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Green synthesis of *Boerhavia diffusa* L. silver nanoparticles against multidrug-resistant bacteria

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Abstract

This study demonstrates the environmentally friendly production of silver nanoparticles (AgNPs) utilizing extract from *Boerhavia diffusa*, which is high in bioactive substances like caffeic acid and quercetin. These spherical, monodispersed AgNPs showed strong, concentration-dependent antibacterial action against *Bacillus tropicus* and *Bacillus cereus*, two Multi-Drug Resistant (MDR) soil microorganisms. The plant's phytoconstituents, especially quercetin, successfully inhibit important bacterial enzymes like beta-lactamase, as further proven by molecular docking. All things considered, the study offers a viable framework for creating plant-mediated nanomaterials as potent substitutes for synthetic antibiotics in the fight against diseases that are resistant to them.

Key words : *Boerhavia diffusa*, Polyphenols, Antibiotics , Antibacterial, Aggregation, Multidrug resistantance, Nanoparticles.

Due to the abuse of conventional antibiotics, multi-drug resistant (MDR) bacteria have become more prevalent worldwide, necessitating the development of novel and sustainable treatment options[20]. In order to enable the green production of silver nanoparticles (AgNPs)[13], this study takes a multidisciplinary approach by utilizing the extensive phytochemical profile of *Boerhavia diffusa*, a medicinal plant recognized for its variety of secondary metabolites. This environmentally friendly synthesis produces biocompatible nanomaterials with improved antibacterial qualities by using plant-derived flavonoids and phenolic acids as natural reducing and stabilizing agents, in contrast to traditional chemical methods[18]. The relevance of plant polyphenols in producing stable, non-toxic metallic particles is highlighted by recent developments in green nanotechnology[1]. This study assesses the dual-action potential of AgNPs and their bioactive coatings by combining phytochemical analysis, microbiological testing against MDR soil isolates from pesticide-treated environment[17]. In addition to

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addressing the shortcomings of existing antibiotics, this integrated approach offers a scientific foundation for creating affordable, plant-mediated nanotherapeutics to counter the escalating problem of antimicrobial resistance worldwide[3,6,15].

Plant extraction and LC-MS Analysis :

Boerhavia diffusa plants were first gathered for the study, dried, and powdered into a fine powder. The therapeutic ingredients in this powder were extracted by soaking it in ethanol for a full day. Liquid Chromatography-Mass Spectrometry (LC-MS) was used to examine the extract in order to determine the precise compounds that were present. Bioactive compounds like quercetin and caffeic acid, which are crucial for the production of nanoparticles and their therapeutic effects, were discovered during this process.

Green Synthesis and Characterization of AgNPs :

The plant extract (8 mL) was combined with a silver nitrate solution (40 mL) to produce silver nanoparticles (AgNPs). The liquid turned dark red as a result of the plant's natural compounds reducing the silver ions into solid nanoparticles over the course of 48 hours. High-tech instruments like TEM and FESEM were then used to examine the size and form of these particles, XRD was used to confirm their crystalline structure, and Zeta potential was used to make sure they were stable and wouldn't group together.

Bacterial Isolation and MDR Screening :

To identify hardy, resistant microorganisms, soil samples were taken from agricultural fields that had been treated with pesticides. To cultivate individual bacterial colonies on agar plates, researchers employed serial dilution. To determine whether these isolates were "Multi-Drug Resistant" (MDR), they were subsequently tested against twelve different kinds of antibiotics. For the experiment's subsequent phase, the microorganisms that withstood the greatest number of antibiotics were chosen.

Antimicrobial Testing :

The agar well diffusion method was used to assess the antibacterial efficacy of synthesized *B. diffusa* AgNPs against MDR isolates. After being cultivated in nutrient broth and adjusted to a 0.5 McFarland standard, the isolates were swabbed onto Mueller-Hinton agar plates. Test samples were introduced to wells created with a cork borer, and the plates were incubated for 24 hours at 37 °C. The zone of inhibition (mm) was used to calculate antimicrobial activity.

