

# Biological activities of synthesized silver nanoparticles from *Cardiospermum halicacabum* L.

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**Abstract.** The present study focuses on the green synthesis of silver nanoparticles using aqueous extract of *Cardiospermum halicacabum*. AgNPs were confirmed by UV–Visible spectrophotometer analysis showed SPR at 424 nm. FT-IR analysis revealed biomolecules capping of the AgNPs. XRD pattern of synthesized AgNPs was found in face-centered-cubic crystal structure and average crystal size was 23 nm. SEM analyses of the synthesized AgNPs determine the spherical shape and EDX spectra confirmed the presence of silver ions. DLS studies revealed that the synthesized AgNPs showed the average size as 74 nm and Zeta potential value of AgNPs was  $-34$  mv. The *C. halicacabum* leaf extract synthesized AgNPs efficiency were tested against different bacterial pathogens MTCC-426 *Proteus vulgaris*, MTCC-2453 *Pseudomonas aeruginosa*, MTCC-96 *Staphylococcus aureus*, MTCC-441 *Bacillus subtilis* and MTCC-735 *Salmonella paratyphi*, and fungal pathogens *Alternaria solani* and *Fusarium-oxysporum*. The antioxidant ability of the AgNPs was tested and the results showed significant DPPH, hydroxyl and superoxide, radical scavenging activities.

**Keywords.** *Cardiospermum halicacabum* L.; silver nanoparticles; antimicrobial activity; antioxidant activity.

## 1. Introduction

The modern science has started exploring the term ‘nano’ in 21st Century. Ayurvedic medicinal systems used noble metals such as gold, silver, etc. Nanoparticles are being considered to be the fundamental building blocks of nanotechnology. Nanotechnology is interdisciplinary which includes physics, chemistry, biology, material science and medicine. Nanotechnology is a universal term for the creation, manipulation and application of structures in the nanometre size ranging between 1 and 100 nm [1]. The use of green chemistry (plants) approaches in nanobiotechnology is rapidly gaining much importance due to easy development of clean, nontoxic and environmental friendly procedures for synthesis of nanoparticles. Nanomedicine is being applied in monitoring, repair, construction and control of human biological systems at the molecular level using engineered nanodevices and nanostructures [2]. Among diverse nanomaterials, noble metals (AgNPs) are one of the most marketable nanomaterials, because they are extensively applied as biocides for their strong antimicrobial activity. The AgNPs also possess high surface area which increases the energy and enhancing the antimicrobial activity of nanoparticles [3]. Nanocomposites with antimicrobial function are highly useful for the minimization of the growth of contaminant microorganisms during the processing or storage of food and in that way help

in the extension of shelf life and improvement of food safety [4]. Currently, silver nanoparticles were used into a wide range of medical devices including bone cement, surgical instruments, surgical masks, etc. Synthesis of noble metal nanoparticles, in particular, silver nanoparticles (AgNPs) using plant gum has become a major focus for researchers due to their simplicity of procedures, stability and their potential applications in chemical sensing, biological imaging, antimicrobial, gene silencing and drug delivery [5]. Recently, several studies have reported natural polymers such as chitosan, starch and tannic acid as reducing agents for the synthesis of silver and gold nanoparticles [6,7]. Nanotechnology is the science and engineering of creating structures in the nanometre scale which have been applied in the field of drug delivery, catalysis, optical devices and nanoelectronics. Although nanomaterials may be synthesized using chemical approaches, it is now possible to use biological materials for the same. Recent studies on the use of microorganisms in the synthesis of nanoparticles are a relatively new and exciting area of research with considerable potential for development [8].

The synthesized AgNPs were found to show potential antimicrobial activity against multidrug resistant Gram-positive (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-negative (*Klebsiella pneumoniae* and *Staphylococcus aureus*) from clinically isolated human pathogens [9]. Currently they are used for developing stable silver nanoparticles and their biomedical applications in the areas of microbial resistance, wound healing, surface enhanced Raman

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scattering and metal enhanced fluorescence [10]. Synthesized AgNPs were tested for antioxidant (DPPH, hydrogen peroxide scavenging assay, nitric oxide radical scavenging assay, reducing power assay) and antibacterial (*S. typhi*, *P. aeruginosa*, *Basillus subtilis* and *M. luteus*) activities. It showed good antioxidant activity as compared to butylated hydroxytoluene (BHT) and ascorbic acid as standard antioxidant. *H. isora* root extract can be used efficiently in the production of potential antioxidant and antibacterial AgNPs for commercial application [11]. Most important application of using synthesized AgNPs in medical industry is making tropical ointments to prevent infection against burn and open wounds [12]. Various methods were used to synthesize AgNPs such as sol-gel processes, chemical precipitation, reverse micelle method, hydrothermal method, microwave, chemical vapour deposition and biological methods [13]. Hence, the present study focussed on the synthesized AgNPs from *C. halicacabum* leaf extract to evaluate the characterization (UV-Visible spectroscopy, FT-IR, XRD, SEM, EDX, DLS, Zeta potential), antimicrobial and antioxidant activities.

