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INNOVATIVE APPROACH FOR COMBATING MDR PATHOGENS BY *FICUS CARICA* AgNPs EXTRACT

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Abstract

Plant-mediated synthesis of nanomaterial has been increasingly gaining popularity due to its eco-friendly nature and cost-effectiveness. In the present study, we synthesized silver (Ag) nanoparticles using aqueous extracts of fresh leaves of Ficus carica medicinal plants as bio-reducing agents. Because of the emergence of antibiotic resistance bacteria, our health sector is facing many problems. To overcome these problems, we have tried to synthesize silver nanoparticles fromthe Ficus carica plant due to its antioxidant, anti-inflammatory and antimicrobial properties. This method allowed the synthesis of nanoparticles, which was confirmed by XRD, ultraviolet-visible (UV-Vis) spectrophotometry, SEM and FTIR. UV-Vis spectra and visual observation showed that the color of the fresh leaf extracts turned into grayish brown and brownish yellow, yellow respectively, after treatment with Ag precursors. Moreover, aqueous leaf extracts were separately tested for their antimicrobial activity against Gram-positive S.aureus and Gram-negative Klebsiella bacteria. The results showed that the bacterial growth was inhibited by the extracts containing Ag nanoparticles. Statistical calculation performed using the test showed that zones of inhibition for the two bacteria produced by the aqueous leaf extracts of Ficus carica containing 1mM Ag. There were significant zones of inhibition when using extracts of leaves containing 1mM Ag precursors. It was shown that the aqueous extracts of fresh Ficus carica leaves containing Ag nanoparticles inhibited bacterial growth. In future, we will check the synergistic effect of AgNPs, plant extract and antibiotics.

Keywords: *Eco-friendly, Ficus carica, FTIR, MDR pathogens, SEM, XRD*

INTRODUCTION

The Infectious Diseases Society of America has highlighted ESKAPE pathogens, a group of microorganisms that can escape antibiotic treatment. These pathogens are a major cause of hospital infections. This category, which also comprises *Acinetobacter baumannii, Enterococcus fecium, Pseudomonas aeruginosa*, and Enterobacter species, includes *Staphylococcus aureus* and *Klebsiella pneumoniae* (1). The persistent rise in bacteria that are resistant to current medicines is a severe issue that raises mortality rates and considerably contributes to treatment failure for infections. To get rid of these resistant bacterial strains, new antibacterial agents or chemicals that prevent resistance pathways must be developed immediately (2). Biological agents act as reducers, stabilizers, or both in the process of forming nanoparticles. Secondary metabolites serve as stabilizing agents, which aid in the environmentally friendly production of AgNPs. Silver ions are changed into AgNPs by flavonoids, which also regulate their size. Tannins are crucial for the stability of AgNPs. By making bacterial strains' membranes more permeable, polyphenols boost AgNPs antibacterial effectiveness (3).

The present study synthesized AgNPs using aqueous extracts of fresh leaves of *Ficus carica* and then evaluated its antimicrobial activity, particularly against the growth of *S.aureus* and *Klebsiella* bacteria. Nanotechnology has aided tremendous progress in material science, allowing for the creation of novel goods by manipulating matter at the nanoscale. Novel nanomaterial production strategies are still being investigated due to limitations associated with traditional physico-chemical synthesis protocols (4, 5). Biological manufacturing of nanoparticles utilizing plant extract has been deemed the most environmentally friendly, efficient, and cost-effective of these procedures. The nanoscale material has new, unique, and superior physical and chemical properties compared to its bulk structure, due to an increase in the ratio of the surface area per volume of the material/particle. The most widely studied nanoparticle materials are metal nanoparticles because they are easier to synthesize (6, 7). Moreover, these materials have a wide range of applications: detectors, catalysts, surface coating agents, and antibacterial/antimicrobials, among many others. Some of the most studied metallic nanoparticles include silver (Ag), Iron (Fe), gold (Au), platinum (Pt), and palladium (Pd) (8, 9).

AgNPs is an interesting metal to be studied, especially in the field of health and medicine. Ag is a strong antibacterial and also toxic to cells. Ag has the ability to damage bacterial cell walls, inhibits bacterial cell growth, and disrupts cell metabolism because of the interaction between Ag ions with macromolecules in cells, such as proteins and deoxyribonucleic acid (DNA). The Ag ion that interacts with the cell prevents protein synthesis, further decreases the membrane permeability, and eventually leads to cell death (10, 11). The AgNPs are chemically more reactive than Ag in their bulk. Therefore, AgNPs are indicated to have stronger antibacterial capabilities AgNPs is an interesting metal to be studied, especially in the field of health and medicine (12, 13).

