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Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *Chenopodium murale* leaf extract

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Abstract Silver is known for its antimicrobial effects and silver nanoparticles are gaining their importance due to their antimicrobial activities. The aims of the current study were to use plant extract for the biosynthesis of silver nanoparticles and to evaluate their antibacterial and antioxidant activity *in vitro*. The results indicated that silver nanoparticles (AgNPs) can be synthesized in a simple method using *Chenopodium murale* leaf extract. The TEM analysis showed that the sizes of the synthesized AgNps ranged from 30 to 50 nm. The essential oil of *C. murale* leaf extract was formed mainly of α-Terpinene, (*Z*)-Ascaridole and *cis*-Ascaridole. The total phenolic compounds and total flavonides were higher in AgNPs-containing plant extract compared to the plant extract. AgNPs-containing leaf extract showed a higher antioxidant and antimicrobial activity compared to *C. murale* leaf extract alone or silver nitrate. It could be concluded that *C. murale* leaf extract can be used effectively in the production of potential antioxidant and antimicrobial AgNPs for commercial application.

1. Introduction

The synthesis of noble metal nanoparticles attracts an increasing interest due to their new and different characteristics as compared with those of macroscopic phase, that allow attractive applications in various fields such as antimicrobials [48], medicine, biotechnology, optics, microelectronics, catalysis, information storage and energy conversion [71]. Silver nanoparticles (AgNPs) have the properties of high surface area, very small size (< 20 nm) and high dispersion [40]. Silver is a safe and effective bactericidal metal because it is non-toxic to animal cells and highly toxic to bacteria [33,38,72]. Silver

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nanoparticles (AgNPs) are one of the most commonly used nanomaterials. AgNPs are known to have antioxidant and antimicrobial properties [1]. AgNPs are used in coating or embedding for medical purposes [70]. In addition to their medical uses, AgNPs are also used in clothing, food industry, paints, electronics and other fields [15,37,66]. Several techniques have demonstrated that AgNPs can be synthesized using chemical and physical methods, but due to the fact of usage of a huge amount of toxic chemicals and high temperature conditions, it becomes a mandate to find an alternative method [49].

Green chemistry approach emphasizes that the usage of natural organisms has offered a reliable, simple, nontoxic and eco-friendly [43,54]. Therefore, researchers in the last years have turned to biological systems for nanoparticle synthesis [64]. Synthesis of nanoparticles by biological methods, using microorganisms, enzyme and plant or plant extract, has been suggested as possible eco-friendly alternatives to chemical and physical methods [56,42]. Biosynthesis of nanoparticles by plant surpasses other biological methods by reducing the complicated process of maintaining cell culture [69].

A plant species *Gliricidia sepium* used for the synthesis of silver nanoparticles, showed an absorption maximum at 440 nm [51]. Green synthesis of AgNPs using *Argimone maxicana* leaves broth generated particles of 20 nm and was found to be effective against many bacterial and fungal pathogens [30]. *Cycas* leaf extract was used to prepare silver nanoparticles of 2 to 6 nm [29]. A plant species *Solanum torvum* produced AgNPs of 14 nm dimension and showed the absorbance peak at 434 nm. The antimicrobial activity of synthesized nanoparticles was tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus flavus* and *Aspergillus niger*, showing a zone of inhibition [22]. Weeds such as *Ipomoea aquatica*, *Enhydra fluctuans* and *Ludwigia adscendens* were used as precursors for the synthesis of AgNPs that showed an absorbance peak between 400 and 480 nm [53]. AgNPs synthesized from *Boswellia ovalifoliolata* stem bark showed UV–Vis analysis absorption maxima at 430 nm and their size varied from 30 to 40 nm [6]. Dried leaves of *Cinnamomum camphora* have been implicated in the synthesis of 55–80 nm of AgNPs [26]. *Emblica officinalis* fruit extract was used for fabrication of gold and silver nanoparticles of 10 nm, showed a maximum absorption of light at 430 nm [5]. *Eucalyptus hybrida* (Safeda) leaves have been shown to synthesize AgNPs of 50 nm [16]. Dried leaves of *Pongamia pinnata* (L) Pierre were used to synthesize AgNPs of 20 nm size with an antimicrobial activity against many gram negative and gram positive microorganisms [52]. Bio-reduction of silver using various plant extracts such as *Helianthus annus*, *Basella alba*, *Oryza sativa*, *Saccharum officinarum*, *Sorghum bicolor* and *Zea mays* have been studied by Leela and Vivekanandan [36]. Leaf extract of *Parthenium hysterophorus* synthesized AgNPs of average size of 50 nm [46]. An aqueous extract of *Azadirachta indica* (Neem) leaves too was studied for the biogenic synthesis of AgNPs, showed a maximum absorbance between 440 and 500 nm [41]. *Chenopodium murale* (Nettleleaf goosefoot) is one of the fast-growing annuals of the family Chenopodiaceae and is widespread throughout different habitat types in Egypt [34,57]. It was introduced from Europe and grows best in moist soil. It is an abundant winter to early summer weed and is considered a pest in agroecosystems, roadsides, and waste places. Field observations reveal the failure of some other plant species to establish within pure patches of *C. murale*, as proved by its negative association pattern with many weeds and cultivated species in some community types [19,20]. The hypothesis is that several factors together determines the nanoparticle synthesis, including the plant source, the organic compounds in the crude leaf extract, the concentration of silver nitrate, the temperature and other than these, even the pigments in the leaf extract. Consequently, the longtime aims are to identify those compounds in *C. murale* grown in Egypt and to investigate their efficiency to reduce silver ions as well as the formation of silver nanoparticles. The aims of the current study were to utilize for the first time the *C. murale* grown in Egypt to (1) evaluate the chemical composition, antioxidant activity, total phenolic content and total flavonoids of the plant (2) synthesis of silver nanoparticles using the leaf extract of *C. murale* and (3) evaluate the antibacterial activity of the plant extract alone or with the plant nanosliver.

