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Green synthesis of silver nanoparticles from *Euphorbia milii* plant extract for enhanced antibacterial and enzyme inhibition effects

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ABSTRACT

Objectives: Silver nanoparticles (AgNPs) are gaining increasing attention in biomedical applications due to their unique properties. Green synthesis methods are environmentally friendly and have demonstrated potential for AgNP production. This study explores the green synthesis of AgNPs using the methanolic extract of *Euphorbia milii*, a plant known for its medicinal properties. The primary objectives of this research were to synthesize AgNPs using *E. milii* extract, characterize the nanoparticles (NPs) using various techniques, and evaluate their antibacterial and enzyme inhibitory activities.

Methods: *E. milii* plant extract was utilized for the green synthesis of AgNPs. The characterization of the NPs was performed through ultraviolet-visible spectroscopy (UV-Vis), Fourier-transform infrared spectroscopy, scanning electron microscopy, and energy-dispersive X-ray spectroscopy (EDX). Antibacterial activity was assessed against *Staphylococcus aureus*, while enzyme inhibitory assays were conducted against urease, α-glucosidase, carbonic anhydrase II, and xanthine oxidase.

Results: The synthesized AgNPs exhibited significant antibacterial effects, with a remarkable 20-mm zone of inhibition against *S. aureus*, surpassing the efficacy of the plant extract alone. Furthermore, the AgNPs demonstrated remarkable enzyme inhibition, achieving impressive percentages of 77.98% against α-glucosidase and 88.54% against carbonic anhydrase II. Half-maximal inhibitory concentration values for enzyme inhibition were highly promising, including 78.09 ± 1.98 μM for α-glucosidase, 0.22 ± 0.10 μM for carbonic anhydrase II, and 7.11 ± 0.55 μM for xanthine oxidase.

Conclusion: In this study, AgNPs were successfully synthesized using *E. milii* extract and characterized using various techniques. The AgNPs exhibited significant antibacterial and enzyme-inhibitory activities, showcasing their potential for biomedical applications.

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Introduction

Nanoparticles (NPs) are an emerging field in today's era with a wide range of applications.[1-3] Silver NPs (AgNPs) have appeared as a promising class of NPs with wide applications in a variety of fields, including medicine, electronics, and environmental remediation.[4-7] Their unique characteristics, like their high surface-to-volume ratio and enhanced reactivity, contribute to their exceptional antimicrobial and enzyme inhibitory activities. These properties have attracted significant attention in the search for novel therapeutics and catalysts.[8] However, the conventional methods of synthesizing AgNPs often involve the use of toxic chemicals and high-energy processes, which can have adverse effects on both the environment and living things.[9,10]

To address these concerns, the field of green synthesis has gained prominence as an eco-friendly and sustainable alternative for the fabrication of NPs.[11] Green synthesis involves the utilization of natural resources, such as plant extracts, for the synthesis of NPs, in which these extracts are used as stabilizing and reducing agents.[12] Plant extracts possess a rich repertoire of bioactive compounds, including polyphenols, flavonoids, and terpenoids, which can do the reduction of metal ions and provide stability to the resulting NPs.[13-15] Moreover, green synthesis offers several

advantages, including low cost, ease of scalability, and reduced environmental impact.[16]

Euphorbia milii, commonly known as the crown of thorns, is a medicinal plant widely distributed in tropical and subtropical regions.[17] It has been used conventionally for its therapeutic properties, including wound healing, anti-inflammatory, and antimicrobial activities.[18-20] The presence of bioactive compounds in *E. milii* makes it a promising candidate for the green synthesis of AgNPs and their biological activities.[19] Various medicinal plants have been used for the green synthesis of AgNPs.[15,21,22] The strong reducing capability and stabilizing potential of *E. milii* plant extract, AgNPs can be synthesized in a sustainable and environmentally friendly manner.

