Research Article

Biosynthesis of silver nanoparticles with *Mentha spicata* against *Aspergillus niger*

Fatema M. A. Alkfaji*, Ibtihal Muiz Al Hussaini

ABSTRACT

Background: *Mentha spicata* not modern uses aqueous leaf extract of medicinal plant. Silver nanoparticles(AgNPs) synthesized by leaves of *Mentha spicata* were evaluated for antimicrobial activity and found highly toxic and hazardous to microorganisms. It is found out that the silver nanoparticles have many inhibitory and fungicidal effects and so its application is extended as an antibacterial agent. The antifungal activity of silver nanoparticles is estimated by the zone of inhibition. *Aspergillus* sp. are indoor and outdoor saprophytic fungi that play a significant role in global carbon and nitrogen recycling while negatively in human and animal. **Materials and Methods:** After the addition of $AgNO_3$ within 20 min, the suspension showed change in color and turned dark brown, formation of silver nanoparticles was confirmed using ultraviolet (UV) spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and field emission scanning electron microscopy (FESEM). In this study, 70 samples were collected from sputum, 44 males and 26 females. Samples were positive 32 males and 18 females (50 infection) for the presence of fungal element. **Results:** Structure of synthesized AgNPs were confirmed by FTIR, UV visible, XRD and FESEM studies. Fungal species were isolated from sputum and bronchoalveolar lavage fluids. Many species were recovered and the effect nanoparticles on Aspergillus niger growth were tested. The result showed the effectivity of nanoparticles against fungus with zone of inhibition with 8 mm at concentration of 20 mg. **Conclusions:** The possibility of preparing nanoparticles of *M. spicata* plant using silver nitrate ($AgNO_3$) result revealed that AgNPs have a good activity against aspergillosis.

KEY WORDS: $AgNO_3$, *Aspergillus niger*, Fourier transform infrared, FSEM, *Mentha spicata*, Nanoparticles, X-ray diffraction

INTRODUCTION

Nanotechnology has attracted a great interest in recent years due to its expected impact on many areas such as medicine, agricultures, and industrial.[1] Silver nanoparticles have antimicrobial due to their surface reactivity, biosynthesis of silver nanoparticles preferred on the chemistry and physical method in medical application because it has advantage ecofriendly and low toxic and cost while chemical and physical methods are toxic and not effective and high cost.[2] Plant extracts contain phenolics, terpenoids, polysaccharides, and flavones compound that contributes in reduction and stabilizing of silver nanoparticles.[3]

The historical use of *Mentha* is not different from its use in modern herbal medicine. It can be used for common cold, cough, sinusitis, fever, bronchitis, nausea, vomiting, indigestion, antimicrobial, antioxidant, intestinal colic, and loss of appetite. It is also used for flavoring chewing gums, toothpaste, confectionery, and pharmaceutical preparations.[4,5] Mints are regarded as one of the most important spices throughout the world. The essential oils of mints are widely used as flavorings in the food, cosmetic, and pharmaceutical industries; the chemical composition of the essential oils in spearmint has been studied by different researchers. Carvone is the major component in all cases and is the character impact component in spearmint, followed by limonene and 1,8-cineole.[6-8]

Studies shows that spearmint, followed by limonene and1,8-cineole are the effective compounds of Mentha Spicata and green nanoparticles responsible for anti-microbial, and anti-oxidant activity against pathogenic microbes and fungi.[6-8] *Aspergillus* can cause aspergillosis in patient's chronic lung problems, as well as in patients with immune suppression, the aspergillosis is infection or allergic reaction caused

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by multiple types of mold. It is usually found on the outdoor patio, on plants, soil, or rotting vegetables. Smoked *Aspergillus* is type of mold that often causes or aerial disease in some people when they inhale its spores.[9] The aim of research to the study of *M. spicata* L. and nanoparticle's synthesis of plant extract with the effective compounds in *M. spicata* L. on the growth and pathogenesis of *Aspergillus niger.*