2. Materials and methods

2.1. Plant materials

The leaf of *C. murale* (Family: Chenopodiaceae) was collected from the Dekernis District, Dakahlia governorate, Egypt during December 2011 and January 2012 (Fig. 1).

2.2. Biosynthesis of AgNPs

The fresh leaf extract used for the biosynthesis of AgNPs was prepared from 20 g of thoroughly washed leaf in a 500 ml Erlenmeyer flask, boiled in 50 ml distilled water for 30 min and the produced extract was subjected to freeze drying. Suspensions were filtered with Whatman No. 40 filter paper [17]. Fifty ml of 5×10^{-3} M aqueous solution of silver nitrate was prepared in a Stoppard Erlenmeyer flask and 1 ml of leaf extract (0.2 g/ml) was added at room temperature for 24 h in the dark until the brownish color was developed which indicated the formation of AgNPs [46].

2.3. Characterization of AgNPs

2.3.1. UV–vis adsorbance spectroscopy analysis

The bioreduction of silver nitrate ($AgNO_3$) to AgNPs was monitored periodically by UV–vis spectroscopy (Shimazu 2401PC) after the dilution of the samples with deionized water [51]. A UV–vis spectrograph of the silver and nanoparticles was recorded by using a quartz cuvette with water as reference. The UV–vis spectrometric readings were recorded at a scanning speed of 200–800 nm [36].

2.3.2. TEM analysis of AgNPs

The suspension containing AgNPs of *C. murale* was sampled by TEM analysis using JEOL model 1200 EX electron microscope. TEM samples were prepared by placing a drop of the suspension of AgNP solutions on carbon-coated copper grids and allowing water to evaporate. The samples on the grids were allowed to dry for 4 min. The shape and size of silver

nanoparticles from *C. murale* were determined from TEM micrographs [18].

2.4. Preparation of plant solvent extracts

Fresh leaves of *C. murale* (5, 10 and 20 g/L) were shaken with absolute ethanol and water (80:20 v/v) in a metabolic shaker for 6 h and filtered. The residues were dried overnight and then were extracted with ethanol and water.

2.5. Extraction of Chenopodium murale essential oil

C. murale leaves were mixed with water (1:5 w/v) for hydrodistillation. The distillate was extracted twice with dichloromethane (1:1 v/v). The organic phase was collected and dried with anhydrous sodium sulfate and dichloromethane was evaporated in a rotatory evaporator at 40 °C under reduced pressure.

2.6. Identification of essential oil components

2.6.1. 1. Gas chromatographic analysis (GC)

GC analysis was performed by Hewlett–Packard model 5890 equipped with a flame ionization detector (FID). A fused silica capillary column DB-5 (60 m × 0.32 mm id) was used. The oven temperature was maintained initially at 50 °C for 5 min, and then programed from 50 to 250 °C at a rate of 4 °C/min. Helium was used as the carrier gas at flow rate of 1.1 ml/min. The injector and detector temperatures were 220 and 250 °C, respectively. The retention indices (Kovats index) of the separated volatile components were calculated using hydrocarbons (C7- C22, Aldrich Co.) as described by Adams [2].

For RI data, a mixture of linear saturated hydrocarbons was co-injected and the mixture was analyzed by GC. RIs were calculated using the following equation:

$$RI = 100y + 100(z - y) \times tR(x) - tR(y)/tR(z) - tR(y)$$

where y and z are the carbon numbers in the hydrocarbons eluting before and after a GC peak of interest, respectively; $tR(x)$ is the retention time of the acetate; $tR(y)$ and $tR(z)$ are the retention times of the hydrocarbons eluting before and after a GC peak of interest, respectively [28].

2.6.2. 2. Gas chromatographic–mass spectrometric analysis (GC/MS)

The analysis was carried out using a coupled Varian gas chromatography/mass spectrometery. The ionization voltage was 70 eV, mass range m/z 39–400 amu. The GC condition was carried out as mentioned above. The isolated peaks were identified by matching with data from the library of mass spectra (National Institute of Standard and Technology) and compared with those of authentic compounds and published data [2]. The quantitative determination was carried out based on peak area integration.

2.7. Determination of total phenol content

Phenol was determined by Folin–Ciocalteau reagent in an alkaline medium and was expressed as gallic acid equivalents [59].

2.8. Determination of total flavonoid content

Total flavonoid content was determined as described by Kim et al. [31] and was expressed as catechin equivalents.

2.9. Determination of antioxidant activity

2.9.1. Free radical scavenging ability on 2, 2-diphenyl-2-picrylhydrazyl (DPPH)

To assess the scavenging ability on DPPH, each extract (5–20 mg/ml) in water and ethanol was mixed with 1 ml of methanolic solution containing DPPH radicals (0.2 mM). The mixture was shaken vigorously and left to stand for 30 min in the dark before measuring the absorbance at 517 nm against a blank [11]. Then the scavenging ability was calculated using the following equation:

$$I(\%) = 100 \times (A\text{blank} - A\text{sample}/A\text{blank})$$

Where I (%) is the inhibition percent, A blank is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound.

