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Nanoparticles Synthesis and Antibacterial Study on Anisomeles Malabarica using Manganese Oxide (MnO)

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Abstract: We have investigated the synthesis and structural as well as magnetic properties of composite nanoparticles, including core–shell particles, consisting of iron and manganese oxides. The synthesis is based on thermal decomposition of suitable metaloleates in a high boiling solvent A versatile route has been explored for the synthesis of nanorods of transition metal (Cu, Ni, Mn, Zn, Co and Fe) oxalates using reverse micelles. Transmission electron microscopy shows that the as-prepared nanorods of nickel and copper oxalates have diameter of 250 nm and 130 nm while the length is of the order of 2.5 ml and 420 nm, respectively. The biosynthesis of metallic NPs is the subject of a number of recent reviews, which focus on the various ‘‘bottom-up’’ bio fabrication methods and characterization of the final products. Numerous applications exploit the advantages of biosynthesis over chemical or physical NP synthesis, including low capital and operating expenses, reduced environmental impacts, and superior biocompatibility and stability of the NP products.
Keywords: *Anisomeles Malabarica*, MnNPs,FTIR, XRD, SEM, Antibacterial Activity.

1. Introduction

Nanotechnology is becoming a new area of increasing research and industrial interest since the 1980’s. Nanotechnology can be defined as the manipulation of atom by atom from the material world by the combination of engineering, chemical and biological approaches. Main fields of nanotechnology applications range from catalysis, micro- and Nano-electronics (semiconductors, single electrons transistors), non-linear optic devices, photo-electrochemistry to biomedicine, diagnostics, foods and environment, chemical analysis and others.[1]Nanoparticles possess high surface to volume ratio due to its small size, which gives very distinctive features to nanoparticles. A multiple of research have established that zinc oxide, manganese oxide nanoparticles have antifungal and antibacterial activity. The other metal nanoparticles like silver nanoparticles also carry these properties. Recent inspiring improvements in the field of nanotechnology have been observed because of development of different and efficient methodologies to fabricate nanoparticles of particular shape and size depending on the specific requirements.

Currently, the use of “green” methods for synthesis and production of engineered nanomaterials in both industrial application and the scientific research has achieved a massive amount of interests[2]. Proper utilization of environmentally benevolent solvents and nontoxic chemicals are some of the key issues in green synthesis approach deliberations [3].Nanotechnology is a science and engineering branch of recently well-established technology referring at the nanoscale, i.e., 1 to 100 nm. Generally, metal oxide nanoparticles are inorganic. Various nanoparticles like Fe, Ni, Co, Mn, Zn etc., are known as the enormously accepted magnetic materials for a wide range of applications like various electronic ignition systems, generators, vending machines, medical in plants, wrist watches, inductor core, transformer circuits, magnetic sensors and recording equipment,

telecommunications, magnetic fluids, microwave absorbers, etc. They are also applicable in other high-frequency applications[4].

In the recent years, the various size and shape of different nanomaterials has been realized through a wet-chemical synthesis because of its wide range of application. Owing to this reason researchers showing an increasing interest to fabricate nanostructured materials. In this present work we prepared nano particles and nanocomposites using wet-chemical synthesis[5-6]

Due to their variable oxidation states, manganese oxides have attracted substantial attention because of their superior magnetic, electrical and chemical properties, which promise great potential in superconductivity applications. In recent years, considerable research has been focused on nanostructured manganese oxides. It has been well documented that nanostructured manganese oxides possess unique magnetic[7-8]

At present several nanostructured metal oxide thin films were grown and studiedto explore their applications by different workers. However, to fabricate any device commercially, it is necessary to develop low cost material by economic method. In this regard, manganese oxide may become a functional oxide material in future due to its low cost and eco friendly nature. As manganese oxide has different oxidation states Mn2+, Mn3+, and Mn4+, it shows different phases such as MnO, MnO2, Mn2O3 and Mn3O4**[9].** For the metal oxide electrode materials, depositing the oxide particles in the conductive matrices such as carbon nanotubes or carbon fibers is an efficient strategy to promote the electrochemical performance, because the conformal coating can ensure intimate contact and continuous transportation of electrons between the conductive matrices and the metal oxides **[10-11].**

