International Journal of Pharmacognosy and Herbal Drug Technology (IJPHDT)
ISSN: 3049-1630 | Vol. 03, Issue 05, May-August-2026 | pp. 102-127

ORIGINAL ARTICLE https://www.aktpublication.com/index.php/ijphdt

Anti-Inflammatory Activity of Herbal Extract Using Albumin Denaturation Method

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Abstract:

Inflammation is a protective biological response against harmful stimuli such as pathogens, tissue injury, and toxic substances. However, chronic inflammation may lead to several diseases, including arthritis, cardiovascular disorders, and cancer. Conventional anti-inflammatory drugs are effective but often produce adverse effects during long-term use. Therefore, herbal medicines have gained importance as safer alternatives due to the presence of bioactive phytochemicals with anti-inflammatory potential. The present study was carried out to evaluate the in vitro anti-inflammatory activity of selected herbal extracts using the albumin denaturation method. Herbal drugs such as turmeric (*Curcuma longa*), neem (*Azadirachta indica*), tulsi (*Ocimum sanctum*), and ginger (Zingiber officinale) were selected for the study. The extracts were prepared using Soxhlet extraction with suitable organic solvents. Different concentrations of the extracts were tested against heat-induced denaturation of albumin protein and compared with the standard drug diclofenac sodium. The anti-inflammatory activity was assessed by measuring the percentage inhibition of protein denaturation at 660 nm using a UV-visible spectrophotometer. Among the tested extracts, neem and turmeric showed significant inhibition of albumin denaturation, indicating appreciable anti-inflammatory activity, although lower than the standard diclofenac sodium. The activity may be attributed to the presence of phytoconstituents such as curcumin, flavonoids, tannins, gingerols, eugenol, and limonoids. The study suggests that these herbal extracts possess promising anti-inflammatory potential and may serve as natural therapeutic agents for inflammatory disorders. The albumin denaturation assay proved to be a simple, economical, and reliable method for preliminary screening of herbal anti-inflammatory activity.

Keywords: Inflammation, Herbal extract, Phytochemicals, In vitro evaluation, Anti-Inflammatory Activity, Albumin denaturation assay

Received: May 10, 2026 **Revised: June 21, 2026** **Accepted: July 25, 2026** **Published: August 03, 2026**

DOI: https://doi.org/10.64063/3049-1630.vol3.issue5.000246

https://ijphdt.com

1.Introduction

Inflammation was described as early as 3000 BC in an Egyptian papyrus and is still a common problem despite continuous advancements in prevention and treatment methods. Inflammation is a complex tissue reaction to injury. Injury may be caused not only by living microbes, i.e., bacteria, viruses, or fungi, leading to infection, but also by injurious chemical, physical, immunological, or radiation agents[1]. Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation and acts by removing injurious stimuli and initiating the healing process. Inflammation is therefore a defense mechanism that is vital to health. At the tissue level, inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function, which result from local immune, vascular, and inflammatory cell responses to infection or injury. Important microcirculatory events that occur during the inflammatory process include vascular permeability changes, leukocyte recruitment and accumulation, and inflammatory mediator release[2]. Inflammation is a protective physiological response of the body against harmful stimuli such as pathogens, injury, or irritation. However, excessive or chronic inflammation can lead to various diseases like arthritis, cardiovascular disorders, and cancer. Conventional anti-inflammatory drugs (NSAIDs and corticosteroids) are effective but often associated with side effects such as gastric irritation, ulcers, and long-term toxicity. Hence, there is growing interest in using herbal extracts as safer, natural alternatives for managing inflammation. Many medicinal plants, such as neem, tulsi, turmeric, and aloe vera, contain bioactive compounds like flavonoids, tannins, alkaloids, and phenolics that exhibit significant anti-inflammatory properties. These compounds act by inhibiting inflammatory mediators, stabilizing proteins, and preventing tissue damage. The albumin denaturation method is a widely used in vitro technique for evaluating the anti-inflammatory activity of herbal extracts. Protein denaturation is a well-documented cause of inflammation, where proteins lose their structure due to stress such as heat or chemicals. Denatured proteins can trigger inflammatory responses and auto-antigen formation. In this method, the ability of herbal extracts to inhibit heat-induced denaturation of albumin (egg or bovine serum albumin) is measured. A higher inhibition of protein denaturation indicates stronger anti-inflammatory activity. Thus, this method provides a simple, cost-effective, and reliable way to screen herbal extracts for potential anti-inflammatory properties before conducting in vivo studies[3].

Inflammation can be classified as acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli, and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A series of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells in the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation, such as mononuclear cells, and involves simultaneous destruction and healing of the tissue.

2. Types of Inflammation

1. Acute Inflammation

Acute Resolution, abscess formation, chronic inflammation, reactive oxygen species, hydrolytic enzymes. Delayed up to many months or years. Tissue destruction, fibrosis, necrosis. Acute

inflammation is a short-term process, usually appearing within a few minutes or hours and begins to cease upon the removal of the injurious stimulus. It involves a coordinated and systemic mobilization response of various immune, endocrine and neurological mediators of acute inflammation. In a normal healthy response, it becomes activated, clears the pathogen, begins a repair process, and then ceases. Acute inflammation occurs immediately upon injury and lasts only a few days. Cytokines and chemokines promote the migration of neutrophils and macrophages to the site of inflammation. Pathogens, allergens, toxins, burns, and frostbite are some of the typical causes of acute inflammation. Toll-like receptors (TLRs) recognize microbial pathogens. Acute inflammation can be a defensive mechanism to protect tissues against injury. Inflammation lasting 2–6 weeks is designated subacute inflammation. Acute inflammation may be regarded as the first line of defense against injury. Acute inflammatory response requires constant stimulation to be sustained. Inflammatory mediators are short-lived and are quickly degraded in the tissue. Hence, acute inflammation begins to cease once the stimulus has been removed.

