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Green fabrication and characterization of silver nanoparticles from leaf and bark extracts of *Alstonia* sp.

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Abstract- In this study, leaf extracts of *Alstonia scholaris* (L.) R.Br (E3)., *Alstonia macrophylla* Wall (E1), and *Alstonia venenata* R.Br.(E2) were used to compare by synthesis and characterization of silver nanoparticles from them. Characterization of synthesized nanoparticles were primarily done by significant colour changes and silver nanoparticle characteristic peak of 450 nm in UV-Visible Spectroscopy. Further confirmation were done using FTIR, particles size analysis and zeta potential. Morphology of the synthesized nanoparticles were imaged using Field emission scanning electron microscopy (FE-SEM). Active phytochemical constituents of the leaf extracts of *Alstonia scholaris* and *Alstonia macrophylla* were characterized using Gas Chromatography-Mass Spectrometry (GC-MS) method. The silver nanoparticles synthesized from three species of *Alstonia* showed similar pattern in FTIR spectroscopy with prominent peaks at 3291.33 cm^{-1} (E1), 3304.77 cm^{-1} (E3), and 3303.89 cm^{-1} (E2), 1637.26 cm^{-1} (E1), 1637.15 cm^{-1}(E3), 1637.17 cm^{-1} (E2) 1065.84 cm^{-1} (E1), 1065.86 (E3), 1065.95 (E2) and 591.32 cm^{-1} (E1) 598.44 cm^{-1}, 585.14 cm^{-1}. Zeta potential and particle size analyzer showed Particle size range of 150-201nm and their zeta potential ranged from -19 to -22 mV. The GC-MS profiles of *Alstonia scholaris* and *Alstonia macrophylla* revealed several common phytoconstituents, proving that identical capping and reducing agents resulted in the emergence of similar peaks among the nanoparticles produced from their plant extract. Some phytoconstituents that were observed in both the plant extracts were lupeol, stigmasterol, campesterol, 2(4H)-Benzofuranone 5, 6, 7, 7a-tetrahydro-4, 4, pentane,3-methyl, 7a trimethyl, (-)-Loliolide, ethane,1- chloro-1-fluoro Neophytadiene, Hexahydrofarnesyl acetone.

Key words: Green synthesis, silver nanoparticles, SEM, DLS, Fourier-transform infrared spectroscopy

INTRODUCTION

Nanobiotechnology, a subfield of nanoscience, has played a pivotal role in the 21st century in understanding a variety of challenges, especially in the domains of agriculture, medicine, and electronics. Nanoscience presents a fundamental scientific challenge since it needs control over atomic interactions. All physicochemical characteristics methods of synthesis of nanomaterials have intrinsic constraints that provide a significant barrier to the development of this field of study. The prospect of using biological materials for nanoparticle synthesis has emerged as the most cost-effective and environmentally friendly method.[1]

Physical, chemical, and biological properties of nanomaterials differ significantly from those of their bulk equivalents.[2] Although physical and chemical approaches are more prevalent for nanoparticle manufacturing,[3] the usage of hazardous substances restricts their uses.[4] In fact, plants, algae, fungi, bacteria, and viruses have been employed to produce metallic nanoparticles in recent years.[5] Eco-friendly and non-toxic, green synthesis of metallic nanoparticles from plants is an intriguing aspect.[6]

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When compared to the raw materials, NPs reveal remarkable features. In terms of environmental protection, this is considered one of the most major motivations for the reliance on the usage of NPs over raw materials.[7] The surface to volume ratio of NPs is greater; as the particle's surface area increases, the particle's radius drops. This is one of the favourable qualities of NPs for drug delivery in the medical industry.[8] The ratio of surface to volume, which determines the surface chemistry, has led in more applications for AgNPs than for other metallic NPs.[9] When the NPs' stability is high, they can be employed as an effective antibacterial agent.[10] This stability is a result of the zeta potential, which offers NPs electrostatic mobility; this enables significant applications in pollution management or removal of any environmental pollutant.

