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ORIGINAL ARTICLE

ASSESSMENT OF PHYTOCHEMICAL CONSTITUENTS AND ANTI-INFLAMMATORY POTENTIAL OF HYDROALCOHOLIC STEM EXTRACT OF *MORINGA OLEIFERA* IN ANIMAL MODELS

N. Ramasamy[1], SNVL Sirisha[2], Tanmay Ghosh[3], Sanmati Kumar Jain[4], Anil Kumar[5], Arkaprava Biswas[6], B. S. Ashok Kumar[7], Ekta Pandey[8] and K. S. Bharath[*9]

[1]Department of Pharmacognosy, S.A. Raja Pharmacy College, Vadakkankulam, Tirunelveli - 627116, India.
[2]Department of Pharmacognosy and Phytochemistry, Malla Reddy College of Pharmacy, Maisammaguda, Hyderabad-500 100.
[3]Department of Microbiology, Dinabandhu Andrews College, Baishnabghata, South 24 Parganas, Kolkata - 700 084, India.
[4]Department of Pharmacy, Guru Ghasidas Vishwavidyalaya (A Central University), Koni, Bilaspur - 495 009, India.
[5]Department of Chemistry (PG), Sahibganj College, Sahibganj - 816 109, India.
[6]Department of Pharmacology, Eminent College of Pharmaceutical Technology, Moshpukr, Barbaria, Paschim Khilkapur, Barasat, Jagannathpur - 700 106, India.
[7]Department of Pharmacognosy, R.L. Jalappa College of Pharmacy, Sri Devaraj Urs Academy of Higher Education and Research (A Deemed to Be University), Tamaka, Kolar - 563 101, India.
[8]Department of Applied Science and Humanities, Bundelkhand Institute of Engineering and Technology, Jhansi-284128, India.
[9]Department of Pharmacology, Chettinad School of Pharmaceutical Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam - 603103, India.
*Corresponding author - K. S. Bharath, e-mail : bharathks1111@gmail.comail.com

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ABSTRACT : This study aims to assess the phytochemical constituents and anti-inflammatory activity of the hydroalcoholic stem extract of *Moringa oleifera* using animal models.The primary objectives were to identify the phytochemical components in the hydroalcoholic stem extract and to evaluate its anti-inflammatory effects in both acute and chronic inflammation animal models. Phytochemical screening revealed the presence of flavonoids, tannins, saponins and phenolic compounds. Quantitative analysis demonstrated a high concentration of total phenolics and flavonoids. In the animal models, the extract exhibited significant anti-inflammatory effects in a dose-dependent manner, comparable to diclofenac. The carrageenan-induced paw edema showed a reduction of edema by 40%, and the cotton pellet granuloma model showed a 35% decrease in granuloma weight, indicating strong anti-inflammatory potential. The hydroalcoholic stem extract of *Moringa oleifera* exhibits potent anti-inflammatory activity, likely due to its rich phytochemical content. These findings support the use of the stem as a viable source for developing anti-inflammatory therapeutic agents.

Key words : *Moringa oleifera*, phytochemical analysis, hydroalcoholic extract, anti-inflammatory, carrageenan-induced edema, cotton pellet granuloma, animal models, flavonoids, phenolic compounds.

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INTRODUCTION

Moringa oleifera, commonly known as the "drumstick tree," is a fast-growing, drought-resistant plant native to the Indian subcontinent and widely cultivated in tropical and subtropical regions. The plant has gained global recognition due to its exceptional nutritional and medicinal properties (Anwar *et al*, 2007). Almost every part of the plant—leaves, seeds, pods, flowers and stems—is utilized in traditional medicine to treat various ailments, including inflammation, infections, and malnutrition (Leone *et al*, 2015). The stem, though less studied compared to the leaves and seeds, has been reported to contain significant phytochemicals such as polyphenols, flavonoids, and alkaloids, which contribute to its therapeutic properties (Verma *et al*, 2014).

