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Antifungal Activity Of *Pongamia Pinnata* Leaf Extract Mediated Green Synthesis Of Iron oxide Nanoparticles

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ABSTRACT

In present study, Iron oxide nanoparticles were synthesized using *Pongamia pinnata* leaves and characterised by UV–visible spectroscopy,FTIR,X-ray Diffraction and SEM.The synthesized nanoparticles were spherical in shape with an average particle size of 500 nm and confirmed by Scanning electronic microscopy (SEM).The FT-IR spectrum revealed the functional groups of the phytoconstituents present in *Pongamia pinnata* leaves. Green synthesized iron oxide nanoparticles showed antifungal activityagainst *Candia albicans,Aspergillus flavus,Aspergillus niger* .The results support the advantages of using simple and cost effective bio-green method for synthesizing iron oxide nanoparticles with anti fungal activities.

Keywords*: Pongamia pinnata*; Iron oxide nanoparticle,UV- Visible Spectrophotometry, FTIR, ,SEM.

INTRODUCTION

Nanotechnology is a modern field of science which plays a dominant role in day to day life aspects which deals with production, manipulation and by the use of material ranging in nanometres[1].Nanoparticles are traditionally synthesized by wet chemical techniques, but nowadays number of approaches are available for the synthesis of iron oxide nanoparticles especially by green biosynthesis. 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..

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine. This plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care[2]. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy.[3].

Plant products and their active constituents played an important role in plant disease control by combating growth and development of pathogens and including resistance in plants. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, pods etc.[4]
Pongamia pinnata is a medium-sized, evergreen, perennial and deciduous tree. It is also known as Karanj. *Pongamia pinnata* is a native of India, Myanmar etc., Plants have been widely used as curative agents for variety of oilments.

MATERIALS AND METHOD

Plant collection

Pongamia pinnata leaves used for study was collected from Thiruvallur district, Chennai, Tamil nadu.

Preparation of plant extracts

The fresh leaves of *Pongamia pinnata* were thoroughly washed by normal distilled water to remove impurities. The cleaned leaves were dried under sunshade to remove moisture completely and they were made into powder by using mechanical grinder and then stored for further use.

Solvent extraction method

10 grams of air dried powder of *Pongamia pinnata* leaf was extracted with 100ml of solvents such as ethanol and distilled water by method of maceration.The sample was kept in dark room for overnight incubation without shaking. After the incubation period the prepared solution was initially filtered through filter paper and was again filtered through Whatmann No.1filter paper to get clear solution.

Phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the various alcoholic and aqueous extracts of leaves of *Pongamia pinnata* . [5]

TANNINS

1 ml of sample was taken, to that few drops of 0.1% ferric chloride was added and observed for blue/black colorization/brownish green.

SAPONINS:

1 ml of sample was taken, to that 2 ml of H_2O (shaken vigorously) was added and observed for foaming appearance.

FLAVONOIDS

1 ml of sample was taken, to that concentrated HCL and magnesium chloride was added and observed for pink tomato red colour.

ALKALOIDS

To 1 ml of sample few drops of dragandoff reagent was added and observed for orange red colour.

PROTEINS

1 ml of sample was taken, to that few drops of Bradford reagent aws added and observed for blue colour development.

STEROIDS

To 1 ml of sample 10% concentrated H_2SO_4 was added and observed for green colour.

QUINONES

1 ml of sample was taken, to that aqueous ammonia (shaking) was added and observed for change in colour of aqueous layer (pink, red or violet).

TERPENOIDS

1 ml of sample was taken, chloroform to that concentrated H_2SO_4 was added and observed for reddish brown ring colour.

CARDIO GLYCOSIDES

1 ml extract and glacial acetic acid 0.4 ml and ferric chloride solution and conc H2SO4 – brown ring colour.

PHENOL

To 1 ml of sample few drops of ethanol and 0.1% ferric chloride was added and observed for blue/black colorization/brownish green.

