INTERNATIONAL JOURNAL OF PHARMACEUTICS & DRUG ANALYSIS

VOL.6 ISSUE 1, 2018; 35 – 40 ; http://ijpda.com; ISSN: 2348-8948

Research Article

Antibacterial Effect Of Fe_2O_3 Nanoparticles Synthesized By *B. diffusa* Herbal Extract

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Date Received: 17th January 2018; Date accepted: 22nd January 2018; Date Published: 22nd January 2018

Abstract

The present study was carried out in order to evaluate the potential antibacterial activity of iron oxide nanoparticles (Fe_2O_3) synthesized by green synthesis against gram negative and gram positive bacteria. The synthesized iron oxide nanoparticles were characterized by scanning electron microscope (SEM-EDS), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR). Antibacterial activity was investigated using agar well diffusion method. The characterized nanoparticles were average size 34 nm with spherical shape. The green synthesized iron oxide nanoparticles show good antibacterial effect on gram positive bacteria compare to gram negative bacteria.

Key words: *B. diffusa*, Iron oxide, Green synthesis, antibacterial activity.

INTRODUCTION

The antimicrobial effects are intensively studied due to an enormously increasing bacterial resistance against excessively and repeatedly used classical antibiotics. It is very complex and the evolutionary processes usually occur during antibiotic therapy, leading to the emergence of heritable resistance to antibiotics (1). In the last decades, the treatment involved in infections caused by bacteria has become more complicated due to the emergence of the resistance mechanisms which has resulted in life threatening infections. This led to the search of alternate materials which can be used as antibacterial agents (2). Iron oxide nanoparticles have been of great interest, not only for fundamental properties caused by their multivalent oxidation states but also for their super paramagnetic, high force, low Curie temperature, high magnetic susceptibility, etc. (3). Iron oxide nanoparticles are of particular interest as antibacterial agents, as they can be prepared with extremely high surface areas and unusual crystalline morphologies with a high number of edges and corners, and other potentially reactive sites (4). They have a positive surface charge to facilitate their binding to the negatively charged surface of the bacteria which may result in an enhancement of the bactericidal effect (5, 6). The Green synthesis provides advancement over chemical and physical methods as it is environmental friendly, cost effective, easily scaled up for large scale synthesis and the method doesn't requires the use of high pressure and toxic chemicals (7, 8). The present study we used the herb *B.diffusa* for synthesis of Iron oxide nanoparticles and also tested their antibacterial effect against gram positive and gram negative bacteria like *B.subtilis S. aureus E.coli, K. pneumonia, A. hydrophila, P. fluorescence, F. branchiophilum, E.tarda* and *Yersinia rukeri*.

Materials and methods:

Preparation of herbal Extract of *B. diffusa*

Boerhaavia diffusa is a medicinal plant widely used in the Ayurvedic medicine (9). The plant was named in honour of Hermann Boerhaave, a famous Dutch physician of the 18th century (10). *Boer-*

haavia diffusa belongs to the family Nyctaginaceae, a diffused perennial herbaceous creeping weed of India, known also under its traditional name as Punarnava. *B. diffusa* herbal plants were collected from local area of Andhra University campus, dried under shade for a week and washed with double distilled water and air dried. Then they were cut in to small pieces and a fine powder was prepared, the powder material was placed in a thimble holder and filled with condensed fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solution of the thimble holder and unloads it back into the distillation flask, carrying extracted solutes into the bulk liquid. In the solvent flask, solute is separated from the solvent by distillation. Solute is left in the flask and fresh solvent passes back into the plant solid bed. The operation is repeated until complete extraction is reached. Plant materialwere extracted with appropriate volume of solvent at 80°C for 8 hrs (11). The water extract was then concentrated by distillation off the solvent under reduced pressure and then allowed to cool. The extracts were packed in glass jars and stored at 4°C for analysis. The yield of the extract is 10%.

