Antimicrobial Activity of Silver Nanoparticles Synthesized by Myrtus Communis Extract

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ABSTRACT

In this work, antimicrobial potential of silver nanoparticles synthesized from extract of Myrtus communis on different pathogenic bacteria and yeast was investigated. 10 mL of extract was mixed with 90 mL of 2 mM aqueous $AgNO_3$ and heated at 70 °C for 3 min. A change from greenish to dark brown color was observed. Characterization using UV-VIS spectrophotometery and X- ray diffraction analysis were performed. The UV-Vis spectral analysis showed silver surface plasmon resonance band at 425 nm. X- ray diffraction showed that the particles were crystalline in nature with face centered cubic structure (FCC) of the bulk silver with broad beaks at 38.50° and 44.76°. Antimicrobial activity against six microorganisms was tested using well diffusion method. The synthesized silver nanoparticles efficiently inhibited various pathogenic organisms in a dose-dependent manner was more pronounced against Gram-positive bacteria than Gram-negative bacteria. The approach of green synthesis seems to be cost effective, eco-friendly and easy alternative to conventional methods of silver nanoparticles synthesis. The powerful bioactivity demonstrated by the synthesized silver nanoparticles leads towards the clinical use as antimicrobial.

Keywords: Myrtus communis; Silver Nanoparticles; Antimicrobial activity.

الفعالية المضاده المايكروبية لدقائق الفضة النانوية المتشكلة بواسطة مستخلص نبات الاس

الخلاصة

تم في هذه الدراسة بحث الفعالية المضادة المايكروبيه لدقائق الفضة النانوية المتشكلة من مستخلص نبات الاس على انواع مختلفة من البكتريا المرضية والخميرة . اذ تم مزج ١٠ مل من

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المستخلص النباتي مع ٩٠ مل من محلول نترات الفضة المحضرة بتراكيز ١ ميلي مولاري و ٢ ميلي مولاري وتم تسخين المزيج عند درجة ٧٠ م ولمدة ٣ دقائق فلوحظ تغير اللون من الاخضر الى البني الداكن . لتشخيص الدقائق المتشكلة تم استخدام المقياس الطيفي UV-VIS والتحليل باستخدام جهاز حيود الاشعة السينية . ظهرت حزمة التصوير الطيفي عند القراءه ٤٢٥ نانوميتر واظهر قياس حيود الاشعة السينية بان جزيئات الفضة كانت بشكل تركيب مركزي مكعبي الاوجه عند قمم واسعة ٣٨,٥٠ و٤٤,٧٦ انكستروم .كما درست الفعالية المايكروبية على ستة انواع من الاحياء المجهرية باستخدام طريقة الانتشار بالحفر واظهرت جزيئات الفضة النانوية المتشكلة تثبيط لمختلف الانواع الممرضة اعتمادا على تركيزها , بذلك يعد تخليق دقائق الفضة النانوية بهذه الطريقة البايولوجية سهلة وغير مكلفة وتقود نحو استخدامها سريريا كمضادات مايكروبية.

INTRODUCTION

Nanotechnology is the newest and one of the most promising areas of research in modern medical science. Nanoparticles exhibit new and improved properties based on size, distribution and morphology than larger particles of the bulk materials from which the nanoparticles are made [1, 2]. Silver nanoparticles have found tremendous applications in the area of catalysis, optoelectronics, detection and diagnostic, antimicrobials and therapeutics [3-6]. Silver has long been recognized as an effective antimicrobial agent that exhibits low toxicity in humans and has diverse in vitro and in vivo applications [7]. Recently, silver-based topical dressings are widely used to treat infections in open wounds and chronic ulcers. These dressings also protect the host material from oxidation and discoloration [8, 9]. There is still need for economic, commercially doable in addition environment friendly synthesis route to synthesize silver nanoparticles. Several approaches are out there for the synthesis of silver nanoparticles for example, chemical reduction [10], photochemical [11], thermal decomposition [12], radiation assisted [13], electrochemical [14], and recently via green chemistry method [15].

Biological method of nanoparticles synthesis using microorganisms [16], enzyme [17], and plant or plant extract offers numerous benefits over chemical and physical method [6, 15]. Among the various known synthesis methods, plant-mediated nanoparticles synthesis is preferred as it is cost-effective, environmentally friendly, and safe for human therapeutic use [15, 18]. Many reports are available on the biogenesis of silver nanoparticles using several plant extracts, particularly *Lantana camara* [6], *Moringa oleifera* [19], *Catharanthus roseus* [20] and *Eucalyptus chapmaniana* [21]. However, potential of the plants as biological materials for the synthesis of nanoparticles is still under utilization.