## 2. Materials and methods

### 2.1 Sample preparation

Healthy leaves of *C. halicacabum* were collected from Botanical Garden Department of Plant Science, Tiruchirappalli, Tamilnadu, India. Collected leaves were washed thoroughly 2–3 times under running tap water followed by sterile distilled water. After that, leaves were shade dried at room temperature for two weeks, then powdered using kitchen blender. The leaf broth solution was prepared using 10 g of finely powdered sample in 100 ml distilled water (250 ml Erlenmeyer flask) and then boiling the mixture at 60°C for 30 min. The extract obtained was filtered through a Whatman no.1 filter paper and it was collected in a 250 ml Erlenmeyer flask and stored at 4°C for further use.

### 2.2 Green synthesis of AgNPs using leaf extract

AgNPs were synthesized by the modification procedure of ref. [14]. The AgNPs were synthesized using 10 ml of *C. halicacabum* leaf extract mixed with 90 ml of 1 mM aqueous solution of silver nitrate ( $\text{AgNO}_3$ ). After 30 min, reduction of silver ions was observed by the change of the solution colour from yellow to brown as a confirmation of synthesis of AgNPs and incubated the mixture at room temperature for 16 h. Then, the AgNPs formed were collected by centrifugation at 5000 rpm for 15 min. The collected pellet was washed three times with distilled water, transferred to a Petri plate and dried in hot air oven 80°C.

### 2.3 Characterization of synthesized AgNPs from *C. halicacabum* leaf extract

**2.3a UV-Visible spectroscopy:** Biosynthesized AgNPs were analysed by using UV-Vis spectrum (Lambda 35).

UV-Vis spectral analysis was observed at a resolution of 300–700 nm.

**2.3b Fourier transform infrared spectroscopy (FT-IR):** FT-IR analysis was carried out by FT-IR spectrophotometer in the range of 4000–400  $\text{cm}^{-1}$ . The synthesized AgNPs sample was dried and grinded with KBr pellets and analysed. The various modes of vibrations were identified to determine the different functional groups present in AgNPs.

**2.3c Scanning electron microscope (SEM):** The particles morphology characterization of shape and size (nanometre to micrometre scale) of synthesized AgNPs were measured by scanning electron microscope (SEM) JEOL 6360 TESCAN 10 kV machine. In this experiment, on the carbon-coated copper grid, thin film of the sample was prepared by dropping very little amount of the sample. The extra solution was removed by using blotting paper and the film on the SEM grid was allowed to dry for 5 min under mercury lamp for observation of shape of the particle.

**2.3d Energy-dispersive X-ray spectroscopy (EDX):** EDAX was carried out to confirm the presence of elemental silver in the biosynthesized *C. halicacabum* using synthesized AgNPs and the drop-coated biofunctionalized AgNPs of *C. halicacabum* on to carbon film and analysed using EDAX JEOL 6360 TESCAN.

**2.3e X-ray diffraction (XRD):** The purified silver nanoparticles were freeze-dried and analysed to evaluate their crystalline structure and to determine mean size in diffractometer (Bruker D8 Advance, Germany) equipped with  $\text{Cu K}\alpha$  ( $\lambda = 1.5418 \text{ \AA}$ ) X-ray radiation source, in the  $2\theta$  range from 20° to 80°. The mean sizes of these silver nanoparticles were calculated by using Scherrer equation.

$$D = \frac{k\lambda}{\beta \cos \theta},$$

where  $k$  is the shape factor (0.94),  $d$  the mean diameter of the nanoparticle,  $\lambda$  the wavelength of the X-ray,  $\beta$  the angular width at FWHM of the X-ray diffraction peak at Bragg's diffraction angle  $\theta$ .

**2.3f Dynamic light-scattering measurements (DLS):** Size distribution of bio-reduced silver nanoparticles was measured using DLS (Zetasizer Nano ZS ZEN3600, Malvern, UK). The mean size of particles inside the sample is obtained with this measurement along with correlation between the number of particles of a particular size vs. the size of the AgNPs.

**2.3g Zeta potential:** Zeta-potential measurements were done with a Zetasizer Nano ZS (Malvern Instruments) in a disposable cell at 25°C using Zetasizer 7.01 software. Zeta-potential measurements were used to study AgNPs stability.