Green synthesis methods for synthesizing nanoparticles using natural products can be used to address the problem by utilizing plants or microorganisms. The utilization of plants in the biosynthesis of nanoparticles involves the content of secondary metabolites as reducing agents (14, 15). The biosynthesis of AgNPs has been carried out by utilizing a number of plants and evaluating the antimicrobial activity, such as water extracts from *Ficus carica.* The aqueous extract of *Ficus carica* was obtained from the leaves which were first dried for further analysis of its antimicrobial activity. The aqueous extract of fresh leaf sample to synthesize AgNPs is important to be investigated mainly because it can be used in medicine. Liquids that use water solvents are much safer for health and the environment than using chemical solvents. Therefore, in this study, we used water as a solvent in medicinal plant extracts (16, 17). In some research, plant extracts and antibiotics are used in combination for better results. In future we check the synergistic effect of AgNPs plant extract and antibiotics (18).

MATERIALS AND METHODS

EXTRACT PREPARATION

Fresh leaves of *Ficus carica* were collected from MNSUAM. A 10 g sample of leaves was thoroughly washed with distilled water to remove dust and surface contaminants, and then dried using tissue paper. The cleaned leaves were crushed and mixed with 100 mL of distilled water in a beaker. The mixture was boiled at 100 °C for 1 hour and allowed to cool at room temperature. The extract was then filtered through Whatman filter paper to remove solid residues, and the filtrate was stored in a refrigerator at 4 °C for future use (Fig. 1a-e).

Table I. Materials and reagents used in the experimentations

Sr. No	Reagent	Concentration
1	Beef Extract	0.075 g/25 mL
2	Peptone	0.125 g/25 mL
3	$AgNO_3$	0.017 g/ 100mL
4	MHA	3 g
5	Plant	Ficus Carica (20 g)
6	Bacterial Strains	*S.aureus* and *Klebsiella*

MEDIA PREPARATION

Muller Hinton agar was prepared by dissolving 6 g of the medium in 200 mL of distilled water, followed by sterilization through autoclaving. Sterile agar plates were then prepared aseptically. For bacterial broth preparation, 0.075 g of beef extract and 0.125 g of peptone were dissolved in 25 mL of distilled water. Aliquots of 5 mL were dispensed into individual test tubes, which were subsequently autoclaved for sterilization. The tubes were inoculated with the selected bacterial strains and incubated at 37 °C for 24 hours (Fig. 1 c & d).

