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Phytochemical Analysis, Antinociceptive and Anti-inflammatory Activities of *Mimusops elengi* Bojer Leaf Extract-loaded Chitosan Nanoparticles in Albino Mice

Cletus Anes Ukwubile, Chidi Kaosi Clement

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Abstract: The present study evaluated the antinociceptive and anti-inflammatory activities of *Mimusops elengi* Bojer leaf extract encapsulated in chitosan nanoparticles (CS-NPs) using murine models, to determine whether nanoparticle formulation enhances the biological effects of a methanolic plant extract. Preliminary phytochemical screening was conducted using established qualitative colorimetric assays, which indicated the presence of major secondary metabolite classes, including phenolics and flavonoids; these tests were intended for compositional inference rather than definitive compound identification. Total phenolic and flavonoid contents were subsequently quantified using spectrophotometric methods, yielding 806.12 mg gallic acid equivalents (GAE)/g and 103.08 mg quercetin equivalents (QE)/g of extract, respectively. Antinociceptive activity was assessed using acetic acid–induced writhing and hot-plate assays, while anti-inflammatory effects were evaluated via the carrageenan-induced paw edema model. Animals treated with *M. elengi*–loaded CS-NPs exhibited statistically significant reductions in writhing responses, prolonged pain reaction latency, and decreased paw edema when compared with untreated controls and animals receiving the crude extract ($p < 0.05$). Inflammatory mediator analysis further demonstrated significant downregulation of pro-inflammatory cytokines (TNF-α, IL-1β, and PGE_2) alongside upregulation of anti-inflammatory cytokines (IL-10 and IL-22). Oxidative stress assessment showed reduced malondialdehyde (MDA) levels, indicating attenuation of lipid peroxidation. All experiments were conducted with appropriate replication, and data were subjected to statistical analysis to ensure reproducibility. While the phytochemical screening provides preliminary compositional insights, the observed pharmacological effects are attributed to the combined action of extract constituents and improved delivery via chitosan nanoparticles. Overall, the findings support the hypothesis that nanoparticle-based formulation can enhance the antinociceptive and anti-inflammatory efficacy of *M. elengi* leaf extract, highlighting its potential as a complementary therapeutic approach while underscoring the need for further compound-level characterization and safety evaluation.

Introduction

Plant-derived natural products remain a central source of pharmacologically active compounds, contributing substantially to modern drug discovery and traditional healthcare systems worldwide (1). It is estimated that over 80% of the global population relies partly or wholly on plant-based medicines for primary healthcare, particularly in Asia and Africa, where biodiversity and ethnomedicinal knowledge are extensive. The therapeutic value of medicinal plants stems from the structurally diverse secondary metabolites they contain, which can modulate oxidative stress, inflammation, pain perception, microbial growth, and cell survival pathways (2). However, translating ethnomedicinal claims into scientifically robust evidence requires systematic chemical characterization, biological relevance, and appropriate analytical integration rather than repetitive descriptive screening.

Despite decades of phytochemical investigations, a persistent limitation in medicinal plant research is the fragmented characterization of bioactive constituents, often restricted to preliminary qualitative tests without contextualization of biological function, mineral contribution, or safety relevance. Moreover, many studies reiterate basic phytochemical screening of well-known plants without

articulating how such data advance mechanistic understanding, formulation strategies, or therapeutic applicability (3). Addressing this gap requires studies that integrate phytochemical profiling with complementary analytical techniques and clearly defined biological endpoints.

Mimusops elengi L. (Sapotaceae), commonly known as Bakul or Spanish cherry, is a widely distributed evergreen tree with extensive ethnomedicinal use across South Asia. Various plant parts have been traditionally employed for managing pain, inflammation, wounds, dental disorders, infections, and respiratory conditions. Previous phytochemical studies have reported the presence of alkaloids, terpenoids, steroids, flavonoids, tannins, saponins, and phenolic compounds in the leaves, flowers, bark, and seeds. Isolated constituents such as β-sitosterol, taraxerol, spinosterol, and D-mannitol have been documented, alongside reports of antioxidant, analgesic, antipyretic, cytotoxic, and wound-healing activities (4-7).

However, existing studies on *M. elengi* remain largely descriptive and compartmentalized, with limited critical comparison across extraction systems, poor linkage between phytochemical classes and pharmacological relevance, and minimal attention to elemental composition that may influence efficacy or toxicity (8). Notably, the mineral and trace element profile of *M. elengi* leaf extracts has not been systematically evaluated alongside phytochemical composition, despite evidence that elements such as iron, zinc, copper, and manganese can modulate redox balance, inflammatory signaling, and enzyme activity. The absence of integrated phytochemical–elemental analysis represents a significant knowledge gap, particularly for formulations intended for biological application.

The present study addresses this gap by combining qualitative phytochemical screening with energy-dispersive X-ray fluorescence (EDXRF) analysis, thereby providing a dual chemical perspective: organic secondary metabolites and inorganic elemental composition. While phytochemical screening offers preliminary insight into the classes of bioactive compounds present, EDXRF complements this by enabling non-destructive, multi-elemental assessment relevant to nutritional value, toxicological safety, and formulation considerations (9). The integration of these approaches allows a more holistic evaluation of *M. elengi* leaf extracts than either method alone.

The selection of methanol as the extraction solvent is based on its proven efficiency in solubilizing a broad spectrum of moderately polar to polar phytochemicals, including phenolics, flavonoids, alkaloids, and glycoside compounds strongly implicated in antioxidant, antinociceptive, and anti-inflammatory activities (10-12). Compared with aqueous or non-polar solvents, methanol provides a higher extraction yield and chemical diversity, making it particularly suitable for exploratory phytochemical studies and downstream nanoformulation development.

Importantly, the biological relevance of the targeted phytochemical classes underpins the experimental design of this study. Phenolics and flavonoids are known to scavenge reactive oxygen species and modulate cyclooxygenase and nitric oxide pathways; alkaloids and terpenoids are associated with central and peripheral antinociceptive mechanisms, while saponins and tannins contribute to membrane stabilization and inflammatory mediator suppression (13-15). These mechanistic associations provide a rational basis for evaluating antinociceptive and anti-inflammatory outcomes in relation to chemical composition. Furthermore, despite growing interest in nanoparticle-based delivery systems, there is a paucity of data on chitosan nanoparticle formulations incorporating *M. elengi* leaf extracts, particularly regarding their phytochemical content and pharmacological relevance. The encapsulation of plant extracts in chitosan nanoparticles has the potential to enhance stability, bioavailability, and biological efficacy(16, 17), yet this strategy remains underexplored for *M. elengi*.

