

Research Article:

Antibacterial Potential of Plant-Extract Mediated Silver Nanoparticles Against Pathogenic Bacteria Isolated from Vaginosis

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Abstract

Background: Bacterial Vaginosis (BV) is a prevalent bacterial dysbiosis worldwide. The emergence of multi-drug resistant (MDR) pathogens enhanced interest in nanomedicine as an alternative therapeutic approach.

Aims: This study investigated the incidence and etiology of BV, besides the antibacterial potential of Purslane (*Portulaca oleracea* L) combined silver nanoparticles (AgNPs) was evaluated.

Methodology: Eighty three women patients were screened for BV at public hospitals and private clinics. Bacterial identification and susceptibility were carried out using Vitek-2 system, molecular analysis and disk diffusion assay. Concurrently, Purslane-mediated AgNPs were synthesized by green biosynthesis, characterized by UV-Vis, XRD, FE-SEM, TEM and FTIR, and its antibacterial activity was examined by diffusion and microdilution assays.

Results: It was found that the incidence rate of BV is 57.8%, and the bacteriological analysis yielded 48 isolates across 11 bacterial species. *Staphylococcus aureus* was the most prevalent (20.83%), followed by *Staphylococcus haemolyticus* (18.75%), *Escherichia coli* (16.67%), *Klebsiella pneumoniae* (12.5%), *Staphylococcus epidermidis* (12.5%), *Staphylococcus hominis* (8.33%), and *Klebsiella aerogenes*, *Vaginella massiliensis*, *Corynebacterium amycolatum*, *Enterococcus faecalis* and *Streptococcus agalactiae* at (2.08%). Resistance against cefixime (68.75%), colistin (45.83) and amoxicillin (37.5) was observed across most of the isolates. On the other hand, characterizations confirmed the successful biosynthesis of quasi-spherical Purslane-mediated AgNPs at absorbance peak of 450 nm, with a diameter of 14.89 nm, FTIR spectroscopy revealed that the reduction of Ag⁺ to Ag⁰ and subsequent stabilization were driven by plant phenolics and proteins, characterized by significant intensity shifts in the hydroxyl (3406 cm⁻¹), amide (1631 cm⁻¹), and C–O (1012 cm⁻¹) vibrational bands. Notably, the Purslane-mediated AgNPs exhibited broad antibacterial efficacy against all BV bacterial species, with MIC ranging from 15.6 to 250 µg/ml.

Conclusion: this study reveals the development of MDR bacteria in BV and promising antibacterial activity of Purslane-mediated AgNPs.

Keywords: Bacterial Vaginosis, Green biosynthesized Nanoparticles, Purslane, Antibacterials

1. Introduction

Bacterial vaginosis (BV) is one of the most gynecological complaints that caused by a significant change in the vaginal microbiota. This vaginal dysbiosis is a complex polymicrobial overgrowth, characterized by the replacement of vaginal *Lactobacilli* flora with facultative and

obligate anaerobes [1, 2]. The virulence factors like sialidase and vaginolysin play important roles in the BV pathogenesis. These factors enhance the destruction of the protective mucus layer of vaginal epithelium, enabling strong adhesion, cytotoxicity, and the establishment of a complex, polymicrobial biofilm [2, 3]. The most common etiologic agent of BV is anaerobic *Gardnerella vaginalis*, besides other Gram-positive and Gram-negative facultative

anaerobic bacteria were isolated [4-9]. These bacteria come from either intrinsic (vaginal tract itself) or extrinsic (sexual activity) sources.

Globally, the prevalence of BV ranges from 23–29%, this changes across countries and local populations [10-12]. The majority of epidemiological data, generated from metropolitan or tertiary care institutions, leaving a huge gap in understanding the prevalence, risk factors, optimal therapeutics and local microbiological profiles of BV [13, 14]. The investigation of BV has received little attention in Kurdistan region-Iraq, as few studies are available in the region [4, 15]. The global reports about the health risks of multi-drug resistant (MDR) bacteria mandate urgent innovations in therapeutic medicines [16, 17]. Overuse and misuse of traditional antibiotics have resulted in decreased drug efficacy and made illnesses like BV frequently recurs and extremely difficult to control [18]. Studies revealed that *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* spp, *Enterococcus* spp and *Streptococcus* spp are the most common isolated MDR bacteria in BV [19-22].

Screening for new antibacterial therapies has been prioritized in medical researches. Silver nanoparticles (AgNPs) have emerged as a potent broad-spectrum antimicrobial agent because of their unique physicochemical properties, higher surface area-to-volume ratio, distinct color variations because of Localized Surface Plasmon Resonance (LSPR), high electrical and thermal conductivity and diverse mechanisms of antimicrobial actions [23, 24]. The Moreover, the advantages of physical methods for nanoparticle synthesis are high purity, uniform size and shape, reduced hazardous chemical solvents, versatility and so on[24]. However, the chemical synthesis of AgNPs involves hazardous chemicals, so this has switched the attention to eco-friendly green biosynthesis that employs plant extracts as reducing and capping agents [25]. Purslane (*Portulaca oleracea*) is a medicinal plant used as anti-inflammatory, antioxidant, and antibacterials, due to its rich components of phenolic compounds, including flavonoids, alkaloids, terpenoids and omega-3 fatty acids [26, 27]. Purslane has recently received a lot of attention in the nanotechnology industry [28].

The local clinics and laboratories hypothesized that BV would be prevalent in Chamchamal district, so exploring the etiological properties of BV would be critical for establishing context-specific health interventions. Thus, evaluating the antibacterial efficacy of green synthesized Ag-NPs may provide valuable insights into innovative antibacterials to overcome MDR infections. This study aimed at the synthesis, characterization and assessing the antibacterial activity of Purslane mediated AgNPs against bacteria isolated from BV.

2. Methods and Materials

A. Study design

This is a descriptive epidemiological study to investigate BV among married women patients in Chamchamal district, Sulaymaniyah Governorate, Kurdistan Region-Iraq. Concurrently, this study involved biosynthesis and

determine the antibacterial activity of Purslane-silver Nanoparticles (P-Ag NPs) against the BV bacterial isolates.

B. Data collection

Physical and gynecological examinations were firstly carried out by a gynecologist. A standardized questionnaire was used to gather information about the participants' sociodemographic and clinical variables. The demographic data included age, educational attainment, types of contraceptives used, past medical history, and recurring infections, while the clinical data were symptoms, existence of vaginal discharges, discoloration of the discharge, and the pH of the vagina. The married women who reported experiencing painful urination, painful intercourse, vaginal discharge, or vaginal itching were deemed symptomatic[4, 29, 30].

C. Vaginal discharge sampling

This study involved 83 women patients, who were appointed at the public obstetrics and gynecological hospitals and private out-patient clinics in Chamchamal district between October 2024 and March 2025. A sterile unlubricated speculum was used by a gynecologist to collect high vaginal swabs (HVS). Prior oral consent was taken from the women participants and ethical approval was provided by the Ethics Committee at college of science/Charmo university (Reference No. 20 in 2025).

D. Microscopic examination and bacterial isolation

Two sterile vaginal swabs were used, one of the vaginal discharge specimens was used for pH measurement and microscopic examination (the specimen was placed on a glass slide, covered with regular saline + 10% KOH for the wet mount preparation), and the second specimen was transported under sterile conditions inside a BOENMED transport medium and immediately cultured on Nutrient, Blood and MacConkey agars and incubated at 37 °C for 24 hours. The bacterial colonies were subcultures and stored in glycerol stocks at -20 °C [6, 31].

E. Identification of the bacterial isolates

1. Identification of the bacteria by VITEK® 2 system

The VITEK® 2 automated system (bioMérieux) was initially used as biochemical platform for the bacterial identification, according to the manufactures' instructions[4, 32]. Depending on the Gram stain reaction, identification was performed using either the VITEK® 2 GP (Gram-positive) card (Lot. Number: 2422735503) or the VITEK® 2 GN (Gram-negative) card (Lot. Number: 2412745103).

