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GREEN SYNTHESIS OF SILVER NANOPARTICLES MEDIATED BY RHIZOPHORA APICULATA WHOLE PLANT EXTRACT

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ABSTRACT

Aim: To identify the anti microbial activities of silver nano particles synthesized with *Rhizophora apiculata* whole plant extract against the fungi and bacteria tested. **Methods:** In vitro anti microbial activities and characterization studies such as UV, XRD, EDX, SEM and TEM analysis were carried out for the synthesized silver nano particles. **Results:** The synthesized silver nanoparticles have maximum absorption at 405 nm with the average size of 3.5 nm. The XRD data showed 2θ intense values with various degrees such as 38.28°, 43.18°, 65.64° and 78.04°. SEM and TEM data showed prominent nano particle formation with a particle size of 2 nm and 21 nm respectively. **Conclusions:** The biosynthesis of silver nanoparticles with whole plant extract of *R.apiculata* provides potential source for the antimicrobial activity against bacterial and fungal diseases.

INTRODUCTION

Nanoscale materials have attracted considerable interest for their potential applications in the fields of electronics, catalysis, pharmaceuticals, etc. Most of the applications are based on the quite different chemical and physical properties of the nanomaterials from those of the bulk and atomic species, which stem from their unique electronic structure and extremely large surface area. The properties of nanomaterials strongly depend on their composition, size and shape. So, it is of enormous importance and an essential requirement for nanotechnology to produce nanomaterials with desired composition, size and distribution. Up to now, considerable efforts have been made to develop controlled and reproducible synthetic methods for nanomaterials, especially for metal nanoparticles (NPs), like Au NPs and Ag NPs etc.

In this research work, we adopted biological method for the synthesis of nanoparticles because it is cost effective and environmental friendly compared to physical and chemical methods. Since chemical and physical methods involve use of high pressure, energy, temperature and toxic chemicals, plant extracts are used for the synthesis. The microbial enzymes and phytochemicals with anti oxidant or reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles. Nature has devised various processes for the synthesis of nano and micro-length scaled inorganic materials which have contributed to the development of relatively new and largely unexplored area of research based on the biosynthesis of nanomaterials (Mohanpuria *et al.*, 2000).

In medical and industrial process, Silver has effective inhibition on microbes. The vital application of silver and silver nanoparticles in medical industry are topical ointments to prevent infection against burn and open wounds (Ip *et al.*, 2006). Bioreduction of silver ions to yield metal nano particles using living plants like geranium leaf (Shankar *et al*., 2003) and neem leaf (Shankar *et al.*, 2004a) have been studied.

In this investigation, we report synthesis of silver nanoparticles, reducing the silver ions presents in the solution of silver nitrate by the aqueous extract of *R.apiculata*whole plant for the first time.

2. METHODS AND MATERIALS

2.1. Materials AgNO3 was purchased from Aldrich and used without purification. Filter paper of size 0.45 µ and 0.25 µ were purchased from Fischer scientific. The aqueous solutions used for synthesis were ultrahigh purity (Mill-Q) water

2.2. Collection of R.apiculata sample Rhizophora apiculata is a species of plant in the Rhizophoraceae family. This species is widespread and common within its range. It is threatened by the loss of mangrove habitat throughout its range, primarily due to extraction and coastal development, and there has been an estimated 20% decline in mangrove area within this species range since 1980. Mangrove species are more at risk from coastal development and extraction at the extremes of their distribution, and are likely to be contracting in these areas more than in other areas. The sample was collected by hand picking.

2.3. Preparation of *Aqueous Extraction of prepared R.apiculata* The collected Sample (*R.apiculata*) was brought to laboratory and it was washed with fresh water to separate contaminants such as adhering impurities, sand particles and dust. Then the sample was soaked in distilled water. The plants were shade dried for 14 days. The dried material was ground and stored in air tight containers. The powder obtained was extracted with distilled water. To 5g of powdered sample, 100 mL of distilled water was added and boiled to 60-70 °C for about 10 min. Then the resulting crude extracts were filtered through 0.25µ filter and stored in refrigerator.

2.4 Synthesis of Silver Nanoparticles AgNO3 of 1mM was prepared by adding 0.015g of AgNO3 to 90 mL of distilled water and used for the synthesis of silver nanoparticles. Then 10 mL of *R.apiculata* extract was added into 90 mL of prepared aqueous solution of 1mM AgNO3 for reduction into Ag+ ions and kept in magnetic stirrer for 1hour at room temperature.