MATERIALS AND METHODS

Collect and Synthesis of Sliver Particles

Collection and preparation of aqueous plant extract *M. spicata* plant was completely collected from AL-Hind region in Karbala, dried under room temperature, and preserved in a dry place until use. The plant extract was prepared (20% w/v) by weight 20 g of dry ground of plant *M. spicata* and completed to 200 ml of deionize water and place in flask (500 ml) and boiling for 10 min. The extract filtered using Whatman filter paper while green synthesis of silver nitrate solution was added to plant extract, reduction of silver nitrate to silver nanoparticles, and color change from yellow to red-brown in aqueous solution due to the surface plasmon resonance (SPR) phenomenon give a peak at 450–550 nm in ultraviolet (UV)–visible spectrophotometer nanoparticles.[10]

Purification of Silver Nanoparticles

After the statement of nano piracy was using a device UVS, the mixture was then purified by centrifugation at 1200 rpm up to 30 min for 3 times, where the puncture was put in plat and in oven at 45 cover night and storage until used.[10]

Characterization of Nanoparticles

a. Determination sites effective by technique Fourier transform infrared (FTIR): The dry mixed material was prepared in a paragraph 2 in FTIR spectroscopy for the most effective location
b. Examination of nanoscale nature by technique X-ray diffraction (XRD): The technique XRD measurement was carried out for the detection of the crystal of AgNPs. The biosynthesized AgNPs were freeze dehydrated and powdered to examine XRD pattern. The phase structure and purity of metallic nanoparticles were checked through XRD patterns which were recorded using powder X-ray diffract meter. XRD analysis was achieved using XRD 6000 at a step size of 0.02˚, scanning rate of 2˚ in 2θ/min and a 2θ range from 30° to 80°, a voltage of 40 kV, and a current of 30 mA with Cu[11]
c. Determination of diameter and shape nanoparticles by FESEM used of the diameter of the diameter and its shape.

Fungi Collected and Isolation

A total of 70 specimens of sputum and bronchoalveolar lavage fluids were obtained over an approximate 4 months' period from November 2019 to February 2020. The fungi were isolated from patients visiting the specialized center of chest and pulmonary diseases in Hilla city/Iraq. The specimens were transported by screw-capped cups to the fungi and mycotoxin laboratory of the college of science in Babylon University, and each specimen was inoculated using direct method of inoculation by streaking on two general media, namely, Sabouraud's dextrose agar and potato dextrose agar, then incubated at 25°C for 2–7 days.[12]

Effect of the Nanoparticles on *A. niger*

Antifungal activity of AgNPs plant extract were determined agar disc diffusion method.[24] Wells of 5 mm diameter were made on the PDA surface and filled with the gradual concentrations of 5, 10, 15, 20, and 25 of AgNP colloids. The plates were incubated at 28°C for 2–7 days. PDA medium with the same amount of mycelium, without nanosilver, was used as a control sample. After incubation time, the plates were tested for the mycelial growth inhibitory zones around the well.

RESULTS

Change lotion solution of silver nitrate with water extract of plant *M. spicata* dark brown color appeared with a black deposit in minutes of mixing indicating the formation of silver nanoparticles as mention in the previous studies. Brown color change in the reaction is due to the Plasmon resonance peak observed for the silver nanoparticles located maximum absorbance of 450 nm as shown in Figure 1. The variety and grain size of synthesized silver nanoparticles were determined by XRD spectroscopy. The XRD pattern showed the characteristic Bragg peaks of (111), (200), (220), and (311) sides of the face center cubic (fcc) silver nanoparticles and confirmed that these nanoparticles and crystalline in nature show in Figures 2 and 3..

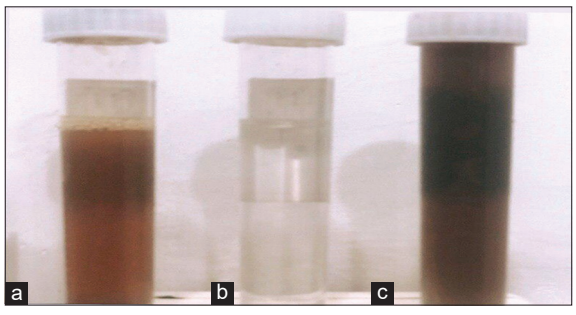

Figure 1: Color change lotion solution of silver nitrate with water extract *Mentha spicata*. (a) Extract of plant *M. spicata,* (b) solution of silver nitrate, (c) mix between solution (a and b) change with the appearance of precipitate

