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Antimicrobial and antioxidant properties of silver nanoparticles from *Moringa oleifera* gum: a green synthesis approach

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Abstract

Plant gums have enormous medicinal potential and have been used in the pharmaceutical and biomedical fields. In the present investigation, *Moringa oleifera gum* (MOG) was collected, and its physical properties and phytochemical composition were investigated. Silver nanoparticles (AgNPs) were produced, and characterization was carried out using UV spectroscopic analysis and scanning electron microscopy (SEM). In this study, the standard method was used for the antioxidant assay and the antimicrobial test. The synthesized nanoparticles (NPs) have irregular shapes and no fixed geometry. The agglomerate shape resembles that of pebble-like structures. UV–vis analysis proved the wavelength of the sample to be 350–470 nm. In antioxidant studies, the synthesized AgNPs exhibited significant DPPH radical scavenging activity values ranging from 21.15 ± 0.017 to 63.46± 0.03 g/mL at concentrations ranging from 100 to 500 g/mL. In an antimicrobial experiment, the maximum incubation zone was 18 mm by 100 µL of AgNPs synthesized from *M. oleifera* gum extract against *S. typhi. P. aeruginosa* expressed a 16 mm zone of incubation at 100 µL of AgNPs synthesized from *M. oleifera* gum. According to the findings of this study, AgNPs derived from *M. oleifera* gum can be employed as a lead chemical in the creation of an effective antimicrobial drug for the treatment of microbial infections. This research establishes the foundation for synthesizing AgNPs from *M. oleifera* gum and its powerful novel pharmacological applications.

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Introduction

Plant gums have tremendous therapeutic importance in pharmaceutical preparations such as tablets, lotions, suspensions, syrups, and ointments. Plant gums are made of polysaccharides that can be used in various formulations and chemical changes to improve their properties [H Zaigham *et al.,* 2019;]. Researchers are actively attempting to develop a wide range of novel synthetic and semisynthetic compounds from natural resources that are incredibly beneficial to humans and animals.

The bioactive compounds derived from plant gum can be extracted using current technological advances. *Moringa oleifera* is a widely distributed plant species used for medicinal purposes. *oleifera* comes under the Moringaceae family and is fast-growing, drought-tolerant, and readily adapted to various habitats and agricultural systems. The plant is native to northeastern India and is commonly found in tropical and subtropical regions [SJS Flora and V Pachauri, 2011;]. In the Indian vegetable industry, it holds a distinct and coherent stance. *M. oleifera* has been used as a food additive because of its high nutritional content and easy digestion of proteins, minerals, vitamins, and carotenoids [JW Fahey, 2005; K Maheshwari *et al.,* 2014; J Mehta *et al.,* 2011].

Pharmaceuticals based on metals, polymers, liposomes, and oxide nanoparticles are being researched for their therapeutic potential in many diseases, including cancer [O C Farokhzad *et al.*, 2006;]. Metal nanoparticle synthesis has emerged as an essential branch of nanotechnology, with a growing commercial demand for NPs due to their numerous applications. Researchers have been interested in AgNPs because of their unique properties. In this research, AgNPs have been characterized as antibacterial agents. Silver's antibacterial action is magnified in the form of NPs due to the increased number of NPs per unit area (increase in area/surface/volume ratio) [M Araujo *et al.*, 2020;]. In the present investigation, *Moringa oleifera gum* (MOG) was collected, and its physical properties and phytochemical composition were analyzed. The green production and characterization of AgNPs from *M. oleifera* gum were then carried out utilizing UV-spectroscopic analysis and SEM. Several studies have reported that *M.* oleifera plant parts such as leaves, stems, and seeds have shown suitable antibacterial activities [S Gupta *et al.*, 2018;]. However, because there has been no previous research on *M. oleifera* gum, the current work focuses on the antimicrobial characteristics of AgNPs generated from *M. oleifera* gum.

