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Green Synthesis of Silver Nanoparticles from Combined Leaf Extracts

Sanjeev kumar[1], Shashi Shankar [2],Vinay PJ [3], Tanuja N Rajaput [4], Deepak Prashar [5], Bharti Sharma [6], Diksha Sharma[7], Nihar Ranjan Kar[8], Dr. Abhishek Suman*

1. *Assistant professor, Kamla Nehru Institute of Management and Technology, Faculty of Pharmacy, Faridipur, Sultanpur*
2. *Assistant professor, Kamla Nehru Institute of Management & Technology, Faculty of Pharmacy, Faridipur, Sultanpur*
3. *Assistant professor, GMS Academy First Grade College, Davanagere*
4. *Assistant professor, GMS Academy First Grade College, Davanagere*
5. *Professor, Shanti Niketan College of Pharmacy*
6. *Assistant professor, KC Institute of Pharmaceutical Sciences, Una*
7. *Associate Professor, Aakash Institute of Medical Sciences, Nalagarh*
8. *Assistant Professor, Centurion University of Technology and Management, Gopalpur, Balasore, Odisha*

Corresponding Author: Dr. Abhishek Suman

howruabhishek@gmail.com

Affiliation: Assistant Professor, Government Pharmacy Institute, Bihar Medical Science University

ABSTRACT

The use of plant extracts in the sustainable production of silver nanoparticles (AgNPs) has gained significant recognition owing to its cost-effectiveness, environmental sustainability, and few adverse effects on living organisms. The present study aims to synthesize silver nanoparticles (AgNPs) using the combination of plant extracts from **Azadirachta indica (Neem) and Aloe flava Pers (Aleo Vera)**. Silver nanoparticles were synthesized utilizing a simple and environmentally friendly method, which involved the use of aqueous leaf extracts from Azadirachta indica and Aloe flava Pers. The synthesized nanoparticles underwent characterization utilizing several techniques, such as the UV-visible spectrophotometer, Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), and X-ray Diffraction (XRD). The measurement of in vitro anti-inflammatory effects involved the

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inhibition of albumin denaturation, whereas the assessment of in vitro antioxidant activity utilised the ABTS free radical scavenging test, DPPH assay, and potassium ferricyanide reducing power assay technique. The targets of the in-vitro antimicrobial trials were Bacillus cereus, Actinomycetes Israeli, Proteus vulgaris, Streptococcus pyogenes, Corynebacterium diphtheria, Pseudomonas aeruginosa, Enterococcus faecalis, and Staphylococcus aureus. These experiments were conducted at various concentrations. The results indicated that the combination of aqueous leaf extracts of Azadirachta indica and Aloe flava Pers with biosynthesised silver nanoparticles (AgNPs) serves as effective stabilising agents. The findings of our study demonstrate the efficacy of phytomediated silver ions as anti-inflammatory, antibacterial, and antioxidant agents in an in vitro setting.

INTRODUCTION

Nanotechnology is widely recognized as a vital area of research in the current day. The use of noble nanoparticles has been increasing across several academic disciplines, primarily due to their diverse and attractive array of potential applications. There are several categories of nanoparticles that may be produced using a range of methods, encompassing hybrid, chemical, physical, and biological techniques. The use of physical and chemical techniques is commonly observed in the production of nanoparticles. However, their potential for biological applications, particularly in clinical contexts, is somewhat restricted as a result of the inclusion of hazardous substances.[1] Nanomaterials have been utilised in tissue engineering, along with several other applications. [2] Silver nanoparticles are of considerable importance as a component within the wider classification of metal nanoparticles. Silver is often employed as a nanomaterial. The application of green synthesis has been generally recognised as a very promising approach for the synthesis of nanoparticles due to its inherent biocompatibility, low toxicity, and ecologically sustainable characteristics. Inflammation is a multifaceted biological process characterised by the degradation and subsequent restoration of tissues, which is triggered as a reaction to tissue damage. A variety of anti-inflammatory medications now on the market provide a multitude of considerations, including their effectiveness and the presence of adverse effects such as gastrointestinal problems, renal impairment, and respiratory depression. The aforementioned scenario highlights the need to progress the advancement of analgesic and anti-inflammatory pharmaceuticals that possess both safety and efficacy. [3] Prior research has established that aloe

