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Original Article

Green Synthesis of Silver Nanoparticles and Antibacterial Properties of Extracts of *Capparis spinosa* Leaves

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ABSTRACT

Today, the production and use of materials with nanometer diversity is increasing day by day due to the unique and fascinating features of these materials. Until now, various physical and chemical methods have been used for the synthesis of silver nanoparticles (AgNPs), but the use of plants for the synthesis of AgNPs is very fast, simple, non-toxic, and environmentally friendly. In this research, the aqueous extract (AE) of *Capparis* plants was used for the biosynthesis of AgNPs. The color of the silver nitrate solution changed to reddish color after adding the extract. The Antimicrobial activity of AgNPs against *Streptococcus pyogenes, Streptococcus pneumoniae, S. saprophyticus, Hafnia alvei, Acinetobacter. baumannii, Enterococcus faecalis, Proteus mirabilis, Serratia marcescens, Staphylococcus aureus* bacteria were investigated by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using microdilution method. The amount of total phenol and flavonoids in the methanolic extract (ME) of capparis leaves was equal to 229.9- 28.09 mg per gram of dry matter. The antioxidant properties of the ME of capparis were 85.18%. The greatest effect of the ME of the medicinal plant capparis was 0.2315 on the inactivity of *E. coli* and the greatest effect of green AgNPs synthesized from the AE of the medicinal plant Capparis with ELISA of 0.3740 was on the inactivity of *S. mutanis*. The maximum diameter of the inhibitor zone (MDIZ) was 5.5 mm due to the inactivity of *H. alvi* bacteria. The results of this research showed that the leaf extract of the *Capparise spinosa* plant is capable of synthesizing AgNPs and the synthetic nanoparticles showed good antimicrobial activity against pathogenic strains in vitro.

Keyword: Biosynthesis, *C. spinosa*, Antimicrobial Activity, Microdilution, AgNPs

INTRODUCTION

Today, cardiovascular diseases, cancer and infectious diseases are the three main causes of global mortality. Although the treatment of infection has provided a good opportunity for drug companies with an annual sales market of about 25.5 billion dollars, most pharmaceutical companies have lost interest in research and development in the field of antibiotics, because these drugs are comparatively. They are less profitable than drugs used in the treatment of chronic diseases, which require long-term treatment. Also, due to its short life cycle (due to bacterial resistance), it is not of interest to pharmaceutical companies. It is clear that the rise of antibiotic-resistant bacteria, along with the significant decrease in the approval of antibacterial agents in the past decades, has caused widespread concern, and bacterial infections are once again emerging as one of the greatest health challenges [1-7].

In this regard, common cancer treatments also face limitations such as adverse drug side effects and drug-resistant cancers. According to published statistics, 2.8 million people worldwide die of cancer every year. Such reports indicate that today we need newer and more effective treatment methods and compounds to deal with these factors. Therefore, a set of biological methods and available tools of nanotechnology can be a new long-term solution for the successful control of such diseases[4, 8-12].

In the last decade, it has been proven that biological systems, including plants and algae, diatoms, bacteria, yeast, fungi, and human cells, can convert metal ions into metal nanoparticles by using the regeneration capacity of proteins and metabolites present in them.At first, the green synthesis of nanoparticles was done in vivo with the help of plants, in such a way that the salt of the desired metal was provided to the plant, and after absorption from the soil and circulation in the plant, it finally reached the place where the compounds present in it by ion regeneration The metal has been converted into nanoparticles and stored. However, the time-consuming, impossibility of controlling the production process, the high cost and difficulty of the process of separating and purifying the nanoparticles produced in this way prompted researchers in this field to use their extracts from plants and other living organisms, which They contain reducing compounds, they can be used for the synthesis of nanoparticles. In this case, in addition to the high speed of the process, it is possible to control the process and produce nanoparticles of different sizes and shapes, and the separation and purification are cheaper and easier[10, 13-18].

It is worth mentioning that during the past years, the production of metal nanoparticles using the principles of green chemistry has attracted the attention of various researchers from all over the world. India can be considered one of the leading countries in this field [4, 19-21].

Mainly, colloidal metal nanoparticles are made by chemical reduction of metal ions by reducing and coating agents. The main advantage of this method is the ease of making nanoparticles of different sizes and shapes (nanorods, nanowires, nanoprisms and nanoplates). Polymers and surfactants act as coating agents and prevent metal nanoparticles from agglomeration. However, many of these regenerating and coating agents are toxic to humans and the environment. To overcome this problem, recent efforts have been made to provide methods based on the principles of green chemistry. In this approach, natural and safe agents such as extracts of plants, bacteria, fungi and single cells are used as regenerating and stabilizing agents in the process of producing nanoparticles. In addition to being easy, cheap and environmentally friendly, this approach also reduces the concerns related to the biomedical applications of the resulting nanostructures. In addition, nanoparticles with different sizes and shapes can be produced by this method [4, 22, 23].

Extraction from biological organisms can be done both as a reducing agent and as a capping agent in the synthesis of silver nanoparticles. The reduction of silver ions by combination with biomolecules present in the cell extract such as enzymes, proteins, amino acids, polysaccharides and vitamins is environmentally friendly, while synthesis with chemicals is not. A large volume of reports shows that the synthesis of silver nanoparticles using bio-organic compounds is successful [20, 24].

In relation to the antimicrobial activity of silver nanoparticles, a clear and definitive mechanism has not yet been proposed, but the reasons proposed in a wide range of articles can be classified into four general categories: 1) binding of nanoparticles to the bacterial wall, 2) changes in membrane permeability 3) gradual penetration nanoparticles enter the cell, which can disrupt the functioning of cellular enzymes and ultimately increase reactive oxygen species (ROS) and can also prevent the transcription process by binding to DNA. 4) In addition to the mentioned cases, some people also believe that silver nanoparticles induce their destructive effects on bacteria by releasing silver ions [4, 12, 20, 22, 25, 26].

Naturally, only one of these processes alone is never able to destroy cells, and usually, these processes occur as a cooperative effect and together with each other and eventually cause cell death [21, 27].

Today, common cancer treatments face limitations, including adverse drug side effects, which require more effective methods to deal with these factors. Biological methods and available tools of nanotechnology can be a new long-term solution for the successful control of such diseases. Nowadays, metal nanoparticles, especially silver nanoparticles, are important in the field of medicine and therapy[28]. Considering the alarming spread of antibiotic resistance to classical antimicrobial agents, it seems necessary to use a new treatment method to fight against resistant pathogens. Silver nanoparticles have been noticed due to their many uses in medical sciences and industries, and their synthesis in a green way has become one of the challenges facing scientists due to their low cost, fast, non-toxic and biocompatibility [29].