2. Chronic inflammation

Chronic inflammation is inflammation that lasts for months or years. Macrophages, lymphocytes, and plasma cells predominate in chronic inflammation, in contrast to the neutrophils that predominate in acute inflammation. Diabetes, cardiovascular disease, allergies, and chronic obstructive pulmonary disease are examples of diseases mediated by chronic inflammation. Obesity, smoking, stress and insufficient diet are some of the factors that promote chronic inflammation.

2.1 Causes of Inflammation

Inflammation is the body's protective response to harmful stimuli. The main causes include:

1. Infectious agents:

Bacteria, viruses, fungi, and parasites trigger immune responses.

2. Tissue injury or damage:

Physical trauma (cuts, burns, fractures) and cell injury activate inflammation.

3. Chemical and toxic substances:

Toxins, pollutants, alcohol, and irritant chemicals can induce inflammation.

4. Immune reactions:

Autoimmune diseases and hypersensitivity reactions cause inflammation against the body's own tissues.

5. Physical factors:

Radiation, extreme heat/cold, and foreign bodies (e.g., splinters).

6. Lifestyle and metabolic factors (chronic inflammation):

Obesity, smoking, stress, poor diet, and lack of exercise[4]

2.2 Pathophysiology of inflammation

The pathophysiology of inflammation is a sequence of vascular and cellular events that occur in response to injury or infection, aimed at eliminating the cause and initiating repair.

1. Initiation (Recognition of Injury)

Harmful stimuli (infection, tissue damage, toxins) are recognized by immune cells.

Release of chemical mediators such as cytokines, histamine, and prostaglandins begins the response.

2. Vascular Changes

Vasodilation → increased blood flow (causing redness and heat).

Increased vascular permeability → leakage of plasma proteins and fluid into tissues → edema (swelling).

3. Cellular Events

Leukocyte migration (mainly neutrophils initially):

Margination → adhesion → diapedesis (movement through vessel walls)

Chemotaxis: leukocytes move toward the site of injury guided by chemical signals. Phagocytosis: engulfment and destruction of pathogens and debris.

4. Amplification by Mediators

Release of inflammatory mediators (histamine, cytokines, leukotrienes, prostaglandins).

These substances:

Sustain inflammation

Recruit more immune cells

Cause pain and tissue effects

5. Resolution or Chronicity Resolution:

removal of cause, tissue repair, and return to normal (homeostasis).

Chronic inflammation: occurs if the stimulus persists → leads to tissue damage, fibrosis, and involvement of macrophages and lymphocytes[5].

2.3 Mediator of inflammation

Mediators of inflammation are chemical substances released from cells or plasma that initiate, amplify, and regulate the inflammatory response.

1. Cell-Derived Mediators

Histamine & Serotonin (Vasoactive amines): Vasoactive amines are substances that change the size of blood vessels. They help control blood flow, blood pressure, and inflammation.

Cause vasodilation and increase vascular permeability

Prostaglandins (PGs)

Cause pain, fever, and vasodilation

Leukotrienes (LTs)

Increase vascular permeability and attract leukocytes

Cytokines (e.g., IL-1, TNF-α)

Regulate immune response and promote inflammation

Chemokines

Induce chemotaxis (movement of WBCs to site of injury)

2. Plasma-Derived Mediators

Complement system (C3a, C5a)

Promote inflammation, chemotaxis, and cell activation

Kinins (e.g., Bradykinin)

Cause pain, vasodilation, and permeability

Coagulation factors

Participate in inflammatory and repair processes

3. Other Important Mediators

Platelet Activating Factor (PAF)
Nitric Oxide (NO)
Reactive Oxygen Species (ROS)[6]

2.4 Mechanism of inflammation or role of protein denaturation in inflammation

Inflammation is a complex biological response triggered by harmful stimuli such as pathogens, damaged cells, or irritants. The process involves several key steps:

1. Initiation Phase

When tissue injury or infection occurs, damaged cells release chemical mediators including histamine, bradykinin, prostaglandins, and cytokines (IL-1β, IL-6, TNF-α). These signals activate the innate immune system and initiate the inflammatory cascade.

2. Vascular Response

Vasodilation increases blood flow to the affected area
Increased vascular permeability allows plasma proteins and leukocytes to migrate to the site of injury
This produces the classic signs: rubor (redness), calor (heat), tumor (swelling), and dolor (pain)

3. Cellular Response

Neutrophils and macrophages are recruited via chemotaxis. They engulf pathogens and release reactive oxygen species (ROS) and proteolytic enzymes, which can cause further tissue damage if unregulated.

4. Resolution or Chronicity

If the trigger is eliminated, anti-inflammatory mediators (lipoxins, resolvins) resolve inflammation. If unresolved, it becomes chronic, contributing to diseases like arthritis, diabetes, and cancer.

