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MICROWAVE ASSISTED SYNTHESIS OF SILVER NANOPARTICLES USING *AZADIRACHTA INDICA* STEM BACK EXTRACTS AND ITS ANTIOXIDANT PROPERTY

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ABSTRACT

Nanotechnology is one of the most active and fastest research field used in modern material science and technology. Green synthesis of nanoparticles is an Eco-friendly method which acts as a joint to the modern research in the field of nanotechnology. The present work was undertaken to synthesize silver nanoparticles from *Azadirachta indica* stem bark extracts using environmental friendly protocols (Microwave). Synthesized nanoparticles were characterized under Fourier-transform infrared and UV-Visible spectroscopy at the range of 200-700 nm. The peak was obtained at 432 nm and the result also showed that DPPH and Hydrogen peroxide Assay of *Azadirachta indica* stem bark extract have higher antioxidant and anti radical properties. The range and size of the particles were confirmed at 48 nm by Particle Size Analyzer. Based on the result obtained it can be recommended that stem bark extracts of *Azadirachta indica* (neem tree) and other plant resources can be used effectively in the production of silver nanoparticles and it could be applied to various fields such as Nanotechnology, Biomedical Sciences and Biotechnology.

Keywords: Antioxidant, azadirachta indica, nanoparticles, stem bark, synthesis.

INTRODUCTION

The field of nanotechnology is one of the most active and fastest developing research fields in modern material science and technology. Nanoparticles are fundamental building blocks of nanotechnology. The most important and distinct property of nanoparticles is their exhibition of large surface area to volume ratio [1]. Physical and chemical methods are popular for nanoparticle synthesis but the use of toxic compounds limits their application to be economically feasible. An array of physical, chemical and biological methods have been used for

synthesis of noble metal nanoparticles of particular shape and size for various applications, but they remain expensive and involve the use of hazardous chemicals [2].

Green synthesis of inorganic nanoparticle is an eco-friendly and fast growing research in the limb of nanotechnology [3]. The biosynthesis method employing plant extracts have drawn attention as a simple and viable alternative to chemical and physical methods. This is because bio-reduction of silver ions has been found to yield metal nanoparticles using living plants such as geranium leaf, neem leaf [4].

Azadirachta indica commonly known as Neem, nim tree or Indian lilac, belongs to the Meliaceae family. It is one of the two species in the genus *Azadirachta* which is native to the Indian sub continent, such as Nepal, Pakistan, Bangladesh, Sri Lanka and Maldives. It is well known in Nigeria and its neighbouring countries for more than 50 years. It is one of the most versatile medicinal plants having a wide spectrum of biological activity. Every part of the tree has been used as a traditional medicine for household remedy against various human ailments from antique times [5].

Azadirachta indica leaf extract has also been used for the synthesis of silver, gold and bimetallic (silver and gold) nanoparticles. The major advantage of using the Neem bark is that it is a commonly available medicinal plant used as a topical or oral medicine for a wide variety of ailments. Clinical data on the effectiveness of Neem bark is lacking, but preliminary trials show tentative support for traditional use of the Neem bark due to its antibacterial, antifungal and insecticidal properties. Therefore biosynthesized silver nanoparticle might have been enhanced as it was capped with the Neem bark extracts [6].

The aim of the study is to synthesize silver nanoparticles using Azadirachta indica stem bark extracts using environmental friendly protocols (Microwave). The objectives of the study are:

1) To characterize synthesized silver nanoperticles from Neem tree stem back.
2) To find out the particle size of the synthesized silver nanoparticles from Neem tree stem bark.
3) To bring to light the antioxidant property of the synthesized silver nanoperticles from Neem tree stem bark.

MATERIALS AND METHODS

Sampling and extract preparation.

Fresh stem bark of Neem tree was collected from Jaipur botanical garden, Jaipur. Rajasthan, India. Sample was washed thoroughly with distilled water and allowed for air dry at room temperature. 25 g of the sample were cut into fine pieces and boiled in a 500 ml Erlenmeyer flask with 100 ml of de-ionized distilled water on hot plate for 30 minutes. The aqueous extracts was filtered with Whatman Number 1 filter paper and then stored at room temperature for synthesis of silver nanoparticles.

