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Green Synthesis of Iron Nanoparticles using Leucas Aspera: Evaluation of Antioxidant, Anti-Inflammatory, and Uv-Protective Properties for Biomedical Applications

S.Suguna[1], Nathiya.A[2], Varsha.S.T[3], Vinothini.V[4], Jayasree.R[5]

[1]*Assistant Professor,* [2,3,4,5]*PG Student, Department of Chemistry, Dhanalakshmi Srinivasan College of Arts and Science for Womens (autonomous) perambalur.621212*

***Abstract*-- Nanotechnology continues to revolutionize the biomedical field, with green synthesis approaches gaining prominence due to their sustainability and biocompatibility. This study presents the eco-friendly synthesis of iron nanoparticles (FeNPs) utilizing Leucas aspera, a medicinal plant recognized for its diverse pharmacological properties. The synthesized FeNPs were systematically characterized through UV-Visible spectroscopy, Scanning Electron Microscopy (SEM), and Fourier Transform Infrared Spectroscopy (FTIR) to elucidate their optical behavior, surface morphology, particle size distribution, and functional biomolecules involved in nanoparticle formation.**

The biosynthesized FeNPs demonstrated notable antioxidant activity, effectively neutralizing free radicals, indicating their potential in mitigating oxidative stress and related pathologies. Anti-inflammatory assays revealed significant suppression of pro-inflammatory markers, highlighting their therapeutic relevance. Furthermore, UV-absorption analysis confirmed the nanoparticles' capability to absorb ultraviolet radiation, suggesting prospective use in dermatological and cosmetic formulations.

This study underscores the potential of Leucas aspera-mediated FeNPs as a sustainable and multifunctional nanomaterial with promising applications in healthcare, particularly in antioxidant therapy, inflammation control, and photoprotection.

***Keywords*-- Leucas aspera, ,(FeNPs), Fourier Transform Infrared Spectroscopy, UV-Vis spectroscopy, Scanning Electron Microscopy (SEM).**

I. Introduction

Leucas aspera, commonly referred to as 'Thumbai', belongs to the Lamiaceae family and is widely distributed across India, ranging from the Himalayan region to the southern parts of the subcontinent, including Sri Lanka. Traditionally, this herb has been extensively utilized for its diverse medicinal applications, particularly as an insect repellent and an antipyretic agent.

The flowers of Leucas aspera are particularly valued in traditional systems of medicine for their multifaceted therapeutic properties. They are known to act as stimulants, expectorants, mild laxatives (aperients), diaphoretics, emmenagogues, and pesticides. The leaves of the plant are also considered beneficial in treating a variety of skin-related disorders, including psoriasis, chronic rheumatism, and other long-standing dermal conditions. In rural health practices, bruised leaves are topically applied as a remedy for snake bites.

As with many members of the Lamiaceae family, Leucas aspera is rich in phytochemicals, which are biologically active compounds that plants produce primarily for defense against pests, microbes, and herbivores. These phytoconstituents also contribute to the plant's medicinal value. Among the key bioactive compounds found in Leucas aspera are fatty acids, terpenoids, ursolic acid, nicotine, glucosides, beta-sitosterol, sterols, diterpenes, and a variety of phenolic substances.

This herb typically grows to a height ranging between 15 and 60 cm and is commonly seen in the plains of India. The broad pharmacological potential of Leucas aspera has made it an integral part of traditional Indian medicinal systems, including Ayurveda and Siddha. Herbal plants like Leucas aspera play a vital role in the healthcare practices of both rural and urban populations, offering natural alternatives to synthetic pharmaceuticals. Their importance is not only cultural but also scientific, as they form the foundation for many modern drugs derived from plant-based molecules.

II. Current Applications And Future Directions

Nanotechnology has emerged as a transformative field, offering wide-ranging applications across industries such as manufacturing, electronics, energy, medicine, and environmental management. Among various nanoparticle synthesis techniques, the bio-mediated combustion approach has garnered attention due to its cost-effectiveness, eco-friendliness, and non-toxic nature.