The secondary metabolites are the main factors for the biosynthesis of silver nanoparticles, the plant extract contains phenol, alcohol, amine, carboxylic acid, alkaloids, and terpenoids that responsible for reduction and stabilizing silver nanoparticles.[20] FTIR spectroscopy showed photochemical analysis of aqueous leaf extract *M. spicata*, it shows prominent bands of absorbance at peaks 3381.33, 2968.55, 1618.33, 1514.17, 1413.87, 1120.68, 1095.60, and 887.28 cm^{-1}. The comparative study of the FTIR spectrum of aqueous leaf extract of *M. spicata* and Silver nanoparticles shown in Figures 4 and 5 respectively. Various peak for silver nanoparticles are observed at wavelength of 3417.86cm^{-1}, 2939.52 cm^{-1}, 1627.92 cm^{-1}, 1411.89 cm^{-1} ,1265.30 cm^{-1}, 111.00cm^{-1} 933.65 cm^{-1} and 609.51cm^{-1}. The FESEM images of the silver nanoparticles are shown in Figure 6. The surface morphology of silver nanoparticles showed even shape and spherical nature.

In this study, 70 samples were collected from sputum, 44 males and 26 females. Samples were positive 32 males and 18 females (50 infection) for the presence of fungal element. Fungi that isolated included *A. niger, Aspergillus terreus*, *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus fumigatus*, *Nigrospora*, *Alternaria,* and *Penicillium* spp Table 1. The samples collected from 20 to 70 years from male and female; the number of fungi was in the age group of 46–69 years (35.8%) followed by the age group of >20 years (8.5%) and then the age group of 21–30 (15.7%) although the least patient was in age group of 31–45 (20%) and age group >70 (20%) Table 1. These results appear a significant difference among group, Table 2, through in this study, it was observed that the percentage of male and female infection was higher than that female where male 65.7% either female 34.3%, and the highest number in the age group of 46–69. *A. niger* appears to be soft or slightly woolen with a black color due to the large quantities of black spores that produce them which are in the form of discrete chains perpendicular to the surface of the gland. Micrometers are surrounded by one row of vial structures. With age, they are surrounded by a second row of vial structures. At their top, spherical are black, with a diameter of 4–5 µm [Figure 7].[23] The cape head is spherically in the early stages of growth with a diameter of 50–500 µm and gradually turns into a cylindrical shape with a diameter of 800 µm or greater in the last stages.[23] The result of the present study showed that five were significant differences between concentration of silver nanoparticles (5, 10, 15, 20, and 25). The antimicrobile activity of AgNps against *Aspergillus niger* in Table 3 The result showed that concentration 20mg most inhibition that rate 8mm, while the concentration 10mg, (7mm),have inhibition 15mg have inhibition (5mm) and 25mghave nhibition (6mm),while concentration 5mg was lowest inhibion 4mm diameter of inhibition zone. Infection due to *Aspergillus* spp. is oneof the most common fungal diseases in human and animals. in the study isolated funges percentage of male and female infection was higher than that female may be belong to smoking cigarette smoking is independently associated with increased mortality after allogeneic Although the effects of cigarette smoking are likely multifactorial, a significantly higher incidence of fungal infections may contribute to the poorer outcomes of smokers after transplantation [Figure 8].[25]

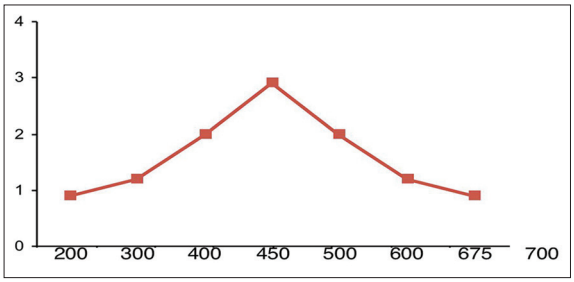

Figure 2: Ultraviolet spectra of a silver nanoparticles synthesized by *Mentha spicata*