2. Molecular identification of bacteria

A polymerase chain reaction (PCR) was utilized to amplify 16S rRNA gene of the isolated bacteria. Firstly, a commercial kit (for better BIO-TECH) was used to extract the bacterial genomic DNA. Nanodrop spectrophotometer (Thermo Scientific) was used to determine concentration and purity of the DNA extracts. Secondly, the PCR reaction mixture (20 µl) was prepared of 10 µl master mix (BIO-Rada), 0.5 µl of each forward (7F:5'-

AGAGTTTGATYMTGGCTCAG-3') and reverse primer (1510R:5'-ACGGYTACCTTGTTACGACTT-3'), 1 μ L DNA template, and 8 μ L ddH₂O. Thirdly, the PCR reaction was run at an initial denaturation (94°C for 5 min), 30 cycles of Denaturation (94°C for 30 sec), annealing (58°C for 30 sec) and extension (72°C for 1 min), and a final extension (72°C for 5 min) [33]. Finally, the ~1.5 kb PCR products were visualized on 1.5% agarose gel under UV transilluminator and sequenced by Sanger DNA sequencing method at Macrogen sequencing facility in Republic of Korea. Moreover, the sequenced 16S rRNA gene of the bacterial isolates were uploaded to Bankit/National Center for Biotechnology Information (NCBI) database. The GenBank accession numbers were allocated to the bacterial strains.

F. Antibiotic Susceptibility Testing

The antimicrobial susceptibility profiles of the BV bacterial isolates were assessed using Kirby-Bauer disc diffusion assay [34]. The pure cultures of each isolate were suspended in sterile saline to make a 0.5 McFarland standard. These suspensions were evenly spread over the surface of Mueller-Hinton Agar (MHA) plates with sterile cotton swabs. Then, 16 distinct antibiotic discs (Liofilchem® S.r.l. and HiMedia Laboratories Pvt. Ltd.) were positioned on the inoculated MHA plates. The tested antibacterials were Amoxicillin (AMX) 25 μ g, Amoxicillin-Clavulanic acid (AMC) 30 μ g, Meropenem (MRP) 10 μ g, Imipenem (IMP) 10 μ g, Amikacin (AK) 30 μ g, Ciprofloxacin (CIP) 5 μ g, Nitrofurantoin (NIT) 300 μ g, Cotrimoxazole (COT) 25 μ g, Rifampicin (RIF) 5 μ g, Colistin (CT) 10 μ g, Levofloxacin (LE) 5 μ g, Novobiocin 30 μ g (NV), Tigecycline (TGC) 15 μ g, Cefixime (CFM) 5 μ g, Ceftriaxone (CTR) 10 μ g, and Piperacillin/Tazobactam (PIP/TAZ) 10 μ g. The plates were incubated at 37°C for 24 hours and the diameters of inhibition zones were measured in millimeters. Finally, the resistant (R) bacterial isolates were identified using Clinical and Laboratory Standards Institute (CLSI, M02) guidelines.

G. Collection and Preparation of Purslane aqueous extract

Common purslane (*Portulaca oleracea* L.) was collected from in the plain of Shwan sub-district, Kirkuk, Iraq (35.61°N, 44.52°E) in August, 2025. The plant taxonomy was confirmed with voucher no. 5072 by college of agriculture herbarum at Sulaimani university. The leaves of the purslane plant were utilized to manufacture Ag-NPs using the previously developed method with some modifications [35, 36]. Fresh purslane leaves were properly rinsed in tap water and then in distilled water. The plant was chopped and dried at room temperature (~25 °C) for 20 days. An aqueous extraction was prepared by dissolving 25 g of coarsely dried powder in 500 mL of ddH₂O (1:20 w/v ratio). This mixture was subjected to thermal-assisted extraction, which was heating at 75 °C for 20 minutes with continuous stirring (at 350rpm). The heated solution was then treated with ultrasonic bath for 30 minutes in order to disturb cellular structures and release phytoconstituents. The resulting crude extract was filtered through Whatman No. 1 filter paper. The filtrate was then centrifuged at 6000 rpm for 10 min, and the final clear supernatant of aqueous extract was collected and

kept at 4 °C. For green synthesis of purslane-mediated silver nanoparticle see section 3.D and Figure. 3.

H. Characterization of Purslane-mediated AgNPs

The physical, structural, and optical properties of the AgNPs were investigated. By using a UV-Vis Spectrophotometric UV Line 9400 (SI Analytics) with a scanning range of 200-800 nm, the UV-Vis absorption spectra of the prepared AgNPs were measured by adding 1 mL of liquid AgNPs to a standard UV-Vis cuvette. Besides, X-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM) and fourier transform infrared spectroscopy (FTIR) were carried out by Photon center for research and study, in Baghdad, Iraq. The crystalline structure and phase purity of the prepared AgNPs were investigated using X-ray diffraction (XRD) on a PW1730 diffractometer (Philips, Netherlands). The system was operated with Cu-K- α radiation (1.5406 Å) at an accelerating voltage of 45 kV and an applied current of 40 mA. Patterns were recorded over a scanning range of $10^\circ \leq 2\theta \leq 70^\circ$ with a step size of 0.0500° and a scan step time of 1.0 s (equivalent to a scan rate of $3^\circ/\text{min}$). The samples were analyzed in a dried powder form, consistent with the preparation methods described by [37]. The average crystallite size (D) of the synthesized AgNPs was determined using the Debye-Scherrer equation [38]. The parameters utilized for this estimation were derived from the most intense diffraction peak at $2\theta = 32.272^\circ$ (Bragg angle $\theta = 16.136^\circ$). The Full Width at Half Maximum (FWHM) was measured as 0.871° , which was converted to 0.0152 radians for numerical consistency in the equation. A shape factor (K) of 0.9 was applied, assuming spherical nanoparticle morphology, with a radiation wavelength (λ) of 1.5406 Å. This calculated value was further corroborated by analyzing the secondary and tertiary reflections at 46.28° ($\beta = 0.900^\circ$) and 27.84° ($\beta = 0.820^\circ$), which consistently confirmed the nanostructured nature of the silver particles.

Morphological characteristics of the synthesized AgNPs were analyzed via field emission scanning electron microscopy (FE-SEM; TESCAN MIRA3, Czechia). The sample for SEM was prepared by placing a drop of the AgNP solution on a gold-coated copper grid, which was then dried at room temperature and transferred for analysis. The FE-SEM was operated at an accelerating voltage of 15 kV to capture high-resolution images of the nanoparticles. The imaging capability of the MIRA3 system allowed for a detailed assessment of the nanoparticle morphology and degree of dispersion, following standard protocols for nanomaterial characterization [39]. Furthermore, the morphological characteristics of the synthesized AgNPs were determined using Transmission Electron Microscopy (TEM; PHILIPS model CM120, The Netherlands), operated at an accelerating voltage of 100 kV. The sample for TEM analysis was prepared by dispersing the AgNPs in distilled water through ultrasonication for 15 minutes to achieve a uniform suspension. Subsequently, a drop of the AgNP suspension was deposited onto carbon-coated copper grids and left to dry at room temperature [40].

The FTIR was used to identify the functional groups involved for the bioreduction of silver ions and the stabilization of the resultant nanoparticles. A Shimadzu FTIR Spectrophotometer (Shimadzu Corp., Japan), which is frequently used for its accuracy in recognizing biomolecular fingerprints in green synthesis, was used to conduct the analysis [41]. The synthesized AgNPs suspension was first purified by centrifugation at 10,000 rpm for 15 min, followed by washing with double-distilled water to remove unreacted plant metabolites. The obtained pellet was then dried at 45 °C to obtain a fine powder, which was subsequently mixed with spectroscopic-grade KBr (1:100) and compressed into a transparent pellet using a hydraulic press [42]. A hydraulic press was used to compress the dried AgNPs and raw plant extract samples into thin, transparent pellets after they had been individually crushed with spectroscopic grade potassium bromide (KBr) in a 1:100 ratios. The spectra were captured at a resolution of 4 cm⁻¹ in the wavenumber range of 4000 to 400cm⁻¹. This wide range makes it possible to identify the distinctive skeletal "fingerprint" region and high-frequency stretching vibrations, both of which are crucial for verifying the chemical interaction between the metallic silver and organic ligands [43].

