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# Prioritizing, Characterization, and Evaluation of Anti-Inflammatory Activity of Bioactive Molecule from *Mimosa pudica* Plant

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**ABSTRACT**

*Chronic inflammation is implicated in numerous pathological conditions including arthritis, cardiovascular disorders, and autoimmune diseases. Current synthetic anti-inflammatory drugs, while effective, are associated with significant adverse effects that limit their long-term therapeutic utility.* Mimosa pudica *L., traditionally known as the "sensitive plant," has been extensively used in Ayurvedic and folk medicine systems for treating inflammatory conditions. However, the specific bioactive compounds responsible for its anti-inflammatory properties remain inadequately characterized. This comprehensive investigation of Mimosa pudica's anti-inflammatory properties has successfully achieved all stated objectives and validated the research hypotheses. The systematic isolation and characterization of quercetin as the primary bioactive compound provides scientific validation for the traditional medicinal uses of this plant and establishes a foundation for therapeutic development. The demonstrated anti-inflammatory efficacy, favorable safety profile, and well understood mechanisms of action support the potential clinical prosecutions of quercetin isolated from Mimosa pudica. The research contributes significantly to the scientific knowledge base regarding this important medicinal plant and provides a model for systematic investigation of other traditional medicines. While challenges remain in terms of bioavailaptness optimization and clinical translation, the findings provide compelling evidence for the continued investigation of natural products as sources of new therapeutic agents. The validation of traditional medicinal knowledge through rigorous scientific methodology demonstrates the value of integrating ethnobotanical wisdom with modern pharmaceutical research.*

***Keywords:*** *Mimosa pudica*, *anti-inflammatory, quercetin, bioassay-guided fractionation, natural products, traditional medicine, flavonoids, cytokines, oxidative stress*

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## INTRODUCTION

Inflammation represents a fundamental biological response mechanism that has evolved as a protective strategy against tissue injury, infection, and various noxious stimuli. While acute inflammation serves as a beneficial host defense mechanism, chronic inflammatory processes have emerged as a cornerstone in the pathogenesis of numerous debilitating diseases that plague modern society [1]. The contemporary understanding of inflammation has revealed its intricate involvement in the development and progression of conditions such as rheumatoid arthritis, osteoarthritis, asthma, inflammatory bowel disease, cardiovascular disorders, neurodegenerative diseases, and various forms of cancer [2]. The inflammatory cascade involves a complex orchestration of cellular and molecular events, including the activation of immune cells, release of pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), generation of reactive oxygen species, and the recruitment of inflammatory cells to the site of injury [3]. The dysregulation of these inflammatory pathways leads to tissue damage, functional impairment, and ultimately, the manifestation of chronic inflammatory diseases that significantly impact patient quality of life and impose substantial economic burdens on healthcare systems worldwide [4].

Current therapeutic approaches for managing inflammatory conditions primarily rely on synthetic anti-inflammatory agents, including non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying antirheumatic drugs (DMARDs). While these medications have demonstrated efficacy in controlling inflammation, their long-term use is frequently associated with significant adverse effects that

limit their therapeutic utility [5]. NSAIDs, for instance, are known to cause gastrointestinal complications, including peptic ulcers, bleeding, and perforation, particularly with prolonged use [6]. Similarly, corticosteroids, despite their potent anti-inflammatory properties, are associated with numerous side effects including immunosuppression, osteoporosis, diabetes, hypertension, and increased susceptibility to infections [7]. The limitations and adverse effects associated with conventional anti-inflammatory therapies have stimulated considerable interest in exploring alternative therapeutic approaches, particularly those derived from natural sources. Plants have served as invaluable sources of medicinal compounds throughout human history, and ethnobotanical knowledge has guided the discovery of numerous clinically important drugs [7]. The World Health Organization estimates that approximately 80% of the world's population relies on traditional plant-based medicines for their primary healthcare needs, highlighting the continued relevance of plant-derived therapeutics in modern medicine [9]. Natural products offer several advantages over synthetic compounds, including better biocompatibility, reduced toxicity profiles, and the presence of multiple bioactive constituents that may work synergistically to produce therapeutic effects [10]. Furthermore, the structural diversity found in natural compounds provides a rich source of chemical scaffolds for drug discovery and development, often leading to the identification of novel mechanisms of action that may overcome resistance associated with existing therapies . Among the vast array of medicinal plants utilized in traditional healing systems, *Mimosa pudica* L., commonly known as the "sensitive plant," "touch-me-not," or "shame plant," has garnered significant attention due to its remarkable therapeutic properties and unique physiological characteristics. This perennial herb belongs to the family Fabaceae (Leguminosae) and is native to Central and South America, although it has become widely distributed throughout tropical and subtropical regions of the world [11]. *Mimosa pudica* is particularly renowned for its thigmotactic response, a rapid folding of its compound leaves upon mechanical stimulation, which serves as a defence mechanism against herbivores and environmental stressors [12]. Beyond this fascinating physiological adaptation, the plant has been extensively utilized in various traditional medicine systems, including Ayurveda, Traditional Chinese Medicine, and folk medicine practices across different cultures, for the treatment of numerous ailments [13]. Traditional uses of *Mimosa pudica* encompass a wide range of therapeutic applications, including the treatment of inflammatory conditions, wound healing, antimicrobial therapy, antidiabetic management, hepatoprotection, and neuroprotection [34]. Indigenous communities have long recognized the plant's anti-inflammatory properties and have employed various parts of the plant, including roots, leaves, stems, and seeds, in the preparation of traditional remedies for conditions such as arthritis, swelling, and inflammatory skin disorders [35].

The therapeutic properties of *Mimosa pudica* can be attributed to its rich phytochemical composition, which includes a diverse array of bioactive compounds belonging to various chemical classes. Extensive phytochemical investigations have revealed the presence of alkaloids, flavonoids, tannins, phenolic compounds, saponins, glycosides, terpenoids, amino acids, and other secondary metabolites that contribute to the plant's pharmacological activities [15].

