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Original Research Article

Phytochemical Studies and Evaluation of Silver Nanoparticles Synthesized from *Solanum elaeagnifolium* Leaves Extract for Antioxidant and Antibacterial Activities

Horace T. Lih[1], Collins O. Airemwen[1,2], Emmanuel M. Halilu[1,3]

[1]*Faculty of Pharmacy, Cyprus International University, Nicosia, Cyprus.*
[2]*Department of Pharmaceutics and Pharmaceutical Technology, Faculty of pharmacy, University of Benin, Benin-City, Nigeria*
[3]*Department of Pharmacognosy and Ethnomedicine, Faculty of Pharmaceutical sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.*

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ABSTRACT

Solanum elaeagnifolium is a medicinal plant in the Solanaceae family and the leaves extract have traditionally been used to treat several diseases, including skin infections, respiratory problems, and digestive disorders. The purpose of the study was to formulate silver nanoparticles (AgNPs) from the leaves extract of *Solanum elaeagnifolium* using the green synthesis method and to evaluate the formulated nanoparticles using scanning electron microscope (SEM) and X-ray diffraction (XRD). The powdered leaves were analyzed for macroscopic, microscopic, and physicochemical characteristics before being extracted using a Soxhlet apparatus. The antioxidant effect was analyzed using 2,2-diphenyl-1-picryl hydrazyl (DPPH). The disk diffusion method was used for assessing the antibacterial activity. Microscopic examinations confirmed the existence of stellate trichomes. Phytochemical screening, confirmed the presence of flavonoids, alkaloids, tannins, saponins, triterpenoids, steroids, and cardiac glycosides. SEM revealed a porous, spherical and crystalline structure with particle sizes of 70-95 nm. The DPPH assay revealed IC_{50} values of 3.40, 2.30 and 1.40 mg/mL for the methanolic extract, silver nanoparticles and ascorbic acid respectively. The results of the antioxidant studies revealed that the formulated silver nanoparticles had higher antioxidant activity than the leaves extract at all concentrations. The leaves extract and silver nanoparticles demonstrated significant antibacterial activity against all the bacteria used in the study with zones of inhibition ranging from 2.9-4.8 mm and 5.7-9.2 mm respectively. The formulated silver nanoparticles demonstrated significant antibacterial and antioxidant effects as a result of the presence of some secondary metabolites such as phenols, saponins, flavonoids and tannins in the leaves extract.

***Keywords*:** Antibacterial, Antioxidant, Green Synthesis, *Solanum elaeagnifolium*.

Introduction

Silver nanoparticles (AgNPs) are nano-sized particles of silver with useful applications in the pharmaceutical, medical and food industries because of their peculiar properties, morphology, size, shape, and high surface area.[1] Recently, there has been an increase in the research areas of AgNPs due to their unique and attractive chemical, physical and biological properties.[2] AgNPs are also known to have distinctive properties in terms of electrical resistance and surface plasmon resonance. Formulation of AgNPs using plant extract is the commonest method of green synthesis of nanoparticles and has several advantages because medicinal plants are abundant in nature, and they are a rich source of secondary metabolites. Due to its safety, faster onset of action, ease of use, affordability and longer duration of action, the formulation of silver nanoparticles utilizing plants extract has gained significant scientific attention.[3] Plant phytochemicals such as tannins, phenols, saponins, and flavonoids have been known to act as capping and reducing agents for synthesizing nanoparticles and also enhance the stability of the silver nanoparticles.[4,5]

