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Original Article

Evaluation of *in vitro* Anti-Inflammatory Activity of *Trichosanthes palmata* against the Denaturation of Protein

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ABSTRACT

Background: *Trichosanthes palmata* Lour. (Cucurbitaceae) is one of the important medicinal plants used traditionally for the treatment of asthma, earache, ophthalmia, leprosy, fever as well as used in the treatment of migraine. To validate folk used of *Trichosanthes palmata* as anti-inflammatory remedy. The leaves of *Trichosanthes palmata* were used for successive extraction (Maceration) with increasing polarity solvents. Ethanolic extract was selected for anti-inflammatory activity. *In vitro* anti-inflammatory activity was evaluated using protein denaturation method at different concentrations. Diclofenac sodium was used as standard drug. **Objectives:** To evaluate the *in vitro* anti-inflammatory effect of ethanolic extract of leaves of *Trichosanthes palmata* against the denaturation of protein. **Materials and Methods:** Inhibition of protein denaturation method was evaluated for anti-inflammatory activity. **Results:** The ethanolic extract of *Trichosanthes palmata* exhibited anti-inflammatory activity by protein denaturation method. **Conclusion:** The ethanolic extract from *Trichosanthes palmata* leaves exhibited significantly enhanced anti-inflammatory activity with increasing concentrations. This effect is likely attributed to the presence of active phytocompounds, including flavonoids, triterpenoids and related polyphenols. Consequently, *Trichosanthes palmata* holds potential as an anti-inflammatory agent.

Keywords: Anti-inflammatory activity, *In vitro*, Protein Denaturation, *Trichosanthes palmata*.

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INTRODUCTION

Inflammation emerges as a sophisticated vascular tissue reaction to diverse deleterious stimuli such as microbial infections, physiochemical factors and exogenous entities. Manifestations of inflammation encompass oedema, nociception, pyrexia, erythema, leucocytosis and localized functional impairment. Consequently, inflammation serves as a fundamental defensive mechanism of the organism, aimed at eradicating injurious triggers while also instigating the commencement of reparative processes.[1] Harmful stimuli can cause inflamed tissues to secrete a variety of bioactive mediators, which interact with a wide range of molecules and cell types to intensify the phlogistic response. This incident may trigger an excessively high inflammatory response, which might start or continue a pathologic process linked to a variety of diseases. Anti-inflammatory pharmaceuticals, both non-steroidal and steroidal, are used to treat inflammation. These medications are effective, but they also have a variety of detrimental side effects.[2]

Inflammation can be categorized into acute and chronic types. Acute inflammation is characterized by heightened vascular permeability, capillary infiltration and the migration of leukocytes. In contrast, chronic inflammation involves the infiltration of mononuclear immune cells, such as macrophages and monocytes, as well as neutrophils. Additionally, it is associated with fibroblast activation, tissue proliferation (angiogenesis) and fibrosis. Inflammation is prevalent in many clinical scenarios, with Rheumatoid Arthritis (RA) being a notable chronic and debilitating autoimmune disorder (Figure 1).[3-4]

The botanical species *Trichosanthes tricuspidata* Lour. (Figure 2), alternatively recognized as *T. palmata* Roxb, *T. bracteata* Lam., *T. puber* Blume, or *Modecca bracteata*, is an ornamental vine found within the Cucurbitaceae family, commonly known as the redball snake gourd. Its native habitat spans regions of China, South and East Asia and tropical Australia. Historical records suggest its traditional use for fever reduction, as a laxative, anthelmintic agent and in alleviating migraines.[5] Fruit of Trichosanthes tricuspidate used in the treatment of asthma, earache and used as carminative agent. Also, fruits are used in the various kinds of the treatments such as in leprosy, epilepsy, used in the treatment of ophthalmia and mostly it is used to reduce the heat of the brain. The holistic utilization of the plant encompasses its role as an

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antipyretic, laxative, anthelmintic and migraine treatment within the realm of medicinal applications. The root extract of the plant exhibits therapeutic efficacy in treating pulmonary ailments in cattle, managing diabetic carbuncles and headaches. Additionally, the root-derived oil serves as an analgesic. Furthermore, the plant demonstrates utility in mitigating snakebite envenomation, while its juice is topically applied for dermatological conditions, particularly skin eruptions, in the Chhattisgarh region.[6]

The present study was designed to evaluate anti-inflammatory potential of leaf extract of *Trichosanthes palmata*.