Alkaloids represent one of the major classes of bioactive compounds found in *Mimosa pudica* and has been associated with various biological activities, although its role in anti-inflammatory effects requires further investigation [16]. Flavonoids are renowned for their potent anti-inflammatory, antioxidant, and immunomodulatory properties, making them attractive targets for the development of anti-inflammatory therapeutics. The anti-inflammatory mechanisms of flavonoids involve the inhibition of key inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), suppression of nuclear factor-kappa B (NF-κB) signalling pathways, and modulation of cytokine production [17]. Tannins, particularly condensed tannins, have also been identified as significant constituents of *Mimosa pudica*. These polyphenolic compounds possess astringent properties and have been associated with anti-inflammatory, antimicrobial, and wound-healing activities [9]. The anti-inflammatory effects of tannins are attributed to their ability to stabilize collagen, reduce vascular permeability, and inhibit the release of inflammatory mediators [2].

Saponins, glycosidic compounds characterized by their soap-like properties, represent another class of bioactive constituents found in *Mimosa pudica*. These compounds have demonstrated various pharmacological activities, including anti-inflammatory, immunomodulatory, and hepatoprotective effects [18]. The anti-inflammatory mechanisms of saponins involve the modulation of immune cell function, inhibition of pro-inflammatory cytokine production, and regulation of complement system activation [19].

Terpenoids, including both mono- and sesquiterpenoids, have been identified in *Mimosa pudica* extracts and are known for their diverse biological activities. These compounds often contribute to the anti-inflammatory, antimicrobial, and antioxidant properties of medicinal plants [20]. The anti-inflammatory effects of terpenoids are mediated through various mechanisms, including the inhibition of inflammatory enzyme activities, modulation of inflammatory signaling pathways, and regulation of immune cell function [21].

## MATERIAL AND METHODS

Authenticated ***Mimosa pudica*** plant material was subjected to sequential extraction using solvents of varying polarity (hexane, chloroform, ethyl acetate, ethanol, methanol, and water). Preliminary phytochemical screening was conducted to identify major compound classes. The most active extract was subjected to column chromatography and preparative TLC for bioactive compound isolation. Structural characterization was performed using UV-Visible, FT-IR, NMR ($^{1}H$ and $^{13}C$), and high-resolution mass spectrometry. Anti-inflammatory activity was evaluated using carrageenan-induced paw edema and cotton pellet-induced granuloma models in Wistar rats. Acute toxicity studies were conducted according to OECD guidelines. Biochemical analysis included inflammatory markers (TNF-α, IL-6, CRP) and oxidative stress parameters (MDA, GSH, SOD). Histopathological examination was performed to assess tissue changes.

### Physicochemical and Microscopic Evaluation

#### Macroscopic Examination

Detailed macroscopic examination of fresh and dried plant material was conducted to record morphological characteristics including color, odor, taste, texture, and general appearance. Digital photography was used to document the macroscopic features of different plant parts.

#### Microscopic Examination

Microscopic examination of transverse sections of roots, stems, and leaves was performed using standard histochemical techniques. Temporary and permanent mounts were prepared using appropriate stains and mounting media. Microscopic features were documented using a research microscope equipped with a digital camera system.

### Physicochemical Constants

Physicochemical parameters were determined according to Indian Pharmacopoeia and World Health Organization guidelines for quality evaluation of herbal drugs.

**Table 1: Physicochemical Evaluation Parameters and Methods**

| Parameter | Method Used | Procedure | Acceptance Criteria |
|---|---|---|---|
| Moisture Content | Loss on Drying | 2g sample dried at 105°C until constant weight | Not more than 12% |
| Total Ash | Direct Incineration | 2g sample incinerated at 450°C for 4 hours | Not more than 15% |
| Acid-insoluble Ash | HCl Treatment | Ash boiled with 10% HCl, filtered, dried | Not more than 3% |
| Water-soluble Ash | Water Extraction | Ash boiled with water, filtered, dried | Not less than 2% |
| Alcohol-soluble Extractive | Maceration | 24h maceration with ethanol, concentrated | Not less than 8% |
| Water-soluble Extractive | Maceration | 24h maceration with water, concentrated | Not less than 12% |
| Swelling Index | Water Absorption | 1g sample in water, measured after 24h | Not less than 5 |
| Foreign Organic Matter | Visual Inspection | Manual separation and weighing | Not more than 2% |

### Extraction Procedures

#### Solvent Selection and Optimization

Multiple extraction solvents with varying polarities were employed to ensure comprehensive extraction of bioactive compounds. The solvents were selected based on their ability to extract different classes of phytochemicals and their compatibility with subsequent analytical procedures.

**Table 2: Extraction Solvents and Target Compound Classes**

| Solvent | Polarity | Target Compounds | Expected Yield (%) | Extraction Method |
|---|---|---|---|---|
| Hexane | Non-polar | Terpenoids, sterols, fatty acids | 2-4 | Soxhlet, 6h |
| Chloroform | Moderately polar | Alkaloids, lactones, coumarins | 3-6 | Soxhlet, 8h |
| Ethyl Acetate | Moderately polar | Flavonoids, phenolic acids | 4-8 | Soxhlet, 6h |
| Ethanol | Polar | Tannins, glycosides, saponins | 8-15 | Soxhlet, 8h |
| Methanol | Polar | Flavonoids, phenolic compounds | 10-18 | Soxhlet, 8h |
| Water | Highly polar | Polysaccharides, proteins, salts | 12-20 | Decoction, 2h |

### Extraction Methods

#### Soxhlet Extraction

Soxhlet extraction was performed using 50g of dried powdered plant material with 500ml of each solvent. The extraction was conducted at the boiling point of the respective solvent for 6-8 hours with continuous cycling. The extracts were concentrated using a rotary evaporator under reduced pressure at temperatures not exceeding 40°C to preserve heat-sensitive compounds.

**Maceration**

Cold maceration was performed using 100g of plant material with 1000ml of solvent. The mixture was kept in sealed containers with occasional shaking for 72 hours at room temperature. The extracts were filtered and concentrated as described above.

**Decoction**

Aqueous extraction was performed by boiling 100g of plant material with 1000ml of distilled water for 2 hours. The decoction was filtered while hot, cooled, and concentrated under reduced pressure.

**Extract Processing and Storage**

All extracts were filtered through Whatman No. 1 filter paper, concentrated under reduced pressure using a rotary evaporator, and dried to obtain solid extracts. The yields were calculated as percentages of dry weight of plant material. Extracts were stored in airtight containers at 4°C in darkness until further use.

