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Research Article

Green Synthesis of Silver Nanoparticles by Using *Simarouba amara* Aubl. Fruit Extract and their Antioxidant and Antibacterial Activities

Guru Kumar Dugganaboyana*, Chaitra Kyasandra Eranna

Postgraduate Department of Biochemistry, JSS College of Arts, Commerce and Science (Autonomous), B.N. Road, Mysuru-570025, Karnataka, India.

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ABSTRACT

The green synthesis of nanoparticles has emerged as a cost-effective and environmentally benign technique for therapeutic applications. Nanomedicine utilizes biocompatible nanomaterials for diagnostic and therapeutic potential for various biomedical applications. Different biological methods are gaining recognition over the physical and chemical methods of synthesis for the production of silver nanoparticles (AgNPs) due to their multiple applications. The present study describes the synthesis of AgNPs using the fruit extract of *Simarouba amara* (*S. amara*)followed by characterization of AgNPs was done using different methods, which include; ultraviolet-visible spectroscopy (UV-Vis) wherein it shows absorption peak at 410 nm confirming the AgNPs, from dynamic light scattering (DLS) the average particle size is 80nm with crystalline structure confirmed by scanning electron microscope (SEM) images and zeta potential analysis shows the positive polarity of the particle favoring the drug targeting. The powder X-ray diffraction study (PXRD) revealed crystalline nature with a face-centered cubic (fcc) structure of AgNPs. The synthesized AgNPs were also tested for antioxidant therein the particles could scavenge the stable free radical 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) of about 80% to that of positive control butylated hydroxytoluene (BHT) and antimicrobial studies indicated its microbicidal efficacy against both Gram positive and negative clinical pathogens. It could be concluded that *Simarouba amara* fruit extract can be used efficiently in the production of potential antioxidant and antimicrobial AgNPs for commercial application.

Keywords: *Simarouba amara* Aubl., AgNPs, Antioxidant activity, Antibacterial activity.

INTRODUCTION

In recent years, researchers are interested in rapid development of nanotechnology which has opened up a world of new possibilities for fabricating nanomaterials of desired particle size usually ranging from 1 to 100 nm, based on the specific characteristics such as size, morphology, shapes suitable for uses in biomedicine, industry and agriculture field[1]. The green synthesis of nanoparticles (NPs) and applications are gaining intense importance in biomedicine, the smaller size of nanoparticles, high surface area and reactivity provide them the ability for therapeutic purpose in different dosage forms and dosing routes. Nanoparticles could be derived from various sources of gas, liquid or solid phases. They can be synthesized using different synthetic methods like physical, chemical, and biological synthesis[2].

The development of green processes for the synthesis of nanoparticles has been evolving into an important branch of nanotechnology as green nanotechnology deals with the safe and eco-friendly methods for nanomaterials fabrication and which is considered as an alternative to conventional physical and chemical methods[3].

Nanomedicine is a rapidly developing and promising field that makes best use of inert metals like silver, gold and platinum to synthesize metallic nanoparticles with high therapeutic potential for various biomedical applications. Among all metal nanoparticles, silver nanoparticles (AgNPs) have much attention due to the surface plasmon resonance (SPR) (strong absorption in the visible region), which can be easily observed by UV-visible spectrophotometer[4]. Silver with its potent antimicrobial activity has been used in the synthesis of silver nanoparticles which finds extensive use in the preparation of food processing, topical ointments and medical implants[5,6].

The green synthesis methods using various plant extracts have been shown to be more advantageous owing to their simple methodology and eco-friendly nature[7,8]. The green synthesis of silver nanoparticles using different medicinal plants including, *Saraca indica*[9], *Pedalium murex*[10], *Nelumbo nucifera*[11], *Azadirachta indica*[12], *Diospyros paniculata*[13] , *Butea monosperma*[14], *Emblica officinalis*[15] has been reported. Such green synthesized silver nanoparticles from *Helicteres isora*[16], *Ruta graveolens*[17], *Aristolochia indica* L[18] and *Cassia tora*[19] have also been shown to exhibit antioxidant and antimicrobial activities.

Considering the vast potentiality of plants as sources, this work aims to apply a biological green technique here is simple, cost effective, easy to perform and sustainable for

*Author for Correspondence: dgurukumar.phd@gmail.com

the synthesis of silver nanoparticles. In this regard, fruit extract of *Simarouba amara* Aublet, popularly known as 'Laxmitaru' or 'paradise tree' a species of family *Simaroubaceae* was used as reductant and stabilizer for silver nanoparticles. The use of *Simarouba amara* has a long history in folk medicine of many countries. Ethnopharmacological data suggest the use of a cup of *Simarouba amara* stem bark decoction, 2-3 times per day, to treat malaria, inflammation, fever, abdominal pain, diarrhea, wounds and as a tonic[20, 21]. With these evidences, this study was designed to synthesize AgNPs using aqueous *Simarouba amara* fruit extract and assess their antioxidant and antimicrobial activity.

