

Antimicrobial Activities of Coriander Seed Essential Oil and Silver Nanoparticles

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Antimicrobial activities of *Coriander* seed essential oil and silver nanoparticles

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Abstract

Bacterial infections are one of the most serious health problems all over the world, which cause need for the discovery of new drug. Since antibiotic resistance is a major threat to both humans and the environment, there is a need for studies on the antimicrobial properties of different forms of traditionally used plants.

Herein, the seeds of coriander were used to isolate essential oil (EO) and to synthesise silver nanoparticles (C-AgNPs). The major oil constituents were characterized by GC-MS as Linalool (79.12%), Camphor (6.16%), γ -Terpinene (2.82%) and α -Pinene (2.67%).

The synthesized AgNPs were characterized by UV-Visible spectrophotometry, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX), X-ray diffraction (XRD) and Fourier Transmission Infrared (FTIR). The surface plasmon resonance (SPR) of C-AgNPs at 437 nm was recorded on the UV-Vis spectrometer. The spherical and homogenous of AgNPs was presented in SEM images. EDX showed elemental distribution and confirmed AgNPs. A characteristic intense peak was at 3.0 keV. The antibacterial activities of the essential oil and AgNPs form of coriander seed against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 bacteria were investigated by broth microdilution test. AgNPs and essential oil of coriander can be expected to provide future opportunities in nanomedicine and materials science. AgNPs can be displayed synergistic antimicrobial effect when used in combination with essential oil.

Keywords: Coriander, essential oil, green synthesized, silver nanoparticles, *Staphylococcus aureus*, *Escherichia coli*.

Introduction

Nanotechnology, with the development of new nanomaterials and nanoparticles, catalysis, electrochemistry, biomedicine, medicine, sensors, food technology, cosmetics, etc. attracts great attention as a new research area for their use [1][2][3][4]. Among all types of nanoparticles, metal nanoparticles have become the focus of science and technology due to their properties such as high surface to volume ratio and high dispersion in solution. In addition, nanoparticles are solid materials with excellent properties in nanometer size (<100 nm) depending on their size and morphology [5].

The use of biocompatible, bio-safe and environmentally friendly nanomaterials is increasing day by day in parallel with the developments in nanotechnology. In addition, silver

nanoparticles (AgNPs) stand out with their applications in medical science in the rapidly developing field of nanotechnology [6]. There are physical and chemical methods for the synthesis of silver nanoparticles [7]. However, these methods are implemented in several stages and usage of toxic chemicals. But in recent years it has been carried out in one stage with aqueous extracts obtained from various parts of plants (leaves, roots, fruits, peel, seeds etc.) [8][9]. This method, called green synthesis, is simple, inexpensive, fast, stable, bio-compatible and nontoxic [10]. the use of plant extract in the biosynthesis of AgNPs is important research area in nano-technology and nanobiotechnology. Until now numerous reports are available about the green synthesis of AgNPs from plant extract. Some examples include *Rhododendron ponticum*, *Hibiscus rosasinensis*, *Cinnamon zeylanicum*, *Tectona grandis*[11][12][13][14][15]

Since multidrug-resistant bacterial infections are one of the biggest health problems in the world, the discovery of new drugs and treatment strategies is urgently needed [16]. Due to the effective use of broad spectrum antibiotics against resistant microbes, there is a lot of interest in the development of new drugs by investigating the antimicrobial effects of essential oils and silver nanoparticles obtained from plants [17].

Essential oils are among the secondary metabolites of plants and their antimicrobial anti-inflammatory properties have been studied extensively and are generally obtained from aromatic plants by hydrodistillation method [18]. The health benefits of aromatic plants and essential oils have been known and reported since ancient times. In addition, these oils are used in the food, medicine and cosmetics industry [17].

Coriander is an annual plant grown primarily for food and medical purposes in the Mediterranean and the Middle East [19]. The plant grown for fruits with a high content of essential oils (0.3-1.1%) has a high economic value due to its use as a flavoring agent in food,

perfumery, cosmetics and medicine. Many studies have reported the antibacterial, anti-inflammatory, anticancer, antidiabetic and antioxidant properties of its various extracts and essential oils [20].

Herein, it was presented the antibacterial activity of essential oil (EO) and silver nanoparticles obtained from coriander (C-AgNPs).

2. Materials methods

2.1. Plant material, reagent, and chemicals

2.1. Reagents and chemicals

Chemicals and reagents were analytical grade. All glasswares were washed with deionized water before any use. Ultrapure deionized water was obtained Milli-Q, Millipore (Bedford, MA, USA) for all synthesis reactions and seed extract preparations.

2.2. Plant materials

2.2.1. Essential oil

Dried seeds of coriander were purchased from local market. Seeds (50 g) after crushing were subjected to hydrodistilled in a Clevenger apparatus for 4-5 hours to give the light yellow essential oil which was stored at refrigerator.