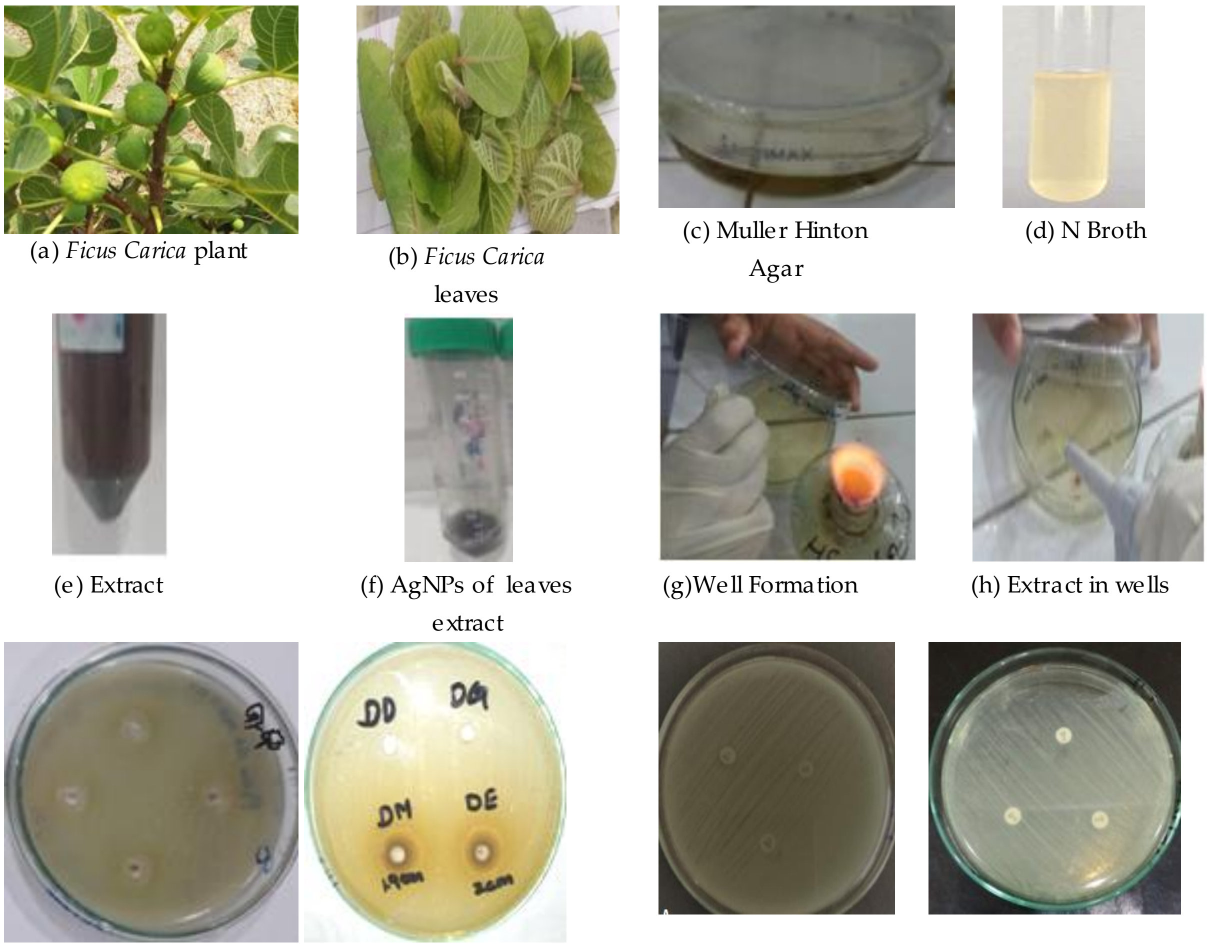
NANOPARTICLE PREPARATION

CENTRIFUGATION METHOD

A 1 mM solution of silver nitrate ($AgNO_3$) was prepared in distilled water. To this solution, 3 mL of *Ficus carica* leaf extract was added to 22 mL of silver nitrate in a sterile Falcon tube. The mixture was incubated in the dark for 15 minutes to facilitate nanoparticle formation. After incubation, the solution was mixed thoroughly and centrifuged at 1000 rpm for 15 minutes. The supernatant was carefully discarded, and the resulting pellet was dissolved in injection-grade water for further use (Fig. 1f).

ANTIBACTERIAL ACTIVITY

Bacterial broth cultures were aseptically spread onto labeled Muller Hinton agar plates using a sterile spreader. Wells were prepared on two agar plates with the aid of a sterile Pasteur pipette. Each well was filled with 100 µL of the prepared plant extract. For comparison, antibiotic discs were placed on two separate Muller Hinton agar plates as positive controls. All plates were incubated at 37 °C for 24 hours, after which the zones of inhibition were observed and recorded (Fig. 1g-j).



(a) *Ficus Carica* plant (b) *Ficus Carica* leaves (c) Muller Hinton Agar (d) N Broth

(e) Extract (f) AgNPs of leaves extract (g)Well Formation (h) Extract in wells

(i) well diffusion assay (j) Disc diffusion assay

Fig. 1. Brief overview of AgNPs synthesis and its antibacterial activity against *K. pneumoniae* and *S. aureus*

Table II. Antibiotic susceptibility pattern

Organisms	AMP	TET	CIP	GEN	AMK	AZM	LVX	DOX
Klebsiella	R	R	R	R	R	R	R	R
Staph aureus	R	R	R	R	R	R	R	R

Table III. Zones of inhibition diameters (cm) for 40 µL of AgNPs against bacteria

