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Original Article

Bio Formation of Silver-Nanoparticles Besides Antimicrobial Activity from *Andrographis beddomei* C.B. Clarke Leaves

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ABSTRACT

Background: Bio formation of the silver nanoparticles are eco-friendly, low cost, within short period could synthesised and widespread significances also performances. **Objectives:** Seldom, the creation of extra-cellular silver nanoparticles utilizing young aqueous leaves extract of *Andrographis beddomei* as a sinking mediator is described here for the first time. But there is no work has not been reported previously using bio nano synthesis and its applications. **Materials and Methods:** The bio formatted silver nanoparticles were synthesised by using *Andrographis beddomei* young leaves extract. The bio synthesised nanoparticles were characterized by following the UV-visible spectroscopy and TEM are accustomed understand size, shape and appearance of the silver nanoparticles. Particles are crystalline in nature, according to energy dispersive X-ray analysis and XRD investigations. An investigation using FTIR spectroscopy confirms the nanoparticles' surface is roofed in bio-moieties. **Results:** The bio formations of the Ag Nps were rapidly i.e., 20 min has occupied time at room temperature. These were followed the characterization values. Where the TEM confirms the sizes from 20 to 30nm Ag Nps. These were further used for the anti-fungal, antibacterial anticay. **Conclusion:** Our research has demonstrated that the bio functionalized silver nanoparticles that resulted from this process have excellent antimicrobial properties. Given the ease of use and eco-friendliness of the synthetic process, high-volume production of these nanoparticles could be explored for use in a variety of pharmaceutical solicitations.

Keywords: *Andrographis beddomei*, Bio-synthesis, Ag NPs, TEM, Anti-microbial activity.

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INTRODUCTION

The creation and stabilization of many types of nanoparticles are the subjects of nanotechnology.[1] Physical and chemical approaches are utilized to quickly manufacture vast amounts of nanoparticles.[2] Biologically produced Silver Nanoparticles (Ag-NPs) have a wide range of applications due to their exceptional chemical and physical properties. Reverse micelles, Metal nanoparticles are commonly produced and immobilized using a variety of techniques, including mechanical and chemical methods,[3] electrochemical reactions methods[4] and a recent green chemistry method.[5]

A growing trend in the synthesis process for nano products is the application of green chemistry which have been identified as possible uses in the following areas: bio labelling,[6] microelectronics,[7] information storage,[8] optoelectronic devices,[9] bio catalysis in chemical reactions,[10,11] Currently, a number of research groups are focusing on biomimetic methods to create metal nanoparticles, also known as "green chemical or phytochemical" methods. These methods include extracts from plants or plant bulbs, nuts, microorganisms and yeast.[12,13]

A well-known source of secondary metabolites have been found from the genus of endogenous plant *Andrographis*. Whereas the *Andrographis beddomi* was reported from Nallamalla forests of Andhra Pradesh, named as *Andrographis nallamalayana*, currently the species has *synonamed* as *A. beddomi*. It has also been shown to have phenolic content[5] due to the intention the antibacterial, antioxidant activities, anti-inflammatory and

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anti-lungs cancer activity were recorded Saiprbha shivakumar[5] *et al.*, There was no previous report were found on the *A. beddomi*. It is also syno-named, *A. nallamalayana* mediated biosynthesis of silver nanoparticles.

In the current work, we investigated the standardized the methodology for the formation of extra-cellular production of Ag Nps at normal temperature utilizing an easy, affordable and repeatable method: the double distilled water filtrate of leaves of *Andrographis beddomei.*

MATERIALS AND METHODS

The fresh, young leaves of *Andrographis beddomei*, were collected from Nallamala forest of Telangana state, which were collected. Authenticated by Dr. Sadashivaiah, Assistant Professor of Dr. BRR Degree and PG College in Jadcharla, Mahabubnagar District, identified and verified. $AgNO_3$, or silver nitrate, is obtained from High Media Laboratories. Utilizing triple-distilled water, solutions were made.

To prepare the plant extract, 25 g of young leaves were repeatedly cleaned with distilled water to get rid of any organic contaminants. After being finely chopped, the *Andrographis beddomei* leaf pieces were placed into the 1000 mL beaker with 500 mL of deionised water, the solution was microwaved for 180 sec to inhibit the enzymes. Two passes through Whatman filter No. 42 paper were performed on the ensuing raw extract. All that remains of the final filter is an extract of *A. beddomei* leaves, which is utilized to convert Ag+ to Ag0. A 10-3 concentration of silver-nitrate solution was used to extravagance the extract.