The objectives of this study were to: (i) qualitatively characterize the major phytochemical classes present in methanolic leaf extracts of *Mimusops elengi*; (ii) determine the elemental composition of the extracts using EDXRF analysis; and (iii) provide a chemical rationale supporting the antinociceptive and anti-inflammatory potential of chitosan nanoparticle–loaded *M. elengi* leaf extracts. It was hypothesized that methanolic leaf extracts of *M. elengi* contain bioactive phytochemical classes and essential trace elements that collectively underpin their traditional analgesic and anti-inflammatory uses, and that integrating phytochemical screening with elemental analysis would yield a more comprehensive chemical framework relevant to biological application

Materials and Methods

Plant Collection and Identification

Fresh leaves of *Mimusops elengi* L. were collected in the evening h of 2024 from Biu, Borno State, Nigeria. Botanical authentication was performed by Dr. C. A. Ukwubile, a taxonomist in the Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri. A voucher specimen (UMM/FPH/SAT/002) was deposited in the departmental herbarium for future reference.

Preparation of Plant Materials and Extraction

The collected leaves were rinsed with distilled water to remove adhering debris and shade-dried at ambient temperature (25–28 °C) for two weeks until constant weight was achieved. The dried material was pulverized into fine powder using a stainless-steel electronic blender to minimize metal contamination. A total of 1200 g of powdered leaves was macerated in 2.5 L of analytical-grade methanol (99.1% v/v; Sigma-Aldrich, USA) for 72 h at room temperature with intermittent stirring. The extract was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 64.5 °C to yield a dark-green crude extract (68.4 g; percentage yield: 5.7%).

The methanolic extract was subjected to bioguided solvent–solvent fractionation using solvents of increasing polarity: n-hexane (1.30 g), ethyl acetate (24.44 g), and n-butanol (8.12 g). Fractions were dried, weighed, and stored at 4 °C until further analysis.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was conducted to qualitatively assess the presence of major secondary metabolite classes, including alkaloids, flavonoids, tannins, saponins, cardiac glycosides, terpenoids, and phytosterols, using standard classical assays (e.g., Dragendorff's, Shinoda, Frothing, Salkowski tests) as described in established protocols (11, 18, 19).

To enhance reproducibility and transparency: 1) All phytochemical tests were performed in triplicate (n = 3) on

independently prepared extract aliquots. 2) Each test included appropriate reagent blanks (solvent + reagent without extract) as negative controls. 3) Visual observations (color intensity, precipitate formation, froth persistence) were recorded independently by two trained analysts, and discrepancies were resolved by consensus to minimize observer bias.

Semi-Quantitative Interpretation

Results were interpreted using a predefined semi-quantitative scoring system based on visual intensity and persistence: High (+++): Strong, immediate reaction with intense coloration/precipitation; Moderate (++): Clear but less intense reaction; Low (+): Weak but observable reaction; Trace (±): Barely perceptible reaction; Absent (–): No observable reaction. These results are screening-level indicators only and were not intended for compound identification or quantification.

Energy-Dispersive X-Ray Fluorescence Analysis

Sample Preparation

For elemental analysis, a portion of the methanolic extract was oven-dried at 40 °C to constant weight, gently ground using an agate mortar and pestle, and sieved through a 200 µm mesh to ensure homogeneity. Approximately 5 g of the powdered extract was mixed with spectroscopic-grade cellulose binder (10% w/w) and compressed into pellets (32 mm diameter) under 10 tons pressure using a hydraulic press. Pellet homogeneity was visually inspected and confirmed by replicate analysis (9).

Instrumentation and Calibration

EDXRF measurements were performed using a calibrated EDXRF spectrometer following standardized operating procedures (9). Instrument calibration was conducted using certified reference materials (CRMs) of plant-based matrices obtained from the International Atomic Energy Agency (IAEA).

Quality assurance includes blank pellet analysis (binder only) to assess background contamination, triplicate measurements per sample (n = 3), and internal standard correction and matrix effect compensation. Limits of detection (LOD) were calculated as three times the standard deviation of blank measurements. Elemental concentrations were expressed as mg/kg dry weight ± standard deviation (9).

Heavy Metal Considerations

Elements classified as potentially toxic (e.g., Pb, Cd) were included strictlyas screening-level indicators (12, 20). No claims regarding safety thresholds or dietary relevance were made. All measurements were interpreted cautiously, acknowledging that confirmatory analysis using ICP-MS or AAS would be required for regulatory or toxicological validation.

Determination of Total Phenolic and Flavonoid Contents

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method, while total flavonoid content (TFC) was assessed using aluminum chloride colorimetry (13, 21, 22). Extract concentrations ranged from 100–800 µg/mL, and absorbance was measured at 765 nm (TPC) and 515 nm (TFC). Gallic acid and quercetin were used as reference standards, respectively. All assays were performed in triplicate (n = 3).

Isolation and Structural Characterization of Bioactive Compound

The ethyl acetate fraction, identified as the most bioactive, was subjected to silica gel column chromatography (60–120 mesh) using gradient elution with n-hexane: methanol. Fifty sub-fractions were pooled based on TLC profiles. A white crystalline compound (10.02 mg) was purified and confirmed by single-spot TLC and melting point determination. Structural elucidation was performed using ^{1}H and ^{13}C NMR spectroscopy (Bruker ASCEND 850 MHz) and GC–MS analysis using an Agilent 7890A system (23-25).

Preparation and Characterization of Extract-Loaded Chitosan Nanoparticles

This process involved dissolving 10 g of *M. elengi* extract into 20 mL of deionized water. Similarly, 4 g of shrimp shell-based chitosan powder was precisely weighed using a Gemini-20 portal milligram scale balance, then dissolved in 100 mL of deionized water with 0.5% v/v glacial acetic acid while being stirred magnetically. The beaker containing CS NPs was continuously stirred at 3000 rpm for two h while a prepared solution of ME was added. A cross-linker called glutaraldehyde was introduced to each formulation (MECS2-MECS4) dropwise (1 mL/5 min) at concentrations of 500, 1000, and 1500 µg/mL. There was no cross-linking of MECS1. A 0.1M NaOH solution was added to the mixtures, and they were stirred for 30 min to correct their pH. In order to improve their targeting, 5 mL of the solution was added to the formulations and agitated for an additional 15 min at 2000 rpm after 20 mg of folic acid had been dissolved in 20 mL of deionized water (18-20). A nanospray drying device (Model B-90 Shanghai Bilon Instrument Co Ltd, China) was used to spray-dry the generated MECS complexes for atomization under the following experimental circumstances: Inlet temperature = 130 °C; Outlet temperature = 55°C- 60 °C; Head temperature = 65 °C- 70 °C; Pressure = 37- 38 mbar.

The *M. elengi* leaf extract-loaded spray-dried chitosan nanoparticles were weighed and kept in sterile containers for further use. Particle size, morphology, entrapment efficiency, yield, and *in vitro* drug release were evaluated in triplicate (26, 27).

Experimental Animals and Ethical Considerations

Healthy Swiss albino mice (50–80 g) were acclimatized for one week under standard laboratory conditions. All experimental procedures complied with institutional animal ethics guidelines.

Pharmacological and Biochemical Assays

Antinociceptive, anti-inflammatory, cytokine, oxidative stress, enzymatic, and cellular infiltration assays were conducted using validated murine models and ELISA-based methods as described. Each experimental group consisted of five animals (n = 5).