Green synthesis of Iron oxide nanoparticles

For the synthesis of iron oxide nanoparticles about 30ml of leaf extract of *Pongamia pinnata* was added to 1mM FeSO4.7H2O Solution in the ratio of 1:5proporations. After the addition of aqueous extract to the salt solution,the colour changes were observed.The reaction was monitored using different concentration of ferrous sulphate of 250mM using leaf extract and the absorbance were measured.

Characterization of Iron oxide nanoparticles

Ultra viloet visible spectroscopy

The Surface Plasmon resonances (SPR) of synthesised iron oxide nanoparticles have been studied by UV – Visible formation of iron oxide nanoparticles. After the addition of *Pongamia pinnata* leaf extract into the aqueous solution of Fe_2SO4,the solution was transferred into glass curvette and was monitored by measuring the reaction in the wavelength ranges from between 300-800nm and DI Water was as an blank and subsequently analysed at room temperature.

FTIR analysis of synthesized Iron oxide nanoparticles:

Fourier Transform Infrared spectroscopy was used to test the physiochemical interaction between leaf extract of *Pongamia pinnata* and iron oxide nanoparticles and also identify the functional groups of the active components based on the peak value in the region of infrared radiation. The infrared spectra are recorded in the mid–infrared region (MIR) within the range (4000-500 cm-1) for reduction and capping of the nanoparticles which is present in the *Pongamia pinnata* and grounded with KBr method to form a pellet.

SEM analysis of synthesized iron oxide nanoparticles:

Morphological studies were performed using field emission scanning electron microscopy (FE-SEM). The FE-SEM micrographs were obtained using a JEOL 6335F FE-SEM microscope equipped with a Thermo Noran energy dispersive spectroscopy (EDS) detector. The samples were spread in the middle of carbon tape and pressure was applied in butter sheet and blown to remove free particles. The sample was kept in vacuum chamber for 1hr and were loaded in SEM instrument and the sample were analyzed.

Antifungal activity

Test microorganism

The Fungal culture used for in vitro antifungal activity are *Candia albicans*, *Aspergillus flavus* ,*Aspergillus niger*

Preparation of inoculum

Stock cultures were maintained at 4°C on slant of potato dextrose agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of potato dextrose broth for fungal that were incubated at 24hrs at 37ºc, and the assay was performed by agar well diffusion method.

Antifungal activity:

Antifungal activity of sample was determined by well diffusion method on potato dextrose agar (PDA) medium. The potato dextrose agar medium was weighed and dissolved in 100ml of distilled water with 1gm of agar. Then the medium is kept for sterilization. After sterilization the media was poured in to sterile petriplates and were allowed to solidify for 1hr. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. Wells were made and subsequently samples (150µl,200µl/wells) were introduced to the wells of inoculated agar plates. DMSO was served as negative control and amphotericin (30 mg/ml)was used as a positive control and then plates were incubated for 24 hrs at 37ºc .Then the microbial growth was determined by measuring the diameter of zone of inhibition.

RESULTS AND DISCUSSION

Collection of study plant material

The leaves of *Pongamia pinnata* was used to study the medicinal and economical values.(Fig1) This plant source has been used as an curative agents. The leaves extract were used for cold,cough, diarrhea,leprosy and inflammations of piles and wounds etc.

Fig.1 *Pongamia pinnata* leaves

Solvent extraction method

The leaves and pods were subjected to solvent extraction using Ethanol and Distilled water. The samples added to 10% ethanol and distilled water. The Colour changes were observed.

Phytochemical analysis

The present study revealed that the various alcoholic and aqueous extracts of leaves of *Pongamia pinnata* contained Tanins, saponin, flavonoids, alkaloids, cardiac glycosides, phenols, steroid , Quinines, terpenoids and protein.In leaf distilled water, the positive result were detected in tannins, saponin, alkaloids, quinines, terpenoids, cardio glycosides, phenol and negative for flavonids, protein, steroids. For ethanolic extract the positive result to be detected in tannins, saponin, steroid, quinines and negative for flavonids, protein, alkaloids, terpenoids, cardio glycosides, phenols.