MATERIALS AND METHODS

Drugs and chemicals

Diclofenac sodium (Standard Drug) purchased from M&B, Sigma or, Fluka. The other chemicals like petroleum ether, Ethanol, and tween 80 were purchased from Qualigens Fine Chemicals, Mumbai.

Plant Material

Leaves of *Trichosanthes palmata* Roxb. were collected in the month of February 2024 from Mahatma Phule Krishi Vidyapeeth Rahuri in the Ahmednagar district of Maharashtra. It was authenticated by R. B. Deshmukh, Head of Botany department at Agricultural Development Trust, Baramati, District Pune of Maharashtra. A voucher specimen (PASR-239) has been deposited in the herbarium section of the Department of Botany, Agricultural Development Trust Baramati, for future reference. The leaves (1000 g) were dried.

Extraction Procedure

In this method, the whole or coarsely powdered crude drug is placed in a sealed container with the solvent and left to stand at room temperature for a minimum of 72 hr with regular stirring until the soluble components have dissolved. Subsequently, the mixture is filtered, the residual solid material is pressed and the resulting liquids are clarified either through filtration or decantation after allowing them to settle.[7]

Defatting of plant material

The powdered plant materials were first defatted by soaking them in petroleum ether at room temperature for 24 hr to eliminate any fatty, oily, or lipid content. After removing the petroleum ether by filtration, the crude drug was dried once more.[8]

Table 1: Result of % yield of Extraction.

Sl. No.	Extract	% Yield (W/W)
	Ethanol	8.5%

Extraction by maceration Method

The defatted plant material was subjected to extraction using three solvents of varying polarity, namely ethanol, ethyl acetate and chloroform, employing the maceration method. The resulting mixture was then filtered using Whatman filter paper no. 1 and the solvent was evaporated to obtain a dry concentrated extract. The weight of the dried crude concentrated extract was measured to determine the extractive yield. Subsequently, the extract was transferred to glass vials (6×2 cm) and stored in a refrigerator at 4°C until required for analysis.[9,10]

Quantitative yield of extract

To ascertain the quantitative yield of the extract, the dish was first weighed while empty and this weight was duly noted. Subsequently, the weight of the dish and its content post-evaporation in the water bath was recorded. The yield of the extract was then calculated using the provided formula:[11]

$$\text{Percentage yield} = \frac{\text{Weight of the sample extract obtained (g)}}{\text{Weight of the powdered sampled used (g)}} \times 100$$

Anti-inflammatory activity by Protein Denaturation Method

The reaction mixture comprised 2 mL of *Trichosanthes palmata* extract at various concentrations ranging from 100 to 500 µg/mL or standard diclofenac sodium at concentrations of 100 to 500 µg/mL, mixed with 2.8 mL of phosphate buffered saline (pH 6.4). To this mixture, 0.2 mL of egg albumin obtained from fresh hen's eggs was added, followed by an incubation period at (27±1)°C for 15 min. Denaturation was induced by subjecting the reaction mixture to a temperature of 70°C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm, with double distilled water serving as the blank. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{At} - \text{Ac}}{\text{Ac}} \times 100$$

Where,

At-Absorbance of test sample;

Ac-Absorbance of control.[12]

Table 2: Absorbance of Diclofenac sodium and ethanolic extract.