Today, nanotechnology, due to its wide application in science and industry, is growing and progressing at a great speed. Nanotechnology is a science based on nanoparticles, particles with a three-dimensional structure between 1 and 100 nanometers in size. These materials have various sizes and shapes such as crystalline, spherical, needle,

rod, etc. [30, 31]. Many methods have been invented for the synthesis of nanoparticles, many of which are inefficient in terms of material and energy consumption [32, 33].

In chemical synthesis methods, the stability of the particles becomes controversial and it is difficult to produce on a large scale. For these reasons, there is a demand for the production of nanoparticles with environmentally friendly methods [34].

Nanomaterials can be useful in fields such as solar energy conversion, drug production and water purification. On the other hand, among the types of nanomaterials, AgNPs play an important role in various sciences such as biology and medicine due to their physicochemical properties. It is also reported that AgNPs have anti-fungal, anti-inflammatory, anti-viral and anti-platelet activity[35].

Due to the increase in the antimicrobial effects of silver on the nano-scale, AgNPs can be applied to fight against different pathogens, therefor nowadays as nanotechnology is being improved and the production of nanoparticles, these nanoparticles have several applications in different sciences for instance medicine, pharmaceuticals, have found toiletries products [36] and the utilization of silver and its nanoparticles as a powerful bactericidal material has been promoted. Of course, recently AgNPs have been used in DNA tracking in addition to being an antimicrobial agent [37, 38].

The antimicrobial properties of AgNPs and their useful use in biotechnology and specific inhibition of microbes have been investigated and proven in various studies. Therefore AgNPs can influence the metabolism and reproductive processes of microorganisms by preventing the respiratory system of bacteria [39, 40] and hurting to the cell membrane of bacteria [41, 42].

C. spinosa belongs to *Caryophyllales* and *Capparidaceae* family. This family includes annual herbaceous plants, perennial or shrub-like, and often covered with glands. In different regions of Iran and different dialects, other names of this plant are Kabar or Caper Legji, Lijin, and Kharo. [43-45]. In a study, the green synthesis of AgNPs (AgNPs) by reducing silver ions in silver nitrate solution by AE taken from *C. spinosa* leaf has been investigated. The antimicrobial effect of nanoparticles produced by *C. spinosa* has been investigated using different pathogenic bacteria such as *Escherichia coli*, *Salmonella typhimurium*, *S. aureus* and Bacillus cereus [35].

Plant extracts, because they are rich in bioactive compounds, have recently been used for the green synthesis of nanoparticles. On the other hand, the potential of biomolecules in plant extracts to reduce metal ions to NPs in a one-step green synthesis process is very important [35, 46, 47]. The aim of this study is the synthesis of AgNPs in the AE of *Capparis* leaves and the investigation of their antimicrobial properties.

MATERIAL AND METHODS

Plant and Extract Preparation

Silver nitrate with AR grade ($AgNO_3$) was purchased from Sigma-Aldrich chemicals and fresh leaves of *C. spinosa* were gathered in the summer of 2022 from the Agricultural Research Institute, Zabol state of Iran and afterward by the Department of Biotechnology and Plant Breeding, University of Zabol (Figure 1).

Aqueous Extract and Methanol Extract of *C. Spinosa* Leaves

C. spinosa leaves were dried and ground. Then, 100 g of powdered plant leaves were soaked separately in water, and methanol (100% per each solvent), and kept on a shaker for 24 h. After one day, the material was passed through No. 1 filter paper. The solvents were removed from the filtered material by a vacuum rotary apparatus. The concentrated extract was stored in an oven at 40°C for 48 h until a pure extract was obtained and the solvent was removed. The extracts were solubilized at a concentration of 100 in a sterile dimethylsulfoxide solution (DMSO; 10% in water) and dissolved in ethanol (1 mg/mL) for phytochemical analysis. Finally, the obtained extracts were dried, and after weighing, they were kept in the refrigerator at 4°C until the experiment.

It used aqueous extract and methanol extract of *C. Spinosa* leaves to Green synthesis of silver nanoparticles and antibacterial properties, respectively.

Fig. 1 Morphological specifications of leaves and stem of *C. spinosa*.

Bacterial Strains and Culture Condition

Bacterial strains have been received from Laboratory of the Veterinary Department, University of Zabol. To evaluate the antibacterial activity the plant extracts have been investigated the usage of stress of microorganism *S. pyogenes* (ATCC® 19615™), *S. pneumonia* (ATCC 49619), *S. saprophyticus* (ATCC®15305), *H. alvei* (ATCC 51873), *A. baumannii* (ATCC 19606), *E. faecalis* (ATCC 29212), *P. mirabilis* (ATCC 35659), *S. marcescens* (ATCC 274), *S. aureus* (ATCC® 25923). The typed cultures of bacteria turned into sub-cultured on Nutrient agar (Oxoid) and saved at 4oC till required for study.

Synthesis of AgNPs

The aqueous leaf extract (ALE) served as the stabilizing and reducing agent during the AgNP preparation process. The appropriate volume of ALE (0.5 mL) was mixed with 50 mL of a 1.75 mM water solution of silver nitrate in a 100 mL conical flask with continual stirring for 15 minutes at 50 °C to create the AgNPs used in this work [48]. It was read when the first color change was observed compared to the control. Finally, Ag_2O was obtained through the listed treatments according to the following reaction:

1) $2Ag^+ + 2NO^{3-} + 2Na^+ + 2OH^- \rightarrow Ag_2O + 2NaNO_3 + H_2O$

The Ag_2O precipitate was isolated and washed with distilled water three times. The precipitate obtained was dried at 50° C for 2 hours. A brown Ag_2O was thus obtained. A color change to brown and opaque was seen as the first sign of nanoparticle synthesis. Finally, the absorption spectra of all samples were measured from 300 to 800 nm [16]. All experiments were performed in triplicate.

The synthesis of AgNPs involved the optimization of many parameters, including pH (which was originally changed individually to 4, 5, 6, 7, and 8, respectively, by adding 1 M sodium hydroxide or 1 M hydrochloric (HCl)) and agitation speed at different rpm ranges (400-800), using a magnetic stirrer. Centrifugation was used to collect the produced AgNPs for 10 minutes at 8000 rpm. The particle was repeatedly cleaned with deionized water, and the supernatant was disposed of. The produced AgNPs were dried for three hours at 60 °C in a hot air oven[48].

