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Amrit Research Journal December 2024, Vol. 5, Issue 1, 82-90

ISSN: 2738-9553
RESEARCH ARTICLE

Green Synthesis of Silver Nanoparticles from *Ocimum sanctum* Linn. and Study of Their Antioxidant Activity

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(Received: August 21, 2024; Received in Revised Form: November 9, 2024; Accepted: November 12, 2024; Available online)

DOI: https://doi.org/10.3126/arj.v5i1.73553

Highlights

- Methanol extract of *Ocimum sanctum* Linn prepared and phytochemically tested.
- Silver nanoparticles were green synthesized using methanol extract.
- FESEM, EDS, and XRD analysis confirm successful synthesis of OCE-AgNPs.
- OCE-AgNPs exhibit remarkable antioxidant activity.

Abstract

Green synthesis of silver nanoparticles (AgNPs) has gained considerable attention due to their unique properties and wide-ranging applications. This study focuses on the synthesis of AgNPs utilizing the methanol extract of aerial parts of *Ocimum sanctum* Linn., commonly known as holy basil or Tulsi. The synthesis process is environmentally friendly and offers a sustainable alternative to conventional methods. Characterization of the synthesized AgNPs is performed using various techniques, including field emission scanning electron microscopy (FESEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD). The results demonstrate the successful synthesis of AgNPs with distinct morphologies and crystalline structures. Additionally, the antioxidant activity of the AgNPs is evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, with ascorbic acid as a reference standard. The synthesized OCE-AgNPs exhibit an IC50 value of 49.71 µg/mL, which is close to that of the standard ascorbic acid (41.34 µg/mL). The results of this study highlight the potential applications of OCE-AgNPs in biomedical and pharmaceutical fields.

Keywords: *Silver nanoparticles, Ocimum sanctum, green synthesis, antioxidant activity, DPPH assay*

Introduction

Silver nanoparticles (AgNPs) have emerged as promising materials in various fields, including medicine, electronics, and catalysis, owing to their unique physical, chemical, and biological properties. [1], [2], [3] These nanoparticles are characterized by their high surface area-to-volume ratio, exceptional electrical and thermal conductivity, high antioxidant activity, and potent antimicrobial properties, making them highly valuable for a myriad of applications. [4], [5] In particular, AgNPs are being explored for their potential in drug delivery systems, diagnostic tools, and as antioxidant and antimicrobial agents in medical

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devices. [6], [7] The increasing demand for multifunctional nanomaterials has driven extensive research into optimizing their synthesis and functionalization.

Ocimum sanctum, commonly known as holy basil or tulsi, is a medicinal plant revered in traditional medicine, particularly in Ayurveda, for its extensive health benefits. [8], [9] This plant is rich in various phytochemicals, including flavonoids, alkaloids, phenolic acids, and essential oils, which confer diverse biological activities such as antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. [10], [11] The high antioxidant value is particularly noteworthy, as it is crucial in neutralizing free radicals. [12], [13], [14], [15] Free radicals are unstable molecules that can cause oxidative stress, leading to cellular damage and contributing to the development of chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders. [16], [17] By scavenging these free radicals, the antioxidants in *O. sanctum* help in preventing oxidative stress and its associated health issues.[18] Moreover, the plant's anti-inflammatory properties aid in reducing inflammation, while its antimicrobial properties help in combating various pathogens, making it a versatile and highly valued plant in traditional and modern medicine. [19], [20]

