RESEARCH ARTICLE

Sustainable Green Synthesis of Polyherbal Silver Nanoparticles and Assessment of their Antibacterial Activity

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ABSTRACT

Objective: The intent of the present endeavor was to formulate nanoparticles by an easy, green, and ecologically friendly synthetic process.

Methods: Nanoparticles were synthesized by reducing the silver ions present in the solution of silver nitrate (0.01M) with aqueous poly herbal extract prepared from Seeds of *Psoralea corylifolia*, roots of *Vetiveria zizanioides* and roots of *Cyperus rotundus*. Silver nanoparticles were characterized using UV, FTIR, SEM and XRD examination. The antibacterial efficacy of yielded silver nanoparticles was examined at 10, and 20 mg ml^{-1} concentrations against two Gram's positive (*E. coli* and *B.subtilis*) and Gram's negative (*P.aeruginosa* and *S.aureus*) bacteria.

Results: The synthesized nanoparticles had shown appreciable antibacterial activity on all the bacteria. Comparatively leading anti-bacterial effect was observed against *Staphylococcus aureus* and *Escherichia coli*.

Conclusion: Thereby, our findings can be implemented to synthesize silver nanoparticles utilizing polyherbal extract in a quick, economical, and environmentally benign manner.

Keywords: polyherbal extract,green synthesis, silver nanoparticles.

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INTRODUCTION

A rapidly developing area of study, nanotechnology has many uses in both science and technology, particularly in the creation of novel nanomaterials and nanoparticles[1]. Because of their numerous applications in a variety of scientific fields, including biomedicine, agriculture, pharmaceutics, textiles, food technology, catalysis, sensors, mechanics, electronics, and optics, nanoparticles, or small particles with sizes ranging from 1 nm to 100 nm, have attracted the attention of scientists[2]. A variety of distinct types of nanoparticles exist, such as those with properties specific to silver, gold, zinc, cadmium sulphide, copper, iron, titanium dioxide, etc. Silver nanoparticles (AgNPs), have garnered significant attention in biomedical science in recent years because of their broad range of applications in various fields, including wound dressings, drug delivery, biosensors, biocatalysis, antibacterial, antifungal, antioxidant, anti-cancer, and anti-inflammatory properties[3].Utilising biological resources for the "green" production of silver nanoparticles (AgNPs) is the most straightforward, affordable, quick, and eco-friendly way to address the shortcomings of chemical and physical approaches. Bioactive AgNPs can be produced sustainably by utilising a variety of biological resources, including bacteria, fungi, algae, plants and their various sections, and so on. Many environmentally friendly methods for the quick, easy, and non-toxic synthesis of AgNPs from biological assets have been published in recent years[4]. Numerous biomolecules included in plant extract, such as vitamins, phenolic compounds, alkaloids, terpenoids, and flavonoids, serve as capping and reducing agents during the course of biosynthesis. Because of their prospective uses in a variety of biomedical science domains, biosynthesised AgNPs have drawn a lot of interest from researchers[5]. AgNPs' bactericidal action is their most common use. AgNPs are being investigated by researchers as possible antibacterial agents due to the rise of multidrug-resistant pathogens. Conforming to an array of assessments, biosynthesised AgNPs showcased notable antibacterial properties against an extensive palette of human ailments. AgNPs can easily pass through bacterial cell walls, disrupt cell membranes, unleash reactive oxygen species, obstruct DNA replication and protein synthesis, and ultimately induce cell death due to their tiny size and huge surface area[6].

