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Antioxidant and Antibacterial activity of Silver Nanoparticles synthesized using *Morinda Citrifolia* Stem extract

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Abstract: The present work focuses the use of the aqueous extract of stem of Morinda folia. In producing silver nanoparticles (AgNPs) from silver nitrate aqueous. Phytochemical analysis of the extract reveals medicinal application. The synthesized silver nanoparticles (AgNPs) were also tested for protein and ascorbic acid. The AgNPs obtained was characterized by UV-visible spectroscopy, FT-IR spectroscopy. SEM images which revealed the presence of various shapes and sizes. In this study, we also investigated antimicrobial and antioxidant activity of green synthesized AgNPs. The antimicrobial activity is investigated by Bauer et al.'method. Antioxidant activity was done by DPPH method.

Keywords: Morinda citrifolia; AgNPs; Antimicrobial; Antioxidant; DPPH; Nanotechnology.

INTRODUCTION

Nano-science is one of the most vibrant growing fields ever known and continues to rigorously reach its branches into various modern technologies such as hydrogen storage, photocatalysis, green energy devices, sensors, biomedical implants, and photovoltaics [1-4]. Though chemical synthesis has been widely adopted for the preparation of a variety of nanostructures, their cost effectiveness, need of sophisticated equipment's, environments, stabilizing and capping agents, environmental hitches fade not only the applications of nanoparticles but also the dream of a green world [5]. For the emphasis of biological protocol applications of nanoparticles, there is also an immense need for synthesizing nanoparticles with a greater compatibility [6]. Hence there is alarming demand for finding cheaper and environmentally friendly nanoparticles synthesis.

The various types of nanomaterials of different metals such as copper, zinc, titanium, magnesium, gold and silver used for improvement of biosensors, gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) were proved to be most effective, as they have good antimicrobial efficacy against a wide variety of bacterial, viruses and other eukaryotic micro-organism [7-15]. Metal NP with at least one dimension approximately 1-100 nm have received considerable attention in both scientific and technological areas due to their unique and unusual physico-chemical properties with that of bulk materials. Due to specific size, shape and distribution nanoparticles are used in the production of novel systems. Literature reveals a promising medical application of AgNPs synthesized using herbal extracts. Silver nanoparticles synthesized using herbal extracts have been reported to have good anti-bacterial, anti-fungal and antioxidant properties [16,17]. The use of plant extracts for synthesis of nanoparticles is potentially advantageous over microorganism due to the ease of scale up, the biohazards, and elaborates process of maintaining cell cultures [18-20]. Metal nanoparticles are of better significance due to their catalytic, optical, electrical and magnetic properties [21] and are most extensively used for their antibacterial properties [22]. Silver nanoparticles were proven to be most efficient as they possess good antimicrobial and antioxidant activities [23-27]. Plant extract was used for the synthesis of silver nanoparticles because it was proved to be less toxic and also need less purification as compared to chemical methods [28, 29]. *Morinda citrifolia* has been known for its medicinal value in the past 2000 years ago. Originated in Tropical Asia, seems to be a much-valued medicinal plant is normally cultivated for its roots, leaves and fruits [30]. In the present work attempts were made for the green synthesis of AgNPs using *Morinda citrifolia.*

MATERIALS AND METHODS

a) Plant materials

The stem of *Morinda Citrifolia* was collected from the bamboo Garden, Amravati, Dist. Amravati during December and January 2021.

b) Synthesis of AgNPs

The fresh stem extract used for the synthesis of AgNPs was prepared from 25gm of thoroughly washed stem in a 500ml flask, boiled in 50ml deionized water for 35 min and the produced extract was subjected to freeze drying. Suspensions were filtered

with Whatman No. 42 filter paper. 50ml of aqueous solution of silver nitrate was prepared in a flask and 1ml of stem extract was added at room temperature for 24h in the dark until the brownish colour was developed which indicated the formation of AgNPs.

c) UV-vis absorbance spectroscopy analysis

The reduction of silver nitrate ($AgNO_3$) to AgNPs was monitored periodically by UV-vis spectroscopy (Shimazu) after the dilution of the samples with deionized water [31]. A UV-vis spectrograph of the silver and nanoparticles was recorded by using a quartz cuvette with water as reference. The UV-vis spectrometric readings were recorded at a scanning speed of 200-800 nm [32].