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Vector-borne diseases such as Zika virus, dengue, chikungunya, malaria, and yellow fever continue to pose significant public health challenges, with mosquitoes acting as primary vectors. One promising strategy to prevent the spread of these diseases involves the use of larvicidal agents and mosquito repellents. While chemical-based repellents have been the conventional choice, they are often associated with toxicity and environmental concerns. In contrast, natural repellents—such as those derived from Leucas aspera—offer a safer and potentially more sustainable alternative. Extracts from Leucas aspera, readily available in many local environments, particularly in rural and agricultural regions, have shown significant larvicidal activity when applied to stagnant water bodies. This positions the plant as an accessible and effective tool for mosquito control, particularly in endemic areas. Beyond vector control, Leucas aspera exhibits potent antivenom properties. With snake bites remaining a major health issue—especially among agricultural laborers—this plant offers a potential first-line remedy due to its widespread availability as a weed in cultivated fields. Research suggests that the active components of the plant could be isolated and developed into plant-based alternatives to commercial antivenoms, which are often costly and not readily available in remote areas. Furthermore, the phytotoxic characteristics of two newly identified compounds from Leucas aspera extract highlight its potential as a natural herbicide. These bioactive molecules may offer environmentally safe options for weed management in sustainable agriculture.

The rich phytochemical profile of Leucas aspera—including compounds with anticancer, antimicrobial, and anti-inflammatory properties—underscores its value in drug discovery. By integrating its bioactive constituents into molecular docking and statistical modeling studies, researchers can identify novel therapeutic leads that target specific disease pathways. The plant continues to serve as a promising candidate in the ongoing search for natural, effective agents in pharmaceutical development.

III. Materials And Methods

Materials Required

Leucas aspera dry powder, Distilled water, centrifuge tubes,. Measuring jars, Eppendorf tubes, Glass rods, Petri plates, Cotton, Conical flasks, Micropipettes and tips, Test tubes, and Sabouraud dextrose agar(SDA) are used for the research.

Chemicals

Sodium hydroxide, Sodium chloride, Copper sulphate, Ferric chloride, Mayer's reagent, Phenol, Sodium nitroprusside, Sodium hypochlorite, Thiobarbituric acid, Hydrochloric acid, Trichloroacetic acid, FRAP reagent, etc.,

Extraction Of Phytochemicals

5 g of Leucas aspera dry powder was weighed and mixed with 50ml of distilled water, mortar and pestle, and heated in a heating mantle at 60°C for 20 mins. The solution was cooled and filtered using Whatman no.1 filter paper, and the extract solution was stored in a falcon tube for further use.

Phytochemical Analysis Of Leucas Aspera

Quantitative Analysis Polyphenols

Where TPC = Total Polyphenol Content (mg/g)

c = Concentration of polyphenols [Gallic acid equivalents (GAE)/ml] V = Volume of the sample (ml) ; M = Weight of the sample (g)

Table 1:
Phenol Estimation: [(c*V)/M]

Protocol for Phenol Estimation by Folin – Ciocalteau assay					
Concentration of working standard (mg\mL)	Volume of Stock standard (in µl)	Volume of distilled water (in µl)	Sample Volume (in ml)	Volume of FC reagent (in ml)	Volume of 7.5% Na2CO3 (in ml)
0	0	1	0	0.25	1
0.005	0.0001	999	0.25	0.25	1
0.01	0.0002	999	0.25	0.25	1
0.015	0.0003	999	0.25	0.25	1
0.02	0.0004	999	0.25	0.25	1
0.025	0.0005	999	0.25	0.25	1
Make up all the reaction volume to 4 ml with distilled water					
Incubate the reaction mixtures at dark for 2 hours					
Measure the absorbance at 725 nm					

