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Research Article

A Green Approach to Phyto-Mediated Synthesis of Silver Nanoparticles Using *Eugenia uniflora* L. Fruit Extract and Their Antioxidant and Antibacterial Activities

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ABSTRACT

In recent years, researchers are interested in rapid development of nanotechnology processes for the synthesis of nanoparticles has been evolving into an important branch of green nanotechnology deals with the safe and eco-friendly methods uses in biomedicine, industry and agriculture field. A green approach to phyto-mediated synthesis of silver nanoparticles using *Eugenia uniflora* L. (*E. uniflora*) fruit extract and their antioxidant and antibacterial activities. The present study describe the synthesis of AgNPs using the fruit extract of *E.uniflora* followed by characterization of AgNPs was done using different methods, which include; AgNPs synthesis was confirmed by UV/visible spectra and characterized by XRD, DLS and SEM analysis. The synthesized AgNPs were also tested for antioxidant and antibacterial activities. The results clearly indicate that, the UV-Visible spectroscopy was employed to understand the biosynthesis of silver nanoparticles by *E. uniflora*. This analysis showed the sharp absorbance at around 440 nm, which was specific for AgNPs. Zeta potential analysis shows the positive polarity of the particle favoring the drug targeting. The dynamic light scattering (DLS) of the average particle size is around 85nm. The scanning electron microscopic (SEM) images showed that AgNPs have been formed and Ag+ ions have been completely consumed and they are crystalline in nature. The synthesized AgNPs were also tested for antioxidant therein the particles could scavenge the stable free radical DPPH of about 85% to that of positive control BHT. The value of the 50% inhibition concentration (IC_{50}) of Standard BHT: 65.55µg/ml and *E. uniflora*: 52.15µg/ml. The antibacterial studies indicated its bactericidal efficacy against clinical pathogens. It could be concluded that *E. uniflora* fruit extract can be used efficiently in the production of potential antioxidant and antibacterial AgNPs for commercial application.

Keywords: *Eugenia uniflora* L., AgNPs, Antioxidant activity, Antibacterial activity.

INTRODUCTION

In the last decade, synthesis and characterization of nanoparticles has received considerable research interest across the globe. Nanomaterials have extensively been synthesized using physical, chemical and biological methods; among them, the bio-based protocols have now turned into promising alternative to the conventional methods of nanoparticle preparation[1].

Nanoparticle has multifunctional properties and very interesting applications in various fields such as biomedicine, nutrition, biosensors, bio-nanotechnology and energy[2,3]. The biogenic syntheses of monodispersed nanoparticles with specific sizes and shapes have been a challenge in biomaterial science. Also, it has created remarkable advantages in the pharmacological industry to cure various bacterial and viral diseases[4]. Nanomedicine makes a huge impact in healthcare sector in treating various chronic diseases. Hence, eco-friendly synthesis of nanoparticles is considered as building blocks of the forthcoming generations to control various diseases[5].

Generally, silver nanoparticles is a nontoxic, safe inorganic antibacterial agent used for centuries and is capable of killing more than 500 types of diseases causing microorganisms. It has a significant potential for a wide range of biological applications such as preventing infections, healing wounds, anti-inflammatory and use as an antibacterial agent for antibiotic resistant bacteria[6]. Silver nanoparticles are mainly synthesized by physical and chemical approaches, which are economically expensive and involve in chemical toxic[7]. Therefore, biogenic synthesis using plant extract has been recently emerged as an alternative method for the synthesis of silver nanoparticles because it is simple, cost effective, environmental-friendly, and easy to scale up for mass production[8]. Plant extract contains novel secondary metabolites such as phenolic acid, flavonoids, alkaloids and terpenoids in which these compounds are mainly responsible for the reduction Ag^+ to Ag^0 and have a strong ability to reduce heavy metals from their higher oxidation to zero oxidation state which may be attributed to the presence of different phytochemicals[9,10].

