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PHYTOCHEMICAL PROFILING AND COMPARATIVE ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF *ENICOSTEMMA LITTORALE* LEAF EXTRACTS USING IN VITRO AND IN VIVO MODELS

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ABSTRACT

The present study was undertaken to evaluate the analgesic and anti-inflammatory activity of methanolic and aqueous leaf extracts of *Enicostemma littorale*. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, and tannins in both extracts. Analgesic activity was assessed using the hot plate method and acetic acid-induced writhing test. In the hot plate method, the methanolic extract showed a significant increase in reaction time from 3.2 ± 0.3 s (control) to 7.9 ± 0.5 s at 400 mg/kg, whereas the aqueous extract showed an increase to 6.5 ± 0.4 s. In the acetic acid-induced writhing test, the methanolic extract exhibited 61.3% inhibition at 400 mg/kg, while the aqueous extract showed 50.0% inhibition compared to control (62 ± 3 writhes). Anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model. The methanolic extract demonstrated 56.7% inhibition of paw edema at 400 mg/kg, whereas the aqueous extract showed 43.3% inhibition, compared to control paw volume of 1.20 ± 0.05 mL. The standard drug (diclofenac sodium, 10 mg/kg) showed higher activity with 71.0% inhibition in the writhing test and 62.5% inhibition in the paw edema model. The results indicate that both extracts possess significant analgesic and anti-inflammatory activity, with the methanolic extract showing superior efficacy. The observed activity may be attributed to the higher content of phenolic and flavonoid compounds, which are known to inhibit inflammatory mediators and modulate pain pathways.

KEYWORDS: *Enicostemma littorale*; Analgesic activity; Anti-inflammatory activity; Writhing test; Carrageenan; Phytochemicals.

1. INTRODUCTION

Pain and inflammation are complex physiological responses that occur as protective mechanisms against tissue injury, infection, or harmful stimuli. Inflammation is characterized by redness, swelling, heat, and pain, and is mediated by the release of chemical mediators such as prostaglandins, histamine, serotonin, and pro-inflammatory cytokines (Medzhitov, 2008). While acute inflammation plays a crucial role in healing, chronic inflammation is implicated in the development of various diseases, including rheumatoid arthritis, cardiovascular disorders, diabetes, and neurodegenerative conditions (Libby, 2007).

Pain is closely associated with inflammation and arises due to the activation and sensitization of nociceptors by inflammatory mediators such as bradykinin and prostaglandins (Julius & Basbaum, 2001). The management of pain and inflammation commonly involves non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics. NSAIDs act by inhibiting cyclooxygenase (COX) enzymes and reducing prostaglandin synthesis, thereby alleviating pain and

inflammation (Vane & Botting, 1998). However, prolonged use of NSAIDs is associated with adverse effects such as gastrointestinal irritation, renal dysfunction, and increased cardiovascular risk (Wallace, 2008). Similarly, opioid analgesics can lead to tolerance, dependence, and respiratory depression.

Due to these limitations, there is growing interest in plant-based medicines as safer alternatives for the management of pain and inflammation. Medicinal plants are rich in bioactive phytoconstituents such as flavonoids, phenolic compounds, tannins, and alkaloids, which have been reported to exhibit significant analgesic and anti-inflammatory activities (Calixto et al., 2000). These compounds exert their effects through multiple mechanisms, including inhibition of inflammatory mediators, suppression of oxidative stress, modulation of enzyme activity, and stabilization of cellular membranes (Middleton et al., 2000).

Enicostemma littorale, a small perennial herb belonging to the family Gentianaceae, is widely distributed in India and other tropical regions. It is traditionally known as "Chota Chirata" and has been extensively used in Ayurvedic medicine for the treatment of fever, diabetes, inflammation, and pain-related disorders (Kirtikar & Basu, 1999). Phytochemical investigations have revealed that the plant contains bioactive compounds such as swertiamarin, flavonoids, phenolic acids, alkaloids, and glycosides, which contribute to its pharmacological properties (Patel & Mishra, 2011).

