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Extraction, Isolation, And Characterization Of Bioactive Phytoconstituents From Medicinal Plants With Anti-Inflammatory Potential

Pawan Ganesh Nayak[1], Dipanwita Chaudhuri Sil[2], Dipanwita Chaudhuri Sil[3], Brijesh Shivhare[4], Tushar B. Gajjar[5], Jyoti Nanda Sharma[6], Mukul Kumar[7], Yash Prashar[*8]

[1]Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal - 576104, Karnataka, India; Email: pawan.nayak@manipal.edu; pawangnayak@gmail.com
[2]Assistant Professor, Department of Botany, Holy Cross College.Aagartala
[3]Associate Professor: Sharda School of Pharmacy, Sharda University (Formerly Anand College of Pharmacy, Keetham, Agra.), Agra-282005, Uttar Pradesh, India.
[4]Assistant Professor, Department of Lifescience, Faculty of Sciences, Baba Mastnath University, Asthal Bohar, Rohtak , Haryana 124021; brijesh@bmu.ac.in
[5]Associate Professor, Faculty of Pharmacy, Gokul Global University, Sidhpur, Gujarat, India, tbgajjar.gphc@gokuluniversitgy.ac.in
[6]Assistant Professor, School of Pharmaceutical Sciences, Chhatrapati Sahuji Maharaj University, Kanpur, jyotinanda954@gmail.com
[7]Assistant Professor, Department of Pharmaceutics ,School of Pharmaceutical Sciences, Faculty of Pharmacy, IFTM University Moradabad , Email: mukuls994@gmail.com
[8]Professor, Chandigarh College of Pharmacy, Chandigarh Group of Colleges, Landran, Sector 112, Mohali, Punjab, PIN 140307 India, yashprashar@gmail.com*

***Corresponding author:** Dr. Yash Prashar, Professor,
Chandigarh College of Pharmacy, Chandigarh Group of Colleges, Landran, Sector 112, Mohali, Punjab, PIN 140307 India, yashprashar@gmail.com*

Abstract
Inflammation is a complex biological response associated with various acute and chronic disorders, including arthritis, asthma, cardiovascular diseases, and neurodegenerative conditions. Although synthetic anti-inflammatory agents such as NSAIDs and corticosteroids are widely used, their long-term administration often leads to adverse effects. Medicinal plants offer a promising alternative due to the presence of diverse phytoconstituents with potent therapeutic efficacy and improved safety profiles. This study focuses on the extraction, isolation, and characterization of bioactive compounds responsible for anti-inflammatory activity from selected medicinal plants. Various extraction techniques, including maceration, Soxhlet extraction, and ultrasound-assisted extraction, were employed using solvents of varying polarity. Isolated constituents were purified using chromatographic strategies such as column chromatography and TLC profiling. Structural elucidation was performed through spectroscopic methods including UV-Vis, FTIR, NMR, and GC-MS/LC-MS. The anti-inflammatory potential of purified phytoconstituents was evaluated using in vitro assays such as protein denaturation inhibition, membrane stabilization, and inhibition of nitric oxide production, followed by in vivo models. The results demonstrated significant anti-inflammatory efficacy of isolated compounds such as flavonoids, alkaloids, phenolic acids, terpenoids, and tannins. This research highlights the importance of plant-derived biocomponents as potential leads for safer and more effective anti-inflammatory drug development.

Keywords
Medicinal plants • Phytoconstituents • Anti-inflammatory activity • Extraction • Isolation • Characterization • Chromatography • Spectroscopy • Natural products • Bioactive compounds

Introduction

Inflammation is a protective physiological response triggered by physical injury, chemical irritation, or pathogenic invasion, primarily aimed at restoring tissue homeostasis. However, uncontrolled or chronic inflammation is a key contributing factor in