In certain cases, targeted therapeutic effects cannot be achieved using the active phytochemical elements of specific plants alone. Herbs can have a greater medicinal impact and lessen toxicity when combined in precise ratios in polyherbal preparations. A compelling pharmacological activity cannot be produced with the active ingredients taken from a single plant. Research indicates that unrefined plant extracts often have higher potencies than their individual components[7]. Whole plants or a combination of

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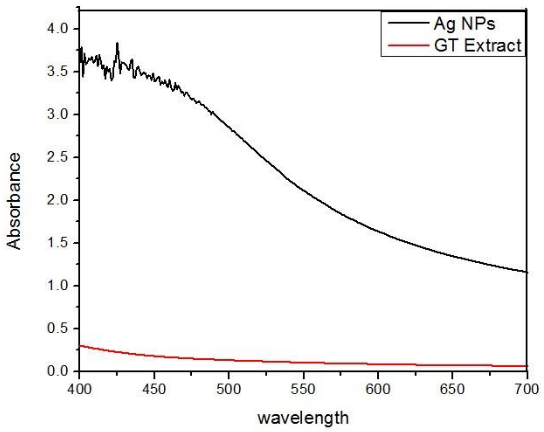

Fig.1: UV spectrum of nanoparticles synthesized from poly herbal extract

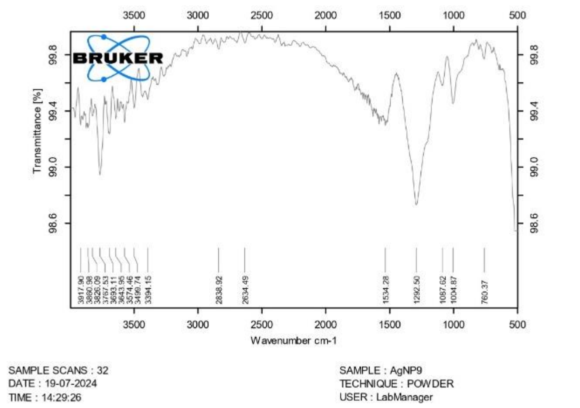

Fig.2: FTIR spectrum of nanoparticles synthesized from poly herbal extract

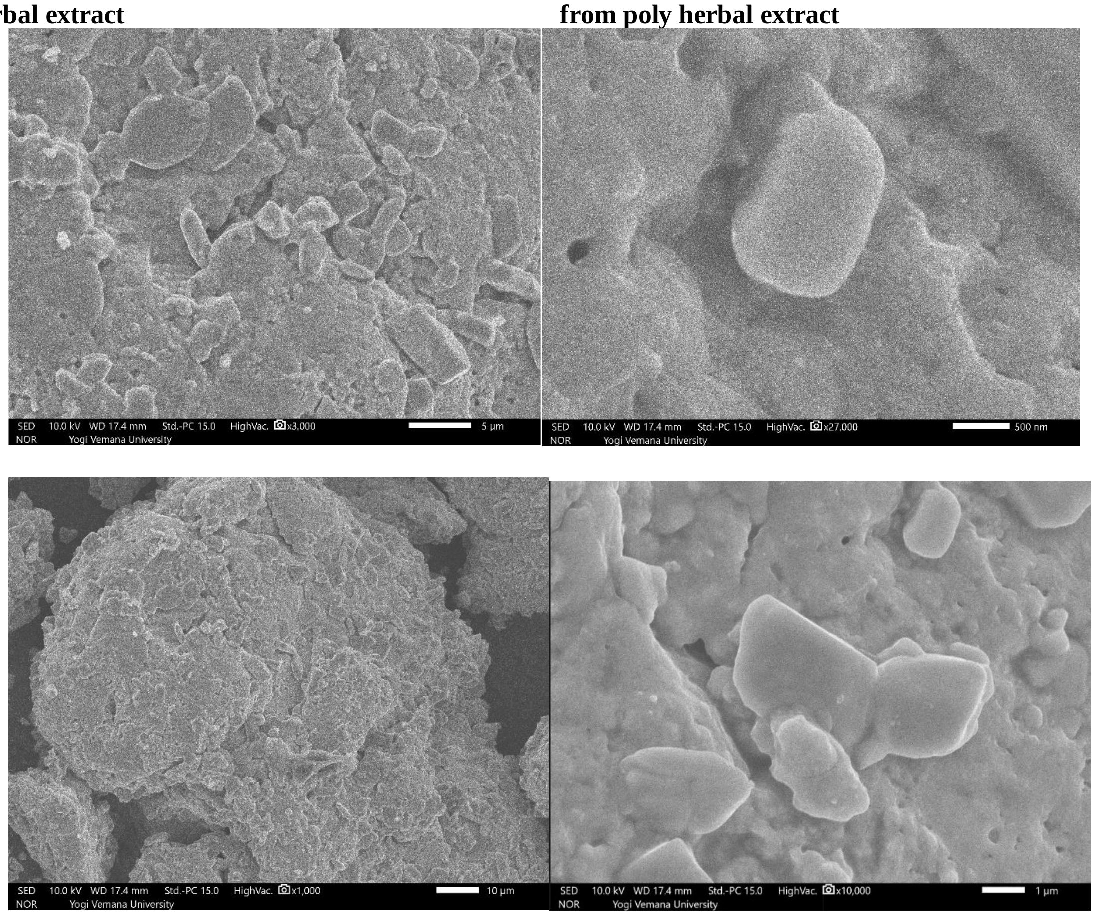

Fig.3: SEM images of nanoparticles synthesized from poly herbal extract

plants are used in traditional medicine instead of specific chemicals. Due to its synergistic effects, polyherbalism has some benefits that aren't seen in single plants. Polyherbal formulations are highly effective in treating a variety of illnesses when used at a safe dosage[8]. Previous studies supported that loading the polyherbal extract into silver nanoparticles enhanced