Volume 66, Issue 1, 2022

Journal of Scientific Research

of

The Banaras Hindu University

Synthesis and Characterization of Silver Nanoparticles using Leaf Extracts of Medicinal plants and its Impact on *Anabaena doliolum*

[1]Darakshan Zabin, [1]Anveshna Shekher, [1]Madhu Yadav, [1]Renu Soni, [1]Garvita Singh*

Department of Botany, Gargi College,University of Delhi, Siri fort road, Siri fort institutional area, New Delhi- 110049, India
dzabin29@gmail.com, anveshnashekher05@gmail.com, madhubunty1999@gmail.com,
renu.soni@gargi.du.ac.in, *garvita.singh@gargi.du.ac.in

***Abstract*: Nanobiotechnology is exhibiting the great potential to be actively utilized in the form of nanoparticles to serve as carriers of biomolecules and benefit human health. In the present work, we have synthesized silver nanoparticles via a green synthesis pathway using medicinal plants *Eucalyptus camaldulensis*, *Azadirachta indica, and Euphorbia heterophylla* (a) separately and (b) in combination for synthesis. The major focus of this study is to assess the efficiency of silver nanoparticles when synthesized from a combination of medicinal plant leaf extracts with enhanced efficiency. The formation of silver nanoparticles was analyzed by the color change of the reaction mixture and Ultraviolet-Visible spectroscopic analysis (UV-Vis) showing characteristic peak of silver nanoparticles. Fourier Transform Infra-red (FTIR) analysis confirmed the various types of secondary metabolites playing active role in synthesis of silver nanoparticles alongwith bio-capping of synthesized silver nanoparticles. When compared with other published results, we concluded that silver nanoparticles synthesized from a combination of leaf extracts from medicinal plants yielded better results and were found to be more promising. Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), and X-Ray Diffraction (XRD) provided key results in terms of particle size, hydrodynamic size, shape, and structure. Our study is the first of its kind where medicinal properties of the aforementioned plants were employed to study their impact on the most commonly found bio-fertilizer of the Indian subcontinent; cyanobacteria *Anabaena doliolum*.**

***Index terms*: *Anabaena doliolum,* Combination of leaf extracts, *Euphorbia heterophylla,* Raman spectroscopy, silver nanoparticles**

I. Introduction

For decades, Nanotechnology, a very exciting field of science, has dealt with particles ranging from 1-100 nm in size. The nanoparticles synthesis, characterization, application, and size has led to a diverse application in industry and other allied fields (Iravani et al., 2014). The advancement in nanotechnology and other allied fields has led to the fabrication of nanoparticles (NPs). Now, nanoparticles from diverse sources can be molded into desired sizes and shapes, and are used for a wide range of applications. Nanobiotechnology is a rapidly growing field that revolves around the synthesis of NPs with varied compositions, sizes, and morphologies intended to be used for various industrial and pharmaceutical applications (Iravani et al., 2014). Apart from its application in industry and pharmaceuticals, Nanotechnology and Nanobiotechnology have tread a path to renovate agricultural and allied fields as well. Nanotechnology in the field of agriculture and biotechnology has the potential to upgrade the efficiency and efficacy of agricultural practices by producing nominal waste contrary to the other prevailing practices. Furthermore, it can detect potential plant diseases, and promote plant growth by incorporating essential nutrients that are lost due to leaching, decomposition, hydrolysis, etc. (Singh et al., 2015).

Generally, the NPs are synthesized using chemical and physical methods. However, these methods are interlinked with hazardous chemicals that exhibit toxicity; chemically synthesized nanoparticles are not suitable to be used for medicinal purposes, whereas the physical method requires high input, space and is heavy on the pocket (Pirtarighat et al., 2018). Alternatively, plant-mediated synthesis provides a breather from the enormous amount of hazardous chemicals associated with the synthesis of NPs (Hemlata et al., 2020). Among various metal nanoparticles, silver nanoparticles (AgNPs) are gaining much importance because they can be successfully incorporated in pharmaceutics and medicine as an antimicrobial agent against a broad range of bacteria (Pirtarighat et al., 2018) and can even serve in cancer diagnostic and therapeutic (Ahmed et al., 2016; Iravani et al., 2014). Furthermore, silver has a broad spectrum of bactericidal and fungicidal properties and thus it is considered the noblest metal used in the fabrication of nanoparticles. Silver can coordinate with numerous ligands and macromolecules in microbial cells and can constrain microbial proliferation.

Several researchers have reported the synthesis of AgNPs using plant extract because it provides the best capping agent for the stabilization of silver nanoparticles (Roy et al., 2017, Vanlalveni et al., 2021). Secondary metabolites from plants such as phenol and flavonoid serve as reducing, stabilizing, and capping agents in the conversion of silver ions to silver nanoparticles (Desai et al., 2016; Hemlata et al., 2020). Biologically synthesized AgNPs from medicinal plants have been widely studied and analyzed over the years. Nanoparticles from medicinal plants are rich in pharmacologically active substances and have been used for different purposes in different industries ranging from healthcare, agriculture, food, textile, and to the cosmetic and fashion industry (Singh et al., 2014; Pirtarighat et al., 2018). However, to the best of our knowledge, there has been no report on the synthesis of AgNPs using leaf extract of *Euphorbia heterophylla* as the raw material. Moreover, this study also focuses on using a combination of leaf extracts from -- *Eucalyptus camaldulensis* (F. Myrtaceae), *Azadirachta indica* (F. Meliaceae), *and Euphorbia heterophylla* (F. Euphorbiaceae) for the synthesis and characterization of AgNPs. *Eucalyptus camaldulensis* possesses ethnomedicinal properties, used to treat several ailments ranging from sore throat, tooth decay, arthritis, wounds and burns to bacterial infection of the upper respiratory tract and urinary tract (Dogan et al., 2017; Salehi et al., 2019). The essential phytochemicals in *Eucalyptus camaldulensis* include terpenes, tannins, isoprenoids, flavonoids, wherein flavonoids are assumed to act as a reducing agent in the biosynthesis of AgNPs (Banerjee et al., 2014; Bashir & Qureshi, 2015). *Azadirachta indica* has been extensively used in the synthesis of nanoparticles because of its use in the treatment of bacterial, fungal, and various kinds of skin ailments since time immemorial. Terpenoids and Flavanones are the main phytochemicals present in the plant that help in stabilizing nanoparticles, act as capping and reducing agents and exhibit antibacterial activity (Banerjee et al., 2014). Additionally, in this study, *Euphorbia heterophylla* was introduced to synthesize silver nanoparticles because AgNPs synthesized from this particular species were yet to be explored. *E. heterophylla* holds essential properties as an antimicrobial and anticancer agent and also aids in bronchitis, asthma, constipation, etcetera (Khandel et al., 2018; Tajbakhsh et al., 2016). Preliminary screening has revealed the presence of flavonoid, alkaloid, tannin, and glycosides as phytochemical constituents (Elshamy et al., 2019).

The emergence of multi-drug-resistant bacteria, with the overuse of antibiotics (Singh et al., 2020a) and insecticides against infectious diseases, required the development of alternative neutral antibacterial molecules having unique properties to control these types of microorganisms (Holmes et al., 2016; Singh et al., 2020b). Pesticides and herbicides conventionally used in the field pose a significant threat to crop production and agricultural yield. Long term use of traditional pesticides causes disease resistance in pathogens and decreases nitrogen fixation in plants due to bioaccumulation of pesticides (Gruere et al., 2011). Nanomaterials can provide a breather for the problems associated with the prolonged use of such harmful pesticides. Current studies suggest that the biosynthesized AgNPs have effective antimicrobial potential and could serve as an alternative to developing antimicrobial compounds to solve the problem of drug resistance (Hamida et al., 2020; Wang et al., 2017). The medicinal plant-mediated AgNPs may be studied for their positive response in controlling such harmful microbes and supporting nitrogen-fixing cyanobacteria. AgNPs aid in the suppression of disease and promote plant growth. It also causes toxicity in bacteria by decaying membrane potential, decreasing ATPase activity, and preventing ATP synthesis at the cellular level (Singh et al., 2015). Recently, with the improvement in the methods of nanoparticle synthesis, the use of nanotechnology has been astonishingly increased. In order to increase the capability of plants to absorb more water and nutrients from the soil (Singh et al., 2020a), there are many advanced tools in nanotechnology with intensified capacity (Singh et al., 2016; Singh et al., 2020b). This suggested that silver nanoparticles promote the growth of certain plants and could be beneficial in blue-green algae nitrogen fixation. This might be due to the uptake of silver nanoparticles, the alteration of membranes and other constituents (Singh et al., 2020b), and protective mechanisms.

This study aims to investigate the possible synergistic and additive interactions of various phytochemicals and medicinal properties from the aforementioned leaf extracts, which help in the reduction of silver ions and act as a capping agent to prevent agglomeration in the conversion of silver ions to silver nanoparticles, leading to the synthesis of stable silver nanoparticles with predefined characteristics. To our best knowledge, this is the first report wherein the combination of three different leaf extracts from medicinal plants has been used to synthesize silver nanoparticles. Furthermore, the role of synthesized nanoparticles from the combination was analyzed in N_2 fixing cyanobacteria *Anabaena doliolum*, which are commonly found in rice fields. The present study is an illustration of how a combination of medicinal plant extract can be more beneficial and effective for nanoparticle synthesis and its application. It has been reported by many research groups that the effects of nanoparticles depend on their size and reactivity (Razzaq et al., 2016). The results obtained suggest that the biological precursors from medicinal plants have a substantial potential for the synthesis of nanomaterials. These nanomaterials show the possibility for extensive applications in the future in the rice field where biofertilizers as cyanobacteria are extensively used, in addition to medical applications.

