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Green Synthesis of Bimetallic Silver-Manganese Nanoparticle and Evalaution of It's Antibacterial Activity

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ABSTRACT

Increasing bacterial resistance due to inappropriate use of antibiotics is one of the most significant problems facing the modern scientific community (1). Moreover, the emergence of new resistant bacterial strains to current antibiotics has become a serious public health issue. This has escalated the need to develop new bactericidal materials (2). Nanomaterials have attracted substantial scientific interest due to their potential technological applications in food storage and preservation, catalysis, environmental protection, and bioassays, as well as in the medical, textile, and cosmetics industries (3 ,4). Cordia macleodii commonly known as Dahiphalas or Dahiman in Hindi is native to India, distributed in moist and dry deciduous forests of India such as Chhattisgarh, Madhya Pradesh, Odisha, Chotanagpur, Maharashtra. The plant is used ethnomedicinally for various purposes like healing wounds (leaf, bark), mouth sores (leaf), treating jaundice (bark) and also as an aphrodisiac (seed). Previous chemical analyses of the plant indicate the presence of alkaloids, amino acids, carbohydrates, flavonoids, glycosides and tannins, phenols, steroids, terpenoids, resin. These components can be used as a reducing agent in the preparation of bimetallic nanoparticles. However, much less study of nanoparticle synthesis has been reported to date using C macleodii extract as a combined reducing and stabilizing agent. In this research, Ag-Mn nanoparticles were synthesized by co-reduction of $AgNO_3$ *and* $MnSO_4$ *solution using C macleodii extract as a combined reducing and stabilizing agent. The antibacterial feature of nanoparticles was evaluated throughout against Gram positive bacteria: Bacillus cereus, Bacillus subtilis, Staphylococcus aureus and Gram negative Proteus vulgaris.*

Among bimetallic Ag-MnNP synthesized from three solvents (methanol, ethanol and water), highest susceptibility was against aqueous Ag-MnNP followed by methanolic Ag-MnNP where most susceptible were Bacillus subtilis, Proteus vulgaris against different concentration (25 ug mL^{-1} *– 100 ug* mL^{-1}*). Highest inhibition zone was observed in Bacillus subtilis (≥15mm) (aqueous Ag-MnNP) followed by Proteus vulgare (≥12mm) (aqueous Ag-MnNP). All the four microorganism showed strong resistance against bimetallic ethanolic Ag-MnNP.*

INTRODUCTION

Green synthesis, which involves using plants resources or different microorganisms, is considered a cost-effective and eco-friendly alternative to chemical and physical methods, of synthesizing nanoparticles. (5). There is an urgent need for new approaches to overcome antibiotic resistance and to develop alternate antimicrobial agents that can control infectious diseases, the spread of pathogens and have long-term effectiveness (6). The main aim of green synthesis is to minimize the use of toxic chemicals to prevent the environment from pollution, using plants and microorganism for biomedical applications. (7,8). Plant-derived nanoparticles have been shown to exhibit unique biological features (9). Bimetallic

Nanoparticles composed of two different metal elements, are of greater interest than monometallic ones from both scientific and technological views due to the synergistic proprieties between the two different metal parts. (10, 11). Another benefit of biological synthesis for NPs is that waste streams of costly materials such as gold or silver salts can be recycled, ultimately reducing the overall costs of production (12). Green synthesis makes use of biological or natural entities such as plant or microbial extracts as reducing, capping, and stabilizing agents in the synthesis of NPs.

Plant Collection

The plant sample of *Cordia macleodii*, were obtained and identified from Van Ropari Nursery, Bhopal, M.P. The plant leaves were removed from the stem, washed with tap water three times and dried for 15 days in the shade.

Preparation of Extract

In soxhlet, the sample (leaves) was placed in a thimble-holder and during operation was gradually filled with condensed 250 ml solvent (Methanol, Ethanol and Water) from a distillation flask. When the liquid reaches an overflow level, a siphon aspirates the whole contents of the thimble-holder and unloads it back into the distillation flask, carrying the extracted analytes in the bulk liquid. This operation is repeated until complete extraction is achieved after six rounds of soxhlet cycle. After the completion extraction process the collected extract was dried at 50°C to achieve to achieve the desired consistency required for future use, extracted material was then stored in airtight container (13).

