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Analgesic and Anti-Inflammatory Effects of Ethanol Leaf Extract of *Guiera Senegalensis* in Murine Models

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Abstract: Pain and inflammation are global public health challenges, often requiring safer and more effective treatments. Medicinal plants like *Guiera senegalensis* have been traditionally used for pain and inflammatory conditions, but scientific validation of their therapeutic potential is limited. This study aimed to evaluate the analgesic and anti-inflammatory properties of ethanol leaf extract of *G. senegalensis* in murine models. Fresh *G. senegalensis* leaves were collected, authenticated, and subjected to ethanol extraction. Phytochemical analysis was conducted to identify bioactive compounds. Acute toxicity studies were performed following OECD guidelines. Analgesic effects were evaluated using the acetic acid-induced writhing test and the hot plate test, while the anti-inflammatory activity was assessed using the formalin-induced paw edema model. Phytochemical analysis revealed the presence of tannins, saponins, and flavonoids. Acute toxicity tests showed no mortality or severe adverse effects at 2000 mg/kg. The extract demonstrated dose-dependent analgesic activity, with 84% inhibition of writhing at doses of 25 mg/kg and 50 mg/kg, outperforming Diclofenac (59%). In the hot plate test, moderate central analgesic effects were observed. Anti-inflammatory tests showed significant reductions in paw edema at doses of 12.5 and 25 mg/kg, comparable to Diclofenac. Ethanol leaf extract of *G. senegalensis* exhibits significant analgesic and anti-inflammatory activities, likely mediated by its flavonoid, saponin, and tannin content. These findings support its traditional use and highlight its potential as a natural alternative for managing pain and inflammation.

Introduction

Pain and inflammation are complex physiological processes that serve as essential protective mechanisms against harmful stimuli. However, when these responses become chronic, they can lead to debilitating health conditions that significantly impact an individual's quality of life and overall well-being (1, 2). The World Health Organization has identified pain management as a critical area in public health, emphasizing the need for effective and safe therapeutic options. This recognition stems from the significant global burden of pain, which affects approximately 20% of adults and is associated with numerous comorbidities, making it a pressing public health issue (3). Traditional analgesics, including non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, are commonly employed to alleviate pain and inflammation. Despite their efficacy, these synthetic medications often come with substantial side effects, such as gastrointestinal complications and the risk of addiction (4, 5). In light of these challenges, there is a growing interest in exploring natural alternatives derived from medicinal plants, particularly in regions with limited access to modern healthcare.

Medicinal plants have long been a cornerstone of traditional healthcare systems, particularly in Africa, where they address many ailments (6). Among these, *Guiera senegalensis* (Family: Combretaceae), commonly known as Sabara in Hausa and Olofun in Yoruba, is a shrub native to the savannah regions of

West and Central Africa (7). This plant has been traditionally utilized in African medicine, with its leaves recognized for their bitter taste and rich composition of essential mineral elements. Historical accounts and recent studies indicate that *G. senegalensis* has been employed for treating many ailments, including coughs, respiratory congestion, fever, malaria, diabetes, hypertension, gastrointestinal disorders, and wounds various inflammatory conditions (8, 9). Furthermore, its branches, leaves, bark, and roots are utilized not only for medicinal purposes but also in dietary practices among local populations to enhance livestock growth and reproductive capacity (8).

Recent studies have validated many of the traditional uses of *G. senegalensis* through pharmacological research, including antimicrobial, antiplasmodial, antiprotozoal, antimalarial, anti-inflammatory, antioxidant, and gastrointestinal effects (9-11). Phytochemical analyses have revealed that *G. senegalensis* contains bioactive compounds such as carbohydrates, alkaloids, phenols, flavonoids, triterpenes, tannins, cardiac glycosides, and saponins, which are believed to contribute to its therapeutic effects (9, 10, 12). The plant's traditional applications align with contemporary research efforts to validate its medicinal properties through scientific investigation. This study uses established pharmacological models in murine subjects to evaluate the analgesic and anti-inflammatory effects of ethanol leaf extract from *G. senegalensis*.

