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**PEEL OF PUNICA GRANATUM
(POMEGRANATE) EXTRACT FOR THE
GREEN SYNTHESIS OF IRON
NANOPARTICLES AND ITS
ANTIMICROBIAL ACTIVITY****¹K. N. PORCHELVI *, ²R.SNEHA**¹Assistant Professor, ²Student

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Abstract: . In the present work, we tried to get extract from the peel of Punica granatum fruit. Formation of FeNPs was confirmed by UV-Visible spectroscopic absorption and Infra-red absorption spectrometry. The morphology of FeNPs was confirmed by SEM. The formation of FeNPs using peel of Punica granatum extract was unique work reported so far. Atomic silver was reduced with aqueous solution of peel of Punica granatum extract. Phytochemical test analysis of the peel of Punica granatum extract was done for the presence of natural products which imparts unique properties to the extract synthesised. Antimicrobial studies and antifungal studies confirmed its potentiality to act against the microbes and fungus.

Index terms: Green synthesis, Iron nanoparticles, peel of Punica granatum extract, characterization techniques, Antimicrobial studies and antifungal studies

I. INTRODUCTION

Nanoparticles are now being used in the manufacture of scratchproof eyeglasses, crack-resistant paints, anti-graffiti coatings for walls, transparent sunscreens, stain-repellent fabrics, self-cleaning windows and ceramic coatings for solar cells [1,2,3]. Iron nanoparticles are significant and employed for the remediation of organic and inorganic pollutants in polluted water, soil and sediments. With in-depth research, various modified iron nanoparticles have been prepared to further overcome the original defects of aggregation or oxidation, and improve the reaction efficiency.

Medical applications were vast and listed as below. As contrast agents for Magnetic Resonance Imaging (MRI) , As therapeutic agents for hyperthermia based cancer treatments, As a primary colorant in glass and ceramics and as a catalyst and magnetic data storage and resonance imaging (MRI).

1.1 ANTIMICROBIAL STUDIES

Infections and diseases may be caused by different types of organisms like bacteria, fungi, and viruses, etc., in humans and animals. The drug used to prevent the pathogenicity of microorganisms is called an **antimicrobial agent**.

Antibiotics, antiseptics, and disinfectants were also working the same way.

Antimicrobial agents are used to preventing infections and diseases caused by pathogens. Different types of antimicrobial drugs are commonly available. Antibacterial drug Zithromax and Antifungal drug Miconazole were highly used. We attempted to explore the potentiality of iron nanoparticles in the present work.



Fig.1. picture of peel of *Punica granatum* extract.

II. MATERIALS AND METHODS

Silver nanoparticles were synthesized according to the chemical reduction method by using moringa oleifera seed extract. This method can easily be performed in any chemical laboratory and economical, thus a cheaper method when compared with other methods of synthesizing silver nanoparticles. [4,5]



Fig.2. .Picture of iron sulphate, peel of *Punica granatum* extract colour of the formation of FeNPs.

III. EXPERIMENTAL SECTION

3.1 PREPARATION OF IRON SOLUTION

About 0.1519g of Ferrous Sulphate (FeSO_4) from our laboratory was taken and weighed accurately. It is made upto 250ml in a 250ml standard flask using distilled water to obtain 0.01N FeSO_4 .

3.2 PREPARATION OF PEEL OF PUNICA GRANATUM EXTRACT

Fresh Punica granatum have been collected from the shop. It was washed well and the Peel of Punica granatum is taken separately. It is well cleaned with ordinary water and rinsed thoroughly with distilled water. It is then dried. Exactly 5g of the peel is weighed in analytical balance. It is crushed well using Morter and Pestle and transferred in a 250ml beaker. About 50ml of distilled water is added to it and boiled for 10mins till the extract is obtained. The extract is then filtered through Whatmann 40 Filter Paper. Then it is collected in a well cleaned beaker and stored for future reactions. This filtrate is used as the reducing agent to reduce the Iron.