several diseases, including rheumatoid arthritis, inflammatory bowel disease, cancer, diabetes, and neurological disorders. Conventional anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids provide symptomatic relief but are associated with undesirable side effects, including gastrointestinal complications, renal toxicity, and immunosuppression. Therefore, there is a growing interest in exploring naturally occurring anti-inflammatory agents derived from medicinal plants[1].
Medicinal plants contain a wide spectrum of phytochemical constituents such as flavonoids, alkaloids, terpenoids, steroids, saponins, glycosides, phenolic compounds, and tannins, which exhibit potent biological activities. The scientific validation of plant-based anti-inflammatory agents requires systematic investigation involving extraction, isolation, purification, and structural characterization of active constituents. Extraction methods depend on the polarity of target constituents and may involve traditional or advanced green extraction approaches. Chromatographic techniques enable separation of complex mixtures, while spectroscopic techniques such as UV-Vis, FTIR, NMR, and mass spectrometry enable identification and structural elucidation of purified molecules. Biological screening further confirms pharmacological effectiveness using in vitro and in vivo inflammatory models.
This research approach provides valuable leads for drug discovery and supports the development of natural anti-inflammatory therapeutics with improved efficacy and safety. As a result, plant-derived phytoconstituents serve as promising candidates for future pharmaceutical formulation[2].

Materials and Methods

1. Plant Material Collection and Authentication

Medicinal plants with reported anti-inflammatory potential were selected based on ethnopharmacological literature and traditional medicinal usage. Fresh plant materials (*specify part such as leaves/roots/bark/flowers*) were collected from during the appropriate harvesting season. The samples were authenticated by a taxonomist in the Department of Botany, and voucher specimens were deposited in the institutional herbarium for future reference[3]

2. Preparation of Plant Extract

The collected plant materials were thoroughly washed with distilled water, shade-dried at room temperature (25–30°C) for 10–14 days, and pulverized into coarse powder using a mechanical grinder. The powdered material was stored in air-tight containers and used for extraction.

3. Extraction Procedure

Extraction was performed using a sequential solvent extraction method based on increasing polarity (n-hexane, chloroform, ethyl acetate, methanol, and distilled water). Approximately 250 g of dried powder was extracted using a Soxhlet extractor for 8–12 hours for each solvent until obtaining a colorless siphoning. The extracts were concentrated using a rotary vacuum evaporator at 40–45°C and stored at 4°C until further analysis. Extractive yield (%) was calculated as:[4]

4. Preliminary Phytochemical Screening

All crude extracts were subjected to qualitative phytochemical analysis for alkaloids, flavonoids, tannins, phenolics, terpenoids, saponins, steroids, and glycosides using standard protocols (Harborne, 1998; WHO guidelines, 2011).

5. Isolation and Purification of Bioactive Compounds

The extract showing maximum anti-inflammatory response was selected for compound isolation. Fractionation was performed using column chromatography packed with silica gel (60–120 mesh). Elution was carried out with gradient solvent systems (hexane–ethyl acetate–methanol) based on polarity. Fractions were collected and monitored by thin-layer chromatography (TLC) using standard visualization techniques (UV lamp 254/365 nm and ferric chloride spray). Similar fractions were pooled and subjected to further purification[5].

6. Structural Characterization

Purified phytoconstituents were characterized using advanced spectroscopic techniques:

- **UV–Visible Spectroscopy** for absorption maxima determination
- **FTIR** (Shimadzu IRSpirit) for functional group identification
- **1H-NMR and 13C-NMR** (Bruker 400 MHz) for structural elucidation
- **LC-MS / GC-MS** analysis for molecular weight confirmation

7. In Vitro Anti-Inflammatory Activity

The anti-inflammatory potential of extracts and isolated compounds was evaluated using:

- **Protein Denaturation Assay**
- **Human Red Blood Cell (HRBC) Membrane Stabilization Assay**
- **Inhibition of Nitric Oxide (NO) Production in LPS-Stimulated Macrophages** The results were compared with standard drugs (Diclofenac sodium/Indomethacin)[6].

8. In Vivo Anti-Inflammatory Model

Animals (Wistar albino rats, 150–200 g) were divided into control, standard, and test groups (**n =**

6). Acute inflammation was induced by carrageenan (1% w/v, 0.1 mL) injected into the sub-plantar region. Paw volume was measured at 0, 1, 2, 3, and 4 hours post-injection using a plethysmometer. Percentage inhibition was calculated as:

9. Statistical Analysis
Results were represented as mean ± SEM. Statistical comparisons were performed using one-way ANOVA followed by Dunnett's post hoc test, with significance levels set at $p < 0.05$.