d) FT-IR analysis

PerkinElmer spectrometer FT-IR Spectrum one in the range of 4000-400cm^{-1} at a resolution of 4cm^{-1} was used. The sample was mixed with KBr procured from Merk chemicals. Thin sample pellet was prepared by pressing with the Hydraulic pellet press and subjected to FT-IR analysis.

e) SEM analysis

Morphological characterization of the samples was done using FEI Quanta 200scanning Electron microscope. A pinch of dried sample was coated on a carbon tape. It was again coated with platinum in an auto fine coater and then the material was subjected to analysis.

f) Antimicrobial activity

The synthesized products (SSAM-3 and SSAM-4) were screened for their antimicrobial activity by using cup plate diffusion method. The bacterial organisms used included both gram-positive as well as gram negative strain like *Staphylococcus aures*, *Enterococci*, *Escherichia Coli* and *Pseudomonas fluoresscens*. The zones of inhibition were recorded after incubation for 24 hr at 37^0C, using vernier caliper. Inhibition zone record of the compound clearly indicated that SSAM-3 highly active against *Staphylococcus aures*, *Enterococci*, *Escherichia Coli* and *Pseudomonas fluoresscens* and SSAM-4 moderately active.

For Antimicrobial Sensitivity Test (After 24 hrs at 37^0)

	ZONE OF INHITION IN MM			
Tested Compounds	GM +VE BACTERIA		GM –VE BACTERIA	
	Staphylococcus aures	*Eterococci*	*Escherichia Coli*	*Pseudomonas fluorescens*
SSAM-3	12mm	15mm	12mm	15mm
SSAM-4	….	15mm	….	15mm
Reference Antibiotic	39mm (Ofloxacin)	35mm (Ofloxacin)	38mm (Ofloxacin)	38mm (Ofloxacin)

g) Antioxidant Activity

In Vitro Methods

DPPH Radical Scavenging Activity

- ***Principle:*** DPPH [1, 1-Diphenyl-2-picryl hydrazyl] is a stable free radical, which shows absorbance at 517 nm. The antioxidant reacts with DPPH and converts it to 1, 1-Diphenyl-2-picryl hydrazine which do not absorb at 517 nm.
- ***Reagent***: Ethanolic solution of DPPH, sample/s stock, standard drug
- ***Procedure:*** To 1 ml of DPPH solution, equal amount of test compound at various concentrations (20-100 ug/ml) were added in a final volume of 2.0 ml. After incubation for 20 minutes at room temperature, absorbance due to changes in colour from deep violet to light yellow were recorded at 517 nm. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). Lower absorbance of the reaction mixture indicated higher free radical activity. The experiment was performed in triplicate.
- ***Calculation:***

Percentage Scavenging activity =Absorbance of Control–Absorbance of sample/ Absorbance of Control × 100

CONTROL:-

Concentration	Absorbance
Ethanol (3.5 mL) and DPPH radical solution (0.3 mL)	0.648

Ascorbic Acid (standard): -

Concentration	Absorbance	Reading
20 ug/ml	0.388	94.44
40 ug/ml	0.368	97.53
60 ug/ml	0.350	100.3
80 ug/ml	0.330	103.39
100 ug/ml	0.306	107

Sample:-

Concentration	Absorbance	Reading
20 ug/ml	0.360	91.14
40 ug/ml	0.350	94.34
60 ug/ml	0.348	96.24
80 ug/ml	0.316	99.44
100 ug/ml	0.302	102

