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ANTI-ARTHRITIC ACTIVITY OF HYDRO-ALCOHOLIC EXTRACTS OF *TINOSPORA CORDIFOLIA* WILLD. AND *CALOTROPIS GIGANTEA* LINN BY IN-VITRO METHOD

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ABSTRACT

Arthritis, particularly Rheumatoid Arthritis, is a chronic inflammatory disorder characterized by joint pain, swelling, and progressive degeneration, requiring safer and more effective therapeutic alternatives. Medicinal plants have gained attention due to their multi-targeted pharmacological actions and lower adverse effects. The present study evaluates the anti-arthritic potential of hydro-alcoholic extracts of *Tinospora cordifolia* and *Calotropis gigantea*, which are widely used in traditional systems of medicine. Literature reports indicate that *Tinospora cordifolia* possesses significant immunomodulatory, anti-inflammatory, and antioxidant properties, while *Calotropis gigantea* exhibits notable anti-inflammatory and analgesic activities due to the presence of bioactive Phyto-constituents such as alkaloids, flavonoids, glycosides, and terpenoids. These phytochemicals play a crucial role in modulating inflammatory mediators and reducing oxidative stress associated with arthritis. *Tinospora cordifolia* showed higher inhibition of protein denaturation compared to *Calotropis gigantea*, while Ibuprofen exhibited maximum activity.

KEYWORDS: *Tinospora Cordifolia*, *Calotropis Gigantea*, Anti-arthritic activity, Hydroalcoholic Extract, Protein denaturation assay, UV spectrophotometric method.

INTRODUCTION

Arthritis is a chronic inflammatory disease that causes pain, swelling, stiffness, and progressive joint deterioration which reduces mobility and worsens the quality of life**.** Among the many types of arthritis, Rheumatoid Arthritis (RA) is one of the most prevalent autoimmune conditions linked to cartilage degradation and persistent inflammation. The disorder is frequently treated with traditional medication such as cortico-steroids, Non-steroidal anti-inflammatory drugs (NSAIDs), Disease-modifying Anti-rheumatic drugs (DMARDs). Long term adverse effects from these treatments, however, could include decrease immunity liver damage, gastro-intestinal distress. As a result, there is growing interest in using medicinal plants as safer and more effective alternative for managing Arthritis.[1]

Tinospora Cordifolia or Guduchi, is a significant medicinal plant that is frequently used in Ayurveda to treat immune-related and inflammatory conditions. Alkaloids, flavonoids, glycoside and terpenoids are among the bio-active components of the plant that have anti-inflammatory, anti-oxidant and immune-modulatory qualities. In a similar vein, *Calotropis Gigantea* (Arka) has long being used to treat inflammatory conditions, discomfort and swelling. because it contains flavonoids, tannins, glycosides, it has been shown to have strong anti-inflammatory, analgesic, anti-oxidant properties.[2]

Because hydro-alcoholic extraction effectively removes both polar and non-polar molecules, increasing therapeutic efficacy, it is frequently utilized for the isolation of the Phyto-constituents. UV-Visible spectrophotometry analysis of protein denaturation test can be used to assess in vitro anti-arthritic activity. Inflammatory illnesses are significantly influenced by protein denaturation inhibition may have anti-arthritic properties.[3]

Based on traditional claims and available scientific evidence, the present review focuses on the anti-arthritic potential of hydroalcoholic extracts of *Tinospora cordifolia* and *Calotropis gigantea* and highlights their phytochemical and pharmacological significance in arthritis management.[4]

Taxonomic Classification

Calotropis Gigantea

- Kingdom: Plantae
- Clade: Tracheophytes, Angiosperms, Eudicots, Asterids

- Order: Gentianales
- Family: Apocynaceae
- Subfamily: Asclepiadoideae
- Genus: *Calotropis*
- Species: *Calotropis gigantea*[5]

Tinospora Cordifolia

- Kingdom: Plantae
- Clade: Tracheophytes, Angiosperms, Eudicots
- Order: Ranunculales
- Family: Menispermaceae
- Genus: *Tinospora*
- Species: *Tinospora cordifolia*[4]

Materials and Methods

Plant Materials

Calotropis gigantea leaves and fresh *Tinospora cordifolia* stem were gathered in Yavat, Maharashtra. After being cleaned of contaminant with distilled water, material was shade

dried at room temperature, grind into a powdered using a mechanical grinder, and kept in airtight container for more research.

