INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
22–0241/2022/28–3–131–140
DOI: 10.17957/IJAB/15.1961
http://www.fspublishers.org

Full Length Article

Green Synthesis Nanosilver Particles from *Ziziphus spina-christi* and *Mentha pulegium* Aqueous Leave Extracts and Evaluation of their Antimicrobial Potential

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Received 06 June 2022; Accepted 03 August 2022; Published 23 September 2022

Abstract

The current study was carried out to prepare silver nanoparticles using *Ziziphus spina-christi* (L.) Desf. and *Mentha pulegium* (L.) aqueous leave extracts and to study their inhibitory effects against bacterial and fungal species. Bioactive compounds (phenols and flavonoids) in plant extracts were estimated by HPLC and bioactive group by FTIR. Characterization of prepared silver nanoparticles were described using Transmission Electron Microscopy (TEM), Zeta potential, and Energy Dispersive Analysis of X-ray **(**EDX). The size distribution of silver NPs was between 8.70–16.2 nm and 7.37–13.6 nm with *Z. spina-christi* and *M. pulegium*, leaves extract respectively. Antimicrobial activities of the biosynthesized silver nanoparticles were studied against some pathogenic bacteria (Gram +ve, Gram -ve) and fungi and the results showed the higher antimicrobial efficacy of extracts with nanoparticles more than the efficacy of free extracts The results conclude that Nanoscale silver particles with high surface area to volume ratio (size below 100 nm) showed high antimicrobial actions against both Gram-positive and Gram-negative bacteria. © 2022 Friends Science Publishers

Keywords: Antimicrobial activity; Green synthesis; Silver nanoparticles; *Ziziphus spina-hristi*

Introduction

In recent times, nanotechnology has received a remarkable amount of interest in advanced fields of biology, physics and chemistry. Nanoparticles, which may be of any shape, have at least one dimension of 100 nm or less (Lee and Jun 2019; Bayda *et al.* 2020; Um-e-Aiman *et al.* 2021). Nanotechnology is unique to the majority of prepared technologies of the 21st century. It is the power to convert the nanoscience notion to advantageous applications by observation, measurement, manipulating, aggregation, commanding and industrialization cases at the nanometer size (NNI 2019).

Silver nanoparticles (AgNPs) are very popular for their antimicrobial activity against multidrug-resistant pathogens (Allawadhi *et al.* 2021). For a long time, AgNPs have been applied as broad-spectrum antimicrobial agents against many pathogenic and nonpathogenic microorganisms in various industrial applications like food packaging and textiles industries, *etc*. (Kumar *et al.* 2021). Biosynthesis of silver nanoparticles (AgNPs) shows potential applications in many areas because of their safe and environmental suitability. The reported research on the green synthesis of AgNPs has been summarized using different parts of the stem, fruits, and seeds of different plants along with an effect on morphological properties (Ijaz *et al.* 2020). The major advantage of using AgNPs for antimicrobial purposes is their higher toxicity against microorganisms with high permeability at low dosage due to the size and shape of nanoparticles (NPs) (Hashemi *et al.* 2020).

There are several methods for preparing silver nanoparticles, including physical and chemical methods (Iravani *et al*. 2014). Green synthesis of silver nanoparticles is a safe and environmentally friendly method (Zahoor *et al.* 2021) that is prepared using some plant extracts (Niluxsshun *et al.* 2021), Green synthesis results in stable and biocompatible nanoparticles. The advantage of green nanoparticles is not only restricted to stability and biocompatibility; in addition, green nanoparticles also exert better antimicrobial action than the chemical or physically synthesized nanoparticles. This enhancement in action is undoubtedly due to biological corona surrounding green nanoparticles, which comes from the source of synthesis. Biological corona comprises various biomolecules, including plants metabolites, flavonoids, carbohydrates,

To cite this paper: Abd-Elraheem MA, MHE Mourad, AM Baiomy, MM Helal, MA Elshaer, MM Adel (2022). Green synthesis nanosilver particles from *Ziziphus spina-christi* and *Mentha pulegium* aqueous leave extracts and evaluation of their antimicrobial potential. *Intl J Agric Biol* 28:131–140

sugar residues, proteins and amino acids (Ghasemi *et al.* 2015; Siakavella *et al.* 2020). Antimicrobial properties of nanomaterials are dependent on several factors including stability, size and their concentration in the growth medium (Duran *et al*. 2016). In addition to the surface coating of NPs, morphology and surface charge also play a critical role in determining the antimicrobial properties of nanomaterials. Ravindran *et al.* (2013) Moreover, Hajipour *et al*. (2012) attributed the super antibacterial properties of NPs to their excellent physicochemical properties, including a high surface-to-volume ratio.

