

Antibacterial Activity of Green Synthesis Silver Nanoparticles Using Some Wild Edible Plants Commonly Used in Al Baha, Saudi Arabia

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How to cite this paper: Al-Ghamdi, A.Y. (2018) Antibacterial Activity of Green Synthesis Silver Nanoparticles Using Some Wild Edible Plants Commonly Used in Al Baha, Saudi Arabia. *Advances in Microbiology*, 8, 938-949.

<https://doi.org/10.4236/aim.2018.812063>

Received: November 3, 2018

Accepted: December 14, 2018

Published: December 17, 2018

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Abstract

In the present study, aqueous extract of *Cissus rotundifolia* (Wild edible plants) was used as a reducing and capping agent in the formation of silver nanoparticles (AgNPs). UV-visible spectroscopy (Uv-Vis) was used to monitor the formation of AgNPs in the aqueous medium. The green-prepared AgNPs investigated using Fourier-transform infrared spectroscopy (FTIR) and X-ray powder diffraction (XRD). The morphology and size of the benign silver nanoparticles were carried out by the transmission electron microscope (TEM) and field emission scanning electron microscope (FE-SEM). The susceptibility of bacteria strains against the green synthesis AgNPs was determined using the disk diffusion method. The microorganisms employed were *E. coli*, *K. pneumoniae*, *B. cereus*, *S. aureus*, *C. lbicans* and *Aspergillus*. The results showed the characteristic surface plasmon resonance peak of the AgNPs appeared at approximately 418 - 446 nm. XRD revealed peaks at 38.2, 44.16, 64.24 and 77.22 θ , and the intensity of these peaks enhanced when using microwave curing compared to ambient temperature. SEM and TEM results showed that the silver nanoparticles have a spherical shape and the particle size for samples is less than 37 nm. FTIR spectroscopy measurements showed the binding of organic compounds on the surface of the silver nanoparticles. The highest antibacterial activity was enhanced with increasing of AgNPs dose and with increasing of extract ration against most of microorganisms.

Keywords

Green Synthesis, AgNPs, FTIR, XRD, SEM-EDX & TEM, Biological Activity

1. Introduction

Wild edible plants are growing freely in the wild habitat without any agricultural

treatments [1]. These plants are rich in minerals, vitamins, dietary fiber, fatty acids and amino acids [2]. However, the presence of anti-nutritional principles in some species of wild plants, such as phytic acid, tannins, saponins, alkaloids and oxalates, can limit their exploitation [3]. *Cissus rotundifolia* is a climbing or prostrate shrub found throughout Africa, Egypt and the Arabian Peninsula and is used as a vegetable [4] [5]. *C. rotundifolia* (Forsk) Vahl is known as a common Arabian Wax Cissus. This wild plant is commonly used as food thickeners and their leaves only are widely consumed after cooking to prepare various dishes according to traditional dietary culture of locals. Tubers of *C. rotundifolia* are added to food as a flavoring agent [6]. Korish [7] evaluated the nutrition of *C. rotundifolia* leaves. They contain an appreciable amount of protein (high level of essential amino acids), fat (about 55% unsaturated fatty acids), crude fiber and minerals. The anti-nutritional factors: oxalate, phytate, tannins and cyanogenic glycosides, are present at very low concentrations. *C. rotundifolia* from Africa and Asia showed anti-diabetic [8] as well as anti-parasitic properties [9]. It has minor economic importance as a medicinal plant [10]. An animal study has already shown that these plant materials have hypocholesterolemic activity [11]. In Yemen, this plant is used in many traditional or popular medical applications, such as loss of appetite, anti-malarial, gastrointestinal troubles, skin diseases and burns [12] [13] [14] [15]. Siddiqui *et al.* [16] used the useless and discarded leaves of *C. rotundifolia* for preparing bio adsorbent to the removal of heavy metal pollutant of water. Al-Mamary [17] reported that *C. rotundifolia* has significant antioxidant efficiencies that could be attributed to the presence of phenolics.