#### 2.4 Antimicrobial activity of synthesized AgNPs from *C. halicacabum* leaf extract

Antimicrobial activity of synthesized AgNPs was determined using disc diffusion method [14]. The overnight inoculated bacterial cultures (MTCC-426 *Proteus vulgaris*, MTCC-2453 *Pseudomonas aeruginosa*, MTCC-96 *Staphylococcus aureus*, MTCC-441 *Bacillus subtilis* and MTCC-735 *Salmonella paratyphi*) and four days-inoculated fungal cultures (*Alternaria solani* and *Fusarium oxysporum*) were spread over the freshly prepared nutrient agar and potato dextrose agar plates. The 6-mm sterile discs (Himedia) were kept on centre of the plate and different concentrations of AgNPs (20, 40, 60  $\mu\text{g ml}^{-1}$ ) were poured on each disc. Streptomycin, ampicillin and ketoconazole disc (reference disc) were kept on the same plate and incubated at 37°C. The antimicrobial property of AgNPs was determined by measuring the zone of inhibition around the discs in diameter (millimetre) after incubation.

#### 2.5 Antioxidant activity of synthesized AgNPs from *C. halicacabum* leaf extract

**2.5a DPPH radical scavenging activity:** The free radical scavenging activity of the synthesized AgNPs was determined by using DPPH method [15]. Briefly, DPPH solution of 0.1 mM was prepared in 95% methanol and 1 ml of this solution was added to 3.0 ml of synthesized AgNPs solution of 10–80  $\mu\text{g ml}^{-1}$ . The solution was incubated for 30 min at dark conditions at room temperature and absorbance was measured at 517 nm using a UV–Vis spectrophotometer. Ascorbic acid was used as a standard. The experiment was repeated triplicate and the DPPH scavenging activity was calculated by

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100,$$

where  $A_0$  is the absorbance of the control and  $A_1$  the absorbance of the AgNPs solution.

**2.5b Hydroxyl radical scavenging activity:** Hydroxyl radical scavenging activity was measured by the salicylic acid method [16]. The synthesized AgNPs solution at different concentrations (10 to 80  $\mu\text{g ml}^{-1}$ ) was dissolved in 1 ml of distilled water. One millilitre of AgNPs mixed with 1 ml of 9 mM salicylic acid, 1 ml of 9 mM ferrous sulphate and 1 ml of 9 mM hydrogen peroxide. The reaction mixture was incubated at 37°C in a water bath for 60 min after incubation. The absorbance of the mixtures was measured at 510 nm using a UV–Vis spectrophotometer. Ascorbic acid was used as a standard and a control was prepared without adding AgNPs. The experiment was repeated in triplicate and the hydroxyl scavenging activity was calculated by

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100,$$

where  $A_0$  is the absorbance of the control and  $A_1$  the absorbance of the AgNPs solution.

**2.5c Super oxide scavenging activity by alkaline DMSO method:** Super oxide scavenging activity is generated by the addition of sodium hydrochloride using air saturated DMSO method [17] with some modifications. The reaction mixture contains 0.1 ml of nitro blue tetrazolium (1  $\text{mg ml}^{-1}$ ), 0.3 ml of AgNPs at different concentration of (10 to 80  $\mu\text{g ml}^{-1}$ ) and 1 ml of alkaline DMSO (1ml of alkaline DMSO containing sodium hydrochloride 5 mM in 0.1 ml of water) to prepare final volume of 1.4 ml. The absorbance was measured as 560 nm using a UV–Vis spectrophotometer. Ascorbic acid was used as a standard and a control was prepared without adding AgNPs. The experiment was repeated in triplicate and the super oxide scavenging activity was calculated by

$$\% \text{ Inhibition} = \frac{A_1 - A_0}{A_1} \times 100,$$

where  $A_1$  is the absorbance of the AgNPs solution and  $A_0$  the absorbance of the control.

### 3. Result and discussion

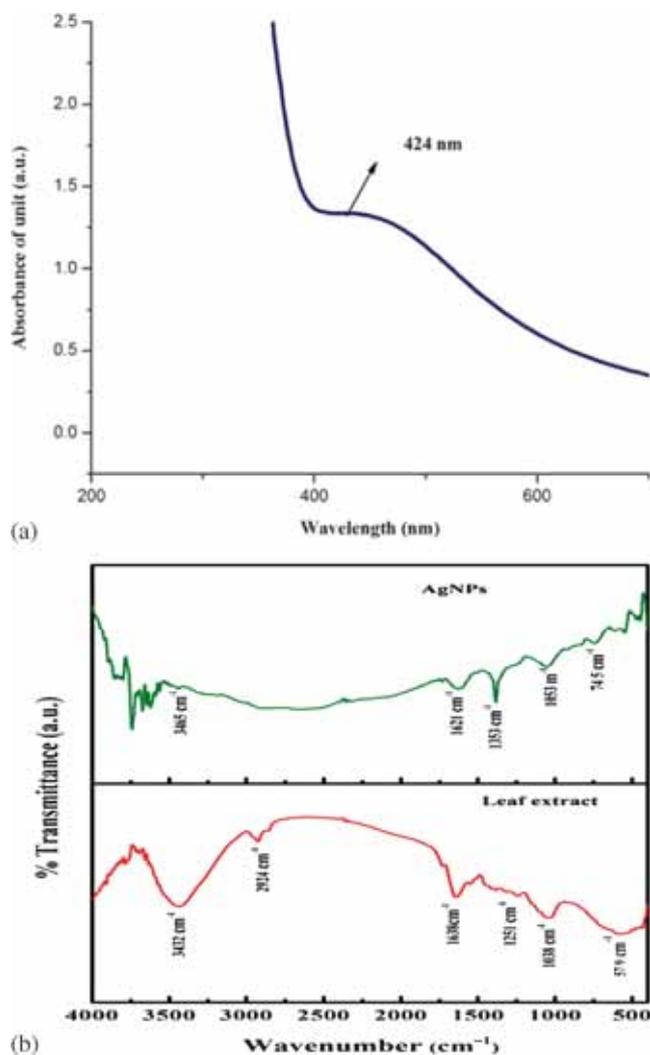
#### 3.1 Green synthesis of AgNPs using leaf extract