2.2.2. GC-MS analysis of volatile oils

The oil Gas chromatography coupled with mass spectrometry (GC-MS) analysis carried out using a Trace 1310 gas chromatograph equipped with an ISQ single quadrupole mass spectrometer (Thermo Fischer Scientific, Austin, TX). The GC oven temperature was adjusted an initial temperature 70 °C for six minutes then heated up to 235 °C at 3° C/min and finally 10 min 235 °C. the ion source and detector temperature were set at 250 °C. A thermos TG-WAXMS GC column (60m×0.25mm×0.25µm) was used for identification. The carrier

gas was helium with a flow rate 1.2mL/min. the chemical constituents of the obtained oil were determined according to their retention times in mass spectroscopy, and corrected by comparison of the known compounds using mass spectral library search against the National Institute of Standards and Technology (NIST).

2.2.3. Seed extract preparation

To remove dust dried seeds were washed two times with deionized water. 25 g dried seeds were boiled 500 ml deionized water for 20 min under reflux. The mixture solution was cooled at room temperature and filtered with Whatman filter paper. The resulting solution was stored in polypropylene bottles at refrigerator.

2.3. Synthesis of Cs-AgNPs

20 ml of coriander seed extract was added to 100 ml of 1 mM AgNO₃ solution drop by drop and stirred in the flask at room temperature. End of the 30 min the color changed which is indicated of C-AgNPs formation. The solution was centrifugated at 5000 rpm for 15 min. After unreacted impurities was removed, brown precipitate was lyophilized.

2.4. Characterization of C-AgNPs

UV-Vis spectroscopy (Perkin Elmer Lambda 35 UV/VIS Spectrometer) was performed in the wavelength range of 200-800 nm. The Fourier Transform Infrared FT-IR spectra were recorded on Shimadzu FTIR 8400 spectrometer. Scanning electron microscope (SEM) analysis was carried out on Quanta Feg 450. The elemental analysis was performed by EDAX detector. X-ray diffraction (XRD) pattern was obtained by an Empyrean, Malvern Panalytical X'pert PRO diffractometer (PXRD).

2.5. Antibacterial assay

The antibacterial activities of the EO and C-AgNPs form of coriander seed against *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* (ATCC 25922) bacteria were investigated by broth microdilution test as previously described [21][22][23]. Bacteria were incubated in nutrient broth (Merck-Cat No. 105443) at 37 ° C for 24 hours, then inoculated into nutrient agar (Merck-Cat No. 105450) and incubated at 37 ° C for 24 hours.

For broth microdilution test, 100 µl of cation-adjusted Mueller-Hinton broth (Oxoid, Basingstoke, Hampshire, England) was added to all 96 wells of a microplate. Later, 100 µl of plant extract was added to the wells and plant extract concentrations between 5 mg mL⁻¹ and 0.0098 mg mL⁻¹ were obtained by diluting in two-fold serial dilution from left to right. From the bacterial suspensions, 5 µL was added to all wells at a final concentration of 5x10⁵cfu mL⁻¹. For each bacterium, a well without plant extract was used for growth control, and a well containing plant extract and Mueller-Hinton broth was used for sterility control. All samples were studied in 4 parallel. In order to ensure the bacterial concentration, it was inoculated from the growth control well at a dilution of 1: 1000 in 0.1 mL of Nutrient agar. The microplate were incubated at 37 °C for 24 hours. At the end of the incubation, the final dilution wells without turbidity were determined as the minimal inhibitory concentration (MIC) value. 10µL Mueller Hinton agar (Merck-Cat No. 105437) was inoculated from wells with MIC values and above concentrations, incubated at 37 ° C for 24 hours, colony count was made and the minimum bactericidal concentration (MBC) value was determined. MBC is the concentration that kills 99.9% of the initial living bacteria.

3.Result and discussion

Although the essential oil composition of aromatic plants is widely dependent on ecological conditions with their genetic structure, planting season, plant part, and extracting proces, the amounts of basic components are almost the same. The seed of coriander afforded light

yellow oil. The composition of the constituents determined by GC-MS analyses of essential oil are listed in Table 1. The relative amounts (%) of the contents of coriander essential oils, calculated from GC-MS peak areas. there were fifteen compounds of 99.98%. while linalool (79.12%) was major compounds in coriander, second compound was camphor (6.16%)[20] [24] . The results of the other studies showed that the linalool is predominant in the seeds of coriander and our findings are in line with those reports. Linalool (3,7-dimethyl-octa-1,6-dien-3-ol) is a naturally happening monoterpene alcohol found in many aromatic species as essential oils that responsible for the plant odor [25]. Linalool has the many biological activities such as antiinflammatory, antimicrobial, and antioxidant [20] . Moreover recent studies showed linalool has potential neuroprotection that may be used for treatment of neurological disorders, including Alzheimer's and Parkinson diseases, anxiety, depression [26].

