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Sustainable Solution to Antibiotic Resistance: Pavetta indica-Based Nanomedicine

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The escalating global health crisis, characterized by the emergence of antimicrobial-resistant pathogens, necessitates innovative strategies. This study explores the potential of *Pavetta indica,* a previously underutilized plant, as a multifaceted resource for addressing this critical challenge. A comprehensive phytochemical investigation unveiled a rich repertoire of bioactive compounds within *P. indica*, including essential metabolites Moisture (9.14%), Crude Protein (10.93%), and Crude Fiber (15.46%). Additionally, significant levels of Calcium, Phosphorus, and Ether Extract were identified, suggesting its potential contribution to a balanced diet and a diverse array of secondary metabolites identified through GC-MS analysis identified diverse bioactive compounds including Dodecanoic acid, Lupeol, and β-D-Glucopyranose among others, highlighting its potential health benefits and paving the way for further research on specific bioactivities. P. indica extract exhibited concentration-dependent ABTS and DPPH scavenging activities, comparable to ascorbic acid. This

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underscores its potential as a natural antioxidant source to combat oxidative stress-related diseases. Leveraging the bio-reducing capabilities of *P. indica* extract, we embarked on the green synthesis of silver nanoparticles. P. indica extract successfully synthesized silver nanoparticles, confirmed by UV-Vis spectroscopy (SPR band at 420 nm) and TEM (spherical particles, 25-50 nm). These nanoparticles showed potent antimicrobial activity against Bacillus subtilis, E. coli, and Pseudomonas aeruginosa, comparable to Gentamicin. The synthesized silver nanoparticles demonstrated concentration-dependent antifungal activity against *Candida albicans, Aspergillus fumigatus, and Sporothrix schenckii,* suggesting their potential as effective antifungal agents. Our findings underscore the significance of exploring underutilized plant resources for developing innovative solutions to pressing global health challenges. By integrating phytochemistry, nanotechnology, and microbiology, this research offers a promising approach to combating antimicrobial resistance. The study's outcomes lay the groundwork for future investigations into the underlying mechanisms of antimicrobial action, optimization of nanoparticle properties, and preclinical evaluation of their therapeutic efficacy.

Keywords: Pavetta indica; antimicrobial resistance; silver nanoparticles; green synthesis; phytochemicals; antioxidants; nanomedicine.

1. INTRODUCTION

Traditional medicine systems worldwide have harnessed the power of plants to promote health and well-being. Among the vast, and often unexplored, botanical resources lies *P. indica*, an unassuming plant with the potential to unlock a treasure trove of health-promoting compounds. This multifaceted investigation embarks on a journey to unveil the secrets hidden within *P. indica*, shedding light on its nutritional composition and potential as a source of nutraceuticals – natural substances with both nutritional and medicinal benefits. The relentless pursuit of natural solutions to maintain optimal health and prevent chronic diseases has propelled the exploration of plants as a rich source of bioactive compounds [1]. Scientific evidence increasingly emphasizes the critical role of dietary factors in this pursuit. Plants serve as the cornerstone of a healthy diet, providing essential nutrients like carbohydrates, proteins, fats, vitamins, and minerals. Delving into the nutritional profile of *P. indica* can offer valuable insights into its potential contributions to a balanced dietary regimen. Beyond its basic nutritional value, *P. indica* might harbor a wealth of bioactive compounds with significant health benefits. Recent research highlights the importance of phytochemicals, naturally occurring plant compounds, in disease prevention and health promotion [2]. These diverse bioactive components possess a range of properties, including antioxidant activity, anti-inflammatory effects, and immune system modulation. Exploring the phytochemical profile of *P. indica* can unlock its potential as a source of natural health-promoting substances, paving the way for the development of novel nutraceuticals. This study employs a multifaceted approach to unlock the hidden potential of *P. indica.* We begin by deciphering the plant's nutritional fingerprint, identifying the presence and quantifying the amounts of essential metabolites that contribute to a balanced diet. Subsequently, we delve deeper into the realm of bioactive compounds [3]. Utilizing advanced analytical techniques like Gas Chromatography-Mass Spectrometry (GCMS), we aim to create a comprehensive metabolic profile. This will allow for the precise identification and characterization of health-promoting phytochemicals present within the plant. Additionally, the antioxidant activity of *P. indica* extracts will be assessed to understand their potential role in combating free radical damage, a major contributor to various chronic diseases. By unraveling the hidden treasures within *P. indica*, this study aims to contribute significantly to the growing body of knowledge on the potential health benefits of plants. Through a meticulous exploration of its nutritional value and comprehensive analysis of its bioactive profile, we hope to pave the way for future research investigating the nutraceutical applications of this remarkable plant. The human quest for natural solutions to promote health and well-being has fueled a surge in exploring plants as a rich source of bioactive compounds. Traditional medicine systems across the globe have long recognized the power of botanical resources, utilizing them for centuries to treat various ailments [4]. They bridge the gap between food and medicine, offering a potential approach to promoting health and preventing diseases. Research on various nutraceuticals derived from plants has shown promising results,

highlighting their potential applications in various health conditions. The escalating global health crisis, characterized by the emergence of antimicrobial resistance, underscores the urgent need for novel and effective antimicrobial agents. Conventional synthetic antibiotics are grappling with the challenge of bacterial adaptation, necessitating the exploration of alternative strategies. Nature, with its unparalleled repository of chemical diversity, offers a promising avenue for the development of next-generation antimicrobials [5]. In recent years, nanotechnology has emerged as a powerful tool in the fight against microbial infections. Silver nanoparticles (AgNPs), in particular, have garnered significant attention due to their potent antimicrobial properties. However, traditional chemical methods employed for AgNP synthesis often involve hazardous chemicals and generate toxic byproducts, raising environmental concerns [6]. To address these challenges, a paradigm shift towards green and sustainable approaches is imperative. Plant-mediated synthesis of nanoparticles has emerged as an eco-friendly alternative. By harnessing the reducing capabilities of plant extracts, it is possible to synthesize AgNPs in a benign environment. The phytochemicals present in these extracts act as both reducing and capping agents, resulting in the formation of stable nanoparticles. This study leverages the reducing potential of P. indica extract to synthesize AgNPs through a green and sustainable approach. By employing this botanical approach, we aim to contribute to the development of environmentally friendly antimicrobial agents. The antimicrobial efficacy of the synthesized AgNPs will be rigorously evaluated against a panel of microorganisms, including both Gram-positive and Gram-negative bacteria, as well as fungi. This research endeavors to expand the knowledge base on plant-based nanomaterials and their potential applications in addressing the growing antimicrobial resistance crisis.

2. MATERIALS AND METHODS

2.1 Plant Material Collection

Fresh leaves of *P. indica* were collected from Kolli Hills, Namakkal District, Tamil Nadu, India. Plant authentication was confirmed by comparison with authentic specimens housed at the Rapinat Herbarium, St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India. A voucher specimen (No. T.B.003) has been deposited in the herbarium for future reference.