Fig.1: Synthesized AgNPs

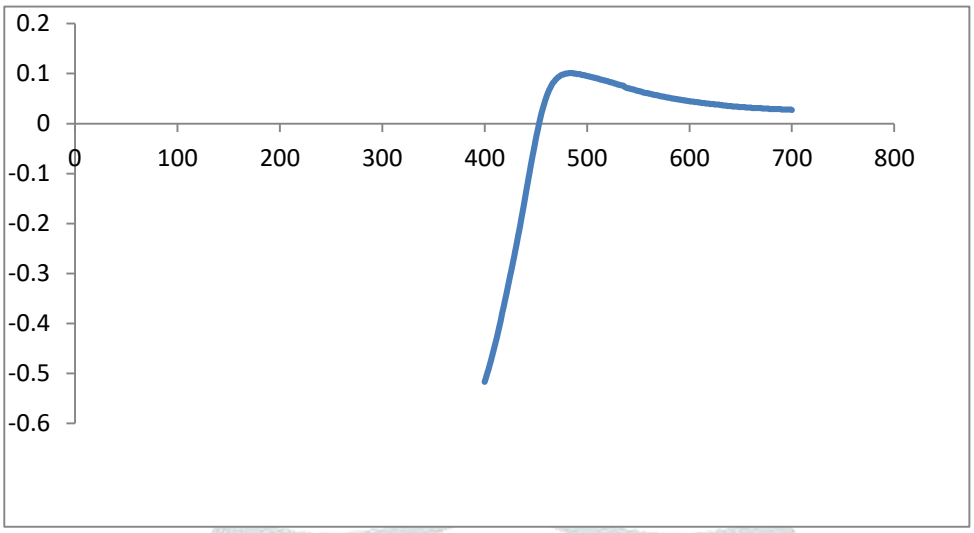

Fig.2: UV-vis spectra of synthesized AgNPs

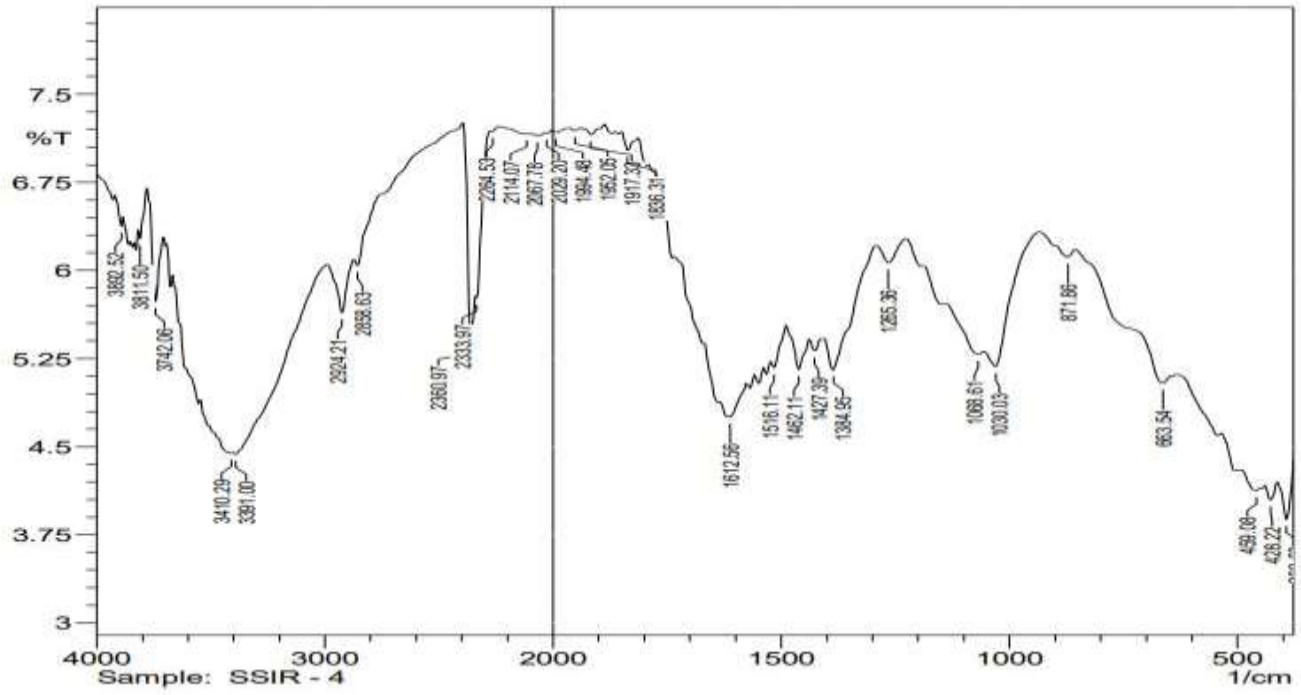

Fig.3: FT-IR absorption spectra of synthesized AgNPs.