Synthesis of Iron Oxide Nanoparticles:

In this study, iron chloride (99.99+ % purity,) was used as the starting chemical material for the synthesis of iron oxide nanoparticles. Aqueous extract powders (500 mg) *B. diffusa,* were dissolved in 100 ml of distilled water. For the preparation of iron oxide nanoparticles, 10 g of iron chloride was first dissolved in 100 ml plant extract solution and pored into RB flask under reflection stir at 60°C for several hours and later filtered through filter paper. The obtained precipitate was washed thoroughly three times with double distilled water to remove all the ions and then heated in a hot air oven at 200°C for 3 hrs. The resulting dried precursor was crushed into powder and stored in a air tight container for further analysis.

Characterization of Iron Oxide Nanoparticles:

The morphological, structural, chemical composition of nanoparticles were analyzed with SEM – EDX (JEOL JSM-6610-LV- with OXFORD EDS), XRD (PANalytical: XPERT-PRO) and FTIR (Shimadzu FT-IR 21) Prestiage equipment.

Antibacterial effect of Iron oxide Nanoparticles

The nanoparticles are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition is measured in millimeters. The modified agar-well diffusion method of Cappuccino and Sherman (12) was employed to study the antibacterial activity of the nanoparticles. Muller Hinton Agar was mixed with hot distilled water and autoclaved at 15 lbs pressure for 15 minutes. After autoclaving, it was allowed to cool down to 45° C-50° C. Then 25 ml of MHA medium was poured onto sterilized petri dishes with a uniform depth of approximately 4 mm. The MHA medium was allowed to cool to room temperature. The bacterial culture standardize was equivalent to 0.5 Mac Farland standards (1.5 X 10^8 CFU/mL). A lawn of bacterial culture was prepared by spreading 50 µL culture broth, MHA medium must be seeded with 24hr culture of bacterial strains. Spread the bacteria on the media in a confluent lawn with a glass spreader. The plates were allowed to stand for 10–15 minutes to allow for culture absorption. The 6 mm size wells were punched into the agar with the head of sterile cork borer. Using a micropipette, 50 µL (100 µg/ml) of the iron oxide nanoparticles solution sample was poured into each of wells on all plates after labeling. Plates were incubated for 24 hours at 37°C. After 24 hours the plates were examined. Results were recorded based on the as the presence or absence of inhibition zone and the size of the zone was measured. Antimicrobial activities of the various bio synthesized iron oxide nanoparticles were determined using Gram-negative bacteria (*E. coli, K. pneumonia, A. hydrophila, P. fluorescence, E. tarda, F. branchiophilum* and *Y. rukeri*) and Gram-positive bacteria (*B. subtilis and S. aureus*). The effect of nanoparticles were compared with that of standard antibiotic rifampicin. Each experiment was repeated 6 times, and the resulting bacterial growth on plates corresponding to a particular sample was averaged and mean, SD values were analyzed.

Result and Discussion:

To determine the morphology and the average size of Fe_2O_3 particles, scanning electron microscopy (SEM) is used. The SEM image shows that magne-

tite nanoparticles have a mean diameter of about 35-40 nm and a nearly spherical shape. The SEM image of iron oxide nanoparticles synthesized by *B. diffusa* aqueous extract was shown in figure 1a. This is comparable to the early findings of vijay kumar et al. [13] who reported the size of iron nanoparticles by using *A. racemosus* root was diameter ranging from 30 to 40 nm. The increase in the size of nanoparticles confirms the presence of iron oxide nanoparticles with agglomerated in its structures. The elemental composition of nanoparticles is examined by energy dispersive spectroscopy. Figure 1(b) exhibits the typical EDS spectrum of nanoparticles which shows well defined peaks for iron (Fe) and oxygen (O). Except, Fe and O, no other peak related with any other element is found in the spectrum which revealed that the synthesized nanoparticles are made of iron and oxygen.