The plant is known as medicine because they contain active substances that cause certain reactions from relenting to the cure of diseases on the human organisms. Plants are the main sources of food. They are rich in nutrients. They are also rich in compounds which have pain relieving and healing abilities. In ancient times, the plants were used for the treatment of disease without knowledge about the compounds present and their mode of actions. *A. malabarica* is a medicinal plant that has been used as a folkloric medicine to treat amentia, anorexia, fevers, swelling and rheumatism **[12]**.Herbal plants have high medicinal values as they have the rich source of bioactive compound and have no side-effects. The bioactive compounds from the herbal plants reduce the virulence of the microorganisms, thereby preventing and protecting from the infections. *A.malabarica* (L) is a traditional medicinal plant, distributed throughout India. It has been used in folk medicine for the treatment of cancer, liver disorder, stomach ailment, fever, cold and cough **[13]**.

The antimicrobial activity of medicinal and aromatic plants has been known and described for several centuries[14].Most of their properties are due to the secondary metabolites present in essential oils. [15]. In recent years a large number of essential oils and their constituents have been investigated for their antimicrobial properties against bacteria and fungi[16].Essential oils and extracts from several plant species are able to control microorganisms related to skin, dental caries and food spoilage, including gram-negative and gram-positive bacteria**.**

A. malabarica (Lin) is a traditional medicinal plant found in tropical and subtropical regions of India, belongs to family Lamiaceae. It is erect shrub commonly known as 'Malabar catmint', commonly found in Western Ghats from Maharashtra to Kerala in India[17]. The herb is reported to possess anticancer, allergenic, antihelmintic[18], antiallergic, antianaphylactic, antibacterial[19-21], anticarcinomic, anti-inflammatory[22] antileukemic, antinociceptive, antiplasmodial, and antiseptic, antiperotic properties[23-24].*Anisomelic* acid is one of the major compounds in *A. malabarica (L.) R. Br.* is a membrane type diterpenoid, which can be synthesized chemically. The present study describes antibacterial effects of biosynthesized ZnO nanoparticles using an leaves extract of *A. malabarica*. This is the new report about manganese oxide from *A. malabarica.*In this study, we first report about green leaf synthesis of MnONps from *A. malabarica* which characterized by UV-Vis spectroscopy, XRD, FT-IR, SEM study and their antimicrobial assessment was performed.

2. Materials and Methods

2.1 Collection of Sample

The healthy plant samples were collected from the Kolli hills (lattitude 11.2485' E and longitude 78.3387' N) near Sethamangalam, dry rocky region of Namakkal district, Tamilnadu. The leaves were separated from the collected plant and dried under room temperature.

Fig.1 ***Anisomeles malabarica***

2.2 Preparation of *Anisomeles malabarica* Leaf Extract

Aqueous extract was prepared by mixing 50 g of dried leaves powder with 500mL of water (boiled and cooled distilled water) with constant stirring on a magnetic stirrer. The suspension of dried leaves powder in water was left for 3 h, filtered through Whatman no. 1 filter paper, and the filtrate was stored in amber coloured air tight bottle at 10 ◦C and used within a week.

2.3 Synthesis of MnO nanoparticles by *Anisomeles malabarica* Leaf Extract

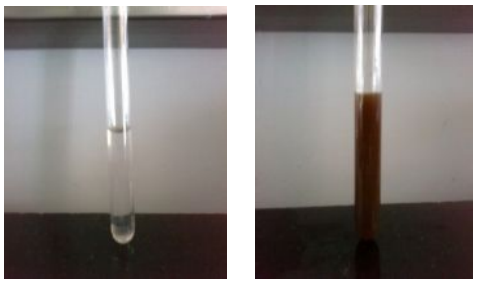

Fig.2 ***A. malabarica*** **Aqueous Extract and Formation of MnONPs**

0.2M Aqueous solution of manganese oxide (MnO) was prepared and used for the synthesis of manganese nanoparticles. 10ml of *A. malabarica* Leaves Extract was added into 90ml of aqueous solution of 0.2M manganese oxide for reduction into Mn+ ions and kept at room temperature for 30min.

2.4 UV-Vis Spectral Analysis

Synthesized silver nanoparticles was confirmed by sampling the aqueous component of different time intervals and the absorption maxima was scanned by UV-Vis spectrophotometer at the wavelength of 200-800 nm on Perkin-lambda 25 spectrophotometer.