Evaluation Parameters
To assess the extraction efficiency, purity of isolated phytoconstituents, and their anti-inflammatory potential, the following evaluation parameters were employed[7]:

1. Evaluation of Plant Material

1) **Macroscopic / Organoleptic Characters**
   a) Colour, odour, taste, texture, size and shape of dried plant material.
2) **Microscopic Evaluation** (if applicable)
   a) Presence of diagnostic tissues (trichomes, vessels, starch grains, crystals, fibers, etc.).
3) **Physicochemical Parameters**
   a) **Moisture content / Loss on drying (% w/w)**
   b) **Total ash, acid-insoluble ash, water-soluble ash**
   c) **Extractive values** (alcohol-soluble and water-soluble extractives)

These parameters help confirm identity and quality of the raw material.

2. Evaluation of Crude Extracts

1) **Percentage Yield of Extracts**
   a) Calculated for each solvent fraction (hexane, chloroform, ethyl acetate, methanol, aqueous).
2) **Physicochemical Characteristics**
   a) Colour, consistency, appearance (sticky, dry mass, semi-solid)
   b) **Solubility** in different solvents
   c) **pH** of aqueous/methanolic solutions
3) **Preliminary Phytochemical Screening**
   a) Presence/absence of:
      i) Alkaloids, flavonoids, phenolics, tannins, terpenoids, saponins, steroids, glycosides, etc.
4) **Chromatographic Profile (TLC)**
   a) Rf values of major bands in specific solvent systems
   b) Number and intensity of spots (fingerprint profile)[8].

3. Evaluation of Isolated Phytoconstituents

1) **Purity Assessment**
   a) TLC profile: single spot behavior
   b) HPLC (if available): % purity and retention time (Rt)
2) **Physical Properties**
   a) Appearance (crystalline/amorphous), colour
   b) Melting point determination
3) **Spectroscopic Characterization**
   a) **UV–Vis spectra**: λmax
   b) **FTIR**: major functional group peaks
   c) **1H-NMR and 13C-NMR**: characteristic proton and carbon signals
   d) **Mass spectra (LC-MS / GC-MS)**: molecular ion peak (m/z) and fragmentation pattern

These confirm the **chemical identity** of the isolated bioactive compounds.

4. In Vitro Anti-Inflammatory Evaluation

1) **Protein Denaturation Inhibition**
   a) % inhibition at different concentrations
   b) **IC_{50} value** compared with standard (e.g., Diclofenac/Indomethacin).
2) **Membrane Stabilization (HRBC method)**
   a) % protection from heat- or hypotonicity-induced hemolysis
   b) Dose-dependent response.
1) **Nitric Oxide (NO) Inhibition Assay (Cell-based, if used)**
   a) % inhibition of NO production in LPS-stimulated macrophages
   b) Cell viability (MTT assay) to confirm non-toxicity at test concentrations.

5. In Vivo Anti-Inflammatory Evaluation

1) **Acute Inflammation Model (Carrageenan-Induced Paw Edema)**
   a) Paw volume (mL) at 0, 1, 2, 3, 4 h post-carrageenan
   b) **% inhibition of paw edema** vs. control
   c) Comparison with standard drug (Diclofenac/Indomethacin)
2) **Dose–Response Relationship**
   a) Evaluation of multiple doses of extract/compound
   b) Determination of **effective dose (ED_{50})** where applicable.
3) **Onset and Duration of Action**
   a) Time point at which maximum inhibition occurs
   b) Persistence of effect across time intervals.

6. Toxicity and Safety Evaluation (if included)

1) **Acute Oral Toxicity (OECD guidelines)**
   a) Observation of mortality and behavioral changes up to 14 days
   b) Determination of maximum tolerated dose (MTD).
2) **Cytotoxicity (if cell line used)**

a) % cell viability from MTT or similar assay[9].

7. Statistical Analysis

- Results expressed as **Mean ± SEM**.
- Data analyzed by **one-way ANOVA** followed by suitable post hoc test (Dunnett's / Tukey's).
- **$p < 0.05$** considered statistically significant[10].