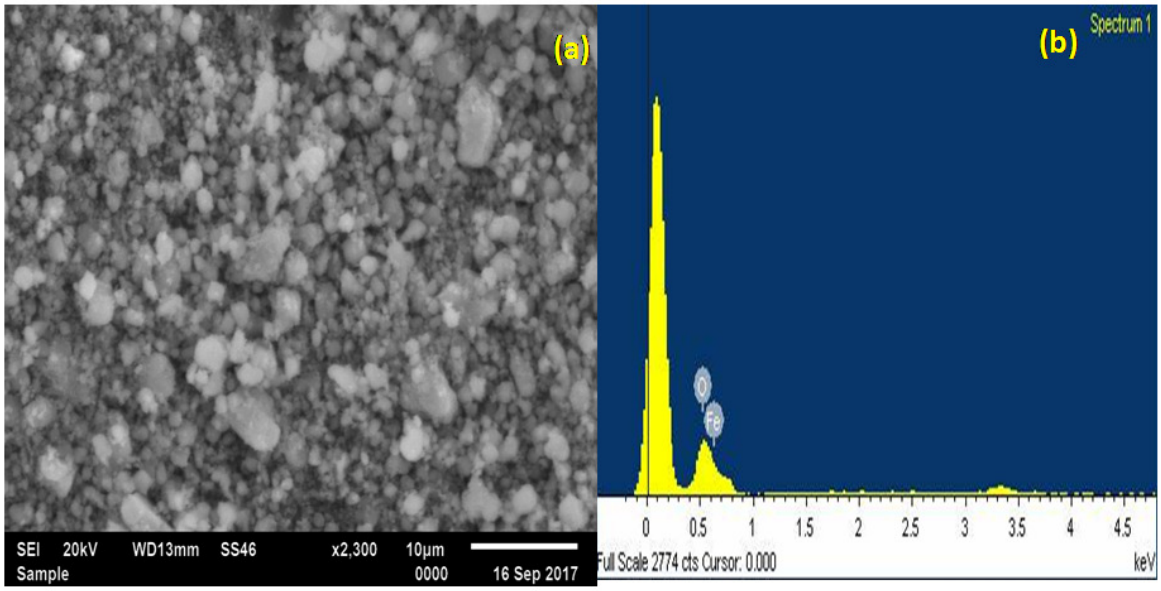

Figure 1: SEM image and EDS spectra of Fe_2O_3

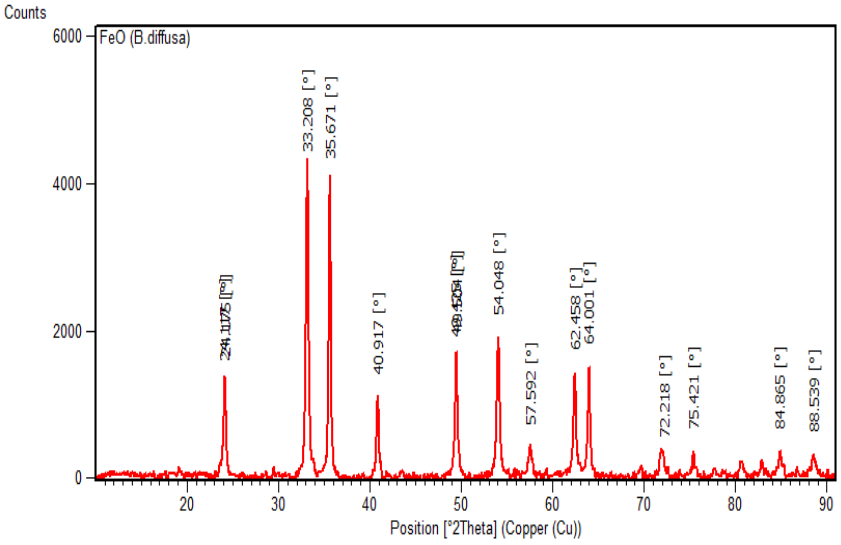

Figure 2: XRD pattern of α-Fe_2O_3

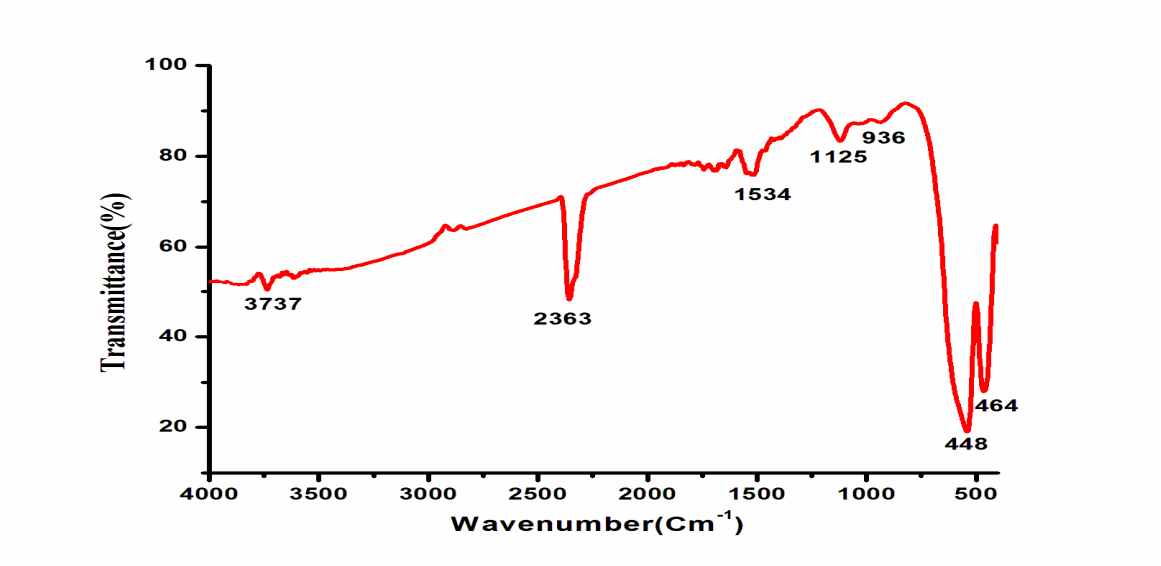

Figure 3. FTIR spectra of Fe_2O_3

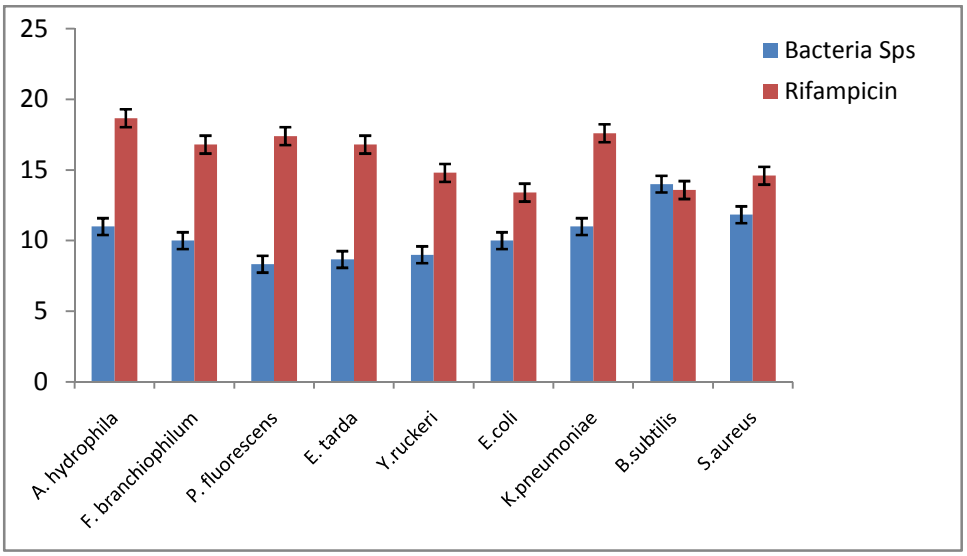

Figure 4. Zone of Inhibition of various bacterial pathogens against iron oxide

X-ray diffraction patterns have been widely used in nanoparticle research to characterize critical features such as a crystal structure, crystallite size, and strain. The XRD pattern of FeO NPs is shown in (Fig.2). Sharp diffraction peak emerges at 2θ angles of 24.1 °, 33.2 °, 35.6 °, 40.9 °, 49.5 °, 54.0 °, 57.5 °, 62.4 ° and 64.0 ° correspond to the hkl values from (012), (104), (110), (113), (024), (116), (112), (214) and (300) crystal planes respectively (JCPDF # 89-8104) representing rhombohedral crystalline phase (13) of α-Fe_2O_3. From the maximum intensity peak, average particle diameter was found to be around 33nm.

FTIR analysis was performed to identify the interaction between different species and changes in chemical composition of the of the Fe_2O_3 nanoparticles were recorded to identify the functional groups of the phytoconstituents responsible for the reduction of the metal precursors (Fig.3) shows the IR spectra of FeO NPs recorded between 400-4000 cm^{-1}. The formation of Fe_2O_3 nanoparticles is cha-

