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CHARACTERIZATION AND CYTOTOXICITY EVALUATION OF IXORA COCCINEA-DERIVED IRON OXIDE MICROPARTICLES FOR BIOMEDICAL APPLICATIONS

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ABSTRACT

Background: This study aimed to synthesise and characterise iron oxide microparticles (IOMPs) using *Ixora coccinea* flower extracts and evaluate their antioxidant, anti-inflammatory, cytotoxic, and antidiabetic activities. **Methodology:** IOMPs were synthesised using *Ixora coccinea* flower extract and characterised using XRD, UV-Vis, EDAX APEX, and SEM. Bioactivity evaluations included anti-inflammatory and antioxidant activities via egg albumin, BSA, and DPPH assays; cytotoxicity through Brine Shrimp Lethality and zebrafish embryonic toxicity assays at 5, 10, 20, 40, and 80 µg/ml; and antidiabetic activity via alpha-amylase and alpha-glucosidase inhibition.

Results: Fe_2O_3MPs demonstrated potent anti-inflammatory (83% protein denaturation inhibition at 50 µg/ml), antioxidant (94.26% inhibition at 50 µg/ml), and antidiabetic (86% α-amylase and 84% α-glucosidase inhibition at 50 µg/ml) properties, surpassing diclofenac sodium and ascorbic acid. Cytotoxicity tests revealed low toxicity, with LC50 values of 80.5 µg/ml (Brine Shrimp) and 82.4 µg/ml (zebrafish).

Discussion: This study presents an eco-friendly synthesis of Fe_2O_3 microparticles using *Ixora coccinea* extract as a reducing and stabilising agent. These microparticles hold promise for biomedical applications, including drug delivery, MRI contrast enhancement, and hyperthermia treatment. Further research must optimise the synthesis process and assess the in vivo biocompatibility and therapeutic efficacy.

Conclusion: This study addresses the need for eco-friendly nanoparticles. Conventional iron oxide microparticle synthesis uses toxic chemicals, but *Ixora coccinea* flower extract offers a sustainable alternative. Evaluating Fe_2O_3MPs' cytotoxicity and bioactivity provides insights into biomedical applications, supporting future investigations that link nanotechnology and therapeutics.

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INTRODUCTION

Ixora coccinea, commonly called the flame of the woods, is an evergreen shrub native to India with significant medicinal properties. Various parts of the plant are utilised in Ayurveda for their antimicrobial, hepatoprotective, cytoprotective, antimutagenic, and chemopreventive effects, attributed to bioactive compounds such as ursolic acid and anthocyanins [1]. Research indicates that methanolic and aqueous extracts exhibit anti-inflammatory, antioxidant, antihistamine, and antidiabetic properties, reducing carrageenan-induced paw edema and blood glucose levels in animal models [2,3]. Additional Ixora species also demonstrate anti-inflammatory and anticancer activities [4,5]. *Ixora pavetta*, *I. pavetta indica,* and *I. brachiate* exhibit anti-inflammatory and antidiabetic activity [6–9]. Methanolic leaf extract and water-soluble extracts of roots and leaves of Ixora demonstrate efficacious anti-inflammatory and blood glucose control properties [10,11]. Iron oxide microparticles (IOMPs) are employed in biomedical applications due to their biocompatibility and magnetic properties, contributing to advancements in MRI, magnetic particle imaging, biosensors, and circulating tumor cell detection. In therapeutics and drug delivery, IOMPs are utilised in targeted drug delivery, cancer hyperthermia, neurotherapeutics, and stem cell therapy. They are also applied in tissue engineering, exhibiting antimicrobial, anticancer, cardioprotective, and wound-healing properties. Additional applications encompass blood purification, gene delivery, personalised medicine, and theranostics [12,13]. Green synthesis utilising plant extracts enhances their bioactivity [14,15]. Spinacia oleracea-synthesized IOMPs demonstrate cardioprotective effects in atherosclerotic rat models, while IOMPs with Phoenix dactylifera and Ficus carioca extracts exhibit enhanced antioxidant activity [16–18]. Ixora species demonstrate strong antioxidant potential through DPPH, FRAP, and ABTS assays, with *I. coccinea* and *I. alba* ICLEA displaying cytotoxicity against malignant melanoma cell lines [19–24]. Hydroalcoholic *I. coccinea* leaf extract protects against testicular damage in CP-treated rats, while *I. brachypoda* leaves exhibit moderate cytotoxicity in brine shrimp lethality tests [25,26]. Despite extensive studies on *Ixora coccinea* and IOMPs, limited research has explored the therapeutic synergy between plant-derived IOMPs and their antidiabetic, antioxidant, and anti-inflammatory potential. Moreover, the availability of selected flowers is abundant in many countries with a history of Chinese medicine. This study aims to evaluate the antidiabetic, cytotoxic, antioxidant, and anti-inflammatory effects of IOMPs synthesised from *Ixora coccinea* flowers, contributing to more targeted therapeutic applications. The novelty of utilising *Ixora coccinea* in green synthesis lies in its rich phytochemicals, which function as natural reducing and stabilising agents. It presents an environmentally favorable alternative to chemical methods, ensuring enhanced biocompatibility for biomedical applications.

MATERIAL AND METHODS

Preparation of Microparticles

To prepare the extract, 50 g of freshly harvested *Ixora coccinea* flowers were cleaned, shade-dried or heated at 50–60°C for 1–2 days, and ground into fine powder. The powder was mixed with 200–250 mL distilled water, heated to 60–70°C, agitated for 60 min, and filtered, yielding 56.72% extract. A 0.1M iron oxide microparticle (IOMP) solution was synthesised using 0.01622 g $FeCl_3$ in 1 L water, mixed with the extract, pH adjusted to 10–11, and agitated at 80°C for two hours. After cooling, the solution was incubated for 1–2 hours, followed by centrifugation, washing, drying (60°C, 2 h), and calcination (400°C) to form IOMPs. UV-Vis spectra confirmed stability. Specifying enzymatic assay incubation time ensures precision. This green synthesis method ensures controlled size, stability, and purity, advancing biogenic nanoparticle research.

