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# GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES (AGNPS) USING *ARTEMISIA ANNUA* L. AND ITS ANTI-BACTERIAL, ANTI-FUNGAL ACTIVITY.

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## ABSTRACT

In the recent times, biomedical and pharmacological applications of Silver nanoparticles (AgNPs) have gained much attention owing to its array of pharmacological effects like anti-bacterial, anti-fungal and anti-cancer activities. In the present study we have attempted to evaluate and endorse the pharmacological effects of green synthesis of AgNPs using *Artemesia annua* L plant extract. Characterization of *Artemesia annua* L AgNPs were performed using UV-vis spectroscopy, Energy dispersive X-ray analysis (EDX), Dynamic light scattering (DLS), X-ray diffraction analysis (XRD), Transmission electron Microscopy (TEM) and Fourier Transform infrared spectroscopy (FTIR). A band at 425nm was observed using UV-vis spectra and TEM denotes that the synthesized AgNPs are about 30-35 nm in size and are spherical in nature. Further, its antibacterial activity was assessed using gram-positive bacteria strains, such as *B. subtilis and S. aureus* and Gram-negative bacteria, such as *P. aeruginosa* and *E. coli* and anti-fungal activity was also demonstrated against fungi such as *C. albicans* and *A. niger* The results obtained show that the synthesized AgNPs using *Artemesia annua* L, exert significant biological functions and hence it can be applied in therapeutic applications like anti-cancer activities. Further, we are also interested to study its mechanistic actions, for which studies are currently in progress.

**KEYWORDS:** ***Artemesia annua, silver nanoparticles, metallic nanoparticle, TEM, anti-bacterial activity***

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# INTRODUCTION

Nanosized metallic particles are unique and can considerably revolutionize many areas of human needs like engineering, nano electronics, medicine, health care, energy, food, and textile and information technology[1]. Integrated development of innovative nanoparticle encapsulated drugs shows theranostic action against many infectious disease and diseases associated with ageing like cancer, neurodegenerative conditions[2-4]. Metallic nanoparticles are unique as they can be targeted to various biochemical and physiochemical properties of the cells, due to their diversity in exhibiting variations in characteristics such as size, distribution and morphology[5] which facilitate the transport of complex molecular cargoes to the target of interest in cell. Recently, nanosilver AgNPs have gained wide applications in biomedical sciences like diagnosis, treatment, medical devices and drug deliveries[6]. Further, the documented biological functions of AgNPs includes anti-bacterial, anti-fungal, anti-viral and anti-inflammatory activities, however its mechanisms of actions in context with biological interactions and modulating intracellular activities remains elusive[7]. Indeed, in recent decades synthesis of metal nanoparticles with significant biological activities has warranted a greater demand and it has been widely performed using bacteria, fungi, yeast, actinomycetes and importantly plant extracts[8]. Recently a number of researchers have successfully attempted to synthesize AgNPs using plant extracts (derived from aerial part to roots) in order to exploit a significant biological efficacy in a synergistic mode, which enhances biological functions of both AgNPs and the plant derivatives as well[9-10]. In this study, we have used *Artemesia annua L*. plant for the synthesis of AgNPs and intended to study its various biological functions including anti-oxidant, anti-bacterial, anti-fungal and ultimately its functioning as an anticancer drug and its mechanisms using in vitro models of ovarian and breast cancer cell lines. Regarding AgNPs synthesis, conventional methods like physical and chemical methods including photochemical methods, lithotheraphy and photochemical reductions are very expensive and hazardous[11]. Hence, biological methods that are eco-friendly, highly stable, non–toxic, and less expensive with high catalytic activity are highly warranted to enhance and exploit the biological functions of AgNPs. Such green synthesis of plant extract derived nanoparticles offers a better avenue for the development of new alternative anti-cancer and anti-bacterial agents[12]. This work describes a plant mediated approach for the preparation of AgNPs using leaf extract of *Artemisia annua* L [sweet wornwood] an ethno-mediated plant, popular for its anti malarial activity[13]. Green synthesis of AgNPs using aqueous extract of shade dried leaves of *Artemisia annua* was carried out and their characteristics such as EDX, XRD, FTIR, TEM were carried out for assessing its stability, biocompatibility and specificity effects like antioxidant, antibacterial and antifungal activities. Though artemisinin a constituent of *Artemisia annua L is* a sesquiterpene lactone containing an unusual peroxide bridge, it was known for its potent anti-malarial activity, anti-bacterial activity, anti-inflammatory activity and anti-tumour activities[14, 26]. It exhibits low bioavailability and poor pharmacokinetics properties which are considered as a major drawbacks in their therapeutic applications. Hence, we aimed to synthesize AgNPs using plant formulation of *Artemisia annua* L for examining and as well as to increase its bioavailability and pharmacokinetics and the preliminary findings of this study is described via characterization studies and analytical methods.

# MATERIALS AND METHODS

The leaves of *Artemisia annua* L was collected from Bangalore and was authenticated by Prof. P. Jayaraman, father of Indian Plant Anatomy, Plant Anatomy Research Center (PARC), Tambaram, Chennai - 600 045, Tamilnadu, India. Following authentication, specimen of the plant was also deposited in the herbarium of PARC. [PARC 2017/3596]. Antibiotic Disc, Mueller-Hinton Agar (MHA), Mueller-Hinton broth (MHB), $H_2SO_4$, copper acetate, ferric chloride, ninhydrin, HCl, Fehling's A and B solutions, iodine and potassium iodide were purchased from Sigma Aldrich, St. Louis, USA. All the other chemicals used were of analytical reagent grade unless otherwise stated. Milli-Q water was utilized in all the experiments.