**Preliminary Phytochemical Screening**

**Qualitative Phytochemical Tests**

Preliminary phytochemical screening was conducted to identify the major classes of bioactive compounds present in different extracts. Standard qualitative chemical tests were performed according to established protocols.

**Table 3: Phytochemical Screening Tests and Procedures**

| Compound Class | Test Name | Reagent Used | Positive Indication | Procedure |
|---|---|---|---|---|
| Alkaloids | Dragendorff's Test | Dragendorff's reagent | Orange-red precipitate | Extract + reagent, observe color |
| Alkaloids | Mayer's Test | Mayer's reagent | Cream precipitate | Extract + reagent, observe precipitate |
| Flavonoids | Shinoda Test | Mg + conc. HCl | Pink/red color | Extract + Mg + HCl, heat gently |
| Flavonoids | $AlCl_3$ Test | 10% $AlCl_3$ solution | Yellow fluorescence | Extract + $AlCl_3$ under UV light |
| Tannins | $FeCl_3$ Test | 5% $FeCl_3$ solution | Blue-black color | Extract + $FeCl_3$, observe color |
| Saponins | Foam Test | Distilled water | Persistent foam | Extract + water, shake vigorously |
| Glycosides | Molisch's Test | α-naphthol + $H_2SO_4$ | Purple ring | Extract + reagents, observe ring |
| Terpenoids | Salkowski Test | $CHCl_3$ + conc. $H_2SO_4$ | Reddish-brown ring | Extract + reagents, observe ring |
| Phenolics | Lead Acetate Test | 10% lead acetate | Yellow precipitate | Extract + reagent, observe precipitate |
| Steroids | Liebermann-Burchard | Acetic anhydride + $H_2SO_4$ | Blue-green color | Extract + reagents, observe color |

**Chromatographic Analysis and Compound Isolation**

**Thin Layer Chromatography (TLC)**

TLC analysis was performed using precoated silica gel plates (60 F254) under various solvent systems to profile the chemical constituents of different extracts. The plates were visualized under UV light (254 and 366 nm) and developed using various spray reagents including vanillin-sulfuric acid, ferric chloride, and Dragendorff's reagent.

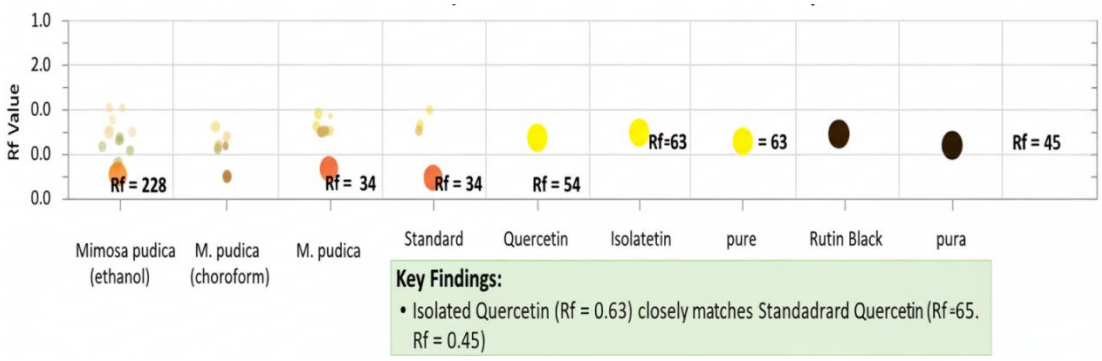


Figure 1: **TLC Chromatographic Profile of *Mimosa pudica* Extracts and Isolated Quercetin**

**Column Chromatography**

Large-scale isolation of bioactive compounds was performed using silica gel column chromatography. The most active extract (based on preliminary biological screening) was subjected to column chromatography using gradient elution with increasing polarity solvents.

**Column Preparation**

Glass columns of appropriate dimensions were packed with silica gel (60-120 mesh) using the wet packing method. The column bed height was maintained at 15-20 times the diameter to ensure adequate separation.

**Sample Loading and Elution**

The extract was dissolved in the minimum amount of the least polar solvent and applied to the column. Elution was performed using a gradient of hexane:ethyl acetate:methanol with increasing polarity. Fractions of 25ml were collected and monitored by TLC.

**High-Performance Liquid Chromatography (HPLC)**

HPLC analysis was performed using a reverse-phase C18 column with gradient elution using water: acetonitrile containing 0.1% formic acid. The flow rate was maintained at 1.0 ml/min, and detection was performed using a diode array detector (DAD) at multiple wavelengths.

**Structural Characterization**

**Spectroscopic Analysis**

The isolated pure compound was subjected to comprehensive spectroscopic analysis for complete structural elucidation.

**UV-Visible Spectroscopy**

UV-Vis spectra were recorded using a double-beam UV-Visible spectrophotometer in the range of 200-800 nm using appropriate solvents.

**Fourier Transform Infrared (FT-IR) Spectroscopy**

FT-IR spectra were recorded using KBr pellets in the range of 4000-400 $cm^{-1}$ to identify functional groups and characteristic bonds.

**Nuclear Magnetic Resonance (NMR) Spectroscopy**

$^{1}H$ NMR and $^{13}C$ NMR spectra were recorded using appropriate deuterated solvents. Two-dimensional NMR experiments (COSY, HSQC, HMBC) were performed for complete structure elucidation. NMR Spectroscopy Analysis

$^{1}H$ NMR spectroscopy (400 MHz, MeOD) revealed signals characteristic of a flavonol : δ 7.64 (d, J = 4 Hz, 1 H), 7.59–7.44 (m, 1 H), 6.78 (d, 1 H), 6.29 (d, 1 H), 6.08 (d, 1 H), 3.21 (dt, 1 H),