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gel exhibits the capacity to mitigate inflammation induced by various substances by enhancing prostaglandin production and leucocyte infiltration. However, it has comparatively reduced effectiveness in reducing inflammation caused by substances. [4] The antibacterial characteristics of silver nanoparticles have been extensively acknowledged in the scientific community due to their tiny size and large surface area, which enable them to effectively combat various pathogens such as bacteria, viruses, and fungus. The assessment of the scavenging capacity of stable free radicals, namely 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), has been widely employed to investigate the antioxidant capabilities of AgNps. The antioxidant characteristics of silver nanoparticles (AgNPs) have been recognised as a result of silver's capacity to exist in two oxidation states (Ag+1 and Ag+2), which is dependent on the precise reaction conditions. As a result, silver nanoparticles (AgNPs) possess the ability to counteract the effects of free radicals through the process of electron transfer, either by accepting or donating electrons. [5] In a study conducted by Bedlovikova et al. (year), it was shown that the enhanced antioxidant activity exhibited by silver nanoparticles (AgNPs) in comparison to plant extracts might potentially be ascribed to the existence of phenolics, flavonoids, and terpenoids on the surface of the nanoparticles. These substances facilitate the nanoparticles' ability to act as quenchers of singlet oxygen, donors of hydrogen, and agents for reduction. [6] In the manufacture of nanoparticles, it is customary to utilise a single botanical extract. This work focuses on the utilisation of plant extracts to synthesise nanoparticles, aiming to increase the medicinal qualities of two specific botanical species. The present investigation entailed the production of silver nanoparticles by a green synthesis method, employing aqueous leaf extracts derived from Azadirachta indica and Aloe flava Pers. Plant extracts have the dual capacity to serve as both a reducing agent and a capping agent. Moreover, the use of plant-mediated techniques for the production of nanoparticles has several benefits, including cost-effectiveness, a faster biosynthetic process, environmental friendliness, and appropriateness for medicinal applications in humans. [7]

MATERIAL AND METHODS:

Materials:

Fresh neem leaves (*A. indica*) and Aloe flava Pers (Aleo Vera) were collected from Government Pharmacy Institute, Bihar Medical Science University, and washed in distilled water till the

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surface dust is completely removed and dried under shade. The aqueous leaf extract was prepared as described earlier; briefly, 50 gm of neem leaf powder was mixed with 500 ml of distilled water and boiled for about 30 min. The boiled solution was filtered using Whatman No. 1 filter paper and clear aqueous leaf extract was obtained. The extract was stored at 4°C until further use. The 1 mM $AgNO_3$ powder was added in 100 ml of distilled water and used within 24 h for the assay. The 2,3-DHS and QDH pure compounds were procured from the M/s Natural Remedies, Bangalore, India.

METHODS:

Preparation of Aloe flava Pers leaf extract:

Leaf of Aloe flava Pers, is a polysaccharide composed of β-(1,4)-linked highly acetylated mannoses, β-(1,4)-linked glucose and α-(1,6)-linked galactose. Since the bioactive components of aloe including Aloe flava Pers always have considerably different exaction methods. Generally, extraction in hot water and ethanol is the classic, most convenient method of laboratory extraction, and has been widely used in industry. Briefly, the water exaction method includes cleaning, homogenization, separation, and centrifugation of *Aloe vera*. The supernatant was collected and mixed with absolute ethanol at a ratio of 1:3 for 12 h. The white precipitate was collected and centrifuged.[8] Aloe flava Pers was collected by dialysis and freeze-drying as white opaque particles. The ratio of liquid to solid has an important effect on the yield of conventional water extraction. The range of extraction temperature with time is usually in the range of 80–100 °C, 0.5–6 h. However, water extraction, the most popular approach, always has disadvantages of long times and high temperatures, low efficiency, and possible polysaccharides degradation, resulting in large consumptions of energy and time. Therefore, it is necessary to improve the extraction conditions. Numerous studies have proved aloe polysaccharide was partially digested with cellulose. [9]

Separation and Purification of Aloe flava Pers:

After extraction, the crude extracts are mainly obtained by ethanol precipitation. As such polysaccharides usually contain proteins, pigments and small molecular substances, further separation and purify is necessary to get Aloe flava Pers. Separation and purification of crude extracts by anion exchange chromatography coupled with gel permeation chromatography

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(DEAE-Sephadex A-25 column) and normal membrane separation were done.[10] However, considering the long fractionation time, low cost, membrane vulnerability, gradient ethanol precipitation method and gradient ammonium sulfate precipitation method were adopted in recent years. Besides, the membranes with special structure and function are also used in the separation and purification of acemannan. Aloe flava Pers was fractionated with ultrafiltration cells from an Amicon with the corresponding molecular weight cut-off membrane according to the molecular size. Moreover, electrospun cellulose acetate membrane was used as an alternative carrier for separation of crude extracts by electrophoresis. By controlling the porosity and pore size of membrane, it was used as a simple technical advantage for separation of high molecular weight and near molecular weight polysaccharides. [11]

Preparation of Azadirachta indica leaf extract:

Fresh *Azadirachta indica* A. Juss (neem) leaves were shade-dried for days at room temperature and powdered with a grinder. The dried powder of *Azadirachta indica* A. Juss (neem) was soaked in HPLC-grade methanol and 45℃. The mixture was filtrated, condensed using a rotary evaporator, and finally lyophilized (Modul Spin 40, Biotron Co.). This methanol extract of *Azadirachta indica* A. Juss (neem) leaves was obtained from the Plant Extract Bank (Daejeon, Korea) and was dissolved in dimethylsulfoxide (DMSO) for the subsequent studies. [12]

Synthesis:

Ag NPs were synthesised as per the earlier descriptions. The Ag NPs were synthesised using neem leaf extract and Aloe flava Pers (10 ml of 10% w/v in distilled water), 2, 3-dehydrosalanol (2, 3-DHS) (10 ml of 0.03% w/v in alcohol) and QDH (10 ml of 0.06% w/v in alcohol). Each of the above compounds were added drop-wise to 90 ml of 2 mM $AgNO_3$ solution and maintained at 80°C on a magnetic stirrer. After addition, the solution was kept at room temperature for 24 h. Synthesis of NPs was ascertained by a distinct change of the hydrosol. The reduction of silver ions (Ag^+) was confirmed by UVV is spectrum of the hydrosol. [13] The Ag NPs were collected by centrifugation at 10,000 rpm for 4 min. of the hydrosol for further characterisation. The concentration of Ag NPs was determined using inductively coupled plasma optical emission spectrophotometer (Prodigy XP, Leeman labs, USA). The samples were diluted 10 times (w/v)

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with distilled water and an aliquot of 20 ml was loaded to the racks of automatic sampler. The average of three readings was used to obtain the concentration of Ag NPs in the sample. [14]

Characterization:

For the characterisation of the synthesised nanoparticles, UV-Vis spectroscopy, Fourier transformed infrared, X-ray diffraction, dynamic light scattering (DLS), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analyses were adopted. [15]

UV-Vis spectroscopy:

The localised surface Plasmon resonance of the formed Ag NPs was recorded using UV-Vis spectrophotometer (UV-2450, SHIMADZU). A 5 ml of sonicated sample of the hydrosol was scanned from 200 to 800 nm to obtain the peak absorption. [16]

Fourier transformed infrared analysis (FTIR):

The FTIR analysis was used to identify the organic functional groups involved in the synthesised Ag NPs. The FTIR spectrum was taken in the mid IR region of 400–4000 cm_1 using attenuated total reflectance technique. The dried sample was mixed with the potassium bromide crystal in the ratio of 1:200 and the spectrum was recorded in transmittance mode.

X-ray diffraction (XRD):

The XRD analysis determines the crystalline structure of Ag NPs. The XRD analysis was carried out on Ag NPs synthesised by the neem leaf extract. The XRD pattern was recorded using computer controlled XRD-system (JEOL, and Model: JPX-8030 with CuKα radiation (Ni filtered = 1.3418 A°) in the range of 40 kV and 20 A. The built in software (Syn-master 7935) program was used to identify XRD peaks corresponds to Bragg's reflections. [17]

Dynamic light scattering:

The particle size and zeta potential were determined for the synthesised compounds by DLS technique. The aqueous suspension of the synthesised nanoparticles was filtered through a 0.22 μm syringe-driven filter unit and the size and distribution of the nanoparticles and zeta potential was measured (Nanopartica, HORIBA, SZ-100). [18]

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Transmission electron microscopy:

The morphology and size of the nanoparticles were studied by TEM (JEOL-JEM-1010 instrument) with an accelerating voltage of 80 kV. A drop of aqueous Ag NPs was dried on carbon-coated-copper TEM grids under vacuum in a desiccator and loaded into the specimen holder. The particle size and surface morphology of NPs were evaluated using ImageJ 1.45 s software. [19]

Scanning electron microscopy:

The surface morphological study of the Ag NPs was studied with SEM. Dried Ag NPs were used for SEM (JEOL-JSM6610LV) at a magnification ranging from 10 to 600,000× operated at an acceleration voltage of 30 kV. [20]

Biological activities of Sliver Nanoparticles containing Azadirachta Indica and Aloe flava Pers:

In vitro anti-inflammatory activity (Inhibition of albumin denaturation):

The anti-inflammatory efficacy of AgNPs biosynthesized from A. indica and A. barbadensis leaf extracts was investigated in vitro utilizing suppression of albumin denaturation. The reaction mixture contained test extracts and 1% aqueous solution of bovine albumin fraction, with the pH adjusted with a trace of 1N HCl. After incubating at 37oC for 20 minutes, the sample extracts were heated to 51oC for 20 minutes before being measured at 660nm. [21] The experiment was repeated three times. The percentage inhibition of protein denaturation calculated was as follows:

$$Percentageinhibition = \frac{(Abscontrol - Abssample)\ \text{X}\ 100}{Abscontrol}$$