Table 1. The Phytochemical constituents of Distilled water extract and ethanol Extract of leaves of *Pongamia pinnata*

S.No	Phytochemicals	Distilled Water Extract	Ethanol Extract
1.	Tanins	+	+
2.	Saponin	+	+
3.	Flavonoids	-	-
4.	Alkaloids	+	-
5.	Proteins	-	-
6.	Steroid	-	+
7.	Quinines	+	+
8.	Terpenoid	+	-
9.	Cardio glycosides	+	-
10.	Phenols	+	-

The therapeutic value of plants depends on the presence of phytochemicals and The similar experiental results indicates the presence and absence tannins, steroids, flavonids, phenols, terpinoids, saponins [6] on the ethanol extract of *Pongamia pinnata.*

Green synthesis of Ironoxide nanoparticles

The ironoxide nanoparticles were synthesized using various concentration of ferrous sulphate solution from 25mM, 50mM, 100mM, 150mM, 200mM, 250Mm. Reaction was visible in 250Mm as a colour change from light Brown to Black were seen,within 24 hrs of the addition of reaction mixtur [7] The result showed the color changed from colorless to black on the aqueous leaf extract of *Ocimum sanctum* .

UV-Visible spectroscopic analysis

Absorption spectra of iron oxide nanoparticles formed in the reaction media has absorbance maximum at 310nm.Figure 2 indicates the presence of iron oxide nanoparticles in between the range of 300- 800nm.[8].

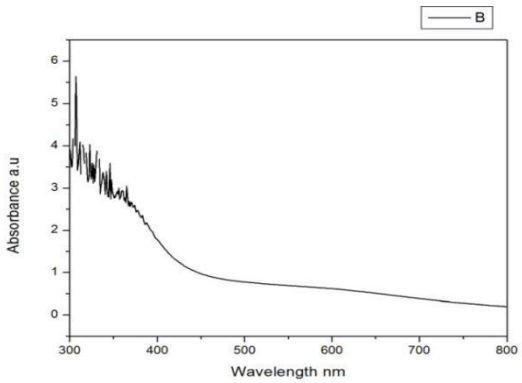

Figure 2: UV-Visible absorption spectra of Iron oxide nanoparticles

FTIR Analysis

FT-IR spectroscopy was performed to determine the possible biomolecules of *Pongamia pinnata which* act as a stabilizer and capping agent in the synthesis of FeSO4–NPs. The spectra of the P*ongamia pinnata* extract revealed strong magnetite absorption bands at 3853.77 cm^{-1}, 3844.13 $cm^{-1,}$ 2953.02 $cm^{-1,}$ 2922.16cm^{-1}, 2852.72 cm^{-1},2108.20 cm^{-1}shown in (Figure 3)form H-C-H Stretching and bending vibrations of phenol group, and the FTIR spectrum of plant extract with iron oxide nanoparticles also shows H-C-H

Stretching vibration at 2953.02cm^{-1}, 2922.16cm^{-1}, 2852.7295 cm^{-1}, and H-C-H bending vibration is seen at 1375.25 and 1076.28 cm^{-1},.725.23cm$^{-1.}$ [9].

