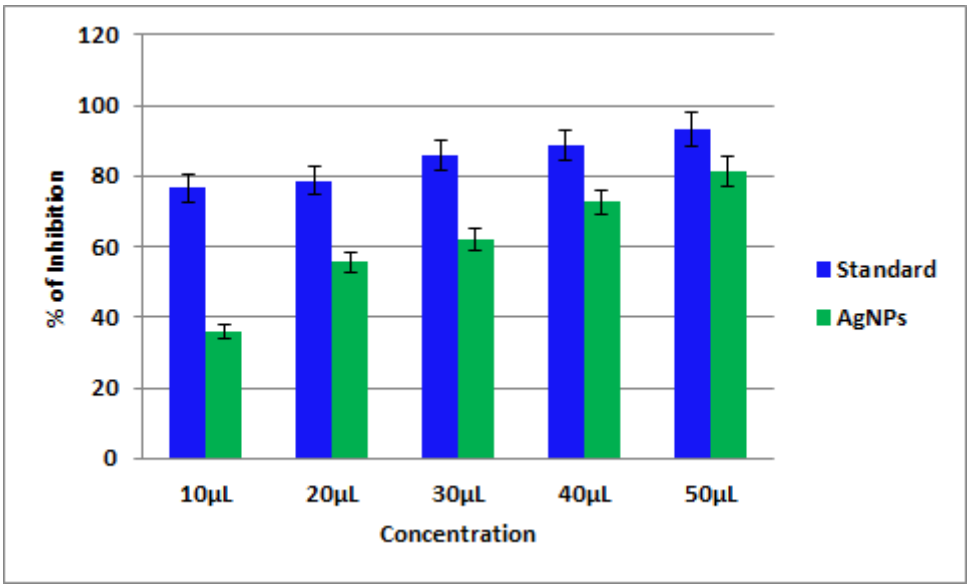

Fig. 4. *F. benghalensis* showed increased DPPH inhibition with increase in concentration of extract. Inhibition was measured after 48 hours of incubation

X- axis shows concentrations in microlitre, Y- axis shows percentage of inhibition

4. CONCLUSION

In this study, a simple, biological and low-cost approach was done for the preparation of silver nanoparticles using *F. benghalensis*. Thus the synthesized *F. benghalensis* mediated silver nanoparticles which was shown to have potent antioxidant property can be subjected to the various other biological activities such as antibacterial, antifungal and cytotoxic evaluation to know the efficiency of these nanoparticles. So that they can be developed as a substitute for conventional formulations in the treatment of oral diseases and help in the betterment of life.

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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