Biosynthesis of iron nanoparticles from *Andrographis Paniculata* leaf extract and its antimicrobial assay

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Abstract

Andrographis paniculata, also called *Chiretta* or *Kalmegh,* is a medicinal plant which is traditionally used for the treatment of cold, fever and several infectious diseases. It is claimed that this plant contains immunological, antibacterial, anti-inflammatory, antithrombotic and hepatoprotective properties. Biological synthesis of nanoparticles is one of the most eco-friendly methods as it replaces the use of toxic chemicals for the production of nanoparticles. In the present study, FeONPs were synthesized using *A. Paniculata* leaf extract, characterization of FeONPs was performed using UV–vis spectroscopy analysis which showed visible peak at 430 nm and Scanning Electron Microscopy (SEM) analysis revealed hexagonal structure and the size of nanoparticle to be 90 nm. FeONPs were more active considering antibacterial activity against *Escherichia coli, Klebsiella pneumoniae* and lesser antibacterial activity against *Staphylococcus aureus*. This activity may be due to the incorporate effect of the isolated arabinogalactan proteins and andrographolides. Thus, FeONPs produced using *A. Paniculata* could play a vital role in killing the bacterial pathogens and also in bioremediation.

KEYWORDS: *Andrographis Paniculata*, Green synthesis, nanoparticles, antimicrobial activity, UV-Vis, SEM.

1 Introduction

Nanotechnology is a developing field of science that deals with synthesis and development of various nanomaterials. Nanoparticles are the particles ranging in size from 1- 100 nm that may differ in size. There are various uses of nanoparticles such as in medical treatments, in various branches of industry production such as solar and oxide fuel batteries for energy storage, in cosmetics, paints, clothes, electronics, etc. [1] Nanoparticles can be synthesized by using chemicals or by biological means. There are various pernicious effects that are associated with chemical synthesis methods which is due to the presence of toxic chemicals on the surface. [2] Nanoparticles can be synthesized biologically using microorganisms, enzymes, fungus, and plants or plant extracts. [3-7] There are two processes through which nanoparticles can be synthesized- “Top-down process” and “Bottom-up process”. In top-down process, satisfactory bulk material is taken and broken into fine particles by grinding, milling, etc. [8]

Researches have been conducted and it was proved that the green methods of synthesis of NPs are more productive and had the advantage of less chances of failure. [9] Apart from that it was found to be cost-effective, non-toxic, sustainable, economical and eco-friendly. The introduction of nano medicine presents many opportunities to fight all types of cancer, neurodegenerative disorders and other diseases. It has been proved through various studies that the plant extracts act as a precursor for the synthesis of the nanomaterials in harmless ways. In the ninth century in Mesopotamia, nanoparticles were used by artisans for generating a glittering effect on the surface of pots. [10,11] There are three main steps that are followed for the synthesis of nanoparticles biologically- the choice of solvent medium, the choice of an environment-friendly reducing agent and the choice of a nontoxic material as a capping agent which is used to stabilize the synthesized nanoparticles. [12] There are two properties of nanoparticles which makes it advantageous for use in drug delivery. Firstly, nanoparticles can penetrate through small capillaries and can be taken up by cells, which makes it easier for drug accumulation at target sites. Secondly, sustainable drugs are released within the target site over a period of time by the use of biodegradable materials for nanoparticle preparation. [13]

Andrographis Paniculata which is also called Kalmegh or "King of Bitters" belongs to the family of Acanthaceae. It has been used for centuries for the treatment of gastro-intestinal tract and upper respiratory infections, fever, herpes, sore throat, chronic diseases, etc.

TAXONOMICAL CLASSIFICATION

Kingdom: Plantae, Plants;

Subkingdom: Tracheobionta, Vascular plants;

Super division: Spermatophyta, Seed plants;

