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Original Article

MUNTINGIA CALABURA SILVER NANOPARTICLES DETERIORATE OXIDATIVE IMPAIRMENT WITH POTENT ANTIBACTERIAL ACTIVITY

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ABSTRACT

Objective: The current study exemplifies the synthesis of silver nanoparticles using *Muntingia calabura* L. (Mc-AgNP's) fruit extract utilizing a green approach and testing the efficacy of synthesized NP's.

Methods: The green synthesize approach was used to synthesis Mc-AgNP's followed by characterization using Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Energy Dispersive X-ray Spectroscopy (EDX), and Field Emission Scanning Electron Microscopy (FESEM). Radical scavenging activity was assessed using DPPH, FRAP, and H_2O_2, followed by antibacterial activity.

Results: The characteristic features of synthesized *Muntingia calabura* silver nanoparticles (Mc-AgNP's) were analyzed using FT-IR which particularizes different functional groups with a broadband at 3408 cm-1 representing hydroxyl (-OH) stretching a peak at 1593.27 cm-1 corresponds to C = O groups in amide whereas a dip at 1383 cm-1 represents C-N amine and C-O stretching of alcohol groups were found. The Crystallinity of synthesized Mc-AgNP's exhibited face-centered cubic (fcc) crystalline structure and the bio-reduction of the silver ions in solution was monitored by Energy dispersive X-ray spectroscopy (EDX). The FESEM analysis indicates that Mc-AgNP's were dispersed in the solution using micrographs and the size ranged from 10 to 60 nm. The synthesized Mc-AgNP's efficiently scavenged free radicals in a dose-dependent manner with 69% for DPPH, 59.9% for FRAP, and 64% for H_2O_2 respectively. Further, the synthesized Mc-AgNP's demonstrated a potent antimicrobial agent against tested bacterial and fungal strains with a maximum zone of inhibition observed in *S. aureus, K. pneumonia*, and *P. vulgaris* with 14.6, 13.8, and 12.4 mm. Similarly, antifungal activity with *Trichoderma harzianum* demonstrated the highest zone with 18 mm followed by *Aspergillus oryzae* with 7 mm.

Conclusion: These results highlight the interesting potential of synthesized Mc-AgNP's as an effective source of bioactive compounds with potent antioxidant and antibacterial activity.

Keywords: Silver nanoparticles, Antioxidant, Antimicrobial

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INTRODUCTION

For several years, nanotechnology had a big influence on industries and in the field of science. Investments in research are supported by governments and corporations with several billion dollars worldwide to develop a good product [1]. Metal nanoparticles are distinguished as the most popular in biology and medicine with silver (AgNP's) nanoparticles playing a prominent role where the sources were mostly from natural origin [2]. Silver nanoparticles are found with a varied size between 1-100 nm [3] in which they are composed of a large percentage of silver oxide due to their large ratio of surface-to-bulk silver atoms. Numerous shapes of nanoparticles can be constructed depending on the application but commonly used are spherical silver nanoparticles also diamond, octagonal, and thin sheets are popular.

Nanoparticle synthesis has been elaborated in dependence on their shape, and size obtained based on various methods and using multiple solvents [4, 5]. The use of toxic substances was minimized by replacing them with natural ones in various sectors [6, 7]. "Green synthesis" of nanoparticles makes use of environmentally friendly, non-toxic, safe reagents like plants with their extracts found to be advantageous over other biological synthesis processes, which involve the very complex procedures of maintaining microbial cultures [8]. The applications of nanotechnology in biological molecules undergo highly controlled assembly for metal nanoparticle synthesis, which was found to be reliable and eco-friendly [9].

Muntingia calabura L., the sole species in the genus *Muntingia*, is a flowering plant native to southern Mexico and found globally in most continents. The fruit is edible, sweet, and juicy and contains a large number of tiny (0.5-mm) yellow seeds. It is a pioneer species that thrives in poor soil, able to tolerate acidic, alkaline, and drought conditions. It is grown for its edible fruit and cultivated in other parts of the tropics, including south-east Asia. The synthesis of AgNP's using aqueous fruit extract containing primary and secondary metabolites consisting of bioactive compounds like alkaloids, terpenoids, flavonoids, phenolic compounds, tannin, and saponins which will reduce $AgNO_3$ into AgNP's and also acts as capping agent. Though studies on the synthesis of AgNP's using aqueous leaf extract of *Muntingia calabura* [10] are reported, the present study focuses on the synthesis of AgNP's from fruits and tests the efficacy to overcome the common pathological conditions, identify the radical scavenging capacity of the AgNP's which are mainly responsible to induce pathogenicity [11].

