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Synthesis, Characterization and Evaluation of Antimicrobial Potency of Silver Nanoparticles using *Ziziphus spina-christi* L. Leaf Extract

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Biosynthesis of nanoparticles is considered a new approach of research in nanotechnology that has recently gained immense interest worldwide. Biologically synthesized silver nanoparticles (AgNPs) are being fashionably widely used particularly in the medical field. In the current investigation, the green chemistry of silver nanoparticles synthesis from the aqueous solution of silver nitrate as a rapid, feasible and eco-friendly technique was described. Extracellular biosynthesis of silver nanoparticles was carried out using bio-extracts of *Ziziphus spina-christi* leaves for the reduction of aqueous silver ions in a short period. The silver nanoparticles formation was detected by the colour change of bio-extracts and further confirmed with the help of UV-VIS spectroscopy where the peak values were in the range of 415-420 nm. In this study the aqueous extract and silver nanoparticles phytosynthesized by *Ziziphus spina-christi* leaves was tested for its antibacterial activity using agar well diffusion method against bacterial species: *Pseudomonas aeruginosa, Bacillus subtili,* and *Staphyllococcus aureus*. Our results showed that, the inhibition zone ranged between (15 ± 1.08 – 20 ± 0.9 mm) for AgNPs mediated by *Ziziphus spina-christi* leavesagainst tested bacteria. Furthermore, *P.aeruginosa* displayed the highest zone of inhibition compared with others. The observed differences between the bactericidal activities of AgNPs against the tested microorganisms might be attributed to the type of bacterial species treated in this study. Our findings indicated that silver nanoparticles synthesis mediated *Ziziphus spina-christi* leaf extract has an efficient bactericidal activity against the bacterial species tested. However, the aqueous extract showed no inhibition zone. It would really be a challenging task in the future investigations to perform experiments at a molecular level to explore the unknown mechanism whereby AgNPs are biologically synthesized. Answers to such mysterious questions will open further avenues to gain deeper insight for better understanding of the potential role of bio-extract in silver nanoparticles formation.

Key words: AgNPs, bactericidal, *Ziziphus spina-christi*, nanotechnology.

Infectious diseases caused bymicro-organisms have become a critical problem constituting a real threat to the well-being of human, plants and other biological communities. The accumulated knowledge from the ever-continuous experimental research has led to application of different techniques with the purpose of combating and mitigating the harmful effects of these pathogenic microorganisms. One of the most popular techniques that has recently gained an immense research concern is the use of silver nanoparticles focusing on their antimicrobial potentialand nowadays their application is quite common in public places[1].

It is thoroughly known that chemical compounds-containing silver are extremely toxic to several bacteria. Generally, it has become well

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established that the extremity of silver toxicity to microorganisms has ranked silver ions and silver-containing compounds an excellent option for the numerous applications in medical fields[2]. The toxic property of silver led to an extensive application of silver in the form of silver nitrates in manymedical treatments to induce antimicrobial action. Silver nanoparticles are customarily synthesized by means of various physical and chemical methods. However, these methods areextremely costly and the chemicals employed in the process are toxic, which might lead to potential environmental and biological risks[3]. In consequence, synthesis of silver nanoparticles by such methods has become much less accepted and gradually abandoned. Thus, biological methods have been used as a feasible alternative for the synthesis of silver nanoparticles where bacteria, fungi and plant extracts are used as major biological systems[1].Plant extracts have been the most popularly used for synthesis of silver nanoparticles for the advantages that they are easily available, safe, and nontoxic in most cases, contain a variety of metabolites and are faster than microbes in the synthesis process[4]. In the process of extracellular biosynthesis of silver nanoparticles different plant extracts are used for the rapid reduction of aqueous silver ions leading to the formation of stable crystalline silver nanoparticles in the solution[5]. The mechanism for the process of plant-assisted reduction of silver ions to silver nanoparticles is generally considered to involve phytochemicals mainly terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids as the key players[6]. Therefore, the biological method of silver synthesis – termed "*green synthesis*" is described as a feasible and environmentally-friendly technique that can be applied in a relatively short time period[5,7]. More important is the fact that green synthesis is compatible for pharmaceutical and biomedical applications since no toxic chemicals are involved in the synthesis process[8,9]. Such green synthesis of nanoparticles is considered an emerging branch of nanotechnology[10] and currently constitutes a matter of serious concern. However, the major drawback of silver nanoparticles is the nontoxicity of silver[1]. In this respect, previous studies have demonstrated the difficulty of complete removal of silver ions deposited in human and animal bodies; however, conversion of silver ions to silver nanoparticles has greatly reduced this risk as nanosilver can be discarded via hair, urine and faeces[11].