Phytochemical Screening

Extracts were subjected to various phytochemical tests such as alkaloids, tannins, saponins, carbohydrates, proteins, phenols, in order to determine the secondary plant constituents, present by employing standard procedures.

Biosynthesis of Bimetallic Nanoparticles

A well-mixed aqueous solution of silver nitrate (0.1M) and manganese sulphate (0.1M) was prepared. The leaf extract was then added to this solution with vigorous stirring for 8-10 h; where spontaneous reduction results in the formation of bimetallic NPs. The solution was centrifuged at 10000 rpm for 20 min, filtered and washed with PBS buffer, dried at room temperature and stored in airtight container for further analysis.

Characterization of Ag-MnNP nanoparticles

The colloidal brown solution was monitored by absorption measurements carried out on UV–Visible Spectrophotometer (UV 1900 I spectrophotometer Schimadzu) between 300 to 600 nm (which is a characteristic wavelength absorption range for nanoparticles) wavelength range for confirming the synthesis of Ag-MnNP nanoparticles in the solution. For absorption measurements, different brown colloidal solutions were poured in cuvette and placed in sample holder where wavelength of specific range is passed through it and absorption values are displayed in the form a spectra. Maximum absorption at a particular wavelength was depicted as a peak.

Well Diffusion Assay

The antibacterial activity of synthesized Ag-MnNPs was evaluated using the agar well diffusion method against Gram-positive *Bacillus subtilis, Bacillus cereus*, *Staphylococcus aureus* and Gram-negative *Proteus vulgaris*. Pure cultures of these microorganisms were obtained from Department of Biotechnology, Barkatullah university, cultured on nutrient agar medium and incubated at 37°C for 24 h. Fresh overnight cultures were inoculated on Mueller Hinton agar (MHA) plates using sterile swabs and allowed to stand for 20 minutes. Sterile cork borer was used to bore 8 mm diameter wells in the agar plates. The wells were loaded with 25 µl of 25 ug ml^{-1}, 50 ug ml^{-1}, 75 ug ml^{-1} and 100 ug ml^{-1} concentration of bimetallic nanoparticle solution and 10 ug ml^{-1} of an antibiotic (Gentamycin) was used as positive control and DMSO was used as negative control. The incubation of the plates at 37 °C for 24 h was done. The zones of inhibition around the well impregnated with bimetallic nanoparticle were measured to determine the antibacterial activity of the synthesized gold nanoparticle. The antibacterial investigation was on triplicate analysis (14).

RESULTS

Phytochemical screening of extracts

Previous information about study on preliminary phytochemical analysis have documented the presence of various phytoconstituents in the *C macleodii* leaves using a single solvent but in the present study, the successive extracts in varying polarity solvents were obtained using methanol, ethanol and water. The phytochemical tests shows presence of alkaloids, tannins, flavonoids, carbohydrates, flavonoids, proteins, steroids, terpenoids (Table1).

Table 1: Phytochemical analysis of extracts of *C macleodii* leaves

S.No	Test	SOLVENT		
		Methanol	Ethanol	Aquous
1	Alkaloid	+	+	+
2	Tannins	+	+	-
3	Flavonoids	+	+	+
4	Carbohydrates	+	-	-
5	Proteins	+	+	-
6	Steroids	+	+	-
7	Phenols	-	+	+
8	Saponins	+	-	-

Biosynthesis of Bimetallic Nanoparticles

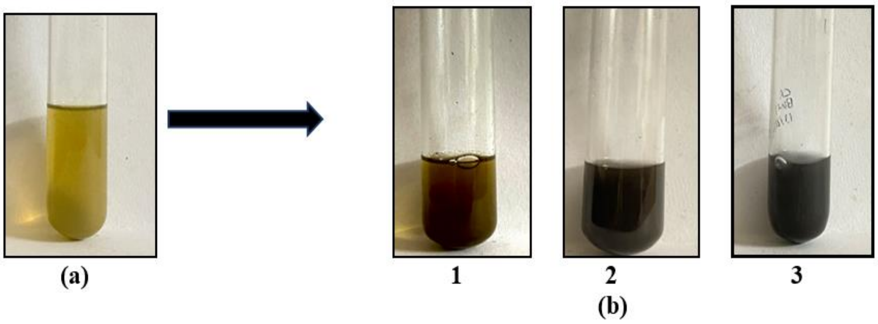


Fig 1. C macleodii nanoparticle solution at (a) 0 minutes (b) after 8 h of reaction time at 40 ºC

