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Research Article
Pharmaceutical Properties of Synthesized Silver Nanoparticles from Aqueous Extract of *Solanum incanum* L. Fruits against Some Human Pathogenic Microbes

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Abstract

Background and Objective: Recently researchers are using bionanotechnology techniques as environmentally safe and cost-less means for processing nanoparticles. This study is designated to document characters of silver nanoparticles synthesized from cold and hot water fruits extract of *Solanum incanum* and investigating their antimicrobial activity. **Materials and Methods:** Silver nanoparticles formation from cold and hot water fruits extract of *Solanum incanum* was characterized by UV-Vis spectral analysis, X-Ray diffraction and scanning electron microscopy. Nanoparticles (NPs) antimicrobial activity had been investigated through Well-diffusion methods. **Results:** UV-Vis spectroscopy showed broad absorption peaks located at 428.66 nm for NPs of cold water extract and 445.73 nm for hot water extract of *S. incanum* fruits. NPs of cold water extract showed some agglomerated nanoparticle in the form of nanoclusters, while hot water extract has a spherical shape of a very low dimension. Antimicrobial assay confirmed the bactericidal and candidacidal activity of biosynthesized AgNPs toward Gram-negative bacteria, Gram-positive and *Candida albicans. S. aureus* is the most sensitive microorganism to the activity of NPs from cold and hot aqueous extracts as the percent differences found to be (21.81%), followed by *S. flexneri* (18.74%), *M. luteus* by (11.69%) and *C. albican* by (11.60%). On the other side, percentage differences between NPs from cold and hot aqueous fruit extracts found to be low on *K. oxytoca* by (0.783%), followed by *P. mirabilis* (4.515%) and *P. aeruginosa* (4.713%). **Conclusion:** There were obvious differences between the synthesized nanoparticle's cold and hot extract of *Solanum incanum* fruits with remarkable antimicrobial properties.

Key words: *Solanum incanum* L., aqueous extract, antimicrobial, NPs, scanning electron microscope (SEM), x-ray diffraction (XRD), UV-Vis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The nanoscale stuff has new, unique and superior chemical and physical properties particularly in comparison to its bulk structure due to an increase in the surface area ratio per material volume/particle size[1]. Metal nanoparticles are the most often studied nanoparticle as they are very simple to synthesize. Besides, such materials have a broad variety of applications, for example, surface coating agents, detectors, catalysts and antimicrobials, etc. Among the most reported metallic nanoparticles are silver (Ag)[2,3], gold (Au)[4] platinum (Pt)[5,6] and palladium (Pd)[7]. Ag nanoparticles are chemically more reactive in their bulk than Ag itself, therefore suggested having greater antimicrobial capabilities[2,3,8-10]. On the other hand, the literature showed that medicinal plants have secondary substances that are of great benefit to human life in terms of functioning as antioxidants, anti-inflammatory, modulating detoxification enzymes, stimulating the immune system, anticancer and in treatment of various types of Diabetes[11-13]. Study results also support the concept that several plants are used in the treatment of different diseases, the symptoms of which may include microbial infection leading to the discovery of novel bioactive compounds[14-16]. Some botanical families, like Solanaceae, have had their therapeutic effects mentioned for a long time. It consists of 2800-3000 herb species belonged to about 85-90 genera of a few trees, shrubs and herbs[17]; globally, occurring around the world, except in the Arctic zone. Freire Allemao, in Medical Gazette of Rio de Janeiro[18], clarified the term *Solanum* derives from the Italian term *Solari*, which implies "relieving". Active phytochemical constituents found in *Solanaceae* are protoalkaloids; glycoalkaloids tropane, cardenolides; capsaicinoids; pyrrolidine pyrrolic alkaloids; nicotine; steroid glycosides; with asteroids, physalins and withanolides, etc.[19]. *Solanum incanum* is one of the most frequently used plants in many cultures[20]. In Africa, it can be used for the treatment of angina, dandruff, fever, colic or indigestion, general infection, liver pain, painful menstruation, skin diseases, snake bites, headache, sore throat, stomach or wounds and abdominal pain. Medications are provided by consuming root, leaf and fruit decoctions either by chewing or swallowing sap, washing sore areas with leaf sap and topical application of ash combined with fat. Seeds and fruits of the plant are usually applied in curdling milk and in cheese production[21,22]. The plant is a rich source by a lot of bioactive compounds used to combat pests and predators, as well as many human and animal diseases[14,21,23]. Fruits and leaves of *S. incanum* have been found to be the source of many classes of phytochemicals, including flavonoids, bioflavonoid, terpenes, xanthenes, tannins, saponins, cyanates, oxalate, anthraquinones, glycoalkaloids, steroid glycosides and minerals such as K and Ca as well in the form of solasonine and solanine[23-26]. Nowadays the utilization of plants for the biosynthesis of Ag nanoparticles involves the content of secondary chemicals as reducing agents is of great interest as eco-friendly and cost less and has less or no side effect than chemicals[27-29].

