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Synthesis and Biological Applications of Roasted Foxnut-Mediated Silver Nanoparticles

Beenish Khanzada[1*], Abdul Nabi Mirjat[2], Aftab Ahmed Khand[3], Nimra Faiz[1], Muzammil Latif[1], Mahnoor Ali[1] and Areeba Ali[1]

[1]Institute of Biochemistry, University of Sindh, Jamshoro, Pakistan
[2]Institute of Microbiology, University of Sindh, Jamshoro, Pakistan
[3]Department of Physiology, University of Sindh, Jamshoro, Pakistan

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***Corresponding Author:**
Beenish Khanzada
Institute of Biochemistry, University of Sindh, Jamshoro, Pakistan
beenish@usindh.edu.pk

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ABSTRACT

Nanotechnology is developing into a fast-expanding discipline with extensive use in medicine, pharmaceuticals, and environmental sciences. AgNPs have been of particular interest because of their potent antimicrobial and antioxidant effects. **Objectives:** To synthesize silver nanoparticles using roasted foxnut (*Euryale ferox*) extract, which was utilized as an eco-friendly and cost-effective green method. **Methods:** A green method involving the synthesis of silver nanoparticles using roasted foxnut (*Euryale ferox*) extract was utilized in the current study as an eco-friendly and cost-effective green method. The roasted foxnut extract acted as a natural reducing and stabilizing agent. Silver nanoparticles were prepared by combining foxnut extract with silver nitrate solution, and the formation of silver nanoparticles was observed by a change in color of the light-yellow solution to brown. To ascertain the formation of nanoparticles, also the functional groups involved in their formation, UV-Visible spectroscopy and FTIR assisted in characterizing the nanoparticles. To determine the biological potential of the nanoparticles synthesized, antioxidant and antibacterial assays were conducted. **Results:** The antioxidant activity of nanoparticles was tested at 500 ppm and 1000 ppm with the assistance of the DPPH free radical scavenging assay. For statistical analysis, one-way ANOVA was used, and SPSS version 22.0 was used. The results were statistically significant ($p<0.05$) with higher scavenging efficiency observed at 1000 ppm. The agar well diffusion method was used to test the antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. **Conclusions:** Roasted foxnut extract-derived silver nanoparticles used in biomedical and pharmaceutical fields should be emphasized owing to significant antioxidant and antibacterial properties.

INTRODUCTION

Nowadays, much attention has been given to metal nanoparticles due to their extraordinary structural features and useful applications in fields of medicine, environment, diagnosis, agriculture, sensing, and so on. Silver, gold, and copper nanoparticles have also been widely researched, especially because of their strong antibacterial and antioxidant powers [1]. In the majority of nanoparticle types, reduction of metal salt is a key step towards their synthesis. Silver nanoparticles (AgNPs) are at the forefront of nanotechnology due to offering small size, large surface area-to-volume ratio, and unique electrochemical properties. Moreover, silver nanoparticles are reported to have distinct benefits, such as inbuilt antibacterial, antioxidant, and antifungal characteristics imparted by the metal silver [2]. A variety of physical and chemical methods have been reported so far for the formation of AgNPs, but these have limitations such as toxicity and environmental hazards. Alternatively, natural sources of AgNPs currently employed include bacteria, algae, viruses, and plants [3]. However, comparatively, plant extract-mediated synthesis of AgNPs offers a minimally toxic and cost-effective methodology with greater stability, less incubation time, and much control of physicochemical properties. Plants' secondary