Green nano silver particles have been used in the fields of medicine, agriculture and others (Ge *et al.* 2014; Castillo-Henríquez *et al.* 2020) they can be used as antimicrobial, antiviral, and antifungal (Mohammed *et al.* 2020). Many studies showed that medicinal plants and natural products are mostly used safely which have no harmful chemical effects, have great biological effects, and are valuable against some fungal pathogens, bacteria, viruses and as antioxidants (Anand *et al.* 2019; Khan and Javaid 2019, 2020; Khadka *et al.* 2021). The Egyptian environment has a lot of plants that are used as antifungal and antibacterial agents because they contain phenolic compounds and flavonoids (Joaquín-Ramos *et al.* 2020) which also enable them to prepare silver nanoparticles (Siakavella *et al.* 2020). *Z. spina-hristi* and *M. pulegium* are among those plants whose leaf extracts are used as antibacterial and antifungal agents (Gad *et al.* 2019) and in the preparation of green nanosilver particles (El-Ansary *et al.* 2018; Rizwana and Alwhibi 2021). This research aimed to investigate the impact of green silver nanoparticles synthesis from *Z. spina-christi* aqueous and *M. pulegium* aqueous leave extract as an antimicrobial agent and compare those results to free plant extracts.

Materials and Methods

Plant material collection

Ziziphus spina-christi leaves were collected from the Research Farm, Faculty of Agriculture, Al-Azhar University, Sadat City, Menoufia, Egypt in May 2021 while *Mentha pulegium* leaves collected from the Mamoura agricultural area, Alexandria. The leaves of *Z. spina-christi* and *M. pulegium* were washed for distilled water and then dried for three days before being ground with an electric mixer for later use in the study.

Preparation of aqueous plant extracts

The aqueous extracts of *Z. spina-christi* and *M. pulegium* leaves were prepared by mixing 10 g of powder leaves into 100 mL of distilled water in a 250 mL conical flask. The mixture was heated at 50°C for 2 h with stirring. The extract was cooled to room temperature then filtered through Whatman No. 1 filter paper. The extract filtrated was stored at -4°C until used for the synthesis of AgNPs.

Phytochemical screening to aqueous plant leaves extracts

Certified chemical methods were used to detect biologically active compounds: flavonoid, tannin, saponin, terpenoid, Polyphenols and alkaloid in *Z. spina-christi* and *M. pulegium* leaves extract according to (Harborne 1998; Ahmad *et al*. 2014; Sharaf *et al.* 2021).

HPLC analysis of aqueous plant leave extracts

Analysis was performed by HPLC – (Agilent 1100) is composed to LC – pumps pump, UV/Vis detector, C18 column (125 mm × 4.60 mm, 5 µm particle size) Chromatograms were obtained and analyzed using the Agilent Chem Station. Chromatographic conditions for polyphenolic and flavonoid. The chemical content of phenolic compounds and flavonoids in *Z. spina-christi* leaves extracts, were chosen with HPLC analysis and were used methods both of Lin *et al.* (1996) and Kuntić *et al.* (2007) with some modifications.

Green synthesis of silver nano-particles

Silver nitrate ($AgNO_3$) was obtained from El-Gamhouria Trading Chemicals and Drugs Company, Egypt. Silver nanoparticles are prepared using 10 mL of aqueous leaves extract from *Z. spina -christi* and *M. pulegium* was taken to 90 mL of 1 mM $AgNO_3$ solution. The solution was heated to 60–70°C for 30 min with stirring. And observed the color changes from green light color to brown color is an indicator of the formation of silver nanoparticles in samples. This method was according to Evanoff and Chumanov (2005) with some modifications (Fig. 1).

Characterization of AgNPs

TEM analysis: To visualize the shape and morphology of the green Nanosilver transmission electron microscopy (TEM) at the EM National Research Centre. Univ.was carried out. One drop of emulsion was negatively stained with ethanol and was positioned on a copper grid. The TEM micrographs were acquired using a transmission electron microscope (JEOL JEM-1400Plus) with a tungsten source and operating at 80 kV (Jain *et al.* 2011).

FT-IR analysis: The characterization of functional groups on extracts and the surface of AgNPs by plant extracts were investigated by FTIR analysis (Shimadzu) and the spectra were scanned in the range of 400–4000 cm^{-1} range at a resolution of 4 cm^{-1} using a device FTIR of type spectrum one.

Zeta potential: The surface charge present of green Nanosilver was measured using Malvern Zetasizer ZS (Malvern Instruments, Worcestershire, UK) by laser Doppler electrophoresis at room. Samples were diluted with

deionized water before measurement. The samples were then injected into a capillary cell for charge measurement. Zeta potential values provide information on the repulsive forces between particles in the emulsion system (Honary and Zahir 2013).