2.2 Plant Sample Preparation for Metabolite Screening

The *P. indica* leaves were shade-dried to remove moisture content. This step is crucial to preserve the integrity of the metabolites within the plant material. Once dry, the leaves were ground into a fine powder to increase the surface area and facilitate efficient extraction [7]. A Soxhlet apparatus was employed for solvent extraction. 15 grams of powdered plant material was placed in the thimble of the apparatus, and solvents Aqueous, Methanol, Ethanol, Acetone, Hexane, Petroleum ether of each (250 ml) were used as the solvents. The process relies on the continuous cycling of the heated solvent through the thimble, efficiently extracting the metabolites from the plant material. After extraction, the concentrated extract in the round-bottom flask was transferred to pre-weighed vials. The weight difference between the empty vials and the vials containing the extract provided the weight of the extracted material. This weight was then divided by the initial weight of the dry plant powder to determine the percentage yield of the extraction process. The extracted solution was concentrated by drying in a hot air oven at 50°C for 48 hours. This step removes residual solvent, leaving behind the concentrated metabolites for further analysis.

2.3 Estimation of Plant Nutritive Values

Sample Preparation: A representative sample of the food or ingredient was obtained and homogenized or finely ground to ensure uniformity.

Moisture: A sample of the food (approximately 5-10 grams) was weighed and recorded as the initial weight (W_1). The sample was dried in an oven at a specified temperature (typically 105-110°C) until a constant weight (W_2) was achieved. Moisture content was calculated using the formula: Moisture content (%) = $(W_1 - W_2) / W_1 * 100$

Crude Protein Analysis: The Dumas method was employed for protein determination. The sample was digested with concentrated sulfuric acid to release nitrogen from proteins. The released ammonia was captured in a known volume of acid and subsequently titrated with a standard acid solution. Nitrogen content was calculated, and then converted to crude protein using a conversion factor (typically 6.25 for most foods).

Crude Fiber Determination: Soluble carbohydrates, proteins, and fats were removed through acid and alkaline digestion. The residue was filtered, washed, dried, ashed, and weighed to determine crude fiber content.

Ether Extract (Crude Fat): Lipids were extracted using a non-polar solvent (ether). A known amount of sample was extracted with anhydrous ether in a Soxhlet apparatus for a specified time. The ether was evaporated, and the residue was dried to constant weight. Ether extract percentage was calculated based on the dry weight of the sample.

Total Ash: Organic matter was burned off in a muffle furnace at a specified temperature (usually 550-600°C). The remaining inorganic residue was weighed, and total ash percentage was calculated based on the dry weight of the sample.

Acid Insoluble Ash (Sand and Silica): Soluble ash components were removed from the total ash using hydrochloric acid. The insoluble residue was filtered, washed, ignited, and weighed. Acid-insoluble ash percentage was calculated based on the dry weight of the sample.

Calcium: Calcium content was determined using Atomic Absorption Spectroscopy (AAS) or titration. The plant material was digested with a suitable acid (e.g., nitric acid) to solubilize calcium. Calcium concentration was determined and calculated based on the dry weight of the sample.

Phosphorus: Phosphorus was converted into a colored complex and measured spectrophotometrically or colorimetrically. The plant material was digested with a suitable acid (e.g., nitric acid and perchloric acid) to convert phosphorus into inorganic phosphate. A colored complex was formed, and its absorbance was measured to determine phosphorus concentration. Phosphorus content was calculated based on the dry weight of the sample.

Salt (Sodium Chloride): Chloride ions were determined through titration. The plant material was extracted with water, and the extract was titrated with silver nitrate solution. Salt content was calculated as sodium chloride equivalent.

Gross Energy: The heat released upon complete combustion of the sample was measured using bomb calorimetry. The plant material was dried, ground, pressed into a pellet, and burned in a bomb calorimeter. The temperature rise of the calorimeter water was used to calculate gross energy.

2.4 Quantitative Estimation of Primary Metabolites

Following the extraction process, various methods were used to quantify the primary metabolites present in the *P. indica* leaves: **Chlorophylls:** The Arnon method was employed to estimate the chlorophyll content. This method involves homogenizing fresh leaf material in chilled acetone, followed by measuring the absorbance at specific wavelengths using a spectrophotometer. The chlorophyll pigments absorb light at these wavelengths, allowing for their quantification. **Proteins:** The Lowry method was used to determine the protein content. The plant extract was reacted with an alkaline copper solution and Folin-Ciocalteu's reagent, resulting in a color change. The absorbance of this colored solution is then measured at a specific wavelength and compared to a standard curve to determine the protein concentration. **Carbohydrates:** The Anthrone reagent method was used to estimate the total carbohydrate content. The plant extract was reacted with the Anthrone reagent, leading to the formation of a colored complex. The absorbance of this complex is measured at a specific wavelength and compared to a standard curve prepared using a known carbohydrate source, allowing for the quantification of total carbohydrates. **Lipids:** The Barned and Blackstock method was employed to estimate the total lipid content. The plant tissue was homogenized in a chloroform-methanol mixture, followed by filtration and evaporation. The remaining residue was then treated with sulfuric acid and vanillin reagent, resulting in a colored solution. The absorbance of this solution is measured at a specific wavelength and compared to a standard curve prepared using cholesterol, allowing for the quantification of total lipids [8].

2.5 Identification Tests for Secondary Metabolites

The solvent extracts underwent standard qualitative chemical analysis to determine the types of phytochemical constituents present in the sample. Triterpenoids: Mix 3 ml of the test

solution with a piece of tin and 2 drops of thionyl chloride. The appearance of a violet or purple color confirms the presence of triterpenoids. Sugars: Mix 3 ml of the test solution with a small amount of anthrone reagent and a few drops of concentrated sulfuric acid (H_2SO_4), then heat. The formation of a green or purple color indicates the presence of sugars. Catechins: Combine 3 ml of the test solution in alcohol with Ehrlich reagent and a few drops of concentrated HCl. The appearance of a pink color indicates the presence of catechins. Flavonoids: Combine 3 ml of the test solution in alcohol with magnesium and one or two drops of concentrated HCl, then heat. The appearance of a red or orange color confirms the presence of flavonoids. Saponins: Mix 3 ml of the test solution with water and shake vigorously. The formation of a foamy lather indicates the presence of saponins. Tannins: Combine 3 ml of the test solution with water and lead acetate. The presence of a white precipitate indicates the presence of tannins. Anthraquinones: Mix 3 ml of the test solution with magnesium acetate. The formation of a pink color indicates the presence of anthraquinones. Amino Acids: Add 3 ml of the test solution to 1% ninhydrin in alcohol. A blue or violet color indicates the presence of amino acids. Steroids: To 3 ml of the test solution, add a small amount of chloroform, 3-4 drops of acetic anhydride, and one drop of concentrated sulfuric acid (H_2SO_4). The formation of a purple color changing to blue or green indicates the presence of steroids. Carbohydrates: The Anthrone reagent method was used to estimate the total carbohydrate content. The plant extract was reacted with the Anthrone reagent, leading to the formation of a colored complex [9].

2.6 Gas Chromatography- Mass Spectrometry (GCMS) Analysis

Ethanol extracts from plant samples were subjected to sonication for 20 minutes. Subsequently, a 20 µL aliquot of the sonicated extract was filtered through a 0.45 µm filter. The filtrate was then analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS is a technique employed for the identification and quantification of volatile and semi-volatile organic compounds. In this method, a gas chromatography separates the components of a mixture based on their boiling points. The maximum allowable temperature for this analysis method is 300°C. with smaller molecules eluting faster than larger ones. The separated compounds are then introduced into a mass spectrometer for structural determination. The GC-MS analysis was performed using an Agilent 7890A GC coupled to a QToF 7200 series mass spectrometer. Separation of compounds was achieved using an INNOWAX column (30 m x 0.250 mm x 0.25 µm) under ambient temperature conditions. A 2.5 mL syringe was used for headspace solid-phase microextraction (HS-SPME) injection. Helium served as the carrier gas. Prior to injection, samples were filtered through a 0.45 µm ultra-membrane filter. The retention times and concentrations of metabolites were determined using the in-built GC-MS software [10].