the drug bioavailability. Hence, this research hypothesized to design a polyherbal extract with the combination of three medicinal plants and to investigate the anti-microbial potential of green-synthesized Ag-nanoparticles notably assessing the pharmacological effectiveness of the nanoparticles made with polyherbal extract.

Of the three approached plant materials the Seeds of *Psoralea corylifolia* belonging to family Leguminosae are used in indigenous medicine systems for healing of various ailments[9]. *Vetiveria zizanioides* (Linn.) Nash, is a perennial grass belonging to the Poaceae family[10]. Its thick, fibrous adventitious roots are highly appreciated and have a fragrant flavour. Cyperus rotundus. Is one of the first herbs utilized for therapeutic purposes dates back to Charaka's time, and it's roots have long been utilized in traditional medicine to treat a wide range of illnesses and to create

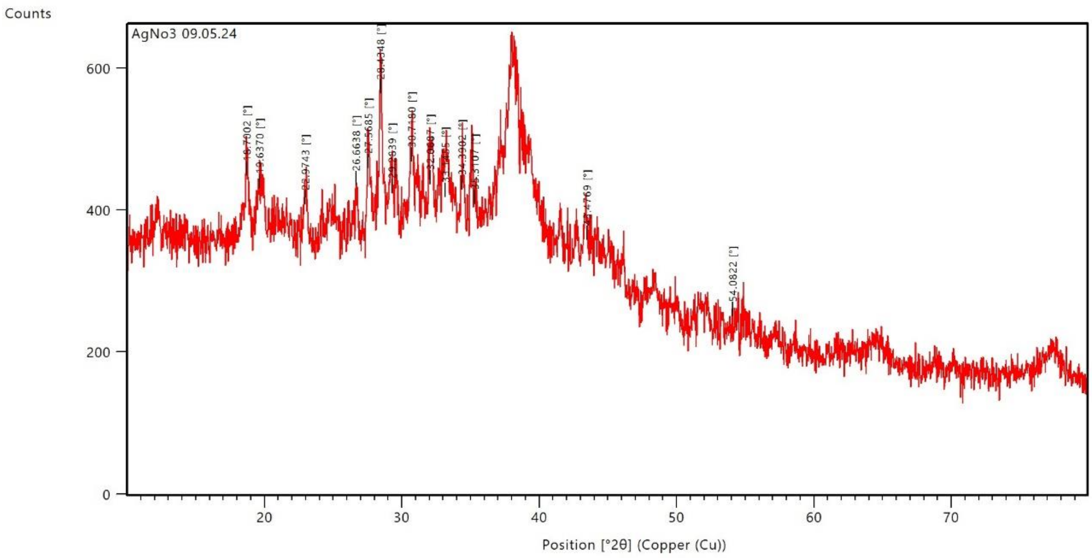

Fig.4: XRD of nanoparticles synthesized from poly herbal extract

pharmacological and nutraceutical products[11]. Due to their antimicrobial potentials, this research hypothesized to synthesize poly herbal silver nanoparticles (PH AgNps) using combination of these three plants and to investigate their antibacterial effect.