Nanoparticles of metals have unique properties, such as surface area and particle size, which are mainly different from those of bulk materials [18] [19]. Solar energy systems, optics, catalytic and antibacterial capacities are main applications fields of AgNPs [20]. Recently, many studies conformed that silver nanoparticles (AgNPs) display effective antimicrobial capacity against both gram-positive and gram-negative bacterial strains. Whereas, toxicity and hazard of chemicals, in addition to high cost and power consumption, are the main drawbacks of chemical methods to preparing silver nanoparticles [21]. AgNPs were synthesized using a green, rapid, one-step, cost-effective and environmentally friendly method using *Ziziphus Jujuba* leaf extract [22]. A bio-synthesis of Ag-NPs using water extract of *M. pendan* was successfully carried out. Whereas, the water soluble flavonoids in the water extract were responsible in the reduction of Ag^+ to Ag^0 [23]. The synthesized silver nanoparticles using autoclave assisted gum extract of neem (*Azadirachta indica*) are exhibited antibacterial activity against clinical isolates of *Salmonella enteritidis* and *Bacillus cereus* [24]. Rajeshkumar [25] investigated the antibacterial activity of biosynthesized silver nanoparticles using the fresh bark extract of *Pongamia pinnata* against gram positive (*Klebsiella planticola*) and gram negative (*Staphylococcus aureus*) bacteria. Khan *et al.* [26] were attributed the antimicrobial activities of silver nanomaterials to the structural changes in the protein cell wall. Antibacterial tests using four bacteria

strains showed that the AgNPs/Camomile extract performed five times better compared to pure Camomile extract and AgNPs/Glucose samples [27]. The antibacterial activity of bio capped Ag NPs with leaf extracts of *Commiphora caudata* shows that these particles have higher inhibitory action for *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus flavus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus* [28].

The current investigation was carried out to screen the antibacterial activity of green synthesis silver nanoparticles using wild edible plants extract against some pathogenic bacterial strains.

2. Material and Methods

2.1. Collection of Plant Materials

Sample of fresh *C. rotundifolia* leaves were collected from a farm at Al-Mikhwah city, Al Baha region in as shown in **Figure 1**. About 1000 g of plant were washed and rinsed with distilled water. Sample was dried indoor and grinded to fine powder using kitchen blender (grinder of Moulinex blender, 400 W) for 2.5 min at high speed and then stored in plastic bags.

2.2. Microorganisms

Bacterial strains were isolated from food samples, which included *E. coli* and



Figure 1. (a) Google earth image of location of Wild plant (*C. rotundifolia*), (b) image of Arabian Wax Cissus leaves and (c) AgNPs prepared using aqueous extract of plant.

Klebsilla (gram negative); *Bacillus cereus* and *Staph aureus* (gram positive); *Candida* (yeast) and *Aspirigullus* (mold). The strains were kept at 4°C on agar slant and sub cultured at 37°C for 24 h on nutrient agar before any susceptibility test.

2.3. Extraction of Material and Synthesis of Silver Nanoparticles

100 g of powdered sample with 750 ml of deionized water poured into pressure cooker and kept for 2 hr on the hot plate at 350°C. The solid residues were removed by filtration and the extract kept in refrigerator to use time [29]. 99.9% pure AgNO₃ was purchased from Sigma-Aldrich, Cairo, Egypt. **Table 1** shows the different conditions of green synthesis of AgNPs. A 20 mM solution of AgNO₃ was prepared for production of AgNPs. Green reduction of Ag⁺ was monitored by visual observation and UV-visible spectroscopy.

2.4. Antimicrobial Assay

The antimicrobial activity of AgNPs was investigated by the disk diffusion method. The pure cultures of each strain were swabbed uniformly on the individual plates using sterile cotton at 35°C on a rotary shaker at 200 RPM. Sterile filter paper discs (5 mm in diameter) impregnated with 25, 50, 100, 150 and 200 µL of the samples of silver nanoparticles solution then sited on the surface of these agar plates. The plates were then incubated at 37°C for bacterial growth after which growth was determined by measuring the diameter of the inhibition zone (mm) using a digital caliper. Each extract was analyzed in triplicate, the mean values are presented. Tetracycline (30 mg/disc) was used as a gram-positive control.

2.5. Characterization Tools

The reduction of silver ions into silver nanoparticles monitored by UV-visible spectroscopy (SHIMADZU MODELUV 1800, Japan) at a wavelength of 350 - 700 nm. A diffractometer XRD thin film PANalytical X pert PRO, Cu target, wave length 1.54 Å, 45 kV, 40 mA made in Holland was used to determine the crystallinity of prepared AgNPs. Scanning electron microscopy (SEM) examination was performed using JEOL JSM 6360 DLA, Japan, at 30 kV, and the SEM-EDX analysis was performed by FEI Company, Quanta FEG250, Holland. Transmission electron microscopy image were taken using a Hitachi, H-800 TEM. TEM

Table 1. Different concentration of silver nitrate and Arabian Wax Cissus extract used for green synthesis of AgNPs.

