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BIOSYNTHESIS OF SILVER NANOPARTICLES USING MINTS LEAF EXTRACT AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT : The aim of this study is to synthesize an easy, non-toxic and eco-friendly method. Silver nanoparticles which were synthesized by leaf extract of mint were characterized by UV-Visible Spectroscopy which appears UV-Visible spectrum of demonstrated a peak 448 nm corresponding to surface Plasmon resonance of silver nanoparticles, Fourier Transform Infrared Spectroscopy (FTIR); functional groups involved in the silver nanoparticles synthesis were identified, the presence of silver nanoparticles was confirmed by X-ray diffraction (XRD) and Atomic Force Microscope (AFM) analysis clearly illustrated that the shape of silver nanoparticles was spherical and the size of the silver nanoparticles has been measured as 55- 85 nm. Evaluation of its antimicrobial activity, the resulted showed that efficiency inhibitory activity against bacteria and fungi, silver nanoparticle showed a greater effect on *Staphylococcus aureus* at a concentration of 150 µg/ml which reached in diameter of the inhibition zone 18.5 mm. whereas in fungi, *Candida utilis*at a concentration of 150 µg/ml, which reached in diameter of the inhibition zone 18mm.

Key words : Silver nanoparticles, antimicrobial, mints, AFM, XRD.

INTRODUCTION

Nanotechnology is the concept that research and development on new material in size between 1-100 nm, this technology is capable of providing different novel applications that range fromfood processing and agricultural production to sophisticated medicinal techniques (Bawa *et al*, 2016). Nanoparticles have been used as therapeutic agent, imaging diagnosis and delivery vehicles for drugs and genes. It can interact with biological systems as the molecular level and allow targeted delivery and passage through biological barriers (Sahoo *et al*, 2017). In recent years, synthesis of metal nanoparticles has been demonstrated by many physical and chemical means, but the importance of biological synthesis is being emphasized globally at present because chemical methods are toxic, expensive, non eco-friendlyand have low productivity (Shah *et al*, 2015). Biological methods involve the synthesis of silver nanoparticles using extracts from organisms as reductant and capping agents (Li *et al*, 2007). There are some examples of synthesizing nanomaterials using plants, including the use of live alfalfa to synthesize gold and silver nanoparticles, and *Acorous calamus* rhizome extract to synthesize silver nanoparticles, the rhizome has a rich profile of bioactive compounds including alkaloids, flavonoids, triterpenes and phenolic compounds (Gardea-Torresdey *et al*, 2003; Nakkala *et al*, 2014).In addition to silica nanoparticles were synthesizedby extract of *Thuja orientalis* leaf (AL-Azawi *et al*, 2019). Silver nanoparticles are one of the fastest growing product categories due to wide range of applications (Marambio-Jones and Hoek, 2010).*Mentha longifolia* L. is genus (Lamiaceae) comprises more than25 species. The cultivation of mint is principally in temperate regions of Europe and Asia but also in South Africa, Australia and the United States. *Mentha* species is an important aromatic plantwith economical benefits in food, medicine and cosmeticsas well as antiseptic, anticarcinogenic,expectorant, calming, diuretic effects,and effects against common cold, indigestion, nausea,and sore throat(Russo *et al*, 2015; Sevindik *et al*,2017, Babaeian *et al*, 2017). Mentha is known to produce a wide range of natural terpenoids named menthol ($C_{10}H_{20}O$) found in the essential oils of the mints family (*Mentha* spp.). Flavonoid, terpenoid and alkaloids compounds present in the extract were claimed to be responsible for reduction and stabilization of nanoparticles (Fatiha *et al*, 2015; Santhoshkumar *et al*, 2017; Huang *et al*, 2007). The mechanism of the bactericidal effect of silver nanoparticles may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell, antibacterial properties of silver particles are size dependent with smaller particles exhibiting a greater effect

due to surface area of the particles, for instance smaller silver nanoparticles having the large surface area available for interaction would give more bactericidal effect than the larger silver nanoparticles (Sahayaraj *et al*, 2011; Fadeel, 2014; Anbukkarasi *et al*, 2017).

The aim of this study issynthesis of silver nanoparticles by ecofriendly method and study characteristic and evaluation antimicrobial activity of silver nanoparticles.

