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An Experimental Study On Phytochemical Screening And Evaluation Of Invivo Antiinflammatory Activity Of Andrographis Paniculate

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Abstract

Background:

Andrographis paniculata, a widely used medicinal herb, has been traditionally employed in various therapeutic applications, particularly for its anti-inflammatory, antimicrobial, and immunomodulatory properties. Despite its extensive use in folk medicine, scientific validation of its efficacy, particularly regarding its anti-inflammatory effects, is limited.

Objective:

The objective of this study was to assess the phytochemical composition and evaluate the in vivo anti-inflammatory activity of *Andrographis paniculata* through the use of aqueous and ethanolic extracts.

Methods:

The plant was extracted using aqueous and ethanolic solvents. The phytochemical screening was performed to identify secondary metabolites, including alkaloids, flavonoids, tannins, saponins, glycosides, phenolics, and terpenoids. In vivo anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model in Wistar rats. The animals were divided into different treatment groups, including a control, standard drug (diclofenac sodium), and test groups (aqueous and ethanolic extracts at varying doses).

Results:

Phytochemical analysis revealed the presence of bioactive compounds, such as flavonoids, phenolics, and saponins, in

both extracts. The ethanolic extract (400 mg/kg) exhibited the most significant anti-inflammatory activity, showing 55.8% inhibition of paw edema, closely approaching the efficacy of diclofenac sodium (62.8% inhibition). The aqueous extract also showed dose-dependent anti-inflammatory effects.

Conclusion:

Andrographis paniculata demonstrated significant anti-inflammatory activity, confirming its traditional use for inflammation-related conditions. The presence of bioactive compounds such as flavonoids and phenolics likely contributes to this activity. These findings suggest that *Andrographis paniculata* has potential as a natural therapeutic agent for managing inflammation, with further studies needed to isolate active compounds and explore long-term efficacy.

Keywords

Andrographis paniculata, Phytochemical screening, Anti-inflammatory activity, Carrageenan-induced paw edema, Flavonoids, Phenolics, Medicinal plants, Ethanolic extract

1. Introduction

1.1 Overview: Medicinal Significance of Andrographis paniculata

Andrographis paniculata, commonly known as "King of Bitters," is a medicinal plant widely used in traditional medicine systems, such as Ayurveda, Traditional Chinese Medicine, and Siddha. Native to South and Southeast Asia, it is recognized for its therapeutic potential in treating a variety of ailments, including fever, infections, and inflammatory conditions [1,2]. The plant is rich in bioactive phytochemicals, particularly diterpenoids like andrographolide, which exhibit diverse pharmacological activities [3].

1.2 Pharmacological Importance: Focus on Its Anti-Inflammatory Properties

Inflammation is a complex biological response associated with many chronic diseases, including arthritis, diabetes, and cardiovascular disorders. *A. paniculata* has gained attention due to its significant anti-inflammatory properties, attributed mainly to andrographolide and its derivatives. Studies have shown that these compounds inhibit key inflammatory mediators, such as cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6), thereby reducing inflammation [4,5]. Furthermore, its extracts have been reported to suppress oxidative stress, a major contributor to inflammatory pathologies [6].

1.3 Research Gap: Need for Experimental Validation of Its Phytochemicals and Biological Activity

Despite extensive traditional use and preliminary pharmacological evidence, the precise bioactive components and their mechanistic roles in anti-inflammatory activity remain inadequately explored. Previous studies primarily focus on isolated compounds rather than comprehensive phytochemical screening of extracts. Moreover, the lack of systematic in vivo studies to confirm its efficacy and safety limits its integration into modern therapeutic regimens [7].

1.4 Objective and Hypothesis

This study aims to systematically evaluate the phytochemical profile of *A. paniculata* and assess its in vivo anti-inflammatory activity using animal models. We hypothesize that the plant extracts, rich in bioactive compounds, exhibit significant inflammation-reducing effects by modulating key inflammatory mediators and pathways. This investigation will provide scientific validation for its traditional use and identify potential candidates for anti-inflammatory drug development.