Previous studies have demonstrated that *Enicostemma littorale* possesses significant antioxidant, antidiabetic, hepatoprotective, analgesic, and anti-inflammatory activities (Maroo et al., 2003). The analgesic effect is attributed to the inhibition of peripheral pain mediators, whereas the anti-inflammatory activity may involve suppression of prostaglandin synthesis and inhibition of inflammatory pathways such as cyclooxygenase (COX) and lipoxygenase (LOX) enzymes (Calixto et al., 2000). Furthermore, the presence of phenolic and flavonoid compounds enhances its ability to reduce oxidative stress, which plays a critical role in inflammation.

Despite its traditional importance and reported pharmacological activities, comparative studies evaluating the analgesic and anti-inflammatory potential of different solvent extracts of *Enicostemma littorale* remain limited. Since the extraction solvent significantly influences the yield and composition of bioactive compounds, it is essential to compare different extracts to identify the most potent one.

Therefore, the present study aims to evaluate and compare the analgesic and anti-inflammatory activity of methanolic and aqueous leaf extracts of *Enicostemma littorale* using established experimental models and to correlate the observed effects with their phytochemical constituents.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Fresh leaves of *Enicostemma littorale* were collected from Odisha, India, authenticated by a botanist, and preserved as a voucher specimen.

2.2 Preparation of Extracts

2.2.1 Methanolic Extract

The dried powdered leaves were extracted using methanol in a Soxhlet apparatus for 6–8 hours. The extract was concentrated using a rotary evaporator and stored for further use.

2.2.2 Aqueous Extract

The powdered material was subjected to maceration with distilled water for 48 hours, filtered, concentrated, and stored under refrigeration.

2.3 Phytochemical Screening

Both extracts were subjected to qualitative phytochemical tests confirming the presence of:

- Alkaloids
- Flavonoids
- Phenolics
- Tannins

2.4 Evaluation of Analgesic Activity

2.4.1 Hot Plate Method

The analgesic activity of the extracts was evaluated using the hot plate method as described by Eddy and Leimbach (1953). Experimental animals were placed individually on a hot plate maintained at a temperature of 55 ± 1°C. The reaction time, defined as the latency to paw licking or jumping, was recorded before and after administration of the test extracts. A cut-off time was maintained to prevent tissue damage. An increase in reaction time compared to the control group was considered indicative of analgesic activity.

2.4.2 Acetic Acid-Induced Writhing Test

Peripheral analgesic activity was assessed using the acetic acid-induced writhing method (Koster et al., 1959). Animals were administered 0.6% acetic acid intraperitoneally to induce writhing. The number of writhes, characterized by abdominal constrictions and stretching, was counted over a period of 20 minutes.

The percentage inhibition of writhing was calculated using the following formula:

- Where represents the number of writhes in the control group and represents the number of writhes in the treated group.

2.5 Evaluation of Anti-Inflammatory Activity

2.5.1 Carrageenan-Induced Paw Edema

The anti-inflammatory activity of the extracts was evaluated using the carrageenan-induced paw edema model, as described by Winter et al. (1962). Acute inflammation was induced by injecting carrageenan into the subplantar region of the hind paw of experimental animals. Paw volume was measured at predetermined time intervals using a plethysmometer.

The percentage inhibition of edema was calculated using the following equation:

- Where denotes the paw volume of the control group and denotes the paw volume of the treated group.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Preliminary phytochemical analysis of the methanolic and aqueous extracts of *Enicostemma littorale* revealed the presence of alkaloids, flavonoids, phenolic compounds, and tannins. These phytoconstituents are known to possess significant analgesic and anti-inflammatory activities (Calixto et al., 2000). The presence of flavonoids and phenolics, in particular, suggests the potential of the extracts to inhibit inflammatory mediators and reduce pain perception.

3.2 Analgesic Activity

3.2.1 Hot Plate Method

The analgesic activity assessed by the hot plate method showed a significant increase in reaction time (latency) in treated groups compared to the control group.

Table 3.1: Effect of Extracts on Reaction Time (Hot Plate Method).

Treatment	Dose (mg/kg)	Reaction Time (sec)
Control	—	3.2 ± 0.3
Methanolic Extract	200	5.8 ± 0.4
Methanolic Extract	400	7.9 ± 0.5
Aqueous Extract	200	4.9 ± 0.3
Aqueous Extract	400	6.5 ± 0.4
Standard (Diclofenac)	10	9.2 ± 0.6

Interpretation

The methanolic extract exhibited a greater increase in latency time compared to the aqueous extract, indicating stronger central analgesic activity. This effect may be attributed to the presence of flavonoids and alkaloids, which are known to modulate pain pathways.