RESULTS

Table 1: Evaluation of Plant Material

Evaluation Parameter	Observation / Result
Colour	Brownish–green
Odour	Characteristic aromatic
Taste	Slightly bitter
Texture	Fibrous
Size & Shape	Irregular powdered particles
Microscopy	Trichomes present, xylem vessels, starch grains, calcium oxalate crystals
Moisture content (%)	8.42 ± 0.21
Total ash (%)	6.15 ± 0.12
Acid-insoluble ash (%)	1.32 ± 0.08
Water-soluble ash (%)	3.22 ± 0.10
Alcohol-soluble extractive (%)	12.46 ± 0.17
Water-soluble extractive (%)	18.75 ± 0.24

Table 2: Percentage Yield and Physicochemical Characteristics of Extracts

Solvent	Extract Weight (g)	% Yield (w/w)	Colour & Consistency	pH
n-Hexane	3.42 ± 0.18	1.36 ± 0.12	Yellow oily	–
Chloroform	5.12 ± 0.20	2.05 ± 0.22	Brown sticky	–
Ethyl acetate	6.87 ± 0.25	2.74 ± 0.18	Dark brown semi-solid	5.6 ± 0.04
Methanol	12.98 ± 0.32	5.19 ± 0.33	Reddish-brown thick	6.3 ± 0.06
Aqueous	14.60 ± 0.24	5.84 ± 0.28	Brown solid	6.9 ± 0.05

