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Research Article

GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING AQUEOUS EXTRACT OF *MORINGA CONCANENSIS* NIMMO LEAVES

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ABSTRACT

Background and Objective: *Synthesis of silver nanoparticles was broadly studied by utilizing chemical and physical methods, but the development of reliable technology to produce nanoparticles is an imperative aspect of nanotechnology. The objective of this work was to synthesize and characterize the silver nanoparticles by green route method by using aqueous extract of M.concanensis Nimmo leaves.* ***Material and Methods:*** *The green route method was employed to synthesis the silver nanoparticles. The synthesized AgNPs were characterized by UV–Visible spectroscopy, Fourier transform infra-red spectroscopy (FTIR), X-ray diffraction (XRD), and Scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS).* ***Results:*** *The surface plasmon resonance found at 865 nm confirmed the formation of AgNPs. SEM images reveal that the particles are spherical in nature. The EDS analysis of the AgNPs, using an energy range of 3 keV, confirmed the presence of elemental silver without any contamination.* ***Conclusion:*** *It was concluded that the leaves of M.concanensis Nimmo were good source for the synthesis of AgNPs and have many medicinal advantages.*

KEYWORDS: *M.concanensis, Silver nanoparticles, Green route method, UV visible spectrum and SEM.*

INTRODUCTION

In recent days nanotechnology has induced great scientific advancement on the field of research and development. Various physical and chemical methods have been formulated for the synthesis of nanoparticles of desired shape and size. However, these methods are not economically feasible and ecofriendly. Although chemical and physical methods may successfully produce pure and well defined nanoparticles, these methods were quite expensive and potentially dangerous to the environment [1]. Biological synthesis consumes naturally occupying reducing agent such as plant extracts, microorganisms, enzymes and polysaccharides which are simple and viable, which is the alternative to the complex and toxic chemical processes [2]. Therefore, green synthesis has been considered as one of the promising method for synthesis of nanoparticles because of their biocompatibility, low toxicity and ecofriendly nature [3]. Bionanotechnology combines biological principles with physical and chemical approaches to produce nano sized particles with specific functions. This also represents an economic substitute for chemical and physical methods of nanoparticles formation [4]. Green synthesis of nanoparticle are cost effective, easily available, ecofriendly, nontoxic, large scale production and act as reducing and capping agent compared to the chemical methods, which is a very costly as well as it emits hazardous byproducts, which can have some deleterious effect on the environment [5]. Herbal plants as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The plant *M. Concanensis* Nimmo occurs in tropical dry forest from southeastern Pakistan almost to the southern tip of India. This plant recently has been found in western Bangladesh. Indigenous knowledge of this plant in that region has not been so far studied. Around twenty types of human ailments can be cured by using *M. Concanensis* Nimmo with simple preparations. The therapeutic values of *M. Concanensis* Nimmo are described with disease cured, part used, mode of drug preparation and method of consumption [6]. The leaves and bark are the most potent part of the plant for medicinal use. This plant was used for treatment of skin tumour, tiredness, to reduce blood pressure, aphrodisiac, jaundice, eyecare, diabetes and bloat [7]. Seed oil can be used in sunscreen [8]. Hence the present study was designed to synthesize and characterize the silver nanoparticles from *M. Concanensis* Nimmo leaves. Aqueous extract of *Moringa Concanensis* Nimmo leaves was used to synthesize the silver nanoparticles on the basic of cost effectiveness, ecofriendly sources, easy available and its medicinal values.

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MATERIALS AND METHODS

1. Collection and identification of plant:

The healthy, matured and insect bites free leaves of *Moringa concanensis* Nimmo plant (Family - *Moringaceae*) was collected from Esanai village, Perambalur district, Tamilnadu, India (Latitude – 11.2982° N, Longitude – 78.8298° E) from the month of December, 2016. The plant sample was identified and authenticated by Dr. C. Murugan, Scientist, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The identification number BSI/SRC/5/23/2016/Tech-152.