II. MATERIAL AND METHODS

A. Collection of Plant materials

Silver Nitrate ($AgNO_3$) was purchased from Sigma-Aldrich chemicals with ≥99.5% purity. Fresh Leaves from *Eucalyptus camaldulensis*, *Azadirachta indica*, and *Euphorbia heterophylla* were harvested from the botanical garden of Gargi College, University of Delhi.

B. Preparation of Leaf Extract

The aqueous leaf extracts were prepared by first cleaning the surface of leaves with running tap water to remove debris and organic contents, followed by double distilled water to eliminate dust and other foreign particles until no impurities remained (Ponarulselvam et al., 2012). After being washed thoroughly, leaves were dried with a laboratory dryer to remove excess water. The fresh leaves were then cut into fine pieces, and 20g each of *E. camaldulensis, A. indica, and E. heterophylla* were weighed and added to 200ml of distilled water in a 500ml Erlenmeyer flask. The mixture was heated for 1 h at 60°C in a water bath to avoid degradation of the proteins while stirring occasionally (Castillo-Henríquez et al., 2020). The extract was finally cooled and filtered using Whatman filter paper (Grade 1).

C. Synthesis of Silver Nanoparticles

Each leaf extract having a concentration of 20% was obtained by mixing pure leaf extract with distilled water. $AgNO_3$ (0.169g) powder was dissolved in 80ml distilled water to prepare a 1mM $AgNO_3$ stock solution. Now, 20ml aqueous leaf extract was made to react with the prepared 1 mM $AgNO_3$ stock solution in a 250

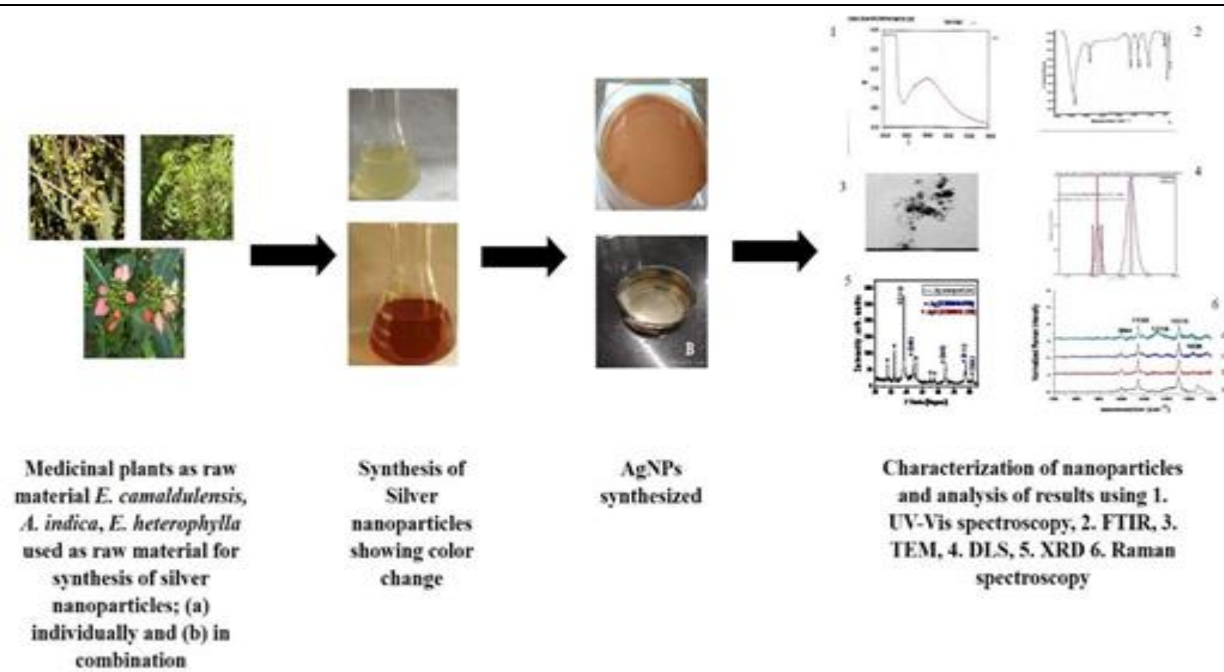


Fig. 1: Overall representation of the material and method used in this research work

ml Erlenmeyer flask. Using this method, a reaction mixture for each species was obtained. To synthesize nanoparticles from a combination of all three medicinal plants, i.e. *Eucalyptus camaldulensis*, *Azadirachta indica*, and *Euphorbia heterophylla*, an equal amount of aqueous leaf extract of each species at 1:1:1, v/v/v was mixed with 80ml of 1mM $AgNO_3$ solution.

This reaction initiated a color change from pale yellow to deep brown in the solution (Fig. 2 A1-D2). The change in color of the reaction mixture suggests a reduction of Ag^+ to Ag^0 and possible deposition of silver nanoparticles (Dolatabadi et al., 2017). The setup was wrapped with aluminium foil to ensure complete darkness to prevent auto-oxidation of silver nitrate and incubated at 25℃ in a shaker (150 rpm) for 72 h. UV-Visible spectroscopy was carried out to confirm the synthesis of silver nanoparticles (Saxena et al., 2016).

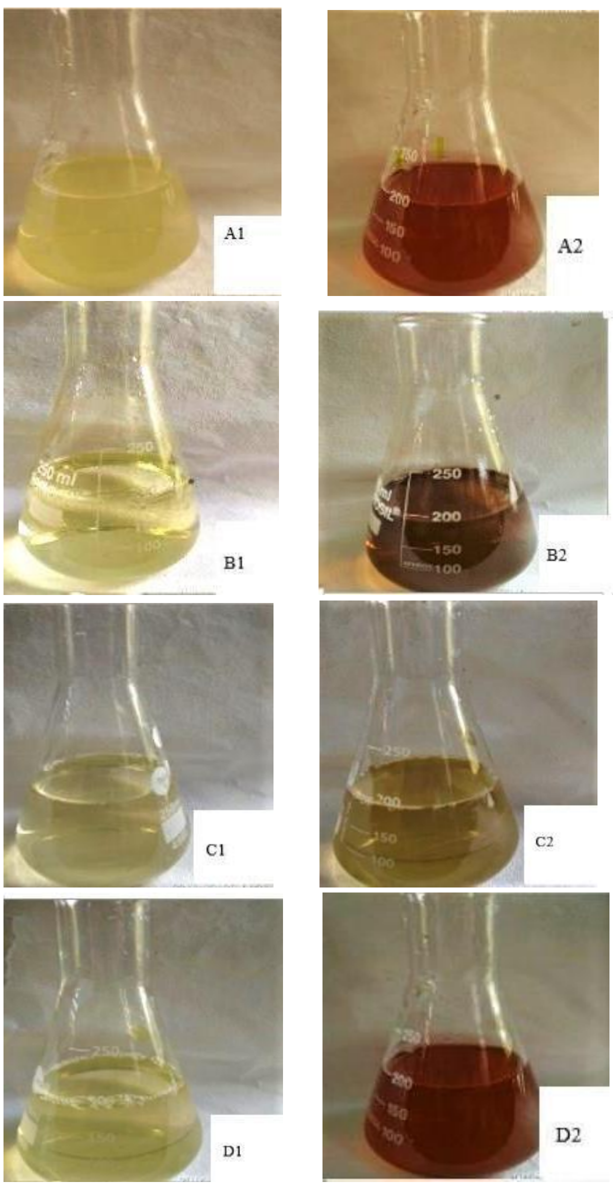


Fig. 2: Manifestation of Color Changes that occurred during the synthesis of silver nanoparticles indicating different stages of reaction; 1. Precursor solution prior to the addition of $AgNO_3$, 2. Change in color of the solution after addition of $AgNO_3$ stock solution; (A_1-A_2) Color change in *E. camaldulensis* extract solution, (B_1-B_2) Color change in extract solution of *A. indica*, (C_1-C_2) Color change in extract solution of *E. heterophylla*, (D_1-D_2) Color change in extract solution of a combination of above leaf extracts.

D. Purification and Concentration of AgNPs

The reaction mixture obtained from individual and combination of leaf extract was purified and concentrated by centrifuging the solution at 10,000 rpm for 10 min. The supernatants were discarded and pellets were washed twice in distilled water to remove contaminating plant residues and cellular metabolites (Fagier, 2021) (Fig. 3 A). The now purified sample was oven-dried at 37°C for 24 h to obtain a dry and concentrated mass of AgNPs (Fig. 3 B).

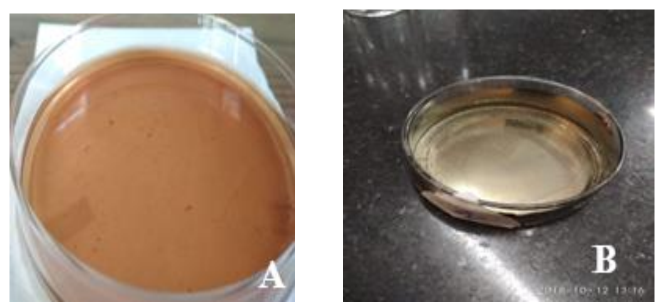


Fig. 3: Collection of purified and concentrated AgNPs sample synthesized from the combination of leaf extracts; (A) The purified AgNPs pellets were decanted in a petri dish after removal of contamination, (B) The concentrated AgNPs obtained after oven drying the pellets for 24 h.

E. Characterization of synthesized Silver Nanoparticles

1) UV-Visible Spectroscopy

UV-Visible spectral analysis was carried out using a UV-Visible spectrophotometer (Lambda 850+ Perkin-Elmer, University of Delhi) with a resolution of 1 nm between 210 and 900 nm. The bioreduction of Ag^+ ions were monitored at room temperature by withdrawing 1 ml aliquots of AgNPs reaction mixtures from *E. camaldulensis, A. indica, E. heterophylla*, and a combination of leaf extracts in a cuvette at known time intervals lasting up to 60 h (Adebayo-Tayo et al., 2019).