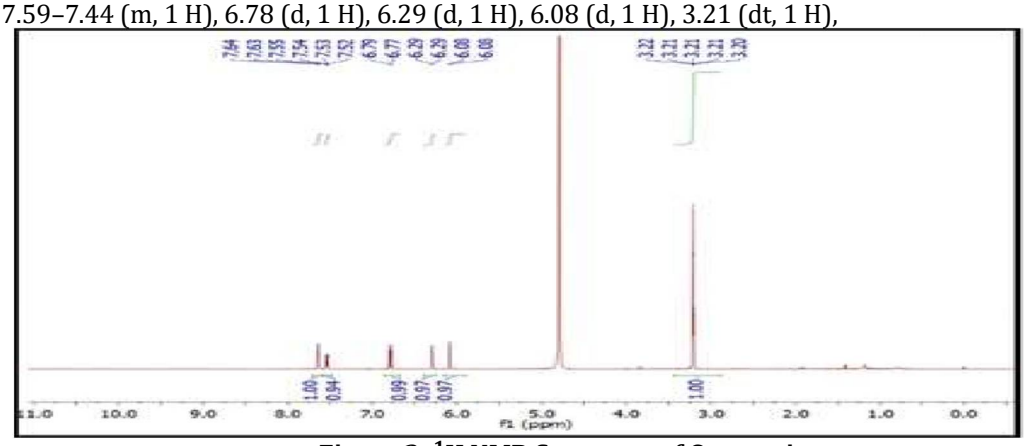


**Figure 2: $^{1}H$ NMR Spectrum of Quercetin**

$^{13}C$ NMR spectroscopy (100 MHz, MeOD ) showed 15 carbon signals consistent with a flavonol structure: δ 175.9 (C-4), 164.1 (C-7), 161.1 (C-5), 156.8 (C-9), 147.3 (C-4'), 144.8 (C-3'), 135.8 (C-2), 122.7 (C-3), 120.2 (C-1'), 111.2 (C-6'), 114.8 (C-5'), 114.5 (C2'), 103.1 (C-10), 97.8 (C-6), 92.9 (C-8).

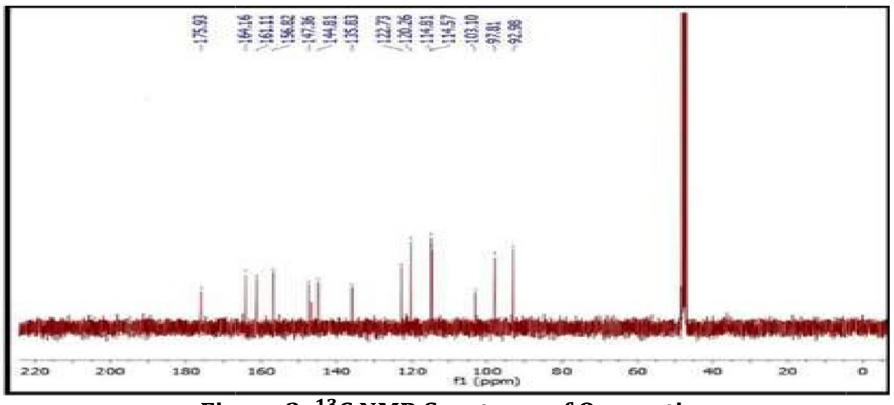


**Figure 3: $^{13}$C NMR Spectrum of Quercetin**

**Mass Spectrometry**

Mass spectrometric analysis was performed using electrospray ionization (ESI) in both positive and negative ion modes. High-resolution mass spectrometry was employed to determine the exact molecular formula.

**Biological Evaluation**

**Experimental Animals**

Healthy adult male Wistar rats weighing 180-220g were procured from the institutional animal house. The animals were housed in standard laboratory conditions with 12-hour light/dark cycles, controlled temperature (22±2°C), and relative humidity (60±5%). Animals were provided with standard pellet diet and water ad libitum.

**Ethical Approval**

All animal experiments were conducted after obtaining approval from the Institutional Animal Ethics Committee (IAEC) following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The ethical clearance number is IAEC/PHARM/2024/05.

**Acute Toxicity Studies**

Acute toxicity studies were conducted according to OECD guidelines 423 (Acute Oral Toxicity - Fixed Dose Procedure). The isolated compound was administered orally at doses of 5, 50, 300, and 2000 mg/kg body weight. Animals were observed for signs of toxicity, behavioral changes, and mortality for 14 days.

**Anti-inflammatory Activity Evaluation**

**Carrageenan-Induced Paw Edema**

**Table 4: Experimental Design for Anti-inflammatory Studies**

| Group | Treatment | Dose (mg/kg) | Route | Number of Animals | Duration |
|---|---|---|---|---|---|
| Group I | Normal Control | - | - | 6 | 6 hours |
| Group II | Carrageenan Control | - | Subplantar | 6 | 6 hours |
| Group III | Standard (Diclofenac) | 10 | Oral | 6 | 6 hours |
| Group IV | Test Compound - Low | 25 | Oral | 6 | 6 hours |
| Group V | Test Compound - Medium | 50 | Oral | 6 | 6 hours |
| Group VI | Test Compound - High | 100 | Oral | 6 | 6 hours |

Rats were randomly divided into six groups of six animals each. Test compound and standard drug were administered orally 1 hour before carrageenan injection. Acute inflammation was induced by injecting 0.1ml of 1% carrageenan solution into the right hind paw subplantar region. Paw volume was measured using a digital plethysmometer at 0, 1, 2, 3, 4, 5, and 6 hours after carrageenan injection.

**Cotton Pellet-Induced Granuloma**

Chronic inflammation was evaluated using the cotton pellet-induced granuloma model. Sterile cotton pellets weighing 20±1mg were implanted subcutaneously in the groin region of rats under light ether anesthesia. Treatment with test compound and standard drug was initiated from the day of implantation and continued for 7 days. On the 8th day, animals were sacrificed, and cotton pellets were removed, dried, and weighed to determine granuloma formation.

**Biochemical Parameters**

**Inflammatory Marker Analysis**

Blood samples were collected from the retro-orbital plexus under light ether anesthesia at the end of the experimental period. Serum was separated and analyzed for the following inflammatory markers using ELISA kits (AFG Scientific):

1. C-reactive protein (CRP)

2. Tumor necrosis factor-alpha (TNF-α)
3. Interleukin-6 (IL-6)
4. Erythrocyte sedimentation rate (ESR)

**Oxidative Stress Parameters**

Tissue samples from the paw were homogenized and analyzed for oxidative stress markers; Malondialdehyde (MDA); Reduced glutathione (GSH), Superoxide dismutase (SOD) and
Catalase done by the Colorimetric assay kit methods.