2. Synthesis of silver nanoparticles:

2.1. Preparation of plant extract:

Ten grams of the dried *M.concanensis* Nimmo leaves powder were kept in a beaker containing 100 ml double distilled water and boiled at 80˚C for 10 minutes to obtained bioactive compounds from *Moringa concanensis* Nimmo leaves. The extract was cooled and filtered through normal filter paper followed by Whatmann filter paper No.1. The final extract was used to synthesis silver nanoparticles.

2.2. Synthesis of silver nanoparticles:

The silver nanoparticles were synthesized using previously described method by Aravinthan et al. (2015) with few modifications. The aqueous solution of silver nitrate at concentration of 0.001 M was prepared to synthesize silver nanoparticles from filtered aqueous leaves extract of *Moringa concanensis* Nimmo. Five ml *Moringa concanensis* Nimmo leaves aqueous extract was slowly added to 95 ml of aqueous solution of $AgNO_3$ while stirring for reduction into Ag ions. The formation reddish brown colour was observed after 3hrs incubation at room temperature AgNPs solution was purified by repeated centrifugation (Remi CM 12-C, Mumbai, Maharashtra, India) at 10,000rpm for 20 minutes to isolate AgNPs free from other bioorganic compounds present in the reaction medium. After centrifugation the obtained particles were washed with distilled water for 10 to 20 minutes and kept in hot air oven for drying at 100˚C for 1 hour.

2.3. Characterization of silver nanoparticles:

2.3.1. UV-Visible spectroscopy analysis: The optical measurement is the prime technique for characterizing the biological synthesis of nanoparticles. The formation and stability of silver nanoparticles in aqueous solution was confirmed by UV-Visible spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan) analysis. The reduction of pure Ag^+ ions was monitored by measuring the UV-Visible spectrum of the reaction medium at 540 nm for 12 hours. UV-Visible spectral analysis was done by UV-Visible spectrophotometer (UV- 2450, Shimadzu, Kyoto, Japan).

2.3.2. Scanning electron microscope (SEM): Scanning electron microscope (SEM) analysis was analyzed using Hitachi S – 4500 SEM machine. The silver nanoparticles were centrifuged at 10,000 rpm for 30 minutes and the pellet was redispersed in 10 ml of ethanol and washed 3 times with sterile distilled water to obtain the pellet. The pellet was dried in an oven and thin films of dried samples (10 mg / ml) were prepared on carbon coated copper grid and analyzed for size determination. The particle size and texture of nanoparticles can be analyzed by using image magnification software compatible with SEM and helps in determining the presence and formation of silver nanoparticles.

2.3.3. Nanoparticle size distribution analysis: Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of silver nanoparticles. The prepared sample was dispersed in deionized water followed by ultra sonication. Then solution was filtered and centrifuged for 15 mins at 25˚C with 5000 rpm and the supernatant was collected. The supernatant was diluted for 4 to 5 times and then the particle distribution in liquid was studied in a computer controlled particle size analyzer (SALD-3101, Shimadzu, Kyoto, Japan).

2.3.4. Energy dispersive X-ray analysis (EDX analysis): Energy dispersive X-ray analysis, thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid in to carbon film and performed on JEOL-MODEL 6390 SEM instrument equipped with a thermo EDX attachments. The energy dispersive X-ray analysis used to identify the signal characteristics of silver nanoparticles.

