



# Eco-friendly synthesis of metal nanoparticles using ginger and garlic extracts as biocompatible novel antioxidant and antimicrobial agents

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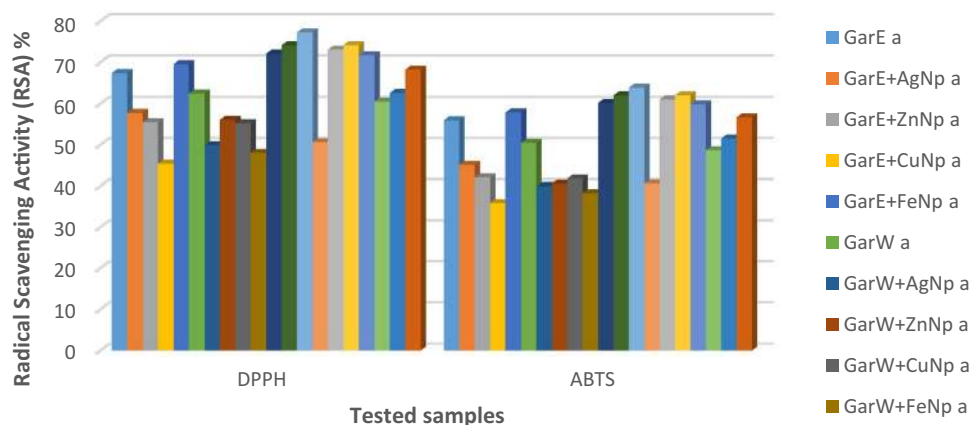
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## Abstract

Ginger and garlic plants were used for the preparation of water and ethanolic extracts. The silver, copper, iron and zinc nanoparticles were eco-friendly synthesized using ginger and garlic extracts and their antioxidant and antibacterial activities were evaluated. The growth of nanoparticles was complemented with characterization using TEM and UV–Vis spectroscopic analysis. Transmission electron microscopy revealed the presence of mono-dispersed metals nanoparticles and silver nanoparticles of ginger have the smallest particle size around 10.10–18.33 nm. The investigated samples were evaluated as antibacterial agents against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans*, *Bacillus subtilis*, *Erwinia carotovora* and *Proteus vulgaris* and as antifungal agents against *C. albicans*. The results of antimicrobial tests indicate that these nanoparticles possess significant activities compared to the results obtained by antibiotic standard. In addition, the total polyphenols, fractionation of phenols, flavonoids by high-performance liquid chromatography systems and antioxidant activity were evaluated.

## Graphical Abstract

Comparison of the DPPH and ABTS antioxidant results.



**Keywords** Garlic and ginger nanoparticles · Chemical composition · DPPH and ABTS assays · TEM · Antibacterial activity

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## Introduction

Spices are the most common antimicrobial and antioxidant agents used in foods. The addition of spices to foods not only confers tasty stimuli but also antimicrobial properties. Spices have been used in traditional medicines for thousands of years

and have been verified as a source of components required for the development of new drugs [1]. Many medicinal plants produce antimicrobial and antioxidant properties that protect the host from cellular oxidation reactions and other pathogens underlining the importance of the search for natural antimicrobial drugs [2]. Spices have a unique flavor which derived from compounds known as phytochemicals or secondary metabolites [3]. Phytochemicals are antimicrobial substances present in spices that can attract profits and resist pests, serve as photo protectors and respond to environmental changes. Many classes of phytochemicals, including isoflavones, anthocyanins and flavonoids, are associated with spices [4].

The scientific name of ginger is *Zingiber officinale* which belongs to *Zingiberaceae* family containing 800 species. Ginger has an active components such as curcumin [5], 6-gingerol, 6-shogaol and 6-paradol [6, 7]. The mixture of honey and ginger has a high antimicrobial activity against Gram-positive bacteria and Gram-negative bacteria [8]. Ginger extracts show antibacterial activity against *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Staphylococcus pneumoniae* and *Haemophilus influenza* pathogens. In addition, the ginger extracts may contain compounds having therapeutic activity.

*Allium sativum* commonly known as garlic, it is a species in the family of onions Alliaceae and belongs to the order of the plants liliales [9]. Garlic (*Allium sativum* L.) exhibits broad antibiotic activity against Gram-negative and Gram-positive bacteria comprising *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus*, *Clostridium*, *Helicobacter* and *Pylori* species. Han et al. [10] have reported that the antibiotic activity of 1 mg of allicin, which is a sulfoxide (+)-*S*-methyl-1-cysteine, was assimilated to that of 15 IU of penicillin. Recent studies have also demonstrated an inhibitory effect by aqueous extracts on many bacterial and fungal species [11–13].

Nanoparticles containing antimicrobial and antioxidant substances could be considered as a new trend of antimicrobial therapeutic agents for the prevention and reduction of deterioration of food and pathogenic microorganisms [14, 15].

The present work is focused on discovering a new technique for green synthesis of metal nanoparticles of ginger and garlic water and ethanolic extracts. The investigated samples were used to evaluate the antioxidant capacity and antimicrobial activity against different microorganisms.