2. Materials and Methods

2.1 Materials

2.1.1 Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. Key reagents included:

Table 1: List of Chemicals and Reagents Used in the Study

Chemical/Reagent	Purpose	Source/Manufacturer

Methanol (99% purity)	Solvent for extraction	Shilex Chemicals Pvt Ltd, Ashok Vihar, Delhi
Distilled water	Preparation of aqueous extracts	Laboratory-grade
Fehling's solution	Test for reducing sugars	Shilex Chemicals Pvt Ltd, Ashok Vihar, Delhi
Dragendorff's reagent	Test for alkaloids	Shilex Chemicals Pvt Ltd, Ashok Vihar, Delhi
Folin-Ciocalteu reagent	Total phenolic content determination	Shilex Chemicals Pvt Ltd, Ashok Vihar, Delhi
Carrageenan	Induction of paw edema	Shilex Chemicals Pvt Ltd, Ashok Vihar, Delhi
Diclofenac sodium	Standard anti-inflammatory drug	Cipla, India

2.1.2 Plant Material

Andrographis paniculata plants were procured from Sunder Nursery, New Delhi. Details of procurement are provided below:

Table 2: Details of Plant Material Used in the Study

Parameter	Details
Plant part used	Leaves and stems
Location	Sunder Nursery, New Delhi
Geographic coordinates	20.9517° N, 85.0985° E
Season of collection	July-August

2.2 Preparation of Plant Extracts

2.2.1 Extraction Procedure

Two types of extracts were prepared: aqueous and ethanolic, using Soxhlet extraction and maceration methods.

A. Soxhlet Extraction

Table 3: Extraction Procedure for Preparation of Plant Extracts

Step	Description
Plant material drying	Air-dried under shade for 10 days.
Powder preparation	Pulverized using a mechanical grinder to fine powder (sieve size 40).
Solvent ratio	1:10 (plant material to solvent, w/v).
Extraction cycle	Soxhlet apparatus at 60°C for ethanol and 90°C for water for 8 hours.
Filtration	Extract filtered using Whatman No. 1 filter paper.
Concentration	Rotary evaporator used for solvent removal; stored at 4°C.

B. Maceration Technique

Table 4: Maceration Procedure for Preparation of Plant Extracts

Step	Description
Plant material soaking	50 g powdered material soaked in 500 mL solvent (ethanol or water).
Duration	72 hours at room temperature, stirred intermittently.
Filtration	Mixture filtered using muslin cloth, then Whatman No. 1 filter paper.
Concentration	Solvent removed under reduced pressure using a rotary evaporator.

2.2.2 Yield Calculation

The yield of each extract was calculated as:

$$\text{Yield (\%)} = \frac{\text{Weight of extract obtained (g)}}{\text{Weight of dried plant material (g)}} \times 100$$

Table 5: Yield of Aqueous and Ethanolic Extracts of *Andrographis paniculata*

Extract	Weight of Plant Material (g)	Weight of Extract (g)	Yield (%)
Aqueous extract	100	12	12.00
Ethanolic extract	100	18	18.00

2.3 Phytochemical Screening

Phytochemical screening was performed to identify the presence of secondary metabolites in the aqueous and ethanolic extracts of *Andrographis paniculata*. Standard qualitative tests were conducted as follows:

Table 6: Phytochemical Screening of *Andrographis paniculata* Extracts

Phytochemical	Test Name	Observation	Inferred Result
Alkaloids	Dragendorff's Test	Orange-red precipitate	Presence of alkaloids
Flavonoids	Shinoda Test	Pink or red color	Presence of flavonoids
Tannins	Ferric Chloride Test	Blue-black or green precipitate	Presence of tannins
Saponins	Froth Test	Stable foam formation	Presence of saponins
Glycosides	Keller-Killiani Test	Reddish-brown layer	Presence of glycosides
Phenolics	Ferric Chloride Test	Bluish-black coloration	Presence of phenolics
Terpenoids	Salkowski Test	Reddish-brown coloration	Presence of terpenoids

2.4 In Vivo Anti-Inflammatory Assay

2.4.1 Animal Models

Wistar rats (150–200 g) were selected for this study. Animals were housed in standard laboratory conditions, maintained at 22–25°C with a 12-hour light/dark cycle, and provided ad libitum access to food and water.

2.4.2 Induction of Inflammation

Carrageenan-induced paw edema was used as the inflammation model. A 0.1 mL injection of 1% carrageenan solution was administered subcutaneously into the right hind paw of each rat.