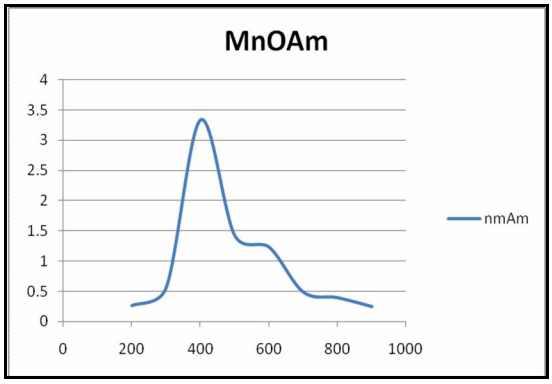

Fig: 3 UV-Vis spectrum of synthesized MnO NPs

2.5 FTIR Spectrum Analysis

The bio reduced silver nitrate solution was centrifuged at 10,000 rpm for 15min and the dried samples were grinded with KBr pellets used for FTIR measurements. The spectrum was recorded in the diffuse reflectance in the range of 4000-400 cm^{-1} using Thermo Nexus 670 spectrometer in the diffuse reflectance mode operating at resolution of 4 cm^{-1}.

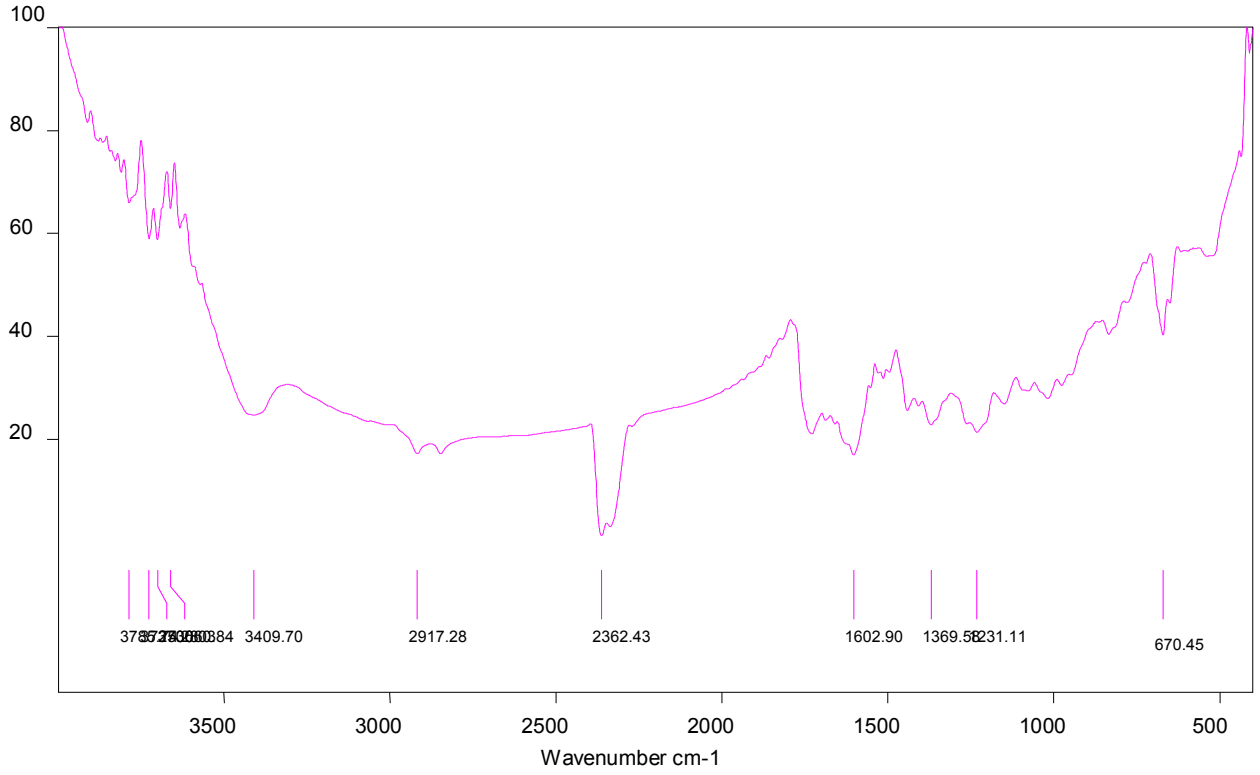

Fig:4 FTIR analysis of MnO nanoparticles.