Experimental Section

Drugs and Chemicals

The chemicals used were normal Saline (Fidson Health Care Nigeria), acetic acid (Oak Chemicals Allied and Inter Trade Ltd), ethanol (Sigma Chemical Co. St Louis, USA), pentazocine (Rambaxy), diclofenac (Hovid), indomethacin (Kapit Pharmaceutical Limited, Nigeria), and formalin.

Experimental Animals

Sixty-six mice weighing 24-28 g were obtained from the animal housing facility at the Department of Pharmacology, Sa'adu Zungur University, Bauchi. These mice were kept under typical laboratory conditions, adhering to a protocol sanctioned by the Faculty of Basic Medical Sciences Research and Ethics Committee (FBMSREC) (BASUG/FBMS/REC/VOL.3/0098).

Collection and Identification of Plant Material

Fresh leaves of *G.senegalensis* were obtained from Gadau village within the Itas-Gadau Local Government Area of Bauchi State, Nigeria, in April 2024. The plant was authenticated and identified at the Herbarium Unit within the Department of Biological Sciences at Sa'adu Zungur University. Abdullahi Ishaka verified the plant as *G. senegalensis* and assigned a voucher number 00156 for future reference.

Extraction of Plant Material

The leaves were thoroughly washed, air-dried, and ground into a coarse powder using a mortar and pestle. A portion of the coarse powder, weighing 100 g, was immersed in 70% ethanol through cold maceration for 72 h. The resultant blend was filtered using filter paper and evaporated at a temperature range of 45–50 °C until a consistent extract weight was achieved.

Preliminary Phytochemical Analysis

The phytoconstituents in the ethanol extract of *G.senegalensis* were identified through established conventional protocols for qualitative phytochemical tests (13).

Acute Toxicity Studies

Swiss Albino mice of both genders, weighing between 25 and 28 g, were selected for acute toxicity studies following the guidelines outlined by OECD 423 (14). The animals were randomly divided into two groups, each consisting of three animals: Group 1 served as the control and received only normal saline (5 mL/kg). At the same time, Group 2 was administered the extract at a dose of 2000 mg/kg. Over 14 days, the animals were observed for any signs of toxicity or notable changes in behavior.

Analgesic Studies

Acetic Acid-Induced Pain

The acetic acid-induced pain test was conducted following the protocol outlined by (15). Twenty mice were randomly assigned to five groups, with four per group. Group 1 (negative control) received normal saline (5 mL/kg), while Groups 2, 3, and 4 were administered *G.senegalensis* extract at doses of 12.5, 25, and 50 mg/kg, respectively. Group 5 was the positive control group, and the group was treated with Diclofenac sodium at 10 mg/kg. Treatments were administered orally. Thirty minutes after treatment, all animals had 0.06% acetic acid at 10 mL/kg intraperitoneally. Five minutes later, the number of writhing was recorded for each mouse during a 10-minute observation period. Results were compared to those of the negative control group, and the percentage inhibition was calculated using **Equation 1** with N being number of writhing.

$$\% \, Inhibition = \frac{N_{control} - N_{sample}}{N_{control}} \times 100 \quad \textbf{Equation 1}$$

Thermally Induced Pain (Hot Plate Test)

The hot plate test was conducted as described by Eddy and Leimbach (1953) (16). Mice were positioned on a hot plate maintained at a temperature ranging between 50-55 °C, and each mouse's paw licking or

jumping duration was noted. Mice displaying an initial nociceptive response within 20 s were included in the study. Group 1 received normal saline at 5 mL/kg, while Group 2 was administered Pentazocine at 10mg/kg. Conversely, Groups 3, 4, and 5 were treated with *G. senegalensis* at 12.5, 25, and 50 mg/kg, respectively. Each mouse was placed on the hot plate repeatedly at 0, 15, 30, and 60 min post-injection, and the reaction time to pain was recorded in seconds. Subsequently, the results were compared with those of the negative control group.

