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Review Article

In vitro and *In vivo* Screening of Medicinal Plants for their Anti-Inflammatory Activity

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ABSTRACT

Inflammation is a natural defense mechanism of the body in response to harmful stimuli such as pathogens, injuries, or irritants. However, chronic inflammation is associated with many disease including arthritis, asthma, and cardiovascular disorders. Although synthetic anti-inflammatory drugs are widely used, they often have adverse effects and limitations. Therefore, medicinal plants have gained attention due to their traditional use and natural therapeutic potential. This review focuses on both *in vitro* and *in vivo* screening methods used to evaluated the anti-inflammatory activity of various medicinal plants. *In vitro* methods such as membrane stabilization, protein denaturation and nitric-oxide inhibition provide rapid and cost-effective preliminary results. *In vivo* models, including carrageenan induced paw edema and formalin induced paw edema offer a more comprehensive evaluation in living systems. This article summarize the finding of several studies that have 0the development of safer and effective anti-inflammatory agents (Phanse, 2012).

Keywords: Anti-inflammatory activity, Herbal Medicine, *In vitro* model, *In vivo* model, Medicinal Plants.

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INTRODUCTION

Inflammation is a complex biological response of the body's immune system to harmful stimuli such as pathogens, damaged cells, or irritants. While acute inflammation is a protective mechanism essential for healing, chronic inflammation is a pathological condition implicated in numerous diseases, including arthritis, cancer, cardiovascular disorders, and neurodegenerative conditions. Conventional anti-inflammatory drugs, including non-steroidal anti-inflammatory drugs and corticosteroids, are effective in managing symptoms but are often associated with significant side effects such as gastrointestinal bleeding, renal toxicity, and cardiovascular risks when used long-term (Yada *et al.*, n.d.).

Inflammation is a vital defense mechanism that protects the body against infections, injuries, and foreign substances. It involves a coordinated cascade of molecular and cellular events that aim to eliminate the initial cause of cell injury and initiate tissue repair. While acute inflammation is typically short-lived and beneficial, chronic inflammation can contribute to the development of several pathological conditions including rheumatoid arthritis, asthma, inflammatory bowel disease, cardiovascular disorders, and neurodegenerative diseases (Oladejo and Akingbade, 2024; Yada *et al.*, n.d.).

This review aims to explore the current advancements in nano formulated anti-inflammatory agents, emphasizing the relevance and methodology of both *in vitro* and *in vivo* assessments. It also highlights recent studies, models used, and the potential of nano formulations in overcoming the limitations of conventional anti-inflammatory therapies.

The evaluation of nano formulated drugs requires a dual approach-*in vitro* studies help understand the cellular mechanisms, cytotoxicity, and anti-inflammatory potential, while *in vivo* models are crucial to assess pharmacokinetics, efficacy, and safety in living systems. Together, these assessments provide a comprehensive understanding of the therapeutic potential of nano formulated anti-inflammatory agents (Oladejo and Akingbade, 2024).

Inflammation can be categorized into

- **Acute inflammation:** A short-term response of the body to harmful stimuli such as microbial infection, physical injury, or chemical irritants. It typically develops within minutes to hours and lasts for few days.

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The goal of acute inflammation is to remove the cause of injury, eliminate dead cells, and initiate tissue repair (Murt Huza and Manjunatha, 2018a).

Chronic inflammation

Chronic inflammation is a prolonged and persistent [inflammatory response that occurs when the acute phase fails to eliminate the cause of injury or when the harmful stimulus continues over time.

Although initially protective, chronic inflammation can lead to tissue destruction, fibrosis, and organ dysfunction (Murt Huza and Manjunatha, 2018a).

Important of inflammation

- Helps in fighting infection and injuries.
- Removes damaged tissues and toxins.
- Initiates tissues repair and regeneration.

Why Anti-Inflammatory Drug Are Needed

Inflammation, though a natural and protective response of the body, can become harmful when it is excessive, uncontrolled, or chronic. In such conditions, the body immune response starts damaging healthy tissues, leading to pain, swelling, organ dysfunction, and chronic disease. To manage this, anti-inflammatory drugs are necessary (Xie and Huang, 2024).