Energy dispersive analysis of X-ray (EDX)

The existence of elemental silver was assured through EDX. The EDX microanalysis was carried out by an X-ray microanalyzer (Oxford 6587 INCA) attached to JEOL JSM-5500 LV scanning electron microscope at 20 kV. The EDX spectrum is recorded in the status of patches from one of the dignified silver nanoparticles on the film. The silver nanoparticles were analyzed using Quanta 200 FEG according to (Devi *et al*. 2012).

Assay for antimicrobial activity of plant leave extracts

The antimicrobial activity of investigated samples was evaluated against 24 h-old also cultures of pathogenic Gram-positive strains, *Staphylococcus aureus* (RCMB 010010), *Bacillus subtilis* RCMB 015 (1) NRRL B-543 and *Methicillin-Resistant Staphylococcus aureus* (MRSA) and Gram-negative strains *Escherichia coli* (RCMB 010052) ATCC 25955, *Salmonella typhimurium* RCMB 006 (1) ATCC 14028 and *Klebsiella pneumonia* RCMB 003 (1) ATCC 13883. In addition to examining their activity against 48 h-old cultures of pathogenic fungi *Aspergillus fumigatus* (RCMB 002008), *Aspergillus niger* (RCMB 002005), *Candida albicans* RCMB 005003 (1) ATCC 10231, *Syncephalastrum racemosum* RCMB 016001 (1) and *Fusarium moniliform* (RCMB 008005) Tests were performed according to NCCLS recommendations (NCCLS 1993).

Antimicrobial activities assessment as inhibition zone determination by the good diffusion method (Hindler *et al*. 1994). The inoculums suspensions were taken from colonies grown on agar plates and inoculated into Mueller-Hinton agar for bacteria plates and incubated for 24 h at 37°C then inhibition zone was measured around each well by millimeter. Fungal inoculation also was prepared from malt agar plates with fungal colonies and incubated for 48 h at 28◦C for inhibition zone determination.

Results

Phytochemical screening to aqueous plant leaves extracts

Phytochemical screening of the aqueous *Z. spina-christi* and *M. pulegium* leaves extract showed the presence of flavonoids, tannins, saponosides, terpenes, polyphenols and alkaloids (Table 1).

Table 1: Qualitative detection of some active compounds in crude aqueous *Z. spina-hristi* and *M. pulegium* leaves extract

Active Compounds	*Z. spina-hristi*	*M. pulegium*
Flavonoids	+	+
Tannins	+	+
Soponosides	+	+
Terpenes	+	+
Polyphenols	+	+
Alkaloids	+	+

+ positive (Presence)

Table 2: Chemical composition analysis of phenolic and flavonoid compounds of water extract from aqueous *Z. spina-hristi* and *M. pulegium* leaves extract by HPLC

Sample	Compound	RT#	Concentration mg mL^{-1}
Mentha pulegium	*p-coumaric*	6.0	13.22
	Caffeic	8.0	8.33
	Pyrogallol	9.0	9.78
	Ferulic	11.0	3.16
	Catechol	12.5	5.39
	Rutin	3.0	5.66
	7-OH flavone	4.0	3.21
	Naringin	5.0	4.16
	Quersestin	7.0	11.14
	kampferol	8.0	3.26
	Apeginin	9.0	4.33
Zizyphus spina-christi	Ferulic	11.0	5.09
	Gallic	10.0	4.26
	Pyrogallol	9.0	17.39
	Eugenol	7.0	5.23
	Syringeic	5.2	23.66
	Rutin	3.0	5.76
	Naringin	4.8	4.88
	Myrecetin	6.0	5.33
	Querstin	7.0	2.14
	Kampferol	8.0	3.26
	Hesperidin	10.0	6.05

HPLC analysis of aqueous plant extracts

Concentrations of phenolic and flavonoids compounds appeared in the aqueous *Z. spina-christi* and *M. pulegium* leaves extract (mg mL^{-1}) in Table 2 and Fig. 2. The Syringic was the most abound phenolic compound in aqueous *Z. spina-christi* leaves extract (23.66 mg mL^{-1}) followed by pyrogallol (17.39 mg mL^{-1}), eugenol (5.23 mg mL^{-1}), ferulic (5.09 mg mL^{-1}) and gallic (4.26 mg mL^{-1}). The *p*-coumaric was the major phenolic compound in aqueous *M. pulegium* L. leaves extract (13.22 mg mL^{-1}) followed by pyrogallol (9.78 mg mL^{-1}), caffeic acid (8.33 mg mL^{-1}), catechol (5.39 mg mL^{-1}) and ferulic (3.16 mg mL^{-1}). The hesperidin was the great flavonoid compound in aqueous *Z. spina-christi* leaves extract (6.05 mg mL^{-1}) then Rutin (5.76 mg mL^{-1}), Myrecetin (5.33 mg mL^{-1}), Naringin (4.88 mg mL^{-1}), Kampferol (3.26 mg mL^{-1}) and Quercetin (2.14 mg mL^{-1}). Quercetin was major flavonoid compound in aqueous *M. pulegium* leaves extract (11.14 mg mL^{-1}) then Rutin (5.66 mg mL^{-1}), Apeginin (4.33 mg mL^{-1}), Naringin (4.16 mg mL^{-1}), Kampferol (3.26 mg mL^{-1}) and 7-OH flavone (3.21 mg mL^{-1}).