Anti-Inflammatory Study

Formalin-Induced Paw Edema in Mice

The anti-inflammatory effects of *G.senegalensis* were evaluated using the formalin-induced paw edema model described by Dauda *et al*. (2024) (17). Five groups, each consisting of four Swiss albino mice, were established. Groups one and five were the control (administered normal saline) and the reference (administered indomethacin) groups. Groups two, three, and four received different doses of extracts (12.5, 25, and 50 mg/kg). The administration of normal saline, indomethacin, and plant extract was done via oral route. Inflammation was induced 30 min after extract administration by injecting 0.1 mL of 2.0% formaldehyde into the sub-plantar tissue of the mice's right hind paw. The thickness of the paw was measured using a digital vernier caliper at 0, 30, 60, and 90 min. The percentage inhibition was calculated using **Equation 1** with N being the thickness of paw edema.

Statistical Analysis

Statistical analysis was conducted using one-way ANOVA followed by the Dunnett post hoc test, with a significance level set at $p < 0.05$ (SPSS, 2010: Version 22). All test samples and standard drug parameters were compared with those of the control group at their respective time points.

Results and Discussion

Phytochemical Screening

The phytochemical analysis of the ethanol leaf extract of *G. senegalensis* revealed the presence of tannins, saponins, and flavonoids (**Table 1**). These bioactive compounds are well-known for their roles in anti-inflammatory and analgesic activities, highlighting the plant's therapeutic potential. Flavonoids, in particular, are potent antioxidants that mitigate oxidative stress and inflammation by inhibiting the synthesis of pro-inflammatory mediators such as prostaglandins and leukotrienes. This inhibition contributes to pain relief and tissue repair (18). Tannins possess astringent properties, stabilizing membranes and reducing capillary permeability, alleviating edema and inflammation (19). Saponins have been reported to exhibit anti-inflammatory effects through the modulation of cytokine production, particularly by suppressing tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) (20). Interestingly, the absence of alkaloids and steroids in the extract may refine the focus of its pharmacological action, directing attention to the detected compounds as the primary contributors to its efficacy. These results align with earlier phytochemical studies on *G. senegalensis*, which have consistently validated its bioactive profile and therapeutic potential (9).

Table 1. Phytochemical constituents' analysis of the crude ethanol extract of *G. senegalensis*.

No.	Phytoconstituents	Result
1	Alkaloids	-
2	Tannins	+
3	Saponins	+
4	Flavonoids	+
5	Steroids	-
Note: (+) = present and (-) = absent		

Acute Toxicity Test

Acute toxicity testing revealed that the ethanol extract was non-lethal at a dose of 2000 mg/kg, with no significant adverse effects observed during the 14-day monitoring period. Behavioral changes such as sedation and reduced food and water intake were transient, suggesting a systemic adjustment rather than toxicity. This finding demonstrates a wide therapeutic window, supporting the plant's safety for therapeutic use. The results are consistent with the OECD 423 guidelines, which recommend assessing safety margins before advancing to detailed pharmacological evaluations (14). Such data are critical for future development, ensuring the extract is safe for further preclinical and clinical investigations.

Analgesic Studies

Acetic Acid-Induced Writhing Test

The acetic acid-induced writhing test, as shown in **Table 2**, demonstrated that the ethanol extract significantly reduced the number of abdominal writhes in mice in a dose-dependent manner. The percentage inhibition ranged from 66% at 12.5 mg/kg to 84% at 25 mg/kg and 50 mg/kg, indicating strong peripheral analgesic activity. Interestingly, the efficacy of the extract at 25 mg/kg and 50 mg/kg was equivalent, suggesting a possible plateau effect at higher doses. The reduction in writhing can be attributed to the inhibition of cyclooxygenase (COX) enzymes, which mediate the production of prostaglandins during tissue injury and inflammation (21). The extract outperformed the standard drug Diclofenac (59% inhibition), highlighting its potential as a natural alternative with possibly fewer side effects.

Table 2. Effect of ethanol extract of G. senegalensis on acetic acid-induced writhing test in mice.

Treatment (mg/kg)	Abdominal Writhing	%Inhibition
Normal saline 5 mL/kg	14.50±2.39	-
GSEE 12.5	5.00±1.08*	66%
GSEE 25	2.25±0.64*	84%
GSEE 50	2.25±0.61*	84%
Diclofenac 10	6.00±1.08*	59%

Note: Values are presented as Mean ± SEM. *Denotes a significant difference ($p < 0.05$) compared to the normal saline control group (one-way ANOVA followed by Dunnet post hoc test). GSEE= *G. senegalensis* ethanol extract

Table 3. Effect of ethanol extract of *G. senegalensis* on pain latency in hot plate test in mice.