## Materials and methods

### Chemicals and materials

Bulbs of garlic and rhizomes of ginger were purchased from local market, Mansoura city, Egypt. Raw materials

were dried away from sunlight to final moisture content of the sample which was set to  $9.5 \pm 0.3\%$  and crushed to fine powder using Braun GmbH Grinding (Model, KSM2; Type, 4041). Grinded plant powder was separated to be fine enough to pass through sieve size (75–100  $\mu\text{m}$ ). DPPH (Aldrich Chemistry, Germany), ABTS (Sigma Chemical Co.), Folin-Ciocalteu phenolic reagent (Fluka, Biochemica, Germany), anhydrous aluminum chloride LR (sd fine-Chem limited, India), potassium acetate and sodium carbonate (El-Nasr Pharmaceutical Chemicals Company of Egypt), silver nitrate ( $\text{AgNO}_3$ , BDH Chemical Co., England), zinc sulphate ( $\text{ZnSO}_4$ , Andenex-Chemie, England), copper sulphate and ferric chloride ( $\text{CuSO}_4$  and  $\text{FeCl}_3$ , Alpha Chemika, India) were used.

### Chemical composition

The following experiments were carried out at the Micro-Analysis Unit, Faculty of Agriculture, University of Mansoura, Mansoura, Egypt. The moisture, ash, crude protein, lipid and fiber content were determined in garlic and ginger according to the method described by AOAC [16] using 6.25 as a protein factor. In addition, total carbohydrates and energy were calculated.

Absorbed air-dried samples were dissolved individually in 1 mL of a concentrated hydrochloric acid solution and the volume was made up to 100 mL with distilled water. Sodium and potassium were determined using a flame photometer according to Castanheira et al. [17]. Magnesium, calcium, copper, zinc, manganese and iron were determined using atomic absorption (Perkin-Elmer 2380) according to Abbas et al. [18]. The phosphorus was determined colorimetrically as described by Moonrungssee et al. [19]. Previous determinations were obtained at the Agricultural Research Center, Mansoura.

Rubber, cobalt, chromium, lead, copper, iron, manganese, nickel and zinc were determined using the Atomic Absorption Spectrometer Buck Scientific 214 Accusystem Series air/acetylene flames. The concentration of elements in plant samples was considered ppm according to Shirin et al. [20]. Previous determinations have been obtained in the applied and multi-purpose experimental integrated unit for biotechnology and genetic engineering at Mansoura University.

### Extraction of active ingredients

Extraction of the selected plants was prepared according to the previously reported method [21]. Accurately 5 g of plant powder was extracted separately using 100 mL of deionized water and/or ethanol (30%) performed at 60 °C for 30 min on a horizontal water bath shaker (Memmert WB14, Germany). The extracts were then filtered through Whatman no. 1 filter paper (Whatman International Ltd., Kent, UK) using

a Büchner funnel and the filtrates were adjusted to 100 mL in volumetric flasks with appropriate deionized water. The extracts were stored at  $-18\text{ }^{\circ}\text{C}$  till analyses.

### Synthesis of metal nanoparticles

Metals of silver, zinc, copper and iron nanoparticles were eco-friendly synthesized using the method reported by Patanayak and Nayak [22] with a slight modification. Aqueous solution of metal salts (1 mmol) such as silver nitrate, zinc sulfate, copper sulfate and ferric chloride (20 mL) was prepared using deionized water and added to 20 mL of prepared plants extract. The obtained nanoparticles were synthesized in an equimolar ratio of (1:1) of plant extracts to ion solutions. The reaction mixture was kept under stirring for additional two hours at room temperature (Devasenan et al. [23]). The mixtures were subjected to UV irradiation using reduction factor lamp (Vilber Lourmat-6.LC, France) at wavelength ( $\lambda = 254\text{ nm}$ ) for 10 min according to Supraja et al. [24] (Fig. 1).

### Characterization of metal nanoparticles

#### UV–Vis spectroscopic analysis

The reduction of pure  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{Zn}^{2+}$  ions and capping of the resulting silver, zinc, copper and iron nanoparticles were monitored according to El-Shahaby et al. [25] using ATI Unicom UV–Vis Spectrophotometer vision software V 3.20, by detecting the UV–Vis spectra of the reaction mixture at different wavelengths. The UV–Visible spectra of the synthesized metal nanoparticles were recorded around 240–440 nm. The analysis was accomplished at  $25\text{ }^{\circ}\text{C}$  using quartz cuvettes (1 cm optical path), and the blank was the corresponding garlic and ginger extracts.

#### Transmission electron microscope (TEM) measurements

The size, shape, surface, crystal structure and morphological data of the obtained nanoparticles were characterized using TEM (JEOL TEM-2100) connected with a CCD camera with voltage of 200 kV. Each sample of synthesized metal

nanoparticles was prepared by involving a suspension of the sample on copper-coated carbon grids and the solvent was evaporated slowly before recording the TEM images. TEM measurements were recorded at the Central Laboratory, Electron Microscope Unit, Faculty of Agriculture, Mansoura University.

#### Statistical analysis

The statistical analysis of the obtained data was determined using the Co-statistical software package [26] by analyzing the variance (one way completely randomized, ANOVA). The significant differences between treatment means were calculated using Duncan's multiple range test at  $p < 0.05$  as the significance level [27].