Test Organism	Sr. No	Zone of inhibition (cm)	
		Well Diffusion	Disk Diffusion
	1	1.9	0
S. aureus	2	2	0
	3	1.8	0
Average		1.9	0
	1	1.3	0
K. pneumoniae	2	1.4	0
	3	1.2	0
Average		1.3	0
	AgNPs concentration	**Zone of inhibition (cm)**	**Antibacterial activity result**
S. aureus	40 µL	1.8 - 2.0	Strong Inhibition
K. pneumoniae	40 µL	1.2 - 1. 4	Moderate Inhibition

RESULTS

The present study revealed that the tested leaf extracts of *Ficus carica* showed antibacterial activity against two bacterial strains: Gram-negative *Klebsiella* and Gram-positive *S.aureus.* Aqueous extracts of *Ficus carica* containing AgNPs showed antibacterial activity against antibiotic resistant bacteria. The results of antibiotic susceptibility test indicated that the bacterial isolates showed multidrug resistant pattern. The zone of inhibition was greater for concentrated dilution of silver nanoparticles. Using 100 µL of *Ficus Carica* AgNPs extract resulted in a larger zone of inhibition measuring 2 cm against *S.aureus* and 1.4 cm against *Klebsiella* in the well diffusion assay. In comparison with the disc diffusion results, there was no zone of inhibition observed against the bacteria. However, AgNPs showed better results against MDR bacterial strains.

X-RAY DIFFRACTION

X-ray diffraction is a potent method for characterizing nanoparticles that can provide details about their crystal structure, crystalline phases, and crystallographic characteristics. It is used to distinguish phases, ascertain the crystal structure, and calculate the size of the crystallites in nanoparticles. Based on the XRD graph, the characterization of *Ficus Carica* AgNPs reveals important details regarding their characteristics. The strongest peak in the XRD is located at 110 on the y-axis and 35 on the x-axis. This peak can be used to calculate the average particle size of the nanoparticles. XRD data confirms that the crystalline structure of AgNPs have average size of 55 nm (Fig. 2).

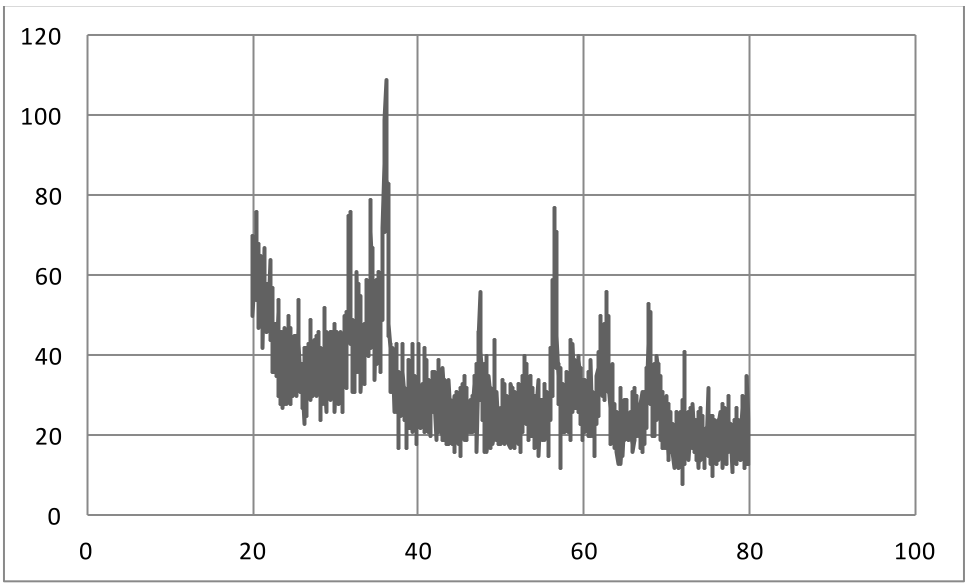


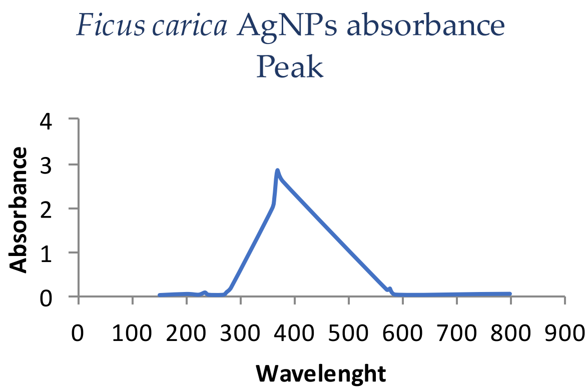
Fig. 2. X-ray Diffraction Patterns of *Ficus Carica* AgNPs

