

Green Synthesis of Silver Nanoparticles from *Mentha spicata* L. Aqueous Extract and Evaluation of Its Antioxidant, Antibacterial and Anticancer Activities

Abdulrahman Abdullateef (✉ abdulrahmanabdullateef06@gmail.com)

Cyprus International University <https://orcid.org/0000-0002-9064-7400>

Immaculata Amarachi Unegbu

Cyprus International University

Emmanuel Mshelia Halilu

Cyprus International University

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Abstract

Mentha spicata L. (Lamiaceae) has been used in traditional medicine to cure indigestion, stomach aches, intestinal weakness, and diarrhea. This research was designed to synthesize silver nanoparticles from an aqueous extract of *M. spicata* and to investigate its antioxidant, antibacterial, and anticancer activities. The extract was obtained by maceration with water and silver nanoparticle MAgNPs were prepared using 5 mM of AgNO₃ solution. The DPPH free radical and hydrogen peroxide scavenging assays were conducted to determine the antioxidant activity. The antibacterial activity was determined using the agar disk diffusion method on *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, and *Escherichia coli*. Anticancer studies were carried out, to determine the cytotoxic and anti-proliferative effect of MAgNPs. The phytochemical screening of the extract revealed the presence of phenolic compounds (flavonoids and tannins). The aqueous extract and MAgNPs scavenged the DPPH free radical, with IC₅₀ values of 12.62 µg/mL and 1.556 µg/mL respectively, while the IC₅₀ of the aqueous extract and MAgNPs against H₂O₂ were 1.695 mg/mL and 5.865 mg/mL. The bacterial strains were susceptible to the extract and MAgNPs with a zone of inhibition ranging between 4–11 mm. The zeta analysis of MAgNPs reveals a size of 70.08 nm and a potential of -13.17 mV. The SEM revealed spherical shape particles. The XRD showed the crystalline structure of MAgNPs. The FTIR revealed the N-H stretching frequency in the MAgNPs indicating the stretching of secondary amine. There was an *in vitro* cytotoxic effect on the viability of MDA MB 231 cancer cells with a significant difference among means ($p < 0.05$). The MTT Assay showed an anti-proliferative effect against the cells. The MAgNPs were found to be potent against MDA MB 231 breast cancer cells with IC₅₀ values of 11.67 µg/mL and 31.57 µg/mL obtained after 24 hours and 48 hours respectively. Therefore, the ability of the extract to scavenge the free radicals and inhibit the growth of the bacteria may be due to its chemical constituents.

INTRODUCTION

The application of nanotechnology in drug delivery and development research has substantially profited in recent years. In general, technological improvements have made it possible to develop systems that can control the surface characteristics of drugs that are delivered to achieve site-specific action, including surface area, particle size, and surface properties (Verma *et al.*, 2018). To manage the release of pharmaceuticals into the bloodstream, certain particles are utilized in drug delivery to transport active medications across the administration route. Additionally, they are crucial for enhancing a dose's efficacy, the drug's selectivity, and ultimately its safety. Active medications are released routinely from these carrier substrates in a fashion that can be controlled by design or affected by the organism's natural biological environment (Sanna *et al.*, 2011).

Nano drugs close the gap in cancer treatment that has always existed. Nanodrugs are more soluble than the majority of previous chemotherapies and have better cancer cell selectivity. This particular selectivity reduces the toxicity of the drug to healthy cells greatly (Mukhopadhyay, 2019). Research has concentrated on biosynthesized silver nanoparticle preparation to address this issue because physiochemically created silver nanoparticles have a significant cytotoxic effect on people. Since 2015, a major silver nanoparticle production method has used green and biosynthesized silver nanoparticles because they offer greater biological applications and lower cytotoxicity. In recent years, plant extracts have been used to create silver nanoparticles. Most of these environmentally friendly silver nanoparticles have improved antibacterial capabilities compared to regular silver and have various biological uses with lower cytotoxicity for people (Kaylani *et al.*, 2018).

Mentha spicata L. belongs to the Lamiaceae family, it produces long, slender spikes with smooth, grey-haired leaves that are topped with light blue flowers. It was also used in ancient medicine because it is effective for conditions

including the common cold, sinusitis, cough, bronchitis, nausea, vomiting, indigestion, and intestinal parasites. Loss of appetite and cramps (Yousuf *et al.*, 2013). Due to the abundance of polyphenolic chemicals in this family, they may have potent antioxidant capabilities. Members of the genus are distinguished by their volatile oils, which have significant economic value and are used by the food, beverage, cosmetics, pharmaceutical, and cosmetics industries. As a result, they are grown as industrial crops in many different nations. Few studies have looked at the use of *M. spicata* as an antioxidant in meat (Dorman *et al.*, 2003).

Due to their ability to enter cells, break proteins and DNA, and release silver from the silver nanoparticles, nanoparticles prevent the growth of bacteria and fungi. This is why they are effective against these microorganisms. Plant chemicals have been used for the synthesis of nanoparticles due to their reductive properties (Halilu *et al.*, 2023). A wide range of compounds known as phytochemicals found in medicinal plants have antibacterial, antioxidant, and anticancer properties (Giao *et al.*, 2007; Tepe, 2008; Yousuf *et al.*, 2013).

MATERIALS AND METHODS

Collection of Plant Material

The plant was collected from Cyprus International University (CIU) staff quarters, Haspolat/Nicosia, North Cyprus, in July 2022. It was identified by Asst. Prof. Dr. Emmanuel Mshelia Halilu (Pharmacognosist), the voucher specimen with number CIU/PHARM/LAMI/003 was deposited in the herbarium at the Faculty of Pharmacy in CIU for future reference.

Plant Extraction

The powdered plant material (50 g) was extracted by maceration method with 500ml of distilled water. The powdered plant leaf was kept in contact with the solvent for 2 days with frequent agitation until the soluble components of the plant were extracted by the solvent. The extract was subjected to pre-filtration three times using cotton and then finally filtered through Whatman No.1 filter paper and then concentrated over a water bath for one hour at 70°C. The solid extract obtained was kept for further studies.

Phytochemical Screening

Phytochemical screening of the aqueous plant extract was carried out according to the procedures described by Halilu *et al.* (2008).

Preparation of silver nitrate solution

The 5 mM silver nitrate (AgNO₃) solution was prepared by dissolving 0.424 g of silver nitrate in sufficient distilled water to make up to 500 mL in a volumetric flask (Narchin *et al.*, 2018).

Preparation of MAgNPs from the Aqueous Extract

Silver nitrate (Sigma-Aldrich, St. Louis, USA) solution was used for the synthesis of MAgNPs. For the synthesis of MAgNPs, leaf extract and 5mM AgNO₃ solution was prepared. 90% of the AgNO₃ solution was mixed with 10% leaf extract with constant stirring using a magnetic stirrer of 300rpm for 16hrs at room temperature, then there was a change in colourless to dark, then kept for 72hours in the dark at room temperature. The solution was taken into a test tube and was centrifuged at 5000rpm for 20 minutes. The supernatant layer was decanted and the sedimented particles at the bottom of the centrifuge tube were transferred to a watch glass and then dried in the oven for 24 h at 50°C (Narchin *et al.*, 2018).