LC-MS Profiling of Boerhavia diffusa:

As shown in Table-1, LC-MS analysis revealed a wide range of bioactive phytochemicals. Important components including caffeic acid (m/z 205.32) and quercetin (m/z 290.27) are essential natural antioxidants that help reduce silver ions (Ag^+) into nanoparticles[7]. Furthermore, organic acids such as ferulic and fumaric acid function as stabilizers, improving colloidal stability and avoiding particle aggregation[19]. According to recent research, these particular metabolites offer a capping layer that keeps the silver core biologically active and stops oxidation[9,11].

Table-1. List of Phytocompounds identified from LC-MS profiling of B. diffusa.

Retention Time (min)	m/z (ESI-)	Compound Name	Chemical Formula
0.746	128.20	Pyroglutamic acid	$C_5H_7NO_3$
1.076	290.27	Quercetin	$C_{15}H_{10}O_7$
1.258	117.20	Fumaric acid	$C_4H_4O_4$
2.664	205.32	Caffeic acid	$C_9H_8O_4$
2.924	256.36	Chlorogenic acid	$C_{16}H_{18}O_9$
4.209	239.35	Ferulic acid	$C_{10}H_{10}O_4$
2.01	153.22	Protocatechuic acid	$C_7H_6O_4$
2.01	153.22	Gentisicacid	$C_7H_6O_4$

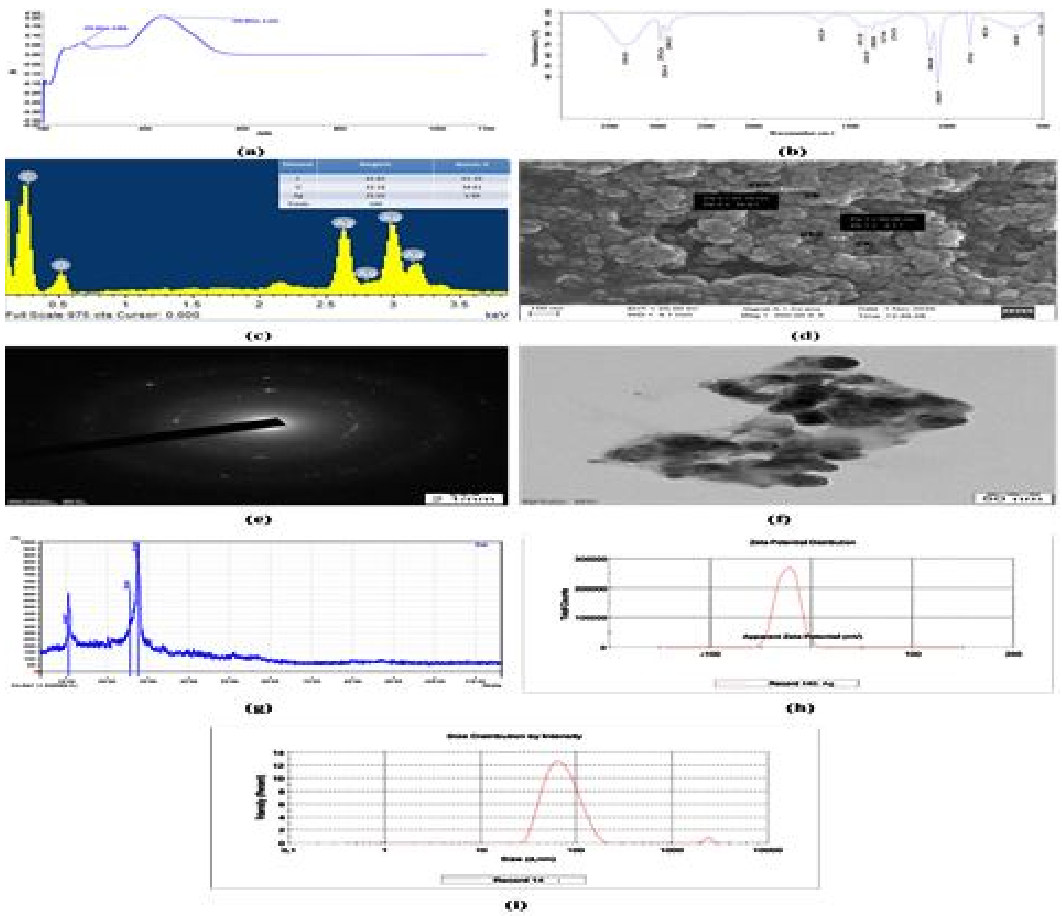

Fig. 1. Characterization of B.diffusa mediated Silver nanoparticles by (a) UV- Vis Spectrophotometric analysis; (b) FTIR analysis; (c) EDX analysis; (d) FE-SEM; (e) Selected Area Electron Diffraction; (f) TEM analysis; (g) XRD analysis; (h) and (i) DLS analysis.