The novelty of this work is to synthesize AgNPs using the methanolic extract of the *E. milii* plant. Green synthesis represents an innovative and eco-friendly approach, utilizing natural extracts as reducing and stabilizing agents for NP formation and avoiding the use of harmful chemicals prevalent in conventional synthesis methods. The rationale behind selecting *E. milii* lies in its abundance of bioactive compounds that may play a pivotal role in the reduction and stabilization of silver ions, leading to the formation of NPs with enhanced biomedical applications.

In this study, we aim to explore the ecofriendly green synthesis of silver NPs using the methanolic extract of *E. milii* and evaluate their antibacterial and enzyme inhibitory activities. The green synthesized AgNPs will be characterized by ultraviolet (UV), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX). The bactericidal activity of the AgNPs will be assessed against the pathogenic bacterium *Staphylococcus aureus*, a common cause of various infections. In addition, the enzyme inhibitory potential of the AgNPs will be investigated against key enzymes, including urease, α-glucosidase, carbonic anhydrase II, and xanthine oxidase.

Experimental

Plant collection identification and extract preparation

E. milii is an ornamental plant cultivated for decorative purposes and was gifted by Dr. Abdur Rauf of the Department of Chemistry at the University of Swabi, Khyber Pakhtunkhwa, Pakistan. The plant was identified and confirmed by Dr. Abdur Rashid, a taxonomist, and a voucher specimen (UOP-545) was deposited in the herbarium of the Department of Botany at the University of Peshawar, Pakistan. The plant material, comprising leaves and stems, was carefully selected and collected in a sterile container. Upon collection, the plant material was transported to the laboratory and washed thoroughly with distilled water to remove any surface contaminants. Subsequently, the plant material was air-dried under shade to preserve its bioactive components, as reported in the literature.[23,24]

Grinding and extraction

The dried plant material was ground into a fine powder. The obtained powder was stored in an airtight container for further use. For the extraction process, 10 g of the powdered plant material was added to 100 mL of methanol in a round-bottom flask. The flask was then sealed and subjected to periodic stirring at room temperature for 12 h. After the extraction period, the mixture was filtered using a filter paper to obtain the methanolic extract of *E. milii*.

Synthesis of AgNPs

The green synthesis of AgNPs was carried out using the methanolic extract of *E. milii,* as described in the literature.[23,25,26] Briefly, 10 mL of the extract was mixed with 90 mL of aqueous silver nitrate ($AgNO_3$) solution at a concentration of 1 mM. The mixture was stirred continuously at room temperature for several hours until a color change from pale yellow to dark brown was seen, showing the reduction of silver ions and the formation of AgNPs.[27]

Instrumental characterization

The synthesized AgNPs were characterized extensively using different techniques to assess their size, morphology, and elemental composition. UV spectroscopy was performed by recording the absorbance spectrum of the AgNPs using a UV-visible spectroscopy (UV-Vis) spectrophotometer. The FTIR KBr pellet method was performed to obtain spectra to identify the functional groups present in the plant extract and their potential role in AgNP stabilization and reduction. SEM was utilized to examine the morphology and texture of the AgNPs. EDX analysis was conducted to check the elemental makeup of the AgNPs.

Bactericidal activity

The antibacterial activities of the synthesized AgNPs were assessed against the pathogenic bacterium *S. aureus* using the agar-well diffusion method. Sterile agar plates were prepared, and wells were created using a sterile cork borer. A standardized suspension of *S. aureus* was spread uniformly on the agar surface. Subsequently, 100 µL of the synthesized AgNPs solution was employed to the wells. The plates were incubated at the appropriate temperature for 24 h, and the inhibition zones were measured to evaluate the antibacterial activity of the AgNPs.[28]

Enzyme inhibitory activity

The enzyme inhibitory capabilities of the synthesized AgNPs were examined against selected enzymes, including urease, α-glucosidase, carbonic anhydrase II, and xanthine oxidase.

For each enzyme, the appropriate assay method was followed to evaluate the inhibitory effects of the AgNPs.[29-32] The percentage inhibition was calculated by comparing the enzyme activity in the presence and absence of the AgNPs. The half-maximal inhibitory concentration (IC_{50}) values, representing the concentration required to inhibit 50% of enzyme activity, were determined using a suitable concentration range of the AgNPs.

