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Rapid phytosynthesis of silver nanoparticles using *Chlorophytum borivilianum* root extract and its antimicrobial activity

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Abstract
Biological synthesis of nanoparticles is a relatively new emerging field, which offers economic and ecofriendly benefits over chemical and physical methods. Herein, we synthesized silver nanoparticles (AgNPs) using root extract of *Chlorophytum borivilianum*, a plant widely found in southeast regions of Indian subcontinent. The nanoparticles were engineered via reduction of silver nitrate ($AgNO_3$) solution with aqueous root extract of *C. borivilianum* at 50 °C. The characteristic surface Plasmon band was confirmed ~430 nm using UV-Vis spectrophotometer. The FTIR study revealed that the nanoparticles were stabilized by non-aromatic compounds present in aqueous extract. The nanoparticles were physicochemically characterized in terms of size using Dynamic Light Scattering (DLS). The morphological characteristics were determined from TEM (transmission Electron microscopy) and SEM (Scanning Electron Microscopy). XRD (X-ray diffraction) pattern suggested the crystalline nature of the nano-particles. These biologically synthesized AgNPs were found to highly toxic against some clinically pathogenic bacteria such as coagulase positive *Staphylococcus* sp., *Enterococcus faecalis*, *Pseudomonas species*, and *Proteus mirabilis* with reference to commercially available antibiotics. Thus, we believe that this rapid green synthesis of CB-AgNPs is a valuable addition to the applications of *Chlorophytum borivilianum.*

Keywords: biosynthesis, silver nanoparticles, characteristics, *Chlorophytum borivilianum*, antibacterial

1. Introduction
In the recent years, silver nanoparticles (Ag NPs) are continuously gaining limelight due to their wide range of application in almost every field from catalysis, photonics, biology, clothing, and optics to pharmaceutics and drug delivery system [1]. Interestingly Ag NPs have received great attention because of their antimicrobial and anticancer activities [2]. Due to these versatile applications, large number of studies is focusing on the fast and efficient synthesis of Ag NPs.

There are number of methods for the synthesis of silver nanoparticles including physical, chemical, electrochemical reduction, photochemical reduction and thermal evaporation [3], [4]. Compared to these methods, natural systems capable of reducing silver ions and producing nanoparticles at moderate conditions have promising future of generating large amounts with limited energy input and positive impact on the environment. Torresday *et al.*, (2002) [5] first reported that plant materials could be used for the synthesis of Nano scale metals; after this study, plant parts such as leaf, roots, latex, seed and stem are extensively used for metal nanoparticles synthesis.

The synthesis of Ag NPs using specifically root extract obtained from *Glycyrrhiza glabra* [6], *Croton sparsiflorus* [7], *Catharanthus roseus* [8], *Ipomoea pes-caprae* [9], *Rubia cordifolia* [10], *Coleus forskohlii* [11] etc. have been reported worldwide. Thus the green chemistry route is more advantageous because it does not require elaborate process such as multiple purification steps.

It is universally known, that silver is an effective antibacterial agent and possesses a strong antibacterial activity against bacteria, viruses and fungi; this antibacterial activity is a result of well-developed surface, providing maximum contact with the environment [12].

The antibiotic resistance in human pathogens is a big challenge in fields like pharmaceutical and biomedicine. These pathogens require treatment of broad-spectrum antibiotics, which are less effective, more toxic, and more expensive. Therefore, with advancement in Nano science there is a need to develop or modify antimicrobial compounds to improve bactericidal properties.

A number of plants are suitable for preparation of Ag NPs but we have selected *Chlorophytum borivilianum*, which is in use for centuries in Ayurvedic because of its anti-oxidative,

anti-inflammatory, anti-diabetic and antifungal actions [13]. On the basis of the available literature [13, 14, 15] we hypothesized that *Chlorophytum borivilianum* extracts, are inherently rich in various phytochemicals, which could be used in the synthesis of silver nanoparticles.

