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SYNTHESIS OF SILVER NANOPARTICLES FROM PERICARP OF *ZIZIPHUS JUJUBA* MILL. AND THEIR *IN VITRO* ANTIMICROBIAL ACTIVITY

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Abstract

Silver nanoparticles (AgNPs) used in this study were prepared from *Ziziphus jujuba* Mill. aqueous pericarp extract. Potent antibacterial activity was recorded from AgNPs–*Z. jujuba* pericarp against *P. Pseudomonas aeruginosa* > *K. Klebsiella pneumoniae* > *M. Micrococcus luteus* > *S. Staphylococcus aureus* > *P. Proteus mirabilis* > *S. Shigella flexneri*. Less antifungal activity was found against phytopathogenic fungi with inhibition efficacy of *P. Penicillium expansum* > *M. Macrophomina phaseolina* > *A. Alternaria alternata* > *D. Drechslera halodes*, *F. Fusarium oxysporum* f. sp. *lycopersici*. UV-Vis spectral analysis of formed AgNPs– *Z. jujuba* was recorded at 450 nm and three characteristic diffraction peaks observed at 2θ =38.235, 43.95 and 64.42. FTIR patterns were located in the region of 4000–400 cm^{-1} and AgNPs particle size were noticed in between 20 and 71 nm with spherical shape embedded in bio-materials. These findings indicate that AgNPs–*Z. jujuba* pericarp aqueous extract could be utilized to control various bacterial infections to human and as well as against phytopathogenic fungi.

Introduction

Ziziphus jujuba Mill. belonging to Rhamnaceae is a spiny plant and distributed in many countries all over the world (Sun *et al.* 2011). The most common synonyms are: *Z. celata*, *Z. lotus* (L.) Lam., *Z. mauritiana* Lam., *Z. nummularia* (Burm.f.), *Z. obtusifolia*, *Z. parryi* Torr, *Z. reticulata* (Vahl) DC., *Z. rignonii* and *Z. taylorii* (Britton). It is a deciduous tree that can grow very well in the Mediterranean climate and can withstand in hot and in arid environmental conditions. The Gulf countries had a lot of *Z. jujuba* which gives shade to different types of livestock during the summer. It bears fruits of various shapes and sizes that are of great medicinal and nutritional values (Golmohammadi 2013). The leaves also have medicinal properties and are usually applied in traditional medicine to treat different ailments (Khare 1995). It is well known that the dried fruits of *Z. Jujuba* are applied as anticancer, refrigerant, pectoral, sedative, stomachic, liver, tonic, styptic and as immune response enhancer (Vahedi *et al.* 2008).

Pharmacological and phytochemical studies have shown that the principal active ingredients in *Z. jujuba* are flavonoid, polysaccharide and triterpenic acid that account for jujuba's antioxidant impact (Li *et al.* 2011). In addition, jujube polysaccharides have been suggested as the key active

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ingredients leading to their immune-modulating and hematopoietic roles. Triterpenic acids have been considered to be active ingredients as anti-inflammatory and anti-cancer agents (Tahergorabi *et al.* 2015). Jujuboside B and betulinic acid may also be active ingredients that have protective effects on the cardiovascular system (Seo *et al.* 2013).

Interestingly, as silver ions are easily transformed metalic silver nanoparticles (AgNPs) using biomimetic and biological techniques of synthesis, their toxicity is found to decrease while their antimicrobial potency are greatly enhanced (Jain and Mehata 2017, Moustafa *et al.* 2021b). These features make AgNPs excellent tools for controlling various microbial diseases especially as their selectivity against pathogenic cells has been demonstrated and no case of antimicrobial resistance has been recorded so far (Negm *et al.* 2020). Plant products had been applied to be used as reducing and capping agents which have the features for the amalgamation of metal ions into nanoparticles (Makarov *et al.* 2014).