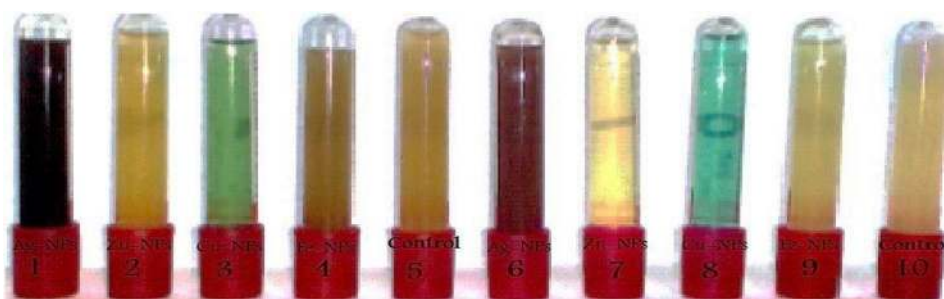
#### Total polyphenols contents

The total phenolic contents were determined using the modified Folin-Ciocalteu indicator technique [28]. Aliquots of 0.1 mL of the solution were taken and mixed with exactly 2.8 mL of distilled water, 2.0 mL of 2% (w/v) sodium carbonate and finally 0.1 mL of 50% (v/v) of Folin-Ciocalteu reagent was added. The mixture was incubated for 30 min at room temperature and the resulting color absorbance was measured at 750 nm against distilled water in the form of a blank using a spectrophotometer. For the quantitative determination, a standard curve of gallic acid (0–200 mg/L) was prepared in the same manner. Total phenol contents were expressed as milligram gallic acid (GAE)/g as a function of dry weight.

#### Total flavonoids contents

The colorimetric method of aluminum chloride [28] was used with slight modifications to determine the content of flavonoids. Approximately, 0.1 g of air dried leaves were dissolved in 1 mL of distilled water. The resulting solution (0.5 mL) was mixed with ethyl alcohol (1.5 mL, 95%) of aluminum chloride (10%, 0.1 mL), potassium acetate (1 M, 0.1 mL) and distilled water (2.8 mL). After incubation at  $25\text{ }^{\circ}\text{C}$  for 40 min, the reaction mixture absorbance was

**Fig. 1** Different colors of ginger-NPs from 1 to 4, garlic-NPs from 6 to 9, ginger and garlic extracts control 5 and 10, respectively



measured at  $\lambda = 420$  nm against distilled water as a white, using a spectrophotometer. Quercetin was chosen as a standard of flavonoids to make the standard curve (0–50 mg/L). The concentration of the total flavonoid contents was expressed as quercetin equivalent of milligram (EQ)/g as a function of dry weight.

### Antioxidant activity

**DPPH radical scavenging assay** The DPPH scanning capacity of each extract was determined according to the method of Li et al. [29]. A stock solution containing 40 mg/mL DPPH (in anhydrous ethanol, w/v) was prepared. One milliliter of sample was mixed with 4 mL of ethanol and DPPH solution. The mixture was shaken vigorously and incubated at 25 °C for 30 min in the dark. Absorbance was determined at 517 nm. A control was prepared without a sample. The scanning capacity of the DPPH radicals was determined from (Eq. 1):

$$\text{RSA}(\%) = \frac{A_0 - A}{A_0} \times 100, \quad (1)$$

where “ $A_0$ ” expresses the control absorbance and “ $A$ ” is the sample absorbance.

**ABTS<sup>+</sup> radical scavenging assay** The appropriate amount of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) [30], diammonium salt and potassium persulfate was transferred to a 10.0 mL volumetric flask and diluted so that the final concentrations were 7.0 and 2.45 mM, respectively. The solution was left in the dark for 12–16 h to form the ABTS<sup>+</sup> radical. Subsequently, the solution of the ABTS<sup>+</sup> was diluted with ethanol until the absorbance of the solution was 1.0. The radical scavenging activity of the oil against ABTS<sup>+</sup> was determined after mixing 0.5 mL of the examined sample diluted in 1-butanol with 2.00 mL of alcoholic solution ABTS<sup>+</sup> and measuring the reduction of absorbance at 734 nm after 15 min. At least five measurements were made for each sample tested, and the percentage of RSA was also estimated by equation (Eq. 1).

### Antimicrobial activity

Antimicrobial activity was estimated by a filter paper disc in the applied experimental and multi-purpose experimental unit for Biotechnology and Genetic Engineering, Faculty of Science, University of Mansoura using inoculations of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans*, *Bacillus subtilis*, *Erwinia carotovora* and *Proteus vulgaris* containing 106 bacterial and fungal cells or 108 yeast cells/mL to spread on nutrient agar, agar Czapek Dox and Agar Sabouraud, respectively. The stored cultures of

the organisms tested were obtained from the Microbiology Laboratory, Faculty of Science, University of Mansoura. The sterilized filter paper discs (Whatman No. 1, 6 mm in diameter) were saturated with 20  $\mu$ L of 20 mg/mL solution of the tested samples that were soaked in control water. The discs were placed on the surface agar of the plates seeded with the test microorganisms. The plates were incubated at 28 °C for the fungi, 37 °C for the bacteria and 30 °C for the yeast. The inhibition zone diameters were measured in mm after 18–24 h for the bacteria and 24–48 h for the yeast.

## Results and discussion

The preparation of stable nanoparticles of garlic and ginger extracts is useful and eco-friendly route to prevent metal ions from agglomeration to bio-reduce  $\text{Ag}^+$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  ions to silver, zinc, copper and iron nanoparticles instead of any artificial or chemical stabilizers such as PVA (polyvinyl alcohol), TPP (tripolyphosphate), PAA (polyacrylic acid), MSA (mercaptosuccinic acid), MPA (3-mercaptopropionic acid), etc. Zero Valence of metals aggregated around bioactive compounds to form nanoparticles. Furthermore, anti-cancer effects were carried out using the aqueous extract of the investigated plants and their nanoparticles. The garlic and ginger extracts were prepared in concentrations of 20 mg/mL.