The fabrication of silver-nanoparticles was agreed out by deionised solution of 1 mM $AgNO_3$. For the reduction into Ag+ ions, 10 mL of *Andrographis beddomei* young leaves extract was added to 490 mL of an aqueous solution containing 1 mM $AgNO_3$. The mixture was then incubated for 50 min. at room temperature. In this case, the filtrate assists like stabilizing and reducing mediator for 1 mM $AgNO_3$.

Characterization of Ag Nps using UV-visible spectra, X-ray-diffraction (XRD), Transmission Electron Microscopy (TEM), Atomic Force Microscope (AFM), Fourier Transforms Infra-Red Spectroscopy (FTIR)

Using a UV-visible 5704SS ECIL spectrophotometer with an ocular length of 10 mm and 1 nm resolution, the synthesis of AgNPs is confirmed. A 300 sec observation was made of the reaction mixture's UV-visible analysis.

In order to analyze crystallinity, X-ray Diffraction (XRD) was utilized to examine films of colloidal Ag Nps that were created on Si (III) substrates using drop-coating. Ricago X-ray Diffractrometer (Japan) operating at 30 kV and 20 mA current with Cu Ka (I=1.54 A°) was used to collect the results.

The Technai-20 Philips instrument was used to capture the Transmission Electron Microscopy (TEM) pictures at 190 keV. Silver nanoparticles were quickly biosynthesised to provide a sample for this assay. Aqueous AgNPs drops coated with *Andrographis beddomei* 109 were applied to carbon-covered copper grids and let to sit for 5 min. Any remaining solution was wiped off using blotting paper. To dry, the TEM grid film is subjected to infrared light.

On a Veeco-Innova scanning probe microscope with etched Si-nano probe tips (RTESPA-M), the Atomic Force Microscopy (AFM) pictures were obtained in ambient circumstances.

Centrifuging the synthesized Ag Nps solu[n] at 10000 rpm for 20 min. produced the AgNPs powder sample. After a solid residue forms, it is cleaned with deionized water to get rid of any biological moieties that aren't linked to the nanoparticle surface and aren't involved in bio functionalization or capping. After thoroughly drying the residue, the nanoparticles were used for FTIR dimensions using a Nicolet iS5 FTIR equipped with diamond ATR (Attenuated Reflectance Technique).

Ag Nps formation as of the aqueous leaves extract of *Andrographis beddomei* exhibits antimicrobial action

Three fungi *Candida albicans, Fusarium oxysporum* and *Aspergillus flavus* the current study employed three bacterial pathogens *Bacillus subtilis, Staphylococcus aureus* and *Escherichia*

Figure 1: *Andrographis beddomei* (Acanthaceae) *Young* leaves used for the synthesis of AgNPs.

coli. The examined strains were procured from SVS Medical College located in Mahabubnagar, Telangana state, India. These test cultures were maintained at 4°C on nutrition and potato agar slants after being cultivated in PDA, NA (Himedia, M002) at 37°C.

The agar well diffusion technique was cast-off to follow the experiment. The disinfected petri dishes were filled with 15 to 20 mL of PDA medium, which was then left to harden. The bacterial strains were diluted to a turbidity of 0.5 Mac Farland standards (108 CFU/mL) and suspended into salted solution (0.85% NaCl). An Ailed glass spreader was recycled for proper mix 1 mL of trial strains through the medium. The culture medium was perforated with 4 mm diameter wells using a flamed sterile borer. The wells were filled with the necessary conc. (20 µL, 40 µL, 60 µL, 80 µL). Following an hour in the refrigerator to allow the extracts to diffuse into the media, the petri-plates remained nurtured at 37°C.

The petri-plates were observed for zones of inhibition 48 hr afterward incubation. A measurement was made and the diameter inhibition zones were recorded in millimetres in diameter. Plant aqueous extract and $AgNO_3$ solution were employed as negative controls. Three duplicates of each experiment were carried out. The antibacterial activity of NA medium nurtured at 37°C for 18 hr was tested using the same methodology.

