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Antioxidant, antimicrobial, and anticancer properties of silver nanoparticles biosynthesized using artichoke waste extract

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Abstract

In the present work, a green method is proposed for the biosynthesis of silver nanoparticles (AgNPs) using artichoke processing waste extract as a reducing agent. The formation of AgNPs was spectrophotometrically detected by the appearance of a maximum peak at 430 nm. Transmission electron microscopic results confirmed the formation of AgNPs with different shapes with average particle size (88.94 nm). Phytochemical and gas chromatography/ mass results indicated the presence of essential compounds, mainly phenols and flavonoids, in the artichoke waste extract. AgNPs biosynthesized using artichoke waste extract were tested to determine their potential antioxidant, antibacterial and anticancer activities. The results showed that AgNPs have a high antioxidant capacity (179.93 mgGAE/ml) and potent free radical scavenging activity (45.94 %). The results also showed that AgNPs have significantly high antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella*, and *Escherichia coli* with inhibition zone diameters 17, 21, 17, and 17 mm, respectively. In addition, AgNPs showed anticancer activity against breast cancer cell line with a decline in cells viability with an increase of AgNPs concentration and IC_{50} (144.29µmole/ml). Based on these results and the benefits of phytochemicals detected in artichoke waste extract, this waste could be effectively used for silver nanoparticles preparation.

Keywords: Antibacterial; artichoke; cytotoxicity; green synthesis; silver nanoparticles

1. Introduction

In recent years, nanotechnology has emerged as an innovative research branch, and nanomaterials have extended to cover almost all application fields. Nanomaterials have unique characteristics such as their size, shape, and morphology. Amongst the various types of nanomaterials, silver nanoparticles (AgNPs) are of particular interest because of their beneficial impacts when utilized as antimicrobials, antioxidants, or anticancer agents, in addition to their uses in painting, clothing, electronics, and food packaging industries (Salaheldin *et al.*, 2019; Calderón-Jiménez *et al.*, 2017).

The conventional method for the preparation of silver nanoparticles involves using chemical reducing agents to initiate the reduction of Ag^+ to Ag^0 (Iravani *et al.*, 2014). However, nowadays,

as the world is seeking a better environment, the use of such chemicals is not preferable. In this respect, the use of plant extracts as reducing agents in nanoparticles preparation is a desirable approach that not only reduces chemicals usage but also results in adding more value to the biosynthesized nanomaterial which is acquired from the bioactive compounds usually present in plant extract (Florkiewicz *et al.*, 2019). Several green extracts such as *Portulacaria Afra* extract (Salaheldin *et al.*, 2019), *Spirulina platensis* extract (Rashad *et al.*, 2019), rind extract of watermelon (Patra *et al.*, 2016), *Fusarium oxysporum* extract (Husseiny *et al.*, 2015) and others were successfully used as reducing agents for the biosynthesis of silver nanoparticles, and the produced nanoparticles revealed several types of bioactivity.

The artichoke plant (*Cynara scolymus*) is a plant originating from the Mediterranean region (Emanuel *et al.*, 2011) with plenty of bioactive compounds and much health potential, especially for its anticancer hepatoprotective and hypocholesterolemic activities (Florkiewicz *et al.*, 2019). Egypt is one of the main Mediterranean countries that produces artichoke, and artichoke-food processing-based industry yields vast amounts of waste consisting mainly of the outer and inner bracts (Ibrahim *et al.*, 2013; Seida *et al.*, 2011). Since integrated waste management is essential environmentally (Mazandaranizadeh *et al.*, 2017), artichoke waste could be reused for extract preparation. Moreover, Artichoke waste extract was previously proven to be a prospective source of phytochemicals and bioactive compounds (Zuorro *et al.*, 2016). Thus the objectives of the present study were to investigate the use of artichoke waste extract for the biosynthesis of silver nanoparticles and determine the antioxidant, anticancer, and antimicrobial activities of the biosynthesized AgNPs by different assays.