Thus, the present study was aimed to biosynthesizing of AgNPs using the pericarp aqueous extracts of *Z. jujuba* and was therefore axed on characterization of physicochemical properties of the biosynthesized nanoparticles using Scanning electron microscope (SEM), UV-Visible spectroscopy, Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). The antibacterial potentials of the greenly synthesized AgNPs were evaluated against six human pathogenic bacterial strains namely *P. aeruginosa, K. pneumoniae, S. flexneri, P. mirabilis, S. aureus* and *M. luteus*. As well, antifungal activity had been examined against six plant pathogenic fungal strains namely, *A. alternata, P. expansum, D. halodes, F. oxysporum* f. sp. *lycopersici* and *M. phaseolina*.

Materials and Methods

Fruits of *Z. jujuba* were collected from special market for fruit and vegetable in city Abha, Asir region, Saudi Arabia. The fruits were cleaned very thoroughly using distilled water (DW) and the seeds were separated from the pericarp and then air-dried for three hours. Fifteen grams from pericarp of the fruit was crushed into fine particles using mortar and pestle. Silver nitrate (99.7 %) was purchased from Fisher Scientific (Leicestershire, UK). All bacterial and fungal strains were obtained from the Biology Department, Faculty of Science, King Khalid University, Saudi Arabia.

$AgNO_3$ powder was dissolved in deionized water to prepare 60 mM $AgNO_3$ solution. The $AgNO_3$ solutions were mixed with 1 g from prepared *Z. jujuba* pericarp to a volume of 10 ml in a flask. The flask was heated in a water bath at 70°C for 3 min. Furthermore, the solution was kept in the refrigerator (-4°C) for the antibacterial and antifungal activities examinations and additional analyzed by using UV-Vis spectrophotometer, SEM, XRD and FITR.

All equipment and media were sterilized for 30 min by autoclaving at 115°C and 15 psi. The antibacterial and antifungal activities were investigated against various pathogenic bacterial to human and fungal strains to plant. The antimicrobial activity was evaluated by well diffusion method. Preparations of the bacteria and fungi stocks were done to rejuvenate the tested microbial strains. To rejuvenate various bacterial strains, one inoculation loop of pure culture of *P. aeruginosa, K. pneumoniae, S. flexneri, P. mirabilis, S. aureus* and *M. luteus* was inoculated into 5 ml of nutrient agar solution and then incubated at 37°C for 48 hrs. All bacterial strains were vortexed and their turbidity was adjusted to 0.5 McFarland solution turbidity ~10^8 CFU/mL using the cultured bacteria . *A. alternata***,** *P. expansum, D. halodes, F. oxysporum* f. sp. *lycopersici* and *M. phaseolina* were cultured at 30°C on potato dextrose agar (PDA) (OXOID, Hampshire, England). Spores of fungal strains were collected from agar plate cultures after 7 days. The suspension concentration of each fungal strains was estimated using a cell count chamber and

adjusted to 2×10^6 spores/ml. Fungal spore suspensions had been kept in 20% glycerol at 4°C for further use.

Sterilized 20 ml of nutrient agar solution and 20 ml of potato dextrose agar (PDA) for bacterial strains and fungal strains, respectively were poured into a Petri dish and kept for 15 min until the solidification of agar solution. Bacterial and fungal strain (0.1 ml) was applied separately to the agar growing medium. Holes of 6-mm diameter were punched in the solidified media and filled with 150 µl of the previously prepared AgNPs of extracts of *Z. jujuba* (Moustafa *et al.* 2013). Dimethyl sulfoxide (DMSO*)* at 20% solution was used as negative control, Nystatin (10 µg disc) was used as a positive control for fungal strains and Cefoxitin (30 µg disc) for bacterial strains. Samples of 3 replicates from each were placed, next it was incubated at 37°C for 48 hrs and for 7 days for fungal strains before the clear zone diameter was measured using a metric millimeter (mm).