2.7 Antioxidant Activity

Stock solutions of ascorbic acid and P. indica extracts were prepared in appropriate solvents (e.g., methanol, ethanol). Serial dilutions of these stock solutions were prepared to obtain different concentrations (e.g., 20, 40, 60, 80, 100 µg/mL). The ABTS radical cation solution was prepared. Equal volumes of the ABTS radical cation solution and sample/standard solutions were mixed and incubated for a specific time (e.g., 6 minutes). Absorbance was measured at 734 nm. The percentage of ABTS scavenging activity was calculated using the following formula: % ABTS Scavenged = [(Absorbance of control - Absorbance of sample) / Absorbance of control] x 100 The DPPH solution was prepared. Equal volumes of DPPH solution and sample/standard solutions were mixed and incubated for a specific time (e.g., 30 minutes). Absorbance was measured at 517 nm. The percentage of DPPH scavenging activity was calculated using the same formula as for ABTS. The percentage of ABTS and DPPH scavenged was plotted against the concentration of ascorbic acid and P. indica extracts. IC50 values (concentration required to scavenge 50% of the radicals) for both compounds were calculated. The specific incubation times, solvents, and concentrations used in the study might vary. Other antioxidant assays (e.g., FRAP, CUPRAC) could also be employed for a comprehensive assessment of antioxidant activity. It is essential to consider the limitations of in vitro assays and validate the findings through in vivo studies [11].

2.8 Synthesis of Silver Nanoparticles

A specific concentration 1 mM of silver nitrate (AgNO3) solution was prepared using deionized water. A fixed volume 5 mL of the prepared plant aqueous extract was added to a specific volume

95 mL of the AgNO3 solution. The reaction mixture was incubated at 95° C for 5 Minutes with constant stirring. The formation of silver nanoparticles was monitored visually by a color change from colorless to brown [12].

2.9 Characterization of Silver Nanoparticles

UV-Visible Spectroscopy: The synthesized silver nanoparticles were characterized using UV-Visible spectroscopy to confirm the formation and determine the surface plasmon resonance (SPR) band.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR analysis was performed to identify the functional groups involved in the reduction and stabilization of silver nanoparticles.

Transmission Electron Microscopy (TEM): TEM analysis was conducted to determine the size, shape, and morphology of the synthesized silver nanoparticles.

2.10 Antimicrobial Activity

The antimicrobial efficacy of the synthesized silver nanoparticles was evaluated against a panel of pathogenic microorganisms comprising both Gram-positive (Bacillus subtilis) and Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa), as well as fungal species (Candida albicans, Aspergillus fumigatus, and Sporothrix schenckii). The agar well diffusion method was employed to assess the antimicrobial activity. Bacterial cultures were cultivated on nutrient agar, while fungal cultures were grown on potato dextrose agar. Wells of a standardized diameter were created (5mm) on inoculated agar plates. 10% of the synthesized silver nanoparticles (50, 100, 250, and 500 µg/mL) were aseptically introduced into these wells. The plates were subsequently incubated at 35°C for 24 hours for bacteria and 28°C for 48 hours for fungi. The zones of inhibition surrounding the wells were measured to determine the antimicrobial potential of the silver nanoparticles [13].

3. RESULTS AND DISCUSSION

3.1 Unveiling the Nutritional Composition

The analysis of *P. indica*'s nutritional profile revealed a harmonious composition of essential metabolites, crucial for a balanced diet. Specific nutrient levels, including moisture (9.14%), crude protein (10.93%), and crude fiber (15.46%), were quantified (Table 1). The presence of essential minerals like calcium and phosphorus, along with ether extract, indicated *P. indica*'s potential contribution to a healthy diet.

3.2 Nutritional Composition: Context and Potential Synergies

The presence of essential secondary metabolites like Triterpenoids, Catachins, Flavonoids, Saponins, Tannins, Aminoacids etc., in *P. indica* paints a promising picture. However, it's important to consider the overall macronutrient profile (carbohydrates, proteins, fats) and micronutrient content (vitamins, minerals) Qualitative and Quantitative measures to fully understand its potential contribution to a balanced diet Tables 2 & 3, Figs. 1 & 2. Furthermore, investigating the digestibility and bioavailability of these identified compounds is crucial. Not all nutrients are readily absorbed by the body, and understanding this aspect is essential for determining the true nutritional value of *P. indica*. Interestingly, the presence of gallic acid alongside carbohydrates like sucrose suggests a potential synergistic effect. Gallic acid may possess properties that regulate blood sugar metabolism, potentially mitigating the rapid rise in blood sugar associated with simple sugars like sucrose [14,15]. This warrants further investigation to understand the interplay between these compounds within the *P. indica* matrix.

3.3 A Tapestry of Bioactive Compounds: Unveiling the Hidden Melody

The GCMS analysis of the *P. indica* extract unraveled a captivating tapestry of bioactive compounds, each with the potential to play a unique melody in promoting health. The presence of Dodecanoic acid, 3-hydroxy, β-D-Glucopyranose, 4-O-β-D- glucopyranosyl, Lupeol etc., (Table 4, Fig. 3) list the identified classes of bioactive compounds, e.g., fatty acids, organic acids, phenolic acids, steroids, terpenoids suggests a diverse range of potential health benefits. The GCMS analysis revealed a diverse range of bioactive compounds in the *P. indica* extract. While identifying these classes is a significant step, further research is needed to pinpoint the specific compounds responsible for the observed bioactivities. Isolating and characterizing individual compounds or synergistic combinations within the extract is crucial for understanding their functionalities and potential health benefits. Additionally, exploring

the potential mechanism of action of these bioactive compounds is essential. For example, understanding how the identified fatty acids influence cellular processes or how the phenolic acids exert their antioxidant activity can provide valuable insights for future research and nutraceutical development [16,17].

3.4 A Powerful Crescendo: Unveiling Antioxidant Activity

The *P. indica* extract demonstrated a remarkable free radical scavenging activity against ABTS compared to DPPH. **ABTS Scavenging Activity:** Both ascorbic acid and *P. indica* extracts showed concentration-dependent ABTS scavenging activity. Ascorbic acid exhibited higher ABTS scavenging activity compared to *P. indica* at all concentrations tested. The ABTS scavenging activity of *P. indica* increased significantly ($p < 0.001$) as the concentration increased from 20 to 100 µg/mL. **DPPH Scavenging Activity:** Similar to ABTS scavenging, both ascorbic acid and *P. indica* extracts demonstrated concentration-dependent DPPH scavenging activity. Ascorbic acid exhibited slightly higher DPPH scavenging activity compared to *P. indica* at all concentrations tested (Fig. 4). The DPPH scavenging activity of *P. indica* increased significantly ($p < 0.001$) as the concentration increased from 20 to 100 µg/mL. Both ascorbic