### Chemical composition of the tested samples

Data in Table 1 showed that moisture, ash, crude protein, raw fat and total carbohydrate contents were identified in garlic and ginger. All results were calculated as (g/100 g dry weight). It was clear that the garlic bulbs contain fat ratio 0.5%. But ginger rhizomes were 3% and the minerals were the lowest in garlic 3.5% than the ginger 9.5%. In addition of that, fiber content in ginger is 16.5% which is higher than garlic 8.5%.

The results showed that garlic contained moisture contents 64.58, crude protein 7.87, crude fat 0.52, crude fiber 2.3, ash 2.46 and NFE 22.27, whereas dry matter in garlic sample was calculated to be 35.42% [31].

Mariam and Devi [32] have mentioned that garlic chemical composition contained: moisture 3.91, protein 19.75, fat 0.49, crude fiber 1.73, Total ash 3.39, Acid insoluble ash, 0.09 g/100 g, Volatile oil content on dry basis 0.49, Energy 348.85 k cal/100 g and Carbohydrate 66.36 g/100 g.

### Minerals contents of ginger and garlic samples

Elements play an important role in human nutrition. For example, phosphorus is an essential element for human bones skeleton and iron represents an important

**Table 1** Chemical composition of garlic and ginger as dried samples

Extracts	Chemical compositions	
	Garlic	Ginger
Moisture %	10.50	12.50
Ash %	3.50	9.50
Protien %	12.42	10.56
Lipid %	0.50	3
Fiber %	8.50	16.50
Total carbohydrate %	64.58	47.94
Energy kJ/kg	3125	2610

**Table 2** Mineral contents of garlic and ginger powders (ppm)

Element	Mineral contents of plants extract	
	Ginger (ppm)	Garlic (ppm)
Na	2.59	4.16
Ca	29.36	49.53
K	20.72	108.45
Mg	34.94	34.94
P	2202.3	3335.77
Cd	2.665	1.977
Co	3.454	1.833
Cr	0.525	0.078
Cu	0.499	0.22
Fe	0.334	0.39
Mn	2.178	1.258
Ni	0.071	0.808
Pb	0.658	0.229
Zn	0.149	0.085

physiological function in the hemoglobin; other elements are important for activity of some enzymes and vitamins. From Table 2, it could be seen that phosphorus values were 2202.3 and 3335.77 ppm while iron values were 0.3903 and 0.3343 ppm in garlic and ginger, respectively.

Akinwande and Olatunde [33] have declared that the mineral content of garlic bulbs is (K, Ca, P, Mg, Na, Fe, Al, Zn, Cu, Mn, Ni, As, B, Mo, Se, Co, Cd and Pb) by concentrations 16675.75, 694.41, 4777.88, 710.12, 341.04, 30.16, 23.3, 66.08, 28.83, 12.26, 4.48, 5.17, 5.82, 0.26, 1.08, 0.19, 1.21 and 0.54 mg/kg, respectively.

Otunola et al. [34] have found that garlic and ginger plants contain the following minerals (Na, Ca, Fe, P, K, Zn, Cu, Mn and Mg) by concentrations 40, 263, 52.9, 101.9, 540, 3.4, 0.01, 0.01 and 41 ppm, respectively, for garlic plant and 50, 257.6, 34.6, 125.6, 2150, 0.4, 0.01, 0.02 and 50 ppm, respectively, for ginger rhizomes.

## UV–Vis spectroscopy

The synthesis of the silver, copper, iron and zinc nanoparticles has been elucidated by scanning the UV–Vis spectra. As shown in Figs. 2 and 3, the maximum absorption peak that recorded at 280 nm is due to the characteristic surface plasmon resonance of the produced metal nanoparticles. The prepared metal nanoparticles were found to be very stable due to possible presence of the polyphenolic components in the extract that prohibited accumulation (Figs. 2 and 3). Garlic and ginger extracts are rich source of flavonoids and phenolics which have an essential role in the reduction process for synthesis of metal nanoparticles [35, 36]. Thus, the high flavonoid and phenolics contents in garlic and ginger water extracts revealed in the phytochemical analysis strongly support the potential of garlic and ginger to bioreduce  $\text{Ag}^+$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  ions to AgNPs, CuNPs, FeNPs and ZnNPs.

The properties of synthesized nanoparticles were examined as a function of UV irradiation. The use of UV–Vis spectroscopic analysis is an effective method for demonstrating the presence of metal nano-structures [37–39]. The UV irradiation role was confirmed to describe the progress of the silver, copper, iron and zinc salts reduction in the presence of garlic and ginger extracts at ambient temperature without UV irradiation (Figs. 2 and 3). No significant change occurred in the UV–Vis absorption spectrum of the prepared sample without UV irradiation. It was then found that in the garlic and ginger extracts solution, UV irradiation displayed an essential role for the synthesis of the silver, copper, iron and zinc nanoparticles.