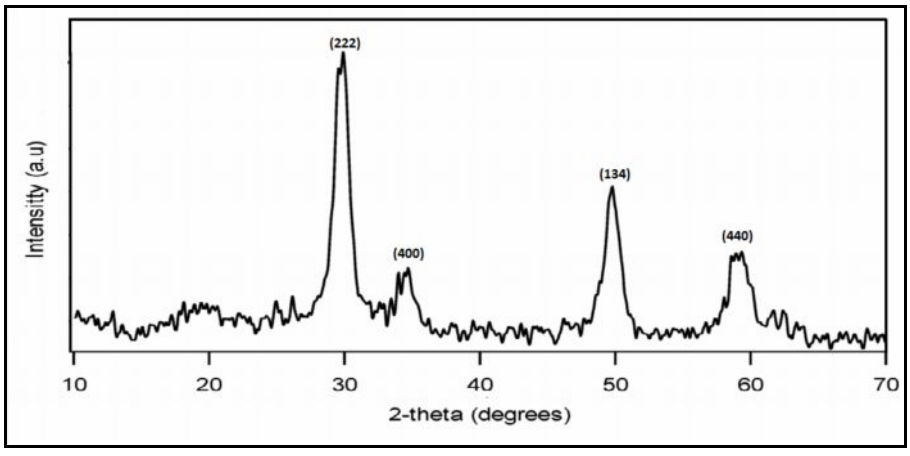

Fig: 5 XRD pattern of MnO nanoparticles of heated sample.

2.6 XRD (X – ray diffraction):

X-ray diffraction studies were carried out using an Altima IV [Make Regakul] X-ray diffractometer. BET surface area was measured on a NOVA 4200e surface area using a pore size analyzer. Thermo gravimetric analysis was carried out using a Perkin-Elmer Thermo gravimetric Analyser.

2.7 Scanning Electron Microscopy (SEM)

Scanning Electron microscopy is another commonly used method of characterization [25] Cao G., Nanostructures and nanomaterials: synthesis, properties and applications. London: Imperial College Press; 2004. Scanning electron microscopy and Fourier transmission electron microscopy are used for morphological characterization at the nanometre to micrometer scale [26].

2.8 Antimicrobial Activity

The antimicrobial activity of the various extracts was studied systemically against different strains of bacteria (Staphylococcus aureus, Proteus vulgaris and E.coli) by cup diffusion plate method. The medium was prepared by dissolving the specified quantity of the dehydrated medium water by heating one water bath and were dispensed in 100 ml conical flask. The conical flask was closed with cotton plugs and sterilized by autoclaving at 121°c for fifteen minutes. The contents of the conical flask were poured ascetically into sterile Petri dishes and allowed to solidify. These sterilized media was used to sub culture the bacterial culture. The Petri dishes were especially with flat bottom and were placed on level substance so as to ensure that the layer of medium is in uniform thickness the Petri dishes were sterilized at 160 degree to 170°C in hot air oven for 30 minutes before use. One loop full of the original bacterial culture was transferred to the conical flask containing the autoclave Muller- Hinton agar medium under bearable heat condition. The flask was shaken to effect though mixing of inoculums contains of the flask were poured in to the separate Petri dish simultaneously along with the media. The various extract was poured into corresponding Petri plates. Control was done along with the experiment that contains the bacterial suspension along with the media. After the inoculation period at 24-48 hours they were examined for bacterial growth.

3. Results and Discussion

3.1 UV-Visible Spectroscopy

Green synthesis of MnONPs was synthesized from the aqueous manganese oxide and A. malabarica leaves extract at room temperature. Initially, the colourless reaction mixture slowly changed to dark brown at 30min with the formation of MnONPs. UV–Vis absorbance spectral value at 420nm has confirmed the reduction of manganese ions to metallic by using the leaf extract .The surface Plasmon resonance band at 420nm confirmed the green synthesis of MnONPs at ***A. malabarica*** leaves extract. The formation of MnONPs was monitored by UV–Vis spectra at different time intervals; after 4 hr the maximum reduction and formation of MnONPs was observed which is established in the absorbance intensity.