After the addition of 10 ml aqueous leaf extract of *C. halicacabum* into 90 ml of 1 mM silver nitrate ( $\text{AgNO}_3$ ), the reaction mixture exhibits yellowish to brown colour in aqueous solution due to excitation of surface plasmon vibrations (SPR) after 16 h incubation. The colour appearances in the reaction mixture (yellowish to brown colour) confirms the generation of stable AgNPs [18–20].

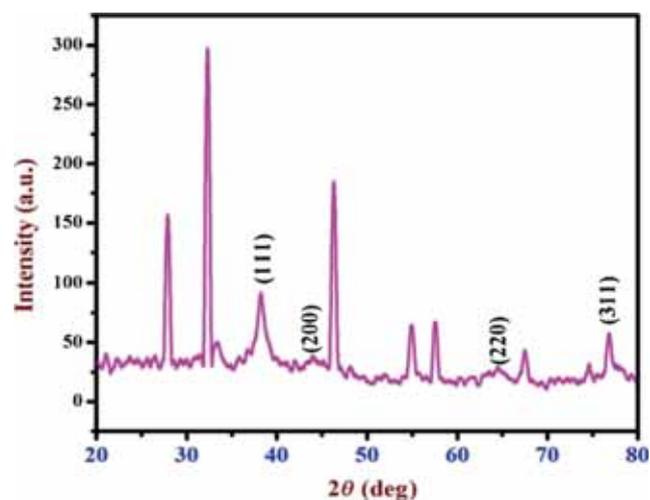
#### 3.2 Characterization of synthesized AgNPs from *C. halicacabum* leaf extract

**3.2a UV–Vis spectroscopy:** Synthesis of AgNPs by reducing aqueous metal ions during exposure of *C. halicacabum* leaves extract was monitored at different time intervals by using UV–Vis spectrophotometer. The maximum absorbance was noticed at 424 nm which clearly shows the generation of AgNPs (figure 1a). It also confirmed that incubation time has direct correlation with size, shape dispersity and synthesis rate of AgNPs. Similar phenomenon has been reported earlier, in which AgNPs synthesized at optimal reaction conditions produces an intense absorbance spectra at 420 nm due to the collective oscillations of surface electrons [21].

**3.2b Fourier transform infrared spectroscopy (FT-IR):** FT-IR transmittance of synthesized AgNPs and *C. halicacabum* leaf extract shows the possible biomolecules responsible for capping and efficient stabilization of nanomaterials. The FT-IR spectrum of synthesized AgNPs shows intense bands at 3465, 1621, 1353, 1053 and 745  $\text{cm}^{-1}$ , respectively, these intense bands correspond to the strong stretching free hydroxyl O–H of alcohols and phenols,  $\text{C}=\text{C}$  alkenes, C–H rock alkanes, C–O strong stretching ethers, C–H strong



**Figure 1.** (a) UV-Vis spectrophotometer analysis of synthesized *C. halicacabum* AgNPs. (b) FT-IR spectral analysis of synthesized *C. halicacabum* AgNPs.



**Figure 2.** XRD pattern analysis of synthesized *C. halicacabum* AgNPs.

**Table 1.** Mean size of synthesized *C. halicacabum* AgNPs estimated by Scherrer equation.

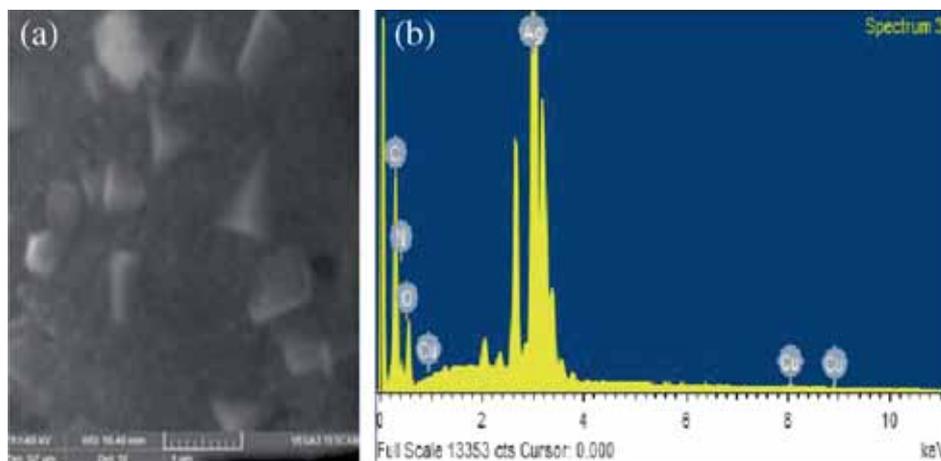
Peak (hkl)	2θ	d spacing (Å)	FWHM	Lattice constant (Å)	Crystallite size (nm)
111	38.249	2.3512	1.044	4.1075	22.4