Chemicals and Reagents

➢ **Apparatus**

- Soxhlet apparatus
- Beaker
- Test tube
- Stirrer
- Test tube stand
- Measuring cylinder
- Conical flask

➢ **Equipment**

- UV-Visible spectrophotometer
- Incubator
- Heating Mantle
- Analytical Balance
- Hot Air oven
- Vacuum Dryer

➢ **Chemicals**

- Egg albumin
- Phosphate buffer
- Distilled water
- Ethanol
- Ibuprofen

➢ **Reagents**

- Dradendorff's Reagent
- Mayer's Reagent
- Hager's Reagent
- Molisch Reagent
- Fehling Reagent

Pharmacognostic Investigation of *Tinospora cordifolia* and *Calotropis gigantea*

A. Microscopic Study

Microscopic evaluation of the powdered and leaf material was performed according to standard pharmacognostic procedures to determine diagnostic characteristics of the plant material.

a) Stomatal Number

Leaf epidermal peel was prepared and mounted on a glass slide using glycerin.

A compound microscope was used to examine the prepared slides.

The number of stomata present per unit area of epidermis was counted and recorded.

b) Vein Islet Number

Small portions of leaf material were cleared using chloral hydrate solution and mounted on a glass slide.

The number of vein islets per square millimeter of leaf surface was counted under microscopic observation**.**

This study helped in authentication and quality control of crude drugs.[6]

3. Microscopic Study of *Calotropis gigantea* and *Tinospora cordifolia*

B. Physicochemical Evaluation

Physicochemical evaluation of plant materials was carried out to determine purity, quality, and standardization parameters.

a) Total Ash Value

A silica crucible was used to burn the precisely wet powder sample until carbon-free ash was reduced.

The proportion of total ash was computed.

b) Acid insoluble ash

After boiling all ash with diluted hydro-chloric acid, it was filtered.

After being gathered, the insoluble material was weighed.

c) water soluble ash

distilled water was used to treat and filtered the total ash. The weight differential was completed

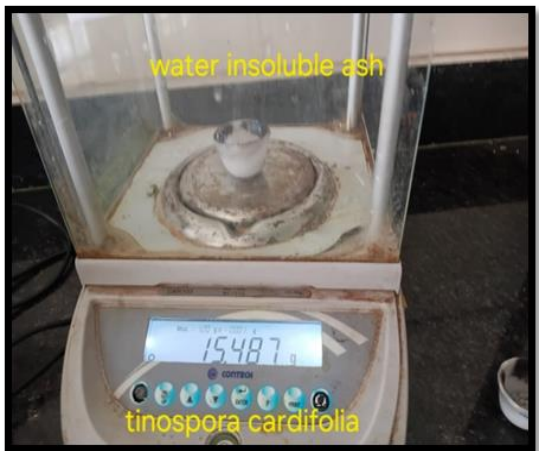
d) Moisture Content (Loss on Drying)

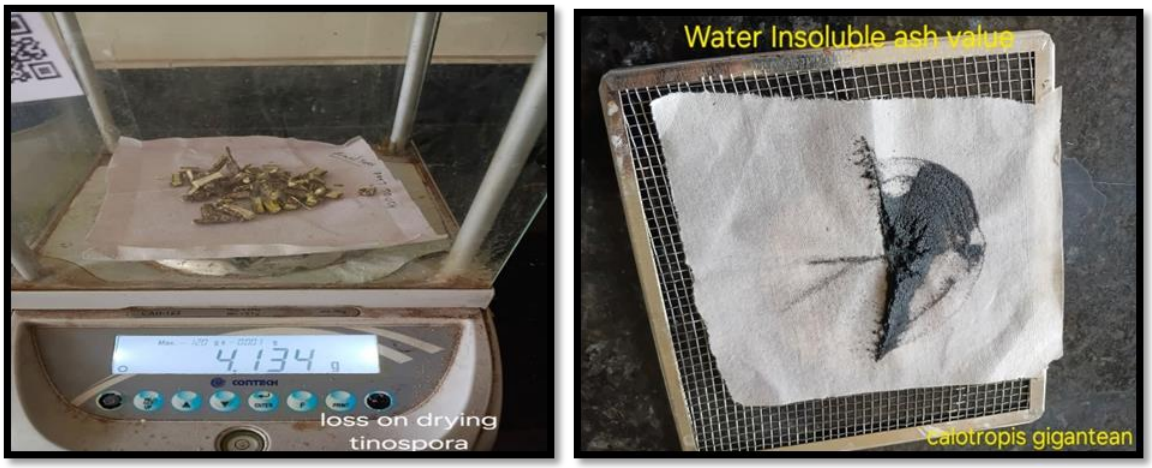
The sample was dried at a specified temperature until constant weight was obtained.