Methanolic Ag-MnNP 2) Ethanolic Ag-MnNP 3)Aqueous Ag-MnNP

Metallic nanoparticles display characteristic optical absorption spectra in the UV-visible region called Surface Plasmon Resonance (SPR) which is due to the physical absorption of light by metallic nanoparticles and this leads to a coherent oscillation of the conduction electron (15). This is a small particle effect since it is absent in individual atoms as well as in their bulk structures. UV-visible spectra of nanoparticles show the characteristic fingerprint of the Surface Plasmon Resonance (SPR) spectra with absorbance at 400-470 nm. The analysis of the colloidal brown solution (fig 1) by UV-Vis spectrophotometer showed a characteristic absorbance peak between 400 and 450 nm which can be attributed to the formation of Ag-Mn nanoparticles (Fig 2, Fig 3 and Fig 4).

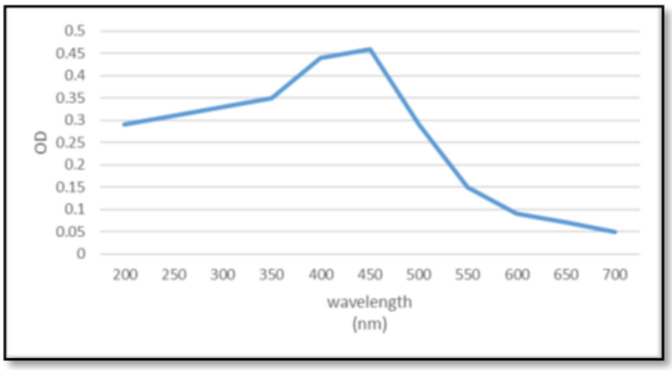


Fig 2: UV-Vis spectra of methanolic Ag-MnNP of C macleodii

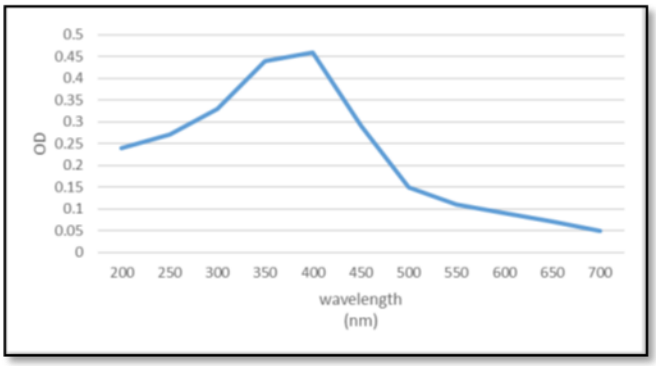


Fig 3 : UV-Vis spectra of ethanolic Ag-MnNP of C macleodii

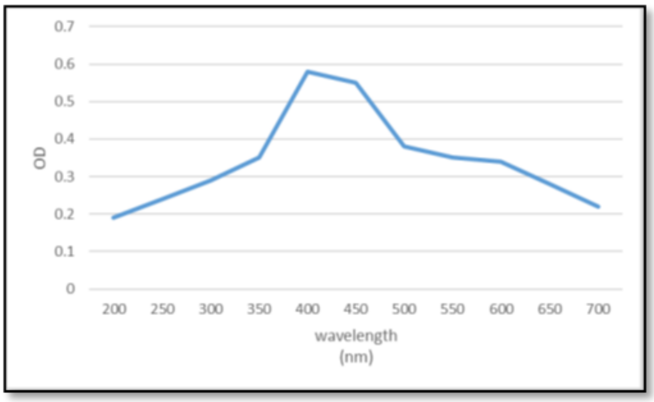
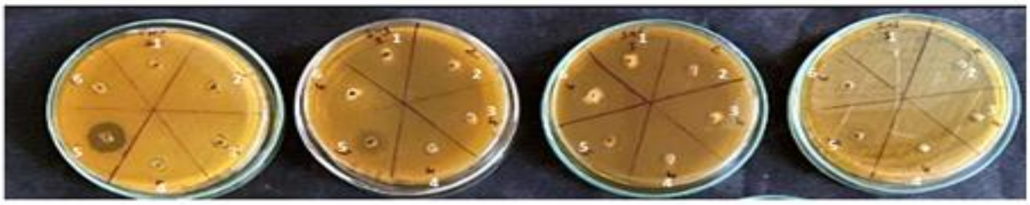