Antinociceptive Activity of Extracts Using Acetic Acid-Induced Writhing in the Mouse Model

Twenty-five Swiss albino mice weighing between fifty and eighty grams of each sex were divided into five groups at random. Group I animals received 10 mL of distilled water intraperitoneally (i.p.), Group II animals received 10 mg/kg b.w. Ibuprofen (i.p.), and Group III animals received 200

mg/kg of chitosan NPs, Group IV animals received 200 mg/kg of M. elengi leaf extract, and Group V animals received 200 mg/kg of chitosan loaded with M. elengi isolated compound (orally). Following a 5-minute stimulation session, the animals were administered 0.6% v/v acetic acid after 30 min, and their abdominal muscles were examined for contractions using a hand lens for 10 min (28). The percentage inhibition of writhing was determined using **Equation 1**.

Evaluation of Antinociceptive Effects by the Hot Plate Method

The purpose of this experiment was to evaluate M. elengi extract's central analgesic effects. Each mouse was kept in an open cylindrical chamber with a metal floor that was heated to 50 ± 2°C during the operation. Paw licking and jumping, two quantifiable behavioral responses that are thought to need greater nervous system processing, were made possible by this arrangement. To avoid paw damage, the mice were exposed to the hot plate for no more than 15 s after receiving three different doses of the extracts, a conventional medication, and a vehicle. At 0, 30, 60, 90, and 120-minute intervals, the mice's reaction times were measured by how long it took them to hop off the plate or lick their paw, and these times were noted (29, 30).

Anti-Inflammatory Activity Using Carrageenan-Induced Paw Oedema in Mice Method

To induce oedema, carrageenan was applied to the animal's paw. Test extracts were then given while a positive control (aspirin) was present. Additionally, the animals were divided into five groups, each consisting of five animals. Group I received 10 mL of distilled water intraperitoneally (i.p.), Group II received 10 mg/kg b.w. of ibuprofen intraperitoneally (i.p.) Group III received 200 mg/kg of chitosan NPs, Group IV received 200 mg/kg of *M. elengi* leaf extract, and Group V received 200 mg/kg of *M. elengi*-loaded chitosan NPs by oral administration. The animals in each group were subsequently given 0.1 mL of 1% carrageenan subplanarly in their right hind paw, which caused acute inflammation. Vernier calipers were subsequently used to measure the paw diameters at 0, 1, 2, 3, and 4 h following the carrageenan injection (29, 31). The percentage inhibition of paw oedema was determined using **Equation 1**. Where, Wc = mean diameter of paw oedema in the negative control group, and Wt = mean diameter of paw oedema in the treated groups.

Evaluation of Pro- and Anti-Inflammatory Markers

The levels of the pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), as well as the anti-inflammatory cytokine interleukin-10 (IL-10), in mice serum were assessed using commercially available ELISA kits (ThermoFisher Scientific, UK) in accordance with the manufacturer's instructions. The ELISA method involves diluting the mouse serum samples (1:2) for IL-1β, IL-6, IL-10, IL-22, TNF-α, and cyclooxygenase-2 (COX-2), as well as prostaglandins (PGE2), along with internal controls and test standards, and then applying them to a solid phase containing antibodies specific to the respective cytokines (32-34).

$$\%\,\text{inhibition} = \frac{\mathbf{W_c} - \mathbf{W_t}}{\mathbf{W_c}} \times \mathbf{100}$$

Equation 1 | Wc = mean number of writhing negative control, and Wt = mean number of writhing treated groups.

Evaluation of Effects on Pro-Inflammatory Cytokines

TNF-α: Tissue lysate from the treated and control mouse groups was obtained using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. The anti-mouse TNF-α antibody was applied to the wells of an ELISA plate. After that, the samples in different concentrations were put into the wells and left to incubate for a whole day. Distilled water was used to cleanse the unbound materials. Following the addition of a substrate, 3,3',5,5'-tetramethylbenzidine (TMB), a detection antibody linked to the enzyme Horseradish Peroxidase (HRP) was added. A microplate reader was then used to measure the absorbance at 450 nm (32, 33, 35)

Interleukin-1 beta (IL-1β) assay: An ELISA plate coated with an anti-IL-1β antibody was added to the prepared sample tissue that had been removed from the treatment and control mouse groups. The standards and samples were placed in the wells and left to incubate for twenty-four h. The enzyme-substrate solution and the detecting antibodies were then added. A microplate reader was then used to measure the absorbance at 450 nm to quantify the amounts of IL-1β (32).

Level of interleukin-6 (IL-6): Mice's serum was extracted and put into a 96-well plate that had been coated with IL-6 antibodies beforehand. After a 24-hour incubation period, the material was cleaned to get rid of any unbound proteins. Subsequently, the HRP-linked detection antibody and substrate were added. IL-6 levels were quantified using a microplate reader set to 450 nm (32, 35).

Evaluation of Effects on Inflammatory Mediators

Cyclooxygenase-2 (COX-2) level: Mice in the treatment and control groups had their tissues removed. After homogenizing the tissues, proteins were extracted. Proteins were transferred to a membrane using SDS-PAGE and Western blot. An anti-COX-2 antibody, COX-2 (D5H5) rabbit monoclonal antibody, was then used for probing. Chemiluminescence was then used to detect the signal (36).

Prostaglandin E2 (PGE2) level: Using the ELISA method, tissue lysates were collected from mice. The samples were then added to PGE2 antibody-coated wells and incubated with the secondary antibody conjugated to an enzyme. Thereafter, substrate was added, incubated, and the colorimetric change was measured using a microplate reader at 450 nm (37).

Evaluation of Effects on Oxidative Stress Markers

Malondialdehyde (MDA) levels: The mice's homogenized tissue and extracts were combined with Thiobarbituric acid (TBA) and incubated at 84.2 °C using the Thiobarbituric Acid Reactive Substances (TBARS) method. A UV-vis spectrophotometer was used to detect absorbance at 532 nm to evaluate lipid peroxidation after the MDA and TBA reacted to produce a pink chromogen (38, 39).

*Catalase (CAT), Glutathione (GSH), Glutathione peroxidase (GPx), and Superoxide dismutase (SOD):*The ELISA method was used to determine the effects of extracts on levels of these oxidative stress markers following the manufacturer's instructions (40).

Nitric Oxide(NO) concentration: Tissue samples were taken from the mouse groups and combined with Griess reagent (sulfanilamide and NEDD) using the Griess assay. After that, the material was allowed to develop color at room temperature. Nitrite content (NO indicator) was then determined by measuring the absorbance at 540 nm (41).

Evaluation of Effects on Cellular Infiltration

Leukocyte count: In this method, a blood sample was collected from the animals by cardiac puncture. The blood was then diluted in a buffer, and cells were counted on an INCYTO number 1 disposable hemacytometer (Benrock Scientific, Nigeria). The concentration of leukocytes per microliter of blood was then calculated (42)

Neutrophil count: In this procedure, a blood smear was made from the animals and stained with a neutrophil-specific marker (LY6G, ThermoFisher Scientific, USA). Then a differential neutrophil count was performed to identify neutrophils using flow cytometry (42, 43).

