*Section A-Research paper*

# GREEN SYNTHESIS OF SILVER NANOPARTICLE FROM POLYHERBAL EXTRACTS

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## ABSTRACT:

**Background:** Silver nanoparticles are harmful to bacteria and are frequently used in a variety of scientific fields. The purpose of this work was to examine the antioxidant and antibacterial properties of a polyherbal acetonic extract used to synthesize silver nanoparticles.

**Materials and methods:** The 90 ml of 1 mM silver nitrate solution and 10 ml of acetonic polyherbal extract were combined in a 250 ml conical flask. The mixture was heated for 10 minutes in a water bath that was set to 80°C. A color change from yellow to brown was observed as the reduction of $Ag^+$ to $Ag^0$ occurred. The synthesis of silver nanoparticles was confirmed by UV-Vis spectrophotometer. The silver nanoparticles were characterized by EDS, Transmission Electron Microscope, and Fourier Transform Infra-Red spectroscopy. The antioxidant

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property of silver nanoparticles was analyzed by the 2, 2-diphenyl-1- picrylhydrazyl, hydrogen peroxide, hydroxyl radical and superoxide radical scavenging methods. The bacteriostatic activity of silver nanoparticles against *Pseudomonas aeruginosa (Pa)*,and *Staphylococcus aureus (Sa)* was determined using bacterial growth inhibition method. The antibacterial sensitivity and Minimum Inhibitory Concentration (MIC) of silver nanoparticles was determined against the bacteria.

**Results:** The results confirmed that the silver nanoparticles synthesized by polyherbal extract were crystalline in nature, average particle size was 8 -70 nm and were mostly spherical in shape. The antioxidant methods confirmed that the silver nanoparticles have more antioxidant activity. The silver nanoparticles have strong antibacterial activity. The MIC value of silver nanoparticles was 50µg/ml.

**Conclusion:** Green synthesized silver nanoparticles have strong antioxidant and antibacterial activity due to the presence of bioactive molecules on the surface of silver nanoparticles.

**KEYWORDS:** Green synthesis, Nanoparticle, Antioxidants, *Pseudomonas aeruginosa*

## INTRODUCTION:

Numerous diseases caused by bacteria frequently result in death and disruption from ruined food, ruined crops, and contaminated tools. Due to prolonged hospital stays, unsuccessful treatments, persistent infections, and delayed wound healing, which frequently results in amputation and increased mortality, bacterial infections raise medical expenditures and put stress on healthcare systems. New antibiotics and fresh approaches to battling bacterial illnesses are urgently needed. Due to their accessibility, lack of toxicity when used therapeutically, and capacity to effectively treat bacterial infections, medicinal plants are being investigated for their potential as a cure for diseases that are brought on by chemically produced medications. The ability of metal nanoparticles (1-100 nm) to effectively battle infectious diseases is an intriguing field of study with numerous applications. The pricey equipment, reagents, high voltage, high temperatures, and toxic solvents used in physical and chemical methods to create nanoparticles raise questions about the environment's and people's safety and health. Phyto-nanotechnology, which produces

nanoparticles using extracts from plants, viruses, algae, fungi, and bacteria, has drawn a lot of interest as a substitute method that is quick, easy to scale up, inexpensive, and alternative. Utilizing plant extracts has the benefit of being biocompatible because they are abundant in bioactive compounds that are amenable to extraction by water as an inert solvent and also function as reducing and capping agents in the synthesis of nanoparticles. Due to its intrinsic antibacterial qualities, silver is used in medicinal applications more frequently than other metals including gold, copper, iron, titanium, and zinc. There is evidence that silver nanoparticles made from plants, which are frequently found in secondary metabolites, have an inherent ability to suppress the growth of bacteria that are resistant to antibiotics and foodborne pathogens.

Furthermore, *Allium sativum* is one of the most significant traditional medicinal plants in India [1-4]. Medicinal characteristics such as anticancer, antitumor, antidiabetic, anti-aging, antimicrobials, etc. have been fully investigated scientifically abroad because of several commercial products currently available [5]. *Emblica officinalis* is a medicinal plant of the *Euphorbiaceae* family, widely distributed throughout the world. It has been considered an important remedy in Indian traditional plant medicine for the treatment of several diseases. The plant exhibits antiproliferative, antipsoriatic, antitumor, larvicidal, contractile muscle, hepatoprotective, anticonvulsant, antifilarial activity, etc. Phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids [6]. *Aloe vera* is an herbaceous and perennial plant that belongs to the *Liliaceae* family and used for many medicinal purposes. Due to its compounds, it can be used to retain skin moisture and integrity. It also prevents skin ulcers as it contains mucopolysaccharides, amino acids, zinc, and water. In terms of quality and speed of wound healing, Aloe vera is much more effective and less costly compared to the currently available alternative treatments. Considering the tendency to promote traditional medicine as well as rare side effects of Aloe vera, the use of this medicinal plant to improve wound healing is recommended as the complementary treatment alongside other methods. Fenugreek is a medicinal plant that is used in disease therapy. This plant is used for