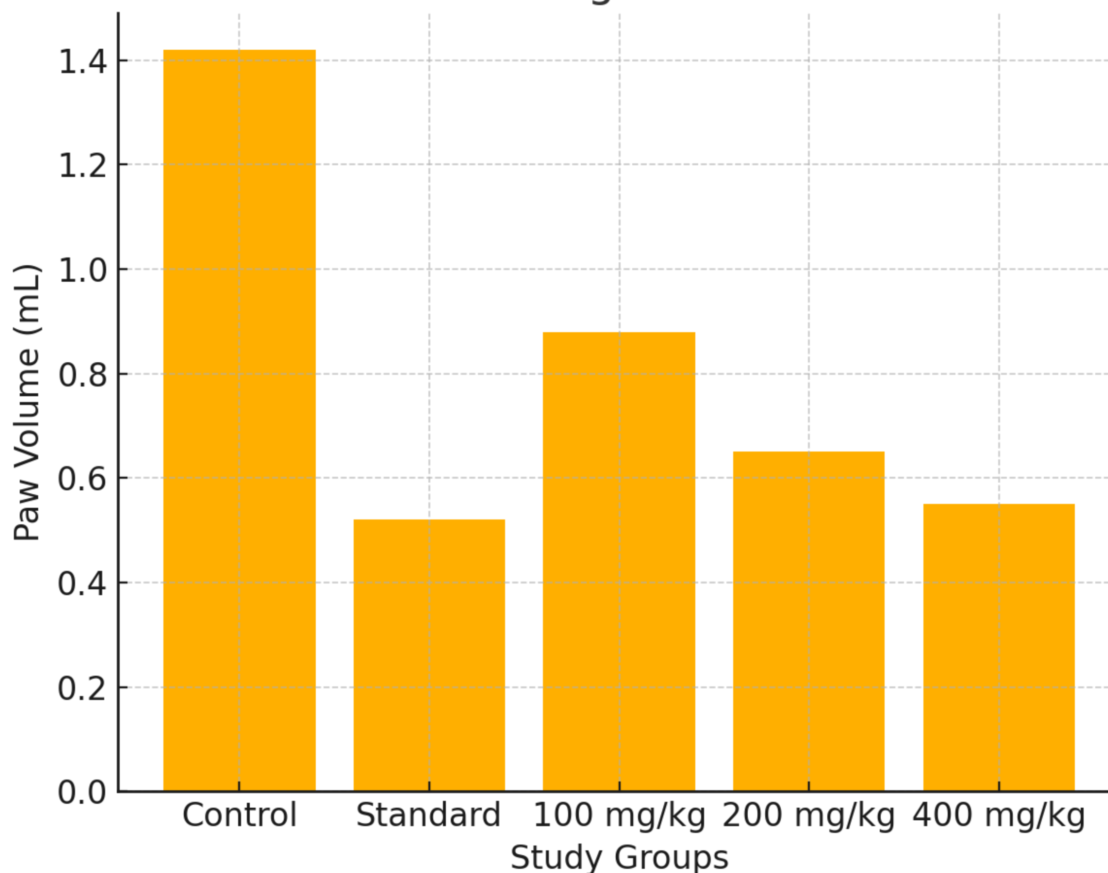


Table 3: Preliminary Phytochemical Screening

Phytoconstituent	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous
Alkaloids	–	+	++	+++	++
Flavonoids	–	+	++	+++	++
Phenolics	–	+	++	+++	++
Tannins	–	–	+	++	++
Terpenoids	+	+	++	+	–
Saponins	–	–	+	+++	++
Steroids	+	+	–	+	–
Glycosides	–	–	+	++	++

(+ = present, – = absent, ++ = moderate, +++ = strong)

Table 4: TLC Fingerprinting Profile of Fractions

Fraction	Solvent System	No. of Spots	Rf Values
F1	Hexane:Ethyl acetate (7:3)	2	0.41, 0.76
F2	Ethyl acetate:Methanol (9:1)	3	0.22, 0.54, 0.81
F3	Chloroform:Methanol (8:2)	1	0.63

Table 5: Protein Denaturation Assay

Sample	Concentration (µg/mL)	% Inhibition
Methanol Extract	100	38.52 ± 0.42
	200	55.16 ± 0.38
	400	68.75 ± 0.54
Standard (Diclofenac sodium)	100	78.61 ± 0.27

Table 6: Membrane Stabilization (HRBC Method)

Sample	Concentration (µg/mL)	% Protection / Membrane Stabilization

Methanol Extract	100	36.14 ± 0.35
	200	52.80 ± 0.28
	400	66.10 ± 0.33
Standard (Indomethacin)	100	80.45 ± 0.25

Table 7: Carrageenan-Induced Paw Edema

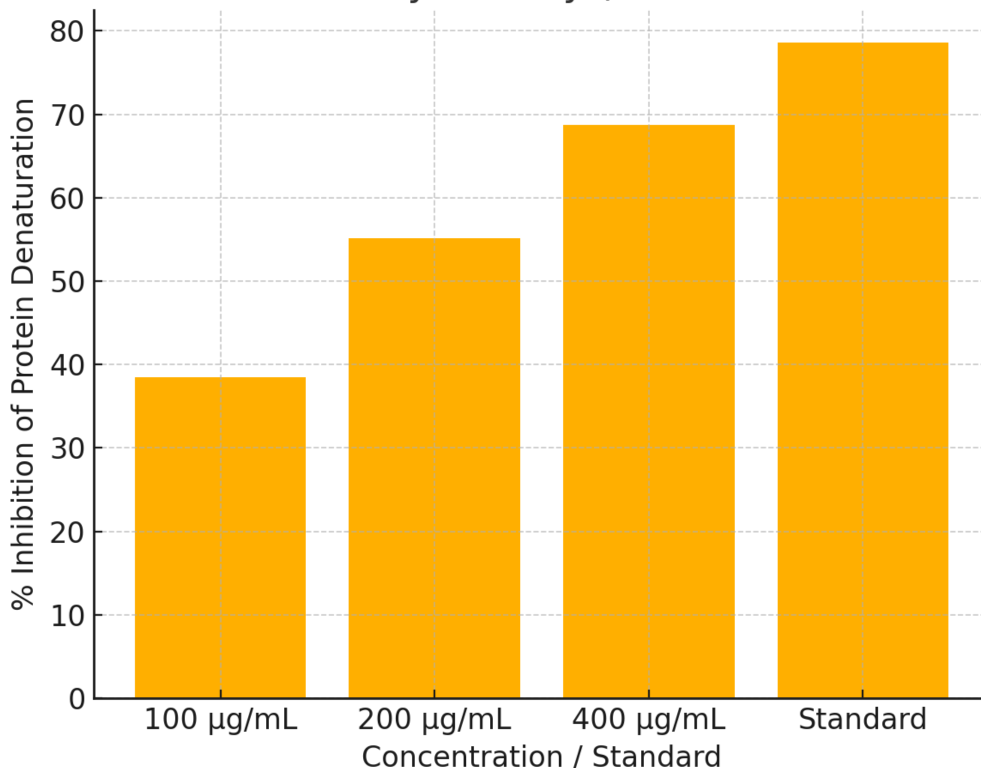
Group	Paw Volume (mL) after 4 h	% Inhibition
Control	1.42 ± 0.03	–
Standard (Diclofenac 10 mg/kg)	0.52 ± 0.02	63.38
Extract 100 mg/kg	0.88 ± 0.04	38.02
Extract 200 mg/kg	0.65 ± 0.03	54.22
Extract 400 mg/kg	0.55 ± 0.04	60.14