2.9.2. β-Carotene bleaching assay

The determination of β-carotene was carried out according to the method developed earlier [68]. In brief, 2 ml of β-carotene solution (0.2 mg/ml in chloroform) was pipetted into a round-bottom flask containing 20 linoleic acid and 200 µl nonionic detergents Tween 20. The mixture was then evaporated at 40 °C for 10 min to remove the solvent, immediately followed by the addition of distilled water (100 ml). After agitating vigorously the mixture, 5 ml aliquots of the resulting emulsion were transferred into test tubes containing different concentrations (5–20 mg/ml) of extracts. The mixture was vortexed and placed in a water bath at 50 °C for 2 h while the absorbance of the tested sample was repeatedly measured every 15 min at 470 nm using a UV–VIS spectrophotometer. The blank solution contained the same concentration of sample without β-carotene. All determinations were performed in triplicates and the total antioxidant activity was calculated based on the following equation:

$$AA = 1 - (A_0 - A_t)/(A_0^0 - A_0^t)$$

where AA is the antioxidant activity, A_0 and A_0^0 are the absorbance values measured at initial time of the incubation for samples and control, respectively, while A_t and A_0^t are the absorbance in the samples and control at $t = 120$ min.

2.10. Determination of antibacterial activity of AgNPs

The cup-plate agar diffusion method described by Srinivasan et al. [61] was adopted to assess the antibacterial activity of the prepared water extract and plant-nanosilver. One ml of standardized *Staphylococcus aureus* bacterial stock suspensions (10^8–10^9) colony forming units (cfu) per ml was thoroughly mixed with 250 ml of sterile nutrient agar. Twenty ml of the inoculated nutrient agar was distributed into sterile petri dishes. The agar was left to set and in each plate 3–4 cups, 10 mm in diameter was cut using a sterile cork borer No.4 and the agar disks were removed. Cups were filled with

Figure 1 *Chenopodium murale* plant.

Figure 2 Color change of silver nitrate to silver nanoparticles (brown, left) by the addition of *C. murale* leaf extract (yellowish green, right).

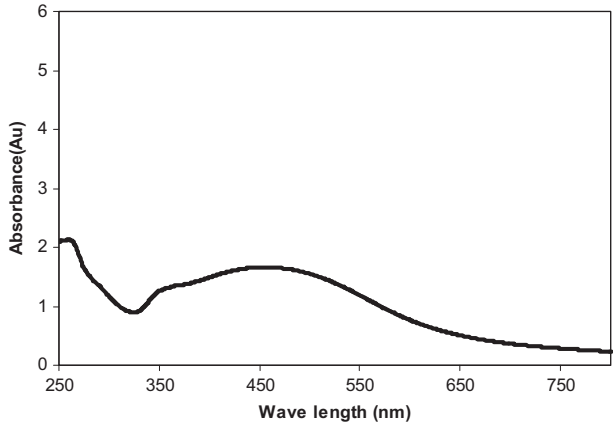

Figure 3 The UV/Vis spectrum of the silver nanoparticles synthesized by *C. murale*.

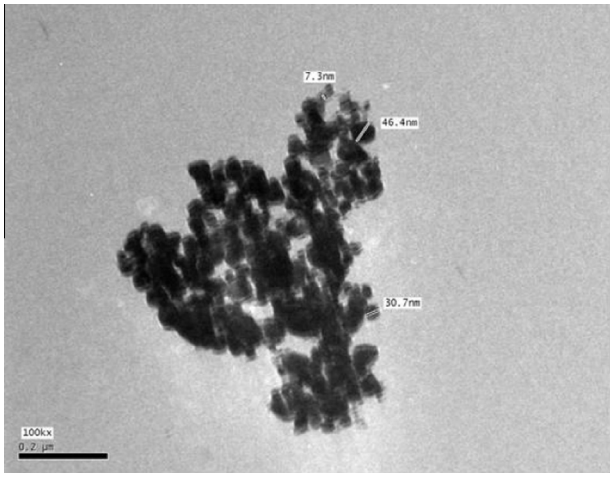

Figure 4 TEM analysis of AgNPs revealed that the size of the nanoparticles ranged between 30 and 50 nm.

0.1 ml of samples and were allowed to diffuse at room temperature for 2 h. The plates were then incubated in the upright position at 37 °C for 18 h. After incubation the diameter of the results and growth inhibition zones were measured averaged and the mean values were recorded.

3. Results

The current results indicated that addition of 0.2 g of the extract to 50 ml of 5 mM aqueous silver nitrate ($AgNO_3$) resulted in the formation of the brown solution after the overnight incubation at 40 °C in the dark which indicated the biosynthesis of silver nanoparticles (AgNPs) (Fig. 2). Spectrophotometric study of the produced brown colored solution through the rage spectra 190–800 nm using Shimadzu UV/VIS 2401PC showed a maximum absorption at 440 nm (Fig. 3). The TEM analysis revealed that the size of AgNPs ranged between 30 and 50 nm (Fig. 4).

The results of chemical composition of plant extract alone or plus AgNPs (Table 1) showed that hydrodistillation of the *Chenopodium* leaves yielded 0.02% essential oil (EO) on a fresh weight basis. The GC–MS and GC analysis using RI revealed that a total of 21 components were identified and represented 93.6% of the crude EO. These included α-Terpinene (40.01%), (*Z*)-Ascaridole (32.21%), *trans*-Ascaridole (4.2%), *p*-Cymene (2.11%), β-Myrcene (1.72%), (E)-2-Hexenal (1.72%), (*E*)-Ascaridole (1.83%) and *cis*-Ascardole (2.02%), constituting 85.82% of the EO (Table 1).

