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Biosynthesis, Characterization and Antibacterial Activity of Silver Nanoparticles (AgNPs) Using *Balanites aegyptiaca* (Heglig) Seed Kernel Oil and Aqueous Extract

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A B S T R A C T

This study presents an eco-friendly and sustainable approach for the biosynthesis of silver nanoparticles (AgNPs) using *Balanites aegyptiaca* (Heglig) seed oil and aqueous extract as dual reducing and stabilizing agents. The objective of this research was to synthesize AgNPs using *B. aegyptiaca* extracts and evaluate their physicochemical properties and antibacterial efficacy. AgNPs were successfully synthesized by reducing silver ions from an aqueous silver nitrate solution with the *B. aegyptiaca* seed oil and aqueous extract. The formation and characteristics of the biosynthesized AgNPs were rigorously confirmed using UV-Vis, FTIR, and SEM. UV-Vis analysis revealed a strong surface Plasmon resonance peak at 430 nm, indicating stable AgNPs formation. FTIR spectroscopy confirmed the role of bioactive compounds from the plant extracts in both reduction and capping, with characteristic shifts in absorption bands. SEM images showed the AgNPs to be spherical and irregular in shape, with some agglomeration. Furthermore, the biosynthesized AgNPs exhibited potent antibacterial activity against both Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacterial strains, with notably higher efficacy against *E. coli*. This research highlights the viability of *Balanites aegyptiaca* as a sustainable and cost-effective platform for nanoparticle synthesis, offering significant potential for various biomedical applications, particularly in the development of novel antimicrobial agents.

Introduction

Nanotechnology has emerged as a rapidly evolving field with diverse applications in medicine, agriculture, environmental science, and materials engineering. Among various nanomaterials, silver nanoparticles (AgNPs) have garnered significant attention due to their remarkable physicochemical properties and broad-spectrum antimicrobial activities (Rai, Yadav et al. 2009, Ahmed, Ahmad et al. 2016). Conventionally, AgNPs are synthesized through physical and chemical methods, which often involve the use of toxic reagents and generate hazardous by-products, posing environmental and health risks (Singh, Kim et al. 2016). In contrast, green synthesis has emerged as an eco-friendly, cost-effective, and sustainable alternative that utilizes biological resources such as plant extracts, bacteria, and fungi to facilitate nanoparticle formation (Iravani 2011, Mittal, Chisti et al. 2013).

Plant-mediated synthesis of nanoparticles has gained particular prominence due to its simplicity, scalability, and the rich diversity of phytochemicals that can act as reducing, capping, and stabilizing agents (Singh, Dutta et al. 2018). Various plant parts, including leaves, roots, seeds, and fruits, have been successfully employed in the green synthesis of AgNPs, leveraging bioactive compounds such as flavonoids, alkaloids, saponins, and tannins (Kharissova, Dias et al. 2013). These phytochemicals play a crucial role in reducing silver ions (Ag^{+}) to metallic silver (Ag^{0}) while also stabilizing the formed nanoparticles (Khalil, Ismail et al. 2014). *Balanites aegyptiaca* (L.) Delile, commonly known as "Heglig" or "Desert Date," is a well-known medicinal plant widely distributed across arid and semi-arid regions of Africa and Asia. It has been traditionally utilized for its pharmacological properties, including antimicrobial, anti-inflammatory, and antioxidant activities (Koko, Mesaik et al. 2008, Murthy, Yadav et al. 2020). The seed kernels of *B. aegyptiaca* are rich in bioactive compounds such as saponins, alkaloids, flavonoids, and steroids, making them suitable candidates for nanoparticle synthesis (Harikrishnan and Balasundaram 2020, Dey and Shaili 2023). Both aqueous and oil extracts of the seed kernels offer diverse phytochemical profiles, which may influence the morphology, stability, and bioactivity of the resulting nanoparticles.

Despite the documented medicinal benefits of *B. aegyptiaca*, there is limited literature on its application in nanotechnology, particularly in the green synthesis. This study aims to explore the potential of *B. aegyptiaca* seed kernel aqueous and oil extracts for the biosynthesis of AgNPs, followed by comprehensive characterization using UV–Vis spectroscopy, FTIR, and SEM techniques. Furthermore, the antibacterial efficacy of the synthesized nanoparticles will be evaluated against selected Gram-positive and Gram-negative bacterial strains, contributing to the ongoing search for alternative antimicrobial agents in the face of rising antibiotic resistance.

Material and Methods

Chemicals

All reagents and solvents used in this work were used as received without further purification.