In recent years, there has been growing interest in the green synthesis of AgNPs using plant extracts. This method offers several advantages over conventional chemical synthesis, which often involves toxic solvents, hazardous reducing agents, and complex procedures. [21], [22] Green synthesis is not only cost-effective and eco-friendly but also scalable, making it suitable for large-scale production. Plant extracts serve as natural reducing and stabilizing agents, simplifying the process and eliminating the need for harmful chemicals. [23] This approach leverages the inherent biological activity of the plant extracts, which can enhance the functional properties of the synthesized nanoparticles. The use of plant-based synthesis aligns with the principles of sustainable development, reducing the environmental footprint of nanomaterial production. Additionally, green synthesis methods contribute to the circular economy by utilizing renewable resources and generating less hazardous waste. This environmentally benign approach also opens the possibility of integrating traditional medicinal knowledge with modern nanotechnology, potentially leading to the discovery of novel therapeutic agents and treatments. The integration of green chemistry principles in nanoparticle synthesis is a significant step forward in achieving sustainable and responsible nanotechnology development. All in all, the utilization of *O. sanctum* for the green synthesis of AgNPs not only takes advantage of the plant's rich phytochemical profile and diverse biological activities but also aligns with the global shift towards more sustainable and eco-friendly synthetic processes. This holistic approach not only enhances the therapeutic potential of AgNPs but also underscores the importance of integrating traditional medicinal knowledge with cutting-edge nanotechnology.

Taking into consideration of the above-discussed advantages, we utilized methanol extract of aerial parts of *O. sanctum* for the green synthesis of silver nanoparticles (AgNPs). The synthesized nanoparticles were extensively characterized and evaluated for their antioxidant activity. The extract served as a natural reducing and stabilizing agent in the synthesis process. The IC50 value of OCE-AgNPs is comparable to that of L-ascorbic acid, demonstrating its potent antioxidant efficacy. This study confirms the successful formation of AgNPs with significant antioxidant properties, highlighting the potential of *O. sanctum* in eco-friendly nanoparticle synthesis and suggesting promising applications in biomedical research and healthcare

Experimental Section

Chemicals and plant materials

Silver nitrate ($AgNO_3$), L-Ascorbic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and methanol were purchased from Sigma–Aldrich Co., St. Louis, MO, USA. Deionized water was utilized in the present analysis. Fresh and healthy aerial parts of the experimental plant, *Ocimum sanctum L.* were collected from Dhading, Nepal. The plant parts were thoroughly rinsed with tap water, followed by distilled water, to remove dust particles, pathogens, and adhered matter. After that, it was shade-dried for 4 days and ground to a fine powder.

Preparation of the *O. sanctum* extract

The grounded powder was subjected to a cold percolation technique using methanol as the solvent (500 mL × 24 hours × 5 cycles). The methanol extract was then concentrated using a rotary evaporator. The concentrated extract was dried and stored in a sealed glass vial in a refrigerator until further experimentation.

Green synthesis of silver nano particles

Silver nanoparticles were synthesized using a slightly modified version of the previously reported methods. [24], [25], [26] Initially, the biological reduction of $AgNO_3$ was performed as follows: 3 mL of plant extract was added to 2 mL of 0.02 M $AgNO_3$ solution, and the volume was adjusted to 20 mL with deionized water in a 50 mL volumetric flask. This mixture was kept at ambient temperature (25±0.5 °C) for 24 hours. The mixture's color transitioned from light to yellowish, then to reddish, and finally to colloidal. This color change was monitored periodically. After the synthesis and completion of the reaction, the solution was centrifuged at 10,000 rpm for 20 minutes and the silver nanoparticles were collected. The as-synthesized *O. sanctum* extract-derived silver nanoparticles were abbreviated as OCE-AgNPs.

Phytochemical screening

The methanol extract (1 g) was completely dissolved in 100 mL of methanol to prepare a stock solution. This stock solution was then used for phytochemical screening, following established protocols with slight modifications. [13], [27], [28]

Physical characterization

The morphology and the constituent elements of the synthesized OCE-AgNPS were studied using a Field Emission Scanning Electron Microscopy (FESEM, Hitachi, Japan) equipped with an energy dispersive spectrometer (EDX). The crystallinity of the nanoparticles was studied with an X-ray diffractometer (Rigaku Corporation, Japan).