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metabolites serve as capping agents for the synthesis of silver nanoparticles. Furthermore, plant-based nanoparticle synthesis has gained much importance due to imparting superior antioxidant, anti-microbial, and other biological activities into nanoparticles. Literature supports the synthesis of a variety of nanoparticles synthesized in different dimensions and morphologies using various parts of diversified medicinal plants [4-6]. In this connection, this study explored for the first time the nanoparticle synthesizing capacity of roasted fox nut. Fox nuts, also known as "makhana," are the seeds of the lotus flower (*Euryale ferox*). Protein, fiber, and micronutrients like calcium, magnesium, iron, and phosphorus are abundant in foxnut [7]. Research has shown that fox nuts exhibit strong antioxidant activity and help in controlling blood sugar, body weight, heart health, and digestive health [8]. Various bioactive agents, such as phenolic, terpenoids, flavonoids, etc., are all abundant in fox nut and have anti-inflammatory and antioxidant properties [9]. Roasting actually enhances the phytonutrients of food items. Roasting of foxnut is reported to increase its phenolic and flavonoid profile, as when the fox nut kernals are roasted, reactions such as browning (Millard) or non- enzymatic browning can be observed. Such browning reactions actually cause a shift in texture, color, and phenolic profile.

To date, there is no research carried out on the use of roasted foxnut for synthesizing silver nanoparticles. Studies are reporting the silver nanoparticle synthesis from *Euryale ferox* (Makhana) leaves; however, the use of roasted *Euryale ferox* in developing silver nanoparticles is scarce. Therefore, to fill this gap, the present study used roasted fox nut seed extract to synthesize and characterize silver nanoparticles. Moreover, this study aimed at analyzing the antioxidant potential and antibacterial activity of synthesized roasted foxnut nanoparticles against selected microbial strains to evaluate their biomedical potential.

METHODS

An experimental study was conducted in the Institute of Biochemistry, University of Sindh, Jamshoro, Pakistan. The study duration was from August 2025 to January 2026. Using a previously reported method [10], 5 g of fox nut (*Euryale ferox*) seed powder was accurately weighed and transferred into a clean beaker. The powder was added to a solvent mixture comprising 20 mL of acetone and 20 mL of distilled water. The mixture was stirred and mixed gently just to make sure that it was mixed properly; it was then covered by aluminum foil. The 72 hours (3 days) allowed the beaker to be kept in a dark place to allow the beaker to be effectively soak and extract the bioactive compounds. The mixture was then subjected to a magnetic stirrer to continue mixing during the 6 hours at room temperature to increase the extraction efficiency. After the magnetic stirring, the mixture was filtered through Whatman filter paper to isolate the solid residues. The obtained filtrate was collected and considered the fox nut extract. Silver nanoparticles were made by adding silver salt solution to the fox nut extract (1ml, 2ml, 4ml), which served as a reducing agent to produce silver nanoparticles. The mixed solution was incubated in the dark at 37°C for 24 hours. The next day, a color change of the solution gradually (usually yellow to brown which confirms the silver nanoparticles synthesis. The separation of the nanoparticles was obtained through centrifugation, washing of the nanoparticles with water, and drying of the nanoparticles. For characterization, UV–Visible spectroscopy analysis was carried out on the fox nut nanoparticles to study their light-absorbing compounds. Three dilutions of the extract (1 mL, 2 mL, and 4 mL) were prepared and scanned in the UV–Visible range 400-800nm. The absorbance values and λmax were recorded. Origin 2020 software was used to draw UV –Visible graphs. To determine the functional groups of foxnut extract that are involved in reducing and stabilizing silver nanoparticles, FTIR analysis was performed. Potassium bromide (KBr) was added to dried foxnut nanoparticles and pressed into pellets. The spectra were collected with a spectrophotometer, PerkinElmer, in the 4000-400 cm-1. For the assessment of antioxidant activities, a fresh 0.1Mm DPPH solution was made in methanol and stored in the dark. 1 ml DPPH solution was allowed to mix with 1ml nanoparticle suspension (500 &1000 parts per million) were combined for the assay. The reaction was done in triplicate. The negative control was made by mixing one milliliter of distilled water with one milliliter of DPPH solution. Positive control was ascorbic acid. The mixtures of the reaction were gently vortexed and incubated in the dark at a temperature of 37°C. After incubation, the absorbance was measured at 517 nm [11]. The antioxidant activity was determined by the following formula: DPPH Scavenging Activity (%) = (Control Absorbance – Sample Absorbance / Control Absorbance) × 100. Next, the antibacterial activity of the Foxnut-mediated nanoparticles was tested against human pathogens, i.e., *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive), using the agar well diffusion bioassay [12]. Powder form of foxnut nanoparticles in 500 ppm and 1000 ppm concentration was dissolved in methanol of HPLC-grade level for antibacterial analysis (with triplicate samples). Primarily, all the bacterial cultures were grown on nutrient agar media for 24 hours at 37°C. Standardized *E. coli* and *S. aureus* bacterial suspensions were evenly swabbed across the agar surface. All the Media were autoclaved at 121°C. For 15-20 min at 15lbs. Antibacterial activity was achieved using fresh culture on agar media by the well diffusion method. Equal-diameter wells were created in the agar using a sterile corn borer (6mm in diameter). Approximately 60 µl of the