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The green synthesis of silver nanoparticles using various medicinal plants fruit extract including *Tinospora cordifolia*[11], *Achras sapota* L.[12], *Emblica officinalis*[13], *Piper longum*[14] and *Piper pedicellatum*[15] has been reported. Considering the vast potentiality of plants as sources this work aims to apply a biological green technique for the synthesis of silver nanoparticles as an alternative to conventional methods. With these evidences, fruit extract of *Eugenia uniflora* L. belongs to the family *Myrtaceae*, popularly known as Surinam cherry is used as reductant and stabilizer for silver nanoparticles[16]. The use of *E. Uniflora* has a long history in folk medicine of many countries. *E. uniflora* fruits and leaves are used as an antioxidant, hypertensive, anti-inflammatory and hypoglycemic agent[17]. It may also reduce weight, blood pressure and serve as a diuretic[18]. With these evidences, this study was designed to synthesize AgNPs using aqueous *E. uniflora* fruit extract and assess their antibacterial effects and antioxidant activity.

MATERIALS AND METHODS

Plant collection and Authentication

The unripe fruits of *Eugenia uniflora* L. were collected from College of Horticulture, Horticulture form Yelawala, Mysuru, 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./882*).

Sample preparation and phytochemical screening

Fresh fruit *Eugenia uniflora* L. 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 *Eugenia uniflora* was performed as per published procedures[19, 20].

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)

Synthesis of AgNPs methodology was developed according to[21] with minor modifications. 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 nanoparticle synthesis and applications[22]. The formation of AgNPs 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[23]. 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.

Evaluation of the bactericidal activity

The bactericidal activity of the AgNPs was evaluated against the clinical isolates *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus* (Gram-positive) and *Escherichia* coli, *Pseudomonas aeruginosa* (Gram-negative) obtained from the Postgraduate Department of Biochemistry, JSS College of Arts, Commerce and Science (Autonomous), Mysuru-570025, Karnataka, India. The bactericidal activity was carried out with 24 h active cultures by employing the disc diffusion method[24]. About 150 CFU/mL of inoculums was swabbed onto nutrient agar plates uniformly and allowed to dry in a sterile environment. Sterile disc of 6 mm (HIMEDIA) was loaded with different concentration of AgNPs (5, 10 and 15 µg/ml) solutions, and another disc was dipped in 1 µg/ml of antibiotic ampicillin was used as positive control. The plates were incubated at 26˚C for 2 days to

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

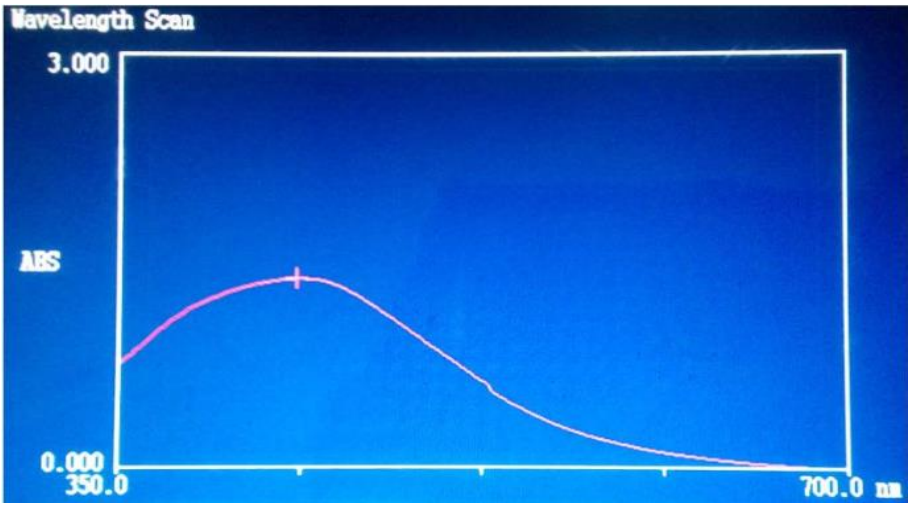

Figure 2: UV-Vis absorption spectrum of synthesized AgNPs.