Characterization of MAgNPs

Zeta Potential and Average Particle Diameter Analysis

Following a 30-minute centrifugation at 4100 g, the samples were repeatedly rinsed with distilled H₂O before being suspended in the water. The pellet was moved into a spotless evaporating dish and dried in the oven at 30°C for 24 hours in preparation for zeta size and charge characterization. Malvern Zetasizer Nano ZSP (Malvern Instruments Ltd. Malvern, UK), was used to detect the zeta potential after the supernatant had gone through two rounds of filtering (Wei *et al.* 2012). A particle size distribution system handles the charge of the dispersed particles as well as the particle size distribution.

Ultra-violet (UV) spectroscopy analysis of plant extract and silver nanoparticles

The aqueous extract and the silver nanoparticle solution were analyzed separately using the UV spectrophotometer. The cuvette was rinsed and filled with methanol to obtain the baseline for the methanol extract and the silver nanoparticle solution. The samples were scanned between 200 to 800 nm to obtain the absorbance of extract and silver nanoparticle solution.

FTIR analysis

The liquid aqueous extract, silver nanoparticle solution, and the synthesized powder silver nanoparticles were analyzed using FTIR. The samples were scanned between 4000 – 400 cm⁻¹. The spectra obtained were compared with a reference chart to identify the functional groups present in each sample.

X-Ray Diffraction (XRD) Analysis

The powdered silver nanoparticles (MAgNPs) were placed in a square grating glass slide and arranged until it was leveled out and evenly squared, then placed in the XRD machine for analysis.

DPPH Scavenging Activity of The Plant Leaf Extract and MAgNPs

The antioxidant activity of the leaf extracts was determined by DPPH scavenging assay according to (Esmaeli *et al.*, 2010) with some modifications. 0.1g of concentrated plant was weighed and dissolved in a test tube of 10mL of ethanol and vortex for 5 minutes. A 5-fold serial dilution was done into 5 test tubes giving different concentrations (10mg/mL, 5mg/mL, 2.5mg/mL, 1.25mg/mL, 0.625mg/mL). 0.024g of DPPH was weighed and transferred into a 50mL volumetric flask, 1mL of DPPH was added to each test tube and allowed to incubate for 30 minutes. The absorbance of each sample was measured at 517nm wavelength using a UV-Spectrophotometer. The DPPH scavenging effect percentage was calculated using the formula below:

$$\%DPPHActivity = \frac{AbsorbanceControl - AbsorbanceSample}{AbsorbanceControl} \times 100$$

Hydrogen Peroxide scavenging assay of Leaf Extract and MAgNPs

The antioxidant activities of *M.spicata* were determined using Hydrogen peroxide according to a method reported with little modifications (Pavithra and Vadivukarasi, 2015). A solution of hydrogen peroxide (43mM) is prepared with 10x Dulbecco's phosphate-buffered saline (DPBS)- 1M pH 7.4. Concentrations of (10, 5, 2.5, 1.25, and 0.625) mg/mL were prepared through serial dilution (5fold) in 4mL of PBS each, and the hydrogen peroxide solution (0.6mL, 43mM)

was added into each test tube, then allowed to incubate in a dark place for 10 minutes and the absorbance was taken at 230nm wavelength, against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as the standard. The free radical scavenging activity was determined by % inhibition with the formula below:

$$\% \text{HydrogenperoxideActivity} = \frac{\text{AbsorbanceControl} - \text{AbsorbanceSample}}{\text{AbsorbanceControl}} \times 100$$

Antibacterial Activities

The antimicrobial activities were determined by disc diffusion method 15.2g of Mueller–Hinton was dissolved in 400ml of distilled water and sterilized in an autoclave at a temperature of 121⁰C for 2 hours and then allowed to cool, 20ml each was then poured into different Petri dish. After solidifying bacteria like *Escherichia coli*, *Staphylococcus aureus*, *Bacillus Subtills*, and *Salmonella* were inoculated on the Mueller–Hinton plate.

A concentration of 20 mg /mL of the leaf extract and MAgNPs was prepared, and then distilled water and dimethyl Sulfoxide (DMSO) were prepared as a negative control. The Levofloxacin antibiotic and 5 mM AgNO₃ was placed as the positive control. A sterilized disc was prepared in different parts of the Petri dish at 6mm, the disc was soaked with leaf extract, MAgNPs, 5mM AgNO₃, distilled water, and DMSO, respectively, and placed on the Petri dish that has been inoculated with the bacteria. It was then allowed to incubate for 24 hours at a temperature of 37⁰C. The experiment was performed in triplicate. The antimicrobial activities were determined by measuring the diameter zone of inhibition.

Anticancer Studies

Two anticancer studies were carried out, to determine the cytotoxicity and anti-proliferative effect of MAgNPs.

Cell Culture and Cytotoxicity

Cell culture media consisted of Dulbecco's Modified Eagle Medium (DMEM)), fetal bovine serum (FBS), penicillin, and streptomycin solutions from Ebsdorfergrund, Germany. A complete DMEM growth medium was prepared by adding 5% FBS, 1% penicillin and streptomycin, and 4mmol/L L-glutamine. The metastatic breast cancer MDA MB 231 cells were obtained from Imperial College London, United Kingdom. They were grown in DMEM supplemented with 5% FBS and maintained in a humidified atmosphere of 5% CO₂ at 37°C. MDA MB 231 cell lines were maintained for 24 hours to allow cell stabilization (Naidu *et al.*, 2016). Cells were trypsinized and plated in petri round-bottom tissue culture plates for cytotoxicity test. Cytotoxicity index (CI) percentage was calculated by using the following equation.

$$\% \text{Cellviability} = \frac{\text{numberofliveCells}}{\text{totalnumberofcells}}$$

MTT Assay

MTT assay is based on the ability of a mitochondrial dehydrogenase enzyme present in the viable cells to reduce tetrazolium rings of the MTT (pale yellow) and form formazan crystals (dark blue) which are largely impermeable to cell membranes, thus resulting in the accumulation within the healthy cells. The crystals are solubilized by the addition of detergent to the cells. The number of surviving cells is directly proportional to the level of the formazan product (Mosmann, 1983). MTT Assay was determined by using MTT as the cytotoxic indicator with some modifications (Narchin *et al.*, 2018). MDA MB 231 cells were diluted with complete DMEM medium to give a density

of 2×10^4 cells and 18µl of this suspension were placed in a 24-well microplate and incubated for at least 4 hours before treatment. To this, 500 µl of the MAgNPs at a concentration range of 5µg/mL, 25 µg/mL and 50 µg/mL. Cells were incubated at 5% CO₂ at 37°C for 24hrs. Dimethyl sulfoxide (DMSO) was used as a negative control and calibration with MTT reagent was used as a positive control. All tested samples were performed in three replicates. After 24h incubation, cell viability was determined by adding MTT (100 µl, in DMEM 400 µl) to each well and following 5 minutes of shaking, and then the plates were incubated for 2 hrs. About 500 µl of DMSO was added to wells to dissolve the formazan crystals and 75 µl glycine buffer. The plates were kept at room temperature for 10 minutes. Finally, the absorbance was read at 490 nm with a scanning multi-well spectrophotometer ELx800 (Thermolab system 354, Finland). Cells were observed in an inverted microscope (Rasti *et al.*, 2022).

Statistical Analysis

Results are expressed as mean ±S.E.M. The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Turkey's test. All analyses were done using Graph Pad Prism Software Version 8.00 and p values (< 0.05) were considered statistically significant.

RESULTS

Extraction

M. spicata in water yielded about 8% as dark brown solid (w/w).

Phytochemical screening

The phytochemical screening (Table 1) revealed the presence of flavonoids, tannins, and saponins which are responsible for the bio-reduction of Ag⁺ to silver nanoparticles (Halilu *et al.*, 2008).