Characterisation of Microparticles

Fe_2O_3MPs are characterised using a variety of scientific methods. XRD was used to determine the particle polymorphs. Chemical bonds and functional groups were detected using FTIR. The optical characteristics were analysed using UV-visible spectroscopy (UV). SEM-EDX provides detailed images of the morphology, particle size (Scanning Electron Microscopy), and elemental composition (Energy Dispersive X-ray Spectroscopy). The particle size and polydispersity index, which indicate colloidal stability, were measured using a zeta sizer.

Anti-inflammatory Activity

Bovine serum albumin denaturation assay

For the bovine serum albumin denatured protein assay, 0.45 mL of bovine serum albumin at a pH of 6.3 was mixed with Fe_2O_3MPs made from Ixora flowers at various dosages (10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50 µg/mL). This mixture was incubated for 30 min at 55°C in a water bath after standing at room temperature for 10 min. The control agent was diclofenac sodium; the reference standard was dimethyl

sulfoxide. Based on spectrophotometric measurements at 660 nm, the percentage of protein denaturation was calculated using the following formula [27]

$$\%\ inhibition = \frac{Abs\ (control) - Abs\ (sample)}{Abs\ (control)} \times 100$$

Egg albumin denaturation assay

To investigate egg albumin denaturation, mix 2.8 mL of phosphate buffer with 0.2 mL of fresh egg albumin. Ixora-mediated Fe_2O_3 MPs were added at concentrations of 10, 20, 30, 40, and 50 µg/ml to maintain a pH of 6.3. The mixture was incubated at room temperature for ten minutes, followed by incubation in a water bath at 55°C for 30 minutes. Diclofenac sodium was the control, and dimethyl sulfoxide was the reference standard. The percentage of protein denaturation was measured spectrophotometrically at 660 nm using a specified formula [28].

$$\%\ inhibition = \frac{Abs\ (control) - Abs\ (sample)}{Abs\ (control)} \times 100$$

Antioxidant activity

DPPH radical scavenging assay

A stock solution of DPPH containing 0.1 mg was extracted in methanol. To prepare the working solution for the tests, the original stock solution was adjusted to the maximum yield of 20 µM in methanol. Two hundred microliters of ixora-mediated Fe_2O_3MPs and DPPH working solutions at varying concentrations (10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, and 50 µg/mL) were combined in a plate with 96 wells. The plates were spent for the plate at room temperature in the dark. The absorbance at 517 nm was measured using a spectrophotometer, with methanol as the empty solution. The ratio of DPPH scavenging activity was calculated using the following formula:

$$\%DPPH\ Scavenging\ Activity = \frac{Acontrol - Asample}{Acontrol} \times 100$$

The absorbency of the DPPH solution without any sample is represented by "A control." In contrast, the DPPH solution's absorption rate, including iron oxide microparticles made by green synthesis, is represented by "A sample." Ascorbic acid served as the benchmark and was used in different proportions [29–31].

Hydrogen peroxide radical scavenging assay

We assessed the biosynthesised iron oxide microparticles' (IOMPs) ability to scavenge hydrogen peroxide ($H2O_2$). A 40 mM buffered phosphate solution (pH 7.4) dissolved H2O. This was achieved by mixing 0.6 mL of an $H2O_2$ solution with test samples of IOMPs at various concentrations (10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, and 50 µg/mL). After 10 min of incubation in the dark, the absorption intensity at 230 nm was measured using a spectrophotometer with vitamin C as the reference standard. The percentage of H2O2 scavenging capability was determined using a subsequent method [29].

$$\%inhibition = \frac{Acontrol - Asample}{Acontrol} \times 100$$

Cytotoxic Activity

Brine shrimp lethality assay

The brine shrimp lethality test involved immersing two grams of iodine-free sodium in 200 mL of filtered water. Six-well immunoassay plates were filled with 10–12 mL of saline solution in each well. Different amounts of microparticles (5µg/ml, 10µg/ml, 20µg/ml, 40µg/ml, and 80 µg/mL) and ten nauplii were present in each well. The total number of viable nauplii was counted when the plates were incubated for one day. The fatality rate was calculated using the following equation [32,33].

Number of dead nauplii / Number of dead nauplii + Number of live nauplii×100

Zebrafish toxicity

Four hours after fertilisation, the zebrafish embryos were incubated at 26°C. At the spherical stage, healthy embryos were randomly chosen and inserted into six-well plate cultures with 0.2 mL of culture water. The formulation involved the addition of different quantities of Fe_2O_3 MPs (5, 10, 20, 40, and 80 µg/mL) to each well. Embryos in the culture medium were used as controls in triplicate trials. The development of larvae and embryos at different fertilisation periods was observed by incubating the plates at 26°C. Every 12 h, the percentage of embryos that hatched and died was noted, and anomalies in the formulation of the embryos were examined under a microscope [34].

Antidiabetic activity

Determination of Alpha-Amylase Enzyme Inhibition

A 0.1% starch solution was prepared by dissolving 27.5 mg alpha-amylase in 100 ml of double-distilled water. Sodium potassium tartrate and 3,5-dinitrosalicylic acid (96 mM) were combined to produce a colorimetric reagent. Alpha-amylase

activity at 25°C was assessed by adding starch to the control and iron oxide microparticle (IOMP) solutions at five concentrations (10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml). Acarbose, the positive control, was evaluated after three minutes. Maltose production was measured by the conversion of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. The absorbance at 540 nm was measured using an ELISA reader to calculate the α-amylase inhibition.

$$I\% = 100- As-Ab/Ac-Ab *100$$

The average absorbance of the sample, blank, and control is denoted As, Ab, and Ac, respectively, whereas the percentage of the inhibitory proportion is represented by I% [35].