blood lipids and sugar decreasing in diabetic and non-diabetic peoples and has antioxidant and antibacterial activity. The plant contains active constituents such as alkaloids, flavonoids, steroids, Saponins etc. It is an old medicinal plant. It has been commonly used as a traditional food and medicine. Fenugreek is known to have hypoglycemic, and hypocholesterolemia, effects, Anti-inflammatory effects [7].

Based on the above investigation of the medicinal plants, this study has a main objective to propose an efficient biosynthesis of silver nanoparticles with proven antibacterial activities. Synthesis of silver nanoparticles using medicinal plants are environment friendly and offers an excellent alternative to the development of antibiotics. Most of the previous research work is done by using a single plant and not the synergistic effect of the different medicinal plants for an antibacterial activity. The present study aims to evaluate the synergistic effect of antibacterial activity of the green synthesized silver nanoparticles using polyherbal formulation of selected medicinal plants against the human pathogens.

## METHODOLOGY:

The leaves of *Allium sativum* and *Emblica officinalis* (1kg) were collected from the garden. The leaves were dried under a hot air oven at 50ºC. *Aloe barbadensis* (Aloe vera peels), *Trigonella foenumgraecum* were picked up from the market, respectively. The fresh leaves and aloe vera peels were thoroughly cleaned under running tap water. The cleaned peels were further dried at 50ºC for 48 hours in a hot air oven and allowed to shade dry for two days. Peels and leaves were powdered and mixed in a ratio of 1:1:1:2 (*Allium sativum, Emblica officinalis, Aloe barbadensis,* and *Trigonella foenumgraecum)* to prepare the extract [8].

## EXTRACT PREPARATION:

The acetone plant extract was prepared following procedure. In brief, a mass of 10.01 ± 0.15 g of the above prepared powder was stirred in 100 ml of 50% acetone. The mixture was heated in a

magnetic stirrer on a hot plate at 60 °C for 10 min. The crude extract was filtered using Whatman No.1 filter paper. The polyherbal solution was allowed to cool at room temperature. The extract to be used was stored at room temperature [9].

**SYNTHESIS OF SILVER NANOPARTICLES USING POLYHERBAL EXTRACTS:**

AgNPs were prepared according to modified literature methods. In brief, 10 ml of the acetonic polyherbal extract was added to 90 ml of 1 mM silver nitrate solution in a 250 ml conical flask. The mixture was heated in a water bath set at 80°C for 10 min. A color change from yellow to brown was observed as the reduction of Ag+ to $Ag^0$ occurred. The particles were separated by centrifuge at 4500 rpm for 20 min. The supernatant was collected by decanting. The silver nanoparticles were precipitated with 80% ethanol and collected using centrifuge at 4500 rpm for 15 min. The final washing was done with absolute ethanol and dried in an oven at 40 °C for 15 min. The synthesized nanoparticles from the distilled water extract were labelled AgNP [10].

**CHARACTERIZATION OF SILVER NANOPARTICLES BY UV–VIS SPECTROPHOTOMETRY:**

The formation of silver nanoparticles was monitored by a UV–Vis Spectrophotometer with a resolution of 1 nm between 200 and 800 nm. A volume of 4 ml of the nanoparticles sample were transferred into a cuvette and analyzed at room temperature [11].

**FOURIER TRANSFORM INFRARED ANALYSIS:**

Identification of the groups acting as reducing and capping agents in the plant extract was done using FTIR. A mass of 0.2105 g potassium bromide was placed in a mortar with 0.0021 g of polyherbal plant extract. The powders were crushed using a pestle till a homogeneous powder was formed, placed in a sample holder, and compressed tightly to form a transparent disc for analysis [12].

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**ANTIBACTERIAL ACTIVITIES:**

An antibiotic sensitivity test was performed to check the antimicrobial action. Broth dilution was used to check the antimicrobial activity and minimum inhibitory concentration against *Pseudomonas aeruginosa (P.a)* and *S aureus* [13].