Table 1 Phytochemical screening		
S/NO	Phytochemical/Test	Inference
2.	Phenols	Present
3.	Flavonoids	Present
4.	Alkaloids	Present
6.	Tannins	Present
7.	Triterpenes	Present
9.	Quinones	Present
10	Coumarins	Present
12.	Anthocyanins	Present

Synthesis of silver nanoparticles (MAgNPs)

The synthesis of the silver nanoparticles occurred progressively with a series of visible colour changes in addition of plant extract to the silver nitrate as shown in Fig. 1.

UV Spectroscopy of Plant Extract and MAgNPs

UV-VIS spectrophotometry was used to determine the presence of chromophoric groups and aromatic rings in plants and nanoparticles based on measurement of the electronic transition of π -bonds, σ -bonds, and lone pair of electrons as well. It indicated the absorbance and wavelength of aqueous extract Table 2, Fig. 2 and MAgNPs, as shown in Table 3 and Fig. 3.

Table 2
UV-VIS peak values of extract of *Mentha spicata*

S/NO	Wavelength (nm)	Absorbance
1.	407	1.9769
2.	502	1.1262
3.	535	1.093
4.	609	1.1421
5.	665	1.6506

Table 3
UV-VIS peak values of extract of MAgNPs

S/NO	Wavelength (nm)	Absorbance
1.	454	1.2208
2.	353	0.9207
3.	343	0.9398
4.	340	0.9265

Zeta analysis

The Zeta potential revealed the electrical potential at the slipping plane and the average size of the MAgNPs as revealed in (Fig. 4).

FTIR

The FTIR result of the MAgNPs is shown in Table 4. and Fig. 5, while the FTIR of *M.spicata* is shown in Table 5 and Fig. 6 respectively.

Table 4
Showing the result of FTIR analysis for
MAGNPs

Wavenumbers (cm ⁻¹)	Functional Group
3226	N-H, OH Stretch
2914	C-H Stretch
2343	COOH Stretch
2104	C-N Stretch
1437	C-O Bend

Table 5
Showing the result of FTIR analysis for *M.spicata*

S/NO	Wavenumbers (cm ⁻¹)	Functional Group
1.	3252	OH Stretch
2.	2905	C-H Stretch
3.	2032	C = C terminal alkyne
4.	2025	C = C terminal alkyne
5.	1563	N-H bend secondary amine
6.	1143	C-F Stretch aliphatic fluoro compounds
7.	902	C-H 1,3 dis-substitution
8.	647	C-H bend

XRD

The XRD showed various peaks of the nanoparticle at different degrees and intensities as shown in Fig. 7

DPPH free radical scavenging activity of Extract and Silver Nanoparticles

The results of the quantitative antioxidant assay were expressed in terms of percentage inhibition and IC₅₀. The result revealed that ascorbic acid showed higher activity than the silver nanoparticles and aqueous leaf extract. The activity of the ascorbic acid, silver nanoparticles, and aqueous extract was generally high at the lowest concentration and was found to be concentration-dependent. The results are presented in Fig. 8. The IC₅₀ of MAGNPs and leaf extract and ascorbic acid were 1.556 mg/mL, 12.62 mg/mL, and 0.333 mg/mL respectively.

Quantitative Antioxidant Activity Determination Using Hydrogen Peroxide (H₂O₂) Against Aqueous Extract and MAGNPs

The results of the quantitative antioxidant assay were expressed in terms of percentage inhibition and IC₅₀. The result revealed that ascorbic acid, MAgNPs, and aqueous leaf extract all showed high scavenging activity H₂O₂. The activity of the ascorbic acid, MAgNPs, and aqueous extract were generally high at the lowest concentration and were found to be concentration-dependent. The results are presented in Fig. 9. The IC₅₀ of MAgNPs and aqueous leaf extract and ascorbic acid were 5.865 mg/mL, 1.695 mg/mL, and 5.449 mg/mL respectively.

Antibacterial Studies

The MAgNPs and *Mentha spicata* leaf extract showed comparable antibacterial activity relative to the levofloxacin which was a standard drug deployed. The zone of inhibition is presented in Table 6 and Fig. 10. The results showed that the standard drug had the highest activity ranging from 26.33 ± 1.213 to 31.89 ± 0.8407, while MAgNPs and the precursor AgNO₃⁺ demonstrated higher susceptibility against all the bacteria tested when compared to the aqueous extract. The activity ranged between 9.889 ± 0.3889mm to 11.44 ± 0.3378mm for MAgNPs and 10.44 ± 0.2940mm to 12.22 ± 0.5212mm for AgNO₃⁺. The results for aqueous leaf *M. spicata* were between 1.889 ± 0.3889mm to 4.667 ± 1.236.

Table 6
Average zones of inhibition (mm)

Bacteria	Levofloxacin	MAgNPs	<i>M.Spicata</i>	AgNO ₃ ⁺	DMSO	WATER
<i>Bacillus. subtilis</i>	26.33 ± 1.213	10.78 ± 0.5212	3.333 ± 0.5528	10.67 ± 0.333	0.000 ± 0.000	0.000 ± 0.000
<i>Staphloccus.aureus</i>	29.67 ± 0.500	9.889 ± 0.3889	1.889 ± 0.3889	11.67 ± 0.4410	0.000 ± 0.000	0.000 ± 0.000
<i>Escherichia. coli</i>	31.89 ± 0.8407	11.44 ± 0.3378	0.000 ± 0.000	12.22 ± 0.5212	0.000 ± 0.000	0.000 ± 0.000
<i>Salmonella. typhi</i>	30.33 ± 0.3727	10.78 ± 0.3643	4.667 ± 1.236	10.44 ± 0.2940	0.000 ± 0.000	0.000 ± 0.000

There was a significant difference across the groups at p < 0.001 when the levofloxacin group was compared to other groups. Values are mean ± SD n = 3.

DISCUSSION

Phytochemical Screening of Aqueous Extracts of *M. spicata* (Table 1) revealed the presence of phenols, flavonoids, alkaloids, tannins, triterpenes, quinones, coumarins, and anthocyanins.

Figure 1 shows the formation of MAgNPs caused by the reduction process; its formation is conclusively indicated in the presence of the solution color and its gradation from brown to dark brown. The color changed to a yellow-dark brown color aqueous solution due to the excitation of the Surface Plasmon Resonance phenomenon (Joy and Johnson, 2015) 2.1g of MAgNPs was obtained.

The plant extract mediates the production and stabilizes the silver nanoparticles by acting as both reducing and capping agents (Thombre *et al.*, 2012). The results of the phytochemical screening indicate the presence of tannins, saponins, and flavonoids, all of which have the hydroxyl groups necessary to reduce silver ions (Ag⁺) to silver

nanoparticles. The phenolic hydroxyl group in phenolic compounds such as flavonoids and tannins are effective in the reduction of silver ions to silver nanoparticles. This study indicated that higher amounts of silver nanoparticles could be produced when the reaction mixture is allowed to incubate in a dark place for more days. Also, the concentration and incubation time are important factors in nanoparticle formation. The higher the concentration of AgNO_3 , the higher the nanoparticles formed (Halilu *et al.*, 2023).