METHODS

Synthesis of Poly herbal silver nanoparticles:

1 g of dried, ground poly herbal tandem was mixed with around 30 ml of water. The mixture was heated at 500 C for 30 minutes. Next, 2.5 ml of NaOH (0.1 M) and 10 ml of silver nitrate solution (0.01 M) were dropwise added to the 20% aqueous produced polyherbal extract solution. The yield of AgNPs was then increased by heating the resultant solution for 30 minutes at 500C. Ultimately, centrifugation at 6000 rpm was used to extract the Poly herbal silver nanoparticles (PH AgNPs), after which they were cleaned with 50% acetone and left to dry at room temperature[12].

Characterization of PH AgNPs:

UV-Vis Spectra analysis:

After diluting a tiny portion of the sample with distilled water, the UV-Vis spectra of the reaction medium were measured to track a reduction of pure Ag+ ions in the supernatant solution[13].

Fourier-transform infrared (FTIR) Analysis:

The silver nanoparticle solution was centrifuged for 10 minutes at 15,000 rpm in order to get FTIR readings. To remove any loose proteins or enzymes that are not encasing the silver nanoparticles, the pellet was rinsed three times with 20 millilitres of de-ionized water. The samples were subjected to KBr pellet grinding and drying before being examined using a Brucker FT-IR[14].

SEM Analysis:

The Hitachi S-4500 scanning electron microscope (SEM) was used for the purpose of evaluation. A small fraction of the sample was dropped onto a carbon-coated copper grid to create thin films. Any excess solution was then blotted out with blotting paper, and the SEM grid's film was allowed to dry for five minutes underneath a mercury lamp[15].

XRD analysis

The developed silver nanoparticles were centrifuged for 15 minutes at 10,000 rpm, and the pellets were then redistributed in sterile double distilled and centrifuged for 10 minutes at 10,000 rpm. The purified pellets were

Table.1: Zone of inhibition of PH AgNPs

S.no	Test Organism	10mg/mL	20mg/mL	Control (Ampicillin)
1	*E.coli*	0.2±0.08	0.4±0.08	0.8±0.16
2	*E.coli*	0.2±0.08	0.4±0.08	0.8±0.16
3	*E.coli*	0.2±0.08	0.4±0.16	0.8±0.16
4	*B. subtilis*	0.1±0.08	0.3±0.12	0.9±0.08
5	*B.subtilis*	0.1±0.08	0.3±0.12	0.9±0.12
6	*B.subtilis*	0.1±0.08	0.3±0.12	0.9±0.08
7	*P.aeruginosa*	0.2±0.12	0.3±0.06	1.0±0.12
8	*P.aeruginosa*	0.1±0.08	0.3±0.06	1.0±0.12
9	*P.aeruginosa*	0.2±0.12	0.3±0.06	1.0±0.12
10	*S.aureus*	0.3±0.09	0.4±0.16	0.8±0.12
11	*S.aureus*	0.3±0.09	0.4±0.12	0.8±0.12
12	*S.aureus*	0.2±0.06	0.4±0.16	0.8±0.04