Role of Protein Denaturation in Inflammation

Protein denaturation plays a central role in the pathophysiology of inflammation:
Autoantigens from denatured proteins: When proteins are denatured due to heat, oxidative stress, or microbial enzymes, they lose their native conformation and expose hidden epitopes, triggering an immune/inflammatory response.
Denaturation of serum albumin: Albumin is the most abundant plasma protein. Under inflammatory conditions, albumin undergoes conformational changes (denaturation), which leads to the production of autoantibodies and activation of complement pathways, amplifying inflammation.
Lysosomal damage theory: Denatured proteins destabilize lysosomal membranes, causing release of hydrolytic enzymes into the cytoplasm, which further perpetuates tissue damage and inflammation.
Relevance to arthritis: In conditions like rheumatoid arthritis, protein denaturation within joint tissues contributes to the chronic inflammatory process. Anti-arthritic drugs are known to inhibit protein denaturation as one of their mechanisms[7].

3. Anti-inflammatory drugs:
Classification of Anti-Inflammatory Drugs
Anti-inflammatory drugs are broadly classified into major categories:

1. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)
NSAIDs are the most commonly used anti-inflammatory agents. They act primarily by inhibiting cyclooxygenase (COX) enzymes (COX-1 and COX-2), thereby blocking prostaglandin synthesis.
Sub-classifications:

a) Salicylates
Aspirin (Acetylsalicylic acid)
Sodium salicylate

b) Propionic Acid Derivatives
Ibuprofen
Naproxen
Ketoprofen

c) Acetic Acid Derivatives
Diclofenac sodium (most used standard in albumin denaturation assay)
Indomethacin
Ketorolac

d) Oxicam Derivatives
Piroxicam
Meloxicam

e) Fenamates
Mefenamic acid
Flufenamic acid

f) Selective COX-2 Inhibitors (Coxibs)
Celecoxib
Rofecoxib
Etoricoxib

2. Steroidal Anti-Inflammatory Drugs (Corticosteroids)
Act by inhibiting phospholipase A2, blocking the entire arachidonic acid cascade.

Drugs	Route
Prednisolone	Oral/IV
Dexamethasone	Oral/IV/topical
Hydrocortisone	Topical/IV
Betamethasone	Topical/oral

Triamcinolone	Intra-articular injection

3.Disease-Modifying Anti-Rheumatic Drugs (DMARDs)
Used for chronic inflammatory conditions like rheumatoid arthritis.

Drug	Mechanism
Methotrexate	Inhibits folate metabolism
Hydroxychloroquine	Stabilizes lysosomal membranes
Sulfasalazine	Inhibits NF-κB and prostaglandin synthesis
Leflunomide	Inhibits pyrimidine synthesis

4. Biological Anti-Inflammatory Agents
Modern targeted biologics acting on specific inflammatory mediators.

Drug	Target
Infliximab, Adalimumab	TNF-α inhibitors
Tocilizumab	IL-6 receptor antagonist
Anakinra	IL-1 receptor antagonist
Rituximab	Anti-CD20 (B-cell depletion)
Abatacept	T-cell co-stimulation blocker[8]

Diclofenac is Used as Standard in Albumin Denaturation Assay
Mostly Diclofenac sodium is the most preferred standard drug because:

Reason	Detail
Protein stabilization	Strongly inhibits heat-induced albumin denaturation
Well-documented	Extensively validated in BSA denaturation assays
Comparison baseline	Provides reliable % inhibition for comparing herbal extracts
Dual COX inhibition	Inhibits both COX-1 and COX-2 pathways
Availability	Easily available as a pharmaceutical-grade standard[9]

4. Herbal medicine

1. curcuma longa (Turmeric)
2. Zingiber officinale (Ginger)
3. Ocimum sanctum (Tulsi)
4. Azadirachta indica (Neem)
5. Aloe vera (Aloe vera)
6. Allium sativum (Garlic)

5. Profile of Turmeric (Curcuma longa)

Phytochemicals include curcumin (a curcuminoid), diarylheptanoids like demethoxycurcumin essential oils such as turmerone and zingiberene, along with alkaloids, terpenoids, tannins, flavonoids, steroids, carbohydrates, and saponins[11].

Fig1 : Turmeric

These compounds contribute to turmeric's yellow color and health benefits, detected across solvent extracts like methanol and chloroform[11].

Biological Source

Turmeric consists of dried rhizomes of *Curcuma longa*.

Family : Zingiberaceae

Common Names

- Haldi
- Indian saffron

Chemical Constituents

- Curcumin
- Demethoxycurcumin
- Volatile oils
- Turmerone

Medicinal Uses

- Anti-inflammatory

- Antioxidant
- Antimicrobial
- Wound healing
- Hepatoprotective

Mechanism of Anti-inflammatory Action
Curcumin inhibits inflammatory mediators such as prostaglandins, cytokines, and cyclooxygenase enzymes.

Pharmacological Activities
- Anticancer
- Antidiabetic
- Antiarthritic
- Immunomodulatory

6. Profile of Neem (Azadirachta indica)
Major phytochemicals are limonoids such as azadirachtin, nimbin, and salannin, plus flavonoids, tannins, glycosides, steroids, saponins, and terpenes[12].

Fig 2: Neem Leaves

These are found in leaves, bark, seeds, and other parts, supporting antimicrobial and insecticidal effects[13].

Biological Source
Neem consists of leaves and bark of *Azadirachta indica*.