Table 1. Essential oil composition coriander seeds, %.

Retention time	Compound name	^a RI	^b RI	%
7.79	α -Pinene	948	955	2.67
9.20	Camphene	953	960	0.49
10.89	2- α -Pinene	956	965	0.25
11.56	Sabinene	973	970	0.15
14.07	α -Myrcene	986	990	0.43
16.50	l-Limonene	1018	2021	1.28
20.66	γ -Terpinene	1059	1061	2.82
23.25	Benzene,methyl (1-methylethyl)	1073	1078	1.12
24.46	α -Terpinolene	1088	1092	0.30
58.95	Linalool	1112	1118	79.12
66.41	Camphor	1121	1129	6.16
69.71	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	1191	1198	0.33
77.42	(+)- α -Terpineol	1207	1215	0.88

81.71	Geraniol	1228	1232	1.88
87.34	Geranyl acetate	1352	1355	2.10

^aRetention Index of references; ^bRetention Index calculated from retention times relative to that of *n*-alkane series; ^c%: Area of compounds, percentages obtained by FID peak-area.

The seed extract was turned to brown within 30 min after the addition of aqueous silver nitrate. The change of color indicates formation of C-AgNPs and is responsible of surface plasmon resonance (SPR) [27]. After centrifuge of C-AgNPs were washed by deionized water to remove some water soluble bio-molecules. C-AgNPs were dried for characterize the structure, composition, and morphology.

The bioreduction of silver ions in the solution was monitored by measuring the UV-Vis spectroscopy (200-700 nm). UV-Vis is a simple analytical technique to display the formation of AgNPs [28]. SPR peak at 437 nm were confirmed the reduction of silver nitrate in to AgNPs (Figure 1.). Different biological functional groups in the structure of plants: alcohols, aldehydes, amines, carboxyl, ketones, hydroxyl, are used to convert Ag⁺ ions into zero-valence silver nanoparticles.

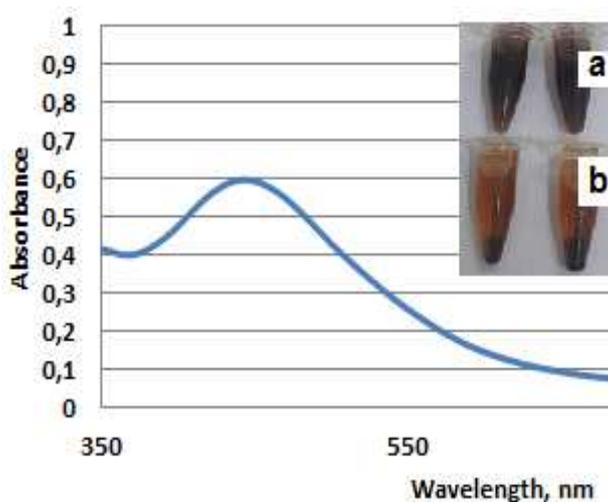


Figure 2. UV-Visible adsorption spectrum of synthesized C-AgNPs at 437 nm. C-AgNPs solution a) before b) after centrifuge

In general, spherical NPs have only a single SPR band in their uv absorbance spectrum [29]. Obtaining a sharp peak indicates that silver nanoparticles are homogeneous and formed in large quantities [30].

F-TIR analysis was carried out to recognize the functional groups involved in the responsible for the reduction and stabilisation of AgNPs existing in the aqueous extract of coriander seed.

The F-TIR spectrum of AgNPs and aqueous extract is shown in Figure 2.

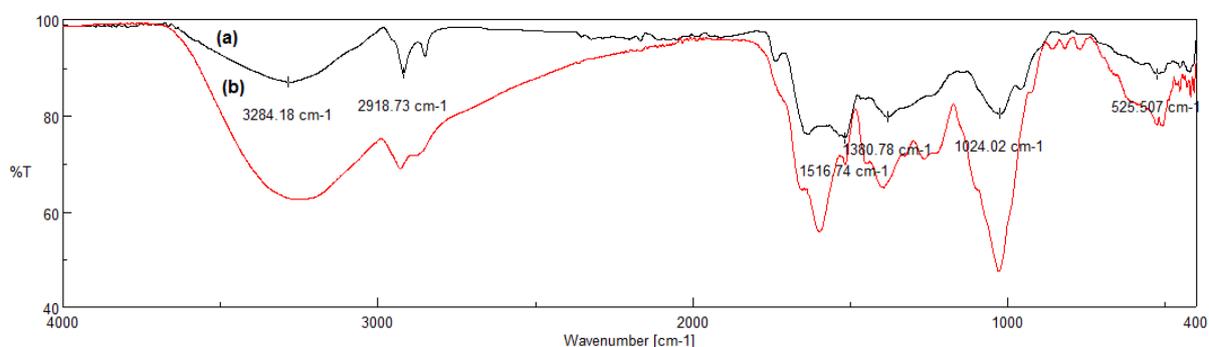


Figure 2. F-TIR spectroscopic graph of C-AgNPs (a) and water extract of coriander (b)