Antioxidant activity test (DPPH radical scavenging activity)

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the methanol extract and OCE-AgNPs was assessed following our previous study with slight modifications.[29], [30], [31], [32] DPPH (7.886 g) was dissolved in 100 mL methanol to prepare a 0.2 mM solution. Various concentrations (10, 30, 50, 70, 90, and 100 µg/mL) of methanol extract, OCE-AgNPs, and ascorbic acid (positive control) were tested. Each concentration (2 mL) was mixed with 2 mL of 0.2 mM DPPH solution, and absorbance was measured at 517 nm using a UV-Visible spectrophotometer (Shimadzu UV professional double beam) after 30 minutes. The experiment was performed in triplicate, and radical scavenging activity was calculated using the equation 1:

$$\% \text{ radical scavenging activity} = [(Abs._{control} - Abs._{sample}) / Abs._{control})] \times 100\% \quad (1)$$

where, $Abs._{control}$ is the absorbance of the control (1 mL MeOH + 0.5 mL DPPH) and $Abs._{sample}$ is the absorbance of the sample. IC50 values, representing the concentration needed to scavenge 50% of free radicals, were determined from concentration versus percentage scavenging activity curves for each sample.

Results and Discussion

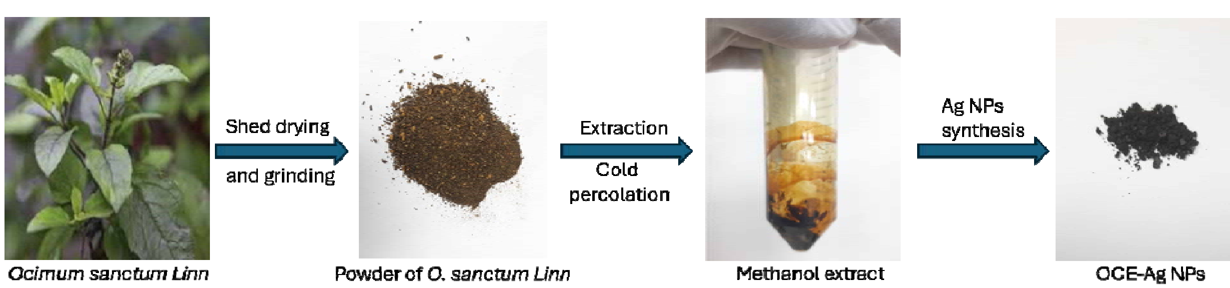

Fig 1. Digital photographs of the synthetic steps involved in the preparation of OCE-AgNPs.

Figure 1 depicts each step involved in the synthesis of the OCE-AgNPs. As illustrated in the figure, cleaned and dried aerial parts of the *O. sanctum* were shed dried, and ground into powder. The methanol extract was extracted from the dried powder and finally, Ag NPs were synthesized using the as-prepared extract. Phytochemical screening of methanol extract of *O. sanctum Linn.* was carried out according to the standard procedure. The results indicated that the extract is rich in flavonoids and polyphenols, along with the presence of alkaloids, terpenoids, tannins, and steroids. The high content of flavonoids and polyphenols suggests that the plant has significant antioxidant potential. However, the extract lacks glycosides, quinones, proteins, and saponins.

Table 1. Phytochemical screening of the methanol extract

S.N.	Class of compounds	Methanol extract
1	Alkaloids	+
2	Flavonoids	++
4	Terpenoids	+
5	Glycosides	-
6	Quinones	-
7	Polyphenols	++
8	Tannins	+
9	Steroids	+
10	Proteins	-
11	Saponins	-

(+) indicates present and (-) indicates absent.

FESEM analysis (**Figure 2a-d**) revealed the formation of almost spherical AgNPs with a uniform size distribution. The EDX color mapping images and EDX spectrum (**Figure 3a-k**) confirmed the homogeneous distribution of elemental silver, along with other elements such as C, N, O, Mg, Cl, and K, which are originated from the plant extract. The XRD analysis (**Figure 4**) identified the crystalline nature of the synthesized AgNPs, which perfectly matched the JCPDS card of Silver (PDF#04-0783). The characteristic peaks at 38.12 º, 44.27 º, 64.43 º, 77.47 º, and 81.54 º corresponding to the (111), (200), (220), (311), and (222) planes of face-centered cubic (fcc) silver nanoparticles. Furthermore, the broad peak at around 25 º is due to the (002) peak of carbon which arises from the secondary metabolites present in the plant. Furthermore, the carbon content as seen in the XRD peaks is supported by the intense carbon peak and elemental color mapping in the EDX results.