Sample Collection and Preparation

Fresh fruits of *B. aegyptiaca* were purchased from the Ebin Masuod Market in Elobeid, Sudan. The seeds were separated and spread under shade at ambient temperature (25–30 °C) for 48 hours to allow for drying. Following this, the seeds were manually sorted to remove any extraneous materials. The dried seeds were then cracked manually to obtain the kernels from the husk. The kernels were subsequently ground into a fine powder using an electric blender. The resulting seed kernel powder was stored in sealed plastic bags at 4 °C until further analysis.

Preparation of Aqueous Extract

Aqueous extract of the seed kernels was performed by macerating 100 g of the powdered *B. aegyptiaca* kernels in 500 mL of distilled water (1:5 w/v ratio) for 48 hours at room temperature, following the cold maceration method. The mixture was subsequently filtered using Whatman No. 1 filter paper. The filtrate (aqueous extract) was stored in the dark at 4 °C until further use.

Oil Extraction

Oil extraction from the seed kernels of *B. aegyptiaca* was carried out according to the method described by (Mortadha, Tahseen et al. 2015). Soxhlet extraction was employed, using hexane as the solvent. Specifically, 10 g of the crushed seed kernels were placed in a porous thimble within the Soxhlet apparatus. Extraction was performed using 250 mL of hexane (boiling point 60–65 °C) for 7 hours, repeated until a sufficient quantity of

oil was obtained. The solvent was subsequently removed under reduced pressure using a rotary evaporator. The extracted oil was stored at 4 °C for subsequent analyses.

Phytochemical Screening of the Seed Kernel Oil

Phytochemical screening of the oil were carried out using standard procedures prescribed by (Harborne 1998). The phytochemicals determined include; alkaloid, saponin, tannin, triterpenoid, glycoside, flavonoids, phenols and steroid.

Biosynthesis of Ag Nanoparticles

In this study, silver nanoparticles (AgNPs) were successfully synthesized by reducing silver ions from an aqueous silver nitrate ($AgNO_3$) solution. Heglig extracts and seed oil (HESO) served as both the reducing and capping agents. The synthesis involved adding 2 ml of a seed oil solution (prepared by diluting approximately 200 µl of seed oil in 2 ml of methanol to a total of 10 ml with distilled water) to 80 ml of a 3 mM $AgNO_3$ aqueous solution. This mixture was continuously stirred magnetically at 80 °C, with heglig extract drops added until a visual change to dark brown indicated the formation of AgNPs.

Characterization of the Biosynthesized AgNPs

The bioreduction of the silver ion by the seed kernel extracts and oil of *B. aegyptiaca* as reducing and capping agents was verified by UV–Vis spectrophotometer (UV-2550 Shimadzu, Japan) at a resolution of 1 nm in the wavelength of 200–700 nm. For further characterization, an aqueous solution of AgNPs was centrifuged at 14,000 rpm for 20 min. The FTIR spectrum analysis was collected at the resolution of 4 cm^{-1} in the range of 500–4000 cm^{-1} region using Fourier transform infrared spectrometer model (Vector 22, Bruker, Germany). Scanning Electron Microscopic (SEM) (TM-1000, Hitachi, Japan) analyzed the size of the synthesized AgNPs. Thin-film samples were prepared on a carbon-coated copper grid by dropping the sample on the grid, and an excess solution was removed via a blotting paper.

Antibacterial Activity

The antibacterial activity of biosynthesized AgNPs was assessed against Gram-positive *S. aureus* and Gam-negative *E. coli* by well diffusion technique. Briefly, bacterial suspensions at concentrations (approximately 5×105 CFU/mL) were uniformly spread on Mueller–Hinton Agar (MHA) plates. Three wells about 6 mm diameter were created in each of these plates using sterile borer. 50 µL of the biosynthesized AgNPs solution were added at various concentrations (25, 50 and 100 µg/mL) to the wells at aseptic conditions, and the tested plates were incubated at 37±2 °C for 24 h. After it, the diameter of an inhibitory zone was measured. This study was carried out in triplicates.

Statistical Analysis

The data were expressed as the standard deviation (mean±SD) obtained from at least three independent experiments. The statistical analysis was evaluated by one-way ANOVA test (SPSS 16) followed by Tukey's HSD test ($p < 0.05$).