- **To Control Swelling and Redness:** anti-inflammatory drugs reduce the vasodilation and capillary permeability that lead to redness, swelling, and warmth in inflamed tissues. This helps in restoring normal tissue function.
- **To Relieve Pain and Discomfort:** Inflammation is often accompanied by pain due to the release of prostaglandins and other chemical mediators. Anti-inflammatory drugs (like NSAIDs) help reduce pain, making the patient more comfortable.
- **To Prevent Tissue Damage:** In chronic inflammation, prolonged immune response can lead to destruction of healthy cells and tissues, as seen in diseases like rheumatoid arthritis, asthma, and IBD. Anti-inflammatory drugs prevent further tissue damage.
- **To Treat inflammatory Diseases:** Anti-inflammatory drugs are the mainstay of treatment in several diseases where inflammation is the root cause, such as:
- Rheumatoid Arthritis,
- soriasis,
- Ulcerative Colitis,
- Asthma,
- To Improve Quality of Life: By controlling chronic inflammation, these drugs reduce symptoms, disability, and complications, leading to improved quality of life for patients.

Limitations of conventional drugs

Despite their importance, many conventional anti-inflammatory drugs (e.g., NSAIDs, corticosteroids) have side effects such as:

- Gastric Ulcers,
- Kidney Damage,
- Increased Risk of Infections,
- Poor Targeting,
- This has led to a growing interest in nanoformulation-based anti-inflammatory therapies, which can enhance drug delivery, reduce dose, and minimize systemic side effects (Xie and Huang, 2024).

Pathophysiology of Inflammation

Inflammation is a protective and coordinated biological response of the body's immune system to harmful stimuli such as pathogens, damaged cells, toxins, or physical injury. Its main objective is to eliminate the causative agent, remove dead or damaged tissue, and initiate tissue repair and regeneration. However, if uncontrolled or unresolved, inflammation itself may cause tissue injury and contribute to various chronic diseases.

The pathophysiological process of inflammation involves a sequence of events that include vascular changes, recruitment of immune cells, and release of chemical mediators (Li, Chen, and Zhang, 2023).

Phase of Inflammation

1. Recognition of the injurious agent: By Pattern Recognition Receptors (PRRs) on macrophages and dendritic cells,

2. Recruitment of inflammatory cells,

3. Neutrophils, macrophages, and other immune cells migrate to the site of injury,

4. Activation of immune cells and mediators,

5. Release of cytokines, prostaglandins, and other signaling molecules,

6. Elimination of the cause of injury,

7. Resolution and tissue repair.

Chemical mediators of inflammation

Chemical mediators are central to the initiation, amplification, and resolution of the inflammatory response. They can be derived from.

- Cellular Sources (e.g., Mast Cells, Macrophages, Neutrophils).

- Plasma Proteins (e.g., Complement System, Coagulation Factors).

Need for Herbal Alternatives in Inflammation Management

Inflammation is a critical component of the body defense system however, when prolonged or dysregulated, it contributes to chronic conditions such as rheumatoid arthritis, inflammatory bowel disease, asthma, cancer, and metabolic syndromes. While modern pharmacological agents like non-steroidal anti-inflammatory drugs and corticosteroids are effective in managing inflammation, their long-term use is frequently associated with serious adverse effects, including gastrointestinal ulcers, renal impairment, hypertension, and increased risk of infections (Mohseni *et al*., 2020).

Limitations of Conventional Anti-Inflammatory Drugs

- **Side effects:** Prolonged use of NSAIDs can cause gastric bleeding, ulceration, and renal toxicity.
- **Tol**erance and resistance: Long term corticosteroid therapy can lead to reduced efficacy.
- **Cost and accessibility:** Many synthetic drugs are expensive and inaccessible in rural or undeveloped areas.
- **Symptomatic treatment:** these drugs often address symptoms, not the underlying cause.

Herbal Alternatives: A Safer Option

- **Rich Source of Bioactive Compounds:** Flavonoids, alkaloids, terpenoids, phenolics,
- **Multitargeted Mechanisms:** Herbal extracts often act on multiple inflammatory mediators,
- **Reduced Toxicity:** Generally associated with fewer and less severe side effects,
- **Accessibility and affordability:** Especially in developing countries (Jayasuriya *et al*., 2017).

Evidence of Efficacy

Several medicinal plants such as *Curcuma longa* (turmeric), *Zingiber officinale* (ginger), *Boswellia serrata* (salai guggul), and with *Ania somniferous* (ashwagandha) have shown significant to standard drugs like indomethacin or diclofenac (Jabn, 2017; Jayasuriya *et al*., 2017).

In vitro Models for Anti-Inflammatory Screening

In vitro screening methods are widely used in the early stages of drug discovery to screen and evaluate the anti-inflammation potential of medical plants. These models are simple, cost-effective, and a to allow for high-throughput screening. They primarily asses the ability of plant extracts or compounds to inhibit inflammation related biological processes at the cellular or biochemical level (Yada *et al*., n.d.).