### *Preparation of Artemisia annua extract*

Extraction was done with four different solvents based on their polarity by using Soxhlet extractor. The methanolic, hexane, acetone and aqueous extracts obtained were concentrated in a lyophilizer for further analysis.

### *Phytochemical and biochemical analysis*

Qualitative and quantitative analysis were done to check the presence of active compounds in different extracts of *Artemisia annua L*.

### *Synthesis of Silver nanoparticles*

Aqueous solution of *Artemisia annua* L was prepared by dissolving 3.0g of leaf powder in 50ml of deionised distilled water and heated at 60º C for 15 minutes. The extract obtained is filtered with Whatman No 1 filter paper and stored at 4ºC for further use. Aqueous solution of 1mM $AgNO_3$ was prepared and *Artemisia annua* L extract was carefully added. Different dilutions were prepared find out the optimum concentration in $T_1$(9+1) 1.0ml of extract with 9.0ml of distilled water with 20µl of AgNO3, T2(8.5+1.5), T3(8.0+2.0), T4(7.5+2.5), T5(7.0+3.0) while 20µl of $AgNO_3$ is kept constant. Occurrence of bio reduction within a short duration indicated by colour change as dark brown shows the presence of silver nanoparticles. The nanoparticles obtained were purified by centrifugation at 10,000rpm for 10 minutes. The pellet was collected and redispersed in distilled water to remove any interactive biological molecules. This step was repeated thrice to ensure better separation of silver nanoparticles which was then used for carrying out characterization studies.

### *Characterization of the synthesized SNPs*

### *UV-Vis spectral analysis*

Different dilutions of tubes from $T_1$-$T_5$ shows bioreduction of silver nanoparticles which was monitored by full scan UV-Vis spectrophotometer in nanometer ranging from 300-700 nm.

***Fourier Transform infrared spectroscopy (FT-IR)***
The FTIR spectrum explains the interaction of AgNPs with biomolecules of *Artemisia annua* L. The so formed silver nanoparticles are washed twice with deionised distilled water to remove proteins and other constituents. The pellets so obtained were dried using KBr and the disc shaped crystals were examined in FT-IR spectrophotometer.

***Ultrastructural analysis using Transmission Electron Microscopy***
A drop of $AgNO_3$ was placed on carbon-coated copper grid and allowed to stand for 2 minutes and allowed to dry at room temperature (RT). The morphology, size and shape of SNPs were determined by transmission electron microscope. TEM measurements were performed using a FE1 Tecnai $G_2$ T-30 S-Twin instrument. The chemical composition of silver nanoparticles was analysed using a Genesis liquid nitrogen coated energy-dispersive X-ray analysis [E-DAX] detector using an ultrathin window.

***X ray diffraction analysis***
Silver nanoparticles from *Artemisia annua* L. were subjected to X-ray diffraction. The lyophilized $AgNO_3$ coated on XRD grid under SEIFERT ISO DEBYEILEX operated at 40KV and a current of 30mA with Cu-k alpha radiation of wavelength 1.5 to 6Å at 2ɵ angle configuration and scan range selected was $30^0$ to $70^{0.}$

***Energy dispersive X-ray analysis (EDS)***
EDX analysis was conducted in the above instrument attached with thermo EDX to analyze the different elemental composition of the sample. The biosynthesized AgNPs were isolated by centrifugation for 20 min at 10,000 rpm. The pellets were collected and dried in the oven at 50 °C to remove any excess water and cooled to room temperature and observed for EDX analysis.

***Dynamic light scattering (DLS)***
The hydrodynamic diameter of the synthesized AgNPs was measured under Dynamic Light Scattering Particle sizes (hydrodynamic diameters) and polydispersity index were measured on a Zetasizer Nano-S (Malvern Instruments Ltd, Malvern, UK) operating with a He-Ne laser at a wavelength of 633 nm using backscattered light.

***Zeta potential***
The AgNPs samples were vortexed and transferred into a 1.0 mL zeta potential cuvette (DTS1060, Malvern). The electrophoretic mobility of the sample was measured by using a Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK) and converted into the zeta potential by applying the Henry equation.

***Antimicrobial screening of different solvent extracts and biosynthesized AgNPs***
The different solvent extracts, silver nitrate solution and the biosynthesized AgNPs obtained from leaf extract of *Artemisia annua L.* was tested for its antibacterial potency [15]. Gram-positive bacteria, such as *B. subtilis and S. aureus* and Gram-negative bacteria, such as *P. aeruginosa* and *E. coli* and its antifungal potent against fungi such as *C. albicans* and *A. niger.* The antibacterial and antifungal activities of the samples were determined by agar well diffusion method using 30mL of nutrient agar medium and potato dextrose agar medium in sterilized petri plates. The agar plates were seeded with different pathogens. The wells were made with diameter of 6mm with the help of a stainless steel cork borer. The wells were labeled as A, B, C and D. Then the wells were loaded with 40µl of methanolic extract, aqueous extract, silver nitrate solution (1 mM) and biosynthesized silver nanoparticles. The plates were incubated for 24 to 48 hours at $37^oC$ respectively, and the zone of inhibition (ZOI;mm) appearing around the wells was recorded.

***Minimum inhibitory concentration***
The microbial activity of different extracts and the biosynthesized AgNPs was recorded by the determination of minimum inhibitory concentration (MIC) according to this method [16]. The selected six different bacterial and fungal suspensions were prepared and seeded on the agar medium. Then the wells were loaded with 25µL, 50µL, 75µL and 100µL of methanolic, and aqueous extracts of silver nitrate solution (1 mM) to biosynthesize the silver nanoparticles. The control well was loaded with 20µl of gentamicin for bacteria and fluconazole for fungi. The plates were incubated for 24 to 48 hours at 37 $^oC$ respectively, and the zone of inhibition (ZOI;mm) appearing around the wells was recorded.