Myrtus communis L. (Family: *Myrtaceae*), is an aromatic evergreen perennial shrub or small treeFigure (1). It is native to Southern Europe, North Africa and West Asia. It is distributed in South America, North western Himalaya and Australia and widespread in the Mediterranean region [22, 23]. It is also cultivated in Iraqi gardens for its fragrant flowers, facing wall and makes an excellent hedging plant.

Figure (1) Arial parts of M. communis L.

In folk medicine, the fruit of this plant is used in the treatment of many types of infectious disease, including diarrhea and bloody diarrhea, and the leaves are used as antiseptic and anti inflammatory agent, and as a mouthwash, for the treatment of candidiasis [23]. Many plants from the Myriaceae family are reported to have antibacterial or antifungal activities [24, 25]. The aromatic and medicinal qualities of *M. communis* contribute to its use in pharmaceutical, cosmetics and food products [26]. To date, there is no report on the synthesis of silver nanoparticles by utilizing the aqueous leaf extract of *M. communis*. Thus, this work was aimed to synthesize and characterize silver nanoparticles, and to investigate the antimicrobial activities.

MATERIALS AND METHODS

Materials

The chemical silver nitrate ($AgNO_3$; 99.98%), Mueller-Hinton agar (MHA) and Sabouraud Dextrose agar (SDA, Oxoid) were purchased from Merck, Germany.

All organic solvents used were of HPLC grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of the extract

Leaves plant extract was prepared by Soxhlet extraction method. About 100 g of powder material was uniformly packed in to a thimble and run in Soxhlet extractor. It was exhaustible extracted with methanol for the period of about 48 hour or till the solvent in the siphon tube of an extractor became colourless. After that extracts were filtered with the help of filter paper and solvent was evaporated from extract in rotary evaporator to get the syrupy consistency. Then extract was kept in refrigerator at 4 °C for future experiments.

Synthesis of silver nanoparticles

Aqueous solution of silver nitrate was prepared by adding 2mM of $AgNO_3$ to 90 ml of distilled water at room temperature. The aqueous solution was mixed with 10 ml of leaf extracts at a temperature of 70 °C while stirring magnetically at 1000 rpm for 3 min. The bio-reduced aqueous component was used for the UV–Vis spectroscopy characterization.

Characterization of silver nanoparticles

UV-Vis spectral analysis was done by using UV-Vis spectrophotometer (PG-T80$^+$ UV/Vis spectrophotometer, England) from 350-700 nm at a resolution of 1 nm. XRD spectrums of the silver nanoparticles solution drop-coated on glass were done on Shimadzu XRD-6000 model with 40 kv, 30 mA with Cu k α radiation at 2 θ angel. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula.

$$D = 0.94 \lambda / \beta \cos \theta$$

Evaluation of antimicrobial activity

The silver nanoparticles synthesized using *M. communis* extract was tested for antimicrobial activity by agar well diffusion method against different pathogenic microorganisms Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumonia, Proteus volgaris, Klebsiella pneumoniae (Gram Negative), Staphylococcus aureus (Gram Positive) and Candida albicans (Yeast). The pure cultures of bacteria were subcultured on MHA and SDA for yeast. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Wells of 8 mm diameter were made on nutrient agar plates using gel puncture. Using a micropipette, 50 µL of nanoparticle solution was poured onto each well on all plates. After incubation at 37◦C for 24 hours, the diameter of zone inhibition was measured in millimeter, and was recorded as mean ± SD of the triplicate experiment.

Statistical analysis

The grouped data were statistically evaluated using ANOVA with SPSS/14 software. Values are presented as the mean ± S.D. of the three replicates of each experiment.

Results and Discussion

Green synthesis of silver nanoparticles using 2mM $AgNO_3$is shown in Figure (2). The fresh suspension of M. communis was greenish in colour. However, after

addition of $AgNO_3$ and heated at 70 °C for 3 min, the suspension turned dark brown. Formation of silver nanoparticles was confirmed using UV-Vis spectral analysis and showed silver surface plasmon resonance band at 425 nm Figure (3). Raut rajesh et al. [27] reported that reduction of silver ion into silver nanoparticles during exposure to the plant extracts could be followed by colour change due to excitation of surface plasmon vibrations in silver nanoparticle. Silver nanoparticles exhibit interesting optical properties directly associated with localized surface plasmon resonance which is highly depends on the morphology of the nanoparticles [15].