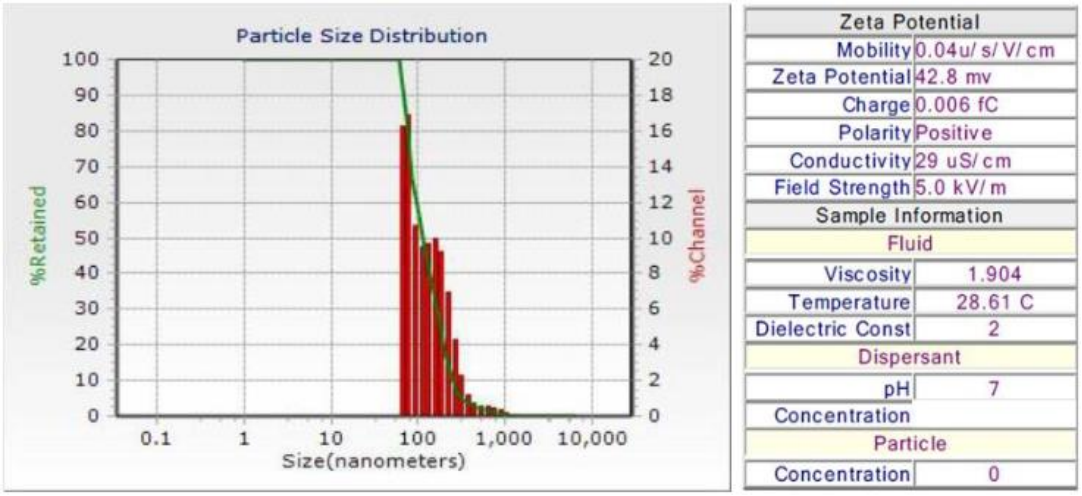

Figure 3: DLS size distribution pattern and Zeta potential analysis of synthesized AgNPs using *Eugenia uniflora* fruit extract.

measure the zone of inhibition. The mean was calculated by performing the experiments in triplicates.

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.

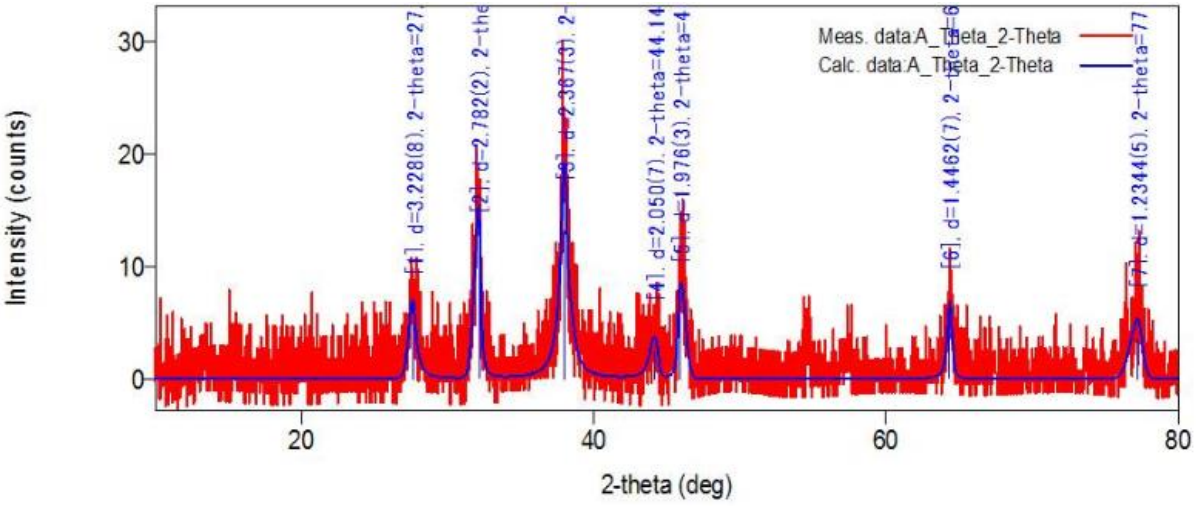

Figure 4: XRD pattern of biosynthesized silver nanoparticles using fruit extract.