Various kinds and types of metallic NPs have been utilised in a variety of fields. The group of noble metals includes gold (Au), silver (Ag), and platinum (Pt), while the group of metal oxides includes silicon oxide (SiO_2) and zinc oxide (ZnO).[11] Among the metal NPs, silver nanoparticles (AgNPs) have the broadest range of applications, and numerous research groups are now investigating additional uses for AgNPs. Ag is regarded one of the earth's fundamental elements because it exists naturally, and Ag is harder than Au. The thermal and electrical conductivity of AgNPs is superior than that of Au. Metallic Ag is currently utilised in numerous surgical operations, as well as fungicides and preservatives. Consequently, AgNPs have garnered greater interest than other metallic NPs.

Alstonia scholaris (L.) R.Br. (Apocynaceae) is a tropical evergreen tree indigenous to the Indian subcontinent and South East Asia with greyish, rough bark and milky, alkaloid-rich sap. The bark, also known as dita bark, is utilised by various ethnic groups of Northeast India and other areas of the world as an antibacterial agent fighting fungal infections, malarial fever, toothache, rheumatism, snake bite, diarrhoea, etc. The latex is used to cure coughs, oral sores, and fever.[12,13] Only the *scholaris* species of the *Alstonia* genus has been investigated for antibacterial activity.[14] Silver has long been known to suppress the growth of certain bacterial strains and germs prevalent in medical and industrial settings.[15] Numerous attempts to employ silver nanoparticles as an anticancer agent have all shown favourable results.[16,17]

Biota with the single most powerful biocidal properties include bacteria, viruses, fungi, algae, and higher nematodes and helminths. In addition, it is non-toxic to animal cells and compatible with human cell lines, providing numerous applications for biological products.[18] There are reports of silver nanoparticles synthesized from leaf and bark extracts of *Alstonia scholaris* (L.) R.Br. that have showed substantial antibacterial activity against various pathogenic microbes. This study aims to further compare the synthesis and characterization of nanoparticles from leaf of *Alstonia scholaris* (L.) R.Br., *Alstonia macrophylla* Wall, and *Alstonia venenata* R.Br.

MATERIALS & METHOD

Preparation of plant extract

All of the chemicals were supplied by Merck. The leaves of *Alstonia macrophylla* Wall (E1), Alstonia *venenata* R.Br. (E2) and *Alstonia scholaris* (L.) R.Br. (E3) were collected from Rambagh, Bengaluru. The leaves were shade dried and powdered using electric grinder. Extract of each sample were prepared by mixing 0.5 gm of sample with 50 ml of distilled water. This mixture was boiled for 15 minutes before being chilled. Mixture was left for 25 hours shaking at 200 rpm. Whatman No. 1 filter paper was used to filter the incubated solution.

Biosynthesis of Silver nanoparticles

To synthesize AgNPs, 2ml of plant extract was added to a 98ml (1mM) aqueous solution of $AgNO_3$ and the mixture was heated to 80°C. The reduction of metal ions was initially established by observing a shift in hue from light brown to dark brown, followed by spectrophotometric absorption between 200 and 800 nm. Several reaction parameters, including the amount of plant extract, the concentration of the silver nitrate solution, and the reaction time, were altered to increase the nanoparticle yield. During the reaction time, spectrophotometric absorption and DLS measurements were taken for each trial. The reaction medium containing AgNPs was centrifuged for 15 minutes at 10000rpm to eliminate unreacted extract from the final solution.

Characterization of Synthesized Nanoparticles

UV–Vis spectroscopy (UV-1900, 190-110 nm) was used to evaluate the reduction of Ag ions. For the morphology, SEM (JEOL JSM-7500F SEM) with EDX was used. The biomolecules were identified using FTIR spectroscopy (Bruker Alpha II) and the XRD was used to identify the crystalline structure of the AgNPs.

[A] UV-Vis absorption spectra

Characteristic spectra were acquired using a double beam spectrophotometer 1800 (Shimadzu, Tokyo, Japan) in the wavelength range of 300-600 nm with a resolution of 2 nm.