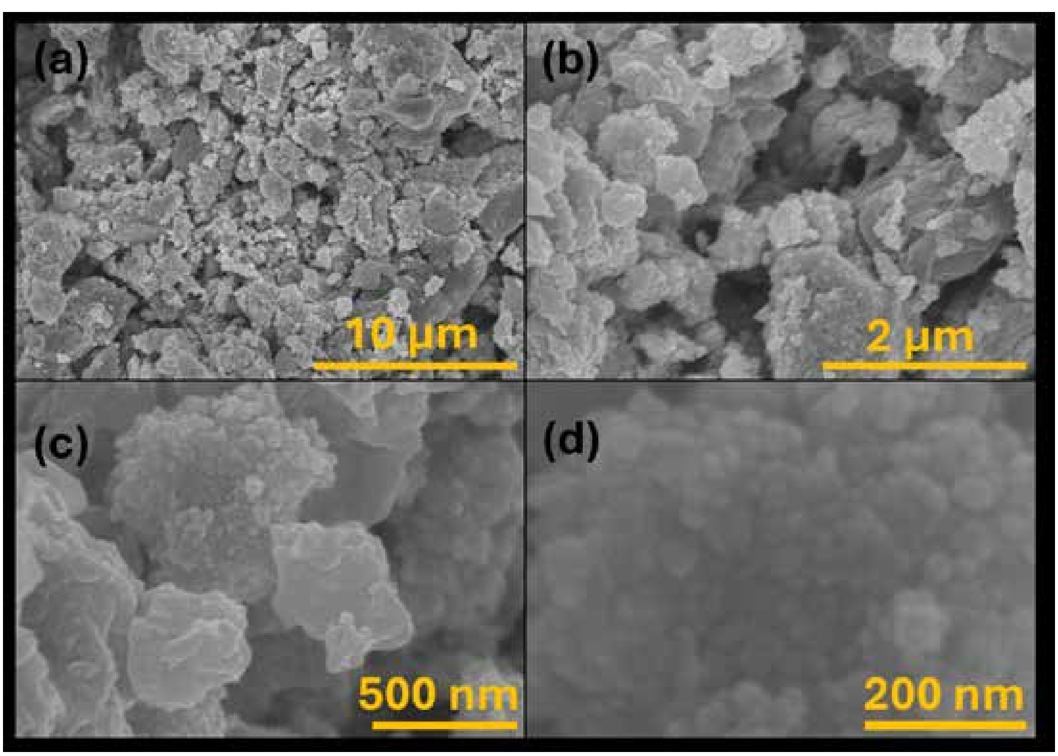

Fig 2. (a-d) OCE-Ag NPs at increasing magnifications.