Evaluation of Cytokine Inhibitors

Interleukin-10 (IL-10) and IL-22 levels: The animals' serum was collected. After coating the ELISA plate with particular IL-10 and IL-22 antibodies, the sample was added. After a 24-hour incubation period, the material was cleaned. Substrates for colorimetric detection and detection antibodies were then added. A microplate reader was then used to measure the absorbance at 450 nm (44).

Evaluation of Enzymatic Activities

Inducible nitric oxide synthase (iNOS): The mice's tissue was homogenized. The homogenized tissue was put in the ELISA plate after it had been coated with an anti-iNOS antibody. After 24 h of incubation, it was measured with a microplate reader at 453 nm (29).

Arginase levels: Mice's homogenized tissues were prepared and treated with a substrate called L-arginine. L-arginine is hydrolyzed by the enzyme arginase to produce urea and ornithine. The contents were then mixed with two drops of thiosemicarbazide (TSC), which reacted with the urea to provide a color signal. Arginase was then calculated by measuring the absorbance at 540 nm (45).

Statistical Analysis

Data were expressed as mean ± standard deviation. Statistical significance was evaluated using one-way ANOVA followed by Dunnett's post-hoc test (GraphPad Prism v9), with $p < 0.05$ considered significant.

Results

Preliminary Phytochemical Profile of *Mimusops elengi* Leaf Methanol Extract

The preliminary phytochemical screening of the methanolic leaf extract of *M. elengi* (SM1) indicated the presence of several major classes of secondary metabolites, including flavonoids, tannins, phenolic compounds, saponins, alkaloids, terpenoids, and phytosterols (**Table 1**). These findings are qualitative and semi-quantitative, reflecting reaction intensity rather than compound concentration or abundance. Importantly, these results should not be interpreted as definitive indicators of phytochemical levels, as the employed assays are non-specific screening tests subject to cross-reactivity and observer interpretation. Instead, the phytochemical profile provides contextual chemical information that supports subsequent quantitative (TPC/TFC) and biological assays. Despite these limitations, the detected phytochemical classes are mechanistically relevant to the observed biological outcomes. Flavonoids and phenolics are widely implicated in redox modulation, cyclooxygenase inhibition, and cytokine suppression, while saponins and triterpenoids are known to influence immune signaling and membrane stability. Alkaloids and phytosterols have been associated with central and peripheral antinociceptive mechanisms. Thus, although qualitative, the phytochemical profile provides a biological plausibility framework rather than proof of efficacy.

Table 1. Preliminary phytochemical profile of *Mimusops elengi* leaf methanol extract.

Phytochemical Class	Test performed	Observed reaction	Inference*	Remarks
Flavonoids	Shinoda / Alkaline reagent test	Development of yellow to reddish coloration	+	Indicates presence of flavonoid-type compounds; intensity reflects reaction strength, not concentration
Tannins	Ferric chloride test	Blue–black/greenish coloration	+	Suggests the presence of hydrolysable or condensed tannins
Phenolic compounds	Ferric chloride test	Dark blue or green coloration	++	Supports an abundance of phenolic-type moieties but remains non-quantitative
Saponins	Frothing test	Persistent froth formation	+	Indicative of surface-active glycosides
Alkaloids	Dragendorff's / Mayer's test	Orange or cream precipitate	+	Preliminary indication only; prone to cross-reactivity
Terpenoids	Salkowski test	Reddish-brown interface	+	Suggests terpenoid skeletons; does not confirm structure
Phytosterols	Liebermann–Burchard test	Greenish-blue coloration	+	Consistent with sterol-type compounds
Glycosides	Keller–Killiani test	Brown ring formation	±	Weak reaction; requires confirmatory analysis
Fixed oils/fats	Spot test	Permanent translucent spot	−	Not detected under current assay conditions

Note: Key:++ = relatively stronger reaction intensity, + = detectable reaction, ± = weak or ambiguous reaction, and − = not detected. *Inference is based on reaction intensity, not compound concentration or abundance.

Table 2. GC-MS composition of *M. elengi* leaf.

Compound Name	Retention time (min)	Peak Area (%)	Molecular Formula
β-Sitosterol	36.42	18.45	$C_{29}H_{50}O$
Stigmasterol	35.76	12.38	$C_{29}H_{48}O$
Oleic acid	27.85	10.56	$C_{18}H_{34}O_2$
Linoleic acid	27.18	9.82	$C_{18}H_{32}O_2$
Palmitic acid	25.42	7.29	$C_{16}H_{32}O_2$
Methyl stearate	28.10	6.41	$C_{19}H_{38}O_2$
2,4-Di-tert-butylphenol	21.90	5.87	$C_{14}H_{22}O$
Squalene	41.23	4.62	$C_{30}H_{50}$
Phytol	37.55	3.89	$C_{20}H_{40}O$
Hexadecanoic acid, methyl Ester	24.12	3.67	$C_{17}H_{34}O_2$

Table 3. Characterization of *M. elengi*-loaded chitosan NPs.

Parameter	Value	Description/Method
Particle Size	220 nm	Determined by dynamic light scattering (DLS)
% yield	64.2%	Calculated based on the total weight of nanoparticles
Entrapment efficiency (%EE)	84.24%	Indicates the percentage of extract encapsulated within the nanoparticles
In vitro drug release	98.64%	Percentage of extract released in a dissolution medium
Zeta potential	25 mV	Indicates nanoparticle stability and surface charge
Kinetic model	First-order kinetic	Describes the drug release profile from the nanoparticles
Swelling index	62.04	Degree of swelling of nanoparticles in aqueous medium

Total Phenolic and Flavonoid Contents

Quantitative assays revealed a total phenolic content (TPC) of 516.22 mg gallic acid equivalents (GAE)/g extract and a total flavonoid content (TFC) of 106.18 mg quercetin equivalents (QE)/g extract (**Table 1**). These results indicate a phenolic-dominant chemical profile, which is consistent with the stronger antioxidant and anti-inflammatory responses observed in downstream assays. Unlike the preliminary screening, these assays provide quantitative, reproducible measurements and therefore represent the most reliable chemical indicators in this study. However, it should be noted that Folin–Ciocalteu and aluminum chloride methods measure total reducing capacity and flavonoid equivalents, respectively, rather than individual compounds. Consequently, comparisons with literature were restricted to studies employing similar colorimetric methodologies.

The predominance of phenolics over flavonoids suggests that non-flavonoid phenols may be major contributors to the observed suppression of oxidative stress markers (MDA, NO) and inflammatory mediators (COX-2, PGE_2).

EDXRF Elemental Profile and Analytical Considerations

EDXRF analysis of the methanolic leaf extract revealed the presence of both essential and non-essential elements (SM2; SM3). Notably, lead (Pb) exhibited the highest apparent percentage concentration (9.13%), a finding that warrants scrutiny.

This observation is analytically and toxicologically significant and should not be interpreted as definitive evidence of plant safety. EDXRF applied to organic-rich matrices such as plant extracts is susceptible to matrix effects, including X-ray absorption and scattering by carbon-rich components, which can artificially inflate peak intensities. In addition, Pb Lα emissions overlap with other elemental lines, increasing the risk of false positives when signal-to-noise thresholds are not rigorously controlled.