## TEM characterization of metal nanoparticles

Samples of Ag (I), Cu (II), Fe(II) and Zn (II) nanoparticles of garlic and ginger extracts were examined with TEM [40–43] to confirm the presence of AgNP, CuNP, FeNPs and ZnNP and to estimate the shape, aggregation and particles size of synthesized nanoparticles. As shown in Fig. 4, the TEM was performed for the synthesized nanoparticles at 100 nm. The size of the particles for the AgNPs of garlic is between 13.13 and 22.69 nm, while the size of the AgNPs of ginger is around 10.10–18.33 nm. The shape of the particles is spherical and the small numbers are tetragonal. The particles in the case of the AgNPs of garlic are more aggregated than the AgNPs particles of ginger. Under the given conditions ( $200 \text{ mgL}^{-1}$  Ag (I), Cu (II), Fe(II) and Zn (II) at  $20^\circ\text{C}$ ), the smaller AgNPs were formed using the extract d. On the other hand, the size of the CuNPs particles in garlic is around the range 14.62–22.80 nm lower than those obtained from ginger CuNPs (14.92–27.41 nm). Ginger CuNPs contain a larger number of small size particles than garlic CuNPs and this is why they have had a greater effect on the different



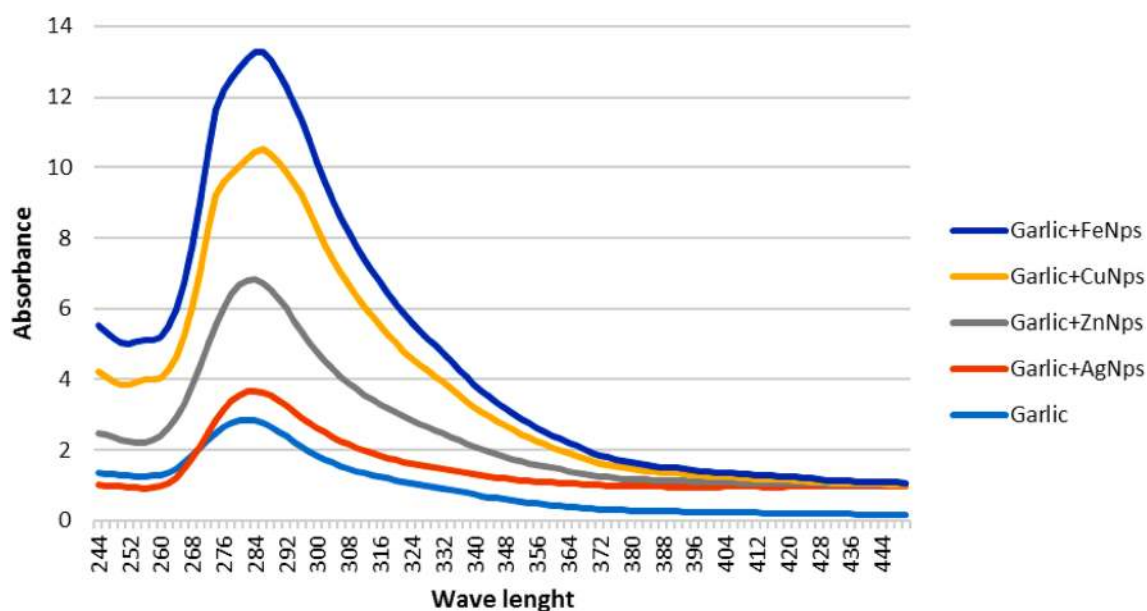


Fig. 2 UV–Vis spectroscopic measurements of garlic and its prepared metal nanoparticles

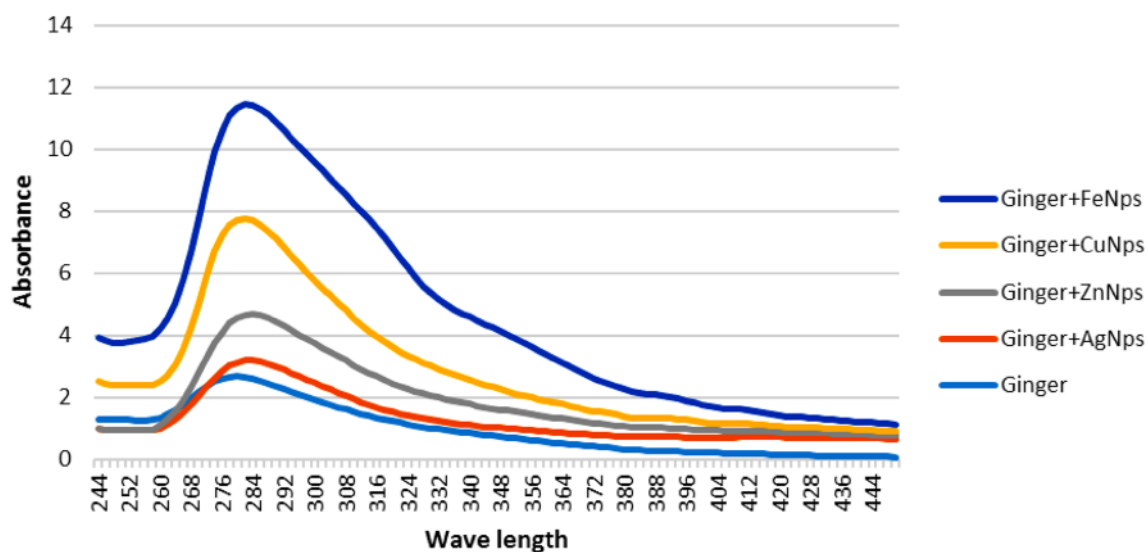
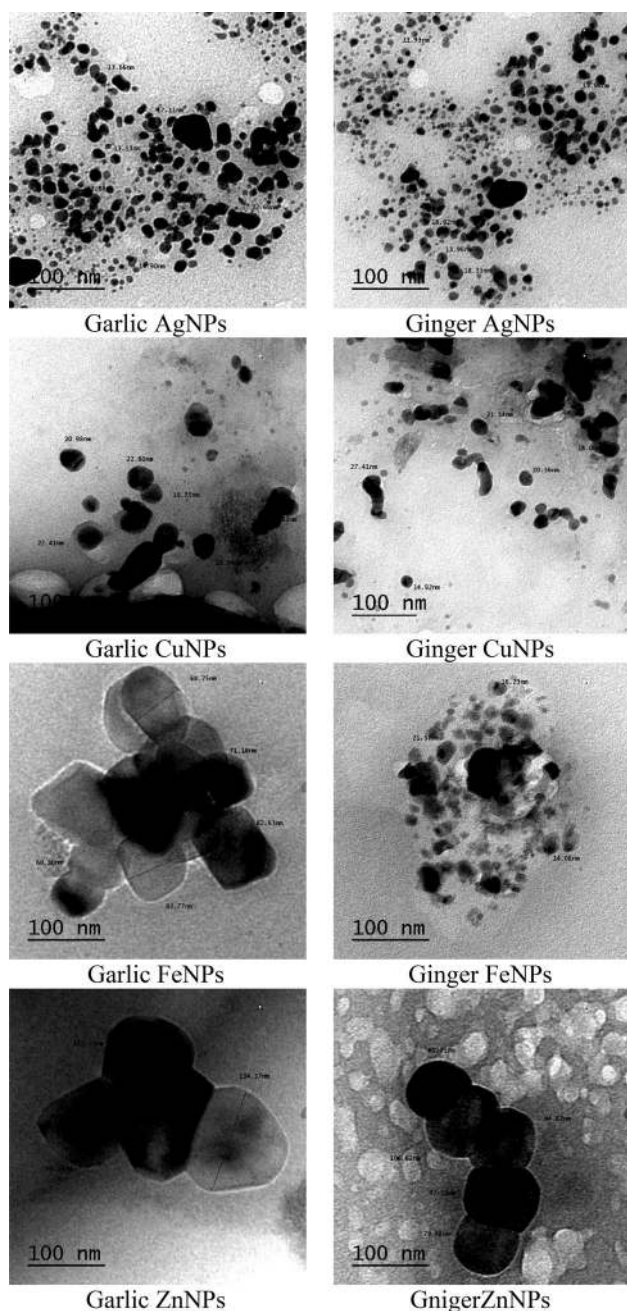