Statistical analysis

The findings in this study are presented as the mean value with the corresponding standard error of the mean (SEM) to assess statistical significance ($P < 0.05$ or 0.01). Statistical analyses were conducted using the GraphPad Prism software.

Results

Biogenic synthesis of AgNPs

In this study, the methanolic extract of the *E. milii* plant was employed as the green reducing agent for the synthesis of AgNPs. The plant extract contains a rich repertoire of bioactive compounds, such as flavonoids, polyphenols, and terpenoids, which can serve as potent reducing agents capable of converting silver ions (Ag^+) into AgNPs (Ag^0). The process is initiated by mixing the plant extract with an aqueous silver nitrate ($AgNO_3$) solution. The bioactive compounds in the extract interact with Ag^+ ions, leading to the reduction of Ag^+ to Ag^0. This reduction reaction is followed by the nucleation and growth of Ag^0 particles, ultimately resulting in the formation of AgNPs. In addition, the various functional groups found in the plant extract play a crucial role in stabilizing the synthesized AgNPs, preventing their aggregation, and maintaining their colloidal stability.[33]

Characterizations

UV-Vis spectroscopy

UV-Vis spectroscopy is a fundamental technique used to analyze and confirm the formation of NPs. In this study, the synthesized AgNPs exhibited a characteristic peak at 430 nm, which confirmed the successful synthesis of AgNPs. The absorption peak observed in the UV-Vis spectrum relates to the surface plasmon resonance (SPR) of the AgNPs. The SPR phenomenon is a result of the collective oscillation of conduction electrons in the NPs on excitation by light. The position and intensity of the SPR peak can provide valuable information about the size, shape, and aggregation state of the NPs. In our case, the observed peak at 430 nm suggests the presence of well-dispersed AgNPs with a particular size and shape, as shown in Figure 1a. The UV-Vis spectroscopy results are consistent with previous studies on AgNPs, where the SPR peak in the range of 400–450 nm is typically observed.[34,35] The specific peak observed in our study further confirms the successful formation of AgNPs using the methanolic extract of the *E. milii* plant.

FTIR spectroscopy

FTIR spectroscopy is a crucial tool for determining the functional groups present in compounds. In this study, the FTIR spectra were recorded for both the *E. milii* plant extract and the synthesized AgNPs to analyze their respective functional groups. The FTIR spectrum of both AgNPs and the plant extract exhibited some common peaks, indicating the presence of similar functional groups. A broad peak at 3406 cm^{-1} was observed in both the plant extract and AgNPs, suggesting the presence of O-H stretching vibrations of alcohol or phenolic groups. However, it is worth noting that the broadening of this peak in the AgNPs was somewhat weaker compared to the plant extract. This difference in broadness may indicate a modification in the hydrogen bonding or interactions of the functional groups upon the formation of AgNPs. Another common feature observed in both the plant extract and AgNPs was a small peak at 2044 cm^{-1}. This peak corresponds to the stretching vibrations of C≡N groups, which may indicate the presence of nitrile functional groups. In addition, a sharp peak at 1628 cm^{-1} was observed in both the plant extract and AgNPs, which can be attributed to the stretching vibrations of C=O bonds, showing the presence of carbonyl groups. In the plant extract, additional small peaks at 1377 cm^{-1} and 1257 cm^{-1} were observed, indicating the presence of C-H bending vibrations and C-O stretching vibrations, respectively. However, in the AgNPs, the intensity of these peaks increased, suggesting a potential interaction or adsorption of these functional groups on the surface of the NPs. Interestingly, a sharp and narrow peak at 1016 cm^{-1} was observed exclusively in the FTIR spectrum of AgNPs. This peak could be attributed to the stretching vibrations of metal-oxygen (Ag-O) bonds, indicating the formation of AgNPs. The FTIR spectrum of the plant extract and AgNPs is shown in Figure 1b.