I. Antimicrobial Bioassay of Purslane-mediated AgNPs

The well diffusion method was used to assess the antibacterial activity of the Purslane-mediated AgNPs. Three different treatments including raw purslane plant extract, silver nitrate (AgNO₃) and green-synthesized AgNPs (4 mg/mL) were tested against two common BV pathogens, *S. aureus* and *E. coli*. The MHA plates were first inoculated with bacterial suspension (0.5 McFarland), then three wells (6–8 mm) were punched into the agar and the above treatments were added to the wells. After overnight incubation at 37°C, the antibacterial potential was determined by measuring the diameter of the inhibition zones.

In addition, the minimum inhibitory concentration of Purslane-mediated AgNPs was determined for 11 bacterial species, isolated from BV, using broth microdilution method in sterile 96-well microtiter plate. A twofold serial dilution (7.8, 15.6, 31.25, 62.25, 125, 250 µg/mL) of the Purslane-Ag NPs were prepared [44]. New batches of bacterial isolates were diluted with Mueller-Hinton broth (MHB) to make 0.5 McFarland. A 120 µl of bacterial cultures were added to the 96-well plates, and then 80 µl of the diluted P-Ag NPs were added. The growth control wells contained 120 µl of bacteria and 80 µl of medium, while the blank wells contained P-Ag NPs and medium. The plate was incubated at 37°C for overnight in a shaking incubator. The end-point optical density (OD₆₀₀) was measured using a BioTek (ELx800) microplate reader (USA) [45].

J. Statistical analysis

All experimental data were processed in triplicate where applicable. Data management, including the calculation of means, standard deviations (SD), and the generation of error bars, was performed using Microsoft Excel 2010. Statistical analysis was conducted using IBM SPSS Statistics (Version 27.0.1). Significant differences and

relationships between the questionnaire variables were determined using one-way analysis of variance (ANOVA). Statistical significance was ascertained at a *p*-value of < 0.05.

3. Results and Discussion

A. Incidence of Bacterial vaginosis

The BV cases were ordinarily diagnosed based on the abnormal vaginal discharge, vaginal pH, the positive clue cell and whiff tests (data not shown), and microbiological analysis. Among the 83 women patients, only 48 were diagnosed as BV (**Figure 1**), this indicated an incidence rate of 57.83%. Similarly, a study found an incidence rate of BV (62.03%) among 108 women in Kalar district [4]. However, another study showed lower incidence of BV (27.33%) among women in Zakho district [15]. Moreover, current findings showed a higher BV incidence rate than the prevalence rates in Nepal [6], India [46] and Egypt [47], with values of 24.4%, 24%, and 33%, respectively. The discrepancy in the results of above researches may be related to numerous aspects such as geographical distribution, size of population, data processing, behavioral differences, and socioeconomic position [6].

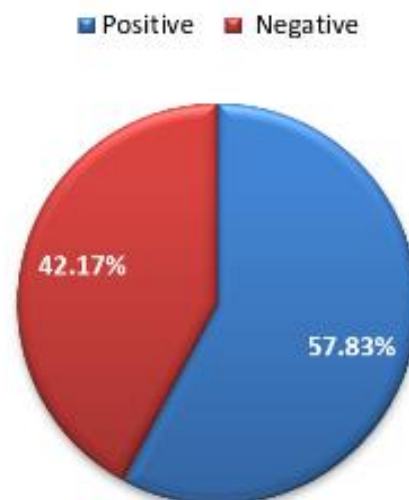


Figure 1: The incidence of BV in Chamchamal district.

B. Etiology of bacterial vaginosis

From the 48 BV cases, a total of 48 isolates of Gram-positive and Gram-negative bacteria were recovered and belonged to 11 distinct species (**Figure. 2, Table 1**). Regarding the frequency of the bacterial species, *S. aureus* (20.83%) was the most common cause of BV. Subsequently, *S. haemolyticus* (18.75%), *E. coli* (16.67%), *K. pneumoniae* (12.5%), *S. epidermidis* (12.5%), and *S. hominis* (8.33%) were detected. The other bacterial species of *K. aerogenes*, *Vaginella massiliensis*, *Corynebacterium amycolatum*, *Enterococcus faecalis*, and *S. agalactiae*, individually accounted for a very low percentage (2.08%) (**Figure. 2, Table 1**).

Current findings are consistent with the studies by Maghsoudi et al. [7], Tiyyagura et al. [8], and Ranjit et al. [6], who found *Staphylococcus aureus* (12.33%) as the most

prevalent Gram-positive cocci, and *E. coli* (13.70%) and *Klebsiella sp.* (8.22%) as the frequently isolated Gram-negative bacteria. However, our findings contradicted the results of Marrazzo et al. [48] in Seattle, Razzak et al. [9] in Iraq, Larsen and Monif [49] in Omaha, and Ranjit et al. [6] in Nepal, who isolated *Pseudomonas spp.* as the most

prevalent species in BV. The appearance of faecal flora in vaginal discharge can be attributed to bad bowel practices [50]. Again, a study in kalar district found Coagulase-negative staphylococci (CoNS) as the predominant cause of BV (32.84%), followed by *E. coli* (14.93%), *S. aureus* (13.43%), *K. pneumonia* (8.96%), *Micrococcus luteus* (7.46%) and *Proteus spp.* (1.49%) [4].

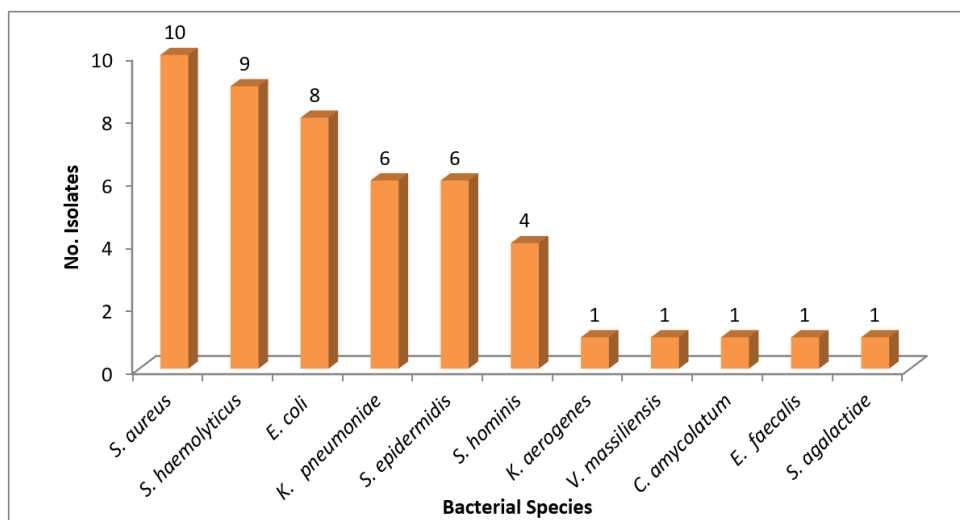


Figure 2: Frequency of bacterial species isolated bacterial vaginosis in Chamachaml district

Table 1: Molecular and bioinformatic data of BV bacterial isolates based on the 16S rRNA gene sequence.