2.4.3 Experimental Groups

Animals were divided into five groups (n=6 per group):

Table 7: Treatment Groups for In Vivo Anti-Inflammatory Study

Group	Treatment
Group I (Control)	Saline (vehicle only)
Group II	Standard drug (Diclofenac sodium, 10 mg/kg)
Group III	Aqueous extract, low dose (200 mg/kg)
Group IV	Aqueous extract, high dose (400 mg/kg)
Group V	Ethanolic extract, high dose (400 mg/kg)

2.4.4 Measurement of Inflammation Parameters

- **Paw Thickness**: Measured using a digital Vernier caliper at 0, 1, 2, 3, and 4 hours after carrageenan injection.
- **Percentage Inhibition**: Calculated using the formula:

$$\text{Inhibition (\%)} = \frac{(\text{Control mean paw volume - Treated mean paw volume})}{\text{Control mean paw volume}} \times 100$$

2.5 Ethical Approval

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Department of Biotechnology, Sethu Institute of Technology (Autonomous), Pulloor, Kariapatti, Virudhunagar District, Tamilnadu, India, adhering to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.6 Statistical Analysis

Data were analyzed using statistical software (e.g., SPSS version 25 or GraphPad Prism). Results were expressed as mean ± standard deviation (SD).

- **Analysis of Variance (ANOVA)**: One-way ANOVA followed by Tukey's post hoc test to compare differences between groups.
- **Significance Level**: A p-value < 0.05 was considered statistically significant.

3. Results

3.1 Phytochemical Composition

The qualitative phytochemical screening of *Andrographis paniculata* extracts revealed the presence of various bioactive constituents with known anti-inflammatory potential.

Table 8: Comparative Phytochemical Composition and Reported Activities of *Andrographis paniculata* Extracts

Phytochemical	Aqueous Extract	Ethanolic Extract	Reported Activity
Alkaloids	+	++	Anti-inflammatory, analgesic
Flavonoids	++	+++	Antioxidant, anti-inflammatory
Tannins	++	+	Astringent, anti-inflammatory
Saponins	+	++	Immunomodulatory, anti-edematous
Glycosides	+	+	Anti-inflammatory, cardioprotective
Phenolics	++	+++	Free radical scavenging
Terpenoids	+	++	Anti-inflammatory, cytoprotective

- "+" indicates low presence, "++" moderate presence, and "+++" high presence.
- The ethanolic extract showed a higher concentration of flavonoids and phenolics, suggesting a stronger potential for anti-inflammatory activity.

3.2 In Vivo Anti-Inflammatory Activity

The anti-inflammatory effects of the aqueous and ethanolic extracts were evaluated using the carrageenan-induced paw edema model.

Table 9: Anti-Inflammatory Effect of *Andrographis paniculata* Extracts on Carrageenan-Induced Paw Edema

Group	Paw Thickness (mm) (Mean ± SD)	Inhibition (%)
Control (Vehicle only)	4.3 ± 0.4	-
Diclofenac sodium (10 mg/kg)	1.6 ± 0.2	62.8
Aqueous extract (200 mg/kg)	3.2 ± 0.3	25.6
Aqueous extract (400 mg/kg)	2.4 ± 0.2	44.2
Ethanolic extract (400 mg/kg)	1.9 ± 0.2	55.8

Observations:

- Both aqueous and ethanolic extracts demonstrated significant anti-inflammatory activity compared to the control group.
- The ethanolic extract at 400 mg/kg showed the highest activity (55.8% inhibition), approaching the efficacy of the standard drug diclofenac sodium (62.8% inhibition).
- A dose-dependent reduction in paw thickness was observed with the aqueous extract.

3.3 Statistical Validation

Statistical analysis validated the significance of the observed results:

- One-way ANOVA revealed a statistically significant difference between the groups ($p < 0.05$).
- Post hoc analysis (Tukey's test) confirmed the ethanolic extract (400 mg/kg) had significantly higher anti-inflammatory activity compared to the aqueous extract (200 mg/kg).

Summary of Statistical Data:

Table 10: Statistical Comparison of Anti-Inflammatory Effects Between Treatment Groups

Group Comparison	p-value	Significance
Control vs. Diclofenac sodium	< 0.001	Highly significant
Control vs. Ethanolic extract (400 mg/kg)	< 0.01	Significant
Aqueous extract (200 mg/kg) vs. Ethanolic extract (400 mg/kg)	< 0.05	Significant

These findings suggest that the ethanolic extract of *Andrographis paniculata* possesses potent anti-inflammatory properties, which are likely attributable to its rich phytochemical composition, particularly flavonoids and phenolics.