Division: Angiosperma

Class: Dicotyledonae

Sub class: Gamopetalae

Order: Personales

Family: Acanthaceae

Genus: Andrographis

Species: paniculata

Andrographis is considered as the herb which possess an important "cold property" which is useful in treating the heat of body during fevers. *Andrographis Paniculata* is an annual, branched, herbaceous plant with a height ranging from 30-110 cm mostly found in moist shady places. It is found extensively in southeastern Asia, Pakistan and Indonesia but it is cultivated widely in China and Thailand, the East and West Indies and Mauritius. The leaves of Andrographis Paniculata comprises of the highest amount of andrographolide (2.39%) which is the most medicinally active phytochemical while the seeds contain andrographolide in lowest amount. [14] *Andrographis Paniculata* acts as an analgesic, antimicrobial and antipyretic agent. It has a property of purifying blood which enables its use in diseases such as skin eruptions, boils, scabies, and chronic fevers, etc. *A. paniculata* has a bitter nature which makes it a herbal agent against the growth of parasitic organisms. [15]

2 Methodology

2.1 Preparation of leaf extract of A. Paniculata

Dried leaves of *A. Paniculata* were taken and grinded/ground into powdered form. About 10 gm of leaves were taken and boiled in distilled water at 50 degrees Celsius for 15 minutes. The solution obtained was then filtered using Wattman filter paper, collected and stored at room temperature for further experiments.

2.2 Pellet formation

In this process, ferrous sulphate served as a precursor. 250 mL of distilled water was added to around 50 mL of leaf filtrate. To the 50 mL of the $FeSO_4$ solution, the plant extract (50 mL) and sodium hydroxide pellets (1 g) were combined. After centrifuging the mixture, the supernatant was discarded. The pellet was dried and saved for later research.

2.3 Production of Iron oxide nanoparticles

In this reaction, the precursor used was ferrous sulphate. 50 mL of leaf filtrate was taken and made up to 250 mL with distilled water. In 50 mL of $FeSO_4$ solution, plant extract and sodium hydroxide pellets were mixed. After that, the mixture obtained was centrifuged and the supernatant was discarded. The brownish black colour pellet was obtained which was dried and stored for further studies.[16]

2.4 Characterization of Iron nanoparticles

The synthesis of Iron nanoparticles was confirmed by using UV-Vis spectrophotometer. The peak was obtained at 430 nm. SEM was performed at BSIP (Birbal Sahni Institute of Palaeosciences) laboratory, Lucknow to check the size, shape and morphology of the nanoparticles. The size of the nanoparticles was found to be 90 nm which proved it to be a nanoparticle and the shape of the nanoparticle was found to be hexagonal.

2.5 Antimicrobial activity of the synthesized nanoparticles

Using the disc diffusion method, the antibacterial potency of FeONPs produced from A. paniculata was evaluated. Gram positive (*S. aureus*) and Gram negative (*K. pneumoniae, E. coli, and P. aeruginosa*) bacteria were cultivated overnight in nutrient broth at 37 °C for the experiment. The coated nanoparticles were added to a swab that had been filled with bacterial inoculum. To determine the bactericidal effectiveness of FeONPs, the zone of inhibition was calculated after a 24-hour incubation period.

3 Results and Discussion

3.1 Raw material collection

The main raw material used for this project was dry leaves of *Andrographis Paniculata (Kalmegh)* which were purchased online, shown in (Fig.3.1)

Fig.3.1 Andrographis Paniculata (Kalmegh)

3.2 Leaf extract preparation

For the leaf extract preparation, we have taken 10 grams of leaves which were boiled in 100 ml of water at 50 degrees Celsius after which the filtrate was extracted from the solution as shown in (Fig.3.2 (a) and (b))