Materials and methods

Collection of moringa gum

The exudate gum of *Moringa oleifera* was collected from the Arunachala hills, Tiruvannamalai, Tamil Nadu, India. Thirty grams of gum material was macerated in 100 mL of distilled water using a magnetic stirrer until completely dissolved. The temperature was kept between 25 °C and 30 °C for 24 h to absorb the water. The mixture was filtered through Whatman filter paper and evaporated at ambient temperature. The prepared sample was further used.

Physical Characterization, Phytochemistry, and Phytoconstituent Analysis of Moringa Gum

The collected *M. oleifera* gum was evaluated for solubility, melting point, bulk density, loss on drying, viscosity, pH, and tap density [R S Kumar and R Renuka,2019; E E Jarald *et al.*,2012].

The qualitative phytochemical investigation of the gum extract was carried out using conventional procedures [JB Harborne, 1998; C.K. Kokate 2005; G Shanmugavel *et al.*, 2018] for the qualitative identification of alkaloids, tannins, phytosterols, triterpenoids, flavonoids, glycosides, and saponins. The results obtained are as follows, indicating the presence and absence of phytoconstituents.

Synthesis and characterization of AgNPs

To eliminate all moisture from the *M. oleifera* gum, it was dried for two weeks in the shade. A powdered sample was prepared from the dried gum using a

household blender. The silver nitrate solution (9 mg/ml) was mixed with the gum mixture (3 mg/ml) and mechanically whirled for 2 hours at 60 °C at 200 g. A color change evidenced the synthesis of AgNPs from colorless to pale yellow, which was eventually transformed to light brown. At 60 °C for 4 h, the resulting mixture was stirred continuously. The color changed from colorless to light, and a dark brown color was formed. The production of AgNPs was validated using a UV visible spectrophotometer to determine the surface plasmon resonance (UV-1800, Shimadzu). A scanning electron microscope was used to examine the structure of the NPs (Hitachi S-4500).

Antioxidant activity

A radical scavenging test using 1-diphenyl-2-picryl-hydrazine (DPPH) was used by Chrzczanowicz *et al.* [J Chrzczanowicz., *et al* 2008;]. The results were computed as a % of scavenging activity using ascorbic acid as the standard and deionized water as the blank solution. The absorbency of the resulting solution was calculated at 517 nm using a spectrophotometer.

The percentage of inhibition was determined using the formula.

$$\text{Inhibition activity (\%)} = \frac{\text{Abs}_{\text{Sample}} - \text{Abs}_{\text{Control}}}{\text{Abs}_{\text{Sample}}} \times 100$$

Abs Sample indicates the test sample absorbance, and Abs Control denotes the control response absorbance. Where Abs Sample is the absorption of the test samples and Abs Control is the absorption of the control response. All experiments were performed in triplicate.

Antimicrobial activity

The antimicrobial action was assessed using Prasad and Swamy's agar diffusion method [J Narenkumar *et al.*, 2018;]. The AgNPs derived from *M. oleifera*gum extract were evaluated for antimicrobial efficacy against the five bacterial strains listed below: *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, and Vibrio cholera*. These cultures were obtained from the Department of Microbiology, Drs Bio Research Centre, Thanjavur. A 5 mm well was drilled into the agar plates using a sterile cork borer. Gum and AgNP-formulated gum were loaded into the wells at suitable test concentrations (100 g/mL). All Petri dishes were maintained at 37 °C for 24 h. After 24 h, the plates were checked for distinct growth inhibition around the well, indicating the existence of antimicrobial properties in the investigated samples.

Results and discussion

M. oleifera is a rapidly growing tree, and it is well known for its horseradish tree, bean oil, and drumsticks [D Panda *et al.*, 2006;]. It is believed to have spread in the Western and Sub-Himalayan regions of Africa, India, Asia, and Pakistan. It has spread across Central America, Cambodia, the Caribbean Islands, the Philippines, and North and South America [M. Dekker, 2002]. In this study, 52 g of dry weight *M. oleifera* gum was collected from the Arunachala hills in Tiruvannamalai, Tamil Nadu, India. In nature, the obtained gum was brownish-red and hardened. Gum exudates were tested for solubility in different solvents and distilled water at room temperature.