MATERIAL AND METHODS

Preparation of plant leaf extracts

Fresh leaves of mint were collected from region Baghdad and washed with distilled water separately, then dried and grinded to form powder using motor and pastel. 5gram of powderwasadded to 100 ml sterile distilled water,it was boiled for ten minutes. After cooling suspensionswere filtered with filter paper (Parashar *et al*, 2009).

Synthesis of silver nanoparticles

1ml of mint leaves extract was added into 9 ml of silver nitrate (1mM) purchased from BDH for reduction silver ions and stabilizing, taking place in dark ambient after 12 hours the colour change from pale yellow to brown colour, then centrifuging at 12000 rpm for 20 minutes and pellet obtained after discarding the supernatant was washed three times, finallydried via oven (Parashar *et al*, 2009).

Characterization of the synthesized silver nanoparticles

Silver nanoparticles were monitored by measuringUV-Visible spectrophotometer in the range of a wavelength from 200 - 900 nm.For Fouriertrans forms Infrared spectroscopy (FTIR) analysis, the silver nanoparticles powder and leaf extract of spearmint was ground with KBr, Spectra was recorded by using a spectro-photometer. The scans recorded were in the range between 4000- 400 cm^{-1}. The size and shape of silver nanoparticles wasmeasured using Atomic force microscope. The crystalline structure of the particles was determined by X- ray diffraction (XRD)using Cu Kα1 radiation (λ= 1.540562 Aº) at 30 mA current and 40 kV.

Antimicrobial activity of silver nanoparticles

The antibacterial activity of silver nanoparticles was tested againstsix bacteria*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus* spp., *Salmonella*spp., *Pseudomonas aeruginosa* and *Escherichia coli*by agarwell diffusion method. Inoculate wasprepared by suspending colonies of bacteria in normal saline and the turbidity was adjusted to 0.5McFarland standard which is approximately1.5×10^{8} CFU/ml. inoculums were cultured on muller hinton agar and made wells of 3 mm diameterusing sterile gelborer. 50 ìl silver nanoparticles (with different concentrations25, 50, 75 and 100, 125, 150 µg/ml)was added onto eachwell on all plates using sterile dropping pipette. The plant extract was used ascontrol for this experiment. Finally, the in oculated plates were in cubatedat 37 °C for 24h for all the bacterial strains. The zones of in hibitions was observe dand measure din millimeters (Cheesbrough, 2006). The same steps previousapplies on four fungiisolated (*Candidautilis*, *Candida*spp., *Saccharomyces cerevisiae* and *Saccharomyces*spp.) except using sabouraud dextros eagar instead muller hinton agar.

RESULTS AND DISCUSSION

Characterization of the synthesized silver nanoparticles

Visual Inspection

After the addition of aqueous plant extract of *M. longifolia* to AgNO3(1mM) solution, the reaction mixture is kept for 24 hr. After 24 hr,a lightyellowish color was observed which changed to dark brown color. This change of color indicates that the formation of silver nanoparticles hastaken place.

UV-Visible spectrophotometer

Indicates the UV-Vis absorption spectra for the synthesized silver nanoparticles from *M. longifolia* plant extracts,the colourless silver nitrate changed to brown within 12 hr. Uv-visible spectra of silver nanoparticles revealed a peak 448 nm.