Table 1. Nutrition values of *P. indica*

S. No.	Test	Result	Unit
1	Moisture	9.14	%
2	Crude Protein	10.93	%
3	Crude Fibre	15.46	%
4	Ether Extract	4.35	%
5	Total Ash	6.96	%
6	Acid Insoluble Ash (Sand and Slica)	0.29	%
7	Calcium	1.60	%
8	Phosphorus	0.14	%
9	Salt	0.29	%
10	Gross Energy	3773	Kcal/kg

Table 2. Qualitative analysis of *P. indica*

Test	Aqueous	Methanol	Ethanol	Acetone	Hexane	Petroleum ether
Triterpenoids	-	+	+	+	-	-
Sugars	+	+	+	+	+	+
Catachins	-	-	-	-	-	-
Flavonoids	-	-	+	+	-	-
Saponins	+	+	+	+	+	-
Tannins	+	+	-	+	+	-
Anthroquinones	-	-	-	-	-	-
Aminoacids	-	-	-	-	-	-
Sterols	-	+	+	+	+	+
Carbohydrates	-	-	-	+	-	-

Table 3. Quantitative analysis of *P. indica*

Name of the test	Quantitative values
Chlorophyll ‘a’ (mg/g) dwt.	2.48 ± 1.2
Chlorophyll ‘b’. (mg/g) dwt.	4.35 ± 2.1
Total Chlorophyll (mg/g) dwt.	6.82 ± 0.9
Total Carotenoid (mg/g) dwt.	1.73 ± 1.1
Total sugar (mg/g) dwt.	2.3 ± 1.6
Total protein (mg/g) dwt.	0.8 ± 1.4
Total lipids (mg/g)	90 ± 0.2
Total free amino acid (mg/g) dwt.	0.2 ± 0.4
Total phenolics (mg/g)	267.6 ± 0.7
Total tannin (mg/g)	133.2 ± 1.4

Table 4. GCMS analysis for Compounds identified in the *P. indica* plant powder

No	RT (min)	Name of the compound	Molecular Formula	Molecular Weight	Peak Area %
1	4.18	Cyclohexene, 1-(3-ethoxy-1-propenyl)-, (Z)-	$C_{11}H_{18}O$	166	0.13
2	5.38	Catechol	$C_6H_6O_2$	110	1.39
3	5.72	1,2-Benzenedimethanol	$C_8H_{10}O_2$	138	0.69
4	7.26	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.98
5	8.86	3-O-Benzyl-d-glucose	$C_{13}H_{18}O_6$	270	3.66
6	9.08	Benzeneacetic acid, 2-nitro-	$C_8H_7NO_4$	181	0.24
7	9.33	Benzenemethanol, α-ethyl-4-methoxy-	$C_{10}H_{14}O_2$	166	0.54
8	9.54	Falcarinol	$C_{17}H_{24}O$	244	1.12
9	11.33	Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	216	10.75
10	11.43	6,9-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	5.83
11	12.08	β-D-Glucopyranose, 4-O-β-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	16.79
12	13.44	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.70
13	14.00	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	312	3.17
14	14.31	11-Hexadecynal	$C_{16}H_{28}O$	236	0.46
15	14.41	Phenol, 2-(3,7-dimethylocta-2,6-dienyl)-	$C_{16}H_{22}O$	230	0.66
16	14.55	Z,Z-4,16-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	308	0.74
17	17.66	Phytol	$C_{20}H_{40}O$	296	1.52
18	27.78	Squalene	$C_{30}H_{50}$	410	6.02
19	32.79	Vitamin E	$C_{29}H_{50}O_2$	430	5.16
20	34.95	Retusine	$C_{19}H_{18}O_7$	358	4.60
21	37.28	Lupeol	$C_{30}H_{50}O$	426	27.64
22	39.75	Betulin	$C_{30}H_{50}O_2$	442	7.20

Table 5. Evaluation of Antibacterial Potential of AgNPs through Zone of Inhibition

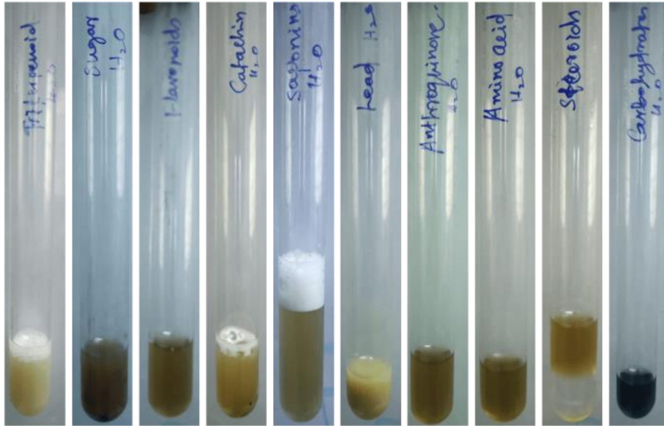
S. No.	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	Antibiotic (Ab) Gentamycin
1.	*Bacillus subtilis*	AgNPs	8±1.0	5.5±0.5	4.3±0.3	2.1±0.1	12±1.0
2.	*E. coli*		14±2.0	6.5±0.5	5.4±0.4	2.2±0.2	15±1.0
3.	*Pseudomonas aeruginosa*		9±1.0	6.4±0.4	3.2±0.2	2.1±0.1	11±1.0

The results were provided in mean ±SD and the significance was also notified (p<0.05)

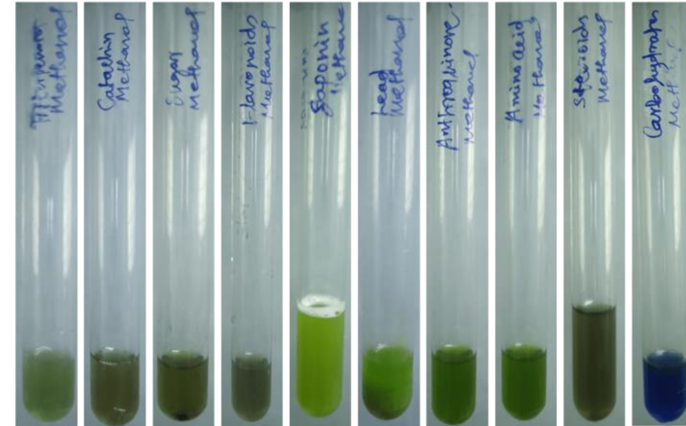
Table 6. Evaluation of Antifungal Potential of AgNPs through Zone of Inhibition

S. No.	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	AM (Antimycotic drug-Amphotericin B)
1.	*Candida albicans*	AgNPs	6±1.0	4.5±0.5	3.3±0.3	2.2±0.2	8±1.0
2.	*Aspergillus fumigatus*		5±1.0	3.3±0.3	2.2±0.2	2.1±0.1	7.5±0.5
3.	*Sporothrix schenckii*		6±1.0	4.4±0.4	3.2±0.2	2.1±0.1	7±1.0

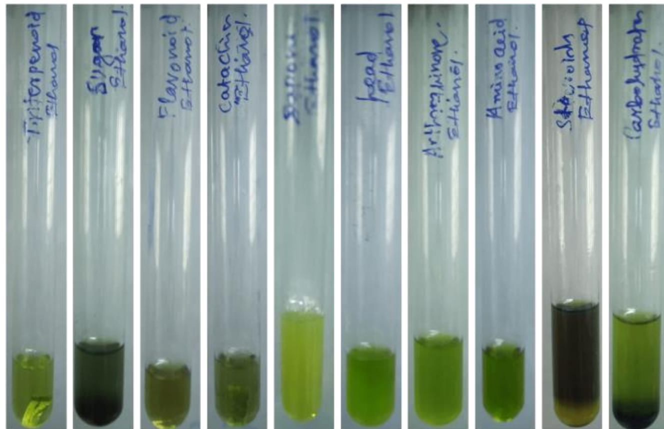
The results were provided in mean ±SD and the significance was also notified (p<0.05)