Flavonoid

Table 2.
Flavonoid Estimation

Protocol For Flavonoid Estimation By Aluminium Chloride Assay					
Concentration Of Working Standard (Mg\Ml)	Volume Of Stock Standard (In µl)	Volume Of Distilled Water (In µl)	Volume Of 0.3 M AlCl3 (In Ml)	Volume Of 0.5 M NaNo2 (In Ml)	Volume Of 1 M NaOH (In Ml)
0	0	1	0.15	0.15	1
0.2	0.004	999	0.15	0.15	1
0.4	0.008	999	0.15	0.15	1
0.6	0.012	999	0.15	0.15	1
0.8	0.016	999	0.15	0.15	1
1.0	0.02	999	0.15	0.15	1
Make Up All The Reaction Volume To 4 Ml With Distilled Water					
Incubate The Reaction Mixtures At 37°C For 5 To 8 Minutes					
Measure The Absorbance At 510 Nm					

International Journal of Recent Development in Engineering and Technology
Website: www.ijrdet.com (ISSN 2347-6435(Online) Volume 14, Issue 11, November 2025)

Reducing Sugars

Table .3
Reducing Sugar Estimation

Protocol for Reducing sugar Estimation by DNS assay			
Concentration of working standard (mg\mL)	Volume of Stock standard (in µl)	Volume of distilled water (in µl)	Volume of DNS reagent (in ml)
0	0	1	1
0.2	0.005	999	1
0.4	0.01	999	1
0.6	0.015	999	1
0.8	0.02	999	1
1.0	0.025	999	1
Boil the reaction mixture for 5 to 10 minutes			
Measure the absorbance at 540 nm			

Proteins

Table 4
Total Protein Content in Leucas aspera

Concentration of working standard(mg/ml)	Volume of stock standard (ml)	Volume distilled (ml) Of water	Volume working standard(ml) of	Volume reagent (ml) of
0	0	1	0.05	0.1
0.2	0.2	0.8	0.05	0.1
0.4	0.4	0.6	0.05	0.1
0.6	0.6	0.4	0.05	0.1
0.8	0.8	0.3	0.05	0.1
1	1	-	0.05	0.1

Total Carbohydrates

The total carbohydrate content was quantified using the Phenol – Sulphuric acid assay.

Table 5

Table 5 : Protocol for Phenol – Sulphuric Acid Assay				
Concentration of working standard (mg\mL)	Volume of Stock standard (in ml)	Volume of distilled water (in ml)	Volume of 80% Phenol (in ml)	Volume of Conc.H2SO4 (in ml)
0	0	1	0.05	1.5
0.02	0.01	0.09	0.05	1.5
0.04	0.02	0.08	0.05	1.5
0.06	0.04	0.06	0.05	1.5
0.08	0.08	0.02	0.05	1.5
0.1	0.1	-	0.05	1.5

The total carbohydrate content for the sample was estimated by the following protocol.

The concentration of glucose (in mg\ml) and the OD values at 490 nm were plotted in MS excel and the line graph was created. Based on the standard graph, the unknown concentration of the test samples was calculated.

Synthesis Of Iron Nanoparticles Requirements

The plant extract (reducing agent) and precursor (ferric chloride FeCl3) were added to the test tube. the solution was incubated for 24h at room temperature and centrifuged at 5000 rpm for 20 mins. After centrifugation, the pellet was settled at the bottom of the tubes and washed with distilled water The nanoparticles were added with ethanol in the tubes and mixed well, and the pellet was collected.

10 mg of iron nanoparticles were collected and dissolved in 1ml of Dimethyl sulfoxide (DMSO) in the Eppendorf tube. From the stock solution, 5mg/ml was prepared and used as the working standard (Stock solution – 10mg/ml).

Characterization Of Nanoparticles

The synthesized iron nanoparticles were subjected to certain instrumental analyses to determine the shape, size, structure, and particle size of the synthesized nanoparticles. The structural, chemical, and optical properties of the synthesized iron nanoparticles were determined by various analyses.

ANTI-OXIDANT ACTIVITY

Reagent preparation:

Ferric chloride stock solution – Prepare 20 mm of FeCl3 solution by adding 0.135 g of anhydrous ferric chloride in 25 ml of distilled water.