## STATISTICAL ANALYSIS

Statistical analyses were carried out using SPSS software, Version 10.0 program. Datas are expressed as mean ± standard deviation (S.D.). Probability of error less than 5% was considered as threshold for statistical significance ($P<0.05$).

## RESULTS

***Phytochemical analysis***
Phytochemicals are the most potent bio components for the reduction and are known as important natural resources for the synthesis of AgNPs[17]. In this study qualitative analysis was performed in crude leaf extract of *Artemisia annua L.* in different solvents which ensures the presence of alkaloids, flavonoids, terpenoids, phenolic compounds, tannins, glycosides and steroids (Table 1), while quantitative analysis of leaf extract in various solvents shows a remarkable variation (Table 2). Methanolic and acetone extracts of *Artemisia annua* L. contains high concentration of alkaloids and terpenoids than in hexane and aqueous extracts, whereas flavonoid and phenolic compounds are found to be high in aqueous and acetone extracts.

**Table 1**
***Qualitative analysis of phytochemicals in the leaf extracts of Artemisia annua L.***

| Plant extracts | Hexane extract | Acetone extract | Methanol extract | Aqueous extract |
|---|---|---|---|---|
| Alkaloids | - | + | + | + |
| Carbohydrates | - | - | + | - |
| Glycosides | - | - | + | - |
| Proteins | - | - | - | - |
| Phenolic compounds | - | + | + | - |
| Flavonoids | - | + | + | - |
| Terpenoids | - | + | + | - |
| Steroids | - | + | + | - |
| Saponins | - | + | - | + |
| Tannins | + | + | + | - |
| Fats and oils | + | - | - | - |
| Amino acids | - | - | - | - |
| Quinones | + | - | - | - |
| Phytosterols | - | + | - | - |
| Coumarins | + | - | + | - |
| Cardiac glycosides | - | - | - | - |

***"+" sign indicates Positive, "-" sign indicates Negative***

**Table 2**
***Quantitative analysis of phytochemicals in the leaf extracts of Artemisia annua L.***

| S.No. | Extract | Alkaloids (% mg of extract) | Total Flavonoid Content (mg QE /mg dry weight of extract) | Total Phenolic Content (mg GAE /mg dry weight of extract) | Terpenoid (%/mg of extract) |
|---|---|---|---|---|---|
| 1 | Methanol | 96.36±6.03 | 0.82±0.03 | 0.82±0.10 | 99.89±11.16 |
| 2 | Acetone | 90.39±2.24 | 1.64±0.11 | 1.64±0.11 | 90.32±6.55 |
| 3 | Hexane | 71.63±3.25 | 0.23±0.02 | 0.32±0.04 | 65.76±4.94 |
| 4 | Aqueous | 83.12±5.55 | 1.73±0.22 | 1.73±0.11 | 72.87±5.56 |

***Values are expressed as Mean ±.SD. Values are statistically significant at P<0.05.***

***Synthesis of silver nanoparticles***

*Artemesia annua* aqueous leaf extract added with 1mM silver nitrate showed a change in colour from pale yellow to dark brown. This visual observation confirmed the formation of silver nanoparticles (Figure 1A). This was due to the excitation of surface plasmon resonance (SPR) by AgNPs[18]. The free electrons of AgNPs, gives rise to SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with the light waves[19].

***Characterization of the synthesized silver nanoparticles***

***UV-Vis spectral analysis***

The reduction of AgNPs in the aqueous solution of silver complex during the reaction with the aqueous extract of *Artemesia annua L* was confirmed by the UV visible spectra. Free electron in AgNPs show SPR absorption band at 425nm (Figure 1B) which is characteristic of these nobel metal particles[20].

***Stability of biosynthesized silver nanoparticles***

The stability of AgNPs in the present attempt revealed that there was no alteration in the peak even after 2 months of incubation under dark room at 37$^{o}$C (Figure 1C). Mies theory spherical nanoparticles of the silver showed a single surface plasmon resonance with strong absorption in the range that shows stability to proceed further. The sharper peak after 60 days of incubation indicated the formation of monodisperse nanoparticles from the extract of *Artemesia annua L*. These results showed that there is no alteration in the peak even after 2 months of incubation, thus indicating the higher stability of the biosynthesized AgNPs of *Artemesia annua L.*