The F-TIR analysis of the coriander showed the presence of a peak at 3284 cm-1, which agreed with that for phenols, alcohols and -OH vibration bands, 2918 cm-1, the extension of the -C-H bond with alkanes vibration and aldehyde -C-H stretching, 1516 cm-1 related to the -C-C stretching peaks with carbonyl stretching, 1380 cm-1 related to the presence of C-H, C-H scissoring bending (alkenes). The weak band around the 525.507 cm-1 due to Me-O stretching vibrations confirm to formation of AgNPs.

SEM images and EDX profile of C-AgNPs synthesized using aqueous extract of coriander seed are depicted in the Figure 3. From the SEM images, the synthesized C-AgNPs are spherical and in the nanometer region. The size variability is due to the content of secondary

metabolites in the extract. EDX peaks showed that C-AgNPs are composed mainly of Ag, O, C are attributed to phenolics and other C-including compounds in the seed extract.

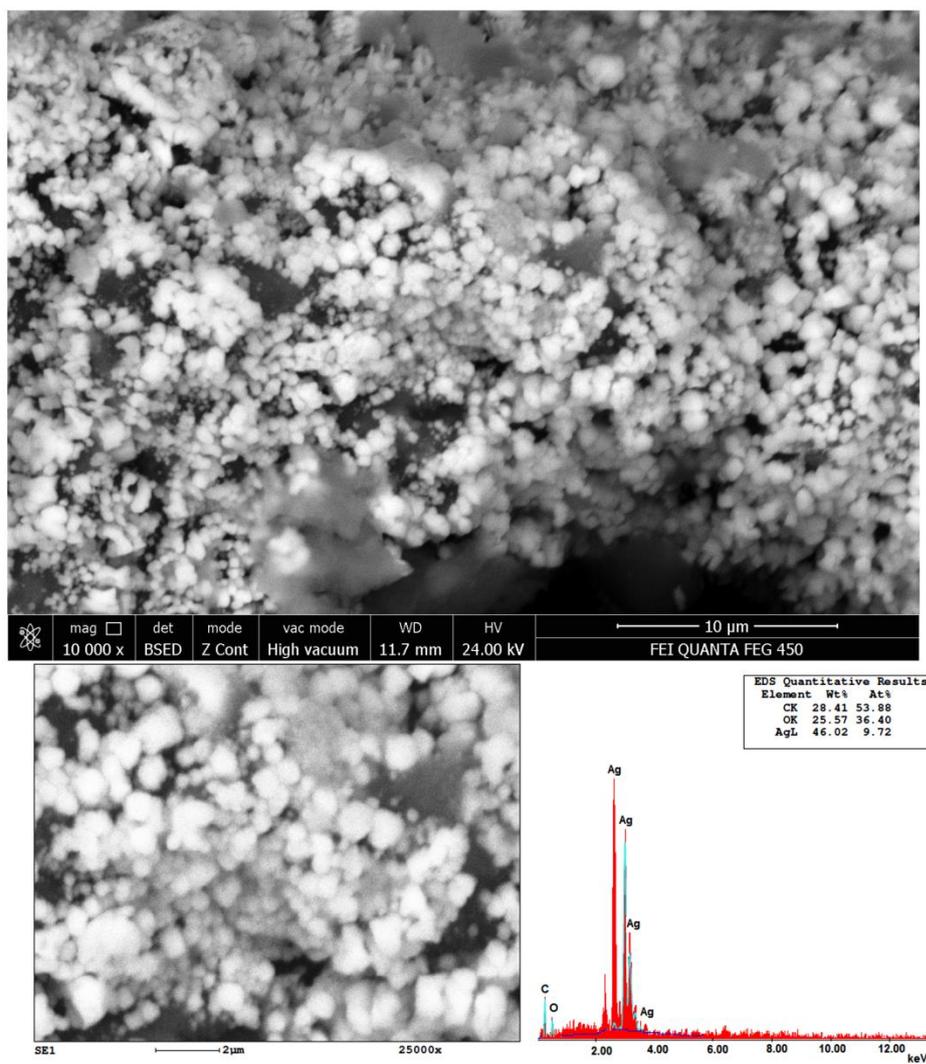


Figure 3. SEM images and EDX spectra of AgNPs synthesized using aqueous extract of Coriander seed.