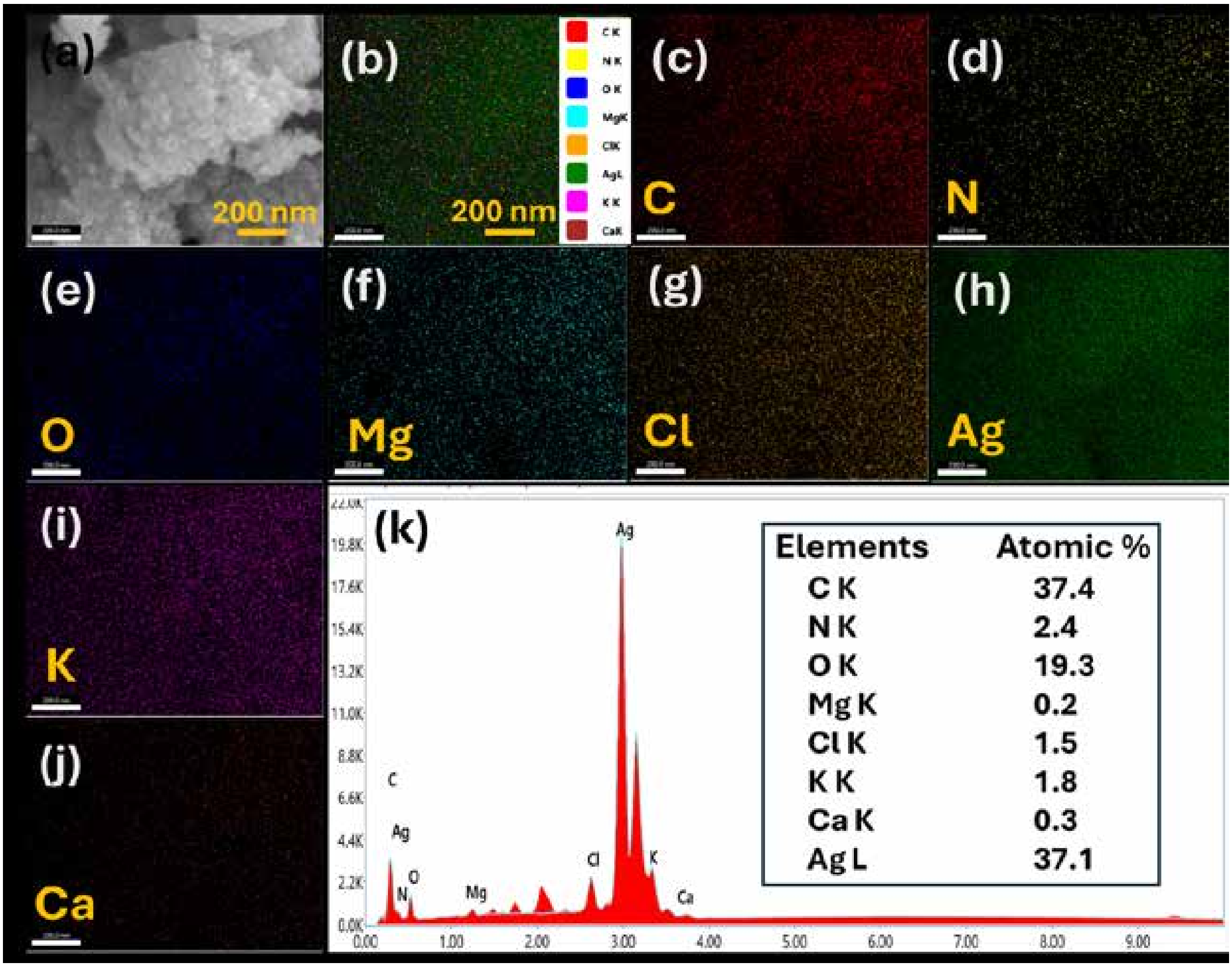

Fig 3. EDX analysis of OCE-Ag NPs (a) EDX color mapping area, (b) superimposition of all elements. EDX color mapping image for (c) C, (d) N, (e) O, (f) Mg, (g) Cl, (h) Ag, (i) K, and (j) Ca. (i) EDX spectrum (inset: element percentage).

The DPPH assay demonstrated that the synthesized OCE-AgNPs exhibited significant antioxidant activity, as evidenced by their ability to scavenge DPPH radicals in a concentration-dependent manner. The antioxidant activity of OCE-AgNPs was found to be comparable to that of ascorbic acid, indicating their potential as natural antioxidants.

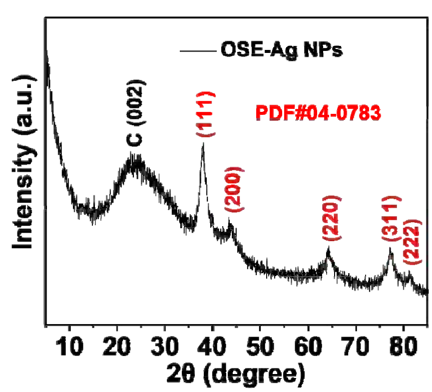

Fig 4. XRD pattern of OCE-Ag NPs.