Human Red Blood Cell (HRBC) Membrane stabilization Assay

Plant Used: *Abutilon indicum* Linn.

Family: Malvaceous.

Part Used: Leaves.

Solvent: Ethanol-4.5% wt/wt, Chloroform-0.45% wt/wt and distilled water-3.7% wt/wt.

Dose: 50 mg/100 mL.

Chemical Used: Diclofenac, phosphate buffer, HRBC suspension.

Principle

During inflammation, lysosomal enzymes are released, which can cause further tissue damage. The HRBC membrane is similar to the lysosomal membrane, and its stabilization indicates anti-inflammatory activity (Daram *et al*., 2021).

Procedures

The HRBC membrane stabilization has been used as method to study the anti-inflammation activity. Blood was collected from healthy volunteer. The collected blood was mixed with equal volume of sterilized Al sever solution (2% dextrose, 0.8% sodium citrate acid and 0.42% sodium chloride in water). The blood was centrifuged at 300 r.p.m. and paced cell were washed with isohaline. The assay mixture contained the drug. 1 ml of phosphate buffer (0.15 m, pH), 2 mL of hyposaline (0.36%) and 0.5 mL of HRBC suspension. Diclofenac was used as reference drug. Instead of hyposaline 2 mL of distilled water was used in the control. All the assay mixture were incubated at 37°C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in presence of distilled water of as 100% (Daram *et al*., 2021).

Protein Denaturation Assay

Plant Used: *Curcuma longa* Linn.

Family: Zingiberene.

Part used: Rhizomes.

Solvent: Ethanol, 70% wt/wt.

Chemical Used: Bovine Serum Albumin (BSA), Hydrochloride Acid (HCl), Indomethacin.

Principal

Inflammation often involves denaturation of protein due to heat or stress. Compounds that prevent protein Denaturation can potentially reduce inflammation. In this assay, BSA is used as a model protein, and inhibition of its denaturation by the test extract indicates anti- inflammation activity (Ewig *et al*., 2025).

Procedures

Prepare 1% aqueous solution of BSA. Mix 0.45 mL of BSA solution with 0.05 mL of plant extract (various concentration). Adjust pH to 6.3 using 1 N HCl. Incubate the sample at 37°C for 5 min. Cool the sample and measure absorbance at 660 nm using UV spectrophotometer. Calculation % inhibition of protein denaturation using the formula (Oladejo *et al*., 2024).

% Inhibition = Absorbance Control-Absorbance Sample×100

Absorbance Control

Albumin Denaturation Assay

Plant Used: *Abutilon indicum* Linn.

Family: Malvaceous.

Part Used: Leaves.

Solvent: Ethanol, 70% wt/wt.

Chemical Used: Fresh egg albumin (BSA as alternative), Diclofenac (standard), Phosphate buffer saline, HCl (for pH adjustment).

Principle

Heat or chemical stress causes denaturation of albumin, denatured albumin increase turbidity. Substances that prevent or reduce this denaturation show anti-inflammatory potential. This assay measures inhibition of albumin denaturation by the test sample spectrophotometrically (El-Shivani and Eid, 2019).

Procedure

Prepare albumin solution if using egg albumin dilute fresh egg white with distilled water to obtain 1% (wt/wt) albumin solution. Or prepare 1% BSA in distilled water as alternative.

Prepare test extract solution at required concentration (example: 10, 50, 100, 200 g/mL) in ethanol or phosphate buffer saline. Prepare standard solution diclofenac at appropriate concentration.

Prepare a control containing albumin + buffer (no extract). Prepare a blank for each sample (same as sample but without albumin) to zero instrument for solvent absorbance. Mixing in labeled test tube, add 0.5 mL of test extract (or standard/control solvent). 0.45 mL of albumin solution (1%). 0.05 mL of phosphate buffer (to adjust volume/Ph). For control 0.5 mL solvent+0.45 mL albumin + 0.05 mL buffer.

For blank 0.5 mL test extract+0.5 mL buffer. Incubate the mixtures at 37°C for 15-20 min. Heat the tube at 70°C for 5 min. Allow to cool to room temperature (Murt Huza and Manjunatha, 2018b).

% Inhibition = Absorbance control-Absorbance sample×100

Human keratinocyte HaCaT

Family: Zingiberene.

Plant used: *Acorus calamus* L.