Synthesis and Characterization of AgNPs:

Figure 1's results indicate that the synthesis was verified by a color change to dark brown, and UV-Vis spectroscopy revealed a surface plas mon resonance (SPR) peak at 434.80 nm, which is characteristic for spherical AgNPs[8]. TEM and FESEM morphological investigation showed spherical particles (50–57 nm), while XRD signals verified a crystalline, face-centered cubic structure[2]. The nanoparticles have enough repulsive forces to stay stable in solution, which is essential for long-term biomedical application, according to a zeta potential of -23.8 mV[4,16].

Isolation of Bacteria from Pesticide-Treated Soil :

Pesticide-contaminated Brinjal root soil was used to isolate seventeen different bacterial colonies. The diversity in colony coloration and textures points to adaptive microbial responses to chemical stress, including the synthesis of exopolysaccharides for biofilm defense[12]. Bacillus-type species are highly prevalent, which emphasizes their special capacity to detoxify organophosphates and last in habitats frequently treated with agrochemicals[14].

Table-2 Antibiotic susceptibility pattern of Pesticide Treated bacterial isolates

Isolates	M	Ox	P	Amp	Ka	Va	E	C	Cip	Tet	Rif
BR 1	-	-	-	7	19	10	22	19	26	20	12
BR 2	-	-	-	7	17	15	16	16	30	18	10
BR 3	-	-	-	7	19	11	20	18	30	21	12
BR 4	-	-	-	-	17	15	22	20	24	17	13
BR 5	-	-	-	7	20	17	22	18	28	22	14
BR 6	NM	16	14	20	20	18	26	26	26	30	14
BR 7	-	-	-	10	13	-	12	10	14	20	14
BR 8	NM	14	20	NM	20	14	22	26	14	26	24
BR 9	-	-	-	7	19	17	20	16	30	20	14
BR 10	-	12	12	22	28	18	22	24	26	28	18
BR 11	-	14	14	NM	26	22	28	30	36	34	24
BR 12	-	16	16	28	28	22	26	30	34	30	20
BR 13	-	-	-	12	7	9	20	26	32	30	28
BR 14	-	-	-	16	24	8	8	24	36	24	17
BR 15	-	-	-	16	16	12	12	20	30	24	8
BR 16	-	NM	NM	NM	30	22	24	30	NM	36	20
BR 17	-	14	-	NM	18	24	20	30	26	36	30

** M – Methicillin; Ox – Oxacillin; P – Penicillin; Amp – Ampicillin; Ka – Kanamycin; Va – Vancomycin; E – Erythromycin; C – Chloramphenicol; Cip – Ciprofloxacin; Tet – Tetracycline; Rif – Rifampicin; NM – Not Measured; "–" indicates no inhibition zone.*

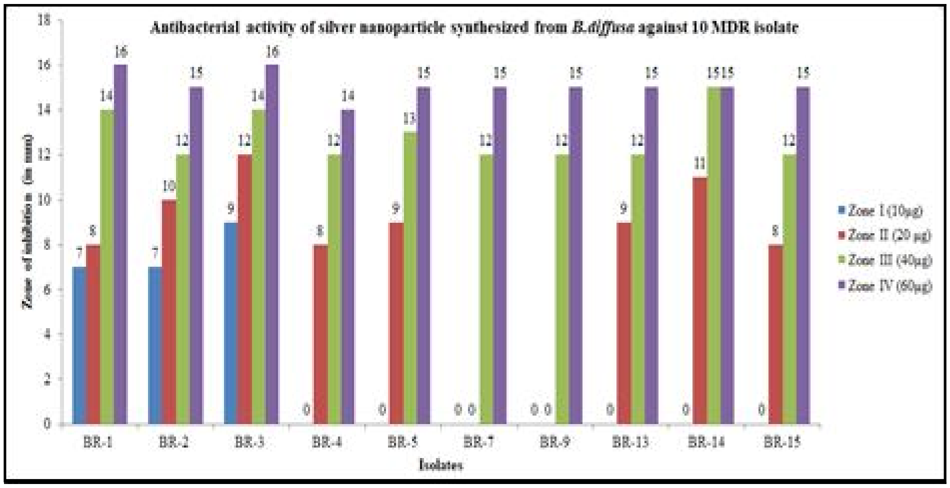

Fig. 2. Graph showing the concentration dependent antibacterial activity of B.diffusa mediated AgNPs against MDR isolates from pesticide treated brinjal root soil

