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Silver Nanoparticles synthesized from Bark extract of *Morinda citrifolia* and Investigated their Antibacterial and Anticancer activity

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ABSTRACT: The plant situated in rare area for the biosynthesis of silver nanoparticles, especially it shows great efficiency in biological activity. Due of its eco-friendly and quick synthesis processes, plant-mediated nanoparticle synthesis is currently gaining significant attention. The utilization of *Morinda citrifolia* bark aqueous extract is the main focus of the current investigation. in the process of turning aqueous silver nitrate into silver nanoparticles (AgNPs). An examination of the extract's phytochemistry suggests its potential as medicine. Protein and ascorbic acid levels in the produced silver nanoparticles (AgNPs) were also examined. By using FT-IR and UV-visible spectroscopy, the AgNPs were identified. SEM pictures showed the presence of different sizes and forms. In this study, we also looked at the antibacterial and anticancer properties of AgNPs produced sustainably. The goal of the current study was to create and describe silver nanoparticles.

Keywords: *Morinda citrifolia*, Bark extract, AgNPs, Antimicrobial, Anticancer, Nanotechnology.

INTRODUCTION

According to Li, *et al.* (2011), nanotechnology is the process of creating nanoparticles of the nanoscale scale length that have regulated size, shape, and material dispersion. Metal nanoparticles (MNP) with at least one dimension of 1-100 nm have received a lot of attention in both scientific and technical domains due to their unique and exceptional physico-chemical properties compared to those of bulk materials. Nanoparticles are utilized in the creation of innovative systems because of their precise size, shape, and dispersion. Nanoparticles are utilized in the creation of innovative systems because of their precise size, shape, and dispersion. AgNPs created utilizing herbal extracts have a promising medical application, according to the literature. According to studies (Banerjee *et al.*, 2011; Elumali *et al.*, 2010). Herbal extract-derived silver nanoparticles exhibit potent antibacterial, antifungal, and antioxidant properties. Silver nanoparticles that are produced by medicinal plants have greater advantages and may increase the antibacterial activity of silver nanoparticles because the medicinally advantageous active biomolecule present in the plants may bind on the surface of the nanoparticles and reduce the silver ions to silver nanoparticles. Due to their strong antimicrobial efficacy against a wide range of bacteria, viruses, and other eukaryotic microorganisms, gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) have been demonstrated to be the most effective nanomaterials for enhancing biosensors (Wang *et al.*, 2009; Litwac *et al.*, 1953).

A number of products, including cosmetics (Kokura *et al.*, 2004), animal feed (Hojberg *et al.*, 2005), coating of catheters, wound dressing (Fernandez *et al.*, 2008), and water purification (Choi *et al.*, 2008), have recently used metallic silver and silver nanoparticles as antimicrobial agents with little risk of toxicity to humans. Using plant extracts rather than microorganisms for the synthesis of nanoparticles may be preferred due to the ease of scaling up, the biohazards, and the challenging process of maintaining cell cultures (Ankamwar *et al.*, 2005; Hoag *et al.*, 2009; Bar *et al.*, 2009). As opposed to chemical methods, plant extract has been demonstrated to be less hazardous and require less purification (Deshpande *et al.*, 2011). According to Bhattacharya and Gupta (2005), it was used to create silver nanoparticles. Silver nanoparticles have been demonstrated to be the most efficient due to their potent antibacterial and antioxidant capabilities (Dwivedi and Gopal 2010; Bauer *et al.*, 1996; Darun *et al.*, 2010; Rai *et al.*, 2008; Sharma *et al.*, 2009).

Since, 2000 years ago, the *Morinda citrifolia* plant has been appreciated for its therapeutic properties. The plant, which appears to have its origins in tropical Asia, is valued for its roots, leaves, and fruits and has been widely utilized in folk medicine (Zin *et al.*, 2009). Anthraquinone compounds, which are typically found in the form of glycones and to a lesser extent, glycosides, are said to be abundant in the roots of these plants (Thomsan, 1971; Zenk *et al.*, 1975). Bowel issues, diabetes, liver diseases, urinary tract infections, menstrual cramps, skin sores and abscesses, mouth and throat issues, and respiratory conditions can all be treated using *M. citrifolia* plant extract (Mactherson *et al.*, 2007). We effectively documented the creation silver nanoparticles used in this research utilizing *Morinda citrifolia* bark extract. The antibacterial and anticancer activities of synthetic silver nanoparticles was assessed.