Fig. 3 UV–Vis spectroscopic measurements of ginger and its prepared metal nanoparticles

cancer cell lines. The shape of the garlic CuNPs particles has spherical and tetragonal shapes, while the shape of the ginger CuNPs particles is a spherical and linear shape. The particles are more aggregated in the case of garlic CuNPs than ginger CuNPs. The particle size of FeNPs in garlic is in the range 60.30–82.63 nm with the tetragonal forms of the particles, while the particle size of FeNPs in ginger is in the range of 14.08–21.57 nm with almost spherical forms for the particles. In general, the particles appear more aggregated in the two extracted plants, with a larger size for FeNP of ginger. Finally, the particle size for both nanoparticles with zinc appears to be

greater than other metals. The ZnNP size of the garlic is in the range of 99.34–134.57 nm, while the range of ZnNP ginger is 79.88–100.62 nm. The particles are aggregated into tetragonal and hexagonal forms for garlic ZnNP and spherical and tetragonal forms in the case of ginger ZnNP (Fig. 4).



**Fig. 4** TEM micrographs characterization illustrating the size and morphology of AgNPs, CuNPs, FeNPs and ZnNPs obtained with garlic and ginger extracts at 100 nm

## Biological applications

### DPPH and ABTS<sup>+</sup> Antioxidant Activities

The antioxidant activity of the extracted garlic and ginger and their eco-friendly synthesized nanoparticles was evaluated using free radical scavenging ABTS<sup>+</sup> and DPPH assays. The results of antioxidant inhibition of the two

**Table 3** DPPH and ABTS<sup>+</sup> antioxidant assays for garlic and ginger extracts and their nanoparticles

Extract and NPs	Total poly-phenols (mgGAE/g)	Total flavonoids (mgQE/g)	Radical scavenging activity (RSA) %	
			DPPH	ABTS <sup>+</sup>
GarE	52.55 <sup>g</sup>	17.21 <sup>d</sup>	67.5 <sup>h</sup>	55.98 <sup>o</sup>
GarE+AgNps	25.36 <sup>p</sup>	11.56 <sup>j</sup>	57.77 <sup>n</sup>	45.13 <sup>t</sup>
GarE+ZnNps	40.39 <sup>j</sup>	12.81 <sup>i</sup>	55.52 <sup>p</sup>	42.09 <sup>u</sup>
GarE+CuNps	13.46 <sup>r</sup>	10.68 <sup>k</sup>	45.4 <sup>t</sup>	35.89 <sup>y</sup>
GarE+FeNps	39.54 <sup>k</sup>	16.53 <sup>e</sup>	69.6 <sup>f</sup>	57.89 <sup>n</sup>
GarW	24.44 <sup>q</sup>	12.32 <sup>j</sup>	62.54 <sup>j</sup>	50.47 <sup>r</sup>
GarW+AgNps	34.38 <sup>m</sup>	13.4 <sup>h</sup>	49.91 <sup>r</sup>	39.99 <sup>w</sup>
GarW+ZnNps	32.22 <sup>n</sup>	12.76 <sup>j</sup>	56.09 <sup>o</sup>	40.61 <sup>w</sup>
GarW+CuNps	35.55 <sup>l</sup>	12.11 <sup>i</sup>	55.21 <sup>p</sup>	41.81 <sup>v</sup>
GarW+FeNps	29.41 <sup>o</sup>	12.29 <sup>j</sup>	48.05 <sup>s</sup>	38.3 <sup>x</sup>
GinE	56.9 <sup>d</sup>	15.21 <sup>f</sup>	72.14 <sup>d</sup>	60.2 <sup>l</sup>
GinE+AgNps	55.14 <sup>e</sup>	16.3 <sup>e</sup>	74.18 <sup>b</sup>	62.05 <sup>j</sup>
GinE+ZnNps	63.44 <sup>b</sup>	21.31 <sup>a</sup>	77.31 <sup>a</sup>	63.9 <sup>i</sup>
GinE+CuNps	45.07 <sup>i</sup>	12.68 <sup>i</sup>	50.62 <sup>f</sup>	40.63 <sup>w</sup>
GinE+FeNps	54.22 <sup>f</sup>	18.44 <sup>e</sup>	73.11 <sup>c</sup>	61.08 <sup>k</sup>
GinW	67.09 <sup>a</sup>	19.31 <sup>b</sup>	74.19 <sup>b</sup>	62.06 <sup>j</sup>
GinW+AgNps	52.26 <sup>g</sup>	16.31 <sup>e</sup>	71.78 <sup>e</sup>	59.87 <sup>m</sup>
GinW+ZnNps	49.84 <sup>h</sup>	12.59 <sup>j</sup>	60.55 <sup>l</sup>	48.66 <sup>s</sup>
GinW+CuNps	55.39 <sup>e</sup>	12.82 <sup>j</sup>	62.61 <sup>j</sup>	51.53 <sup>q</sup>
GinW+FeNps	61.8 <sup>c</sup>	14.63 <sup>g</sup>	68.29 <sup>g</sup>	56.7 <sup>o</sup>