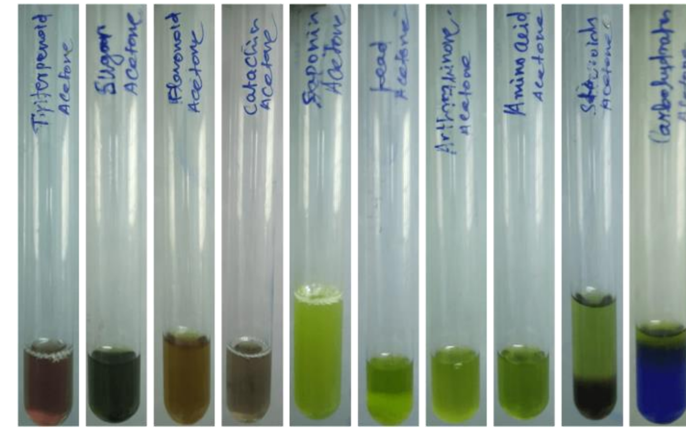
Aqueous extract



Methanolic extract



Ethanolic extract



Acetone extract

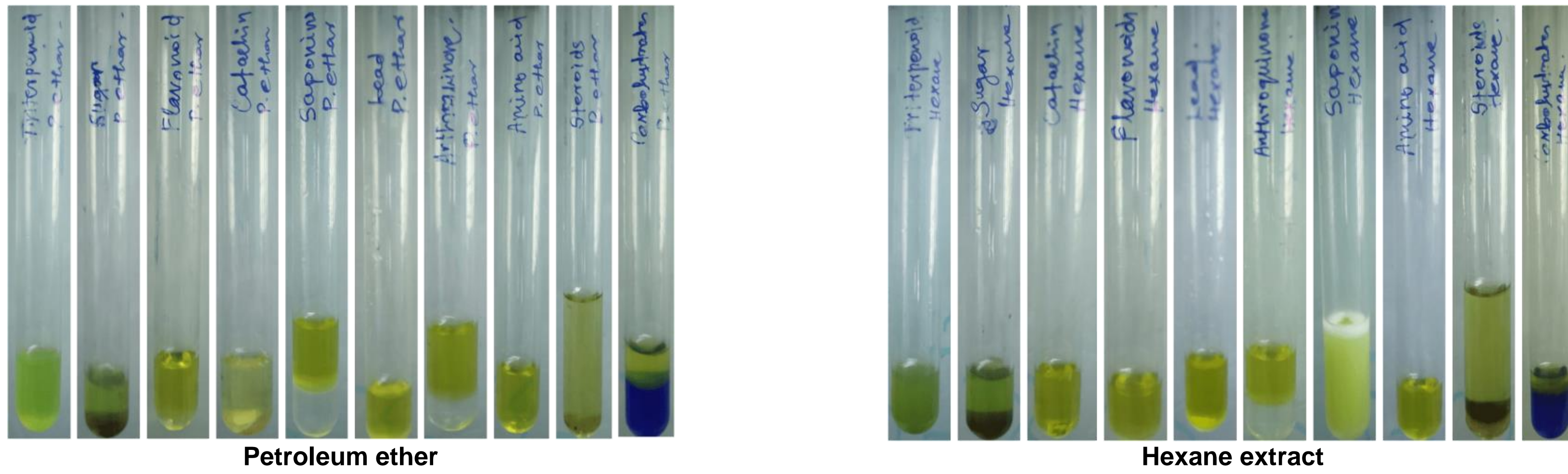


Fig. 1. Phytochemical screening of *P. indica* using different solvent extracts

acid and *P. indica* extracts possess significant antioxidant activity. Ascorbic acid exhibits slightly higher antioxidant activity compared to *P. indica*. The antioxidant activity of *P. indica* increases with concentration, suggesting a dose-dependent effect. This observation suggests the potential of *P. indica* as a source of natural antioxidants with the ability to combat oxidative stress, a major contributor to various chronic diseases [18,19]. Further investigation into the specific antioxidant mechanisms of these compounds can provide valuable insights for future nutraceutical development. The observed superior free radical scavenging activity against ABTS compared to DPPH is promising. However, it's important to compare this activity to known antioxidant standards like ascorbic acid (Vitamin C) to gain a clearer understanding of the extract's relative potency. Additionally, investigating the underlying mechanisms responsible for the observed antioxidant activity is crucial. Understanding the mechanism can guide future research on optimizing the extract's activity and identifying potential targets for therapeutic applications [20,21].