The UV-Vis spectrum of the reaction medium was applied to investigate the reduction of pure Ag^+ ions. UV-Vis spectral investigation analysis was accomplished by using (HITACHI, Model U-2800 spectrophotometer) at the wavelength of 300–600 nm. Also, changing in the color reaction mixture had been recognized through visual observation by the addition the metal ion solution to the *Z. jujuba* extract. Morphology images of sample containing Ag nanoparticles were studied via scanning electron microscopy (SEM) (JSM-7500 F; JOEL-Japan. The scale of AgNPs, SMILEVIEW software attached to the SEM device was used. The FTIR spectrum was recorded using the FTIR diamond ATR platform spectrometer (Agilent, Cary 630, USA) over the range of 400-4000 cm^{-1}. Phase identification, grain size and the crystalline nature had been done by XRD (Shimadzu, 6000 Diffractometer, Japan) which was controlled at 30 mA and 40 kV by using Cu Kα radiations with 1.54 A^0. The average crystallite size corresponding to the observed peaks was calculated from the Debye–Scherrer relation (Cullity 1978), $D = (0.9\ \lambda)/(\beta\cos\theta)$, (D, λ and β are the average size of the crystals, the wavelength of radiation and the full width at half the maximum height (FWHM), respectively. θ is the position of the maximum diffraction peak.

By using IBM SPSS statistics software, the statistical analysis of the results was carried out for variance analysis (One way – ANOVA, post hoc analysis using Tukey's test).

Results and Discussion

The present study revealed that NPs from *Z. jujuba* pericarp extract had potent antibacterial activity against *P. aeruginosa*, *K. pneumoniae*, *S. flexneri*, *P. mirabilis*, *S. aureus* and *M. luteus* (Fig. 1A). *P. aeruginosa* isolate showed the highest susceptibility to the AgNPs-*Z. jujuba* extract with inhibition zone (3.25 ± 0.14cm) followed by *K. pneumoniae* (3.10 ± 0.26 cm) and *M. luteus* (3.06 ± 0.25 cm). *S. aureus*, *P. mirabilis* and *S. flexneri* showed less level of susceptibility with inhibition zone (2.90 ± 0.20 cm), (2.73 ± 0.35 cm) and (2.67± 0.05cm), respectively. In contrast, AgNPs-*Z. jujuba* pericarp showed less antifungal activity against *A. alternata*, *P. expansum*, *D. halodes*, *F. oxysporum* f. sp. *lycopersici* and *M. Phaseolina* compared with the standard drugs (Fig. 1B). *P. expansum* isolate showed the maximum susceptibility to the AgNPs- *Z. jujuba* extract by (1.51± 0.16 cm), whereas *A. alternata*, *D. halodes*, *F. oxysporum* f. sp. *lycopersici* and *M. Phaseolina* having inhibition zone between 1.37 ± 0.08 cm and 1.23 ± 0.13 cm. No inhibition activities were observed against any of bacterial and fungal strains for dimethyl sulfoxide (DMSO) when it is used as a negative control. Positive control either against bacterial strains (Cefoxitin, 30 µg-disc) or fungal strains (Nystatin, 10µg-disc) had variable inhibition activities. From these results, it is obvious that Nystatin had potent inhibition zone than AgNPs- *Z. jujuba* pericarp. Nystatin inhibition zone found to be more than AgNPs-*Z. jujuba* pericarp by 47.43% against *F. oxysporum* f. sp. *lycopersici*, 44.45% against *D. halodes* and 42.38% against *M. Phaseolina*. *A.*

alternata and *P. expansum* had less susceptibility from NPs-*Z. jujuba* pericarp than Nystatin by 36.32 and 33.14% respectively. *Vise-versa*, Cefoxitin, (30 µg-disc) had less inhibition zone than AgNPs -*Z. jujuba* pericarp against all bacterial strain except for *S. flexneri*. Cefoxitin (30µg-disc) showed inhibition zone activities less than AgNPs-*Z. jujuba* fruits by 22.37% against *K. pneumoniae*, 19.71% against *P. aeruginosa* and 11.38% against *M. luteus*. The least differences between AgNPs-*Z. jujuba* pericarp inhibition activities and Cefoxitin was found against *S. aureus* and *P. mirabilis* by 7.80 and 0.74%, respectively.