*Corresponding author. E mail: acollins@ciu.edu.tr
Tel: +2348133737933

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As a result of their peculiar qualities, such as potent antimicrobial activities, chemical stability, high thermal and electrical conductivity, AgNPs are among the most significant nanoparticles receiving attention from scientists globally.[6,7] *Solanum elaeagnifolium* also known as silver leaf nightshade, is a medicinal plant in the Solanaceae family. The plant can be found in North and South America, Africa, and Asia. *Solanum elaeagnifolium Cav* has been widely studied for its ethnobotanical uses, phytochemistry, and pharmacological activities. The leaves have been used conventionally to treat several disease conditions such as peptic ulcer, hypertension, skin infections, and digestive disorders.[8] Previous studies have reported that *Solanum elaeagnifolium* leaves extract possess high amount of phenolic compounds and it is a rich source of organic antioxidants. Rajalakshmi and Pugalenthi[9] reported that *Solanum elaeagnifolium* leaves extract possess potent antibacterial activity against a number of harmful microorganisms, such as *Staphylococcus aureus* and *Escherichia coli*. Uladag *et al.*,[10] performed a review of *Solanum elaeagnifolium* traditional uses, photochemistry, and pharmacological activities highlighting the plant as a rich source of secondary metabolites, natural antioxidants, anti-inflammatory agents, and antimicrobial compounds. Goyal *et al.*,[11] also conducted a research on the photochemistry, pharmacology, and toxicology of *Solanum elaeagnifolium* and reported that the medicinal plant possessed significant anti-cancer, anti-diabetic, and neuroprotective properties. Abubakar *et al.*, [12] also carried out an in-depth analysis of *Solanum elaeagnifolium, its e*thnobotanical uses, photochemistry, and pharmacological activities and reported that leaves extracts possessed anti-inflammatory, anti-diabetic, and antinociceptive properties. Current literature review shows that there are limited studies on the green synthesis of silver nanoparticles using *Solanum elaeagnifolium leaves extract hence the aim of this study was to formulate AgNPs* of

Solanum elaeagnifolium and evaluate its phytochemical components, antioxidant and antibacterial activities.

Materials and methods

Materials

Silver nitrate (BDH Chemical Ltd, UK), DPPH, Sigma Aldrich, Germany, Methanol, (Emsure, India), ascorbic acid, hydrogen peroxide, Iodine (Ranbaxy, India). Analytical grade chemicals were used in the study.

Collection Identification and Preparation of Plant Material

Mature and uncontaminated *S. elaeagnifolium* leaves were collected in July, 2022 in Lefkosa, Cyprus (Latitude: 35.1856º N; Longitude: 33.3823 E), and the botanical identification was performed by Dr Emmanuel Halilu; a Pharmacognosist in the Faculty of Pharmacy, Cyprus International University. The collected leaves were washed with tap water to eliminate any undesirable particles and allowed to air dry in the shade. To produce smooth particles, the leaves were finely diced and air-dried once more for 7 days in the shade. A blender was used to turn it into powder and stored in an airtight container for future use. A voucher specimen was created, given the voucher number CIU/PHARM/SOLA/001, and stored as a reference in the herbarium of the Pharmacognosy Department.

Macroscopy

The odour, colour, texture, shape, size, surface properties, and taste of the dried powdered leaves were used in the microscopical identification by the procedure previously described by World Health Organization (WHO).[13]

Microscopy

The microscopic examinations of the powdered leaves were done using standard method described by WHO.[13] This was used to investigate structures such as trichomes and stomata, which provided valuable information on the type and distribution of trichomes in the plant material. The powdered leaves (0.5 g) was placed in a clean glass test tube, 1 ml of chloral hydrate was added, and the mixture was heated over a hot water bath for 5-10 min at 100°C before being removed and allowed to cool. The sample was placed on a clean slide, few drops of diluted glycerol was added, the slide was covered with the help of a slide cover, and the specimen was viewed under the microscope at an objective of 10x and a magnification of 100.

Phytochemical screening

Qualitative phytochemical screening was done using previous methods described by Halilu *et al.*, [14]. The presence of tannins, saponin, alkaloids, steroids, terpenoids, glycosides, phenols, and flavonoids were analyzed in the crude methanol leaves extracts.