Table 1. Phytoconstituent presence in Moringa Gum.

S.No	Phytochemical	Gum
1	Alkaloids	+
2	Phenol	+
3	Saponins	+
4	Flavonoids	+
5	Steroids	+
6	Phytosterol	-
7	Glycosidase	+
8	Carbohydrates	+
9	Protein	+
10	Lipid	+

The components were partially solubilized and swelled in exudates of water and gum. Similar results have been published for Grewia gum, Malva nut gum, and Kondagogu gum [E I Nep, and B R Conway., 2010; P Somboonpanyakul *et al.*, 2006; VTP Vinod *et al.*, 2008]. Most gum exudates are complicated combinations of polysaccharides and terpenes, which play a vital role in their characteristics and significantly lower their solubility [A Nussinovitch, 2009].

Table 2. Free radical scavenging activity of gum, AgNPs and ascorbic acid.

S.No	Conc. ug/mL	Gum		AgNPs		Ascorbic acid	
		OD	%	OD	%	OD	%
1.	50	0.21	27.58	0.11	15.38	0.21	64.40
2.	100	0.19	34.48	0.09	30.76	0.19	67.79
3.	150	0.18	37.93	0.07	46.15	0.16	72.88
4.	200	0.16	44.82	0.04	69.23	0.13	77.96

The occurrence of insoluble cell wall substances is also the main component that reduces the solubility of gum exudates [E I Nep, and B R Conway., 2010]. In this study, the melting points of *M. oleifera* gum alone were estimated to be approximately 80 °C.

There is no prior research on the melting issues of *M. oleifera* gum. However, many studies have been performed on *M. oleifera* materials such as seeds and oil. Previously, similar photochemical properties were analyzed from the leaves, flowers, stems, and roots of *Moringa* oleifera. Mohammed *et al.* [F AA Mohammed, 2015] investigated propranolol hydrochloride, gum, and combinations. Because of its amorphous nature, propranolol hydrochloride displayed a sharp endothermic peak at 167.51 °C, leading to its melting point, whereas gum showed a broad endothermic peak at 153.02 °C. Abdulkarim *et al.* [S M Abdulkarim *et al.*, 2005] investigated the physical characteristics of *M. oleifera* seed oil. In this investigation, a frozen oil sample was melted in an oven at 60 °C.

