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# *In-Vitro* Analysis of *Dracaena trifasciata* (Prain) Mabb. Extracts: Potential for Anti-Inflammatory and Anti-Arthritic Effects

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**Abstract**

The objective of this research is to investigate the potential antiarthritic and anti-inflammatory effects of Dracaena trifasciata leaves using in vitro methods. The antiarthritic activity was assessed using the protein denaturation method, while the anti-inflammatory activity was evaluated using the HRBC membrane stabilization method. Results showed that the hydroalcoholic extracts exhibited significant antiarthritic effects against egg albumin denaturation with 41.56% and against standard drug Diclofenac sodium with 88.22% inhibition at 800µg/ml concentration. Leaves extract shows 53.48 % stabilization of HRBC membrane at 800 µg/ml concentration 95.64% by Diclofenac sodium at the same concentration. These findings suggest that the hydroalcoholic extract demonstrates promising potential comparable to the standard drug Diclofenac Sodium in both antiarthritic and anti-inflammatory activities, as indicated by the in vitro studies conducted using the aforementioned methods.

**Keywords:** *Dracaena trifasciata*, *In-vitro* activity, Antiarthritic, Anti-inflammatory, hydroalcoholic extract.

## Introduction

Herbal medicine, deeply rooted in the cultural and traditional practices of many developing countries, has long been utilized as a form of herbal medicine. It has shown considerable efficacy in treating arthritic conditions. However, the prolonged use of modern drugs for

these disorders often leads to severe side effects. Consequently, there is a pressing need to develop new therapeutic agents with minimal adverse effects.

Rheumatoid arthritis (RA) is a persistent autoimmune condition marked by joint pain and inflammation, particularly affecting the hands and feet, often leading to physical disability. Globally, approximately one-fifth of the elderly population, predominantly women, experience arthritis.[1] As a systemic disorder, RA impacts the functioning of vital organs such as the heart, liver, and lungs, and is often associated with additional health complications.[2]

The root cause of the disease remains unknown. However, the presence of autoantibodies contributes to disease advancement alongside inflammation in both the joints and throughout the body.[3] The innate and humoral immune responses, as well as the production of cytokines, matrix metalloproteinases (MMPs), prostaglandins (PGs), cyclooxygenase (COX), and leukotrienes (LTs), are crucial in the development and prognosis of the disease.[4] Pannus formation in the synovial membrane leads to bone destruction, while enzymes released degrade cartilage.[5] Immune responses are orchestrated by Th cells, with Th1 cells activating macrophages and releasing pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α), interleukin (IL)-1, -6, -1β, and -7, crucial in cellular immunity. Conversely, Th2 cells, associated with humoral immunity, inhibit macrophages and prompt the release of anti-inflammatory cytokines such as IL-4, -10, and -13.[6] The excessive presence of reactive oxygen moieties (ROS) contributes to the onset and progression of various diseases including diabetes, cancer, and RA.[7]

Typically, rheumatoid arthritis (RA) is managed symptomatically, often necessitating lifelong treatment involving Non-steroidal anti-inflammatory drugs (NSAIDs), steroids, Disease-modifying anti-rheumatic drugs (DMARDs), immunosuppressants, and cytotoxic drugs.2 Adverse effects of these therapies are numerous that necessitate the development of safer and cost-effective anti-arthritic agents for lifelong use. Side effects associated with NSAIDs therapy include stomach problems, including pain, constipation, diarrhea, gas, nausea, and stomach ulcers. [8] Side effects associated with DMARD's therapy include anemia, leukopenia, myopathy, cardiomyopathy, nausea, abdominal pain, diarrhea, rash/allergic reaction, bone marrow suppression, hepatotoxicity etc.[9] The potential of herbal medicines as promising alternatives for developing safe and effective treatments for arthritis stems from their ready availability and cost-effectiveness compared to conventional allopathic medications. [10]

*Dracaena trifasciata* is locally called as snake plant belongs to Asparagaceae family. [11] Phytochemical screening of plant shows the presence of alkaloid and flavonoids. [12] A review of existing literature indicates a lack of scientific verification regarding the anti-arthritic and anti-inflammatory properties of *Dracaena trifasciata*. Consequently, this study was designed to investigate the plant's potential for alleviating arthritis through a series of *in-vitro* experiments.

**Materials and method**

**Collection and Authentication of Plant Material**

The fresh leaves of *Dracaena trifasciata* were collected from local area of Malwa region of Madhya Pradesh, and it was authenticated by Dr. S.N. Dwivedi, Retd. Professor Janata PG College & Visiting Professor, APS University, Rewa, M.P., Voucher specimen No. J/Bot/DTP-12 was alloted. The collected leaves were shade dried and coarsely powdered. The Plant material was subjected to successive extraction using hydroalcoholic solvent (50:50) by

cold maceration process. The solvents were distilled under reduced pressure using rotary evaporator.