MATERIALS AND METHODS

Collection of sample and synthesis of Mc-AgNP's

The fully ripened fruits of *Muntingia calabura* were collected from Erode District, Tamil Nadu, India during the month of December 2016. The fruits were pooled and were kept in cold (–4 °C) storage for further analysis. For the extraction of secondary metabolites, 100 g of fruits were taken and homogenized with 400 ml of methanol. Then, the extracts were centrifuged thrice (3000 g, 15 min) then, the clear supernatants were collected separately from the solvent [12] and the pellet was dried in a lyophilizer. AgNP's were synthesised following the earlier protocols [13].

Chemicals and solvents

All chemicals and solvents were procured from SD Fine Chemicals, Mumbai and Fischer Inorganic and Aromatic Limited, Chennai, India

Characterization of synthesized Mc-AgNP's

The synthesized nanoparticles were subjected to characterization using UV-Vis JASCO V-700 spectroscopy recorded at wavelengths ranging from 200 to 1000 nm, Fourier Transform-Infrared Spectroscopy (FTIR 8400) analysis of the dried powder of AgNP's by scanning it in the range of 450–4000 at a resolution of 4 cm^{-1}. The morphology of the Silver nanoparticles was examined using Field Emission Scanning Electron Microscopy (FESEM), and the images were operated at 15 kV on a 0° tilt position, crystallinity of AgNP's was observed using Bruker AXS D8 Advance X-ray Diffractometer (XRD) and Energy-Dispersive X-Ray Spectroscopy Analyser (EDXA: Oxford Link ISIS-300).

***In vitro* radical scavenging and antimicrobial activity of Mc-AgNP's**

In vitro antioxidant property of the AgNP's was carried out following the methods with slight modification. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay [14], FRAP (ferric reducing antioxidant power) assay [15], and H_2O_2 (hydrogen peroxide) scavenging activity was carried out following the method described earlier [16].

The synthesized Mc-AgNP's were analyzed for antibacterial and antifungal activity tested against five bacterial strains (*Escherichia coli*, *Klebsiella pneumonia, Staphylococcus epidermidis, Staphylococcus aureus*, *and Proteus vulgaris*) and two fungal strains (*Aspergillus oryzae* and *Trichoderma harzianum*). The activity was performed by determining the inhibitory effect of AgNP's by agar well disc diffusion method and the antimicrobial activity was determined by measuring the zone of inhibition observed on the plates and the standard antimicrobial agent such as Choremphanicol was used as control [17].

RESULTS AND DISCUSSION

Characterization of synthesized Mc-AgNP's

The Characteristic absorption peaks of silver nanoparticles can be seen at around 360–440 nm as revealed in the fig. 1, which was identified as the "surface Plasmon resonance band". Similarly, the silver nanoparticles produced from the *Muntingia calabura* leaf extract exhibited absorption peaks at 440 nm [10]. This indicates the preliminary confirmation of the presence of silver nanoparticles. The dried nanoparticle samples were analyzed in FTIR to identify the possible bio-molecules responsible for the reduction of the Ag^{+}ions by *Muntingia calabura* fruit extract. The FTIR spectrum is presented in fig. 2. The peak at 3408 cm^{-1}, shows the presence of hydroxyl (-OH) stretching groups caused by inter-molecular hydrogen bonding compounds such as phenols, alcohols, and carboxylic acids. The peak at 1593.27 cm^{-1} is a spectrum of C = O groups in amide. The dip at 1383 cm^{-1} peak of the cluster C-N amine and C-O stretching of alcohol groups were found. It is clearly understood that FTIR provides information on the vibrational and rotational modes of motion of a molecule. The Infrared spectrum of an organic compound provides a unique fingerprint, which is readily distinguished from the absorption patterns of all other compounds. In this regard, the peak at 1026.17 cm^{-1} indicates the C-N bond stretching. Saware *et al.* stated that the distinct peak in the range of 1640 and 1540 cm−1, which represents amide I and amide II of proteins, arises due to carbonyl stretch and – N–H stretch vibrations in the amide linkages [16]. The Carbonyl group of amino acid residues and peptides of proteins has the stronger ability to bind metal, so proteins could be the most possible organic molecule for stabilizing the AgNP's in the medium [17].