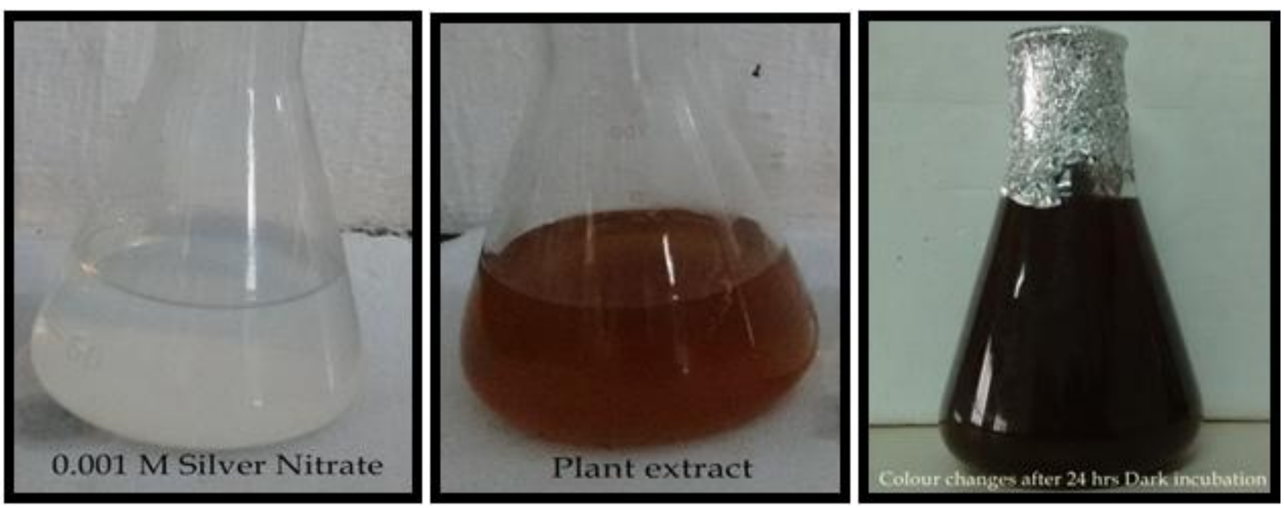
2.3.5. Fourier transform – Infra red analysis (FT-IR): The FT-IR spectroscopy measurements are carriedout to identify the biomolecules that bound specifically on the silver surface and local molecular environment of capping agent on the nanoparticles. To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 10,000 rpm for 10 mins and the resulting suspension was redispersed in 10 ml sterile distilled water. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FT-IR spectrophotometer.

RESULTS

1. Results of green synthesis of silver nanoparticles from *Moringa concanensis* Nimmo leaves:

1.1. Visual observation:

The silver nanoparticles were synthesized from aqueous extract of *Moringa concanensis* Nimmo leaves. The formation of silver nanoparticles from reaction medium was confirmed by color change. The reaction mixture contains the aqueous extract of *M.concanensis* Nimmo leaves and aqueous silver nitrate solution. After the 24 hrs of dark incubation the color of the reaction medium was changed from light brown to black (Figure 1). This result indicates the formation of silver nanoparticles by the reduction of Ag ions.



A B C

Fig. 1: Synthesis of silver nanoparticles from *M.concanensis* Nimmo leaves

A *– Aqueous silver nitrate solution (0.001M);* ***B*** *– Aqueous extract of Moringa concanensis Nimmo leaves;* ***C*** *– Reaction medium after 24hrs dark incubation*

1.2. UV-Visible Spectroscopy analysis of synthesized silver nanoparticles from aqueous extract of M.concanensis Nimmo leaves:

The UV spectrum showed (Figure 2) the surface plasma AgNPs at increasing concentration was taken and the color changes were observed for nanoparticles. For silver color changes from colorless to dark brown color. Metal nanoparticles can be synthesized by reducing metal ions using some chemical molecules. In green synthesis, this is observed that natural material extract act as reducing agent for generation of metal nanoparticles**.**

1.3. Scanning electron microscopic analysis of synthesized silver nanoparticles from aqueous extract of M.concanensis Nimmo leaves:

The SEM image showed individual silver nanoparticles as well as a number of aggregates, SEM images of silver nanoparticles derived from the leaf extracts of *M.concanensis* Nimmo showed particles to be in spherical shape with size ranging from 0.2 to 1 µm (Figure 3).