Synthesis of silver nanoparticles (AgNPs)

A microwave irradiation was used for the synthesis of AgNPs. 100ml/1mM silver nitrate ($AgNO_3$) solution was taken in 250 ml conical flask and 10 ml of aqueous Neem tree stem bark extract was added., The mixture was kept in the microwave oven (model: Ms- 2049UW, japan) input power (230 v, 50 Hz) for 180 s at different intervals. The colour of the mixture changed from yellow to brown which indicates the formation of silver nanoparticles (Fig. 1). Then the content was incubated in the darkness for 24 hours, it was then centrifuged at 10,000 rpm for 10 minutes to remove the biological admixtures present.

UV- visible spectroscopy

The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the reaction medium from the wavelength of 200 -700 nm by using Spectro UV 2080 Double beam 1200 L/mm, China. The result signifies that UV-visible spectra show an absorption band at 432 nm which corresponds to the absorbance of silver nanoparticles after 180 s (figure 2), while the a study illustrated that *Azadirachta indica* leaves has an absorption peak of 351 nm [7].

DPPH assay

The prepared solution of the stem back extracts was taken with different concentrations from 50-250 µg, 1 ml of prepared 1 mM DPPH in ethanol and 0.95 ml HCl were added. The reaction mixture was incubated at room temperature for 20 minutes and the absorbance was measured at 517 nm after generating a yellow colour. The DPPH free radical scavenging activity was subsequently calculated [8].

$$\%\, DPPH\ radical\ scavenging = \frac{absorbance(cont) - absorbance(sam) x\ 100}{absorbance(cont)} \text{---------- i}$$

Hydrogen peroxide assay

The stem bark extracts were taken in a test tube with varying concentrations starting from 20-100 ug. 0.6 ml of hydrogen peroxide, prepared phosphate buffer (pH 7.4) was added. The reaction mixture was incubated at room temperature for 10 minutes. After incubation absorbance was measured at 270 nm against the blank solution with phosphate buffer the DPPH hydrogen peroxide assay was calculated [8].

$$\%\ inhibition = \frac{absorbance(cont) - absorbance(sam) x\ 100}{absorbance9cont0} \text{---------- ii}$$

Fourier Transform Infrared Spectroscopy

Infrared Spectroscopy gives information on the vibrational and rotational modes of motion of a molecule and hence an important technique for identification and characterisation of a substance. The particles were analyzed under FT-IR for the size conformation.

Particle size analyser

The particle size analysis was carried out for the sample which is lyophilized and dispersed by ultrasonicator for the determination of size.

RESULTS AND DISCUSSION

Eco-friendly synthesis of silver nanoparticles from *Azadirachta indica* stem bark extracts was formulated and the stem bark extracts is very much suitable for the synthesis of small size silver nanoparticles. The colour changed from yellow to brown, indicating the presence of different phytochemicals responsible for reduction, stabilization and capping of silver nanoparticles, which is confirmed by UV-Visible and FT-IR spectroscopy.

Plate 1: Colour change of reduced silver metal in synthesis of AgNPs at different time intervals from 30 s, 60 s, 90 s, 120 s, 150 s and 180 s.