The primary aim of the present study was to develop a simple and environment friendly approach for the synthesis of Ag NPs using *Chlorophytum borivilianum*. Furthermore, characterization of the synthesized Ag NPs using UV-visible spectroscopy, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Dynamic Light Scattering (DLS) and Fourier Transform Infrared Spectroscopy (FTIR) was also done. The antibacterial activity of the biologically prepared AgNPs was also analyzed against a panel of human pathogens (which are extensively antibiotic resistant), including coagulase positive *staphylococcus* sp, *Enterococcus faecalis*, *Pseudomonas aeroginosa* and *Proteus mirabilis.*

2. Material and Methods

2.1 Materials

The *C. borivilianum* roots were collected directly from local market. $AgNO_3$ (99.98%) was used as a silver precursor, and was provided by Sigma Aldrich (USA). All reagents used were of analytical grade. All solutions were freshly prepared and kept in dark to avoid any photochemical reactions.

2.2 Root Extract Preparation

The *C. borivilianum* tubers were washed to remove the adhering mud particles and possible impurities. Later, it was dried under shade for a week to completely remove the moisture. The root was cut into small pieces, powdered in a mixer grinder and then sieved to get uniform size range. The final sieved powder (CB) was used for all further studies.

For the production of extract, 5 g of *C. borivilianum* tuber powder was added to 100 ml double distilled water and then mixed for 1 hour at 50 ^{0}C, further final aqueous extract (CBE) was obtained by filtering it through whatmann filter paper No. 1 and stored at 4^{0}C in refrigerator.

2.3 Synthesis of *C. borivilianum* conjugated silver nanoparticles (CBE-Ag NPs)

For the synthesis of silver nanoparticles from aqueous *C. borivilianum* powder extract, 10ml of plant extract was added drop wise to 100 ml of 2mM de-ionized aqueous $AgNO_3$ solution. These flasks were then placed on a magnetic stirrer up to color change at 50 ^{0}C. The resulting solution became reddish brown (orange) in color. Throughout the reduction process, all solutions were kept at a room temperature in the dark to avoid any photochemical reactions. The silver nanoparticles obtained were centrifuged at 10,000 rpm for 7 min and subsequently dispersed in sterile distilled water to get rid of any uncoordinated biological materials.

2.4 Physicochemical characterization of silver nanoparticles

2.4.1 Ultraviolet visible (UV-vis) spectroscopy

After formation of silver nanoparticles (CBE-AgNPs), the absorbance of the *C. borivilianum* root extract and CBE-AgNPs was measured over the wavelength range of 300 to 800 nm. The preparation of Ag-NPs was studied by UV-Vis spectroscopy (Systronics Double beam spectrophotometer 2203).

2.4.2 FT-IR spectroscopy

After complete bio-reduction of Ag+, the *C. borivilianum* root-powder extract was centrifuged at 15,000 rpm for 20 minutes to isolate the Ag-NPs from proteins and other compounds present in the solution. FTIR analysis was carried out through the potassium bromide (KBr) pellet (FTIR grade) method in 1:100 ratios and spectrum was recorded. The FTIR spectra were recorded over the range of 400-4000 cm^{-1} using Shimadzu IR affinity-instrument.

2.4.3 X-ray diffraction analysis

For XRD measurements, the AgNPs were dried in hot air oven at 60°C and such dried powder was analyzed using Panalytical X'pert^3 Powder diffractometer to know the crystallinty of sample. The Cu-α radiation (k= 1.5418 Å) was selected and their diffractogram was obtained in the 2θ range of 25°-80°.

2.4.4 Dynamic Light Scattering (DLS) measurements

In addition to UV spectroscopy and FTIR, DLS was used to measure particle size of *Chlorophytum borivilianum* synthesized nanoparticle. Zetasizer Nano ZS90 (Malvern Instruments Limited, Malvern, WR, UK) was used to measure the size distribution of AgNPs.

2.4.5 TEM and SEM measurements

Transmission electron microscope (TEM) analysis of silver nanoparticles was done using the Tecnai G^2 20 (FEI) S-Twin operating Machine accelerating at 200 kV. On the other hand, SEM analysis of silver nanoparticles was done using Nova nano FE-SEM 450 (FEI) instrument. Thin films of the silver nanoparticles were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the grid were allowed to dry by putting it under a mercury lamp for 5 mins.

2.4.6 Antibacterial activity of synthesized silver nanoparticles

The antibacterial activity of AgNPs was evaluated against clinical isolates of *Pseudomonas aeruginosa, Proteus mirabilis,* Coagulase positive *Staphylococcus sp.* and *Enterococcus faecalis* by disc diffusion method. Approximately 10^6 colony-forming units (CFU) of the microorganisms were inoculated on Mueller Hinton agar plates and the activity was studied by disc diffusion method. All the plates were incubated at 37°C overnight. After incubation, the plates were observed for the presence of a zone of inhibition.