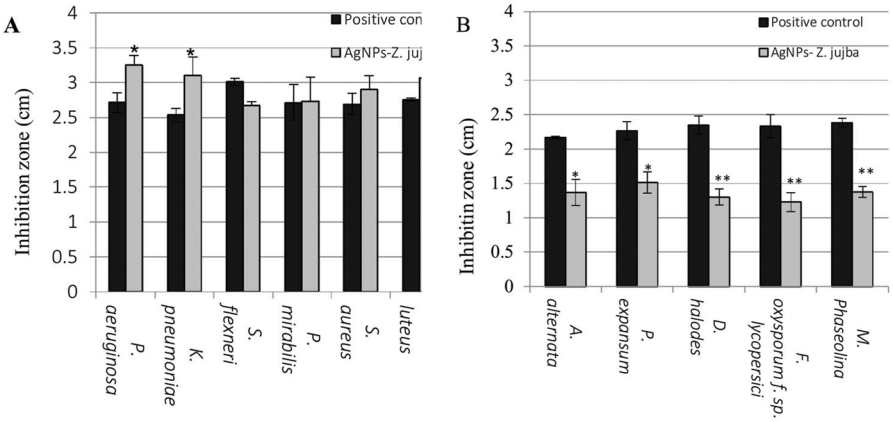

Fig. 1. Panel A; Antibacterial activities of AgNPs-*Z. jujuba* pericarp extract. Panel B; Antifungal activities of AgNPs-*Z. jujuba* pericarp extract. $^{*}P < 0.05$, $^{**}P < 0.01$.

Color of AgNPs-*Z. jujuba* pericarp had been changed from bale yellow at 60 second to bale brown at 90 sec to intense brown at 180 sec. Figure 2 showed the absorption spectrum of NP-*Z. jujuba* which is located in the range between 300 and 650 nm and the maximum absorbance peak was seen at 450 nm of the formed AgNPs.

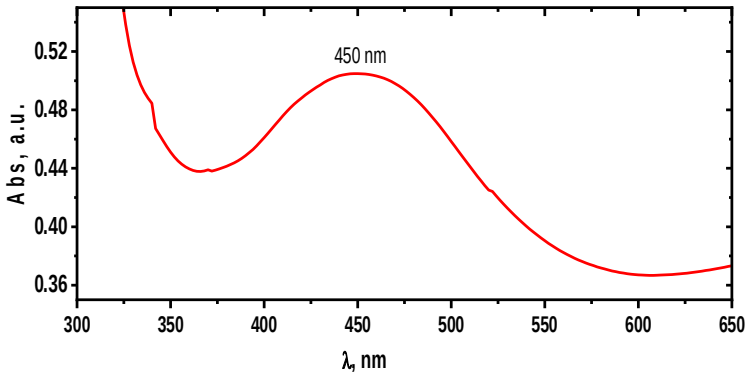

Fig. 2. Ultraviolet visible analysis of AgNPs-*Z. jujuba* pericarp extract.

Figure 3 and Table 1 showed XRD patterns for silver nanoparticles synthesized from *Z. jujuba* pericarp extract. Three characteristic diffraction peaks for Ag were observed at 2θ =38.235, 43.95 and 64.42 which correspond to the main (111), (200) and (220) diffraction planes,

respectively, of face-centered cubic (fcc) Ag NPs crystals according to Joint Committee on Powder Diffraction Standards (JCPDS File No: 04-0783) (Das *et al.* 2013). The average crystallite size of silver nanoparticles synthesized is found to be 70 nm.