The current study also revealed that AgNPs EO synthesized by the *C. murale* leaves was formed mainly of α-Terpinene (36.8%), (*Z*)-Ascaridole (30.4%) and *cis*-Ascaridole (4.21%). The results of total phenolics content (TPC) presented in Table 2 indicated that TPC was higher in plant-AgNPs (80.83 ± 0.15 mg/g GAE) compared to the aqueous extract alone (74.9 ± 0.23 mg/g GAE). The results presented in Table 2 also revealed that total flavonids were higher in plant-AgNPs compared to those found in the plant extract alone

Table 1 Chemical composition of the *Chenopodium murale* leaf and AgNPs.

No.	% Area		RI	Compounds	Method of identification
	Plant extract	AgNPs			
1	1.72	1.48	847	(E)-2-Hexenal	RI & MS
2	1.72	1.9	1160	β-Myrcene	RI & MS
3	40.01	36.8	1020	α-Terpinene	RI & MS
4	2.11	3.49	1030	*p*-Cymene	RI
5	1.25	1.38	1033	Benzyl alcohol	RI & MS
6	0.39	0.43	1075	*p*-Cresol	RI & MS
7	0.46	0.35	1113	*p*-Mentha-1,3,8-triene	RI & MS
8	0.44	0.62	1191	*p*-Cimen-8-ol	RI & MS
9	1.03	1.29	1200	α-Terpineol	RI
10	32.21	30.4	1249	(*Z*)-Ascaridole	RI & MS
11	0.95	1.25	1260	Piperitone	RI & MS
12	1.83	2.1	1309	(*E*)-Ascaridole	RI & MS
13	0.49	0.58	1345	(*E*)-Piperitol acetate	RI & MS
14	0.69	0.42	1363	(*Z*)-Carvyl acetate	RI
15	0.7	0.9	1558	β-Caryophyllene	RI
16	0.21	0.34	1627	*trans-p*-Mentha-2,8-dien-1-ol	RI
17	0.95	1.4	1662	Citronellyl acetate	RI
18	0.12	0.18	1686	γ-Curcumene	RI
19	2.02	4.21	1714	*cis*-Ascaridole	RI
20	0.1	2.22	1732	*trans-p*-Mentha-1(7),8-dien-2-ol	RI & MS
21	4.2	0.42	1828	*trans*-Ascaridole	RI
Total	93.6%	94.26%			

Table 2 Characterization of the aqueous and AgNP extracts of *Chenopodium* Spp.

Extract	TPC	TF	Antioxidant activity					
			DPPH (IC_{50})			*β*C (IC_{50})		
			5 g/L	10 g/L	20 g/L	5 g/L	10 g/L	20 g/L
Aqueous	74.9 ± 0.23	12.77 ± 0.07	12.63 ± 0.15	22.97 ± 0.12	59.43 ± 0.15	15.47 ± 0.09	30.2 ± 0.12	51.13 ± 0.12
AgNPs	80.83 ± 0.15	14.1 ± 0.12	13.27 ± 0.12	21.3 ± 0.2	65.43 ± 0.18	13.97 ± 0.09	29.11 ± 0.06	53.38 ± 0.04

TPC, total phonic content; TF, total flavoniods; *β*C, *β* carotene assay.

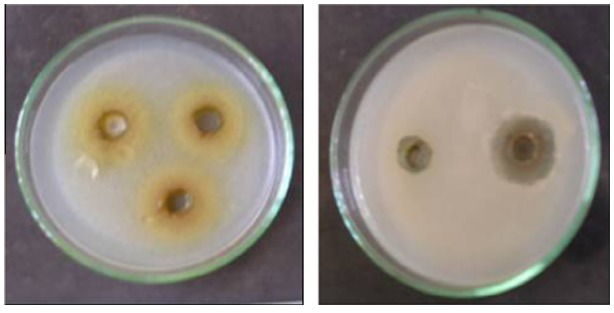

Figure 5 The antibacterial effect of *C. murale* leaf extract (a), silver nitrate (b, left) and the biosynthesized AgNPs (b, right) using the test bacterium *Staphylococcus aureus*.

and the recorded values were 12.77 ± 0.07 and 14.1 ± 0.12 mg/g respectively.

The antioxidant activity of the aqueous extract and plant-AgNPs was evaluated using DPPH scavenging and β-carotene bleaching assays. As shown in Table 2, a significant difference was observed among the respective values obtained. The DPPH values were increased in a dose dependent manner. The recorded value for the lowest concentration of the aqueous extract (5 mg/L) was 12.63 ± 0.15 and this value was increased to 59.43 ± 0.15 when the concentration was increased to 20 mg/L. However, these values recorded 13.27 ± 0.12 and 65.43 ± 0.18 for the two concentrations of the plant-AgNPs respectively indicating that plant-AgNPs possessed a higher scavenging activity compared to the plant extract alone. Interestingly, the results of β-carotene oxidation demonstrated also a higher antioxidant activity of plant-AgNPs than the extract alone and this activity increased in a dose dependent manner (Table 2).

The antimicrobial activity of *C. murale* leaf extract, silver nitrate ($AgNO_3$) and plant-AgNPs was determined by the cup plate method as a simple and fast method to distinguish the antimicrobial activity of the tested samples and to explain the increment of the clear zone using the same concentration before (AgNO3) and after nanoparticle (AgNPs) formation. The results indicated that the leaf extract alone did not exhibit antimicrobial effect (Fig. 5) against *S. aureus* (G + ve bacteria). Silver nitrate (5 m M) showed an appreciable positive effect against the tested microorganisms (low clear zone). However, plant-AgNPs showed the greatest antimicrobial activity against the tested microorganism.