The qualitative UV-VIS profile of aqueous extract of *M. spicata* (Fig. 2) was taken at the wavelength of 200 nm to 800 nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 407, 502, 535, 609, and 665nm with the absorption 1.9769, 1.1262, 1.093, 1.1421 and 1.6506 respectively. This is similar to other findings (Jain *et al.*, 2016) with the use of the methanolic extract of *M. spicata*, which had the profile of peaks shown at 353, 407, 504, 535, 609 and 665 nm with the absorption 1.309, 1.463, 0.1, 0.066, 0.108 and 0.625 respectively. UV-VIS shows the absorption spectrum of *M. spicata* extract and these are almost transparent in the wavelength region of 200–800 nm. Absorption bands observed about *M. spicata* plant extract are displayed in Table 2.

In the UV–Vis Spectra of MAgNPs showed peaks at 454, 353, 343, and 340nm. Although these peaks were different in the silver nanoparticles synthesized with *M. spicata* (Qaeed *et al.*, 2023), their work revealed peaks at 455, 458, 459, and 479nm. It is suspected that electrons move very freely because the valence bond is close to the conduction band, increasing the surface plasmon resonance (SPR) absorption, which causes the MAgNPs electrons to resonantly oscillate with the incident light. All of the electrons on the surface oscillate as a dipole with the same phase as a result of this. At certain values, the resonance between the absorption and extinction frequencies occurs, which is attributable to the difference in the size of the particles, as shown in (Table 3 and Fig. 3). The UV spectra of the MAgNPs show a gradient of increasing absorption, this is due to the varying sizes and shapes of the particles as well as the availability of phytochemical compounds in the extract (Qaeed *et al.*, 2023).

It was discovered that the produced MAgNPs zeta size was 70.08 nm, with a -13.17 charge. The negative surface charges of the suspended MAgNPs prevent their aggregation according to Coulomb's law. Additionally, the stable possession of the biomolecules in *M.spicata* leaf extract can be credited to the negative charge of the biogenic nanoparticles. The MAgNPs' measured negative surface charge demonstrated their electrostatic attraction to one another in (Fig. 4).

The concentrated form of MAgNPs and plant extract were all analyzed using FTIR spectrometry. The IR peaks of the MAgNPs were observed at 3226 cm^{-1} , 2914 cm^{-1} , 2343 cm^{-1} , 2104 cm^{-1} , and 1437 cm^{-1} (Table 4; Fig. 5). The IR peaks of the aqueous extract were observed at 3252 cm^{-1} , 2905 cm^{-1} , 2148 cm^{-1} , 2032 cm^{-1} , 2025 cm^{-1} , 1563 cm^{-1} , 1143 cm^{-1} , 902 cm^{-1} , and 647 cm^{-1} (Table 5; Fig. 6). IR analysis was used to study the nature of capping ligands that stabilize the silver nanoparticles, formed by the bio-reduction process (Halilu *et al.*, 2023). The N-H bend observed in the MAgNPs indicated the stretching of secondary amine. Furthermore, in the extract, the absorption band at 2023 cm^{-1} and 2025 cm^{-1} indicated the presence of terminal alkyne. Also, the absorption band at 1143 cm^{-1} observed in the extract indicated the presence of an aliphatic fluoro compound. The OH stretching at 3252 cm^{-1} shows the presence of phenolic groups and this agrees with the result of the qualitative phytochemical screening (Nandiyanto *et al.*, 2019).

The result of the XRD showed the atomic arrangement, crystalline structure, and crystallite size of silver in MAgNPs. The XRD peaks (Fig. 7), confirmed the presence of five significant peaks between $30\text{--}81^\circ$. The XRD peaks obtained from this study conform with the patterns of silver nanoparticles. The peaks of diffractions were observed at 38.4° , 43.7° , 77.3° , 81.5° relating to the facets 111, 200, 220, 311, and 222 respectively of the face-centered cubic (FCC)

which shows that the silver nanoparticles were crystalline. Our results are similar to previous work, which claimed that the planes (111), (200), (220), (311), (222), and (400) of the Joint Committee on Powder Diffraction Standards (JCPDS), file No. 04-0783, which corresponded to Bragg reflections at 28.0, 32.4, 46.4, 54.9, 57.7, and 64.7, were responsible for the creation of the structure of AgNPs (Al Aboody, 2019).

The activity of the extract and MAgNPs have been compared with ascorbic acid (standard), the percentage inhibition increases as the concentration decreases. The IC_{50} of MAgNPs and leaf extract and ascorbic acid were 1.556 mg/mL, 12.62 mg/mL, and 0.333 mg/mL respectively as regards the IC_{50} , MAgNPs had better activity than the extract in the DPPH scavenging activity (Fig. 8).

The IC_{50} of MAgNPs and aqueous leaf extract and ascorbic acid were 5.865 mg/mL, 1.695 mg/mL, and 5.449 mg/mL respectively in the H_2O_2 scavenging activity. The lower the IC_{50} , the higher the activity (Halilu *et al.*, 2023). The ascorbic acid being a standard drug had higher activity than extract and nanoparticles. However, these outcomes are summarized in (Fig. 9).

The results of the present study suggest that MAgNPs and aqueous leaf extracts of *M. spicata* have significant free radical scavenging activity, which was a similar result (Emre *et al.*, 2021). The primary secondary metabolites that serve as natural antioxidants in plants are phenolic and flavonoid molecules (Kanatt *et al.*, 2007). Due to their hydroxyl group and capacity to serve as hydrogen donors, they are primarily responsible for antioxidant activity (Hosseinimehr *et al.*, 2007; Benabdallah *et al.*, 2016). According to the findings of this work, *M. spicata*'s aqueous extract had both phenolic content.

It has been explained that *M. spicata* has anti-oxidative properties. Two bioactive molecules, carvone, and cis-carvone, were found to be prevalent in the polyphenolic characterization of extracted essential oil. Our results are in tandem with supercritical extraction of spearmint leaves which was reported to possess up to 71% antioxidant potential calculated using DPPH radical scavenging assay (Mandana *et al.*, 2011). Therefore, the efficiency of these plant extracts' antioxidant and antidiabetic properties may be correlated with their phenolic concentration. The strong pharmacological potentials of these plants may be explained by the presence of phenolics and other antioxidant chemicals in the plants. It should be taken into cognizance that depending on the polyphenolic concentration, the polarity of the solvent used for extraction, and the portion of the plant from which they are being extracted, extracts from different plants employing different solvents may have varying scavenging abilities (Ade-Ajayi *et al.*, 2011).

The present results revealed that *M. spicata* showed major activity against gram-positive and gram-negative bacteria, this was determined through the antibacterial inquiry using test organisms that the crude extracts of the specified plants have extraordinary antibacterial potentials. MAgNPs were excellently effective against *Escherichia coli* (11.44 ± 0.3378 mm), *Bacillus subtilis* (10.78 ± 0.5212 mm), *Staphylococcus aureus* (9.889 ± 0.3889 mm) and *Salmonella typhi* (10.78 ± 0.3643) respectively. Results are summarized in (Table 6). The aforementioned inhibition of the plant extract was also in conformity with previous work, where these compounds were known to possess significant anti-bacterial activities (broth micro-dilution & disc diffusion methods) against *Escherichia coli*, *Candida albicans*, *Candida tropicalis*, and *Staphylococcus aureus* (Sarer *et al.*, 2011). The results of our present investigation are in harmony with earlier findings of Hossain *et al.*, [30] who investigated the anti-bacterial activity of spearmint oil against *S. aureus*, *E. coli*, *B. subtilis*, & concluded that these strains were positively affected by the application of *Mentha spicata* extracts. In another study, several different types of medically harmful bacterial species have been reported to be inhibited by hydrodistilled oils of *Mentha spicata* leaves (Aggarwal *et al.*, 2002).