Part Used: Leaves.

Solvent: Water.

Dose: 50 mg/kg.

Route of administration: intraperitoneally,

Chemical Used: Immunofluorescence staining, polyinosine, polypyridyl acid.

Principle

Human keratinocytes, such as the HaCaT cell line, are skin derived epithelial cells that play a crucial role in inflammation and immune responses. In an *in vitro* anti inflammation assay, test compound such as plant extract or drug are applied to keratinocyte cultures before or after inducing inflammation. The ability of the compound to reduce inflammatory. The ability of the compound to reduce inflammatory mediator level, inhibit COX/LOX enzymes, or down regulate inflammatory gene expression is measured. This model mimics skin inflammation and allows screening of anti-inflammatory potential at the cellular level without using animal (Kedi *et al*., 2018).

Procedure

HaCaT cells treated with polyinosine: Polypyridyl acid and peptidoglycan induced the inflammatory reaction. The anti-inflammatory activities of ACL were investigated using RT-PCR, ELISH assay, western blotting, and immune fluorescence staining (Kedi *et al*., 2018).

Lipoxygenase Inhibition Assay

Plant Name: *Alepidea amatymbica.*

Family: Apiaceae.

Part Used: Roots.

Solvent for Extraction: Acetone.

Chemical Used: Soybean lipoxygenase enzyme (EC 1.13.11.12), sodium phosphate buffer 0.1 m, pH 6.8 (or pH 7.0 as per protocol), linoleic acid substrate.

Principle

Lipoxygenase inhibition assay involves measuring the enzymes ability to catalyze the oxidation of unsaturated fatty acid, typically

linoleic acid, into a hydroperoxide. Inhibitors of LOX will reduce this reaction, which is then quantified through various methods, often involving a color change. Lipoxygenases are present in the human body and play an important role in the stimulation of inflammatory reactions (Morass *et al*., n.d.).

Procedure

Buffer 0.1 M sodium phosphate, pH 6.8. Enzyme dilute soybean LOX to working concentration (example: 1,000 U/mL stock → dilute to provide ~10 mL/well optimize enzyme amount so the control gives linear increase at 234 nm). Substrate linoleic acid 10 mM stock. Prepare fresh and keep on ice. 160 mL sodium phosphate buffer (0.1 M, pH 6.8)10 mL lipoxygenase enzyme solution 10 mL test sample solution (or 10 mL buffer for control mL standard for positive control). Pre-incubate enzyme + sample mixture at 25°C for 5 min. Record absorbance increase at 234 nm every 15-30 sec for 3-5 min or measure initial rate. Use control wells no inhibitor to get enzyme activity (Morass *et al*., n.d.).

DPPH assay

Plant Name: *Curcuma longa*.

Family: Zingiberaceae.

Part used: Rhizomes.

Solvent: 70% ethanol.

Chemical: DPPH (0.1 mm in methanol), methanol (HPLC/AR grade), ascorbic acid (standard), phosphate buffer, microplate/ UV.

Principle

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay is an antioxidant activity test based on a color change reaction involving a stable, violet colored free radical. When DPPH reacts with an antioxidant, it becomes a reduced, pale- yellow molecule. The degree of color change is measured using a spectrophotometer to quantify the sample antioxidant capacity (Peiris *et al*., 2025).

Procedure

This experimental procedure in an ethanol solution of 2,2-diphenyl-1-picrylhydrazyl radical (final concentration was 1.0×10^{-4} M), test extract at different concentration were added. The reaction mixture were shaken vigorously and then kept in the dark for 30 min. The absorbance of the resulting solutions was measured in 1 cm cuvettes, using a PerkinElmer Lambda 40 UV/visible spectrophotometer at 517 nm, against blank without DPPH. Decreasing of DPPH solution absorbance indicates an increase of DPPH radical scavenging activity. This activity is given as %DPPH radical scavenging that calculated in the equation.

%DPPH Radical Scavenging = Sample Absorbance - Control Absorbance ×100

In vivo Models for Anti-Inflammatory Screening

In vivo model are essential tools for evaluating the anti-inflammatory potential of plant extracts and synthetic compounds under physiological conditions. They help in understanding the integrated response of cells, tissues, and organ systems to inflammatory stimuli. Both acute and chronic inflammation can be studied using various models, each specific pathways and mediators involved in the inflammatory process (Chen *et al*., 2018).

Carrageenan-Induced Paw Edema

Plant used: *Hydrocotyle Batrachium*.

Family: Apiaceae.