bending alkene and then leaf extract shows intense bands at 3432, 2924, 1638, 1251, 1038 and 578 cm<sup>-1</sup>, respectively, these intense bands correspond to the strong stretching free hydroxyl O-H of alcohols and phenols, C-H strong stretching alkanes, C=C strong stretching alkene, C-N variable stretching amine, C-O weak stretching ethers, C-Br stretching alkyl halides (figure 1b). Variation in transmittance level clearly reveals that the metal nanoparticles were functionalized with plant biomolecules. This result correlates with the result of Jacob *et al* [22] where O-H stretches at 3397 cm<sup>-1</sup> demonstrating the presence of polyphenols as capping agents on the surface.

**3.2c X-ray diffraction (XRD):** The XRD pattern of synthesized AgNPs using *C. halicacabum* leaf extract is shown in figure 2 and table 1. In this pattern, four 2θ peaks are identified at 38.12, 44.30, 64.45 and 77.41°, which are corresponding to (111), (200), (220) and (311) planes of face-centered-cubic crystal structure of AgNPs (JCPDS card no: 89-3722). For cubic system, the calculated lattice constant values (a=b=c= 4.1075 Å). Using the Scherrer formula, the average crystallite size found to be 22.4 nm (table 1). They reported that the synthesized AgNPs were crystalline in nature and the calculated average crystallite size is 13 nm [23]. Some additional peaks were reported the presence of bioorganic matters and capping agent for AgNPs formation [24,25]. This result proves that the reduced silver ions were crystalline in nature and also found to be identical with those reported for the standard silver metal (Ag<sup>0</sup>) [26].

**3.2d Scanning electron microscope (SEM):** SEM micrographs of synthesized AgNPs at different magnifications such as 500 nm, 5 and 10 μm display uniformly distributed spherical AgNPs with the size of below 100 nm. Although agglomeration of nanoparticles were noticed at some places, there is no direct fusion was seen, it is mainly because of the presence of capping agents (figure 3a). This result highly agree with many earlier reports where biosynthesized AgNPs using different plant entities have shown the spherical-shaped particles [27–29].

**3.2e Energy-dispersive X-ray spectroscopy (EDX):** EDX spectroscopy shows strong metal signal for Ag which confirms the presence of pure Ag metal as the major constituent without impurities. The appearance of optical adsorption peak at ~14.140 keV is the characteristic for the adsorption



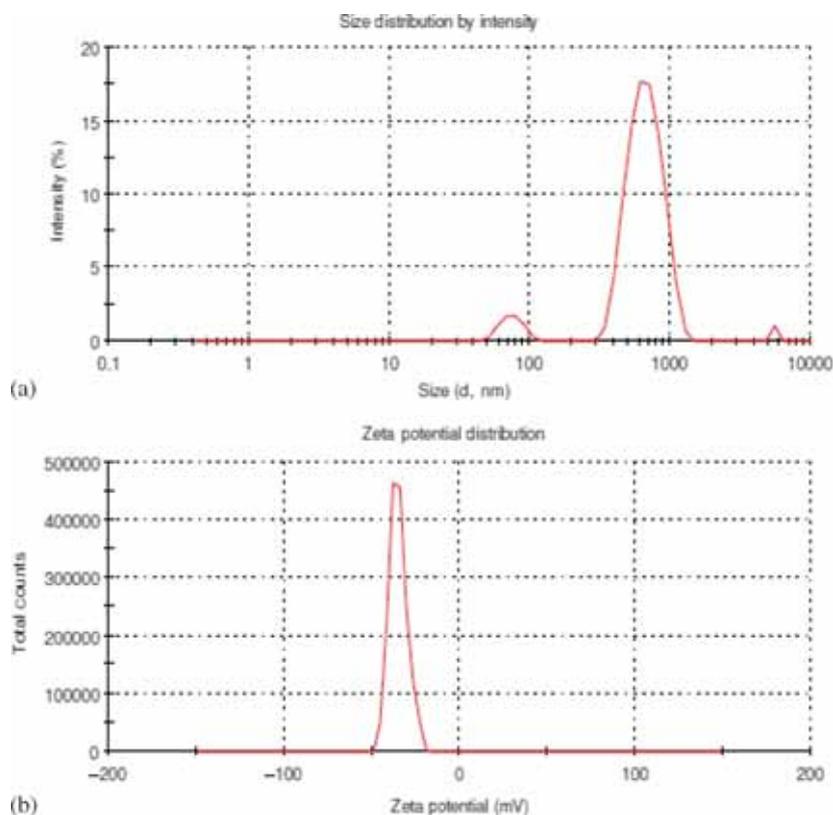
**Figure 3.** (a) SEM and (b) energy-dispersive X-ray spectroscopy.