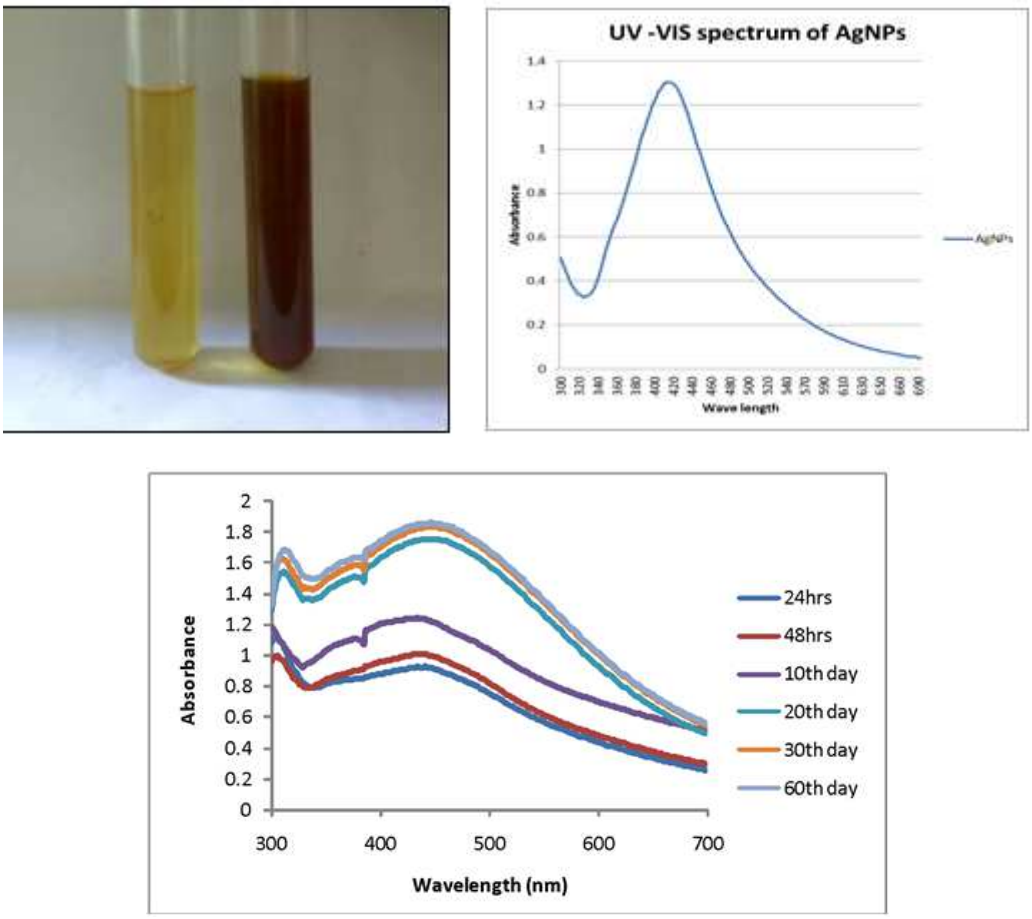



**Figure 1**

***(A) Optical photographs of synthesized AgNPs from Artemesia Annua L. (B) UV–visible spectrum of the AgNPs. (C) UV–visible spectrum of the AgNPs at different time durations.***

***Fourier Transform infrared spectroscopy (FT-IR)***

Presence of various functional groups in biomolecules responsible for the bioreduction of $Ag^{+}$, capping and stabilization of nanoparticles was studied using FTIR measurements (Figure 2). The observed intense bands were compared with the standard values in order to identify the presence of functional groups. Here, FTIR spectrum showed absorption bands at 3728, 2970, 1600, 1392, 1274, 1078 $cm^{-1}$, indicating the presence of capping agent with the nanoparticles. The bands at 3728 $cm^{-1}$ in the spectra correspond to O-H stretching vibration indicating the presence of alcohol and phenol bands. Bands at 2970 $cm^{-1}$ indicate the presence of C-H bonds from aromatic compounds. The bands at 1392 $cm^{-1}$ were corresponding to C-C stretching for C-C. The bands at 1274 $cm^{-1}$ was assigned for N-H and C-C from proteins and the bands at 1078 $cm^{-1}$ wasassigned forN-H present in the amide linkage of proteins. Reports denote that these functional groups are highly responsible for the capping and stability of AgNPs[21,22].

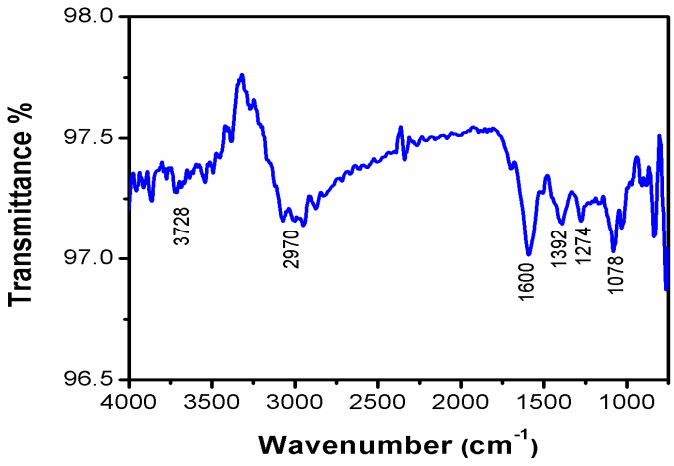



**Figure 2**

***Fourier transform infrared spectrum of synthesized AgNPs from Artemesia Annua L.***

***Ultra structural analysis using Transmission Electron Microscopy***

The particle size, distribution, shape and morphology of AgNPs were characterized using TEM and were predominantly spherical in shape with average particle size of 3--35nm. Transmission electron spectroscopy images of AgNPs (Figure 3 (A-C)) (**A**) 200nm, (**B**) 100nm and (**C**) 50nm.