1.4. Nanoparticle size distribution analysis of synthesized silver nanoparticles from aqueous extract of M.concanensis Nimmo leaves:

The average particles size was determined by DLS method, and it was found to be 865 nm as revealed in the size distribution graph (Figure 4).The size distribution histogram of dynamic light scattering (DLS) indicated that the size of these silver nanoparticles is 100 nm to 1000 nm. Some distribution at lower range of particle size indicates that the synthesized particles are also in lower range of particle size. The DLS pattern of the suspension of Ag nanoparticles synthesized using *M.concanensis* Nimmo aqueous leaf extract as shown in figure 4.

1.5. Energy dispersive X-ray analysis of synthesized silver nanoparticles from aqueous extract of M. Concanensis Nimmo leaves:

The energy dispersive X-ray analysis gives qualitative as well as quantitative status of elements that may be involved in formation of nanoparticles. The elemental profile of synthesized nanoparticles using aqueous extract of *M.concanensis* Nimmo leaves showed higher counts at 3 keV due to silver, confirms the formation of silver nanoparticles (Figure 5). The elemental analysis of the synthesized silver nanoparticles from aqueous extract of *Moringa concanensis* Nimmo leaves showed in figure 5 revealed highest proportion of silver followed by chloride.

1.6. Fourier Transform – infrared red (FT-IR) analysis of synthesized silver nanoparticles from aqueous extract of M. Concanensis Nimmo leaves:

The FT-IR spectroscopy measurements were carried out to identify the biomolecules that bound specifically on the silver surface and local molecular environment of capping agent on the nanoparticles. The synthesized silver nanoparticles were analyzed by FT-IR spectrophotometer. The FT-IR spectra of biosynthesized AgNPs from *M.concanensis* Nimmo leaves extract, showed in figure 6, indicates the presence of carboxylic, hydroxyl, alkanes, alkenes and carbonyl groups. Display of strong broad O–H stretch carboxylic bands in the region 3273.34 cm^{-1} and carbonyl stretching bands in the region 1070.54 cm^{-1} was observed. The peaks appearing in the region 1630.88 cm^{-1} are attributed by the alkene group. The alkanes stretching bands was observed in the region of 2847.05 cm^{-1} and 2915.53 cm^{1} in FT-IR spectrum.