Numerous studies have demonstrated the polyphenolic components' propensity to change the properties of bacterial membranes, mostly as a result of variations in the hydrophobicity, integrity, and surface charge of the membranes. These alterations result in membrane rupturing and intracellular substance leakage, which ultimately induce cell death (Lopez-Romeo et al., 2018; Monte *et al.*, 2014). Additionally, according to other researchers, the anti-bacterial impact of phenolic-rich extract may be caused by its inhibition of the activity of essential enzymes and nucleic acid synthesis (Thirumurugan, 2010). Significant correlations between the molecular size, structure, and hydrophobicity of polyphenols (saponins and terpenoids) and their antibacterial effects have been found. The results of this study (Fig. 10) will open up new possibilities for the use of *Mentha spicata* leaf extract as a clinical pathogen-fighting anti-bacterial agent.

The cytotoxicity potential of MAgNPs was determined using an MTT assay against Human cancerous cell lines (MDA MB 231 breast cancer cell lines). A significant increase ($p < 0.05$) in the Cytotoxicity value of the MAgNPs treated cells was noted when compared to the negative control cells. The Cytotoxicity was found to increase with increasing dose tested in the MAgNPs, but there was no significant difference ($p < 0.05$) that was observed between all the concentrations tested for the MAgNPs investigated.

The IC_{50} for cytotoxicity was found to be the lowest for the cells treated with MAgNPs after 24 hours (11.67 $\mu\text{g/mL}$) being the most potent inhibitor. MAgNPs treated cells after 48 hours indicated an IC_{50} value of (31.57) $\mu\text{g/mL}$. The findings for the MAgNPs were in agreement with a recent study that demonstrated that extracts and essential oils from six mentha species had cytotoxic effects against Vero, Hela, and HepG2 cell lines at various concentrations (IC_{50} 28.1-166.2 $\mu\text{g/mL}$); the IC_{50} value for HepG2 was determined to be 94.3 $\mu\text{g/mL}$ (Rahimifard *et al.*, 2009).

Morphological changes such as cell shrinkage and clumping, vacuolation, distorted cells, cell adhesion, and cell growth inhibition were observed in the MDA MB 231 cells treated with MAgNPs indicating the signs of apoptosis. Cell shrinkage, chromatin condensation, DNA fragmentation, and apoptotic bodies are significant signs of apoptotic cell death. Chemoprevention is one of the cutting-edge methods to control cancer as an alternative to therapy, which has its drawbacks. It is defined as the application of a specific chemical agent (natural or synthetic) to suppress the process of carcinogenesis. The metabolic activation of procarcinogens and the interaction of cellular DNA are both inhibited by flavonoids, which fall under the category of blocking agents (Surh, 1999; Naidu *et al.*, 2016). MAgNPs after 24 hours indicated an IC_{50} value of (11.67 $\mu\text{g/mL}$) and (31.57) $\mu\text{g/mL}$ after 48 hours can be potential candidates for further development as a cancer therapeutic agent (Naidu *et al.*, 2016).

For acquiring nutrient molecules, cancer cells' membranes are bigger than those of normal cells, and they feature gap junctions that are downregulated, making them prepared for metastasis. Ineffective nano-drug delivery systems are caused by the poor lymphatic system and its wide gaps; these qualities make it easier for nanostructures to penetrate cancer cells and prevent them from escaping, which increases their effectiveness (Rasti *et al.*, 2022).

CONCLUSION

The results of the current study's investigation showed that the bio-reduction of the Ag^+ ions to silver nanoparticles has been attributed to flavonoids, tannins, and saponins. The conclusion was that the silver nanoparticle had excellent antibacterial potential against all the pathogenic test species and could be used to produce a variety of antibacterial medications to combat emerging multi-resistant infectious species. The significant antioxidant capabilities found in this study may be useful in establishing baseline information for the manufacture of natural, non-toxic, and environmentally friendly goods in the food, health, and pharmaceutical industries. MAgNPs were

found to possess moderate cytotoxic potential and effective antiproliferation. MAgNPs can be potential candidates for further development as cancer therapeutic agents.

Declarations

FUNDING

Not applicable

ETHICAL APPROVAL

Not applicable

CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

INFORMED CONSENT

It is not applicable

AUTHORS' CONTRIBUTION

This work was carried out in collaboration among all authors. Author AA carried out the investigation, performed the statistical analysis, and wrote the first draft of the manuscript. Author IAU managed the analyses of the study and the literature searches. Author EMH designed the study and wrote the protocol. All authors read and approved the final manuscript.

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Figures

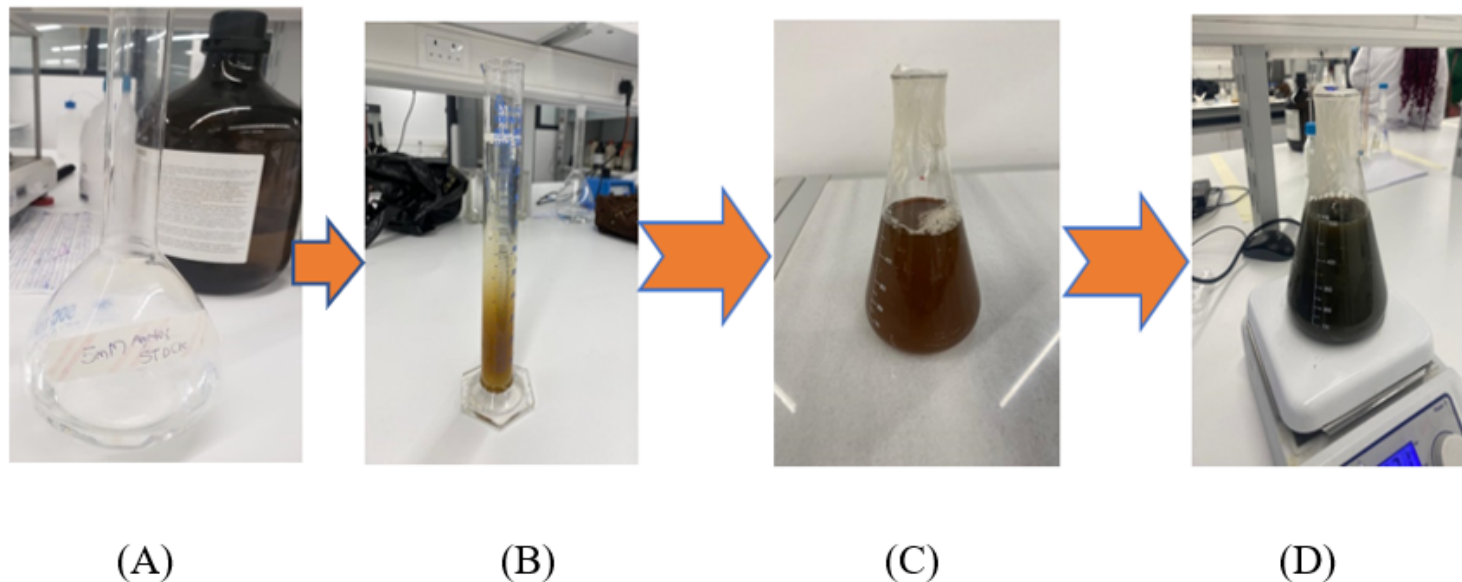


Figure 1

Change in the color of silver nitrate solution after addition of *Mentha spicata* aqueous leaf extract. (A): colorless silver nitrate solution; (B): *Mentha spicata* aqueous leaf extract; (C) and (D): dark brown color indicates formation of silver nanoparticles.