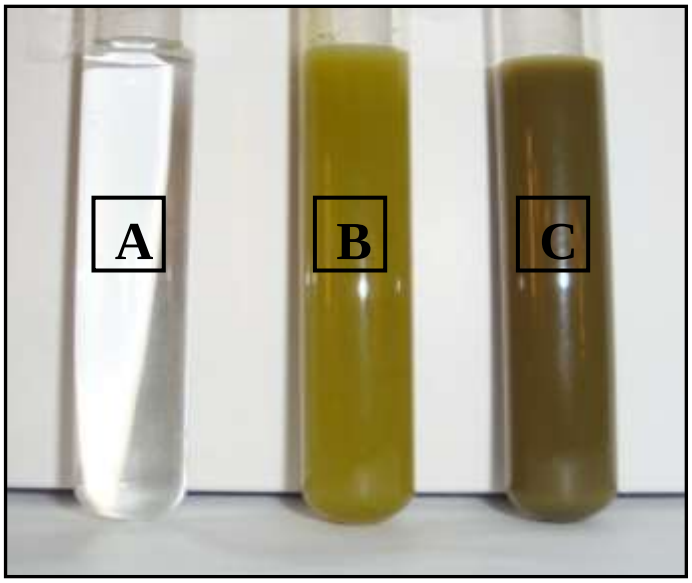

Figure (2) Photograph showing colour changing. (A) 2mM AgNO3 without M. communis extract (B) Aqueous leaf extract of M. communis (C) Changing colour from greenish to dark brown after adding 2mM AgNO3 and heated at 70 °C for 3 min.

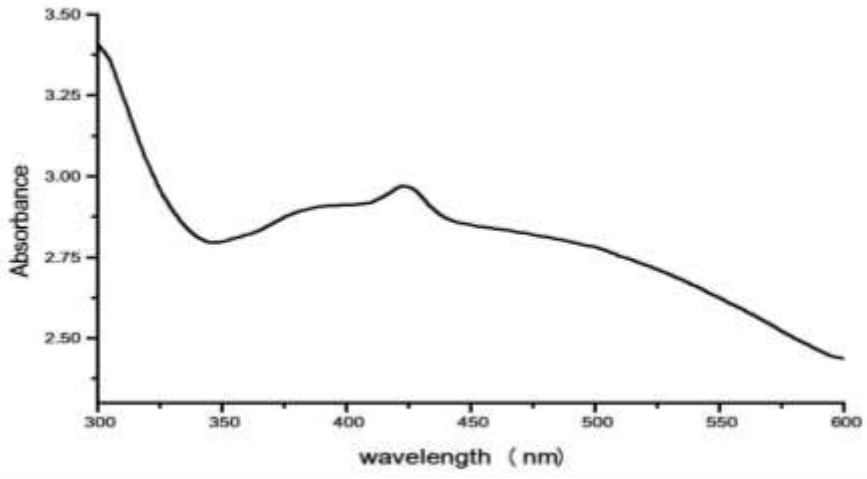

Figure (3) UV/Vis absorption spectra of reduction of silver ions to silver nanoparticles after heating at 70 °C for 3 min.

The X-ray diffraction pattern of the biosynthesized silver nanostructure produced by the leaf extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image Figure (4). The Braggs reflections were observed in the XRD pattern at 2 θ of 38.50° and 44.75° in the whole spectrum of value ranging from 20 to 60 and indicated that the structure of silver nanoparticles is Face-Centered Cubic (FCC). These are corresponding to (111) and (200) planes for silver, respectively. The lattice constant calculated from this pattern was a = 4.086 A° and the data obtained was matched with the database of Joint Committee on Powder Diffraction Standards (JCPDS) file No. 04-0783. The average grain size of the silver nanoparticles formed in the bioreduction process was determined using Scherr's formula and was estimated as 60-100 nm.

The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium [28, 29]. It is generally recognized that UV–VIS spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions [19, 30]. XRD is commonly used for determining the chemical composition and crystal structure of a material; therefore, detecting the presence of silver nanoparticles in plants extracts can be achieved by using XRD to examine the diffraction peaks of the plant [20]. In present study the X-ray pattern of synthesized silver nanoparticles matches the FCC structure of the bulk silver. The X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag^+ ions by the *M. communis* might are crystalline in nature.

