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Research Article

IN VITRO ASSESSMENT OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF GREEN SYNTHESIZED SILVER NANOPARTICLES FROM *DIGITARIA RADICOSA* LEAVES

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ABSTRACT

Objective: The present study endeavors the synthesis of green silver nanoparticles (SNPs) from the methanolic leaf extract of medicinally important herb, *Digitaria radicosa* and ascertain *in vitro* antioxidant and antibacterial activity.

Methods: Green SNPs synthesized by adding 1 ml of methanolic extract of *D. radicosa* and 99 ml of 1 mM silver nitrate solution acts as a reducing and stabilizing agent for SNPs. Further, the nanoparticles were characterized by ultraviolet-visible (UV-Vis), Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM) analysis. The free radical scavenging activity of SNPs was evaluated *in vitro* and the antibacterial activity against foodborne pathogens, such as *Staphylococcus aureus* and *Escherichia coli,* were determined by disc diffusion method.

Results: UV-Vis spectroscopic analysis confirmed the synthesis of green SNPs indicated by the peak observed at 442 nm due to the excitation of surface plasmon resonance (SPR) in the SNPs. FT-IR spectra revealed the availability of functional groups which may involve in the SNPs synthesis. XRD pattern illustrated the characteristic peaks of (111), (122), (231) facets of the center crystalline and cubic face centered nature of SNPs. SEM analysis showed that synthesized green SNPs were of spherical in shape and size of around 90 nm. The free radical scavenging potential was evident from *in vitro* antioxidant profile where SNPs scavenges free radicals quite comparable to that of standard ascorbic acid. Disc diffusion assay revealed the significant antibacterial activity of SNPs against *S. aureus* and *E. coli*.

Conclusion: The biosynthesized green SNPs from *D. radicosa* acts as a potent free radical scavenger and possess significant antibacterial activity toward food pathogens.

Keywords: Silver nanoparticles, *Digitaria radicosa*, Ultraviolet-visible, X-ray diffraction, Fourier transform infrared, Scanning electron microscopy, *In vitro* antioxidant assays, Antibacterial activity.

INTRODUCTION

Noble metallic nanoparticles have been the subject of focused research in recent years, owing their unique optical, mechanical, magnetic, thermal, and chemical properties that are extensively different from those of bulk materials [1]. The metallic nanoparticles including gold, silver, and metal oxide nanoparticles have shown great promise in terms of biological applications due to their large surface area to volume ratio. Numerous physical, chemical and biological methods have been used for the synthesis of noble metal nanoparticles of particular shape and size, but they remain expensive and involve the use of hazardous chemicals [2]. The green synthesis of metallic nanoparticles is cost effective, easily available, eco-friendly, non-toxic, and large scale compared to that of chemical synthesis which is quite expensive and has an adverse effect in medical applications [3]. There is a strong demand for "green synthesis," synthesis that is clean, mere toxic, and environmentally friendly for the synthesis of nanoparticles [4]. Microorganisms and plant extracts are extensively used in the synthesis of inorganic nanoparticles either intracellular or extracellular [5]. The plant-mediated biological synthesis of nanoparticles is significant owing its ease and eco-friendly nature [6]. Nanoparticles synthesized by plants are more stable, and the rate of synthesis is quite faster than that in the case of other biological sources. Moreover, shape and size also differ with those produced by other sources [7,8].

Silver nanoparticles (SNPs) have been the highly focused nanoparticles due to their unusual optical, chemical, electronic, photo-electrochemical, catalytic, magnetic, antibacterial, and biological labeling properties [9]. SNPs were widely used in applications such as biomedical, drug delivery, food industries, agriculture, textile industries, and water treatment as an antioxidant, antimicrobial, and anticancer agent [10-19]. SNPs act as both an electron sink as well as a redox catalyst [5]. Plant materials, such as leaves, fruits, seeds, latex, and barks, were used for the synthesis of SNPs [20-23]. When compared with other plant materials, leaf mediated synthesis gains more advantageous like the simple and rapid synthesis of SNPs [24]. The aqueous silver nitrate solution liberates silver ions due to the presence of phytochemicals in the extract [25]. The secondary metabolites such as flavonoids, alkaloids, and tannins have been reported to play a significant role in the reduction of Ag+ ions into Ag atoms which then link together to form SNPs. SNPs likely to possess significant antibacterial activity due to their interaction with respiratory enzymes which leads to the generation of reactive oxygen species and thereby cell destruction occur [26].