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nanoparticle sample extracts were added to the respective wells with proper labelling. Plates were incubated for 24 hours at 37°C. A zone of inhibition was observed and measured.

One-way ANOVA was used, and SPSS version 22.0 was used. The results were statistically significant ($p<0.05$) with higher scavenging efficiency observed at 1000 ppm.

RESULTS

The color change of the Foxnut and silver nitrate mixed solution during incubation from light yellow to brown, as seen in (B), confirmed the formation of silver nanoparticles. A was before incubation, and B was after incubation (Figure 1).

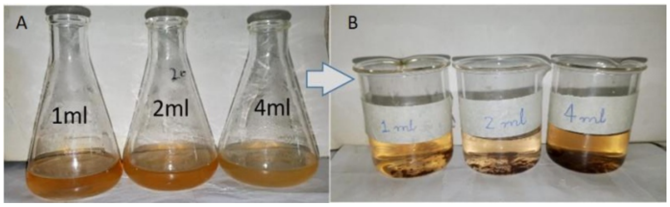

Figure 1: Color Change of the Extract Showing the Synthesis of Foxnut Nanoparticles

UV-Visible spectroscopy was used to confirm the formation of silver nanoparticles prepared using roasted foxnut (*Euryale ferox*) extract. The UV-Visible spectra showed a clear absorption peak between 420–450 nm, which is a typical peak for silver nanoparticles. This peak confirmed that silver ions were successfully changed into silver nanoparticles. However, the extract and the salt solution (silver nitrate) did not show any peaks in this region (Figure 2).

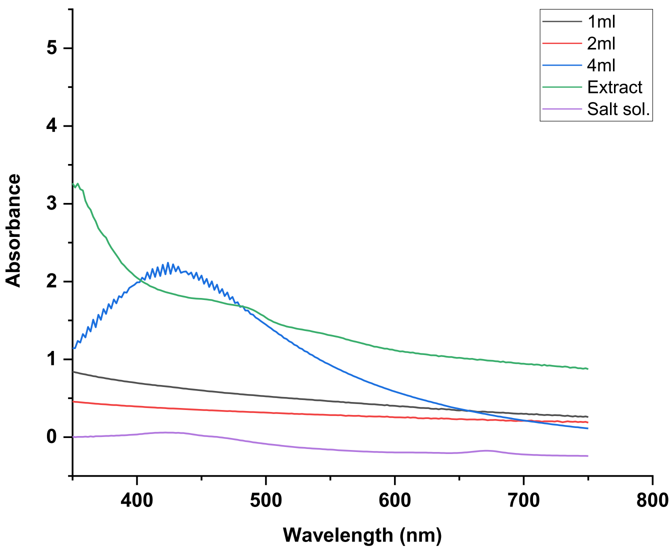

Figure 2: UV-Visible Analysis of Fox Nut Nanoparticles

FTIR spectra of the extract of Foxnut and the synthesized Foxnut nanoparticles are provided. The two spectra show that both contain strong peaks, showing that different phytochemicals are present, which are reduced and stabilized during the process of producing nanoparticles. The Foxnut extract spectrum (b) shows a broad absorption band in the area of about 33003400 cm-1, a noticeable peak in the range of 16001700 cm-1, and other peaks in the range of 10001200 cm-1. In comparison, the Foxnut nanoparticle spectrum (a) showed similar functional group characteristics; however, slight peak shifts and variations in intensity were evident. The broad O-H stretching band near ~3300 cm^{-1} remained detectable but appeared less intense in the nanoparticle spectrum, whereas the carbonyl peak around 1600–1700 cm^{-1} became sharper and showed mild shifting (Figure 3).