Bacterial Species	Strain ID	Accession No.	NCBI link
<i>S. aureus</i>	charmo z6	PX788569	https://www.ncbi.nlm.nih.gov/search/all/?term=PX788569
	charmu z14	PX788577	https://www.ncbi.nlm.nih.gov/search/all/?term=PX788577
<i>S. haemolyticus</i>	charmo z2	PX781777	https://www.ncbi.nlm.nih.gov/search/all/?term=PX781777
<i>E. coli</i>	charmo z6	PX783021	https://www.ncbi.nlm.nih.gov/search/all/?term=PX783021
	charmu z11	PX788573	https://www.ncbi.nlm.nih.gov/search/all/?term=PX788573
<i>K. pneumoniae</i>	charmo z3	PX782497	https://www.ncbi.nlm.nih.gov/search/all/?term=PX782497
	charmu z12	PX788575	https://www.ncbi.nlm.nih.gov/search/all/?term=PX788575
<i>S. epidermidis</i>	charmo z4	PX782577	https://www.ncbi.nlm.nih.gov/search/all/?term=PX782577
	charmu z7	PX788570	https://www.ncbi.nlm.nih.gov/search/all/?term=PX788570
	charmo z9	PX788571	https://www.ncbi.nlm.nih.gov/search/all/?term=PX788571
<i>S. hominis</i>	charmo z13	PX788576	https://www.ncbi.nlm.nih.gov/search/all/?term=PX788576
<i>K. aerogenes</i>	charmo z10	PX788572	https://www.ncbi.nlm.nih.gov/search/all/?term=PX788572
<i>V. massiliensis</i>	charmo z5	PX782592	https://www.ncbi.nlm.nih.gov/search/all/?term=PX782592
<i>C. amycolatum</i>	charmo z1	PX632071	https://www.ncbi.nlm.nih.gov/search/all/?term=PX632071
<i>E. faecalis</i>	charmo z17	PX830358	https://www.ncbi.nlm.nih.gov/search/all/?term=PX830358
<i>S. agalactiae</i>	charmo z15	PX788578	https://www.ncbi.nlm.nih.gov/search/all/?term=PX788578

C. Antibacterial susceptibility of the bacterial isolates

Antibiotic susceptibility test was done for all the 48 bacterial isolates across 11 bacterial species (**Table 2**). Overall, the results showed a diverse pattern of antibacterial susceptibility among the isolates. Most of the bacterial isolates were sensitive for most of the tested antibiotics such as imipenem, amikacin, tigecycline and pip/tazobactam (PIP/TAZ), while a considerable number of the isolates were resistant to cefixime (68.75%), colistin (45.83%) and amoxicillin (37.5%). This high level of resistance to third-generation cephalosporins and penicillin indicates a significant presence of Extended-Spectrum Beta-Lactamase

(ESBL) producers in the bacterial isolates. This is consistent with the worldwide findings, insisted an increasing ESBL prevalence in both Gram-positive and Gram-negative pathogens [51]. Furthermore, intermediate level of resistance was seen to levofloxacin (16.67%) and ciprofloxacin (29.17%), suggesting that fluoroquinolones may be moderately useful in BV treatment. Interestingly, imipenem (IMP), amikacin (AK), tigecycline (TGC) and pip/tazobactam (PIP/TAZ) continued to be quite effective with low resistance rates of 6.25%, 10.4%, 10.4%, and 10.4%, respectively (**Table 2**). Thus, the above results emphasize on the avoidance of empirical antibiotic therapy in order to preserve the effectiveness of last-sort medications [52].

Table 2: Percentage (%) of antibiotic resistance of all the isolated bacteria in BV.

Antibiotic	Symbol	<i>S. aureus</i> (N=10)	<i>S. haemolyticus</i> (N=9)	<i>E. coli</i> (N=8)	<i>S. epidermidis</i> (N=6)	<i>K. pneumoniae</i> (N=6)	<i>S. hominis</i> (n=4)	Others (N=5)	Total (N=48)
Amoxicillin	AMX	30	44.4	50.0	0.0	50.0	25.0	60.0	37.5
Amox-Clavulanic	AMC	10	22.2	12.5	33.3	16.7	0.0	40.0	18.8
Meropenm	MRP	10	11.1	12.0	0.0	0.0	25.0	40.0	12.5
Imipenem	IMP	10	0.0	0.0	0.0	16.7	0.0	20.0	6.3
Amikacin	AK	20	0.0	0.0	0.0	0.0	0.0	60.0	10.4
Ciprofloxacin	CIP	30	22.2	12.5	33.3	16.7	50.0	60.0	29.2
Nitrofurantoin	NIT	20	22.2	12.5	0.0	66.7	0.0	80.0	27.1
Cotrimazole	COT	30	11.1	37.5	0.0	16.7	25.0	80.0	27.1
Refampicin	RIF	30	22.2	25.0	33.3	66.7	25.0	60.0	35.4
Colistin	CT	40	66.7	37.5	50	50.0	25.0	40.0	45.8
Levofloxacin	LE	30	22.2	12.5	0.0	0.0	0.0	60.0	16.7
Novobiocin	NV	20	22.2	50.0	16.7	50.0	0.0	60.0	31.3
Tigecycline	TGC	10	0.0	12.5	0.0	0.0	0.0	60.0	10.4
Cefixime	CFM	70	77.8	75.0	66.7	16.7	100.0	80.0	68.8
Ceftriaxone	CTR	20	22.2	12.5	16.7	16.7	0.0	40.0	20.8
Pip/Tazobactam	P/Z	20	11.1	12.5	0.0	0.0	0.0	20.0	10.4

D. Green synthesis of Purslane-mediated AgNPs

The purslane Ag-NPs were made by adding 2 ml of purslane aqueous extract to 48 ml of 1 mM AgNO₃, using a

previous procedure [36] with modified AgNO₃ concentration and adjusting pH to 6.4. Upon exposed to direct sunshine, the color of the reaction mixture changed from colorless to reddish-brown (**Figure 3**). Finally, the solution was kept in dark at room temperature for overnight.

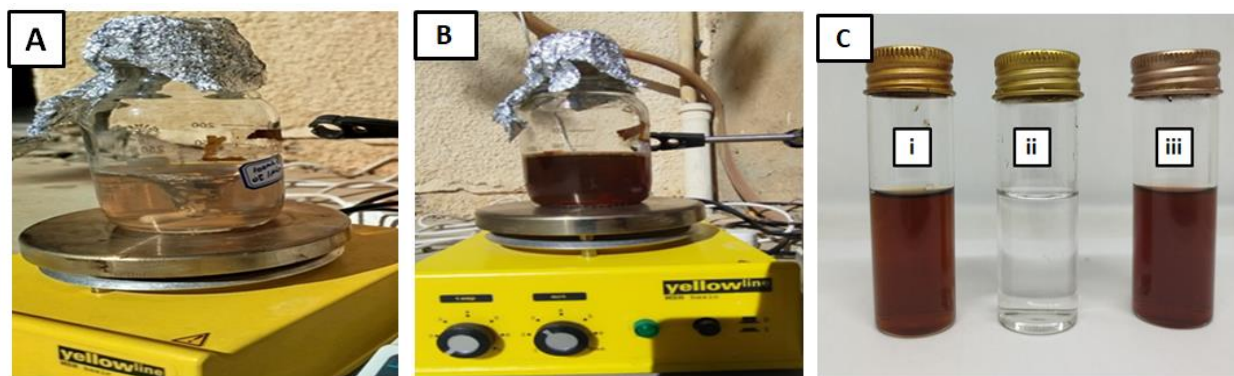


Figure 3: Visual confirmation of AgNP biosynthesis using purslane aqueous extract. (A) Reaction mixture before synthesis; (B) Reddish-brown AgNP solution after sunshine exposure; (C) Comparison of (i) purslane extract, (ii) AgNO₃, and (iii) synthesized AgNPs.