2) Fourier Transform Infra-Red Spectroscopy

The functional group of biosynthesized AgNPs was analysed using the Fourier transform infrared spectrophotometer (FTIR Niclot 6700, University of Delhi) with a scan range of 400 to 4000

cm^{-1} and a resolution of 4 cm^{-1}. Dried AgNPs obtained from *E. camaldulensis*, *A. indica*, *E. heterophylla*, and a combination of leaf extracts were encapsulated with Potassium Bromide (KBr) in a 1:100 ratios to prepare a translucent sample disc for analysis (Lingaraju et al., 2020). The spectra of AgNPs were recorded and the functional groups were categorized corresponding to their peak ratios.

Following the analysis of AgNPs through UV-Visible spectroscopy and FTIR for each leaf extract and also for the combination of leaf extracts, the additional characterization techniques were only carried out for AgNPs obtained from the combination of leaf extracts since it showed better and efficient nanoparticle synthesis.

3) Transmission Electron Microscopy

The morphology and particle size of AgNPS obtained was visualized using Transmission Electron Microscopy (TEM) technique. TEM grids were prepared by dropping a 5 μL aqueous solution of AgNPs obtained (Jain & Mehata, 2017) from the combination of leaf extract on copper-coated grids and air-dried at sterile conditions. Sample images of TEM analysis were obtained using the Transmission Electron Microscope (Tecnai G2 20, Indian Institute of Technology, Delhi) operated at 200 kV.

4) Dynamic Light Scattering

Dynamic light scattering (DLS) was used to observe the average size distribution of biosynthesized silver nanoparticles using a dynamic light scatterer (Malvern Zetasizer Nano ZS, Jamia Millia Islamia, Delhi). To investigate the formation, crystalline structure and quality of synthesized AgNPs from the combination of leaf extracts, the suspension was centrifuged, the pellet was dissolved in deionised water, and passed through Cu-kα radiation of λ= 0.154187 nm.

5) X-Ray Diffraction

The scanning images of AgNPs from combination extract were procured after the diffraction was done at a 2θ angle from 10° to 80° at 0.02/min using X'Pert PRO (PANalytical Netherlands Indian Institute of Technology, Delhi). To calculate the crystal size Debye-Sherrer equation was implemented: $D=0.9\lambda/\beta \cos\theta$ (where D= crystal size, λ is the wavelength, β is full width at half maximum of the peak I radians, and θ is Bragg's angle in radians)

F. Growth conditions of A. doliolum

Anabaena doliolum, a filamentous heterocystous cyanobacterium, was provided by Dr Garvita Singh, (obtained from Microbiology lab, School of Biotechnology, Banaras Hindu University, Varanasi). The identity of the isolate was confirmed by the sequencing of its 16S rRNA gene, where the sequences showed 99% similarity with *A. doliolum* available in the NCBI database (Accession No. JX075257). The axenic culture was routinely grown diazotrophically in BG11 medium in a culture room at 27 ± 2°C and illuminated with Sylvania 40W T12 fluorescent lamps at an intensity of 14.4 ± 1 Wm-2 for a 14/10 h light/dark cycle. Unless otherwise stated, all the experiments were performed with the log phase cultures, having an initial dry weight of approximately 0.15 mg/ml (Singh et al.; 2014).

G. Raman Spectroscopy

The specific change in biomolecules of cyanobacterium *Anabaena doliolum* after interaction with AgNPs (varied concentration) obtained from a combination of leaves was analysed using a Raman spectrophotometer. Raman spectroscopy was recorded using RENISHAW in which a 532 nm Nd Yag laser (incident power ~0.3 mW) is used as an excitation source with a CCD detector. The Raman active molecules near AgNPs from a combination of leaf extracts enhance the Raman intensity, this occurrence is called Surface Enhanced Raman Spectroscopy (SERS).

III. RESULT

A. Visual observation and UV-Visible Spectroscopy

The bioreduction of silver ions in the reaction mixture with plant extracts of *E. camaldulensis*, *A. indica*, and *E. heterophylla* respectively happened in approximately 5 min. Furthermore, the prepared solution of AgNPs obtained from a combination of plant extracts with 1 mM $AgNO_3$ exhibited color change within 1 min suggesting faster bioreduction. The intensity of the reaction mixture's color increases as the reaction proceeds. To corroborate the synthesis and stability of AgNPs, UV-Visible spectroscopic analysis was achieved. UV-Vis spectra for individual leaf extract solution and also the combination of these leaf extracts gives a sharp and concise peak at 420 nm for silver nanoparticles (Fig. 4), this peak indicates the formation of AgNPs in solution.

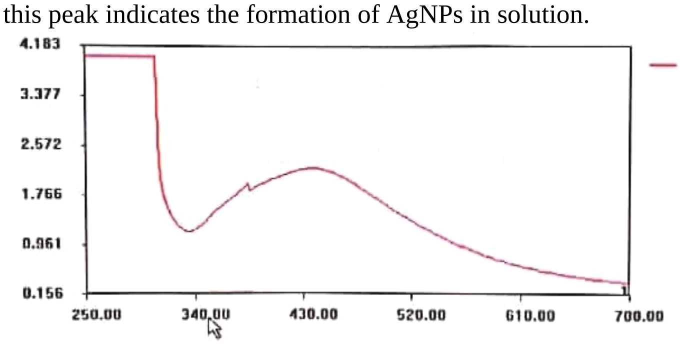
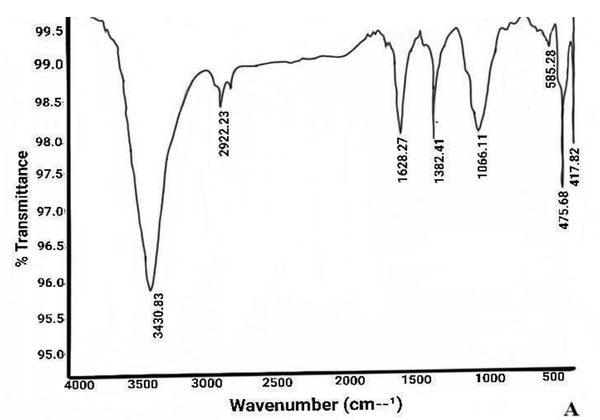


Fig. 4: UV-Visible spectral analysis of reaction mixture during synthesis of AgNPs from the combination of leaf extracts.

B. Fourier Transform Infrared Spectroscopy (FTIR)

The potential phytochemicals that might be responsible for the bioreduction, capping and stabilization of AgNPs are shown in (Fig. 5 A-D). In the FTIR spectra of *E. camaldulensis*, *A. indica*, *E. heterophylla*, prominent peaks were observed at 3725 cm^{-1} (Table I b) and between 3431- 3436 cm^{-1} associated with O-H stretching of alcohol and N-H stretching of amines respectively (Table I a-d). Another peak at 2922 cm^{-1} assigned to alkane C-H stretching but a dip in the band at 2920 cm^{-1} for the combined solution represented O-H stretching of carboxylic acids (Table I d). Bands at 1737- 1735 cm^{-1} are linked to C=O stretching of ester and aldehyde. Peaks between 1621-1629 cm^{-1} vary largely from C=C stretching of alkene, C=O stretching of amide, and N-H bending of amine respectively. FTIR peaks ranging from 1380-1382 cm^{-1} belong to C-H stretching and bending of alkane and aldehyde. 1082-1069 cm^{-1} associate with C-O stretching of compound class primary alcohol. The band at 1066 cm^{-1} is with C-O stretching of primary alcohol (Table I a). Peaks at 954 cm^{-1} represent C-N stretching of amide, 819 cm^{-1} is C-Cl stretching of halo compound, 824 cm^{-1} suggest bending vibration of C=C which indicate the presence of alkane. Lower bands at 670-656 cm^{-1} are of C-Br stretching indicating Halo compound, 556 cm^{-1} C-S out of plane associated with phenyl ring.

The interactions between functional groups and nanoparticles cause a significant shift in the AgNPs absorption peak. The lower spectral band with amide linkage of protein has the ability to form protein layers around silver and prevent agglomeration which in turn stabilize the silver nanoparticles. C=O group was involved in the bioreduction process which is confirmed by the shift in spectral peak to 1628 cm^{-1} in AgNPs preparation (Umeron et al., 2016). Analysis of the spectral peaks suggests that apart from a few reductions in frequency curve, the plant extract and AgNPs IR profile share common biomolecules.



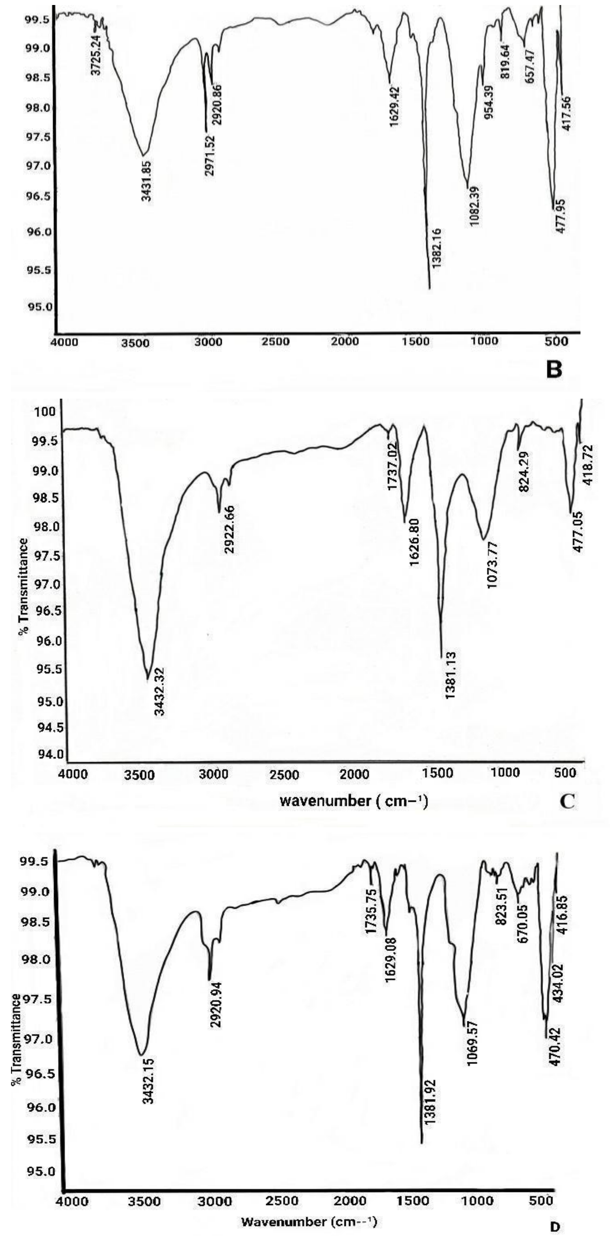


Fig. 5: FTIR Profile of AgNPs synthesized from; (A) *E. camaldulensis* sample with 1 mM $AgNO_3$ stock solution, (B) *A.indica* sample with 1mM $AgNO_3$ after 60 h, (C) *E. heterophylla* in addition with 1mM $AgNO_3$, and (D) Combination leaf extracts of *E. camaldulensis, A.indica, and E. heterophylla*.