RESULTS

Formation of Ag nanoparticles applying extract from the leaves of *Andrographis beddomei*

250 mL of 1 mM $AgNO_3$ solution was assorted with 2 mL of plant (Figure 1) extract to create silver nanoparticles. In 30 min, the solution changed from colourless to brown. Ag nanoparticles' pale yellowish colour in deionised solution is caused by the shallow Plasmon resonance being excited. The extract's colour changed from colourless to brown upon combining with the Ag ion complex's aqueous solution. It resulted from the decrease in Ag^+, which denotes the Ag nanoparticle creation depicted in Figure 2.

Within 15 min, there is a noticeable colour shift from translucent to yellow, which is indicative of the development of Ag Nps were confirmed by UV-visible examination. The colour shift to a shady orange-brown is also caused by the growth and increasing concentration of Ag Nps. After 25 min, the noticeable colour change had not been observed; it recommended that the reduction reaction was complete. (Figure 2).

UV-vis spectra Analysis

UV-vis spectroscopy analysis is a valuable tool for determining the production and stability of metal nanoparticles in aqueous solution. Silver nanoparticles are known to have UV-visible

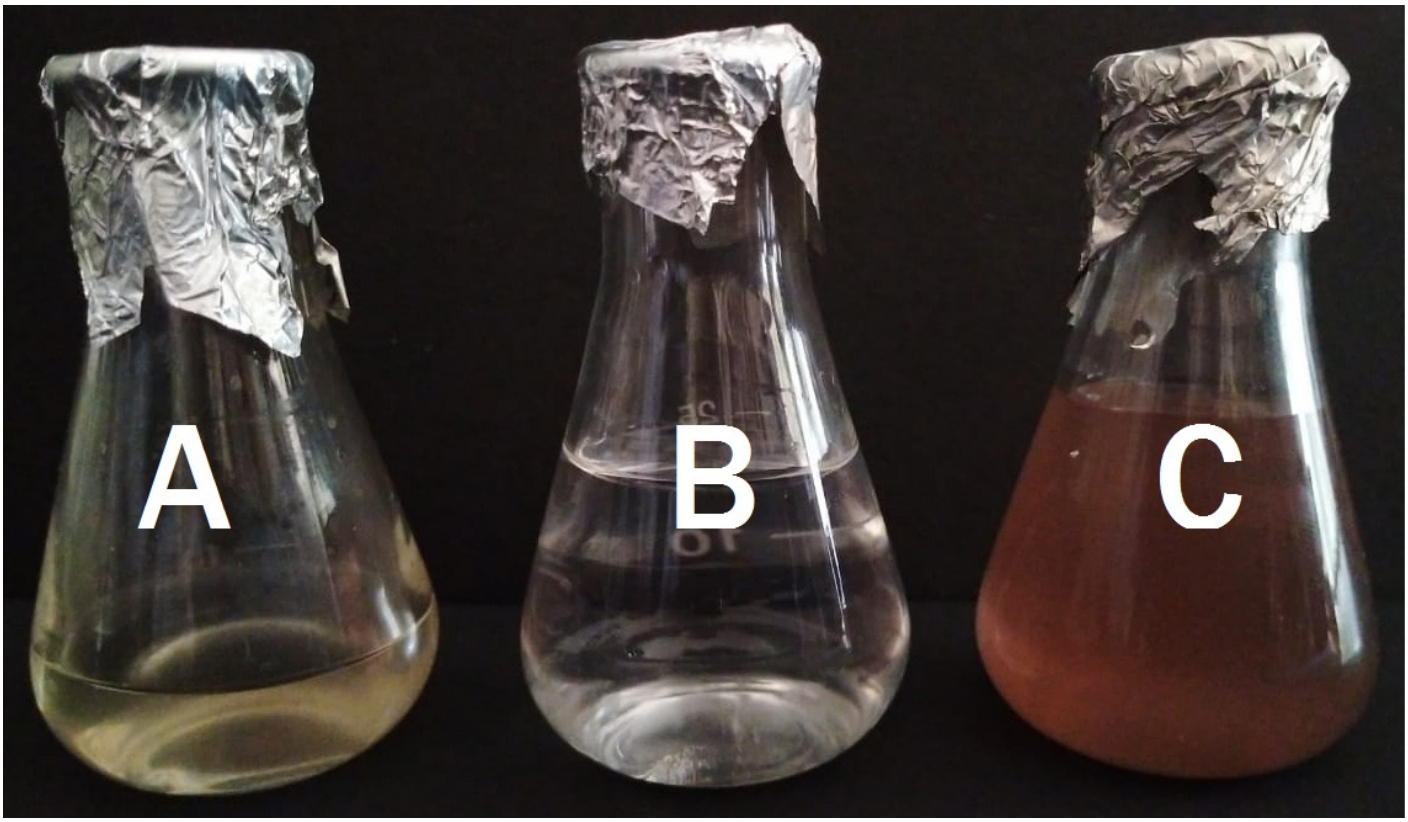

Figure 2: Formation of Ag Nps utilizing an aqueous leaf extract from *Andrographis beddomei* treated with a room-temperature $AgNO_3$ solution. A) *Andrographis beddomei* aqueous leaves extract, B) Silver nitrate ($AgNO_3$) solution, C) Afterward establishment Ag Nps alteration the colour of $AgNO_3$ solution.

absorption maxima in the 400-500 nm region due to this characteristic. In this paper, the creation of AgNPs was first established using UV-visible spectroscopy due to the Surface Plasmon Resonance phenomenon (SPR). Figure 3 shows evidence of SPR. One narrow absorption band was found at 420-440 nm, which is typical of mono-dispersed Ag Nps.

FTIR measurements were performed to determine the potential biomolecules in *Andrographis beddomei* young leaves aqueous extract that are responsible for capping and efficient stability of the silver nanoparticles.

XRD analysis