3. Results and Discussion

The aim of this experiment was to produce smaller sizes of AgNPs using *C. borivilianum* extract, which acts as a reducing and stabilizing agent. When the extract was subjected with $AgNO_3$, the biosynthesis reaction started, in which Ag+ were reduced to Ag-NPs and the color was observed after 3 hrs of reaction. The main mechanism behind the plant-assisted nanoparticles synthesis is the involvement of various phyto-chemicals. Phytochemicals such as saponins, terpenoids, ketones, flavones, amides, aldehydes, organic acids and flavones are water-soluble and immediately reduce ions [16]. It is reported that *Chlorophytum borivilianum* is rich in saponins, flavones, terpenoids, glycoside etc. that in turn act as reducing as well as capping agent [14].

3.1 Visual observation and UV-visible spectroscopy

Visual observation showed that final color deepened with the

increase in time, this is attributed to the fact that noble metals are known to exhibit unique optical properties due to the property of surface plasmon resonance (SPR) [17]. The color of the reaction mixture started changing to reddish brown after 3 hrs, which indicates the generation of silver nanoparticles, due to the reduction of silver metal ions Ag+ into silver nanoparticles Ag° via the active molecules present in the *C. borivilianum* plant extract (Figure 1). The color is attributed to the excitation of SPR. As shown in Figure 2, a characteristic and well-defined SPR band for silver nanoparticles was obtained at around 431 nm. Results of this study goes in accordance with [18] who reported silver nanoparticles exhibit well defined SPR bands at around 433 nm.

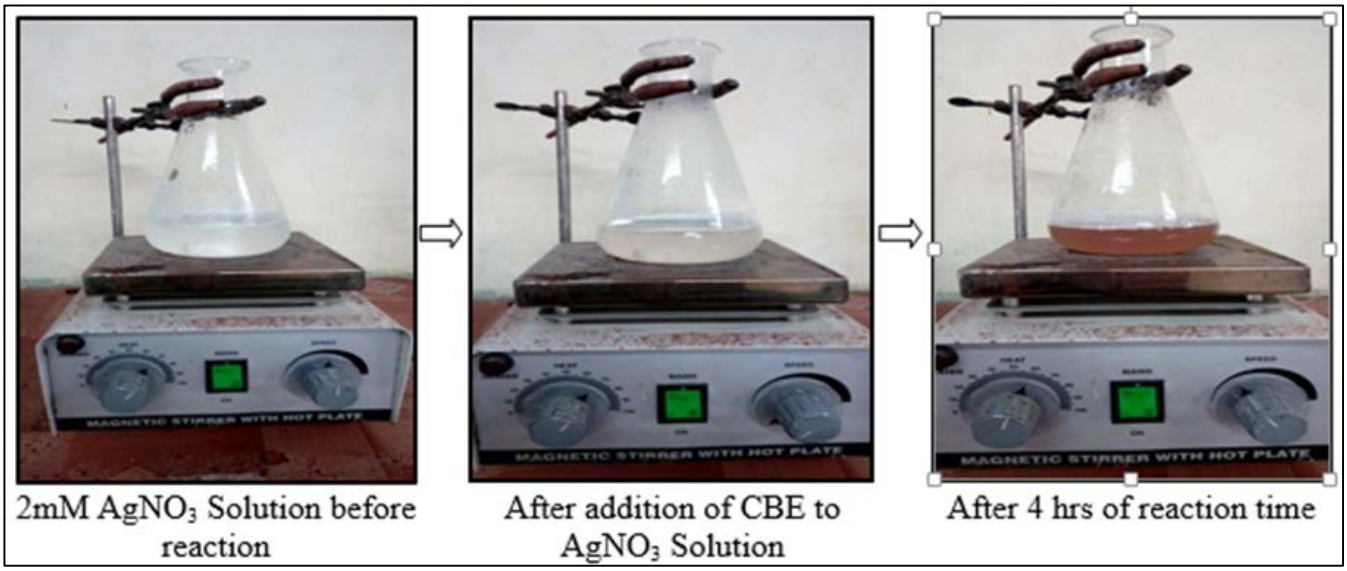

Fig 1: Color of the solution started changing to reddish brown after 3 hrs of reaction