Results and Discussion

Green synthesis of silver nanoparticles using microorganisms and plant extracts has been studied, the latter method being the best alternative to obtain these nanomaterials due to the ease and efficacy in the reduction of metal ions by the biomolecules present in plant extracts (Jafarizad, Safaee et al. 2015). Consequently, the process involves no chemicals that are hazardous for the environment and living organisms.. For instance, the use of biological materials such as plants is usually safe. Plants also contain reducing and capping agents (Jadoun, Arif et al. 2021). The biosynthesis method of nanoparticles is considered one of the most promising methods, as it has gained a very important area due to its economic and environmentally friendly benefits, and it is more effective, which exceeded its formation by physical and chemical methods. Nowadays, considerable attention has been paid to the green synthesis of nanoparticles through plant extract and biological substances, because of hazardous and toxic by-products are usually paired with chemical methods (Kasture, Patel et al. 2008, Kumar, Mamidyala et al. 2010)

The Percentage Yield of Oil Extraction from *B. aegyptiaca* Seed Kernel

In this experiment, the kernel of heglig seed showed a high percentage yield of oil, as the percentage of oil in the heglig seeds reached 43%.

Table.1 Chemical constituents present in oil extraction from *B. aegyptiaca* seed kernel

Phytochemicals	Result	Phytochemicals	Result
Alkaloids	+Ve	Tannins	+Ve
Steroids	+Ve	Phenols	+Ve
Triterpenoid	+Ve	Flavonoids	+Ve
Saponins	+Ve	Glycosides	+Ve

Figure.1 Schematic illustrations for biosynthesizing of HSEO-AgNPs using Heglig seed kernel extracts and Heglig seed kernel oil