Research in nanotechnology has recently shown that the most distinguishing characteristics of nanoparticles are their larger surface area to volume ratio. Such increase in the surface area of nanoparticles will likely lead to dramatic increase in their surface energy and ultimately their biological effectiveness will increase substantially[12,13] particularly against bacteria even at low concentrations. However, the actual mechanism and the mode of action of silver on the microbes is still poorly known and remained rather vague and only few assumptions have tried to explain this effect. It has been speculated that silver nanoparticles when get in contact with the bacterial cell wall, they cause disturbance in permeability of the cell membrane and cellular respiration leading to cell death[3]. Formation of free radicals asa consequence of silver nanoparticles was also believed as a possible mechanism involving damage to cell membrane and ultimately leading to death of bacterial cell[14, 15]. Biochemically, it was also proposed that silver ions released from silver nanoparticles can de-activate many vital enzymes through their interaction with the thiol groups[16]. In a molecular context, it has been assumed that nanoparticles influence the DNA replication of the bacteria via its effect on the soft sulfur and phosphorus bases of the DNA resulting in death of bacterial cell[17]. These explanations partially helped in understanding how silver nanoparticles cause death to bacteria, however there is still a growing need for further research to provide information to confirm these claims and improve our knowledge on such mechanisms.

ZiziphusSpina-christi belongs to the family *Rhamnaceae* has been used in folk medicine. Plant leaves were used in medicine as an antiseptic, antifungal and anti-inflammatory agent and for healing skin diseases such as atopic dermatitis[18].and this might be attributed to the isolated flavonoides, three cyclopeptide alkaloids and four saponin glycosides from the leaves of *Ziziphus Spina-christi*[19, 20]. Furthermore, flavonoids play an important role in the reduction process for biosynthesis of AgNPs[21] which led to use of *Z. spina-christi* leaf extracts to mediate reduction of the silver ions present in the form of aqueous solution of silver nitrateand given special interest in synthesizing silver nanoparticles.

Despite the abundance of antibacterial applications employing silver nanoparticles,the biosynthesis mechanisms and themode of action are not clearly known and remain a matter of conflicting debate.The main objective of this investigation was to evaluate the ability of the bio-extractfrom *Z. spina-christi* leaf for AgNPs formation and to studyits bactericidal impact on some pathogenic bacteria. To realize that goal, inhibition zone was assessed for the treated microorganisms.

MATERIALS AND METHODS

Ziziphus spina-christi L. leaves were collected from Riyadh, Saudi Arabia. Silver nitrate ($AgNO_3$) was purchased from MerckCompany (Darmstadt, Germany). Mueller-Hinton agar, Mueller-Hinton broth, and nutrient broth were purchased from Wateenalhyaa company (Riyadh, Saudi Arabia) for the antibacterial assays.

Synthesis of silver nanoparticles (AgNPs)

The aqueous extract of *Z. spina-christi* leaf was prepared by mixing 10 g of the dry leaf sample with 100 ml of highly purified water. The mixture was heated for 10 minutes at 80°C to denature the enzymes in the extract. The solution was filtered through a Whatman filter paper No. 1 (pore size 125 mm). The supernatant (filtrate) was further filtered through aWhatman filter paper No. 1 (pore size 25 µm) to remove the remaining plant residues. For synthesis of the AgNPs, 12 ml ofaqueous *Z. spina-christi* leaf extractas a reducing agent were mixed with 88 ml of 1 mM $AgNO_3$ solution in an Erlenmeyer flask and allowed to react at room temperature for 24 hours. Ultra-high purity water was used as a reaction medium to avoid the presence of chloride ions and also to prevent precipitation of silver chloride. The AgNPs extract was stored at 4°C until further analysis.

Characterization of AgNPs

The reduction of silver ions to AgNPs in the solution was monitored by measuring the ultraviolet-visible spectrum of the solution using a UV 2450 double-beam spectrophotometer (Shimadzu, Tokyo, Japan) operated at a resolution of 2 nm in the range from 300–600 nm.

Evaluation of the antibacterial activity of AgNPs

The antibacterial activity of the bio-synthesized AgNPs and aqueous extract of *Z. spina-christi* was determined using the standard well diffusion method. Three types of pathogenic bacteria, including Gram-negative bacteria *Pseudomonas aeruginosa* and two Gram-positive bacteria *(Staphylococcus aureus* and *Bacillus subtilis*) were tested. Pure cultures of the micro-organisms were sub-cultured on Mueller-Hinton agar. A sterile cotton swab was then used to spread the resulting suspension on the nutrient agar and allowed to dry for 10 minutes. Subsequently, four adequately spaced wells (holes) of 4 mm diameter each were made per plate at the culture agar surface using sterile metal cup borer. In each hole, 0.2 ml of each extract and control were put under aseptic conditions, kept at room temperature for one hour to allow the biosynthesized extracts to diffuse into agar medium and incubated accordingly. Distilled water was used as a reference negative control. The plates were then incubated for 24 hours at 37°C. At the end of the incubation period the zones of inhibition were measured to the nearest millimeter[22]. The inhibition zone is the area surrounding the hole with no growth of inoculated microorganisms. For confirmation of the results each test was performed in four replicate.