Duncan's letters for statistical analysis: "a" is expressed as the highest values and descending to "z" which is expressed as the lowest values

*GarE* ethanolic extraction of garlic, *GarW* water extraction of garlic, *NPs* nanoparticles, *GinE* ethanolic extraction of ginger, *GinW* water extraction of ginger

assays are compatible to each other and reflect the best activated of the tested samples.

From the results given in Table 3, it is clear that GinE, GinE+AgNp, GinE+ZnNp, GinE+FeNp, GinW and GinW+AgNp have the highest antioxidant activities using DPPH and ABTS assays. The tested GinE+ZnNp is the best antioxidant agent with 77.31 and 63.90% of radical scavenging activity through DPPH and ABTS assays, respectively.

On the other hand, the tested samples of GarE, GarE+FeNp, GarW, GarW+ZnNp and GinW+FeNp have slightly strong antioxidant activity with high percent of radical scavenging activity in the range 56.09–69.60 using DPPH assay and in the range 40.61–57.89 using ABTS assay. The investigated samples are capable of scavenging the free radicals from the mixture due to their rich electron donor. The results of antioxidant activity indicated significant values of radical scavenging activity (RSA) % for each of the tested samples. The function of antioxidant agent is to get rid of free radical through its reduction by donating

hydrogen atoms to free radicals. The tested samples have the ability to reduce DPPH radical (violet color) into diphenylpicrylhydrazine (yellow color) by donating hydrogen or electron, same as  $ABTS^{+}$  (Table 3, Fig. 5).

The results of antioxidant activity using DPPH assay were found to be higher than those obtained by  $ABTS^{+}$  assay. The higher antioxidant capacity of ZnNps of ginger ethanolic and ginger water extracts is due to the high phenolic and flavonoid contents.

### Antimicrobial activity

The antibacterial activity [40, 44] of garlic and ginger extracts and their nanoparticles were evaluated against Gram +ve-bacteria (*B. subtilis* and *S. aureus*) and Gram –ve-bacteria (*E. carotovora*, *P. vulgaris* and *K. pneumoniae*). The antifungal activity of the extracted and prepared nanoparticles was evaluated against *C. albicans* strain.

From the obtained results given in Table 4, the garlic extract itself and GarE+FeNps have no antibacterial and antifungal activity towards all the different bacterial and fungal strains. So, the combination of nanoparticles with garlic extract enhanced the activity depending on the shape, aggregation and particles size. CuNps of garlic is the most effective target against bacterial strains, but with no response against *Klebsiella pneumonia* (KP) and *Candida albicans* (CA). Ag and Zn nanoparticles are effective antibacterial agents against *Klebsiella pneumonia*. At the same group of garlic nanoparticles, the strongest antibacterial agent is found to be garlic extract of AgNps with inhibition zone 12.6 mm of killing *Proteus vulgaris* strain.

In general, the most effective antibacterial and antifungal agents are GarW+AgNp, GinE+AgNp, GinE+CuNp and GinW+AgNp with high inhibition zones against all the tested bacterial and fungal strains. On the other hand,

by comparing the obtained results of the extracted and prepared nanoparticles of ginger and garlic against the same bacterial and fungal strains, it is found that GinE+AgNp has the highest activity against *Erwinia carotovora*. Moreover, GarW+AgNp exhibited high activity against *Proteus vulgaris* (PV), GarW+AgNp has high activity against *Klebsiella pneumonia* (KP), GinW+AgNp showed the highest activity against *Bacillus subtilis* (BS) and GinW+CuNp has the highest activity against *Staphylococcus aureus* (SA). Furthermore, GinE+CuNp showed the highest antifungal results against *Candida albicans* (CA) with inhibition zone 15 mm (Table 4).

From the obtained results (Table 4), it is concluded that the type of metal, the physical properties and the particle nature affected the effectiveness of the tested agents against the bacterial and fungal strains. The smallest particle size and the more aggregated particles are the effective agents to spread faster and kill the microorganisms.