Extraction of powdered leaves of Solanum elaeagifolium using a Soxhlet extractor

The powdered leaves of *Solanum elaeagifolium* Cav. were extracted using a Soxhlet extractor. Methanol (500 mL) was added to a round-bottom flask, which was then connected to an isomantle along with a Soxhlet extractor and condenser. The powdered plant material was placed inside the Soxhlet extractor in a thimble, and the side arm was cleaned with glass wool. As the solvent reached 64°C, it began to evaporate and pass through the apparatus to the condenser. The resulting condensate was observed to drip into the reservoir, which contained the thimble. As the solvent level rose in the siphon, it returned to the flask, and the process continued for 8 h. Each cycle took 3-5 min. The extract obtained was subsequently concentrated using a revolving concentrator and then dried at 50°C for 24 h using a hot air oven. The extract was kept in a tightly sealed container for further studies.[15]

Green synthesis of silver nanoparticles from methanolic Solanum elaeagifolium leaves extract

The silver nanoparticles were prepared by reacting 0.1 M silver nitrate ($AgNO_3$) solution with the *Solanum elaeagifolium* leaves extract in ratio 9:1 with constant agitation using a magnetic stirrer (Isotex, India) at 200 rpm for 12 h at 25°C. After 5 min of addition of the aqueous $AgNO_3$ to the extract, there was a change in colour from green to dark brown which indicates the bio-reduction of silver ion (Ag^+) to silver nanoparticles (AgNPs). The solution was then kept for 48 h in the dark at 24°C and was then centrifuged at 4500 rpm for 35 min and the supernatant layer was separated from the residue and dried in an oven (Beko, Turkey) at 50ºC for 12 h to obtain dry powdered particles.[5]

Characterization of the synthesized nanoparticles

UV-Visible of plant extracts and silver nanoparticles analysis

Validation of the extract was done using a UV/Visible spectrophotometer (Shimadzu, Japan). A serial dilution of each of the aqueous extracts was prepared and the wavelength of maximum absorbance was obtained by scanning over a wavelength range of 800 to 200 nm.

Fourier Transform Infra-Red Spectroscopy (FTIR)

FTIR spectrophotometer (Shimadzu, Japan) was used to determine the chemical functional groups of the formulated nanoparticles and was scanned between 4000-1000 cm^{-1}.

X-Ray Diffraction (XRD) Analysis

XRD analysis of the formulated silver nanoparticles was done using Rikagu generator (XRD Rikagu Rint 2000, Japan) at a voltage of 25kV, 20mA current intensity, 2Θ angle and 3°min^{-1} in the range of 4-50°.

Scanning Electron Microscopy (SEM)

The size and shape of the formulated nanoparticles were examined using scanning electron microscope (Quanta 200 FEG model, Japan). The sample was placed on a sample holder and gold coated before the microscopy was done.

Evaluation of the free radical scavenging activity

The antioxidant activity of the leaves extract and the nanoparticle was determined according to method previously used by Airemwen and Obarisiagbon[5] with some modifications. A 10 mg/mL stock solution was prepared using 0.1 g of nanoparticles and extract which was then diluted serially to obtain 5, 2.5, 1.25, and 0.25 mg/mL solutions. DPPH solution (0.8 mM) was prepared using methanol and then added to the test tubes containing the samples and incubated for 30 min in the dark. The absorbance of each samples was measured at 480 nm using UV spectrophotometer (Shimadzu, Japan).

Antibacterial activity

The agar disk diffusion method was used for the antibacterial study as previously described by Halilu *et al.*,[16]. The zones of inhibition which were indications of anti-bacterial activity were measured in millimeters using a transparent ruler and the results were compared to the controls.

Data analysis

All tests were done in triplicate, and the mean and standard deviations (SD) were determined using Microsoft excel (2019 version, USA). The results were expressed as mean±SD.

Results and Discussion

Macroscopic examination of Solanum elaeagnifolium

Organoleptic evaluation of *Solanum elaeagnifolium* powdered leaves revealed various distinctive qualities as shown in Table 1. The physical features of powdered plant leaves from *Solanum elaeagnifolium* revealed important details about their chemical composition and potential therapeutic effects. The fine grains and smooth texture of the powder may indicate the existence of chemicals that are soluble and easily extracted in solvents. The brownish green colouration indicates the existence of natural pigments, which could have antioxidant or antibacterial activities. The odour and bitter taste may also suggest the presence of specific types of substances known to have medicinal benefits, such as alkaloids or flavonoids. These phytochemical constituents may be responsible for the antioxidant, antibacterial, and anticancer activities of the plant.[14]

Microscopic examination of Solanum elaeagnifolium

The result of the microscopic analysis of the *Solanum elaeagnifolium* powdered leaves samples revealed the presence of stellates trichomes which provides valuable information about the identity, quality, and potential medicinal properties of a particular plant (Figure 1). Furthermore, it can be used for the identification of any impurities or foreign materials present in the plant material which is vital for quality control and standardization of herbal medicines.