**Table 2.** EDX analysis of composition from synthesized *C. halicacabum* AgNPs.

Elements	Weight%	Atomic%
C K	15.04	30.35
N K	19.69	34.07
O K	16.16	24.49
Cu K	0.41	0.16
Ag L	48.70	10.94
Total	100.00	100.00

of Ag nanocrystallites. The small quantities of additional elements, including C, N, O, Cu and Ag were associated with *C. halicacabum* leaf extract reducing the silver ions (figure 3b and table 2). EDX analysis cannot distinguish between elemental Ag and Ag atoms in other compounds. This result only illustrate the increase of the Ag concentration in the nano-films. Increased intensity of Ag line is attributed to the increased content of silver in the nano-film [30].

**3.2f Dynamic light-scattering measurements (DLS):** Dynamic light scattering (DLS) analysis determined the



**Figure 4.** (a) DLS size distribution pattern analysis of synthesized AgNPs using *C. halicacabum* leaf extract. (b) Zeta potential stabilization pattern analysis of synthesized AgNPs using *C. halicacabum* leaf extract.

**Table 3.** Inhibition zones of synthesized *C. halicacabum* AgNPs against different bacterial pathogens.

Microorganisms	Zone of inhibition (mm)			
	Different concentrations of synthesized AgNPs ( $\mu\text{g ml}^{-1}$ )			
	Streptomycin and ampicillin $10 \text{ mg ml}^{-1}$	20 $\mu\text{g}$	40 $\mu\text{g}$	60 $\mu\text{g}$
Control	10.00 $\pm$ 0.00			
<i>Proteus vulgaris</i>		10.66 $\pm$ 0.66	13.00 $\pm$ 0.00	15.33 $\pm$ 0.88
Control	14.00 $\pm$ 0.00			
<i>Salmonella paratyphi</i>		10.00 $\pm$ 0.00	16.33 $\pm$ 0.88	21.00 $\pm$ 0.57
Control	14.00 $\pm$ 0.00			
<i>Pseudomonas aeruginosa</i>		10.00 $\pm$ 0.00	12.33 $\pm$ 0.33	15.00 $\pm$ 0.00
Control	14.00 $\pm$ 0.00			
<i>Staphylococcus aureus</i>		10.00 $\pm$ 0.00	12.33 $\pm$ 0.33	15.00 $\pm$ 0.00
Control	17.00 $\pm$ 0.00			
<i>Basillus subtilis</i>		10.00 $\pm$ 0.00	12.33 $\pm$ 0.33	16.00 $\pm$ 1.52

**Table 4.** Inhibition zones of synthesized *C. halicacabum* AgNPs against different fungal pathogens.

Microorganisms	Zone of inhibition (mm)			
	Different concentrations of synthesized AgNPs ( $\mu\text{g ml}^{-1}$ )			
	Ketoconazole $10 \text{ mg ml}^{-1}$	20 $\mu\text{g}$	40 $\mu\text{g}$	60 $\mu\text{g}$
Control	3.66 $\pm$ 3.66			
<i>Alternaria solani</i>		3.33 $\pm$ 3.33	10.66 $\pm$ 0.66	8.00 $\pm$ 4.04
Control	10.00 $\pm$ 0.00			
<i>Fusarium oxysporum</i>		13.33 $\pm$ 0.33	9.33 $\pm$ 1.76	17.66 $\pm$ 2.96

average particles size distribution profile of synthesized nanoparticles [31]. In our result, we noticed that the synthesized AgNPs were polydispersed in nature with the average size of 74 nm (figure 4a). Few large-sized particles appeared in DLS result was mainly due to the agglomeration AgNPs in the solution. The intensity ratio between the two peaks that correspond to the small particles constituted only 1–3% of the total intensity, while the rest corresponded to the large particles [32].