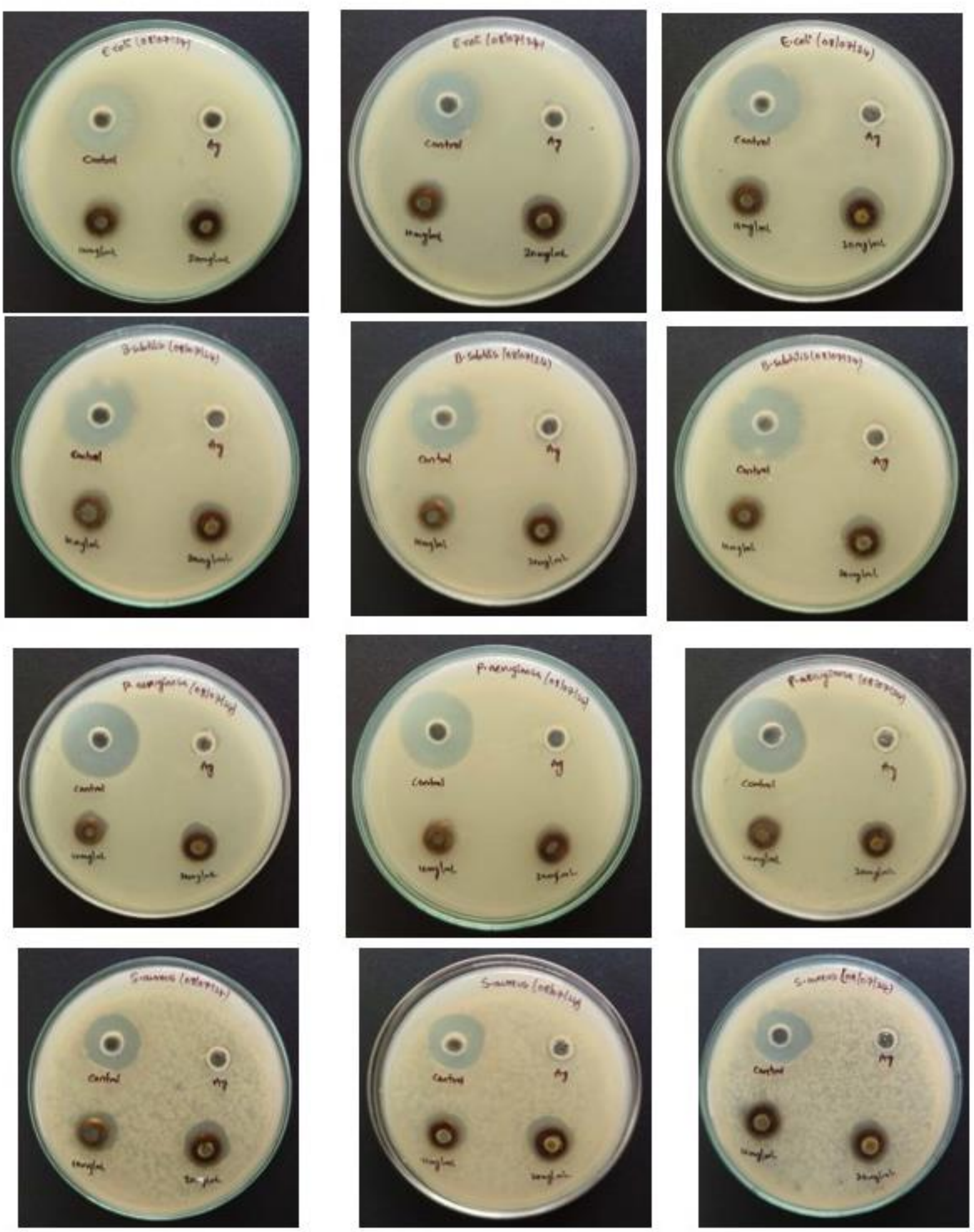

Fig.5: Antibacterial study of synthesized PH AgNPs

examined using an X-ray diffraction unit (XRD) after being baked in an oven at 50° C[16].

Antibacterial Activity of PH AgNPs

The disc-diffusion assay, was employed to assess the extent to which PH AgNPs impeded the development of bacteria. Two Gram-positive and two Gram-negative bacteria were used to investigate PH AgNPs' antibacterial activity. To create the base for plates, around 20 millilitres of Mueller-Hinton agar were put into sterile Petri dishes. Using a sterile cotton swab, 0.1 ml of standard bacterial stock solutions (108–109 cfu ml−1) was scattered onto the base plates. DMSO was utilised to generate a stock solution of 20 mg ml−1 AgNPs; dilutions of 10 and 20 mg ml−1 were employed[17].