Concentration (µg/mL)	Absorbance	
	Diclofenac sodium	Ethanolic extract
100	0.336	0.558
200	0.315	0.485
300	0.224	0.336
400	0.145	0.225
500	0.098	0.118
Absorbance	0.779	

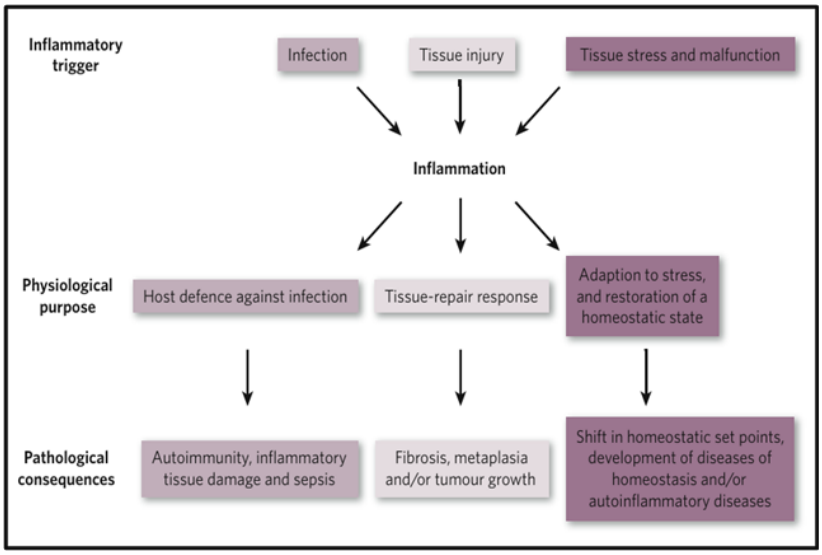

Figure 1: Causes, physiological and pathological outcomes of Inflammation.[4]

Figure 2: *Trichosanthes palmata* leaves.

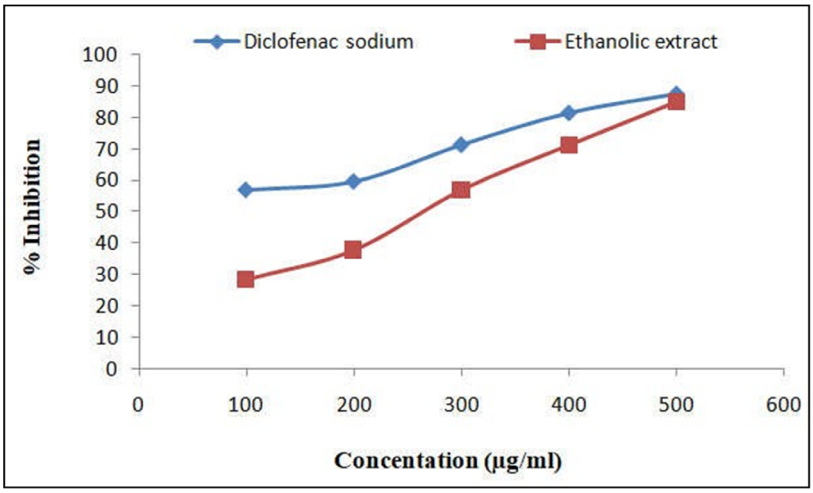

Figure 3: Anti-inflammatosry activity of ethnolic extract *in vitro*.

Table 3: % Inhibition of Diclofenac sodium and ethanolic extract.

Concentration (µg/mL)	Absorbance	
	Diclofenac sodium	Ethanolic extract
100	56.87	28.37
200	59.56	37.74
300	71.25	56.87
400	81.39	71.12
500	87.42	84.85
IC_{50}	43.78	261.09

RESULTS

The percentage yield of the ethanolic extract of *Trichosanthes palmata* was determined to be 8.5%, as shown in Table 1. This indicates a moderate extraction efficiency using ethanol as the solvent.

The *in vitro* anti-inflammatory activity was assessed using the protein denaturation method. The absorbance values for Diclofenac sodium and the ethanolic extract at different concentrations are presented in Table 2 and Figure 3. The results show that Diclofenac sodium exhibited lower absorbance values across all tested concentrations, indicating stronger inhibition of protein denaturation compared to the ethanolic extract.

The percentage inhibition of protein denaturation increased with concentration for both Diclofenac sodium and the ethanolic extract, as shown in Table 3. However, Diclofenac sodium demonstrated significantly higher inhibition at lower concentrations, with an IC_{50} value of 43.78 µg/mL, whereas the ethanolic extract showed an IC_{50} value of 261.09 µg/mL, suggesting that while the extract has anti-inflammatory potential, it is less potent than Diclofenac sodium.