Although background and peak cps/mA values were recorded, these values alone do not establish elemental certainty without comparison to detection limits, blank spectra, and confirmatory techniques. Consequently, the reported Pb concentration should be regarded as a screening-level indicator only, not a validated quantitative measurement. No regulatory or nutritional claims can be made based on these data.

From a safety perspective, the apparent Pb signal highlights the critical need for confirmatory analysis using ICP-MS or AAS, particularly if the extract is intended for pharmacological or nutraceutical application. Environmental contamination, soil uptake, or sample handling cannot be excluded. Therefore, rather than supporting safety, the EDXRF findings emphasize the importance of toxic metal monitoring in medicinal plant research.

GC–MS and NMR Structural Confirmation of Stigmasterol

GC–MS analysis of the methanolic extract identified several phytochemical constituents, predominantly sterols, fatty acids, and related bioactive molecules (**Table 2**). From the ethyl acetate fraction, stigmasterol was isolated and structurally confirmed using ^{1}H- and ^{13}C-NMR spectroscopy. The NMR spectral features, including characteristic methyl resonances, olefinic protons, hydroxylated C-3 signals, and steroidal carbon shifts, were consistent with previously reported stigmasterol spectra (**Figure 1**). This represents confirmatory analytical evidence, distinguishing this result

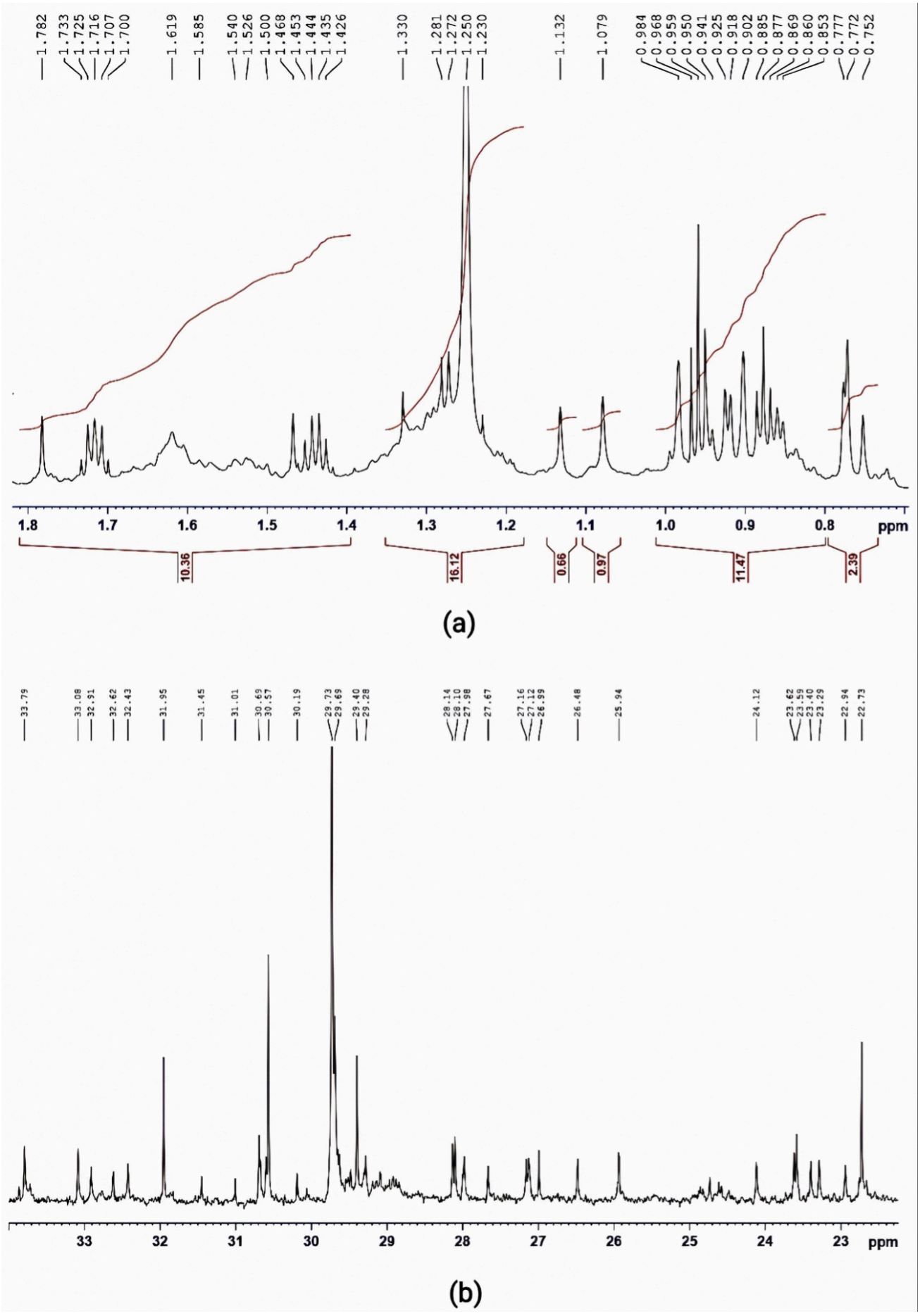

Figure 1. 1H (a) and 13C-NMR (b) spectra of isolated stigmasterol from M. elengi leaf extract.

from the preliminary phytochemical screening (**Table 1**). Stigmasterol has been extensively reported to modulate inflammatory signaling pathways, particularly NF-κB–dependent transcription, and to suppress TNF-α, IL-1β, and COX-2 expression. Its confirmed presence provides a credible molecular link between the chemical composition of *M. elengi* and the observed anti-inflammatory and antinociceptive effects.

Characterization and Biological Performance of Extract-Loaded Chitosan Nanoparticles

The formulated *M. elengi* extract-loaded chitosan nanoparticles exhibited favorable physicochemical characteristics (**Table 3**; **Figure 2**), supporting their suitability as a delivery system. Importantly, biological assays consistently demonstrated that the nanoparticle formulation outperformed the crude extract. In both acetic acid–induced writhing and hot plate models, the nanoparticle-treated group showed the highest inhibition of nociceptive responses, indicating involvement of both peripheral and central analgesic mechanisms (**Tables 4 and 5**; **Figure 3**). These findings correlate with the observed suppression of prostaglandins, nitric oxide, and pro-inflammatory cytokines. The enhanced biological activity is plausibly attributed to improved bioavailability, sustained release, and cellular uptake afforded by chitosan encapsulation rather than increased phytochemical concentration per se. Thus, the biological effects should be interpreted as formulation-dependent, not solely extract-dependent.