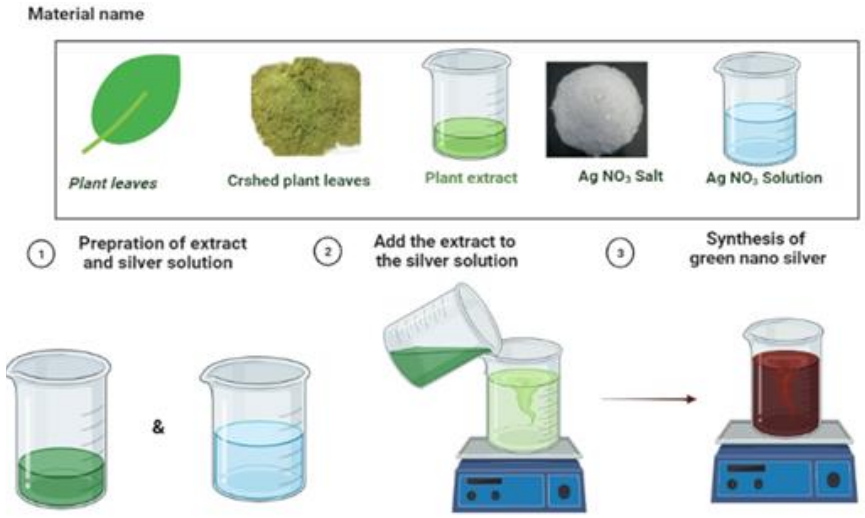

Fig. 1: Green nanosilver synthesis steps

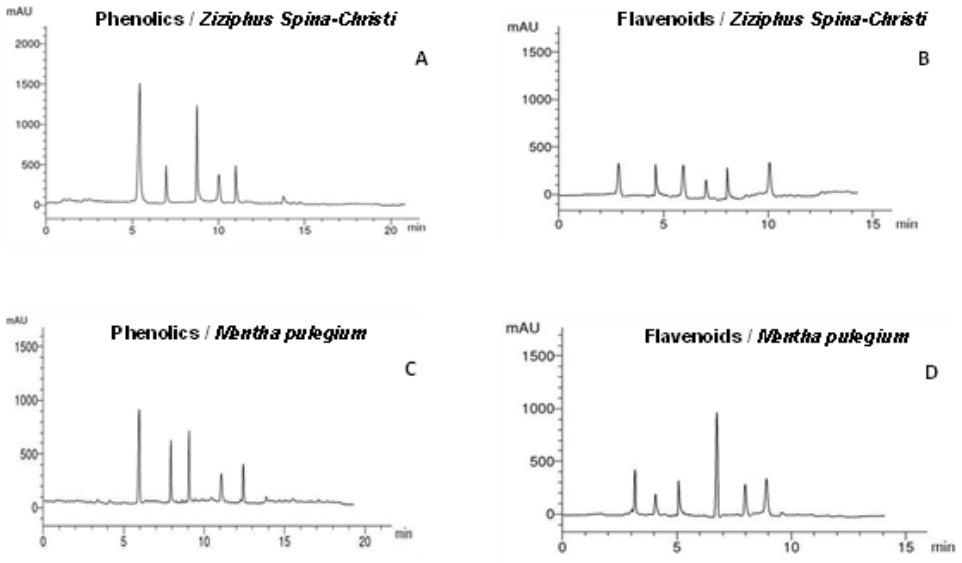

Fig. 2: HPLC chromatograms for Chemical composition analysis of phenolic and flavonoid compounds of water extract from aqueous *Z. spina-christi* and *M. pulegium* leaves extract

FTIR test of plant leaves extract and biosynthesized AgNPs

It describes the infrared absorption spectrum for the *M. pulegium* leaves extract and *Z. spina-christi* leaves extract and the synthesizing of AgNPs. Fig. 3 showed that the FTIR analysis of *M. pulegium* leaves extract it observed a strong wide peak at 3444.86, 2089.62, 1635.15 and 1340.13 cm^{-1}. While the FTIR analysis of *M. pulegium*-AgNPs was peaked at 3453.08, 2090.44, 1635.50 and 1339.71 cm^{-1}. Fig. 4 observed the FTIR analysis of *Z. spina christi* leaves extract it indicated with intense peaks of 3402.24, 2079.22, 1634.63 and 678.50 cm^{-1}. However, the FTIR analysis of *Z. spina-christi*-AgNPs was peaked at 3450.93, 2089.97, 1638.04 and 687.70 cm^{-1} (Fig. 3, 4).