3.2.2 Acetic Acid-Induced Writhing Test

The number of writhes decreased significantly in extract-treated groups.

Table 3.2: Effect on Writhing Response.

Treatment	Dose (mg/kg)	No. of Writhes	% Inhibition
Control	—	62 ± 3	—
Methanol Extract	200	38 ± 2	38.7%
Methanol Extract	400	24 ± 2	61.3%
Aqueous Extract	200	45 ± 3	27.4%
Aqueous Extract	400	31 ± 2	50.0%
Standard (Diclofenac)	10	18 ± 1	71.0%

Calculation (Example)

For methanolic extract (400 mg/kg):

Interpretation

The methanolic extract showed higher inhibition of writhing compared to the aqueous extract, indicating stronger peripheral analgesic activity. This suggests inhibition of prostaglandin synthesis and other inflammatory mediators.

3.3 Anti-Inflammatory Activity

3.3.1 Carrageenan-Induced Paw Edema

Both extracts exhibited significant anti-inflammatory activity by reducing paw edema.

Table 3.3: Effect on Paw Edema Volume.

Treatment	Dose (mg/kg)	Paw Volume (mL)	% Inhibition
Control	—	1.20 ± 0.05	—
Methanol Extract	200	0.78 ± 0.04	35.0%
Methanol Extract	400	0.52 ± 0.03	56.7%
Aqueous Extract	200	0.90 ± 0.05	25.0%
Aqueous Extract	400	0.68 ± 0.04	43.3%
Standard (Diclofenac)	10	0.45 ± 0.02	62.5%

Calculation (Example)
For methanolic extract (400 mg/kg):

Interpretation
The methanolic extract demonstrated greater inhibition of paw edema compared to the aqueous extract, indicating stronger anti-inflammatory activity. The carrageenan-induced inflammation model is biphasic, involving histamine and serotonin release in the early phase and prostaglandins in the late phase. The observed inhibition suggests that the extracts may interfere with prostaglandin synthesis pathways.

3.4 DISCUSSION
The present study demonstrated that both methanolic and aqueous extracts of *Enicostemma littorale* possess significant analgesic and anti-inflammatory activities, with the methanolic extract showing superior efficacy.

The enhanced activity of the methanolic extract may be attributed to its higher content of phenolic and flavonoid compounds, which are known to inhibit key inflammatory mediators such as prostaglandins and cytokines (Middleton et al., 2000). The results of the hot plate test suggest central analgesic activity, possibly mediated through modulation of opioid receptors or central pain pathways. In contrast, the acetic acid-induced writhing test indicates peripheral analgesic activity through inhibition of prostaglandin synthesis.

Similarly, the carrageenan-induced paw edema model confirmed the anti-inflammatory potential of the extracts. The significant reduction in paw volume suggests inhibition of inflammatory mediators involved in both early and late phases of inflammation.

Overall, the findings support the traditional use of *Enicostemma littorale* in the treatment of pain and inflammatory conditions and highlight its potential as a natural therapeutic agent.

4. SUMMARY
The study demonstrated that *Enicostemma littorale* possesses significant analgesic and anti-inflammatory properties. The methanolic extract showed superior activity compared to the aqueous extract in all experimental models.

5. CONCLUSION
The findings confirm that *Enicostemma littorale* is a promising natural source of analgesic and anti-inflammatory agents. The methanolic extract exhibited stronger activity, likely due to higher concentrations of bioactive phytochemicals.

This plant may be developed into herbal formulations for the management of pain and inflammatory disorders. Further studies including isolation of active compounds and clinical evaluation are recommended.

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SUMMARY
The present study was conducted to evaluate the analgesic and anti-inflammatory potential of methanolic and aqueous leaf extracts of *Enicostemma littorale*. Preliminary phytochemical screening confirmed the presence of important bioactive constituents such as alkaloids, flavonoids, phenolic compounds, and tannins in both extracts, which are known to contribute to pharmacological activities.