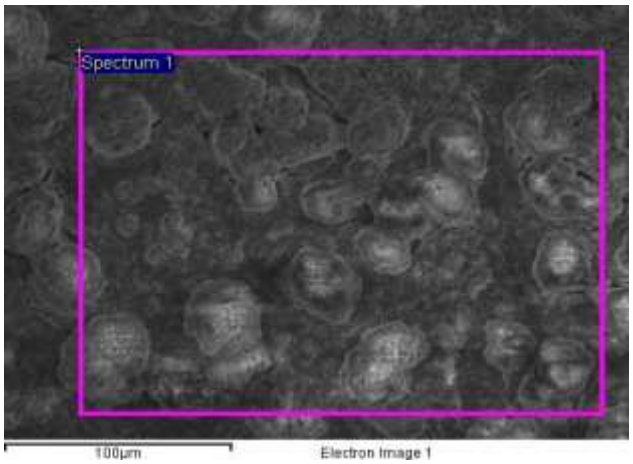

Fig.4.SEM images of silver nanoparticles synthesized from *Morinda citrifolia* stem extract show highly agglomerated spherical shape.

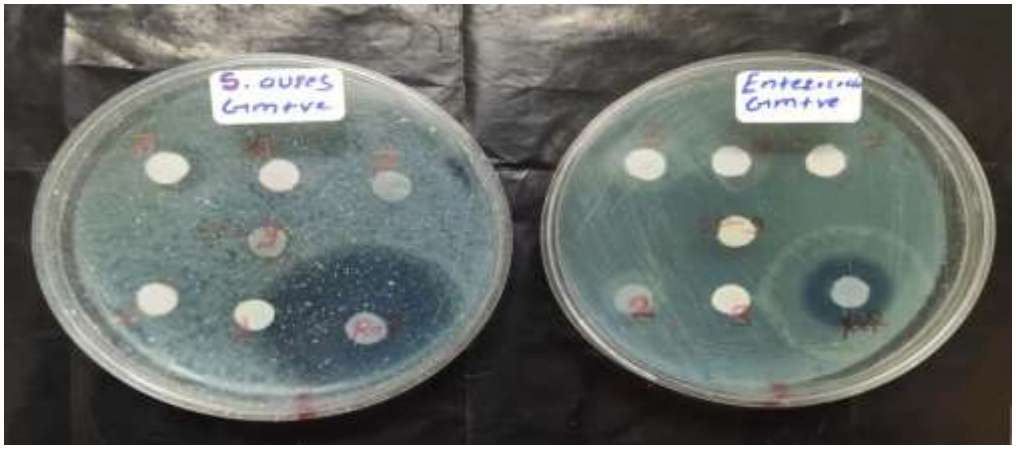

Fig.5. Antimicrobial activity of *Streptococcus aureus* and *Escherichia Coli.*

Fig.6.Free radical scavenging activity

RESULTS AND DISCUSSION

When the Stem extract of *Morinda Citrifolia* was mixed with AgNO3 solution, the pale yellow colour of aqueous extract changed to brownish colour immediately within 10 min, indicating the formation of silver nanoparticles.

The reduction of pure silver ions was confirmed by UV-vis spectra where the maximum absorbance was seen at 465nm (fig.2). FT-IR spectrum peak at 3410cm^{-1}, 3391cm^{-1}, 2924cm^{-1}, 2333cm^{-1}, 1612cm^{-1}, 1516cm^{-1}, 1030cm^{-1}, 663cm^{-1}, 1265cm^{-1} and 1384cm^{-1} which shows many functional groups. SEM images showed aggregation of nanoparticles (Fig.4). Compound showed better anti-microbial activity against the growth of pathogen (fig.5). The pathogens, Escherichia Coli and S. aureus were found to be sensitive against the compound. The free radical scavenging activity (anti-oxidant activity) of the silver nanoparticles was assessed by DPPH assay. The freshly prepared DPPH solution exhibited a deep purple colour with a maximum absorbance at 517nm. The disappearance of purple colour on adding synthesized silver nanoparticles might be due to presence of antioxidant in the medium (fig.4). Free radical scavenging activity of the AgNPs on DPPH radical was found to be decrease with increase in concentration, showing a maximum of 20% at 0.388 ug/ml.

CONCLUSION

The synthesis of silver nanoparticles using stem of *Morinda Citrifolia* extract has been demonstrated. This method is simple, economic, non-toxic and efficient. Nanoparticles synthesis by medicinal plants shows more benefit, they may enhance the antibacterial activity of silver nanoparticles, because the medicinally valuable active biomolecules present in the plants may bind on the surface of the nanoparticles and reduce the silver ions to silver nanoparticles. These silver nanoparticles were of high purity, making them potentially useful for biological applications. This biological method is potentially attractive for large scale synthesis of metallic and metal oxide nanomaterials.

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