Evaluation of the Alpha-Glucosidase Enzyme Inhibition

Different Fe_2O_3 MPs formulations (10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, and 50 µg/mL) were delivered using a 0.2 M compound. Tri's buffer (pH 8.0) and a substrate solution (2% maltose or sucrose starch) were used. One milliliter of alpha-glucosidase enzyme was introduced after five minutes at 37°C. The mixture was incubated for 40 min at 35°C before adding 2 mL of 6N HCl to stop the reaction. Acarbose was the recommended medication. Following measurement of the fluorescence intensity at 540 nm using an ELISA reader, the inhibitory percentage (I%) of the alpha-glucosidase enzyme was calculated using the following procedure:

$$I\% = 100 – As-Ab/ Ac-Ab *100$$

In this instance, I% indicates the percentage of elimination, and As, Ab, and Ac depict the test sample's (IOMPs) average absorbance of the test sample, blank, and control (acarbose), respectively[35].

Statistical Analysis

Statistical analysis was conducted using SPSS 26 software, with a significance level set at 0.05 ($P \leq 0.05$). Results were expressed as mean ± SEM, and the normality of variables was assessed using the Shapiro–Wilk test. For normally distributed data, one-way ANOVA followed by Tukey post hoc tests were performed. In cases where data did not exhibit a normal distribution, the nonparametric Kruskal–Wallis test was employed [36].

RESULT AND DISCUSSION

UV-Vis Spectroscopy:

The stability and green production of iron oxide microparticles (IOMPs) were investigated using a Shimadzu Double Beam UV 1900 UV-visible spectrophotometer. The UV-Vis spectra showed an absorbance peak at 445 nm, aligning with reference values for Fe_2O_3 microparticles (400-450 nm), confirming successful synthesis. Extracellular reduction of Fe^{3+} ions validated formation. Figure 1 depicts the UV-Vis spectra at different time points, with synthesis completing after two hours. The strong match with literature references confirms IOMPs stability and optical properties, highlighting the method's reliability for green synthesis of iron oxide microparticles.

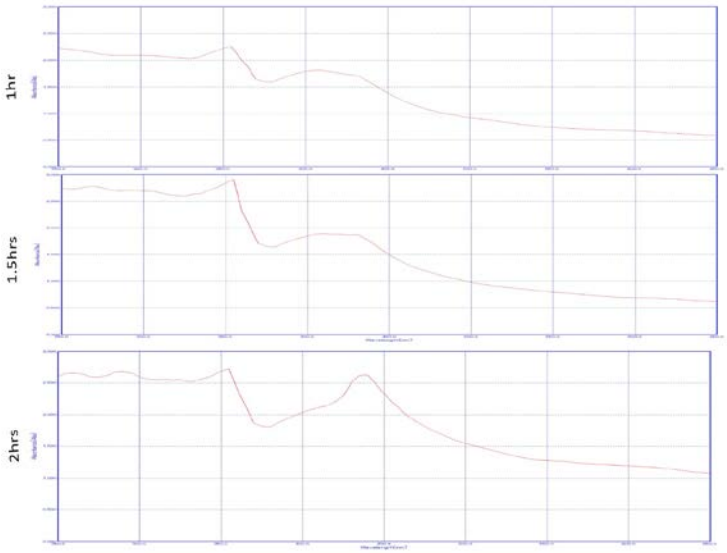
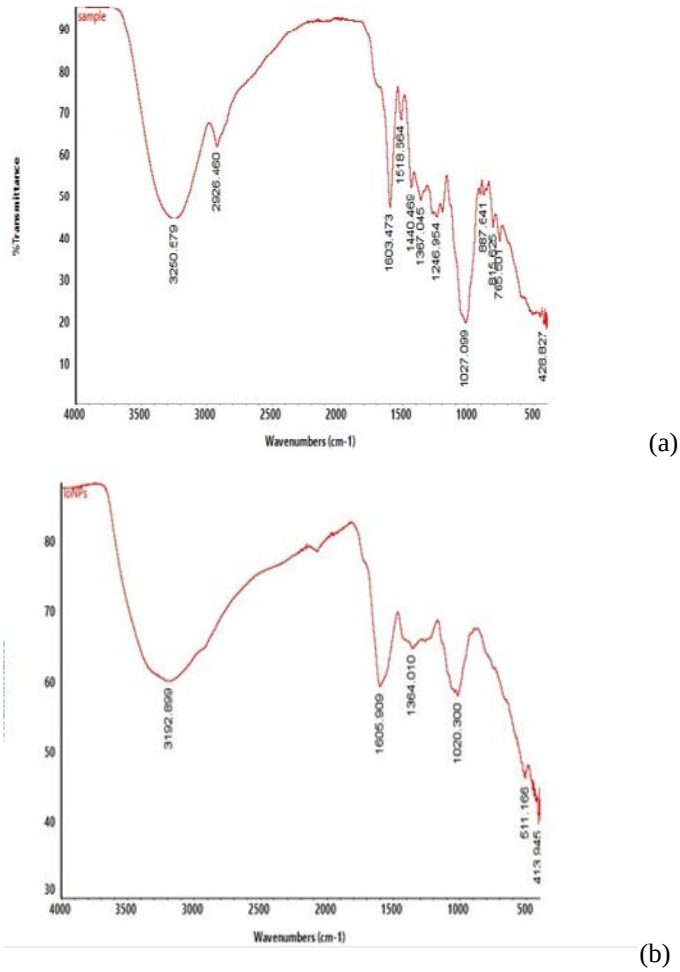


Figure 1: UV-Vi spectra of iron oxide microparticles (IOMPs) synthesized using *Ixora coccinea* flower extract, showing absorbance peaks at 1, 1.5, and 2 h, with the highest peak observed at 455 nm after 2 h.