Family: Meliaceae

Common Names
- Neem
- Indian lilac

Chemical Constituents
- Azadirachtin
- Nimbin
- Nimbidin
- Quercetin

Medicinal Uses

- Antibacterial
- Antifungal
- Anti-inflammatory
- Antipyretic

Mechanism of Action

Neem inhibits inflammatory cytokines and oxidative stress.

Pharmacological Activities

- Antimalarial
- Antiviral
- Antioxidant
- Anticancer

7. Profile of Tulsi (Ocimum sanctum)

Prominent phytochemicals include eugenol, rosmarinic acid, apigenin, luteolin, ursolic acid, oleanolic acid, and isothymusin, alongside flavonoids and other antioxidants.

Fig:3 Tulsi

These support anti-cancer and anti-inflammatory activities in preclinical studies. [14]

Biological Source

Tulsi consists of leaves of *Ocimum sanctum*.

Family: Lamiaceae

Common Names

- Holy basil
- Tulsi

Chemical Constituents

- Eugenol
- Ursolic acid
- Rosmarinic acid
- Linalool

Medicinal Uses

- Anti-inflammatory

8. Profile of Ginger (Zingiber officinale)

Phytochemicals feature gingerols, phenolic acids, flavonoids, alkaloids, tannins, saponins, glycosides, steroids, terpenoids, phenols, and anthraquinones[14].

Fig 4: Ginger

These vary by extract type and provide antioxidant and anti-inflammatory potential. (16)

Biological Source

Ginger consists othe f rhizomes of *Zingiber officinale*.

Family: Zingiberaceae

Common Names

- Ginger
- Adrak

Chemical Constituents

- Gingerol
- Shogaol
- Zingerone
- Essential oils

Medicinal Uses

- Anti-inflammatory
- Antiemetic
- Digestive stimulant
- Antioxidant

Mechanism of Action

Ginger inhibits prostaglandin synthesis and reduces oxidative stress.

Pharmacological Activities

- Anticancer
- Antidiabetic
- Antimicrobial
- Antiulcer[15-17]

9 . Albumin denaturation method

The albumin Denaturation Method is an in vitro anti-inflammatory assay used to evaluate the ability of a substance (plant extract, drug, compound, etc.) to inhibit protein denaturation.

Inflammation is a complex biological response of the body to harmful stimuli such as pathogens, damaged cells, or irritants. It is a protective mechanism, but when it becomes chronic or uncontrolled, it leads to serious diseases including arthritis, cardiovascular disorders, and cancer. The search for effective anti-inflammatory agents, particularly from natural plant sources, has led researchers to develop various in vitro screening models that are simple, reproducible, and cost-effective. Among these, the albumin denaturation method stands out as one of the most widely used and accepted techniques for evaluating the anti-inflammatory potential of drugs, plant extracts, and synthetic compounds.

Protein denaturation is the process by which a protein loses its native three-dimensional structure due to the disruption of non-covalent bonds and sometimes covalent bonds that stabilize the protein's architecture. The native conformation of a protein is maintained by a variety of molecular forces, including hydrogen bonds, hydrophobic interactions, ionic interactions, and van der Waals forces. When these forces are disrupted by physical agents such as heat or chemical agents such as acids, alkalis, or organic solvents, the protein unfolds and loses its functional integrity. In biological systems, denaturation of body proteins is recognized as one of the primary causes of inflammation and associated pathological conditions.

Albumin is the most abundant plasma protein in the human body and plays a crucial role in maintaining osmotic pressure, transporting hormones, fatty acids, and drugs in the bloodstream. In the albumin denaturation method, Bovine Serum Albumin (BSA) is commonly used as the test protein because of its structural similarity to human serum albumin, its ready commercial availability, and its well-documented physicochemical properties. Egg albumin is also used in some experimental protocols as an alternative. The choice of albumin as the model protein is based on its sensitivity to heat-induced denaturation, which can be easily monitored spectrophotometrically.

Principle of the Method

The fundamental principle of the albumin denaturation method is based on the well-established observation that denaturation of tissue proteins is one of the documented causes of inflammation. Any agent capable of inhibiting protein denaturation is therefore considered to possess anti-inflammatory activity. The method exploits the heat-induced denaturation of albumin as a model of inflammation.

When a solution of BSA is heated to approximately 70°C for 10–15 minutes, the protein undergoes denaturation. The denatured albumin molecules aggregate and form insoluble precipitates, resulting in increased turbidity of the solution. This turbidity can be quantitatively measured using a spectrophotometer, typically at a wavelength of 660 nm or 280 nm. A turbid solution with high

absorbance indicates significant protein denaturation, while a clear solution with low absorbance indicates protection of the protein from denaturation.
When a test compound (drug, plant extract, or fraction) is pre-incubated with the albumin solution before heat treatment, and if the compound has anti-inflammatory (anti-denaturation) properties, it will stabilize the protein structure and prevent or significantly reduce the formation of turbidity. The degree of protection conferred by the test compound against albumin denaturation is a direct indicator of its anti-inflammatory potential.

Calculation of Percentage Inhibition
The anti-denaturation (anti-inflammatory) activity of the test compound is expressed as the percentage inhibition of albumin denaturation, calculated using the following formula:
% Inhibition = (1 − Absorbance of sample / Absorbance of control) × 100
A higher percentage inhibition indicates greater anti-inflammatory activity. The IC50 value (concentration required to inhibit 50% of albumin denaturation) is calculated from dose-response curves and compared with that of the standard drug to determine the relative potency of the test compound.