E. Characterization of Purslane-mediated AgNPs

1. Optical Properties by UV-Vis Spectroscopy

The effective biosynthesis of Purslane-mediated AgNPs was first validated by ocular examination. Under sunlight exposure, the color of the mixture changed from pale yellow to deep reddish-brown (**Figure 3**), indicating the reduction of silver ions (Ag⁺) to elemental silver (Ag⁰) and nanoparticle production. This photochemical reduction is driven by photon energy from sunshine, which excites the phytochemicals in the Purslane extract (e.g., flavonoids, polyphenols), boosting their reducing capacity and speeding up the nucleation of silver atoms [53, 54]. Using a UV-Vis Spectrophotometric UV Line 9400 (SI Analytics) with a scanning range of 200–800 nm, the UV-Vis absorption spectra of the prepared AgNPs were measured by adding 1 mL of liquid AgNPs to a standard UV-Vis cuvette. The UV-Vis spectral examination of the colloidal solution revealed a strong and characteristic surface plasmon resonance (SPR) peak at around 450 nm (**Figure 4**), which is typical of spherical or roughly spherical AgNPs. The detected single SPR band at 450 nm is a signature of monodisperse, quasi-spherical AgNPs with sizes typically < 50 nm [55]. The SPR phenomenon results from the collective oscillation of conduction electrons in AgNPs following interaction with light; the peak's position and shape are affected by particle size, shape, and the dielectric constant of the medium [56]. The slight red-shift of the SPR band compared to bulk silver (~400–420 nm) may be attributed to surface adsorption of phytochemicals, which alter the local dielectric environment.

Aggregation usually leads to peak broadening or a red shift [57]. However, the lack of secondary peaks at longer wavelengths (e.g., >500 nm) indicates the absence of considerable aggregation or typical anisotropic forms such as rods or plates, which create numerous or red-shifted SPR bands [58]. The consistency between the visual cue (reddish-brown color) and the spectroscopic results (peak at 450 nm) clearly supports the efficacy of Purslane phytoconstituents as reducing and stabilizing agents. The effective synthesis under sunlight also demonstrates an energy-efficient, green approach that does not require harsh chemical reductants or external heating [59].

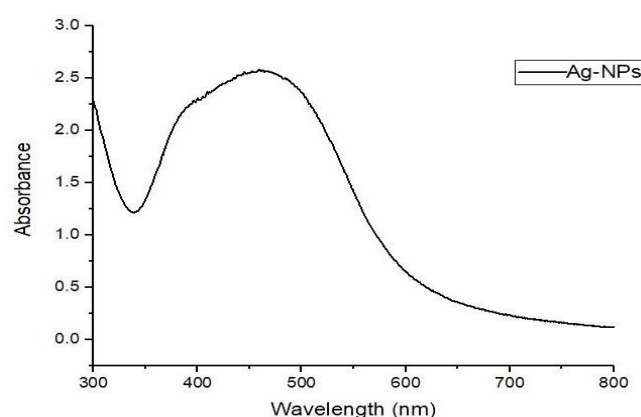


Figure 4: UV-Vis spectroscopy of Purslane-mediated AgNPs.

2. X-ray diffraction (XRD) analysis

The XRD analysis is a crucial technique for identifying Ag-NPs' features and analyzing their structural properties [27]. The **Figure 5** displays the X-ray diffraction (XRD) pattern of the Purslane-mediated AgNPs, where distinct diffraction peaks are observed in the diffractogram within the 2θ range of 20° to 80°. There are notable peaks at 2θ values of roughly 38.2°, 44.3°, 64.5°, and 77.4°. The Miller indices, which are indicative of a face-centered cubic (FCC) crystalline structure of metallic silver, are shown by the peaks (111), (200), (220), and (311) [60]. The peaks' sharpness and high intensity, especially the (111) reflection at 38.2°, show that the produced AgNPs are in high crystallinity [61].

The observed peak positions provide evidence for the presence of crystalline metallic silver (Ag⁰) because they match the standard values supplied by the Joint Committee on Powder Diffraction Standards (JCPDS), specifically file no. 04-0783 [62]. Additionally, the Debye–Scherrer equation ($D_p = 0.94\lambda/\beta\cos\theta$) states that peak width is inversely proportional to crystallite size, meaning that the broadening of the diffraction peaks is a direct function of the particles' "nanoscale" dimensions [63]. The crystal size of the AgNPs were estimated by utilizing the Debye-Scherrer formula ($D_p = 0.94\lambda/\beta\cos\theta$), which depends on the Bragg angle, the X-ray wavelength of the Cu Kα radiation, and the breadth of the peak at half maximum in radians. The crystalline size of

the AgNPs was determined to be 14.89 nm using this approach. This is less than the reported nanoparticle size of 32.24 nm [64] and 50 nm [65]. Other smaller peaks that are visible at lower angles (around 28° and 32°) are commonly

seen in green synthesis techniques and are usually ascribed to the crystallization of bio-organic phases or capping agents that stabilize the surface of the nanoparticles, like proteins or phenolic compounds from the plant extract [62].

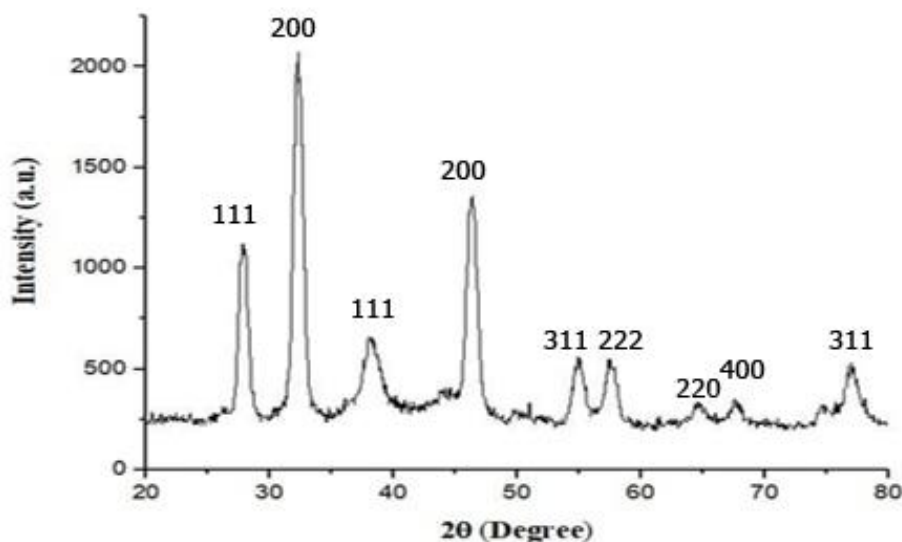


Figure 5: X-ray diffraction (XRD) of Purslane-mediated AgNPs.

3. Field Emission- Scanning Electron Microscopy (FE-SEM)

The FE-SEM is used to examine the morphological properties of the green synthesised- AgNPs. The SEM micrographs of the Purslane-mediated AgNPs predominantly reveal large size, quasi-spherical nanoparticles within the nanoscale range and noticeable aggregation (**Figure 6**). The plant material's dehydration process could be attributed to this aggregation. Certain volatile phytochemicals that are essential for regulating the creation of nanoparticles may be

lost during the drying process [66]. Additionally, the dry extract's lower protein content is probably the cause of the agglomerated form. A reduced protein concentration in the dry extract results in less stability and a greater propensity of the particles to clump together because proteins serve as capping and stabilising agents [67]. Furthermore, the high surface energy per unit volume ratio, which is a common phenomenon in most green synthesised nanoparticles, may also be the cause of this agglomeration [68].