3.2 Fourier Transform Infrared [FTIR]

FTIR measurement was carried out to identify the biomolecules for capping and efficient stabilization of the metal Nano particles synthesis by A. malabarica leaf broth. The FTIR spectrum of MnO is shown fig. The band at 3409 cm^{-1}corresponds to O-H stretching, H-bond and phenols. The peak at 2917cm^{-1} corresponds to C=H stretch alkanes. The arraignment at 1602cm^{-1} corresponds to N=H bonded primary amines group and the bands observed at 1369cm^{-1} corresponds to C-H stretching alkanes groups. Therefore the synthesized Nano particles synthesis were surrounded by protein and mentalities such as terpenoids having function groups of alcohol, ketone aldehydes and carboxylic acids from the analysis of FTIR studies we conformed that the aliphatic amine group from the amino acids residues and protein has the stronger ability to bind metal indicating that the protein could possibility from the metal Nano particles synthesis. This suggests that the biological molecules could possibly perform dual function of formation and stabilization of MnO in the aqueous medium.

3.3 XRD (X-ray diffraction):

From the XRD pattern of the catalyst calcined at 300 c, it was observed that the catalyst is composed of a mixture of trigonal / rhombo hedral MnO (ICSD-80867) and Ortho-rhombic silver manganite (ICDD: 01-076-1584). The XRD pattern of catalyst calcined at 400 and 500 ^{0}c suggests that the catalyst undergoes phase transition as the calcinations temperature increase and the structure changes to higher symmetry, which can be confirmed by the disappearance of the peaks at 31.353^0, 56.26^0, 63.93^{0}in 20. The indices (222), (400), (134) and (440) are attributed to the orthorhombic arrangement which is identical to manganese oxide. The XRD pattern

shows well defined diffraction features characteristic to Nano crystalline with crystallite size about 7.74nm calculated by the Debye-Scherer equation**.**

3.4 Scanning Electron Microscopy (SEM)

Scanning electron microscopic analysis of theMn solution control and reduced from of MnO solution were early distinguishable owing to their sizes difference was clear from the SEM picture. That the manganese particles in the bio reduced colloidal substance measure develop in size [0.5-0.2µm in size is the SEM of analysis of Manganese oxide. The SEM analysis of manganese nanoparticles use synthesized in plant leaf extract showed large density manganese nanoparticles which are highly agglomerated clumped the resolutions ranging from [0.5-0.2µm]. It shows significant dissonant varied Nano structure [crystals], as especially 0.5-0.2µm and differentiates crystals result in *A. malabarica*. SEM analysis shows in the profound distribution of manganese nanoparticle in the surface are absorbed.

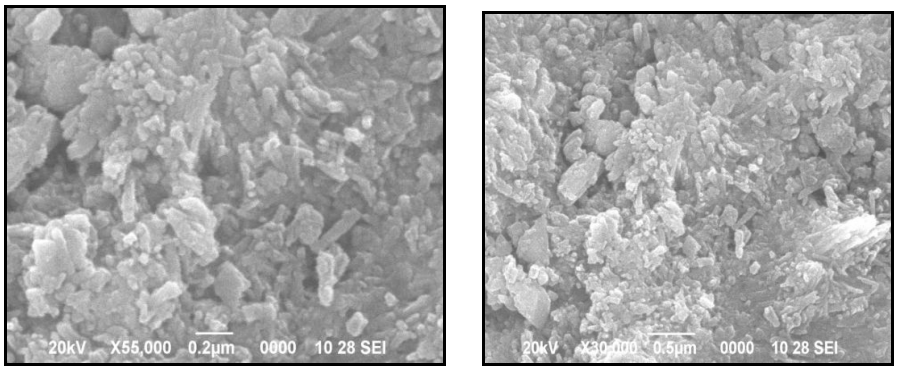

Fig 6: Surface morphology of MnO nanoparticles at different magnifications

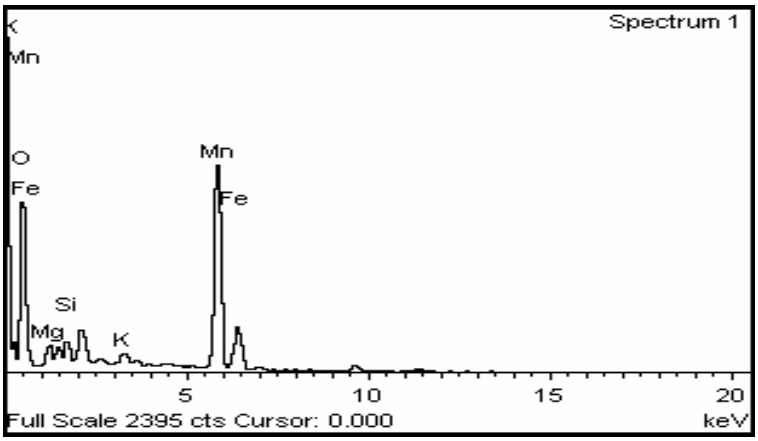

Fig:7 EDX images of MnO Nanoparticles

3.5 EDX analysis of MnO Nanoparticles

Energy dispersive X-ray spectroscopy (EDX) was employed to establish the element identity of the observed particles. In the analysis by Energy Dispersive X-ray spectroscopy (EDX) of the MnO nanoparticles, the presence of elemental metal signal (Manganese and oxygen) was confirmed.