Sample	AgNO ₃ , 20 mM, ml	extract, ml	R, %	Temperature
a	90	10	10	ambient
b	80	20	20	ambient
c	70	30	30	ambient
d	60	40	40	ambient

samples were prepared by placing drops of aqueous dispersion of AgNPs in distilled water on 200 mesh carbon coated copper grids and dried at ambient conditions for 10 to 12 h. The FT-IR spectra of the products recorded on a JASCO Asia Portal - FT/IR-6300 Spectrometer using the KBr pellet method.

3. Results and Discussion

3.1. Characterizations of AgNPs

Green reduction of Ag^+ by *C. rotundifolia* extract was monitored by observing the colour of silver solutions that changed from colourless, to yellow, brown and then reddish brown as evidence of silver ion reduction, **Figure 1(c)**, increasing the silver nanoparticles concentration and changing the particle morphology. It was observed that the colour started to change after 0.5 min. and reached a maximum absorption capacity of UV after 7 min.

This period decreased with increasing the *C. rotundifolia* extract content. In addition, the UV-Vis spectroscopy investigation of the prepared AgNPs showed that the size and shape of AgNPs influence the shape and position of the UV-Vis absorption peaks from plasmon resonance. The UV-Vis absorption spectra of the synthesized AgNPs at ambient temperatures with time intervals up to 3 h are shown in **Figures 2(a)-(d)**. The appearance of a strong peak at 437 nm indicated the formation of AgNPs that related to its surface plasmon resonance phenomena [30]. As shown in **Figures 2(a)-(d)**, the absorbance value for AgNPs increased with an increase in *C. rotundifolia* extract ratio and indicated a higher concentration of AgNPs produced, which was due to the availability of more reducing bio-molecules in *C. rotundifolia* extract. The SPR peaks at a lower substitution of *C. rotundifolia* extract 20:80 (v/v) were broad band whereas with a higher substitution ratio the SPR peaks became sharper and narrower. The intensity of this absorption band increased with time with a slight shift to 441 nm. The organic compounds in the *C. rotundifolia* extract contain numerous functional groups that can interact with silver to form a complex. Furthermore, the cleavage of C-C bonds of organic molecules released electrons required for the reduction of Ag. When the complex compound Ag^{+} *C. rotundifolia* was reduced to Ag^0 cissus, the organic molecules endow AgNPs with excellent dispersibility.

Figure 3 shows XRD patterns of the green synthesized AgNPs. The distinct diffraction bands at approximately 37.97, 44.29, 64.38 and 77.39 2θ correspond to (111), (200), (220) and (311), respectively, were matched to planes of a face-centred cubic lattice of silver (ICSD No. 01-071-4613), which confirm the crystalline structure of prepared AgNPs. The extra peaks near 22.5, 26.2, 29.2 and 32.0 2θ are due to the presence of organic compounds on the surface of AgNPs. The intensity of the peak at the (111) plane was greater than the other peaks, suggesting that this plane was the predominant one. According to Scherrer's formula, the average sizes of AgNPs synthesized by *C. rotundifolia* leaf extracts at room temperature is 28 nm [31] [32].