[B] Dynamic light scattering (DLS)

Analyzer for measuring the particle size and zeta potential of manufactured nanoparticles. The aqueous suspension of the produced nanoparticles was filtered via a 0.22-lm syringe-driven filter unit, and the size and dispersion of the nanoparticles were determined using the dynamic light scattering method (Nanopartical, HORIBA, SZ-100)

[C] Fourier Transform Infrared (FTIR) Spectroscopy

A Fourier transform infrared (FTIR) spectrum was obtained using an FTIR-8400 spectrophotometer (Shimadzu, Tokyo, Japan) with a resolution of 4 cm^{-1} over the range of 500 to 4000 cm^{-1}. The materials were formed into pellets by combining potassium bromide (KBr) as a binding agent.

[D] Field Emission-Scanning Electron Microscopy (FE-SEM)

The surface morphology was evaluated using field emission-scanning electron microscopy (FE-SEM) (Hitachi S-4800, Tokyo, Japan) and energy dispersive X-ray spectroscopy (EDAX) for the elemental analysis. Before viewed under a microscope, the samples were dispersed in ethanol and placed on specimen tubs with a gold coating.

Characterization of the Extract

Extract characterization was done using Gas Chromatography-Mass Spectrometry (GC-MS) technique. Sample was diluted in Methanol and injected into a GC-MS QP2010 model (Shimadzu®), Column, GC, SH-I-5 Sil MS Capillary, 30m x 0.25mm x 0.01um, with Splitless injection mode. The GC–MS operating conditions for the analysis were as follows: 45°C for 2 minutes, then 140°C at a rate of 5°C per minute, then 280°C and kept isothermally for 10 minutes. The sample injection volume was 2 L, and the carrier gas was 1 mL/min of helium. The components of the sample were ionised with 70 eV. The duration of the GC ranged from 9.10 minutes to 52.0 minutes. The NIST14.L library (2020) was then queried to compare the compound structures to the NIST database. The compounds were subsequently identified by comparing their retention periods and mass spectra to those of previously identified compounds in the NIST library (C:DatabaseNIST14.L).

RESULT & DISCUSSION

Surface plasmon oscillations in aqueous media give silver nanoparticles (AgNPs) a yellowish-brown appearance. The solution's color altered from pale light to yellowish brown to reddish brown to colloidal brown when various leaf extracts were added to the aqueous silver nitrate solution, all of which are indicative of AgNP production.[19] Previous research has also validated the completeness of the interaction between leaf extract and by seeing similar shifts in color.[20-24] Figure 1(A-E) displays the colour change observed after 15 min, 30 min, 45 min, 60 min, and 24 hours following the start of the process. Figure 1 (F-H) displays the UV-vis spectra acquired for nanoparticles synthesized from leaves of *Alstonia macrophylla* (E1), *Alstonia venenata* (E2) and *Alstonia scholaris* (E3). Due to their surface plasmon resonance, AgNPs generated in reaction media have absorption maxima between 425 and 475 nm in their absorption spectra.

UV visible spectroscopy was employed to understand the formation of nanoparticles through the change of colour of AgNO solution from colourless to dark brown. Excitation of surface Plasmon resonance and reduction of Ag+ to Ag0 are the two major causes for the change of colour of solution (shown in figure 1(A-E)). Temperature, time and stirring accelerated the reaction. Many studies reported the characteristic peak of AgNPs in the range of 400- 450 nm which was present in all the three synthesized nanoparticles E1, E2 and E3, with a peak at 440 nm observed in UV spectrum as shown in figure 1(F-H). The UV-vis spectra also showed that AgNPs formed fast within the first 15 minutes and were stable in solution for up to 24 hours after the reaction had finished.