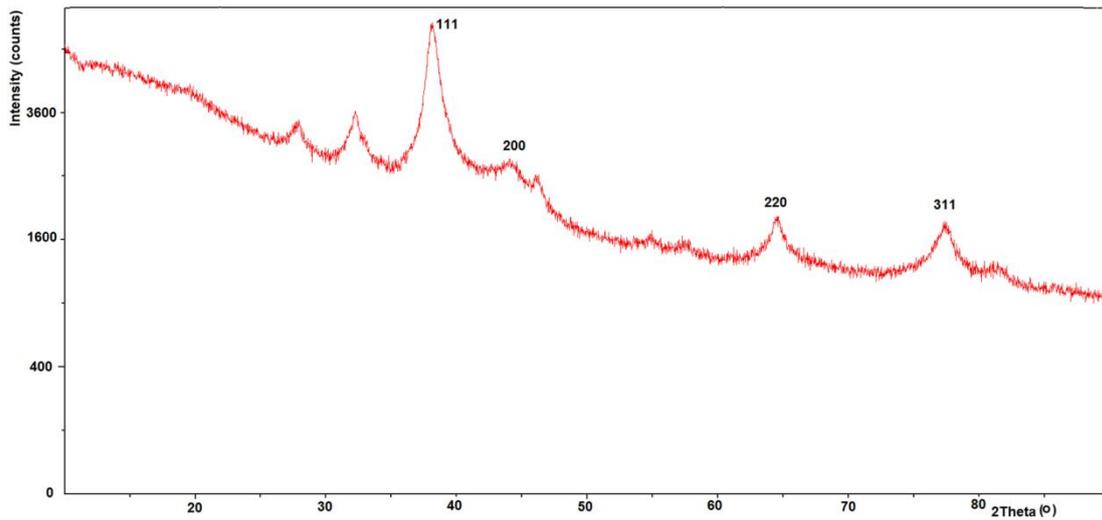


Figure 4. XRD pattern of C-AgNPs synthesized from coriander seed extract.

The crystalline structure of C-AgNPs synthesized using aqueous extract of coriander seeds is depicted in the figure. XRD pattern of C-AgNPs shows the (111) plane at 38.25 θ . Among the different plane (111) is main plane. The obtained results are successfully matched the earlier reports [31][32]. XRD data is proved that the obtained C-AgNPs are face-centered cubic (fcc) structure. The particle size of C-AgNPs was calculated by Scherrer equation

$$D = (k\lambda)/(\beta\cos\theta)$$

Where D is the crystallite size of AgNPs; β is the full width half maximum of (111) plane, λ is the wavelength of X-ray, θ is diffraction angle, and k is a constant. The average crystalline size of the C-AgNPs was calculated as 24.52nm.

So far green synthesized AgNPs and essential oils are reported to exhibit excellent antibacterial property. AgNPs have high surface to volume ratio and small size that facilitate better contact with the microorganisms. NPs attach to and penetrate the pathogenic microbial cell wall, causing structural changes or cell death.

The antibacterial activity of green synthesized C-AgNPs and essential oil was done against one gram positive bacteria such as *S. aureus* and one gram negative bacteria which is *E. coli*. Broth microdilution method was used to test the ability of the antibacterial agent to rupture the bacterial cells.

Table 2. Antibacterial activities of the EO, AE and C-AgNPs form of coriander seed.

Microorganisms	Plant form	MIC	MBC
<i>S. aureus</i>	CEO (mL)	0.312	2.5
	Aqueous extract-AE(mg mL ⁻¹)	0.039	0.009
	C-AgNPs (mg mL ⁻¹)	0.312	1.25
<i>E. coli</i>	CEO (mL)	0.078	0.312
	Aqueous extract-AE(mg mL ⁻¹)	0.039	0.009
	C-AgNPs (mg mL ⁻¹)	0.312	1.25

MIC and MBC values of three different forms of coriander seed such as the essential oil, AE and C-AgNPs are given in Table 2. According to these data, it was determined that all forms of the coriander seed showed antibacterial activity against bacteria.

It has been shown in previous studies that linalool and camphor, which are the main components of Coriander essential oil (CEO), have antibacterial activity [20][33]. In some of the previous studies, linalool and CEO were tested simultaneously with the MBC values and MIC values were found to be equal. It indicates that linalool, the main ingredient in CEO, is effective. This can be explained by the fact that both CEO and linalool similarly cause cell death by damaging the cell wall [34].

In this study, the antibacterial effect of essential oil against *E.coli* and *S. aureus* was determined, especially *E.coli* was found to be more sensitive to the CEO than *S. aureus*. Similar to our results, in a study by Silva et al., It was found that gram-negative bacteria are more pronounced to the CEO than gram-positive bacteria [35]. This result may be attributed to the different cell wall structures of bacteria.