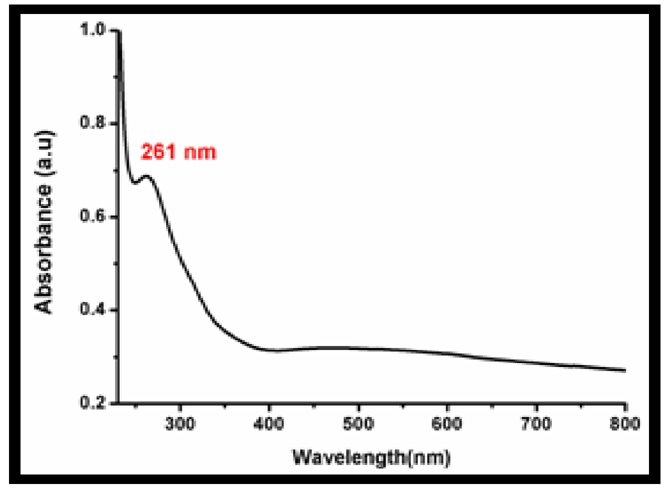


Fig. 2: UV visible spectral analysis of silver nanoparticles

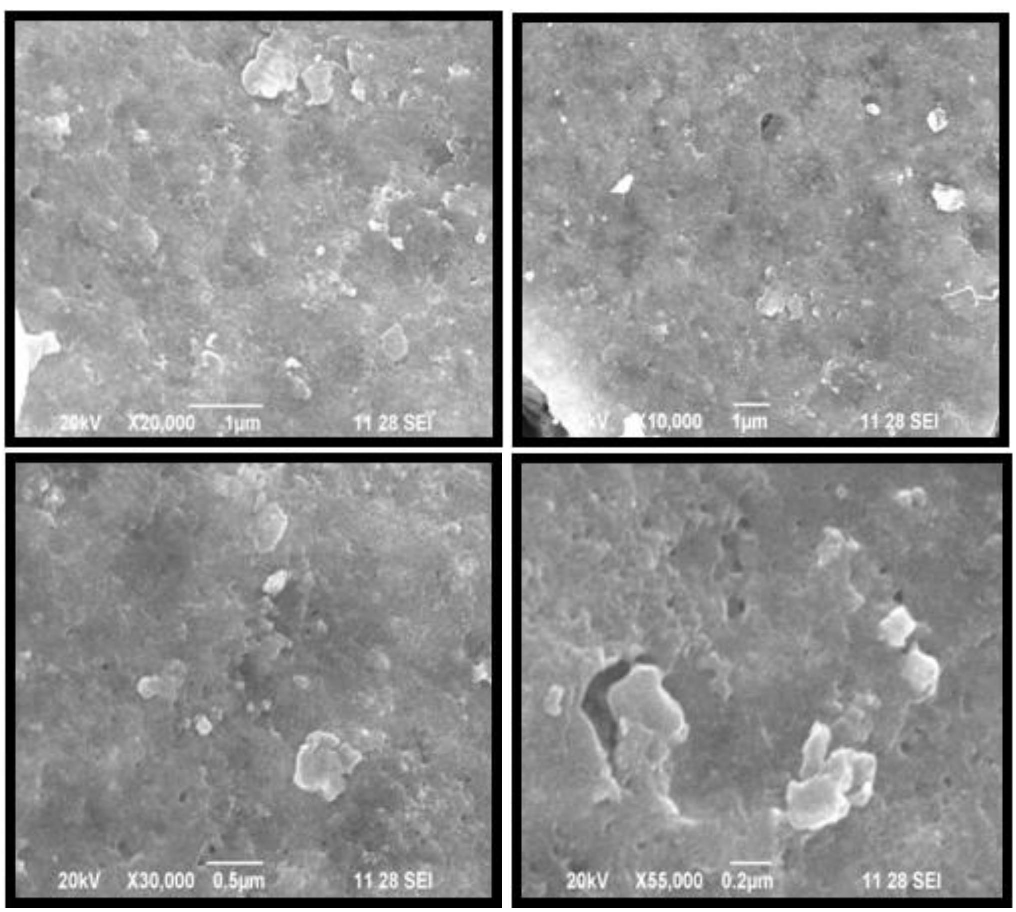


Fig. 3: Scanning electron microscopic analysis silver nanoparticles

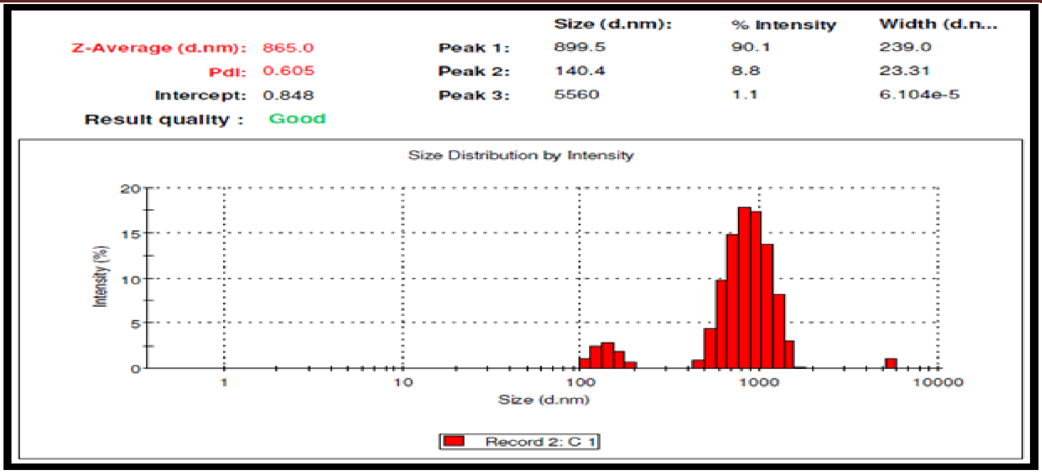


Fig. 4: Nanoparticle size distribution analysis of synthesized silver nanoparticles