RESULTS AND DISCUSSION

UV-Vis Spectra analysis:

A popular spectral tool for verifying AgNP production in a colloidal solution through metallic nanoparticle surface plasmon resonance is the UV-vis spectrometer. The size, shape, concentration, and aggregation condition of the generated nanoparticles all affect this optical property. Figure illustrates the noticeable transition of the yellowish GT to a brown hue, signifying the creation of PH AgNPs. The PH AgNP solution's UV scan revealed a clear peak at 410 nm (Fig.1). Researchers state that the band that represents the absorption by AgNPs is between 400 and 450 nm, and that this is because surface plasmon vibration is being excited.

FTIR Analysis

The FTIR spectrum (Figure) of silver nanoparticles showed strong IR bands characteristic of hydroxyl (3693 cm-1, 3643 cm-1, 3574 cm-1 and 3499 cm-1), alkanes (2,838 and 2,634 cm-1), C=O (1,534 cm-1) and phenols C-O Stretch (1,292cm-1 and 1087 cm-1) functional groups (Figure). The capping pattern of bioreduced silver nanoparticles produced by Poly herbal extract, which in turn contributed to the great stability of the synthesised silver nanoparticles, was strongly corroborated by the FTIR analysis[18]. Consequently, the results of FTIR analysis demonstrated that the biotransformation of silver ions as silver nanoparticles and subsequent stabilization in aqueous media are caused by an assortment biological molecules that exist in poly herbal extract.

SEM analysis

Figure depicts the PH AgNPs, which are primarily distributed as aggregates, are shown in SEM pictures in a range of diameters less than 100 nm. The stabilising effect of the capping agents in the extract can account for the nanoparticles' lack of direct interaction with one another within the aggregates that were generated. It is known that phytochemicals actively contribute to the stabilisation and reduction of metal nanoparticles[19].

XRD analysis

XRD was used to analyse the crystalline size and structure of the produced silver nanoparticles. Diffraction peaks were seen at $2\theta = 18.7°$, 19.6°, 22.9°, 28.4°, 30.7°, 35.3°, 43.4°, and 54.0° in the XRD study of PH AgNPs. The synthesized silver nanoparticles were proven to be crystalline in nature and in nanocrystal form by the acquired XRD spectrum. The active silver composition with indexing was revealed by the strong peaks in the XRD study (Fig.4). Numerous researchers have observed the same result, which suggests that the silver nanoparticles are crystalline, face-centered, and cubic in form[20].

Antibacterial assay

This study investigated the potential antibacterial action of synthesized silver nanoparticles mediated by poly herbal extracts. The antibacterial activity of the synthesized PH AgNPs was evaluated against Gram-positive (*E. Coli and B. subtilis*) as well as Gram-negative (*P.aeruginosa and S. aureus*) bacteria. Significant zones of inhibition demonstrated the antibacterial impact of the synthesised PH AgNPs when tested for antibacterial activity (Table 1 and Fig.5). The observed action could potentially be attributed to the adhesion of the nanoparticles to the bacterial cell membrane and their internal penetration[21]. Silver nanoparticles engage in interactions with sulfur-hold proteins found in the bacterial membrane, in addition with phosphorus-encompassed substances such as DNA. The combined action of AgNPs and naturally occurring phytoconstituents in the poly herbal extract may account for the foremost bactericidal activity of the developed nanoparticles.

CONCLUSION

The current investigation details the synthesis of silver nanoparticles with medicinal plants in reducing silver ions and stabilizing the silver nanoparticles. The primary cause of the creation of silver nanoparticles was the reduction of silver ions to nanosized silver particles by water soluble chemical compounds found in the poly herbal extract. The synthesised silver nanoparticles' crystalline nature and stability were revealed by FTIR, SEM, XRD, and UV-visible spectroscopy investigations. worthwhile antibacterial activity was illustrated by the developed silver nanoparticles and hence may serve as a quick, cost effective and suitable alternative for synthetic antibiotics against multi drugs resistance bacteria.

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