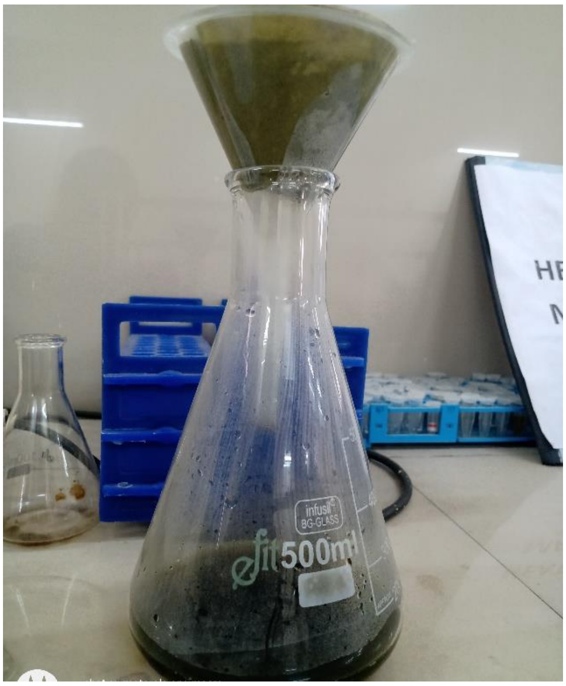


Fig.3.2(a) Filtration process

Fig.3.2(b) Filtrate obtained

3.3 Formation of pellet

The pellet was the main source of synthesizing nanoparticles which contained leaf extract. For the formation of pellet, 250 mL of distilled water was added to 50 mL of leaf filtrate after which, in the 50 mL of the FeSO4 solution, the plant extract (50 mL) and sodium hydroxide pellets (1g) were mixed and centrifuged. The supernatant was discarded and pellet was separated.