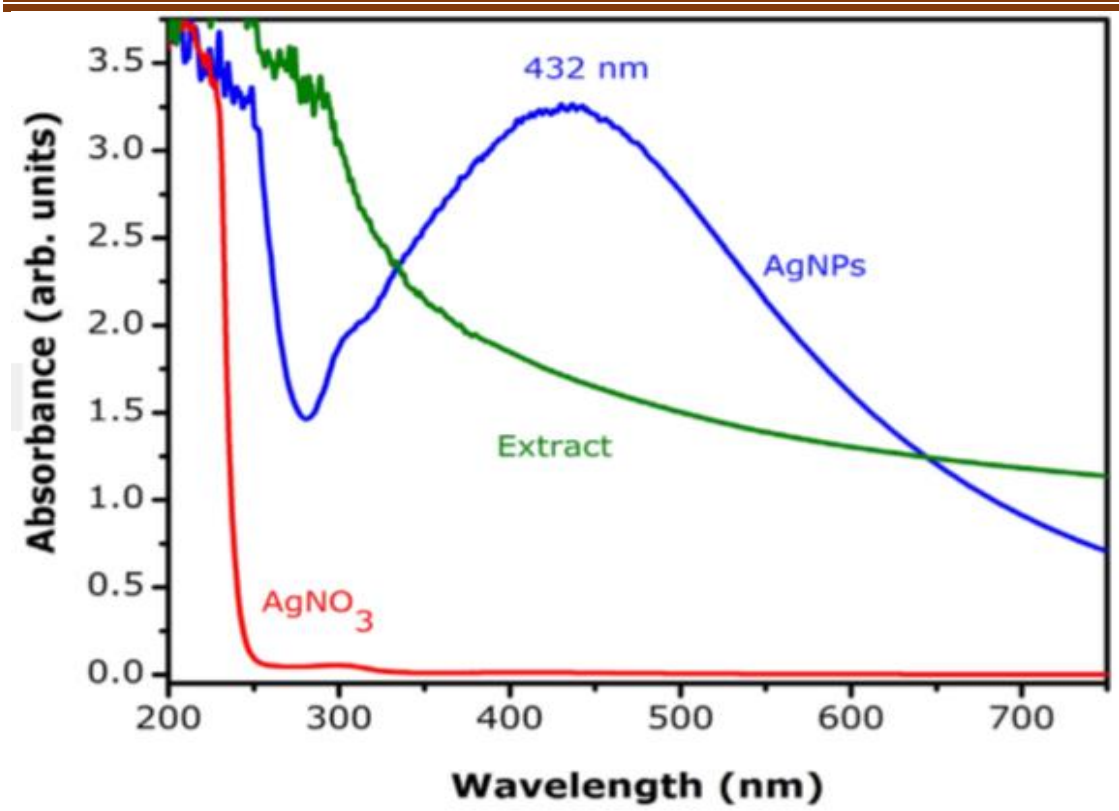

Fig. 1: UV- visible absorption spectra of solution containing AgNPs of Neem tree and $AgNO_3$.

FT-IR spectroscopy

FT-IR spectra of the stem bark extracts revealed the major peaks of some essential functional groups necessary for the formation of bonds between the phytoconstituents and the silver ions in the solution. The functional groups of stem bark extracts and the synthesized nanoparticles were identified by using FT-IR spectroscopy between the scan ranges of 400-4000 cm^{-1}. The synthesized stem bark extracts of *Azadirachta indica* show a strong bands at 3400 cm^{-1} which indicates the presence of phenolic at O-H stretching vibration; 2947 cm^{-1} for C-H stretching vibration of methylene group; 2308 cm^{-1} for N-H bond of Ammonium ions; 1603cm^{-1} for N-H band of amines; 1419 cm^{-1} for O-H band of carboxylic acids; 1316 cm^{-1} for C-H band of alkenes, 1021 cm^{-1} for C-N band of aliphatic amines, 774cm^{-1} for C-H Band of Aromatic Benzene and 654cm^{-1} for C-H deformation of Alkynes reflecting its complex nature. Most of the peaks appeared in the stem bark extract disappeared after the synthesis of AgNPs. Based on the FT-IR analysis, it was confirmed that the broad peaks of Phenols acts as a reducing, stabilizing and

capping agents [9]. According to Niramathi [10] the FT-IR spectra entail that the proteins present in the plant extract acted as a capping agent.