**DETERMINATION OF FREE RADICAL SCAVENGING ACTIVITY:**

**2, 2-diphenyl-1-picrylhydrazyl (DPPH) method:**

With a small adjustment, the procedure was carried out in accordance with Musarrat et al [14]. Using the stable radical DPPH, the ability of AgNPs and regular vitamin C to scavenge free radicals was assessed. AgNPs were diluted in 1 ml amounts of 10, 20, 30, 40, 50, 75, and 100 mg/ml before being combined with 1 ml of newly made DPPH solution (1 mM in methanol) and vortexed vigorously. After that, the mixture was let to sit at room temperature in the dark for 30 minutes. At 517 nm, the absorbance was measured using a UV-Vis spectrophotometer. Methanol was used as a blank solution and DPPH was used as the control (all the reagents were utilized except for the sample). The following formula was used to calculate the percentage of inhibition, which was used to express the free radical scavenging activity.

$$\%\ of \text{scavenging} = \frac{PC - PS}{PC} \times 100$$

where PC is the absorbance of control and PS is the absorption of AgNPs

**Hydrogen peroxide scavenging activity:**

0.3 ml of phosphate buffer (50 mM, pH 7.4), 0.6 ml of hydrogen peroxide solution (2 mM $H_2O_2$ in phosphate buffer, 50 mM, pH 7.4), and 0.1 ml of AgNPs (25–250 mg/ml) were mixed. The mixture was vortexed, and a UV-Vis spectrophotometer (Systronics, AU-2701) was used to

record the observation at 230 nm after 10 minutes [15]. The following formula was used to determine the percentage of hydrogen peroxide scavenging activity.

$$\%\ of\ \text{scavenging} = \frac{OC - OS}{OC} \times 100$$

Where OC represents the absorbance of control (all the reagent except the test sample) and OS absorbance of AgNPs

**Hydroxyl radical (OH) scavenging activity:**

The scavenging activity of OH radicals was assessed in accordance with Keshari et al., 2016 [15]. Deoxyribose (10 mM), 0.150 ml $FeSO_4$, EDTA (10 mM), 0.15 ml $H_2O_2$ (10 mM), 0.075 ml AgNPs (250 mg/ml in methanol), 0.45 ml sodium phosphate buffer (200 mM, pH 7.0), and 0.525 ml deionized water were combined. For 4 hours, the mixture was kept in the digital incubator. 0.75 ml of trichloroacetic acid (2.8%) and 0.75 ml of TBA (1% in 50 mM NaOH) were added to stop the reaction. After 10 minutes in a boiling water bath, the solution was cooled with tap water. At 520 nm, the solution's absorbance was measured. As a blank, methanol was utilized. The percentage of hydroxyl radical scavenging activity was calculated using formula.

$$\%\ of\ \text{scavenging} = \frac{Hc - Hs}{Hc} \times 100$$

Whereas Hc is the absorbance of control (all the reagent except the test sample) and Hs, absorbance of AgNP

**Superoxide radical scavenging activity:**

This activity of AgNPs was determined according to Kesari et al., 2016 [15]. Superoxide radicals are generated by the oxidation of NADH in Nicotinamide adenine dinucleotide (NADH) phenazinemethosulphate (PMS) system and analyzed by the reduction of Nitro blue tetrazolium

(NBT). 0.2 ml Ag-NPs (100-500 mg/ml in methanol), 1 ml Tris-HCl buffer (16 mM, pH 8), 1 ml NBT (50 mM), 1 ml NADH (78 mM) and 1 ml PMS (10 mM) were mixed and kept for 5 min at $25^0$C. With the use of a UV-Vis spectrophotometer (Systronics, AU-2701), the absorbance was measured at 560 nm. Following formula was used to determine the superoxide production inhibition percentage:

$$\%\ of\ inhibition = \frac{Sc - Ss}{Sc} \times 100$$

Whereas Sc is the absorbance of control (all the reagent except the test sample) while Ss is the absorption of AgNPs

**STATISTICAL ANALYSIS:**

The values were expressed as the mean ± standard error of the mean. The data collected was statistically analyzed using one-way analysis of variance with SPSS/20 Software. A p-value < 0.5 was inferred as statistically significant.