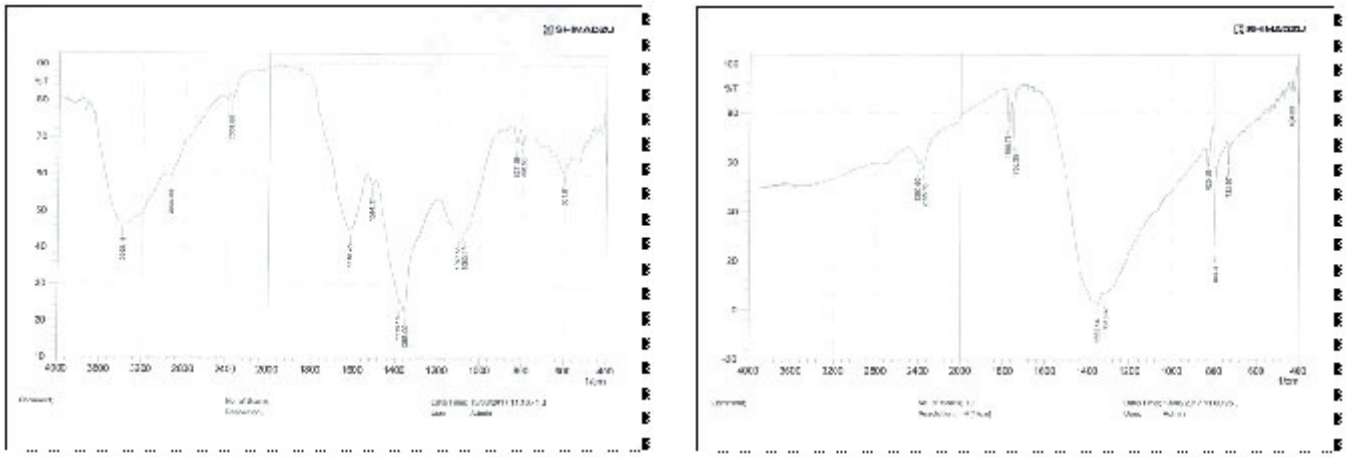
The occurrence of the absorbance peak at 448 nm is due to the surface Plasmon resonance (SPR) which occurs as a result of the excitation of the surface plasmon's on the outer surface of the metal nanoparticleswhich gets excited by electromagnetic field in Uv-visible spectroscopy (Agnihotri *et al*, 2013).

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform spectroscopy analysis is utilizable for characterizing the surface chemistry of silver nanoparticles. To identify functional groups from the leaf extract of *M. longifolia*which take part in the reaction as reducing and capping agents, organic functional groups like O-H, C=O linked to the surface of nanoparticles are found by FTIR (Mittal *et al*, 2013).

FTIR analysis was carried out for powder leaf extract of spearmint and silver nanoparticles. As indicated in fig.1.athe spectrum of leaf extract of *M. longifolia* powder showed several absorption peaks.

The absorbance peak at 3385.18 cm^{-1} corresponds to O-H stretch of alcohols and phenols, 2933.83, 2362.88 cm^{-1} corresponds to C-H stretch alkene and O-H stretch



(a) (b)

Figure 1 : FTIR analysis of leaf *M. longifolia*extract (a) and biosynthesized silvernanoparticles (b).