Acetate Buffer – Add 0.16 g of sodium acetate in 100 ml of 0.28 M acetic acid and maintain the pH at 3.6.

TPTZ (2,4,6-Tripyridyl-S-triazine) stock solution – Prepare 40 mm of TPTZ solution by adding 0.03 g of TPTZ in 10 ml of HCl.

FRAP Reagent:

Prepare the reagent by adding the above three components in the ratio 1:10:1 and use them for further analysis.

Control preparation:

Prepare 1 % ferrous ammonium sulphate (FAS) solution by adding 100 mg of anhydrous ferrous ammonium sulphate in 100 ml of distilled water.The reaction components were added into the microfuge tubes as per the values given in the Table 6.

Table 6

Table 6: Procedure for evaluation of anti–oxidant activity by FRAP assay							
Components	S1 (in µl)	S2 (in µl)	S3 (in µl)	S4 (in µl)	S5 (in µl)	PC (in µl)	NC (in µl)
FRAP Reagent	200	200	200	200	200	200	200
Extract/Nano particles	10	20	30	40	50	50 (1 % FAS solution)	-
Solvent	40	30	20	10	-	-	50
Incubate the tubes for 5 minutes at 25°C							

In-Vitro Anti - Inflammatory Assays Inhibition Of Protein Denaturation Assay Requirements

Egg albumin (freshly collected egg white), Phosphate Buffered Saline (PBS) with pH 7.4, 1% Aspirin (prepared with 95% ethanol), Test samples.

International Journal of Recent Development in Engineering and Technology
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After adding all components, incubate the microfuge tubes at 37℃ for 20 minutes.

Heat the tubes at 50 to 51℃ for 20 minutes after incubation.

Cool the tubes immediately after taking them out of the hot water bath and measure the absorbance at 660 nm.

The following formula is used to calculate the percentage inhibition of protein denaturation:

% Inhibition = [(ODControl - ODTest)/ODControl]*100

Where OD Control - Optical density of control

ODTest - Optical density of test sample

The bar chart is plotted for the sample concentration on the x-axis and the % inhibition on the y-axis using MS excel.

Inhibition Of Haemolysis Assay Introduction

Haemolysis results in the liberation of haemoglobin from the lysis of erythrocytes. The highly cytotoxic pro-oxidant called heme group is released due to the catabolism of haemoglobin. The cellular internalization of the haemoglobin-haemopoxin complex by CD91 cells and its catabolism produces carbon monoxide, iron, and biliverdin catalyzed by haeme oxygenase -1 (HO -1). Inflammation is one of the main reasons for the induction of haeme oxygenase - 1 enzyme. The anti-inflammatory drug should inhibit this haemolysis process.

Requirements

Fresh Blood, Phosphate Buffered Saline (pH 7.4), Test samples, 1% Aspirin solution.

PROCEDURE

Preparation of RBC Suspension

Fresh blood is collected from the healthy volunteers mixed with anticoagulant EDTA.

The blood is centrifuged at 1500 rpm for 5 minutes, and the supernatant is removed from the centrifuge tubes.

RBC pellets are washed with PBS buffer and centrifuged at 1500 rpm for 5 minutes.

The supernatant is removed from the tubes, and the washing is repeated 2-3 times.

Finally, the RBC pellets are suspended with a phosphate buffer solution with pH 7.4 and used for further experiments.

The experiment is conducted, and the following components are added as per the protocol in Table 8.

After adding all the components, the tubes are incubated in water bath at 56℃ for 30 minutes.

The tubes were centrifuged at 1500 rpm for 5 minutes after cooling.

The supernatant is taken, and the absorbance is measured at 540 nm.

The following formula measures the percentage inhibition of haemolysis assay:

% Inhibition = [(ODControl - ODTest)/ODControl]*100

Where ODControl - Optical density of control ODTest - Optical density of test sample

The bar chart is plotted for the sample concentration on the x-axis and the % inhibition on the y-axis using MS excel.