**RESULTS:**

**Spectrophotometric Characterization:**

The main instrument for clarifying the creation of AgNPs during the early synthesis phase is UV-Vis spectrophotometry. The results of this experiment showed that the smaller (spherical) AgNPs efficiently absorbed light photons with a wavelength of 400 nm.These results showed that manufactured AgNPs containing natural extract were stable because as the particles accumulated, the peaks started to weaken and widen with the emergence of secondary peaks at longer wavelengths. Additionally, the color change in the solutions of *Allium sativum, Emblica officinalis*, *Aloe barbadensis*, and *Trigonella foenumgraecum* extracts during synthesis is size and shape-specific, confirming the progress of their reduction to $AgNO_3$ throughout synthesis

processes. These findings were corroborated by Balaji et al. [16], who demonstrated that the creation of AgNPs caused the colourless $AgNO_3$ solution to change from yellow to brown to deep red. In addition, surface plasmon resonance (SPR) excitation is responsible for the brown color's appearance. Additionally, the SPR absorbance was highly sensitive to the type, dimension, and shape of the formed particles as well as their interparticle relations.

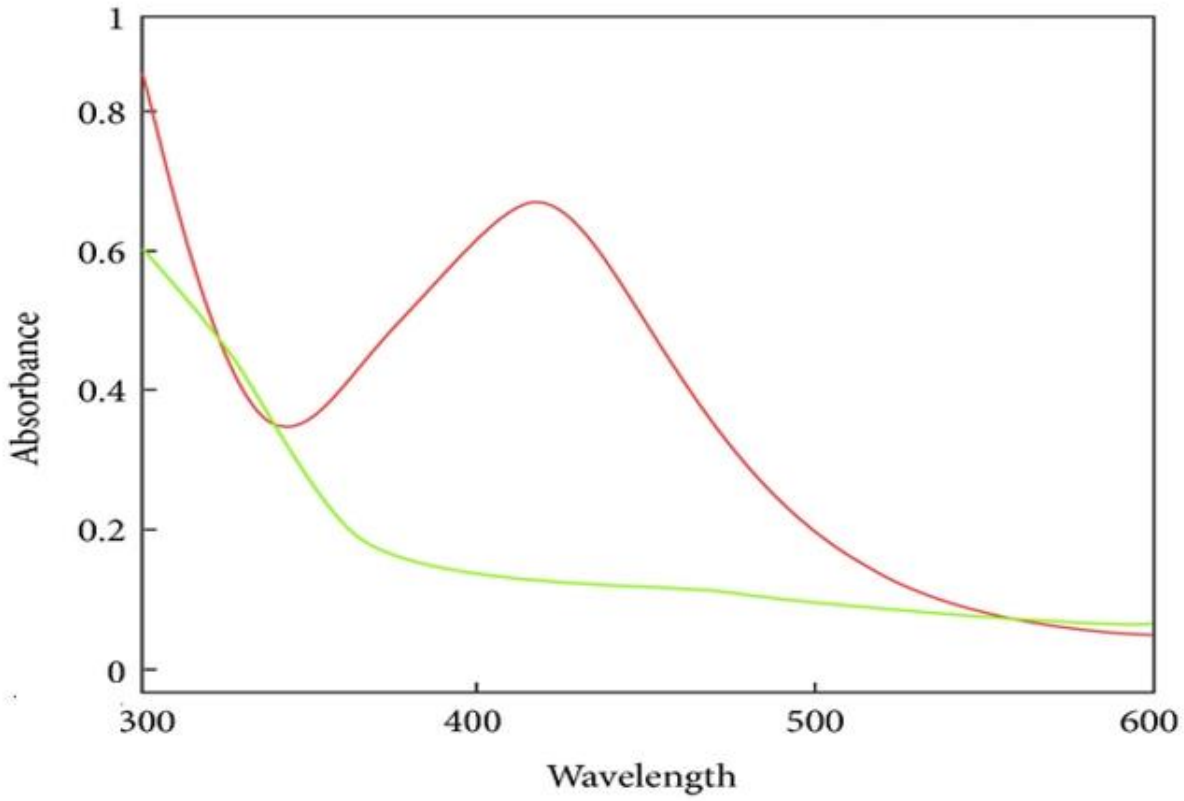


**Figure 1:** UV-visible spectra of AgNPs synthesized were brownish in color. The maximum UV absorption of AgNPs was 430 nm.