Inflammation is a complex biological response to

harmful stimuli, and while it is essential for immune defense, chronic inflammation is implicated in various diseases, including arthritis, cardiovascular disorders, and cancer (Medzhitov, 2008). Current pharmacological interventions often involve non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids, which can cause adverse effects with prolonged use. Therefore, there is an increasing interest in exploring plant-based anti-inflammatory agents as safer alternatives (Ojha *et al*, 2016). The rich phytochemical diversity of plants like *Moringa oleifera* presents a promising avenue for the development of effective natural therapies.

Despite extensive studies on the leaves, seeds, and flowers of *Moringa oleifera*, the therapeutic potential of its stem remains underexplored. Preliminary investigations suggest that the stem may possess bioactive compounds with anti-inflammatory activity, but comprehensive studies, particularly involving animal models are scarce (Awodele *et al*, 2012). Addressing this research gap is crucial to fully harness the medicinal value of the plant. The present study aims to: Analyze the phytochemical composition of hydroalcoholic stem extract of *Moringa oleifera*, and to evaluate its anti-inflammatory potential using animal models, focusing on both acute and chronic inflammation.

MATERIALS AND METHODS

Plant material

Collection and identification : Fresh stems of *Moringa oleifera* were collected from a botanical garden in Tamilnadu during the peak growth season. The plant was authenticated by taxonomist.

Preparation and storage : The collected stems were washed thoroughly with distilled water to remove debris, cut into small pieces, and shade-dried for 10 days at room temperature (~25°C). The dried stems were ground into a fine powder using a mechanical grinder and stored in an airtight container at 4°C until further use.

Preparation of extract

Hydroalcoholic Extraction Process : Powdered stem material (100 g) was mixed with 70% ethanol and distilled water in a 3:1 ratio (500 mL). The mixture was macerated for 48 hours at room temperature with intermittent shaking.

Filtration and concentration : The extract was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C. The resultant semi-solid mass was dried in a vacuum oven to yield the hydroalcoholic extract (yield: 12% w/w).

Phytochemical analysis

Qualitative analysis : The hydroalcoholic extract was screened for the presence of major phytochemical groups using standard protocols (Harborne, 1998):

Quantitative assays

- Total phenolic content (TPC): Determined using the Folin-Ciocalteu method, expressed as gallic acid equivalents (GAE) per gram of extract.
- Total flavonoid content (TFC): Determined using the aluminum chloride colorimetric assay, expressed as quercetin equivalents (QE) per gram of extract.

Experimental animals

Selection criteria : Healthy Wistar rats (150–200 g) of either sex were procured. The study was conducted following ethical guidelines approved by the Institutional Animal Ethics Committee.

Housing and maintenance : The animals were housed in polypropylene cages under controlled conditions of temperature (22 ± 2°C), humidity (50 ± 10%) and a 12-hour light/dark cycle. They were fed a standard pellet

Table 1 : Extract yield of *Moringa oleifera* stem hydroalcoholic extract.

Extract yield	Weight of powder (g)	Volume of solvent (mL)	Yield (% w/w)
Moringa oleifera stem extract	100	500	12.0

Table 2 : Phytochemical screening of *Moringa oleifera* Stem extract.

Phytochemical	Test Performed	Result
Alkaloids	Wagner's test	Present
Flavonoids	Shinoda test	Present
Tannins	Ferric chloride test	Present
Saponins	Froth test	Present
Phenolics	Ferric chloride test	Present

Table 3 : Quantitative phytochemical analysis of *Moringa oleifera* Stem extract.

Content	Concentration (mg/g extract)
Total phenolic content	132.5 ± 2.1
Total flavonoid content	75.8 ± 1.6

Table 4 : Effect of *Moringa oleifera* Stem extract on Carrageenan-Induced Paw Edema.

Group	Dose (mg/kg)	Paw Volume (mL) at 4 hours
Control	-	0.68 ± 0.05
Indomethacin	10	0.20 ± 0.02
Extract (Low)	100	0.40 ± 0.03
Extract (Medium)	200	0.30 ± 0.02
Extract (High)	400	0.25 ± 0.01

Table 5 : Effect of *Moringa oleifera* Stem Extract on Cotton Pellet Granuloma formation.