Modulation of Inflammatory, Oxidative, and Immune Markers

Treatment with *M. elengi*-loaded chitosan nanoparticles significantly reduced TNF-α, IL-1β, IL-6, COX-2, PGE_2, NO, and MDA levels while increasing IL-10 and IL-22 concentrations (**Figure 4**; **Table 6**). This dual modulation indicates both suppression of inflammatory initiation and promotion of resolution pathways. The concurrent reduction of oxidative stress markers (MDA, iNOS) and restoration of antioxidant enzymes (SOD, CAT, GPx, GSH) support the hypothesis that redox regulation is a central mechanism underlying the observed anti-inflammatory and antinociceptive effects (**Figure 4**; **Table 7**). However, while these biological outcomes are robust, they cannot be quantitatively attributed to individual phytochemicals without targeted pharmacokinetic and dose–response analyses. The results, therefore, demonstrate the biological efficacy of the formulation, not the definitive pharmacological superiority of specific compounds.

Discussion

In this study, albino mice were used to evaluate the phytochemical profile and biological activities of Mimusops elengi leaf extract and its chitosan nanoparticle (NP) formulation, with emphasis on antinociceptive and anti-inflammatory effects. The findings provide experimental support for the pharmacological potential of M. elengi at a screening and formulation level, while also highlighting important analytical and interpretational limitations inherent to preliminary phytochemical and elemental analyses.

Preliminary phytochemical screening indicated the presence of alkaloids, flavonoids, saponins, triterpenoids, phenolic compounds, and fixed oils in the methanolic leaf extract of M. elengi. These assays are qualitative and non-specific, designed to indicate the presence of broad classes of secondary metabolites rather than confirm chemical identity or quantify abundance. Consequently, these findings should be interpreted as a supportive chemical context, not definitive compositional evidence.

Despite these limitations, the detected phytochemical classes are consistent with the biological effects observed *in vivo*. Flavonoids and phenolic compounds are widely reported to exert antioxidant, anti-inflammatory, and antinociceptive effects through modulation of cyclooxygenase (COX), lipoxygenase (LOX), and nitric oxide (NO) pathways, as well as through suppression of pro-inflammatory cytokines such as TNF-α and IL-1β (46-49).

Table 4. Antinociceptive effects of Mimusops elengi extracts in mice.

Group	Treatment	Dose	Mean abdominal writhing (± SE)	% Inhibition of writhing
Group I	Normal Control (Saline)	10 mL	15.05 ± 2.1	0
Group II	Ibuprofen	10 mg/kg	5.02± 1.3	66.67
Group III	Chitosan NPs	50 mg/kg	10.23 ± 1.8	33.33
Group IV	*M. elengi* extract	200 mg/kg	8.12 ± 1.5	46.67
Group V	*M. elengi*-loaded chitosan NPs	200 mg/kg	3.24 ± 1.1	80.00
Note: Results are mean ± SE (n = 5).				

Table 5. Effects of M. elengi leaf extracts on hot plate-induced pain in mice.

Animal group	Hot plate latency (sec)	% Pain inhibition
I. Normal control	10.16 ± 1.02	0
II. Standard drug (morphine)	25.04 ± 1.01	67.44
III. Chitosan NPs	14.01 ± 1.12	10.10
IV. Extract only (200 mg/kg)	18.34 ± 1.18	56.14
V. *M. elengi*-loaded Chitosan NPs	28.66 ± 2.01	80.56
Note: Results are mean ± SE (n = 3).		

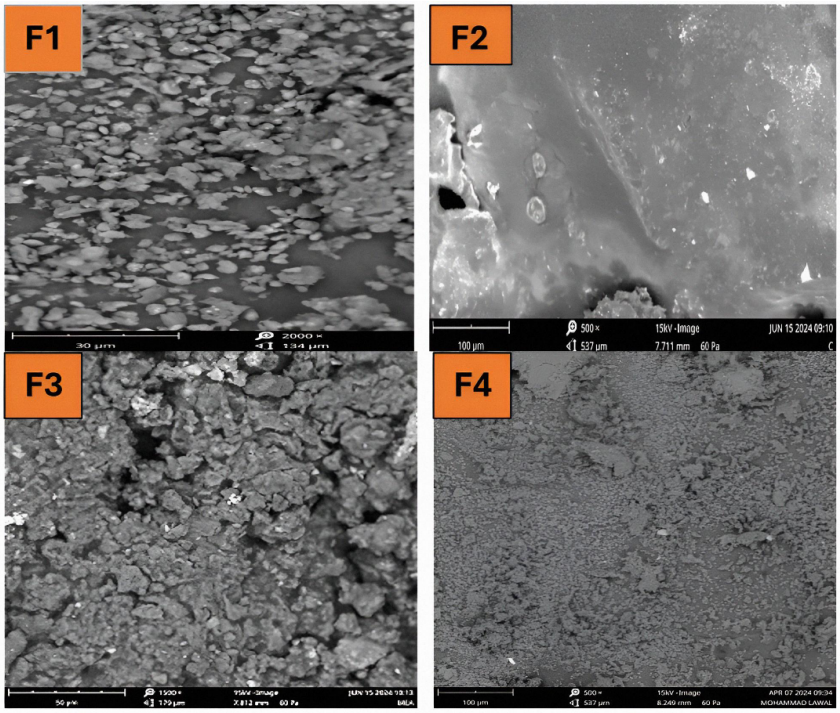

Figure 2. Scanning electron microscopy of formulated M. elengi-loaded chitosan NPs. F2 was used for drug delivery because of its optimum characteristics.

Table 6. Effects of M. elengi-loaded chitosan NPs on inflammatory mediators and markers of mice.

Parameter	Group I (normal control) 10 mL D/H20	Group II (disease model) aspirin	Group III (plant extract) 200 mg/kg	Group IV (chitosan NP) 20 mg/kg	Group V (Plant extract-loaded chitosan NP) 50 mg/kg
TNF-alpha (pg/mL)	35.5 ± 4.3	120.7 ± 8.5**	90.2 ± 6.8*	85.1 ± 6.0*	55.5 ± 5.1**
IL-1B (pg/mL)	25.4 ± 3.2	95.3 ± 7.4**	65.8 ± 5.2*	60.7 ± 4.8*	40.9 ± 3.9**
IL-6 (pg/mL)	18.9 ± 2.8	112.3 ± 10.1**	78.4 ± 7.2*	73.6 ± 6.5*	48.3 ± 4.6**
IL-10 (pg/mL)	10.8 ± 1.5	6.4 ± 0.8**	15.2 ± 2.3**	17.5 ± 2.9**	21.3 ± 3.2**
IL-22 (pg/mL)	5.3 ± 1.1	18.7 ± 3.2**	12.6 ± 1.8*	14.3 ± 2.2*	9.7 ± 1.5*
COX-2 (U/mg protein)	0.45 ± 0.07	1.85 ± 0.15**	1.30 ± 0.13*	1.22 ± 0.10*	0.67 ± 0.09**
PGE2 (ng/mL)	1.2 ± 0.1	4.5 ± 0.6**	3.2 ± 0.5*	2.9 ± 0.4*	1.8 ± 0.3**
MDA (nmol/mg protein)	0.95 ± 0.1	2.5 ± 0.3**	1.8 ± 0.2*	1.6 ± 0.2*	1.1 ± 0.1**
NO (µmol/L)	18.5 ± 2.3	45.6 ± 5.4**	34.2 ± 3.6*	31.5 ± 3.3*	22.1 ± 2.5**
iNOS (U/mg protein)	0.7 ± 0.1	2.8 ± 0.4**	2.0 ± 0.3*	1.8 ± 0.3*	1.1 ± 0.2**
Arginase (U/mg protein)	35.4 ± 5.2	15.2 ± 2.4**	22.8 ± 3.1*	24.5 ± 3.6*	32.1 ± 4.8**
Leucocyte count (×10³ cells/µL)	5.4 ± 0.6	12.8 ± 1.1**	8.5 ± 0.7*	7.9 ± 0.6*	6.2 ± 0.7**
Neutrophil count (%)	20.1 ± 2.3	55.6 ± 6.8**	40.3 ± 5.2*	38.9 ± 5.0*	25.8 ± 3.7**

Note: Results are mean ± SD (n =5). * p<0.05 and ** p< 0.01 were considered statistically significant (one-way ANOVA followed by Dunnett's post hoc test).