3.6 Antibacterial screening

The results of the antibacterial screening by cup plate method using different bacterial strains namely Staphylococcus *aureus* (gram +ve), E.coli (gram –ve) and Proteus *vulgaris* (gram +ve) is shown below. The varying (MnNPs) of *A.malabarica* was used to screen for its antibacterial effect by the above method. Pathogenic bacteria collected from the MTCC were used in this study. The Gram-negative bacteria species, *Escherichia coli* and the Gram-positive with *proteus vulgaris* Gram-positive and *Staphylococcus aureus* had

been used in the assay. 24 h fresh cultures were prepared and the standardized. The antibacterial activities of MnONPs against the studied pathogenic strains are shown in Fig-1. The values of zone of inhibition obtained from the assay are presented in Table 1. All Gram-negative bacteria had shown good sensitivity towards the green synthesized MnONPs for the concentration 22 µl. It is quite interesting to note that all bacterial species tested in this study showed resistance to the synthetic antibiotic drug which in turn indicates the better antibacterial activity of the MnONPs than the commercially available synthetic drug.

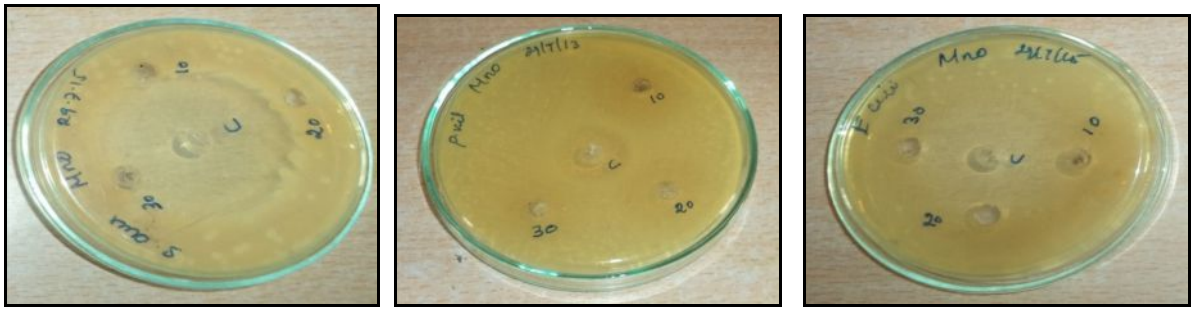

Plate 1.Antimicrobial activity of *A.malabarica* S. aureus, *E. coli* and *P. vulgaris* (MnNPs)

1.Antibacterial activity from MnNPs of *A.malabarica* by Cup Diffusion Method.

S.No	Micro Organism	Gram	Standard 50µl	Zone of inhibition MnNPs		
				100µl	200µl	300µl
1.	Staphylococcus aureus	+ve	15	18	20	22
2.	Escherichia coli	-ve	12	10	18	20
3.	proteus vulgaris	+ve	10	8	12	16

4. Conclusion

The biological Production of metal nanoparticles is becoming a very important field in chemistry, biology and material science. Metal nanoparticles have been produced chemically and physically for a long time; however, their biological production has only been investigated very recently. The rapid biological synthesis of manganese nanoparticles using leaves extract of *A. malabarica* nanoparticles was done. The reduction mechanism and effect of various factors leading to different morphologies of the resulting nanoparticles has scarcely discussed. The rapid biological synthesis of manganese nanoparticles using leaf broth of *A.malabarica* ***provides*** an environmental friendly, simple and efficient mode for the synthesis of being nanoparticles were further characterized using UV-Vis, FTIR, XRD and SEM, EDX spectroscopic techniques. These reduced manganese nanoparticles were surrounded by a faint thin layer of proteins and metabolites such as terpenoids having functional groups of amines, alcohols, ketones, aldehydes and carboxylic acids.

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