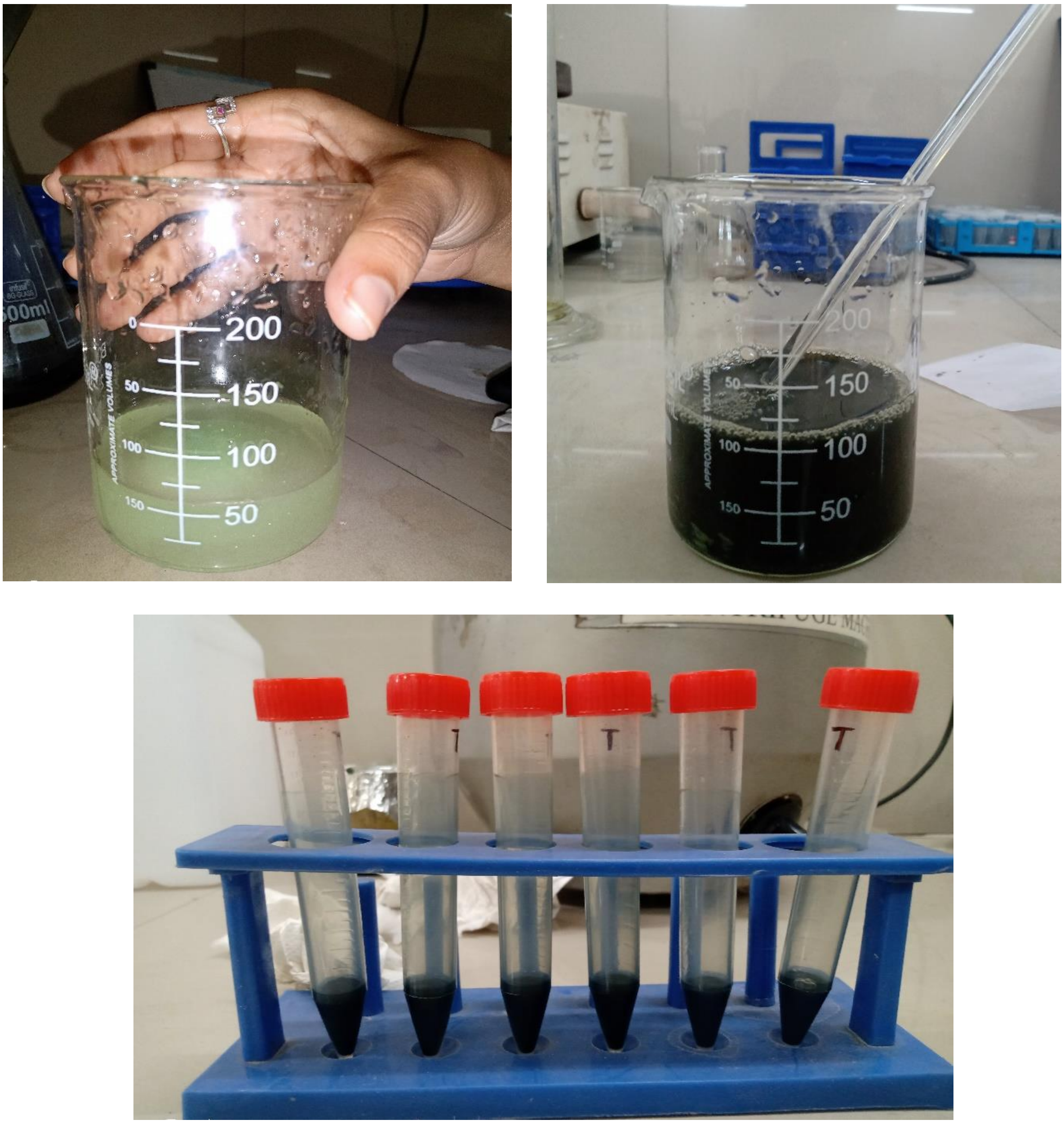


Fig3.3 Results after the centrifugation

3.4 Nanoparticles synthesis

For the synthesis of nanoparticles, pellet was spread uniformly on a glass plate and kept in hot air oven for 30 mins at 80 degrees Celsius and the dried nanoparticles were scratched the next day.

Fig.3.4(a) Pellet spreading

Fig.3.4(b) Nanoparticles

3.5 Characterization of nanoparticles

The characterization of nanoparticles was done using UV-Vis Spectrophotometry which gave peak at 430 nm, confirming it to be an iron nanoparticle.

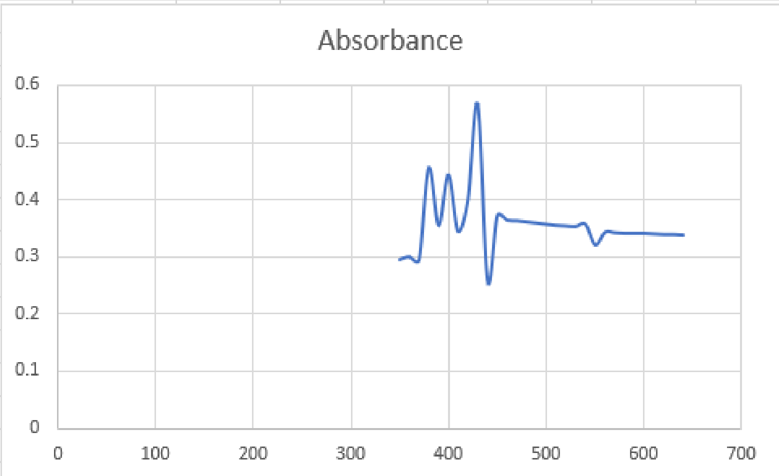
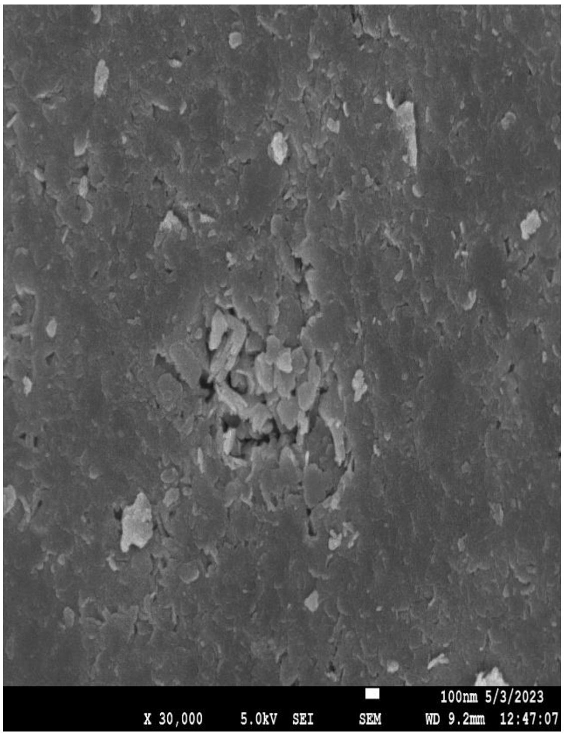