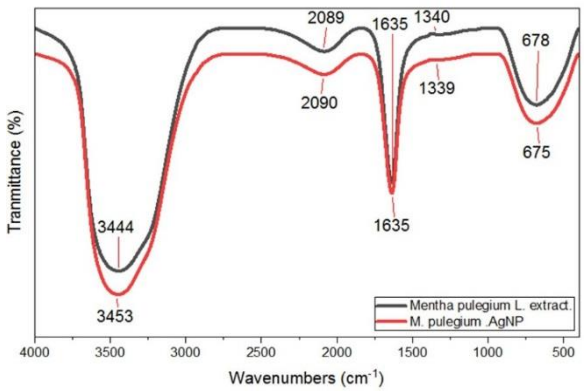

Fig. 3: FTIR analysis of *M. pulegium* leaves extract and M. *pulegium*-AgNPs

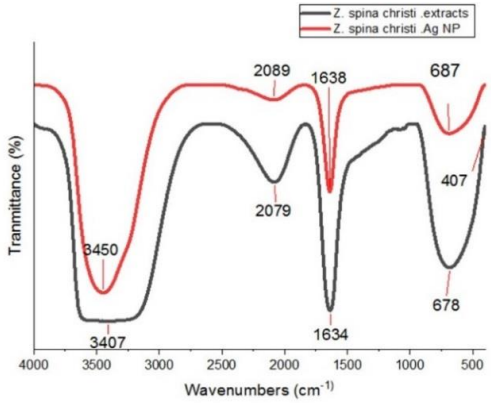

Fig. 4: FTIR analysis of *Z. spina christi* leaves extract and Z. *spina Christi*-AgNPs

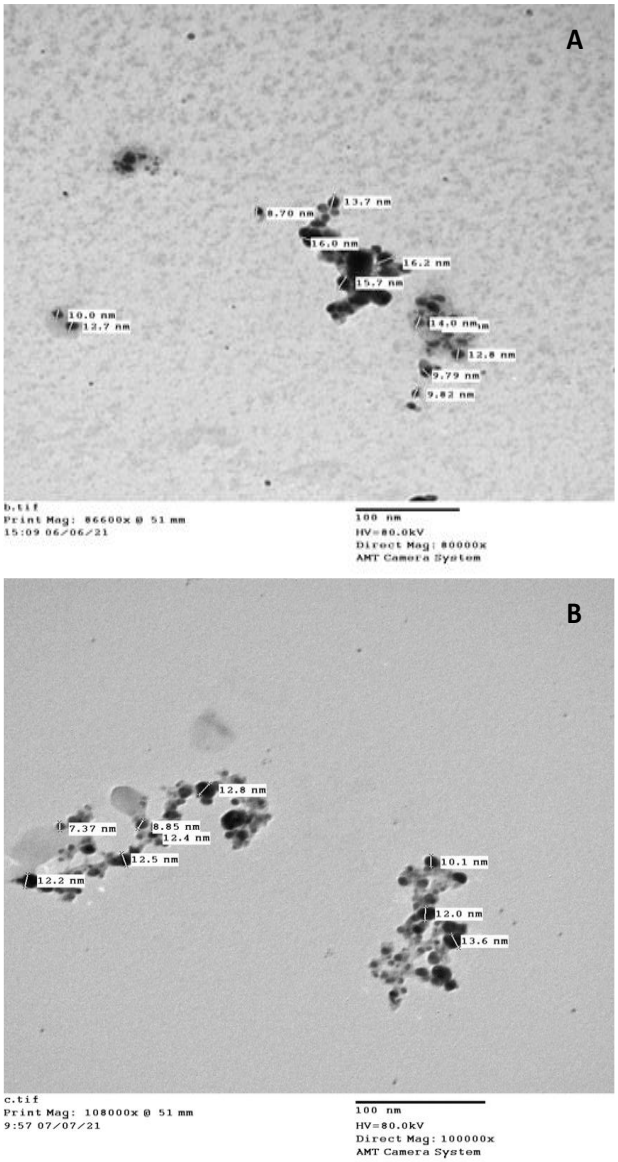

Fig. 5: TEM images of green Nanosilver synthesized using aqueous extract of *Z. spina-christi* (A) and *M. pulegium* (B) leaves