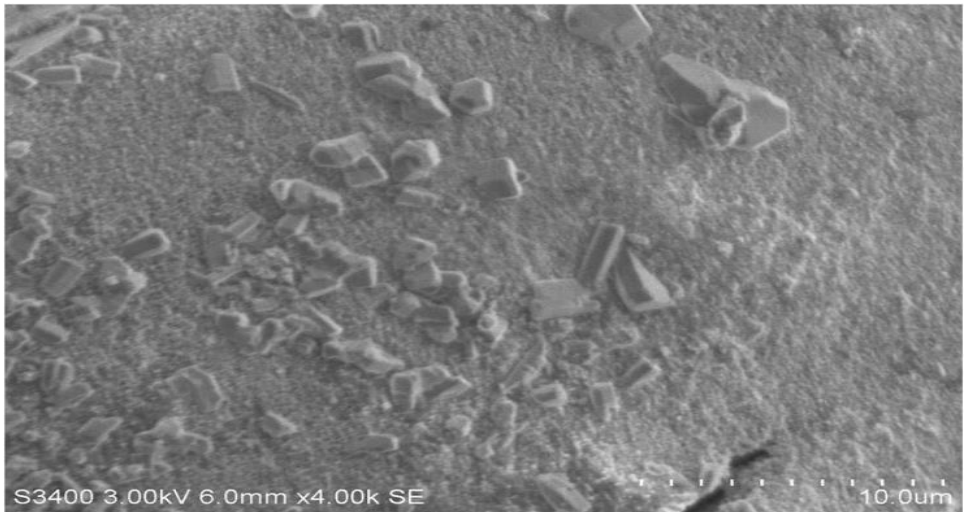

Figure 5: SEM image of synthesized AgNPs using *Eugenia uniflora* fruit extract.

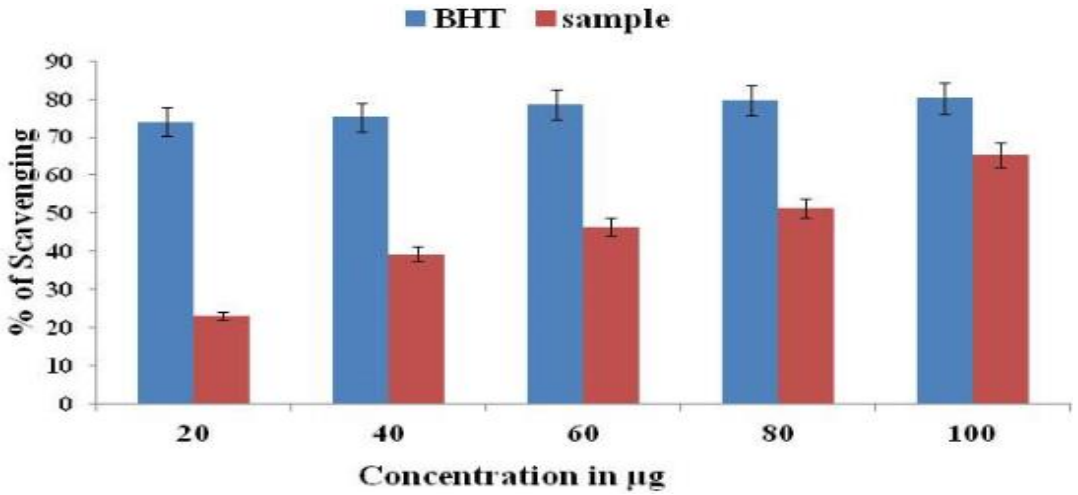

Figure 6: DPPH radical scavenging assay.

RESULTS

UV-Vis absorption spectroscopy analysis

The addition of *Eugenia uniflora* fruit extract to the aqueous $AgNO_3$ solution resulted in the pale yellow to reddish brown color surface plasmon resonance (SPR) (Fig.1). The reduction of aqueous Ag^+ ions to Ag^o by the *Eugenia uniflora* fruit extract was simply analyzed by UV-visible spectroscopy. The UV-Vis spectra of the AgNPs band occur near 440 nm (Fig.2) indicating the