FTIR

FTIR (Shimadzu IRTracer-100) analyzed the Ixora extract and Fe_2O_3MPs to identify functional group changes during reduction. Figure 2 exhibits distinctive peaks of iron oxide microparticles (IOMPs) associated with Fe-O vibratory stretching and surface functional groups, including hydroxyl groups. The FTIR analysis elucidates functional group interactions contributing to the bioactivity of Ixora extract and Fe_2O_3MPs. The spectra show hydroxyl (OH) groups, evidenced by the broad peak at 3192.899 cm^{-1}, indicating enhanced hydrogen bonding and antioxidant potential. Fe-O vibratory stretching peaks at 511.166 cm^{-1} and 413.945 cm^{-1} confirm iron oxide presence, crucial for redox reactions and catalytic activity. The peak at 1364.010 cm^{-1}, associated with carbonate groups or surface modifications, suggests improved biocompatibility, while the 1020.300 cm^{-1} peak linked to hydroxyl or other functional groups enhances interaction with biomolecules. These functional groups facilitate adsorption, stability, and

bioavailability, essential for biomedical applications such as drug delivery, antimicrobial activity, and antioxidant functions. The FTIR findings elucidate how the interaction between Ixora extract and Fe_2O_3MPs enhances their potential bioactivity, making them suitable for therapeutic and environmental applications.



(a)

(b)

Figure 2: (a) FTIR spectra of the sample. (b) FTIR measurement of Fe_2O_3 microparticles synthesized using *Ixora coccinea* flower extract, highlighting the Fe-O stretching vibration and O-H stretching vibration at different wavelengths in cm^{-1}.

XRD

As observed in Figure 3, the X-ray diffraction (XRD) analysis (Bruker D8 Advance X-ray Diffractometer) indicates that the material comprises 24.6% crystalline and 75.4% amorphous phases. Distinct peaks at specific 2θ values (e.g., 24.100°, 26.592°, and 33.160°) corroborate the presence of crystalline regions, while the broad depression in the lower 2θ range signifies the predominance of the amorphous phase. The mixed-phase composition suggests synthesis conditions that limited complete crystallization, resulting in both ordered and disordered structures. This structural combination plays a significant role in bioavailability, stability, and reactivity. The crystalline phase enhances structural integrity, improving mechanical strength and controlled drug release, while the amorphous phase provides higher surface reactivity, increasing solubility, adsorption capacity, and biomolecule interaction. The balance between crystallinity and amorphous content influences biodegradability, rendering the material suitable for drug delivery, tissue engineering, and catalysis-based biomedical applications. Thus, the XRD findings confirm the material's phase composition and elucidate its potential biomedical relevance, particularly in applications requiring enhanced bioactivity, controlled dissolution, and stability.

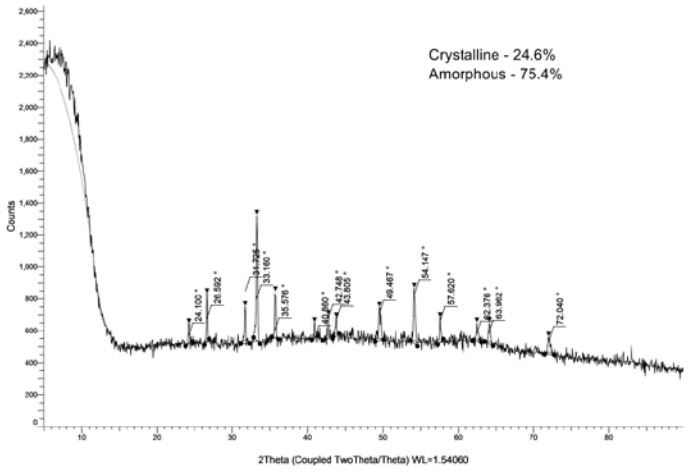
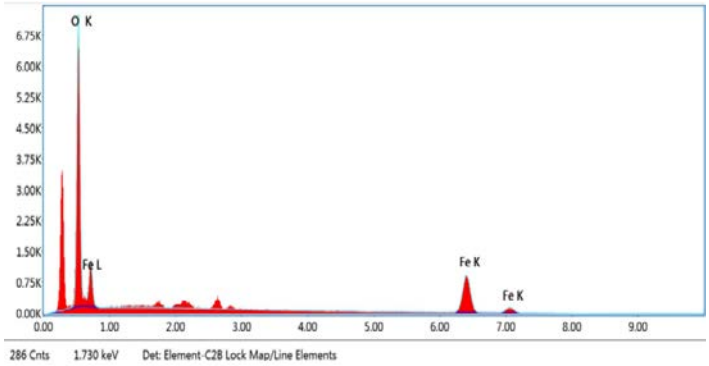


Figure 3: XRD analysis of Fe_2O3 MPs synthesized using *Ixora coccinea* flower extract, indicating a D size value of 403.63 nm. The analysis also reveals that the amorphous phase is more prominent than the crystalline phase

EDX analysis:

Ixora coccinea (IOMPs), chemically manufactured iron oxide microparticles, were confirmed for their chemical composition by using EDAX APEX. The EDX spectra showed prominent peaks for iron (Fe) and oxygen (O), indicating the presence of iron oxide. The sample comprised 40.08% iron and 59.92% oxygen by weight and 70.01% iron and 29.99% oxygen by atomic percentage. Figure 4 illustrates the net intensity values for iron (255.98) and oxygen (78.68), which reflect the higher atomic percentage of iron. Low error rates indicate precise measurement. The absence of notable peaks for other elements, appropriate K-ratios, and correction factors confirmed the purity of microparticles. These percentages align with the predicted iron-oxide stoichiometry, validating the synthesis process and

ensuring the desired chemical composition and structure. The successful biosynthesis and characterization of the iron oxide microparticles were corroborated by the XRD and FTIR results.



Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
O K	40.08	70.01	255.98	4.99	0.3158	1.1583	0.6804	1.0000
FeK	59.92	29.99	78.68	3.61	0.5312	0.8779	1.0056	1.0043

Figure 4: EDX analysis of Fe_2O_3 microparticles synthesized with *Ixora coccinea* flower extract shows peaks for iron (Fe) and oxygen (O), confirming iron oxide microparticles. The sample comprised 40.08% iron and 59.92% oxygen.

Zeta sizer

The Zeta Sizer analysis of iron oxide microparticles using Malvern Zeta sizer (Figure 5) revealed a dominant size peak at 1.617 µm, representing the primary particle size in the sample. The Z-average size of 1.669 µm reflects the weighted average of all particle sizes, indicating a heterogeneous size distribution. The Polydispersity Index (PDI) of 0.323 suggests a relatively uniform particle size distribution, which is crucial for ensuring colloidal stability in biomedical applications. A lower PDI indicates improved dispersion, reducing the likelihood of aggregation and enhancing the stability of the formulation. Peaks 2 and 3, with negligible size and intensity, are likely artifacts that do not significantly impact the overall analysis. The broad size distribution, in conjunction with a controlled PDI, ensures effective suspension stability, rendering the material suitable for drug delivery, biomedical imaging, and catalysis-based applications. The zeta potential was measured at ± 30 mV, indicating good stability. These results confirm that the synthesis process effectively produced a stable colloidal system, which is essential for long-term storage, bioavailability, and application consistency in various fields.

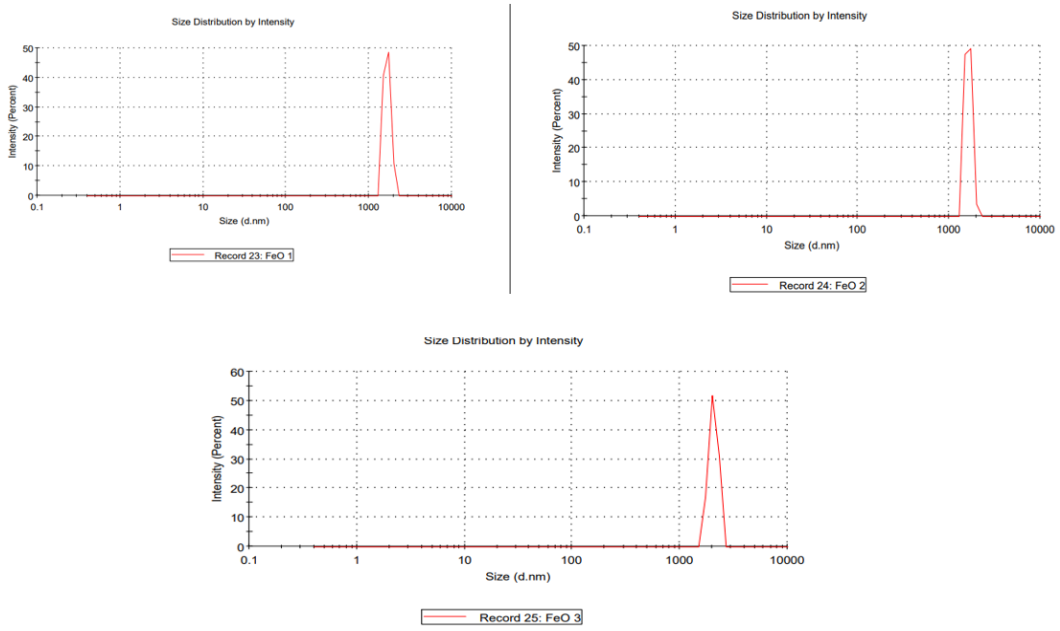


Figure 5: Zetasizer analysis and polydispersity index (PDI) of Fe_2O_3 microparticles synthesised using *Ixora coccinea* flower extract. The study showed a dominant size peak at 1.617 µm and a PDI of 0.323.

Anti-inflammatory activity

Iron oxide microparticles (IOMPs) synthesised from *Ixora coccinea* flower extracts demonstrated superior anti-inflammatory properties compared to diclofenac sodium across five concentrations (10–50 µg/ml). In the Egg Albumin Assay, diclofenac sodium achieved 81% suppression with an IC50 of 10.9 µg/ml, while Fe_2O_3 microparticles attained 83% inhibition at 50 µg/ml. In the BSA assay, IOMPs exhibited 84% inhibition

at 50 µg/ml, surpassing diclofenac sodium's 80% inhibition (IC50 of 15.7 µg/ml) (Figure 6). This effect is attributed to active compounds in the extract, such as tannins, phenols, and flavonoids, which function synergistically with IOMPs. These particles likely interact with inflammatory proteins by binding to key sites on enzymes and cytokines, disrupting their signaling pathways. IOMPs may chelate metal ions, modulate protein conformation, and inhibit pro-inflammatory mediators such as TNF-α, IL-1β, IL-6, and ROS, thus reducing inflammation [37]. The *Ixora coccinea* coating enhances biocompatibility, facilitating efficient cellular uptake and targeted action. These findings suggest that IOMPs could be a potent alternative for managing inflammatory diseases.