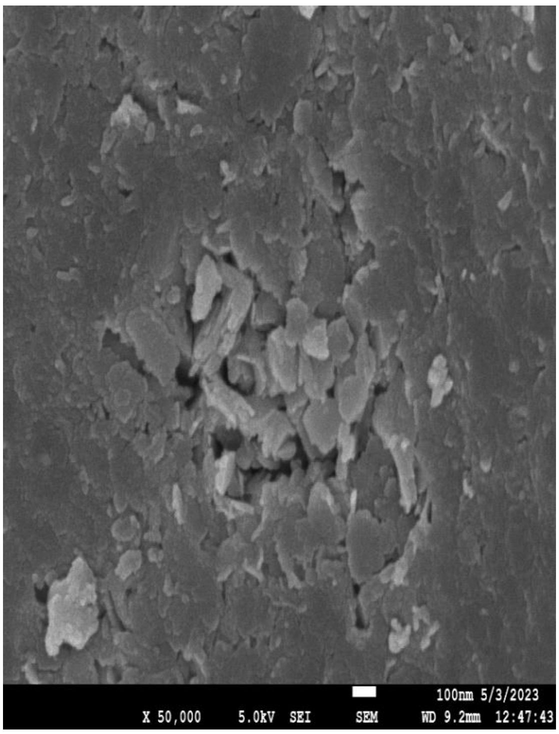
Fig 3.5 Graph of absorbance and wavelength showing peak at 430 nm

3.6 SEM analysis

SEM was performed for iron nanoparticles to check its size, shape and morphology. It was found that the size of iron nanoparticle resulted to be 90 nm and the shape was found to be hexagonal.

3.6(a) Scanning Electron Microscope

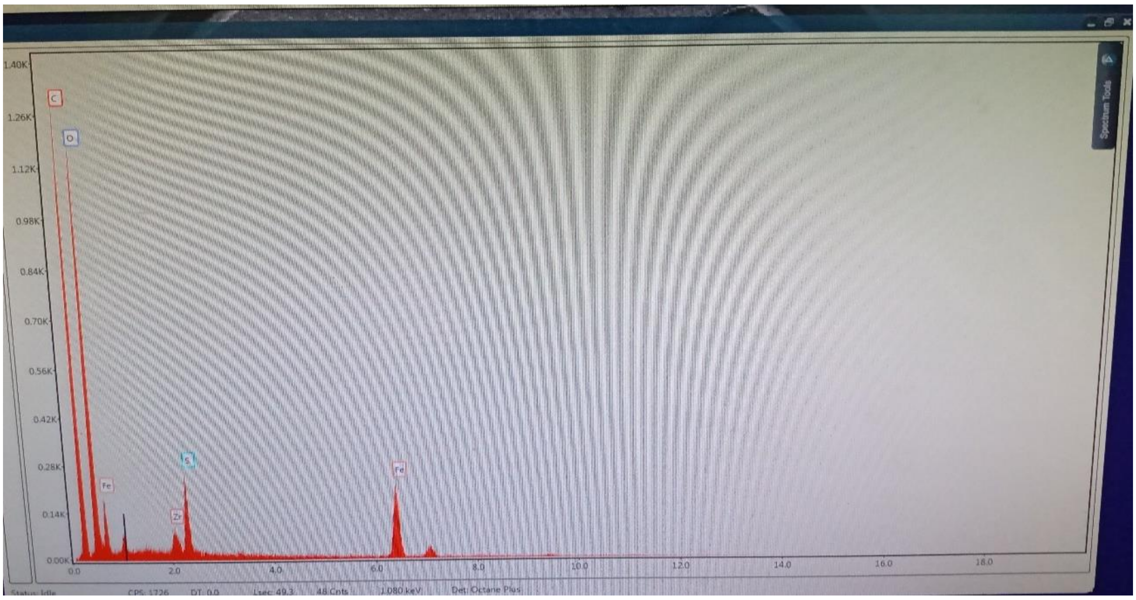




3.6(b) SEM images

<u>Graphical representation of SEM-</u>

The graphs below show the peaks of Fe which confirms the presence of Iron nanoparticles.



3.6 (c) Graphs representing peaks of Fe

3.7 Antimicrobial activity assay

The antimicrobial efficacy of the iron nanoparticles was tested using different bacterial strains including *E. coli, S. aureus, K. pneumoniae and P. aeruginosa*. The results of the same are as follows-