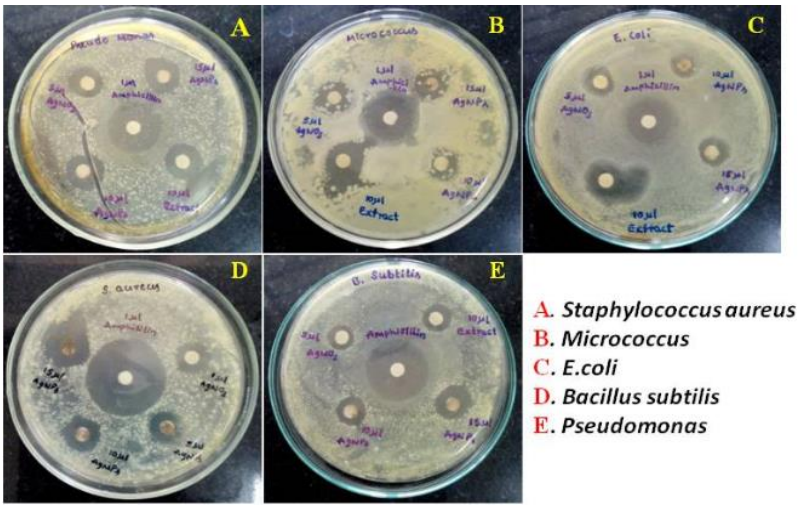

Figure 7: Antibacterial activity of green synthesized AgNPs from *Eugenia uniflora* fruit extract.

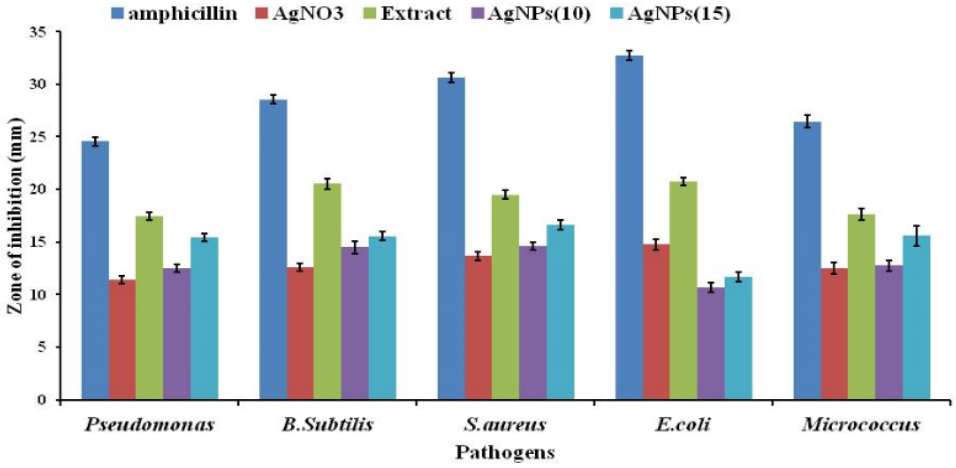

Figure 8: Antibacterial activity of synthesized AgNPs against various pathogenic bacterial strains.

formation of AgNPs due to reduction of silver ions by active molecules present in the fruit extract. In accordance with previous literature studies were also reported by many researchers[21,25]. The brown color confirms that it was due to the reduction of Ag^+ which indicates the formation of AgNPs.

Dynamic light scattering (DLS) measurement

The size distribution histogram of dynamic light scattering (DLS) indicates that the size of these silver nanoparticles is 85nm. 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.3) shows the DLS pattern of the suspension of Ag nanoparticles synthesized using *Eugenia uniflora* fruit extract. Zeta potential analysis has shown the positive polarity of particle favouring the drug targeting.

X-ray diffraction (XRD) measurement

The X-ray diffraction pattern of the biosynthesised AgNPs from the fruit extract is shown in (Fig.4). The intensity data were collected over a 2 theta range of 20˚-80˚. The five diffraction peaks located at 27.61˚, 32.14˚, 37.98˚, 44.14˚, 44.88˚, 64.36˚ and 77.21˚ indicated the (145.32), (173.25), (164.36), (131.08), (159.12), (284.33) and (112.95) reflections of metallic silver. A sharp and strong diffraction peak centered at 32.14˚ was appeared, which can be indexed to the (173.25). The sharp peaks clearly indicate the synthesized AgNPs are crystalline in nature, with a face-centered cubic (fcc) structure.

Scanning electron microscopy (SEM) measurement

The SEM analysis was used to determine the structure of the reaction products that were formed. The morphology of the AgNPs was predominantly showed individual silver particles as well as a number of aggregates. SEM images of AgNPs derived from the unripe fruit extract of *Eugenia uniflora* showed spherical shaped with the average range of particle size distribution from 85 to 100 nm (Fig.5). This result correlated with a previous report obtained using papaya fruit extract which was at a range of 25 to 50 nm[26].