Therefore the present research aimed to synthesize and comparing physical properties of Ag nanoparticles by applying cold and hot fresh aqueous extracts of *S. incanum* and then evaluated its antimicrobial inhibition activity against the growth of *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella flexneri, Klebsiella oxytoca*, *Micrococcus luteus* and *Candida albicans.*

MATERIALS AND METHODS

Study area: This research project was conducted from July 2019-2020 at the Department of Biology, Faculty of Science and Kingdom of Saudi Arabia.

Sample collections and extract preparations: Ripened fruits of *S. incanum* applied in this study were collected from the abandoned area in Abha city, Asir region, KSA during the growing season in June 2019 and the experimental research conducted in the Department of Biology, Faculty of Science, KSA. A total 30 g from fresh fruits were washed thoroughly using Distilled Water (DW) and ground using mortar and pestle by mixing with 15 mL of deionized water until it became a suspension. A total 15 mL was stored in the neatly labeled airtight plastic container until further use. Another 15 mL was boiled in a test tube in 100°C for 5 min, cooled, labeled and stored until further use.

Acquisition of microbial strains: The microbial pathogens viz., *P. mirabilis*, *P. aeruginosa, S. aureus*, *S. flexneri*, *K. oxytoca, M. luteus* and *C. albicans* that used in this research were procured from the Microbiology Laboratory, Faculty of Medicine, at King Khalid University, Kingdom of Saudi Arabia.

Preparation of nutrient media: Various microbial colonies were inoculated in liquid nutrient broths and incubated in 150 rpm shaking incubator at 39°C overnight before inoculation. Each broth culture was then adjusted to match McFarland's 0.50 turbidity standard to approximately[20] 1×10^8 CFU mL^{-1}. Mueller–Hinton (M-H) media were prepared according to procedures defined by its supplier as a growth medium for Agar well diffusion method.

Agar well-diffusion test: Susceptibility test for various microbial strains was accomplished using agar well diffusion method as described previously[30]. Plates containing M-H agar media have been prepared and kept for 12 hrs at room temperature to make sure that the media are free from contamination. Total of 100 µL of standardizing inoculum was evenly smeared over M-H agar media with a sterile swab moistened with the bacterial suspension. The wells (6 mm in diameter) were punched using sterile cork borer and filled with 100 µL of NPs extracts and ensured diffusion at 24°C for 3 hrs. Each plate was then incubated in an upright position at 37°C for 48 hrs. Wells containing the same amount of Dimethyl sulfoxide (DMSO) (10%) have been applied as negative controls and cefoxitin (30 mcg) was used as the positive control. Each plate was examined after incubation and the inhibition zone diameters were determined using a millimeter ruler. Microbial cultures with an inhibition region equal to or more than 7 mm of diameter are considered to be susceptible to NPs extracts [31]. For each sample, three replicates were performed against each of the test microbes. The data were expressed as a mean±standard deviation.

AgNPs form hot and cold extract of *S. incanum* fruits: The stable AgNPs were synthesized by treating 9 mL aqueous solution of $AgNO_3$ (0.5 mM) with 1 mL filtered fruit extracts of *S. incanum*, then heated at temperature (70°C) for 3 min.

Characterization of Ag nanoparticles: The change in color of the reaction mixture having (metal ion solution + *S. incanum* (cold and hot extract)) was reported by visual observation. The reduction of pure Ag^+ ions was tracked by calculating the UV-Vis spectrum of the reaction medium.

UV-Vis spectral: UV-Vis spectral study was performed in the range between 300 and 600 nm using a double-beam spectrophotometer (Hitachi, U-3010) whereas all samples are dispersed in Distilled Water (DW) and held in a quartz cuvette with a length of 10 mm[32].