e) Extractive Values

Water-soluble extractive value

Alcohol-soluble extractive value[7]





4. Physicochemical Evaluation of *Calotropis gigantea* and *Tinospora cordifolia*

Preparation of Hydro-alcoholic Extract

- **Collection of Plant Material**

The plant materials selected for the present study were *Tinospora cordifolia* (stem) and *Calotropis gigantea* (leaves). Fresh and healthy plant parts were collected from a reliable local source during the appropriate growing season to ensure maximum phytochemical content. The collected plant specimens were carefully selected to avoid contamination with diseased or damaged parts. The identity of the plants was confirmed through standard botanical references or authenticated herbarium sources to ensure taxonomic accuracy.[8]

1. Collection of *Tinospora cordifolia* and *Calotropis Gigantea*

Shade Drying of Plant Material

The cleaned plant materials were subjected to shade drying at room temperature (25–30°C) under well-ventilated conditions. Shade drying was preferred over direct sunlight to prevent degradation of heat-sensitive and light-sensitive phytoconstituents. The materials were periodically turned to ensure uniform drying. Drying was continued until a constant weight was obtained, indicating complete removal of moisture content and prevention of microbial growth.[9]

Grinding and Powder Preparation

The completely dried plant materials were separately pulverized using a mechanical grinder to obtain a coarse powder. The powdered materials were then passed through a suitable mesh sieve (No. 40–60) to ensure uniform particle size, which is important for efficient extraction of active constituents. The powdered samples were stored in airtight containers and kept in a cool, dry place to protect them from moisture and environmental contamination until further analysis.[8]

2. Powder of *Calotropis Gigantea* and *Tinospora cordifolia*

Preparation of Hydroalcoholic Extract (Soxhlet Extraction)

A hydro-alcoholic solvent system (ethanol:water:50:50) was used for extraction.

Procedure:

Accurately weighed 250 g of *Tinospora cordifolia* powder

Accurately weighed 250 g of *Calotropis gigantea* powder

Each sample was placed separately in a Soxhlet apparatus

Extracted using hydroalcoholic solvent for 6 hours continuous cycles

Temperature maintained near solvent boiling point

Soxhlet extraction ensured exhaustive extraction of phytoconstituents. [10]

5. Soxhlet extraction of *Calotropis gigantea* and *Tinospora cordifolia*

Filtration and Concentration of Extract

The extracted solution was filtered using Whatman filter paper

The filtrate was concentrated using a water bath at controlled temperature (40–60°C)

Solvent was evaporated until a semisolid crude extract was obtained

Extracts were stored in airtight containers to avoid moisture absorption[6]

Preliminary Phyto-chemical Screening

In order to identify bioactive components like alkaloids, flavonoids, glycosides, saponins, steroids, terpenoids, phenolic compounds, the hydro-alcoholic extracts were first screened for phytochemicals using standard qualitative techniques. The presence of the corresponding phytochemicals was detected by the emergence of distinctive color changes or precipitates.[10]

6. Preliminary Phyto-chemical Screening of *Calotropis gigantea* and *Tinospora cordifolia*

Preparation of Standard and Test Solutions

- Standard drug: Ibuprofen solution prepared at required concentration.
- Test solutions: Plant extracts were prepared in different concentrations such as:

100 ppm

200 ppm

300 ppm

400 ppm

Solutions were freshly prepared using suitable solvent to ensure accuracy.[6]

Evaluation of Anti-Arthritic Activity (Protein Denaturation Method)

Anti-arthritic activity was evaluated using inhibition of protein denaturation assay.

Principle

Denaturation of proteins (like albumin) leads to inflammation-related changes. Substances that inhibit denaturation show anti-arthritic potential.