Significance and Applications
The albumin denaturation method is particularly valuable in early-stage pharmacological screening of plant-derived compounds and new drug candidates. It is simple, rapid, and does not require sophisticated equipment, making it accessible to laboratories worldwide. The method provides a preliminary indication of anti-inflammatory activity that can guide further in vivo studies. It is especially useful in ethnopharmacological research, where large numbers of plant extracts need to be screened quickly and economically for bioactivity[18].

10. Aim And Objective

Aim
To evaluate the anti-inflammatory activity of herbal extracts by using the albumin denaturation method in vitro.

Objectives
1. To prepare herbal extracts from selected medicinal plants.
2. To study the inhibitory effect of herbal extracts on albumin denaturation.
3. To compare the anti-inflammatory activity of different concentrations of herbal extracts.
4. To compare the activity of herbal extracts with a standard anti-inflammatory drug.
4. To calculate the percentage inhibition of protein denaturation produced by the extracts.
5. To assess the potential of herbal extracts as natural anti-inflammatory agents[19].

10.1. MOA

1. Stabilization of Protein Structure
Phytochemicals present in herbal extracts (flavonoids, tannins, alkaloids, phenols) interact with albumin molecules through hydrogen bonding and hydrophobic interactions, thereby preventing conformational changes (denaturation) in albumin induced by heat or chemical agents.

2. Inhibition of Auto-Antigen Formation
By preventing albumin denaturation, herbal extracts suppress the formation of autoantigens, which would otherwise trigger antibody-mediated inflammatory pathways (Type III hypersensitivity reactions).

3. Membrane Stabilization

Bioactive compounds in herbal extracts stabilize lysosomal and cell membranes, preventing the release of pro-inflammatory lysosomal enzymes (proteases, phospholipases) that amplify the inflammatory cascade.

4. Inhibition of Inflammatory Mediators

The phytoconstituents inhibit the release of:
Prostaglandins (via COX pathway inhibition)
Histamine and bradykinin
Cytokines (TNF-α, IL-1β, IL-6)

5. Antioxidant Mechanism

Free radicals generated during inflammation can denature proteins. Herbal antioxidants scavenge ROS (Reactive Oxygen Species), indirectly protecting albumin from oxidative denaturation[20].

11. Materials and methods

Materials Required

Chemicals

- Bovine serum albumin
- Diclofenac sodium
- Distilled water
- Phosphate buffer
- Methanol
- Hydrochloric acid
- Sodium hydroxide

Equipment

- UV-Visible spectrophotometer
- Incubator
- Water bath
- Analytical balance
- Pipettes
- Test tubes
- Beakers
- Measuring cylinders

Plant Materials

- Turmeric rhizomes
- Neem leaves
- Tulsi leaves
- Ginger rhizomes

12.Preparation of solution

Preparation of test solution

1. All Apparatus were clean and dried.
2. Prepared the stock solution of 100 ppm of herbal extract by dissolving in ethanol.
3. Different dilutions of herbal extract were prepared (eg. 100, 200, 300, 400, 500 ug/ml)
4. By using the solvent baseline correction was done.

5. With the help of the highest concentration, scanning was done in the range of 660 nm; ƛ max was determined.
6. Absorbance of unknown dilution of herbal extract was also determined.
7. A graph of absorbance vs. concentration was also plotted[21].

Fig. 5: Volumetric flask

13. Experimental Procedure

Collection of Plant Materials

Fresh turmeric rhizomes, neem leaves, tulsi leaves, and ginger rhizomes were collected from local areas and authenticated.

Drying

The collected plant materials were washed thoroughly with water to remove dirt and impurities. The materials were shade-dried at room temperature for several days until complete drying was achieved.

Pulverization

The dried plant materials were powdered separately using a mechanical grinder. The powdered material was passed through sieve no. 40 to obtain uniform particle size.

Extraction by Soxhlet Apparatus

Principle of Soxhlet Extraction

Soxhlet extraction is a continuous extraction technique used for separating active constituents from plant materials using a suitable solvent. In this method, the solvent repeatedly passes through the plant material and extracts phytoconstituents efficiently.

Materials Required

- Soxhlet apparatus
- Heating mantle
- Round bottom flask
- Condenser
- Whatman filter paper
- Methanol or ethanol
- Powdered crude drug

1. Turmeric (curcumin)

Materials

Turmeric were collected from the area around Kalwan. Soxhlet extractor, Round-bottom flask, Condenser, Heating mantle or water bath, Sample material, Stand and clamps, Glass tubing or rubber pipes for water inlet and outlet. Solvents (Methanol, ethanol, hexane) were supplied by our college. All chemicals used were analytical grade with 95- 98% purity.

Fig:6 Turmeric Powder

Sample preparation

1. Take turmeric roots
- Dry them and grind them into powder using a mortar.
- Air-dry the powder to remove moisture.
2. Weighing the sample
- Take a known quantity of turmeric powder (10g) for extraction.
3. Solvent extraction
- Treat turmeric powder with a suitable organic solvent such as :
-Ethanol
- Turmeric powder is placed inside the Soxhlet extractor.
- A solvent like acetone or ethanol is heated.
4.Distillation
- Heat the mixture to the boiling point of the solvent[22]

Fig:7 Soxhlet Apparatus

General Reaction mixture
-Mix 1 mL of 1% Albumin solution, 1 mL of extract or standard, and 2ml of PBS / Distilled water.