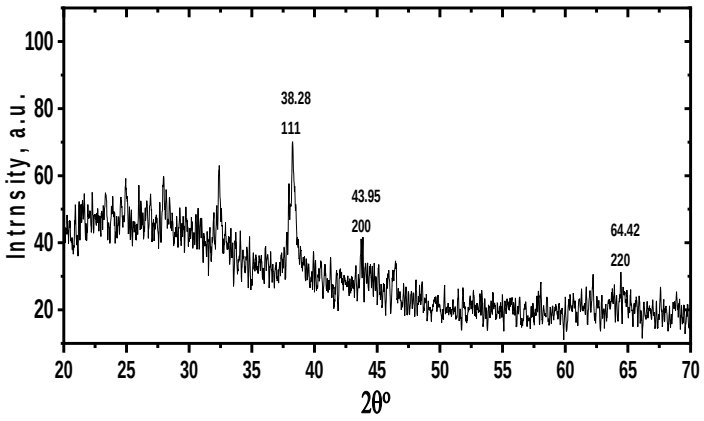

Fig. 3. XRD pattern of NPs- *Z. jujuba* pericarp extract.

Table 1. XRD data analysis of NPs-*Z. jujuba* pericarp extract.

[2theta	FHWM	Lamda (nm)	Crystallite planes	Crystallite size (nm)
38.24	0.2251	0.154056	(111)	37.37
43.95	0.1053	0.154056	(200)	81.39
64.42	0.1030	0.154056	(220)	91.19
The average Crystallite size				69.98

Figure 4 represents FTIR pattern in the region of 4000 - 400/cm. Many peaks were seen at 2922, 2860, 1602, 1362, 1023, 2089.8, 771, 513 cm^{-1} and at 475 cm^{-1}. Scanning electron microscopy (SEM) images of AgNPs via *Z. jujuba* pericarp extract revealed that it had a spherical shape imbedded in bio-materials (Fig. 5). The SMILEVIEW software attached to SEM system was used to obtain the size of AgNPs-*Z. jujuba* pericarp extract found lies between 20 and 71 nm.

It was noticed that AgNPs-*Z. jujuba* pericarp extract had a broad-spectrum antibacterial effect against all bacterial strains more than fungal strains. The obtained results proofed that no remarkable differences were found in inhibition zone between both types of tested bacterial strains either for *P. aeruginosa*, *K. pneumoniae*, *P. strains* and *S. flexneri* as a species of GNB or *M. luteus* and *S. aureus* as a species of GPB. While the components of cell wall of Gram-positive (GPB) and Gram-negative bacteria (GNB) had different pathways for adsorption of NPs (Friehe 2011). This might due to the action of synthesized AgNPs-*Z. jujuba* pericarp which could physically interact with the cell surface of both types of bacterial strains. This finding is in agreement with the previous studies (Liu *et al.* 2015). AgNPs showed a broad spectrum of activities against various microbes. It was reported that the synthesized AgNPs had a potent inhibitory effect comparable to NPs from other sources (Arora *et al.* 2018). In the present study, AgNPs produced from *Z. jujuba* pericarp extract showed antifungal activities against tested fungal

strains less than tested positive control. The reduced antifungal activity could be associated with the nature of the synthesized nanoparticles from *Z. jujuba* which might play crucial role in the degree of inhibition activity. The cell wall nature of fungi had glycoproteins and a small percentage of 1,3-glucans forming a highly dynamic shell surrounding the cell wall surface. These compounds and probably many others in the fungi cell wall may hinder the entry of specific nanoparticles into the cell. The antifungal properties of AgNPs on various phytopathogenic fungi and *Candida* sp., *in vitro* and *in vivo* had been widely documented, for example, *Aizoon canariense* (Moustafa *et al.* 2021b), *Juniperus procera* (Hassan *et al.* 2021), from soil (Moustafa *et al.* 2021a) and virgin olive oil (Negm *et al*. 2020).

