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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *MORINDA CITRIFOLIA* FRUIT SAMPLES AND ITS CHARACTERIZATION

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ABSTRACT

Plants have been an important source of medicine with qualities for thousands of years. Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. The Silver nanoparticles were synthesized using a much simpler and fast means using the *Morinda citrifolia* fruit samples. The Silver ions readily get reduced to Silver nanoparticles hence the fruit extracts of *Morinda citrifolia* can be considered as potential reducing agent. The primary confirmation was done qualitatively with the color change of brownish black. The bioreduction was observed by the change in the color. The color change of the dispersion is attributed to the Surface Plasmon resonance vibration which is shown by the electrons of the nanoparticles synthesized. This was confirmed by the absorbance maximum found around 430 nm using UV-Visible spectral analysis. Scanning Electron microscopy showed the particle size ranging from 12-26nm.

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INTRODUCTION

Nanomaterials are synthesized from the novel, eco-friendly and sustainable techniques of physical, chemical, biological and engineering processes. In this 21st century, the nanotechnology has emerged as an interdisciplinary field with the biosynthesis of metal nanoparticles. Nanotechnology is gaining importance in various fields such as health care, food and feed, cosmetics, environmental health, biomedical science, chemical industries, drug and gene delivery, energy science, electronics, mechanics and space industries. There are many ways to synthesis nanoparticles such as solid reaction, co-precipitation, chemical reaction and sol gel method etc, (Saranyadevi *et al.*, 2014). Bio-based approaches for the synthesis of nanoparticles are rapidly gaining importance due to their ease of synthesis, eco-friendliness and formation of stable and biocompatible nanoparticles. Bacteria, fungi, plants and seaweeds are the potential sources utilized for the synthesis of

nanoparticles (Dhas *et al.,* 2013). The green synthesis with plant extracts are simpler and advantageous over other biological processes as they are safe to handle.

In biological method, the plant extracts has been used as reducing agent and capping agent for the synthesis of nanoparticles (Akl and Nida, 2012) due to their reducing properties (Umesh and Vishwas, 2013). Some properties such as size, distribution, and morphology of the particles are clearly obtained from the nanoparticles (Nethradevi *et al.,* 2012). Silver nanoparticle acts as antimicrobial agent which finds applications in medical field such as Ag NPs coated blood collecting vessels, coated capsules, band aids etc., (Akl and Nida, 2012; Umesh and Vishwas, 2013; Nethradevi *et al.,* 2012; Geoprincy *et al.,* 2011). The silver is non toxic to animal cells and highly toxic to bacteria and other microorganisms such *E coli*, *P. aeroginosa*, *S. aureus* etc. Due to these phenomena it is considered to be a safe and effective bactericidal metal (Kalimuthu *et al.,* 2008) and therefore can be incorporated with several materials such as cloths, ointments etc. The band aids so developed are highly sterile and therefore can be useful in the hospitals to prevent or to minimize the infections with pathogenic bacteria such as *E coli*, *S. aureus* etc, (Wijnhoven *et al.,* 2009).

Synthesis of silver nanoparticles using plant extracts have been reported in *Boswellia ovalifolilata*, *Shorea tumbuggaia Svensoina hyderobadensis, Thespesia populnea, Vinca rosea* , *Cassia auriculata* . *Morinda citrifolia* (Noni) has been extensively used in folk medicine for over 2,000 years. It has been reported to have broad therapeutic effects, including cancer activity, in both clinical practice and laboratory animal models (Wang and Su, 2001). Noni has traditionally been used for colds, flu, diabetes, anxiety, and high blood pressure, as well as for depression and anxiety. The green fruit, leaves, and root/rhizomes were traditionally used in Polynesian cultures to treat menstrual cramps, bowel irregularities, diabetes, liver diseases, and urinary tract infections (Klueh *et al.,* 2000). Hence the present study was demonstrated the synthesis of silver nanoparticles from fruit samples of *Morinda citrifolia* and its characterization.