XRD and SEM: Prepared nanoparticles have been collected by centrifugation at 10,000 rpm for 15 min and then processed according to Jemal *et al.*[33]. The crystalline nature, phase identification and grain size had been achieved by XRD (Shimadzu, 6000 Diffractometer, Japan) which was operated at 40 kV and 30 mA using Cu Kα radiations with 1.54 A^0. AgNPs absorbance spectra were recorded by Jasco V-670 UV-Vis spectrophotometer (Japan) adjusted at 350-600 nm during all reactions. The AgNPs morphology images were studied via scanning electron microscopy (SEM) (JSM-7500 F; JOEL-Japan).

Statistical analysis: One-way Analysis of Variance (ANOVA) was applied to determine statistically significant differences among NPs fruits extracts of *S. incanum* concerning positive control. A post hoc analysis test LSD (least significant difference) was functioned to test the difference means against slandered control ($p = 0.05$ or 0.01).

RESULTS AND DISCUSSION

Antimicrobial activity study: In this study, the analyzed effect of cold and hot NPs fruit extracts of *S. incanum* showed potent inhibition activity against all microbial strains which were characterized by a clear zone around the wells (Fig. 1). It showed potent antimicrobial activities in case NPs of hot water extract than NPs of cold water extract against all tested microbial strains. In the case of *S. aureus*, an increase in the diameter of the inhibitory zone from (21.70±0.26 mm) of cold water extracts to be (26.43±0.35 mm) for NPs of hot water extract. *M. luteus*, *K. oxytoca*, *S. flexneri*, *P. mirabilis* and *P. aeruginosa* were inhibited from NPs cold water extract by (25.67±0.30 mm), (29.76±0.23 mm), (21.16±0.20 mm), (20.00±0.12 mm) and (26.2±0.20 mm) respectively. This inhibition zone increased in case of NPs hot water extract to be for *K. oxytoca* (30.00±0.10 mm); *S. flexneri* by (25.13±0.34 mm), *P. aeruginosa* (27.40±0.20 mm), *M. luteus* (28.67±0.15 mm) and *P. mirabilis* (26.23±0.13 mm). NPs fruit extracts of *S. incanum* also showed potent inhibition zone against *C. albican* in the range between (30.2±0.17 mm) from NPs cold water extract and (33.7±0.21 mm) from NPs hot water extract. From these results, it is obvious that *S. aureus* is the most sensitive micro-organism among other microbes to the effect of hot and cold water NPs fruits extract as the differences in inhibition activities found (21.81%), followed by *S. flexneri* (18.74%), *M. luteus* by (11.69%) and *C. albican* by (11.60%). Vice versa, the differences between hot and cold water NPs fruits extract found to have a very low impact on the inhibition activities of *K. oxytoca* (0.783%) followed by *P. mirabilis* (4.515%) and *P. aeruginosa* (4.713%). No inhibition activity against any microbial strain was observed when dimethyl sulfoxide (DMSO) was used as a negative control, while positive control had an inhibition zone against all microbes tested in the range between (29.87±0.34-24.60±0.03 mm). The antimicrobial activity gained from Ag nanoparticles synthesized from aqueous extract of *S. incanum*