In vitro anti-oxidant activity DPPH Radical Scavenging Activity:

The antioxidant activity of biosynthesized AgNPs derived from A. indica and A. barbadensis leaf extract was assessed by DPPH assay which is popular in natural product antioxidant studies. This assay is based on the theory that a hydrogen donor is an antioxidant which measures compounds that are radical scavengers. It shows the mechanism by which DPPH accepts hydrogen from an antioxidant [19].100µl of 0.1 mM of DPPH solution in methanol was added to 300 µl of sample solution at different concentration 25, 20, 15, 10 and 5µg/mL. [22] The mixtures was shaken

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vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UVVIS spectrophotometer. Ascorbic acid was used as the standard. The effect of radical scavenging activity calculated was as follow:

$$DPPHScavengingactivi = \frac{(Abscontrol - Abssample)\ \text{X}\ 100}{Abscontrol}$$

ABTS free radical scavenging assay:

The ABTs free radical cation decolorization test was used to assess the in vitro antioxidant activity of biosynthesized AgNPs derived from A. indica and A. barbadensis leaf extract [20]. The reaction of 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1) produced a positive cation radical, which was stored in the dark for 12-16 hours at room temperature before use. The absorbance at 734 nm was measured to be 0.700 after diluting the ABTS+ solution with methanol. After 30 minutes of initial mixing, the absorbance was measured at 734 after the addition of 5l of Bio - Ag NP to 4 ml of diluted ABTS + solution. Each experiment was preceded with a solvent blank. Every measurement was taken at least three times. The percent suppression of absorbance at 734 nm was calculated. [23]

Antimicrobial Activity:

The Biosynthesized Sliver nanoparticles was screened for antimicrobial activity by disc diffusion method, using nutrient agar media against gram-positive bacteria namely Bacillus cereus, Actinomycetes Israeli, Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium diphtheria and Enterococcus faecalis, gramnegative bacteria namely Proteus vulgaris and Pseudomonas aeruginosa. Gentamicin was used as a reference standard. Petri plates containing 20 ml nutrient agar medium were seeded with 24 hr culture of bacterial strains and adjusted to 0.5 OD value according to McFarland standard. Well was cut and the different concentration (500, 250, 100 and 50 μg/ml) of the extract are added into the well. [24] Then the plates were incubated at 37°C for 24 hrs till perfect growth was observed. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. The values were calculated using Graph Pad Prism 6.0 software (USA). [25]

Results and discussion:

In this study, green Ag NPs were synthesised using aqueous Azadirachta indica (Neem) and Aloe flava Pers (Aleo Vera) and compounds. By addition of aqueous Azadirachta indica (Neem)

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and Aloe flava Pers (Aleo Vera) extract to AgNO3 solution, the colour has been changed from colourless to brown. The synthesised Ag NPs were characterised to determine their formation. [26]

UV-Vis spectroscopy:

The UV-Vis spectroscopy could be used to examine the size and shape-controlled nanoparticles in aqueous suspensions [11]. The UV-Vis spectroscopy was employed to record the localised surface plasmon resonance of aqueous Azadirachta indica and Aloe flava Pers coated Ag NPs and the peak absorbance was recorded at 370, 430, 230 and 220 nm, respectively, which confirms the formation of green Ag NPs (Fig. 1). The present findings demonstrated that, the reduction of Ag+ into Ag NPs during the exposure to plant extracts was observed. The change in colour was noted and is due to the localised surface plasmon resonance phenomenon of the formed Ag NPs. The metal nanoparticles have free electrons, which give the plasmon resonance peak absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. Earlier research findings also explained that, the Ag NPs showed a surface plasmon resonance peak in a range of 400–450 nm. Based on the characteristic Ag NPs surface plasmon resonance range, it was confirmed that, A. indica has huge potential to reduce Ag+ into Ag NPs. The present observation (430 nm) differed with the previous results [10, 14] who noted a plasmon band at 351 and 370 nm, respectively. This lower absorbance may be influenced by several factors such as concentration and combination of plant extract, pH, incubation temperature, reaction time and electrochemical properties of metal ion. The variation in the values of absorbance confirms the changes in the particle size. [27]