Table 1: Types of fungi, number of sample, and percentage (%) of fungi isolates

Type of fungi	Number of isolates	Percentage (%) of isolates
Aspergillus niger	42	84
Aspergillus flavus	20	40
Aspergillus terreus	5	10
Aspergillus parasiticus	5	10
Aspergillus fumigatus	9	18
Nigrospora	10	20
Alternaria	2	4
Penicillium spp.	17	34
Total number	110	100%

DISCUSSION

Reduction of silver ion into silver nanoparticles when added to the plant extracts leads to color change. In

Table 2: Age and gender distribution of patients

Age/gender	<20	21–30	31–45	46–69	>70	Total number and percentage
Male	4	7	10	16	9	46
Female	2	4	4	9	5	65.7
Percentage	8.5	15.7	20	35.8	20	24
						34.3

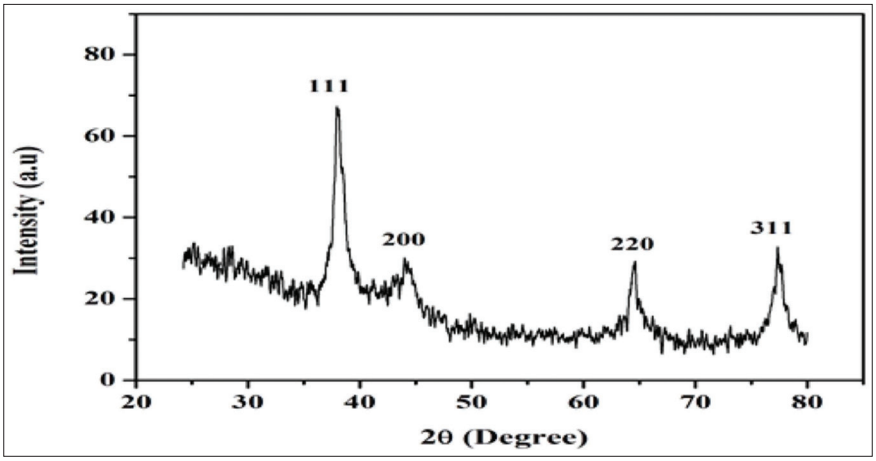

Figure 3: X-ray diffraction result for silver nanoparticles of *Mentha spicata*

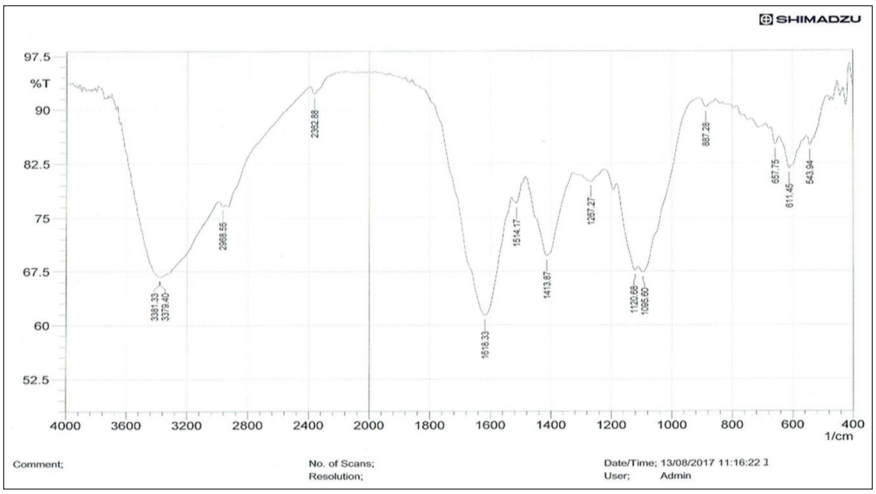

Figure 4: Fourier transform infrared spectrum of aqueous leaf extract of *Mentha spicata*