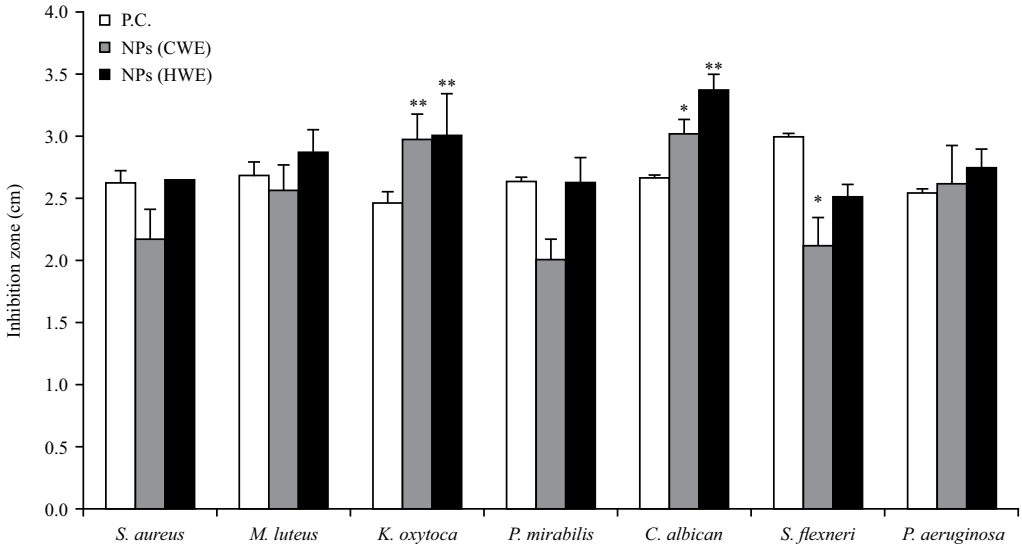

Fig. 1: Antimicrobial activity of nanoparticle of cold and hot water fruits extract of *S. incanum* against *S. aureus*, *M. luteus*, *K. oxytoca*, *P. mirabilis*, *C. albican, S. flexneri* and *P. aeruginosa*

PC: Positive control; NPs (CWE) nanoparticles of cold water extract; NPs (HWE) nanoparticles of hot water extract; significant differences (*p≤0.05, **p≤0.01), between treatments±SD of the mean for n = 3

had been characterized to have strong inhibitory activity against various microbial strains[34]. However, depending on the results of the statistical analysis, only five treatments were shown to be significantly different from positive controls. The variables were cold and hot NPs extracts from *S. incanum* against *K. oxytoca* and *C. albican* and NPs cold water extract against *S. flexneri*. Ours published data previously showed that plant ethanol extract had antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus* sp., *Acinetobacter* sp., *Klebsiella pneumonia*, *Micrococcus* sp., *Bacillus subtilis* and *Staphylococcus epidermidis* [14]. This support that *S. incanum* extracts had remarkable medicinal values either using various solvents for extraction the bioactive materials or nanoparticle synthesis using plant extracts. Inhibition zone resulted from ethanol extract against tested bacterial strains[14] is comparatively very low than the present results, supporting previous studies that the small size of Ag nanoparticles makes it easier for such particles to penetrate the outer wall of the bacteria, infiltrate the body, kill the respiratory chain and thus inhibit cell respiration and finally causing bacterial death[20,35]. The screening results indicated that AgNPs synthesized from *S. incanum* were more active against both gram-negative bacteria and gram-positive. Although, there were differences between Gram-positive and negative in many aspects[36-39]. It was stated that the binding of nanoparticles to bacteria cell wall depends on the surface area for interaction, hence either gram-positive or gram-negative inhibited severely by synthesized AgNPs. The nanoparticles have a larger surface area available for interaction that enhances the bactericidal effect for both types of bacteria than large particles[39]. Therefore, we suggest that AgNPs have more toxicity to the microorganism than the extraction by various solvent from plants. Previous findings have also shown that silver species release Ag^+ ions that interact with bacterial protein namely thiol groups which may alter or delay DNA replication[37,40].

UV-Vis spectral analysis: The aqueous extract of fresh fruits of *S. incanum* changes their colors and appeared brown when boiled for hot extraction. The cold water extract changes color from bale yellow to brown while the brown color of the boiled one changed their color again after adding the $AgNO_3$ solution to be intense dark brown after 2 min. Color changes are likely because some of the Ag ions are beginning to be reduced due to the effects of heat accompanied by plant extract and yielding Ag^+ complex. This complex was mainly responsible for color change from the pale yellow to the brown in case of cold water extraction and from brown to dark brown in case of hot water extraction. As previously reported, this change in the color proof the formation of Ag nanoparticles[41]. The synthesized Ag nanoparticles in each solution either cold or hot water were analyzed using UV-Vis spectroscopy to investigate the characteristics of the peak spectrum of the Ag nanoparticle wavelength (Fig. 2). UV-Vis spectra of synthesized AgNPs using the cold and hot aqueous extract of *S. incanum* are centered on 450-420 nm. The characteristics of Ag

wavelength nanoparticles usually appear at intervals in the range between 400-600 nm [32]. This information shows that Ag nanoparticles have been formed in both extracts and that Ag^+ has probably been reduced to Ag^0 (need more investigation). Proteins and all secondary extract metabolites play a vital role in the reduction and capping process for the formation of nanoparticles[41]. As can be seen, a broad absorption peak located at 428.66 nm and 445.73 nm for NPs of cold water (CWE) and NPs of hot water extract (HWE) of *S. incanum* respectively. These broad peaks are a characteristic peak of Ag-NPs[42], which attributed to the collective oscillation of electrons known as Surface Plasmon Resonance (SPR)[43].