Table I: List of Bands assigned for FTIR Spectra

(a) Prominent FTIR Peaks of *Eucalyptus camaldulensis*

Sl. No.	Wavenumber	Functional groups	Peak assignment
1	3430	N-H stretching	Aliphatic 1° amine
2	2922	C-H stretching	Alkane
3	1628	C=C stretching	Alkene
4	1382	C-H stretching	Alkane
5	1066	C-O stretching	1° Alcohol
6	585	C=C bending	Alkene
7	475	C-Br stretching	Halo compound
8	417	C-S out of plane	Phenyl ring

(b) Prominent FTIR Peaks of *Azadirachta indica*

Sl. No.	Wavenumber	Functional groups	Peak assignment
1	3725	O-H stretching	Alcohol
2	3431	N-H stretching	Phenolic 1° and 2° amine
3	2921	C-H stretching	Alkane
4	2920	C-H stretching	Alkane
5	1629	N-H stretching	Amine
6	1382	C-H bending	Aldehyde
7	1082	C-O stretching	1° Alcohol
8	954	C-N stretching	Amide
9	819	C=Cl stretching	Halo compound
10	657	C=Br bending	Halo compound
11	477	C-S stretching	Phenyl ring
12	417	Out of plane deformation	Phenyl ring

(c) Prominent FTIR Peaks of *Euphorbia heterophylla*

Sl. No.	Wavenumber	Functional groups	Peak assignment
1	3432	N-H stretching	Phenolic 1° and 2° amine
2	2922	C-H stretching	Alkane
3	1737	C=O stretching	Ester
4	1626	C=C stretching	Alkene
5	1381	C-H stretching	Alkane
6	1073	C-O stretching	1° Alcohol
7	824	C=C bending	Alkene
8	477	C-S stretching	Phenyl ring
9	418	Out of plane deformation	Phenyl ring

(d) Prominent FTIR Peaks of a combination of *E. camaldulensis, A. indica and E. heterophylla*

Sl. No.	Wavenumber	Functional groups	Peak assignment
1	3432	N-H stretching	Phenolic 1° and 2° amine
2	2920	O-H stretching	Alkane
3	1735	C=O stretching	Ester
4	1629	N-H stretching	Alkene
5	1381	C-H stretching	Alkane
6	1069	C-O stretching	1° Alcohol
7	823	C=C bending	Alkene
8	670	C-Br stretching	Phenyl ring
9	470	C-S stretching	Phenyl ring
10	432	C-S out of plane	
11	416	Out of plane ring deformation	

C. *Transmission Electron Microscopy (TEM)*

TEM illustrates that particles are spherical with an average size of 10-30 nm and are well dispersed (Fig. 6 A-C). The data obtained using AgNPs from combined leaf extracts showed distinct spherical shaped NPs with small sizes without significant agglomeration. Apart from some irregularly shaped nanoparticles, others were spherical and homogenous which conforms to the shape of the SPR band in UV-Vis spectra. Small size provides nanoparticles with a large surface area, enhancing their reactivity and catalytic properties in various applications. Tem pattern displayed the formation of silver diffraction rings, suggesting the Face cubic centred crystalline nature of AgNPs obtained from a combination of leaf extracts. Similarities in nanoparticle shapes could be attributed to having the same type of bio-reductive and capping agents.

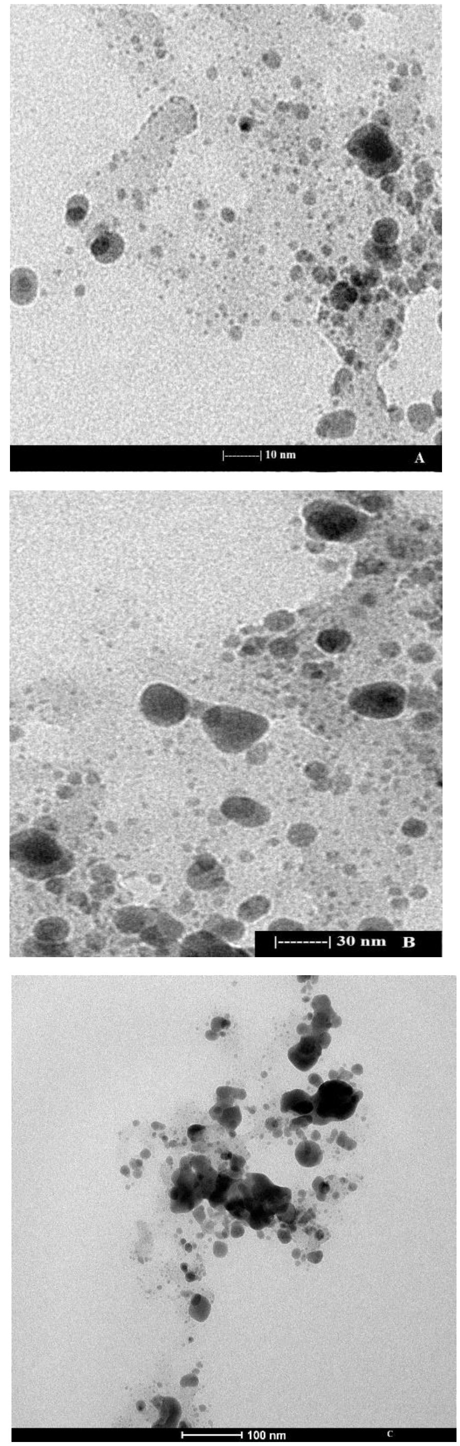


Fig. 6: TEM micrograph showing the formation of Ag nanoparticles from the combination of leaf extracts; (A) TEM micrograph showing an average size of 10 nm, (B) Spherical Ag nanoparticles with an average size of 30 nm formed after addition of 1mM $AgNO_3$ to a solution containing combination of leaf extracts, (C) TEM micrograph showing nanoparticles with average size range 100 nm.

D. Dynamic Light Scattering (DLS)

The hydrodynamic particle size and the polydispersity index (PDI) of synthesized AgNPs are measured using dynamic light scattering. DLS also tells about the population of particles in the sample in a considerably short duration of time with more precision. The average particle size as shown by the size distribution graph is 45 nm with PDI of 0.1 to 0.2 indicating monodisperse particles (Fig. 7 A-B).

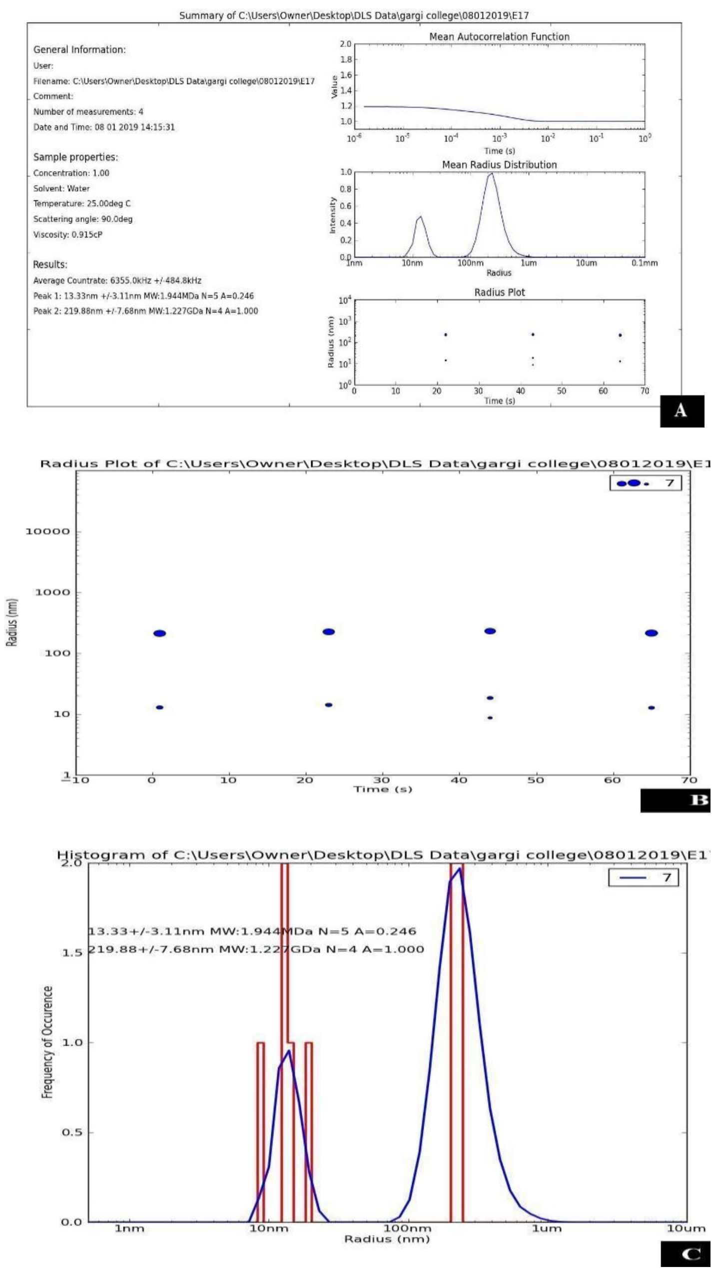


Fig. 7: DLS micrograph of AgNPs from a combination of leaf extracts showing intensity distribution (A) Summary of mean radius distribution; (B) Radius v/s time graph; (C) Frequency v/s radius graph.