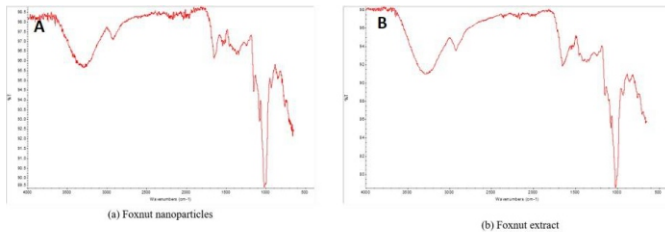

Figure 3: FTIR Spectra Showing the Functional Groups Present in (A) Foxnut Nanoparticles and (B) Foxnut Extract

Using formula mentioned in methods section, percent scavenging was calculated. One-way ANOVA was applied between two concentrations (500 ppm and 1000 ppm) absorbance and blank absorbance (DPPH), which showed significant antioxidant activity with a p-value of <0.01, i.e., less than 0.05 (Table 1).

Table 1: DPPH Free Radical Scavenging Activity of Foxnut Nanoparticles

Nanoparticles Concentration (ppm)	Absorbance of Sample	Blank (DPPH) Absorbance	DPPH Scavenging Activity	p-value
500 ppm	0.588 ± 0.001	0.653	9.95%	<0.01
1000 ppm	0.272 ± 0.001		58.38%	<0.01

Antibacterial activity shows that the nanoparticles exhibited antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*, as shown by clear zones of inhibition around wells containing nanoparticle solutions. For DPPH analysis, two concentrations of foxnut nanoparticles, 500 ppm and 1000 ppm, were analyzed for their free radical scavenging potential using the DPPH assay. A visible color change was observed during the assay. The 500-ppm sample showed only a slight fading of the purple color of DPPH (a), whereas the 1000 ppm sample showed a more pronounced color fading. Overall, both samples demonstrated antioxidant potential; the 1000 ppm sample exhibited significantly higher scavenging efficiency (58%). Ascorbic acid served as the positive control, showing an IC50 of 0.01 mg/ml (Figure 4).

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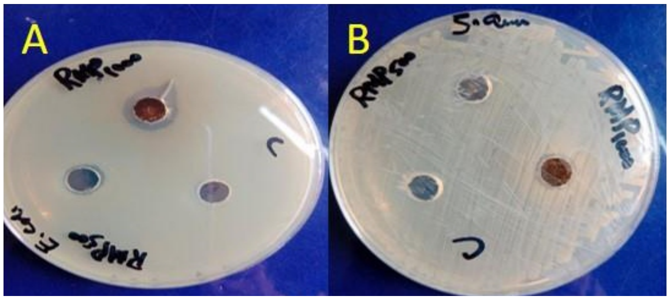

Figure 4: Zones of Inhibition of Foxnut Nanoparticles Against (a) *E. coli* and (b) *S. aureus*

Zones of inhibition were larger at 1000 ppm (17mm and 16mm), indicating a stronger antibacterial effect at the higher concentration, compared to 500 ppm. Negative control (Methanol) showed negligible zones, whereas Ciprofloxacin (20µg/ml) showed the highest zone of inhibition comparatively (Table 2).

Table 2: Zones of Inhibition in (mm)

Samples	Zones of Inhibition in mm	
	E. Coli	*S. aureus*
500ppm	11	09
1000ppm	17	16
Methanol	00	00
Ciprofloxacin	21	19