Part Used: Whole plant.

Common Name: Marsh pennywort.

Solvent: Water.

Dose: 500 and 100 mg/kg.

Route of administration: Orally.

Chemical used: Indomethacin.

Principle

Carrageenan hind-paw injection causes biphasic acute edema. The test drug/extract reduces paw swelling and control. These models mimic acute and chronic inflammatory condition and measure parameter such as edema formation, granuloma development, or vascular permeability. The principle is based on comparing the inflammatory response in treated animals against controls, allowing the determination of anti-inflammatory potential (Umar *et al*., 2010).

Procedure

The anti-inflammatory activity of HBW (*Hydrocotyle batrachium* Hance) was determined by the carrageenan induced edema test. Male and mice were randomly assigned to five groups and then fasted with free access to water for 24 hr before the experiment. Fifty microliters of a 1% suspension of carrageenan in saline, which had been prepared 30 min before each experiments, was injected into the plantar side of the right hind paw of the mice. After 60 min, HBW at dose of 100, 500 and 1,000 mg kg^{-m} were administered orally, and after 90 min, indomethacin at the dose of 10 mg kg^{-m} was administered an intraperitoneal route after the carrageenan treatment. Paw volume was measured before carrageenan injection and at 60, 120, 180, 240, 300, 360 min after the administration of the indomethacin agent using a plethysmometer (Lantz *et al*., 2005).

Cotton Pellet-Induced Granuloma in Rats

Plant Used: *Cássia fístula* Linn.

Family: Caesalpinaceae.

Part used: Bark.

Common Name: Amalltas.

Solvent: Water and methanol.

Dose: 2,000 mg/kg.

Rote of administration: Orally.

Chemical Used: Diclofenac sodium.

Principle

Subcutaneous sterile cotton pellets induce proliferative chronic inflammation (fibroblast proliferation and collagen deposition). Reduced dry granuloma weight indicates anti-proliferative/anti-inflammatory effect. The anti-inflammatory potential of a test compound is assessed by its ability to reduce the dry weight of the granuloma, which reflects inhibition of protein and collagen synthesis during chronic inflammation (Cheng *et al*., 2016).

Procedure

Wistar albino rats (170-200 g) of either six divided into 4 groups of 6 animal in each group. Cotton pellets weighing 30+1 mg were autoclave and implanted subcutaneously into both sides of the groin region of each rat, Group1 served as control and received the vehicle. The extracts CFA and CFM at concentration 500 mg/kg orally for Group 2 and Group 3 animals for same period. On the eighth day the animals were sacrified and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60ºC, weighed and compared with control. Diclofenac sodium, 5 mg kg^{-} was used as standard (Gonfa *et al*., 2023).

Formalin Induced Paw Edema in rats

Plants used: *Crocus sativus* Linn.

Family: Iridaceae.

Part Used: Powder of stigma and petal.

Common Name: Saffron.

Solvent: Water and ethanol.

Dose: 0.8 and 2 g/kg.

Route of administration: Intraperitoneally.

Chemical used: Diclofenac.

Principle

The formalin induced paw edema model evaluates both neurogenic and inflammatory pain responses. Subcutaneous injection of dilute formalin into a rat hind paw produces biphasic edema and pain. The anti-inflammation activity of a drug is measured by its ability to reduce paw swelling (volume or thickness). In the late phase, reflecting inhibition of mediator release and vascular permeability (Al-Khayri *et al*., 2022).

Procedure

Rats were divided into groups of six. The inflammation was produced by subaponeurotic injection of 0.1 of 2% formaldehyde in the right hind paw of the first and third day. The animals were treated daily with the extracts or diclofenac intraperitoneally for 10 days. The daily changes in paw size were measured by wrapping a piece of cotton threads round the paw and measuring the circumference with a meter rule (Al-Khayri *et al*., 2022).

Xylene Induced ear edema

Plant Used: *Crocus sativus* Linn.

Family: Iridaceae.

Part Used: Powder of stigma and petal.

Common Name: Saffron.

Solvent: Water and ethanol.

Dose: 0.8 and 2 g/kg.

Rote of administration: Intraperitoneally.

Chemical used: Diclofenac, dexamethasone.

Principle

Xylene induced ear edema is the topical application of xylene to the pinna produces an acute irritant dermatitis leading to rapid vasodilation, increased vascular permeability, and neurogenic inflammation. This causes plasma extravasation and tissue fluid accumulation in the treated ear (Al-Khayri *et al*., 2022).