Apart from this, some distribution can be clearly observed at the lower particle size range indicating AgNPs might also have lower particle size. As expected, the DLS measured size is slightly larger than the TEM size. The differences possibly reflect the fact that TEM only 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. These ions or other associated molecules make the particle appear larger to the instrument in comparison to TEM. Hence, the hydrodynamic diameter is always greater than the size estimated by TEM (Erjaee et al., 2017). Additionally, phytochemicals that are present in leaf extracts contribute to the size and the particle size varies with the variation in phytochemicals present.

E. X-ray Diffraction (XRD)

XRD also provides a rough idea about the particle size through the Debye Scherrer equation. The XRD pattern displayed five peaks at 2θ angles ranging from 20° to 80° (Fig. 8). In particular, the five distinct reflections correspond to 37.5° (111), 44.13° (200), 63.90° (220), 76.85° (311), and 81.1° (222) reflects the pattern of face-centred cubic (FCC) and crystalline structure of the AgNPs. The crystallinity improved with an increase in temperature; well-defined peaks at 80°C in XRD showed that AgNPs particles were extremely crystallized. The diffraction peaks were consistent with the Joint Committee's standard data files on Powder Diffraction Standards (JCPDS file no. 04-0783). The well-defined crystalline structure is due to the biomolecules in the plant extract that act as reducing agents and stabilize the AgNPs (Ahmed et al., 2016; Singh et al., 2014).

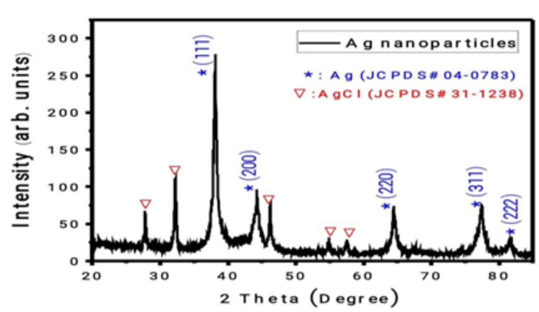


Fig. 8: XRD spectra of Combination leaf extracts of *E.camaldulensis, A. indica, and E. heterophylla*

F. Surface-Enhanced Raman Spectroscopy (SERS)

SERS is a method employed for the chemical structure elucidation of metal nanoparticles and helps in exploring the plausible mechanism of encapsulation of AgNPs by suitable capping agents (Joshi et al., 2018). In this study, the Raman spectra of *A. doliolum* after treatment with AgNPs obtained from the combination of leaf extract is shown in (Figure 9). Four different concentrations; control, 10 µg, 20 µg, and 50 µg preparation were taken for Raman analysis after their application in rice field cyanobacterium *Anabaena doliolum* in their growth media. The cells were pelleted and their active biomolecules were extracted by the freeze-thaw method. These molecules were further analysed with the help of Raman spectroscopy. The spectra obtained showed that AgNPs obtained by a combination of leaf extracts are not harming the active biomolecules of cyanobacteria including Phycocyanin and chlorophyll with increasing concentration up to a certain extent.

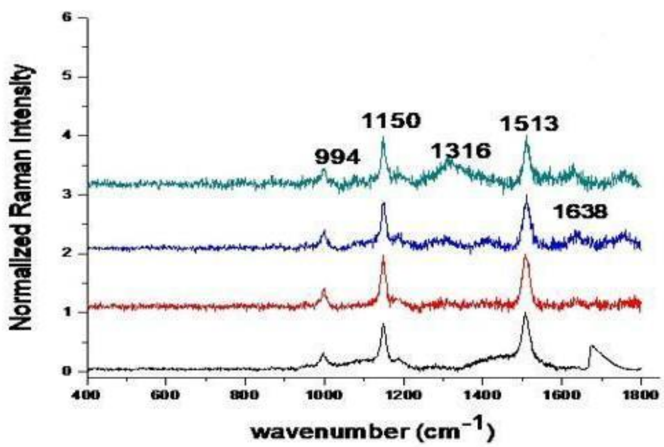


Fig. 9: SERS spectra obtained after interaction of AgNPs at different concentrations on *Anabaena doliolum* (control, 10 µg, 20 µg, and 50 µg); (a) control, (b) 10 µg, (c) 20 µg. (d) 50 µg

Raman analysis of all the aforesaid concentrations shows intense bands at the same wavenumber position demonstrating that the properties of silver nanoparticles are fixed (Fig. 10) and is not affecting the cyanobacterium. The vibration band at 994 cm^{-1} is identified as C-C aliphatic chain vibrations. Two broad bands at 1150 cm^{-1} and 1513 cm^{-1} are indications of asymmetric C-O-C and C=C stretching vibrations respectively. Few weak spectral bands elucidated at 1316 cm^{-1} and 1638 cm^{-1} are assigned to stretching vibrations of C=C and symmetric and asymmetric C=O bonds. In figure 7, it can be observed that there is a selective enhancement of a few Raman bands where the superior SERS signal intensities are projected. This enhancement in spectra around 1150 cm^{-1} and 1513 cm^{-1} indicates that asymmetric C-O-C and C=C are absorbed on the rough surface of silver nanoparticles and is associated with the capping of the nanoparticle and its interaction with the biomolecule phycocyanin of cyanobacterium tested. A peculiar peak position is an indication that no stress molecules are triggered by silver nanoparticles and thus support the growth of cyanobacteria by suppressing the growth of other microbes like bacteria and fungi in growth media.

IV. DISCUSSION

Biosynthesized metal NPs have been studied at length for their promising therapeutic, pharmaceutical, industrial, and agricultural role with significant antimicrobial properties. Several researchers have reported the synthesis of metal nanoparticles through the biological route using microorganisms and plants to incorporate the potential biomolecules present in the microorganisms and plants onto the nanoparticles (Kulkarni & Muddapur, 2014; Li et al., 2011; Masum et al., 2019; Ponarulselvam et al., 2012). However, the synthesis of silver nanoparticles using the combination of leaf extracts from different medicinal plants is unique and has great promise in the field of research. Our study has clearly shown the effectiveness and efficiency of AgNPs when synthesized using the combination of *E. camaldulensis, A. indica, and E. heterophylla* leaf extracts solution. The results obtained from the combination mixture were much more rapid, accurate and the silver nanoparticles synthesized were stable in comparison to other studies where silver nanoparticles were synthesized using a single leaf extract (Alghoraibi et al., 2020; Atarod et al., 2015; Nagar et al., 2016; Vanlalveni et al., 2021). The combination has the cumulative effect of various anti-microbial, anti-fungal, antiviral, antibacterial and antioxidant properties which were present separately in each medicinal plant. The nanoparticles synthesized from a combination of all three medicinal plants contain a range of biologically active components such as polyphenols, proteins, alkaloids etc. as studied with the help of FTIR, which can donate hydrogen to free radicals and thus break the chain reaction of free radicals at the first initiation step (Otunola et al.; 2018). Therefore, this indicates that the antioxidant activity of the silver nanoparticles was enhanced by these bio-components by preventing the formation of reactive oxygen species (ROS). This clearly shows the complete reduction of silver ions (Ag^+) to Silver (Ag^0) without any byproduct formation. Furthermore, among various metals, we preferred to use Silver for the synthesis of nanoparticles because Ag ions and Ag-based compounds are antimicrobial agents, as they possess strong growth inhibitory effects against various microorganisms (Singh et al.; 2014).

The color change of the aqueous solution from pale yellow to deep brown indicated a reduction of silver ions to silver nanoparticles. The deep brown formation occurred due to the oscillation of free electrons in the reaction mixture (Rajeshkumar et al., 2014). The color change in AgNPs obtained from a combination of leaf extracts occurred after 1 min of reaction as compared to that in the previous work (Dipankar & Murugan, 2012; Roy et al.; 2017) where the color change could only be observed after a significant amount of reaction time ranging from 1h up to 7 days. Therefore, it can be concluded that the formation of the nanoparticles is rapid when different leaf extract solutions from medicinal plants are integrated. Furthermore, the succession of nanoparticle formation was justified by UV-Vis Spectroscopy. The most rapid bioreduction leading to the formation of AgNPs from combined solution displayed absorption peak at 48 h of the incubation period, in comparison to peak at 72 h of incubation time in case of each plant extract. Biosynthesized AgNPs obtained from a combination of leaf extract showed a strong and prominent band of absorption at 420 nm as opposed to that of each extract (Deka et al., 2021).