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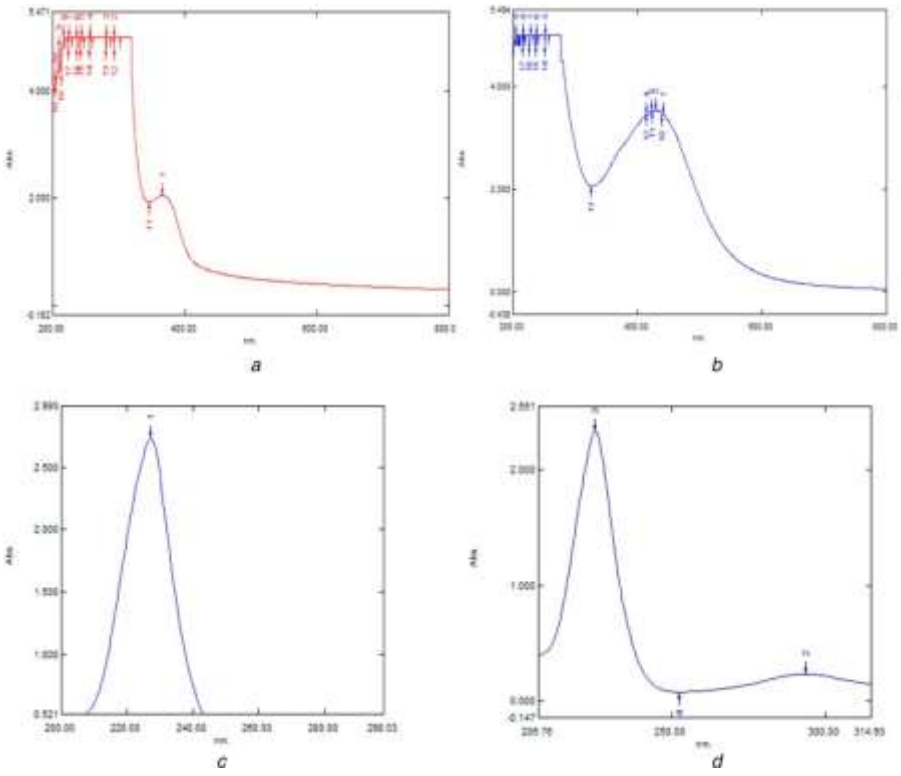

Figure 1: *UV-visible* absorption spectra

(a) **Azadirachta indica** (b) **Aloe flava Pers** (c) **Azadirachta indica** and **Aloe flava Pers** (d) **Azadirachta indica and Aloe flava Pers** Ag NPs

Fourier Transform Infrared (FTIR):

The use of Fourier Transform (FT) infrared spectrum measurement to detect the functional groups responsible for capping and reducing agent for the AgNPs generated by leaf extract has been approved. There were three separate bands visible. The N-H stretching mode generated the intense broad band at 3271 cm^{-1}, and the band corresponding to the O-H stretching mode implies protein connection. The CHO stretching mode in the amine group, which is commonly present in proteins, induces the strong band at 1637 cm^{-1}, suggesting that proteins function as a capping agent for AgNPs, improving the stability of the Nanoparticles generated. A strong and broad peak at 386 cm^{-1}, similar to Ag metal, was anticipated to appear. Figure 4.FT-IR spectra of A. indica and A. barbadensis biosynthesized silver nanoparticles. [28]

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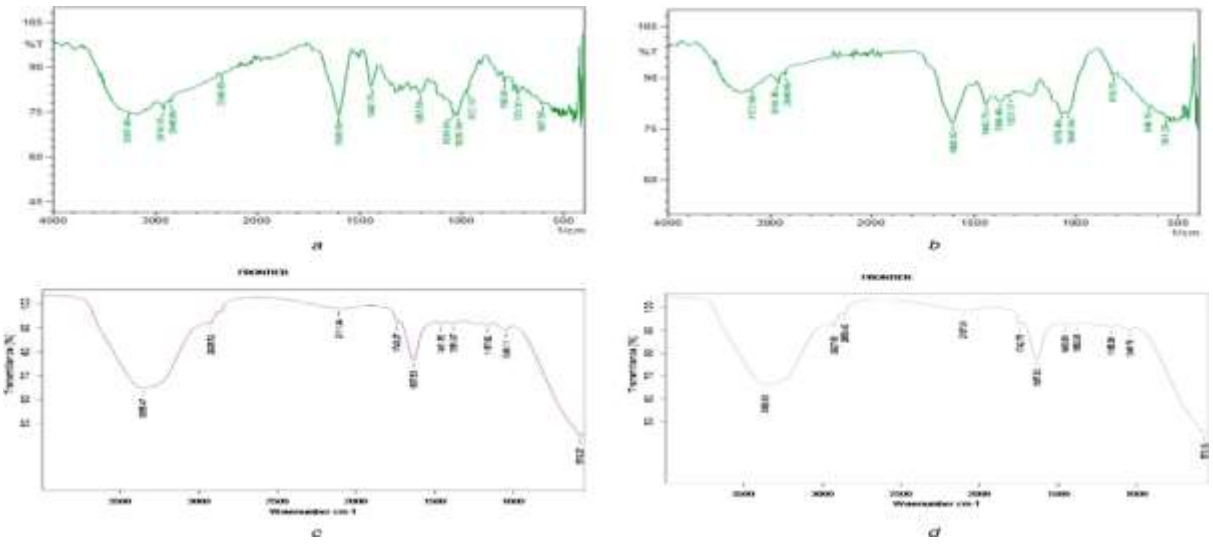