UV-Vis spectroscopy

In order to check the attendance absorption spectrum of AgNPs has been synthesized Nanoparticles were measured by applying PerkinElmer spectrophotometer (UV/Visible-Lambda 45 model, USA). To that end, 1 ml of 200 µl of solution containing extract and nitrate was subjected to UV/Vis quantification at wavelengths between 300 and 700 nm. The treated extracts were collected at different times and absorption spectra were

immediately calculated over the wavelength range of 420–450 nm before the samples were dried. This wavelength range is associated with elemental silver.

XRD Spectroscopy

In this study, X-ray diffraction was used to extract the fidelity of the synthesis of AgNPs, examining the type of crystal lattice and the size of the synthesized silver particles. AgNPs were synthesized using an XRD device, voltage 40 kV, current 40 mA, and its material was copper, performed in the laboratories of Sistan and Balouchestan University. The AgNPs form sharp peaks at 38°, 44°, 67°, and 78°, with specific peaks corresponding to planes (111), (200), (220), and (311) in sequence. [17]. If the peaks at these four angles are perfectly sharp and well-defined, indicating a favorable interaction between the mineral nanoparticles and the extract while the nanoparticles are stabilizing, they exhibit characteristic sharp peaks. Otherwise, the peak is weak [18]. On the other hand, the nanoparticle size cannot be obtained directly from the peak, but its limit can be obtained by the Debye-Scherrer equation [19] as follows: $L = K\lambda/\beta Cos\Theta$.

FTIR Spectroscopy

For FTIR spectroscopy, 25 mL of 1 mM silver nitrate solution was mixed with 5 mL of extract and kept at 20 °C for 24 hours. After completion of the reaction, the samples were centrifuged at 5000 rpm for 30 minutes and the supernatant was removed. This was repeated three times for confirmation. After centrifugation, the samples were dried and the powder was applied for FTIR analysis. A similar procedure was followed for the extract.

Scanning Electron Microscopy (SEM)

In order to check the size of synthesized AgNPs and the morphology of synthesized AgNPs, this analysis was used. For this purpose, the nanoparticle sediment was centrifuged three times at 12,000 rpm and the resulting sediment was photographed by an electron microscope in such a way that the nanoparticles were fixed on a copper grid covered with carbon and after drying with a lamp Infrared photography was done.

Extraction for the Measurement of Phenol, Total Flavonoid and Antioxidant

The ME was qualified by cold maceration method with a ratio of 1:20 dry plant material and 80% methanol solvent. The samples were soaked in the solvent for 48 hours on a shaker at 120 rpm. After that, it was filtered with Whatman No. 1 filter paper and transferred to a rotary evaporator with a temperature of 45 degrees for concentration. One hour after the concentration of the extract, it was transferred under the laminar hood so that the rest of the solvent gradually evaporated and the dry extract was obtained. This dry extract was used to prepare ME for other tests with a concentration of 1 mg/ml.

Measurement of Total Phenol

The amounts of phenolic compounds in methanolic plant extracts were measured by the method of preceding research [49] results were stated regarding milligrams of gallic acid per gram of extract. According to this method, 200 microliters of the extracts (with a concentration of 1 mg/ml) were poured into test tubes. 400 microliters of Folin Ciocalto reagent (diluted in a 1:10 ratio with distilled water) and 400 microliters of 7% sodium carbonate were added to the above mixture. After 30 minutes of storage at ambient temperature, its light absorption was read by a spectrophotometer at a wavelength of 765 nm. Finally, by putting the absorption value of the extract in the linear equation related to the garlic acid standard curve (10, 50, 100, 150, 200 and 250 mg/ml)(Figure 2), the amount of total phenol in the extract was calculated. The data were expressed based on the equivalent of milligrams of garlic acid per gram of extract (mg GAE/g). All calculations were acquired in triplicate.

$Y=0.0001X +0.171$

Y: Absorption number recorded in the spectrophotometer

X: amount of total phenol

Measurement of Total Flavonoid

The flavonoid content of these extracts was determined by the aluminum chloride colorimetric method. In this method, 100 microliters of aluminum chloride solution (10%), 100 microliters of one molar potassium acetate solution and 2.8 milliliters of distilled water were added to 500 microliters of ME. Samples were incubated for 40 minutes at room temperature and the absorbance of the mixture at 415 nm was calculated. The standard curve was drawn based on a solution with different concentrations (50, 150, 250, 350, 450, and 550) -450-350-250-

150-550-450-550 mg/ml) of quercetin (Figure 3) and the amount of flavonoid equivalent to milligrams of quercetin per gram of dry plant powder (mgQUEg-1) was calculated and determined. Also, the solution blank was prepared in the same way without extract [50]. All measurements were performed in three replicates.

Y=0.001x – 0.0442

Y: Absorption number recorded in the spectrophotometer

X: amount of total flavonoids

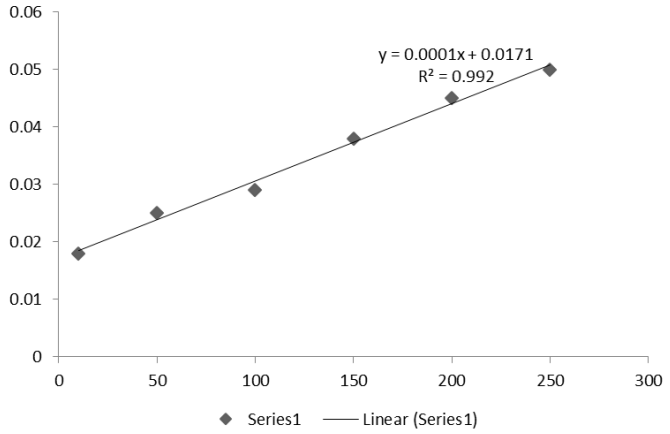

Fig. 2 The calibration of the gallic acid standard curve

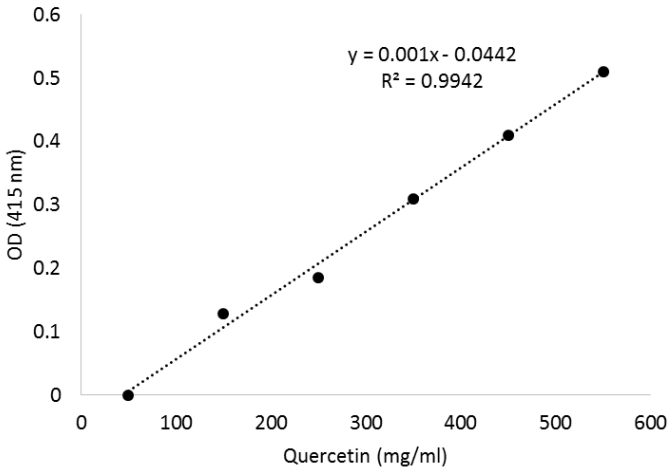

Fig. 3 The quercetin curve to evaluate the flavonoid

Measurement of Antioxidant Activity

The free radical inhibitory activity of DPPH was calculated according to the method of a previous study [22]. This method is based on changing the color of the purple methanolic solution of 2 and 2-diphenyl-1-picryl-hydrazyl to the yellow solution of diphenyl-picrylhydrazine. 250 microliters of the extract was mixed with 750 microliters of DPPH solution (two milligrams of DPPH was dissolved in 50 milliliters of methanol). After incubating these samples for 30 minutes at room temperature in the dark, the absorbance was read at a wavelength of 517 nm using a spectrophotometer. The percentage of inhibition of free radicals was calculated with the following formula:

(Ac-As)/Ac×100 = percentage of free radical inhibition

AC: absorbance for the control sample

AS: absorption rate of plant sample

Determining the Sensitivity of AgNPs Against Human Pathogen

Determination of bacterial susceptibility to AgNPs was performed by the well-dilution method. Six wells were made with solid culture medium and 100 µl of each well was combined with Muller Hinton Broth (MHB) culture

medium. Then 100 ml of the diluted plant extract was added to the first well and after mixing 100 µl of the first well was added to the second well and so on to the last well. 100 µl of medium was removed from the last well and 10 µl of microbial suspension containing 107 units/ml, corresponding to 0.5 McFarland, was placed in a 37°C incubator for 24 hours. The MIC was considered to be the first well that prevented bacterial growth after being placed in the incubator. To ensure clear wells, 10 µl was removed and transferred to the Muller Hinton Agar medium. And after 24 hours, the first dilution that could destroy 99.9% of the bacteria was shown as the MBC.

Statistical Analysis of Data

Three replicates were measured for each treatment. Statistical analysis of data was performed using SAS statistical software version 9.1 and data were presented on a ± standard deviation scale.

Arises

Biosynthesis of AgNPs.

Fresh AE of *C. spinosa* leaves was pink in color and turned dark red upon the addition of 0.001 M silver nitrate solution and exposure to sunlight for 3 minutes.

UV-Vis Spectroscopy

After adding the plant extract to the silver nitrate solution, the complete color change of the reaction solution was achieved after two hours, and during the reaction, the solution turned from pale yellow to dark brown. It was measured from the visible ultraviolet testing device in the wavelength range of 300 to 700 meters, as can be seen in Figure 4, the absorption of nanoparticles at the wavelength of 410 nm was revealed, which indicates the synthesis of AgNPs. In different test treatments, the absorption intensity of the samples was measured and considered as an indicator of the production of nanoparticles.

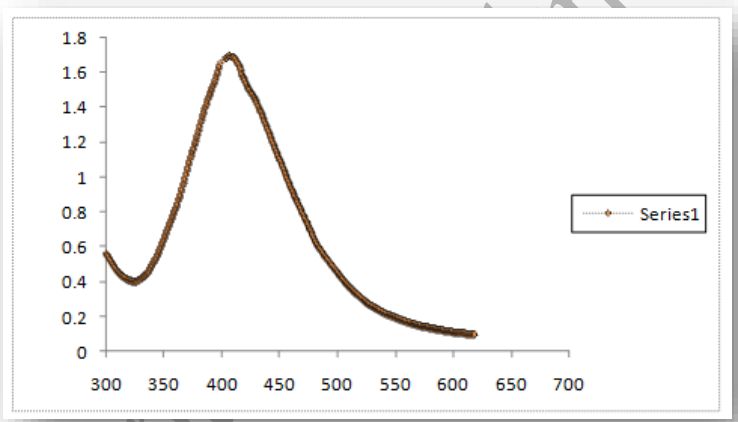

Fig. 4 The absorption spectrum of AgNPs in the range of 300-700 nm.

FTIR Spectroscopy

FTIR spectroscopy analysis was conducted to identify responsible biological molecules as the cover agent of synthesized nanoparticles. The sample of nanoparticles is shown in Figure 6. In this spectrum of peaks in positions 3440-3446 (related to NH Amide bonds) 2922-2924 (related to CH Alkan bonds) 1631-1638 (related to amino acids with NH_2 groups, Amine Type I bonds) 1415-1401 (Due to H-C deformation, ketones and esters) 1147-1154 (related to skeletal vibration of di methyl) 1019-1021 (related to P O tensile bonds). This data has shown that the groups of amide, carboxyl, amino and amino acids that exist in the leaf extract are intricated in the synthesis of AgNPs (Figure 5).

XRD Spectroscopy

X-ray diffraction pattern of synthetic nanoparticles (Figure 6) after washing and removing waste materials showed that the nanoparticles were metallic silver (AgO). Specific peaks were obtained at angles of 36.5, 42.3, 68.4, and 75.4 degrees.