FTIR measurements were conducted to reveal the key functional groups and biomolecules that participated in the bioreduction of Ag^{+} to Ag^{0} and stabilization of AgNPs. FTIR analysis was carried out for AgNPs synthesized from *E. camaldulensis*, *A. indica*, *E. heterophylla*, and a combination of the aforementioned plant extracts. In AgNPs from combined sources, a significant shift in the peak was observed when compared with FTIR spectra obtained from control leaf extract (Qayyum et al., 2017) and AgNPs from each medicinal plant. FTIR spectra of AgNPs from combined sources indicated the presence of various absorption bands ranging from 416 to 3432 cm^{-1} which is an amalgamation of absorption bands observed in AgNPs from each leaf extract. In contrast to the previous studies reported by (Aziz et al., 2019; Masum et al., 2019; Manikandam et al., 2017; Sila et al., 2019) which only showed few absorption bands, the FTIR spectrum in our study revealed various absorption peaks for N-H and O-H stretching vibrations indicating strong hydrogen bonding, C-O and C=O stretching vibrations attributed to carboxylic acid, primary alcohol, aldehyde, and ketone, which are strongly linked with the reduction of silver ion and stabilization of nanoparticles due to oxidation of hydroxyl radical. From the present FTIR analysis, it can be deduced that proteins and carbonyl groups prevent possible agglomeration and help in the stabilization of nanoparticles. The stabilization is a result of the strong metal-binding ability of carbonyl groups and proteins. It can be concluded that biological molecules not only help in the formation but also the stabilization of silver nanoparticles (Mallikarjuna et al.; 2011). From the results of colour change on the reaction of silver nitrate with leaf extract solution, UV-Vis Spectroscopy and FTIR, it was inferred that the results obtained for AgNPs through a combination of leaf extracts are much more rapid, efficient, and promising as compared to the results of AgNPs obtained from individual leaf extract or other biological sources. Therefore, other characterization techniques such as TEM, DLS, and XRD have proceeded only for AgNPs from combined sources.

The TEM micrographs of biosynthesized AgNPs suggest that the sizes of the particles were around 10-30 nm which is comparatively smaller and efficient as compared to that reported by (Anandalakshmi et al., 2016; Rauwel et al., 2015). The particles were spherical and crystalline in nature. While the TEM micrographs suggested smaller sized nanoparticles, we observed the mean diameter size of nanoparticles through DLS to be 45nm for AgNPs from combined sources. The difference in the results of DLS and TEM possibly reflects the fact that TEM only measures a number-based size distribution of the physical size and does not include any capping agent, while DLS measures the hydrodynamic size, which is the diameter of the particle, plus ions or molecules that are attached to the surface that aids in the efficient capping of AgNPs. These ions or other associated molecules make the particle appear larger to the instrument in comparison to TEM. Hence, the hydrodynamic diameter is always greater than the size estimated by TEM. Furthermore, many studies proposed the importance of hydrodynamic diameter for understanding and optimizing the size of nanoparticles and their performance in biological assays. The crystalline nature of Ag-NPs was evident from XRD analysis. The AgNPs diffractogram displayed numerous sharp intense peaks which not only indicate the crystallinity of sustainability of the AgNPs but also confirm the formation of the silver NPs. The XRD results in this paper were compared with standard results (Goudarzi et al., 2016; Roy et al., 2015; Shameli et al., 2012;) and found to be resonating with them. The distinct reflections obtained indicate the formation of the face-centred cubic (FCC) structure of the AgNPs. It has also been reported that the presence of active biomolecules such as amino acids and small secondary metabolites stabilizes the AgNPs for a long duration by avoiding the aggregation of nanoparticles. In conclusion, our study showing the use of a combination of leaf extracts for the synthesis of silver nanoparticles has garnered promising results and are much more effective as compared to the nanoparticles that were traditionally obtained using a single source.

In addition to the synthesis and characterization of silver nanoparticles, our study intensely focuses on the efficiency and application of AgNPs from a combination of leaf extracts on rice field cyanobacteria. The rice field cyanobacteria *Anabaena doliolum* was selected for further studies since it can easily be found in Indian soil conditions and play a pivotal role in efficient nitrogen fixation, soil fertility, and crop growth. *Anabaena doliolum* also acts as a natural biofertilizer for the rice field. Previous studies (Aziz et al., 2019; Lara et al., 2004; Rajeshkumar & Malarkodi, 2014) have scrupulously explored the applications of silver nanoparticles as promising antibacterial, anticancer, anti-inflammatory and antifungal agents. However, their role on the soil microflora is just as important and needs to be explored deeply. Our study is the first of its kind where medicinal properties from three different plants that are well known for their antioxidant properties are employed to study their impact on the most commonly found biofertilizer of the Indian subcontinent; *Anabaena doliolum*. Industrial fertilizers and pesticides adversely affect the soil and the soil microflora by causing mortality, morbidity and prolonged damage but our study presents a very effective role of AgNPs usage as nano pesticides and nano fertilizers without affecting the microbial soil. *A. doliolum* was studied with the help of Raman spectroscopy after being treated with synthesized AgNPs obtained from a combination of leaf extracts, the result showed a very mild effect of AgNPs on the active biomolecules of cyanobacteria, even the phycocyanin and chlorophyll content was found to be undisturbed. With the increasing toxicity level of soil due to the use of traditional pesticides and insecticides, our study is quite relevant in its way to be used as eco-friendly alternatives to harmful pesticides. Now that, with the help of our study, we have established that AgNPs from a combination of leaf extracts have a positive effect in the agricultural sector, a lot of applications need to be explored. Plant growth and its life cycle depend highly on the availability of nutrients but widespread nutrient deficiencies in soil due to leaching, runoff, microbial mineralization, poor uptake and lesser solubility is pertaining to a greater risk for future agricultural demands (Jacoby et al., 2017). Hence, nanomaterial can be employed for the controlled release of agrochemicals, pesticides and insecticides catering to specific demand, desired properties, enhanced activity on target site, and delivering the nutrients in a demand-driven period in a precise manner. Silver nanoparticles along with other metal nanoparticles have the potential to enter roots and leaf surface cuticles to regulate better metabolic activity in plants. Controlled nano pesticide release reduces chemical contamination in the soil and protects (Shang et al., 2019; Yan and Chen, 2019) non-target plant tissue from potential damage. AgNPs has both positive and negative aspects depending on the concentration, size, chemical composition, stability and shape of nanoparticles (Costa and Sharma 2016; Mirzajani et al., 2013; Tripathi et al., 2017). The negative impact of silver nanoparticles disrupts plant growth and productivity but robust and smart engineered techniques are being explored for the betterment of agricultural crop production to cater to different agricultural needs. A significant increase in agricultural production could be possible through the utilization of current knowledge in the field of nanotechnology for the efficient nutrient system, good plant protection practices, efficient photo capturing system in plants, precision agriculture, and many others (Ditta et al.;2015, Javed et al., 2019). Yet, whatever the chemical nature of nanoparticles is, one should remember potential adverse effects, i.e., phytotoxicity at high concentrations and toxicity for unintended non-target organisms. Through a small effort by our study, we have tried to highlight the efficient use of common silver nanoparticles used as fertilizer, pesticides etc. In our attempt to study the impacts of medicinal plants in combination with the consequence of toxicity to important microbes such as nitrogen-fixing cyanobacteria has been done. Further attempts to study the impacts on the proteomic level will explain the metabolic alterations if any by these nanoparticles. More studies are required to elucidate the responsible mechanisms behind the improved growth and productivity of crop plants caused by the application of different types of green synthesized nanoparticles with a variety of medicinal plants.

V. CONCLUSION

The world population is expected to reach 9.6 billion or more in 2050. To feed this ever-increasing population of the world, more pressure will be on land, which cannot be extended further. Conventional fertilizers might not be helpful in this situation as these have become expensive due to high energy requirements and being environmentally unsafe. Nanobiotechnology in the agricultural field has been as effective as in any other field and use of metal nanomaterials is allowing us to advance in the agricultural sector and providing alternatives to conventional practices. Biofertilizers based on nanotechnology are quite useful for plant growth and development. In our study, the efficacy of silver nanoparticles was increased by synthesizing them from a combination of medicinal plants i.e. *E. camaldulensis, A. indica, and E. heterophylla*. This property of combined leaf extract NPs can be used to maintain a healthy population of *A. doliolum* which in turn can increase crop production in paddy fields and also helps to maintain microflora of soil. Many researchers have clearly reported that higher concentrations of various NPs/NMs are toxic for plant growth, which is ultimately dependent on their particle size. So, a checkpoint might be worked out in future studies to elucidate the critical concentration of certain NPs/NMs with a particular size and possible combinations might be searched out. So, for environmental sustainability, green NMs/NPs could also be utilized as a source of nutrients for the crops and could play a key role in greener nano nutrition.

VI. ACKNOWLEDGEMENT

The authors acknowledge the funding provided by the Department of Biotechnology (DBT), Ministry of Science and technology, Star College Scheme. The authors thank the Principal, Gargi College, and the University of Delhi for providing the necessary facilities. The authors also wish to acknowledge the University Science Instrumentation facility, Delhi University, Department of Physics, Gargi College, Banaras Hindu University, Central Research Facility, IIT Delhi, and Central Instrumentation Facility, Jamia Millia Islamia for providing the characterization facilities.