DISCUSSION

In this green synthesis process, the fox nut extract was used as a natural reducing and stabilizing agent. In form concordance to another study, the evidence of the current study revealed that silver nanoparticles had been effectively produced through a green process, which was evident in the change of the visible color of the solution from light yellow to brown. The color change was caused by surface plasmon resonance, which means that silver ions were reduced to silver nanoparticles [13, 14]. These findings are similar to a recent original research article published in ACS Omega (2024), where silver nanoparticles were also synthesized using plant extracts and showed a dark brown color as confirmation of nanoparticle formation and successful synthesis [15]. The comparative FTIR analysis confirms that the phytochemicals found in the Foxnut extract had a dual role to play, as both reducing and stabilizing agents in the synthesis of nanoparticles. The OH stretching vibrations of alcohols and phenolic compounds are at 3300 -3400 cm-1, corresponding to the foxnut extract. These hydroxyl-containing groups are commonly associated with polyphenols and flavonoids naturally present in plant extracts [16]. A noticeable peak in the range of 1600–1700 cm^{-1} represents C=O stretching of carbonyl groups, confirming the presence of aldehydes, ketones, and other secondary metabolites. Additional peaks in the region of 1000–1200 cm^{-1} correspond to C–O stretching vibrations of alcohols, ethers, and ester groups. However, less intense OH peaks in the case of foxnut nanoparticles suggest that hydroxyl groups were involved in the reduction of metal ions and subsequent stabilization of nanoparticle surfaces. The carbonyl peak around 1600–1700 cm^{-1} became sharper and less shifted, which indicates possible binding interactions between carbonyl-containing biomolecules and the nanoparticle surface. Furthermore, the bands around 1000–1200 cm^{-1} persisted, supporting the role of C–O containing phytochemicals as capping agents [17]. The visual color change in the DPPH Assay further supports the spectrophotometric data by indicating stronger free radical scavenging activity at higher nanoparticle concentration. However, the 1000 ppm sample exhibited significantly higher scavenging efficiency (58%), suggesting that foxnut nanoparticles could serve as promising antioxidant agents at higher concentrations. This could be due to the presence of high levels of phenolic and flavonoid compounds, which are further concentrated and stabilized during roasting and nanoparticle formation, allowing efficient antioxidant activity [18]. A concentration-dependent antibacterial response of the nanoparticles was depicted in the antibacterial assay. In contrast, wells containing methanol (negative control) showed no clear zones of inhibition, confirming that the observed antibacterial effects were due to the nanoparticles and not the solvent. Overall, the nanoparticles were effective against both *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). Literature shows that Gram-negative bacteria appear to be more sensitive, likely due to a thicker cell membrane and negatively charged teichoic acids [19]. Silver nanoparticles cause damage to the plasma membrane of bacterial cells, silver ions and nanoelements accumulate and destabilize the cell, increasing the membrane permeability, contents of the cell are released, and cause cell death. Moreover, this activity may be attributed to the presence of bioactive secondary metabolites, particularly polyphenols and flavonoids, which are known to exert antibacterial effects by interacting with bacterial cell walls and inhibiting key biological functions [20].

There are some limitations. This study could not perform SEM, Zeta potential, and XRD analysis of synthesized nanoparticles due to funding limitations; however, this work may be directed in the future towards advanced characterization. Ic50 values of roasted foxnut-mediated silver nanoparticles in antioxidant and antimicrobial assays should be calculated to lead them towards in vivo assays.

CONCLUSION

The present study successfully demonstrated the green synthesis of silver nanoparticles using roasted foxnut

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(*Euryale ferox*) extract. UV-Visible analysis suggested 4ml of foxnut extract to be more effective in making silver nanoparticles. FTIR analysis suggested that hydroxyl, carbonyl, and amine groups present in roasted Fox nut extract served as stabilizing and capping agents in the synthesis of nanoparticles. The biological evaluation revealed that the synthesized nanoparticles possess significant, i.e., 58%, antioxidant activity as confirmed by DPPH free radical scavenging assay validated by statistical findings. However, it needs to be evaluated in detail with IC50 calculation and in vivo assays. The antibacterial analysis showed a concentration-dependent antibacterial effect. against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Therefore, it may be concluded that the roasting process altered the phytochemical profile of fox nut, potentially giving rise to silver nanoparticles with biomedical applications.

Authors' Contribution

Conceptualization: BK
Methodology: ANM, AAK
Formal analysis: NF, ML, AA
Writing and Drafting: BK, AAK, NF, ML, MA
Review and Editing: BK, ANM, AAK, NF, ML, MA, AA

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

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