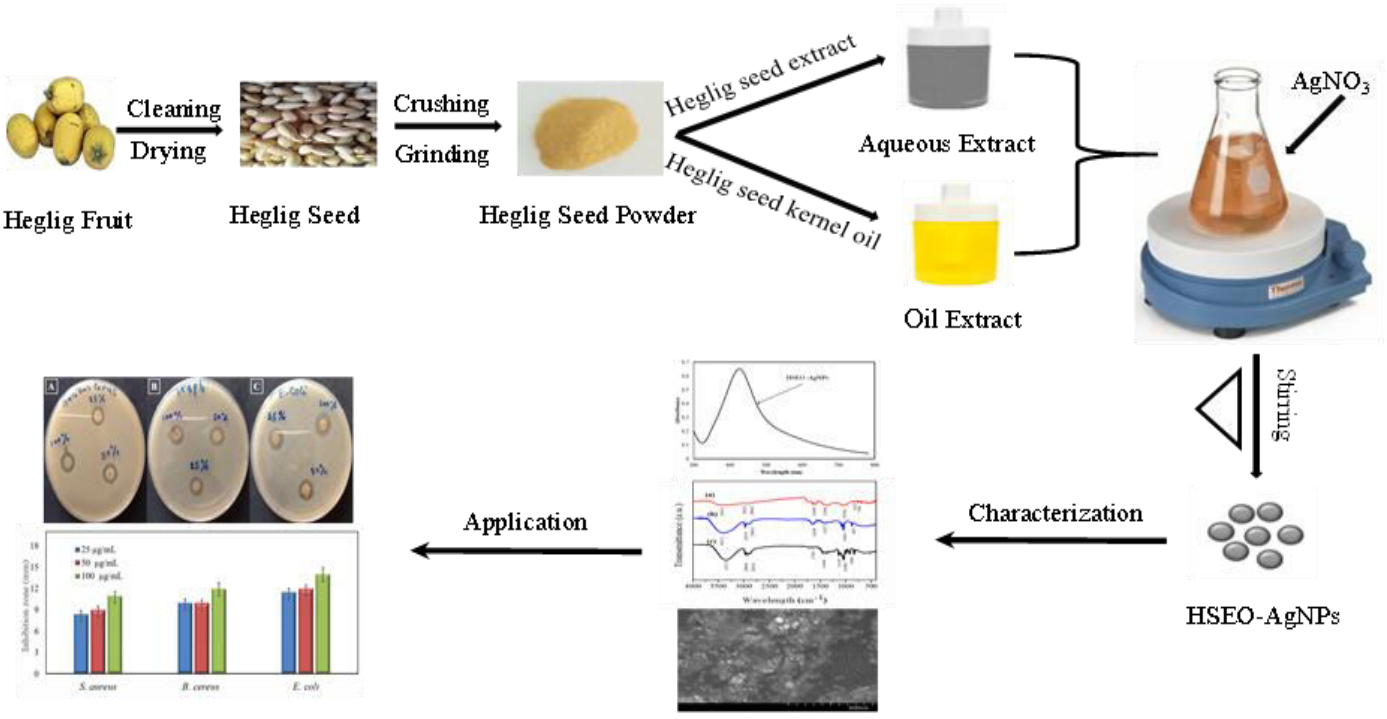


Figure.2 (A) $AgNO_3$ solution (B) Heglig seed kernel extract (C) HSEO-AgNPs

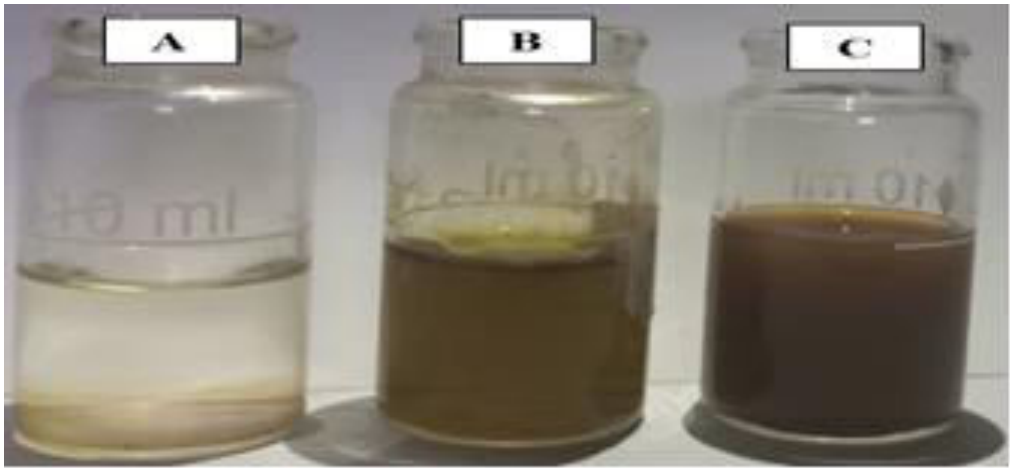


Figure.3 UV–Vis spectra of biosynthesized