UV-VIS SPECTROPHOTOMETRY

The absorption spectra of Ficus carica AgNPs solution were analyzed for the UV-Vis spectrophotometry in the wavelength range of 200-800 nm. After 1 day, the UV-Vis spectrum of the nanoparticle solution was measured. Using UV-Vis spectrum, the optical characteristics of biosynthesized *Ficus Carica* Ag NPs were examined. UV-Vis spectrum shows a prominent absorption band at the wavelength of 400 nm. The absorbance values are quite low 800 nm showing that the nanoparticles have little absorption in the near-infrared range. The UV- Vis spectrum of *Ficus Carica* AgNPs was 2.7 at 400 nm (Fig. 3a).

SCANNING ELECTRON MICROSCOPY

Scanning Electron Microscopy (SEM) is a technique used to obtain high-resolution images of the surface of a sample. We can see that particles are held together due to weak physical forces. The AgNPs displayed a variety of nano sizes and irregular forms, ranging from spherical to hexagonal and elongated structures, according to the SEM pictures. The nanoparticle surfaces seemed to be mostly smooth, with just irregular surface characteristics and aggregation (Fig. 3b).



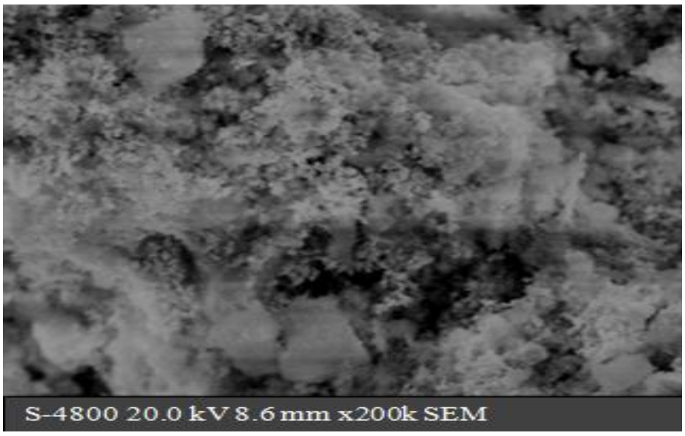


Fig. 3 (a). UV-Vis spectrum of *Ficus Carica* AgNPs; **(b).** SEM images of AgNPs biosynthesized by *Ficus carica*

FOURIER TRANSFORM INFRARED SPECTROSCOPY

FTIR analysis plays a crucial role in the analysis of various materials and compounds. FTIR spectroscopy is a powerful characterization method for the investigation of interactions between plant extract and AgNPs. Functional groups were identified by FTIR spectroscopy that is responsible for the reduction process. The IR bands of leaf extract of *Ficus carica* were identified 3269.62 cm^{-1} for O-H group, 1636.62 cm^{-1} were identified for C=O,958.41 cm^{-1} identified for C=C, 906.80 cm^{-1} and 648.04 cm^{-1} were identified for C-Cl (Fig. 4).