MATERIALS AND METHODS

Plant collection and Authentication

The fruits of *Simarouba amara* Aubl. was collected from University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India. It was authenticated by Dr. G.V.S. Murthy, Director, Botanical Survey of India, Tamil Nadu Agricultural University Campus, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in the laboratory for future reference (*BSI/SRC/5/23/2016Tech./608*).

Sample preparation and phytochemical screening

Fresh fruit *Simarouba amara* extract was used for the bio-reduction of $AgNO_3$ to Ag. 10 g of fresh fruits were washed thoroughly and ground into a fine powder in a 500 ml Erlenmeyer flask along with 100 ml of double distilled water. Further, the pure seed extract was separated by reiterated vacuum filtration and then stored at 4˚C and used for further experiments. The phytochemical screening of fresh fruit *Simarouba amara* was performed as per published procedures[22,23].

Chemicals and preparation of $AgNO_3$ solution

AR-grade silver nitrate ($AgNO_3$) was purchased from Finar Chemicals and fresh 0.01697 g of $AgNO_3$ was dissolved in 100 mL double distilled water (Millipore) to produce 1mM solution of $AgNO_3$.

Synthesis of silver nanoparticles (AgNPs)

30g of fresh unripe fruits in 100ml distilled water (Millipore) are crushed and filtered by using Whatman No.1 filter paper. 1mM of 100ml Silver nitrate solution was prepared in a 250ml beaker covered with aluminium foil, and kept in a magnetic stirrer. With vigorous stirring, 10ml of fruit extract was added drop wise to the silver nitrate solution. With vigorous stirring, the extract was added drop wise to the $AgNO_3$ solution and the total volume was made up to 100 ml by addition of double distilled water. The colour changed from light yellow to dark brown after continuous stirring for 4hrs. The AgNPs synthesis was confirmed by UV/visible spectra at 350-700 nm and λ_{max} was noted.

Characterization of AgNPs

Characterization of nanoparticles is important to understand and control nanoparticles synthesis and application. The formation of Ag-NPs was confirmed by sampling the reaction mixture at regular intervals and the absorption maximum was scanned by UV-Visible spectra, in a range of wavelength between 350 and 700 nm using HITACHI U-2900 Double beam spectrometer. The X-ray diffraction (XRD) patterns of the silver nanoparticles were recorded using SmartLab 3kW, Item (C/N) 2080B211, Rigaku Corporation Made in Japan. DLS measurements were carried out with a DLS particle size analyzer (Microtrac.INC W3231 Made in USA) to estimate the average size distribution of the prepared particles. Scannining electron microscopy (SEM) analysis was performed (HITACHI S-3400N) to study the morphology of the AgNPs.

In vitro antioxidant assay

DPPH free radical scavenging assay

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging potential of the AgNPs was determined using the method by[24]. Various concentrations (20, 40, 60, 80 and 100 µg/ml) of AgNPs and standard butylated hydroxytoluene (BHT) were taken in different test tubes. In the above samples, 1 mL of freshly prepared DPPH (0.1 mM) dissolved in methanol was added and vortexed thoroughly. Finally, the solution was incubated in dark place for 30 min. The absorbance of stable DPPH was recorded at 517 nm. The DPPH (containing no sample) was used as a control prepared using the same procedure. The free radical scavenging activity was expressed as the inhibition percentage. The inhibition percentage was calculated using the following formula

Percentage of radical scavenging activity =

$$\frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

BHT was taken as reference standard. The percentage inhibition vs concentration was plotted and the concentration required for 50% inhibition of radicals was expressed as IC_{50} value.

Assessment of antimicrobial assay

The evaluation of antimicrobial activity was carried out

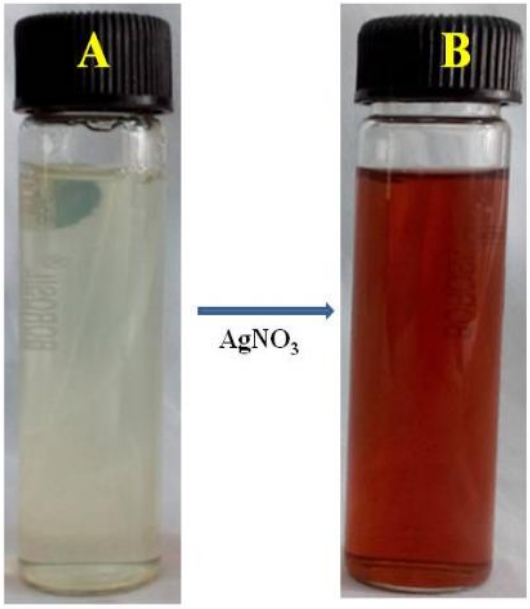

Figure 1: Visual observation of the solution before bio-reduction (A) and after bio-reduction (B)

using five different stains. These following microorganisms were used: *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus* and *Pseudomonas*. The microbial cultures were