HSEO-AgNPs using Heglig seed kernel extracts and Heglig seed kernel oil

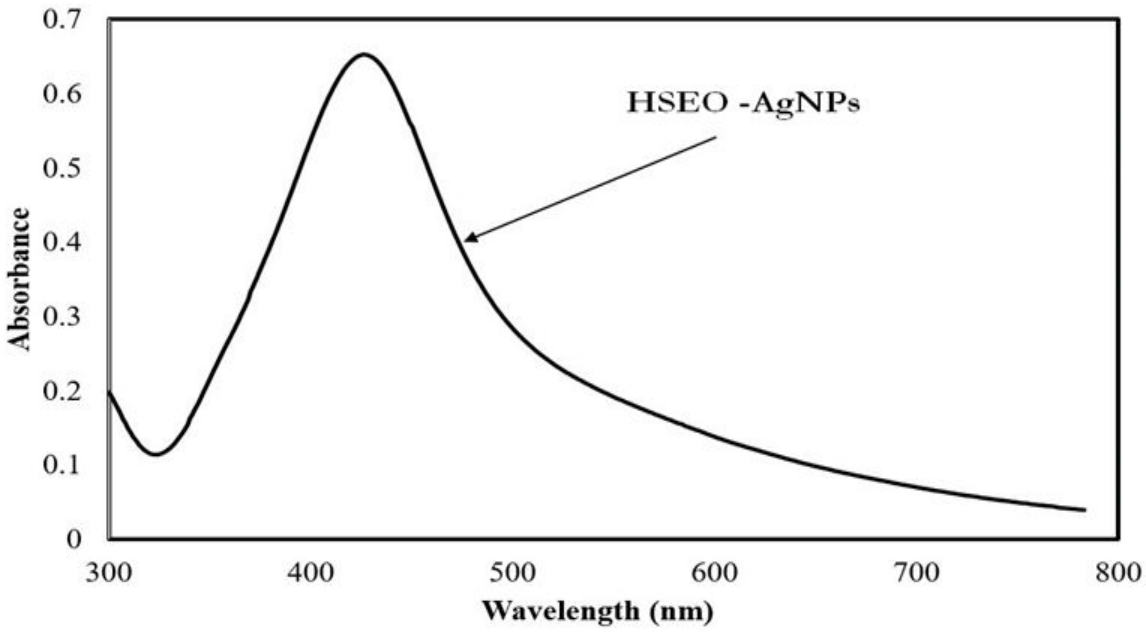


Figure.4 FTIR spectra of (a) HSEO-AgNPs (b) Heglig seed kernel extract (c) Heglig seed kernel oil