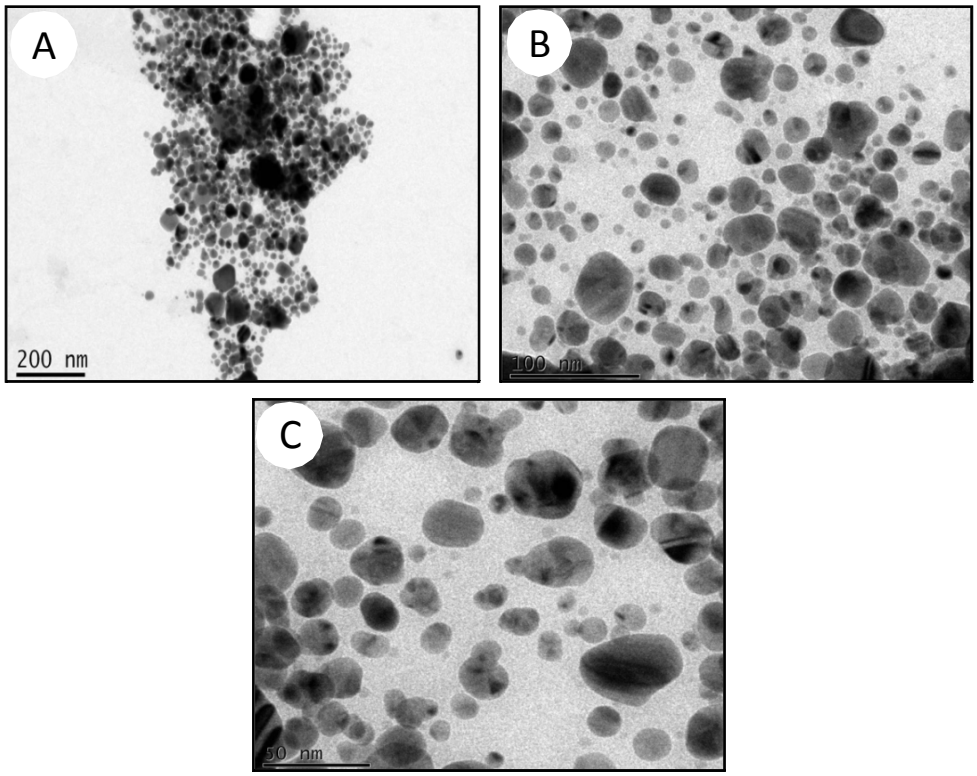



**Figure 3**

***High-resolution transmission electron spectroscopy images of (A) AgNPs (scale bar 200 nm) and (B) magnified AgNPs (scale bar 100 nm). (C) Magnified AgNPs (scale bar 50 nm) (D) X-ray diffraction pattern of AgNPs synthesized from Artemesia Annua L.***

***X-ray diffraction analysis***

The crystalline nature of AgNPs was confirmed by X-ray crystallography. The XRD pattern of synthesized AgNPs is shown in Figure 4. The XRD patterns of AgNPs synthesized from the *Artemesia annua* L extracts were assigned to a face-centered cubic. The Bragg reflections with 2θ values of 32.1°, 38.7° and 47.2° may be indexed as the band for face-centered cubic structures of silver[23]. The XRD pattern, thus, clearly showed that the synthesized AgNPs is crystalline in nature.

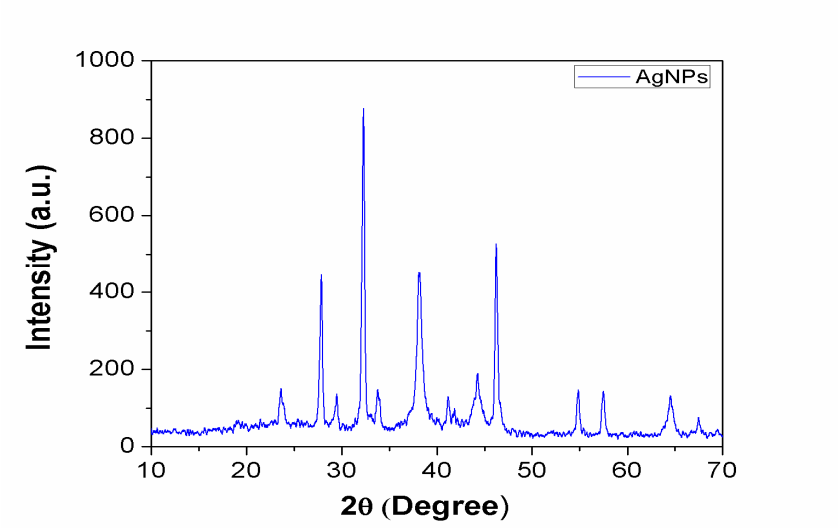



**Figure 4**

***X-ray spectrum of AgNPs.***

***Energy dispersive X-ray analysis (EDX)***

The EDX (Figure 5) spectrum shows a single peak corresponding to silver, indicating that the synthesized AgNPs were free from impurity. The strong signal of the Ag atoms indicated the crystalline nature of AgNPs with a typical optical absorption peak at approximately 3keV

due to surface Plasmon resonance property (Figure 5). Further, the presence of Oxygen peaks along with the Ag signals suggested that the AgNPs are capped by phytoconstituents through oxygen atom.