Fig. 2 FTIR spectra showing the functional groups

((a) **Azadirachta indica** (b) **Aloe flava Pers** (c) **Azadirachta indica** and **Aloe flava Pers** (d) **Azadirachta indica and Aloe flava Pers** Ag NPs

X-ray diffraction (XRD pattern) Analysis:

XRD analysis was used to determine the crystalline structure of AgNPs extracted from the leaves of A. indica and A. barbadensis. The X-ray diffraction pattern (Figure 6.) displays four primary distinctive diffraction peaks, suggesting face-centered cubic Ag crystals (blue lines). 38.12°, 44.35°, 64.56°, and 77.48° are the diffraction peak values at 2. Peaks from other phases were also found, indicating that the nanoparticles are single phase Ag with cubic structure. The average crystallite size computed using Scherrer's equation for the width of the (111) peak is 15-19 nm, which agrees with the particle size measured by SEM images. The unassigned peaks are caused by the crystallisation of bioorganic species found in plant extract. [29]

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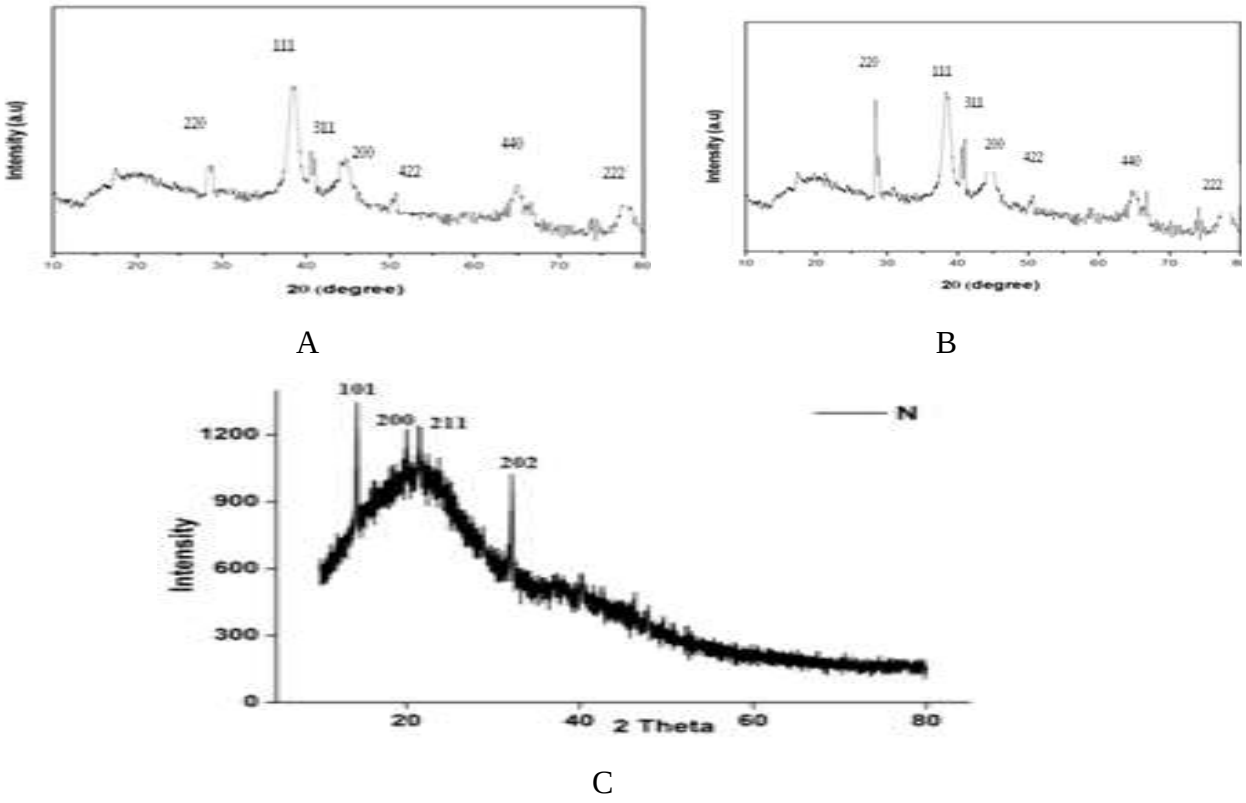