4. Discussion

Previous studies reported that AgNPs can be synthesized by plants such as *Azadirachta indica* [58], *Capsicum annuum* [9], *Carica papaya* [29], *Gliricidia sepium* [52], *Eucalyptus hybrida* [16] and microorganisms such as *Aspergillus fumigatus* [10] *Cladosporium cladosporioides* [8], *Fusarium oxysporum* [3], *Pseudomonas aeruginosa* [27] and *Rhodopseudomonas capsulate* [25]. In the current study, aqueous silver ions were reduced to AgNPs after mixing with *C. murale* leaf extract followed by incubation for 24 h in the dark. The color turned to reddish brown and this change in color has been previously observed by several investigators [65,55,30]. These authors suggested that the color change appeared due to the surface plasmon resonance of deposited AgNPs. In the current study, the mechanism by which the plant extract could be synthesized AgNPs may be explained by the higher total phenolics content in the plant. These plant phenolics are strong antioxidants with high reducing capacity [47] which can be used for AgNPs synthesis [39]. The higher content of total phenolic content in *C. murale* leaf extract facilitates the reduction of silver ions to nano-scale-sized silver particles due to the electron donating ability of these phenolic compounds. Moreover, the quinoid compound produced due to the oxidation of the phenol group in phenolics can be adsorbed on the surface of nanoparticles, accounting for their suspension stabilization [67].

The total yield of crude oil reported in the current study was lower than the reported yield range from Nigeria [45] and Cameroon [62]. However, yields between 0.2% and 0.3% have been reported by Gupta et al. [24]. On the other hand, the chemical composition of the EO is substantially different from those reported by Jardim et al. [28], where α-Terpinene (40.01%), (*Z*)-Ascaridole (32.21%) and *trans*-Ascaridole (4.2%) constituted the major portion of the EO. Moreover, the variation of the volatile components (93.6%) of EO may be due to the differences in extraction and identification method applied. [2]. On the other hand, the chemical composition of EO was quite different from that reported in other studies. α-Terpinene (40.01%) and Ascaridoles (Z, E, cis and trans with 40.26%) were the major components (80.27%) in the present study. However, previous studies indicated that p-cymene and α-terpinene were the major components in EO which represent 15.5% and 56% in Nigerian plants [45], 19.5% and 63.6% in Indian plants [24] and 50% and 37.6% in Cameroon plants [62]. Moreover, the current results showed that (Z) – and (E)-ascaridole were the minor components. In this concern, Cavalli et al. [12] reported that α-terpinene (9.7%), p-cymene (16.2%) and ascaridole (41.8%) were found in a commercial EO available in France. Thus, in all cases, the ascaridoles considered to be quality indicators of *C. ambrosioides* EO were much lower than those obtained in Brazil [23].

It is well documented that the phenolic compounds may contribute directly to antioxidative action [7]. However, antioxidant activities are attributed to the phenolic contents in plants probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [13]. Although no available literature on the total phenolic content in *C. murale*, Laghari et al. [35] reported higher total phenolic contents (3066 mg of GAE/100 g) in *Chenopodium album*. In the same concern, Emam [21] reported that plants in the family *Chenopodiaceae* are rich in phenolic and flavonoid compounds and induced antioxidant potentials. Similar to the current results, Nsimba et al. [44] reported a higher antioxidant activity to other spices of the family *Chenopodiaceae* i.e. *chenopodium quinoa* and *chenopodium album*. Moreover, the results also indicated that the marginal increase in antioxidant activity of plant-AgNPs, compared to the plant extract suggested that the plant extract itself is responsible for the majority of the antioxidant activity and AgNP is not contributing much to the antioxidant activity.

The antimicrobial activity of AgNPs was reported in a series of reports [50,30,29,22]. In the current study, plant-AgNPs were effective against *S. aureus*. Similar to these observations, Govindaraju et al. [22] showed a zone of inhibition when the synthesized nanoparticles were tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus flavus* and *Aspergillus niger*. A number of theories for antimicrobial actions of colloidal silver solution have been proposed. For example, alteration of permeability of cell membrane [60], release of lipopolysaccharides and membrane proteins [4], generation of free radicals responsible for the damage of membrane [32], and dissipation of the proton motive force resulting in the collapse of the membrane potential [14], however; the exact mechanism has not been fully deciphered. Moreover, Tripathi et al. [63] studied the effect of silver nano balls on *Escherichia coli*, *S. typhimurium*, *B. subtilis* and *P. aeruginosa* by colony forming unit (cfu) and growth curve at a concentration of 40 μg/ml and showed a significant reduction of bacterial population and their growth pattern at the studied concentration.