Fig 3.7(a)Zone of inhibition in *E. coli*

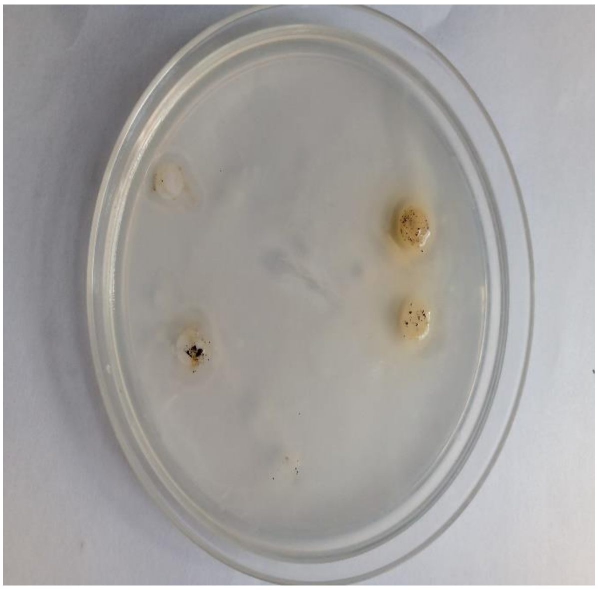


Fig 3.7(b)Zone of inhibition in *S. aureus*

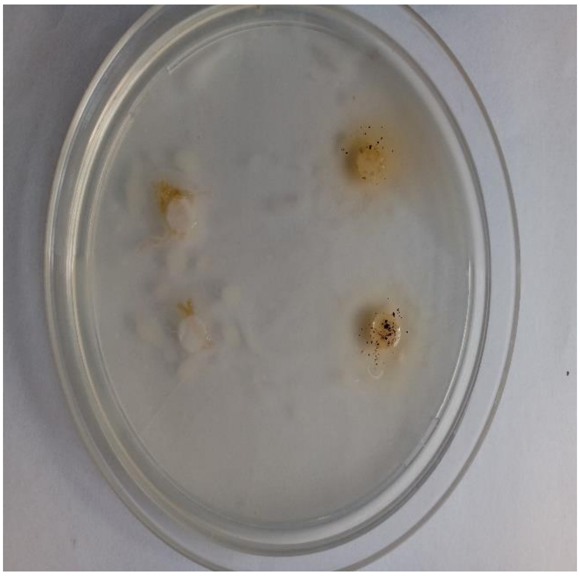


Fig 3.7(c)Zone of inhibition in *K. pneumoniae*

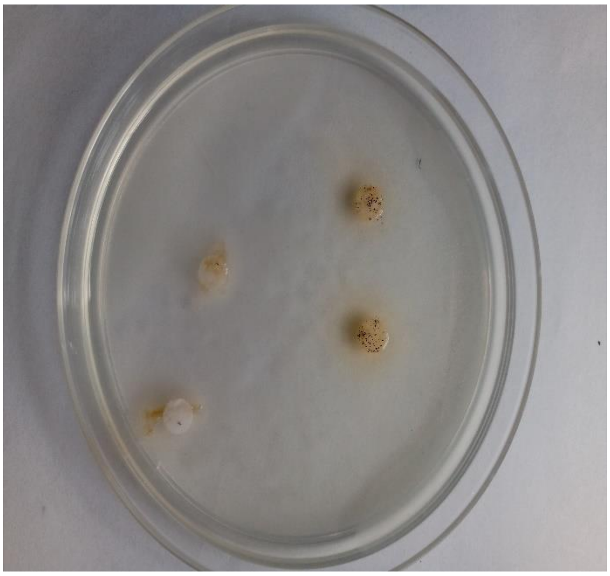


Fig 3.7(d)Zone of inhibition in *P. aeruginosa*

4 Conclusion

Nanoparticle technologies have brought about significant changes which has helped in converting poorly soluble, poorly absorbed and labile biologically active substance into useful assets. Using copper, zinc, titanium, magnesium, gold, alginate and silver, different nanomaterials are being produced. Apart from that, nanomedicine has huge possibility for the improvement in the treatment of human diseases. The development of these eco-friendly methods for the synthesis of nanoparticles is evolving into an important branch of nanotechnology especially silver nanoparticles, which have many applications. The cost-effective and non-toxic method of synthesizing nanoparticle has emerged advantageous to the researchers as well as to the environment. Further researches are going on for the synthesis of Iron nanoparticles and it will prove to be of various advantages in future because of its less cost and easy availability as compared to others. It might be better than other nanoparticles in terms of stability, high antimicrobial activity, high antioxidant activity, etc.

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