Young leaves of *Andrographis beddomei* were used for the synthesised AgNPs' XRD examination and the archetypal XRD configuration is displayed in Figure 4. When associating with JCPDS data, the peaks are indexed as *Andrographis beddomei* silver plans (111), (220), (311) and (222). This might be because the broth made from *Andrographis beddomei* leaves contains crystalline bio-organic substances and/or proteins. S. Shiv Shankar *et al.* reported similar findings about the silver nanoparticles that were made with broth made from *P. graveolens* leaves.[14] A thorough examination of this crystalline phase that coexists with the silver nano-crystals is currently underway.

TEM analysis

An investigation using transmission electron microscopy was conducted at IIT Bombay. The analysis revealed that the particles had an irregular, spherical form and ranged in size from 30 to 50 nm (Figure 5). There were 20% of 30 nm particles, 30% of 40 nm particles and 50% of 50 nm particles found.

AFM Study

The AFM inspection is an efficient approach for determining the morphology of bio-functionalized Ag NP particles. AFM experiments further validated the synthesized Ag Nps. The tapping mode technique was specifically designed to analyse synthesized nanoparticles in three dimensions for the purpose of exploring bio functionalization. Figure 6 depicts Ag Nps that have been bio-functionalized and have an organic layer with many organic moieties on the surface. As a result, we can conclude that the nanoparticles were irregular and spherical in shape. The dispersion of these particles was random.

FTIR analysis

To determine the potential biomolecules in the extract of *Andrographis beddomei* leaves that could be responsible for capping and effectively stabilizing Ag Nps, FTIR measurement was performed (Figure 7). A noticeable absorption band can be seen in the infrared spectrum of silver nanoparticles at 1616, 44 cm^{-1}. The stretching vibration of N-H could be the cause of the strong band at 1386, 30 cm^{-1}. can be designated as stretched vibration fascination bands for C=H, -O-H, -S-H, -N=C=N, -C=O and -S=O. These come from water-soluble substances found in leaves, such as polyphenols, alkaloids and flavonoids.