SEM

SEM, also known as scanning electron microscopy, is a technique commonly used to characterize the surface features of materials. In this study, SEM imaging was employed to examine the morphology of the synthesized silver NPs in solution form. Both high- and low-resolution SEM images were captured to provide a comprehensive understanding of the AgNPs' surface characteristics [Figure 2]. The SEM images of the AgNPs revealed a rough texture and distinctive features, which can be attributed to the occurrence of the NPs in the solution. The rough surface morphology observed in the SEM images suggests the stabilization of the AgNPs, possibly due to the interaction between the NP surfaces and the surrounding medium. These interactions may involve the adsorption of stabilizing agents or the occurrence of functional groups from the plant extract used in the green synthesis process.

EDX spectroscopy

The EDX analysis of the green-synthesized AgNPs derived from *E. milii* extract revealed the occurrence of various elements. Carbon and oxygen were the predominant elements, comprising 49.09% and 38.49% of the composition,

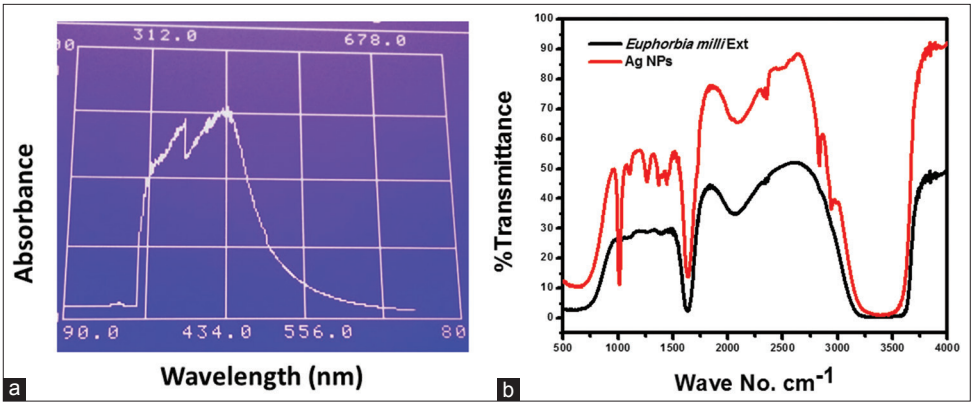

Figure 1: Ultraviolet-visible spectroscopy spectrum of AgNPs (a) and fourier-transform infrared spectroscopy spectrum of plant extract and AgNPs (b). AgNPs: Silver nanoparticles

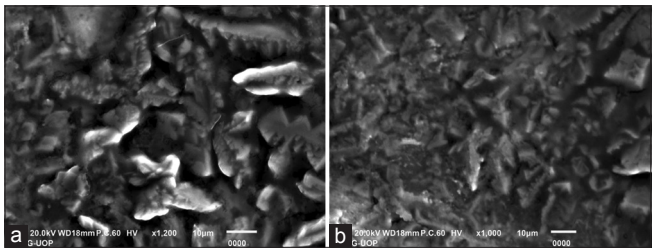

Figure 2: SEM images of silver nanoparticles. High resolution (a) and low resolution (b)

respectively, as shown in the inset of Figure 3. The presence of these elements suggests the involvement of organic compounds, potentially derived from biomolecules present in the extract. In addition, aluminum and potassium were detected in smaller amounts, indicating the occurrence of trace elements or impurities. Notably, the analysis confirmed the successful synthesis of AgNPs, as a small amount of silver (0.13%) was observed. These results highlight the potential role of organic compounds in the extract in the reduction of silver ions and subsequent NP formation.