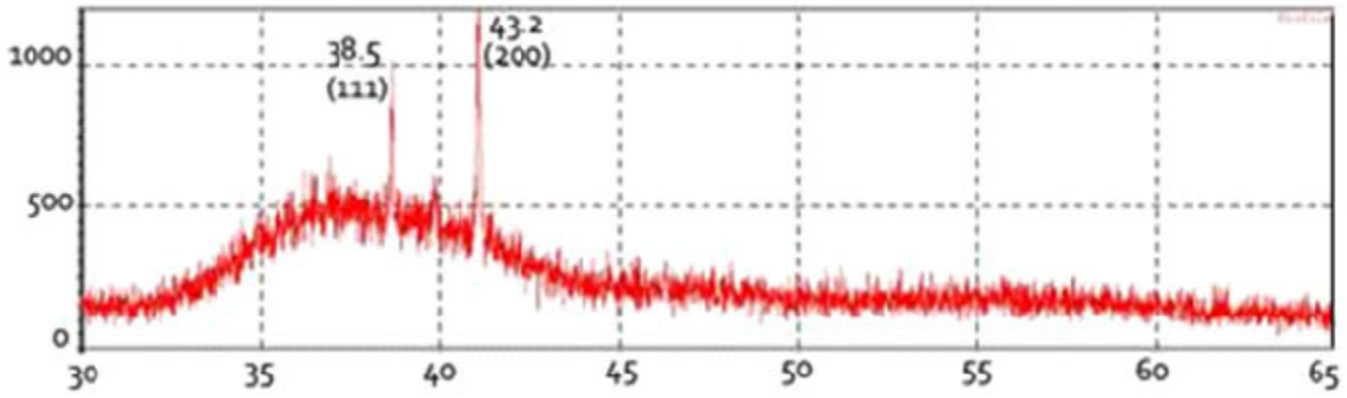
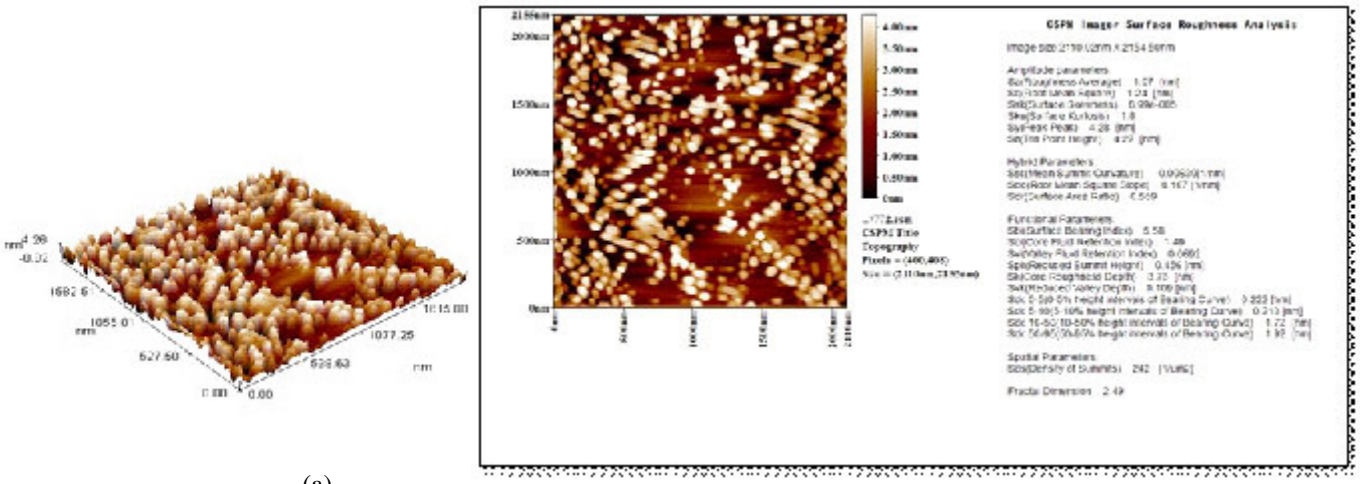


Figure 2 : XRD patterns of silver nanoparticles .



(a) (b)

Figure 3 : Three-dimensional image (a) two-dimensional image (b) for silver nanoparticles was synthesized by leaf extract of *M. longifolia*

carboxylic acids,1616.40 cm^{-1}corresponds to C=O stretch, 1515.17 cm^{-1} corresponds to C=C stretch, 1379.15, 1356.00 cm^{-1} corresponds to C-N stretch of amine, 1097.53, 1080.17 cm^{-1} corresponds to C-O stretch, 827.49, 798.56, 601.81 cm^{-1}corresponds to C-C stretchof alkan. After reaction and synthesized silver

nanoparticles,disappearing or shiftingpeak as shown in fig.1.b.

X-ray diffraction (XRD)

XRD pattern of silver nanoparticles synthesized by *M. longifolia* leaf extract is shown in fig.2 clearly illustrate the crystalline nature, XRD pattern result shows at 2è of 38.5º and 43.2º these are corresponding to (111) and (200) planes for silver, respectively. XRD results clearly show that the silver nanoparticles formed by reduction of silver nitrate by the *M. longifolia* might is crystalline in nature. XRD is used for determining the chemical composition and crystal structure of a material (Ponarulselvam *et al*, 2012).

Atomic Force Microscope (AFM)

AFM analysis revealed the silver nanoparticles mainly had spherical shape figure.The size of particles obtained ranged from 55- 85 nm, fig.3shows AFM images evolutions of the topography of the surface of the silver nanoparticles synthesized by leaf extract of mints. Thesizedistributionof silver nanoparticles wasshownin the table.1.

The researcher found that the average of the sizes of synthesized of silver nanoparticles is varied according to the plant, the resultsshowed that average size of 28 nm, 26.5 nm,65 nm, 22.3 nm and 28.4 nm corresponding to *Ocimum tenuiflorum*, *Syzygium cumini*, *Citrus sinensis*, *Solanum tricobatum*and *Centella asiatica*, respectively (Logeswari *et al*, 2015).