Procedure

Mice were divided into group of seven. 30 min after intraperitoneally. injection of the extract, diclofenac and dexamethasone, 0.03 mL of xylene was applied to the anterior and posterior surfaces of the right ear. The left ear was considered as control. 2 hr after xylene application mice were killed and both ears were removed. Circular section were taken using a cork borer with a diameter of 7 min, and weighed. The increased in weight caused by the irritant was measured by subtracting the weight of the untreated left ear section from that of the treated right ear section (Al-Khayri *et al*., 2022).

Cotton Oil-Induced Mouse Ear Edema

Plant Used: *Anthrium cerrocampanense*.

Family: Araneae.

Part used: Whole plant.

Common Name: Tailflower.

Solvent: Water, ethanol, dichloromethane.

Dose: 100 mg/kg.

Route of administration: Intraperitoneally.

Chemical Used: Indomethacin.

Principle

Croton oil induced ear edema in mice is topical application of croton oil on the mouse ear produce acute and sub acute inflammation, mainly through the release of arachidonic acid metabolites and proinflammatory cytokines. The anti-inflammatory activity of a drug is assessed by its ability to reduce the increase compared to control. This model is especially useful for evaluating topical anti-inflammatory agents (Al-Khayri *et al*., 2022).

Procedure

Cutaneous inflammation was induced to their immer surface of the right ear of anesthetized mice (100 mg kg^{-m} ketamine hydrochloride, intraperitoneally) by application of 15 mL of acetone or acetone-water (1:1) solution contain 80 mg of croton oil as irritant (control animals). For treated animals, appropriate amounts of indomethacin or the tested extract were dissolved in the croton oil containing solution and applied as for controls. After 6 hr mice were sacrificed and a punch (5 mm of diameter) was excised from both ears. Inflammation was measured as edema formation and quantified by the weight difference between the treated and the untreated ear sample (Al-Khayri *et al*., 2022).

Phytoconstituents Responsible for Anti-Inflammatory Activity

Medicinal plants contain a wide range of bioactive phytochemical that contribute to their anti-inflammatory effects. These compounds act through different mechanisms such as inhibition of cyclooxygenase, lipoxygenase, suppression of proinflammatory cytokines and downregulation of NF-κB signaling pathways. Plant based phytoconstituents are knows for their potential sources of anti-inflammatory agents. Various studies reported the potential disease healing efficiency of medical plants. Medicinal plants are the primary source of medicines (Ansari *et al*., 2025).

Alkaloids

Example of Plants: Berberis vulgaris (Berberine), Colchicum autumnal (Colchicine).

Mechanism

Inhibit neutrophil migration, reduce cytokine production, and suppress NF-κB Activity.

Role

Help in reducing acute and chronic inflammation (Al-Khayri *et al*., 2022).

Flavonoids

Example of Plants: *Allium cepa* (Quercetin), *Ginkgo biloba* (Kaempferol), Citrus (Hesperidin).

Mechanism

Inhibit COX/LOX enzymes, scavenge free radicals, stabilize cells membranes and downregulate proinflammatory mediators.

Role

Provide strong antioxidant and anti-inflammatory protection (Ferraz *et al*., 2020).

Phenolic Compound

Example of plants: *Curcuma longa* (Curcumin), *Vitis vinifera*, Syzygies aromaticum

Mechanism

Suppress NF-κB activation, reduce oxidative stress, inhibit release of inflammatory mediators.

Role

Act as dual antioxidants and anti-inflammatory mediators (Ferraz *et al*., 2020).

Glycosides and saponins

Example of plants: with *Ania somniferous*, *Panax ginseng* (Ginsenosides).

Mechanism

Inhibit proinflammatory cytokines stabilizes lysosomal membranes and reduce vascular permeability.

Role

Effective in both acute and chronic inflammatory (Ferraz *et al*., 2020).

Limitations of Current Studies

Despite significant progress in evaluating the anti-inflammatory potential of medicinal plants and their formulations, several limitations restrict their translation into clinical use. Current anti-inflammatory research faces limitations related to the complexity of inflammation, the limitations of *in vitro* and *in vivo* models, and the challenges in translating research findings into effective and safe drugs. Specifically, while various *in vitro* and *in vivo* models exist, they may not fully replicate the complexity of the human inflammatory response (Winter *et al*., 1962).

Lack of clinical data

Most studies are restricted to *in vitro* and animal models, with very few clinical trails. This makes it difficult to confirm the real therapeutic potential in humans. Its is especially large scale a

high-quality studies demonstrating efficacy and safety in humans, are often lacking for promising anti-inflammatory compounds, particularly those derived from natural sources (Itharat *et al*., 2024).