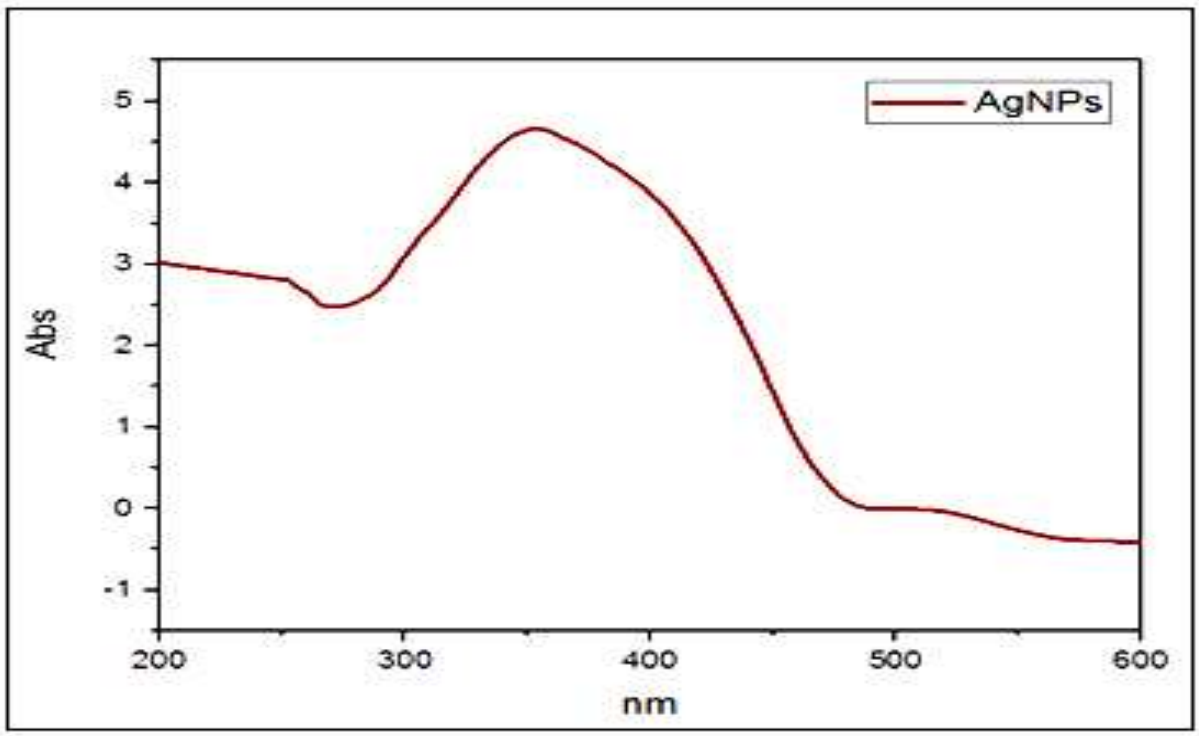

Fig. 1. Physical characterization of the green synthesized silver nanoparticles.

Several researchers investigated the physicochemical characteristics of plant-derived products, and they discovered that, except for bulk density, the physicochemical properties increased with humidity [N A Aviara *et al.*, 2013]. In this study, bulk density was observed at 0.73 gm/ml, loss on drying was 0.43%, viscosity was 11CP, pH was noted at 6.21 ± 0.02, and tap density was 0.92 gm/ml. These physical attributes can help design aeration gear for stored commodities and assess seed susceptibility to airflow during drying [N N Mohsenin, 1986].

The purified gum was subjected to phytochemical characterization in the present study. In this analysis, the gum sample contained alkaloids, flavonoids, tannins, saponins, glycosides, and phenols (Table 1).

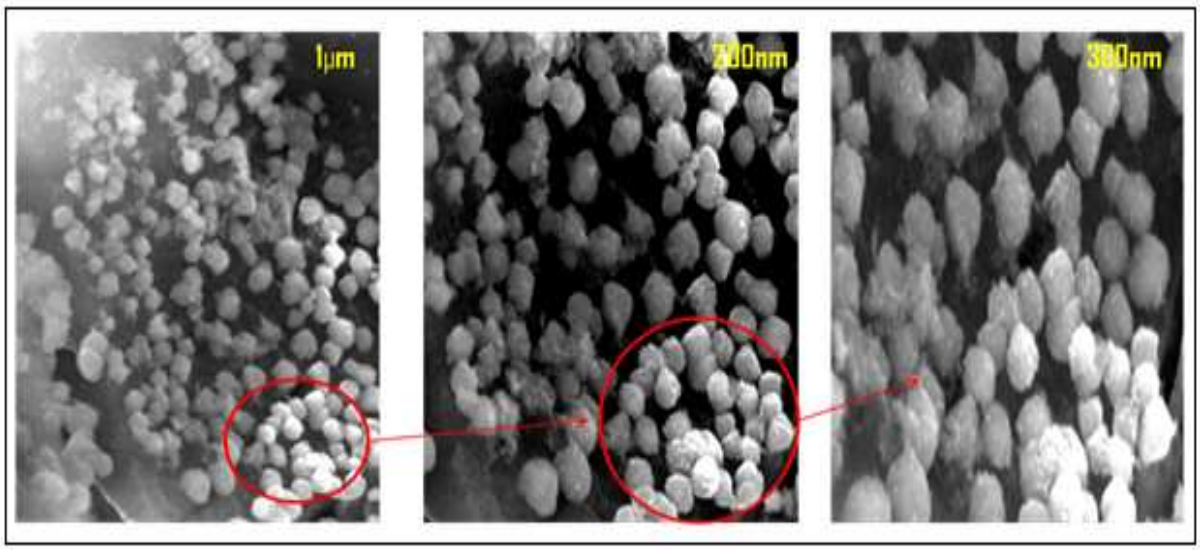

Fig. 2. Scanning electron microscope analysis of green synthesis silver nanoparticles.