Incubation
-Incubate at 37ºC for 20 minutes.
-Heat at 70ºC for 5 minutes in a water bath.

Cooling and measurement
-Cool the tubes and measure absorbance at 660 nm.

2 . Neem
Several materials have been used in the current study, and a few steps were implemented during the preparation of the sample till the analysis was completed.

Materials
Soxhlet extractor, Round-bottom flask, Condenser, Heating mantle or water bath, Sample material, Stand and clamps, Glass tubing or rubber pipes for water inlet and outlet. Solvents (Methanol, ethanol, hexane) were supplied by our college. All chemicals used were analytical grade with 95-98% purity.

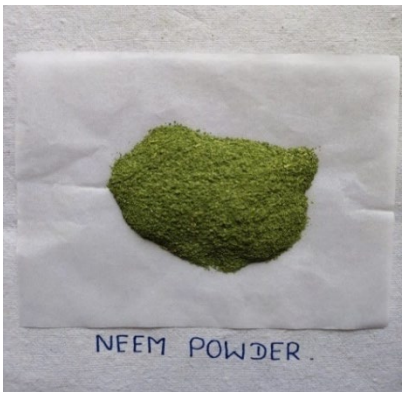

Fig:8 Neem Powder

Sample preparation

1. Collect neem leaves and wash them with distilled water.
2. Dry the leaves in shade for about three days.
3. Grind the dried leaves into fine powder.
4. Prepare the extract using Soxhlet extraction or immersion extraction.
5. For Soxhlet extraction, place the 26.0 g neem leaf powder in a thimble and extract it with 300 mL methanol for several hours.
6. For immersion extraction, soak the powder in methanol for about 24 hours with occasional shaking.
7. Filter the extract to remove solid particles.
8. Evaporate the solvent to obtain the crude neem extract.
9. Store the extract properly for further testing.
10. Use the extract for UV-Vis analysis or other experiments[23].

Fig:9 Soxhlet Apparatus

3. Tulsi

Materials

Soxhlet extractor, Round-bottom flask, Condenser, Heating mantle or water bath, Sample material, Solvent, Stand and clamps, Glass tubing or rubber pipes for water inlet and outlet. Solvent (ethanol) was supplied by our college. All chemicals used were analytical grade with 95- 98% purity.

Fig:10 Tulsi Powder

Sample preparation

1. Put the powdered sample inside the thimble.
2. Place the thimble into the Soxhlet extractor.
3. Add the solvent to the round-bottom flask.
4. Fix the Soxhlet apparatus with the condenser on top.
5. Start heating the flask so the solvent boils and vapour rises.
6. The vapour condenses in the condenser and drips into the extractor.
7. The solvent collects in the chamber and extracts the desired compounds from the sample.
8. When the chamber gets full, the solvent automatically siphons back into the flask.
9. This process repeats several times.
10. After enough cycles, stop heating and collect the extracted solution from the flask[24].

Fig. 11: UV-Visible Spectrometer

4. Ginger

Materials

Soxhlet extractor, Round-bottom flask, Condenser, Heating mantle or water bath, Sample material, Solvent, Stand and clamps, Glass tubing or rubber pipes for water inlet and outlet. Solvent (ethanol) was supplied by our college. All chemicals used were analytical grade with 95- 98% purity.

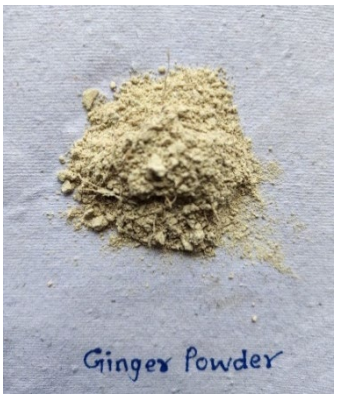

Fig :11 Ginger powder

Sample preparation:

1. Take dried ginger rhizomes and grind them.
2. Take 10 g of crushed ginger.
3. Put it in a Soxhlet apparatus.
4. Add 250 mL of 99.9% ethanol.
5. Run the extraction for 17 hours.
6. The ethanol keeps boiling, condensing, and passing through the ginger to remove compounds.
7. After extraction, remove the ethanol using a rotary evaporator at 60°C under vacuum.
8. Collect the concentrated ginger extract[25].

Fig:12 Rotary Evaporator

14. Observation Table

Dilution Table:

Sr.No.	Required Concentration (ppm)	Required volume	Stock solution concentration (ug/ml)
1	100 ug/ml	1 ml	100 ppm
2	200 ug/ml	2 ml	100 ppm
3	300 ug/ml	3 ml	100 ppm
4	400 ug/ml	4 ml	100 ppm
5	500 ug/ml	5 ml	100 ppm

Sr.No.	Herbal extract/std/control	volume	Concentration ug/ml	Absorbance	% inhibition
1.	Turmeric (extract)	1ml	100 ug/ml	660 nm	33.7%
		2ml	200 ug/ml	660 nm	32.3%
		3ml	300 ug/ml	660 nm	10.7%
		4ml	400 ug/ml	660 nm	52.7%
		5ml	500 ug/ml	660 nm	52.7%
2.	Neem (Extract)	1ml	100 ug/ml	660 nm	75.4 %
		2ml	200 ug/ml	660 nm	39.3 %
		3ml	300 ug/ml	660 nm	63.8 %
		4ml	400 ug/ml	660 nm	79.9 %
		5ml	500 ug/ml	660 nm	59.3 %
3.	Tulsi (Extract)	1ml	100 ug/ml	660 nm	7.80%