Characterization using DLS

The polydispersity index, particle size, and size distribution profile of the colloidal solution of ASPL nanoparticles were all measured using dynamic light scattering (DLS). The potential stability of molecules in colloidal suspension was shown by negatively charged plant nanoparticles with negative zeta potential values. Figure 2A-C (A1, A2, B1, B2, C1 and C2) shows particle size distribution and Zeta potential for silver nanoparticles synthesized from leaf extracts of *Alstonia macrophylla* (leaf), *Alstonia scholaris* (leaf) and *Alstonia venenata* (leaf). Particle size range is between150-201nm and their zeta potential ranged from -19 to -22 mV among 3 NPs.

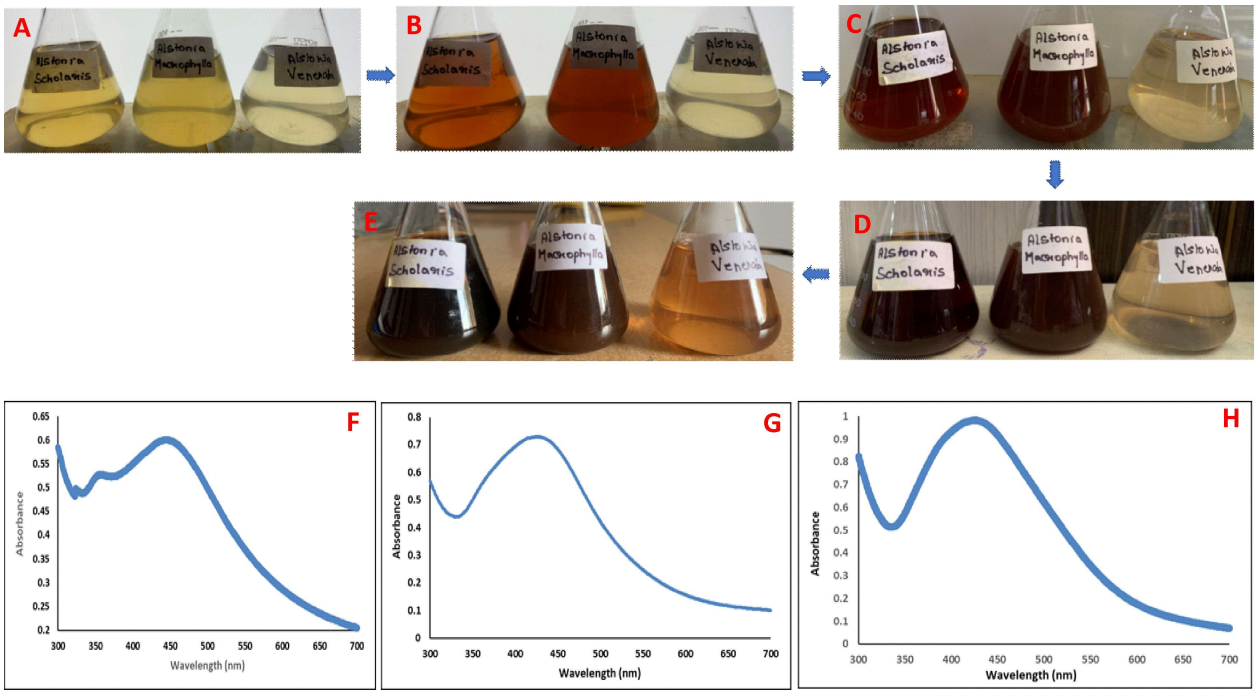


Fig 1: Synthesis of AgNPs using leaves of the plant *Alstonia* and its various species. Colour change during Nanoparticles synthesis at [A]15 min [B] 30 min [C] 45 min [D] 60 min [E] 24 hours. UV–vis absorption spectrum of silver nanoparticles using green synthesis method. [F]E1-*Alstonia macrophylla* (leaf) [G]E3-*Alstonia scholaris* (leaf) and [H] E2- *Alstonia venenata* (leaf)