In this study, it was determined that silver nanoparticles synthesized from coriander seed (C-AgNPs) showed antibacterial effects against both bacteria (inhibitory and bactericidal).

However, the antibacterial effect of the AE of the plant has been found lower. The mechanism of inhibition of the AgNPs on microorganisms is not still well known. It is known that silver has antimicrobial properties against almost 700 different microorganisms including fungi, viruses, gram negative and gram positive bacteria [36]. The antimicrobial activity mechanisms of AgNPs are being studied by many researchers. It could be explained; C-AgNPs have large surface area that offers better contact with bacterial cell. According to some researchers, AgNPs disrupt the structure of the cell wall and enter microbial cells, resulting in cell death by depleting the ATP in the cell by the leakage of essential enzymes, metabolites, nucleic acids and peptides [37].

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4. Conclusion

This study has an important meaning for the treatment of infections by examining the antibacterial properties of essential oil and nanoparticles obtained from coriander. C-AgNPs and EO have a broad spectrum of in vitro activity against *S. aureus* and *E. coli*. These promising results suggest that further in vivo experiments are required to better evaluate the safety issues related with their use as a topical formulation in the form of a nano-emulsion combination. The major compound was determined as linalool (79.1%) which could be responsible for antibacterial effect. Moreover, AgNPs can be displayed synergistic antimicrobial effect when used in combination with essential oil. The high activity of AgNPs is significant due to their morphology which enable them to be used in medicinal materials.

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Figures

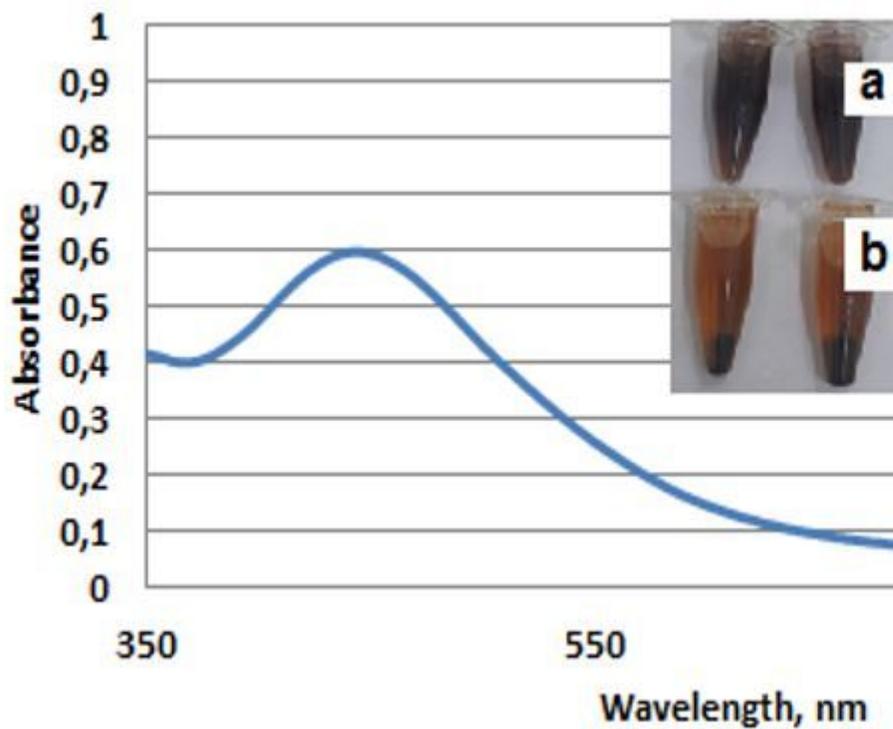


Figure 1

UV-Visible adsorption spectrum of synthesized C-AgNPs at 437 nm. C-AgNPs solution a) before b) after centrifuge