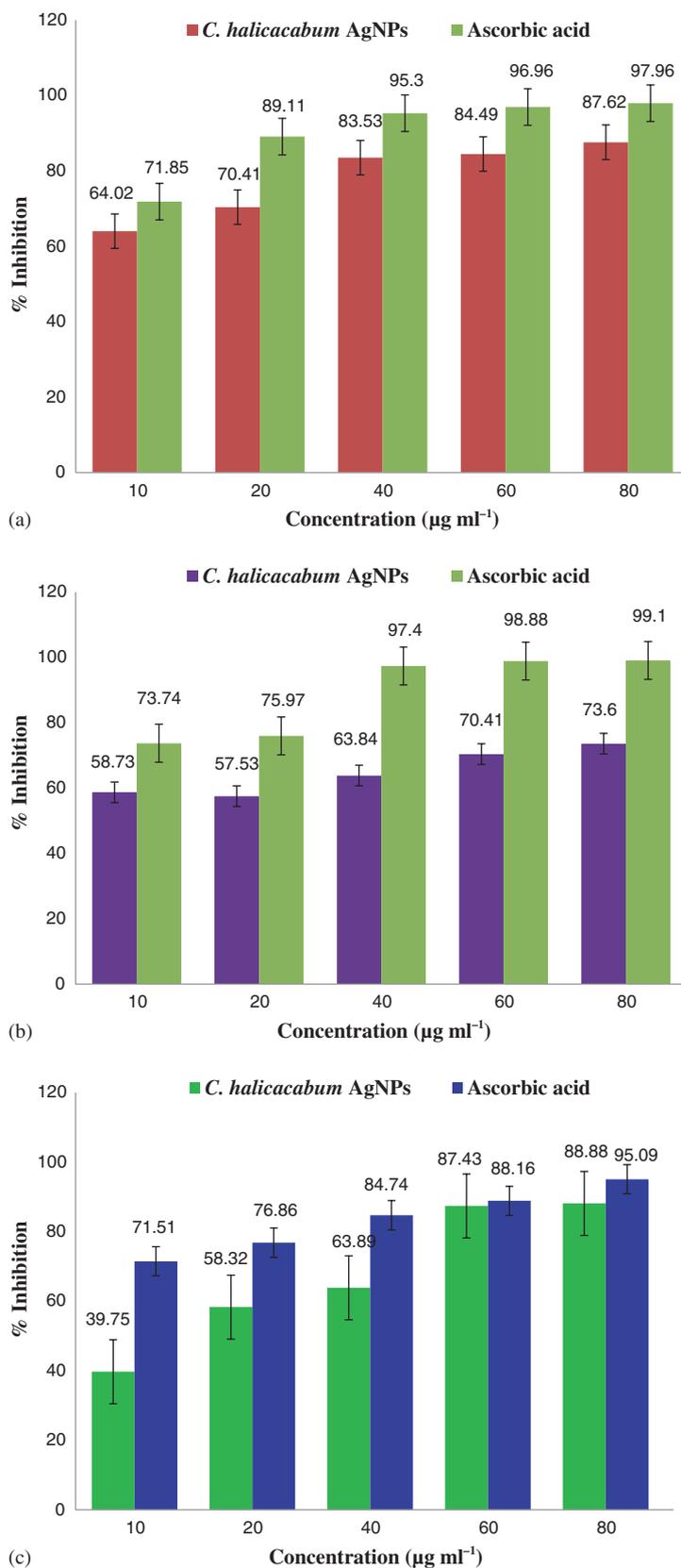
**3.2g Zeta potential:** A zeta potential was used to determine the surface potential of synthesized AgNPs solutions and their stability. The zeta potential was measured as an insinuation of potential stability of colloid. It should be noted that the particles with zeta potential values more positive than +30 mV or more negative than –30 mV are considered to be stable [33]. In our result, the zeta value of synthesized AgNPs was found at –34.8 mV with a peak area of 100% intensity. This value depicts that the generated AgNPs were highly stable in colloidal solution (figure 4b).

### 3.3 Antimicrobial activity of synthesized AgNPs from *C. halicacabum* leaf extract

The inhibitory action of synthesized AgNPs and standard antibiotic drugs viz. streptomycin, ampicillin and ketoconazole

against different pathogens were measured and tabulated in tables 3 and 4. After 24 h (bacteria) and 3 days (fungi) of incubation period, a clear zone of inhibition (mm) was measured which ranges from 10.00  $\pm$  0.00 to 21.00  $\pm$  0.57 and from 3.33  $\pm$  3.33 to 17.66  $\pm$  2.96 for bacteria and fungi, respectively. Biological synthesis of metal NPs is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phytotoxic effects [34]. It is suggested that positively charged nanoparticles will undergo electrostatic interaction with negatively charged cell surface and alter the membrane permeability. This leads to the entry of nanoparticles into cytoplasm and cause cytotoxicity [35]. Recently, AgNPs have been synthesized from various biomasses and their antibacterial potential has been evaluated against different pathogenic bacteria [36]. A similar kind of inhibition was reported in *Sesuvium portulacastrum* [18,19,37] against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E. coli*, respectively. Inhibitory potential of nanoparticles have been reported as concentration-dependent as well as it was found to be variable with respect to the bacterial strains used [36].