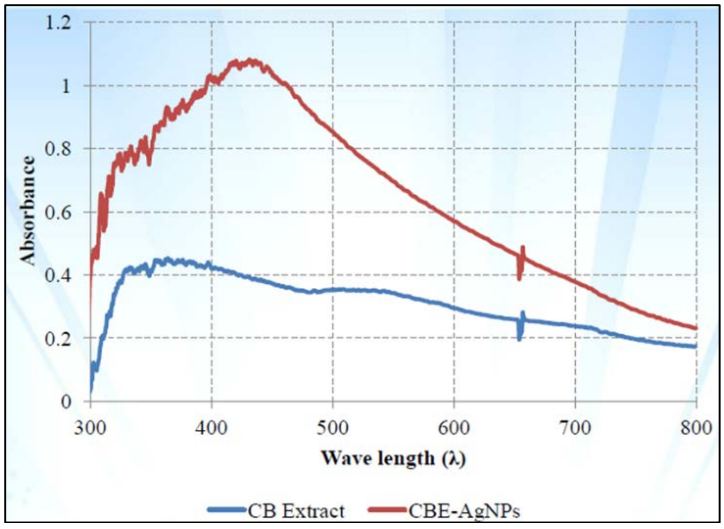

Fig 2: UV-Vis Spectrum showing the characteristic peak around 431 nm for silver nanoparticles

3.2 FTIR Spectra of CB-Ag NPs

The FTIR spectra were recorded to identify potential biomolecules that contributed to the reduction of the Ag^+ ions and to the capping of the bio reduced AgNPs [19]. The FTIR spectrum of our dried plant root extract powder (Figure 3) shows a trough in a range 3400 cm^{-1} to 2400 cm^{-1} corresponding to stretching vibration of –O-H bond of –COOH group [21]. Another strong peak observed ~1732 cm^{-1} is assigned to the stretching vibration of carbonyl group (-C=O) [20]. Peak at 1029 cm^{-1} correspond for stretching in ether linkage (C-O-C). Besides, sharp peaks at 1651 and 1681 cm^{-1} denote the presence of C=C bonds in the compound. Peaks observed at 1459 and 939 cm^{-1} indicate the bending and stretching vibrations in –O-H bond. Peak obtained at 1240 cm^{-1} is due to C-OH bond stretching vibrations. Moreover, peaks around 2856 and 2950 cm^{-1} are due to presence of sp^3 hybridized C-H bond in methyl groups. AgNPs are also showing similar peaks i.e. 1732, 1029, 1459, 939, 1240, 2856, 2748 cm^{-1} and the same trough around 3400-2400 cm^{-1}. Although, the peaks corresponding to C=C bonds were disappeared after reduction of silver nitrate by using plant root extract [22]. Further, the intensities of peaks in FTIR Spectrum of AgNPs were found lower in comparison to plant extract spectrum. The peaks in fingerprint region shows similarity pattern with the compound known as saponins which indicates that saponins could be responsible for the reduction of silver salt during formation of AgNPs [23].