Digitaria radicosa, an endemic herbal species belonging to *Digitaria* genus well known for its medicinal property. So far, there have been no reports on the pharmacological properties and synthesis of nanoparticles from this species. In the present study, we have synthesized SNPs from *D. radicosa* leaf extract through bioreduction of Ag^{+} ions to Ag^{0} nanoparticles using silver nitrate solution. The synthesized nanoparticles were further characterized by ultraviolet-visible (UV-Vis) spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD) pattern analysis, and scanning electron microscopy (SEM) analysis. Moreover, the *in vitro* antioxidant and antibacterial activity of synthesized SNPs were assessed to determine their therapeutic potential.

Green synthesis of SNPs using leaves extract of *D. radicosa* is simple, non-toxic technique to generate economically feasible and eco-friendly SNPs.

METHODS

Materials

Silver nitrate (99.0%) AR, a high grade was purchased from Sigma-Aldrich, India. Milli Q double distilled water was used throughout the study. Filtration was done using Whatman No. 1 filter paper. All glass wares were sterilized using hot air oven.

Preparation of plant extract

Fresh leaves of *D. radicosa* herbs were collected from Kolli Hills, Namakkal, Tamil Nadu. The species was authenticated by a botanist in the Department of Botany, Periyar University. The leaves were washed under running tap water pursued by rinsing with distilled water for several times to remove the dust particles and air dried to remove the residual moisture. About 25 g of dried leaves were chopped into small pieces and pulverized into the powder which was then soaked in methanol for 48 hrs with continuous shaking. The methanolic extract was filtered with Whatman No. 1 filter paper, and the filtrate was condensed to a semi-solid under 40°C by using rotary vacuum evaporator. The obtained extract was further utilized for green SNPs synthesis.

Synthesis of green SNPs

The green SNPs were synthesized by treating 1 ml of methanolic extract of *D. radicosa* with 99 ml of aqueous solution of 1 mM silver nitrate and incubated for a period of 15 hrs at room temperature. The entire reaction was carried out in darkness to avoid the photoactivation of silver nitrate solution. The visual observation from yellow to colloidal brown indicated the completion of SNPs synthesis. The colloidal mass was subjected to high-speed centrifugation at 10000 rpm and the precipitate isolated was washed thrice with ethanol to remove the organic impurities. Then it was air dried in a hot air oven and stored in a photo-resistant container until further use.

Characterization of green SNPs

The synthesized nanoparticles were then characterized by UV-Vis spectroscopy, FT-IR spectroscopic analysis, XRD pattern, and SEM analysis as follows. To confirm the complete synthesis of green SNPs, the absorption spectra were taken 200-800 nm using a double beam UV-Vis spectrophotometer (Systronics, 2202). To identify the functional groups in synthesized SNPs, the FT-IR spectrum was recorded by ART model FT-IR Spectrophotometer (Rukey Co., Germany). The spectra were recorded using attenuated total reflectance technique. The FT-IR spectrum was recorded in the mid-IR region 4000-400/cm at room resolution by KBr pellet technique using Thermo Nicolet FT-IR Nexus spectrometer coupled with triglycine sulfate detector. The interferometer and the detector chamber were purged with dry nitrogen to get rid of spectral interference due to atmospheric carbon dioxide and water vapor.

The particle size and nature of synthesized SNPs were determined by XRD pattern obtained from Shimadzu XRD-6000/6100 model with 30 Kv, 30 Ma with Cu k α radians at 2θ angle. The dried SNPs were lyophilized, and structural analysis was made using SEM (VEGA 3 TESCAN, Anna University). Thin carbon coated film were made from synthesized nanoparticles and used for structural analysis.

In vitro antioxidant potential

To evaluate the antioxidant potential of synthesized SNPs from the methanolic extract of *D. radicosa*; 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, hydrogen peroxide scavenging, metal chelating activity, and reducing power assay were done *in vitro* [27,28].

Antibacterial activity

The synthesized SNPs were examined against well-known foodborne pathogenic microbes such as Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria by the agar disc diffusion method. The pure bacterial cultures were subcultured on nutrient broth. 100 µl of both of the strain was swabbed on culture plate homogeneously using the sterile cotton swab. The sterile discs dipped in different concentrations of SNPs (10, 20, 40, 80 µg) were placed on the nutrient agar plate. The treated plates were then incubated at 37°C for 24 hrs. The antibacterial action of SNPs was evaluated by the extent of the zone of inhibition and their minimal inhibitory concentration.