***In-vitro* Antiarthritic Activity by Protein Denaturation Method**

The reaction mixture (5 ml) comprised egg albumin (0.2 ml), phosphate buffered saline (2.8 ml, pH 6.4), and 2 ml of *Dracaena trifasciata* extract, along with diclofenac sodium at various concentrations (12.5, 25, 50, 100, 200, 400, and 800 µg/ml). An equal volume of double-distilled water was used as a control. These mixtures were then placed in a Biochemical Oxygen Demand (BOD) incubator at 37 ± 2 °C for 15 minutes, followed by heating at 70 °C for 5 minutes. Subsequently, their absorbance was measured at 660 nm.[13]

The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance control} - \text{Absorbance test sample}}{\text{Absorbance Test Control}} \times 100$$

***In-vitro* Inflammatory activity by HRBC Membrane Stabilisation Method**

Maintaining the stability of the lysosomal membrane plays a crucial role in reducing inflammation by preventing the release of harmful substances from activated neutrophils, like bactericidal enzymes and proteases. These substances can exacerbate tissue inflammation and damage if released into the extracellular space. Similarly, stabilizing the lysosomal membrane is vital. The membrane of human red blood cells, or erythrocytes, bears resemblance to the lysosomal membrane. Therefore, stabilizing the erythrocyte membrane suggests that a substance might also stabilize lysosomal membranes. Testing the stabilization of human red blood cell membranes under conditions of reduced tonicity serves as an in vitro method to assess the anti-inflammatory potential of drugs or plant extracts.[14,15]

Blood samples were obtained from healthy human volunteers who had abstained from consuming NSAIDs for a period of two weeks. The collected blood samples were combined with an equal volume of Alsever solution (comprising 2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% sodium chloride) and then centrifuged at 3000 rpm. The resulting packed cells were washed with isosaline, and a 10% suspension was prepared. Various concentrations of the extracts (12.5, 25, 50, 100, 200, 400, and 800 µg/ml) were prepared using DMSO. To each of these solutions, 1 ml of phosphate buffer, 2 ml of hyposaline, and 0.5 ml of HRBC suspension were added. The mixtures were then incubated at 37°C for 30 minutes and subsequently centrifuged at 3000 rpm for 20 minutes. The clear supernatant liquid was assessed using a UV-visible spectrophotometer at 560 nm. Diclofenac sodium was used as the reference standard.[16]

Percentage protection = 100 − [(Absorbance sample/Absorbance control) × 100]

**Results and discussion**

***In vitro* anti-arthritic potential**

**Activity against egg albumin denaturation**

Results of protein denaturation study, shows that hydroalcoholic extracts effectively inhibit protein denaturation (albumin) caused by heat in a concentration dependant manner. Table

1 shows significant inhibition of 41.56% and 88.22%, respectively, for the extract and diclofenac sodium at a concentration of 800$\mu$g/ml.

**HRBC membrane stabilization activity**

The plant extracts demonstrated a dose-dependent stabilization of the RBC membrane, with the highest stabilization observed at a concentration of 800µg/ml. Hydroalcoholic extract shows 53.48 % inhibition of RBC membrane lysis as compared with 95.64% by Diclofenac sodium at 800 µg/ml as shown in figure 2.

**Table 1: Effect of hydroalcoholic extracts of *D. trifasciata* on protein denaturation and stabilization of HRBC membrane**

| Concentration (µg/ml) | % Protein denaturation | | % HRBC membrane stabilization | |
|---|---|---|---|---|
| | Leaves Extract | Diclofenac Sodium | Leaves Extract | Diclofenac Sodium |
| 12.5 | 9.48 | 52.64 | 6.64 | 72.54 |
| 25 | 12.45 | 59.28 | 11.22 | 74.23 |
| 50 | 16.23 | 66.23 | 14.32 | 76.29 |
| 100 | 23.11 | 70.82 | 19.26 | 78.11 |
| 200 | 28.52 | 74.34 | 28.82 | 81.82 |
| 400 | 32.35 | 81.44 | 34.11 | 85.28 |
| 800 | 41.56 | 88.22 | 53.48 | 95.64 |