MATERIALS AND METHODS

A. Plant materials

Using *Morinda citrifolia* bark extract, we effectively documented the creation of in this work, silver nanoparticles. Silver nanoparticles that were created synthetically were used to assess their antibacterial and anticancer activities.

B. Synthesis of AgNPs

The 50gm volume of fresh bark was chopped into small pieces and after being thoroughly cleaned in a 500 ml flask, the material was boiled in 100 ml deionized water for 20 minutes at 600 C, and the resulting extract was then submitted to freeze drying. Whatman No. 42 filter paper was used to filter the suspensions. 1ml of bark extract was added to 50ml of a prepared silver nitrate aqueous solution, and the mixture was left for 24 hours in the dark at room temperature, until a brownish tint appeared, indicating the formation of AgNPs.

UV-vis absorbance spectroscopy analysis. After dilution with deionized water, UV-vis spectroscopy was used to periodically monitor the conversion of silver nitrate ($AgNO_3$) to AgNPs (Shimazu). In order to record the silver and nanoparticles' UV-vis spectrograph, a quartz glass that was half full of water was utilized as a reference. The UV-vis spectrometric data were collected at a scanning rate of 200-800 nm.

FT-IR analysis. APerkinElmer FT-IR Spectrum One spectrometer with a resolution of 4 cm^{-1} and a wavelength range of 4000-400 cm^{-1} was employed. Merk Chemicals' KBr was used to combine the sample. Prior to FT-IR analysis, the material was compressed into a thin pellet using a hydraulic pellet press.

SEM analysis. The samples were characterized morphologically using an FEI Quanta 200 scanning electron microscope. On a piece of carbon tape applied a pinch of the dried sample. Prior to analysis, the sample was re-coated with platinum using an automatic fine coater.

Antimicrobial activity. The synthesized materials were diffused onto cups using the cup plate method. (SSAC-7 and SSAC-8) were tested to see if they are antimicrobial strains that are gram-positive and gram-negative of bacteria such *Staphylococcus aureus*, *Pseudomonas fluoresscens*, *Escherichia coli*, and enterococci were employed in the study. Vernier calipers were used to measure the zones of inhibition following a 24-hour incubation period at 370°C. The compound's inhibition zone record made it evident that SSAM-7 and SSAM-8 were both extremely active against, successively, *Staphylococcus aureus*, *Enterococci*, *Escherichia coli*, and *Pseudomonas fluoresscens*.

Table 1: Test for Antimicrobial Sensitivity (After 24 hrs at 37°).

Zone of Inhibition in Mm				
Tested Compounds	**GM +VE BACTERIA**		**GM –VE BACTERIA**	
	Staphylococcus aures	***Eterococci***	***Escherichia Coli***	***Pseudomonas fluorescens***
SSAC-7	18mm	15mm	18mm	15mm
SSAC-8	12mm	…..	12mm	15mm
Reference Antibiotic	39mm (Ofloxacin)	35mm (Ofloxacin)	38mm (Ofloxacin)	38mm (Ofloxacin)

MATERIALS

Dulbecco's Modified Eagle Media (DMEM) with Glucose, Cat. No. 11965-092 (Gibco, Invitrogen), MDA MBA231 (Human Breast Cancer)

The product's Cat No. is 10270106 (Gibco, Invitrogen).

Thermofisher Scientific's antibiotic-antimycotic 100 × solution, item number 15240062, and the TACS Annexin V-FITC Apoptosis Detection Kit (R&D system), item number 4830-01-K.

Protocol: Cytotoxicity. The cells were plated in a 96-well flat-bottom microplate with 5% CO_2 and 95% humidity, and maintained at 370°C overnight. The concentrations used to treat the samples were 100, 50, 25, 12,5, 6,25, and 3.125 M/ml. The cells were then incubated for a further 48 hours. Each well was rinsed twice with PBS and then 20 L of the MTT staining solution was added. The plate was then incubated at 37°C. To dissolve the formazan crystals after 4 hours, 100 L of DMSO was given to each well. The absorbance was then measured with a 570 nm microplate reader.

Formula. We calculate the IC 50 of compounds using graph pad Prism Version5.1: Surviving cells (%) = Mean OD of test chemical/Mean OD of negative control × 100.