Phytochemical Screening

The presence of phytochemical components or secondary metabolites was indicated by colour change when appropriate reagents were reacted with the leaves extract. The chemical components contained in the plant that are responsible for the biological and antioxidant activities as well as the bio-reduction of silver in the synthesis of nanoparticles were identified using photochemical screening. The test confirmed the presence of tannins, steroids, cardiac glycosides, alkaloids, flavonoids, and saponins, while cyanogenic glycoside and anthraquinones were absent (Table 2). The result obtained is similar to findings by previous studies done by Hossain *et al.*,[3].

Table 1: Organoleptic evaluation

S/N	Parameters	Result
1	Size	Fine powder
2	Colour	Brownish green
3	Texture	Smooth
4	Odour	Woody
5	Taste	Bitter

Table 2: Results of phytochemical screening of *Solanum elaeagnifolium* leaves extract

S/N	Test	Result
1	Alkaloids	+
2	Tannins	+
3	Flavonoids	+
4	Saponins	+
5	Cyanogenic glycoside	-
6	Terpenoids	+
7	Steroids	+
8	Cardiac steroidal glycoside	+
9	Anthraquinones	-

+ = present, - = negative

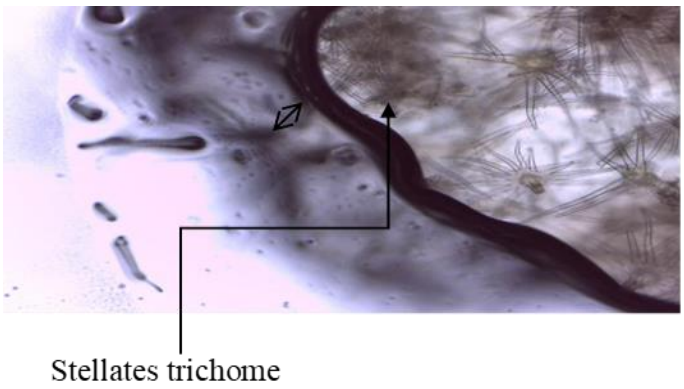

Figure 1: Microscopic features of *Solanum elaeagnifolium* showing stellate trichomes

Synthesis of Solanum elaeagnifolium silver nanoparticles

The chemical interaction between the leaves extract and the silver nitrate ($AgNO_3$) solution resulted in a colour change from green to brown and eventually formed a brownish precipitate that indicate the synthesis of silver nanoparticles. Similar colour change was observed in previous studies done by Nzekekwu and Abosede,[17] which also confirmed that the final reaction between leaves extract and the $AgNO_3$. The methanolic leaves extract served as a capping and reducing agent which resulted in the formation and stabilization of the silver nanoparticles.[5] Previous studies have revealed that the hydroxyl group present in organic compounds like phenols and flavonoids may be responsible for the bioreduction of silver ions (Ag^+) to Ag^o.[2,3] The activation of the surface plasmon resonance (SPR) phenomenon caused the colour change of a mixture of silver nitrate solution and the leaves extract from light green immediately after the addition of the extract to dark green after 30 min and finally to dark brown coloration after 72 h (Figure 2).

UV-Visible spectra analysis

The excitation of surface plasmonic vibrations caused by the reduction of Ag^+ ions were measured spectrophotometrically for the *Solanum elaeagnifolium* AgNPs and it showed maximum absorbance at 480 nm after 30 min of incubation. Surface plasmon resonance (SPR) of AgNPs generated by reduction of aqueous Ag^+ was responsible for the absorption bands in the visible spectra.[5,17] Previous studies have shown that silver nanoparticles generally exhibit distinct optical properties as a result of SPR which depends on the size distribution and shape of the synthesized nanoparticles.[18] Previous researches have also shown that SPR of AgNPs typically occur at about 440 nm and this is as a result of the vibration of free electrons in the conduction band after they have been excited by incident light of a specific wavelength.[17] The UV-Visible analysis revealed that *Solanum elaeagnifolium* leaves extract acted as the reducing agent in the bioreduction of silver ions.