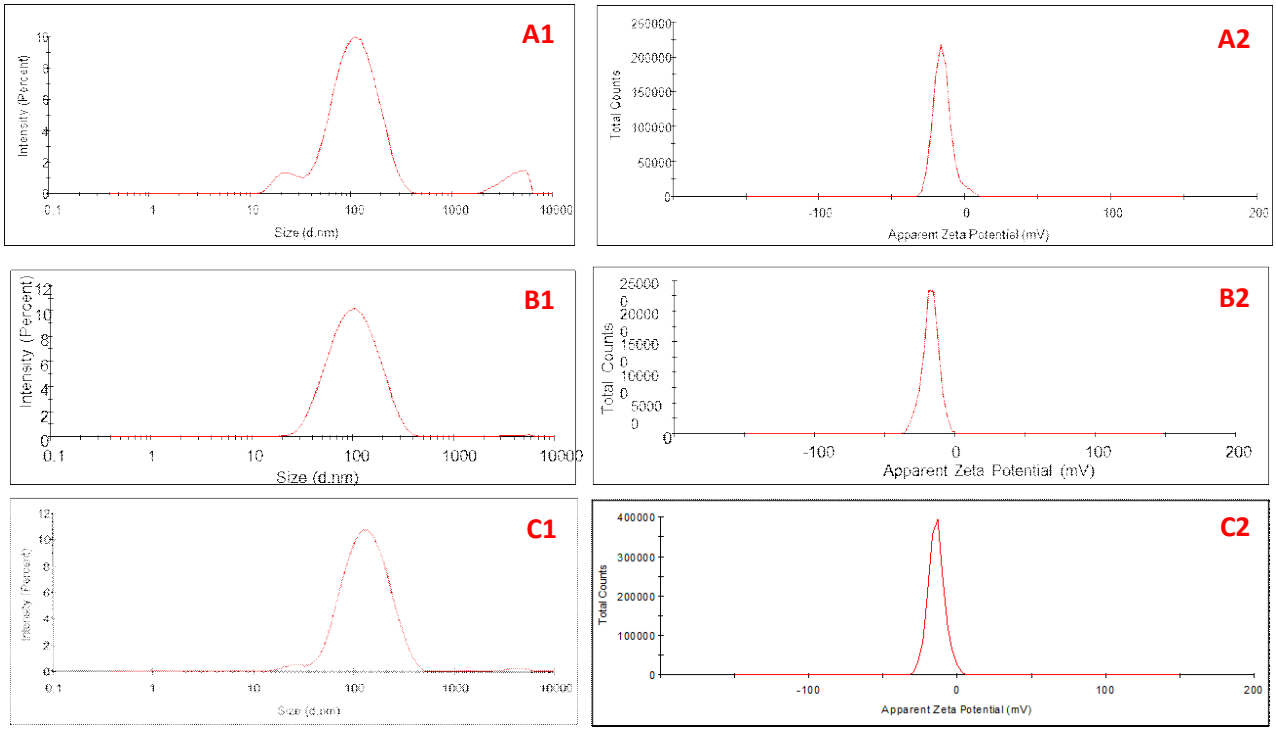
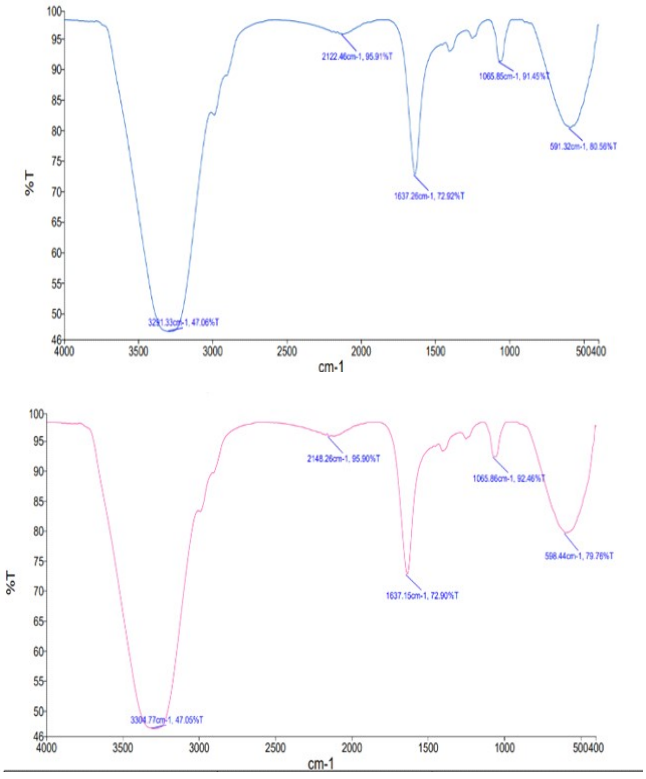