Poor standardization of plant extracts

Variation in extraction methods, solvents, and plant material leads to inconsistent results and reduced reproducibility. The complex nature extracts and the difficulty in isolating pure compounds make it challenging to identify and develop lead compounds (Itharat *et al*., 2024).

Variability in experimental models

Ethical consideration regarding animal welfare, along with inherent difference between animal model and human present challenges in using *in vivo* models for anti-inflammatory.

Difficulty in complexity of inflammation in *in vitro* models

In vitro cell culture models provide a simplified environment and struggle to replicate the intricate interplay of cells, tissues, and systems involved in the inflammatory response observed in living organisms.

Challenges in reproducibility

Difficulties in reproducing experimental results, even within the same laboratory, can limit the reliability of preclinical findings and impede their translation into clinical application.

Toxicity and Safety Concerns

'Long-term safety profiles' are ready studied. Some plants may contain toxic constituents or interact with conventional drugs, posing risk in clinical use (Oppong *et al*., 2024).

Future Prospects of Anti-Inflammatory

Despite the availability of numerous anti-inflammatory drugs, current therapies such as non-steroidal anti-inflammatory drugs and corticosteroids are often associated with severe side effects including gastrointestinal toxicity, cardiovascular complications, and immunosuppression. Therefore, there is a growing need to develop novel and safer anti-inflammatory agents. The future of anti-inflammatory therapy is expected to shift toward targeted, safer, and patient-specific strategies.

Safer Conventional Therapies

The development of next generation NSAIDs with improved selectivity of COX-2 while minimizing cardiovascular risk remains a major focus. Similarly, novel corticosteroid formulations that provide localized delivery (such as inhaled or topical steroids) aim to reduce systemic adverse effects while retaining therapeutic efficacy.

Biologics and Monoclonal Antibodies

Biologics targeting proinflammatory cytokines (e.g., TNF-α, IL-1β, IL-6) have revolutionized the management of autoimmune and chronic inflammatory disorders. Future research is directed toward next generation biologics that are safer, less immunogenic, and potentially available in oral formulations. Additionally, biosimilars and combination therapies are expected to increase accessibility and therapeutic success.

Nanomedicine and Targeted Delivery

Nanotechnology offers a promising approach to enhance drug delivery by enabling site-specific targeting of inflamed tissues. Nano formulations such as liposomes, polymeric nanoparticles, and dendrimers improve drug solubility, stability, and bioavailability while reducing systemic toxicity. Future nanomedicine may employ smart nanocarriers responsive to local stimuli (pH, temperature, enzymatic activity), allowing controlled and sustained release of anti-inflammatory agents.

Plant Based and Natural Anti-Inflammatory

Phytochemicals such as curcumin, quercetin, resveratrol, and *Boswellia* acids have demonstrated significant anti-inflammatory potential through multitarget mechanisms. The future focus lies in standardizing herbal extracts, conducting rigorous clinical validations, and overcoming limitations of poor bioavailability by formulating herbal nano drug delivery systems. Moreover, ethnopharmacological research continues to provide new leads for plants derived therapeutics

Gene and RNA Based Therapies

Advances in molecular biology have opened opportunities to modulates inflammation at the genetic level. RNA based drugs such as siRNA and miRNA can selectively silence proinflammatory mediators. Similarly, CRISPR-Cas9 gene editing technology holds potential for long-term modulation of inflammatory pathways, particularly in genetic or chronic inflammatory conditions.

Microbiome Targeted Therapies

The human gut microbiota has emerged as a crucial regulatory of inflammation and immune responses. Strategies to restore microbiome balance using probiotics, prebiotics, and postbiotics are being actively investigated. Fecal microbiota transplantation represent another 1 innovative approach for the treatment of inflammatory bowel disease and related disorders.

Personalized and Precision Medicine

Interindividual variability in drug response necessitates a move toward precision medicine. Biomarker based therapy can allow selection of the most effective drug for each patient, thereby improving treatment outcomes and minimizing side effects. The integration of artificial intelligence and computational drug

discovery is also expected to accelerate the identification of novel anti-inflammatory agents.

Novel Mechanistic Targets

Emerging research highlights new pathways in inflammation beyond COX enzymes and cytokines. Inhibitors of the NLRP3 inflammasome, modulators of the JAK-STAT pathway, and cannabinoid receptor agonists are being explored as next-generation therapeutic options, particularly in neuroinflammatory and autoimmune conditions.