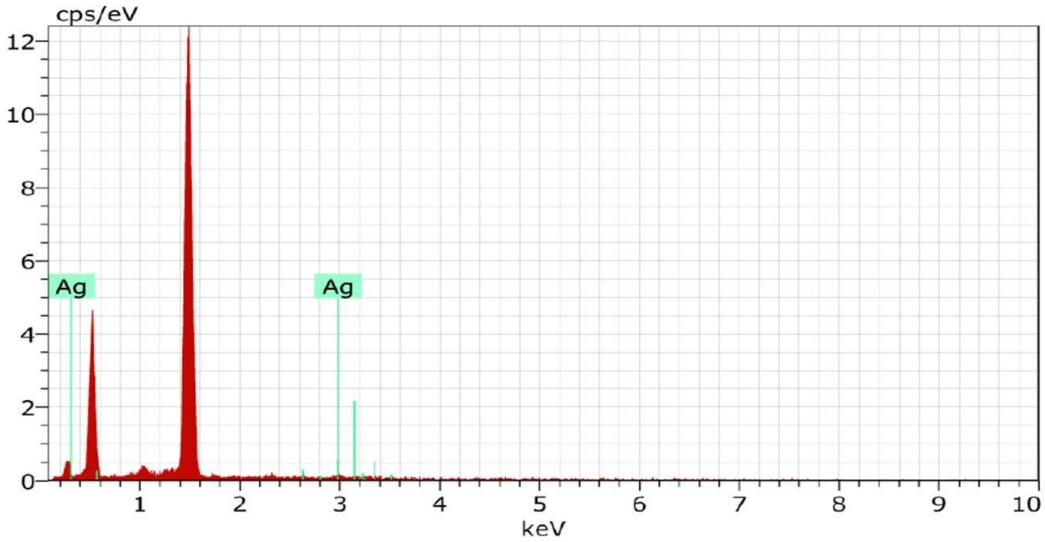



**Figure 5**
***Energy dispersive X-ray analysis (EDX) analysis of AgSNPs***

***Particle Size distribution by Dynamic light scattering (DLS)***

The hydrodynamic diameter using the diffusion coefficient of the mono dispersive colloids of AgNPs and the autocorrelation function are measured using DLS. The size and distribution of AgNPs was recorded at average diameter of 35 nm (Figure 6).[24] Patil *et al.* (2012) reported that the particle size of AgNPs ranged from 15 nm to 30 nm, and more than 90% of the particles were 20 nm.

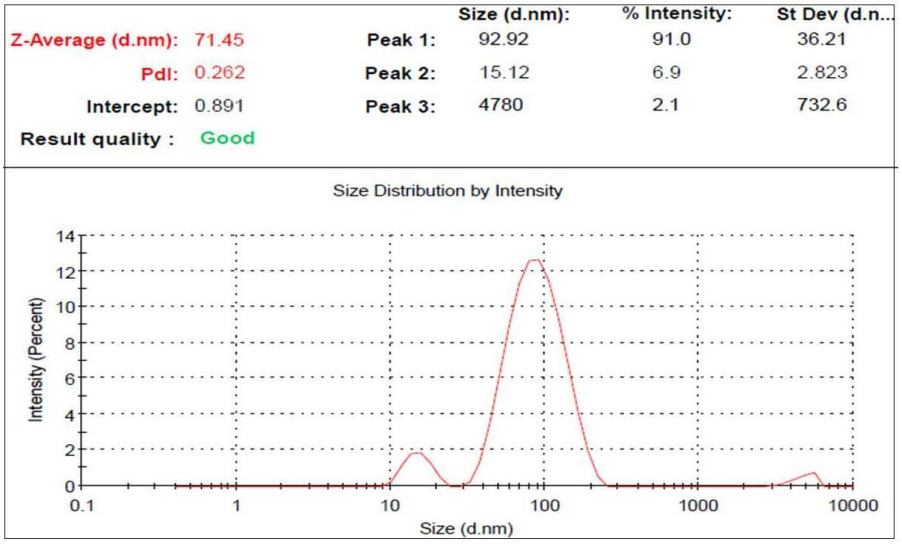



**Figure 6**
***Particle Size distribution by Dynamic light scatting (DLS)***

***Zeta potential measurement***

The higher the zeta potential value, the higher the electrical charge on the surface on the surface of the particles. Here, using water as dispersant zeta potential is found to be -9.3mV for AgNPs are found to be stable (Figure 7). Higher the negative potential value, the higher is the long term stability, good colloidal nature and high dispersity of AgNPs due to the negative-negative repulsion as reported. [25]

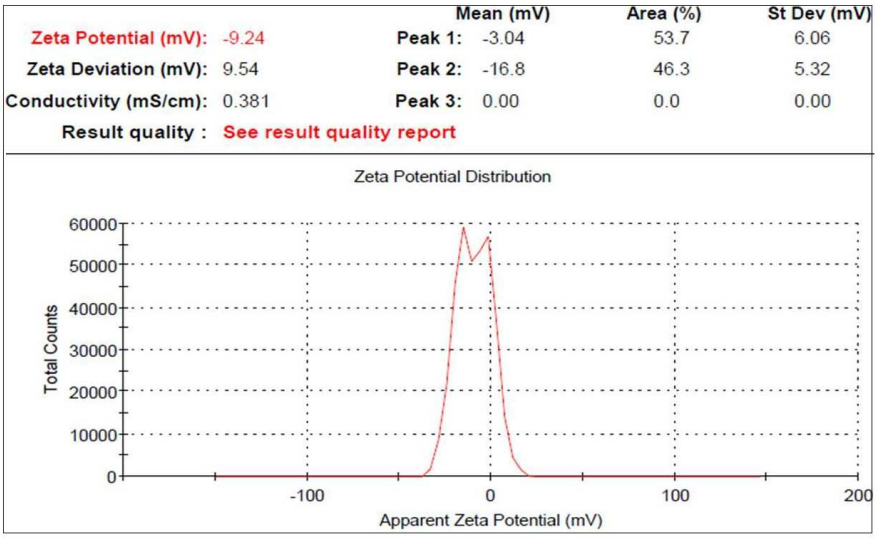



**Figure 7**
***Zeta potential measurement analysis of synthesized silver nanoparticles.***

***Antimicrobial activity of different solvent extracts and biosynthesized AgNPs***

Methanolic extract of *Artemisia annua L* and biosynthesized AgNPs of *Artemisia annua* were studied for their anti-microbial activity against different bacterial and fungal strains. Well diffusion assay method was to demonstrate the anti-microbial activity against bacterial strains like *S. aureus*, *E. coli*, *P. aeruginosa* and *B. subtilis* and fungal strains like *C. albicans* and *A.niger.* (Table 3).