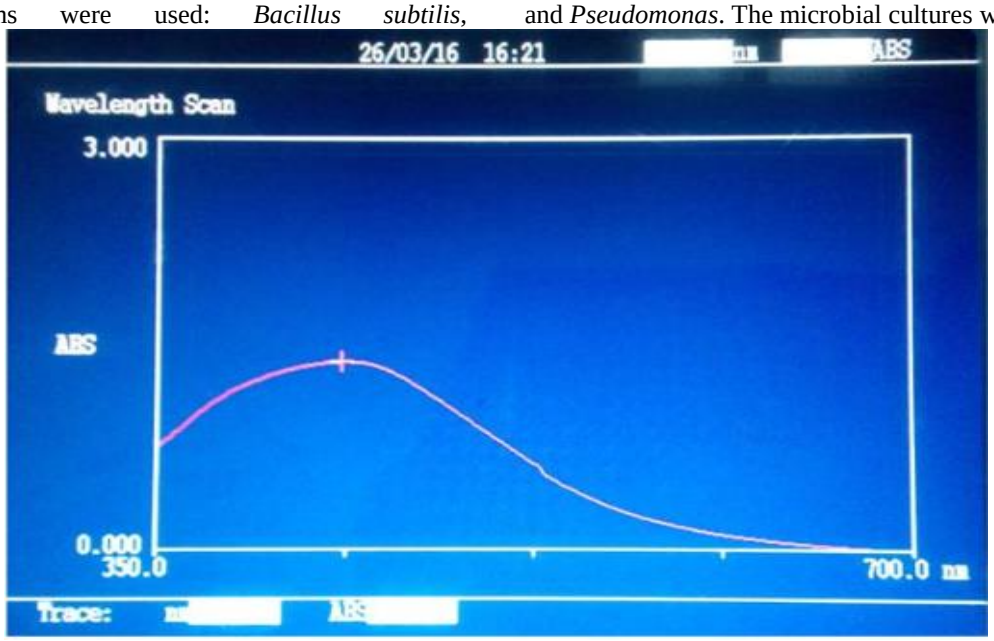

Figure 2: UV-Vis absorption spectrum of synthesized AgNPs.