2. Materials and methods

2.1 Collection of artichoke waste

The waste resulting from artichoke processing was obtained from Egypt's local food processing factory. The debris was ground and then oven-dried at 40°C until constant weight.

2.2 Preparation of artichoke waste extract (AWE)

The extract was prepared by weighing 2.5 g of the previously dried waste in 250 ml of deionized water and then boiling them with continuous stirring for 30 min. The resulting solution was cooled and filtered using Whatman no 1 filter paper. The extract solution was stored at 4°C until further use as a reducing agent (El-Chaghaby *et al.*, 2019).

2.3 Phytochemical analysis of Artichoke waste extract

Different qualitative tests were performed to determine the presence or absence of AWE phytochemicals: Phenols, flavonoids, carbohydrates, protein, Saponin, tannins, and lipids. The Lead acetate test was used to detect the presence of flavonoids. In this test, 10 mg of the extract

were mixed with a few drops of a 10% lead acetate solution, and the formation of yellow-colored precipitate was taken as evidence for flavonoid. Tannins were detected by mixing 2 ml of the extract with two drops of 5% ferric chloride and the appearance of a brown color. Saponin was detected by boiling 1 ml of the extract with 10 ml of deionized water for 15 minutes, then cooling it and vigorously shaking it to record froth formation. For a sodium hydroxide examination, five mg of extract was dissolved in 0.5 ml of 20 percent sulphuric acid solution. It becomes blue after a few drops of aqueous sodium hydroxide solution are added, signaling the presence of phenols. The presence of lipids was confirmed by using the Sudan IV stain test. The yellow precipitate established the existence of proteins formed 2 ml of the extract were heated with 1 ml concentrated nitric acid. Finally, carbohydrates were detected using Molisch's test. These tests were performed as previously reported by (Kamkar *et al.*, 2013; Kushwah *et al.*, 2019).

2.4 GC/Ms analysis of Artichoke waste extract

The chemical composition of AWE was assessed by GC/Ms analysis using GC (Agilent Technologies 7890A) connected to a mass-selective detector (MSD, Agilent 7000) as earlier reported (Santana *et al.*, 2013). The carrier gas was helium, and the analytical conditions were as follows: initial temperature: 100°C (increasing 8°C per minute until a final temperature of 250°C); inlet temperature and mass detector temperatures were 250°C and 300°C, respectively. The mass detector was used in search mode with a scale of 100 to 400 mass units ("scan"). The library index rendered the structural assignments, which selected only specific structures with a 90% or higher probability.

2.5 Preparation of AgNPs using AWE

A solution containing 1mM silver nitrate was prepared using $AgNO_3$ (99%) purchased from Sigma-Aldrich. Then, silver nanoparticles (AgNPs) were prepared by slowly adding 5ml of artichoke extract to 30 ml of $AgNO_3$ (1mM) solution with continuous stirring for 20 min. at 50°C. The formation of AgNPs was monitored visually through color change and then spectrophotometrically by wavelength scanning (200–800 nm) using a UV–vis spectrophotometer.

2.6 Characterization of biosynthesized AgNPs

AgNPs biosynthesized using Artichoke waste extract were characterized using High-Resolution Transmission Electron Microscope (HR-TEM, Tecnia G20, FEI, Netherlands). A droplet of colloidal solution was lowered onto a carbon-coated copper grid, which was then allowed to dry for 45 minutes. The experiment was conducted in bright field imaging mode with a 200 kV electron accelerating voltage and a lanthanum hexabromide (LaB6) electron source gun. An Eagle CCD camera with a resolution of (2k*2k) was used to obtain and capture transmitted

electron images. AgNPs particles size distribution was carried out using a laser diffractometer using Zeta Sizer nano-series (Nano ZS). Measurements were taken in the range between 0.6:6000 nm.