racterized by the adsorption bands around 448 cm^{-1} and 564 cm^{-1} corresponded to Fe-O stretches of Fe_2O_3 (14) and other peaks at 936 cm^{-1}, 1125 cm^{-1} corresponds to stretches O-C stretches, 1534 cm^{-1} to N=O stretches, 2363 cm^{-1} and 3737 cm^{-1} stretches to O-H which are proved to be phyto constituents present in the sample.

Antibacterial studies carried out with green synthesized iron oxide nanoparticles showed against inhibitory activity broad spectrum of strains including gram positive and negative bacteria. MIC was recorded as 12.5 µg/ml for gram positive bacteria and gram negative bacteria. The diameter of inhibitory zone around each well is provided in (Fig. 4). The zone of inhibition highest *B.subtilus* and *S. aureus* flowed by gram negative bacteria *i.e A. hydrophila, K. pneumonia, E. coli, F. branchiophilum, Y. ruckeri, P. fluorescens,* and. *E. tarda,* Fe_2O_3 nanoparticles inactivation of bacteria involves the direct interaction between Fe_2O_3 nanoparticles and cell surfaces, which affects the permeability of membranes where nanoparticles enter and induce stress in bacterial cells, subsequently resulting in the inhibition of cell growth and eventually cell death. Besides that, the more positive charge on the cell surface of gram positive bacteria interacts stronger with the Fe_2O_3 nanoparticles than with gram negative bacteria. Hence the zone of inhibition values probably more against gram positive bacteria than gram negative bacteria during the contact of Fe_2O_3 samples (15). Majority of studies suggest that, nanoparticles cause disruption of bacterial membranes probably by the production of reactive oxygen species (ROS) such as superoxide and hydroxyl radicals.

Conclusion:

Iron oxide nanoparticles synthesis is an easy and less time consuming way using *B. diffusa* herbal extract. The Phytochemicals in *B. diffusa* extract may possess the properties of reducing the ferric cations and also act as capping agent. The method is eco-riendly and provides bio-compatibility in pharmaceutical, biomedical and cosmetic applications as they do not use toxic chemicals for the synthesis protocol. Green synthesized iron oxide nanoparticles could be a potential antibacterial agent to treat diseases caused by bacteria.

Acknowledgements

We are thankful to the DST-PURSE Programme for the financial assistance and Advanced Analytical Laboratory, Andhra University for their support in carrying out in this research work regarding SEM-EDX and XRD, FTIR analysis.

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