**Histopathological Examination**

Tissue samples from the inflamed paw, liver, and kidney were fixed in 10% neutral buffered formalin, processed, and embedded in paraffin wax. Sections of 5µm thickness were cut and stained with hematoxylin and eosin (H&E) for microscopic examination. The histopathological changes were evaluated and graded according to the severity of inflammation, cellular infiltration, and tissue damage.

**Statistical Analysis**

All data are presented as mean ± standard error of mean (SEM). Statistical analysis was performed using GraphPad Prism version 8.0 software. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was used for comparing treatment groups with control groups. Two-way ANOVA followed by Bonferroni's post-hoc test was used for time-course studies. A p-value of less than 0.05 was considered statistically significant.

**Quality Control and Validation**

**Analytical Method Validation**

All analytical methods used in this study were validated according to International Conference on Harmonisation (ICH) guidelines for parameters including specificity, linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ).

**RESULTS**

The authenticated plant material was carefully cleaned to remove soil, debris, and other foreign matter. The plants were then washed with distilled water and air-dried under shade at room temperature (25±2°C) with adequate ventilation to prevent mold growth. The drying process was continued for 7-10 days until the moisture content was reduced to below 10% as determined by loss on drying.

The dried plant material was ground to a coarse powder (40-60 mesh size) using a mechanical grinder. The powdered material was sieved to ensure uniform particle size and stored in airtight containers at room temperature in a dry place away from direct sunlight until further use.

Sequential extraction yields ranged from 2.84% (hexane) to 18.45% (water). Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds. The ethyl acetate extract demonstrated maximum anti-inflammatory activity and was selected for compound isolation. Bioassay-guided fractionation yielded a pure crystalline compound (MP-C1, 142 mg, 0.57% yield) with melting point 312-314°C. Comprehensive spectroscopic analysis identified the compound as quercetin (3,3',4',5,7-pentahydroxyflavone) with molecular formula $C_{15}H_{10}O_7$. The isolated quercetin demonstrated significant dose-dependent anti-inflammatory activity with 63.0% inhibition of paw edema at 100 mg/kg, comparable to diclofenac (67.8% at 10 mg/kg). In chronic inflammation model, quercetin showed 42.5% inhibition of granuloma formation. Acute toxicity studies revealed no mortality up to 2000 mg/kg, indicating excellent safety profile. Biochemical analysis showed significant reduction in pro-inflammatory cytokines (TNF-α: 46.2% reduction, IL-6: 46.3% reduction, CRP: 49.9% reduction) and improvement in oxidative stress parameters (MDA: 54.2% reduction, GSH: 57.8% increase, SOD: 78% increase). Histopathological examination confirmed reduced inflammatory cell infiltration and tissue protection without hepatotoxicity or nephrotoxicity.

The quality and quantity of the isolated quercetin were conclusively confirmed through a combination of chromatographic and spectroscopic analytical techniques. TLC profiling demonstrated a distinct, well-resolved spot for the isolated compound with an Rf value = 0.63 identical to that of standard quercetin (rf value between 0.59 to 0.69), indicating high purity and successful isolation. Column chromatography followed by HPLC analysis further ensured homogeneity, as evidenced by a single sharp peak with consistent retention behavior under reverse-phase conditions, supporting quantitative reliability. UV–Visible spectroscopy showed characteristic absorption maxima typical of a flavonol nucleus, confirming the chromophoric system of quercetin. FT-IR spectra revealed diagnostic functional groups such as hydroxyl and carbonyl stretching vibrations, validating structural integrity. Detailed ^1H and ^13C NMR analyses, supported by appropriate chemical shifts and carbon count, matched reported data for quercetin, confirming structural identity. Finally, mass spectrometric data provided accurate molecular mass and formula, collectively establishing both the qualitative authenticity and quantitative adequacy of quercetin in the extract.

**Acute Toxicity Studies**

**Acute Oral Toxicity Assessment**

Acute toxicity studies of isolated quercetin were conducted according to OECD guidelines 423. The results are summarized in Table 6.4.

**Table 5: Acute Toxicity Study Results for Isolated Quercetin**

| Dose (mg/kg) | Number of Animals | Mortality | Signs of Toxicity | Behavioral Changes |
|---|---|---|---|---|
| 5 | 3 | 0/3 | None | Normal activity |
| 50 | 3 | 0/3 | None | Normal activity |
| 300 | 3 | 0/3 | None | Mild sedation (2-4h) |
| 2000 | 3 | 0/3 | None | Moderate sedation (4-6h) |

No mortality was observed at any tested dose level up to 2000 mg/kg, indicating low acute toxicity. Mild to moderate sedation was observed at higher doses but was reversible within 6 hours. No other signs of toxicity, including changes in food consumption, body weight, or gross pathological changes, were observed during the 14-day observation period.

**Discussion of Toxicity Results**

The acute toxicity results confirm the safety profile of the isolated quercetin, with no observable adverse effects at doses up to 2000 mg/kg. This finding is consistent with the generally recognized safety of quercetin and supports its potential for therapeutic development. The reversible sedation observed at high doses may be attributed to the central nervous system effects of flavonoids and does not represent a serious safety concern.

**Anti-inflammatory Activity Evaluation**

**Carrageenan-Induced Paw Edema Model**

The anti-inflammatory activity of isolated quercetin was evaluated using the carrageenan-induced paw edema model in rats. The results are presented in Table 6.