Fig 4 : UV-Vis spectra of aqueous Ag-MnNP of C macleodii

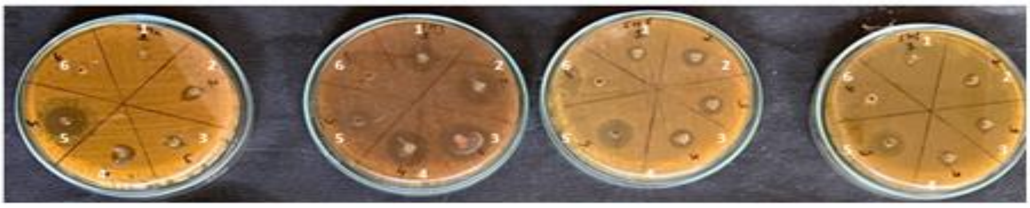
Antibacterial Activity

The results showed high antibacterial activity against both gram (−) and gram (+) bacteria. The potent antibacterial properties of Ag-MnNPs may be attributed to the released metal ions, which could have interaction with microorganisms by means of their attaching to the surface of the cell membranes of bacteria and penetrating into the bacterial cells.

5a



5b

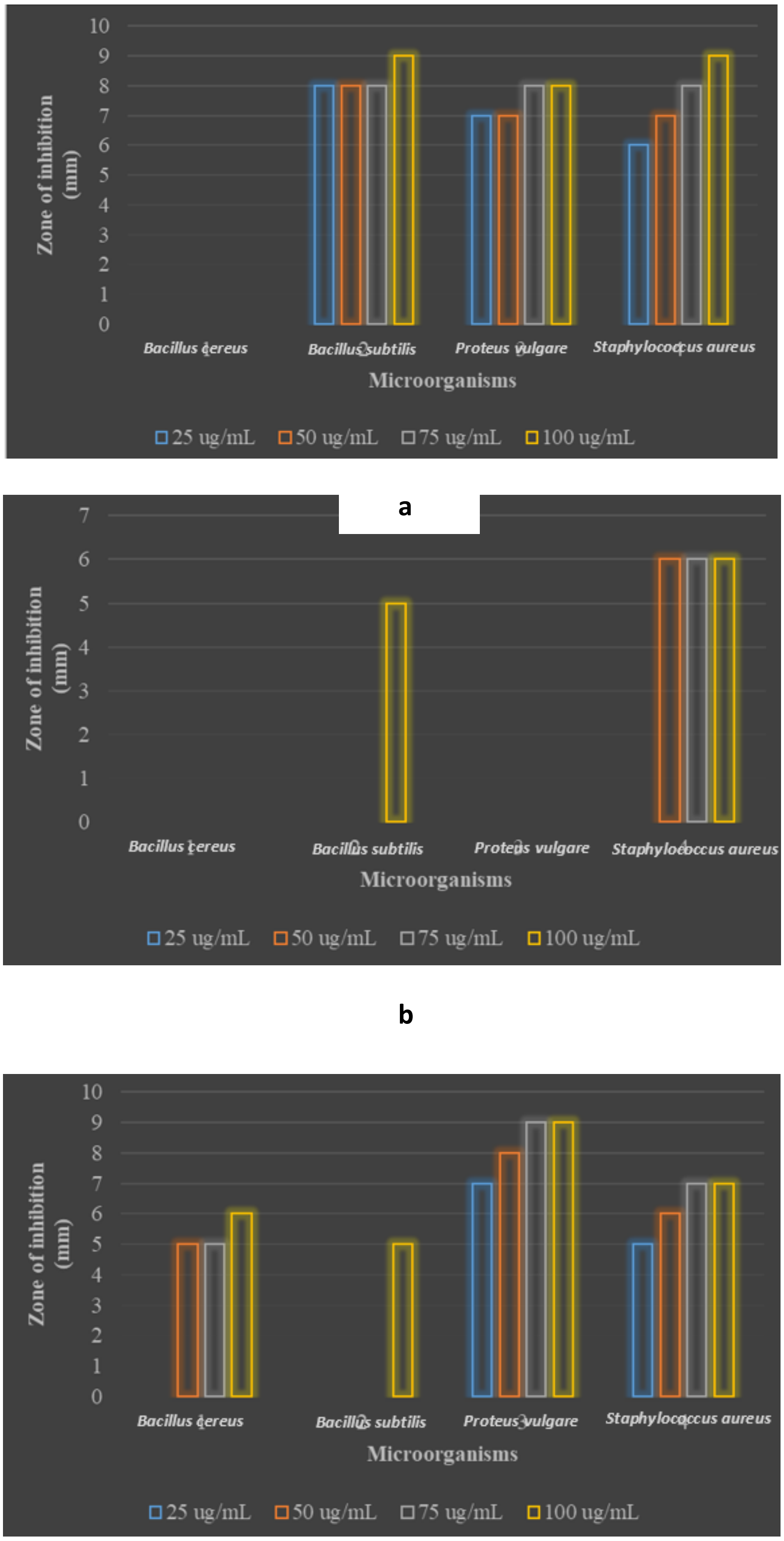


5c

Figure 5: Zones of inhibition against of a) methanolic Ag-MnNPs b) ethanolic Ag-MnNPs c) aqueous Ag-MnNPs at different concentrations against various microorganisms. (1- 25 ug mL^{-1}, 2- 50 ug mL^{-1}, 3- 75 ug mL^{-1}, 4-100 ug mL^{-1}, 5- positive control (Gentamycin), 6- negative control [DMSO])

Among bimetallic Ag-MnNP synthesized from three solvents (methanol, ethanol and water), highest susceptibility was against aqueous Ag-MnNP (Fig 5c) followed by methanolic Ag-MnNP (Fig 5a) where most susceptible were *Bacillus subtilis*, *Proteus vulgaris* against different concentration (25 ug mL^{-1} – 100 ug mL $^{-1}$). Highest inhibition zone was observed in *Bacillus subtilis* (≥15mm) (aqueous Ag-MnNP) followed by *Proteus vulgare* (≥12mm) (aqueous Ag-MnNP). All the four-microorganism showed strong resistance against bimetallic ethanolic Ag-MnNP (Fig 5b).

5b



a

b

c