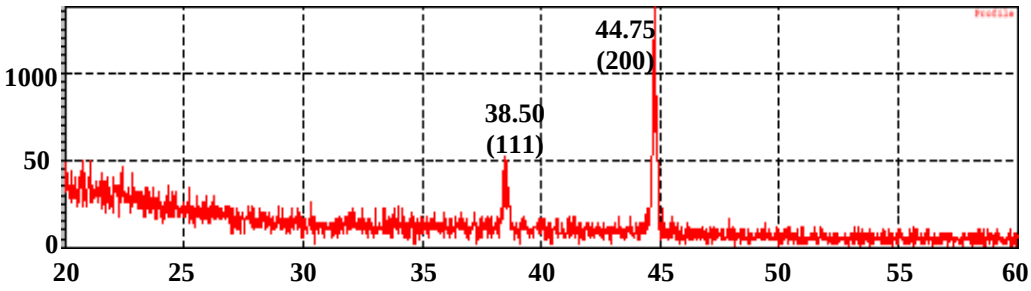

Figure (4) XRD patterns recorded from drop-coated films on glass substrate of silver nanoparticles synthesized by treating M. communis extract with AgNO3 aqueous solutions.

In this study, the application of silver nanoparticles as an antimicrobial agent was investigated and demonstrated that the zone of inhibition increased according to concentration of silver nanoparticles in all human pathogens Figure (5). At 2mM silver nanoparticles, the 20 mm clear inhibitory zone appeared around 100 µl against *S.* aureus, S. pneumoniae after incubation for 24 h, then 19 mm for Gram-negative bacteria *E. coli* and *P. volgaris* followed by K. pneumonia, P. aeruginosa

(17 mm). However, the effect was more pronounced against Gram-positive bacteria than Gram-negative bacteria. Thus, silver nanoparticles could be considered as excellent broad-spectrum antibacterial agents. More importantly, the silver nanoparticles produced by M. communis also exhibited potent antifungal activity against C. albicans. Since the biosynthesized silver nanoparticles showed considerable antifungal activity, they could be potential to be widely used in clinical applications [31]. This observation is in agreement with earlier studies [9, 21, 32].

The mechanism of inhibitory action of silver nanoparticles on microorganisms, though not very clearly understood, could be by their adhesion to the cell membrane and further penetration inside or by interaction with phosphorus containing compounds like DNA disturbing the replication process or preferably by their attack on the respiratory chain. It has also been suggested that a strong reaction takes place between the silver ions and thiol groups of vital enzymes, such as NADH dehydrogenase II in the respiratory system, which is implicated as a candidate for the site of production of reactive oxygen species in vivo [33]. Therefore, inhibition of this enzyme results in an increase in the free radical production. The increase in catalase production in the presence of ROS could be explained by the necessity for cells to reduce the concentration of H_2O_2, which is the source of the free radicals. It is proposed that reactive oxygen species can induce apoptotic pathways in bacteria which could ultimately lead to their death [33, 34].

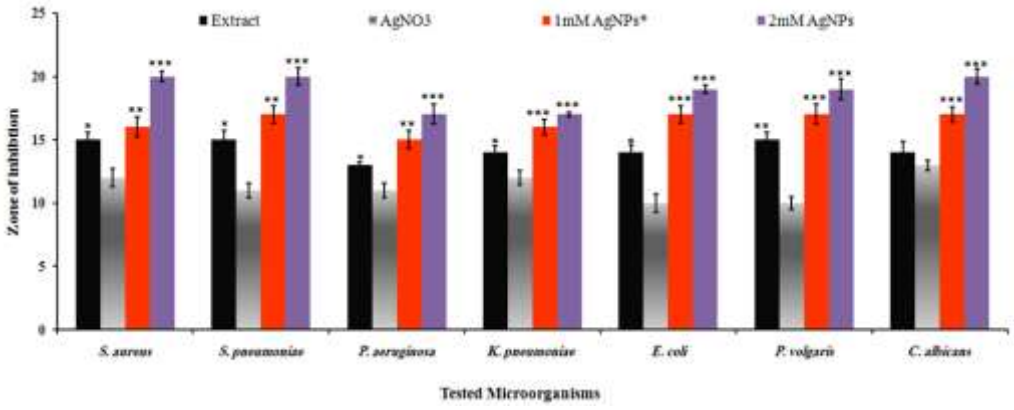

Figure (5) Size of the inhibition zone for silver nanoparticles synthesized by treating M. communis extract against the tested microorganisms. AgNPs = Silver nanoparticles, * p<0.05; ** p<0.01; *** p<0.001.

CONCLUSIONS

In conclusion, the leaves extract of M. communis are capable of synthesize silver nanoparticles has been demonstrated. It is rapid biological synthesis, environmental friendly, simple and efficient route for synthesis of nanoparticles. These obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost effective,

compatibility for medical and pharmaceutical applications. The biosynthesized silver nanoparticles showed excellent antimicrobial activity. Antimicrobial activity concluded that the synthesized nanoparticle more effective for gram positive bacteria when compared with gram negative bacteria. The results of synthesized nanoparticle leads towards the clinical use as antimicrobial agent.

Conflict of interest statement

We declare that we have no conflict of interest.

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