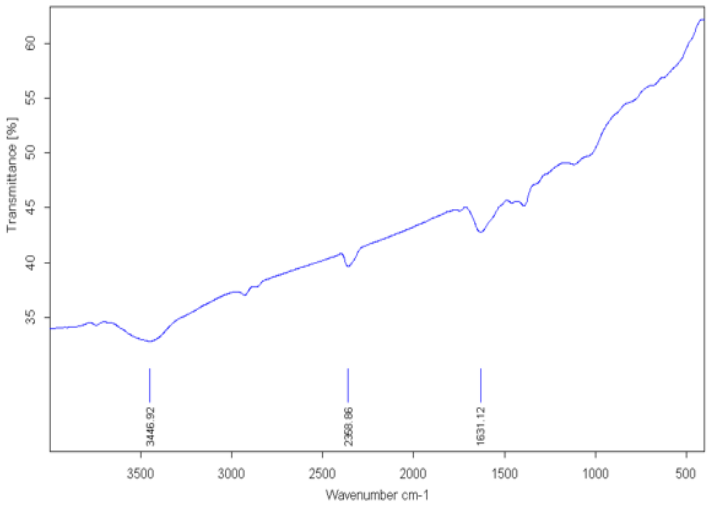

Fig. 5 FTIR Spectrum of Synthesized AgNPs

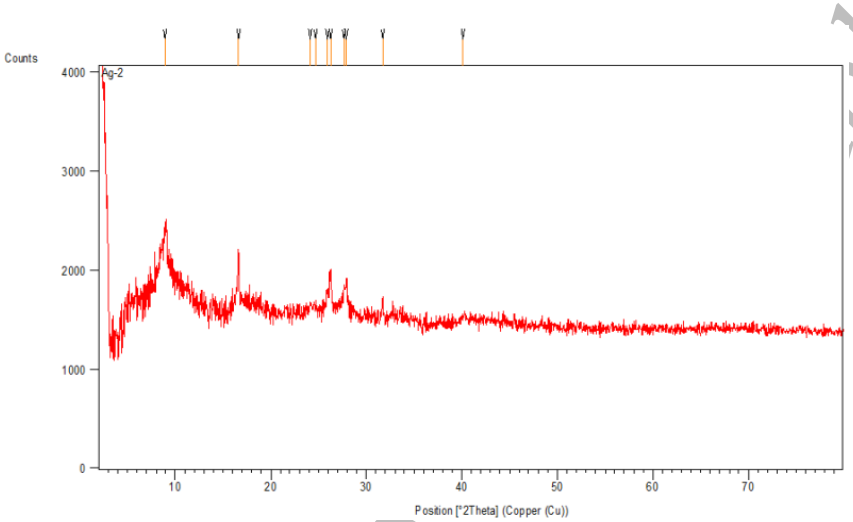

Fig. 6 XRD pattern from Synthesized AgNPs.

FeSEM Analysis

FeSEM analysis of the synthesized AgNPs revealed that the particles were nanoscale in size, especially spherical (Figure 7). The size of the nanoparticles synthesized under optimal conditions was determined to be 21 nm, and the nanoparticle size abundance percentages are shown in the histogram plot.

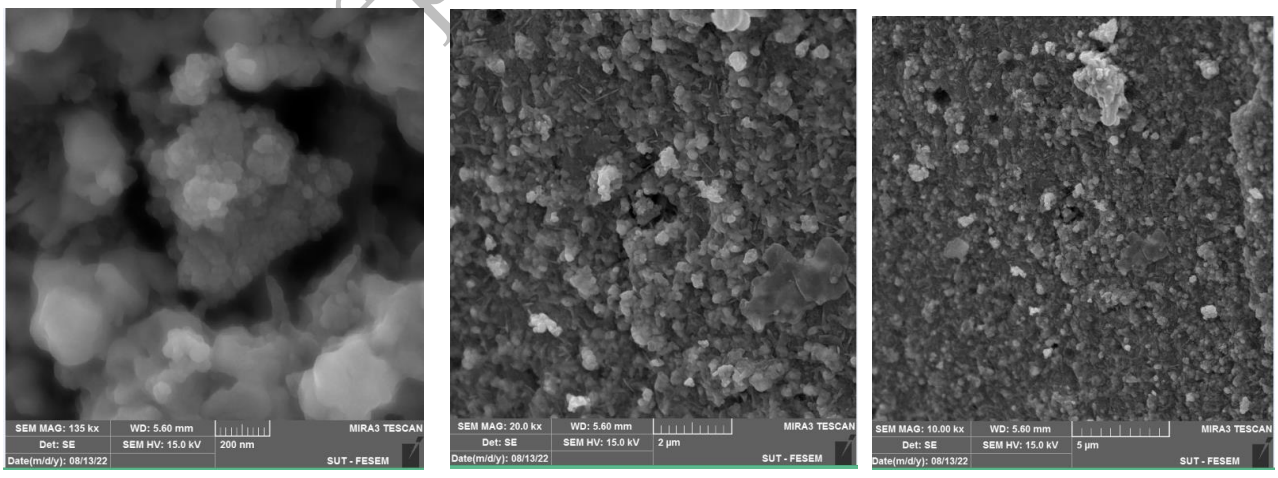

Fig. 7 Fe SEM analysis of the synthesized AgNPs

The amount of total phenol and flavonoids in the ME of capparis leaves was equal to 229.9- 28.09 mg, respectively, per gram of dry matter. The antioxidant properties of the ME of capparis were 85.18%. The results of ELISA showed that the ME of the medicinal plant capparis on the activity of *B.cereus, E.coli, E.fecalis, H.alvi, P.aeruginosa, P.mirabilis, S.aureus, S.mutanis, S. Pneumonia and S. pyogenes* had a different effect (Table 1). The greatest effect of the ME of the medicinal plant capparis was 0.2315 on the inactivity of *E. coli* (Figure 8). The best experts of ME, the presence of 12.5 mg/ml with ELISA 0.2373 was the lack of microbial activity (Figure 9).

Table 1 Analysis of Variance ELISA on the inactivity of bacteria in the ME of the capparis

Source	DF	SS	MS	F
strain	9	0.43223	0.04803	7.5 **
concentration	5	1.01038	0.20208	31.57 **
strain*concentration	45	1.38745	0.03083	4.82 **
Error	120	0.768	0.0064	
Total	179	3.59806		

**Significant at the one percent level

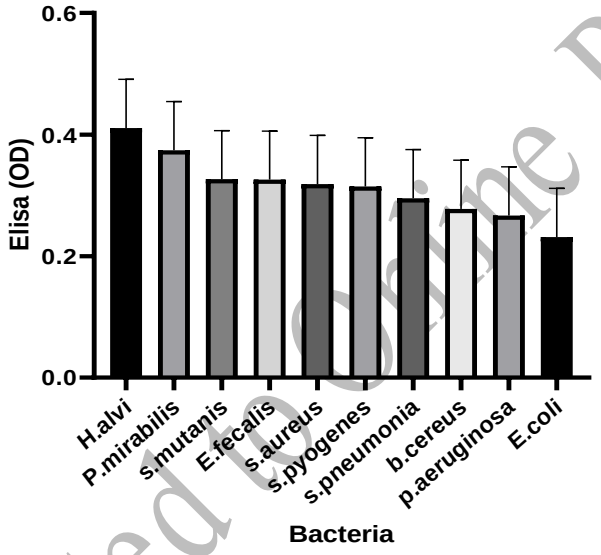

Fig. 8 ELISA of the ME of the medicinal plant Capparis against bacteria

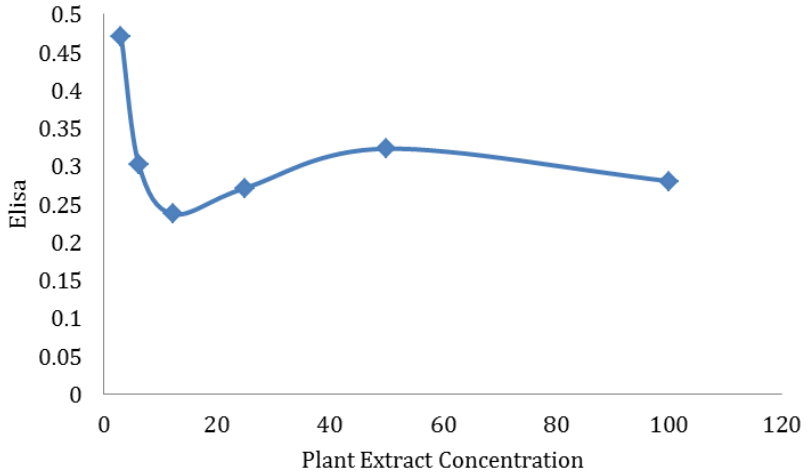

Fig. 9 ELISA of various concentrations of the ME of the medicinal plant Capparis on the inactivity of bacteria