Table 7. Effects on oxidative stress markers in carrageenan-induced mice paw oedema.

Animal group	CAT (U/mg protein)	SOD (U/mg protein)	GSH (µmol/g tissue)	GPx (U/mg protein)
I. Normal control	15.0 ± 1.0	12.0 ± 0.8	6.0 ± 0.01	25.0 ± 1.02
II. Standard Drug (aspirin)	30.0 ± 2.0	25.0 ± 1.5	10.0 ± 1.01	40.0 ± 2.03
III. Chitosan NPs	18.0 ± 1.2	14.0 ± 1.0	7.5 ± 0.01	28.0 ± 1.06
IV. 200 mg/kg extract	22.0 ± 1.5	18.0 ± 1.2	9.03 ± 0.01	35.0 ± 1.14
V. *M. elengi*-loaded Chitosan NPs	35.0 ± 2.5	30.0 ± 1.8	12.5 ± 1.02	45.0 ± 1.04

Note: Results are mean ± SE (n = 5).

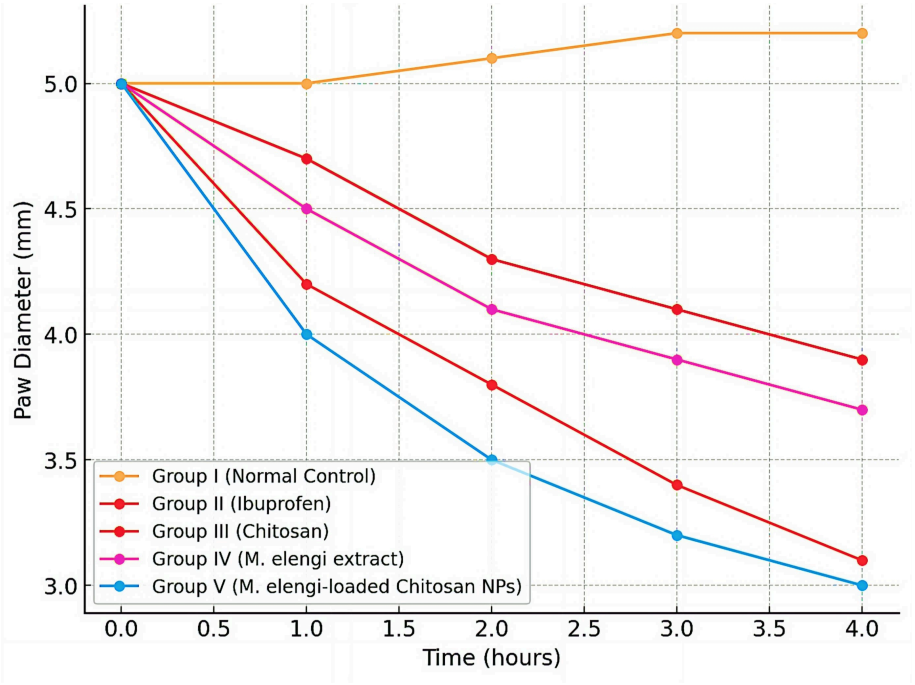

Figure 3. Effects of M. elengi leaf methanol extract-loaded chitosan NPs on paw oedema diameter of mice. Results are mean ± SE (n = 5).

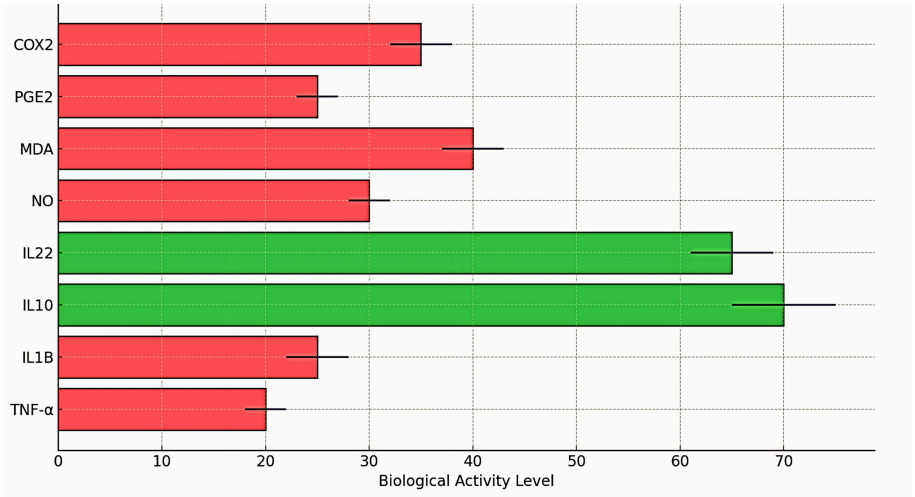

Figure 4. Effects of M. elengi leaf extract-loaded chitosan (Group V) on inflammatory markers of Swiss albino mice. Results are mean ± SD (n = 5).

In the present study, the total phenolic content exceeded the total flavonoid content, suggesting that non-flavonoid phenolics may be major contributors to the observed bioactivity. This aligns with reports indicating that phenolic compounds play a dominant role in reducing oxidative stress and inflammatory mediator production. Saponins and triterpenoids have been associated with immunomodulatory and anti-inflammatory effects, partly through interference with NF-κB and MAPK signaling pathways, while alkaloids have been implicated in both central and peripheral antinociceptive mechanisms, including modulation of opioid and non-opioid pathways (18, 46). However, because individual compounds were not quantified, causal attribution of biological effects to specific phytochemicals cannot be made based on the present data alone.

EDXRF analysis detected several elements in the methanolic extract, including an apparent signal for lead (Pb). While EDXRF is a useful screening tool for multi-element detection, its application to organic-rich plant matrices is subject to matrix effects, peak overlaps, and semi-quantitative limitations(9). As such, the elemental data obtained in this study should be regarded as indicative rather than confirmatory. Importantly, the detection of Pb does not establish safety, nor does it permit comparison with regulatory limits in the absence of validated quantification, detection limits, blanks, and confirmatory analysis (e.g., ICP-MS or AAS). Environmental uptake, soil contamination, or analytical artifacts cannot be excluded. Therefore, the elemental findings underscore the need for rigorous toxic metal validation rather than supporting claims of medicinal safety. This clarification directly addresses concerns regarding overinterpretation of elemental data.