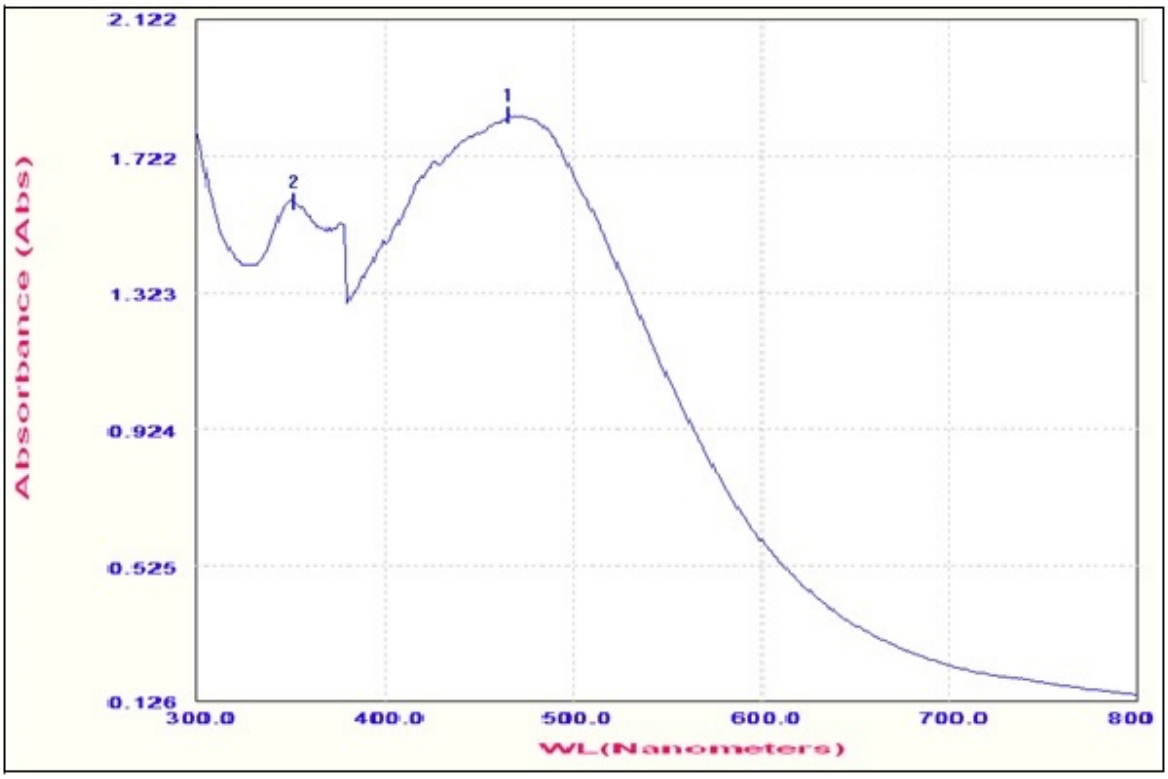

Figure 3: UV-vis spectrum of bio functionalized AgNPs showing surface plasmon peak at 440 nm.

The biological components are known to interact with metal salts via these functional groups, facilitating their reduction to nanoparticles.

Anti-microbial activity of Ag Nps of *Andrographis beddomei* young leaves extract

Ag NPs from *Andrographis beddomei* leaves at 80 µL/well conc. shown the highest antibacterial activity against *E. coli*, with a maximum inhibition sector of 25.00 mm, followed by *Bacillus subtilis* at 24.00 mm and *Staphylococcus aureus* at the lowest inhibition zone of 22.00 mm. Regarding fungi, the highest recorded inhibition zone of 14.00 mm was obtained against *Fusarium oxysporum* and *Candida albicans*, while a zone of 13.00 mm was noted against *Aspergillus flavus*. The concentration of AgNPs determined the antibacterial activity. If Ag Nps concentration was to grow, the inhibition zone would likewise increase. $AgNO_3$ solution and plant aqueous extract, the two negative controls, were unable to exhibit inhibition against any of the bacteria that were tested. As benchmarks against bacteria and fungus, streptomycin sulphate and ketoconazole have an inhibitory zone of 30.00 and 28.00 mm (Table 1).

DISCUSSION

The creation of simple, dependable and environmentally acceptable processes contributes to the growing interest in the synthesis and uses of human-beneficial nanoparticles. A colour shift may occur after exposure to the plant extracts, which reduces silver ions into silver nanoparticles. In our investigation, the colour shift was noticeable at room temperature in less than 30 min. Due to the shallow Plasmon resonance phenomena, silver nanoparticles in aqueous solution displayed a dark reddish-brown

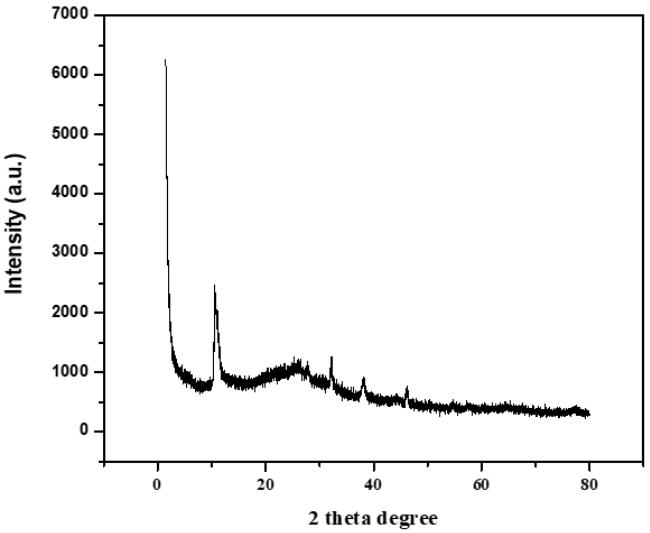

Figure 4: XRD outlines of bio-functionalized Ag Nps.