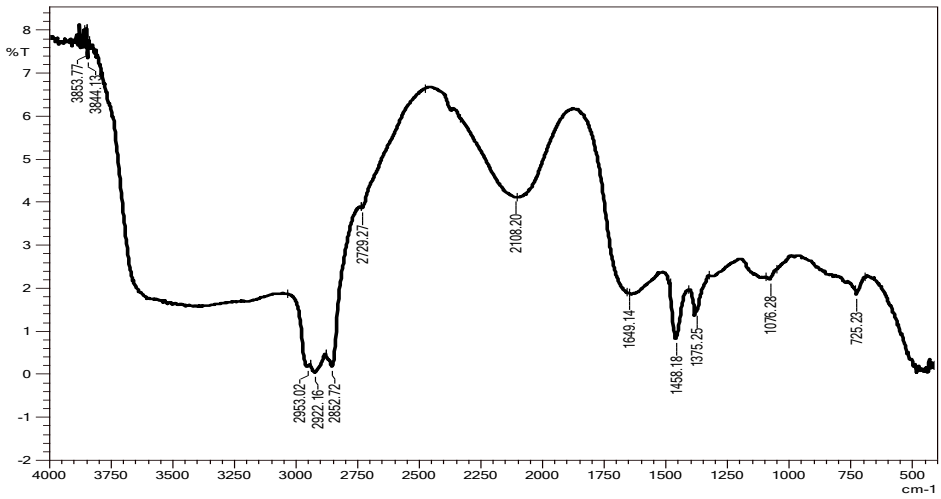

Figure 3:FTIR spectrum of Iron oxide Nanoparticles

Scanning Electron Microscopy analysis

The structure and morphology of the synthesized iron oxide nanoparticles was analysed using SEM at different magnification levels including 20µm and 40 µm,80 µm,160µm.SEM images revealed that the synthesized iron oxide nanoparticles were aggregated as Spherical in shapes with rough surfaces. The morphology of the nanoparticles mostly appeared to be a porous and spongy.[10].Scanning electron microscopy result shows that the particles size of sample was approximately 500nm were observed (Figure 4)

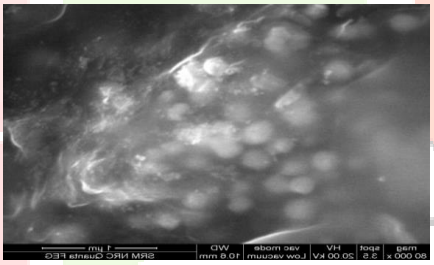

Figure 4:Scanning Electron Microscopic image of iron oxide nanoparticle

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Antifungal activity

The antibacterial assay of iron oxide nanoparticles was done against the fungal pathogens. Synthesised Iron oxide nanoparticles of *Pongamia pinnata* showed inhibition zone against different fungal strains(Figure 5). Iron oxide nanoparticle showed the maximum zone of inhibition in *Aspergillus Flavus (0.7mm,0.4) and Aspergillus niger (0.4mm,0.4mm)* and minimum zone was observed by *Candida albicans (0.6mm,0.3mm).* the similar result for *candida albicans* in leaf extract *Pongamia pinnata* against human pathogens shows its least activity in 0.4mm[11]for Aspergillus flavus *and* minimum inhibition zone was observed in stem (13.6 ± 0.82 mm) ,(14.6 ± 0.39 mm)

Aspergillus niger

Candida albicans

Aspergillus flavus

Figure 5:Antifungal activity of Iron oxide nanoparticles

a - Dimethyl Sulfoxide (Negative control) b - Amphotericin (Positive control)
c – crude extract d- Iron oxide nanoparticles extract

Table 3: Antifungal activity of Iron oxide Nanoparticles

Test sample	***Candida albicans (mm)***	***Aspergillus flavus (mm)***	***Aspergillus niger (mm)***
Amphotericin	0.3	0.6	0.5
Crude	0.6	0.7	0.4
Iron oxide Nanoparticles	0.3	0.4	0.4

Conclusion

The current study was done to evaluate the antifungal activity in leaves of *Pongamia pinnata* using ironoxide nanoparticles. The green synthesis and characterization of iron oxide nanoparticles using leaf extract of *Pongamia pinnata* were performed and confirmed by spectroscopic and microscopic techniques. This synthesis method is uncomplicated, environmentally friendly, non- toxic and low cost due to the availability of the source of reducing agent leaves of *Pongamia pinnata*.

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