2.7 Antioxidant activity

The total antioxidant capacities of AgNPs and artichoke waste extract were determined using the standard phosphomolybdenum method (Prieto *et al.*, 1999). Briefly, an aliquot of 0.3 ml of each sample was mixed with 2.7 ml of the reagent solution composed of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. Ascorbic acid was used as a reference standard, and the results were expressed as mg ascorbic acid equivalent per ml (mgAAE/ml). Also, the free radical scavenging activity of silver nitrate, AgNPs, and artichoke waste extract was investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method (Vongsak *et al.*, 2013). The reaction was performed using 1 mL of each sample at different concentrations and 2 mL of 0.1 mM DPPH solution. Thirty minutes later, the absorbance was measured at 517nm.

2.8 Anticancer activity of AgNPs

The anticancer activity of AgNPs was tested against breast cancer cell line (MCF 7) using the 3-[4,5-dimethylthiazol-2-yl]2,5- di-phenyltetrazolium bromide (MTT) assay as described by Venugopal *et al.*, 2017. The IC_{50} of AgNPs was calculated from the plot of cell viability percentage against AgNPs concentrations (µmole).

2.9 Antimicrobial activity

Disc diffusion assay was used to determine the inhibition zone diameters caused by AgNPs and artichoke extract against four pathogenic bacteria, two-gram positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*), and two Gram-negative bacterial (*Escherichia coli* and *Salmonella*). Briefly, inoculated plates containing Muller-Hinton agar medium were prepared by dispensing 100 µL of 108 CFU/mL inoculum suspension. Sterile filter paper discs (6 mm in diameter) were soaked with each sample extract and placed on inoculated plates. After incubation at 30 °C for 24 h, the inhibition zones surrounding the paper disc were measured. This test was done at the Food Safety Laboratory, Regional Center for Food and Feed, Agricultural Research Center, Ministry of Agriculture, Egypt.

2.10 Statistical Analysis

All measurements were done in triplicates, and the results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was also used to examine the effects at a significance level ($p<0.05$). Statistical analysis was carried out using SPSS software.

3 . Results and discussion

3.1 Phytochemicals screening for artichoke waste extract

Plant materials usually produce a wide range of secondary metabolites with diverse biological properties (Manzoor *et al.*, 2016). The phytochemical composition of artichoke waste extract is depicted in Table (1). AWE's qualitative phytochemical analysis revealed the presence of many important plant secondary metabolites accountable for its several bioactivities, as previously reported by many authors (Ben Salem *et al.*, 2017; Tsevegsuren *et al.*, 2014).

Table 1. Phytochemical constituents of Artichoke extract

Phytochemicals	**Result**
Phenols	Positive
Flavonoids	Positive
Carbohydrates	Positive
Protein	Positive
Saponin	Positive
Tannins	Positive
Lipids	Positive

3.2 Chemical constituents of AWE by GC/Ms

The GC/Ms analysis of AWE, as given in Table (2), revealed its richness with many bioactive compounds such as phenols, flavonoids, and vitamins. The extract contains caffeic acid dimethyl ether, a derivative of caffeic acid metabolized to phenolic acids *in vivo* (Rechner *et al.*, 2001). Furthermore, AWE contains several phenolic acids such as ferulic acid, gallic acid, gentisic acid, dihydroxy-benzoic acid, and also monoterpenoid phenol (thymol), which are all known to possess potent bioactivities such as anti-inflammatory, antigenotoxic, hepatoprotective, neuroprotective, antimicrobial, and antioxidant (Alia and Abdelgayed, 2018; Yang *et al.*, 2020). Also, the glycoside (2-phenyl ethanol) found in AWE possesses high antioxidant activity (Głąb *et al.*, 2016). Moreover, carotenoids such as lycopene and Astaxanthin and the sesquiterpenes (selinene) were found in the GC/Ms results. It is worthy to note that several vitamins, especially vitamin E, A, and B6 were present among the compounds detected in the GC/Ms analysis of AWE. These compounds have the potential to be antioxidants and free radical scavengers, reducing the development of oxidative stress in a variety of diseases (Ben Salem *et al.,* 2017).