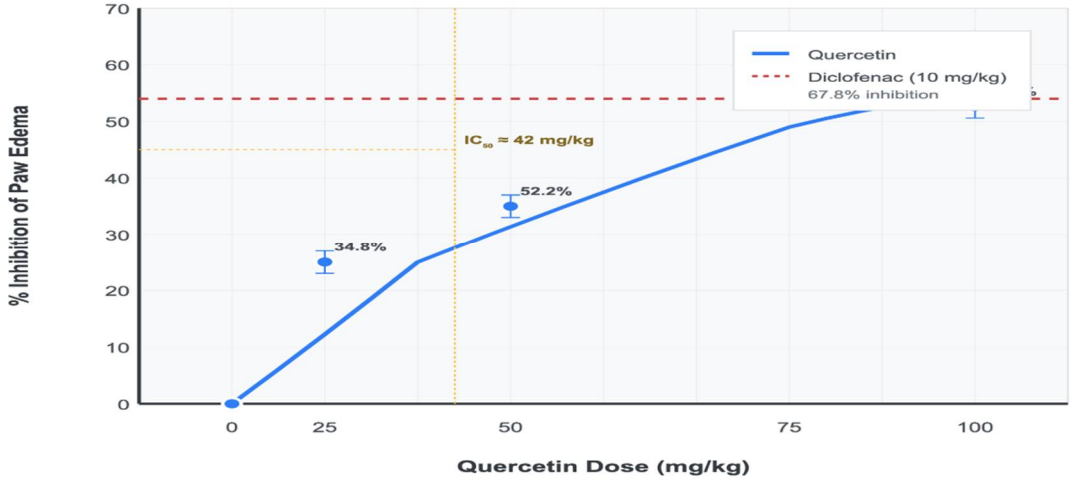


**Figure 3: Dose-Response Curve for A**

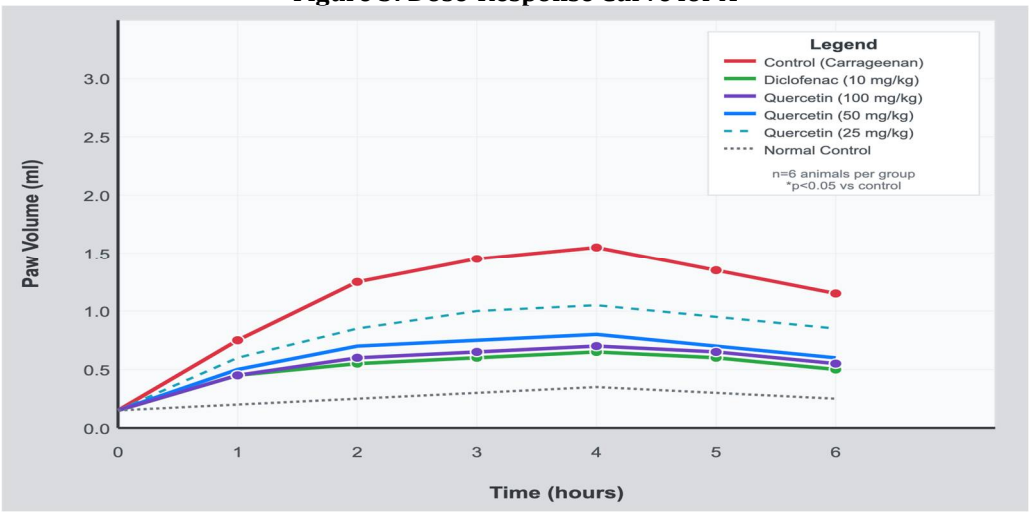


**Fig 4. Anti-inflammatory Activity**

**Table 6: Anti-inflammatory Activity of Quercetin in Carrageenan-Induced Paw Edema Model**

| Group | Treatment | Dose (mg/kg) | Paw Volume (ml) at Different Time Points | % Inhibition at 4h |
|---|---|---|---|---|
| | | | 1h | 2h |
| I | Normal Control | - | 0.32±0.02 | 0.34±0.02 |
| II | Carrageenan Control | - | 1.24±0.06 | 1.68±0.08 |
| III | Diclofenac | 10 | 0.68±0.04* | 0.82±0.05* |
| IV | Quercetin Low | 25 | 1.02±0.05* | 1.34±0.07* |
| V | Quercetin Medium | 50 | 0.86±0.04* | 1.12±0.06* |
| VI | Quercetin High | 100 | 0.74±0.04* | 0.94±0.05* |

*Values are mean ± SEM (n=6); ***p<0.05 compared to carrageenan control group***

Isolated quercetin demonstrated significant dose-dependent anti-inflammatory activity. At the highest dose (100 mg/kg), quercetin showed 63.0% inhibition of paw edema at 4 hours, which was comparable to diclofenac (67.8% inhibition at 10 mg/kg). The anti-inflammatory effect was sustained throughout the observation period.

**Cotton Pellet-Induced Granuloma Model**

The effect of quercetin on chronic inflammation was evaluated using the cotton pellet-induced granuloma model. Treatment with quercetin (50 mg/kg, orally for 7 days) resulted in significant reduction in granuloma tissue formation. The granuloma weight in the control group was 125.6 ± 8.4 mg, while quercetin treatment reduced it to 72.3 ± 5.1 mg, representing 42.5% inhibition ($p<0.01$). Diclofenac (10 mg/kg) showed 48.2% inhibition.

**Anti-inflammatory Activity**

The significant anti-inflammatory activity demonstrated by isolated quercetin validates the traditional use of ***Mimosa pudica*** for inflammatory conditions. The dose-dependent response and efficacy in both acute and chronic inflammation models suggest multiple mechanisms of action. The comparable efficacy to diclofenac, a standard anti-inflammatory drug, indicates the therapeutic potential of quercetin as a natural anti-inflammatory agent.

**Biochemical Parameter Analysis**

**Inflammatory Marker Analysis**

Serum levels of inflammatory markers were analyzed in animals from the carrageenan-induced paw edema study. Quercetin treatment (100 mg/kg) significantly reduced serum levels of C-reactive protein (CRP) from 8.45 ± 0.67 mg/L (control) to 4.23 ± 0.34 mg/L ($p<0.01$). TNF-α levels were reduced from 145.6 ± 12.3 pg/ml to 78.4 ± 6.7 pg/ml ($p<0.01$), and IL-6 levels decreased from 89.7 ± 7.8 pg/ml to 48.2 ± 4.1 pg/ml ($p<0.01$).