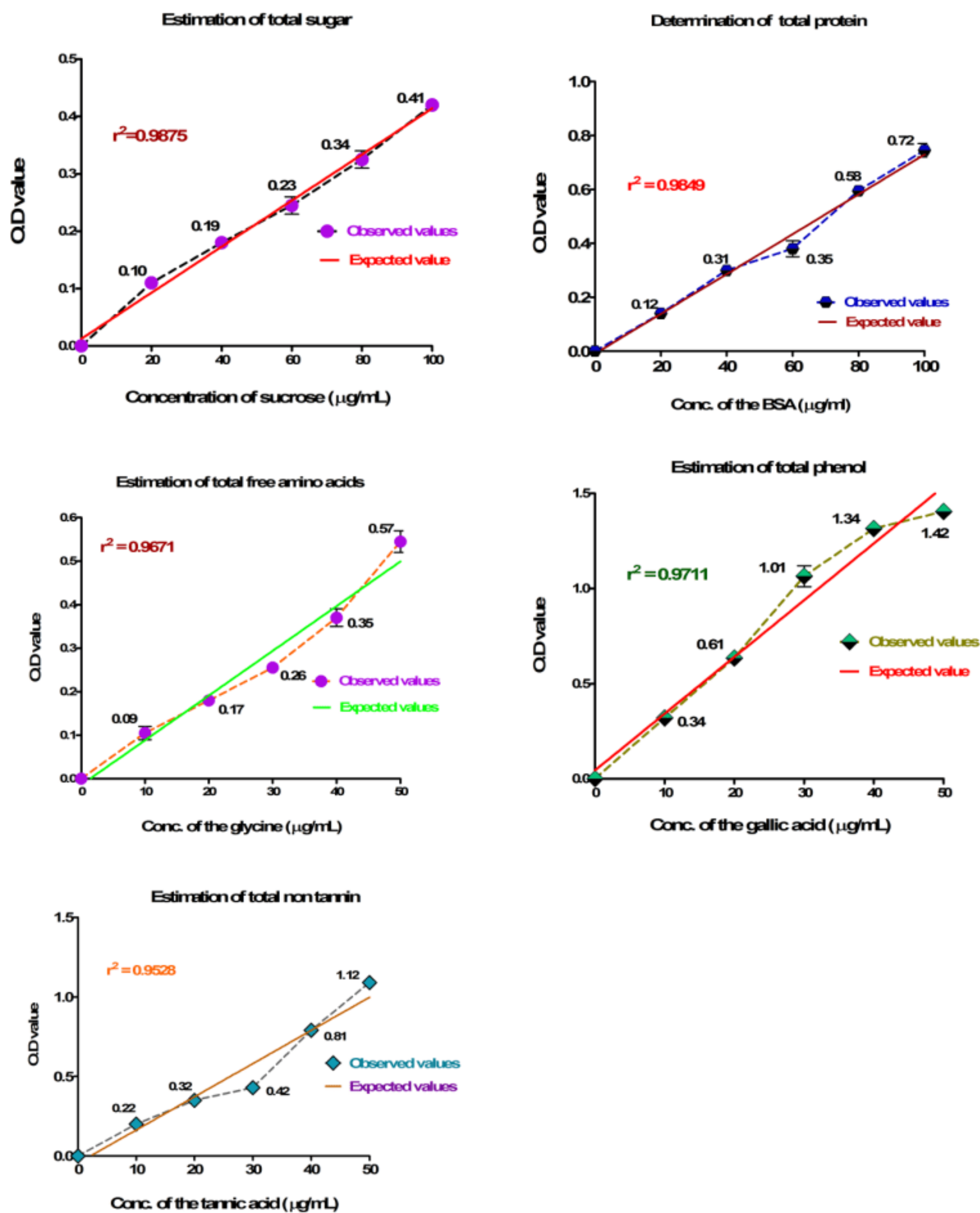


Fig. 2. Phytochemical quantitative analysis Screening of *P. indica*

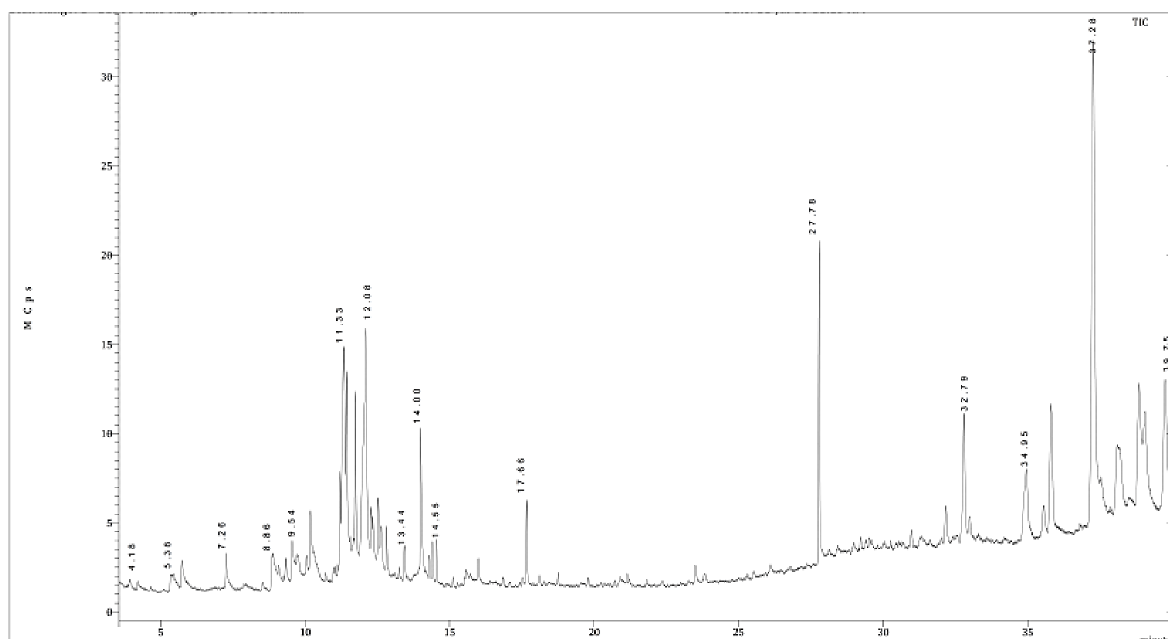


Fig. 3. GC- MS/MS Chromatogram of *P. indica*

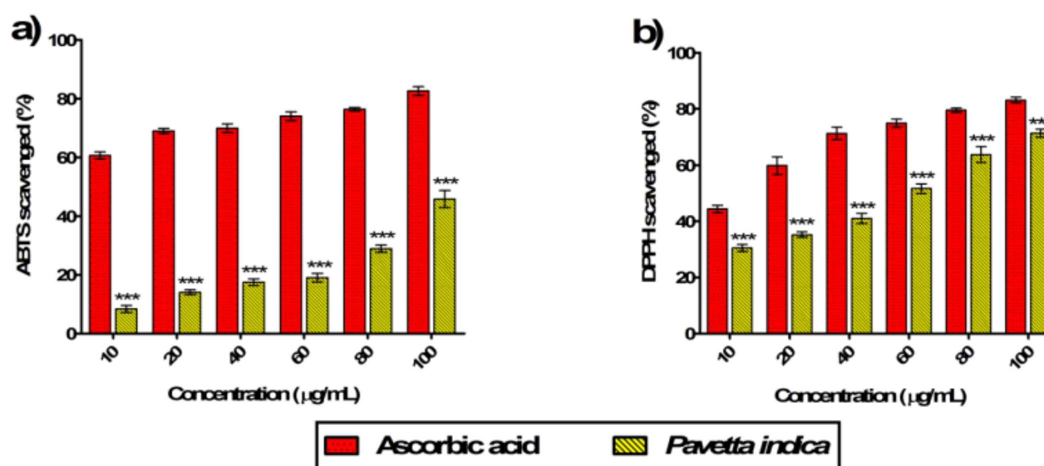


Fig. 4. Antioxidant activity of *P. indica*

3.5 Nanotechnology

Synthesis of Silver Nanoparticles: Silver nitrate was employed as the precursor for silver nanoparticle synthesis in the present investigation. The reducing agent for this green synthesis process was derived from *P. indica*, a plant revered in traditional Indian medicine for its therapeutic properties.

Metal-Plant Interaction: The interaction between silver ions and the phytochemicals present in the *P. indica* extract facilitated the green synthesis of silver nanoparticles. A visual indicator of this process was the distinct color transformation of the reaction mixture from a pale green to various shades of brown. This color change corresponded to the reduction of silver ions into silver nanoparticles. The bioactive compounds within the plant extract acted as reducing agents, promoting the formation of nanoparticles in solution.

3.6 Characterizations of Ag Nanoparticles

UV-Vis Spectral Analysis: UV-Vis spectroscopic analysis revealed a characteristic surface plasmon resonance (SPR) band

centered at approximately 420 nm, indicative of silver nanoparticle formation (Fig. 5). The interaction between the silver nitrate precursor and the *P. indica* extract facilitated the reduction of silver ions into Ag nanoparticles. A visual color change from colorless to brown accompanied this transformation. The UV-Vis spectra exhibited a broad absorption band spanning from approximately 350 to 480 nm, suggesting a polydispersed nature of the synthesized nanoparticles. With increasing reaction time, a blue shift in the peak position was observed, accompanied by an increase in absorbance intensity. This phenomenon is attributed to the growth and aggregation of silver nanoparticles. The emergence and intensification of the SPR band over time confirmed the progressive formation and growth of silver nanoparticles within the reaction mixture [22, 23].