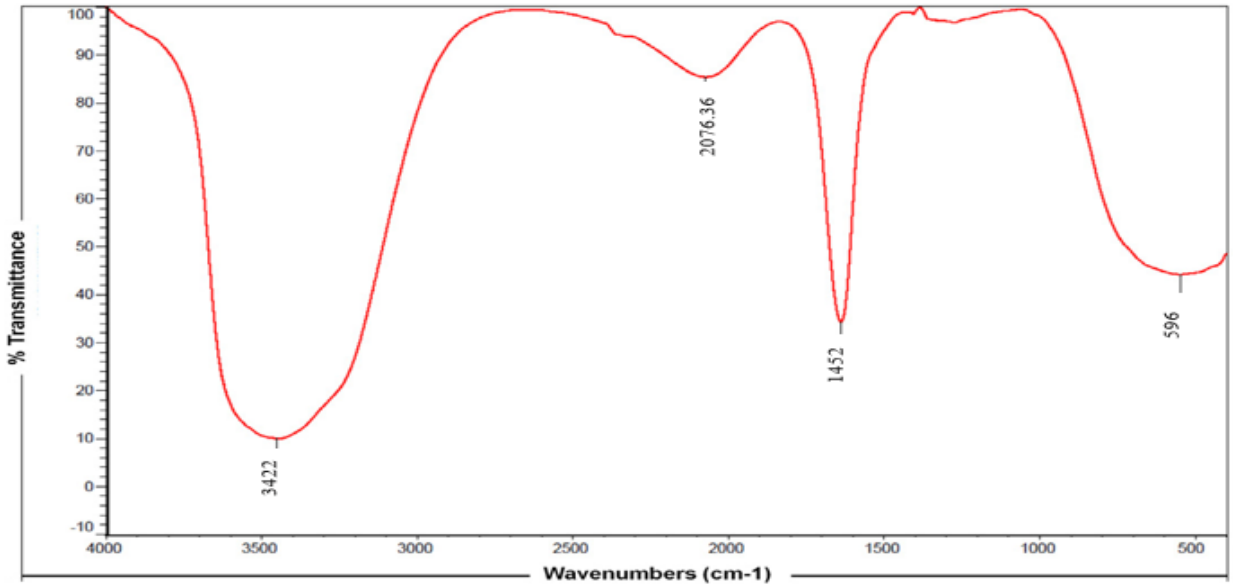


**Figure 2:** FTIR spectra of AgNPs.

The FTIR study was conducted to evaluate the responsible biomolecules for the reduction of Ag+ ions and the bioreduced silver nanoparticles stabilization to avoid lumps of the nanoparticles and their capping in the aqueous medium [17]. The 500–4000 $cm^{-1}$ peak range in the FTIR study corresponded to several functional groups and indicated the existence of chemicals that stabilize proteins. According to the results of the FTIR study, there are various functional groups that correlate to sharp absorption peaks at 596, 1452, 2074.36, and 3422 $cm^{-1}$.The primary amide as well as secondary and the -OH stretch of the common phenolic and alcohol chemicals found in extract match to the strong FTIR peaks. Absorption peak at 1452 $cm^{-1}$ may be designated to the primary amide bond of proteins, similarly the band at 3422 $cm^{-1}$ is close to that reported for -OH stretching in alcohols and phenolic compounds [18]. It is reported that absorption band at 1452 $cm^{-1}$ is found to be close to the native proteins which were reported for the interacting with biosynthesized silver nanoparticles, and it was also confirmed that this protein does not loses its secondary structure when it binds or reacts with silver nanoparticles [19]. This FTIR analysis reports confirms that carbonyl group of amino acid residue have strong binding affinity with the

silver nanoparticles and that may be act as a reducing agent and stabilizing agent to avoid agglomeration and that leads to the providing stability to the silver nanoparticles in the medium [20]. Absorbance peak near at 1630 $cm^{-1}$ is associated with stretch vibration of -C=C- [21] which is assumed for the primary amide bonds of proteins [22]. The presence of the peak in the primary amide and secondary regions were assigned for the proteins and enzymes which are responsible for the reduction of metal ions for synthesis and stabilization process.