		2ml	200 ug/ml	660 nm	32.36%
		3ml	300 ug/ml	660 nm	34.63%
		4ml	400 ug/ml	660 nm	51.55%
		5ml	500 ug/ml	660 nm	52.75%
4.	Ginger (Extract)	1ml	100 ug/ml	660 nm	6.86%
		2ml	200 ug/ml	660 nm	47.16%
		3ml	300 ug/ml	660 nm	35.58%
		4ml	400 ug/ml	660 nm	52.57%
		5ml	500 ug/ml	660 nm	52.48%
5	Standard	1 ml	100 ug/ml	660 nm	96.8%
		2 ml	200 ug/ml	660 nm	88.0%
		3 ml	300 ug/ml	660 nm	91.5%
		4 ml	400 ug/ml	660 nm	90.1%
6	Control	1ml	100 ug/ml	660 nm	2.053
		2ml	200 ug/ml	660 nm	2.027
		3ml	300 ug/ml	660 nm	2.119
		4ml	400 ug/ml	660 nm	2.252
		5ml	500 ug/ml	660 nm	2.252

15. Calculations

Calculations

Formula - % Inhibition = (A control – A sample) / A control × 100

1. Turmeric extract

1. For 1ml - 2.053 - 1.361/2.053 × 100 = 33.7%
2. For 2ml - 2.027 - 1.373/2.027 × 100 = 32.3%
3. For 3ml - 2.119 - 1.893/2.119 × 100 = 10.7%
4. For 4ml - 2.252 - 1.079/2.252 × 100 = 52.7%
5. For 5ml - 2.252 -1.064/2.252 × 100 =52.7%

2. Neem extract:

1. For 1ml: 2.053 - 0.505/2.053 × 100 = 75.4 %
2. For 2ml: 2.027 - 1.230/2.027 × 100 = 39.3 %
3. For 3ml: 2.119 - 0.765/2.119 × 100 = 63.8 %
4. For 4ml: 2.252 - 0.452/2.252 × 100 = 79.9 %
5. For 5ml: 2.252 - 0.916/2.252 × 100 =59.3 %

3. Tulsi extract:

1. For 1ml: 2.053 – 1.893/2.053 × 100 = 7.80%
2. For 2ml: 2.027 – 1.371/2.027 × 100 = 32.36%
3. For 3ml: 2.119 – 1.385/2.119 × 100 = 34.63%
4. For 4ml: 2.252 – 1.091/2.252 × 100 = 51.55%
5. For 5ml: 2.252 -1.064/2.252 × 100 = 52.75%

4. Ginger extract

1. For 1ml: 2.053 – 1.912/2.053 × 100 = 6.86%
2. For 2ml: 2.027 – 1.071/2.027 × 100 = 47.16%
3. For 3ml: 2.119 – 1.365/2.119 × 100 = 35.58%
4. For 4ml: 2.252 – 1.068/2.252 × 100 = 52.57%
5. For 5ml: 2.252 -1.070/2.252 × 100 = 52.48%

Standard (Diclofenac sodium)

1. For 1ml: 2.053 - 0.065/2.053 × 100 = 96.8%
2. For 2ml: 2.027 - 0.244/2.027 × 100 = 88.05%
3. For 3ml: 2.119 - 0.181/2.119 × 100 = 91.5%
4. For 4 mL: 2.252 – 0.222/2.252 × 100 = 90.1%

16. Result and Discussion

Results

The anti-inflammatory activity of the selected herbal extracts was evaluated by the albumin denaturation method and compared with the standard drug diclofenac sodium. The percentage inhibition of heat-induced albumin denaturation was used as the indicator of anti-inflammatory potential. Higher percentage inhibition indicated stronger protection against protein denaturation and better anti-inflammatory activity.

Comparative Anti-inflammatory Activity of Herbal Extracts

Herbal Extract	Highest % Inhibition Observed	Concentration/Volume	Observation
Turmeric (Curcuma longa)	52.7%	4–5 ml	Moderate anti-inflammatory activity
Neem (Azadirachta indica)	79.9%	4 ml	Highest activity among herbal extracts
Tulsi (Ocimum sanctum)	52.75%	5 ml	Moderate activity
Ginger (Zingiber officinale)	52.57%	4 ml	Moderate activity
Diclofenac Sodium (Standard)	96.8%	1 ml	Very strong anti-inflammatory activity

Among all the tested herbal extracts, neem showed the highest inhibition of albumin denaturation (79.9%), indicating strong anti-inflammatory potential. Turmeric, tulsi, and ginger also exhibited considerable inhibitory activity, with maximum inhibition values around 52%. However, the activity of all herbal extracts remained lower than the standard diclofenac sodium, which showed inhibition values ranging from 88% to 96.8%.

The absorbance values decreased in the presence of herbal extracts compared with the control, demonstrating their ability to stabilize albumin protein against heat-induced denaturation. This confirms that the phytoconstituents present in these medicinal plants possess anti-inflammatory properties.