REFERENCES

Adebayo-Tayo, B., Salaam, A., & Ajibade, A. (2019). Green synthesis of silver nanoparticles using Oscillatoria sp. extract, its antibacterial, antibiofilm potential and cytotoxicity activity. *Heliyon*, *5*(10), e02502. https://doi.org/10.1016/j.heliyon.2019.e02502

Ahmed, S., Saifullah, Ahmad, M., Swami, B. L., & Ikram, S. (2016). Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. *Journal of Radiation Research and Applied Sciences*, *9*(1), 1–7. https://doi.org/10.1016/j.jrras.2015.06.006

Alghoraibi, I., Soukkarieh, C., Zein, R., Alahmad, A., Walter, J. G., & Daghestani, M. (2020). Aqueous extract ofEucalyptus camaldulensisleaves as reducing and capping agent in biosynthesis of silver nanoparticles. *Inorganic and Nano-Metal Chemistry*, *50*(10), 895–902. https://doi.org/10.1080/24701556.2020.1728315

Anandalakshmi, K., Venugobal, J., & Ramasamy, V. (2015). Characterization of silver nanoparticles by green synthesis method using Pedalium murex leaf extract and their antibacterial activity. *Applied Nanoscience*, *6*(3), 399–408. https://doi.org/10.1007/s13204-015-0449-z

Atarod, M., Nasrollahzadeh, M., & Sajadi, S. M. (2015). Green synthesis of a Cu/reduced graphene oxide/Fe3O4 nanocomposite using Euphorbia wallichii leaf extract and its application as a recyclable and heterogeneous catalyst for the reduction of 4-nitrophenol and rhodamine B. *RSC Advances*, *5*(111), 91532–91543. https://doi.org/10.1039/c5ra17269a

Aziz, N., Faraz, M., Sherwani, M. A., Fatma, T., & Prasad, R. (2019). Illuminating the Anticancerous Efficacy of a New Fungal Chassis for Silver Nanoparticle Synthesis. *Frontiers in Chemistry*, *7*. https://doi.org/10.3389/fchem.2019.00065

Banerjee, P., Satapathy, M., Mukhopahayay, A., & Das, P. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing*, *1*(1). https://doi.org/10.1186/s40643-014-0003-y

Bashir, T., & QureshiI, M. Z. (2015). Phytosynthesis of Silver Nanaoparticles using E. camaldulensis leaf extractand their characterization. *Journal of the Chilean Chemical Society*, *60*(1), 2861–2863. https://doi.org/10.4067/s0717-97072015000100019

Castillo-Henríquez, L., Alfaro-Aguilar, K., Ugalde-Álvarez, J., Vega-Fernández, L., Montes De Oca-Vásquez, G., & Vega-Baudrit, J. R. (2020). Green Synthesis of Gold and Silver Nanoparticles from Plant Extracts and Their Possible Applications as Antimicrobial Agents in the Agricultural Area. *Nanomaterials*, *10*(9), 1763. https://doi.org/10.3390/nano10091763

Costa, M. V. J., & Sharma, P. K. (2016). Effect of copper oxide nanoparticles on growth, morphology, photosynthesis, and antioxidant response in Oryza sativa. *Photosynthetica*, *54*(1), 110–119. https://doi.org/10.1007/s11099-015-0167-5

Deka, N. J., Nath, R., Tamuly, S., Hazorika, M., Pegu, S. R., & Deka, S. M. (2021). Green synthesis and characterization of silver nanoparticles using leaves extract of Neem (Azadirachta indica L.) and assessment of its in vitro antioxidant and antibacterial activity. *Annals of Phytomedicine: An International Journal*, *10*(1). https://doi.org/10.21276/ap.2021.10.1.17

Desai, P. P., Prabhurajeshwar, C., & Chandrakanth, K. R. (2016). Hydrothermal assisted biosynthesis of silver nanoparticles from Streptomyces sp. GUT 21 (KU500633) and its therapeutic antimicrobial activity. *Journal of Nanostructure in Chemistry*, *6*(3), 235–246. https://doi.org/10.1007/s40097-016-0197-y

Dipankar, C., & Murugan, S. (2012). The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from Iresine herbstii leaf aqueous extracts. *Colloids and Surfaces B: Biointerfaces*, *98*, 112–119. https://doi.org/10.1016/j.colsurfb.2012.04.006

Ditta, A., Arshad, M., & Ibrahim, M. (2015). Nanoparticles in Sustainable Agricultural Crop Production: Applications and Perspectives. *Nanotechnology and Plant Sciences*, 55–75. https://doi.org/10.1007/978-3-319-14502-0_4

Dogan, G., Kara, N., Bagci, E., & Gur, S. (2017). Chemical composition and biological activities of leaf and fruit essential oils from Eucalyptus camaldulensis. *Zeitschrift Für Naturforschung C*, *72*(11–12), 483–489. https://doi.org/10.1515/znc-2016-0033

Dolatabadi, S., Shokufe, E., Elnaz, M., & Rahele, Z. (2017). Green synthesis and antibacterial effect of silver nanoparticles using Eucalyptus camaldulensis. *Journal of Neyshabur University of Medical Sciences*, *5*(3), 74–85. http://journal.nums.ac.ir/article-1-398-en.html

Elshamy, A. I., Abd-ElGawad, A. M., El Gendy, A. E. G., & Assaeed, A. M. (2019). Chemical Characterization of Euphorbia heterophylla L. Essential Oils and Their Antioxidant Activity and Allelopathic Potential on Cenchrus echinatus L. *Chemistry & Biodiversity*, *16*(5), e1900051. https://doi.org/10.1002/cbdv.201900051

Erjaee, H., Rajaian, H., & Nazifi, S. (2017). Synthesis and characterization of novel silver nanoparticles using Chamaemelum nobile extract for antibacterial application. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, *8*(2), 025004. https://doi.org/10.1088/2043-6254/aa690b

Fagier, M. A. (2021). Plant-Mediated Biosynthesis and Photocatalysis Activities of Zinc Oxide Nanoparticles: A Prospect towards Dyes Mineralization. *Journal of Nanotechnology*, *2021*, 1–15. https://doi.org/10.1155/2021/6629180

Goudarzi, M., Mir, N., Mousavi-Kamazani, M., Bagheri, S., & Salavati-Niasari, M. (2016). Biosynthesis and characterization of silver nanoparticles prepared from two novel natural precursors by facile thermal decomposition methods. *Scientific Reports*, *6*(1). https://doi.org/10.1038/srep32539

Gruere, G., Narrod, C., & Abbott, L. (2011). Agricultural, food, and water nanotechnologies for the poor: Opportunities, constraints, and role of the Consultative Group on International Agricultural Research. International Food Policy Research Institute. https://ideas.repec.org/p/fpr/ifprid/1064.html

Hamida, R. S., Abdelmeguid, N. E., Ali, M. A., Bin-Meferij, M. M., & Khalil, M. I. (2020). Synthesis of Silver Nanoparticles Using a Novel Cyanobacteria *Desertifilum* sp. extract: Their Antibacterial and Cytotoxicity Effects. *International Journal of Nanomedicine*, *Volume 15*, 49–63. https://doi.org/10.2147/ijn.s238575

Hemlata, Meena, P. R., Singh, A. P., & Tejavath, K. K. (2020). Biosynthesis of Silver Nanoparticles Using Cucumis prophetarum Aqueous Leaf Extract and Their Antibacterial and Antiproliferative Activity Against Cancer Cell Lines. *ACS Omega*, *5*(10), 5520–5528. https://doi.org/10.1021/acsomega.0c00155

Holmes, A. H., Moore, L. S. P., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P. J., & Piddock, L. J. V. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet*, *387*(10014), 176–187. https://doi.org/10.1016/s0140-6736(15)00473-0

Iravani, S., Korbekandi, H., Mirmohammadi, S. V., & Zolfaghari, B. (2014). Synthesis of silver nanoparticles: chemical, physical and biological methods. *Research in Pharmaceutical Sciences*, *9*(6), 385–406. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4326978/

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., & Kopriva, S. (2017). The Role of Soil Microorganisms in Plant Mineral Nutrition—Current Knowledge and Future Directions. *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.01617

Jain, S., & Mehata, M. S. (2017). Medicinal Plant Leaf Extract and Pure Flavonoid Mediated Green Synthesis of Silver Nanoparticles and their Enhanced Antibacterial Property. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-15724-8

Javed, Z., Dashora, K., Mishra, M., D. Fasake, V., & Srivastva, A. (2019). Effect of accumulation of nanoparticles in soil health-a concern on future. *Frontiers in Nanoscience and Nanotechnology*, *5*(2). https://doi.org/10.15761/fnn.1000181

Joshi, N., Jain, N., Pathak, A., Singh, J., Prasad, R., & Upadhyaya, C. P. (2018). Biosynthesis of silver nanoparticles using Carissa carandas berries and its potential antibacterial activities. *Journal of Sol-Gel Science and Technology*, *86*(3), 682–689. https://doi.org/10.1007/s10971-018-4666-2

Khandel, P., Yadaw, R. K., Soni, D. K., Kanwar, L., & Shahi, S. K. (2018). Biogenesis of metal nanoparticles and their pharmacological applications: present status and application prospects. *Journal of Nanostructure in Chemistry*, *8*(3), 217–254. https://doi.org/10.1007/s40097-018-0267-4

Kulkarni, N., & Muddapur, U. (2014). Biosynthesis of Metal Nanoparticles: A Review. *Journal of Nanotechnology*, *2014*, 1–8. https://doi.org/10.1155/2014/510246

Lara, H. H., Ayala-Núñez, N. V., Ixtepan Turrent, L. D. C., & Rodríguez Padilla, C. (2009). Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. *World Journal of Microbiology and Biotechnology*, *26*(4), 615–621. https://doi.org/10.1007/s11274-009-0211-3

Li, X., Xu, H., Chen, Z. S., & Chen, G. (2011). Biosynthesis of Nanoparticles by Microorganisms and Their Applications. *Journal of Nanomaterials*, *2011*, 1–16. https://doi.org/10.1155/2011/270974

Lingaraju, K., Raja Naika, H., Nagabhushana, H., Jayanna, K., Devaraja, S., & Nagaraju, G. (2020). Biosynthesis of Nickel oxide Nanoparticles from Euphorbia heterophylla (L.) and their biological application. *Arabian Journal of Chemistry*, *13*(3), 4712–4719. https://doi.org/10.1016/j.arabjc.2019.11.003

Mallikarjuna, K., Narasimha, G., Dillip, G. R., Praveen, B., Shreedhar, B., ShreeLakshmi, C., Reddy, B. V. S., & Deva Prasad Raju, B. (2011). Green Synthesis of silver nanoparticles using Ocimum leaf extract and their characterization. *Digest Journal of Nanomaterials and Biostructures*, *6*(1), 181–186.