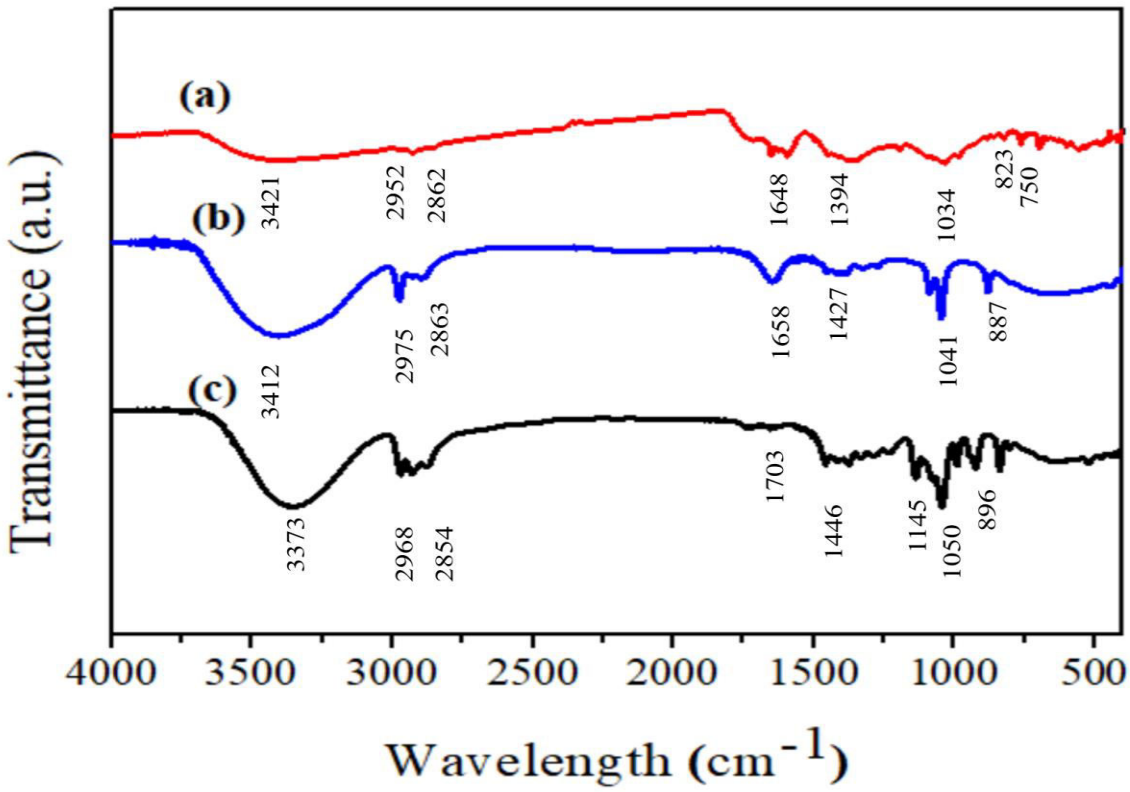


Figure.5 The SEM image of the biosynthesized HSEO-AgNPs

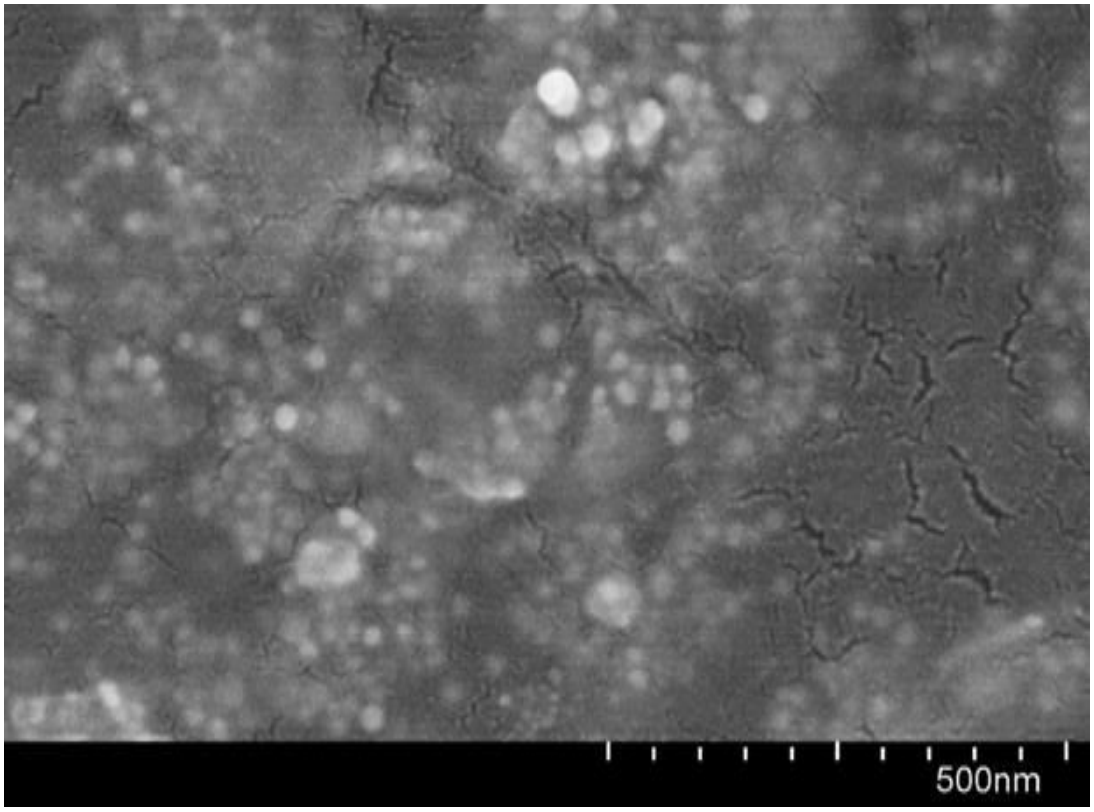


Figure.6 Antibacterial activity of biosynthesized HSEO-AgNPs against *(A) Bacillus subtilis* (B) *Staphylococcus aureus,* and (C) *Escherichia coli*