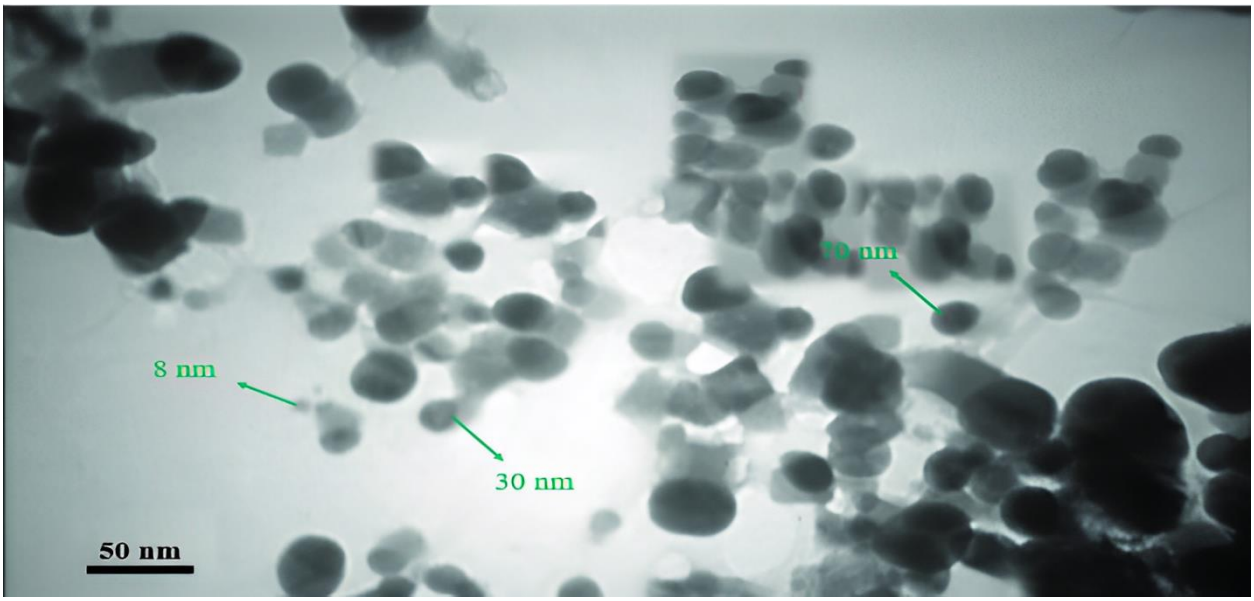


**Figure 3:**TEM images of the synthesized AgNPs

The development of silver nanostructures is confirmed by TEM investigation, which also provides images of synthesized silver nanoparticles at scale of 50 nm. According to TEM images, synthesized silver nanoparticles have a non-specific distribution and are typically spherical and near spherical in shape. The range of 8–70 nm was discovered to be the average diameter of synthesized silver nanoparticles. In TEM images, a sizable fraction of mainly spherical silver nanoparticles around 30 and 70 nm were noted. We also noted that there are only

very minor amounts of clustered silver nanoparticles, which may explain some of the particle size variation.

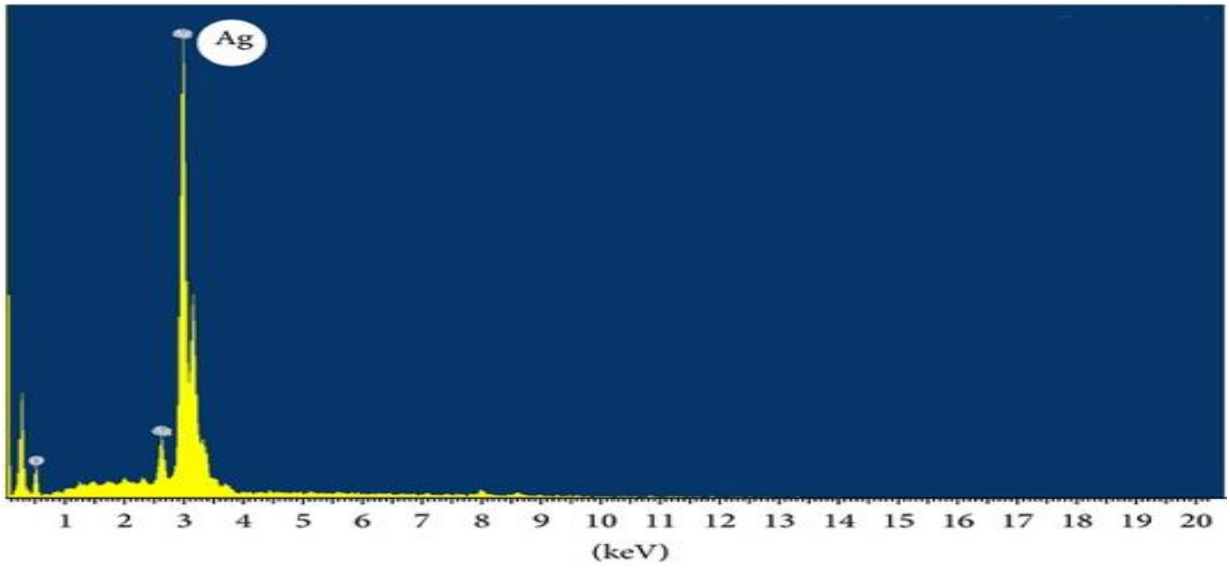


**Figure 4:** EDX spectrum of synthesized AgNPs.

The silver nanoparticles synthesized by the polyherbal extract are predominantly spherical in shape. The EDX analysis had peaks at 3 keV confirmed the presence of silver element. The qualitative and quantitative status of the component elements in AgNPs was recognized by the EDX spectrum. Figures 4 show the elemental mapping of synthesized AgNPs that demonstrates the existence of silver. The existence of an intense peak at 3 keV of Ag° with the highest counts confirms the formation of silver nanoparticles [23].