Results of analysis of variance analysis of green AgNPs synthesized from an AE of the medicinal plant Capparis on the inactivity of *B.cereus, E.coli, E.fecalis, H.alvi, P.aeruginosa, P.mirabilis, S.aureus, S. mutanis, S.pneumonia* and *S.pyogenes* had a different effect (Table 2). The greatest influence of green AgNPs synthesized from the AE of the medicinal plant Capparis with ELISA of 0.3740 was on the inactivity of *S. mutanis* (Figure 10). The most effective concentration of synthesized green AgNPs was 1000 µg/ml with 0.3250 ELISA on microbial inactivity. In addition, with the rise in the concentration of synthesized green nanoparticles, the activity of bacteria has decreased (Figure 11).

Table 2 Analysis of Variance ELISA of Green AgNPs Synthesized from AE of capparis Medicinal Plant on Inactivity of Used Microbes

Source	DF	SS	MS	F
Source	9	1.76487	0.1961	24.21 **
concentration	4	1.51495	0.37874	46.76 **
Source*concentration	36	2.04597	0.05683	7.02 **
Error	100	0.81	0.0081	
Total	149	6.13579		

** Significant at the one percent level

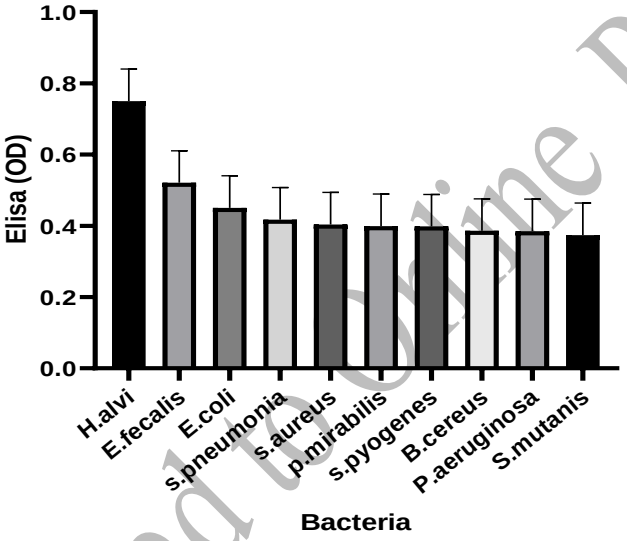

Fig. 10 ELISA of synthesized green AgNPs obtained from the AE of the medicinal plant snake grass on the inactivity of the microbes used.

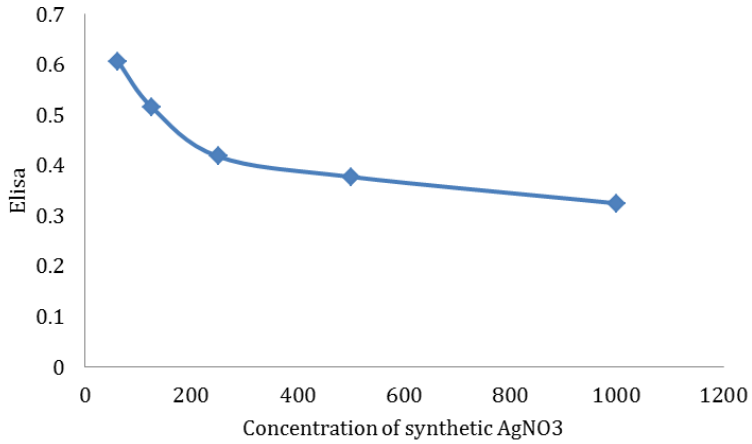

Fig. 11 ELISA of different concentrations of synthesized green AgNPs obtained from the AE of the medicinal plant Capparis on the inactivity of the microbes applied

Synthetic nanoparticle non-growth zone diameter variance analysis results of the leaves of the medicinal plant Capparis on the inactivity of the microbes *B.cereus, E.coli, E.fecalis, H.alvi, P.aeruginosa, P.mirabilis, S.aureus, S.mutanis , S.pneumonia* and *S.pyogenes* had a different effect (Table 3). The MDIZ was 5.5 mm due to the inactivity of *H. alvi* bacteria (Figure 12).

Table 3 Analysis of the variance of the diameter of the inhibitor zone diameter of synthetic nanoparticles of the leaves of the medicinal plant snake grass on the inactivity of the microbes used.

Source	DF	SS	MS	F
bacteria	9	24.675	2.74167	30.46**
Error	20	1.8	0.09	
Total	29	26.475		

** Significant at the one percent level

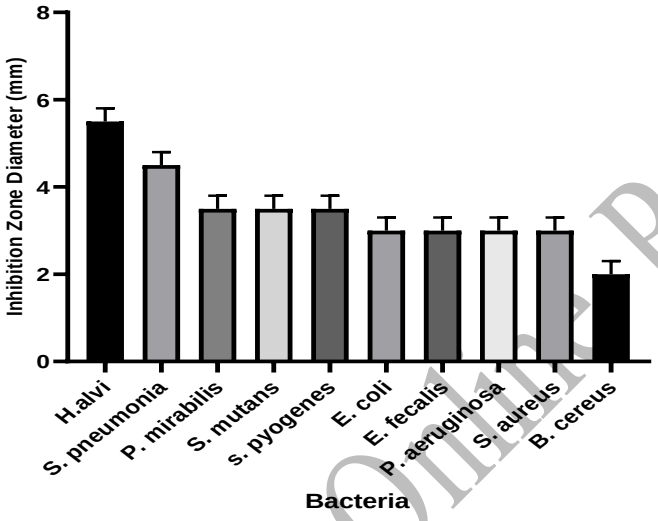

Fig. 12 The inhibitor zone diameter of the absence of growth of synthetic nanoparticles of the leaves of the medicinal plant snake grass on the inactivity of the microbes used

DISCUSSION

It has been confirmed. The dark red appearance of biosynthetic AgNPs after reaction with silver ions is a clear indicator of the reduction of metal ions and the formation of AgNPs, possibly caused by changes in the surface plasmon resonance of metal nanostructures [23]. This is an early color change indicative of the formation of a colloidal suspension of AgNPs [24], making it an excellent tool for detecting silver nanoparticle formation. As the concentration of silver ions increases, so does the adsorption rate. As the amount of metal ions increases, more ions are reduced and thus more nanoparticles are synthesized [25, 26]. In this study, green synthesis of AgNPs using an AE of the *C. spinosa* plant was carried out due to the widespread use of AgNPs and the general environmental concerns in chemical synthesis methods of these nanoparticles. The color of synthesized nanoparticle was changed to pink.