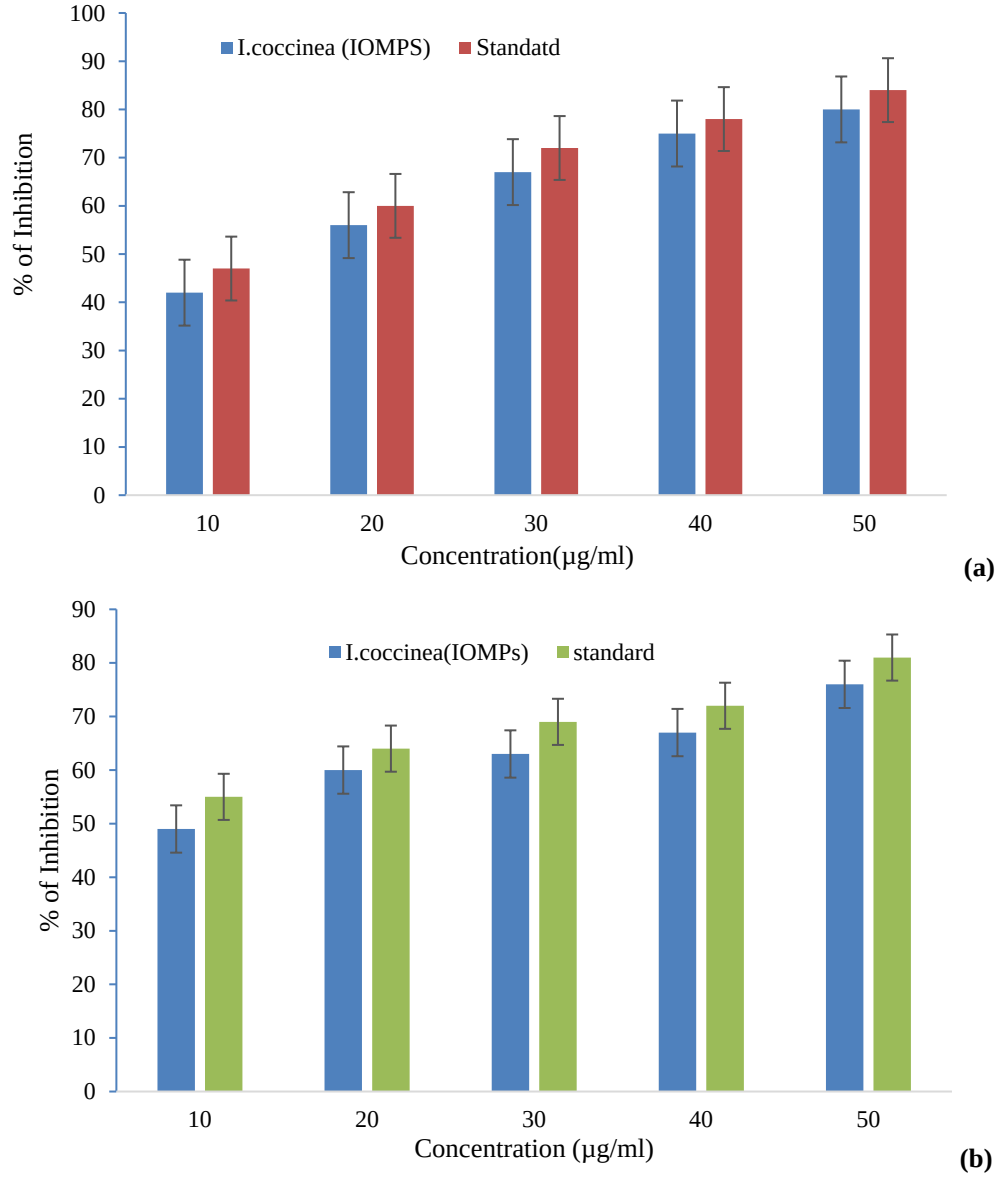


Figure 6: (a) BSA assay indicates that Fe_2O_3 microparticles (Fe_2O_3MPs) at 10-50 µg/ml inhibit protein denaturation by 84%, while diclofenac sodium achieves 80% inhibition. (b) ESA assay shows 83% inhibition by Fe_2O_3MPs at 10-50 µg/ml, compared to 81% by diclofenac sodium.

Antioxidant activity:

This study evaluated the antioxidant activity of plant extracts, ascorbic acid (control), and Fe_2O_3 microparticles (Fe_2O_3MPs) using DPPH and H_2O_2 scavenging assays. Antioxidants reduced DPPH absorbance at 630 nm, indicating free radical neutralisation. The IC50 of Fe_2O_3MPs from *Ixora coccinea* was 7.99 µg/ml, comparable to the standard IC50 of 7.55 µg/ml. Fe_2O_3MPs exhibited higher antioxidant activity than ascorbic acid, achieving 94.26% inhibition in the DPPH assay at 50 µg/ml, surpassing ascorbic acid's 93.15%. In the H_2O_2 assay,

Fe_2O_3MPs showed 87.5% inhibition at 50 µg/ml, exceeding ascorbic acid's 86.4%. The antioxidant mechanism of Fe_2O_3MPs involves electron transfer, neutralising free radicals and ROS, while Fe^{3+}/Fe^{2+} redox cycling breaks down superoxide and hydroxyl radicals. Metal ion chelation by phytochemicals prevents Fenton reactions, reducing oxidative stress. Bioactive compounds enhance Fe_2O_3MPs stability and efficiency. The *Ixora coccinea* extract coating improves biocompatibility and cellular uptake [36]. Future studies should investigate in vivo antioxidant activity, bioavailability, and applications in oxidative stress-related diseases, pharmaceuticals, and food preservation. These findings elucidate Fe_2O_3MPs as a promising antioxidant agent with potential therapeutic applications.