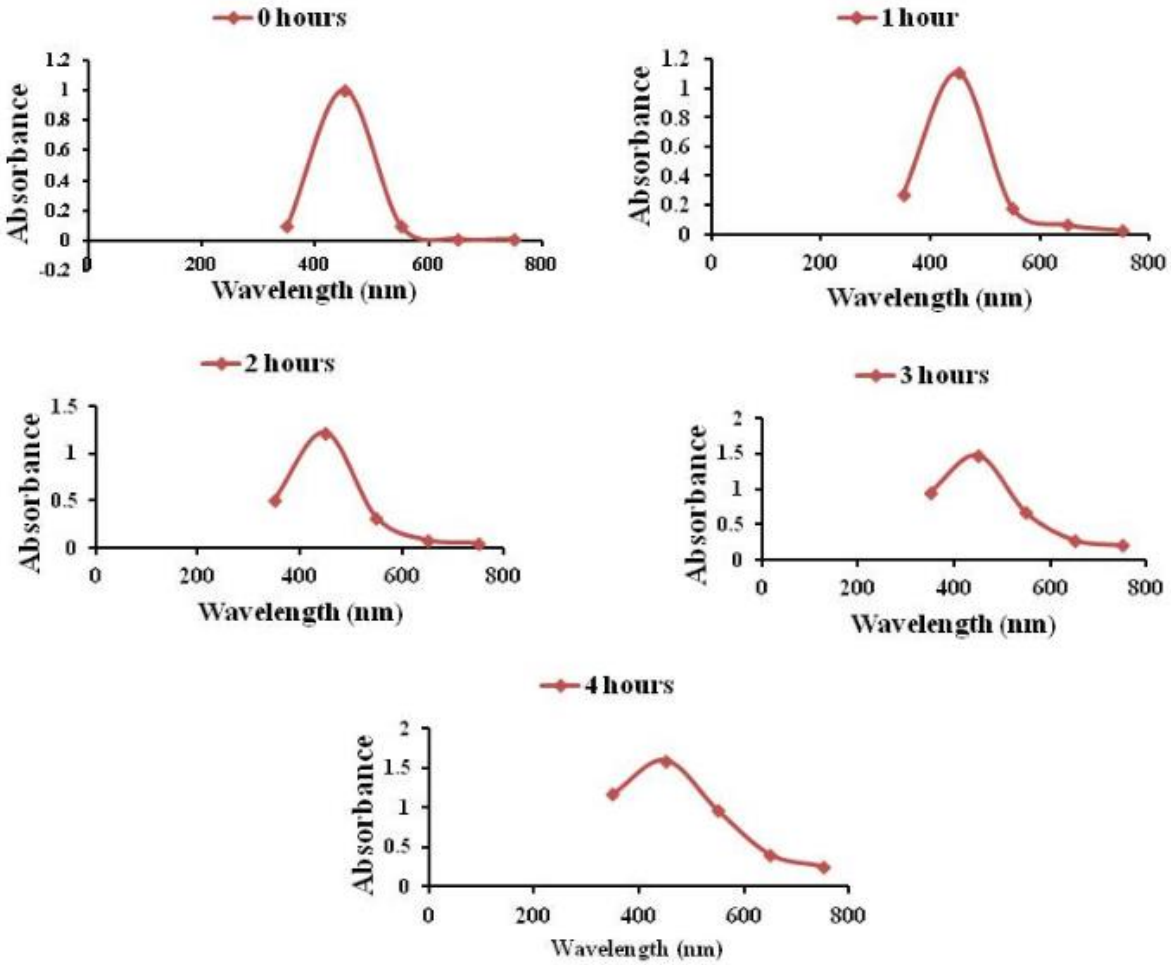

Figure 3: Synthesis of Silver nanoparticles at varying stirring times.

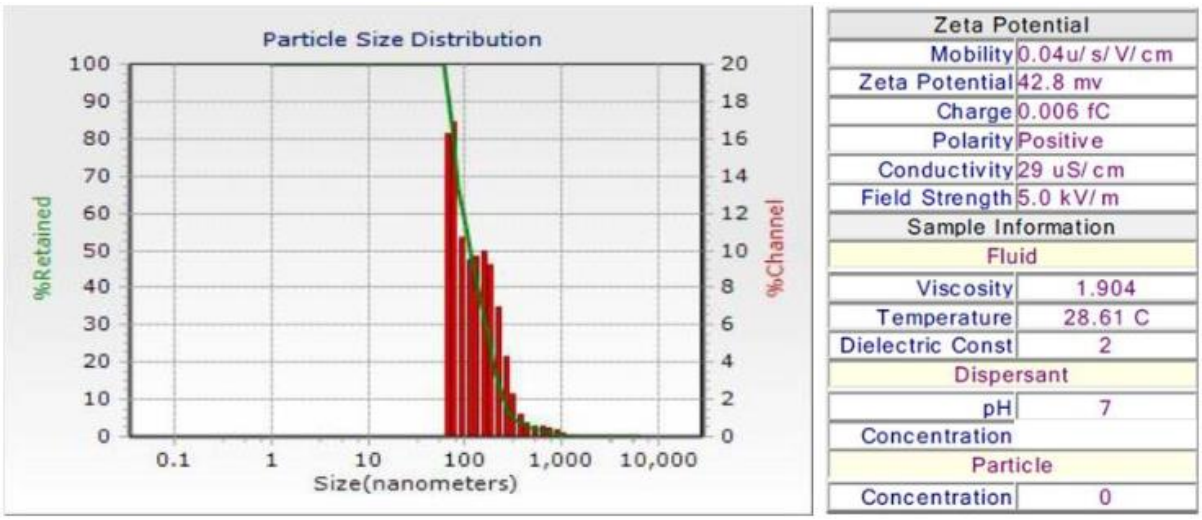

Figure 4: DLS size distribution pattern and Zeta potential analysis of synthesized AgNPs using *Simarouba amara* fruit extract.

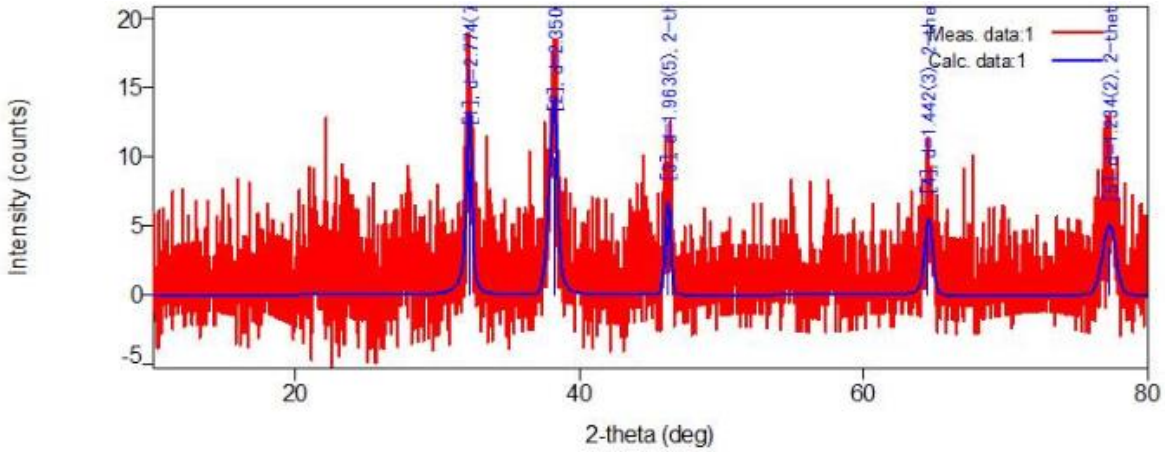

Figure 5: XRD pattern of biosynthesized silver nanoparticles using fruit extract.