IV. Result And Discussion

Phytochemicals Screening Analysis Of Plants And Roots Extract Qualitative Analysis Of Leucas Aspera Extract

The phytochemical analysis of Leucas aspera powdered extract was conducted, and the results are tabulated as follows in. The color change observed in each qualitative analysis is shown in Figure 1

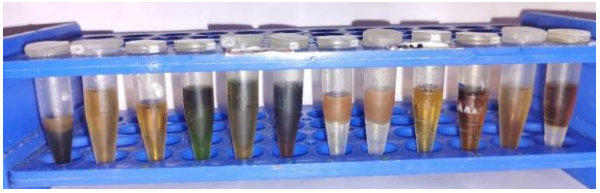

Figure 1 : Qualitative Analysis Of Leucas Aspera Powder

Quantitative Analysis of Leucas Aspera Plant Extract

Polyphenols

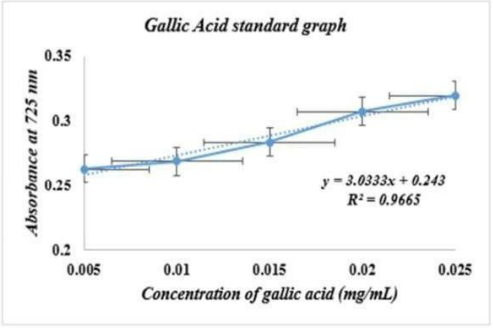

Figure 2: Standard Graph Of Gallic Acid

International Journal of Recent Development in Engineering and Technology
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From the gallic acid calibration curve, the total phenolic concentration in the plant extract was obtained as 0.61mg/ml (expressed as Gallic acid Equivalence).

Flavonoids

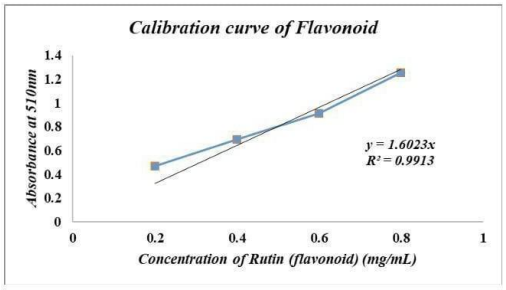

Figure 3: Standard Graph Of Rutin

From the rutin calibration curve, the unknown total flavonoid concentration in the plant extract was obtained as 0.76 mg of flavonoids(expressed using Rutin Equivalence) per mL of plant extract.

Proteins

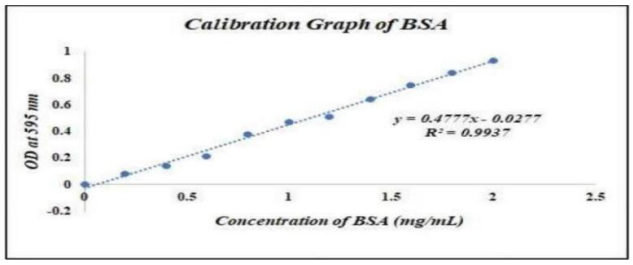

Figure 4 Standard Graph Of Bovine Serum Albumin

From the BSA calibration curve, the total protein concentration of plant extract was obtained as 0.20mg/ml

Total Carbohydrates

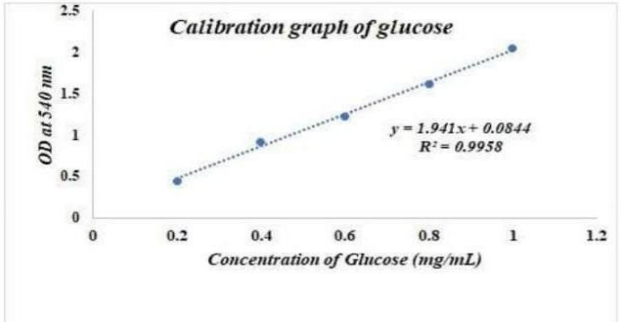

Figure 5 : Calibration Graph Of Glucose

From the glucose calibration curve, the total carbohydrate concentration of plant extract was obtained as 0.96 mg/ml