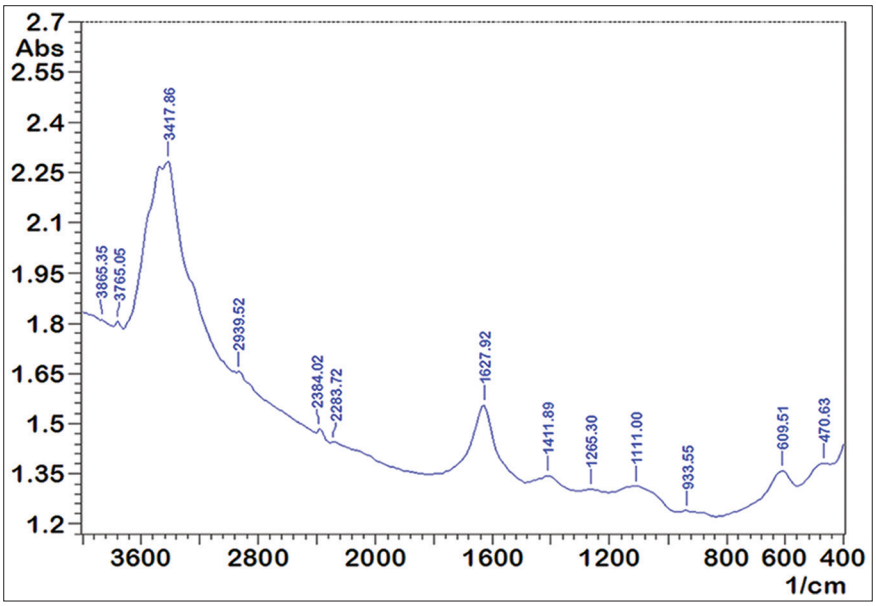

Figure 5: Fourier transform infrared spectrum of silver nanoparticles synthesized by dried leaf extract of *Mentha spicata*

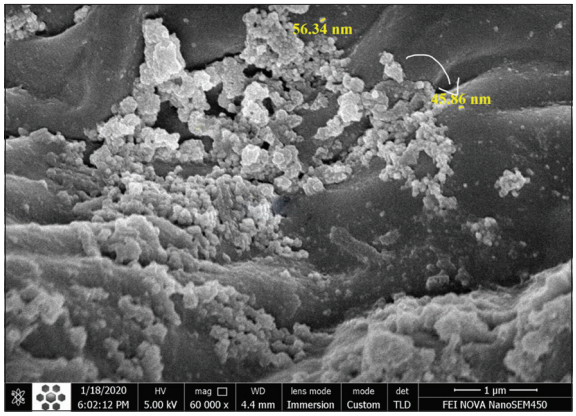

Figure 6: Field emission scanning electron microscopy image of extracellular AgNPs formed *Mentha spicata*

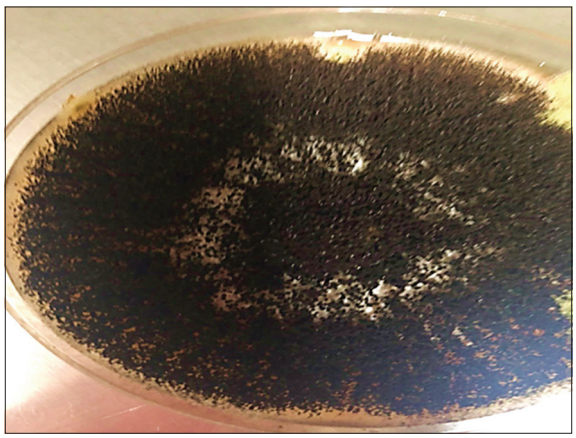

Figure 7: Shape of *Aspergillus niger* colony

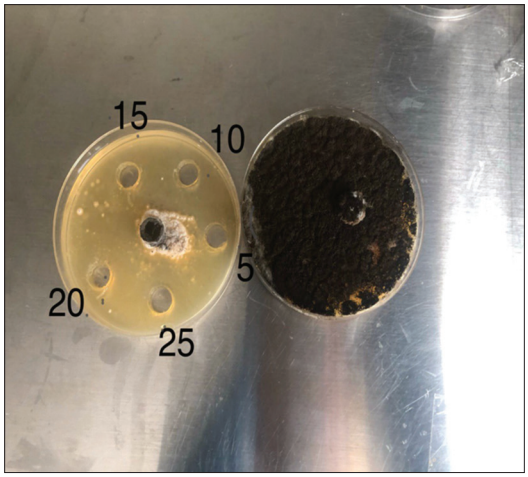

Figure 8: Effect AgNPs on *Aspergillus niger* growth

the present study, silver nanoparticles exhibited dark brown color in aqueous solution.[13,14] The conversion in color is mentioned to SPR, which is an individual land of the nanoparticles. The SPR highest can be responsible for covering and balance of AgNPs formed and might too relate for the spherical shape of AgNPs[15,16,26] UV–visible spectroscopy. The progress of reduction of silver nitrate to SNPs can be easily