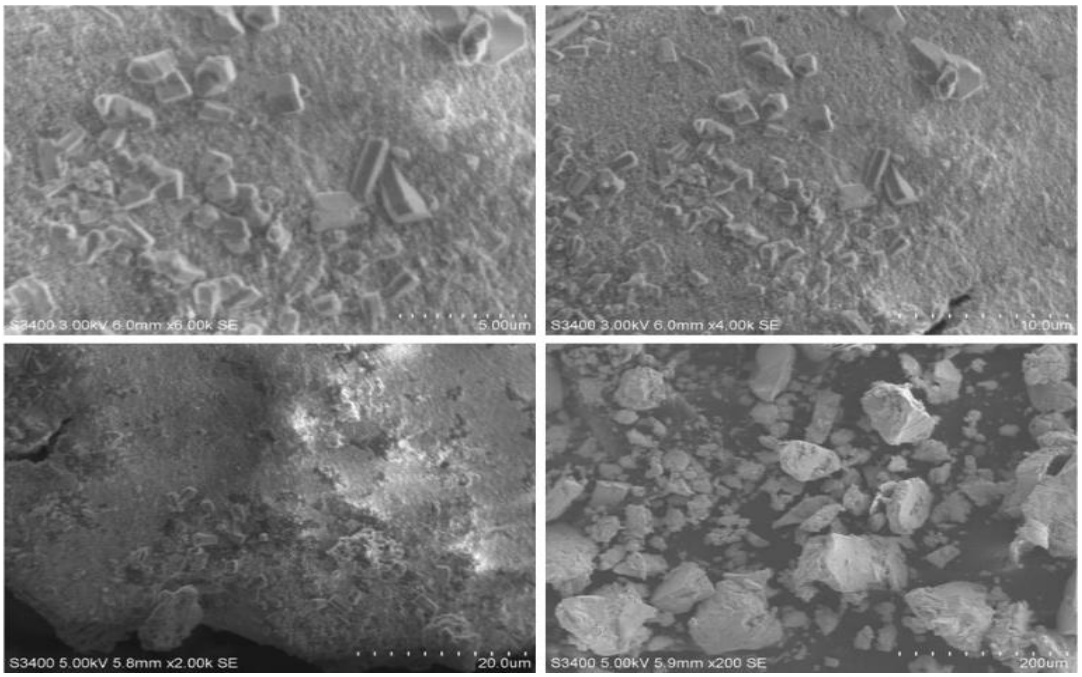

Figure 6: SEM image of synthesized AgNPs using *Simarouba amara* fruit extract.

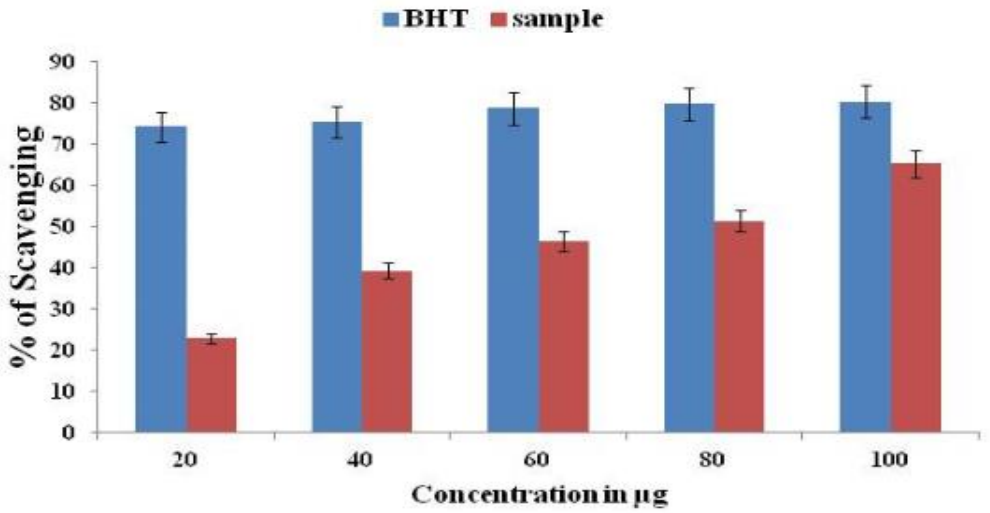

Figure 7: DPPH radical scavenging assay.