GC–MS profiling revealed a diverse range of extractable constituents, including sterols and fatty acid derivatives. Among these, stigmasterol was isolated from the ethyl acetate fraction and structurally confirmed using ^{1}H and ^{13}C NMR spectroscopy. This represents robust chemical evidence, distinguishing stigmasterol from compounds

inferred only through screening assays. Stigmasterol has been reported to exert anti-inflammatory and antinociceptive effects through suppression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and inhibition of NF-κB signaling (50, 51). Its confirmed presence provides a plausible molecular basis for some of the biological effects observed. Nevertheless, its relative contribution to the overall pharmacological activity cannot be quantitatively determined within the scope of this study.

The chitosan NP formulation consistently exhibited greater antinociceptive and anti-inflammatory activity than the crude extract. This enhancement is most plausibly attributed to formulation-dependent effects, including improved solubility, stability, cellular uptake, and sustained release of bioactive constituents. Chitosan is a biocompatible polymer known to enhance mucosal adhesion and drug residence time, thereby improving biological performance without altering the intrinsic chemistry of the encapsulated compounds (26, 27). The superior efficacy of nanoparticle formulation should therefore be interpreted as a delivery advantage, rather than evidence of increased phytochemical potency. This distinction is critical to avoid overstating the pharmacological implications of nanoencapsulation.

Antinociceptive effects were demonstrated using both the acetic acid–induced writhing test and the hot plate model. The writhing test primarily reflects peripheral nociception mediated by inflammatory mediators such as prostaglandins and bradykinin, while the hot plate test evaluates central pain processing (42, 46). The observed reductions in writhing frequency and increased response latency suggest that the extract-loaded NPs may influence both peripheral inflammatory and central nociceptive pathways. However, while these models indicate analgesic potential, they do not establish specific molecular targets. Assertions regarding opioid receptor involvement or direct central nervous system action should therefore be regarded as speculative and require targeted antagonist or receptor-binding studies for confirmation.

The significant reduction in TNF-α, IL-1β, COX-2, PGE_2, NO, and MDA levels indicates effective suppression of inflammatory signaling and oxidative stress. These mediators are central to the initiation and maintenance of inflammation, and their downregulation is consistent with interference in NF-κB–dependent pathways (45). However, NF-κB activity was not directly measured, and thus mechanistic conclusions are inferred rather than demonstrated. The concurrent upregulation of IL-10 and IL-22 suggests activation of anti-inflammatory and resolution pathways. IL-10 is a key regulator that limits excessive inflammatory responses, while IL-22 contributes to tissue repair and epithelial regeneration (32). This dual modulation indicates that the nanoparticle formulation may not only suppress inflammatory initiation but also support resolution processes.

The reduction of oxidative stress markers (MDA, NO) and restoration of antioxidant defenses (SOD, CAT, GPx, GSH) further support the role of redox regulation in the observed biological effects. Oxidative stress is closely linked to both inflammation and nociception, and attenuation of ROS-mediated damage likely contributes to reduced pain perception and inflammatory burden (52, 53). Nonetheless, antioxidant effects were assessed indirectly, and direct ROS-scavenging assays would strengthen this interpretation.

While the findings demonstrate enhanced biological activity of *M. elengi* extract when delivered via chitosan nanoparticles, claims regarding clinical safety, therapeutic equivalence, or superiority to NSAIDs are not supported by the present data. No pharmacokinetic, chronic toxicity, or comparative efficacy studies were conducted. Therefore, the results should be viewed as preclinical proof-of-concept evidence, rather than a basis for therapeutic substitution.

Overall, this study demonstrates that *M. elengi* leaf extract exhibits antinociceptive and anti-inflammatory activity that is significantly enhanced through chitosan nanoparticle encapsulation. The observed effects are supported by preliminary phytochemical profiling, confirmatory identification of stigmasterol, and modulation of inflammatory, oxidative, and immune markers. However, the qualitative nature of phytochemical screening and the screening-level elemental analysis necessitate cautious interpretation. Further quantitative chemical profiling, mechanistic validation, and safety assessment are essential before translational or therapeutic claims can be made.

Conclusion

This study provides a multi-level screening evaluation of *Mimusops elengi* leaf methanol extract and its chitosan nanoparticle formulation, integrating preliminary phytochemical profiling, quantitative phenolic assays, elemental screening, compound identification, and *in vivo* biological assessment. Phytochemical screening confirmed the presence, but not abundance, of major bioactive classes, while quantitative TPC and TFC assays demonstrated a phenolic-dominant profile consistent with antioxidant and anti-inflammatory activity. GC–MS and NMR analyses provided confirmatory evidence for stigmasterol, establishing a credible molecular basis for the observed biological effects. EDXRF analysis revealed an apparent Pb signal that raises important safety and analyticalconcerns. Due to known matrix effects, spectral overlaps, and lack of confirmatory validation, these findings must be regarded as preliminary indicators only, underscoring the necessity for ICP-MS or AAS confirmation before any safety or nutritional conclusions can be drawn. Biological assays demonstrated that chitosan nanoparticle encapsulation significantly enhanced the antinociceptive and anti-inflammatory efficacy of the extract, likely through improved bioavailability and sustained delivery rather than increased chemical potency. Nevertheless, the results do not establish therapeutic equivalence or safety relative to conventional drugs. Overall, the findings support *M. elengi* as a promising source of bioactive compounds at a screening level, while clearly delineating the limitations of qualitative phytochemistry and EDXRF in isolation. The future studies should: employ LC-MS/MS or HPLC-DAD for quantitative phytochemical profiling; validate elemental findings using ICP-MS/AAS, with strict contamination controls; conduct dose–response and pharmacokinetic studies of isolated compounds; perform sub-chronic toxicity and metal bioaccumulation assessments, and investigate molecular mechanisms using targeted pathway assays (e.g., NF-κB, COX-2). These steps are essential to transition *M. elengi* from preliminary screening toward evidence-based therapeutic development.

Abbreviations

All abbreviations have been interpreted in the manuscript on first use.

Declarations

Author Informations

Cletus Anes Ukwubile
Corresponding Author
Affiliation: Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria.
Contribution: Conceptualization, Data Curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing - Original Draft.

Chidi Kaosi Clement
Affiliation: Department of Human Physiology, College of Health Sciences, Faculty of Basic Medical Sciences, Nile University of Nigeria, Abuja, Nigeria.
Contribution: Formal analysis, Investigation, Methodology, Resources, Visualization, Writing - Review & Editing.

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The data generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Statement

The animals used in this study were approved by the Animal in Research Ethical Committee guidelines of PJ Rat Farms, Nigeria, prohibiting harsh treatment of laboratory animals, with approval number PJRF/RA525/2024. Other international guidelines on the use of laboratory animals, such as ARRIVE, were also followed.

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Additional Information

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