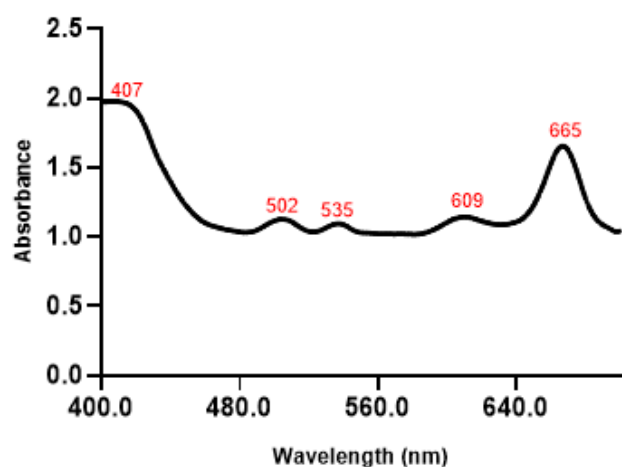


Figure 2

UV Spectrophotometry of *M. spicata* aqueous extract

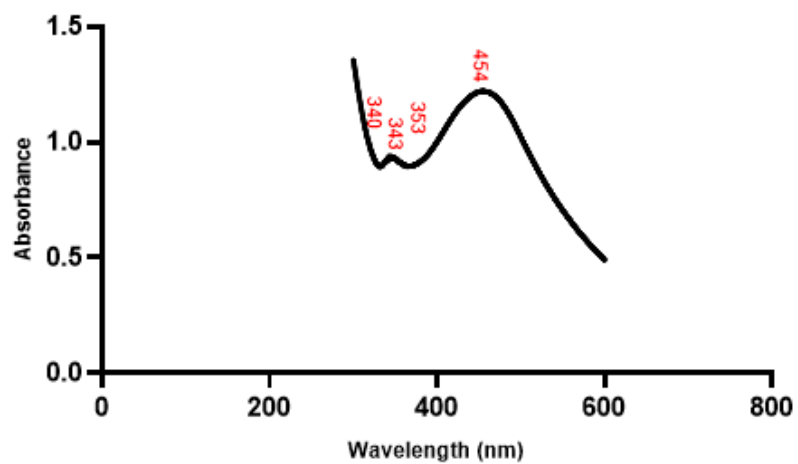


Figure 3

UV Spectrophotometry of MAgNPs

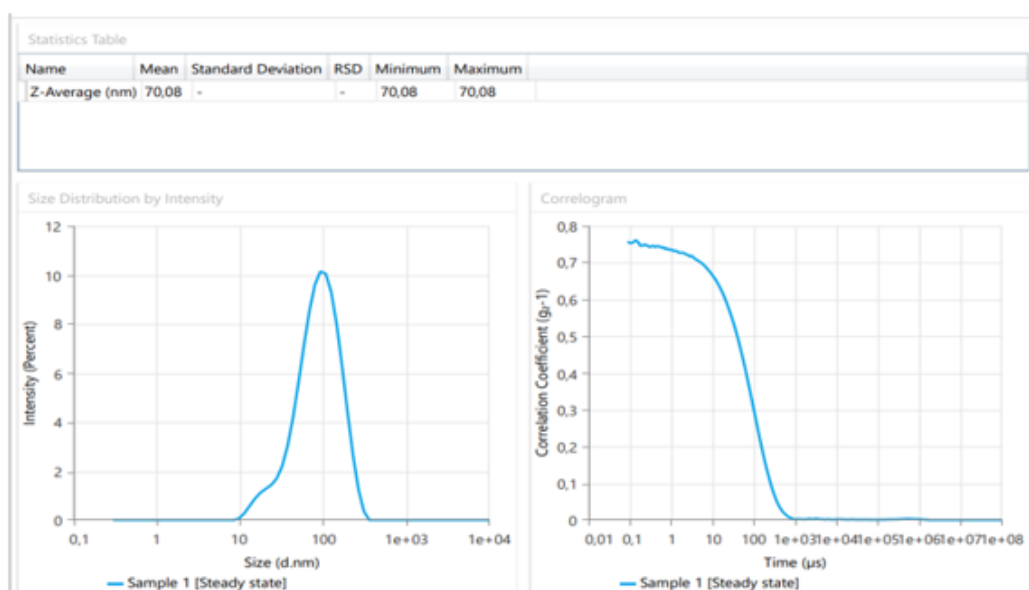


Figure 4

Zeta size of MAgNps

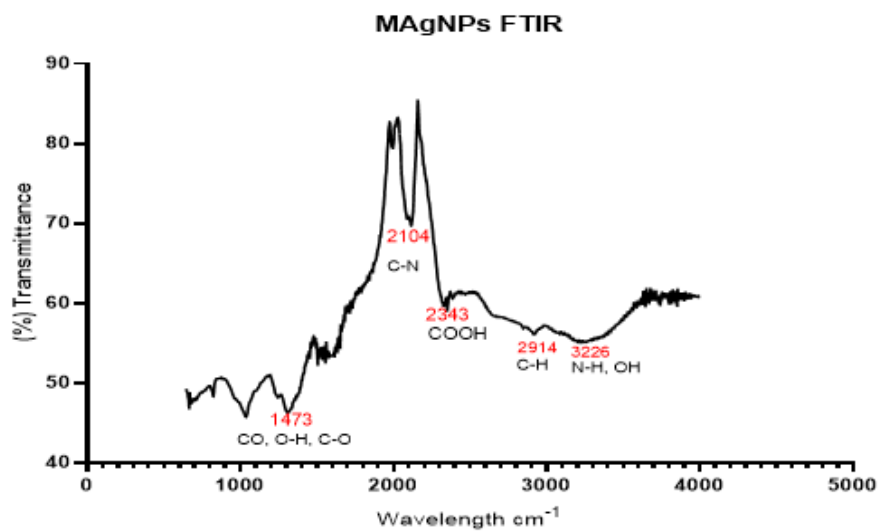


Figure 5

FTIR of MAgNPS

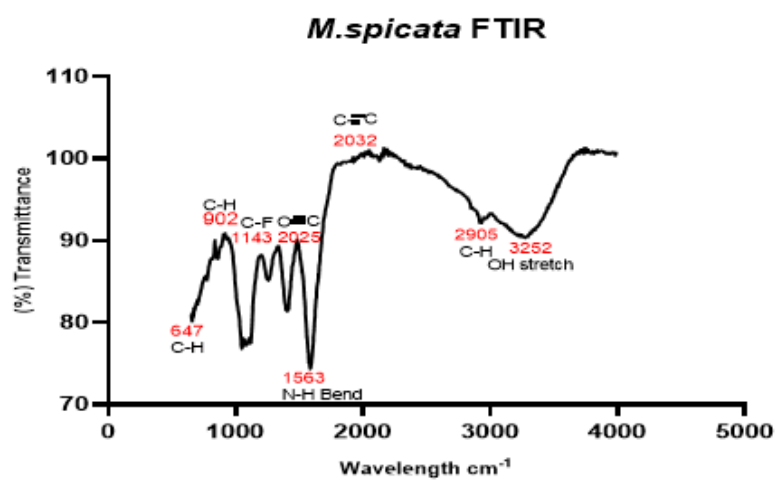


Figure 6

FTIR of *M.spicata*

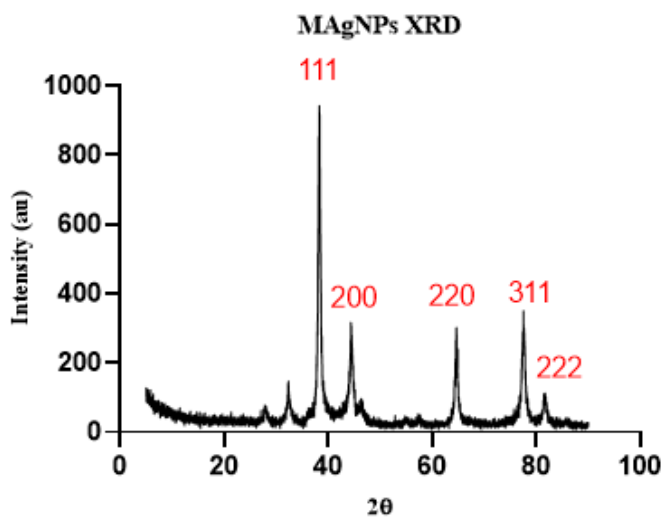


Figure 7

XRD of MAgNPs

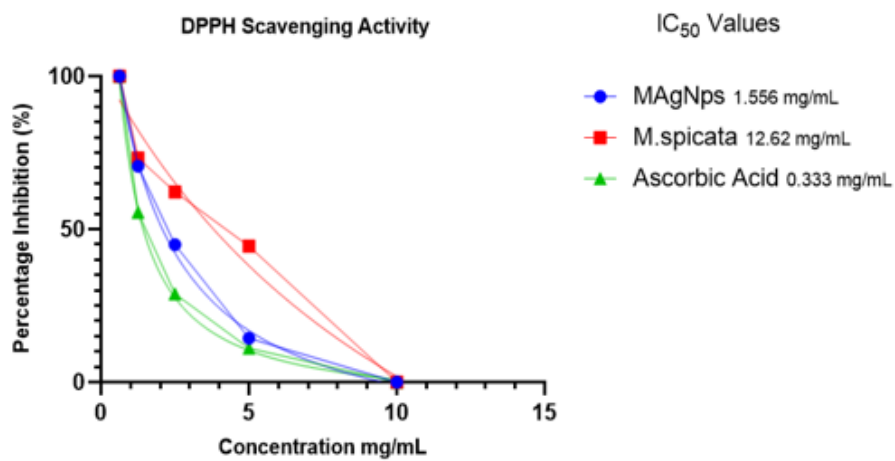


Figure 8

DDPH% inhibition of MAgNPs, Extract and Ascorbic Acid