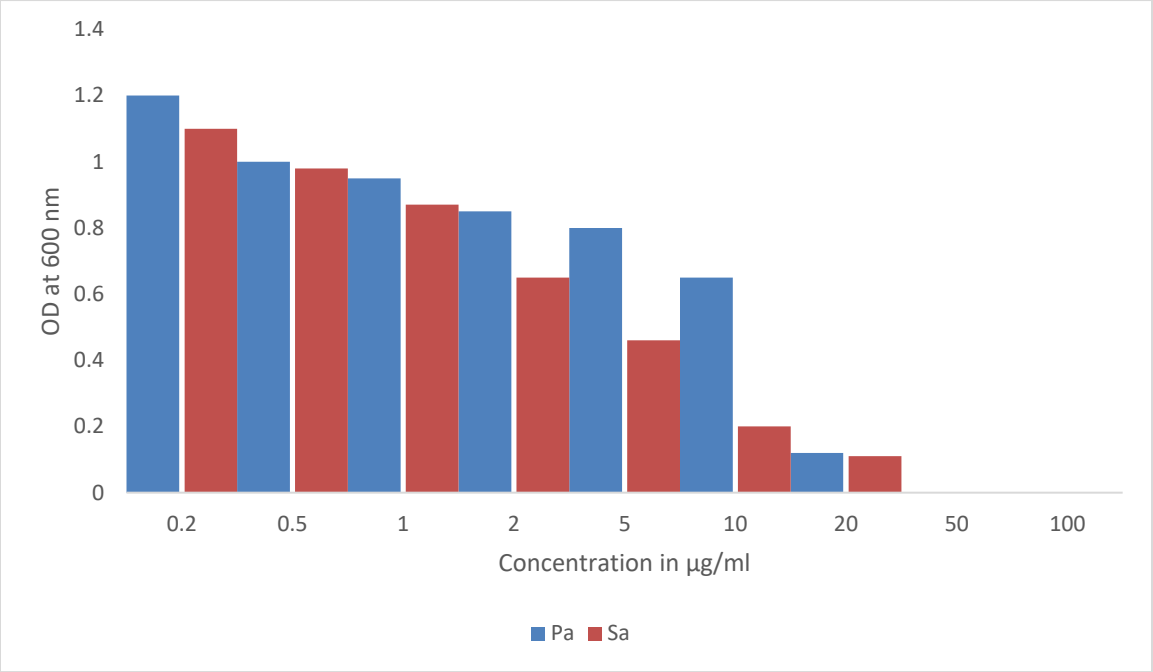


**Figure 5:** Antimicrobial and minimum inhibitory concentration of silver nanoparticle.

The antimicrobial activity of AgNP was evaluated using the MIC method. The antimicrobial effectiveness was determined against the bacterial concentration of $10^6$ CFU/ml with different concentrations of AgNP (0.2, 0.5, 1, 2, 5, 10, 20, 50, and 100 µg/ml). The cultures were incubated at 37°C at 120 rpm. Bacterial concentrations were determined by measuring optical density (OD) at 600 nm. With the increase of concentration of nanoparticles, the final bacterial concentration decreased. Data illustrated that on increasing the concentration of silver nanoparticle optical density at 600 nm was decreased which indicated that prepared silver nanoparticle has good antimicrobial properties. When the concentration of AgNP was 50 µg/ml, growth of *Pa* and *Sa* was completely inhibited, which indicated that the MIC of AgNPs50 µg/ml. Result was supported by Rashid et al. [24] who reported the increased antibacterial activity of AgNPs synthesized from the water extracts of the roots of *Bergenia ciliata*, *Bergenia stracheyi*, *Rumex dentatus*, and *Rumex hastatus* from Pakistani origin against six pathogenic

bacteria including *E. coli*, *S. aureus*, *S. haemolyticus*, *Bacillus cereus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*.