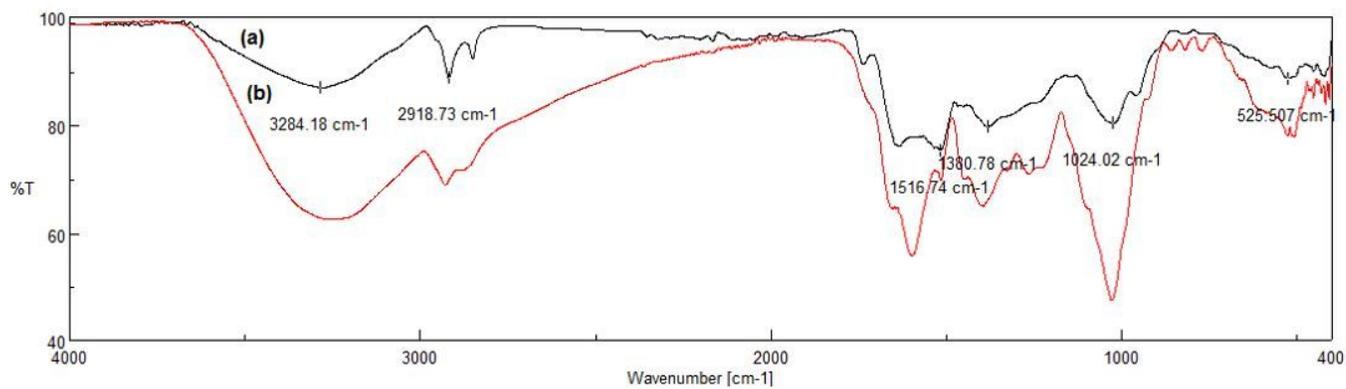


Figure 2

F-TIR spectroscopic graph of C-AgNPs (a) and water extract of coriander (b)

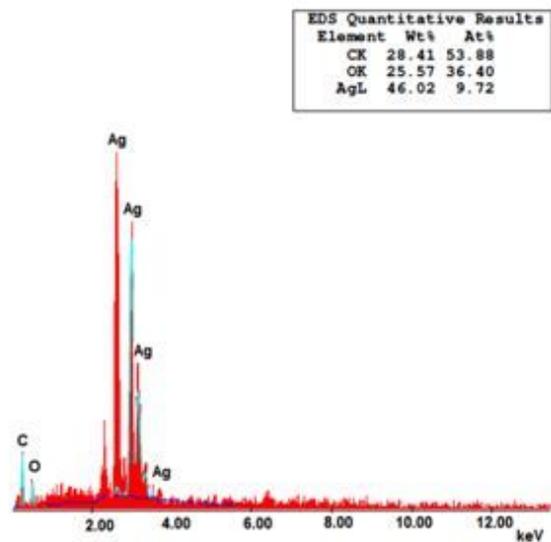
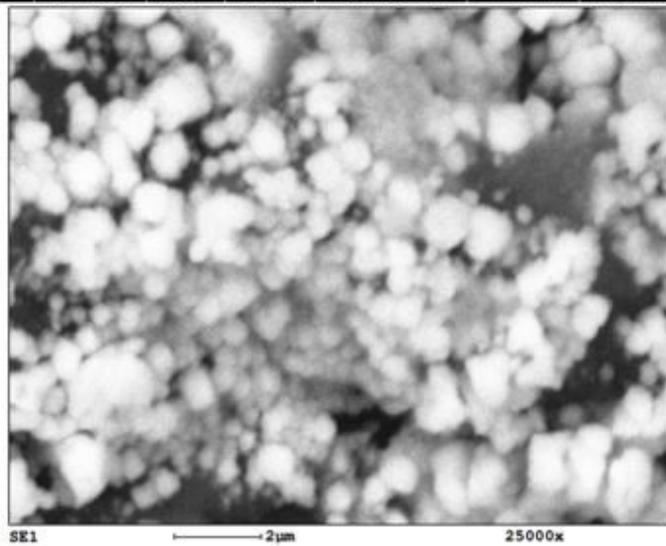
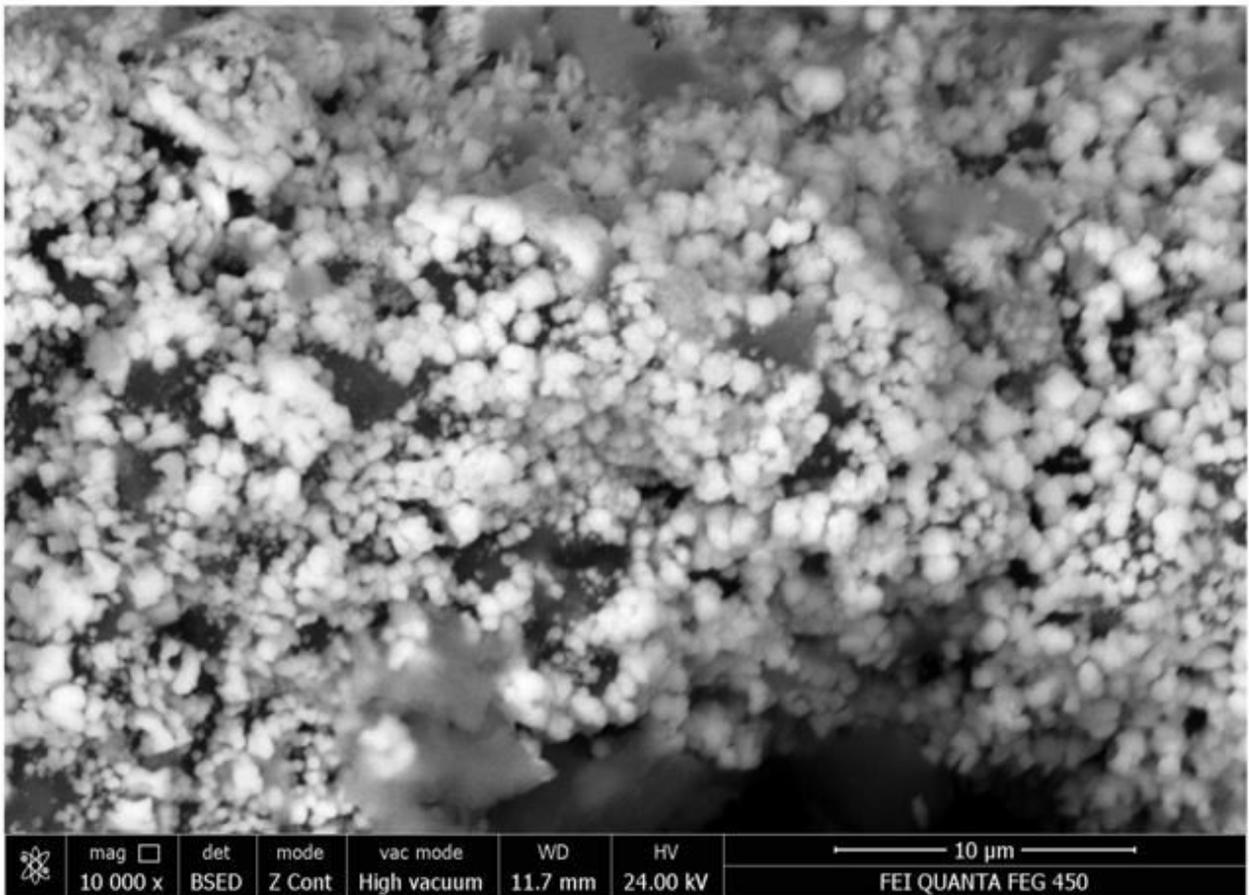