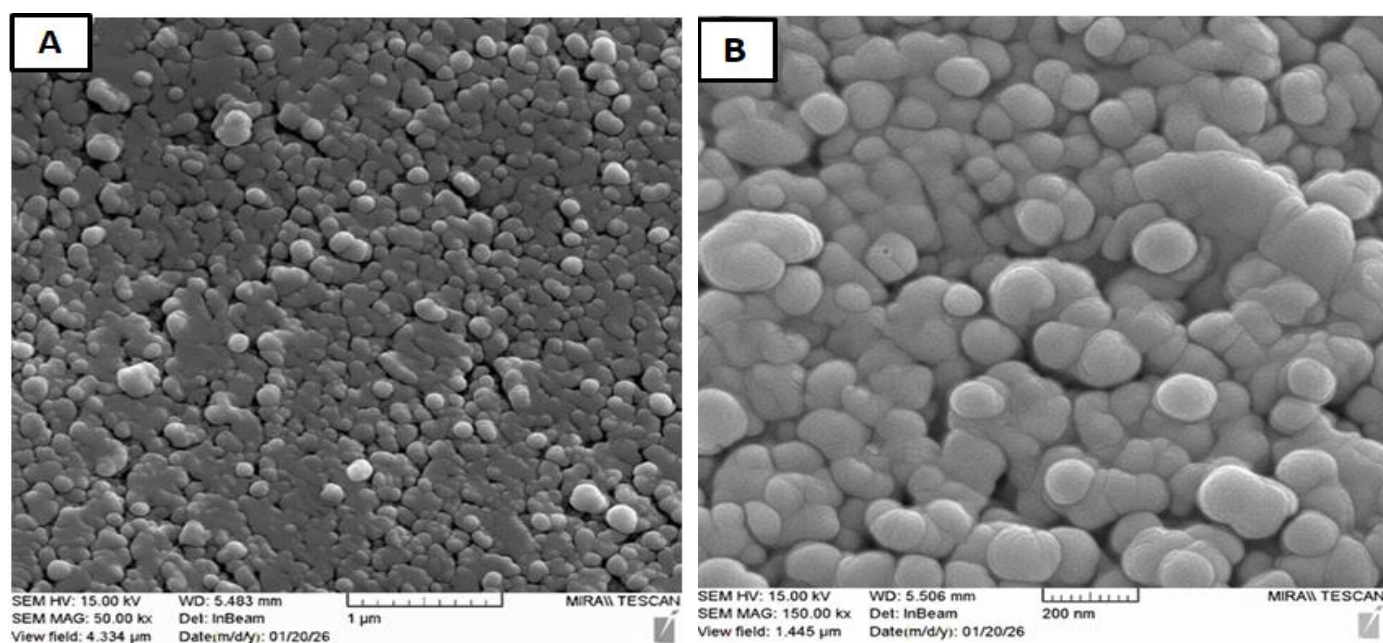


Figure 6: Field Emission-Scanning Electron Microscopy (FE-SEM) of Purslane-mediated AgNPs demonstrating their quasi-spherical shape and tendency to agglomerate, viewed at (A) 1 µm and (B) 200 nm scale bars.

4. Transmission electron microscopy (TEM) analysis

The TEM was used to analyse the biosynthesised AgNPs' size, shape, and crystalline morphology. **Figure 7** exhibits the TEM micrographs of the Purslane-mediated AgNPs. Visual examination of the micrographs shows that the synthesised nanoparticles have quasi-spherical shape. The particles vary in size and show a polydispersed distribution. The particle diameter typically falls between 10 and 50 nm, which is a hallmark of AgNPs produced by biological pathways, according to the scale bars of 50 nm and 100 nm [69].

Significantly, the TEM pictures show some degree of particle agglomeration, in which separate nanoparticles have joined together to create more asymmetrical aggregates. The characteristics of the dry plant extract utilised as the capping and reducing agent may be responsible for this aggregation behaviour. Certain volatile phytochemicals and

heat-sensitive proteins that normally serve as strong stabilising factors in fresh extracts may degrade or be lost as a result of the drying process of *purslane* leaves [70]. As a result, the nanoparticles combine to achieve a thermodynamically stable state because the decrease in accessible capping biomolecules raises the surface energy per unit volume [71].

Additionally, a faint, lighter halo or thin layer surrounds the electron-dense dark cores of the AgNPs, when closely examining the high-magnification TEM images (**Figure 7b**). The observed halo may suggest the presence of a possible organic capping layer, produced by the secondary metabolites of *P. oleracea*, including polysaccharides, alkaloids, and flavonoids [72]. The two functions of this coating are to reduce Ag^+ to Ag^0 and to provide steric hindrance, which stops the full fusing of particles, even within the observable agglomerates [73].

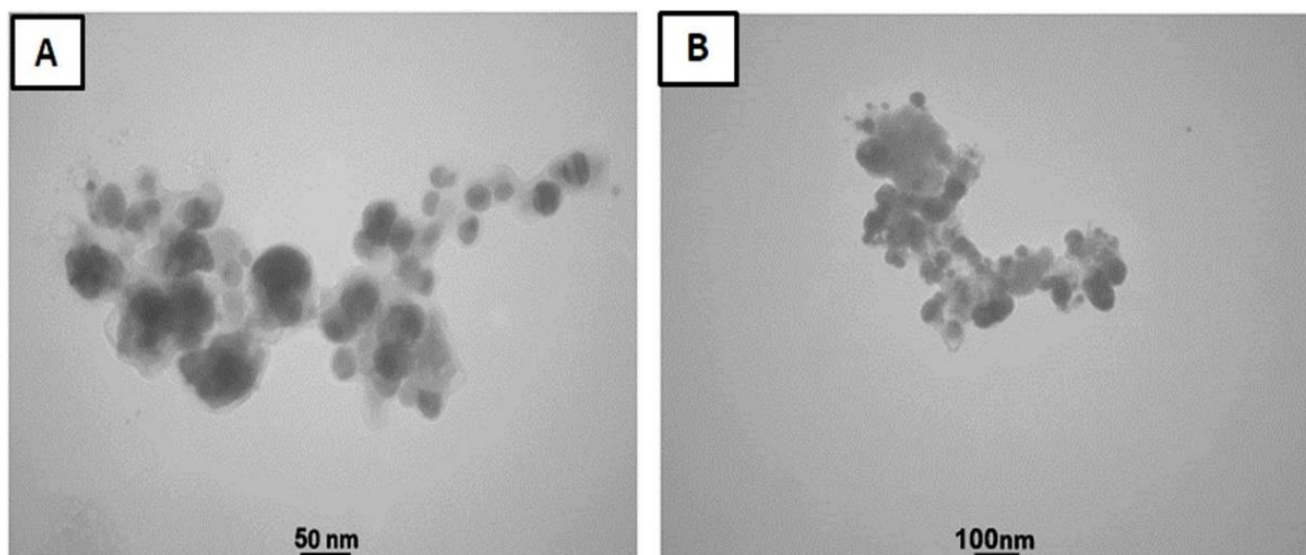


Figure 7: TEM images of the synthesized Purslane-mediated AgNPs at (A) 50 nm and (B) 100 nm scale bars, displaying their predominantly quasi-spherical shape and agglomeration behavior.

5. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a crucial analytical method for determining the functional groups on the surface of synthesis nanoparticles. The two main functions of FTIR in the context of green synthesis are to identify the particular biomolecules from the plant extract that remain as a "capping" layer and supports the involvement of biomolecules in reduction and stabilization [43]. A "molecular fingerprint" of the interface between the organic stabilizing shell and the inorganic silver core can be obtained by measuring the absorption of infrared radiation, which allows us to observe the vibrational frequencies of chemical bonds.

The FTIR spectra of the plant extract and the synthesized AgNPs (**Figure 8**) were analyzed to determine which functional groups were involved in the synthesis. The raw plant extract (red line) showed a strong absorption band at 3406 cm^{-1} , which corresponded to hydroxyl (-OH) stretching. Aliphatic C-H stretching vibrations were found between 2920 and 2814 cm^{-1} . The fingerprint region (1500 – 400 cm^{-1}) showed substantial peaks at 1367 cm^{-1} , 1012

cm^{-1} , and 615 cm^{-1} , as well as a strong peak at 1631 cm^{-1} . Following AgNPs synthesis, the spectra (black line) retained the key peak positions but displayed notable intensity variations. The intensity of the hydroxyl (-OH) band at 3406 cm^{-1} decreased significantly. However, the peak at 1631 cm^{-1} the band remained sharp and well resolved. The fingerprint region saw significant refinements and slight alterations, especially in the C-O-C and C-N stretching vibrations between 1300 and 1000 cm^{-1} .