Group	Dose (mg/kg)	Granuloma weight (mg)
Control	-	45.5 ± 2.1
Indomethacin	10	18.0 ± 1.5
Extract (Low)	100	30.2 ± 1.8
Extract (Medium)	200	22.5 ± 1.3
Extract (High)	400	19.8 ± 1.0

diet and water ad libitum.

Anti-inflammatory assays

Acute Inflammation Model: Carrageenan-induced Paw Edema

- Method: Inflammation was induced by injecting 0.1 mL of 1% carrageenan solution into the subplantar region of the right hind paw.
- Groups (n=6):
- Control (normal saline)
- Standard (indomethacin, 10 mg/kg)
- *Moringa oleifera* extract (100 mg/kg, 200 mg/kg and 400 mg/kg)
- Measurement: Paw volume was measured using a plethysmometer at 0, 1, 2, 3 and 4 hours post-injection.

Chronic Inflammation Model: Cotton Pellet Granuloma test

- Method: Sterile cotton pellets (50 ± 1 mg) were implanted subcutaneously into the dorsum of the rats.
- Groups (n=6): Same as the acute inflammation model.
- Measurement: Granuloma formation was assessed by weighing the dried pellets after 7 days.

Dosage and administration : The extract was administered orally using an oral gavage in volumes not exceeding 1 mL/100 g body weight.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

RESULTS

Phytochemical composition

Qualitative and Quantitative Results

The qualitative analysis confirmed the presence of key phytochemical groups, including alkaloids, flavonoids, tannins, saponins, and phenolics. Quantitative analysis revealed high levels of phenolic and flavonoid content, which are known for their antioxidant and anti-inflammatory properties.

Table 6 : Phytochemical Screening and Quantitative content of *Moringa oleifera* Stem extract.

Phytochemical	Qualitative test result	Quantitative Content (mg/g extract)
Alkaloids	Present	-
Flavonoids	Present	75.8 ± 1.6
Tannins	Present	-
Saponins	Present	-
Phenolics	Present	132.5 ± 2.1

Discussion on Major Phytochemical constituents

The high phenolic and flavonoid contents are indicative of the extract's potential for mitigating oxidative stress and inflammation. These compounds are known to modulate inflammatory pathways by scavenging free radicals and inhibiting pro-inflammatory mediators.

Anti-Inflammatory activity

Acute Inflammation Model : In the carrageenan-induced paw edema model, the hydroalcoholic stem extract demonstrated a dose-dependent reduction in paw volume. At the highest dose (400 mg/kg), the extract's effect was comparable to the standard drug, indomethacin.

Chronic Inflammation model : In the cotton pellet granuloma test, the extract significantly reduced granuloma formation in a dose-dependent manner. The reduction was most pronounced at 400 mg/kg, approaching the efficacy of indomethacin.

Table 7 : Percentage Paw Volume reduction in Carrageenan-induced Paw Edema.

Group	Dose (mg/kg)	Paw Volume Reduction (%)
Control	-	0
Indomethacin	10	70.6
Extract (Low)	100	41.2
Extract (Medium)	200	55.9
Extract (High)	400	63.2

Table 8 : Percentage Granuloma weight reduction in Cotton Pellet Granuloma test.

Group	Dose (mg/kg)	Granuloma Reduction (%)
Control	-	0
Indomethacin	10	60.4
Extract (Low)	100	33.6
Extract (Medium)	200	50.6
Extract (High)	400	56.5

Comparison with Positive control : The anti-inflammatory effects of the hydroalcoholic stem extract were dose-dependent, with the high-dose group showing results comparable to indomethacin. This suggests the extract's potential as a natural anti-inflammatory agent.

Dose-dependent effects : Both acute and chronic inflammation models demonstrated that increasing doses of the extract significantly enhanced anti-inflammatory activity, underscoring its therapeutic potential.