Biological activities

The antibacterial activity of the prepared AgNPs was evaluated against the pathogenic bacterium *S. aureus* using the agar-well diffusion method. The experiment included a negative control using distilled water, a positive control using the standard antibiotic drug linezolid, and an additional control using the *E. milii* extract without AgNPs. The results of the agar-well diffusion method demonstrated the inhibitory effect of both the standard drug linezolid and the synthesized AgNPs against *S. aureus*. The zone of inhibition observed for linezolid was 25 mm, indicating its potent antibacterial activity. The AgNPs also exhibited significant antibacterial activity, with a zone of inhibition measuring 20 mm. These results indicate that the synthesized AgNPs possess promising antimicrobial properties. Furthermore, the *Euphorbia* extract without AgNPs showed a smaller zone of inhibition, measuring 18 mm. This suggests that the extract itself may possess some inherent antibacterial activity, albeit weaker than that of the synthesized AgNPs or linezolid. The negative control using distilled water did not exhibit any inhibitory effect, confirming the absence of contamination or interference. Table 1 shows the zone of inhibition in mm.

Enzymes inhibition

The enzymatic inhibitory activities of the tested extracts and AgNPs were evaluated against four enzymes: urease, α-glucosidase, carbonic anhydrase II, and xanthine oxidase. The results, including the concentration used, percentage inhibition, and IC_{50} values, are presented in Table 2.

Urease activity was significantly inhibited by both the extract and AgNPs. The extract exhibited a 45.98% inhibition at a concentration of 0.2 µg, while the AgNPs showed a higher inhibition of 49.32% at a concentration of 0.25 mM. Notably, the standard inhibitor thiourea demonstrated potent urease inhibition with 98.76% efficacy and an IC_{50} value of 22.01 ± 0.94 µM. The α-glucosidase activity was also effectively inhibited by the extract and AgNPs. The extract displayed a moderate inhibition of 33.09% at 0.2 µg concentration, while the AgNPs exhibited a significant inhibition of 77.98% at 0.25 mM concentration. The standard inhibitor, acarbose, showed robust inhibition with 95.09% efficacy and an IC_{50} value of 30.11 ± 0.97 µM.

Carbonic anhydrase II enzyme activity was notably inhibited by both the extract and AgNPs. The extract exhibited a 43.98% inhibition at a concentration of 0.2 µg, while the AgNPs showed a higher inhibition of 88.54% at a 0.25 mM concentration. The standard inhibitor, acetazolamide, demonstrated potent inhibition with 91.09% efficacy and an IC_{50} value of 0.18 ± 0.87 µM.

In the case of xanthine oxidase activity, both the extract and AgNPs demonstrated significant inhibitory effects. The extract exhibited a 59.65% inhibition at a concentration of 0.2 µg, while the AgNPs showed a higher inhibition of 84.23% at a 0.25 mM concentration. The standard inhibitor, allopurinol, displayed strong inhibition with 97.98% efficacy and an IC_{50} value of 2.76 ± 0.05 µM.