Reducing Sugars

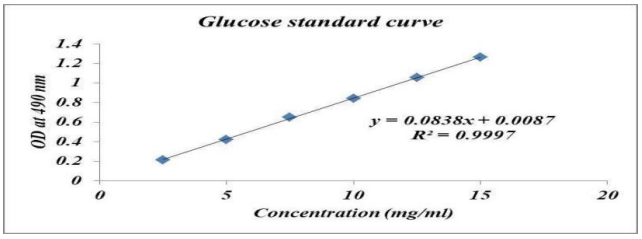

Figure 6: Standard Graph Of Glucose

From the glucose calibration curve, the total glucose concentration of plant extract was obtained as 0.04mg/ml.

The experimental findings of phytochemical screening revealed the presence of various phytochemicals in the plant extract of Leucas aspera both qualitatively and quantitatively.

Characterization Of Nanoparticles

The synthesized Iron nanoparticles were characterized to determine their optical and structural properties. The analysis results are shown below:

UV - Visible Spectroscopy

The synthesized silver nanoparticles were analyzed for the maximum absorption wavelength in UV-Spectrophotometer in the range of 200-1100nm, which was found to be 440 nm.

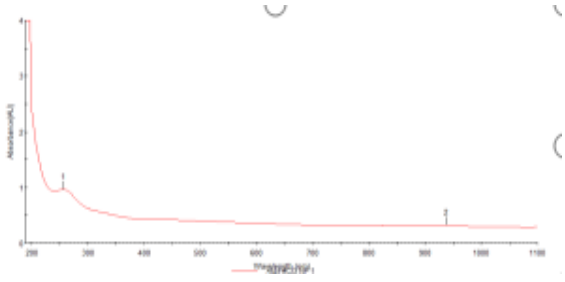

Fig 7:UV-Visible spectrum of iron nano particle

The maximum absorption wavelength (λmax) of iron nanoparticles was found to be 440 nm, which indicates the formation of silver nanoparticles. It was similar to the Surface Plasmon Resonance (SPR) peak of iron nanoparticles obtained in the 230-350 nm range

International Journal of Recent Development in Engineering and Technology
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Fourier Transform Infrared Spectroscopy

The synthesized iron nanoparticles were subjected to FTIR spectroscopy, and the chemical bonds in the nanoparticles were identified. A spectrum was obtained in the wave number range 400 - 4000 cm-1. The FTIR spectrum for the iron nanoparticles is shown in below Figure 8.

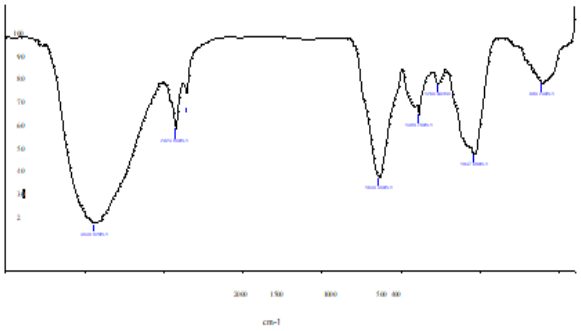

Figure 8: FTIR Spectrum Of Iron Nanoparticles

The wavenumber 3434.52 cm-1 corresponds to the O-H stretching vibrations formed by the organic ligands.The C-H stretching vibrations correspond to the wavenumber 2923.84cm-1 (Kayani et al., 2014). The N-H bond of amines corresponds to 1634.34 cm-1, whereas wavenumbers between 1384.75 cm-1 also indicate the CH3-CH bonds of alkane and alkyl groups. The wavenumber 2854.19 cm-1 corresponds to symmetric or asymmetric stretching of C-H bonds in aliphatic alkanes, whereas 1264.43 cm-1 indicates C-O stretching vibrations in esters, ethers, and carboxyl groups. Peaks at 1037.60 and 604.23 cm-1 indicates C-O-C stretching vibrations of aromatic ethers or alcohols often linked with phenols, whereas the latter indicates the formation of metal oxygen interactions and characteristic bonds of haematite phase (Naseem & Farrukh, 2015).