Results of FTIR analysis

The FTIR spectra of the *Solanum elaeagnifolium* leaves extract showed absorption bands at 3333, 1640, 1005 and 652 cm^{-1} attributed to the presence of N-H, -C=C-, CO-O-CH and C-H functional groups respectively while the spectrum of the synthesized AgNPs showed absorption bands at 3348, 2912 and 1582 cm^{-1} as a result of the presence of -OH, C-H and NO_2 functional groups respectively (Figure 3). Hence, the results FTIR analysis showed that these functional groups from the aqueous leaves extracts of *Solanum elaeagnifolium* were responsible for the reduction Ag^+ to Ag^o as well as the synthesis of the AgNPs. These findings are similar to previous studies done by Nzekekwu and Abosede.[17]

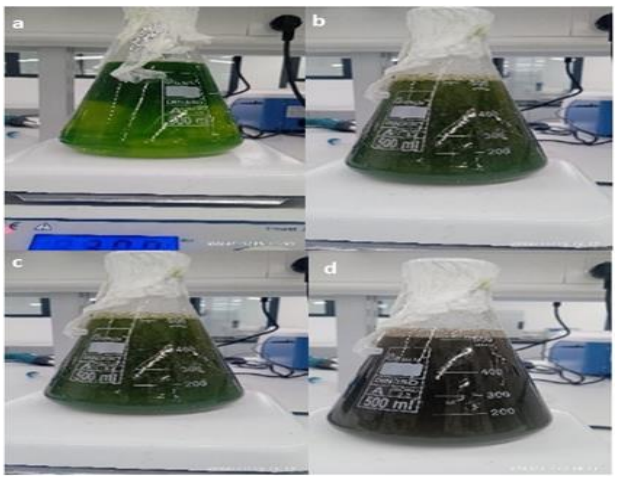

Figure 2: Synthesis of silver nanoparticles and sequence of colour change with time (a) immediately after mixing the silver nitrate solution with the leaves extract and (b) After 30 min (c) after 48 h and (d) after 72 h.

Table 3: Percentage scavenging activity with DPPH

Concentration (mg/mL)	% SCA extract	% SCA NPs	% SCA Ascorbic acid
0.625	28.41	38.42	46.31
1.25	34.52	44.16	57.48
2.5	48.13	57.94	75.27
5	67.72	76.32	89.27
10	81.24	88.41	95.49

SCA= Scavenging activity

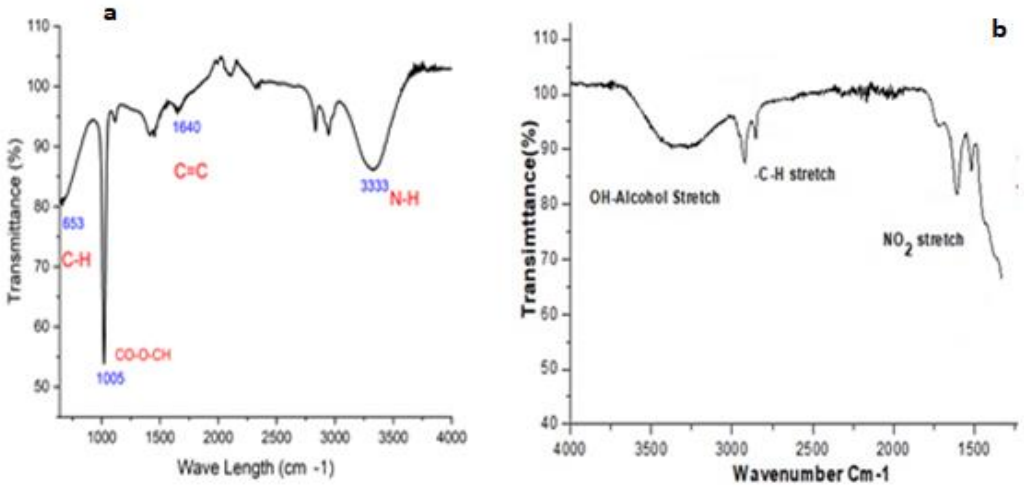

Figure 3: FTIR spectra of (a) *Solanum elaeagnifolium* powdered leaves and (b) silver nanoparticles