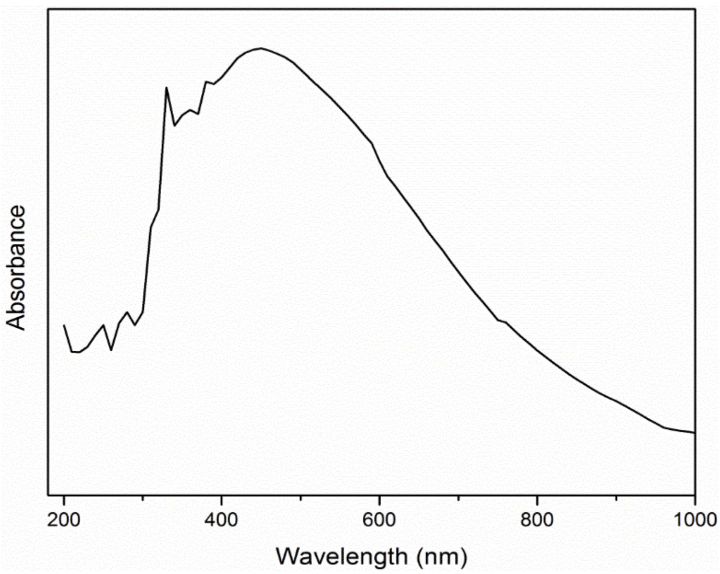

Fig. 1: UV-visible spectrum of Mc-AgNP's

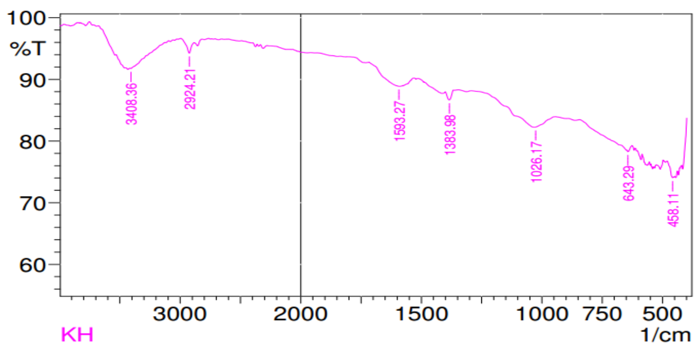

Fig. 2: FTIR spectra of Mc-AgNP's

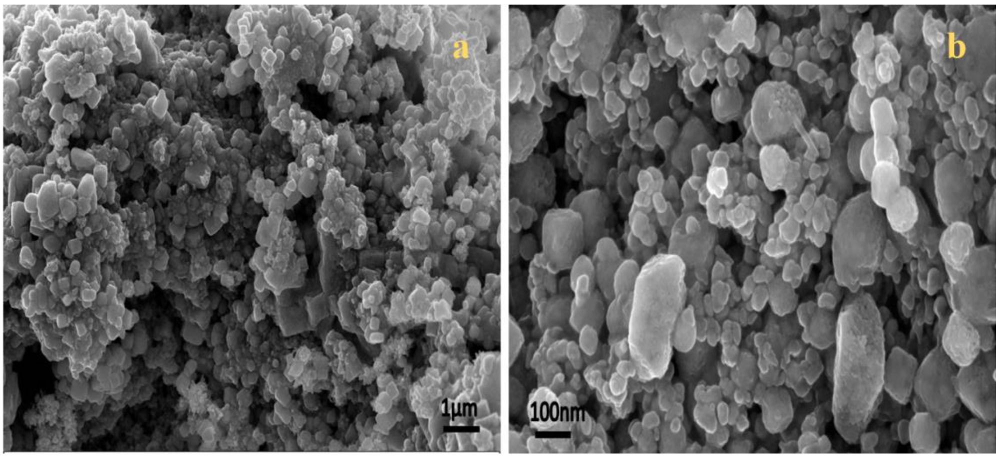

Fig. 3: FESEM images of Mc-AgNP's

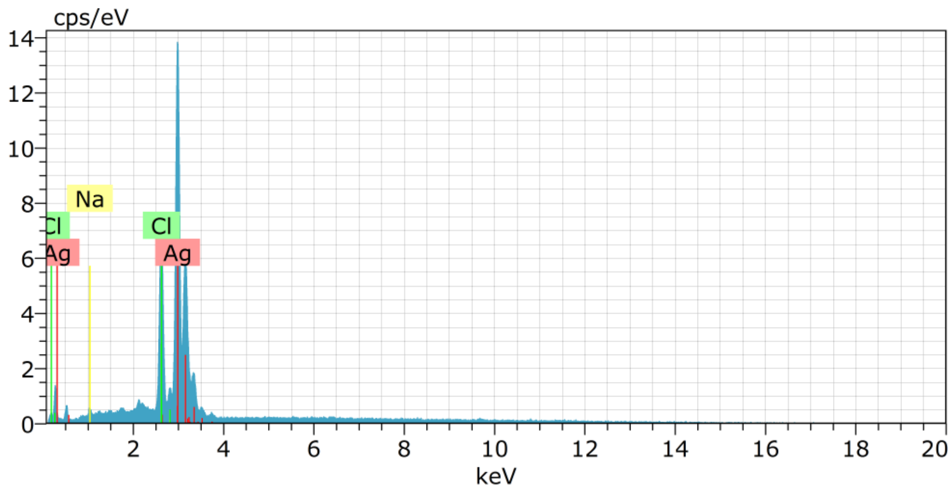

Fig. 4: EDX spectrum of Mc-AgNP's

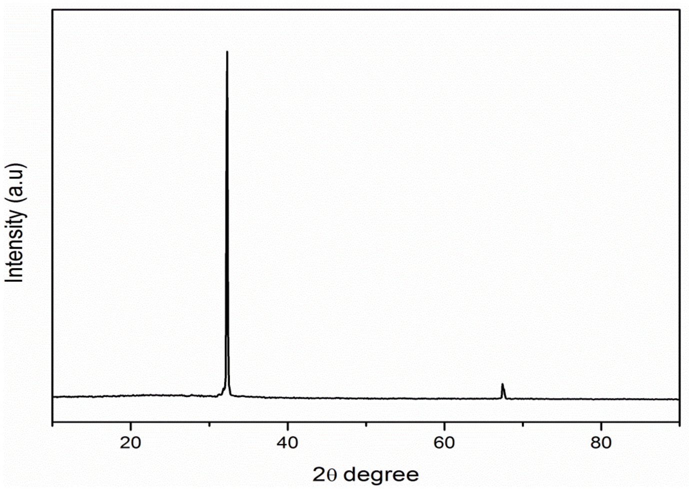

Fig. 5: XRD pattern of Mc-AgNP's

The size and shape of the Mc-AgNPs were assessed using the FESEM technique. Fig. 3 exhibits that the particles were spherical, some were irregular and few particles were present individually. Earlier studies have reported similar results, stating that the particles were spherical and few were aggregated [20]. The energy dispersive X-ray analysis (EDX) reveals a strong signal in the silver region and confirms the formation of silver nanoparticles. The EDX data from fig. 4 reveals the presence of elements like Ag, cl and Na was confirmed in synthesized silver nanoparticles with *Muntingia calabura* fruit extract. The quantitative analysis of atomic ratio represented Ag (66.1%) was found higher than Cl (31.15%) and Na (2.74%) as in fig. 4. Metallic silver nanocrystals generally show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance [18]. This analysis revealed that the nano-structures were formed solely of silver (fig. 4).

The X-ray diffraction pattern of the biosynthesized silver nanoparticles produced by the *Muntingia calabura* fruit extract is shown in fig. 5. The XRD pattern showed two intense peaks (32.30° and 67.40°) in the whole spectrum of 2 θ values ranging from 20 to 80 and indicated that the structure of silver nanoparticles is