Discussion

The present investigation demonstrated that the selected herbal extracts possess significant in vitro anti-inflammatory activity by inhibiting heat-induced albumin denaturation. Protein denaturation is one of the important causes of inflammation because denatured proteins can trigger auto-antigen production and inflammatory responses. Therefore, compounds capable of preventing protein denaturation are considered potential anti-inflammatory agents.

Neem extract exhibited the highest anti-inflammatory activity among the tested herbal drugs, with a maximum inhibition of 79.9%. The strong activity of neem may be attributed to the presence of bioactive phytochemicals such as nimbidin, nimbin, azadirachtin, quercetin, flavonoids, and terpenoids. These compounds are known to inhibit inflammatory mediators, reduce oxidative stress, and stabilize proteins and cell membranes.

Turmeric extract showed moderate anti-inflammatory activity with a maximum inhibition of 52.7%. The activity may be due to curcumin and related curcuminoids present in turmeric. Curcumin is well known for inhibiting cyclooxygenase (COX) enzymes, prostaglandin synthesis, and inflammatory cytokines such as TNF-α and IL-6.

Tulsi extract also demonstrated moderate inhibition of albumin denaturation, with a maximum inhibition of 52.75%. The anti-inflammatory effect of tulsi may be due to phytoconstituents such as eugenol, rosmarinic acid, ursolic acid, and flavonoids, which possess antioxidant and anti-inflammatory activities.

Ginger extract showed appreciable anti-inflammatory activity with a maximum inhibition of 52.57%. Ginger contains active constituents such as gingerols, shogaols, and zingerone that inhibit prostaglandin synthesis and reduce oxidative stress, thereby contributing to anti-inflammatory action.

The standard drug diclofenac sodium showed the highest inhibition values (88–96.8%), confirming the validity and reliability of the albumin denaturation method. Diclofenac stabilizes proteins by inhibiting cyclooxygenase pathways and reducing inflammatory mediator formation.

The findings of the present study support the traditional medicinal use of neem, turmeric, tulsi, and ginger in the treatment of inflammatory conditions. The observed activity may be associated with the synergistic action of phytochemicals such as flavonoids, phenolics, tannins, alkaloids, and terpenoids present in these herbal extracts.

Overall, the albumin denaturation assay proved to be a simple, rapid, economical, and reliable in vitro screening method for evaluating anti-inflammatory activity of herbal drugs. Further investigations involving isolation of active constituents, detailed pharmacological studies, toxicity evaluation, and in vivo experiments are recommended to establish their therapeutic efficacy and clinical applications.

17. Advantages and limitations

Advantages

1.Simple and rapid method.
2.Cost effective and economical technique.
3.Requries simple laboratory equipment.
4. Suitable for evaluation of herbal extract.
5. Requires less time for analysis.
6.Sensitive method for detecting protein stabilization activity.

Limitations

1.Limited to evaluation of protein denaturation only.
2.It is only an in vitro method and does not completely mimic in vivo contitions.
3.Variation in phytochemical composition may affect results.

4.Cannot determine pharmacokinetic properties of herbal compounds.
5.Results may vary depending on concentration and solvent used. [26]

18.Conclusion

The present study demonstrated that selected herbal extracts possess considerable anti-inflammatory activity as evaluated by the albumin denaturation method. The extracts of turmeric, neem, tulsi, and ginger showed the ability to inhibit heat-induced protein denaturation, which is one of the important mechanisms involved in inflammation. Among the tested herbal extracts, neem and turmeric exhibited comparatively higher percentage inhibition, indicating stronger anti-inflammatory potential. However, the activity of all herbal extracts was lower than that of the standard drug diclofenac sodium. The observed activity may be due to the presence of bioactive phytochemicals such as curcumin, flavonoids, phenolic compounds, terpenoids, and tannins, which stabilize proteins and inhibit inflammatory mediators. The study supports the traditional use of these medicinal plants in the treatment of inflammatory conditions. Furthermore, the albumin denaturation assay was found to be a simple, rapid, and cost-effective in vitro method for screening anti-inflammatory activity of herbal drugs. Further studies including isolation of active constituents, detailed pharmacological evaluation, and in vivo investigations are recommended to establish their therapeutic efficacy and safety.

19. Precautions

1. All glassware and apparatus should be properly cleaned and dried before use.
2. Use analytical grade chemicals and freshly prepared reagents.
3. Maintain accurate concentrations while preparing stock and test solutions.
4. Albumin solution should be prepared freshly to avoid contamination.
5. Avoid exposure of herbal extracts to direct sunlight and excess heat.
6. Proper calibration of the UV-visible spectrophotometer should be done before analysis.
7. Maintain the incubation temperature at 37°C accurately.
8. Heating temperature should be controlled carefully at 70°C to prevent excessive denaturation.
9. Pipetting should be done carefully to avoid volume errors.
10. Use separate pipettes or tips for different samples to avoid cross-contamination.
11. Samples and standards should be mixed uniformly before incubation.
12. Absorbance should be measured immediately after cooling.
13. Air bubbles should be avoided while filling cuvettes.
14. Use clean and scratch-free cuvettes for accurate absorbance readings.
15. Perform the experiment in triplicate for reliable and reproducible results.
16. Proper labeling of all test tubes and solutions should be maintained.
17. Dispose of solvents and chemical waste according to laboratory safety guidelines.
18. Wear a laboratory coat, gloves, and protective eyewear during the experiment. [27]

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