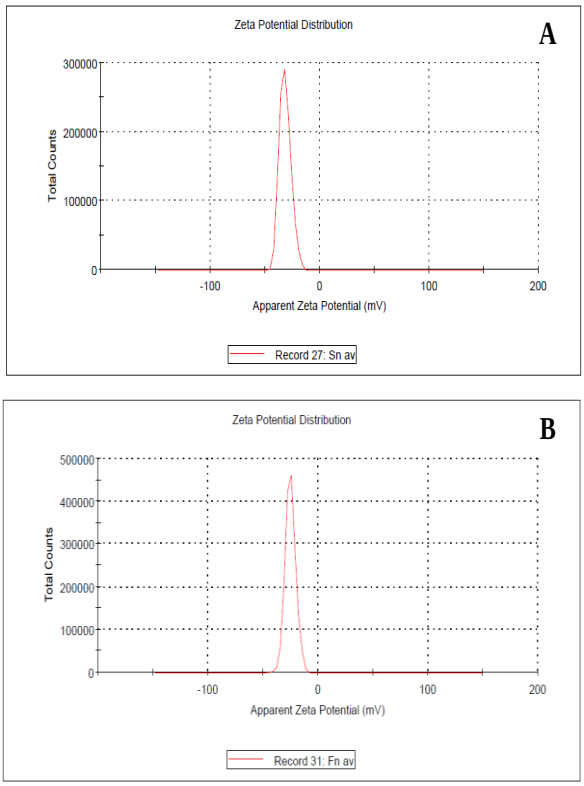

Fig. 6: Zeta potential value of silver nanoparticle with *Z. spina-christi* and *M. pulegium.* leaves extract

TEM analysis: TEM micrograph was examined the morphology of silver nanoparticles. The data obtained from TEM images found distinct shapes and sizes of polydisperse nanoparticles. These images suggest that the majority of nanoparticles which were prepared with *M. pulegium* leaves and *Z. spina-christi* leaves extract were spherical for silver and the size distribution of silver NPs between 8.70–16.2 nm for silver NPs with *Z. spina-christi* leaves extract and 7.37–13.6 nm for silver NPs with *M. pulegium* leaves extract (Fig. 5a, b).

Zeta potential: The value of the zeta potential is given a negative value of (-31.6 and -25.2 mV) for nanoparticles that were prepared with *Z. spina-christi* leaves and *M. pulegium* leaf extract, respectively (Fig. 6A and B).

EDX analysis: EDX spectrophotometer analysis determined the presence of Ag element indicative of AgNps. The EDX analysis detected a powerful signal from Ag area of silver nanoparticles with *Z. spina Christi* (Fig. 7a) and with *M. pulegium* (Fig. 7b). Metallic silver nanoparticles usually show a standard optical absorption peak almost between at 3–4 keV approximately and the average concentration of elemental silver was 70.53%, 65.56% for *Z. spina-christi* and *M. pulegium*, respectively. There were other peaks of K, Ca, Cl, S, Si, Na and Cu indicating that they were mixed deposits found *Z. spina-christi* in plant extract.

Antimicrobial activity of plant extracts with and without silver nanoparticles

The values of inhibition zone of microorganisms exposed to leave extracts alone or with nanoparticle concentrations of 0.01, 0.02 and 0.03 (mg mL^{-1}) in case of *F. moniliform* were 9, 12, 12 and 12 mm, with *S. racemosum* were 0, 10, 14 and 14 mm and with *S. aureus*, (MRSA), were 0, 13, 15 and 16 mm, respectively (Table 3).

Silver nanoparticles produced based on *M. pulegium* exhibited antimicrobial active against both *S. aureus* and *B. subtilis*, the promising result was observed with *S. typhimurium* where the inhibition zone values were 13, 20, 20 and 20 mm, while with MRSA were 0, 16, 16, 16 after exposure to leave extracts alone and extracts with nanoparticle concentrations of 0.01, 0.02 and 0.03 mg mL^{-1}, respectively (Table 4).

Discussion

The data represent a promising strategy as an antifungal and antibacterial by using green synthesis of silver nanoparticles using plant extracts. The morphological features of the surface, surface charge, particle size and functional groups were confirmed by TEM, HPLC, FT-IR, EDX and zeta potential measurements. Results detected the public of flavonoids, tannins, saponosides, terpenes, polyphenols, and alkaloids in the aqueous *Z. spina-christi* and *M. pulegium* leaves extract. These results agree with previous studies carried out on the same plant (Suliman and Mohammed 2018; Eftekhari *et al*. 2021; Hussein and Hamad 2021).

The results of phenolic and flavonoids compounds in leaves of *Z. spina-christi* and *M. pulegium* showed pyrogallol (12.86 mg 100 g^{-1}), ferulic (5.38 mg 100 g^{-1}), gallic (0.16 mg 100 g^{-1}). Alharbi *et al*. (2021) mentioned that caffeic acid (0.3 mg g^{-1}) and hesperidin (0.5 mg g^{-1}). Abdulla *et al.* (2016) reported that hisperidin (3.4 mg 100 g^{-1}), Rutin (1.52 mg 100 g^{-1}), naringin (0.39 mg 100 g^{-1}), kampferol (0.22 mg 100 g^{-1}) and quercetin (8.48 mg 100 g^{-1}).