Statistical analysis

All the experiments were done in triplicates. The data of experimental study were expressed as the mean±standard deviation (n=3). Statistical analysis was performed using Graph Pad Prism Software, Version 5.0 (Graph Pad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

The present study emphasizes the use of the medicinal plants for synthesis of green SNPs with potent antioxidant effect and their characterization study. SNPs synthesized from different parts of plant extracts were considered to be environment-friendly, economically feasible, and safe. In the present study, an attempt was made to synthesize SNPs from the methanolic extract of *D. radicosa* leaves extract.

Synthesis of SNPs

Phytochemicals in the extract play a significant role in the formation of green SNPs. *D. radicosa* plant extract acts as a reducing agent whereby the aqueous silver ions were reduced to SNPs.

The complete synthesis of green SNPs was signified by the color change from watery to yellowish brown after 15 hrs incubation in dark at room temperature as shown in Fig. 1 and there was no significant change afterward. A similar observation has also been reported earlier [29,30]. The yellowish brown observed is due to the excitation of SPR vibrations in the SNPs. SPR occurs due to the in-phase oscillation of conducting electrons through excitation of all free electrons on irradiation with the visible light [31,32].

Characterization of SNPs

The synthesized biogenic SNPs were subjected to determine their size and morphological characteristics with the help of UV-Vis spectroscopy, FT-IR spectroscopy, XRD analysis, and SEM.

UV-Vis spectroscopy

The formation and stability of green SNPs in aqueous suspension were primarily characterized by UV-Vis spectrophotometer. UV-Vis spectroscopy is a generally recognized technique to examine the size and shape controlled particles in aqueous suspensions [33]. UV-Vis absorption spectrum of the colloidal SNPs has been recorded as a function of time by using a quartz cuvette with silver nitrate as the reference shown in Fig. 2. The excitation of SPR vibrations was evidently shown by the absorption spectra at 379 nm and 442 nm indicating the formation of SNPs. It could be referred from the previous reports that SPR peaks around 400-480 nm are characteristic of noble SNPs [34]. The SPR peaks depend on the size and shape of the metal nanoparticles [35]. The broad SPR band observed in the spectra has shown that nanoparticles are large and polydispersed in the aqueous solution [36].

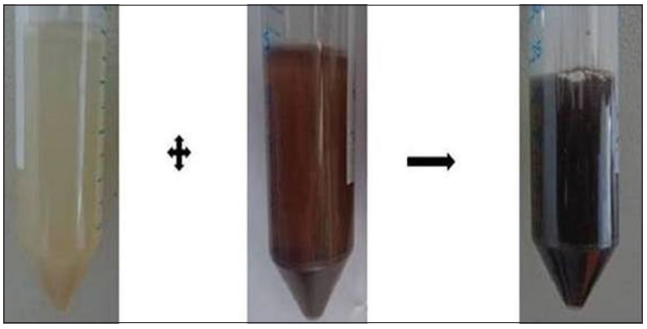

Fig. 1: Color change indicates the formation of silver nanoparticles. (a) 1 mM silver nitrate solution, (b) *Digitaria radicosa* methanolic extract, and (c) silver nanoparticles

FT-IR spectroscopy

FT-IR spectroscopy analysis was accomplished to determine the functional groups responsible for the reduction of Ag^+ ions to Ag^0 nanoparticles. The FT-IR absorption spectra obtained was given in the Fig. 3. The intense peaks in FT-IR pattern observed between 1500 and 500/cm eventually shows the stretching vibrations for C=O, -C-N, -C-NH_2, -C-H, and -C=C- functional groups. The absorption band at 1514/cm represents the characteristics for N-H group of amines. The bands observed at 1645/cm and 1725/cm indicates the stretching vibrations of alkenes (-C=C-) and α, β-unsaturated aldehydes, and ketones (C=O). The absorption bands above 3411/cm and 3388/cm show the presence of O-H and N-H stretching vibrations. The absence of FT-IR bands between 2400 and 3300/cm confirms protein precipitation. FT-IR results suggest that protein nanoparticle interaction occurs by free amine groups and electrostatic interaction of carboxyl groups. Earlier reports also state that proteins stabilize nanoparticles via precipitation thereby preventing agglomeration [7].