In FTIR spectroscopy, oxidized components are present in the alcoholic and phenolic substances of the extract and act as one of the active stabilizers to reduce the AgNPs [27]. Although the exact mechanism of nanoparticle formation as green synthesis is still unknown, it seems that terpenoids, sugars, phenols, and other plant-extracted compounds can be used to synthesize metal nanoparticles [28]. In the present study, The adsorptions at 1638 cm -1 and 3449 cm -1 in the FTIR analysis correspond to the affinities of the C-O-H and C=O groups of the extracted compounds, the tensile vibrations of the alkyl groups and the C-C bonds to the benzene ring. This assignment indicates the presence of a CHO group that may be the major reagent that reduces Ag+ to Ag0 and is converted to CO after the reaction.

It has been reported in research that synthesized AgNPs have excellent antibacterial properties and high antimicrobial activity compared to ionic silver [35]. Silver attaches to the cell walls and membranes of bacterial

cells and inhibits the respiratory process. In E. coli, silver inhibits the uptake of phosphate, mannitol, succinate, proline, and glutamine from bacterial cells. Silver's high affinity for sulfur and phosphorus is the main key to its antibacterial properties. Sulfur and phosphorus are abundant throughout the bacterial cell membrane. AgNPs react with sulfur-containing proteins inside or outside the cell membrane, affecting cell survival. Capparis has antioxidant and antibacterial properties [51], so the antibacterial effects of polysaccharides from the leaves of this plant are more effective on Gram-negative bacteria (*E. coli*, *Shigella* and *Salmonella*) than Gram-positive bacteria (Bacillus Panis, and *S. aureus*). Also, the antimicrobial properties of the root of the snake grass plant have been investigated [52], which is widely used in traditional medicine in southern Italy. The antibacterial properties of snake grass root are attributed to its heterocyclic compounds. In the present study, The greatest effect of the ME of the medicinal plant capparis was 0.2315 on the inactivity of *E. coli* and the greatest effect of green AgNPs synthesized from the AE of the medicinal plant capparis with ELISA of 0.3740 was on the inactivity of *S.mutanis*. The MDIZ was 5.5 mm due to the inactivity of *H. alvi* bacteria. Nanoparticles are more symmetrical due to their higher surface-to-volume ratio and easier absorption. In addition, silver ion release potential also increased by decreasing the size of AgNPs. Due to their smaller size, AgNPs have a greater contact surface with the environment and microorganisms, which leads to an increase in their biological and chemical activity and, as a result, has a greater effect on the cell membrane. In low concentrations of nano-particles, due to the small number of nanoparticles, the amount of interaction of the particles with the bacterial cell membrane is low, and as a result, the inhibitory power of nano-particles is low, as a result, the amount of inhibition and inhibitor zone diameter is lower. For this reason, a balanced concentration of nanoparticles is always desirable to deal with the growth of bacteria. The mechanism of action of silver is related to its interaction with the compounds of the thiol group present in the respiratory enzymes of bacterial cells.

It has succeeded in synthesizing AgNPs with dimensions of 2 to 23 nanometers using tea extract [53]. It was investigated [53] the combined effect of AgNPs with ampicillin and gentamicin antibiotics against Salmonella typhi. The antibacterial test results confirmed the increase in the antimicrobial activity of these antibiotics in combination with AgNPs against Salmonella typhi. Also, the increase in microbicidal activity in the combination of AgNPs with ampicillin antibiotic is more than gentamicin [53]. In 2012, Saxena and his colleagues succeeded in synthesizing AgNPs using fig extract (*Ficus benghalensis*). These nanoparticles showed good resistance against *E. coli* bacteria [54]. Boqa et al. showed the antimicrobial activity of snake grass root extract on the growth of *Deinococcus radiophilus* [55]. In the research of Kalpana and Prakash, who studied the antimicrobial activity of the leaves and fruits of snake grass, the results illustrated that the diameter of the inhibition inhibitor zone diameter of the ethanolic extract of snake grass with a concentration of 1000 ppm against the bacteria *B.subtilis*, *E.coli*, *E.faecalis*, *Klebsiella*, *P. aeruginosa*, and *S.aureus* was equal to 1.2, 1.5, 2, 0.9, 1.6 and 1 cm, while the diameter of the inhibitor zone diameter of the ethanolic extract of snake grass fruit against these bacteria was equal to 1.7, 2.3, 2.1, 1.5, 2.4 and 1.6 cm [55]. In the present study, The results show that the size of the synthesized nanoparticles was measured at 21 nm under optimal conditions. Frequency percentages of nanoparticle sizes are shown in histogram plots.

In a study, the synthesis of AgNPs from the AE of *Eucalyptus camaldulensis* flower and their antibacterial effect has been investigated. In this research, an environmentally compatible extracellular biosynthesis method, i.e. regenerating agents of AE of *Eucalyptus camaldolensis* flower, has been used to produce AgNPs. The production of AgNPs has been confirmed using color change, ultraviolet spectroscopy, X-ray diffraction pattern and scanning electron microscopy. The antibacterial activity of the resulting AgNPs against gram-positive and gram-negative bacteria has been investigated by micro-broth dilution and minimum bacterial lethal concentration methods. The presence of AgNPs has been confirmed by the maximum absorbance at 413 nm, color change to dark brown, infrared spectroscopy and X-ray diffraction pattern. The average size of nanoparticles has been measured between 67-80 nm. Finally, the minimum growth inhibitory concentration and the MBC on the bacteria *S. aureus* 3.12 and 6.25, *Bacillus subtilis* 6.25 and 6.25 and *Pseudomonas aeruginosa* 50 and 100 mg/ml have been measured. The results of the research showed that the AgNPs synthesized by the AE of *Eucalyptus camaldolensis* flower had good antibacterial activity against the tested bacteria and can be used as an antibacterial agent in different fields [29]. In this study, the nanoparticles synthesized under optimal conditions was determined to be 21 nm.