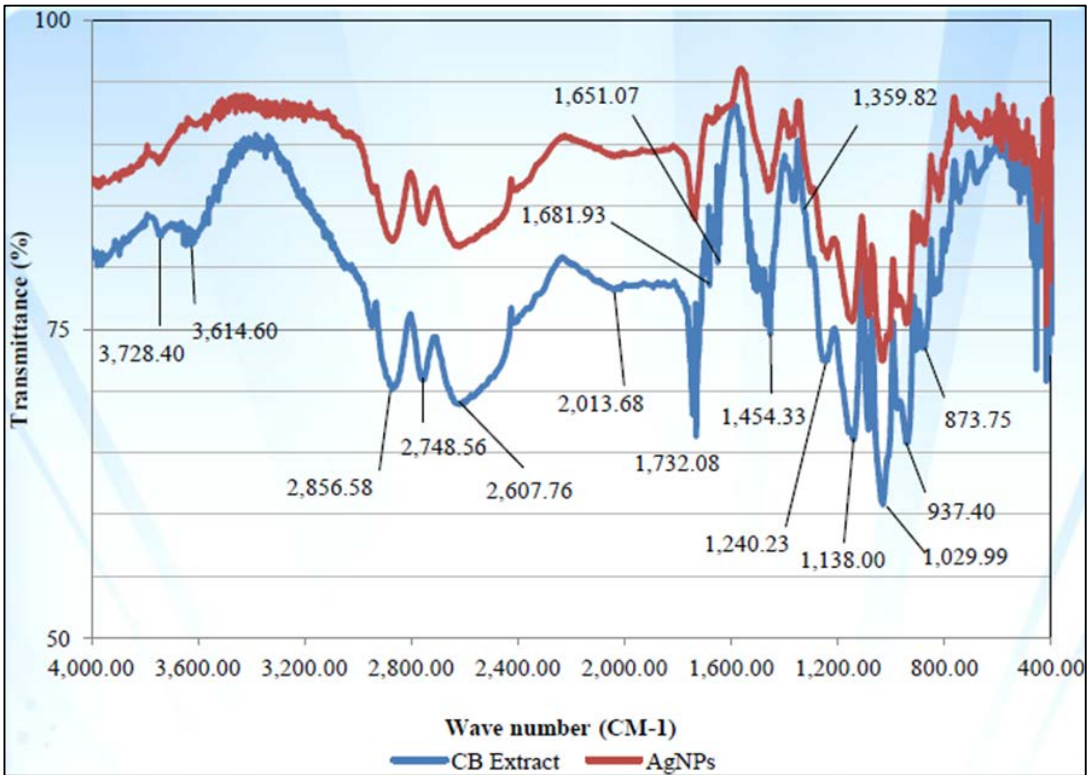

Fig 3: Comparative FTIR spectrum of CBE-AgNPs and CB Extract

3.3 Particle size distribution analysis of CB-AgNPs by DLS

DLS was carried out to determine the particle size of AgNPs in aqueous solutions; this characterization is essential before assessing the *in vitro* toxicity [24]. DLS was used in conjugation with TEM, to evaluate the size distribution of CB-AgNPs. The average size obtained was found <100 nm (as shown in Figure 4). The poly dispersity index (PDI) was found near to 0.3 which indicated mostly mono-dispersive nature of synthesized nanoparticles. As expected, the DLS measured size is slightly larger than the TEM size. The difference between DLS and TEM is due to the fact that TEM measures a number based size distribution of the physical size and does not include any capping agent, while DLS measures the hydrodynamic diameter, which is the diameter of the particle, plus ions or molecules that are attached to the surface and moves with the AgNPs in solution [25]**.** Thus, these ions or other associated molecules make the particle appear larger by DLS in comparison to TEM.

