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

Mohamed S. Abdel-Aziz, Mohamed S. Shaheen, Aziza A. El-Nekeety, Mosaad A. Abdel-Wahhab

Microbial Chemistry Department, National Research Center, Dokki, Cairo, Egypt
Flavour & Aroma Department, National Research Center, Dokki, Cairo, Egypt
Food Toxicology & Contaminants Department, National Research Center, Dokki, Cairo, Egypt

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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 flavonoids 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
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]. Cicus 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 hybridra (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 nanosilver.

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 (AgNO₃) to AgNPs was monitored periodically by UV–vis spectroscopy (Shimazu 2401PC) after the dilution of the samples with deionized water [51]. A UV–vis spectrophotograph 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 spectrometry. 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:

\[
I(\%) = 100 \times (A_{blank} - A_{sample}/A_{blank})
\]

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 agitation 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:

\[
AA = 1 - (A_0 - A_t)/(A_0^b - A_t^b)
\]

where AA is the antioxidant activity, \( A_0 \) and \( A_0^b \) are the absorbance values measured at initial time of the incubation for samples and control, respectively, while \( A_t \) and \( A_t^b \) 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 (10⁵–10⁶) 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
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 (AgNO₃) 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 range 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-Ascaridole (2.02%), constituting 85.82% of the EO (Table 1).

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 flavonoids were higher in plant-AgNPs compared to those found in the plant extract alone.
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 (AgNO3) 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 mM) 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], Gliciridia sepium [52], Eucalyptus hybrida [16] and microorganisms such as Aspergillus fumigatus [10], Cladosporium cladosporioides [8], Fusarium oxysporum [3], Pseudomonas aeruginosa [27] and Rhodopseudomonas capsulata [23]. 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, Eman [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 species 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 flavonoids 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


Biosyntheses of silver nanoparticles using green chemistry