3.3 GREEN SYNTHESIS OF IRON NANOPARTICLES

For the synthesis of Iron Nanoparticles, about 5ml of freshly prepared 0.01N FeSO_4 is taken in a cleaned 100ml beaker. Then 3ml of Punica granatum extract is measured using the measuring jar and poured into the beaker containing Iron solution. While adding the extract we observed the change in the colour of the solution indicating the reaction. Yellow colour of Ferrous Sulphate when it get reacts with the Punica granatum extract, the colour changes to Black colour. The reaction will be completed within a fraction of second. The colour change indicates the formation of Iron Nanoparticles.[6,7].



Fig.3. Colour of the formation of FeNPs

IV. RESULT AND DISCUSSION

4.1 ULTRAVIOLET VISIBLE SPECTRUM

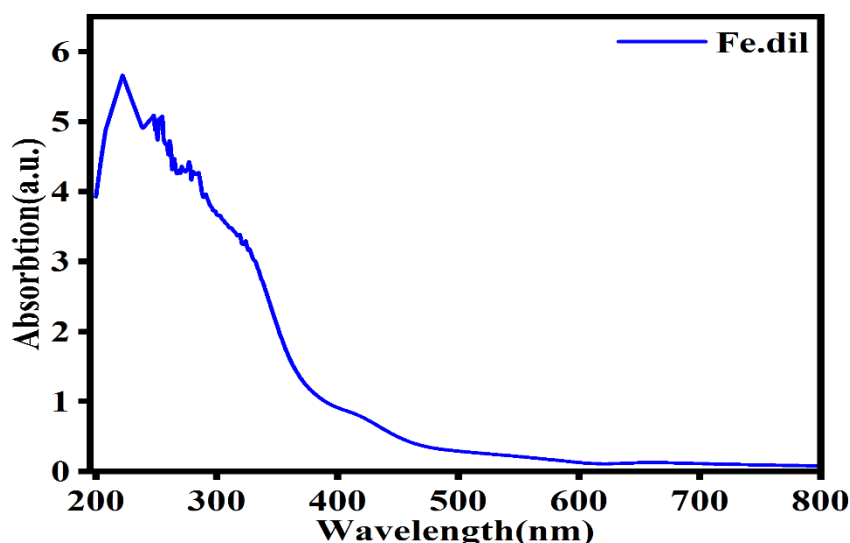


Fig. 4. Ultra Violet Visible spectrum of the FeNPs formed.

In Iron Nanoparticles the test undergone by UV Visible spectroscopy, we observe the peak at 200 to 250 nm. We can find the approximation value of the Iron Nanoparticles by the taken sample. This peak confirms the formation of Iron Nanoparticle (FeNPs) by UV-Visible Spectrum. [8,9]