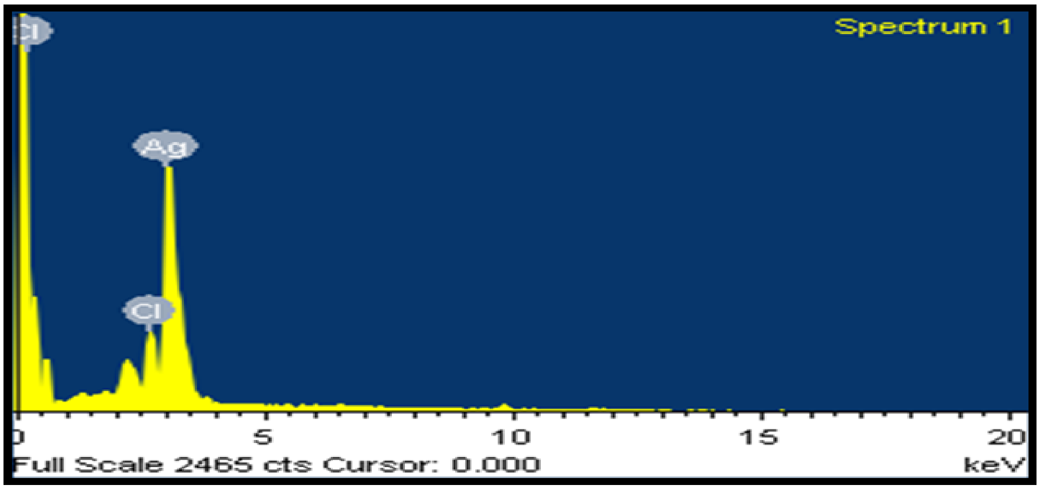


Fig. 5: Energy dispersive X-ray analysis of synthesized silver nanoparticles

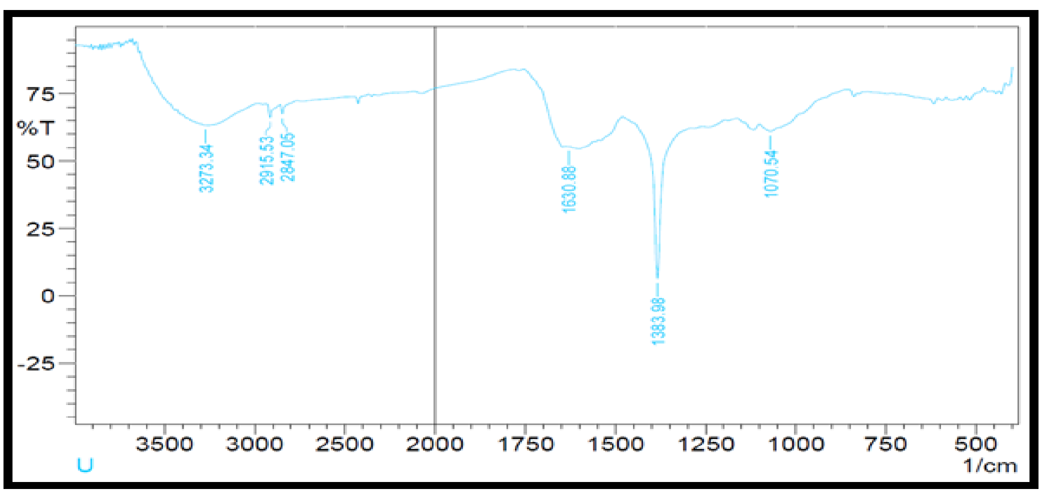


Fig. 6: Spectrum of FT-IR analysis of synthesized silver nanoparticles

DISCUSSION

The utilization of medicinal plant materials, microbes, enzymes for synthesis of nanoparticles has been revolutionized in recent years [9]. Noble metals are known to exhibit unique optical properties due to the property of surface plasmon resonance [10]. The formation of silver nanoparticles was monitored with color change and UV-Vis spectroscopy. The color of the reaction mixture started changing to brown within 10 min and to black after 1 hr, indicating the generation of silver nanoparticles, due to the reduction of silver metal ions Ag into silver nanoparticles AgNP via the active molecules present in the plant extract [11]. The UV–Visible spectroscopy was an important technique for analyzing the formation of silver nanoparticles (AgNPs) in the reaction medium. Aqueous solution of AgNPs has free electron, which gives rise to plasma resonance absorption band, due to combined vibration of metal nanoparticles in resonance with the light wave. A surface plasma resonance spectrum of AgNPs was obtained at 261 nm after 5 min colour changing to light yellowish colour. This may be due to the excitation of Surface Plasmon Resonance (SPR) of the synthesized Ag NPs [12]. The SPR band at 410 to 430 nm confirmed the synthesis of AgNPs at plant products [13]. UV-Vis spectrophotometer allows identification, characterization and analysis of metallic nanoparticles. In general 200-800 nm light wavelength was used for the characterization of size range 2-100 nm [14].

The morphology of the silver nanoparticles was predominantly spherical and they appear to be monodispersed. Further, analysis of the silver particles by energy dispersive spectroscopy confirmed the presence of the signal characteristic of silver. In SEM analysis, there were observed few traces of AgNPs clusters due to aggregation of nanoparticles (Figure 3), which might be induced by the evaporation solvent during sample preparation [15]. The scanning electron microscopy (SEM) is a common method for surface and morphological characterization. Scanning electron microscopy (SEM) is used for the morphological characterization at the nanometer to micrometer scale [16]. The dynamic light scattering (DLS) was used to characterize the surface charge and the size distribution and quality of nanoparticles. This is also very useful to know the polydispersity index of the