Fig. 3 X-ray diffraction pattern

(a) **Azadirachta indica** (b) **Aloe flava Pers** (c) **Azadirachta indica and Aloe flava Pers** Ag NPs

SEM analysis:

The SEM was employed to study the surface morphology, size and shape of synthesised Ag NPs. It was noted that, the morphology of neem mediated Ag NPs was nearly spherical, predominately cuboidal, rectangular, poly-dispersed and aggregated into large irregular structures with no well-defined morphology. The SEM images gave a clear indication about the morphological evidence regarding the A. indica and A. barbadensis coated Ag NPs (Fig. 6). The observations of the present study for neem mediated Ag NPs were in accordance with earlier reports [17, 20] in contrast to other [16], who reported as triangular shape. It is evident by scanning electron microscopic analysis, the morphology of 2, 3 DHS coated Ag NPs was predominately polydispersed and aggregated into large irregular structures without a well-defined morphological features. The morphology of QDH coated Ag NPs was spherical, predominately cuboidal, rectangular,

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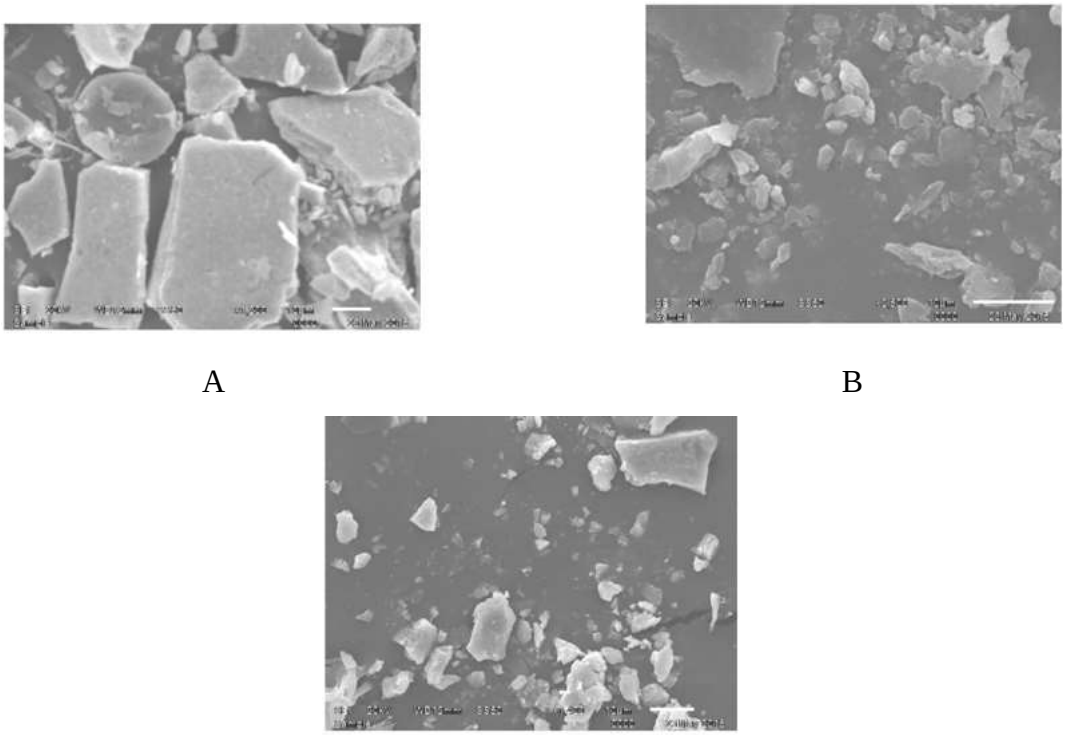
polydispersed and aggregated. Published literature is not available for comparison and discussion of the present observations. [28]



A B C

Fig. 6 SEM microphotograph

(a) **Azadirachta indica** (b) **Aloe flava Pers** (c) **Azadirachta indica and Aloe flava Pers** Ag NPs

Biological activities of Sliver Nanoparticles containing Azadirachta Indica and Aloe flava Pers:

The synthesized silver NPs are characterized and it is very essential to study the biological activity of these particles to exploit commercially for pharmaceutical applications. In the present work, anti-inflammatory and anti-oxidant activities of the Bio-synthesized Ag NP of combined extract are evaluated. [30]

In vitro anti-inflammatory activity (Inhibition of albumin denaturation):