The presence of AgNPs has been confirmed by the maximum absorbance at 420 nm, color change to dark brown and infrared spectroscopy, and AgNPs synthesized with peppermint extract and edible have better inhibition on the activity of *S. aureus* bacteria compared to *E. coli* bacteria. Regarding the culture medium of *S. aureus*, aqueous extract and alcoholic extracts with 10 grams of extracted material have the largest and smallest diameters of non-growth halo, respectively. The results of the research showed that the AgNPs synthesized by the AE of edible mint and peppermint have good antibacterial activity against the tested bacteria and can be used as an antibacterial agent in different fields [56]. In this study, Fresh AE of *C. spinosa* leaves was pink in color and had more effect on some bacteria.

It has investigated the antimicrobial effects of the ethanolic extract of snake grass on *S. typhimurium*, the results illustrated that the lowest inhibitory concentration was equal to 2.5 µg/ml, and 3 strains were inhibited at this concentration. While the highest inhibitory concentration was equal to 10 µg/ml [57]. In Mahboubi's study, the antimicrobial activity of water, ethanolic, ethyl acetate and methanol extracts of snake grass leaf and root extracts were investigated. The arises indicated that the AE of snake grass root showed more inhibition against bacteria than the AE of snake grass [58]. The methanol extract of the stem and branch of snake grass was tested against Bacillus subtilis and the results showed that the diameter of the inhibition zone was 26.8 and 24.6 mm, respectively [59]. Mazarei et al. showed that the polysaccharides of snake grass leaves have the most antimicrobial activity against *E. coli*, Salmonella typhi and Salmonella dysentery bacteria [51]. It has revealed that the extract of snake grass had an inhibitory effect against bacteria 28.33±0.66, 26.25±0.16, 25.15±0.60, 22.31±0.26, 11 20.22±0.00 against *S. aureus*, *Staphylococcus epidermidis*, *S. pyogenes*, Pseudomonas aeruginosa, *E. coli* bacteria [60]. In the study of Azizian Sharme et al., who investigated the biosynthesis of AgNPs using the AE of mace plant leaves and investigated their antimicrobial activity, the results showed that after adding the extract to the silver nitrate solution, the color of the solution changed to brown. AgNPs showed the highest absorption at 405 nm and have a spherical shape and their average size is between 8-12 nm. These nanoparticles showed significant antimicrobial activity on the tested samples so that they prevented the growth of bacteria and fungi at very low concentrations [61].

It synthesized green nanoparticles of silver using *Allium paradoxum* plant extract and investigated its antimicrobial activity, the results of optimizing the reaction conditions showed that the best temperature was 85 degrees Celsius, the best pH was 12, and the best concentration was 5 mM. Silver nitrate and within 30 minutes. The nanoparticles were stable for at least two months and their approximate size was 30 to 40 nm. Also, nanoparticles showed strong antimicrobial effects on Pseudomonas aeruginosa bacteria. These particles also showed good antibacterial effects on *S. aureus* and *E. coli* [62]. It has synthesized AgNPs in the medicinal plant Curcuma longa, the results showed that the particle size is between 5-25 nm and inhibits *E. coli*, *S. aureus*, Klebsiella pneumoniae, and *S. pyogenes* bacteria [63]. In another study that synthesized AgNPs in garlic extract, the results showed that the diameter of the inhibition zone against *E. coli* bacteria was equal to 15 mm, and against *S. aureus* was equal to 9.5 mm at a concentration of 5 µm [64]. In the study of Ying loo et al., who synthesized AgNPs in the AE of tea leaves, the results showed that the size of the nanoparticles is 4.06 nm and the MBC and the MIC of the nanoparticles against *E. coli*, and Klebsiella bacteria. pneumoniae, *S. typhimurium* and Salmonella were equal to 7.8, 3.9, 3.9, 3.9 and 7.8, 3.9, 7.8 and 3.9 µg/ml [65]. In a research, it has been reported that the survival rate in fish treated with *C. spinosa* extract was significantly higher compared to the control group. Results have shown that *C. spinosa* extract stimulates innate immunity through cytokine responses and promotes the growth of rainbow trout[66].

It has investigated the synthesis of nanoparticles in Acinetobacter baumannii species, the results showed that the MIC and the MBC against the bacteria *E. coli*-Klebsiella pneumoniae- and Pseudomonas aeruginosa equal to 8-32-8 micrograms/ It was 64-32 and 64 micrograms per milliliter [67]. In the study of Rautela et al., who synthesized AgNPs in Tectona grandis plant, the results showed that the size of the particles is 10-30 nm and the diameter of the inhibitor zone diameter against *S. aureus* bacteria is 16, Bacillus cereus is 12, and *E. coli* is 17 mm in concentration. 50 micrograms [68]. In has synthesized AgNPs in the leaf and root extracts of Berberis vulgaris, the results showed that the size of the particles was between 30-70 nm and inhibited the bacteria *E. coli* and *S. aureus* [69].

The proteins in the extract provide a dual function of reducing silver and controlling their shape in the synthesis of silver nanoparticles. Carboxyl groups in aspartic residue, glutamine and hydroxy groups in tyrosine residue in proteins have been suggested as silver ion-reducing agents. The reduction process is carried out by a simple bifunctional tripeptide which is mostly identified in the amino acid residue. This synthesis process produces small silver nanoplates with low dispersion[70, 71].

AgNPs are used as a medicinal tool for infections against microbes. Most bacteria have become resistant to antibiotics, and in the near future, there is an urgent need to replace antibiotics with new substances with antibacterial properties. The results showed that synthesized silver nanoparticles have more significant biological effects than commercial silver nanoparticles, and therefore these nanoparticles can be considered as drug candidates[28]. Although there have been reports about the toxicity of silver nanoparticles and the toxicity caused by silver nanoparticles has been associated with an increase in inflammatory indicators in heart tissue, it seems that regular anaerobic exercises can have a protective effect against the increase of some inflammatory factors. It has chemical and biological toxicity of silver nanoparticles[72].

CONCLUSION

This data has shown that the groups of amide, carboxyl, amino and amino acids that exist in the leaf extract are intricate in the synthesis of AgNPs. The size of the nanoparticles synthesized under optimal conditions was determined to be 21 nm. The amount of total phenol and flavonoids in the ME of capparis leaves was equal to 229.9- 28.09 mg per gram of dry matter. The antioxidant properties of the ME of capparis were 85.18%. Synthetic nanoparticle non-growth zone diameter variance analysis results of the leaves of the medicinal plant Capparis on the inactivity of the microbes *B.cereus, E.coli, E.fecalis, H.alvi, P.aeruginosa, P.mirabilis, S.aureus, S. mutanis, S.pneumonia* and *S.pyogenes* had a different effect. The MDIZ was 5.5 mm due to the inactivity of *H. alvi* bacteria.

Naturally, Iranian scientists have also had significant activities in this field and the results of their work have been published in the form of valid scientific articles

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