prepared nanoparticles [17]. Dynamic light scattering (DLS) measurements were done to determine the size of the SNPs formed. The particles size distribution curve of the synthesized silver nanoparticles was showed in (Figure 4). This showed various sizes of the particles ranging from 100 nm to 1000 nm and had an average particle size of 865 nm. Energy dispersive spectroscopy (EDS) was used to know the elemental composition of metal nanoparticles, which gives the elemental knowledge of synthesized silver nanoparticles [18]. Generally metallic silver nanocrystals showed typical optical absorption peak approximately at 3 keV due to their surface Plasmon resonance [19].

Fourier transform infrared spectroscopy (FT-IR) analysis was used for functional characterization of nanoparticles. The FT-IR was very useful for the surface chemistry because the organic functional groups can be determined which are attached to the surface of nanoparticles [20].

The crystalline silver nanoparticles have been found tremendous applications in the fields of diagnostics, antimicrobials and therapeutics. However, there is still need for economic commercially viable as well as environmentally clean synthesis route to synthesize the silver nanoparticles. Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes [21].

Silver was the only material that the plasmon resonance can be turned to any wavelength in the visible spectrum [22]. Studies carried out in the last few decades shows that silver nanoparticles exhibit a rare combination of valuable properties like catalytic activity, high electrical double layer capacitance etc. Nanosilver has also been used extensively as antibacterial agent in the health industry, food storage, textile and a number of environmental applications [23].

The findings of the above investigation was clearly indicates that the plant *Moringa concanensis* Nimmo leaves was an excellent source for the synthesize of silver nanoparticles that may have various medicinal properties.

CONCLUSION

The green synthesis and characterization of silver nanoparticles synthesized from aqueous extract of *Moringa concanensis* Nimmo leaves was done and confirmed by UV–visible spectrophotometer, FT-IR, SEM, TEM, XRD and EDX techniques. The nanoparticles appeared to be spherical in shape with smooth surface and the size of the particles varied from 100 to 1000 nm. Based on this study it was concluded that the plant *Moringa concanensis* Nimmo is a good source for synthesize of silver nanoparticles in eco-friendly manner.

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