4.2 INFRARED SPECTRUM

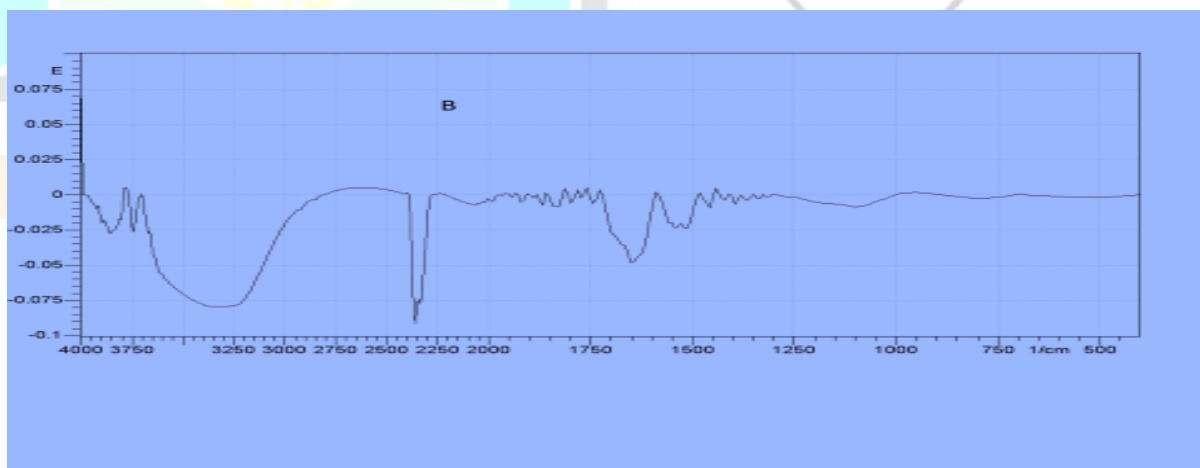


Fig. 5. Infra red spectrum of the FeNPs formed.

In Iron Nanoparticles the test undergone by Infrared Spectroscopy, we observe peak between 3750 – 4000 cm⁻¹, 3000 – 3250 cm⁻¹, 2000 – 2500 cm⁻¹, 1500 – 1750 cm⁻¹, 1250 – 1500 cm⁻¹. We can find the exact Functional group which is present in Iron Nanoparticles by using this sample. The FTIR spectrum of Iron Nanoparticles obtained by thermal decomposition process. This analysis was used to determine the functional organic groups in the surface of the nanoparticles generated by oleic acid.

Two bands between 3750 – 4000 cm⁻¹ can be seen, and they are attributed to stretching of OH amide group and terminal groups which correspond to oleic acid. Next Two bands between 3000 - 3250 cm⁻¹ can be seen and they are attributed to C-H group. Then the 2000 – 2500 resembles the C-H₂ of stretching symmetric group. The band at 1500 – 1750 cm⁻¹ represents the –C-H group. And the band at 1250 - 1500 cm⁻¹ represents the presence of =C-H group. [10.11]

4.3. PHYTOCHEMICAL CONSTITUENTS OF THE EXTRACT

Various test with the extract solution is done as mentioned in the table. The presence of Flavanoids, Alkaloids, Tannins, Saponins and Steroid was confirmed. The ability of the extract solution may be due to the presence of this components to synthesise the Iron Nanoparticles. The photographs of the test tube and its colour are shown [11]

(i). Shinoda's test

2ml of the extract was dissolved in 5ml of ethanol and to this 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicates the presence of **Flavanoids**.

(ii). Molisch's test

2ml of extract was shaken with 10ml of water, filtered and the filtrate was concentrated. To this 2 drops of freshly prepared 20% alcoholic solution of alpha-naphthol was added. 2ml of conc. sulphuric acid was added so as to form a layer below the mixture. Red-violet ring indicates the presence of **Carbohydrates**.

(iii). Tannins

To 1-2ml of the extract, few drops of 5% FeCl_3 solution was added. Presence of brown colour indicates the presence of **Tannins**.

(iv). Salkowski reaction

2ml of dry extract was shaken well with Chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of the test tubes. Formation of red colour indicates the presence of **Steroids**.

(v). Saponins

In a test tube containing 5ml of the extract, a drop of the sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3minutes. Formation of honeycomb like froth indicates the presence of **Saponins**.



Fig.6. Images of the phytochemical tests of the extract.

The Flavonoid present in extract of Peel of *Punica granatum* may be responsible for the reduction of Iron atom to Nanoparticles. This result is obtained from the studies of the Phytochemical tests.

4.4. SCANNING ELECTRON MICROSCOPE

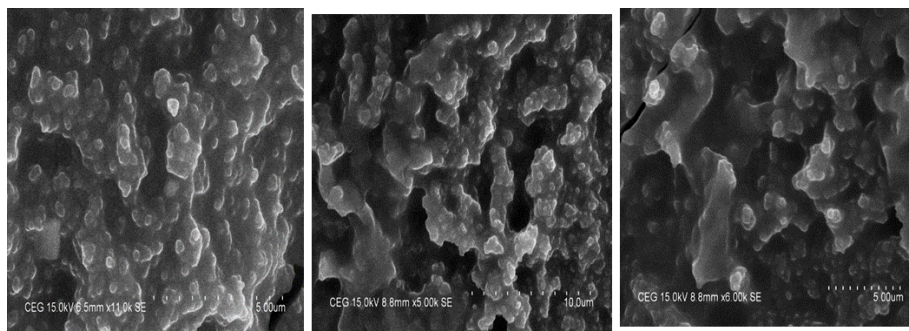


Fig. 7. Scanning Electron Microscope Images

SEM picture shows the presence of spherical shaped iron nanoparticles present and agglomeration occurred.[12,13]