Figure 3

SEM images and EDX spectra of AgNPs synthesized using aqueous extract of Coriander seed.

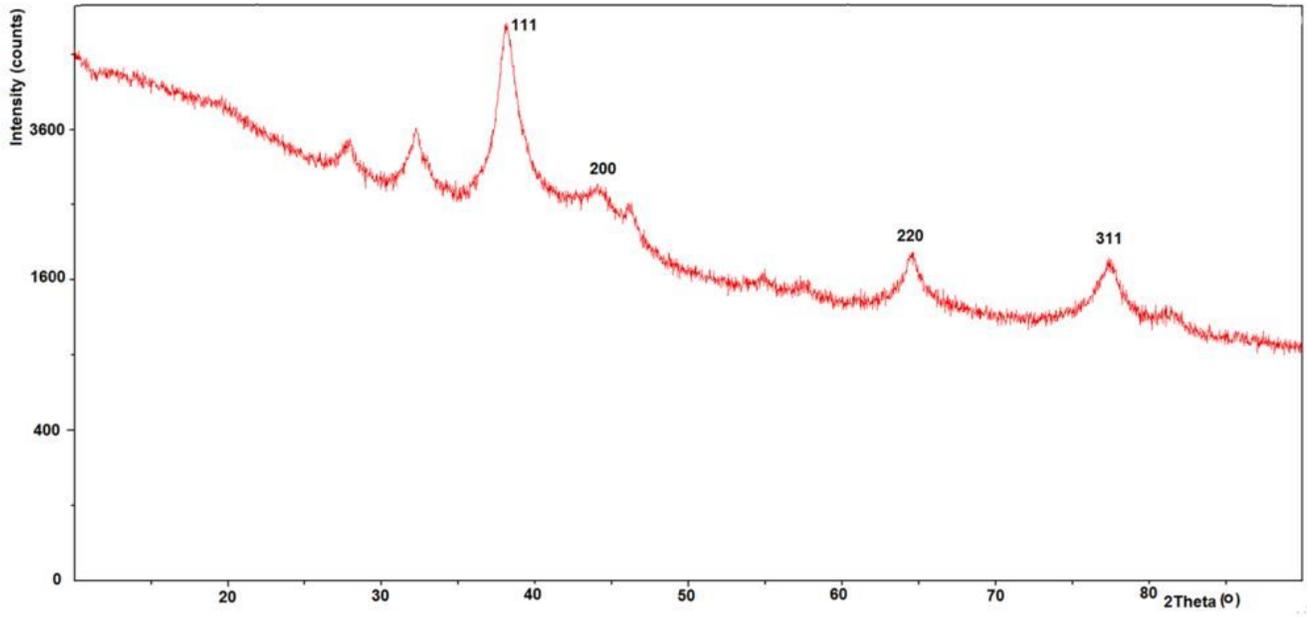


Figure 4

XRD pattern of C-AgNPs synthesized from coriander seed extract.