The % inhibition of Diclofenac sodium and an ethanolic extract was assessed across various concentrations, with absorbance values serving as indicators of their inhibitory effects. Comparing the two substances, Diclofenac sodium consistently demonstrated higher % inhibition than the ethanolic extract at each concentration tested. As concentrations increased, both substances exhibited a corresponding increase in % inhibition, reflecting a typical concentration-response relationship seen in pharmacological studies.

However, the IC_{50} values revealed a significant difference in potency between Diclofenac sodium and the ethanolic extract. Diclofenac sodium displayed a much lower IC_{50} value of 43.78 µg/mL compared to the ethanolic extract's IC_{50} value of 261.09 µg/mL, indicating Diclofenac sodium's superior potency in inhibiting the biological process under investigation. These findings suggest that while both substances possess inhibitory properties, Diclofenac sodium is more effective at lower concentrations.

DISCUSSION

The % inhibition of Diclofenac sodium and an ethanolic extract was assessed across various concentrations, with absorbance values serving as indicators of their inhibitory effects. Comparing the two substances, Diclofenac sodium consistently demonstrated higher % inhibition than the ethanolic extract at each concentration tested. As concentrations increased, both substances exhibited a corresponding increase in % inhibition, reflecting a typical concentration-response relationship seen in pharmacological studies.

However, the IC_{50} values revealed a significant difference in potency between Diclofenac sodium and the ethanolic extract. Diclofenac sodium displayed a much lower IC_{50} value of 43.78 µg/mL compared to the ethanolic extract's IC_{50} value of 261.09 µg/mL, indicating Diclofenac sodium's superior potency in inhibiting the biological process under investigation. These findings suggest that while both substances possess inhibitory properties, Diclofenac sodium is more effective at lower concentrations. Further research is warranted to elucidate the specific mechanisms of action and potential therapeutic applications of the ethanolic extract, as well as to explore any synergistic effects that may arise from combining Diclofenac sodium with the extract.

CONCLUSION

The current study's findings revealed that various parts of *Trichosanthes palmata* contain several bioactive phytochemical compounds in their ethanolic extract. The *in vitro* anti-inflammatory activity of this extract was evaluated by testing its effect on protein denaturation using egg albumin. Results indicated that the ethanolic extract from different parts of *Trichosanthes palmata* exhibited significantly higher anti-inflammatory activity with increasing concentrations. This activity is likely due to the presence of active phytocompounds such as flavonoids, triterpenoids and related polyphenols. Therefore, *Trichosanthes palmata* shows potential as an anti-inflammatory agent. This investigation underscores the importance of sourcing potent anti-inflammatory agents from natural products, which may offer effective alternatives with fewer side effects compared to synthetic drugs.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PUD: Peptic Ulcer Disease; **GRDDS:** Gastro retentive Drug Delivery System; **NSAIDs:** Non-Steroidal Anti-Inflammatory Drugs; **RA:** Rheumatoid Arthritis; **IV:** *In vitro*; **TP:** *Trichosanthes Palmata*; **PDM:** Protein Denaturation Method; **DS:** Diclofenac Sodium; **QYA:** Quantitative Yield Analysis; **IC_{50}:** Half Maximal Inhibitory Concentration; **PBS:** Phosphate Buffered Saline.

SUMMARY

The study demonstrated that the ethanolic extract of *Trichosanthes palmata* leaves exhibits significant *in vitro* anti-inflammatory activity by inhibiting protein denaturation. The extract's effectiveness increased with higher concentrations, likely due to the presence of bioactive phytocompounds such as flavonoids, triterpenoids and polyphenols. Although Diclofenac sodium showed greater potency with a lower IC_{50} value, the extract still demonstrated promising inhibitory effects, suggesting its potential as a natural anti-inflammatory agent. These findings highlight the importance of exploring plant-based alternatives to synthetic anti-inflammatory drugs, which may provide effective therapeutic benefits with fewer side effects. Further studies are needed to elucidate the specific mechanisms of action and assess its potential in clinical applications.

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