Three different mechanisms of antimicrobial action of AgNPs have been proposed: First AgNPs attach to the surface of the cell membrane and disturb its power functions, such as permeability and respiration. Plasmolysis (cytoplasm



**Figure 5.** (a) DPPH radical scavenging activity of *C. halicacabum* AgNPs and ascorbic acid. (b) Hydroxyl radical scavenging activity of *C. halicacabum* AgNPs and ascorbic acid. (c) Super oxide radical scavenging activity of *C. halicacabum* AgNPs and ascorbic acid.

separated from bacterial cell wall) in *P. aeruginosa* and the inhibition of bacterial cell wall synthesis in *S. aureus* bacteria were reported by Song *et al* [38]. Second, AgNPs are able to penetrate the bacteria and cause further damage, possibly by interacting with sulfur- and phosphorus-containing compounds such as DNA. Third, AgNPs release silver ions, which make an additional contribution to the bactericidal effect [39].

### 3.4 Antioxidant activity of synthesized AgNPs from *C. halicacabum* leaf extract

**3.4a DPPH radical scavenging activity:** The scavenging ability of synthesized AgNPs is shown in figure 5a. The antioxidant efficacy of synthesized AgNPs from *C. halicacabum* leaf extract was quantified spectrophotometrically by changing the DPPH colour from purple to yellow. The DPPH was considered to be a model of lipophilic radical. A chain reaction in lipophilic radicals was initiated by lipid auto-oxidation. Being a stable free radical, DPPH is regularly used to determine radical scavenging activity of natural compounds. In its radical form, DPPH absorbs at 517 nm and its absorbance decreases upon reduction with an antioxidant [40]. The IC<sub>50</sub> value for DPPH scavenging activity of synthesized AgNPs was found to be 4.30 µg ml<sup>-1</sup>, while the IC<sub>50</sub> value for ascorbic acid was 4.23 µg ml<sup>-1</sup>, respectively.

**3.4b Hydroxyl radical scavenging activity:** The scavenging ability of synthesized AgNPs was measured through hydroxyl radical scavenging assay as shown in figure 5b. The radical scavenging capacity of the sample might be attributed to the phenolic compounds in the sample. In this present study, the percentage inhibitions were increased with increasing concentrations of the synthesized nanoparticles. The IC<sub>50</sub> value for hydroxyl radical scavenging activity of synthesized AgNPs was found to be 5.08 µg ml<sup>-1</sup>, while the IC<sub>50</sub> value for ascorbic acid was 5.91 µg ml<sup>-1</sup>, respectively. In an earlier report, *S. torvum* fruits were used for the synthesis of AuNPs and AgNPs, further the hydroxyl scavenging activity site-specific hydroxyl (·OH) radical deoxyribose degradation inhibition by gold and silver nanoparticles in dose dependent manner was observed. Synthesized nanoparticles with large surface area (spherical in shape) can easily accept electrons from ·OH radical. When compared to gold nanoparticles, hydroxyl scavenging activity inhibition (68.8%) of silver nanoparticles exhibit (50%) less inhibition due to the large surface area [41]. Takeki Hamasaki *et al* [42] was reported that synthesized Pt NPs sizes tested from 1 to 5 nm, 1 nm Pt NPs showed the highest O<sub>2</sub><sup>-</sup> scavenging ability.

**3.4c Super oxide scavenging activity by alkaline DMSO method:** The scavenging ability of synthesized AgNPs was confirmed by super oxide scavenging assay as shown in figure 5c. Superoxide (O<sub>2</sub><sup>-</sup>) radicals easily react with DNA and protein which necessitate their immediate clearance in living systems. Fluorescent light illumination of serum bovine milk was generated a superoxide anion [43] and superoxide radical inhibition has been reported for

platinum and selenium nanoparticles [44]. The potential superoxide scavenging activity of gold and silver nanoparticles reported earlier by ref. [41]. Superoxide radicals are known to be very harmful to the cellular component. These were formed by alkaline DMSO which reacts with NBT to produce coloured diformazan. The synthesized AgNPs scavenges super oxide radicals and thus inhibits diformazan formation illustrates increase scavenging of superoxide radicals in dose-dependent manner due to the scavenging ability of the *C. halicacabum* synthesized AgNPs IC<sub>50</sub> value is 15.59 µg ml<sup>-1</sup> and IC<sub>50</sub> value of ascorbic acid is 3.24 µg ml<sup>-1</sup> accordingly.

## 4. Conclusion

In this study, AgNPs were synthesized successfully using the leaf extract of *C. halicacabum* as a novel bioreductant. Plant extract being very eco-friendly and cost effective can be used for the large scale synthesis of AgNPs in nanotechnology processing industries. The present study synthesized AgNPs from *C. halicacabum* leaf extract seem to be promising and effective antibacterial agent against bacterial and fungal strains. On the other hand, it also possesses stupendous antioxidant activity which can be useful for the development of a new drug for biomedical applications. This biological chemistry approach towards the synthesized AgNPs is highly essential effort being addressed in nanomedicine because of its varied advantages.

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