Table 2. GC/Ms analysis of artichoke waste extract

Retention Time (min.)	Compound	Chemical formula
5.516	Caffeic acid dimethyl ether	$C_{11}H_{12}O_4$
7.376	Ferulic acid	$C_{10}H_{10}O_4$
7.813	Gallic acid	$C_7H_6O_5$
9.572	Gentisic acid	$C_7H_6O_4$
10.726	Phloroglucinol	$C_6H_6O_3$
11.126	Fisetin	$C_{15}H_{10}O_6$
13.374	alpha-tocopherol	$C_{29}H_{50}O_2$
13.991	dihydroxy-benzoic acid	$C_7H_6O_4$
15.112	Pyridoxine	$C_8H_{11}NO_3$
15.195	vitamin A	$C_{20}H_{28}O_2$
16.1	2-phenyl ethanol	$C_6H_5CH_2CH_2$
18.838	Thymol	$C_{10}H_{14}O$
22.689	Lycopene	$C_{40}H_{56}$
24.031	Astaxanthin	$C_{40}H_{52}O_4$
25.9	Selinine	$C_{15}H_{24}$

3.3 Formation and characterization of AgNPs

After adding AWE to silver nitrate solution (under the previously mentioned conditions), the solution color changed to faint brown, which was considered the first evidence for the reduction of $AgNO_3$ and formation of Ag-nanoparticles (Mohammed *et al.*, 2018). Further confirmation for AgNPs formation was done by recording the spectrum of the solution by spectrophotometer, and the result is shown in Figure (1). The maximum peak was observed at 430 nm, which is characteristic of surface plasma resonance of the formed AgNPs (Kwon *et al.*, 2020).

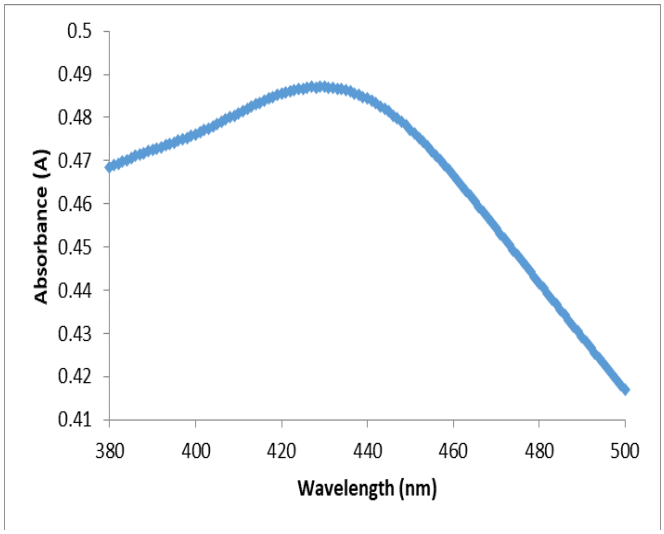

Fig. 1. Spectrophotometric spectral scan of AgNPs

The silver nanoparticles prepared using AWE as a reducing agent were characterized by a transmission electron microscope (Figure 2), and their particle size distribution was also determined (Figure 3). The TEM of the biosynthesized AgNPs revealed different sizes of particles with irregular shapes. At the same time, the average size of the particles was found to be 88.94 nm.

Several authors have considered TEM images as proof for AgNPs synthesize extracellularly (Husseiny *et al.,* 2015; Salaheldin *et al.,* 2019). The average particle reported in the present study was in the same range written by Elamawi *et al.,*(2018) (80-100nm) for AgNPs biosynthesized using *T. longibrachiatum.* Also, Chand *et al.,* (2020) reported the biosynthesis of AgNPs ranging between 5 and 100 nm using onion peels, tomato, and acacia extracts. In the same line, the mean particle size of AgNPs produced using leaf extracts of *Cynara scolymus* was 98.47 nm (Aramkitphotha *et al.,* 2019).