4.5. ANTIMICROBIAL STUDIES

4.5.1. ANTIBACTERIAL ACTIVITY

Sample test was carried out in a 96 well Plates under aseptic conditions. A sterile 96 well plate was labeled. A volume of 100 µl of sample was pipetted into the first well of the plate. To all other wells 50 µl of nutrient broth was added and serially diluted it. To each well 10 µl of resazurin indicator solution was added. 10 µl of bacterial suspension was added to each well. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37 °C for 18–24 h. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.[14,15]

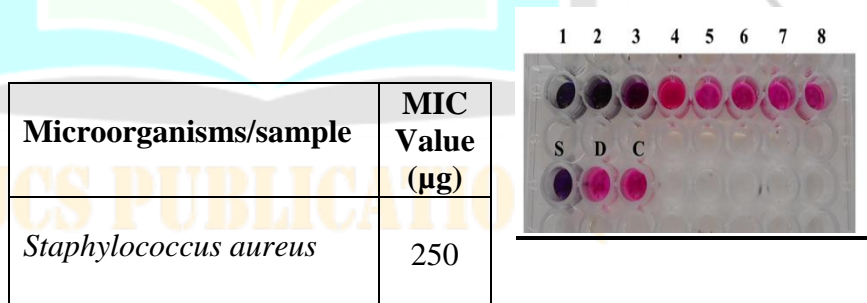


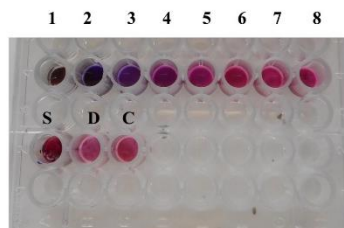
Fig.8. photo picture of antimicrobial studies.

Table 1. Antibacterial studies data

S.No	Growth of inhibition									STD Ketocanzole 10µg	DMSO	Culture
	1000 µl	500 µl	250 µl	125 µl	62.5 µl	31.2 µl	15.6 µl	7.8 µl				
1	-	-	-	+	+	+	+	+		-	+	+

From the above data, the synthesized nanoparticle Iron have the ability to act as an Antibacterial agent. The MIC value Minimum Inhibitor Concentration of Iron Nanoparticles in the gram positive bacteria *Staphylococcus aureus* is 250 μ g

Microorganisms/sample	MIC Value (μ l)
<i>Candida albicans</i>	125



4.5.1. ANTIFUNGAL ACTIVITY

	1000 μ l	500 μ l	250 μ l	125 μ l	62.5 μ l	31.2 μ l	15.6 μ l	7.8 μ l	STD Ketocanazole 10 μ g	DMSO	Culture
<i>Candida albicans</i>	-	-	-	-	+	+	+	+	-	+	+

From the above data, the synthesized nanoparticle Iron have the ability to act as an Antifungal agent. The MIC value Minimum Inhibitor Concentration of Iron Nanoparticles in the Fungal *Candida albicans* is 125 μ g.[16,17]

V. CONCLUSION

We tried the green synthesis of Iron Nanoparticles(FeNPs) using Peel extract of *Punica granatum*. The green synthesis of Iron Nanoparticles using peel extract of *punica granatum* provides an eco-friendly, cost effective and simple route for synthesis of FeNPs nanoparticles. The method of synthesis is found to be efficient in terms of reaction time as well as stability of synthesized FeNPs. The synthesized iron nanoparticles were characterized using UV, FTIR, SEM and its Phytochemical constituents of the extract. The Antimicrobial activities and the antifungal studies confirmed its efficiency.

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