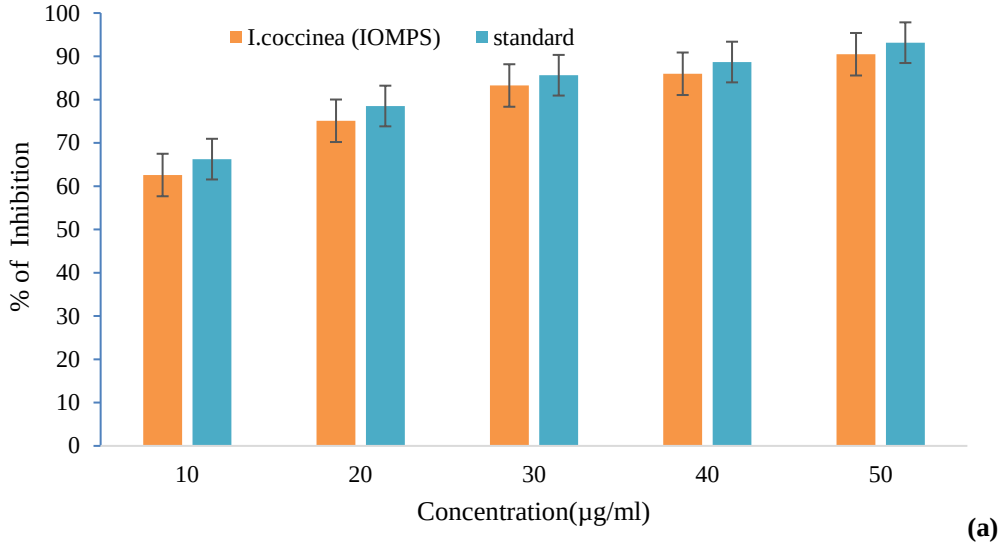


Figure 7: (a) Radical scavenging activity in the DPPH assay showing the percentage inhibition of Fe_2O_3 microparticles (Fe_2O_3MPs) and standard ascorbic acid at 10-50 µg/ml, with Fe_2O_3MPs achieving 94.26% inhibition compared to 93.15% with ascorbic acid. (b) Radical scavenging activity in the H_2O_2 assay showing the percentage inhibition of Fe_2O_3MPs and ascorbic acid at 10-50 µg/ml, with Fe_2O_3MPs reaching 87.5% inhibition versus 86.4% with ascorbic acid.

Cytotoxic Activity

Brine shrimp lethality assay

This technique analyses Fe_2O_3 microparticles (Fe_2O_3-MPs) for cytotoxicity and provides a reliable and user-friendly method to assess potential harmful effects. The graph (Figure 8) illustrates Fe_2O_3-MPs cytotoxicity at various doses. Ten nauplii and a control group in six wells were exposed to 5, 10, 20, 40, and 80 µg/mL concentrations. No mortality was observed on day 1 at

any concentration. However, by day 2, cytotoxic effects were observed, particularly at higher doses. At 40 µg/mL, live nauplii were reduced by 40%, and at 80 µg/mL, survival decreased by 60%, indicating dose-dependent cytotoxicity. The control group maintained a 100% survival rate throughout the experimental period. With an estimated LC50 value of 80.5 µg/mL, our results indicate that Fe_2O_3-MPs exhibit low cytotoxicity within the tested range of 5–80 µg/mL, suggesting that they are relatively safe at lower doses and that significant cytotoxicity occurs only at higher concentrations. According to the standard regulatory dosage, 1000 mg/kg/day has demonstrated no cytotoxicity; therefore, based on our study, this is considered a safe dosage for in vivo application.

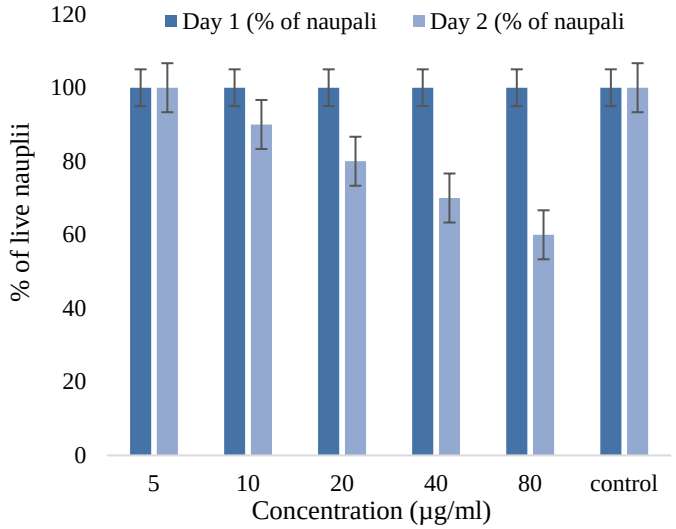
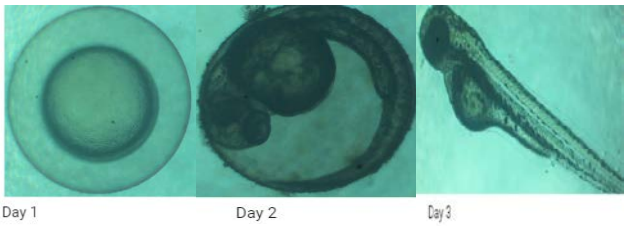


Figure 8: Cytotoxic effect of Fe_2O_3MPs using *Ixora coccinea* flower extract against nauplii, showing 20% mortality at 40 µg/ml and 30% mortality at 80 µg/ml with LC50 value of 80.52 µg/ml.

Zebrafish toxicity

A Zebrafish embryonic toxicity assay was used to assess the toxicity of Fe_2O_3 microparticles (Fe2O3 MPs) from *Ixora coccinea* flower extract at 5, 10, 20, 40, and 80 µg/mL. Figures 9a shows the developmental stages at 24, 48, and 72 h post fertilisation. Embryo and larval mortality rates were monitored, showing no mortality on day 1 but a dose-dependent increase by day 2, with viability dropping below 80% at 20 µg/mL and significantly at 40 and 80 µg/mL, remaining above 50%. Figure 9b shows an LC50 value of 82.4 µg/mL, with viability below 50% at concentrations above 80 µg/mL. Hatching rates at 5–80 µg/mL were 0% on day 1 and reached 100% at lower concentrations by day 2 but fell below 50% at 80 µg/mL, as indicated in Figure 9c, with an LC50 value of 59.33 µg/mL. Fertilised embryos were observed at doses above 40 µg/mL on day 3.