The comparison of the two spectra gives supporting evidence conclusive proof of the synthesis's chemical mechanism. The plant extract's phenolic compounds and saccharides were actively oxidized, serving as the main reducing agents by providing electrons to enable the conversion of reduction Ag^+ ions to Ag^0 . This is indicated by the decrease in intensity and the slight shift of the broad peak at 3406 cm^{-1} (O-H stretching) and the 1012 cm^{-1} band (C-O stretching)[74]. Concurrently, the band at 1631 cm^{-1} may be attributed to C=O stretching of amide groups or C=C stretching of aromatic compounds, with a possible contribution from bending vibrations of adsorbed water, while the band

at 1519 cm^{-1} may correspond to N–H bending or aromatic vibrations. The presence of these bands suggests that proteinaceous or nitrogen-containing biomolecules may be associated with the AgNPs surface and could contribute to nanoparticle stabilization [25]. The C–H band at 2885 cm^{-1} suggests the presence of organic residues or capping molecules. The fingerprint region (below 1500 cm^{-1}) validates the particular chemical interaction between the silver core

and the plant metabolites, with shifts in the 1367 cm^{-1} and 1012 cm^{-1} peaks characterizing the C–N and C–O–C stretching commonly found in plant glycosides and amino acids. These groups act as ligands, binding the organic shell to the metal [28]. However, the low-frequency bands at 615 and 491 cm^{-1} may be linked to metal–oxygen or metal–nitrogen interactions, indicating that the plant extract's biomolecules are involved in the stabilization of the AgNPs [75].

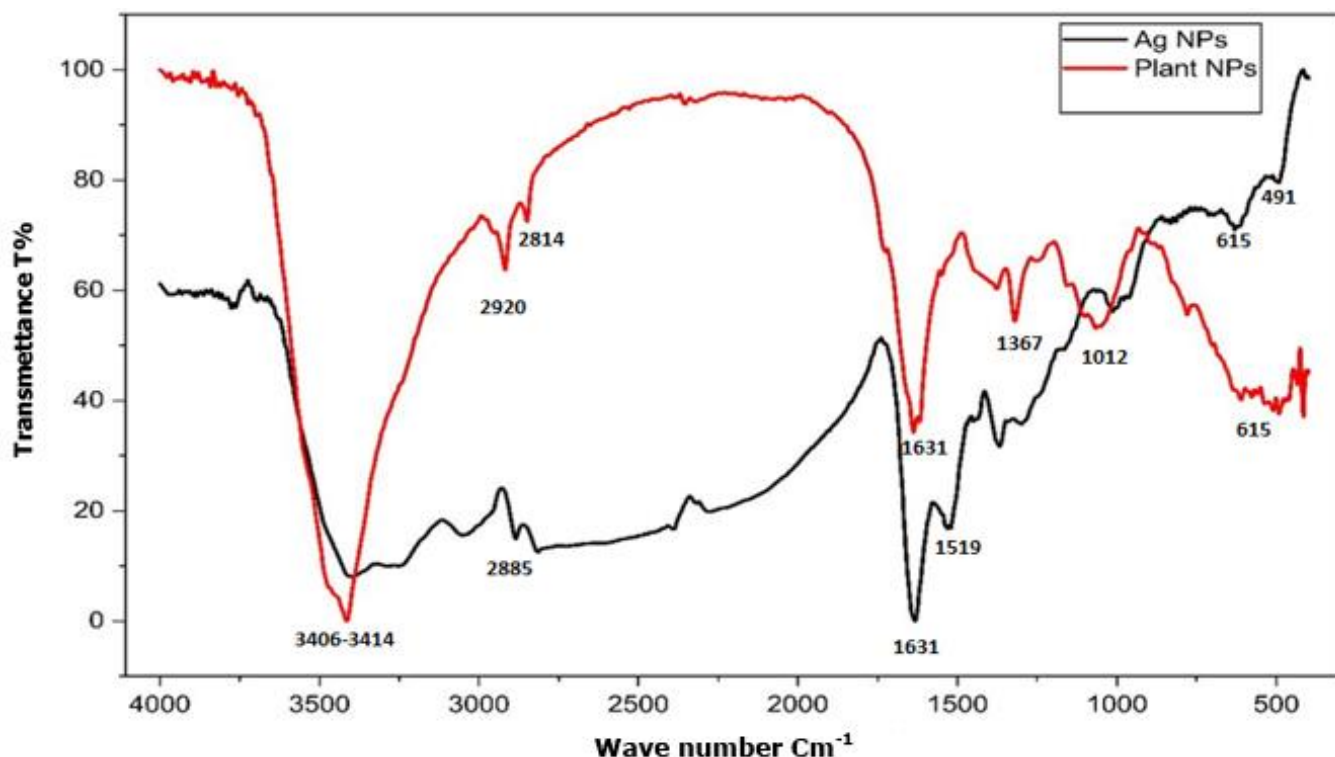


Figure 8: FTIR spectra of the plant extract (red) and synthesized AgNPs (black).

F. Antibacterial activity of Purslane-mediated AgNPs

The antibacterial efficacy of Purslane-AgNPs was evaluated using a well diffusion experiment against two common clinically important bacteria, *Escherichia coli* and *Staphylococcus aureus*. The results demonstrated that the Purslane-Ag NPs seems to have broad spectrum and better antibacterial activity over the crude extract and its ionic counterpart (**Figure 9**). Purslane-mediated AgNPs (4.0 mg/ml) produced a zone of inhibition with estimated 21 mm diameter against *E. coli*, while the AgNO_3 displayed a smaller zone with an 15 mm diameter. Likewise, Purslane-mediated AgNPs (4.0 mg/ml) generated a distinctive 24 mm inhibition zone against *S. aureus*, as opposed to 14 mm inhibition zone by AgNO_3 . However, complete lack of inhibitory action (0 mm) was observed for the Purslane aqueous extract

against both the bacteria. This demonstrates that the antibacterial ability is not innate to the plant extract it self, but it results from the effective bio-reduction and stabilization of silver into nanoparticle form. Moreover, the purslane-mediated AgNPs' better performance than AgNO_3 indicates that the nanoparticle structure offers a more effective way to distribute silver ions. Purslane-mediated AgNPs probably gained from the capping of the organic molecules in the plant extract, which may improve stable contact with the bacterial cell membrane, whereas AgNO_3 depends on the instantaneous release of silver ions [76]. The slight difference in zone of inhibition seen between *S. aureus* (24 mm) and *E. coli* (21 mm) (**Figure 9**), may be related to the differences in bacterial cell wall structure, the gram-negative bacteria have an outer lipopolysaccharide membrane that may serve as a barrier to some antimicrobial drugs [23].

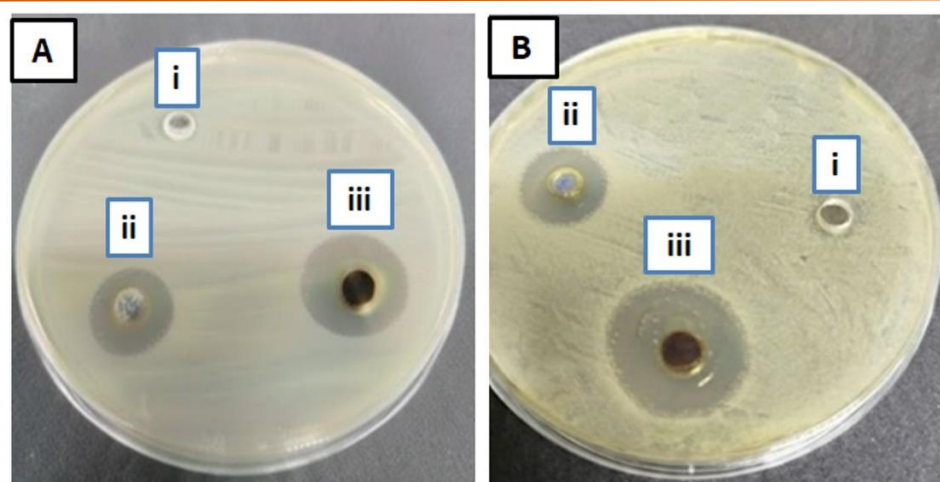


Figure 9: Agar well diffusion assay of antibacterial activity against *Escherichia coli* (A) and *Staphylococcus aureus* (B). The wells represent: (i) Purslane aqueous plant extract, (ii) Silver Nitrate (AgNO_3) solution and (iii) Purslane-mediated silver Nanoparticles (P-Ag NPs).