5. Conclusion

The current study revealed that silver nanoparticles can be synthesized in a simple method using *C. murale* leaf extract. The TEM analysis showed that the sizes of the synthesized AgNps ranged from 30 to 50 nm. The essential oil of plant-AgNPs was formed mainly of α-Terpinene, (*Z*)-Ascaridole and *cis*-Ascaridole. The total phenolic compounds and total flavonides were higher in plant-AgNPs compared to the plant extract alone. Plant-AgNPs showed a higher antioxidant and antimicrobial activity compared to *C. murale* leaf extract alone or silver nitrate.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

[1] K.M. Abou El-nour, A. Eftaiha, A. Al-Warthan, R.A. Ammar, Synthesis and applications of silver nanoparticles, Arab. J. Chem. 3 (2010) 135–140.
[2] R.P. Adams, Identification of essential oil components by gas chromatography/mass spectroscopy, Allured Publishing Corporation, Carol Stream, 1995.
[3] A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M.I. Khan, R. Kumar, M. Sastry, Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium Oxysporum*, Colloid Surf. B 28 (2003) 313–318.
[4] N.A. Amro, L.P. Kotra, K. Wadu-Mesthrige, A. Bulchevy, S. Mobashery, G.Y. Liu, High resolution atomic force microscopy

studies of the E. coli outer membrane: the structural basis for permeability, Langmuir 16 (2000) 2789–2796.
[5] B. Ankamwar, C. Damle, A. Ahmad, M. Sastry, Biosynthesis of gold and silver nanoparticles using *Emblica officinalis* fruit extract, their phase transfer, transmetallation in an organic solution, J. Nanosci. Nanotechnol. 5 (2005) 1665–1671.
[6] S. Ankanna, T.N.V.K.V. Prasad, E.K. Elumalai, N. Savithramma, Production of biogenic silver nanoparticles using *Bosvellia ovalifoliolata* stem bark, Digest J. Nanomater. Biostruct. 5 (2010) 369–372.
[7] J.M. Awika, L.W. Rooney, X. Wu, R.L. Prior, L.C. Zevallos, Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products, J. Agric. Food Chem. 51 (2003) 6657–6662.
[8] D.S. Balaji, S. Basavaraja, R. Deshpande, Bedre, D. Mahesh, B.K. Prabhakar, A. Venkataraman, Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium Cladosporioides* fungus, Colloids Surf B 68 (1) (2009) 88–92.
[9] H. Bar, D.K. Bhui, G.P. Sahoo, P. Sarkar, S.P. De, A. Misra, Green synthesis of silver nanoparticles using latex of *Jatropha curcas*, Colloids Surf. A 339 (2009) 134–139.
[10] K.C. Bhainsa, S.F. D'Souza, Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*, Colloids Surf B 47 (2006) 160–164.
[11] A. Braca, Nunziatina De Tommasi, Lorenzo Di Bari, Cosimo Pizza, Mateo Politi, Ivano Morelli, Antioxidant principles from *Bauhinia terapotensis*, J. Nat. Prod. 64 (2001) 892–895.
[12] J.F. CavallI, F. Tomi, A.F. Bernardini, J. Casanova, Combined analysis of the essential oil of chenopodium ambrosioides by GC, GC–MS and 13C-NMR spectroscopy: quantitative determination of ascaridole, a heat-sensitive compound, Phytochem. Anal. 15 (2004) 275–279.
[13] S.T. Chang, J.H. Wu, S.Y. Wang, P.L. Kang, N.S. Yang, L.F. Shyur, Antioxidant activity of extracts from *Acacia confusa* bark and heartwood, J. Agric. Food Chem. 49 (2001) 3420–3424.
[14] L. Chun-Nam, C.M. Ho, R. Chen, Q.Y. He, W.Y. Yu, H.P. Sun, K.H. Tam, J.F. Chiu, C.M. Che, Proteomic analysis of the mode of antibacterial action of silver nanoparticles, J. Proteome Res. 5 (2006) 916–924.
[15] M.S. Cohen, J.M. Stern, A.J. Vanni, R.S. Kelley, E. Baumgart, D. Field, J.A. Libertino, I.C. Summerhayes, In vitro analysis of a nanocrystalline silver-coated surgical mesh, Surg. Infect. (Larchmt.) 8 (2007) 397–403.
[16] M. Dubey, S. Bhadauria, B.S. Kushwah, Green synthesis of nanosilver particles from the extract of *Eucalyptus hybrid* (Safeda leaves), Digest J. Nanomater. Biostruct. 5 (2009) 537–543.
[17] A.D. Dwivedi, K. Gopal, Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extracts, Colloids Surf. A 369 (2010) 27–33.
[18] T. Elavazhagan, K.D. Arunachalam, *Memecylon edule* leaf extract mediated green synthesis of silver and gold nanoparticles, Int. J. Nanomed. 6 (2011) 1265–1278.