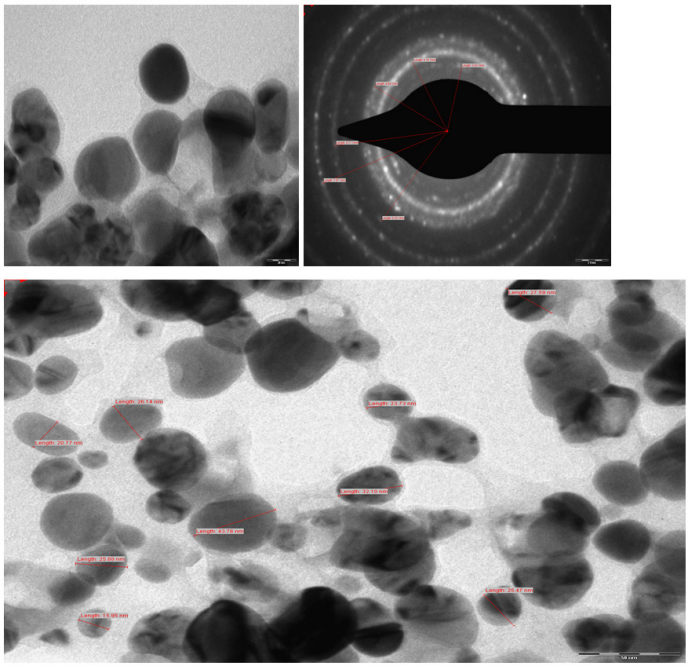

Figure 5: TEM photo of bio functionalized Ag Nps, with SAED pattern.

Table 1: Ag Nps produced from aqueous young leaf extract of *Andrographis beddomei* have antimicrobial action.

Tested Fungi and bacterial strains	Changed conc. of AgNPs and inhibition zone in mm						
	80 µL/ well	60 µL/ well	40 µL/ well	20 µL/ well	Young leaves aqueous extract	1 mM $AgNo_3$ Sol^n	Standard K and S (1000 µg/ mL conc.)
Fussarium oxysporum	16.00	10.00	09.00	07.00	-	-	24.00
Aspergillus flavus	15.00	12.00	06.00	04.00	-	-	22.00
Candida albicans	15.00	10.00	07.00w	05.00	-	-	20.00
Staphylococcus aureus	19.00	17.00	13.00	10.00	--	-	28.00
Bacillus subtilis	18.00	15.00	11.00	08.00	--	-	27.00
Escherichia coli	20.00	18.00	16.00	09.00	-	-	30.00

Std.: K=Ketoconazole in contradiction of fungi, S=Streptomycin in contradiction of bacteria.