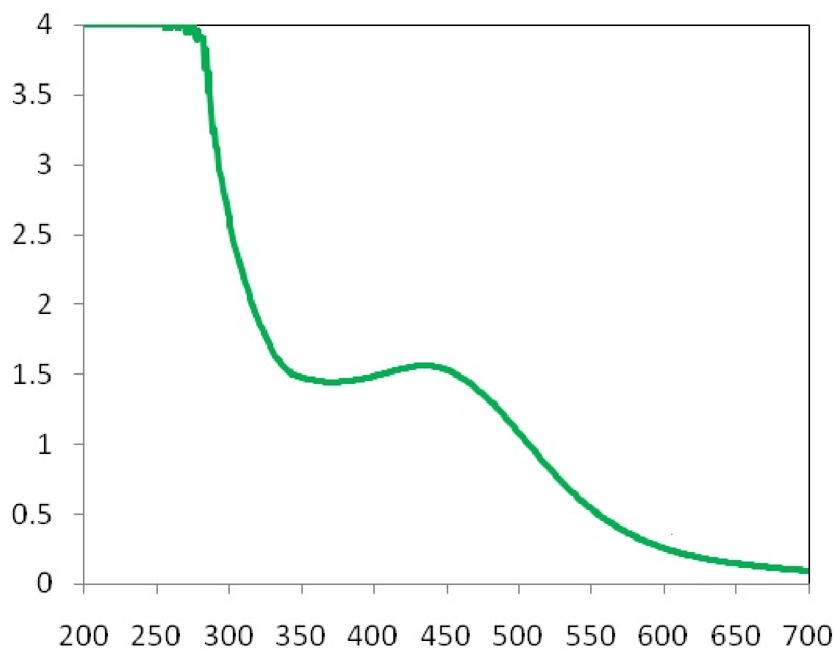


Fig. 5. UV Spec data of AgNPs

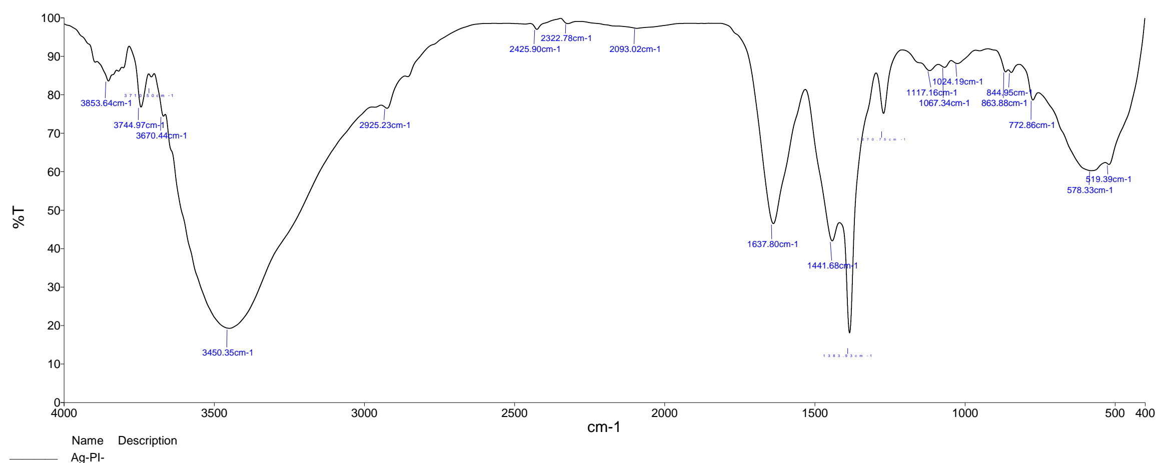


Fig. 6. FTIR data of AgNPs

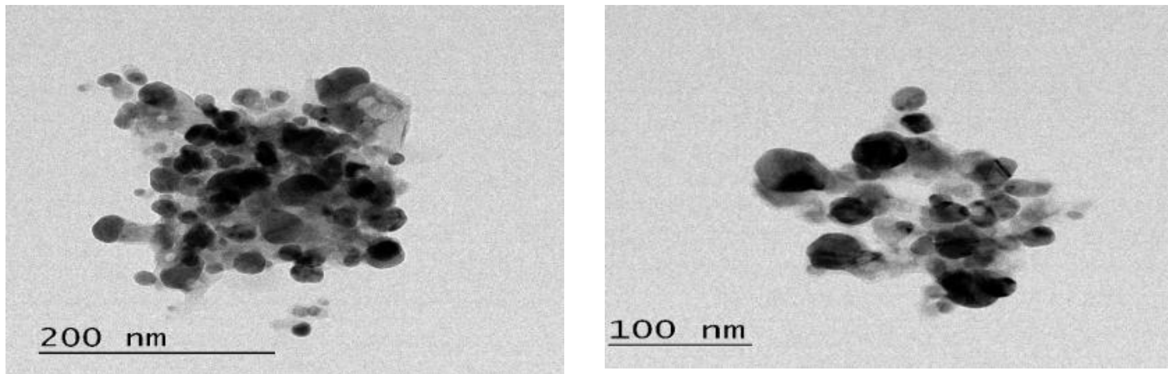


Fig. 7. TEM image of AgNPs synthesized using leaves of *Pavetta indica*

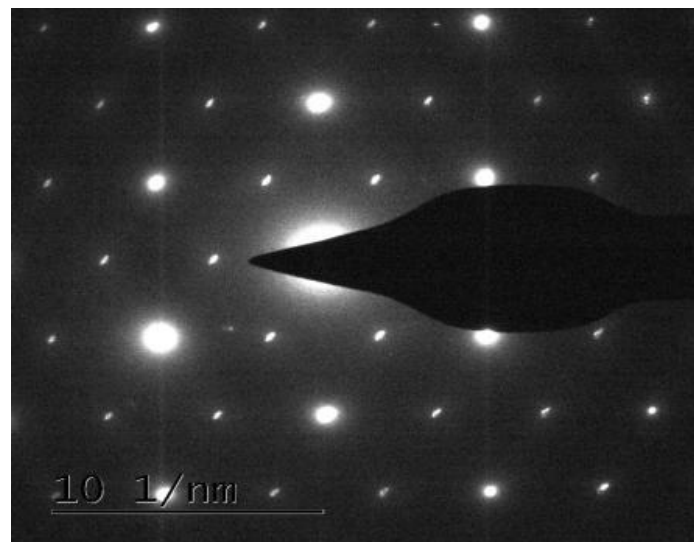
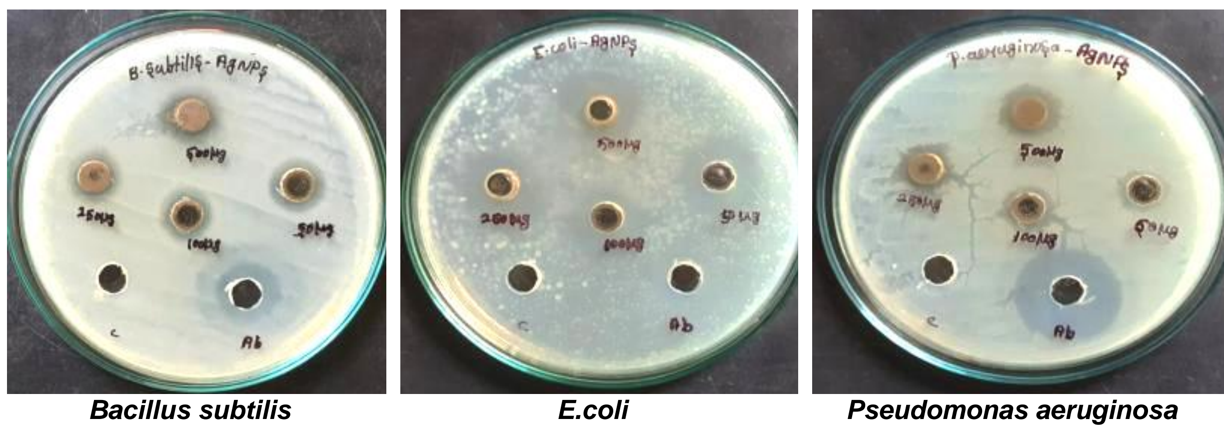
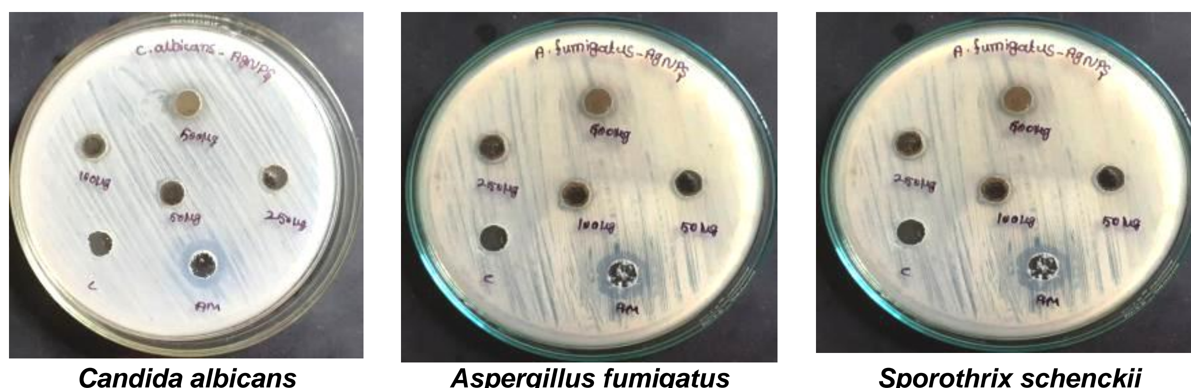


Fig. 8. SAED pattern of AgNPs synthesized using *Pavetta indica* leaves extract



Bacillus subtilis ***E.coli*** ***Pseudomonas aeruginosa***

Fig. 9. Antibacterial activity of AgNPs



Candida albicans ***Aspergillus fumigatus*** ***Sporothrix schenckii***

Fig. 10. Antifungal activity of AgNPs

3.7 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was employed to elucidate the functional groups present in the *P. indica* extract. The obtained spectrum exhibited characteristic absorption bands within the spectral range of 4000-400 cm^{-1}. Prominent peaks were observed at 3450 cm^{-1} (O-H stretching), 2925 cm^{-1} (C-H stretching of alkanes), 1637 cm^{-1} (C=C stretching of alkenes), 1441 cm^{-1} (aromatic C-C stretching), and 1378 cm^{-1} (C-H bending of alkanes) (Fig. 6). These findings suggest the presence of hydroxyl, aliphatic, aromatic, and other functional groups within the plant extract, which might contribute to the reduction of silver ions and stabilization of the synthesized nanoparticles [24,25].

Transmission electron microscopic (TEM) and Selected area electron diffraction (SAED) pattern study: Transmission electron microscopy (TEM) was employed to characterize the morphology and size distribution of the synthesized silver nanoparticles. Micrographs obtained at varying magnifications revealed spherical nanoparticles with an average size ranging from 25 to 50 nm (Fig. 7). Evidence of agglomeration among the particles was observed, likely attributed to the capping effect of biomolecules present in the *P. indica* extract. To further elucidate the crystalline nature of the nanoparticles, selected area electron diffraction (SAED) patterns were recorded (Fig. 8).

3.8 Antibacterial Activity- Silver Nanoparticles

The agar well diffusion method was employed to assess the bactericidal efficacy of *Pavetta indica*-mediated synthesized AgNPs at various concentrations. Fig. 9 illustrates the antibacterial activity of the AgNPs against *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Observed inhibition zones indicated the antibacterial potential of the synthesized AgNPs. The largest inhibition zone of 14 ± 2.0 mm was recorded for *E. coli*. *Bacillus subtilis* and *Pseudomonas aeruginosa* exhibited inhibition zones of 8 ± 1.0 mm and 9 ± 1.0 mm, respectively. Bactericidal activity was concentration-dependent for the test sample. These results were compared to the standard drug, gentamicin (Table 5). The emergence of antibiotic-resistant microbial strains poses a significant challenge to global health. While antibiotics have been instrumental in combating infections, their overuse has led to the proliferation of resistant pathogens. Nanotechnology offers a promising alternative with silver nanoparticles exhibiting broad-spectrum antimicrobial properties at relatively low concentrations [26,27].