This discovery suggests that *M. oleifera* gum rice comprises phytochemical components. Synthesized AgNPs were used in this analysis. The outcome of AgNPs is partially shown by the incremental transition in the color of the solution from brown to dark brown, indicating the development of AgNPs. The production of AgNPs was confirmed using a UV visible spectrophotometer (UV-1800, Shimadzu) to measure the surface plasmon resonance (Figure 1).

The UV–visvisible spectrum of the AgNPs with an intense peak at 420 nm, characteristic of silver

The absorption peak of the UV–Visible spectra of the aqueous medium containing AgNPs was approximately 350–450 nm. The synthesized NPs are polydisperse spherical, according to the scanning electron microscopy illustration. The NPs that were synthesized have irregular shapes and no fixed geometry. The agglomerate shape resembles that of pebble-like structures. The scanning electron microscopy image is depicted in Figure 2. The DPPH radical is a widely used reagent to study antioxidant substances and their radical-scavenging abilities. When an antioxidant component is combined with a DPPH radical reagent, the color of the reagent fades, and it turns yellow; the respective OD values are presented in Table 2. By collecting the hydrogen atoms emitted by antioxidant molecules, the DPPH free radical is reduced [I J Goldstein *et al.*,1965;]. The proportion of inhibition was dose-dependent DPPH-radical scavenging ability at concentrations of 100 to 500 g/mL^{-1}.

The synthesized AgNPs from *M. oleifera*gum exhibited significant DPPH radical scavenging activity values ranging from 21.15± 0.017 to 63.46± 0.03 g/mL at concentrations ranging from 100 to 500 g/mL (Table 2). This investigation proves that AgNPs synthesized *from M. oleifera*gum have strong antioxidant properties. Similar studies have been carried out previously by Raja *et al.* [W Raja *et al.*, 2016].