According to Prasad *et al.*,[19], the amine (N-H), hydroxyl (-OH), and carboxyl (-C=O) groups of the leaves extract are primarily involved in the synthesis of silver nanoparticles. Peaks at certain wavelengths were seen in both the leaves and the AgNPs revealing the presence of diverse organic chemicals and functional groups that contributed to the stabilization and reduction of the silver nanoparticles.

Result of XRD analysis

This was done to elucidate the underlying polymorphic structure of the AgNPs. The spectrum revealed crystalline nanoparticles that are capped by biomolecular components which were responsible for the reduction of silver ions (Figure 4). Similar findings were also obtained by Nzekekwu and Abosede.[17]

SEM analysis

SEM is a sophisticated imaging method that scans the surface of a sample with an electron beam to obtain high-resolution images of its topography, morphology, and composition. It investigates the surface features of nanoparticles to learn about their physical qualities, such as stability, uniformity, and surface chemistry.[20] SEM was used to analyze the size, shape, distribution, and aggregation conditions of the nanoparticles. Figure 5 shows the SEM image of the nanoparticles and it revealed a porous, spherical and crystalline structure with particle sizes of 70-95 nm.

DDPH scavenging activity of Solanum elaeagnifolium leaves extract and silver nanoparticles

The fundamental property of an antioxidant is its capacity to scavenge free radicals. Antioxidant compounds such as flavonoids and polyphenols scavenge free radicals such as lipid peroxyl, hydroperoxide, peroxide, and reactive oxygen species (ROS) thus inhibiting the oxidative pathways that cause degenerative illnesses. The commonest technique for evaluating antioxidant activity is the DPPH test. When reacted with antioxidant compounds, DPPH can accept an electron or hydrogen ion from an antioxidant scavenger molecule to form a more stable DPPH compound. The results of the antioxidant test were expressed as half-maximal inhibitory concentration (IC_{50}) and percentage inhibition (Table 3 and Figure 6). The result revealed that the formulated silver nanoparticles had higher antioxidant activity than the leaves extract at all concentrations. The results showed that ascorbic acid (standard) had greater free radical scavenging activity than both the silver nanoparticles and leaves extract. On the basis of the percentage inhibition, the antioxidant effects demonstrated by all the samples were concentration dependent. From the graph of the percentage inhibition against the concentration, the IC_{50} of the leaves extract, silver nanoparticles and ascorbic acid were 3.40 mg/mL, 2.30 mg/mL and 1.40 mg/mL respectively. The free radical scavenging activity demonstrated by the silver nanoparticles and leaves extract was found to be as a result of the presence of phenolic compounds. Similar findings were obtained by Kero *et al.*,[21].

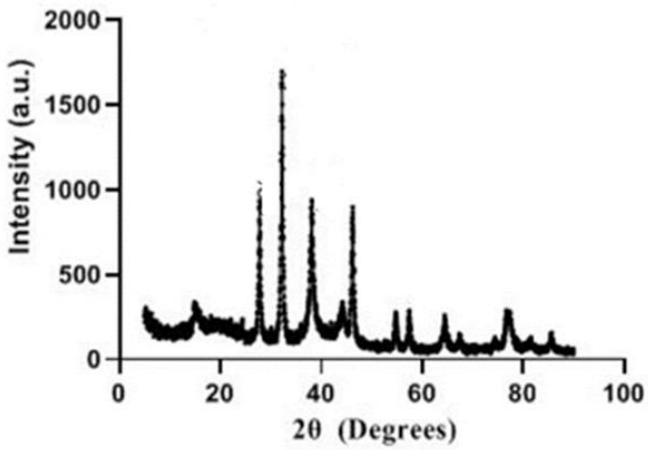

Figure 4: XRD spectrum of *Solanum elaeagnifolium* nanoparticles

Table 4: Mean zone of inhibition of growth

Organism	Silver NP (mm)	SE Extract (mm)	Gentamicin (mm)	Silver Nitrate (mm)	Water
S. aureus	9.2	4.8	22.7	8.6	0
E. coli	6.2	3.1	18.6	5.2	0
B. subtilis	8.9	4.2	19.7	7.4	0
S. typhi	5.7	2.9	16.5	4.8	0