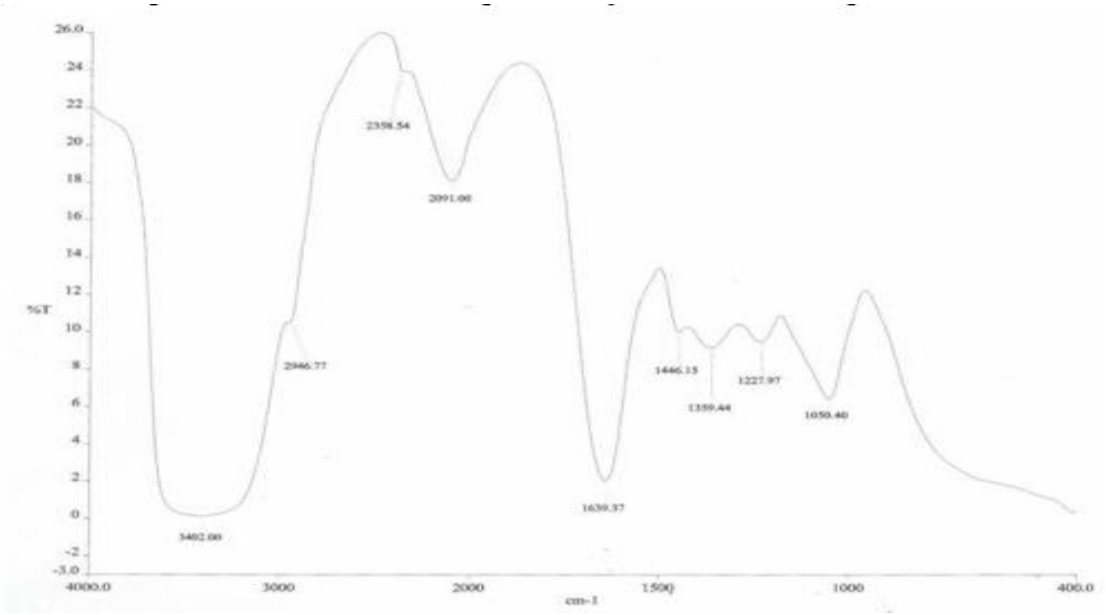

Fig. 2: FT-IR of synthesized silver nanoparticles from *Azadirachta indica* stem bark extracts.

The result obtained also show that DPPH and Hydrogen peroxide Assay of *Azadirachta indica* stem bark has high antioxidant and anti radical properties by donating electrons to free radicals which neutralized and prevents them from causing harm. Therefore they fight any damage caused by free radicals, and unstable molecules that can harm cellular structure. This also correlated positively with the number of hydroxyl groups bonded to the phenol structure and mode of substitution of hydroxyl groups in aromatic ring.