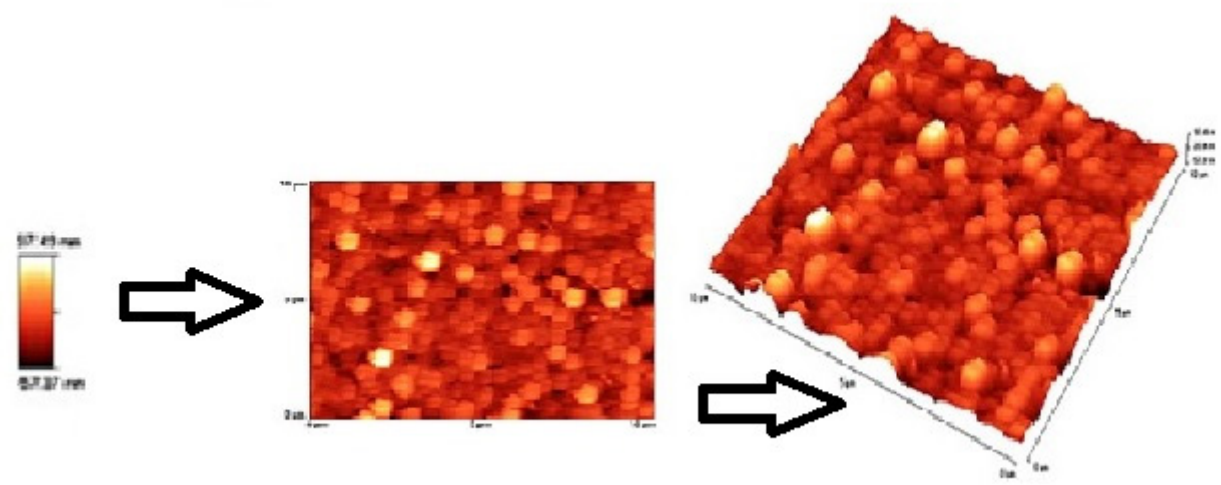

Figure 6: A.F.M. image of bio functionalized Ag Nps.