Antioxidant activity of AgNPs

In the present study, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), a stable free radical with a characteristic absorption at 517nm, was used to study the radical-scavenging effects. The DPPH activity results showed the effective free radical % scavenging potential of *E. uniflora* fruit extract AgNPs, the *E. uniflora* extract as 65.55% and 52.15% respectively (Fig. 6). The previous literature revealed that, using various medicinal plant

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

Sl. No	Name of the organism	Inhibition zone (mm)				
		Amphicillin (1µg/ml)	$AgNO_3$ (1µg/ml)	Extract (5µg/ml)	AgNPs (10µg/ml)	AgNPs (15µg/ml)
1	*Pseudomonas*	24.5±0.42	11.4±0.34	17.4±0.37	12.4±0.37	15.4±0.34
2	*B.subtilis*	28.5±0.45	12.6±0.43	20.5±0.40	14.4±0.35	15.5±0.45
3	*S.aureus*	30.6±0.45	13.6±0.48	19.4±0.36	14.6±0.44	16.6±0.44
4	*E.coli*	32.7±0.56	14.7±0.56	20.7±0.53	10.6±0.51	11.6±0.96
5	*Micrococcus*	26.4±0.40	12.5±0.40	17.6±0.49	12.7±0.57	15.6±0.43

extract AgNPs showed enhanced scavenging activity with the increase in DPPH scavenging potential of silver nanoparticles[27, 28] have been reported.

Antibacterial activity of AgNPs

The bactericidal activity of the green synthesized *E. uniflora* fruit extract AgNPs has potent antibacterial activity against both Gram-negative and Gram-positive human pathogens. AgNPs displayed antibacterial activity against Gram positive and Gram negative bacteria, with varying degrees, as suggested by the diameter of inhibition zone, while *E. uniflora* fruit extract show low antibacterial activity compare to AgNPs (Fig. 7, 8 & Table.1). The results showed that AgNPs are effective antibacterial 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).

In this summery, *E. uniflora* was taken for synthesis of AgNPs because of its medicinal values. Various studies have been done by many researchers which confirm that use of *E. uniflora* has a long history in folk medicine of many countries and their leaves are used in popular medicine as an antioxidant, hypotensive, anti-inflammatory, hypoglycaemic agent and also used in the treatment of fever, rheumatism, stomach diseases, disorders of the digestive tract, hypertension, yellow fever, and gout. It may also reduce weight, blood pressure, and serve as a diuretic[18, 29]. The previous literature revealed that, green synthesized AgNPs using various plant extracts has potent bactericidal activity[30]. Therefore *E. uniflora* from fruit extract AgNPs showed remarkable potent antibacterial activity.

DISCUSSION

The present study is the first report green synthesis of silver nanoparticles by using *Eugenia uniflora* L. fruit extract and their antioxidant and antibacterial activities. Herbal plants are very ancient and true natural medicines which are useful for the treatment of different diseases. The addition of *Eugenia uniflora* fruit extract to the aqueous $AgNO_3$ solution resulted in the pale yellow to reddish brown color surface plasmon resonance (SPR). Noble metals are known to exhibit unique optical properties due to the property of surface plasmon resonance[32]. 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 phytoconstituents, which have found very important applications in the fields of agriculture, human and medicine[31]. A *Eugenia uniflora fruit has* been to synthesize silver nanoparticles and it has shown potent antioxidant activity and antibacterial activities. It is generally assumed that use of plant derived phytoconstituents may contribute to the stability in the direction of a sufficient antioxidant status. 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[25].

CONCLUSION

We demonstrate an eco-friendly and low cost synthesis protocol of small size AgNPs using *E. uniflora* fruit extract solution. The present study clearly indicate that, the production of environmentally benign AgNPs using *E. uniflora* 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. Crystalline AgNPs of average diameter 85 nm are successfully employed in both antioxidant and antibacterial activity. Based on these findings, AgNPs may lead to valuable applications in various fields such as medicinal and as antimicrobial agents. Thus, we have effectively synthesized AgNPs using the green route and have demonstrated their potential therapeutic uses. 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

Authors declare no conflict of interest.

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