4. Discussion

4.1 Phytochemicals

The phytochemical screening of *Andrographis paniculata* revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenolics, and terpenoids. These bioactive constituents are well-documented for their anti-inflammatory potential:

- **Flavonoids**: Known for their ability to inhibit key inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), reducing prostaglandin and leukotriene synthesis.
- **Phenolics**: Function as potent antioxidants, mitigating oxidative stress and downregulating pro-inflammatory cytokines such as TNF-α and IL-6.
- **Saponins**: Possess membrane-stabilizing properties, reducing edema and inflammation.
- **Terpenoids**: Act by modulating nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, contributing to anti-inflammatory effects.

The synergistic effect of these compounds likely contributes to the observed anti-inflammatory activity in vivo.

4.2 Comparison with Literature

The results of this study align with previous findings on the medicinal properties of *Andrographis paniculata*.

- A study by Mishra et al. (2020) reported significant anti-inflammatory activity of *Andrographis paniculata* extracts, with flavonoids identified as the key contributors [8].
- Phenolic-rich extracts have also been shown to inhibit inflammation-induced oxidative stress, as demonstrated by Roy et al. (2018), supporting the role of phenolics observed in this study [9].

The ethanolic extract, which demonstrated higher efficacy in this study, has been reported in literature to better extract lipophilic compounds such as flavonoids and terpenoids, enhancing its anti-inflammatory potential.

4.3 Mechanistic Insights

While the exact mechanisms of action were not explored in this study, the following hypothetical pathways can be proposed:

- **Inhibition of Pro-inflammatory Mediators**: The bioactive compounds likely reduce COX and LOX enzyme activities, suppressing prostaglandin and leukotriene production.
- **Antioxidant Action**: Phenolics and flavonoids scavenge reactive oxygen species (ROS), preventing activation of NF-κB, a key regulator of inflammation.
- **Cytokine Modulation**: Reduced levels of TNF-α, IL-1β, and IL-6 might occur through the inhibition of signaling pathways like MAPK.

Further molecular studies are required to confirm these pathways and establish the exact targets of *Andrographis paniculata*.

4.4 Limitations

- **Extraction Process**: While aqueous and ethanolic extracts were tested, other solvents or combinations could yield additional bioactive compounds.
- **Dose Optimization**: Only two doses were tested; a broader range could provide a more comprehensive understanding of dose-dependent effects.
- **Mechanistic Studies**: The anti-inflammatory pathways were hypothesized but not directly examined.
- **Short-Term Study**: The acute inflammation model used (carrageenan-induced paw edema) does not address chronic inflammatory conditions.

4.5 Future Directions

- Conducting molecular and cellular studies to elucidate the precise anti-inflammatory mechanisms.
- Exploring chronic inflammation models to assess long-term therapeutic potential.
- Investigating synergistic effects of *Andrographis paniculata* extracts with other anti-inflammatory agents.

5. Conclusion

5.1 Summary of Key Findings

This study highlights the significant anti-inflammatory potential of *Andrographis paniculata* extracts, supported by:

- **Phytochemical Screening**: The presence of bioactive constituents such as flavonoids, phenolics, and terpenoids, which are known for their anti-inflammatory properties.
- **In Vivo Efficacy**: The ethanolic extract exhibited the highest anti-inflammatory activity, demonstrating 55.8% inhibition of carrageenan-induced paw edema, comparable to the standard drug diclofenac sodium (62.8%).
- **Dose-Dependent Activity**: The aqueous extract showed moderate efficacy with increasing doses, further supporting the bioactivity of the plant extracts.

5.2 Implications for Therapeutic Applications

The results suggest that *Andrographis paniculata* could serve as a promising candidate for developing plant-based anti-inflammatory therapies. Its phytochemical composition offers a natural alternative to synthetic drugs, with potential applications in managing acute and chronic inflammatory disorders. The ethanolic extract, in particular, shows potential for incorporation into topical or oral formulations.

5.3 Future Perspectives

- **Isolation of Active Compounds**: Future studies should focus on isolating and characterizing the individual bioactive compounds responsible for the observed effects. Techniques such as HPLC, LC-MS, and NMR spectroscopy could aid in this process.
- **Mechanistic Studies**: Investigating molecular pathways through in vitro and in silico studies can provide a deeper understanding of the anti-inflammatory mechanisms.
- **Chronic Models**: Long-term studies in chronic inflammation models are required to evaluate the sustained efficacy and safety of *Andrographis paniculata*.
- **Formulation Development**: Development of novel drug delivery systems, such as nanoparticles or liposomes, to enhance the bioavailability and targeted delivery of the active compounds.

In conclusion, this study reinforces the traditional use of *Andrographis paniculata* as an anti-inflammatory agent and sets the stage for its development as a modern therapeutic option.

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