Scanning Electron Microscopy

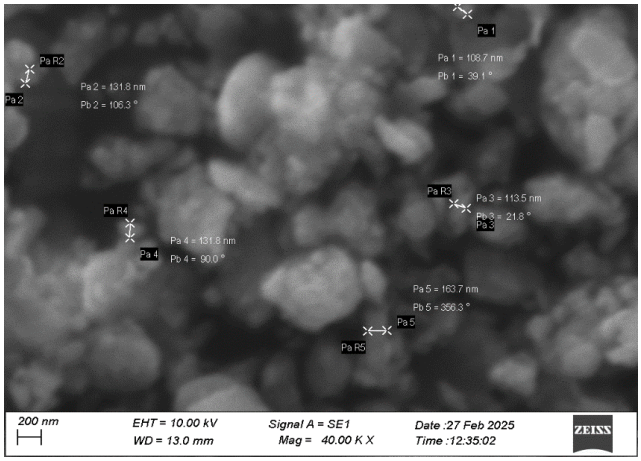

Figure 9: SEM Analysis Of Iron Nanoparticles From Leucas Aspera Plant

The SEM image of synthesized Fe nanoparticles is shown in Figures 5.13 & 5.14 , 5.15 which reveals the structure and size of the obtained nanoparticles. The SEM result shows that the morphology of the Fe nanoparticles is found to be spherical. The size of the nanoparticle exceeds 131.nm. The size of the Fe particles should be reduced by optimizing the nanoparticleprocess to get maximum application efficiency.

Anti -Oxidant Activity

The anti-oxidant activity of the plant extract iron nanoparticles was determined, as mentioned in the procedure. The results obtained are shown in Figure.

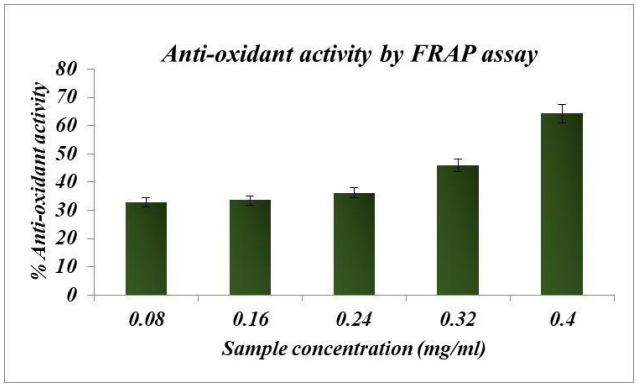

Figure 10: Anti -Oxidant Activity By Frap Assay Graph

International Journal of Recent Development in Engineering and Technology
Website: www.ijrdet.com (ISSN 2347-6435(Online) Volume 14, Issue 11, November 2025)

Based on the results, the percentage of anti-oxidant activity was found to be maximum at 62% at 0.4 mg/mL nanoparticle concentration. The percentage activity decreases as the concentration of nanoparticles increases. Maximum activity is achieved at even lower concentrations of iron nanoparticles.

Anti-Inflammatory Activity

Inflammation is an innate defence mechanism caused by body tissues in response to the attack caused by pathogens or by entering any foreign substances inside the body. One such response is a protein denaturation process, where the protein loses its biological function due to inflammation. This protein denaturation leads to the synthesis of auto antigens inside our body, resulting in inflammatory disorders such as rheumatoid arthritis, diabetes, and cancer. Hence, the sample which is evaluated for its anti-inflammatory ability should inhibit the protein denaturation process.