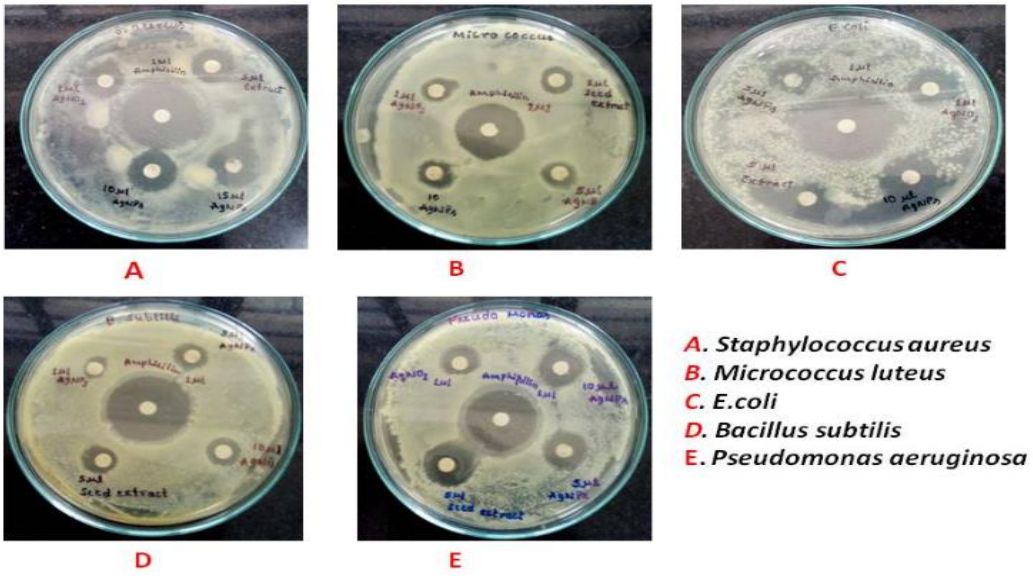

Figure 8: Antibacterial activity of green synthesized AgNPs from *Simarouba amara* fruit extract.

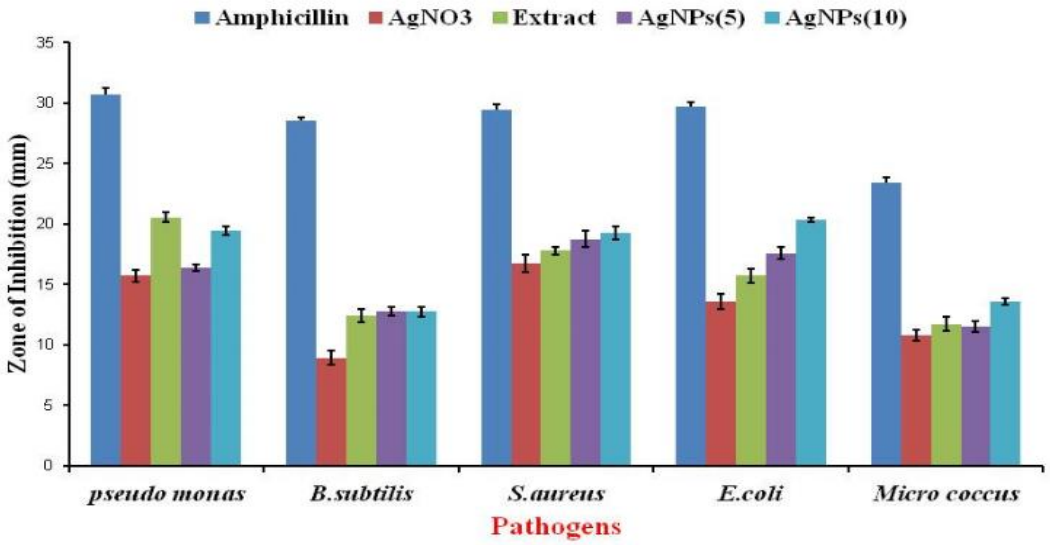

Figure 9: Antibacterial activity of synthesized AgNPs against various pathogenic bacterial strains.

Table 1: Effect of synthesized AgNPs against various pathogenic bacterial strains. .

S. No	Name of the organism	Zone of Inhibition (mm)				
		Amphicillin (1µg/ml)	$AgNO_3$ (1µg/ml)	Extract (5µg/ml)	AgNPs (5µg/ml)	AgNPs (10µg/ml)
1	*Pseudomonas*	30.6±0.50	15.6±0.51	20.5±0.40	16.3±0.25	19.4±0.33
2	*B.subtilis*	28.5±0.40	8.9±0.73	12.4±0.30	12.7±0.66	12.7±0.52
3	*S.aureus*	29.4±0.34	16.7±0.61	17.7±0.57	18.7±0.51	19.2±0.17
4	*E.coli*	29.6±0.48	13.5±0.45	15.7±0.61	17.5±0.42	20.3±0.25
5	*Micrococcus*	23.3±0.28	10.7±0.58	11.7±0.51	11.4±0.36	13.5±0.41

Values are average of triplicates, ± indicates standard error

maintained in the Postgraduate Department of Biochemistry, JSS College of Arts, Commerce and Science (Autonomous), Mysuru-570025, Karnataka, India.