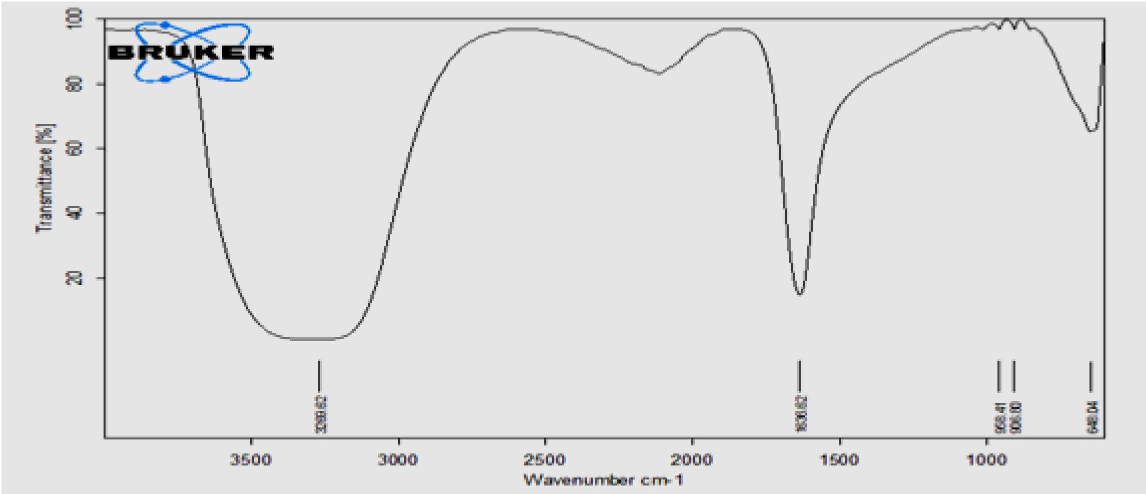


Fig. 4. FTIR spectrum of *Ficus carica* leaf extract

DISCUSSION

Antibiotic-resistant bacteria are a major problem in healthcare today. To overcome these problems, we have tried to synthesize AgNPs from *Ficus carica* plant due to its antioxidant, anti-inflammatory and antimicrobial properties (19). Antibacterial activity was shown by an inhibition zone which was characterized by a clear zone between the wells (containing samples) and a certain distance. Formation of inhibition zones around the wells shows bacterial sensitivity to antibacterial ingredients. It was clear that the *Ficus Carica* extracts containing AgNPs that had the antibacterial activity. Biosynthesized AgNPs were analyzed for their antimicrobial activity against *Klebsiella* and *S.aureus* by well diffusion method. It was observed that microbial growth of *Klebsiella* and *S.aureus* was dependent on AgNPs concentration to some extent.

According to earlier research, AgNPs are chemically produced that is hazardous to the environment. In this study, we created NPs using environmentally beneficial *Ficus carica*, which inhibits the growth of bacteria. The zone of inhibition ranged from 1.8 to 2 cm for S.aureus and 1.2 to 1.4 cm for *Klebsiella*. These results indicated that the AgNPs synthesized using mixture of extract have stronger activity than standard antibiotics (20). AgNPs were synthesized from silver nitrate solution using *Ficus carica* leaf extract. AgNPs were prepared and characterized by color change from greenish to brownish black. The prepared AgNPs were characterized by XRD, UV- Vis spectrophotometry, SEM and FTIR analysis (21, 22). The strongest peak in the XRD is located at 110 on the y-axis and 35 on the x-axis. This peak can be used to calculate the average particle size of the nanoparticles. XRD data confirms that the crystalline structure of AgNPs have average size of 55 nm (23, 24).

The absorbance values are quite low 800 nm showing that the nanoparticles have little absorption in the near-infrared range. The UV-Vis spectrum of *Ficus Carica* AgNPs was 2.7 at 400 nm (25, 26). Scanning Electron Microscopy (SEM) is a technique used to obtain high-resolution images of the surface of a sample. The AgNPs displayed a variety of nano sizes and irregular forms, ranging from spherical to hexagonal and elongated structures, according to the SEM pictures. The nanoparticles surfaces seemed to be mostly smooth, with just irregular surface characteristics and aggregation (27, 28). Results of FTIR analysis were recorded in the range of 3500-100cm-1. Functional groups were identified by FTIR spectroscopy that is responsible for the reduction process. The IR bands of leaves extract of *Ficus carica* were identified 3269.62cm^{-1} for O-H group, 1636.62cm^{-1} were identified for C=O,958.41cm^{-1} identified for C=C, 906.80cm^{-1} and 648.04cm^{-1} were identified for C-Cl (29, 30). These bacterial isolates were further re-used in antimicrobial activity test of disc diffusion assay and well diffusion assay. The results of antibiotic susceptibility test indicated that the bacterial isolates showed multidrug resistant pattern. The results of well diffusion test indicated that the bacterial isolates show antibacterial activity against multidrug resistant pattern. In future we check the synergistic effect of AgNPs plant extract and antibiotics (31).

Conflict of interest:

The authors have no conflict of interest.

Authors' contribution:

MN Conceptualization, experimental design; MBA Assisted in experimental work; SMSP Microbiological assays and data interpretation; SK Assisted in statistical analysis, literature review; SM Antibacterial assay, laboratory support, and data collection; SS Microbial culture preparation and handling; FB Manuscript drafting, figure/table preparation, proof editing; UQ methodology, referencing, formatting of the manuscript; MB plant collection, extract preparation, laboratory support; MU microbiology experiments, critical manuscript review.

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