XRD and SEM analysis: XRD patterns for silver nanoparticles synthesized by both types of extract shown in Fig. 3. Three characteristic diffraction peaks were observed at 2θ = 38.26, 44.44 and 64.61 for cold water extract and 2θ = 38.11, 44.17 and 64.29 for hot water extract of *S. incanum*. These Angles can be well-indexed to 111, 200 and 220 diffraction planes of face-centered cubic (fcc) Ag crystals (JCPDS00-004-0783). The width of XRD peaks is related to crystallite size. Debye-Scherrer formula was applied to calculate the mean of crystallite diameter from half-width of the diffraction peaks:

$$D = (k\lambda)/(\beta\cos\theta)$$

where, D is mean crystallite size of the powder, λ is the wavelength of Cukα , β is the full width at half-maximum, θ is the Bragg diffraction angle and k is a constant. The 111 plane was chosen to calculate the crystalline size. Using the Scherrer formula, the particle size of AgNPs was estimated and found 21.50 nm and 9.78 nm for NPs of cold water extract and hot water extract respectively as shown in (Table 1). The obtained broad peaks further indicate that the present bio-synthesized samples are in the nanoscale range. Other unsigned peaks

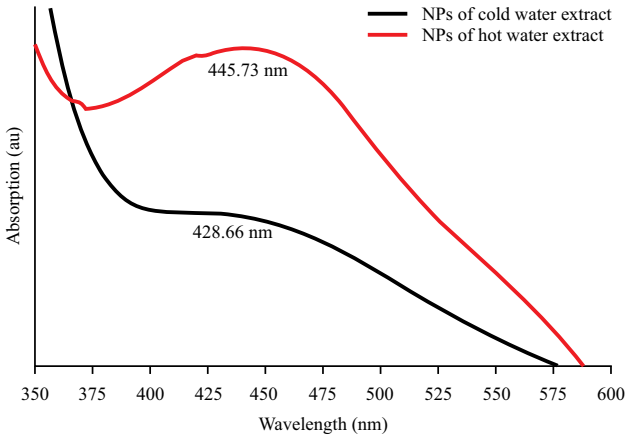

Fig. 2: The UV/Vis absorption spectra of Ag nanoparticles extracted by cold water and hot water of *S. incanum*

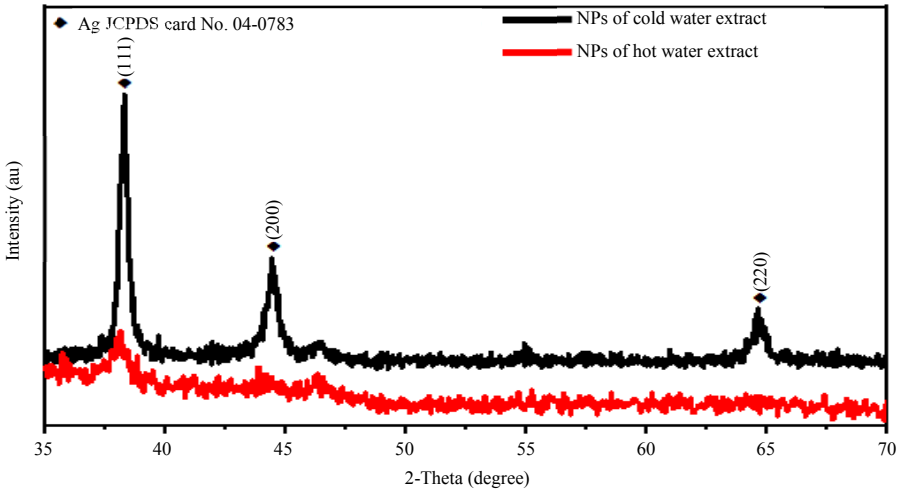

Fig. 3: X-ray diffraction pattern of Ag nanoparticles extracted by cold water extract and hot water extract of *S. incanum*

Table 1: Standard and experimental diffraction angles, FHWM, d spacing, diffraction plane, Ag particles Size from cold water extract and hot water extract of *S. incanum*