Procedure

Reaction mixture containing albumin solution + test/standard drug

Reaction's mixture was incubated at 37^0 C for 15 mins

Heated at 70^0 C for 5 mins temperature to induce denaturation

Reaction mixtures cooled to room temperature[11]

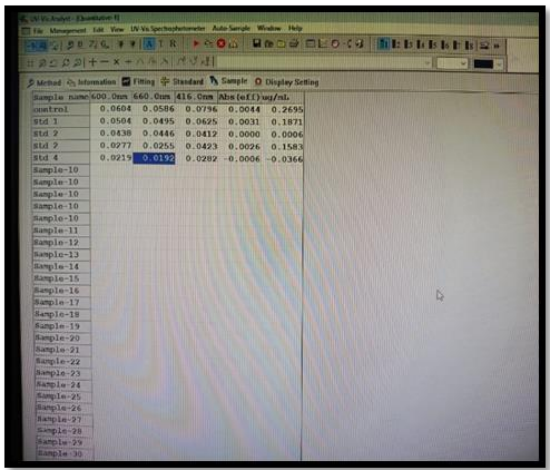
7. Preparation of Dilutions of standard and test solution

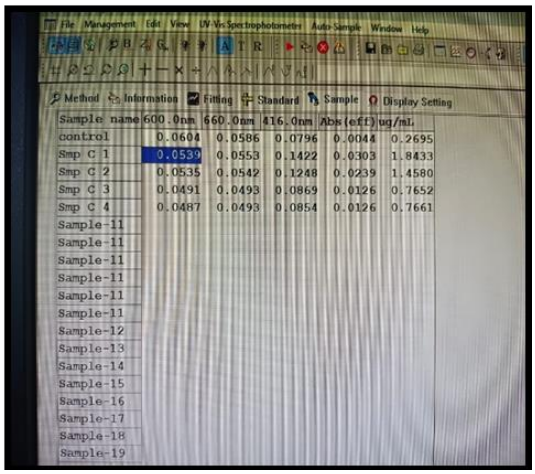
UV-Visible Spectrophotometric Analysis

Absorbance of each sample was measured using a UV-Visible spectrophotometer

660nm wavelength was selected based on albumin assay requirements

Blank and control were used for calibration[12,13]





8. Analysis of *Calotropis gigantea* and *Tinospora cordifolia* by UV-Visible Spectrophotometer

Calculation of Percentage Inhibition

The anti-arthritic activity was calculated using:

% Inhibition of protein denaturation =

$$\frac{\textbf{Absorbance (control)} - \textbf{Absorbance(sample)} \times 100}{\textbf{Absorbance(control)}}$$

This helped to determine the effectiveness of plant extracts compared to standard drug.[14]

Comparison with Standard Drug (Ibuprofen)

The activity of *Tinospora cordifolia* and *Calotropis gigantea* extracts was compared with Ibuprofen, and results were analyzed based on percentage inhibition values.[15]

Interpretation of Results and Conclusion

The results were interpreted based on

- Dose-dependent activity
- Comparison with standard drug[15]

RESULT

The physicochemical evaluation of *Tinospora cordifolia* and *Calotropis gigantea* was carried out.

The results indicated that *Calotropis gigantea* possessed a higher loss on drying and total ash value compared to *Tinospora cordifolia*, whereas *Tinospora cordifolia* showed comparatively higher moisture content, water soluble ash value, and acid insoluble ash value. These physicochemical parameters provide useful information regarding the quality, purity, and standardization of the plant materials used in the study.

Table 1: Physico-chemical Analysis of *Tinospora cordifolia* and *Calotropis Gigantea.*

Sample identify	***Tinospora cordifolia***	***Calotropis gigantea***
Loss on drying	72.46%	84.26%
Moisture content	0.99%	0.49%
Total Ash value	6.92%	14.6%
Water soluble ash value	5.33%	3.33%
Acid insoluble ash value	4.6%	2.66%

- Preliminary phytochemical screening of *Tinospora cordifolia* and *Calotropis gigantea* revealed the presence of various phytoconstituents. Both plant extracts showed the presence of carbohydrates, saponins, flavonoids, and certain alkaloids and glycosides. *Tinospora cordifolia* exhibited a comparatively higher number of positive phytochemical tests, while *Calotropis gigantea* showed the presence of tannins and selected phytocompounds. These findings indicate the presence of bioactive constituents that may contribute to their pharmacological activities.