DISCUSSION

The findings of this study highlight the potential anti-inflammatory properties of the hydroalcoholic stem extract of *Moringa oleifera*. This aligns with previous research demonstrating the pharmacological relevance of *Moringa oleifera* in managing inflammation and oxidative stress (Anwar *et al*, 2007; Leone *et al*, 2015). The phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolics, tannins, and saponins, which are known to exhibit anti-inflammatory activities (Harborne, 1998; Verma *et al*, 2014). These bioactive compounds likely contribute to the extract's efficacy in both acute and chronic inflammation models.

Phenolics and flavonoids, identified in significant concentrations in the extract are known for their antioxidant and free radical scavenging properties, which help mitigate inflammatory responses (Verma *et al*, 2014). Flavonoids have been previously reported to inhibit the synthesis of inflammatory mediators such as prostaglandins, leukotrienes, and nitric oxide (Ojha *et al*, 2016). Similarly, tannins contribute by modulating inflammatory signaling pathways, such as NF-κB and COX-2 (Awodele *et al*, 2012). These findings are consistent with the observed reduction in paw edema in the carrageenan-induced acute inflammation model and decreased granuloma formation in the cotton pellet granuloma model.

The anti-inflammatory effect of the *Moringa oleifera* stem extract in this study was comparable to the reference drug, diclofenac sodium, particularly in the carrageenan-induced paw edema model. This model reflects the early phase of inflammation mediated by histamine and serotonin, as well as the late phase involving prostaglandins (Medzhitov, 2008). The ability of the extract to suppress both phases suggests its broad-spectrum anti-inflammatory potential.

Previous studies have focused predominantly on the leaves, seeds, and pods of *Moringa oleifera*, with limited attention given to the stem (Anwar *et al*, 2007; Leone *et al*, 2015). However, this study demonstrates that the stem also holds therapeutic value, particularly for conditions associated with chronic inflammation. By utilizing the underexplored stem, this research expands the pharmacological applications of *Moringa oleifera*, emphasizing its sustainable use as a multi-purpose plant.

Although the study provides valuable insights, certain limitations must be addressed. First, the extract's specific mechanisms of action remain unclear, necessitating molecular studies to elucidate its effects on inflammatory pathways. Second, while the study used rodent models, human clinical trials are essential to validate these findings and determine the extract's safety and efficacy in humans (Awodele *et al*, 2012). Future studies should also explore the synergistic effects of the identified phytochemicals to optimize therapeutic formulations.

Overall, the hydroalcoholic stem extract of *Moringa oleifera* exhibits promising anti-inflammatory properties, underscoring its potential as a natural alternative to conventional NSAIDs. Its phytochemical composition plays a pivotal role in its efficacy, supporting the broader medicinal use of *Moringa oleifera*. Further research will enhance our understanding of its mechanisms and clinical relevance.

CONCLUSION

This study highlights the significant anti-inflammatory potential of the hydroalcoholic stem extract of *Moringa oleifera*, which can be attributed to its rich phytochemical composition. The extract demonstrated dose-dependent efficacy in both acute and chronic inflammation models, showing comparable results to the standard anti-inflammatory drug, indomethacin. High concentrations of phenolic and flavonoid compounds, which have well-established antioxidant and anti-inflammatory properties, are likely responsible for the observed bioactivity. These compounds are known to inhibit inflammatory mediators such as cyclooxygenase (COX) and lipoxygenase (LOX) enzymes (Gupta *et al*, 2019; Ramesh *et al*, 2021).

The findings are consistent with previous studies on other parts of *Moringa oleifera*, which have shown similar anti-inflammatory effects, particularly in leaves and seeds (Das *et al*, 2020; Choudhary *et al*, 2020). This study, however, addresses the relatively underexplored potential of the stem, adding new evidence to its medicinal value.

Future studies should aim to isolate and identify specific bioactive compounds in the extract, investigate molecular mechanisms underlying its effects and assess long-term safety through clinical trials. Such research could pave the way for developing safe, plant-based anti-inflammatory agents to address conditions like arthritis and other chronic inflammatory diseases.

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