Sample	Standard diffraction angle [2θ in degrees]	Diffraction angle (degree)	FHWM in radians	Lambda	d- spacing (nm)	Diffraction plane	Particle size (nm)
NPs of (CWE)	38.116	38.26	0.0068260	0.154	0.235	111	21.50
NPs of (HWE)	38.116	38.11	0.0150022	0.154	0.236	111	9.87

NPs of (CWE): Nanoparticles of cold water extract of *S. incanum*; NPs of (HWE): Nanoparticles of hot water extract of *S. incanum*; d-Spacing: Distance between planes of atoms, FHWM: Full width at half maximum

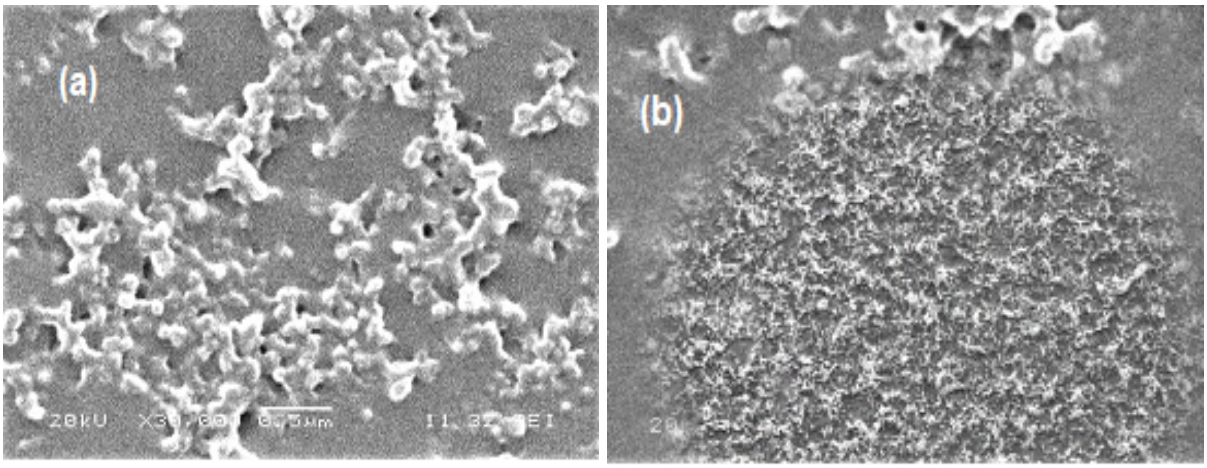

Fig. 4: SEM of NPs from cold water extract (a) and hot water extract (b) of *S. incanum*

have also been found in the XRD pattern, which can be attributed to the crystallization of bio-organic phases at the surface of the NPs[44,45]. Figure 4a-b displays the Scanning Electron Microscopy (SEM) images for the synthesized AgNPs extracted by cold water extract and hot water extract of *S. incanum* fruits respectively. Figure 4a shows some agglomerated nanoparticle in the form of nanoclusters whereas Fig. 4b shows that AgNPs are almost having a uniform spherical shape of very low dimension. The size of AgNPs recalculated from both figures using SMILEVIEW software attached with SEM system and found it has 27 nm for NPs of cold water and 15 nm for NPs of hot water of *S. incanum*. The agglomeration of AgNPs in the form of nanoclusters may be due to the effect of plant extract during synthesis. The other reason also maybe during the preparation of the sample for SEM, as the initial sample is a colloidal solution.

CONCLUSION

Based on our study, there was a difference between the physical characters of synthesizing NPs from cold and water extract of *S. incanum* fruits and their antimicrobial potency against various human pathogenic microbes. Ag nanoparticles formed from the extract were able to inhibit the growth of tested microbial stain with various degrees of potency that could be used as promising agents in pharmaceutical applications.

SIGNIFICANCE STATEMENT

This study revealed that NP_S from aqueous extracts of *S. incanum* fruits can be applied as a natural alternative to control multidrug-resistant microbes, which is potentially effective against various microbial strains whereas healthy risks of use of chemically antimicrobial agents may be avoided. In future NPs synthesized from aqueous extract *S. incanum* fruits and from other parts like stem, root and leaves should be optimized to identify, isolate and scale up the yield of a specific compound against specific microbes.

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