Table 2: Phyto-chemical Screening of *Calotropis gigantea* and *Tinospora cordifolia.*

Chemical constituents	Tests	*Tinospora cordifolia* Hydro-alcoholic extract	*Calotropis Gigantea* Hydro-alcoholic extract
Alkaloids	Mayer's test	+	-
	Hager's test	+	+
	Dragendorff's test	+	+
Glycosides	Killer kilani test	+	-
	Borntrager's test	+	+
Carbohydrates	Fehling's test	+	+
	Benedict's test	+	+
Saponins	Foam test	+	+
Steroids	Salkowski test	+	-
Tannins	Ferric Chloride test	-	+
Flavonoids	Sulphuric Acid test	+	+
	Alkali test	+	-

- The anti-arthritic activity of the hydroalcoholic extract of *Tinospora cordifolia* was evaluated by the protein denaturation assay using UV spectrophotometric method. The extract showed a concentration-dependent increase in inhibition of protein denaturation. At concentrations of 100, 200, 300, and 400 ppm, the percentage inhibition was found to be 12.45%, 12.96%, 14.33%, and 15.52%, respectively. The standard drug Ibuprofen showed 23.89% inhibition, indicating higher anti-arthritic activity compared with the plant extract. The findings suggest that *Tinospora cordifolia* possesses moderate anti-arthritic potential due to its ability to inhibit protein denaturation.

Table 3: Effect of *Tinospora cordifolia* on Protein Denaturation Assay.

Tinospora cordifolia	concentration	absorbance	Percent inhibition of denaturation of protein
Sample 1	100 ppm	0.0513	12.45%
Sample 2	200 ppm	0.0510	12.96%
Sample 3	300 ppm	0.0502	14.33%
Sample 4	400 ppm	0.0495	15.52%
Ibuprofen	-	0.0446	23.89%
Control	-	0.0586	-

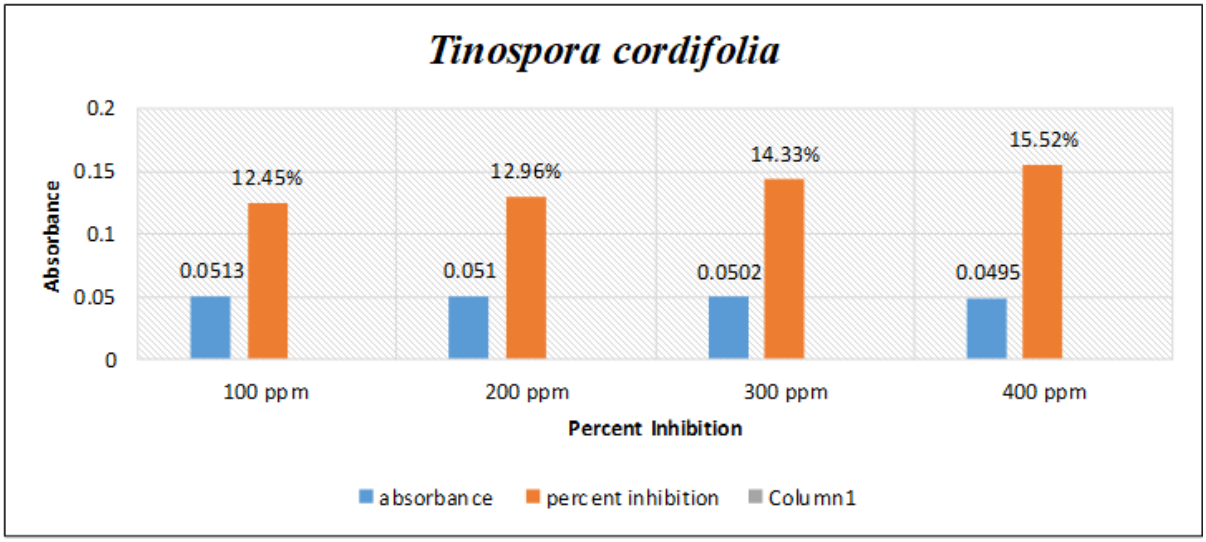


Fig. 1: Anti-arthritic activity of *Tinospora cordifolia.*

- Hydroalcoholic extract of *Calotropis gigantea* showed anti-arthritic activity by inhibiting protein denaturation in a concentration-dependent manner. The percentage inhibition increased from 3.83% at 100 ppm to 15.87% at 400 ppm. The standard drug Ibuprofen showed higher inhibition of 23.89%, indicating stronger activity compared to the extract. These results demonstrated significant concentration -dependent inhibition of protein denaturation.