[19] A.A. El-Khatib, A.K. Hegazy, H.K. Galal, Allelopathy in the rhizosphere and amended soil of *Chenopodium murale* L, Weed Biol. Manag. 4 (2004) 35–42.
[20] A.A. El-Khatib, A.K. Hegazy, H.K. Galal, Does allelopathy have a role in the ecology of *Chenopodium murale*?, Ann Bot. Fennici. 41 (2004) 37–45.
[21] S.S. Emam, Bioactive constituents of *Atriplex halimus* plant, J. Nat. Prod. 4 (2011) 25–41.
[22] K. Govindaraju, S. Tamilselvan, V. Kiruthogs, G. Simgaravelu, Biogenic silver nanoparicles by *Solanum torvum* and their promising antimicrobial activity, J. Biopest. 3 (2010) 394–399.
[23] E. Guenther, The essential oils, Vol. VI, Van Nostrand, New York, 1952.
[24] D. Gupta, R. Charles, V.K. Mehta, S.N. Garg, S. Kumar, Chemical examination of the essential oil of *Chenopodium ambrosioides* L from the southern hills of India, J. Essent. Oil Res. 14 (2002) 93–94.
[25] S. He, Z. Guo, Y. Zhang, S. Zhang, J. Wang, N. Gu, Biosynthesis of gold nanoparticles using the bacteria *Rhodopseudomonas capsulata*, Mater. Lett. 61 (18) (2007) 3984–3987.
[26] J. Huang, Q. Li, D. Sun, Y. Lu, Y. Su, X. Yang, H. Wang, Y. Wang, W. Shao, N. He, J. Hong, C. Chen, Biosynthesis of silver and gold nanoparticles by using novel sun-dried *Cinnamomum camphora* leaves, Nanotechnology 18 (2007) 105104–105115.
[27] M.I. Husseiny, M.A. El-Aziz, Y. Badr, M.A. Mahmoud, Biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa*, Spectrochim. Acta Part A 67 (3–4) (2007) 1003–1006.
[28] C.M. Jardim, G.N. Jham, O.D. Dhingra, M.M. Freire, Composition and antifungal activity of the essential oil of the Brazilian *Chenopodium ambrosioides* L, J. Chem. Ecol. 34 (2008) 1213–1218.
[29] A.K. Jha, K. Prasad, Green synthesis of silver nanoparticles using Cycas leaf, Int. J. Green Nanotech. Phy. Chem. 1 (2010) 110–117.
[30] N. Khandelwal, A. Singh, D. Jain, M.K. Upadhyay, H.N. Verma, Green synthesis of silver nanoparticles using *Argimone maxicana* leaf extract and evaluation of their activity, Digest. J. Nanomater. Biostruct. 5 (2010) 483–489.
[31] D. Kim, S.W. Jeong, C.Y. Lee, Antioxidant capacity of phenolic phytochemicals from various cultivars of plums, Food Chem. 81 (2003) 321–326.
[32] J.S. Kim, E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.Y. Huwang, Y.K. Kim, Y.S. Lee, D.H. Jeong, M.H. Cho, Antimicrobial effects of silver nanoparticles, Nanomed. Nanotechnol. Biol. Med. 3 (2007) 95–101.
[33] U. Klueh, V. Wagner, S. Kelly, A. Johnson, J.D. Bryers, Efficacy of silver-coated fabric to prevent bacterial colonization and subsequent device-based biofilm formation, J. Biomed. Mater. Res. 53 (6) (2000) 621–631.
[34] A. Kosinova, Weed communities of winter crops in Egypt, Preslia 47 (1975) 58–74.
[35] A. Laghari, S. Memon, A. Nelofar, M.K. Khan, A. Yasmin, Determination of free phenolic acids and antioxidant activity of methanolic extracts obtained from fruits and leaves of *Chenopodium album*, Food Chem. 126 (2011) 1850–1855.
[36] A. Leela, M. Vivekanandan, Tapping the unexploited plant resources for the synthesis of silver nanoparticles, Afr. J. Biotechnol. 7 (2008) 3162–3165.
[37] Q. Li, S. Mahendra, D. Lyon, L. Brunet, M. Liga, Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications, Water Res. 42 (18) (2008) 4591–4602.
[38] C. Marambio-Jones, E.M.V. Hoek, A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment, J. Nanopart. Res. 12 (5) (2010) 1531–1551.
[39] G.A. Martinez-Castanon, N. Nino-Martinez, F. Martinez-Gutierrez, J.R. Martinez-mondeza, F. Ruiz, Synthesis and antibacterial activity of silver nanoparticles with different size, J. Nanopart. Res. 10 (2008) 1343–1348.
[40] T.V. Mathew, S. Kuriakose, Studies on the antimicrobial properties of colloidal silver nanoparticles stabilized by bovine serum albumin, Colloids Surf. B 101 (2013) 14–18.
[41] A. Mukherjee, N. Chandrashekharan, A.M. Raichur, A. Tripathi, Antibacterial activity of silver nanoparticles synthesized by aqueous extract of *Azardirachta indica* (Neem) leaves, J. Biomed. Nanotechnol. 5 (2009) 93–98.
[42] B. Nair, T. Pradeep, Coalescence of nanoclusters and formation of submicron crystallites assisted by *Lactobacillus* Strains cryst, Growth Des. 2 (2002) 293–298.