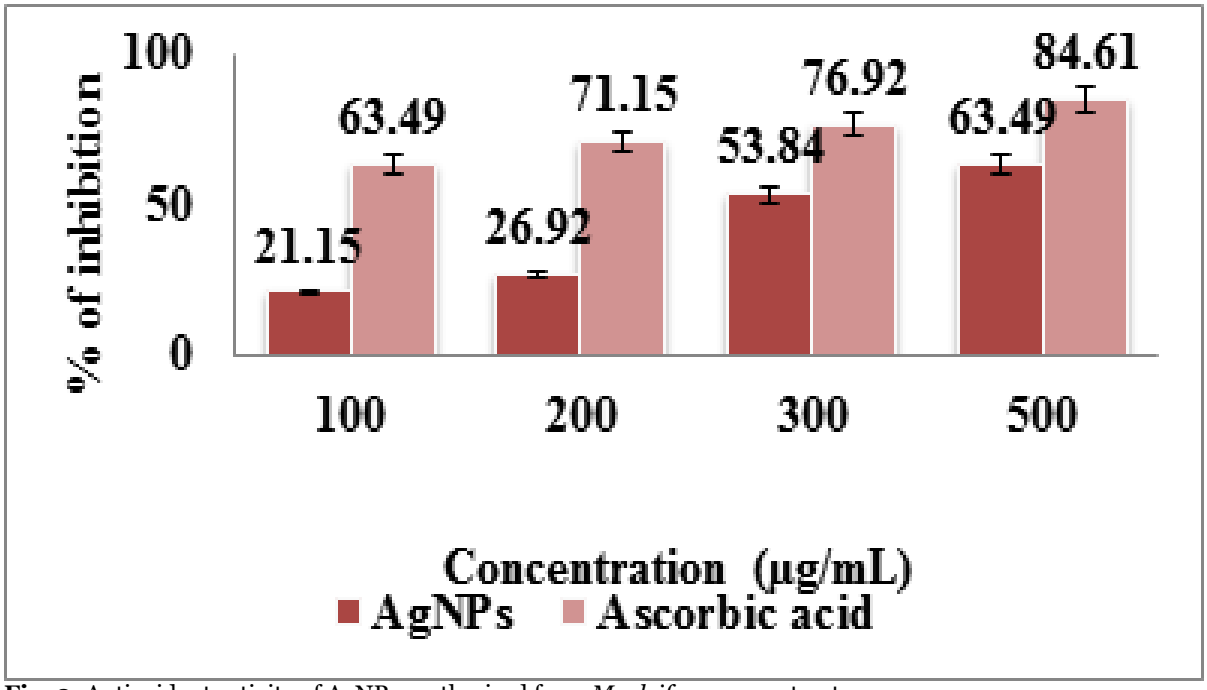

Fig. 3. Antioxidant activity of AgNPs synthesized from *M. oleifera* gum extract.

They discovered that *M. oleifera* gum polysaccharide had high radical scavenging potency at 300 g/mL-1 (860.3%), comparable to normal antioxidants (BHA and BHT demonstrated 92.3% and 92.33% operation, respectively). Siddhuraju and Becker [P Siddhuraju, and K Becker, 2003] investigated the antioxidant properties of crude extracts of *M. oleifera* leaves from three distinct agroclimatic origins. The antioxidant activity of the three moringa samples, both methanol and ethanol extracts from India, was the highest, at 65.1 and 66.8%, respectively (Figure 3). According to Luqman *et al.* [S Luqman *et al.*,2012], as the concentration of the extracts increased, the scavenging capacity of the extracts also increased, preventing the generation of the DPPH free radical. Previous study results indicate that Moringa plant derivatives have good antioxidant properties. These antioxidant properties can aid in cancer treatment. More research is needed to determine the viability of Moringa plants. The antimicrobial effect of the AgNPs produced was tested using *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli,* and *Vibrio cholera*. At a volume of 100 L/well, Figure 4 demonstrates strong antibacterial efficacy against all tested bacterial strains. The antibacterial property was saturated with produced AgNPs based on the discs' inhibitory zones in this experiment (Figure 4).

The maximum incubation zone was 18 mm by 100 µL of AgNPs synthesized from *M. oleifera* gum extract against *S. typhi. P. aeruginosa* expressed a 16 mm zone of incubation at 100 µL of AgNPs synthesized from *M. oleifera* gum. There have been no investigations on the antibacterial properties of AgNPs synthesized from *M. oleifera* gum. Previously, several studies have been carried out on the antimicrobial activity of AgNPs synthesized from plant derivatives. Devendra *et al.* [B N Devendra *et al.*, 2011] investigated the antimicrobial properties of *M. oleifera* leaf extract against several pathogen strains. According to his research, chloroform extract exhibits antibiotic properties against a broad range of microorganisms. Elgamily *et al.* [H Elgamily *et al.*, 2016] performed a microbiological study of *M.* oleifera extracts. In this investigation, the ethanol extract of *M. oleifera* showed the most significant inhibitory zone values against S. aureus and S. mutans. In southwestern Nigeria, Oluduro [An O Oluduro, 2016] evaluated the antibacterial and nutritional capabilities of *M. oleifera* leaf extracts. In this investigation, aqueous and methanolic extracts had substantial inhibitory effects on orthopedic wound bacteria at 30 mg/ml.