The observed components of each extract are well known as vital active vehicles with antioxidants, microbes, and anti-inflammatory and sustainability. This guide justifies the traditional and popular use of its flights. Phenolic and flavonoids compounds are also related to antioxidant capacity, especially quercetin and glycoside conjugates, which can chelate metal ions and free radicals to inhibit the oxidation process (Lu *et al*. 2011). Some studies have shown that plant flavonoids have high antioxidant activity Pontis *et al*. (2014) and Sohaib *et al*. (2015).

The results related to characterization of the infrared absorption spectrum for the *M. pulegium* leaves extract and *Z. spina-christi* leaves extract and the synthesizing of AgNPs agree with various previous studies (Zayed *et al*. 2015;

Table 3: Antimicrobial activity of *Z. spina christi* extracts as mean inhibition zone produced on a range of pathogenic microorganisms

Sample code Tested microorganisms	*Z. spina Christi*	*Z. spina Christi* with AgNPs	*Z. spina Christi* with AgNPs 0.02	*Z. spina Christi* with AgNPs 0.03
FUNGI				
Aspergillus fumigatus (RCMB 002008)	NA	NA	NA	NA
Aspergillus niger (RCMB 002005)	NA	NA	NA	NA
Candida albicans RCMB 005003 (1) ATCC 10231	NA	NA	NA	NA
Syncephalastrum racemosum RCMB 016001 (1)	NA	10	14	14
Fusarium moniliform (RCMB 008005)	NA	9	12	12
Gram Positive Bacteria:				
Staphylococcus aureus (RCMB010010)	NA	12	12	12
Bacillus subtilis RCMB 015 (1) NRRL B-543	NA	NA	NA	NA
Methicillin-Resistant Staphylococcus aureus (MRSA)	NA	13	15	16
Gram Negatvie Bacteria:				
Escherichia coli (RCMB 010052) ATCC 25955	NA	11	12	13
Salmonella typhimurium RCMB 006 (1) ATCC 14028	NA	10	11	11
Klebsiella pneumonia RCMB 003 (1) ATCC 13883	NA	10	12	12

The test was done using the diffusion agar technique, well diameter: 6.0 mm (100 μL was tested), NA = no activity

Table 4: Antimicrobial activity of *M. pulegium* L. extracts as mean inhibition zone produced on a range of pathogenic microorganisms

Sample code Tested microorganisms	*M. pulegium*	*M. pulegium* with AgNPs	*M. pulegium* with AgNPs	*M. pulegium* with AgNPs
Aspergillus fumigatus (RCMB 002008)	NA	NA	NA	NA
Aspergillus niger (RCMB 002005)	NA	NA	NA	NA
Candida albicans RCMB 005003 (1) ATCC 10231	NA	NA	NA	NA
Syncephalastrum racemosum RCMB 016001 (1)	NA	NA	NA	NA
Fusarium moniliform (RCMB 008005)	NA	NA	NA	NA
Gram Positive Bacteria:				
Staphylococcus aureus (RCMB010010)	10	12	12	13
Bacillus subtilis RCMB 015 (1) NRRL B-543	NA	NA	12	12
Methicillin-Resistant Staphylococcus aureus (MRSA)	NA	16	16	16
Gram Negatvie Bacteria:				
Escherichia coli (RCMB 010052) ATCC 25955	NA	NA	NA	NA
Salmonella typhimurium RCMB 006 (1) ATCC 14028	13	20	20	20
Klebsiella pneumonia RCMB 003 (1) ATCC 13883	NA	10	11	11

The test was done using the diffusion agar technique, well diameter: 6.0 mm (100 μL was tested), NA = no activity