Inflammation is a key event in many infections and is the first line of defence. However, the inflammation if chronic may lead to disorders and pose a major health issue. Currently, the drugs in use for inflammation are palliative in nature but mostly exhibit side effects. Nanoparticles may

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aid in decreasing toxicity and adverse effects by reducing dose and size. As a result, we examined the anti-inflammatory efficacy of bio-AgNPs produced from A. indica and A. barbadensis. Protein denaturation is a condition in which the natural structure of a protein is destroyed owing to chemical, stress, or heat action. Inflammation has been linked to protein denaturation and the loss of biological activities. A. indica and A. barbadensis bio-AgNPs inhibited albumin denaturation in a dose- dependent manner, with 66 and 68 percent inhibition, respectively for20 and 25 µg/ml. The inhibition was on par with the standard drug aspirin (68%) at 100 µg/ml. Figure 7 .Effect of biosynthesized AgNP of A. indica and A. barbadensis on albumin denaturation. Values represent mean ± SD of triplicates. [31]

In vitro anti-oxidant activity:

Antioxidant activities might be related by the presence of flavonoids, alkaloids in the extract of plants used for the study. This means a reduction in antioxidant activity may result in a reduction in the metabolite concentration of plants during Nanoparticles formation. The surface area of silver is large, which means more plant chemical substances are added to the active surface. As a result, the shell response phenomenon in the NP is elevated by bio AgNPs (due to an adsorbed antioxidant moiety on the surface). [32]

Potassium Ferricyanide Reducing Power Assay:

The antioxidant activity is complex mechanism and cannot be evaluated by single method. It is essential to carry out more than one method to assess the anti-oxidant potential. The biosynthesized AgNP of A. indica and A. barbadensis showed reducing power activity however lesser than the standard Butyl Hydroxy Toluene (BHT).The reducing power of the sliver nanoparticles indicates its potential antioxidant activity by reduce ferric salt (Fe^{3+}) to ferrous salt (Fe^{2+}) by electron transfer reaction. Figure 8. Reducing power activity of biosynthesized AgNP of A. indica and A. barbadensis. Values represented as mean ± SD of triplicates. [33]

Antimicrobial activity:

The biosynthesized AgNP of A. indica and A. barbadensis extracts exhibited marked antimicrobial activity against all the microorganisms, Among the gram positive bacteria tested in the present experiment Bacillus cereus, Actinomycetes Israeli, and Enterococcus faecalis was more susceptibility to the extract, where Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium diphtheria was moderately susceptibility to the extract. In the gram negative bacteria Proteus vulgaris was more susceptibility to the extract, where Pseudomonas aeruginosa

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was moderately susceptibility to the extract. Antimicrobial activity for Gram positive bacteria of biosynthesized Ag NPs of A. indica and A. barbadensis and Figure 9b shows antimicrobial activity for Gram negative bacteria of biosynthesized Ag NPs of A. indica and A. barbadensis. The zones of inhibition produced by AgNPs against the different eight species. [34]

CONCULSION

The in vitro anti-inflammatory, antioxidant, and antibacterial activities of AgNP synthesised from Azadirachta indica and Aloe flava Pers were assessed. The results of in vitro experiments, including the assessment of albumin denaturation suppression, reduction of free radicals in the DPPH assay, ABTS radical scavenging test, and Potassium Ferricyanide reducing power assay, suggest that the AgNP derived from A. indica and A. Barbadensis have anti-inflammatory and antioxidant characteristics. The antibacterial activity shown significant suppression against both gram-negative and gram-positive bacteria. Based on the findings presented, it can be inferred that silver nanoparticles derived from A. indica and A. Barbadensis exhibit accelerated synthesis rates, increased yields with reduced particle sizes, pronounced antibacterial efficacy against both gram-negative and gram-positive bacteria, as well as notable antioxidant and anti-inflammatory properties. These observations collectively suggest that the bio-synthesized AgNPs possess significant biological activity. In the current investigation, it was shown that the combined leaf extract of Azadirachta Indica and Aloe flava Pers had notable efficacy in the synthesis of Silver Nanoparticles, exhibiting a dose-dependent response. The combination leaf extract of Azadirachta Indica and Aloe flava Pers AgNP, which is synthesised in a green manner, has remarkable anti-inflammatory, antioxidant, and antibacterial properties in comparison to solo extracts. The synthesis technique was verified to be simple, rapid, environmentally benign, and non-toxic. The results of our study indicate that the utilisation of plant-derived biosynthesized silver nanoparticles has significant promise for the progress of Nano medicine. Furthermore, our bio AgNPs have considerable potential for development into pharmaceutical products intended for biomedical applications.

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