XRD pattern

Fig. 4 has shown the typical XRD pattern of synthesized nanoparticles with different diffraction peaks in the whole spectrum of 2θ values ranging from 0° to 80°. The four intense diffraction peaks at 37.8°, 24.9°, 31.8°, and 44.2° indexed as 111, 200, 240, and 311 planes for the face centered cubic silver as per the JCPDS file No. 03-0921. The crystalline nature of the synthesized nanoparticles confirmed using Debye-Scherrer equation and the average peak is 37.8°. The broadening of Bragg's peaks around bases indicating the formation of nanosized particles. The noise peaks might be due to the crystallinity of bioorganic phase on the nanoparticle surfaces [29,37]. Thus, XRD pattern clearly shows that the synthesized SNPs were crystalline in nature similar to the earlier studies [29,37,38].

SEM imaging

The size and shape of the nanoparticles were ascertained using SEM. The air-dried powder of SNPs were prepared and subjected for analysis [39]. The obtained SEM image confirms the spherical shape of the nanoparticle and size was about 90 nm (Fig. 5). It is evident that the morphology of SNPs is spherical in agreement with the shape of SPR band in the UV-Vis spectra [40]. The particles were also found to be slightly agglomerated similar to that of nanoparticles synthesized from *Acacia nilotica* seeds [41].

***In vitro* antioxidant activity**

The *in vitro* free radical scavenging activity of the synthesized SNPs was determined using different assays. The reducing power of compounds increases analogous to their antioxidant ability. A highly stable lipophilic DPPH radical is reliable to accept hydrogen or electrons from the synthesized SNPs. Hydroxyl radical serves to be one of the potent reactive oxygen species which disrupts plasma membrane and causes cell damage. The metal chelating assay involves color reduction which in turn determines their chelating ability of synthesized nanoparticles for ferrous ions [42]. The mean IC50 value of nanoparticles synthesized from methanolic extract *D. radicosa* for scavenging DPPH and H_2O_2 free radicals; for chelating metals was shown in the Fig. 6. Percentage of inhibition increases in a dose dependent manner which is evident from

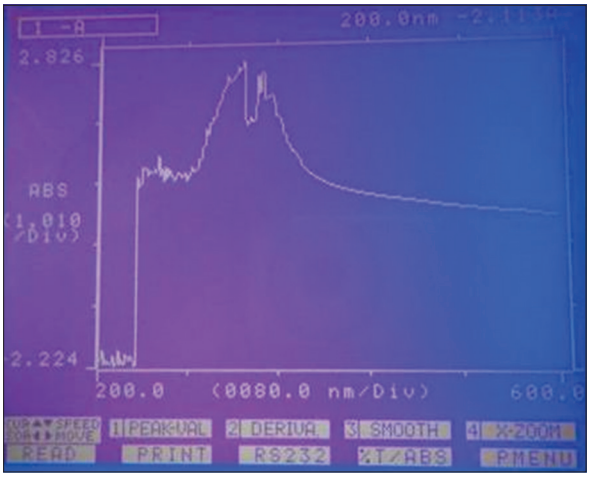

Fig. 2: Ultraviolet-visible spectra of biosynthesized silver nanoparticles

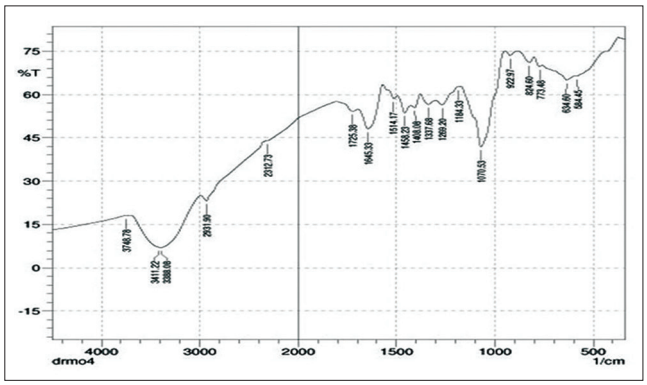

Fig. 3: Fourier transform infrared spectrum of biosynthesized silver nanoparticles