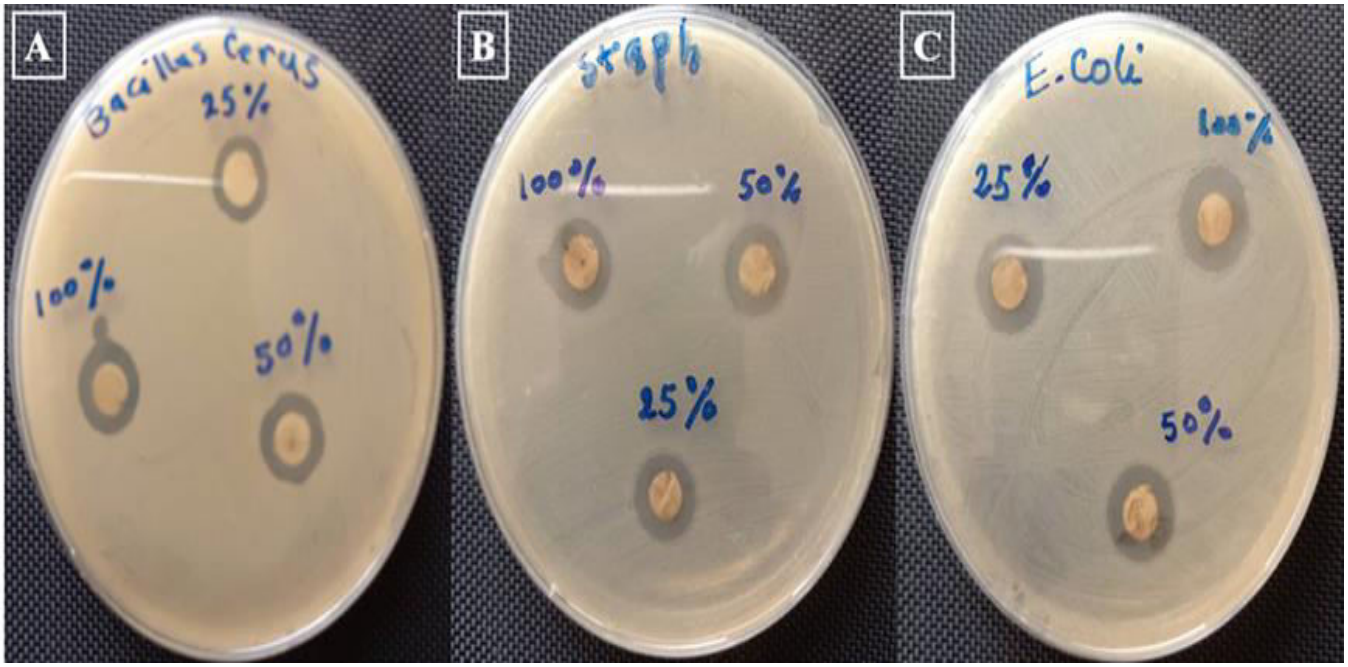
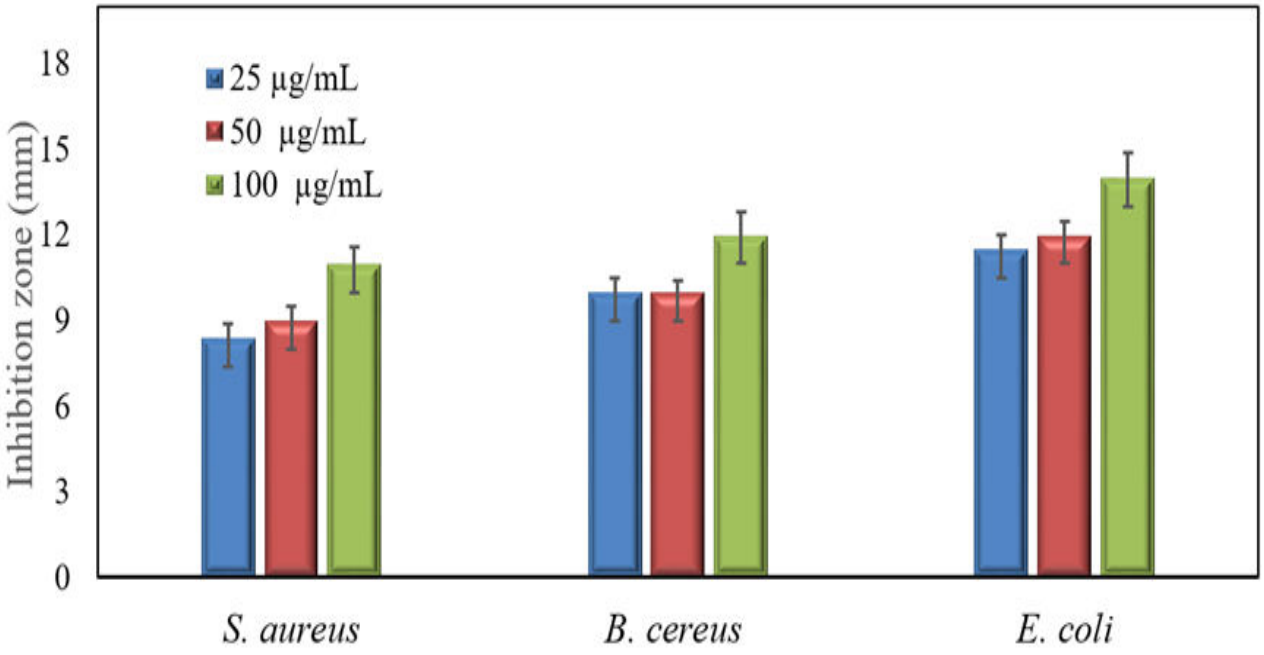


Figure.7 Zone of inhibition (mm) for various concentrations of biosynthesized HSEO-AgNPs against bacteria.



The *Balanites aegyptiaca* oil was bright yellow in color, Liquid at room temperature with palatable flavor and this result is consistent with other work (Ahmed and Alshareef 2018).

Phytochemical Estimation

Phytochemical evaluation was conducted for the extract obtained to detect the presence of chemical constituents such as alkaloids, glycosides, phenols, steroids triterpenoid, saponins, tannins, flavonoids. Various chemical tests were performed to detect the presence of these chemical constituent, the results obtained from these test are compiled in the Table 1

Characterization of biosynthesized Ag nanoparticles

UV-Visible Spectrophotometer Analysis

In the current work, the reduction of Ag ions into Ag nanoparticles was successfully accomplished after optimization of the experimental parameters, by the aqueous solution of $AgNO_3$ and seed kernel extracts and oil as reducing and capping agents, respectively. The change of colors to dark brown visually observed and indicated the formation of AgNPs Figure 2. This phase was followed by UV-vis absorption spectroscopy as shown in Figure 3. The strong surface plasmon resonance (SPR) was located at 430 nm, suggesting the formation of stable AgNPs by seed kernel oil. Similar results were reported by Awad, El Dib et al. (2013).