2.5 Charaterization UV–Vis Spectra analysis was carried out to confirm the silver nanoparticles formation in Shimadzu 1800 UV spectrophotometer (Kyoto, Japan). The functional group responsible for the silver nanoparticles was analysed using FTIR (Perkin Elmer spectrum, USA). Further the size of synthesized nanoparticle was determined by SEM (JEOL Ltd, Tokyo, Japan)

2.6. **X Ray Diffraction studies** The air dried nanoparticles were coated onto XRD grid and analyzed for the formation of silver nanoparticle by Philips X-Ray Diffractometer with Philips PW 1830 X-Ray Generator operated at a voltage of 40kv and current of 30mA with cu kal radiation. The diffracted intensities were recorded from 10° to 80° 0f 20 angles.

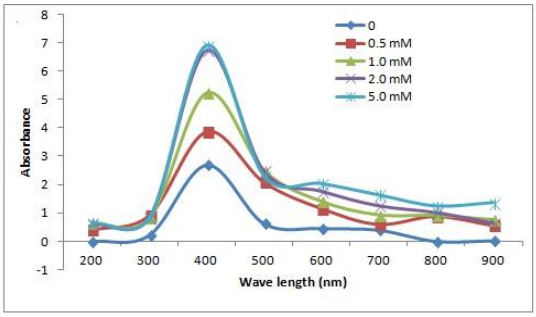
2.7 Antibacterial study by the zone of inhibition assay All complexes were tested for their antibacterial activity by the zone of inhibition assay. For this purpose, filter paper discs of 5 mm diameter were prepared from Whatman No. 1 filter paper, 5, 10, and 20 µmol/L complexes were loaded onto the disc. The discs were placed on PDA plates spread with bacterial culture. The plates were incubated at 30 °C for 2 days after which the zone of inhibition was measured.

2.8 Antifungal assay by disc diffusion method
The antifungal testing was conducted using standard disc diffusion assay, according to the procedures of Conner and Beuchat (1984) and Elgayyar et al. (2001) on PDA medium. After sterilization, plates were prepared at room temperature. Filter paper discs of 5 mm diameter and 5, 10, and 20 µmol/L complexes were loaded onto the agar plates seeded with fungal spores. The plates were incubated at 30 °C for 4 days and the zone of inhibition was measured

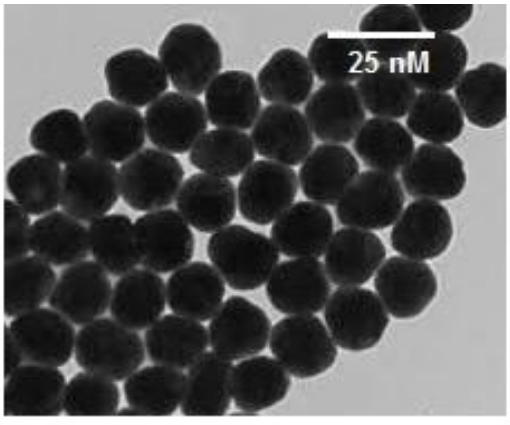
3. RESULTS AND DISCUSSIONS

3.1. UV-Vis Spectra Analysis

Synthesized silver nanoparticles were yellowish orange in aqueous solution due to excitation of surface plasma vibrations in silver nanoparticle (Shankar *et al.*, 2004b). The colourless AgNO3 solution changed to yellowish orange when *R.apiculata* extract was added. This colour change was due to the reduction of Ag+ into Ag0 which leads to the formation of silver nanoparticles. In general, morphology of the nanoparticles are greatly influenced by the SPR, since it is the basis for measuring adsorption of material onto the surface of metal nanoparticles. SPR in nanometer-sized structures is called Localized Surface Plasmon Resonance (LSPR). UV-Vis absorption spectrophotometer was used to investigate the LSPR phenomenon. The spectra displayed the characteristic surface plasmon resonance (SPR) band of silver nanoparticles at about 405 nm, indicating the formation of silver nanoparticles (Fig. 1)

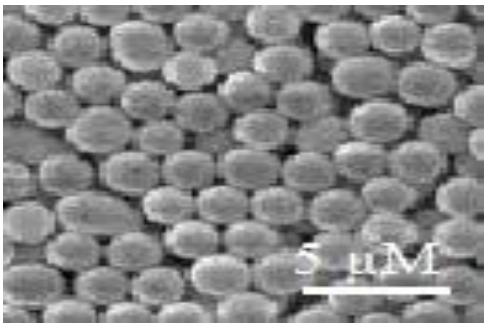


3.2 TEM Transmission electron microscopy (Fig. 2) has been employed to characterize the size, shape and morphologies of formed silver nanoparticles. TEM image is the evident that the morphology of silver nanoparticles is nearly spherical shape (Fig. 2) of SPR band in the UV–vis spectra. The average particle size measured from the TEM images histogram is observed to be 21 ± 3 nm (Fig. 2) which is in good agreement with the particle size calculated from XRD analysis.