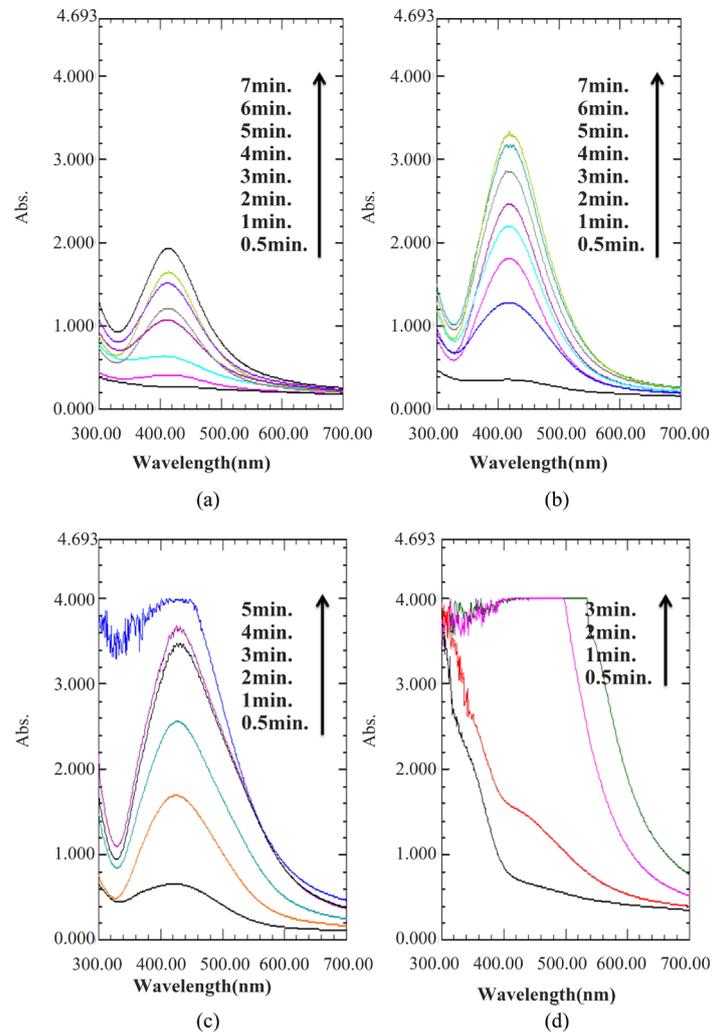


Figure 2. Absorption spectra of prepared AgNPs at ambient temperature (a) 10%, (b) 20%, (c) 30% and (d) 40% of *Cissus* leaf extract.

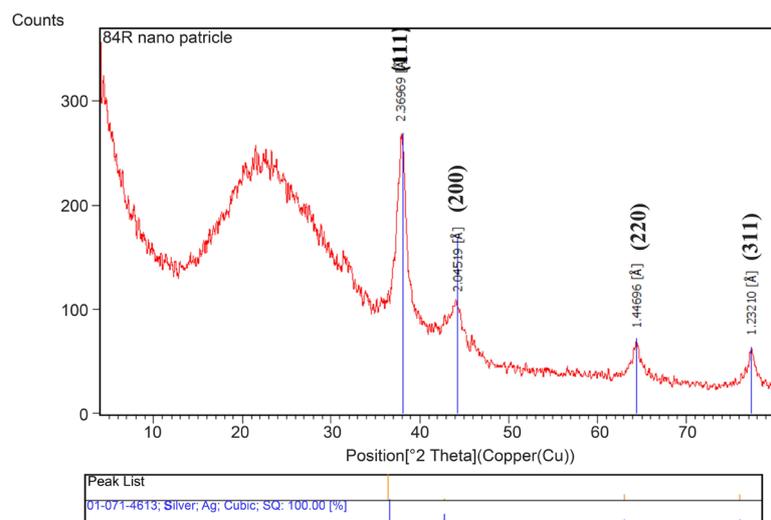


Figure 3. XRD patterns of synthesized AgNPs at ambient temperature (d) 40% of *Cissus* leaf extract.

The presence of organic compounds of *C. rotundifolia* extract on the surface of the nanoparticles was investigated using FTIR. The FTIR spectra of silver nanoparticles prepared with 40:60 (v/v) leaf extract: Ag⁺ (d) at ambient is shown in **Figure 4**. The band at 3432 cm⁻¹ is attributed to the hydroxyl group of organic compounds, the small bands appearing at 2927 and 2852 cm⁻¹ may be due to C-H stretching of vibration of the -CH₂ group from the aliphatic chains, and a relatively strong band can be noted at 2065 cm⁻¹ due to CN stretching vibration [33]. Additionally, the band at 1635 cm⁻¹ is related to stretching of the carbonyl groups and bands at 1384 cm⁻¹ and 1106 cm⁻¹ related to the carboxylic groups. These absorption bands indicate the absorption of different organic.

Figure 5 shows SEM images of the nanoparticles synthesized with 40:60 (v/v) BLE leaf extract: Ag⁺ (d sample) at ambient. Mostly spherical and nearspherical shapes were observed for AgNPs in the 20 - 40 nm size range. TEM analysis gives actual information about the morphology of the surface of the AgNPs.