Manikandan, R., Beulaja, M., Thiagarajan, R., Palanisamy, S., Goutham, G., Koodalingam, A., Prabhu, N., Kannapiran, E., Basu, M. J., Arulvasu, C., & Arumugam, M. (2017). Biosynthesis of silver nanoparticles using aqueous extract of Phyllanthus acidus L. fruits and characterization of its anti-inflammatory effect against H 2 O 2 exposed rat peritoneal macrophages. *Process Biochemistry*, *55*, 172–181. https://doi.org/10.1016/j.procbio.2017.01.023

Masum, M. M. I., Siddiqa, M. M., Ali, K. A., Zhang, Y., Abdallah, Y., Ibrahim, E., Qiu, W., Yan, C., & Li, B. (2019). Biogenic Synthesis of Silver Nanoparticles Using Phyllanthus emblica Fruit Extract and Its Inhibitory Action Against the Pathogen Acidovorax oryzae Strain RS-2 of Rice Bacterial Brown Stripe. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.00820

Mirzajani, F., Askari, H., Hamzelou, S., Farzaneh, M., & Ghassempour, A. (2013). Effect of silver nanoparticles on Oryza sativa L. and its rhizosphere bacteria. *Ecotoxicology and Environmental Safety*, *88*, 48–54. https://doi.org/10.1016/j.ecoenv.2012.10.018

Nagar, N., Jain, S., Kachhawah, P., & Devra, V. (2016). Synthesis and characterization of silver nanoparticles via green route. *Korean Journal of Chemical Engineering*, *33*(10), 2990–2997. https://doi.org/10.1007/s11814-016-0156-9

Otunola, G. A., & Afolayan, A. J. (2018). In vitro antibacterial, antioxidant and toxicity profile of silver nanoparticles green-synthesized and characterized from aqueous extract of a spice blend formulation. *Biotechnology & Biotechnological Equipment*, *32*(3), 724–733. https://doi.org/10.1080/13102818.2018.1448301

Pirtarighat, S., Ghannadnia, M., & Baghshahi, S. (2018). Green synthesis of silver nanoparticles using the plant extract of Salvia spinosa grown in vitro and their antibacterial activity assessment. *Journal of Nanostructure in Chemistry*, *9*(1), 1–9. https://doi.org/10.1007/s40097-018-0291-4

Ponarulselvam, S., Panneerselvam, C., Murugan, K., Aarthi, N., Kalimuthu, K., & Thangamani, S. (2012). Synthesis of silver nanoparticles using leaves of Catharanthus roseus Linn. G. Don and their antiplasmodial activities. *Asian Pacific Journal of Tropical Biomedicine*, *2*(7), 574–580. https://doi.org/10.1016/s2221-1691(12)60100-2

Qayyum, S., Oves, M., & Khan, A. U. (2017). Obliteration of bacterial growth and biofilm through ROS generation by facilely synthesized green silver nanoparticles. *PLOS ONE*, *12*(8), e0181363. https://doi.org/10.1371/journal.pone.0181363

Rajeshkumar, S., & Malarkodi, C. (2014). In Vitro Antibacterial Activity and Mechanism of Silver Nanoparticles against Foodborne Pathogens. *Bioinorganic Chemistry and Applications*, *2014*, 1–10. https://doi.org/10.1155/2014/581890

Rajeshkumar, S., Malarkodi, C., Paulkumar, K., Vanaja, M., Gnanajobitha, G., & Annadurai, G. (2014). Algae Mediated Green Fabrication of Silver Nanoparticles and Examination of Its Antifungal Activity against Clinical Pathogens.

International Journal of Metals, *2014*, 1–8. https://doi.org/10.1155/2014/692643

Rauwel, P., Küünal, S., Ferdov, S., & Rauwel, E. (2015). A Review on the Green Synthesis of Silver Nanoparticles and Their Morphologies Studied via TEM. *Advances in Materials Science and Engineering*, *2015*, 1–9. https://doi.org/10.1155/2015/682749

Razzaq, A., Ammara, R., Jhanzab, H. M., Mahmood, T., Hafeez, A., & Hussain, S. (2016). A Novel Nanomaterial to Enhance Growth and Yield of Wheat. *Journal of Nanoscience and Technology*, *2*(1), 55–58. http://www.jacsdirectory.com/jnst

Roy, P., Das, B., Mohanty, A., & Mohapatra, S. (2017). Green synthesis of silver nanoparticles using Azadirachta indica leaf extract and its antimicrobial study. *Applied Nanoscience*, *7*(8), 843–850. https://doi.org/10.1007/s13204-017-0621-8

Salehi, B., Sharifi-Rad, J., Quispe, C., Llaique, H., Villalobos, M., Smeriglio, A., Trombetta, D., Ezzat, S. M., Salem, M. A., Zayed, A., Salgado Castillo, C. M., Yazdi, S. E., Sen, S., Acharya, K., Sharopov, F., & Martins, N. (2019). Insights into Eucalyptus genus chemical constituents, biological activities and health-promoting effects. *Trends in Food Science & Technology*, *91*, 609–624. https://doi.org/10.1016/j.tifs.2019.08.003

Saxena, J., Sharma, P. K., Sharma, M. M., & Singh, A. (2016). Process optimization for green synthesis of silver nanoparticles by Sclerotinia sclerotiorum MTCC 8785 and evaluation of its antibacterial properties. *SpringerPlus*, *5*(1). https://doi.org/10.1186/s40064-016-2558-x

Shameli, K., Ahmad, M., Shabanzadeh, P., Zamanian, A., Sangpour, P., Abdollahi, Y., & Mohsen, Z. (2012). Green biosynthesis of silver nanoparticles using Curcuma longa tuber powder. *International Journal of Nanomedicine*, 5603. https://doi.org/10.2147/ijn.s36786

Shang, Y., Hasan, M. K., Ahammed, G. J., Li, M., Yin, H., & Zhou, J. (2019). Applications of Nanotechnology in Plant Growth and Crop Protection: A Review. *Molecules*, *24*(14), 2558. https://doi.org/10.3390/molecules24142558

Sila, M. J., Nyambura, M. I., Abong'o, D. A., Mwaura, F. B., & Iwuoha, E. (2019). Biosynthesis of Silver Nanoparticles from *Eucalyptus corymbia* Leaf Extract at Optimized Conditions. *Nano Hybrids and Composites*, *25*, 32–45. https://doi.org/10.4028/www.scientific.net/nhc.25.32

Singh, A., Gautam, P. K., Verma, A., Singh, V., Shivapriya, P. M., Shivalkar, S., Sahoo, A. K., & Samanta, S. K. (2020a). Green synthesis of metallic nanoparticles as effective alternatives to treat antibiotics resistant bacterial infections: A review. *Biotechnology Reports*, *25*, e00427. https://doi.org/10.1016/j.btre.2020.e00427

Singh, A., Singh, N. B., Hussain, I., Singh, H., & Singh, S. C. (2015). Plant-nanoparticle interaction: An approach to improve agricultural practices and plant productivity. *International Journal of Pharmaceutical Science Invention*, *4*(8), 25–40. http://ijpsi.org/Papers/Vol4(8)/E0480025040.pdf

Singh, G., Babele, P. K., Shahi, S. K., Sinha, R. P., Tyagi, M. B., & Kumar, A. (2014). Green Synthesis of Silver Nanoparticles Using Cell Extracts of Anabaena doliolum and Screening of Its Antibacterial and Antitumor Activity. *Journal of Microbiology and Biotechnology*, *24*(10), 1354–1367. https://doi.org/10.4014/jmb.1405.05003

Singh, S., Tripathi, D. K., Dubey, N. K., & Chauhan, D. K. (2016). Effects of Nano-Materials on Seed Germination and Seedling Growth: Striking the Slight Balance Between the Concepts and Controversies. *Materials Focus*, *5*(3), 195–201. https://doi.org/10.1166/mat.2016.1329

Singh, Y., Kaushal, S., & Sodhi, R. S. (2020b). Biogenic synthesis of silver nanoparticles using cyanobacterium Leptolyngbya sp. WUC 59 cell-free extract and their effects on bacterial growth and seed germination. *Nanoscale Advances*, *2*(9), 3972–3982. https://doi.org/10.1039/d0na00357c

Tajbakhsh, M., Alinezhad, H., Nasrollahzadeh, M., & Kamali, T. A. (2016). Green synthesis of the Ag/HZSM-5 nanocomposite by using Euphorbia heterophylla leaf extract: A recoverable catalyst for reduction of organic dyes. *Journal of Alloys and Compounds*, *685*, 258–265. https://doi.org/10.1016/j.jallcom.2016.05.278

Tripathi, D. K., Tripathi, A., Shweta, Singh, S., Singh, Y., Vishwakarma, K., Yadav, G., Sharma, S., Singh, V. K., Mishra, R. K., Upadhyay, R. G., Dubey, N. K., Lee, Y., & Chauhan, D. K. (2017). Uptake, Accumulation and Toxicity of Silver Nanoparticle in Autotrophic Plants, and Heterotrophic Microbes: A Concentric Review. *Frontiers in Microbiology*, *08*. https://doi.org/10.3389/fmicb.2017.00007

Umoren, S., Eduok, U., Solomon, M., & Udoh, A. (2016). Corrosion inhibition by leaves and stem extracts of Sida acuta for mild steel in 1M H2SO4 solutions investigated by chemical and spectroscopic techniques. *Arabian Journal of Chemistry*, *9*, S209–S224. https://doi.org/10.1016/j.arabjc.2011.03.008

Vanlalveni, C., Lallianrawna, S., Biswas, A., Selvaraj, M., Changmai, B., & Rokhum, S. L. (2021). Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: a review of recent literature. *RSC Advances*, *11*(5), 2804–2837. https://doi.org/10.1039/d0ra09941d

Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: present situation and prospects for the future. *International Journal of Nanomedicine*, *Volume 12*, 1227–1249. https://doi.org/10.2147/ijn.s121956

Yan, A., & Chen, Z. (2019). Impacts of Silver Nanoparticles on Plants: A Focus on the Phytotoxicity and Underlying Mechanism. *International Journal of Molecular Sciences*, *20*(5), 1003. https://doi.org/10.3390/ijms20051003

***