SE = *Solanum elaeagnifolium*, NP = Nanoparticles

Antimicrobial activity of Solanum elaeagnifolium silver nanoparticles

The *Solanum elaeagnifolium* leaves extract and nanoparticles showed comparable antimicrobial activity relative to the positive control (gentamicin) and the zone of inhibition is presented in Table 4. The results showed that nanoparticles demonstrated significant antibacterial activity against all test bacteria, with the highest activity demonstrated against *S. Aureus* ($P<0.05$). The results of the antibacterial studies showed that the AgNPs inhibited the growth of both the gram positive and negative bacteria with mean zone of inhibition of growth ranging between 5.7-9.2 mm. The extract in water showed mean zone of inhibition between 2.9-4.8 mm (Table 4). Gentamicin (standard) showed mean zone of inhibition ranging between 18.6-22.7 mm while the silver nitrate solution had a zone of inhibition of 4.8-8.6 mm. The antibacterial activity of the formulated silver nanoparticles was significantly greater than the leaves extract and this may be due to the relatively larger surface area of the nanoparticles ($P<0.05$). The exact mechanism of action of the antibacterial effects of silver nanoparticles is unknown however, previous studies have postulated that it may be due to the ability of the silver nanoparticles to penetrate the bacterial cell wall and induce a cellular damage in the cell membrane leading to increased cell permeability.[16,22] This can result in an unhindered penetration of the nanoparticles through the cytoplasmic membrane and thus result in apoptosis[23]. Other researchers have also proposed that it may be due to the interaction between the silver nanoparticles, DNA and proteins within the bacterial cell, causing cellular damage. The release of silver ions from the AgNPs could also be responsible for the antibacterial effect.[24,25]

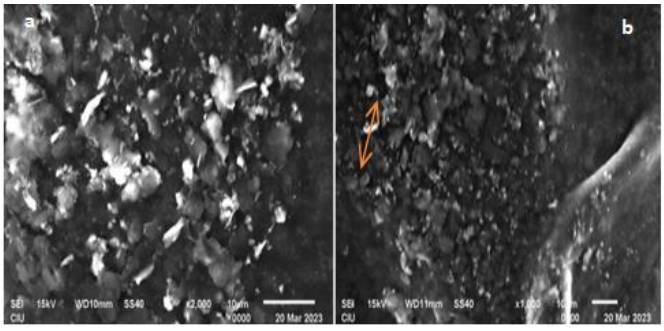

Figure 5: SEM images of Solanum elaeagnifolium nanoparticles (a) x1000 and (b) x15000

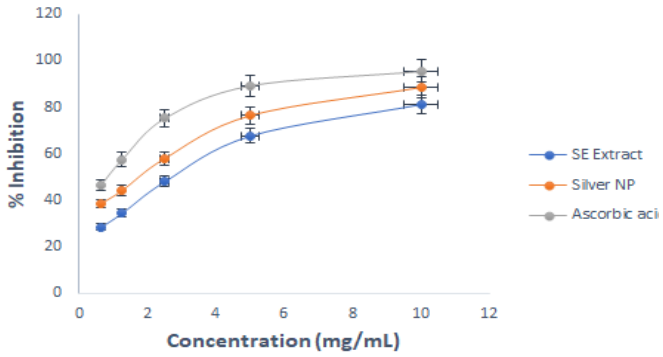

Figure 6: DPPH free radical scavenging activity of extract, nanoparticles and ascorbic acid

Conclusion

Silver nanoparticles of *Solanum elaeagnifolium* were synthesized in this study from the methanol leaves extract using a green and eco-friendly method. The formulated silver nanoparticles demonstrated significant antibacterial and antioxidant effects due to the presence of some secondary metabolites such as phenols, saponins, flavonoids and tannins. The silver nanoparticles could have potential pharmaceutical applications after further research such as animal studies have been done.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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