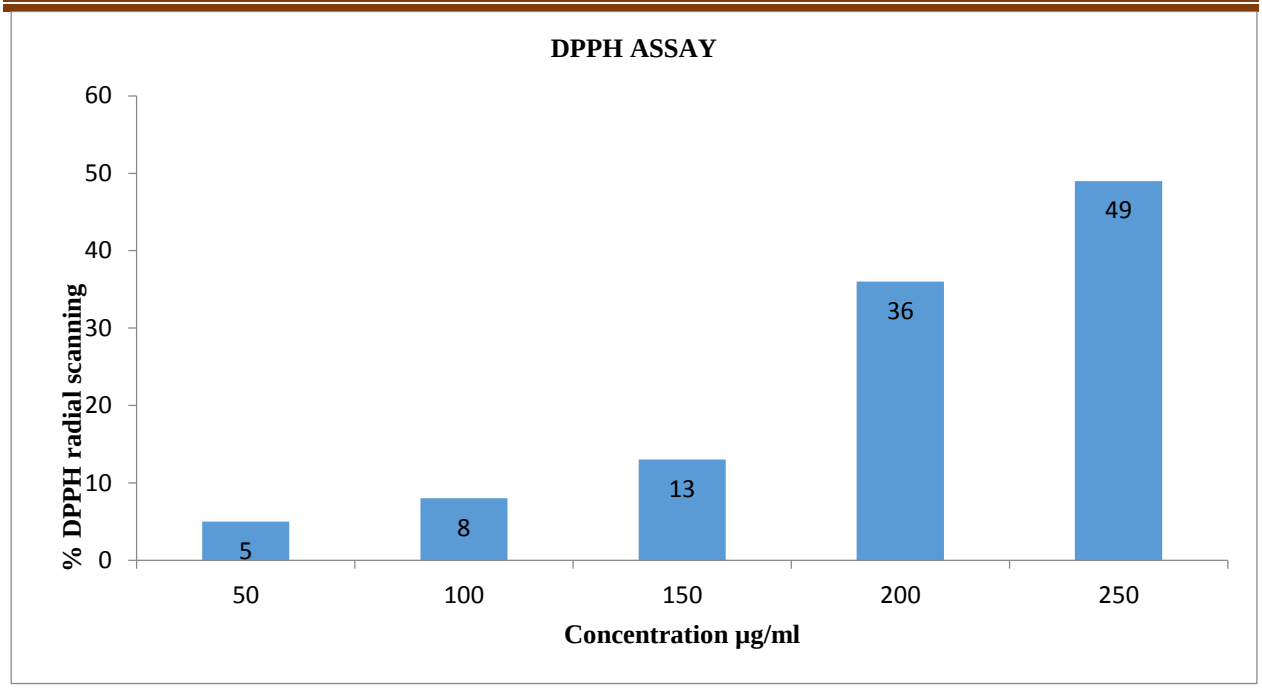

Fig. 3: DPPH Assay of the synthesized AgNPs of Neem tree stem bark extracts.

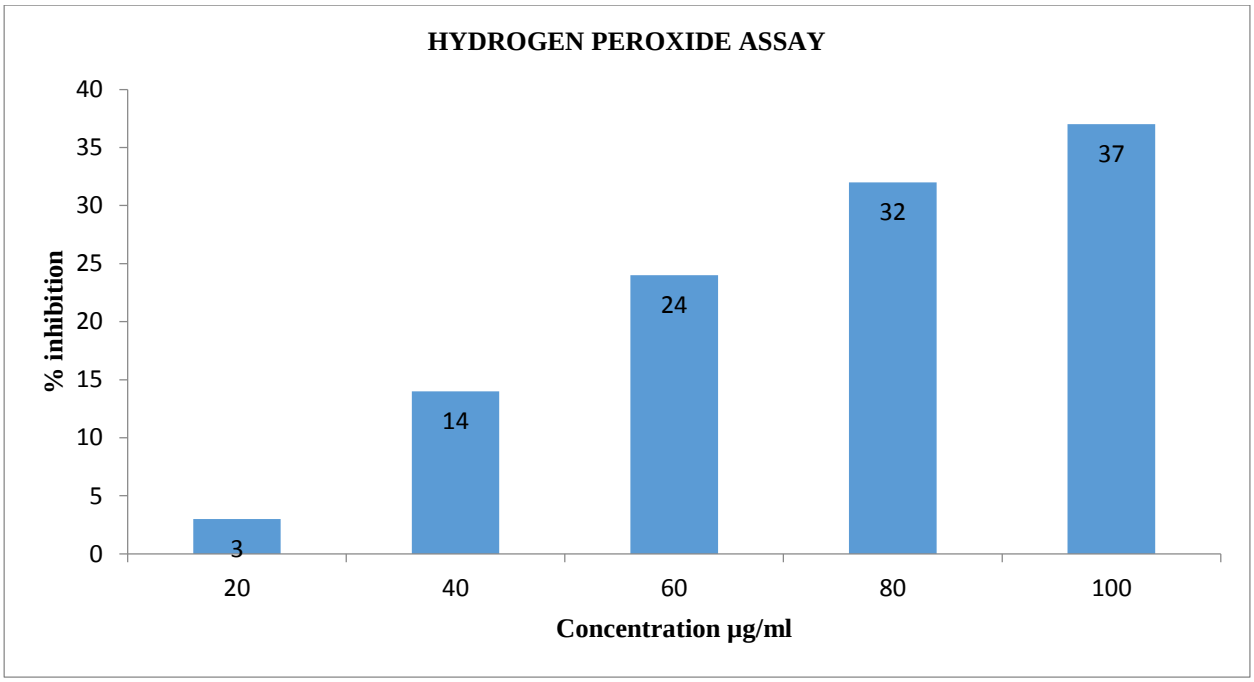

Fig.4: Hydrogen peroxide Assay of the synthesized AgNPs of Neem tree stem bark extracts