***Table 3***
***Antibacterial activity of the AgNPs, $AgNO_3$ and Methanolic extract.***

| S.No. | Name of the organism | Zone of inhibition (in mm) | | |
|---|---|---|---|---|
| | | AgNPs | $AgNO_3$ | Methanolic extract |
| 1 | *Staphylococcus aureus* | 12±0.42 | - | 10±0.28 |
| 2 | *Bacillus subtilis* | 17±1.24 | - | 13±0.86 |
| 3 | *Escherichia coli* | - | - | 10±0.15 |
| 4 | *Pseudomonas aeruginosa* | 14±0.92 | 13±0.59 | 11±0.92 |
| 5 | *Candida albicans* | 8±0.47 | - | - |
| 6 | *Aspergillusniger* | 7±0.74 | - | - |

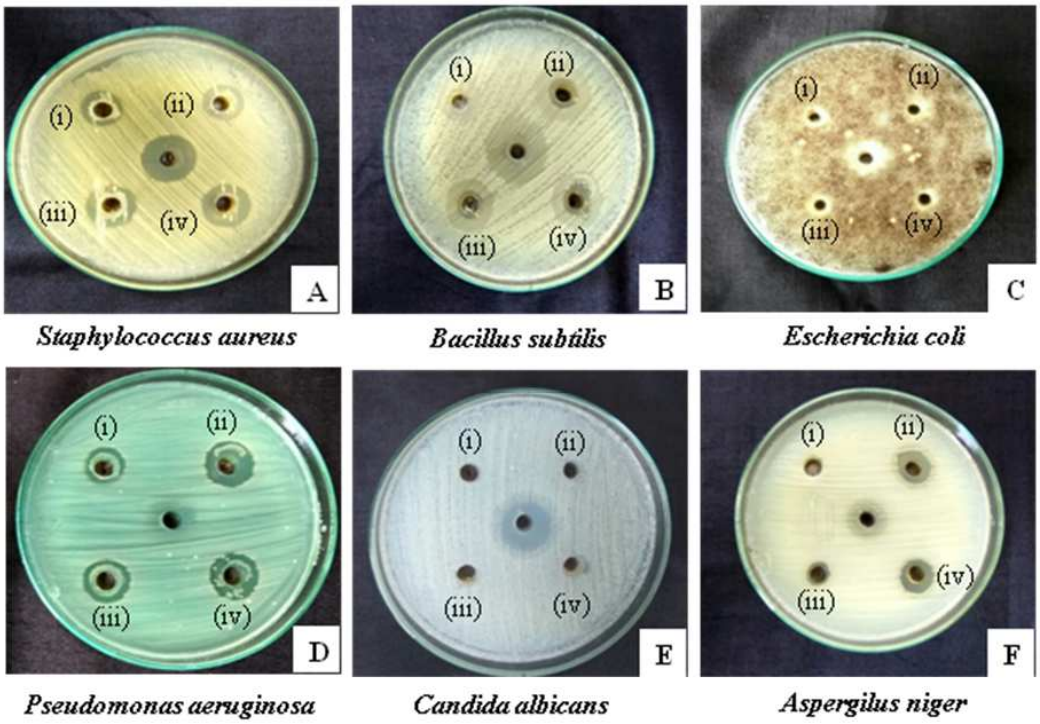
***Values are expressed as Mean ±.SD. Values are statistically significant at P<0.05.***

This study has showed that the synthesized *Artemisia annua L* AgNPs have maximum activity against bacterial strains than fungal strains. The zone of inhibition was recorded and it was found that *B. subtilis* was highly sensitive forming a zone with diameter 22 mm followed by *S. aureus* (20mm), *P.aeruginosa* (16 mm) and *E. coli* with 17mm, whereas the fungal strains used in our study exhibited a very lowzone of inhibition (Figure 8, Table 4).

**Table 4**
***Antimicrobial activity of Artemesia Annua L synthesized silver nanoparticles against human pathogenic microorganisms by well diffusion assay.***

| S.No. | Name of the Organism | Synthesized silver nanoparticles | | | | |
|---|---|---|---|---|---|---|
| | | Zone of inhibition (mm in diameter) | | | | |
| | | 25 µL | 50 µL | 75 µL | 100 µL | control |
| 1 | *Staphylococcus aureus* | 11±0.33 | 14±0.25 | 17±0.47 | 21±0.51 | 17±0.43 |
| 2 | *Bacillus subtilis* | 16±0.56 | 18±0.84 | 21±0.55 | 23±0.34 | 23±0.59 |
| 3 | *Escherichia coli* | - | 9±0.32 | 13±0.43 | 18±0.26 | 14±0.11 |
| 4 | *Pseudomonas aeriginosa* | 13±0.95 | 15±0.11 | 16±0.31 | 17±0.77 | 12±0.34 |
| 5 | *Candida albicans* | - | - | - | - | 17±0.56 |
| 6 | *Aspergillus niger* | 6±0.44 | 10±0.14 | 11±0.23 | 12±0.47 | 10±0.51 |

***Values are expressed as Mean ±.SD. Values are statistically significant at P<0.05.***




***(a) S.aureus, (b) B.Subtilis, (c) E.Coli, (d) P.aeruginosa, (d) C.albicans, (e) A.niger. (i) 25µl, (ii) 50µl, (iii) 75µl (iv) 100µl of synthesized AgNPs.***