**Antioxidant activity:**

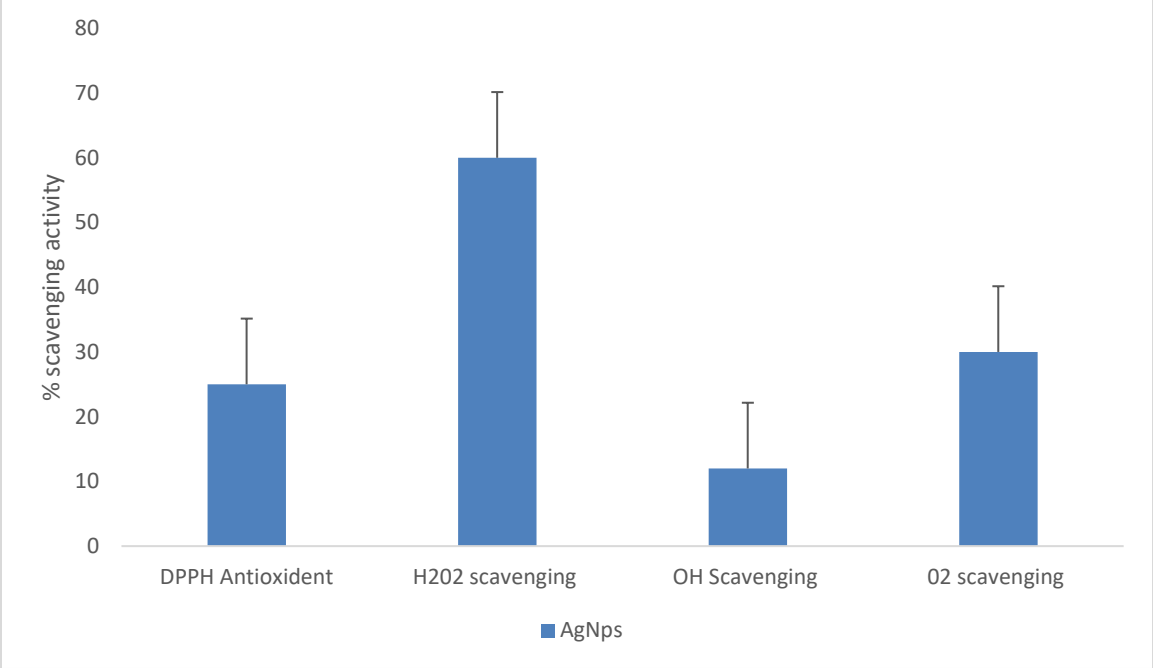


**Figure 6:** Percentage of Antioxidant (DPPH), and Free radicals (hydrogen peroxide, hydroxyl, and superoxide radical) scavenging activity of silver nanoparticles.

The outcomes demonstrated that AgNPs have antioxidant action. The DPPH antioxidant activity of the AgNPs was 25%. The hydrogen peroxide scavenging activity of AgNPs was 60 %. The hydroxyl radical scavenging activity of AgNPs is 12%. AgNPs has 30% superoxide scavenging activity.The DPPH, Hydrogen peroxide, hydroxyl radicals, superoxide scavenging methods confirmed the silver nanoparticles has antioxidant, hydrogen peroxide, hydroxyl radicals and superoxide scavenging activities.These properties of silver nanoparticles occur due to the presence of functional groups on the surface of silver nanoparticles.The advanced activity of the

synthesized AgNPs is explained due to the simultaneous mechanisms of hydrogen atom transfer (HAT) and single electron transfer (SET) of flavonoids and silver ions present in the AgNPs.

The secondary metabolites such as phenolics, flavonoids, terpenoids, and soluble proteins act as capping agents for the synthesis of nanoparticles [25]. The electron-donating potential of polyphenols in the plant extracts facilitated the bio reduction of $Ag^+$ to $Ag^\circ$ and stabilized the AgNPs. Similarly, the water-soluble flavonoids in plants are also involved in the reduction of silver ions for the synthesis of AgNPs [26]. Important phytochemicals like flavonoids, polyphenols, saponins, terpenoids, and vitamins are responsible for the antioxidant activity of the plant extracts. The compounds may have absorbed the larger surface area of the spherical AgNPs and amplified the antioxidant potential. The negatively charged phytochemicals exhibit an electrostatic attraction to the positively charged or neutral AgNPs which also significantly improved the bioactivity.

## CONCLUSION:

The present work describes the formation of silver nanoparticles with the help of polyherbal extract. The extract of polyherbal has bioactive compounds which are responsible for the reduction and capping of silver nitrates into silver nanoparticles. The capping agent provides stability to the AgNPs. The synthesized AgNPs have antioxidant, hydrogen peroxide, hydroxyl radicals, and superoxide scavenging activity. This activity occurs due to the presence of functional groups on the surface of AgNPs. Moreover, AgNPs have strong antibacterial activity against the selected bacteria. These silver nanoparticles might be used as antibiotics in future due to their being non-toxic, cheap, eco-friendly, and highly effective against the bacteria.

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