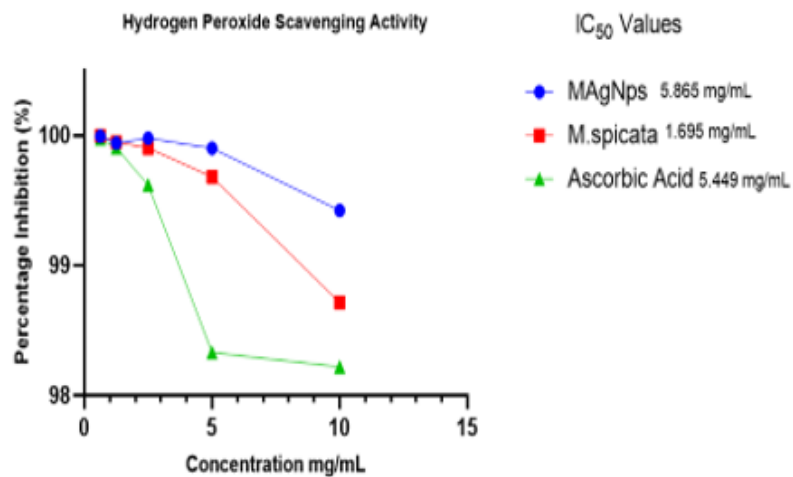


Figure 9

Hydrogen Peroxide % inhibition of MAGNPs, *M.spicata* and Ascorbic Acid

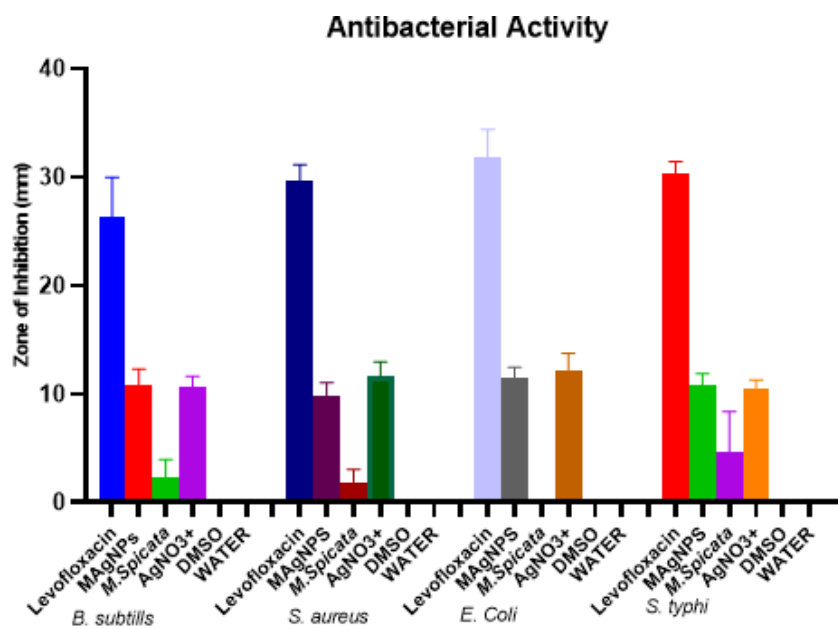


Figure 10

Zone of inhibition of tested samples against *Bacillus. Subtills*, *Staphylococcus aureus*, *Escherichia. Coli*, *Salmonella typhi*. Values are mean $\pm$ SD n=3. across the groups at ($p < 0.001$).

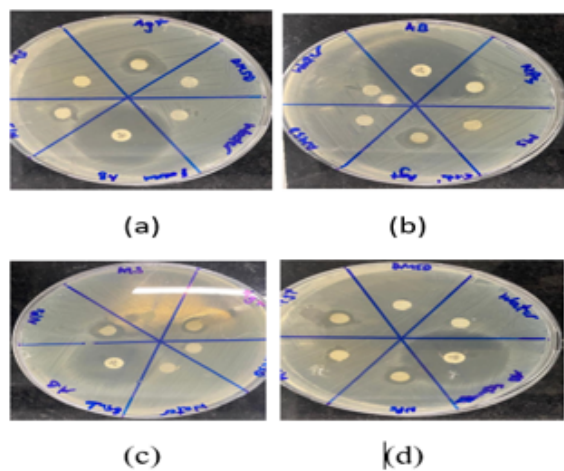


Figure 11

Antibacterial activity of *Mentha spicata* using (a) *Staphylococcus aureus*, (b) *Escherichia coli* (c) *Bacillus subtilis* (d) *Salmonella typhi*