The particle size analyser result shows that the size range of the synthesized nanoparticles is 48 nm in size. The study carried out by Lalitha *et al* [6] stated that the size of nanoparticles was 38 nm for *Azadirachta indica* leaves, and that of ponmurugan [7] was 21.17 nm.

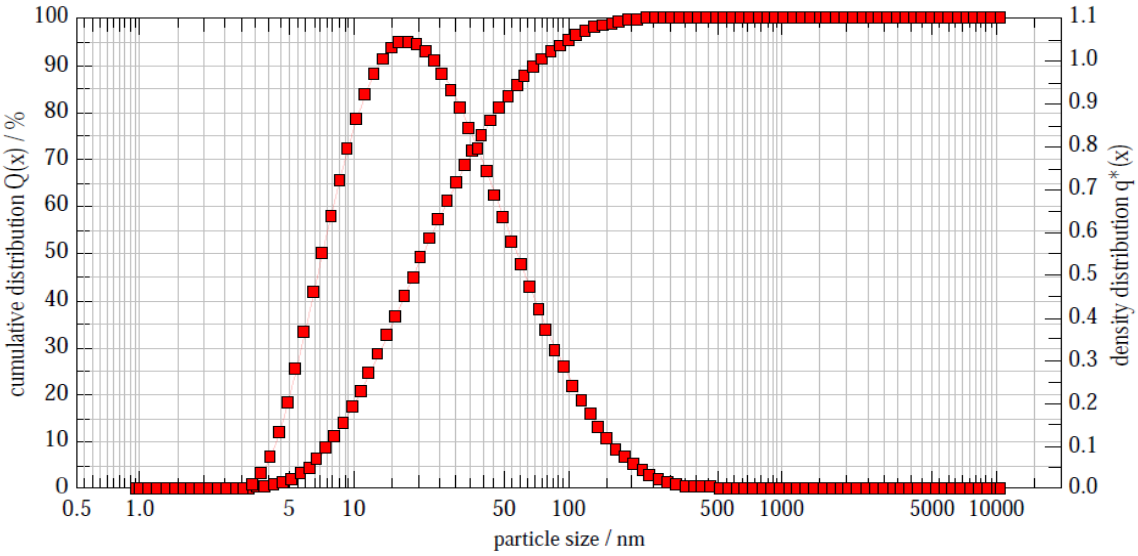

Fig. 5: Particle Size Analysed of synthesized silver nanoparticles from *Azadirachta indica* stem bark extracts**.**

CONCLUSION

The colour change pattern of stem bark extracts of *Azadirachta indica* from yellow to brown shows the preliminary conformation of synthesized silver nanoparticles. The intense peak obtained at 432 nm using UV-visible spectroscopy indicates the presence of silver nanoparticles in stem bark of *Azadirachta indica.* Further studies of FT-IR confirmed the synthesis of nanoparticles from stem bark extracts of *Azadirachta indica.* Also FT-IR studies indicate phenols and proteins are most responsible for reducing, stabilizing and capping agents towards the formation of AgNPs. The same type of FT-IR result for *Azadirachta indica* leaves was obtained by Padmanabhan *et al* [10]. The particle size analyser result shows that the size range of the synthesized silver nanoparticle is 48 nm .Therefore it can be recommended that stem bark extracts of *Azadirachta indica* and other plant resources can be used effectively in the synthesis of silver nanoparticles and it could be applied to various fields such as nanotechnology, biomedical sciences, pharmacy, biotechnology and so on.

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