Screening for Multi-Drug Resistance (MDR) Strains :

A substantial incidence of multi-drug resistance was found through susceptibility testing against 11 types of antibiotics, as shown in Table-2. Ten isolates demonstrated resistance to several broad-spectrum substances, confirming that soil contaminated by pesticides can serve as a reservoir for MDR bacteria[5]. These findings highlight the necessity of using several antimicrobial drugs to fight environmental resistance.

Antimicrobial Activity of Silver Nanoparticles:

Against the ten MDR isolates, the green-synthesised AgNPs showed strong, concentration-dependent antibacterial activity. Figure 2 illustrates that maximum inhibition was attained at 60 µg, with isolates BR-1 (*B. tropicus*) and BR-3 (*B. cereus*) exhibiting the highest sensitivity (16 mm). AgNPs most likely cause resistant bacterial cells to lyse by rupturing cell membranes and producing reactive oxygen species (ROS)[10].

With the help of important metabolites like quercetin and caffeic acid, this study identifies *Boerhavia diffusa* as an efficient bio-source for the environmentally friendly manufacture of solid, crystalline silver nanoparticles (AgNPs). The produced AgNPs showed strong, concentration-dependent antibacterial action against *Bacillus tropicus* and *Bacillus cereus*, two soil isolates that are multi-drug resistant (MDR). This inhibitory impact is driven by quercetin's strong binding affinity to vital bacterial enzymes including beta-lactamase 2, according to complementary molecular docking. In the end, this study offers a green, sustainable nanotechnological foundation for creating powerful antimicrobial compounds to counter the growing problem of antibiotic resistance.

References :

1. Abeyrathne EDNS, KC Nam, and DU Ahn (2022). *Antioxidants 11*(11): 2267.
2. Bamal D, A Singh, and G Chaudhary (2021). *Sci Rep 11:* 17450.
3. Dedeh GY, AK Apetorgbor, DA Koomson, and NO Boadi (2021). *Toxicol Rep 8:* 1167–1174.
4. Edayadulla N, S Kumar, and N Basavegowda (2024). *J Cluster Sci 35:* 412–425.
5. Hombach M, FP Maurer and GV Bloemberg (2024). *Clin Microbiol Rev 37:* e00055.
6. Hu Y, Y Zhang, Y Yin, and J Wang (2022). *Environ Pollut 293:* 118544.
7. James P, R Kumar, and V Arul (2025). *Mater Lett 356:* 135541.
8. Khurana S, P Chawla, and R Chaudhary (2021). *Nano Express 2*(2): 025008.
9. Lima MCN, MJS Paiva, and RC de Oliveira (2021). *Colloids Surf A Physicochem Eng Asp 612:* 125954.
10. Liu Y, L He, and A Mustapha (2020). *LWT 127:* 109372.
11. Meshram S, S Shailajan, and A Singh (2024). *Chemosphere 349:* 140812.
12. Orlandi VT, E Martegani, and P Barbieri (2022). *Microorganisms 10*(5): 1014.
13. Patel MK, S Pandey, and V Kumar (2025). *Plant Nano 9:* 100084.
14. Paul A, S Kumar, and J Das (2023). *Arch Microbiol 205:* 112.
15. Poudel S, M Gautam and BP Gaire (2022). *ACS Omega 7*(21): 18000.
16. Shaik MR, M Khan, and M Kuniyil (2021). *Molecules 26*(9): 2459.
17. Sinan KI, G Zengin, and R Ceylan (2021). *Ind Crops Prod 161:* 113182.
18. Singh J, T Dutta, KH Kim, M Rawat, P Samddar and P Kumar (2023). *Nanoscale Res Lett 18:* 45.
19. Sudheer S, N Chauhan, and R Kumar (2025). *J Environ Manage 374:* 121102.
20. Ventola CL (2025). *J Clin Pharmacol 65*(2): 112–125.