TEM images of the prepared AgNPs with different magnifications are shown in **Figures 6(a)-(c)**. They clearly show the formation of the best AgNPs with spherical and oval shapes in the size range of 22 - 38 nm. These perfect particle sizes with various shapes of AgNPs may be related to different components of the plant extract [34]. ATEM image of AgNPs is shown in **Figure 6(d)**. It clearly shows a lattice spacing of 0.21 nm related to the (111) plane of Ag that matches with the XRD pattern. The polycrystalline nature of prepared silver nanoparticles was confirmed by SAED, **Figure 6(e)**, as the FCC structure of silver [35]. Furthermore, EDX analysis confirmed the presence of silver as a single element.

3.2. Antimicrobial Activity of Green Prepared AgNPs

Antibacterial activities of the prepared AgNPs using extract of *C. rotundifolia* against the tested organisms are shown in **Table 2**. All the AgNPs samples tested

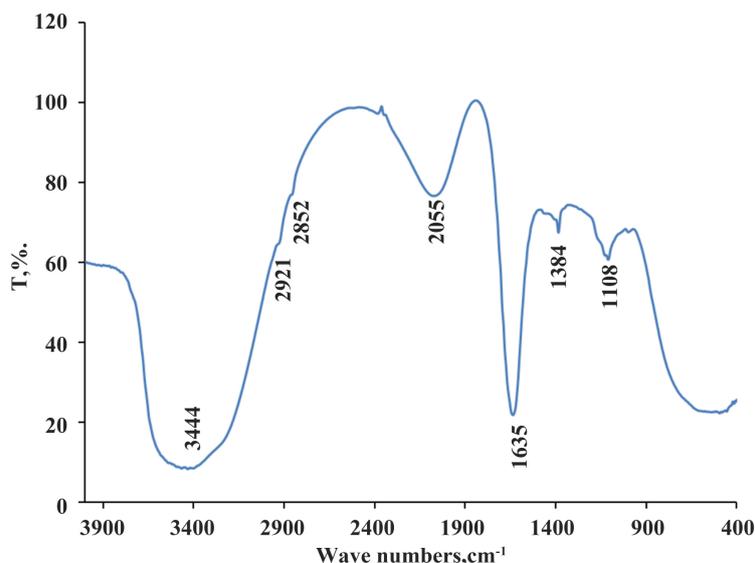


Figure 4. FTIR spectra of synthesized AgNPs at ambient temperature (d sample) 40% of *C. rotundifolia* leaf extract.

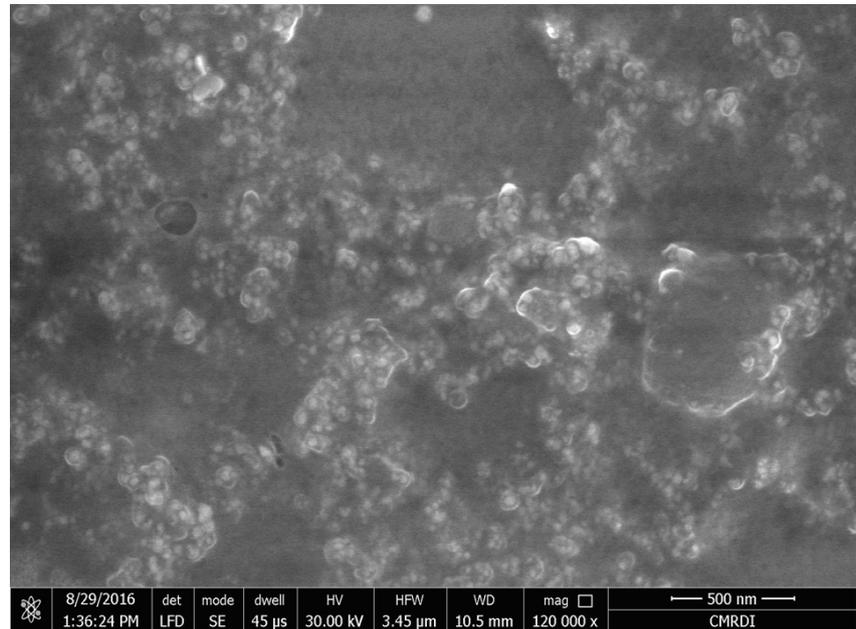


Figure 5. (a) FESEM micrograph of synthesized AgNPs at ambient temperature (d sample) 40 % of *C. rotundifolia* leaf extract.