MATERIALS AND METHODS

Preparation of fruit extract

5 grams of fresh fruit samples *Morinda citrifolia* was collected and washed with distilled water 3-4 times to remove the dust particles. Fruit samples were chopped into small pieces and mixed into 150 ml distilled water separately. Mixture was stirred at 800 rpm for 3 hrs in magnetic stirrer. The aqueous extract was separated by filtration with whatmann No. 1 filter paper. The filtrate was collected and stored at 4^{o}C.

The extract stored was used for biosynthesis of silver nanoparticles from silver nitrate. 25 ml of the prepared extract was added to 25 ml of aqueous $AgNO_3$(0.1M in 100 ml) at room temperature. The mixture was stirred continuously for 5-10 minutes.

$$Ag\ NO_3 \rightarrow Ag + NO^{3-}$$

$$Ag\ 2H_2O \rightarrow Ag0 + 4H + O_2$$

Synthesis of $AgNO_3$

45 ml of 1 mM aqueous $AgNO_3$ solution added to 5 ml of plant extract to the conical flask. The flask was then incubated in the dark at 4 hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without plant extract. The Ag nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. (Arunachalam *et al*., 2012). Then the Ag nanoparticles air dried in desiccator for 6 hrs. Dried nanoparticle was used for biological activities.

(a) $AgNO_3$ (b) AgNPs

Fig.1: Aqueous solution $AgNO_3$ with fruit extract (a) before adding the extract and (b) after addition of extract at 4 h.

UV and FTIR Spectroscopic analysis

The reduction of pure Ag+ ions was examined under visible and UV light for proximate analysis. For UV and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vaccum pump. The sample is diluted to 1:10 with the same solvent. The reduction of pure Ag+ ions were scanned in the wavelength ranging from 260-800 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected.

FTIR analysis

FTIR analysis was performed using Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm -1 and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

Scanning Electron microscopy (SEM) analysis of silver nanoparticles

ZEEISS-SEM machine were used to characterize mean particle size and morphology of nanoparticles. The freeze dried sample of AgNPs solution was mixed with deionized water, small drop of this sample was placed on glass slide allowed to dry. A thin layer of platinum was coated to make the samples conductive ZEEISS-SEM machine was operated at a vacuum of the order of 10-5 torr. The accelerating voltage of the microscope was kept in the range 10kV

RESULTS AND DISCUSSION

The reduction was completed with the appearance of brownish-black color which confirms the formation of silver nanoparticles. The contents were centrifuged at 8, 000 rpm for 10 minutes. The supernatant was used for the characterization of Silver nanoparticles. The bioreduction of Silver ions was studied the UV-VIS spectrophotometer.

During the process of biosynthesis the Silver nanoparticles were formed giving a brownish black color to the aqueous solution which is due to the excitation of Surface Plasmon Resonance. In course of time, the mixture grew darker and darker but remained to be highly stable within a few hours. This color change clearly indicated the reduction of Ag+ ions confirming qualitatively the formation of Ag- Nanoparticles. (Karthick and Avimanyu, 2011; Savithramma (*et al.*, 2012).

From the visual observations it was clear that the plant extract of *M. Citrifolia* was a good reducing agent for Ag+ ions to Silver nanoparticles. The UV-Vis absorption spectra of the Silver nanoparticles formed is shown in the Figure 2. Absorption spectra of both the extract and the nanoparticles are shown which shows the formation of Silver nanoparticles with an absorption maximum at ≈ 430 nm. The results of the present study was in accordance with the findings of (Velavan *et al.*, 2012; Wang and Su, 2001; Wang *et al.*, 2002).

FTIR spectral analysis showed the presence of phenolic compounds, amines, alcohol, ether and esters. (Figure 3)

The particle size distribution exposed to particle size diameter in the nano silver liquid was given by the Figure. 4 a & b. The Particle size of the nanosilver was ranging from 12nm-26nm.