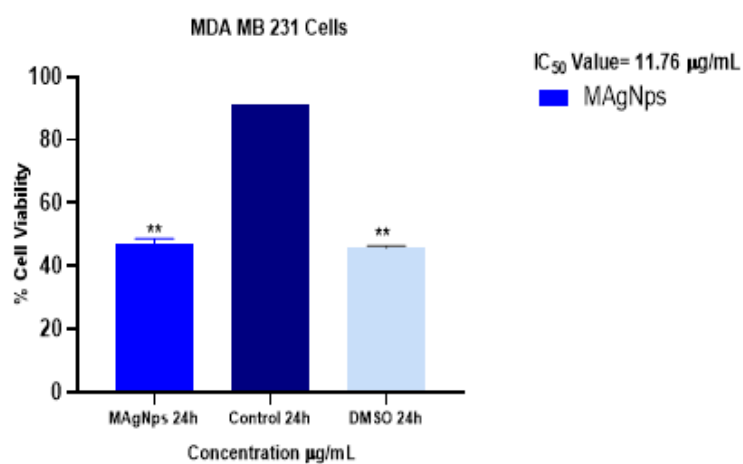


Figure 12

Graph showing the in vitro cytotoxic effect of complexes on viability of MDA MB 231 cancer cells after 24 hours. Values are mean $\pm$ SD n=3. **= Significantly different from control (P<0.05)

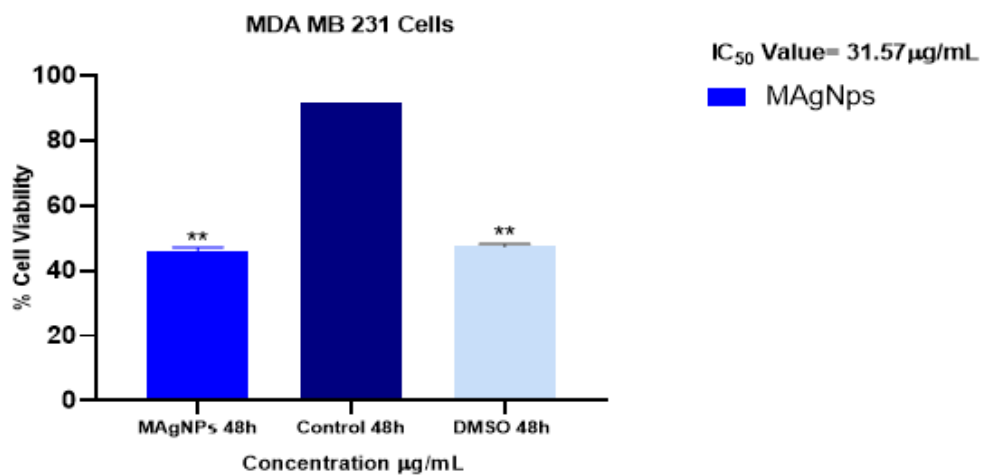


Figure 13

Graph showing the in vitro cytotoxic effect of complexes on viability of MDA MB 231 cancer cells after 48 hours. Values are mean $\pm$ SD n=3. **= Significantly different from control (P<0.05).

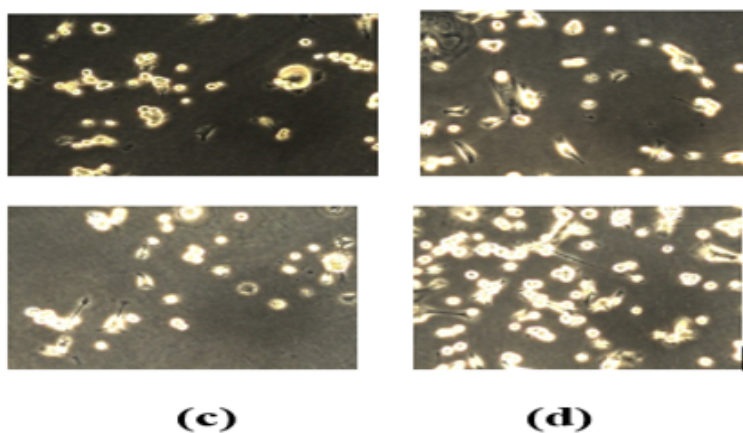


Figure 14

Morphology of MDA MB 231 cells treated with MAgNPs after 24 hours (a) Control (b) (5 µg/mL), (c) 25 µg/mL (d) 50 µg/mL. Cell shrinkage and clumping, signs of apoptosis, macrophage differentiation were observed ($\times 20$).

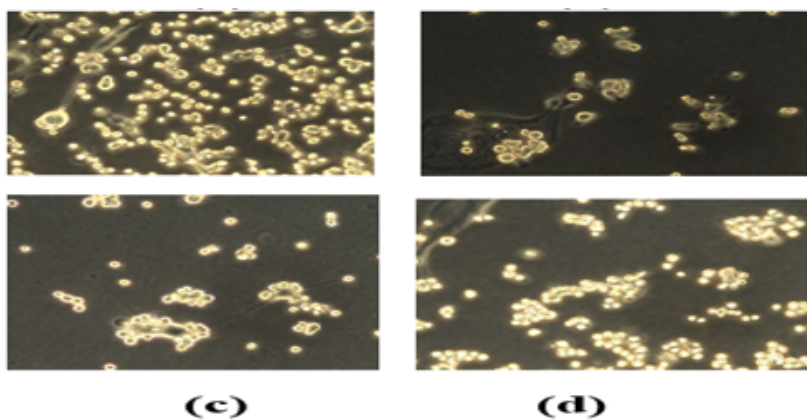


Figure 15

Morphology of MDA MB 231 cells treated with MAgNPs after 48hours (a) Control (b) 5 µg/mL, (c) 25µg/mL (d) 50 µg/mL. Cell shrinkage and clumping, signs of apoptosis, macrophage differentiation were seen (×20).

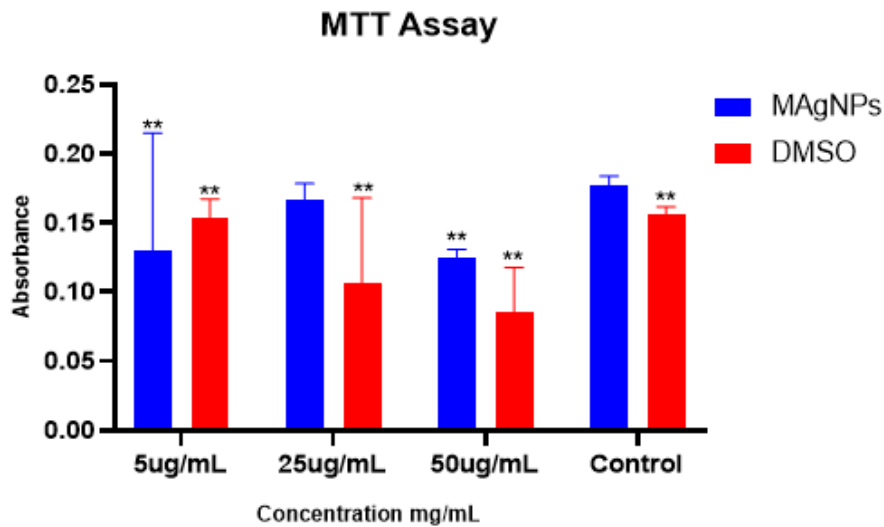


Figure 16

MTT Assay showing the antiproliferative effect of MAgNPs on MDA MB 231 Cancer Cell lines. Values are mean ±SD n=3. **= Significantly different from control (P<0.05).

Supplementary Files

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