Compounds based on metals and their ions are extremely toxic to microbial species and displayed a significant biocidal behavior due to the presence of reactive species with a large surface area [35, 45, 46]. The results of antimicrobial activity revealed that the synthesized nanoparticles are more potent than the plants extract. More recently, it has been shown that the chelation of silver (I) prevents the unfolding of DNA. Silver nanoparticles consist of silver atoms (Ag) and their size is greater than those of the silver ions ( $Ag^{+}$ ), which facilitate the reaction with more molecules, resulting in the enhancement of antimicrobial activity [47].

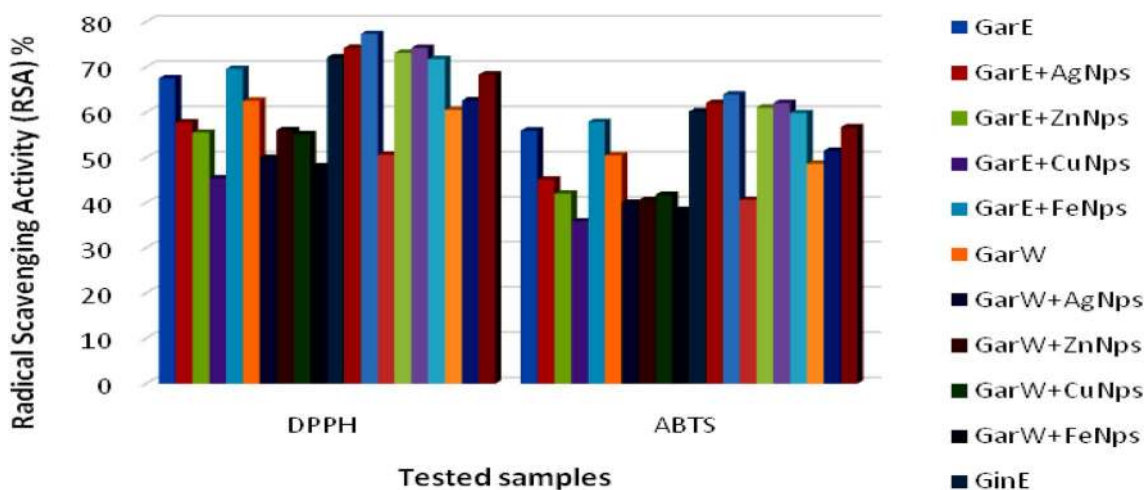


Fig. 5 Comparison of the DPPH and  $ABTS^{+}$  antioxidant results



**Table 4** Antimicrobial activities of garlic and ginger extracts and their nanoparticles

Extracts and NPs	Inhibition zone (IZ) of bacterial strains (mm)					
	Gram (–ve)-bacteria			Gram (+ve)-bacteria		Fungi strains
	EC	PV	KP	BS	SA	CA
GarE	0	0	0	0	0	0
GarE+AgNps	0	12.6	7	0	0	7.6
GarE+ZnNps	0	0	7.6	0	0	0
GarE+CuNps	7.3	7	0	7	7	0
GarE+FeNps	0	0	0	0	0	0
GarW	0	0	0	0	0	0
GarW+AgNps	10	13.6	14.3	9	14	9
GarW+ZnNps	0	0	0	0	0	0
GarW+CuNps	8	7	7.3	8	0	7
GarW+FeNps	0	0	0	0	0	0
GinE	8	0	8	7.3	8	8
GinE+AgNps	10.6	8	9.3	9.3	8	11
GinE+ZnNps	8	0	0	7.6	7	7.3
GinE+CuNps	9	8	7	8	8	15
GinE+FeNps	8	0	7	7.6	7.6	7
GinW	0	0	0	0	0	0
GinW+AgNps	9	12.3	13	11	8	9
GinW+ZnNps	0	0	0	0	0	0
GinW+CuNps	7	12.3	0	0	16.6	9.3
GinW+FeNps	0	0	0	0	0	0

EC, *Erwinia carotovora*; PV, *Proteus vulgaris*; KP, *Klebsiella pneumonia*; BS, *Bacillus subtilis*; SA, *Staphylococcus aureus*; CA, *Candida albicans*

## Conclusion

The moisture, ash, crude protein, raw fat and total carbohydrate contents were identified in garlic and ginger. Samples of Ag(I), Cu(II), Fe(II) and Zn(II) nanoparticles of garlic and ginger extracts were examined with TEM to estimate the shape, aggregation and particles size of synthesized nanoparticles. The tested GinE+ZnNp is the best antioxidant agent with 77.31 and 63.90% of radical scavenging activity through DPPH and ABTS assays, respectively. The antimicrobial results revealed the efficiency of nanoparticles for inhibiting the growth of microorganisms using concentration of 20 mg/mL. The obtained results showed that GarW+AgNp, GinE+AgNp, GinE+CuNp and GinW+AgNp are the most active antimicrobial agents against different microorganisms. The efficiency of garlic and ginger extracts in the rapid synthesis of stable nanoparticles provides a variety of interesting and valuable morphologies due to the collaboration of diverse groups of phytochemicals such as phenolics and flavonoids which have been evaluated. The synthesized AgNPs, CuNPs, FeNPs and ZnNPs of garlic and ginger plants extracts were

characterized by TEM and UV–vis spectra. The particles size, shape, surface area, and crystal structure have the great effects on the obtained anticancer results.

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## Compliance with ethical standards

**Conflict of interest** The authors indicate no potential conflicts of interest.

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