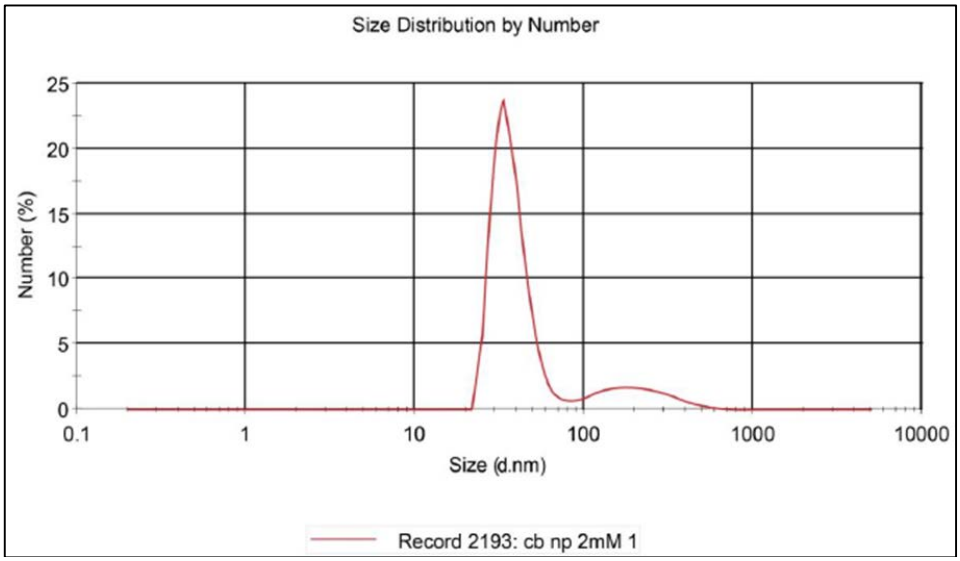

Fig 4: DLS Analysis of synthesized nanoparticles

3.4 XRD analysis

Figure 5 shows XRD patterns of vacuum dried silver nanoparticles synthesized using root extract of *Chlorophytum borivilianum.* A number of strong bragg's reflections with 2θ values of 27.83°, 32.27°, 46.15°, 55.08°, 57.49°, 67.65° and 77.07° are observed which may be indexed to the (111), (200), (202), (311), (222), (400) and (402) facets of the face centered cubic structure matched to silver chloride nanomaterial (JCPDS File No.: 85-1355 or COD file No. 96-9011667). XRD pattern thus clearly indicates that silver nanoparticles are crystalline in nature. The present results was found in good agreement with the observation in [26, 27].

Further, the mean size of nanoparticles for the peak 46.15° (202) was calculated using Debye-Scherrer's equation by determining the width of peak (FWHM) and It was found ~35 nm.

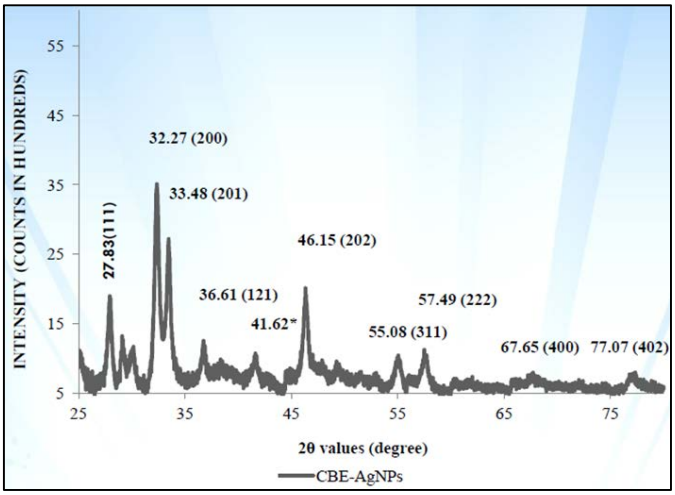

Fig 5: XRD Spectrum of synthesized silver nanoparticles (CBE-AgNPs)

3.5 Size and morphology analysis of AgNPs using SEM and TEM

The shape of the synthesized silver nanoparticles was analyzed by FE-SEM. Representative SEM micrographs of AgNPs magnified are shown in Figure 6. Mono-dispersed spherical silver nanoparticles were formed with the help of phytochemicals present in *Chlorophytum borivilianum* root extract, which is an added advantage.

TEM was used to obtain essential information about nanoparticle size and morphology. TEM micrographs revealed that AgNPs were distinct, uniformly spherical shapes and were well separated from each other. The average particle size was estimated from measuring more than 100 particles from TEM images. The sizes ranged between 30-50 nm with an average particle size of 35 nm (Fig 7). The crystalline nature of CB-AgNPs was confirmed by the Selected Area Electron Diffraction (SAED) pattern (Fig 7d).