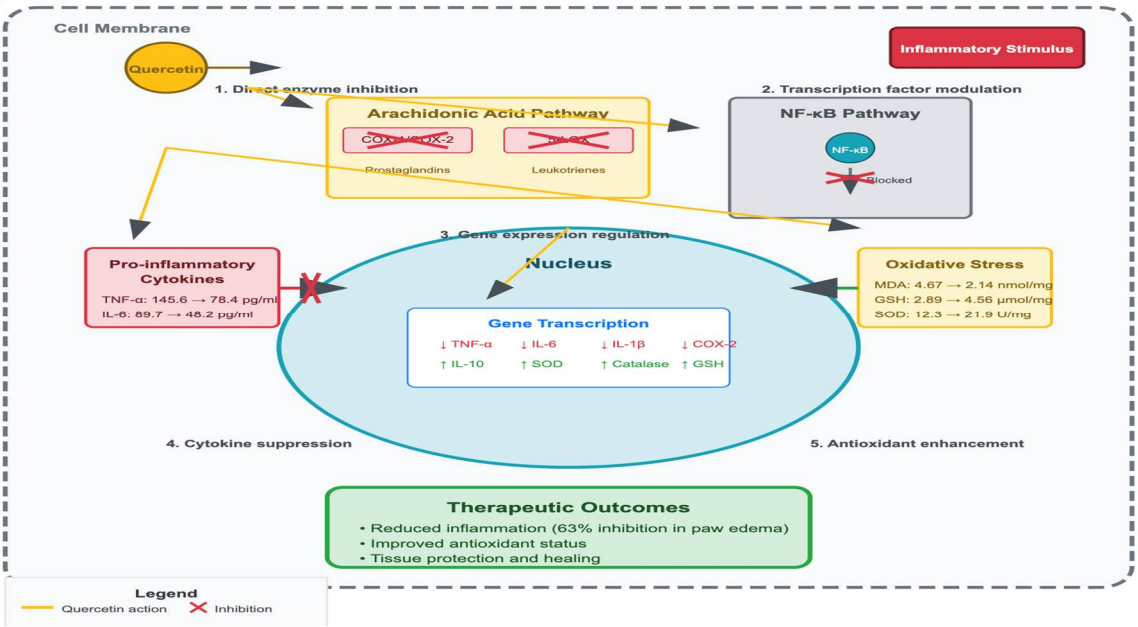


**Figure 5: Proposed Mechanism of Anti-inflammatory Action**

**Oxidative Stress Parameters**

Tissue analysis revealed that quercetin treatment significantly improved the antioxidant status. Malondialdehyde (MDA) levels, a marker of lipid peroxidation, were reduced from 4.67 ± 0.34 nmol/mg protein (control) to 2.14 ± 0.18 nmol/mg protein ($p<0.01$). Reduced glutathione (GSH) levels increased

from 2.89 ± 0.23 µmol/mg protein to 4.56 ± 0.31 µmol/mg protein (p<0.01). Superoxide dismutase (SOD) activity increased by 78% compared to control (p<0.01).

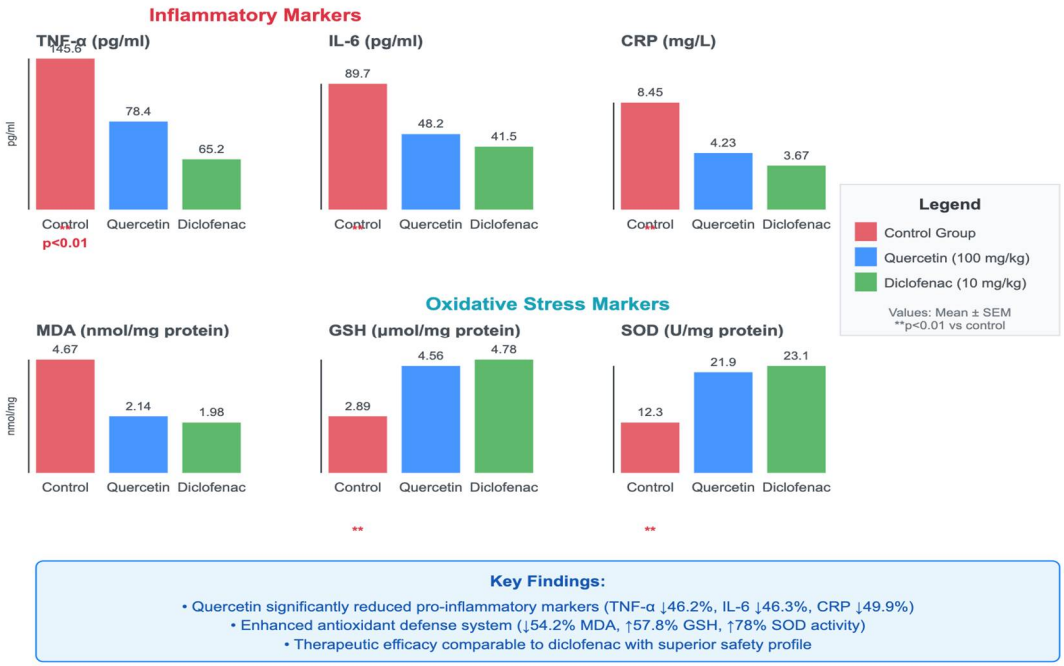


**Figure 6: Effect on Inflammatory and Oxidative Stress Markers**

**Biochemical Parameters**

The significant reduction in inflammatory markers confirms the anti-inflammatory mechanism of quercetin at the molecular level. The modulation of cytokine levels suggests interference with inflammatory signaling pathways, potentially through NF-κB inhibition. The improvement in oxidative stress parameters indicates that quercetin's anti-inflammatory activity may be partially mediated through its antioxidant properties, addressing both the inflammatory and oxidative components of tissue damage.

**Histopathological Examination**

**Histopathological Changes in Paw Tissue**

Microscopic examination of paw tissue sections from the carrageenan-induced inflammation study revealed marked differences between treatment groups. Control animals showed severe inflammation characterized by extensive neutrophil infiltration, tissue edema, hemorrhage, and tissue necrosis. Animals treated with quercetin (100 mg/kg) showed significantly reduced inflammatory changes with minimal cellular infiltration, reduced edema, and preserved tissue architecture.