The analgesic activity was assessed using the hot plate method and acetic acid-induced writhing test. The results demonstrated a significant, dose-dependent increase in latency time in the hot plate method and a reduction in the number of writhes in the writhing test. The methanolic extract exhibited greater analgesic activity compared to the aqueous extract, indicating both central and peripheral mechanisms of pain inhibition.

The anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model. Both extracts showed a significant reduction in paw edema, with the methanolic extract demonstrating higher percentage inhibition. This suggests effective suppression of inflammatory mediators involved in both early and late phases of inflammation.

Overall, the study indicates that *Enicostemma littorale* possesses significant analgesic and anti-inflammatory properties, with the methanolic extract showing superior activity, likely due to higher concentrations of phenolic and flavonoid compounds.

CONCLUSION
The findings of the present investigation clearly demonstrate that *Enicostemma littorale* is a potent natural source of analgesic and anti-inflammatory agents. Among the two extracts studied, the methanolic extract exhibited superior efficacy in all experimental models, including the hot plate test, acetic acid-induced writhing test, and carrageenan-induced paw edema model.

The enhanced pharmacological activity of the methanolic extract may be attributed to its higher content of bioactive phytoconstituents, particularly flavonoids and phenolic compounds, which are known to inhibit inflammatory mediators such as prostaglandins and cytokines. The study also suggests that the plant exerts both central and peripheral analgesic effects.

These results scientifically validate the traditional use of *Enicostemma littorale* in the management of pain and inflammatory conditions. However, further studies

involving isolation of active compounds, detailed mechanistic investigations, and clinical evaluations are necessary to establish its safety and therapeutic efficacy for pharmaceutical applications.

REFERENCES

1. Calixto, J. B., Beirith, A., Ferreira, J., Santos, A. R. S., Filho, V. C., & Yunes, R. A. Naturally occurring antinociceptive substances from plants. *Phytotherapy Research*, 2000; *14*(6): 401–418.
2. Julius, D., & Basbaum, A. I. Molecular mechanisms of nociception. *Nature*, 2001; *413*(6852): 203–210.
3. Kirtikar, K. R., & Basu, B. D. (1999). *Indian medicinal plants* (2nd ed.). International Book Distributors.
4. Libby, P. Inflammatory mechanisms: The molecular basis of inflammation and disease. *Nutrition Reviews*, 2007; *65*(12): S140–S146.
5. Maroo, J., Vasu, V. T., Gupta, S., & Gupta, Y. K. Dose dependent hypoglycemic effect of aqueous extract of *Enicostemma littorale* in alloxan-induced diabetic rats. *Phytomedicine*, 2003; *10*(2–3): 196–199.
6. Medzhitov, R. Origin and physiological roles of inflammation. *Nature*, 2008; *454*(7203): 428–435.
7. Middleton, E., Kandaswami, C., & Theoharides, T. C. The effects of plant flavonoids on mammalian cells. *Pharmacological Reviews*, 2000; *52*(4): 673–751.
8. Patel, D. K., & Mishra, S. K. Antidiabetic potential of *Enicostemma littorale*: A review. *International Journal of Pharmaceutical Sciences and Research*, 2011; *2*(6): 1257–1265.
9. Vane, J. R., & Botting, R. M. Mechanism of action of anti-inflammatory drugs. *American Journal of Medicine*, 1998; *104*(3A): 2S–8S.
10. Wallace, J. L. Prostaglandins, NSAIDs, and gastric mucosal protection. *Gastroenterology Clinics of North America*, 2008; *37*(2): 255–264.
11. Eddy, N. B., & Leimbach, D. Synthetic analgesics II: Dithienylbutenyl and dithienylbutylamines. *Journal of Pharmacology and Experimental Therapeutics*, 1953; *107*(3): 385–393.
12. Koster, R., Anderson, M., & De Beer, E. J. Acetic acid for analgesic screening. *Federation Proceedings*, 1959; *18:* 412–417.
13. Winter, C. A., Risley, E. A., & Nuss, G. W. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proceedings of the Society for Experimental Biology and Medicine*, 1962; *111*(3): 544–547.
14. Calixto, J. B., et al. *Phytotherapy Research*, 2000; *14:* 401–418.
15. Middleton, E., et al. *Pharmacological Reviews*, 2000; *52:* 673–751.