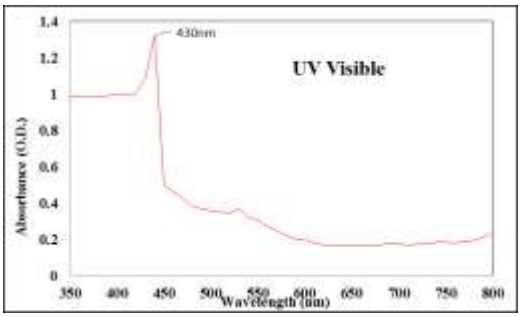

Fig.2: UV Spectroscopic analysis of silver nanoparticles

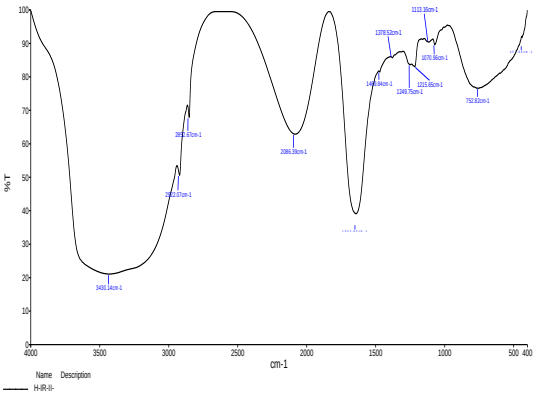

Fig.3: FTIR Spectroscopic analysis of silver nanoparticles

Table.1: Functional group identification from FTIR analysis

Peak Value	Bond	Functional Groups
3430.14	O–H Stretch, H–Bonded	Alcohols, Phenols
2912.07, 2852.67, 1469.84	C–H Stretch	Alkane
1642.63	N–H Bend	Primary Amines
1249.75,1215.65, 1113.16, 1070.96	C–O Stretch	Alcohols, Carboxylic Acids, Esters, Ethers
752.82	C–H "oop"	Aromatics

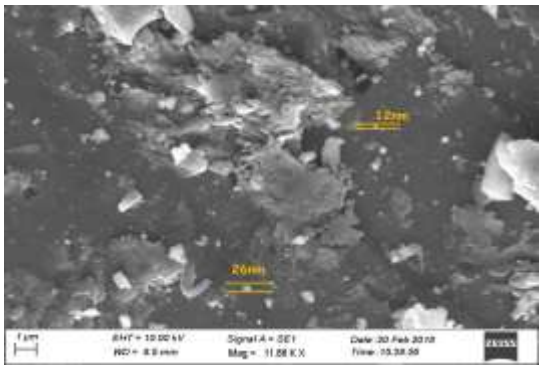

Fig.4(a): Scanning Electron microscopy (SEM) analysis of silver nanoparticles

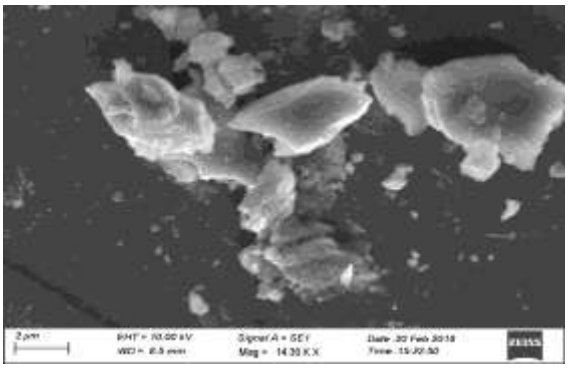

Fig.4(b): High resolution of individual AgNPs focused by Scanning Electron microscopy

CONCLUSION

The Silver nanoparticles were synthesized using a much simpler and fast means using the *Morinda Citrifolia fruit samples*. The Silver ions readily get reduced to Silver nanoparticles hence the fruit extracts of *Morinda Citrifolia* can be considered as potential reducing agent. The primary confirmation was done qualitatively with the color change of brownish black. The bioreduction was observed by the change in the color. The color change of the dispersion is attributed to the Surface Plasmon resonance vibration which is shown by the electrons of the nanoparticles synthesized. This was confirmed by the absorbance maximum found around 430 nm using UV-Visible spectral analysis.Scanning Electron microscopy showed the particle size ranging from 12-26nm.

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