face-centered cubic (FCC). These are corresponding to (111) and (220) planes for silver, respectively. A sharp and strong diffraction peak centered at 32° appeared, which can be indexed to the (1 1 1) reflection of the metallic silver (JCPDS File No. 04-0783). The results of the present study are in agreement with the previous reports stating the presence of silver in different plant extracts [7].

In vitro radicals scavenging and antimicrobial activity of Mc-AgNP's

It is evident and clear that the human system is triggered by free radicals causing oxidative damage by ROS, RNS produced by activated macrophages and neutrophils leading to several diseases like diabetes, arthritis, autism, cancer, cataracts, aging, Parkinson's disease, Alzheimer's dementia and most important is heart disease [21]. The dose-response radical-scavenging activity of synthesized AgNP's using *Muntingia calabura* fruits was observed and represented in table 2. Many plant and microorganism-based products and their secondary metabolites are proven scientifically to be potent radical scavengers [11] and most of the experimental studies on radical scavenging ability are observed using DPPH a common nitrogen-centered free radical. DPPH readily accepts electrons from antioxidant compounds with the change in color from violet to yellow and the intensity of the color change is measured spectrophotometrically [22, 23]. The results of the DPPH assay revealed significant radical scavenging properties with an inhibition of 69% and IC_{50} of 1.4933 mg\ml, which is significant and correlates with the results of previous studies with fruit extract of *Muntingia calabura* [12].