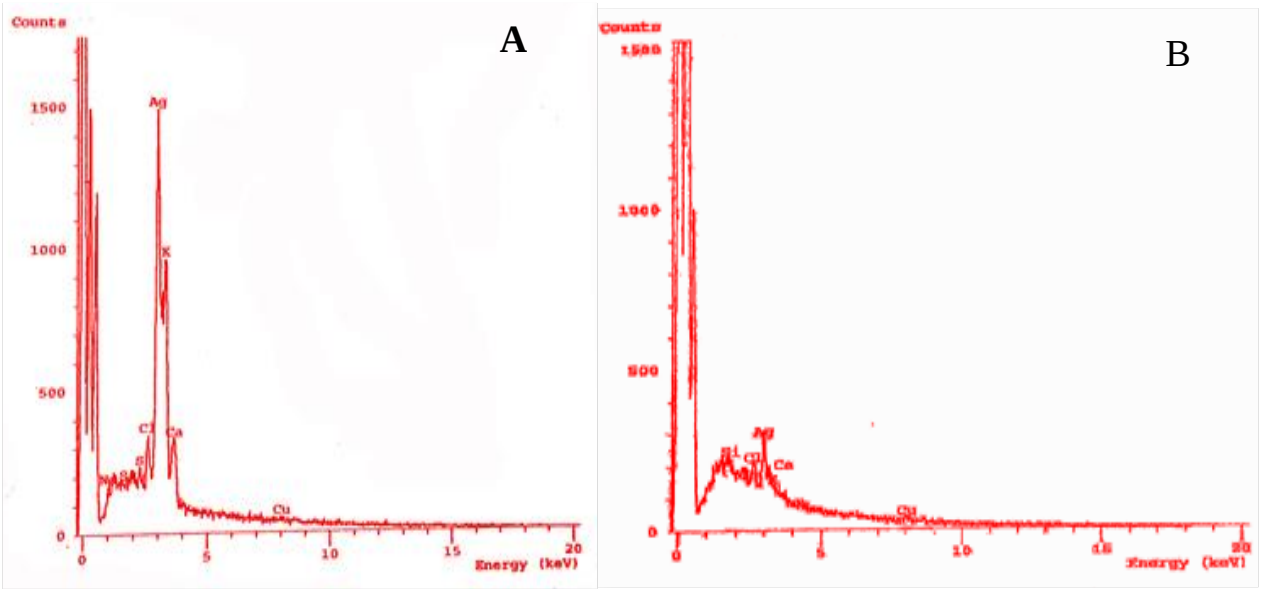

Fig. 7: EDX spectrum of silver nanoparticles with A: *Z. spina christi*. B: *M. pulegium*

Halawani 2016; Rad *et al*. 2019; Rizwana and Alwhibi 2021). The results improved that Ag^+ is linked to bioactive groups such as (phenol, alcohol and C=O of ester or aldehydes) and also a confirm crowned and reduced Ag^+ to AgNPs noticeable modifications in seismic bands and extending in infrared radiation from *M. pulegium*-AgNPs indicate that functional groups play a role in prominent in reduction and reduction modes during *M. pulegium*-AgNPs synthesis. The peaks observed in each of the spectra in the current study indicate the presence of many biologically active molecules such as phenols, proteins, amines and aromatic vehicles. This dynamic is well recognized for its role in different processes during NP syntheses, such as reduction, activation and stability. Previous research showed the role of carbon and hydroxyl in the above operation involving biodegradation from green AgNPs. Furthermore, the biomolecules in the plant extract prevent the NPs mass (Kora *et al*. 2012; Gupta *et al*. 2019).

Zeta potential is an important parameter to analyze the long-term stability of the nanoparticles. It refers to the surface charge of the particles. Zeta potential of nanoparticles is of significance on stability in suspension through the electrostatic repulsion between the particles. The high zeta potential value leads to a more stable emulsion than the low zeta potential of the particleszeta potentials indicated stability of the particle size distribution (Donga and Chanda 2021).

In this investigation, silver nanoparticles gave *Z. spina-christi* leaves extracts antimicrobial activity if it was compared with the same extract alone that do not affect tested pathogenic microorganisms where, it gave the remarkable activities with both fungi and bacteria moreover its efficacy was increased by increasing the nanoparticle concentration in most cases where the inhibition zone diameter values increased. These results approved by (Yin *et al.* 2020) found that silver nanoparticles possess a broad spectrum of antibacterial, antifungal and antiviral properties. These observations may be achieved by penetration of nanoparticles of silver to cell walls that change the structure of cell membranes and even result in cell death. Their efficacy is due to their nanoscale size moreover to their large ratio of surface area to volume, so the cell membrane permeability may increase the chance of reactive oxygen species production and interrupt replication of deoxyribonucleic acid by releasing silver ions (Yin *et al.* 2020).

Conclusion

The present work was focused on developing of AgNPs from the aqueous leaves extract of *Z. spina-christi* and *M. pulegium*. The TEM, HPLC, FT-IR, EDX analysis showed the AgNPs actual size and its distribution, the range recorded 8.70–16.2 and 7.37–13.6 nm for AgNPs of *Z. spina–christi* and *M. pulegium* leaves extract, respectively. The stability tests concluded that the nanoparticles are stable and well dispersed. The antimicrobial activity was evidenced by the change in morphology of treated pathogens at very less concentrations. Thus, the AgNPs can show strong antibacterial effects if the nanoparticles are applied. This effect is most likely correlated to the Phenolic and Flavonoids compounds.

Acknowledgement

The authors acknowledge the faculty of Agriculture, Biochemistry Department, Al Azhar University for facilitating the work in this research. Also for the Central Lab, National Research center to do characterization of nanomaterial.

Author Contributions

MAE: Conceptualization, investigation and methodology; MHE, BAM and HMM: Microbial experiments; MMA: Supervision, review and editing.

Conflict of Interest

The authors declare that they have no competing interests.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approvals

This work does not involve animals hence ethics approval not required.

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