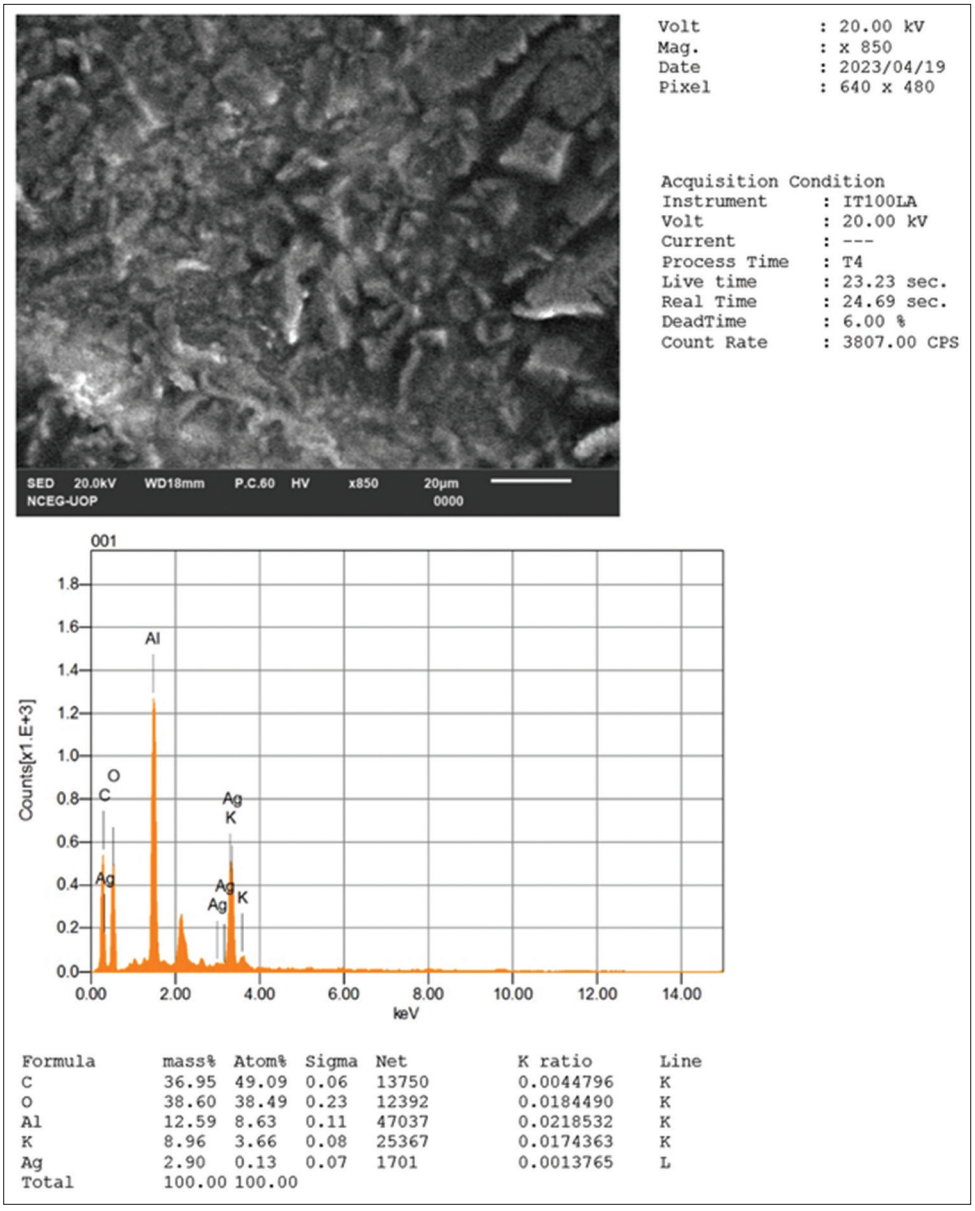

Figure 3: EDS spectrum of the synthesized silver nanoparticles

Table 1: Antibacterial activity of synthesized AgNPs, linezolid, euphorbia extract, and distilled water

Sample	Zone of inhibition (mm)
Linezolid	25
Synthesized AgNPs	20
Euphorbia extract	18

AgNPs: Silver nanoparticles

Discussion

The biogenic synthesis of AgNPs involves a sustainable and eco-friendly approach, utilizing natural extracts or biomaterials as reducing and stabilizing agents for the formation of NPs.[15,25] The outcomes of this research will shed light on the effectiveness of *E. milii* plant extract in synthesizing AgNPs with enhanced antimicrobial and enzyme inhibitory properties. The green synthesis approach offers a sustainable alternative to conventional methods, reducing the reliance on toxic chemicals and minimizing the ecological footprint.[26,33] The results from this study will contribute to the emerging body of knowledge on green synthesis techniques and broaden the scope of AgNPs' applications in biomedicine and enzyme inhibition. The green synthesis approach not only ensures the sustainable production of AgNPs but also offers the potential for enhanced biomedical applications due to the presence of bioactive components from the plant extract, which may impart additional therapeutic properties to the NPs.