A DPPH assay was conducted to analyze the antioxidant activity of methanol extract and OCE-AgNPs, using ascorbic acid as a standard reference. Different concentrations of methanol extract, OCE-AgNPs, and ascorbic acid were incubated at room temperature, and their absorbance was measured with a spectrophotometer. The percentage of free radical scavenging and the IC50 values for each samples were calculated and are presented in **Table 2** and **Figure 5**.

Table 2: DPPH free radical scavenging activity and IC_{50} values of different samples.

Concentration (µg/mL)	% free radical scavenging activity		
	Methanol extract	OCE-Ag NPs	Ascorbic acid
0	0	0	0
10	27.36	42.69	48.57
30	40.2	53.72	57.14
50	48.61	58.62	62.85
70	53.61	63.52	67.14
90	56.11	67.3	74.28
110	57.75	69.89	77.14
IC_{50} value(µg/mL)	72.78	49.71	41.34

values are expressed as mean ± SD (n=3)

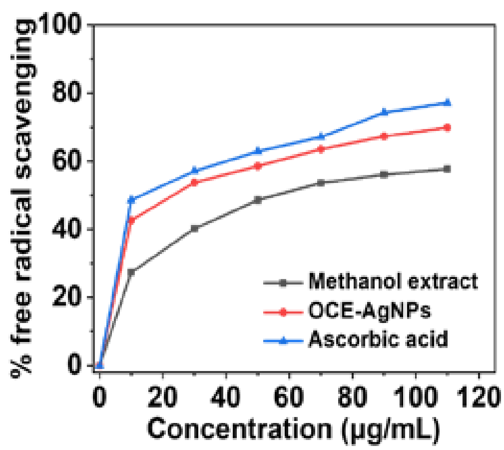

Fig 5. % radical scavenging activity of methanol extract, OCE-AgNPs and ascorbic acid at different concentrations.

From the calculations, the inhibitory concentration for 50% inhibition (IC50) value of OCE-AgNPs is 49.71 **µg/mL**, which is close to that of standard ascorbic acid (41.34 µg/mL). The IC50 value of methanol extract is 72.78 **µg/mL**, substantially lower than that of both OCE-AgNPs and ascorbic acid. The closer the IC50 value of a sample is to that of ascorbic acid, the higher its antioxidant activity. The antioxidant activity of OCE-AgNPs is highly effective, being comparable to or even surpassing the results reported in earlier studies on *Ocimum sanctum* extracts and green-synthesized silver nanoparticles. This highlights the enhanced potential of OCE-AgNPs, which combine the bioactive properties of the plant extract with the unique capabilities of silver nanoparticles for superior free radical scavenging.[8], [33], [34], [35], [36] The presence of secondary metabolites such as flavonoids and polyphenols, which have the ability to scavenge free radicals, might explain the antioxidant activity exhibited by the OCE-AgNPs. [5], [37] Furthermore, the close IC50 value of OCE-AgNPs to that of ascorbic acid can be attributed to several factors. First, silver nanoparticles themselves can exhibit antioxidant properties. Second, when synthesized with plant extracts, the nanoparticles are often capped and stabilized by phytochemicals from the plant, potentially enhancing their antioxidant capacity. Third, the combination of silver nanoparticles with flavonoids, polyphenols, and other bioactive compounds from the extract can lead to higher antioxidant activity compared to the extract alone.[38]

Conclusions

In conclusion, this study successfully synthesized AgNPs using the leaf extract of *Ocimum sanctum* via a green and sustainable approach. The synthesized OCE-AgNPs exhibited distinct morphologies, crystalline structures, and notable antioxidant activity, highlighting their potential for applications in various fields, including the biomedical and pharmaceutical field. Further research is warranted to explore the therapeutic efficacy, toxicity, and stability of OCE-AgNPs for practical applications.

Acknowledgments:

The authors acknowledge the Department of Forests and Soil Conservation, Babarmahal, Kathmandu, for providing the laboratory facilities to conduct this research.

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