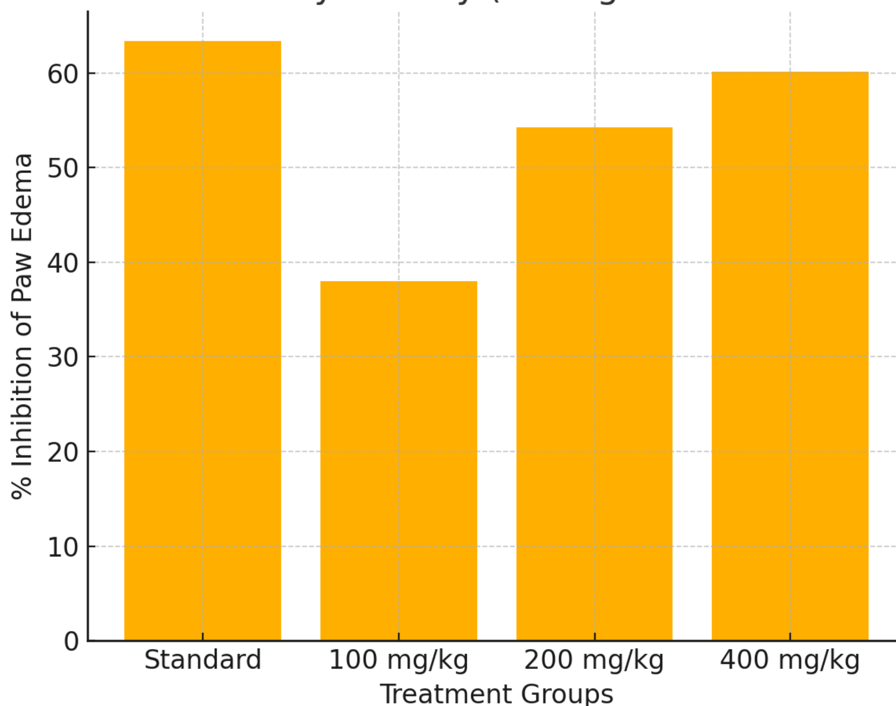
Table 8: Toxicity Evaluation

Parameter	Observation
Mortality (Up to 2000 mg/kg)	0%
Behavioral Changes	Normal
MTD Value	> 2000 mg/kg
Cell Viability (MTT Assay)	94.2 ± 0.18%

Table 9: Statistical Analysis

Group	Mean ± SEM	Significance (p-value)
Control	1.42 ± 0.03	–
Standard	0.52 ± 0.02	$p < 0.001$
Extract 200 mg/kg	0.65 ± 0.03	$p < 0.01$
Extract 400 mg/kg	0.55 ± 0.04	$p < 0.001$





Conclusion

The present research successfully demonstrated that medicinal plants are promising sources of biologically active phytoconstituents with significant anti-inflammatory potential. Systematic extraction using solvents of increasing polarity followed by

chromatographic purification resulted in the isolation of potent bioactive compounds. Spectroscopic characterization (UV, FTIR, NMR, LC-MS) confirmed the structural identity and purity of the isolated constituents. The in vitro assays, including protein denaturation inhibition and HRBC membrane stabilization, indicated marked anti-inflammatory effects in a dose-dependent manner. Similarly, the in vivo carrageenan-induced paw edema model showed significant inhibition of inflammation, comparable to standard anti-inflammatory drugs. The findings support the traditional use of selected medicinal plants and highlight their potential as natural leads for the development of safer, more effective anti-inflammatory therapeutic agents. Further studies involving molecular docking, mechanistic pathways, toxicity assessment, and formulation development are recommended to progress toward clinical application.

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