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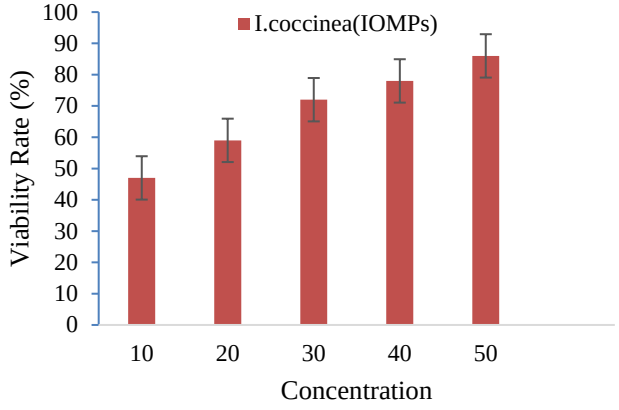

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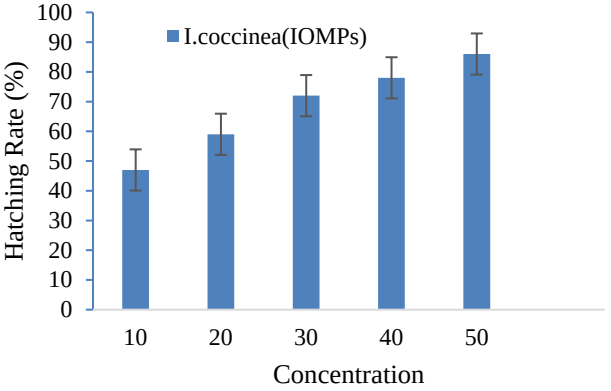

9(c)

Figure 9: (a) represents the toxic level at various concentrations of Fe_2O_3MPs using *Ixora coccinea* flower extract, which was observed under a microscope at different stages in consequent days (1-3 days), where it shows post-fertilisation at 24 h, 48 h, and 72 h. (b) Viability percentage of nauplii, with viability dropping to 80% at 20 µg/ml and showing significant reductions (60%) at 40 and 80 µg/ml. (c) The hatching percentage was 100 %, with 0% mortality on day 1 and < 50% hatch rate and mortality on day 2 at 80 µg/ml, with an LC50 value of 59.33 µg/ml.

Anti diabetic activity

Iron oxide microparticles functionalised with *Ixora coccinea* flower extracts exhibited stronger antidiabetic effects than normal ascorbic acid at five different doses. At 50 µg/mL, the

highest inhibition rates of 86% and 84% were observed for alpha-amylase and alpha-glucosidase, respectively. Figure 10 shows this is marginally less than the 88% inhibition of ascorbic acid at the same dose. The main cause of the enhanced activity was the bioactive ingredients of the floral extract, which included compounds with flavonoid alkaloids and glycosides. These microparticles also increased insulin sensitivity by inhibiting two key enzymes that break down carbohydrates: α-glucosidase (8.93 µg/mL) and alpha-amylase (12.50 µg/mL). Fewer sugars are absorbed and metabolised. Their combined activity makes them a promising alternative to traditional antidiabetic medications.

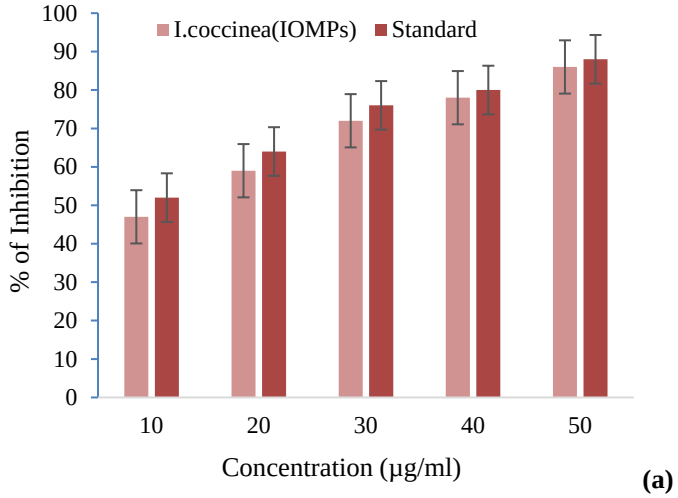

Figure 10: (a) Antidiabetic activity in alpha-amylase assay showing inhibition by Fe_2O_3MPs and standard ascorbic acid at 10-50 µg/ml, with Fe_2O_3MPs exhibiting 90% inhibition compared to 88% inhibition by ascorbic acid. (b) Alpha-glucosidase assay showing inhibition by Fe_2O_3MPs and ascorbic acid at 10-50 µg/ml, with Fe_2O_3MPs demonstrating 88% inhibition versus 84% by ascorbic acid.

CONCLUSION

Iron oxide microparticles (IOMPs) synthesised using *Ixora coccinea* flower extracts showed superior anti-inflammatory, antioxidant, cytotoxic, and antidiabetic activities at 50 µg/ml compared to standard treatments, attributed to bioactive compounds, such as tannins, phenols, and flavonoids. Cytotoxicity tests confirmed their safety, with LC50 values above 80 µg/ml in Brine Shrimp and Zebrafish assays. The microparticles effectively inhibited the key enzymes involved in carbohydrate metabolism, suggesting their potential for diabetes management. These findings indicate that Fe_2O_3 microparticles could be explored as potential therapeutic agents in nanomedicine for inflammatory disorders and diabetes management. They also emphasised the significance of green synthesis using renewable resources such as *Ixora coccinea*, supporting the development of safer and eco-friendly biomedical materials, which can be further validated through clinical studies.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Pavithra Bharathy contributed to conceptualisation, information collection, data curation, and manuscript drafting. Silpa Jayaprakash, Allen Christopher M, and Rajini Prem G conducted the study. Punniyakoti Veeraveedu Thanikachalam drafted, conceptualised, and revised the manuscript critically for important intellectual content.

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