Nanoparticles are now considered a viable alternative to antibiotics and seem to have a high potential to solve the problem of the emergence of bacterial multidrug resistance[25]. In particular, silver nanoparticles (AgNPs) have attracted much attention in the scientific field[26,27]. Silver has always been used against various diseases; in the past it was use as an antiseptic and antimicrobial against Gram-positive and Gram-negative bacteria[28, 29] due to its low cytotoxicity[30].

In the present work, the synthesized AgNPs in aqueous were tested against bacterial pathogens by agar disc diffraction method[31]. The overnight grown bacterial suspensions of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus* and *Pseudomonas* were standardized using McFarland standard Whatman filter paper (No:1) discs of 5 mm diameter were used. The concentrations of biosynthesized AgNPs varying from (5, 10 and 15µg/ml) were prepared with twofold symmetry. 5 g of solidified agar was added with 50 ml of distilled water and sterilized. This mixture was poured equally into five Petri plates and about 150 CFU/ml of inoculums was swabbed onto nutrient agar plates uniformly and allowed to dry in a sterile environment. The five organisms to be tested were inoculated in five discs (5 mm diameter) dipped in different concentration of AgNPs (5, 10 and 15 µg/ml) solutions, and another disc was dipped in 1 µg/ml of antibiotic ampicillin. Each Petri plate was loaded with these five discs. The plates containing the bacterial and AgNPs were incubated at 37˚C and then examined for confirmation, the appearance of a clear area around the disc. The diameter of each zone inhibition was measured using a meter ruler, and the mean value for each organism was recorded and expressed in millimeters.

Statistical analysis

The results were expressed as mean ± SD of three independent experiments ($P<0.01$). IC_{50} values were calculated from DPPH assay and subjected to statistical analysis.

RESULTS

Visual observation and UV-Vis spectroscopy

In accordance with literature studies, AgNPs solution has dark brown colour. The colour of the *Simarouba amara* was pale yellow before its treatment with silver nitrate solution, but after the reaction it turned to dark brown is shown in (Fig.1), indicating the formation of AgNPs due to reduction of silver ions by active molecules present in the fruit extract. This colour is attributed to surface Plasmon resonance, which is a size-dependent property of NPs[32].

The UV-Visible spectroscopy was employed to understand the biosynthesis of silver nanoparticles by *Simarouba amara* is shown in (Fig.2). It is generally recognized that UV-Visible spectroscopy could be used to examine size and shape of controlled NPs in aqueous suspensions. This analysis showed the sharp absorbance at around 410 nm (Fig. 2), which was specific for AgNPs. The UV-Vis absorption band in the current visible light region (400-450 nm) is an evidence of the presence of surface plasmon resonance (SPR) of Ag-NPs[7,8].

Synthesis of AgNPs at varying stirring times

The stirring time effect on the synthesis of silver nanoparticles is shown in the (Fig.3). The color intensity and monodispersity increased with stirring time. The appearance of surface plasmon resonance peak (SPR) at a wavelength range of 400-450 nm corresponds to AgNPs which absorb radiations intensely at a wavelength of 447 nm due to the transition of electrons[33].

Particle size and distribution

The size distribution histogram of dynamic light scattering (DLS) indicates that the size of these silver nanoparticles is 80nm. Some distribution at lower range of particle size indicates that the synthesized particles are also in lower range of particle size is show in (Fig.4) shows the DLS pattern of the suspension of Ag nanoparticles synthesized using *Simarouba amara* fruit extract. Zeta potential analysis shown in (Fig.4) the positive polarity of the particle favouring the drug targeting.

XRD analysis

The X-ray diffraction pattern of the biosynthesised AgNPs from the fruit extract is shown in (Fig.5). The intensity data were collected over a 2 theta range of 20˚-80˚. The five diffraction peaks located at 32.24˚, 38.26˚, 46.21˚, 64.55˚ and 77.28˚ indicated the (2.77), (2.34), (1.96), (1.44) and (1.23) reflections of metallic silver. A sharp and strong diffraction peak centered at 32.24˚ was appeared, which can be indexed to the (2.77) reflection and closely matched the reported reference values of Joint Committee on Power Diffraction Standards (JCPDS pdf

no: 89-3722). The sharp peaks clearly indicate the synthesized AgNPs are crystalline in nature, with a face-centered cubic (fcc) structure.

SEM analysis for AgNPs

The analysis of the scanning electron microscopy (SEM) images predicts the formation and the morphology of stable silver nanoparticles obtained from the current green approach. SEM images showed that AgNPs have been formed and Ag+ ions have been completely consumed. The AgNPs are mainly uniform spherical shaped with the average range of particle size distribution from 78 to 100 nm (Fig.6). This result correlated with a previous report obtained using papaya fruit extract which was at a range of 25 to 50 nm[34].