Fig 6: Zone of inhibition of different microorganisms against a) methanolic Ag-MnNP b) ethanolic Ag-MnNP c) aqueous Ag-MnNP

The study also showed that NPs were more active against Gram (+) bacteria than Gram (-) bacteria (Fig 6). According to Dibrov, positively charged NP attach to the negatively charged cell membrane of bacteria by electrostatic attraction. The gram (+) bacterial cell wall has more peptidoglycan than that of gram (−) bacteria, therefore a large number of silver nanoparticles may attach to the peptidoglycan in gram (+) bacteria than in gram (−) bacteria (16). The antibacterial activity of the bimetallic nanoparticles may be due to the nature of Ag which is highly dispersed in the MnO_2 matrix, so that Ag can be more exposed to bacterial cells and shows potent antibacterial activity. Moreover, the advantage of incorporation of Ag into MnO_2 lattice is the reduction of toxicity of free ions to human cells (17).

CONCLUSION

The present work was focused on developing on bimetallic Ag-Mn nanoparticles from *Cordia macleodii* extract using three solvents (methanol, ethanol and water) and the assessment of the antibacterial activity of synthesized against both Gram (+) and Gram (-) bacteria. The phytochemical constituents such as flavonoids, phenolic acids, and alkaloid groups from *C macleodii* extract played a vital role in the reduction of Ag and Mn ions to bimetallic NPs, as well as the stabilization of the formed NPs.

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