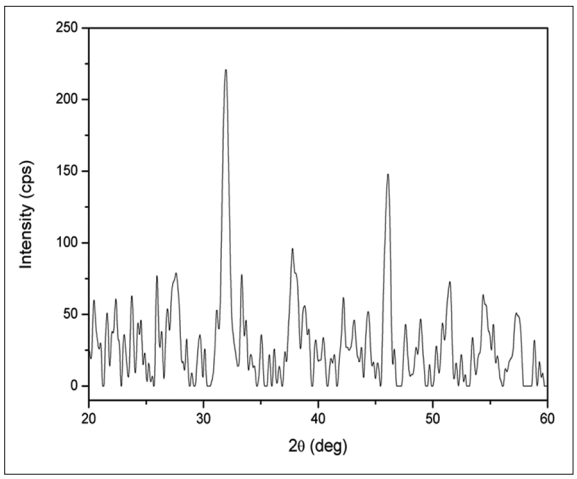

Fig. 4: X-ray diffraction pattern confirms the crystalline nature of green SNPs

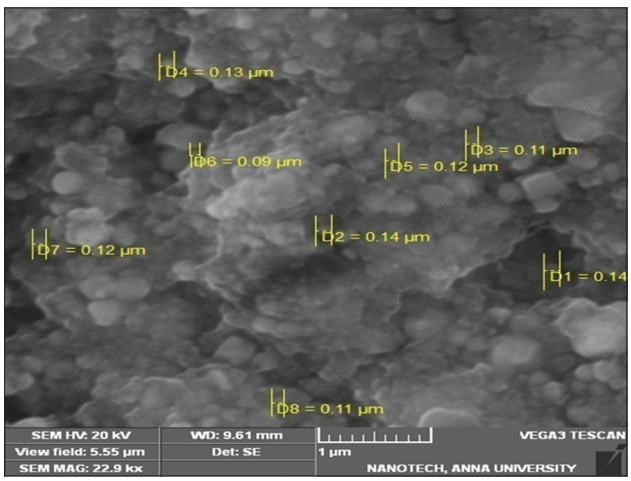

Fig. 5: Scanning electron microscopic image of spherical shaped green silver nanoparticles

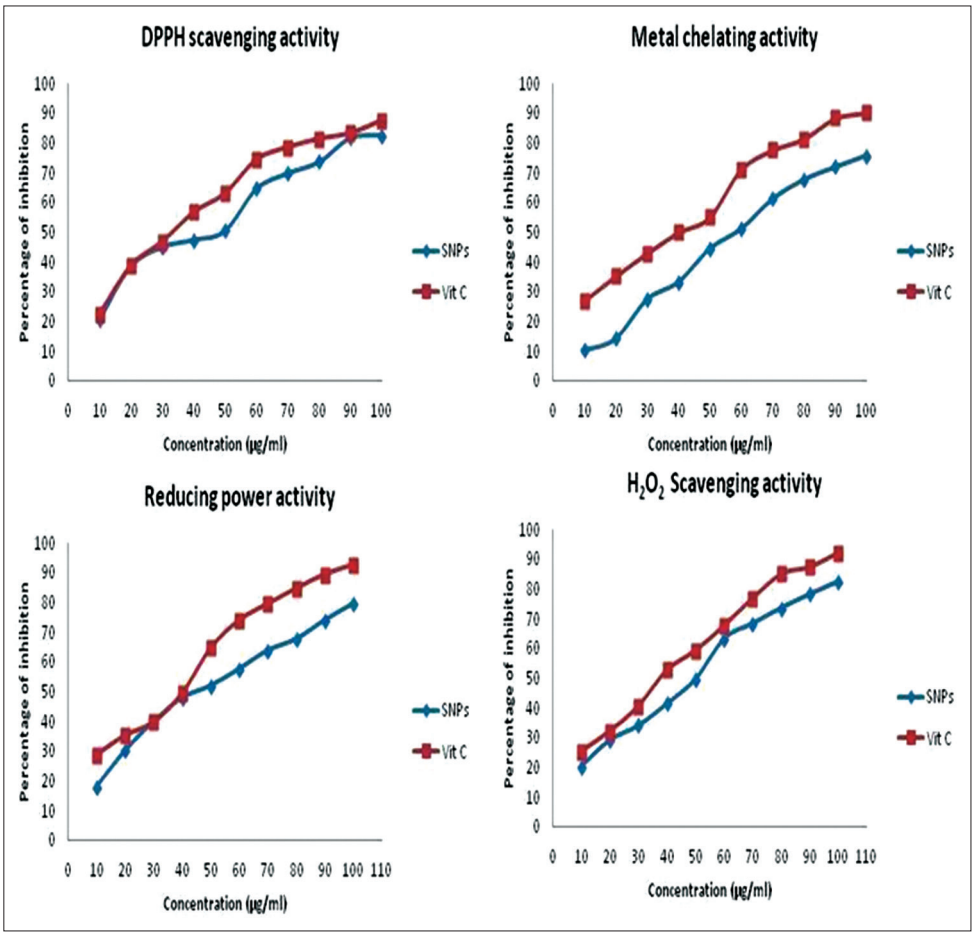

Fig. 6: *In vitro* antioxidant potential of *Digitaria radicosa* synthesized silver nanoparticles. Values are expressed as mean±standard deviation (n=3)