RESULTS AND DISCUSSION

The present study was an attempt to evaluate the capacityof *Z. spina-christi* L. leaf extractas a reducing and stabilizing agent forsilver nanoparticles formation.A further goal from this study was to evaluate the antimicrobial efficacy of the biosynthesized silver nanoparticlesagainst some Gram-positive and Gram-negative bacteria. The bio-materials were chosen for this study on the basis of their rich contents of secondary metabolites since the reduction of silver to nano size is accomplished by the secondary metabolites present in the biomaterials[23].Former investigations on lichensrevealed thatthe content ofphenolic compounds have been associated with anti-oxidative action in biological systems mainly due to their redox properties which can play an important role in absorbing and neutralizing free radicals, quenching single and triplet oxygen or decomposing peroxides[24]. Flavonoids play an important role in the reduction process for biosynthesis of AgNPs[21]. Consequently, thehigh content of phenolic compounds in *Z. spina-christi*

Table 1. Inhibition zone diameters (mm) of bacteria treated with silver nanoparticles synthesized by Z.spina-christi, L. leafextract and aqueous extract

Plant extract	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Staphyllococcus aureus*
AgNPs	17 ± 0.71	21 ± 0.9	15 ± 1.08
Aqueous extract	0.0	0.0	0.0

leaf extract supports the potential bio-reduction of Ag^{+} to Ag^{o}. Nanoparticles are generally considered as particles with a size of up to 100 nm, exhibit completely new or improved properties as compared to the larger particles of the bulk material that they are made up of [25].

The present findings have clearly indicated that silver nanoparticles were successfully synthesized by*Z. spina-christi* leaf extract according to the observed visible dark colour in the treated silver nitrate solution with the biomaterial. Since colour change of the solution from transparent to brown was an indication to production of silver nanoparticles[26,27], the brown colourationraised from the excitation of surface plasmon vibration (SPR) with the silver nanoparticles[28].Bio-reduction of silver ions to AgNPsin this study was also monitored spectrophotometrically with the absorbance wavelength (430 nm) for the biosynthesized material providing a fairly convenient spectroscopic signature for the formation of silver nanoparticles[29].Furthermore, the antibacterial activity of the bioreduced silver nanoparticles and aqueous extract of *Z. spina-christi* leaves in this study were investigated against some bacterial strains, *Staphylococcus aureus* and *Bacillus subtilis* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative) by zone of inhibition using the standard agar well diffusion method. The biosynthesized nanoparticles by *Z. spina-christi*leaf extract displayed inhibition zone against all the bacterial species investigated as follows: *Bacillus subtilis* (17 ± 0.71 mm),*Pseudomonas aeruginosa*(21 ± 0.9 mm), and*S.aureus*(15 ± 1.08 mm)as shownin table 1. Different responsesto the AgNPsof bacterial species were observed dueto differences in the environmental conditions and genetic variations, interestingly,it was observed that Gram-negative bacteria (*Pseudomonas aeruginosa*) had the highest inhibition zones relative to the other treated bacteria when AgNPs prepared by *Z. spina-christi*leafextract was applied. It seems that our findings are in good conformity withprevious findings which showed that the antibacterial effects of silver nanoparticles may be associated with the characteristics of certain bacterial species[13,15]. Early observations also have shown that silver nanoparticles have relatively higher antibacterial activity against Gram-negative bacteria than Gram-positive bacteria, which might be attributed to the thinner peptidoglycan layer and the presence of beta barrel proteins called porins[3].It would be a great challenging task for the future research to elucidate the mechanisms underlying plant-mediated synthesis ofAgNPs.On the other hand, the aqueous extract of *Z. spina-christi* leaves was tested against the three bacterial species mentioned (Table 1). Our results showed no inhibition zone. Same observation was also reported by[30,31]that an aqueous extract of *Z. spina-christi*leaves showed an inhibition zone against *Staphylococcus aureus*only when high concentration of the extract was applied (200mg/ml). Recent investigations[32]had contradictory results when he studied the effect of aqueous extract of *Z. spina-christi* leaves on *Staphylococcus aureus*, but same observation was clear when testing*Pseudomonas aeruginosa*. Differences might be due to the different extraction conditions.

CONCLUSIONS

In the light of the current findings it might be concluded that synthesis of silver nanoparticles mediated by leaf extracts of *Z. spina-christi* has proven to be promising and showed great antibactericidal potency. Since silver nanoparticles might also cause toxicity at varying levels, therefore to avoid this downside of silver nanoparticles application care should be takento finely regulate and optimally maintain the adequately required concentrations for safe use. Hence, with careful

handling of this nanotechnology, silver nanoparticles can be a good friend for the betterment of human life and the environment at large. The antimicrobial screening demonstrated that the synthe-sized AgNPs had a high inhibitory effect on bacteria. These observations may serve as a guide for studying the controlled release of these synthesized AgNPs, which has potential in the field of infectious diseases. These AgNPs may be explored as an option for decreasing the pathogenic potential of infectious bacterial species.

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