Fig. 2: Graph showing particle size distribution and Zeta potential for silver nanoparticles AgNPs synthesized from leaf extracts of [A1- A2] E1-*Alstonia macrophylla* (leaf) [B1-B2]E3-*Alstonia scholaris* (leaf) and [C1-C2] E2-*Alstonia venenata* (leaf) with labelled prominent peaks

Characterization using FTIR

FTIR spectroscopy was used to identify the various functional groups contributing to the production of the AgNPs seen in Figure 3. Bonding structures generated between metal particles and biomolecules may be analyzed via spectrum of FTIR.[25] This FTIR analysis has been used to examine how several phytochemicals contribute to the reduction of inorganic silver nitrate to silver.[26] Nanoparticles from all the three plants E1, E2 and E3 showed similar pattern having significant bands of absorbance at around 3291.33, 2122.46, 1637.26, 1065.84, 591.32 in E1, 3304.77, 2148.26, 1637.15, 1065.86, 598.44 for E3 and 3303.89, 2144.59, 1637.17, 1065.95, 585.14 for E2. The AgNPs formed by using the *Alstonia* leaf extract have a peak at 3291.33 cm^{-1} (E1), 3304.77 cm^{-1} (E3), and 3303.89 cm^{-1} (E2) that indicates the O-H stretch of the phenolic group, 1637.26 cm^{-1} (E1), 1637.15 cm^{-1} (E3), 1637.17 cm^{-1} (E2) corresponds to the C=O stretch of the ketone group, C-O stretch was assigned at 1065.84 cm^{-1} (E1), 1065.86 (E3), 1065.95 (E2) and C-Cl alkyl halide group has a peak at 591.32 cm^{-1} (E1) 598.44 cm^{-1}, 585.14 cm^{-1}. As a result, silver nanoparticles' surfaces often include phenolic and carbonyl chemicals. Flavonoids, alkaloids, and polyphenols, all of which are water-soluble, provided these chemicals. These bands agree with earlier studies.[27-29]



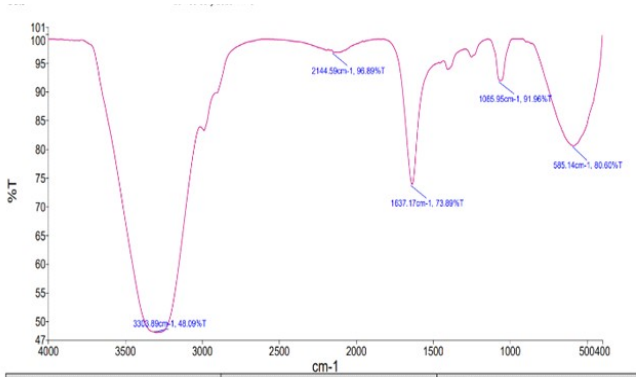


Fig 3: FTIR spectrums of AgNPs synthesized from leaf extracts of [A] E1-*Alstonia macrophylla* (leaf) [B]E3-*Alstonia scholaris* (leaf) and [C] E2- *Alstonia venenata* (leaf) with labelled prominent peaks

Characterization using FE-SEM

The form and size features of the produced nanoparticles have been further elucidated with the use of FESEM as shown in figure 4 [A-C]. It is found that the silver nanoparticles synthesized were mostly spherical in shape and the size is found as 100 nm for *Alstonia macrophylla* (leaf) and *Alstonia scholaris* and 1µm for *Alstonia venenata* (leaf).[25,30] Because of their high surface tension and energy, nanoparticles were also found to exhibit agglomeration characteristics. It was observed that nanoparticles synthesized from three different species of *Alstonia* did not have high difference in morphology evident from the images shown in fig (4A-C). The phytochemicals possessed by three extracts were found to be similar as observed in GCMS profiles (fig 5), thus providing similar quantity and nature of reducing agents for synthesis of nanoparticles. Capping agents were very similar in three cases as the FTIR study, which showed a shift and a variation in peak area, corroborated this.