Antibacterial activity of silver nanoparticles

Inhibitionzones of bacteria were observed in the table1, the results showed that silver nanoparticle had higher inhibitory efficacy at a concentration of 150µg/ml against *S. aureus*,*B. subtilis*,*Bacillus* spp., *Salmonella*spp., *P. aeruginosa* and*E. coli*with a diameter of inhibition zone was 18.5, 17, 16.5, 15.5, 14.5 and 16.5, respectively.

Vitis venifera fruit mediated syntheses of silver nanoparticles are spherical in shape, size range between 30-40 nm having antibacterial activity against *Bacillus subtilis* and *Klebsiella planticola*, 50 ì1 of silver nanoparticleswith a diameter of inhibition zone was measured 13, 11 mm, respectively. In addition studies,

Table 1 : Granularity Cummulation Distribution.

Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)
55.00	8.83	8.83	70.00	15.90	56.54	85.00	9.89	88.69
60.00	18.02	26.86	75.00	14.84	71.38	90.00	7.77	96.47
65.00	13.78	40.64	80.00	7.42	78.80	95.00	3.53	100.00

Table 2 : Antibacterial activity of silver nano particles.

Bacterial isolate	Concentration of silver nano particles (µg/ml)					
	25	50	75	100	125	150
	Inhibition zone rate (mm)					
S. aureus	10	12.5	13	15	16.5	18.5
B. subtilis	10	12	12.5	14	15	17
***Bacillus* spp.**	10	11.5	12	13	14.5	16.5
***Salmonella* spp.**	9	11	12	13.5	15	15.5
P. aeruginosa	9	10.5	11.5	12	13	14.5
E. coli	10	12	12.5	14	15	16.5

Table 3 : Antifungal activity of silver nano particles.

Fungi isolate	Concentration of silver nano particles (µg/ml)					
	25	50	75	100	125	150
	Inhibition zone rate (mm)					
C. utilis	9	13	14	14.5	16	18
***Candida* spp.**	9	11	12	13	15	16.5
S. cerevisiae	9	12	12.5	14	15.5	17
***Sacchar omyces* spp.**	9	11	12	13	14	16.5

*Syzygium alternifolium*fruit extract to synthesized silver nanoparticles which possess spherical shape, size range between 32-68 nmthat having antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*,*Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Bacillus subtilis* and *Staphylococcus aureus* with an inhibition rate of 15.2, 17.5, 19.1, 14.5, 23.2, 13.3 and 14.3 mm in diameter , respectively.*Carica Papaya* peel extract using to synthesis of silver nanoparticles size range 10-30nm which having antibacterial activity against *S. aureus*, *B. subtilis*, *K. Pneumonia* and *E.coli*at concentration 200 mg/disc showed result of inhibition zones 10, 12,16 and15 mm in diameter.(Gnanajobitha *et al*, 2013; Yugandharand Savithramma, 2016; Kokila, 2016).

Antifungal activity showed that silver nanoparticle had higher inhibitory efficacy at a concentration of 150µg/ml against*Candidautilis*, *Candida*spp., *Saccharomyces cerevisiae* and *Saccharomyces*spp. with a diameter of inhibition zone was 18, 16.5, 17 and 16.5, respectively. As shown in table 3.

So other studies found that efficacy of antifungal agentsofsilver nanoparticles, has been reported that have silver nanoparticles activity against *Candida* spp., recently, have been reported that the increase in hydroxyl radicals by silver nanoparticles causes apoptotic cell death in *Candida albicans* (Owaid *et al*, 2015; Kumar *et al*, 2013; Hwang *et al*, 2012).

Mechanism of action of silver nanoparticles, silver nanoparticles attach to the surface of the cell membrane and drastically disturb its proper functioning, such as permeability and respiration; they are able to penetrate inside the bacteria and fungi, in addition destruct membrane integrity of fungal sporesleading to cell death; further damage by interacting with sulfur- and phosphorus-containing in DNA (Salomoni *et al*, 2017; Krishnaraj *et al*, 2012).It has been reported that the size, shape and capping agent of silver nanoparticles significantly effect on their capability to penetrate the cells and also on their toxicity and antibacterial activity (Siddiqi *et al*, 2018).

CONCLUSION

Silver nanoparticles can besynthesizedby green chemistry using leaf extract of mint, which is eco-friendly, cost- efficient and nontoxic. Silver nanoparticles have antimicrobial activity.

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