In addition, the minimum inhibitory concentration (MIC) of purslane-mediated AgNPs was determined for the 11 bacterial strains using broth microdilution assay. The purslane-mediated AgNPs demonstrated broad antibacterial activity, with MIC values ranging from 7.8 $\mu\text{g/mL}$ to 250 $\mu\text{g/mL}$ (Figure 10, Table 3). Depending on the OD_{600} values, the bacterial strains were categorized into three susceptible groups (Table 3). The Group I highly sensitive strains included *S. agalactiae* ($\leq 7.8 \mu\text{g/mL}$), *V. massiliensis* ($\leq 7.8 \mu\text{g/mL}$), *S. haemolyticus* (7.8 $\mu\text{g/mL}$), and *C. amycolatum* (15.6 $\mu\text{g/mL}$). The Group II intermediate sensitive strains consisted mostly of Gram-negative pathogens, such as *K. aerogenes* (31.25 $\mu\text{g/mL}$), *K. pneumoniae* (31.25 $\mu\text{g/mL}$), and *E. coli* ($\sim 31.25 \mu\text{g/mL}$), and a Gram-positive *S. aureus* (62.5 $\mu\text{g/mL}$). The Group III low sensitive strains included only Gram-positive bacteria, such as *S. hominis* (125

$\mu\text{g/mL}$), *E. faecalis* (125 $\mu\text{g/mL}$), and *S. epidermidis* (250 $\mu\text{g/mL}$). These MIC values are consistent with a study observed that AgNPs are effective at low microgram concentrations [77].

The broad antibacterial activity of Purslane-mediated AgNPs is supposedly due to the disruption of cell membrane permeability that causes cellular leakage [78]. The other possible antibacterial mechanisms of the AgNPs are reactive oxygen species (ROS) production that damages DNA and inactivation of vital respiratory enzymes [77, 79]. These findings demonstrated that the Purslane-Ag NPs have potent, dose-dependent and broad-spectrum antibacterial activity, which seemingly bypassed resistance mechanisms as well.

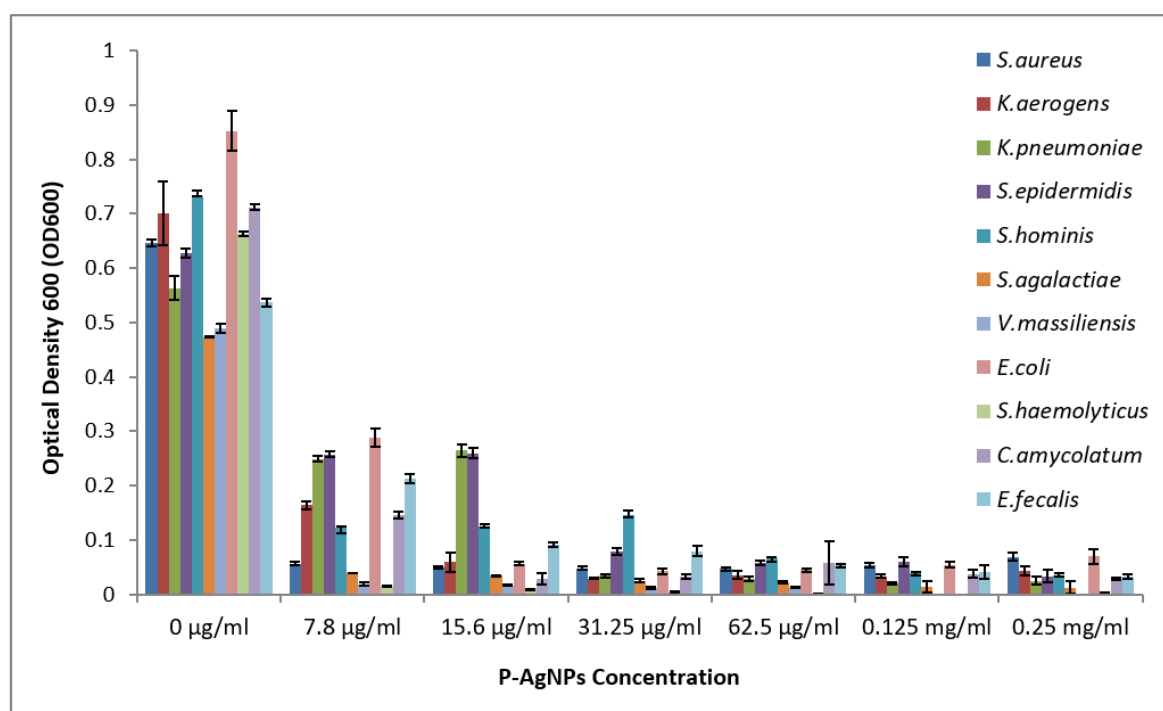


Figure 10: Broth microdilution assay: The BV bacterial species ($n = 11$) were exposed to different concentrations of Purslane-mediated silver nanoparticles (AgNPs). The data are the mean values for three technical replicates with error bars indicating the standard deviation between replicates.

Table 3: Minimum Inhibitory Concentrations (MIC) of Purslane-mediated AgNPs against 11 bacterial species isolated from BV.

Category	Bacterial Species	Strain ID	MIC (μg/ml)	Interpretation
I	<i>Staphylococcus haemolyticus</i>	charmo z2	7.8	High sensitive
	<i>Vaginella massiliensis</i>	charmo z5	7.8	
	<i>Streptococcus agalactiae</i>	charmo z15	7.8	
	<i>Corynebacterium amycolatum</i>	charmo z1	15.62	
II	<i>Escherichia coli</i> <i>col</i>	charmo z11	31.25	Intermediate
	<i>Klebsiella pneumoniae</i>	charmo z3	31.25	
	<i>Klebsiella aerogenes</i>	charmo z10	31.25	
	<i>Staphylococcus aureus</i>	charmo z6	62.5	
III	<i>Staphylococcus hominis</i>	charmo z13	125	Low sensitive
	<i>Enterococcus faecalis</i>	charmo z17	125	
	<i>Staphylococcus epidermidis</i>	charmo z4	250	

4. Conclusion

In conclusion, this study provides important microbiological insights into the burden of bacterial vaginosis (BV) among the studied women patients, who infected with different species of Gram-positive and Gram-negative bacteria. The isolated bacteria differently exhibited resistance against the tested antibiotics, but mostly against cefixime, colistin and amoxicillin. These findings demonstrate a notably high incidence of BV and underscore the polymicrobial nature of BV, with possible difficulty in management of drug resistant cases. Furthermore, this study highlights the successful green synthesis of AgNPs using Purslane leaf extract as a reducing agent. Physicochemical characterization confirmed the formation of crystalline, quasi-spherical nanoparticles with a surface plasmon resonance peak at 450 nm and an average particle size of 14.89 nm and the FTIR analysis confirmed that biomolecules present in the plant extract, particularly phenolic compounds, saccharides, and proteinaceous constituents, played a dual role in reducing Ag⁺ ions and stabilizing/capping the synthesized AgNPs. Antibacterial evaluation demonstrated significant activity of the biosynthesized AgNPs against all the bacterial species. Collectively, this study recommends maintaining good personal hygiene and avoidance of empirical therapy by women to reduce the incidence of BV. Additional investigations of the nanoparticles are required to assess their feasibility in BV management.

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None

CONFILICT OF INTEREST

The authors declare that there are no conflicts of interest related to this publication.

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