3.9 Antifungal Activity

The antifungal potential of the green-synthesized silver nanoparticles derived from *P. indica* leaf extract was evaluated against three clinically significant fungal pathogens: *Candida albicans*, *Aspergillus fumigatus*, and *Sporothrix schenckii*. The agar disc diffusion method was employed using potato dextrose agar as the culture medium. Silver nanoparticle solutions were prepared at four different concentrations for antimicrobial testing (Fig. 10). The obtained results, as depicted in Figures a, b, and c, demonstrated a clear zone of inhibition around the wells containing the silver nanoparticle solutions, indicating antifungal activity against all tested fungal strains (Table 6). The diameter of the inhibition zones was found to be concentration-dependent, with larger zones

observed at higher nanoparticle concentrations. Amphotericin B, a standard antifungal drug, served as a positive control in this assay. These findings suggest that the green-synthesized silver nanoparticles possess promising antifungal properties, warranting further investigation into their potential as a therapeutic agent against fungal infections [28]. Silver nanoparticles exhibit a multifaceted approach in their antifungal activity. Initially, they adhere to the fungal cell surface, creating structural disruptions and facilitating subsequent internalization [29,30].

4. CONCLUSION

This multifaceted investigation unveiled the potential of *P. indica* as a promising source of both nutritional and bioactive compounds. The identified essential metabolites and antioxidant properties underscore its role in promoting overall health and well-being. Future research can delve deeper into the specific health benefits of these compounds through in vitro and in vivo studies. A significant aspect of this research involved the green synthesis of silver nanoparticles using *P. indica* extract. The resulting nanoparticles exhibited promising antimicrobial properties against both bacterial and fungal pathogens. This study contributes to the growing body of research highlighting the potential of plant-mediated synthesis as an eco-friendly approach to developing antimicrobial agents. While this research provides valuable insights, it also presents opportunities for further exploration. Characterizing the complete phytochemical profile of *P. indica* and investigating the mechanisms underlying the antimicrobial activity of the synthesized silver nanoparticles are crucial next steps. Additionally, optimization of nanoparticle synthesis parameters and evaluation of their toxicity profile are essential for potential applications. In conclusion, this study demonstrates the potential of *P. indica* as a versatile resource with applications in both nutrition and antimicrobial development. By harnessing the power of nature, this research contributes to the development of sustainable and effective solutions for human health challenges.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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