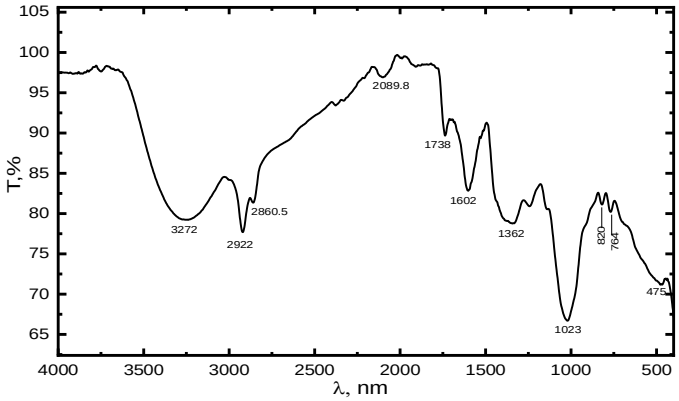

Fig. 4. Fourier-transform infrared spectrum of synthesized AgNPs- *Z. jujuba* pericarp extract.

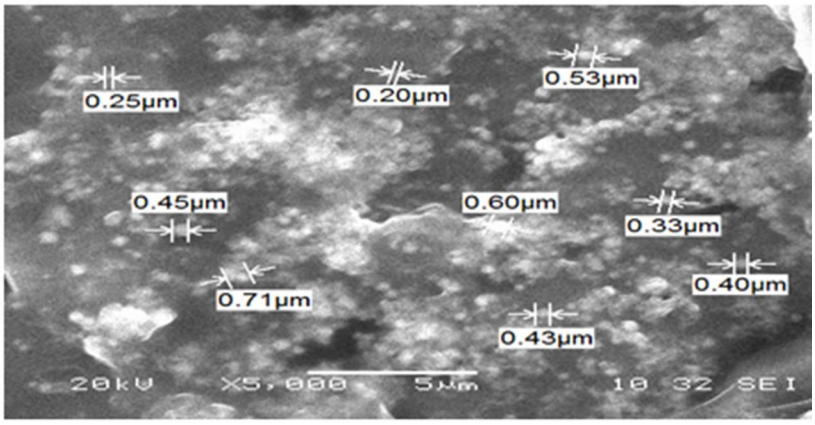

Fig. 5. SEM image of AgNPs-*Z. jujuba* pericarp extract.

The brown colour of AgNPs- *Z. jujuba* pericarp is owing to the activation of surface plasmon vibrations (Elechiguerra *et al.* 2005). A breadth absorption peak was observed at 450 nm, which proves the synthesized items of Ag (Mude *et al.* 2009, Waghmode *et al.* 2013). The absorbance band around 300 to 650 nm suggests that spherical or nearly spherical of AgNPs had been formed (Pal *et al.* 2007).

The obtained SEM micrographs showed the high density of nanostructures, confirming the presence of AgNPs, as it had a spherical shape imbedded in bio-materials. AgNPs-*Z. jujuba*

calculated by SMILEVIEW-SEM had been found to be in between 20 to 71nm and this is in agreement with the particle size calculated from XRD analysis. This confirms that plant extract is essential in production the fine AgNPs (Kouvaris *et al*. 2012). The width of XRD peaks is related to crystallite size whereas other peaks found probably due to organic impurities present in the *Z. jujuba* pericarp extract (Mickymaray 2019, Moustafa *et al*. 2021b).

FTIR spectrum showed the broad band at 3272 cm^{-1} which is due to N–H and O–H stretching mode in the linkage of the proteins. Weak intense peaks at 2922 and 2860/cm could be assigned to presence of secondary amines and C–H stretching vibrations. The strong band at 1602 and 1362/cm was mainly attributed to the amide bands, –C–N stretching vibrations of proteins. The peak located at 1023/cm can be assigned as the absorption peak of C–O stretching (Waghmode *et al*. 2013). The weak band at 2089.8 cm^{-1} is due to the C = N stretching of R – N = C = S (Mickymaray 2019). The small intense band at 1738 cm^{-1} arises from the C = O stretching mode in amine group which is commonly found in the protein , indicating the presence of proteins as capping agent for silver nanoparticles which increases the stability of the nanoparticles synthesized.

In conclusion, AgNPs-*Z. jujuba* pericarp exhibited antibacterial and antifungal activities and could be applied in biomedicine for the treatment of bacterial infections of humans and are also important for agricultural biotechnology to control phytopathogenic fungi.

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