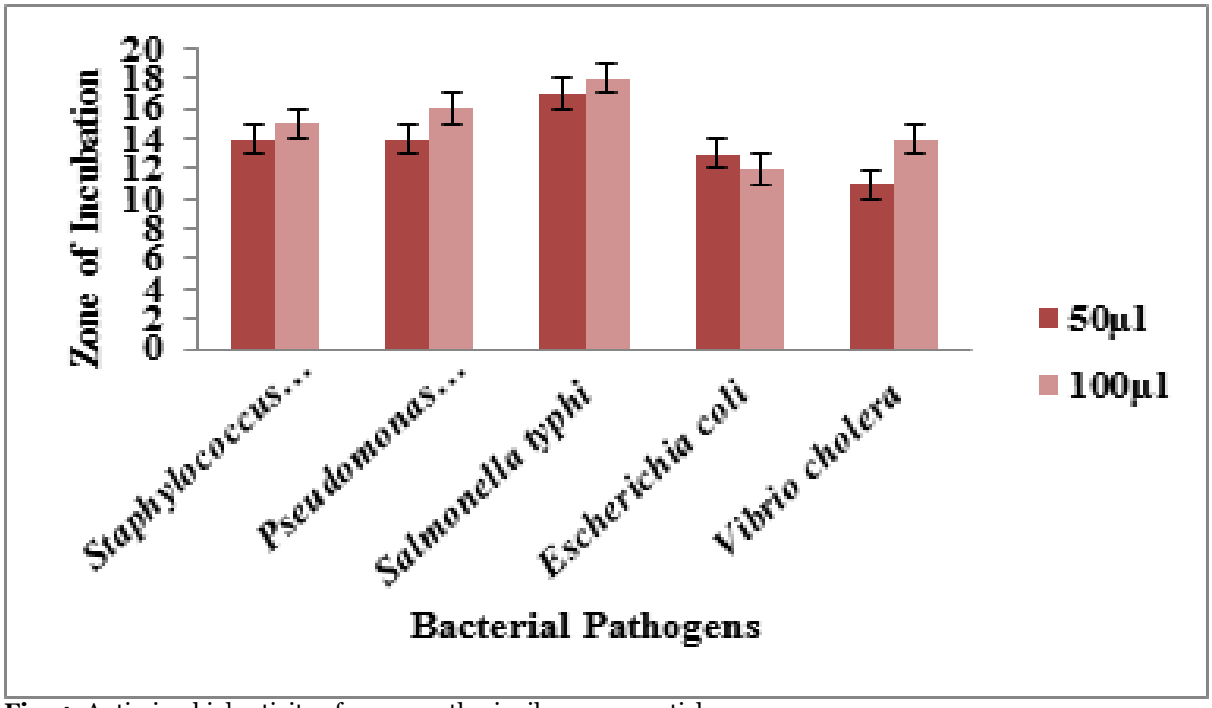

Fig. 4. Antimicrobial activity of green synthesis silver nanoparticles.

The present findings of this study validated the antibacterial characteristics of NPs made from *M. oleifera* plant gum and its derivatives, suggesting that medicinal herbs might be used to control pathogenic diseases in the pharmaceutical sector.

Conclusions

In conclusion, in the present study, *M. oleifera* gum was collected, and its physical properties and phytochemical composition were investigated. Then, green synthesis and characterization of AgNPs were carried out from the collected *M. oleifera* gum. AgNP-synthesized gum material was analyzed for antioxidant and antimicrobial properties. The antioxidant investigation proved that AgNPs synthesized *from M. oleifera*gum had strong antioxidant properties. Antimicrobial experiments demonstrated that they could be used as lead compounds in producing potent antimicrobial medications to treat infectious diseases. Previous study results indicate that Moringa plant derivatives have good antioxidant and antimicrobial properties. These properties provide a good path and encouragement to researchers to find new metabolites that can cure cancer and microbial infectious therapy. This study lays the groundwork for the synthesis of AgNPs from *M. oleifera* gum and its novel pharmacological applications. More research is necessary to determine the viability of Moringa plants.

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