Antioxidant activity of AgNPs

Antioxidants are micro-constituents that can be act as a scavenger of reactive oxygen species (ROS) by terminating the oxidizing chain reaction. ROS play a fundamental role in the pathogenesis of a variety of degenerative conditions including cardiovascular diseases and carcinogenesis. DPPH assay are widely used to evaluate the radical-scavenging ability of green synthesized nanoparticles. In the present study, DPPH, a stable free radical with a characteristic absorption at 517nm, was used to study the radical-scavenging effects. The DPPH scavenging assay exhibited effective inhibition activity of AgNPs when compared with the standard BHT (butylated hydroxytoluene). The antioxidant activity (DPPH approach) suggest that, *S. amara* fruit extract of IC_{50} value was 38±1.25 µg/ml which was compared with standard Ascorbic acid (65.55 ±1.02 µg/ml) was shown in (Fig.7). The *S. amara* fruit extract percent of inhibition was near to the standard. The previous study reveals that, *Helicteres isora* root extract AgNPs showed good antioxidant activity as compared to standard butylated hydroxytoluene by[16].

Antimicrobial activity of AgNPs

The *Simarouba amara* fruit extract AgNPs exhibited good antimicrobial activity against both Gram-negative and Gram-positive bacteria is shown in (Fig. 8, 9 & Table. 1). But it showed higher antimicrobial activity against *Staphylococcus aureus* (Gram positive), *Escherichia* coli (Gram negative) and *Pseudomonas aeruginosa* (Gram negative) than *Bacillus subtilis* (Gram positive) and *Micrococcus luteus* (Gram positive). This result is possible due to the difference in the structure of the cell wall between Gram-positive and Gram-negative bacteria. However, zone of inhibition was observed less in *Bacillus subtilis* (Gram-positive) and *Micrococcus luteus* (Gram-positive), these results indicate that AgNPs show very less antibacterial activity against these microorganisms. Peptidoglycan is composed of a thick layer of bacterial cell wall, consisting of linear polysaccharide chains cross-linked by short peptides thus forming more rigid structure leading to difficult penetration of the AgNPs[35]. This high bactericidal activity is certainly due to the silver cations released from AgNPs that act as reservoirs for the Ag^+ bactericidal agent[36]. Therefore, AgNPs were widely used in antibacterial coatings in processing of medical instruments[37] and food industries for packaging[4]. The biologically synthesized AgNPs using various plant extracts also showed a similar potent bactericidal activity[7,38]. AgNPs had superior antibacterial activity than silver nitrate[39,40].

DISCUSSION

To the best of existing information, the present study is the first report green synthesis of silver nanoparticles by using *Simarouba amara* Aubl. Fruit extract and their antioxidant and antibacterial activities. Medicinal plants are very ancient and true natural medicines which are useful for the treatment of different diseases. They can be used directly or in extracted forms for the management of various ailments due to the presence of various secondary metabolites[41]. Many plants contain a variety of phyto-pharmaceuticals, which have found very important applications in the fields of agriculture, human and veterinary medicine[42]. *A Simarouba amara fruit has* been to synthesize silver nanoparticles and it has shown potent antioxidant activity and antibacterial activities. It is generally assumed that frequent use of plant- derived phytochemicals may contribute to shift the stability in the direction of a sufficient antioxidant status. As a result, attention in natural antioxidants, in particular plant origin, has gained prominence in recent years. Nanomedicine is a rapidly developing and promising field that makes best use of inert metals like silver, gold and platinum to synthesize metallic nanoparticles with high therapeutic potential for various biomedical applications. Silver with its potent antimicrobial activity has been used in the synthesis of silver nanoparticles which finds extensive use in the preparation of food processing, topical ointments and medical implants[5]. Hence, the anticancer activity of *S. amara* fruit extract can be attributed to the secondary metabolites present in the fruit extract.

CONCLUSION

Green synthesis is an effective way to synthesize silver nanoparticles due to its eco-friendly, simple, cost-effective and efficient protocol. The present study clearly indicate that, the production of environmentally benign AgNPs using *Simarouba amara* fruit extract contain more phenols, alkaloids that play major roles as reducing agents for use in synthesis of AgNPs, in which biomolecules act as stabilizing agent. The extract acts as both reducing and stabilizing agent which was confirmed by UV-visible spectrophotometer, DLS, XRD and SEM techniques reports revealed that synthesized AgNPs were crystalline in nature with an average particle size of 78-100nm. The synthesized AgNPs possess more potent antioxidant and antimicrobial activities against both Gram-negative and Gram-positive bacteria. The AgNPs have emerged as a typical antimicrobial nanomaterial applied in industry, daily life, and medicine. Hence, this method can be employed in large-scale nanoparticles can be synthesized and can be used in many medicinal and technological applications.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

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