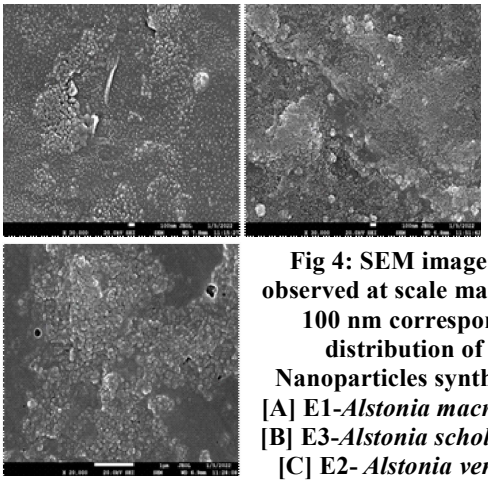
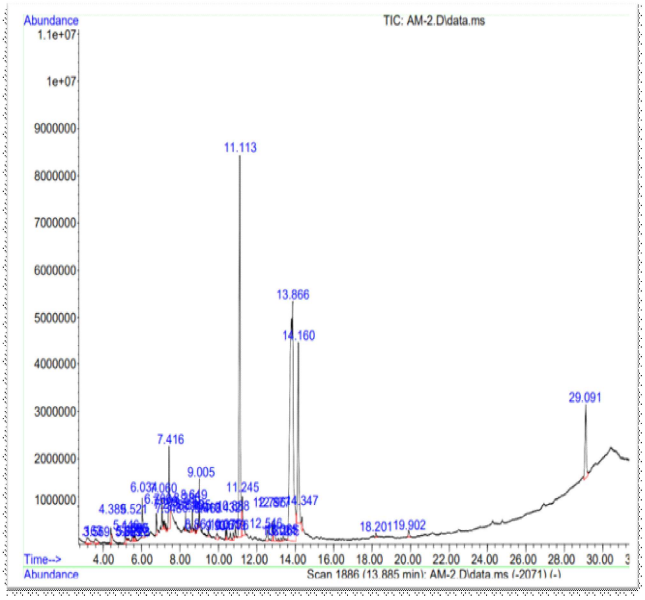


Fig 4: SEM images of AgNPs observed at scale magnification of 100 nm corresponding size distribution of AgNPs. Nanoparticles synthesized from [A] E1-*Alstonia macrophylla* (leaf) [B] E3-*Alstonia scholaris* (leaf) and [C] E2- *Alstonia venenata* (leaf)

Characterization of Extract using GC-MS

Extraction and characterization of phytoconstituents in leaf extracts of *Alstonia macrophylla* and *Alstonia scholaris* were evaluated using GC-MS technique. The total ion chromatogram (TIC) of the both extracts are shown in the figure 5(A-B). *Alstonia scholaris* extract showed presence of oxime-methoxy-phenyl, 2-methoxy 4-vinyl phenol, pentane,2-methyl-, 2(4H)-Benzofuranone 5, 6, 7, 7a-tetrahydro-4, 4, pentane,3-methyl-, 7a trimethyl, (-)-Loliolide, ethane,1- chloro-1-fluoro Neophytadiene, Hexahydrofarnesyl acetone, Phytol, 4, 8, 12, 16-Tetramethylheptadecan-4-olide, 1,3-propanediol, 2-(hydroxymethyl)-2-nitro-, 9,12-Octadecadienoic acid (Z,Z)-, γ-sitosterol, 24-methylenecycloartanol, lupeol, stigmasterol. The antiarthritic properties of *Alstonia scholaris* may be linked to its analgesic, anti-inflammatory, immunosuppressant, and anti-oxidant actions.[31] Previous studies have also reported the antiepileptic and sedative potential properties in the ethanolic extracts of the plant.[32] In another study it was revealed that the antivenom property of leaf extract of the plant and therefore highlighting their significance in snakebite treatment.[33] The extensive medicinal role of *A.scholaris* have been further explained by their *in-vitro* cytotoxic and antiproliferative properties.[34] His findings were also supported by Malick *et al.* in his study.[35]