Table 4: Effect of *Calotropis gigantea* on Protein Denaturation Assay.

Calotropis gigantea	**Concentration**	**absorbance**	**Percent inhibition of denaturation of protein**
Sample 1	100 ppm	0.0553	3.83%
Sample 2	200 ppm	0.0542	7.50%
Sample 3	300 ppm	0.0493	15.87%
Sample 4	400 ppm	0.0492	15.87%
Ibuprofen	-		23.89%
Control	-		-

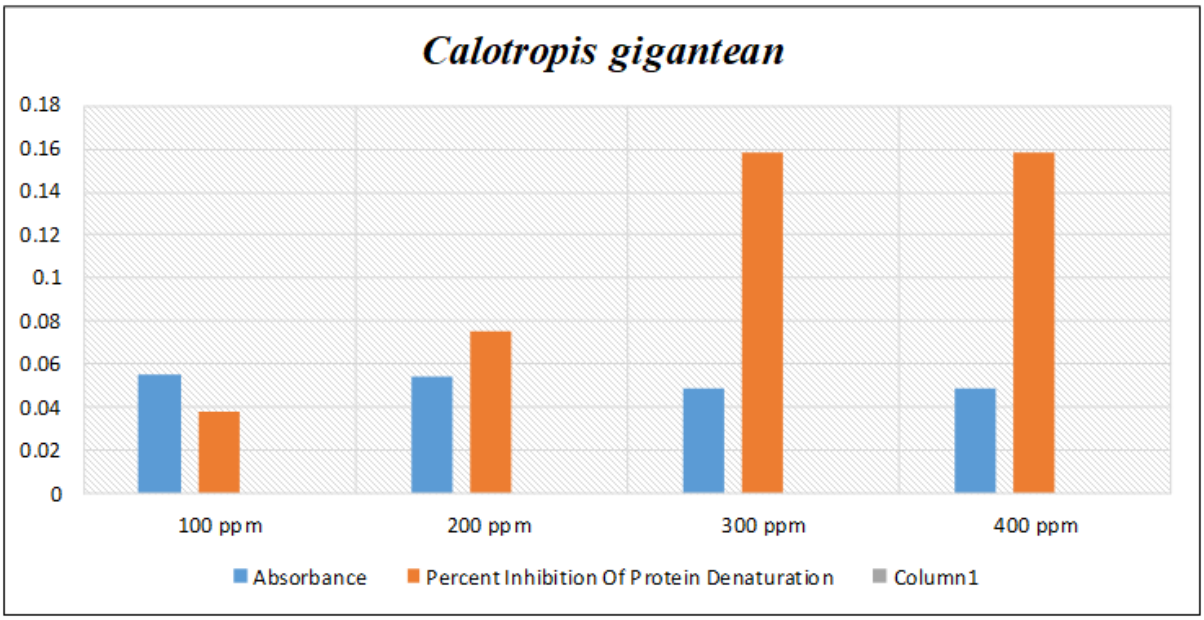


Fig. 2: Anti-arthritic activity of *Calotropis gigantea.*

5. DISCUSSION

Microscopic and physicochemical evaluation

Microscopic parameters such as stomatal number and vein islet number were studied for identification of plant materials. Physicochemical parameters including ash values and extractive values were also evaluated to determine purity, quality, and standardization of the crude drugs.

Phyto-chemical screening and extraction

Hydroalcoholic extraction using the Soxhlet apparatus effectively extracted bioactive constituents from the plant materials. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, tannins, terpenoids, and saponins, which are known to possess anti-inflammatory and antioxidant properties. The observed anti-arthritic activity may be due to flavonoids and alkaloids reported in previous studies.

Anti-arthritic activity

The anti-arthritic activity was evaluated using the UV spectrophotometric protein denaturation method. The extracts showed inhibition of protein denaturation, indicating their potential role in reducing inflammatory responses associated with arthritis. The findings are in agreement with previous reports on *Tinospora cordifolia* showing anti-inflammatory and immunomodulatory activity."

Overall findings

The observed anti-arthritic activity may be attributed to the synergistic effect of phytoconstituents present in *Tinospora cordifolia* and *Calotropis gigantea*, suggesting their possible use as natural alternatives for arthritis management.