FTIR Spectra Analysis

The biomolecules in the heglig seed kernel extracts and seed kernel oil that caused Ag+ reduction and acted as capping agents for efficient stabilization were recognized by FTIR spectrum measurements. The Figure 4 represents a comparison of the FTIR spectra of (a) HSEO-AgNPs (b) Heglig seed kernel extract (c) Heglig seed kernel oil. Figure 4. (a) represents the FT-IR spectra of biosynthesized HSEO-AgNPs showing absorption peaks at 3421, 2952, 2862, 1648, 1394, 1034, 823 and 750 cm^{-1}.

As a result, the characteristic bands in HSEO-AgNPs shifted to a lower frequency region in comparison with Heglig seed kernel extract and Heglig seed kernel oil Figure 4. (b and c) which clearly indicates the presence

of residual Heglig extract and oil compounds as the reducing and stabilizing (capping) agent to the AgNPs. In the spectrum, the peak at approximately 3421 cm^{-1} was related to the stretching of N-H bonds resulting from aliphatic primary amines. The peaks at 2952 and 2862 cm^{-1} were attributed to the asymmetric CH_2 stretch and the symmetric CH_2 stretch, respectively, while the 1648 cm^{-1} peak of N-O stretch derived from a nitro compound. The band at 1394 cm^{-1} corresponded to the C-H stretching frequencies of the alkene group. The peak at 1034 cm^{-1} represented a S=O stretch, consistent with sulfoxides, Band at 750 cm^{-1} is assignable to C=C bend of alkenes. These bands are consistent with other's work (Jain and Mehata 2017, Nallal, Prabha et al. 2021, Dhaka, Raj et al. 2022). Therefore, FTIR amine (N-H) and sulfinyl (S=O) groups in the heglig seed kernel extract and oil bioactive compound led to a reduction of. silver ions

Scanning Electron Microscopic Analysis

The SEM image shows surface morphology of the biosynthesized HSEO-AgNPs in a spherical and irregular in shape, with small clusters of particles due to agglomeration through sample preparation as shown in Figure 5. Similar results were stated by (Dhaka, Raj et al. 2022)

Antibacterial Activity Study

Figure 6 shows the antibacterial activity of biosynthesized HSEO-AgNPs against different selected strains of gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative Escherichia coli (*E. coli*) at the concentration of 25, 50, and 100 µg/mL using disc diffusion method, and the results of the zone of inhibition are presented in Figure 7. Generally, all prepared samples showed potential antibacterial activity against all pathogens. Interestingly, HSEO-AgNPs displayed a highest antibacterial activity against gram negative bacteria *Escherichia coli* (*E. coli*) compared to gram positive (*Staphylococcus aureus* and *Bacillus subtilis*). These findings are in good agreement with another work (Gasmalla, Idris et al. 2016).

Several mechanisms have been proposed for the antibacterial activity of silver NPs. For instance, silver NPs may adhere to the surface of the cell membrane disturbing permeability and respiration functions of the cell (Kvítek, Panáček et al. 2008) but can also penetrate inside the bacteria (Jose Ruben, Jose Luis et al. 2005). Other hypothesis states that the deadly effect of Ag, resulting from the interaction of ionic silver with thiol groups, inhibits vital enzymes (Gupta, Maynes et al. 1998, Matsumura, Yoshikata et al. 2003).

In conclusion, this study successfully demonstrated the eco-friendly synthesis of stable, uniformly shaped silver nanoparticles (AgNPs) using Balanites *aegyptiaca* seed oil and aqueous extract as both reducing and stabilizing agents. Characterization via FTIR, UV-Vis spectroscopy, and SEM confirmed the formation of AgNPs with excellent colloidal stability. These biosynthesized AgNPs showed potent antibacterial activity against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*) pathogens, indicating their potential for biomedical applications. The research highlights *Balanites aegyptiaca* as a sustainable platform for nanoparticle synthesis, with future studies suggested to explore mechanistic insights, scalability for industrial applications, and broader biomedical evaluations.

Author Contributions

Monira Abdalla Ajabeldoor: Investigation, formal analysis, writing—original draft. Wafa Omer Ahmed: Validation, methodology, writing—reviewing. R. S. M. Bashier:—Formal analysis, writing—review and editing. Abdelmoneim Bakur: Resources, investigation writing—reviewing. Awad Mohammed Babeker: Validation, formal analysis, writing—reviewing. Mohammed Bahreldin Hussein: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript. Ahmed Abdalla Agab Eldour : Investigation, formal analysis, writing—original draft.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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