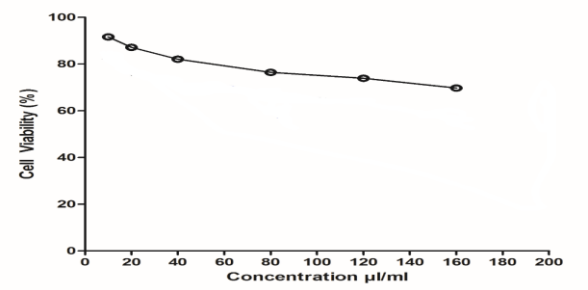

Fig. 1. Prepared AgNPs.

RESULTS

Table 2: IC50 value of Compounds (µM/ml).

Sample Code	MDA MBA231	
	Mean	SD
SSAC-7	825.4	17.6

Cell Viability of MDA MBA231		
Concentration µM/ml	SSAC-7	
160	70.35	69.10
120	74.72	73.03
80	75.28	77.53
40	81.46	82.58
20	87.64	86.52
10	90.45	92.70
Negative Control	100	

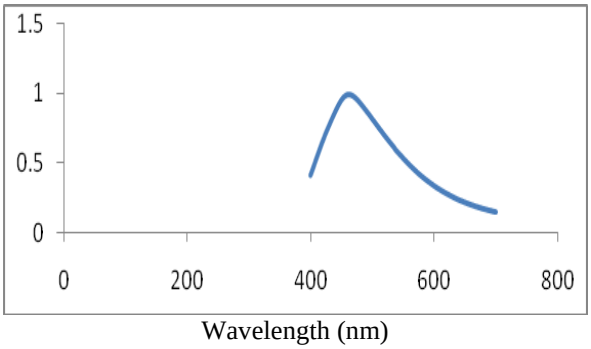

Fig. 2. UV-vis spectra of AgNPs.

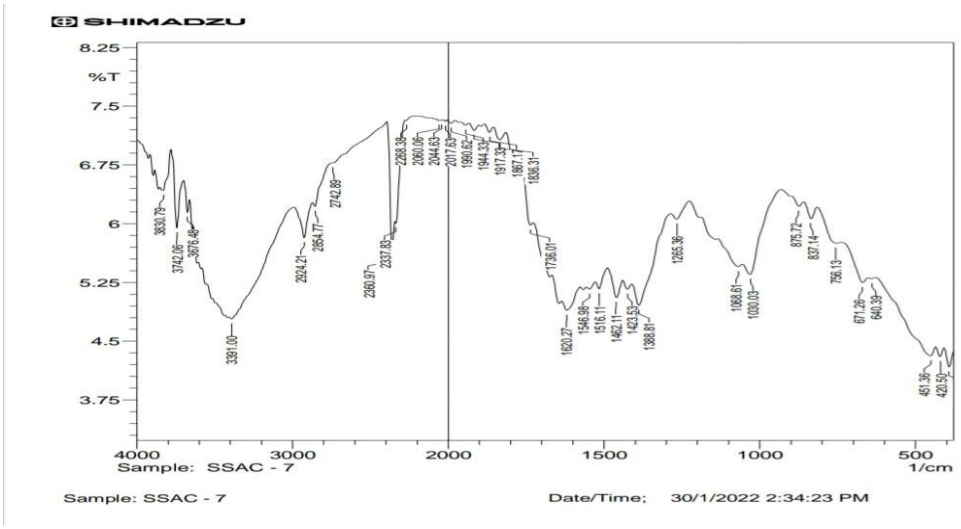

Fig. 3. FT-IR absorption spectra of AgNPs.