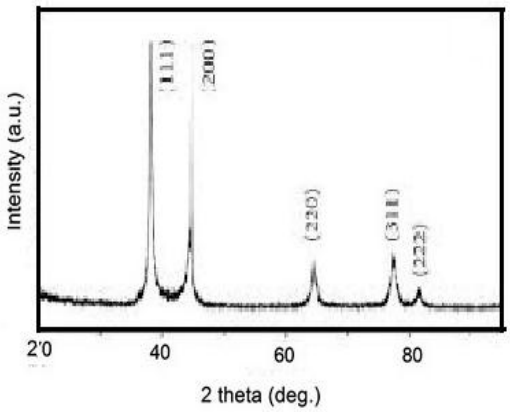


3.3 SEM SEM images provided information about the morphology and size of the biosynthesized silver nanoparticles. The silver nanoparcticles were found to be spherical in shape. The diameter of synthesized silver

nanoparticle was identified as 2-3.5 nm (Fig .3). Further SEM image showed the high density silver nanoparticles synthesized by the *R.apiculata* extract. This confirmed the development of silver nanostructures by the plant extract.



3.4 XRD analysis XRD analysis showed four distinct differaction peaks at 38.28°, 43.18°, 65.64° and 78.04° and can be indexed 20 values of (111), (200), (220), (311) crystalline planes of cubic Ag. The average grain size of the silver nanoparticles formed in the bioreduction process is determined using scherr s formula d=(0.9 * 180)/ Cos and is estimated to be 5.2nm (Fig.4).



3.5 Antibacterial activity

The above Ag-NP complexes were dissolved in water and tested for their antimicrobial activity. Complex concentrations ranging from 0 to 100 mol/L were used in the initial antibacterial assay. Based on the results of this preliminary test, three different concentrations were chosen (5, 10, and 20 mol/L) to check their efficiency in inhibiting microbial growth. The results presented in Table 1 indicate that Ag-NP complexes had high antibacterial activity, however, they were found to have no effect on the growth of the bacteria.

3.6 Antifungal activity of Co(III) ethylenediamene complexes

Preliminary experiments were conducted on the antifingal activity of Ag-NP complexes against *A. niger*, *N. crassa*, and *F. oxysporum* by the disc diffusion method as described by Elgayyar et al. (2001) and Conner and Beuchat (1984). The effective concentration and the diameters of zones of inhibition are shown in Table 2. Of all the three fungi tested, the activity was observed in terms of inhibition of fungal growth around the discs loaded with complexes (Table 2). Ag-NP Complexes [did not inhibit *A. niger* and *F. oxysporum.* However, these complexes showed little activity against *N. crassa* in terms of growth inhibition.

Table-1 Antibacterial activity of Ag-NP complex extracted from *Rhizophora apiculata*

Organism	Zone of Inhbition		
	5 µmol/L	10 µmol/L	20 µmol/L
E.coli	1.5	2.7	3.1
E. coli HB 101	3.4	4.1	5.5
Salmonella typhimurium	0.65	0.7	1.1
Proteus vulgaris	1.6	2.8	4.7
Pseudomonas aeruginosa	0.7	1.3	2.2
Staphylococcus aureus	0.8	1.3	2.4
Streptococcus faecalis	0.4	0.9	1.7
Bacillus subtilis	0.6	0..7	1.8
Xanthomonas oryzae	1.4	2.6	4.1

Table-2 Antifungal activity of Ag-NP complex extracted from *Rhizophora apiculata*

Organism	Zone of Inhbition		
	5 µmol/L	10 µmol/L	20 µmol/L
Aspergillus niger	0.6	0.7	0.9
Fusarium oxysporium	0.9	1.1	1.2
Neurospora crassa	3.2	4.7	5.1

4. CONCLUSIONS

In conclusion, green synthesis of silver nanoparticles from *R.apiculata* extract was studied. The reduction of the metal ions led to the formation of silver nanoparticles of fairly well-defined dimensions using the extract. Further, antimicrobial activity of *R.apiculata* extract has been enhanced on synthesizing silver nanoparticles from it. This green chemistry approach towards the synthesis of silver nanoparticles has many advantages such as environmental friendly, cost effective and easily scaled up to large scale synthesis. Antimicrobial studies of silver nanoparticles on human pathogen opens a door for a new range of antibacterial agents.

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