6. CONCLUSION

The present study demonstrated that hydroalcoholic extracts of *Tinospora cordifolia* and *Calotropis gigantea* possess anti-arthritic activity as evaluated by the protein denaturation method using UV spectrophotometry. Both extracts showed concentration-dependent inhibition of protein denaturation, suggesting their ability to reduce inflammatory responses associated with arthritis. Among the tested extracts, *Tinospora cordifolia* exhibited comparatively higher activity than *Calotropis gigantea*. However, the standard drug ibuprofen showed greater inhibition compared to both plant extracts. The observed activity may be attributed to the presence of phytoconstituents such as alkaloids, flavonoids, tannins, and glycosides. These findings indicate that the studied plants have potential as natural anti-arthritic agents and may be useful for further pharmacological investigations.

8. REFERENCE

1. Kaur, C., Mishra, Y., Kumar, R., Singh, G., Singh, S., Mishra, V., Et Al. (2024). Pathophysiology, Diagnosis, And Herbal Medicine-Based Therapeutic Implication Of Rheumatoid Arthritis: An Overview. Inflammopharmacology, 32: 1705–1720. Https://Doi.Org/10.1007/S10787-024-01445-8.
2. Singh, D., & Chaudhuri, P. K. (2023). Chemistry And Pharmacology Of *Tinospora Cordifolia* And Calotropis *Gigantea*: A Review Of Phytochemical Constituents And Therapeutic Potential. Journal Of Herbal Medicine, 41: 100684. Https://Doi.Org/10.1016/J.Hermed.2023.10068
3. Singh, D., Nainwani, R., Sharma, T., & Gautam, R. K. (2013). In-Vitro Anti-Inflammatory And Anti-Arthritic Activity Of Hydroalcoholic Extract Of *Pongamia Pinnata* (L.) Pierre Seed. International Journal Of Pharma Research And Review, 2(12): 20–25.
4. Upadhyay, A. K., Kumar, K., Kumar, A., & Mishra, H. S. (2010). *Tinospora Cordifolia* (Guduchi): Validation Of The Ayurvedic Pharmacology Through Experimental And Clinical Studies. International Journal Of Ayurveda Research, 1(2): 112–121.
5. Kirtikar, K. R., & Basu, B. D. (2005). Indian Medicinal Plants (2nd Ed., Pp. 1606–1609). Dehradun: International Book Distributors

6. Khandelwal, K. R. (2015). Practical Pharmacognosy: Techniques And Experiments (23rd Ed.). Pune: Nirali Prakashan.
7. Indian Pharmacopoeia Commission. (2018). Indian Pharmacopoeia. Ghaziabad: Indian Pharmacopoeia Commission.
8. Kokate, C. K., Purohit, A. P., & Gokhale, S. B. (2014). Practical Pharmacognosy (54th Ed.). Pune: Nirali Prakashan.
9. World Health Organization. (2011). Quality Control Methods For Herbal Materials. Geneva: World Health Organization.
10. Harborne, J. B. (1998). Phytochemical Methods: A Guide To Modern Techniques Of Plant Analysis (3rd Ed.). London: Chapman And Hall.
11. Sakat, S., Juvekar, A. R., & Gambhire, M. N. (2010). In-Vitro Antioxidant And Anti-Inflammatory Activity Of Methanol Extract Of *Oxalis Corniculata* Linn. International Journal Of Pharmacy And Pharmaceutical Sciences, 2(1): 146–155.
12. Beckett, A. H., & Stenlake, J. B. (2002). Practical Pharmaceutical Chemistry (4th Ed.). New Delhi: CBS Publishers.
13. Choudhary, Y., Patil, R. Y., Bohra, S., & Choudhari, A. (2024). Comparative Study Of Cow Urine Extract And Hydroalcoholic Extract For In-Vitro Anti-Inflammatory Potential Of Nyctanthes Arbor-Tristis Leaves. International Journal Of Scientific Research In Science And Technology, 11(2): 908–913. Https://Doi.Org/10.32628/IJSRST24112144
14. Mizushima, Y., & Kobayashi, M. (1968). Interaction Of Anti-Inflammatory Drugs With Serum Proteins, Especially With Some Biologically Active Proteins. Journal Of Pharmacy And Pharmacology, 20(3): 169–173.
15. Winter, C. A., Risley, E. A., & Nuss, G. W. (1962). Carrageenin-Induced Edema In Hind Paw Of The Rat As An Assay For Anti-Inflammatory Drugs. Proceedings Of The Society For Experimental Biology And Medicine, 111: 544–547.