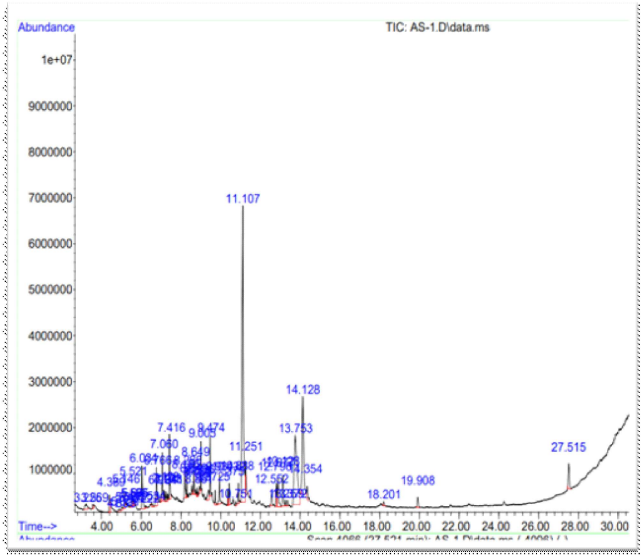


Fig 5: GC-MS Profile of leaf extracts showing Total ion chromatogram (TIC) of [A] *Alstonia macrophylla* (leaf) [B] E3-*Alstonia scholaris*

Whereas the extract of *Alostonia macrophylla* showed presence of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, 2-methoxy-4-vinylphenol, 2,6-dimethoxyphenol, 2,6-dimethoxy-4-(2-propenyl)-phenol, 3,5-dimethoxy-4-hydroxyphenylacetic acid, burnamicine, γ-sitosterol, 24-methylenecycloartanol, lupeol, stigmasterol, campesterol, 2(4H)-Benzofuranone 5, 6, 7, 7a-tetrahydro-4, 4, pentane,3-methyl-, 7a trimethyl, (-)-Loliolide, ethane,1-chloro-1-fluoro Neophytadiene, Hexahydrofarnesyl acetone. There were similarity in pattern of metabolites observed among the two extracts. Some metabolites were common while some were solely present in each of them. Medicinal properties of aqueous and alcoholic extracts of *Alostonia scholaris* are very well studied and reported in comparison to *Alostonia macrophylla*. This study highlights several active constituents from GC-MS profile that can play integral role in drug development and medication.

CONCLUSION

In this work, we provide a biomimetic, single-step, and eco-friendly method for manufacturing silver nanoparticles from extracts of *Alstonia* leaves containing compounds with therapeutic potential. This method exemplifies a safe, green, and efficient strategy for biosynthesizing silver nanoparticles. This research demonstrates that $AgNO_3$ may be efficiently reduced to silver nanoparticles with an average diameter in the range

of 15.14 nm using leaf extracts of the *Alstonia macrophylla, Alstonia scholaris* and *Alstonia venenata.* Other benefits of these nanoparticles are also their stability for a long period of time. The absence of UV-Visible alterations in synthesized silver nanoparticles, even after long term storage, may be owing to the presence of proteins, enzymes, and sugars that may function as a capping agent. Synthesized silver nanoparticles can be further tested for their potential antibacterial activity against pathogenic strains of bacteria. This work provides further evidence that different *Alstonia* species have high content of active constituents as observed from results of GCMS thus it may open up vast opportunities for the development of antibiotics and medicines against a wide range of harmful bacterial types.

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CONFLICT OF INTEREST

There is no conflict with other interest in the manuscript content

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