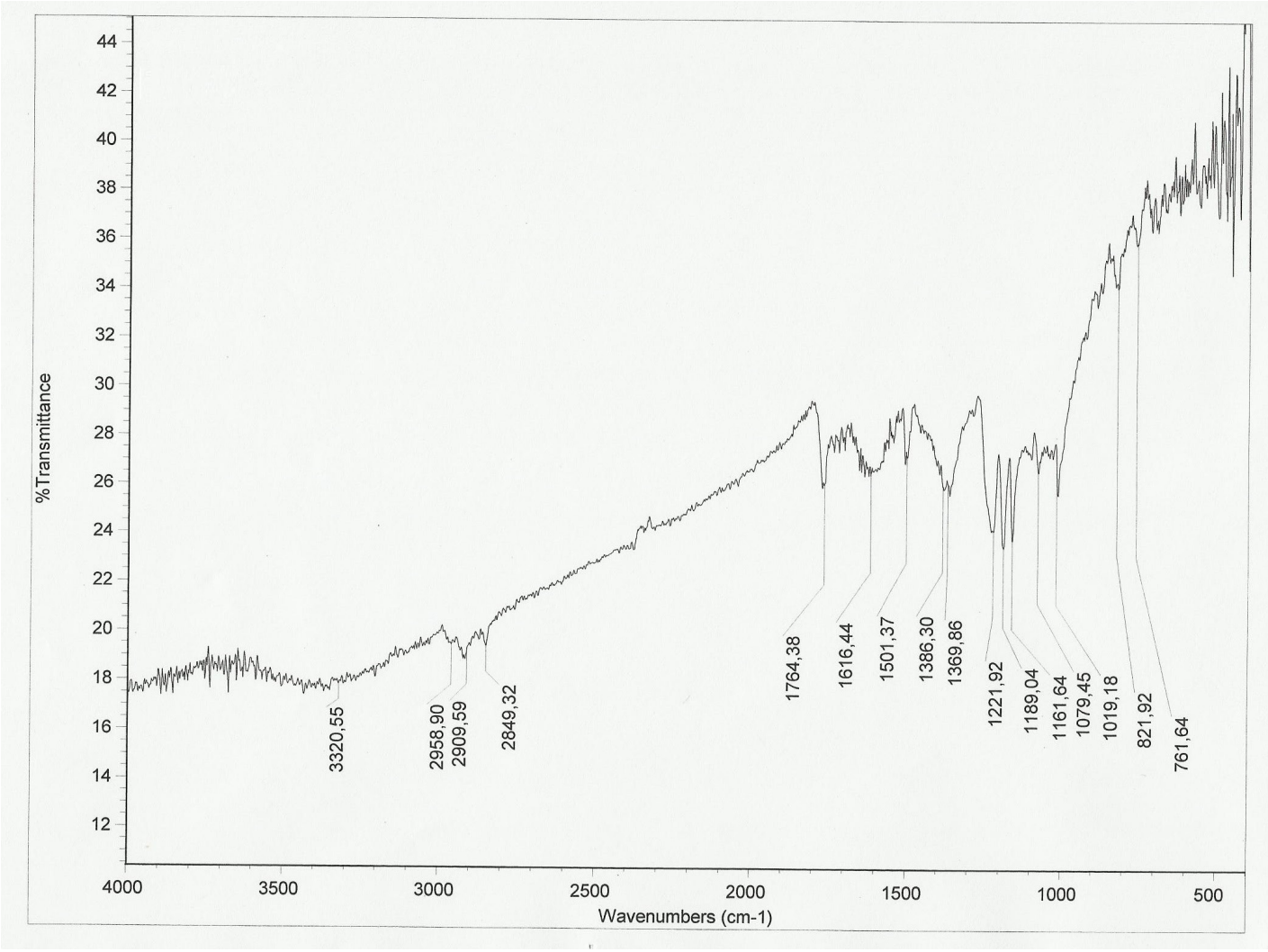

Figure 7: FTIR spectrum of bio functionalized AgNPs.

colour[15] formed silver nanoparticles using *E. hybrida* and reported that the Terpenoid and flavonoid components of the leaf extract may be the chemicals that stabilize the nanoparticle on the surface.

Similar to this, *A. beddomei* leaf extract was used to create silver nanoparticles in the current investigation. It's interesting to note that after 30 min of incubation; silver nanoparticles were created quickly, making this one of the wildest bio-reducing ways to produce silver nanoparticles. After that, nothing much changed.

Spectrophotometric analysis was used to assess the production of silver nanoparticles made from *A. beddomei* leaves aqueous extract at a wavelength range of 400-500 nm. The results showed a distinctive peak for *A. beddomei* Ag Nps at 420 nm for the extract and Ag NO_3 mixture, which verified the formation of the silver nanoparticles. This resembles the distinctive peaks of the surface Plasmon vibrations of the silver nanoparticles made using *Geranium*.[16] Regarding the identification of possible forest plants for the synthesis of silver nanoparticles, the investigation's outcome is quite intriguing. The biological synthesised nanoparticles using ambient biological resources[17] reported by Sinha *et al*.

Metal nanoparticles determine the frequency and width of Plasmon absorption at the surface; XRD can be used to examine the diffraction peaks of plants to determine whether plant extracts contain silver nanoparticles. The X-ray pattern of the produced silver nanoparticles in this study matched the FCC structure of the bulk silver and no appreciable extra phases were seen in the XRD patterns. The spherical nature of the silver nanoparticles generated by the reduction of Ag^+ ions by *Andrographis beddomei* is clearly shown by the X-ray diffraction data.

The current species first time used for the biosynthesis of Ag Nps, but partly from the same genus Andrographis macrobotrys was also used for the green synthesis and confirmed 20 to 50 nanoparticles, whereas here confirmed the 20 to 60 nm of Ag Nps. The antifungal and antibacterial activity of the current report has observed more than effective of past reports from the same genus.[5]

The current bio formation of Ag Nps also focused on the secondary metabolite's groups like phenols or flavonoids. The similar aimed report previous focused on the polyphenolic rich root extract of *Codonopsis clematidea* by Ajay Sharma.[18]

Imran Gajbhiye *et al*., focused on green synthesis of silver nanoparticles using *Andrographis paniculata* leaves extract using antiproliferative activity in human lung adenocarcinoma cells and documented[19] whereas the present report used the same genus but species is different, which could also rapidly bio formed the Ag Nps and their biological significances.

According to this study, Ag Nps are more efficient against fungus and bacteria when used as an antibacterial agent. However, there was an antimicrobial effect that was dose-dependent.

CONCLUSION

Andrographis beddomei extract has been shown to be a rapid and green method for producing silver nanoparticles with erratic crystallization and a spherical shape. After adding *Andrographis beddomei* broth to the silver nitrate solution, silver nanoparticles began to form within 15 min. At 2 hr, the UV-Vis spectrophotometer showed that the reaction was nearly finished, with peaks located in the range of 420,430 and 440. It was shown that the size and agglomeration of the particles reduce and the rate of reduction rises with increasing broth content. It was found that the best ratio for reducing silver ions to silver nanoparticles was leaves broth to 10^{-3} mm silver nitrate solution. The generated particles had a spherical shape and ranged in size from 20 to 60 nm, according to the TEM analysis. The particles also showed a propensity to aggregate, suggesting that they could be useful as antibacterial agents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AgNps: Silver Nano Particles; **TEM:** Transmission Electron Microscopy; **AFM:** Atomic force microscopy; **FTIR:** Fourier-transform infrared spectroscopy; **XRD:** X-ray diffraction; **SEAD:** Selected Area Electron Diffraction.

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