This eco-friendly approach using *E. milii* plant extract represents a promising approach with potential biomedical applications. The successful synthesis and characterization

Table 2: Enzyme inhibitory activity of extracts and nanoparticles

Enzyme	Tested extract	Concentration	% Inhibition	Half-maximal inhibitory concentration (µM)
Urease	Extract	0.2 µg	45.98	-
	AgNPs	0.25 mM	49.32	-
	Thiourea	0.2 µM	98.76	22.01±0.94
α-glucosidase	Extract	0.2 µg	33.09	-
	AgNPs	0.25 mM	77.98	78.09±1.98
	Acorbose	0.2 µM	95.09	30.110.97
Carbonic anhydrase II enzyme	Extract	0.2 µg	43.98	-
	AgNPs	0.25 mM	88.54	0.22±0.10
	Acetazolamide	0.2 µM	91.09	0.18±0.87
Xanthine oxidase	Extract	0.2 µg	59.65	72.01±0.66
	AgNPs	0.25 mM	84.23	7.11±0.55
	Allopurinol	0.2 µM	97.98	2.76±0.05

AgNPs: Silver nanoparticles

of AgNPs were achieved through the reduction of silver ions (Ag^+) by the bioactive compounds present in the plant extract. The presence of various functional groups in the extract played a crucial role in stabilizing the AgNPs and preventing their aggregation.[23,36] The synthesized AgNPs displayed a characteristic peak at 430 nm in the UV-Vis spectrum, confirming their successful formation and well-dispersed nature. The observed antibacterial activity of the synthesized AgNPs can be attributed to the inherent properties of AgNPs, including their high surface area, which allows for enhanced interaction with bacterial cells. The NPs may penetrate the bacterial cell wall, leading to cellular damage and inhibition of bacterial growth.[37,38] The antibacterial mechanism of AgNPs involves the release of silver ions, which exhibit antimicrobial effects by interfering with essential cellular processes. The slightly lower zone of inhibition observed for the synthesized AgNPs compared to the standard drug linezolid could be attributed to differences in their mechanisms of action and concentrations used. Linezolid is a well-established antibiotic specifically designed to target bacterial ribosomes, while AgNPs may have a broader mode of action against various cellular components. Furthermore, the AgNPs exhibited substantial inhibitory effects against enzymes such as urease, α-glucosidase, carbonic anhydrase II, and xanthine oxidase. The results indicate that the tested extracts and AgNPs possess potential enzymatic inhibitory activities. The AgNPs consistently demonstrated higher inhibitory effects compared to the extract for all enzymes tested. This could be attributed to the increased surface area and improved bioavailability of the NPs, allowing for enhanced interaction with the enzymes.[39] Furthermore, the IC_{50} values provide information on the concentration of the extract or AgNPs required to achieve 50% inhibition of the respective enzyme activity. Lower IC_{50} values indicate greater potency. Notably, some of the standard inhibitors, such as thiourea, acarbose, acetazolamide, and allopurinol, displayed higher inhibitory activities with lower IC_{50} values compared to the tested extracts and AgNPs. This highlights the potential of these compounds as reference standards for comparison. The observed enzymatic inhibitory activities of the tested extracts and AgNPs suggest their potential therapeutic applications. Inhibition of urease, α-glucosidase, carbonic anhydrase II, and xanthine oxidase is associated with various physiological processes and can be targeted for the treatment of diseases such as diabetes, cancer, and gout.

Conclusion

The green synthesis of AgNPs using the methanolic extract of the *E. milii* plant resulted in the production of NPs with significant antibacterial and enzyme inhibitory activities. The synthesized AgNPs exhibited a notable inhibition zone against *S. aureus*, surpassing the activity of the plant extract alone. Furthermore, the AgNPs demonstrated potent enzyme inhibition against urease, α-glucosidase, carbonic anhydrase II, and xanthine oxidase. These results highlight the potential of the *E. milii* plant extract for synthesizing AgNPs with valuable biomedical applications. The green synthesis approach offers an eco-friendly and sustainable method for producing NPs, making it a promising avenue for further exploration in nanotechnology and biomedical sciences.

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Conflict of Interest

The author declares no conflict of interest.

Consent for Publication

All the authors have agreed to the published version of the manuscript.

Ethical Approval

Not applicable.

Consent to Participate

Not applicable.

Availability of Data and Materials

Not applicable.

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Author Contributions

SB: conceptualization, investigation, writing-original draft, writing-review, and editing. All authors read and approved the submitted version.

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