[43] K.B. Narayanan, N. Sakthivel, Biological synthesis of metal nanoparticles by microbes, Adv. Colloid Interface Sci. 156 (1–2) (2010) 1–13.
[44] R.Y. Nsimba, H. Kikuzaki, Y. Konishi, Antioxidant activity of various extracts and fractions of *Chenopodium quinoa* and *Amaranthus spp*. seeds, Food Chem. 106 (2) (2008) 760–766.
[45] P.A. Onocha, O. Ekundayo, T. Eramo, I. Laakso, Essential oil constituents of *Chenopodium ambrosioides* L. leaves from Nigeria, J. Essent. Oil Res. 11 (1999) 220–222.
[46] V. Parashar, R. Parashar, B. Sharma, A.C. Pandey, *Parthenium* leaf extract mediated synthesis of silver nanoparticle: a novel approach for weed utilization, Digest J. Nanomater. Biostruct. 4 (2009) 45–50.
[47] P.G. Pietta, Flavonoids as antioxidants, J. Nat. Prod. 63 (2000) 1035–1042.
[48] S. Priyadarshini, V. Gopinath, N.M. Priyadharsshini, D. MubarakAli, P. Velusamy, Synthesis of anisotropic silver nanoparticles using novel strain, Bacillus. flexus and its biomedical application, Colloids Surf. B 102 (2013) 232–237.
[49] P. Quaresma, L. Soares, L. Contar, A. Miranda, I. Osório, P.A. Carvalho, R. Franco, E. Pereira, Green photocatalytic synthesis of stable Au and Ag nanoparticles, Green Chem. Comm. 11 (2009) 1889–1893.
[50] M. Rai, A. Yadav, A. Gade, Silver nanoparticles as a new generation of antimicrobials, Biotechnol. Adv. 27 (1) (2009) 76–83.
[51] R.W. Raut, N.S. Kolekar, J.R. Lakkakula, V.D. Mendhulkar, S.B. Kashid, Photosynthesis of silver nanoparticles using *Gliricidia sepium* (Jecq), Curr. Nanosci. 5 (2009) 117–122.
[52] R.W. Raut, N.S. Kolekar, J.R. Lakkakula, V.D. Mendhulkar, S.B. Kashid, Extracellular synthesis of silver nanoparticles using dried leaves of *Pongamia pinnata* (L) Pierre, Nano-Micro Lett. 2 (2010) 106–113.
[53] N. Roy, A. Barik, Green synthesis of silver nanoparticles using unexploited weed resources, Int. J. Nanotech. Appl. 4 (2010) 95–101.
[54] M. Sathishkumar, K. Sneha, Y.S. Yun, Immobilization of silver nanoparticles synthesized using *Curcuma longa* tuber powder and extract on cotton cloth for bactericidal activity, Bioresource Technol. 101 (2010) 7958–7965.
[55] A. Saxena, R.M. Tripathi, R.P. Singh, Biological synthesis of silver nanoparticles by using onion (*Allium cepa*) extract and their antibacterial activity, Digest J. Nanomater. Biostruct. 5 (2010) 427–432.
[56] S. Schultz, D.R. Smith, J.J. Mock, D.A. Schultz, Single-target molecule detection with nonbleaching multicolor optical immunolabels, Proc. Natl. Acad. Sci. USA 97 (3) (2000) 996–1001.
[57] K.H. Shaltout, A. Sharaf El-Din, R.A. El-Fahar, Weed communities of the common crops in the NileDelta region, Flora 187 (1992) 329–339.
[58] S.S. Shankar, A. Rai, B. Ankamwar, A. Singh, A. Ahmed, M. Sastry, Biological synthesis of triangular gold nonoprism, Nat. Mater. 3 (2004) 482–488.
[59] R.P. Singh, K.N.C. Murthy, G.K. Jayaprakasha, Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models, J. Agric. Food Chem. 50 (2002) 81–86.
[60] I. Sondi, B.S. Sondi, Silver nanoparticles as antimicrobial agents a case study on *E. coli* as a model for Gram-negative bacteria, J. Colloid Interface Sci. 275 (2004) 117–182.
[61] D. Srinivasan, S. Nathan, T. Suresh, P. Lakshmanaperumalsamy, Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine, J. Ethnopharmacol. 74 (2001) 217–220.
[62] L.A. Tapondjou, C. Adler, A.H. Boud, D.A. Fontem, Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetles, J. Stored Prod. Res. 38 (2002) 395–402.
[63] R.M. Tripathi, A. Saxena, N. Gupta, H. Kapoor, R.P. Singh, High antibacterial activity of silver nanoballs against E. Coli MTCC 1302, *S. Typhimurium* MTCC 1254, *B. Subtilis* MTCC 1133 and *P. Aeruginosa* MTCC 2295, Digest J. Nanomater. Biostruct. 5 (2010) 323–330.
[64] N. Tsibakhashvil, T. Kalabegishvili, V. Gabunia, E. Gintury, N. Kuchava, N. Bagdavadze, D. Pataraya, M. Gurielidzse, D. Gvarjaladze, L. Lomidze, Synthesis of silver nanoparticles using bacteria, Nano Stud. 2 (2010) 179–182.
[65] N. Vigneshwaran, A. Kathe, P.V. Varadrajan, R.P. Nachane, R.H. Balasubramanya, Biomimetics of silver nanoparticles by white rot fungi Phaenerochaete chrysosporium, Colloids Surf. B 53 (2006) 55–59.
[66] N. Vigneshwaran, A.A. Kathe, P.V. Varadarajan, R.P. Nachane, R.H. Balasubramanya, Functional finishing of cotton fabrics using silver nanoparticles, J. Nanosci. Nanotechnol. 7 (2007) 1893–1897.
[67] W. Wang, Q. Chen, C. Jiang, D. Yang, X. Liu, S. Xu, One step synthesis of biocompatible gold nanopartcles using gallic acid in the presence of poly-(N-vinyl-2-pyrrolidone), Colloids Surf. A Physicochem. Eng. Asp. 301 (2007) 73–79.
[68] M. Wettasinghe, I.F. Shahidi, Evening primrose meal: a source of natural antioxidants and scavenger of hydrogen peroxide and oxygen-derived free radicals, J. Agric. Food Chem. 47 (5) (1999) 1801–1812.
[69] I. Willner, R. Baron, B. Willner, Growing metal nanoparticles by enzyme, Adv. Mater. 18 (9) (2006) 1109–1120.
[70] X. Xu, Q. Yang, J. Bai, T. Lu, Y. Li, X. Jing, Fabrication of biodegradable electrospun poly (l-lactide-co-glycolide) fibers with antimicrobial nanosilver particles, J. Nanosci. Nanotechnol. 8 (2008) 5066–5070.
[71] S.Y. Yeo, H.J. Lee, S.H. Jeong, Preparation of nanocomposite fibers for permanent antibacterial effect, J. Mater. Sci. 38 (2003) 2143–2147.
[72] G. Zhao, S.E. Stevens Jr., Multiple parameters for the comprehensive evaluation of the susceptibility of *Escherichia coli* to the silver ion, Biometals 11 (1) (1998) 27–32.