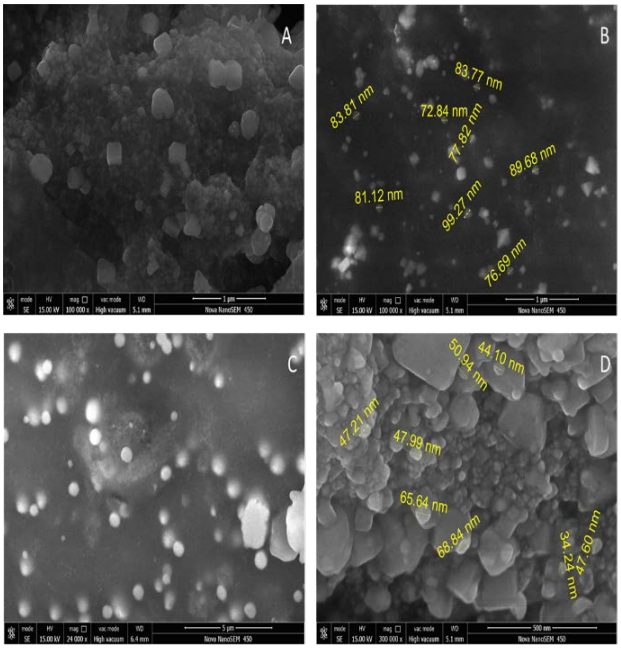

Fig 6: SEM images of *Chlorophytum borivilianum* silver nanoparticles

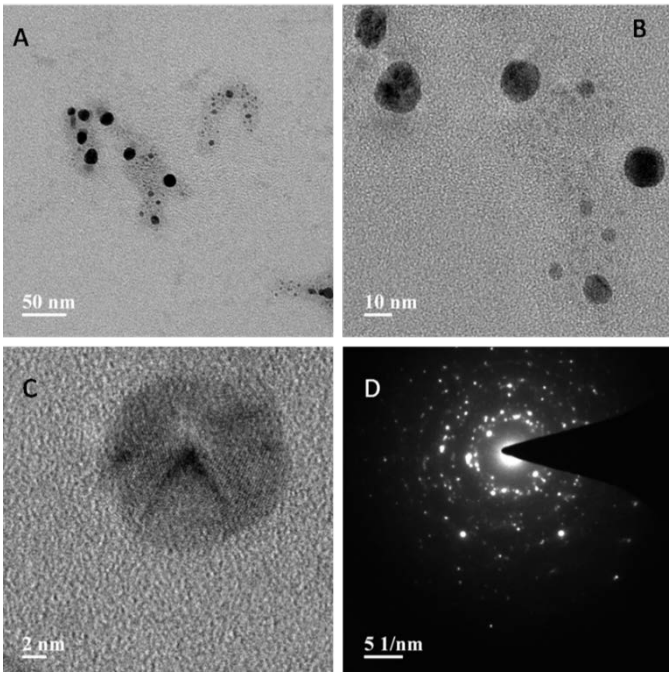

Fig 7: TEM image of CB-AgNPs: (A-C) high resolution image of Nano crystals (D) SAED pattern.

3.6 Antibacterial activity

Disease-causing microbes that have become resistant to drug therapy are increasing public health problems. Many researchers are now engaged in developing new cost effective antimicrobial reagents with the emergence and increase of microbial organisms resistant to multiple antibiotics. Silver has been used for years in the medical field for antimicrobial applications such as burn treatment, prevention of bacteria colonization on catheters etc. but the effects of silver nanoparticles (AgNPs) on microorganisms have not been fully developed. Nano silver, being less reactive than silver ions, is expected to be more suitable for medical applications. Above all, reducing the particle size of metals is also an efficient and reliable tool for improving their biocompatibility, which facilitates their application in different fields such as bioscience and medicine.

The antimicrobial activity of AgNPs was evaluated against clinical isolates of *Pseudomonas aeruginosa*, *Proteus mirabilis*, Coagulase positive *Staphylococcus* sp. and *Enterococcus faecalis* by disc diffusion method (Table 1 describes the various clinical pathogens used for the study).

In the present study, a comparative study between Silver nitrate (A), plant extract (B), distilled water as control (C) and silver nanoparticles (D) is depicted (Figure 8). The antibacterial activity was greater in case of nanoparticles. As per results obtained (Table 2) the gram-negative bacteria (*P. aeoginosa and P.mirabilis*) showed larger zones of inhibition, compared with the Gram-positive bacteria *(E. faecalis and Staphylococcus).* This may be due to the different cell wall composition as Gram positive bacteria possess thick peptidoglycan layer, thus forming more rigid structure leading to difficult penetration of the silver nanoparticles, on the contrary Gram negative bacteria possess thinner peptidoglycan layer [28]. Antibacterial property of silver nanoparticle can also be explained by following facts:

- First, AgNPs attach to the negatively charged cell surface, as a result it effects the physical and chemical properties of the cell membranes and cell wall and disturb important functions such as permeability,

osmoregulation, electron transport and respiration [29, 30, 31].

- Second, AgNPs can cause further damage by penetrating the cell, where they can interact with DNA, proteins and other phosphorus and sulfur containing cell constituents [29, 30, 32]
- Third, AgNPs release silver ions, generating an amplified biocidal effect, which is size, and dose dependant [33]. Thus, CB-AgNPs showed more bactericidal activity compared with the silver salt and the zone of inhibition was 13-15 mm and 10-12 mm, respectively.