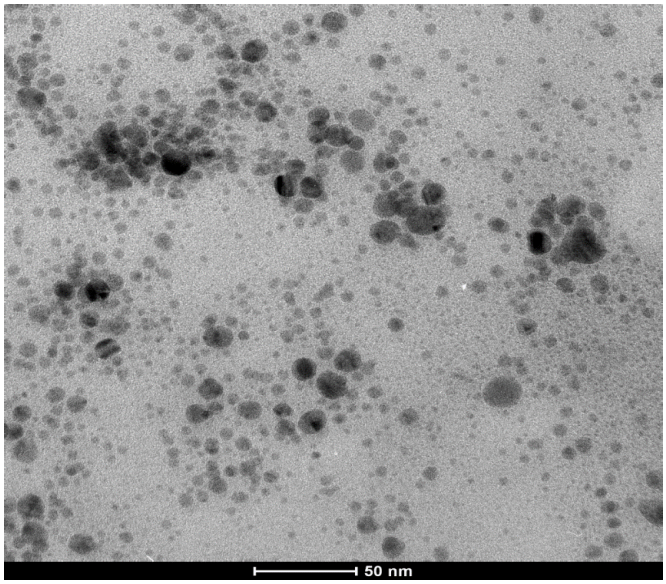

Fig. 2. TEM photo for biosynthesized AgNPs

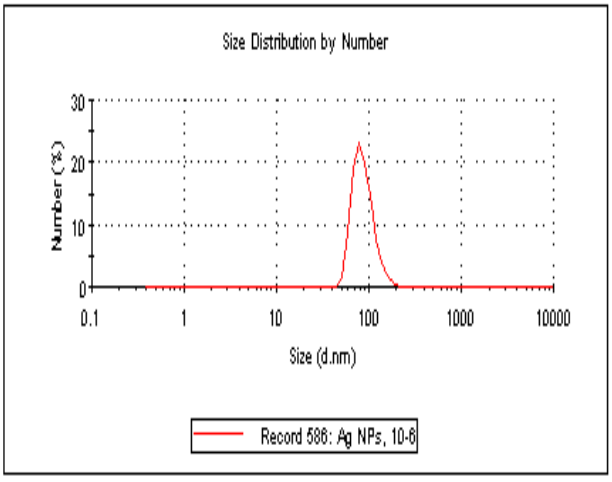

Fig. 3. Particle size distribution of biosynthesized AgNPs

3.4 Antioxidant activity of AgNPs

Table (3) presents the total antioxidant capacity and DPPH scavenging activity of AgNPs prepared using AWE as a reducing agent. The free radical DPPH is well known for its stability and is usually used to determine different extracts' free radical scavenging and antioxidant capacity (Saeed *et al.*, 2020). The present work showed that AgNPs biosynthesized using artichoke waste extract have a higher total antioxidant capacity and higher DPPH inhibition percentage compared to artichoke waste extract and silver nitrate solutions. This higher antioxidant activity reported for the AgNPs prepared using AWE is a direct result related to the composition of the section, which indicated the presence of several phenolic compounds and flavonoids, which are the main phytochemicals accountable for the antioxidant capacity (Salari *et al.*, 2018).

Table 3. Antioxidant activity and DPPH scavenging activity of AgNPs, silver nitrate, and AWE Data are presented as mean ± standard deviations, and the letters a,b,c within the same column signifies that data are significantly different at $p<0.05$.