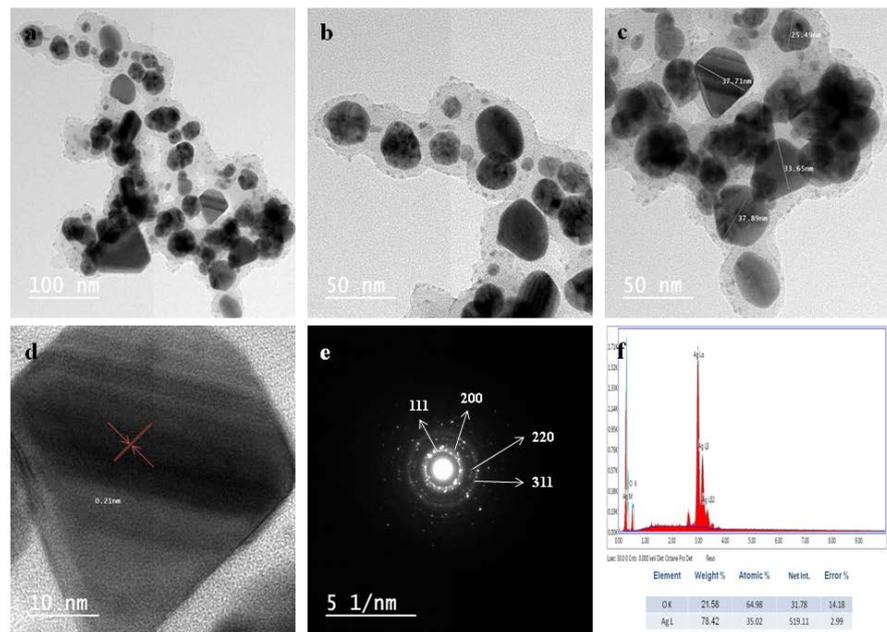


Figure 6. (a-d) HRTEM images of synthesized AgNPs at ambient temperature (d sample) 40 % of *C. rotundifolia* leaf extract, (e) SADE image, (f) EDX Spectrum.

showed antibacterial activity, however, the *C. rotundifolia* extract differ in its activities against the micro-organisms tested. As seen in **Table 2**, the inhibition zone area increased with a higher dose of AgNPs from 25 to 200 μ ml. The highest antibacterial activity was observed with increasing ratio of plant extract (d sample, 40%) against *E. coli*, *K. Pneumoniae* and *B. Cereus*. This is clearly attributed to antimicrobial efficiency of AgNPs, and not to the organic compounds

Table 2. Antimicrobial activity of green synthesis AgNPs against different microorganisms.

Sample	b	c	d	b	c	d	b	c	d	b	c	d	Blank	
Dose, μ L	25			50			100			200			-ve ZOI	+ve ZOI
Micro organism	Zone of inhibition*													
<i>E. coli</i>	0	0	0	14.80	15.08	17.00	12.39	16.52	20.34	12.3	14.07	22.67	0	22.75
<i>K. pneumoniae</i>	0	12.35	13.81	15.60	17.05	26.18	14.73	18.8	20.7	16.27	23.92	27.31	0	20.61
<i>B. Cereus</i>	0	0	0	10.33	12.34	18.23	17.71	22.18	26.37	22.39	23.21	29.34	0	25.5
<i>S. aureus</i>	0	0	11.93	15.71	16.90	18.47	15.67	16.83	19.08	15.81	19.92	20.2	0	27.44
<i>C. lbicans</i>	0	0	0	12.64	12.09	12.93	11.27	14.16	16.1	17.27	20.83	22.24	0	0
<i>Aspergillus</i>	0	0	0	13.54	11.67	11.09	11.6	15.24	15.77	21.42	22.42	23.56	0	0

*Values are mean inhibition zone (mm) \pm S.D of three replicates.

in the extract. The mechanism of AgNPs as an antimicrobial is not understood, but many studies suggest that it occurs through the interaction of silver nanoparticles with the DNA of microorganisms, forming free radicals and destructuring the cell walls [36] [37].

4. Conclusion

From the outcomes of this study, we can conclude that silver nanoparticles can be prepared using an aqueous extract of *C. rotundifolia* leaf. The stability of bio-synthetic silver nanoparticles was monitored for up to six months. The prepared AgNPs showed antimicrobial activity against *E. coli*, *K. pneumoniae*, *B. cereus*, *S. aureus*, *C. lbicans* and *Aspergillus* and it increased with increasing the dose of plant extract.

Acknowledgements

Thanks to the help of Deanship of Scientific Research at Al-Baha University, KSA. This work was financial supported by Deanship of Scientific Research at Al-Baha University, Kingdome of Saudi Arabian (48/1438).

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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