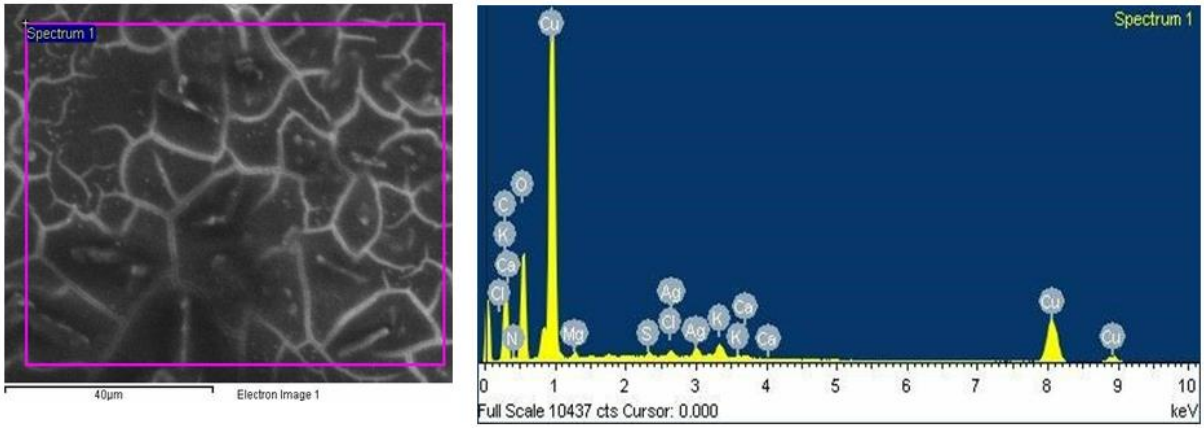

Fig. 4. SEM images of AgNPs show highly agglomerated shape.

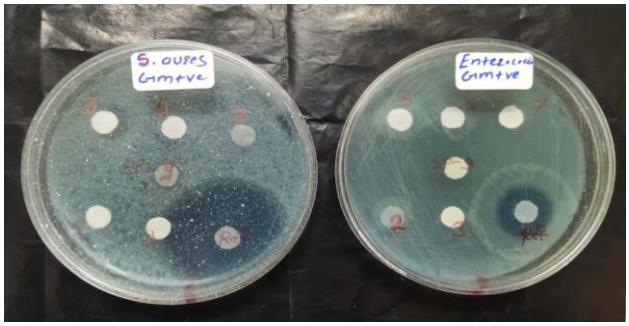

Fig. 5. Antimicrobial activity of *Streptococcus aureus* and *Escherichia coli.*

DISCUSSION

When *Morinda citrifolia* bark extract was combined with $AgNO_3$ solution, the aqueous extract initial yellow color turned to a brownish hue 10 minutes later, demonstrating the synthesis of silver nanoparticles. The greatest absorbance was observed at 475 nm in the UV-vis spectra, which verified the reduction of pure silver ions (Fig. 2). The size of AgNPs can be related to their UV-visible properties. In addition, the biomolecules' bonding to AgNPs has been verified by FTIR characteristics. There are numerous functional groups visible in the FT-IR spectrum peak at 3391cm^{-1} (broad), 2924cm^{-1}, 2337cm^{-1}, 1620cm^{-1}, 1516cm^{-1}, 1462cm^{-1}, 1388cm^{-1}, 1068cm^{-1}, 837cm^{-1}, and 671 cm^{-1} (Fig. 3). Nanoparticle aggregation was visible in SEM images (Fig. 4). Synthesized AgNPs shown excellent antibacterial and anticancer potential. Compound displayed improved antimicrobial efficacy against pathogen growth (Fig. 5). *Escherichia coli* and *S. aureus* were discovered to be susceptible to the substance.

CONCLUSIONS

It has been proven that *Morinda citrifolia* bark extract can be used to create silver nanoparticles. The purpose of this inquiry is to assess how well the method for creating AgNPs from *Morinda citrifolia* works. This approach is straight forward, affordable, non-toxic, and effective. Nanoparticles produced by medicinal plants exhibit more benefit because the medicinally advantageous active proteins in the plants may bind to the surface of the nanoparticles and reduce the silver ions to silver nanoparticles. They might enhance the silver nanoparticles' antibacterial properties. It has been proven that extract from the bark of *Morinda citrifolia* can be used to create silver nanoparticles. This investigation's goal is to assess how well *Morinda cirrifolia* is used in the process of creating AgNPs. This process is easy, affordable, non-toxic, and effective. Silver nanoparticles produced by medicinal plants have more benefits and may increase the antimicrobial activity of silver nanoparticles because the medicinally advantageous active biomolecules present in plants may bind to the surface of the nanoparticles and reduce the silver ions to silver nanoparticles.

FUTURE SCOPE

The synthesized AgNps from bark of *Morinda citrifolia* are used in medicinal nanotechnology for drug design

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Conflict of Interest. None.

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