The detail literature survey was done on various plants which can be used in inflammation including their plant used, family, part used, common name, solvent of extraction, yield, dose, route of administration, chemical used, Procedure mechanism of action, and Conclusion was done. It is cleared from mechanism of action of these extract that almost all drugs work on the principle of inhibition of prostaglandin and other inflammatory mediators synthesis. Inflammation is a complex biological response that plays a central role in the pathogenesis of several acute and chronic diseases. Current synthetic anti-inflammatory drugs, though effective, are often limited by adverse effects, drug resistance, and long-term safety concerns. In this context, the exploration of medicinal plants and novel drug delivery systems, including nano formulations, offers promising alternatives. Evidence from both *in vitro* and *in vivo* studies highlights their ability to modulate inflammatory mediators, stabilize cell membranes, and attenuate tissue damage, thereby supporting their therapeutic relevance. Overall, medicinal plants and nano formulations represent a valuable frontier in anti-inflammatory drug discovery, with the potential to provide safer, effective, and sustainable alternatives for managing inflammation-related disorders (Winter *et al*., 1962).

CONCLUSION

Inflammation is a complex biological response that plays a crucial role in the pathogenesis of numerous acute and chronic diseases. Although conventional anti-inflammatory drugs such as NSAIDs and corticosteroids are effective, their prolonged use is often associated with significant adverse effects and safety concerns. This has led to increasing interest in alternative therapeutic approaches, particularly those derived from medicinal plants.

The present review comprehensively summarizes various *in vitro* and *in vivo* models used for evaluating anti-inflammatory activity. *In vitro* assays such as membrane stabilization, protein and albumin denaturation, lipoxygenase inhibition, DPPH assay, and cell-line-based studies provide rapid, cost-effective, and mechanistic insights into anti-inflammatory potential. *In vivo* models including carrageenan-induced paw edema, cotton pellet-induced granuloma, formalin-induced inflammation, and ear edema models offer a more integrated assessment under physiological conditions. Together, these models form a complementary framework for screening and validating potential anti-inflammatory agents.

Phytoconstituents such as flavonoids, alkaloids, phenolic compounds, glycosides, and saponins demonstrate significant anti-inflammatory effects through multiple mechanisms, including inhibition of cyclooxygenase and lipoxygenase pathways, suppression of pro-inflammatory cytokines, stabilization of lysosomal membranes, and modulation of NF-κB signaling. Furthermore, emerging approaches such as nano-formulations enhance drug delivery, improve bioavailability, and reduce systemic toxicity, representing a promising advancement in anti-inflammatory therapy.

Despite encouraging preclinical findings, limitations such as lack of standardization, insufficient clinical trials, reproducibility challenges, and safety concerns must be addressed before widespread clinical translation. Future research should focus on rigorous clinical validation, improved extract standardization, advanced drug delivery systems, and precision-based therapeutic strategies.

Overall, medicinal plants and nano-based formulations represent a promising and sustainable frontier in anti-inflammatory drug discovery, with the potential to provide safer, more effective, and targeted therapies for inflammation-related disorders.

ABBREVIATIONS

ACL: *Acorus calamus* Linn.; **AR:** Analytical Reagent; **BSA:** Bovine Serum Albumin; **COX:** Cyclooxygenase; **CRISPR:** Clustered Regularly Interspaced Short Palindromic Repeats; **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **ELISA:** Enzyme-Linked Immunosorbent Assay; **HCl:** Hydrochloric Acid; **HBW:** *Hydrocotyle batrachium*; **HO-1:** Heme Oxygenase-1; **HRBC:** Human Red Blood Cells; **IBD:** Inflammatory Bowel Disease; **IL-1β:** Interleukin-1 beta; **IL-6:** Interleukin-6; **JAK-STAT:** Janus Kinase-Signal Transducer and Activator of Transcription; **LOX:** Lipoxygenase; **miRNA:** MicroRNA; **NF-κB:** Nuclear Factor Kappa B; **NLRP3:** NOD-, LRR- and Pyrin Domain-Containing Protein 3; **NSAIDs:** Non-Steroidal Anti-Inflammatory Drugs; **PRRs:** Pattern Recognition Receptors; **RT-PCR:** Reverse Transcription Polymerase Chain Reaction; **siRNA:** Small Interfering Ribonucleic Acid; **TNF-α:** Tumor Necrosis Factor alpha; **UV:** Ultraviolet.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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