Table 3: Mean zone of inhibition (mm) by AgNPs in *Aspergillus niger*

Concentrations (mm)	Control	5	10	15	20	25
Inhibition zone	0	4	7	5	8	6

evaluated using a UV–visible spectrophotometer. This is because SNPs can absorb light in the visible region due to the SPR phenomenon based on their size and shape.[27] The presence of nanoparticles was confirmed by obtaining a spectrum in visible range of 200–700 nm. The optimum concentration suitable for nanoparticles synthesis by mint extract was found to be 450 nm. The appearance of strong band is that the spectral pattern is due to the excitation of the localized surface plasmons which cause strong light scattering by an electric field at a wavelength where resonance occurs, as mentioned.[17] The XRD pattern in result showed the characteristic Bragg peaks of (111), (200), (220), and (311) sides of the fcc silver nanoparticles and confirmed these nanoparticles and crystalline in nature. The angles values appeared (2θ) 37.9A, 44.5A, 64.2A and 77.9A and as per Scherrer equation, size is almost of AgNPS. A study by Anup *et al*.[18] was indicated that the XRD pattern was showed that six strong peaks were observed at 32.21, 38.15, 44.47, 46.38, 64.38, and 77.53 which were corresponding to planes (54.36), (89.13), (52.54), (100), (58.95), and (52.77), respectively.[19] FTIR spectroscopy showed that photochemical analysis of aqueous leaf extract *M. spicata*, it shows prominent bands of absorbance at peaks 3381.33^{-1}, 2968.55^{-1}, 1618.33^{-1}, 1514.17^{-1}, 1413.87^{-1}, 1120.68^{-1}, 1095.60^{-1}, and 887.28 cm^{-1}. The comparative study of the FTIR spectrum of aqueous leaf extract of *M. spicata* and silver nanoparticles shown in Figures 4 and 5 respectively.[21] The surface morphology of silver nanoparticles showed even shape and spherical nature. Similar results were also reported for phytosynthesized silver nanoparticles.[22] This result strongly confirms that *M. spicata* murex leaf extracts might act as a reducing and capping agent in the production of silver nanoparticles. Infection due to *Aspergillus* spp. is one of the most common fungal diseases in human and animals the silver nanoparticles tests against *A. niger*. in the study isolated fungus percentage of male and female infection was higher than that female may be belong to smoking cigarette smoking is independently associated with increased mortality after allogeneic.[25] In this study, the application of silver nanoparticles as an antimicrobial agent was investigated and exhibited better antimicrobial activity against some selected human pathogens. The results suggested that silver nanoparticles have the capacity to inhibit the growth of *A. niger*. The effect was observed in a concentration dependent manner in both fungi. The both fungi showed maximum inhibition at 20 mg

AgNPs concentration. This high antifungal activity of silver nanoparticles is probably related to the high intensity at which AgNPs solution was capable of state and agglutinate to fungal hyphae and to deactivate pathogenic fungi. There are many mechanisms of inhibitory effect of Ag^{+} on microorganisms, such as DNA loses its ability to replicate,[28] resulting in inactivated expression of ribosomal subunit proteins, instead of some other enzymes and cellular proteins necessary to the adenine triphosphate production.[29]

CONCLUSIONS

The possibility of preparing nanoparticles of *M. spicata* plant using silver nitrate ($AgNO_3$). Through the measured result appeared UV 450 nm, FTIR and XRD paper found Ag nanoparticles, FESEM demonstrates the presence of clear spherical crystal indicative of the formation of nanoparticles. FTIR technique showed that the plant has many functional group. The genus of *Aspergillus* was found to be the predominant opportunistic fungal pathogen isolated from patients with pulmonary diseases. Result revealed that AgNPs have a very good activities against aspergillosis.

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