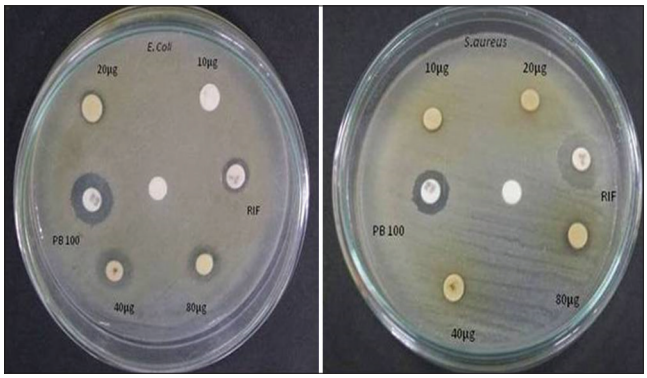

Fig. 7: The antibacterial activity of synthesized silver nanoparticles in food pathogenic microbes *Escherichia coli* and *Staphylococcus aureus*

their elevated degree of inhibition at higher concentration (100 µg/ml) compared with that of lowest concentration (10 µg/ml). The maximum percentage of inhibition of SNPs at 100 µg/ml concentration was recorded to be 82.45±0.84, 75.68±0.19, 79.4±0.7, and 82.3±0.85 for DPPH scavenging, metal chelating, reducing power, and H_2O_2 scavenging assays, respectively.

In vitro antioxidant assays exemplified that SNPs have potential scavenging activity against DPPH and hydrogen peroxide free radicals. They are also likely to possess chelating activity for ferrous ions and liable reducing power of compound. The IC50 values of Vitamin C and SNPs corresponding to DPPH radical, metal chelation, reducing power, and H_2O_2 free radical scavenging were found to be 33, 40, 41, 38 µg/ml and 46, 51, 45, 45 µg/ml, respectively. The results revealed the existence of effective radical scavenging activity by biosynthesized nanoparticles when compared with standard ascorbic acid. The antioxidant activity of biosynthesized SNPs exhibited significant dose-dependent inhibition similar to nanoparticles obtained from *Shorea roxburghii* and *Cassia tora* extracts [43,44]. Thus, the green synthesized SNPs from *D. radicosa* leaf extract act as a potent free radical scavenger and thereby establishing their therapeutic significance.

Antibacterial activity

The antibacterial activity against foodborne pathogens was evaluated by disc diffusion method through the zone of inhibition. After incubation, the zone of inhibition was measured to assess the inhibitory activity of the SNPs (Fig. 7).

SNPs exhibited a slight zone of inhibition at the concentration of 80 µgml in *S. aureus,* whereas in the case of *E. coli,* begins at 20 µg ml and the activity increases with that of increasing concentration of the SNPs. The minimal inhibitory concentration of SNPs against *S. aureus* and *E. coli* were found to be 50 µg/ml and 40 µg/ml, respectively.

Due to their limited size, nanoparticles could easily enter bacterial cells. The presence of thicker peptidoglycan layer in Gram-positive bacteria makes the inhibitory activity be minimal while compared with that of thin layered Gram-negative bacteria [45]. The SNPs supposed to penetrate their inner membrane through loss of permeability in outer membrane followed by the leakage of cellular materials. They prevent bacterial respiration and their reproduction by damaging respiratory chain dehydrogenases [46].

CONCLUSION

The investigations of the study confirm the green synthesis of stable SNPs from the methanolic extract of *D. radicosa* after 15 hrs incubation by UV-Vis spectroscopy. Through FT-IR spectroscopy, the active functional groups which may involve in the bioreduction of silver ions were identified. The crystalline nature of green SNPs was substantiated by the obtained XRD pattern. The SEM image analysis confirmed the spherical shape of the nanoparticles and 90 nm in size.

In the present investigation, the *in vitro* antioxidant potential of the SNPs was briefly illustrated. Furthermore, the biosynthesized SNPs reported to possess substantial antibacterial activity toward both strains and thereby proven to be a potent antibacterial agent which can be further used for coating on food packaging to prevent contamination of foodborne pathogens. Thus, the green synthesis of SNPs from *D. radicosa* is found to be cost effective, eco-friendly and has a multipotent therapeutic value.

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