FRAP is often used to measure the antioxidant capacity of foods, beverages, and nutritional supplements containing polyphenols and also provides an easy and rapid way to evaluate antioxidant activity [12, 24] and the results revealed that synthesized Mc-AgNP's was significantly lower than the ascorbic acid, but the radical scavenging ability improved by increasing the concentration and at higher concentration (60µg\ml) maximum inhibition of 59.9% was observed (table 1). The results specify a marked ferric-reducing ability of the synthesized Mc-AgNP's might be due to the presence of active components of the extract that reacted with free radicals to become a stable product and inhibit the free radical chain reactions [11, 25]. Mc-AgNP's were capable of scavenging hydrogen peroxide radicals in a dose-dependent manner and the maximum scavenging activity was observed at 60µg/ml concentration with 64% inhibition, which was on par with the standard ascorbic acid with 74.7% inhibition (table 1).

Table 1: Radical scavenging potential of MC-AgNP's

Activity	Concentration/% inhibition		
	20 µg\ml	40 µg\ml	60 µg\ml
DPPH	44±0.42	56±0.34	69±0.28
FRAP	42.6±0.36	49±0.28	59.9±0.16
H_2O_2	18.2±0.24	48±0.36	64±0.26

Note: All value represents three individual observations, and data are expressed in mean±SEM calculated by one-way ANOVA

Table 2: Antimicrobial activity of synthesized MC-AgNP's

Microorganism	Zone of inhibition			
	20 µg\ml	40 µg\ml	100 µg\ml	Control (10 µg\ml)
Bacterial strains				
Staphylococcus epidermidis	3.2±0.12	9±0.14	12±1.25	16.8±0.42
Escherichia coli	4.4±0.18	9±0.27	13.5±0.13	14.6±2.24
Klebsiella pneumonia	3.8±0.22	10±0.15	13.8±0.17	14.8±1.14
Staphylococcus aureus	6.2±0.26	11.8±0.23	14.6±0.12	16.8±2.12
Proteus vulgaris	4.6±0.22	11±0.17	12.4±0.19	18.2±2.2
Fungal strain				
Trichoderma harzianum	6.2±0.12	10±0.18	18±0.23	21.6±0.84
Aspergillus oryzae	1.8±0.14	6±0.27	7±0.17	18.6±1.16

Note: All value represents three replicates, and data are expressed in mean±SEM calculated by one-way ANOVA

Table 2 depicts the antimicrobial potential of AgNP's from *Muntingia calabura* and the results represent that *Staphylococcus aureus, Klebsiella pneumonia*, and *Proteus vulgaris* demonstrated maximum ZI with 14.6, 13.8, and 12.4 mm respectively at 100 µg\ml concentration (table 2). Similarly, the tested other bacterial strains also demonstrated dose-dependent activity and the results were on par with the tested standard drug. The anti-fungal activity for the Mc-AgNP's demonstrated dose-dependent activity and the maximum zone was observed by *Trichoderma harzianum* representing ZI with 10 mm and 18 mm for 40 and 100 µg\ml concentrations (table 1) but represented minimum zone even at higher concentration against *Aspergillus oryzae*. The results are in line with the previous research on nanoparticles illustrating effective antimicrobial activity [20, 25].

CONCLUSION

The present study reveals the protective efficacy of synthesized AgNP's from *Muntingia calabura* fruit exhibits potent radical scavenger and inhibit the growth of microorganisms. Thus, the traditional usage with the technology development may pay a better way for the development of drugs against various diseases.

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AUTHORS CONTRIBUTIONS

KSZ: hypothesized, drafted, and coordinated all the experiments and represented to be a correspondence of the manuscript. SS: supervised, literature search, data acquisition, data analysis, manuscript editing. EMV and SRA: experimental studies, synthesis of NP's. MS and MV: experimental studies, antioxidant analysis, data acquisition. NS and DS: antimicrobial studies, data acquisition.

CONFLICT OF INTERESTS

There is no conflict of interest among all the authors.

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