	Total antioxidant (mgAAE/ml)	**DPPH inhibition (%)**
AWE	86.44^{b} ±3.55	22.06^{b} ±1.08
$AgNO_3$	60.23^{c} ±1.99	19.29^{b} ±0.66
AgNPs	179.93^{a} ±2.91	45.94^{a} ±2.33

3.5 Anticancer activity of AgNPs

Anticancer activity analysis was performed to check the efficiency of AgNPs biosynthesized using AWE against MCF-7 cancer cells by MTT assay. The results for cells viability percentage (Figure 4) indicated a decline in cells viability in a silver nanoparticles concentration-dependent manner. Moreover, using different AgNPs concentrations, the IC_{50} value was determined as 144.29µmole/ml. This proven anticancer efficacy of AgNPs may be related to the effect of AgNPs in the stimulation of reactive oxygen species and their action on cellular components, which results in cell death (Venugopal *et al.*, 2017).

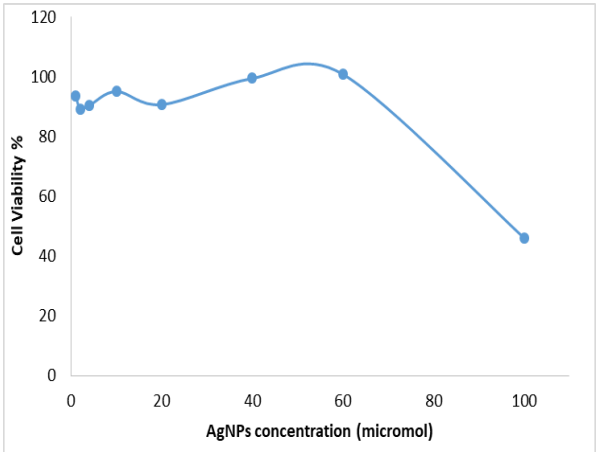

Fig. 4. Effect of AgNPs concentration on cancer cells viability

3.6 Antibacterial activity of AgNPs

Table (4) shows the diameters of inhibition zone diameters for Artichoke extract, silver nitrate, and biosynthesized silver nanoparticles against *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella*, and *Escherichia coli*. The data revealed significant differences ($p<0.05$) in the inhibition zone diameters recorded by the extract, silver nitrate, and AgNPs. It can be noted that artichoke extract did not have any inhibitory effect against the four studied bacteria. While silver nitrate and biosynthesized AgNPs exerted pronounced inhibition against all tested bacteria with significantly ($p<0.05$) higher inhibition zone diameters for AgNPs than those induced by silver nitrate. According to Kushwah *et al.*, (2019), AgNPs exert physical damage to bacteria cells, penetrate the cytoplasm, and inactivate essential bacterial respiratory enzymes and proteins. Also, it was previously shown that AgNPs, due to their small size and their high specific surface area, can easily penetrate inside bacterial cells and cause cell membrane damage (Patra *et al.*, 2016).

Table 4. Antibacterial activity (expressed as inhibition zone diameter)

Bacteria	Inhibition zone (mm/mg sample)		
	Artichoke extract	**$AgNO_3$ (1mM)**	**Biosynthesized AgNPs**
Bacillus cereus **(G^+)**	0.00^c	12.00^b ±0.94	17.00^a ±0.59
Staphylococus aureus **(G^+)**	0.00^c	11.00^b ±0.16	21.00^a ±0.20
Salmonella **(G^-)**	0.00^c	12.00^b ±0.68	17.00^a ±0.26
Escherichia coli **(G^-)**	0.00^c	14.00^b ±0.45	17.00^a ±0.76

Data presented are mean ± standard deviation. a,b,c: different letters signify significance differences ($p<0.05$)

4. Conclusion

The present findings show that the AgNPs biosynthesized using artichoke waste extract demonstrated possible antibacterial efficacy against both Gram positive and Gram negative bacteria. They also exhibited potent antioxidant activity and anticancer activity against the cell line of breast cancer (MCF 7). As a result of their green synthesis, these AgNPs have a range of benefits, including cost-effectiveness and an environmentally friendly process.

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