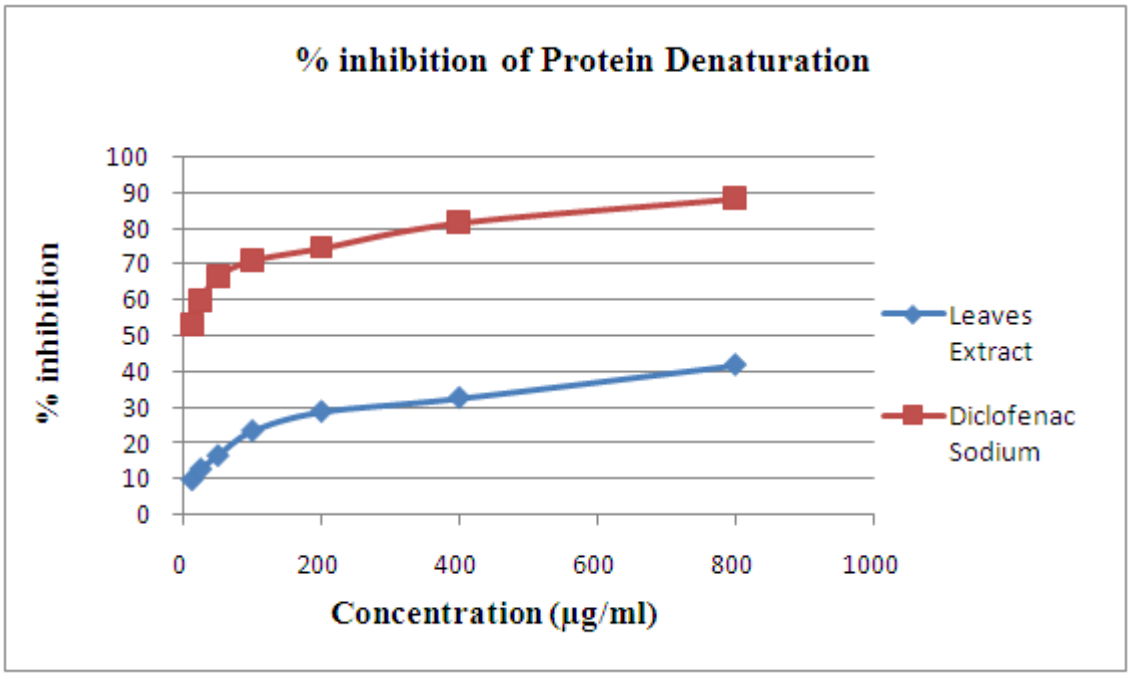


**Figure 1: Effect of hydroalcoholic extracts of *D. trifasciata* on protein denaturation**

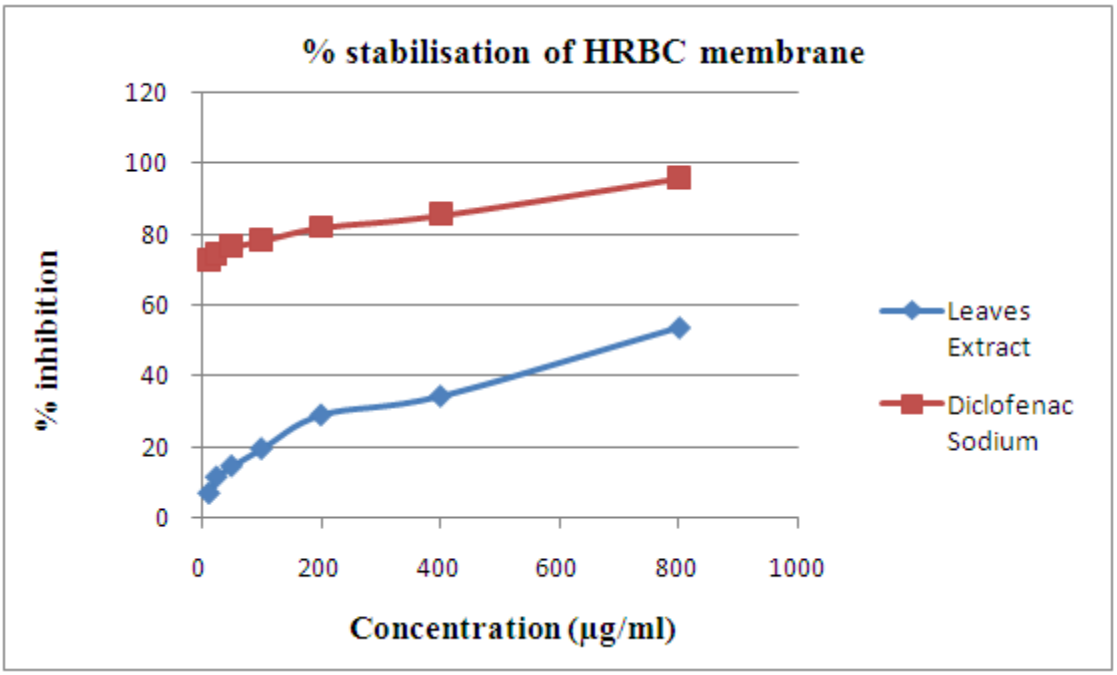


**Figure 2: Effect of hydroalcoholic extracts of *D. trifasciata* on stabilization of HRBC membrane**

## Conclusion

In-vitro studies which were carried out by the above mentioned methods proved that the hydroalcoholic leaves extracts of *D. trifasciata* possess anti-inflammatory and antiarthritic activity which was similar to that of standard. In summary, this contemporary research lends pharmacological support to reported folkloric usage of *D. trifasciata* in the treatment and management of painful arthritic inflammatory conditions. Based on our results, further thorough studies are required for appraisal of exact mechanism of action of *D. trifasciata*, determination of pro-inflammatory cytokines level, isolation of active constituents and cellular characterization that could conclusively establish *D. trifasciata* as a potentially safer disease modifying agent in the treatment of RA.

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