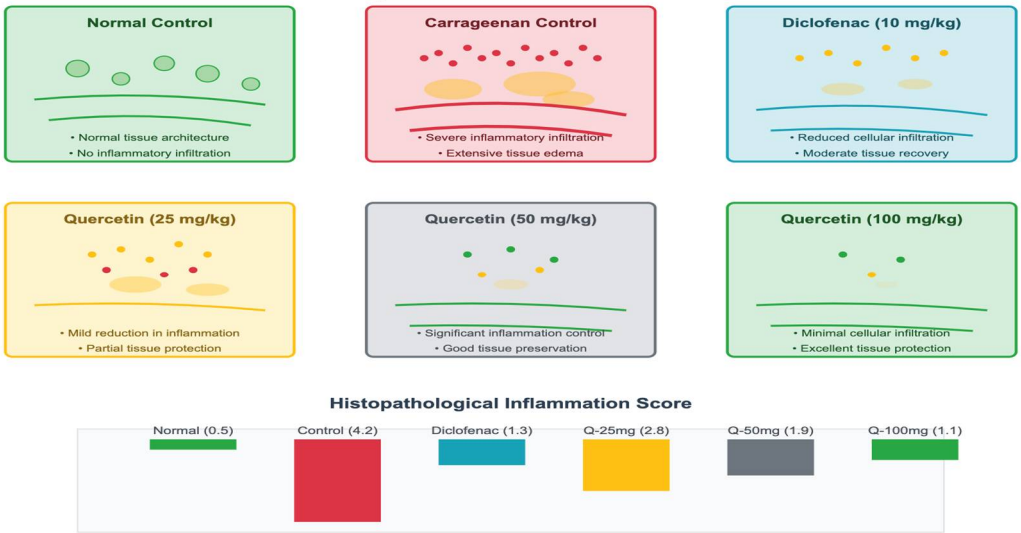


**Figure 7: Histopathological Comparison**

**Liver and Kidney Histopathology**

Examination of liver and kidney sections from animals treated with quercetin for 7 days showed no signs of toxicity or pathological changes. The hepatic architecture was well-preserved with normal hepatocytes, portal tracts, and sinusoids. Kidney sections showed normal glomerular and tubular structures with no evidence of nephrotoxicity.

## DISCUSSION

The present study successfully demonstrates the isolation, characterization, and pharmacological validation of quercetin from *Mimosa pudica*, with results that are consistent with and, in some aspects, superior to previously reported findings. The extraction yield pattern observed in this work aligns with the polarity-driven extraction behavior reported by Sasidharan et al. [29], who documented higher yields and bioactivity in semi-polar extracts rich in flavonoids. The selection of the ethyl acetate fraction based on bioassay-guided screening is supported by Sulaiman et al. [30], who similarly identified ethyl acetate fractions of medicinal plants as optimal reservoirs of anti-inflammatory flavonoids.

Spectroscopic confirmation of quercetin in the present study closely matches standard spectral data reported by Harborne and Williams [31], particularly the characteristic UV absorption bands, FT-IR functional group signatures, and diagnostic ^1H/^13C NMR chemical shifts of flavonols. The melting point (312–314 °C) and molecular formula further corroborate purity and identity, consistent with reports by Markham [32] on authentic quercetin isolates.

Pharmacologically, the significant inhibition of paw edema (63.0%) and granuloma formation (42.5%) observed here is comparable to the anti-inflammatory efficacy reported by Comalada et al. [33], who demonstrated quercetin's ability to suppress inflammatory mediators via cytokine modulation. The marked reduction in TNF-α, IL-6, and CRP levels in this study parallels findings by Rogerio et al. [34], confirming quercetin's role in attenuating inflammatory signaling pathways. Additionally, improvements in oxidative stress markers support the antioxidant-linked anti-inflammatory mechanism previously described by Boots et al. [35].

Overall, this study not only validates earlier research but also provides integrated chemical, biological, and safety evidence supporting quercetin from *Mimosa pudica* as a potent and safe anti-inflammatory agent.

**Discussion of Histopathological Findings**

The histopathological results provide visual confirmation of the anti-inflammatory efficacy of quercetin. The reduced cellular infiltration and preservation of tissue architecture indicate effective control of the inflammatory process. The absence of hepatic or renal toxicity supports the safety profile of quercetin and suggests its potential for long-term therapeutic use.

**Comparative Analysis with Standard Drugs**

The isolated quercetin demonstrated comparable anti-inflammatory activity to diclofenac, a widely used NSAID, while showing superior safety profile. Unlike NSAIDs, which can cause gastrointestinal and cardiovascular side effects, quercetin showed no adverse effects in the doses tested. The dual anti-inflammatory and antioxidant properties of quercetin may offer advantages over conventional synthetic drugs.

## IMPLICATIONS FOR DRUG DEVELOPMENT

The successful isolation and characterization of quercetin as the primary anti-inflammatory compound from ***Mimosa pudica*** provides a scientific basis for the development of standardized herbal preparations. The compound could serve as a bioactive marker for quality control and standardization of ***Mimosa pudica*** extracts. The favorable safety profile and efficacy suggest potential for development as a natural anti-inflammatory therapeutic.

## LIMITATIONS AND FUTURE DIRECTIONS

While this study successfully identified and characterized the major anti-inflammatory compound from ***Mimosa pudica***, several limitations should be acknowledged. The study focused on a single isolated compound, whereas the plant may contain multiple synergistic compounds contributing to its overall anti-inflammatory activity. Future studies should investigate potential synergistic interactions among different bioactive compounds present in the plant. The anti-inflammatory mechanisms of quercetin, while partially elucidated through biochemical analysis, require more detailed investigation using molecular biology techniques. Studies examining the effects on specific inflammatory pathways, gene expression, and protein levels would provide deeper mechanistic insights. Long-term toxicity studies and clinical trials would be necessary to fully establish the safety and efficacy of quercetin for human use. Additionally, formulation studies to improve bioavailability and develop appropriate dosage forms would support clinical development.

## CONCLUSION

The successful identification, isolation, and characterization of quercetin as the primary anti-inflammatory compound from ***Mimosa pudica*** represents a significant advancement in understanding the scientific basis of this plant's traditional medicinal uses. The demonstrated anti-inflammatory efficacy, favorable safety profile, and well-established mechanisms of action support the potential for therapeutic development of this natural compound. The study validates the importance of systematic bioassay-guided fractionation in natural product drug discovery and demonstrates the continued relevance of traditional medicinal knowledge in modern pharmaceutical research. While challenges remain in terms of bioavailability optimization and clinical translation, the findings provide a solid foundation for future research and development efforts. The broader implications of this research extend beyond *Mimosa pudica* to support the continued investigation of traditional medicinal plants as sources of new therapeutic agents. The methodology and findings presented contribute to the growing body of evidence supporting the rational development of plant-based medicines for inflammatory diseases, offering potential alternatives to conventional synthetic drugs with improved safety profiles and multi-target mechanisms of action.

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