Inhibition Of Protein Denaturation Assay (Egg Albumin)

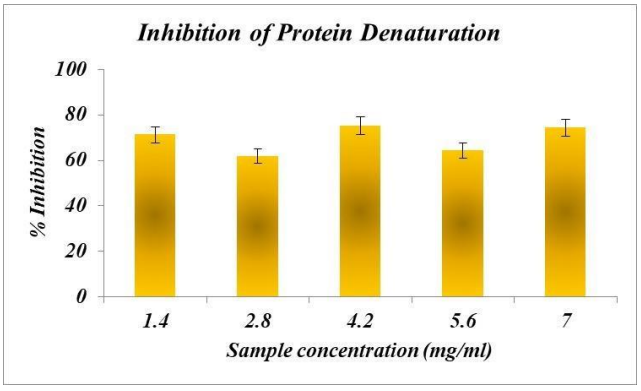

Figure 11: Inhibition Of Protein Denaturation Ability Of Sample

The percentage inhibition increases with an increase in sample concentration. The maximum activity of 76.3% is achieved at a concentration of 4.2mg\mL. The sample has better inhibition, which helps in controlling the protein denaturation thereby reducing the chance of inflammation.

Inhibition of Haemolysis ASSAY

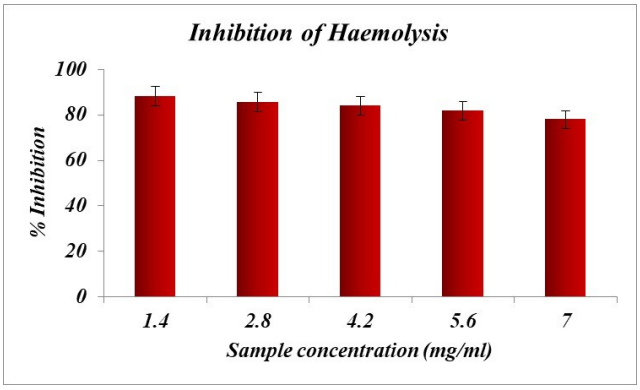

Figure 12: Inhibition Of Haemolysis Ability Of Sample

The percentage inhibition increases with an increase in sample concentration. The maximum activity of 88.5% is achieved at a concentration of 1.4 mg\mL. The sample has modest inhibition towards haemolysis process which facilitates the reduction of inflammation.

V. Conclusion

The plant samples were collected and prepared for extraction. Phytochemical profiling of the plant extracts was conducted through both qualitative and quantitative analysis. Iron nanoparticles were synthesized using the chemical reduction method. The synthesized nanoparticles were characterized using UV-Visible spectroscopy, FTIR analysis, and SEM analysis. The antioxidant activity of the synthesized nanoparticles was evaluated, along with their anti-inflammatory activity.

Based on the experimental analysis carried out, the following observations and outcomes were exhibited by the influence of iron nanoparticles derived from Leucas aspera plant.

The total flavonoid concentration in the aqueous extracts of leaf was obtained as 1.30mg of flavonoids per ml.

The total protein concentration in the aqueous extracts of leaf was obtained as 0.640mg of protein per ml.

The total carbohydrates concentration in the aqueous extracts of leaf was obtained as 2.379 mg of carbohydrates per ml.

The total polyphenols concentration in the aqueous extracts of leaf was obtained as 2.605 mg of polyphenols per ml.

The total Reducing sugars concentration in the aqueous extracts of leaf was obtained as2.448 mg of Reducing sugars per ml.

The synthesized iron nanoparticles were characterized by SEM, FTIR, and UV-Visible spectroscopy.

The anti-oxidant activity was found to be maximum at 62% at 0.4 mg/mL nanoparticle concentration. The percentage activity decreases as the concentration of nanoparticles.

increases. Maximum activity is achieved at even lower concentrations of iron nanoparticles.

The percentage inhibition increases with an increase in sample concentration. The maximum activity of 76.3% is achieved at a concentration of 4.2mg\mL.

The percentage inhibition increases with an increase in sample concentration. The maximum activity of 88.5% is achieved at a concentration of 1.4 mg\mL.

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