Table 1: Description of the various pathogenic bacteria used

Bacteria name	Description
Pseudomonas aeruginosa (pyo)	Gram negative bacilli; it is a multidrug resistant opportunistic pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses and hospital acquired infections.
Proteus mirabilis	Gram-negative bacilli; pathogenic, well known for its ability to swarm across surfaces in a striking bulls-eye pattern. Clinically, this organism is most frequently a pathogen of the urinary tract, causing UTI.
Enterococcus faecalis	Gram positive, pathogenic, it is commensal bacteria inhabiting the gastrointestinal tracts of humans and other mammals. It has both intrinsic and acquired resistance to antibiotics, making them important nosocomical pathogens.
Coagulase positive *Staphylococcus* sp.	Gram positive; pathogenic, common commensal microorganisms and opportunistic pathogens in humans and animals.

Table 2: Zone of inhibition of silver nitrate, plant extract (CBE), distilled water and silver nanoparticles (CBE-AgNPs)

Bacteria	$AgNO_3$Soln.(a)	Plant extract (b)	Distilled water (c)	CBE-AgNPs (d)
Pseudomonas aeruginosa	11 mm	6 mm	6 mm	15 mm
Proteus mirabilis	11 mm	6 mm	6 mm	15 mm
Coagulase-positive *Staphylococcus*	12 mm	6 mm	6 mm	13 mm
Enterococcus faecalis	10 mm	6 mm	6 mm	13 mm

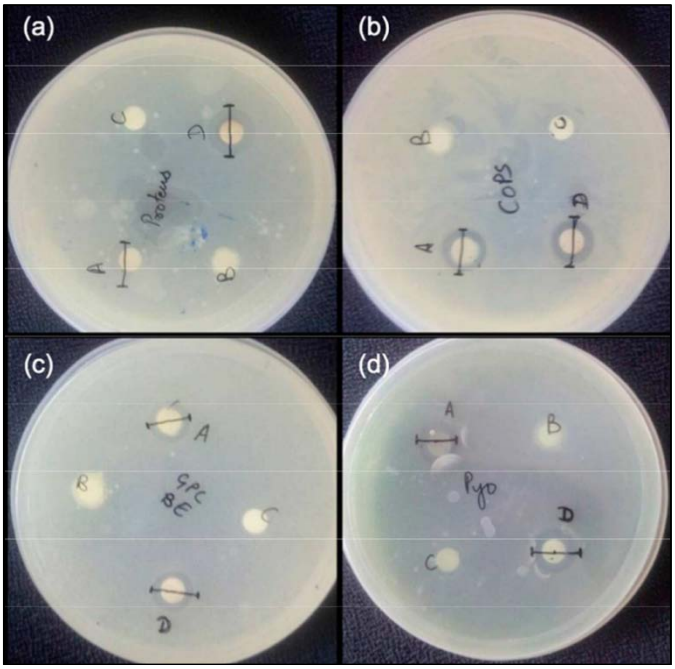

Fig 8: Zone of inhibition of silver nanoparticles against various clinical pathogens (a) *Proteus mirabilis* (b) Coagulase positive *Staphylococcus sp.* (c) *Enterococcus faecalis* (d) *Pseudomonas aeruginosa*

4. Conclusion

This is the first study to report a plant-mediated approach for the synthesis of AgNPs using the root extract of *Chlorophytum borivilianum* as a reducing and capping agent without using any harmful reducing agents such as sodium borohydrate, or any dispersing or capping agents.

Chlorophytum borivilianum, has various beneficial properties and its conjugation with silver increases its affectivity. These nanoparticles can further be used in different anticancer and anti-radiation studies specifically targeting reproductive organs, as *Chlorophytum borivilianum* is a well-known ayurvedic herb for the same.

In summary, CB-AgNPs were synthesized using a green and highly effective preparation technology. This synthesis has enormous potential in industries and also offers an ecofriendly alternate to traditional physical and chemical methods. From the results obtained, we can confirm that *C. borivilianum* conjugated nanoparticles has antibacterial properties. Also, these results suggest that CB-AgNPs could be used for the treatment of various infectious disease caused by Gram negative and Gram-positive bacteria. Interestingly the bacteria used in study are antibiotic resistance and the antibacterial results obtained give an effective treatment against these pathogenic bacteria.

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