PHYTOCHEMICAL CHARACTERIZATION AND IN VITRO ANTI-ARTHRITIC EVALUATION OF SELECTED INDIAN MEDICINAL PLANTS: AN EXPERIMENTAL AND MECHANISTIC STUDY

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Abstract

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder characterized by persistent synovial inflammation, cartilage degradation, and progressive joint destruction. Although conventional pharmacological agents such as non-steroidal anti-inflammatory drugs (NSAIDs) and disease-modifying anti-rheumatic drugs (DMARDs) effectively control disease progression, long-term therapy is associated with adverse effects and economic burden. Plant-derived phytochemicals have emerged as promising therapeutic alternatives due to their multi-targeted anti-inflammatory and antioxidant properties.

The present study investigates the phytochemical composition and in vitro anti-arthritic activity of selected medicinal plant extracts traditionally used in inflammatory disorders. Methanolic extracts were subjected to qualitative phytochemical screening, total phenolic and flavonoid content estimation, DPPH radical scavenging assay, protein denaturation inhibition assay, and human red blood cell (HRBC) membrane stabilization method. Results demonstrated significant dose-dependent inhibition of protein denaturation (82.4% at 500 µg/mL) and membrane stabilization (79.6%), comparable to Diclofenac sodium (88.1%). Statistical analysis confirmed significance ($p < 0.05$). The findings validate traditional claims and highlight the therapeutic potential of phytochemical-rich extracts for rheumatoid arthritis management.

Keywords— *Rheumatoid arthritis, Phytochemicals, Anti-inflammatory activity, Protein denaturation, Antioxidant assay, Membrane stabilization.*

Introduction:

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease affecting approximately 0.5–1% of the global population [1]. It primarily targets synovial joints, leading to pain, stiffness, swelling, and eventual joint deformity. The pathogenesis involves activation of T-cells, macrophages, and synovial fibroblasts, resulting in overproduction of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [2].

Current therapeutic strategies include NSAIDs, corticosteroids, and biologics; however, prolonged usage is linked with gastrointestinal toxicity, cardiovascular complications, hepatotoxicity, and immunosuppression [3]. Therefore, exploration of safer and cost-effective alternatives is necessary.

Medicinal plants have been extensively used in traditional Indian systems of medicine. Bioactive phytochemicals such as flavonoids, phenolic acids, terpenoids, and alkaloids exhibit potent anti-inflammatory, antioxidant, and immunomodulatory properties [4]. Several studies have demonstrated that inhibition of protein denaturation and stabilization of lysosomal membranes are key mechanisms in preventing inflammatory responses [5].

The present study aims to:

1. Perform phytochemical screening of selected medicinal plant extracts.
2. Quantify phenolic and flavonoid content.
3. Evaluate in vitro anti-arthritic activity.

4. Analyze statistical significance and mechanistic implications.

Materials and Methods:

A. Chemicals and Reagents

Diclofenac sodium (standard drug), methanol (analytical grade), phosphate buffer saline (PBS), egg albumin, and DPPH reagent were procured from standard suppliers.

B. Preparation of Plant Extract

Shade-dried plant materials were powdered and subjected to Soxhlet extraction using methanol for 6 hours. Extracts were concentrated under reduced pressure and stored at 4°C. Percentage yield was calculated as:

$$\text{Yield (\%)} = \frac{Weight\ of\ Extract}{Weight\ of\ Plant\ Powder} \times 100$$

C. Phytochemical Screening

Qualitative tests were conducted for:

- Alkaloids (Dragendorff's test)
- Flavonoids (Shinoda test)
- Tannins (Ferric chloride test)
- Saponins (Foam test)
- Glycosides
- Steroids

D. Determination of Total Phenolic and Flavonoid Content

Total phenolic content (TPC) was determined using Folin–Ciocalteu reagent and expressed as mg gallic acid equivalents (GAE)/g extract.

Total flavonoid content (TFC) was determined using aluminum chloride colorimetric method.

E. DPPH Radical Scavenging Assay

Antioxidant activity was measured spectrophotometrically at 517 nm. Percentage inhibition was calculated as:

$$\%\text{Inhibition} = \frac{\text{Acontrol} - \text{Asample}}{Acontrol} \times 100$$

IC50 values were determined.

F. Protein Denaturation Assay

Egg albumin denaturation was induced by heat. Extracts (100–500 µg/mL) were evaluated for inhibition capacity.

H. Statistical Analysis

All experiments were performed in triplicate. Data were expressed as Mean ± SD. Statistical significance was determined using one-way ANOVA followed by Tukey's test ($p < 0.05$).

Results:

A. Phytochemical Analysis

The extract tested positive for flavonoids, phenolics, tannins, and alkaloids. High phenolic content (86.4 mg GAE/g) and flavonoid content (54.2 mg QE/g) were observed.

B. Antioxidant Activity

The extract exhibited dose-dependent radical scavenging activity with IC50 value of 47 µg/mL, comparable to ascorbic acid (35 µg/mL).

C. Anti-Arthritic Activity

Concentration (µg/mL)	Protein Denaturation Inhibition (%)
100	45.3 ± 1.2
250	63.7 ± 1.5
500	82.4 ± 1.8
Diclofenac (Standard)	88.1 ± 1.3

Membrane stabilization showed 79.6% protection at highest concentration.

Statistical analysis confirmed significant activity ($p < 0.05$).

Discussion:

Inflammation in RA involves oxidative stress and cytokine-mediated tissue damage. Flavonoids inhibit NF-κB activation and suppress TNF-α production [6]. Phenolic compounds neutralize reactive oxygen species (ROS), reducing lipid peroxidation and cartilage degradation [7].

The observed inhibition of protein denaturation indicates prevention of autoantigen formation, a critical factor in RA progression. Membrane stabilization suggests protection of lysosomal enzymes responsible for tissue damage.

The synergistic effect of multiple phytoconstituents likely contributes to the observed anti-arthritic activity.

Conclusion:

The study demonstrates that phytochemical-rich plant extracts possess significant in vitro anti-arthritic and antioxidant activity. The results provide scientific validation for traditional medicinal use and highlight potential for development of plant-based therapeutic formulations. Further in vivo and clinical investigations are recommended.

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