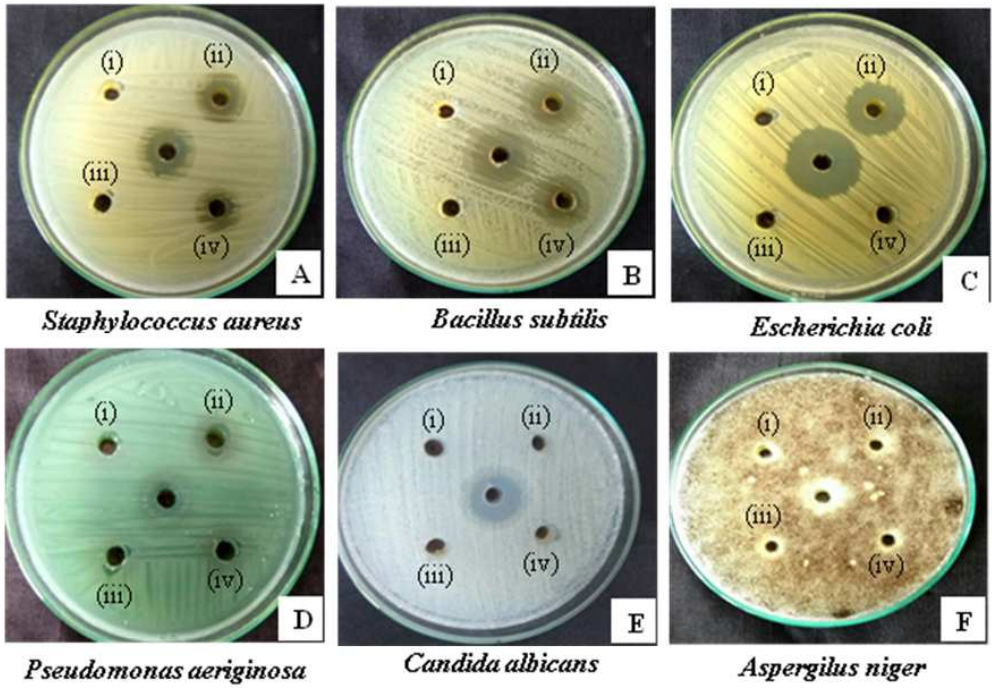
**Figure 8**
***Antimicrobial activity of Artemesia Annua L synthesized silver nanoparticles against human pathogenic microorganisms by well diffusion assay.***

The AgNPs exhibited a zone of inhibition of 23 mm for *B. subtilis* followed by 21mm for *S. aureus,* 18 mm for *E.coli* and 17 mm for *P. aeruginosa.* Biosynthesized AgNPs were also effective for fungal strains while the plant extract showed minimum zone for *A. niger* and *C.albicans.* Aqueous extract of *Artemesia annua L* with silver nitrate as AgNPs acts synergistically against fungal pathogens exhibiting 12mm for *A.niger* and 6mm for *C.albicans.* This remarkable antifungal property shows the necessity for scaling up the usage of this AgNPs for its efficacy against infectious disease (Figure 9, Table 5). However, further studies are needed to investigate if the bacterial and fungal stains can develop resistance towards the synthesized AgNPs from *Artemesia annua L* and the associated cytotoxicity of nanoparticles towards human cells before proposing their therapeutic use.

**Table 5**
***Antimicrobial activity of Artemesia Annua L Methanolic extracts against human pathogenic microorganisms by well diffusion assay.***

| S.No. | Name of the Organism | Methanolic extract | | | | |
|---|---|---|---|---|---|---|
| | | Zone of inhibition (mm in diameter) | | | | |
| | | 25 µL | 50 µL | 75 µL | 100 µL | control |
| 1 | *Staphylococcus aureus* | 10±0.29 | 14±0.37 | 17±0.32 | 20±0.94 | 17±0.55 |
| 2 | *Bacillus subtilis* | 11±0.38 | 14±0.69 | 19±0.34 | 22±0.73 | 23±0.49 |
| 3 | *Escherichia coli* | - | 12±0.63 | 14±0.11 | 17±0.71 | 14±0.28 |
| 4 | *Pseudomonas aeriginosa* | 11±0.18 | 14±0.22 | 15±0.81 | 16±0.33 | 12±0.71 |
| 5 | *Candida albicans* | - | - | - | - | 17±0.64 |
| 6 | *Aspergillusniger* | - | - | - | - | 10±0.34 |

***Values are expressed as Mean ±.SD. Values are statistically significant at P<0.05.***




*(a) S.aureus, (b) B.Subtilis, (c) E.Coli, (d) P.aeruginosa, (d) C.albicans, (e) A.niger.*
*(i) 25µl, (ii) 50µl, (iii) 75µl (iv) 100µl of methanolic extracts of Artemesia annua L.*

**Figure 9**
***Antimicrobial activity of Artemesia Annua L Methanolic extracts against human pathogenic microorganisms by well diffusion assay.***

## CONCLUSION

In this study, we have reported the facile approach for the biosynthesis of AgNPs from *Artemesis annua L* plant extract. The optimization of reaction parameters resulted in the formation of monodisperse spherical AgNPs of size 30-35 nm. AgNPs began to form rapidly within 24 hrs and addition of leaf extract to silver nitrate showed a peak at 420nm UV-vis spectrum. The newly synthesized AgNPs nanoparticles showed a higher anti-bacterial activity compared to *Artemesia annua L* in the methanolic extract. Due to their stability, high monodispersity and higher antibacterial properties, synthesized AgNPs in our study are expected to exhibit significant biochemical activities for which the studies are currently in progress. Our results showed that the extracts of *Artemesis annua L* plants leaves produce usable AgNPs with potent antibacterial and antifungal activities. These nanoparticles also inhibit the growth of pathogenic bacteria, while further preliminary evaluation of cytotoxicity and anti-cancer activity of biosynthesized AgNPs on MDA-MB-231 cells is currently under progress.

## AUTHOR'S CONTRIBUTION STATEMENT

Mrs. Renuga Devi Karthikeyan designed and executed the experiments. Prof. Dr. Julius Amaldas analyzed and provided valuable insights to perform the studies. He also assisted in Manuscript Preparation and provided critical suggestions.

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## CONFLICT OF INTEREST

Conflict of interest declared none.

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