Treatment (mg/kg)	0 min (s)	15 min (s)	30 min (s)	60 min (s)
Normal saline 5mL/kg	1.50 ± 0.29	1.00 ± 0.00	2.00 ± 0.41	1.75 ± 0.25
GSEE 12.5	3.00 ± 0.71	2.25 ± 0.63	3.00 ± 0.41	4.00 ± 1.41
GSEE 25	2.50 ± 0.65	2.25 ± 0.48	3.75 ± 1.44	2.00 ± 0.00
GSEE 50	2.00 ± 0.41	1.00 ± 0.00	4.00 ± 1.41	3.00 ± 0.00
Pentazocine 10	5.25 ± 1.37*	4.00 ± 0.41*	8.00 ± 1.87*	9.25 ± 1.11*

Note: Values are presented as Mean ± SEM. *denotes a significant difference ($p < 0.05$) compared to the normal saline control group (one-way ANOVA followed by Dunnet post hoc test). GSEE= *G. senegalensis* ethanol extract.

Table 4. Effects of the ethanol extract of *G. senegalensis* on formalin-induced edema.

Treatment (mg/kg)	0min (s)	30min (s)	60min (s)	90min (s)
Normal saline 5mL/kg	3.56 ± 0.12	3.73 ± 0.14	3.49 ± 0.04	3.55 ± 0.09
GSEE 12.5	3.79 ± 0.06	4.16 ± 0.02*	3.47 ± 0.09	3.06 ± 0.05*
GSEE 25	3.55 ± 0.11	4.14 ± 0.06*	3.52 ± 0.13	3.09 ± 0.19*
GSEE 50	3.67 ± 0.09	4.01 ± 0.12	3.77 ± 0.15	3.34 ± 0.01
Diclofenac 10	3.75 ± 0.04	4.85 ± 0.09*	3.58 ± 0.09	2.47 ± 0.09*

Note: The data is presented as Mean ± Standard Error of Mean (SEM). The asterisks indicate a significant difference ($p < 0.05$) compared to the Normal Saline control group. After one-way ANOVA, Dunnett's post hoc test for multiple comparisons was used. GSEE = *G. senegalensis* ethanol extract.

Thermal Induced Pain Hot Plate

The hot plate test evaluated the central analgesic effects of the extract. Although the reaction times were prolonged in mice treated with the extract, the effect was less pronounced than Pentazocine, a well-established central analgesic (see **Table 3**). The results suggest that the ethanol extract has mild central analgesic activity, likely mediated through modulation of opioid receptors or central pain pathways. Notably, the time-dependent increase in latency to pain response indicates sustained efficacy, beneficial for managing persistent or chronic pain conditions. These results, combined with the findings from the acetic acid test, suggest that the extract primarily acts through peripheral mechanisms, with some contribution from central pathways.

Anti-Inflammatory Study

The anti-inflammatory effects of the extract were assessed using the formalin-induced paw edema model, a robust method for evaluating both acute and chronic phases of inflammation. The extract demonstrated a dose-dependent reduction in paw thickness, with the 12.5 mg/kg and 25 mg/kg doses showing significant inhibition of edema at 30 and 90 min (**Table 4**). These findings indicate the extract's ability to modulate inflammatory responses by possibly inhibiting the release of histamine, serotonin, and prostaglandins, key mediators in the early phases of inflammation (22). The reduction in paw edema at later times suggests its influence on neutrophil infiltration and other cellular processes involved in the resolution of inflammation. The performance of the extract at 25 mg/kg was comparable to Diclofenac, the standard anti-inflammatory drug, emphasizing its potential therapeutic value.

Conclusion

The combination of analgesic and anti-inflammatory activities observed in this study underscores the potential of *G. senegalensis* as a multi-faceted therapeutic agent. Its bioactive compounds, including flavonoids, saponins, and tannins, likely act synergistically to relieve pain and control inflammation. This dual action is particularly beneficial for conditions such as arthritis, where pain and inflammation coexist. Moreover, these findings further validate the plant's traditional use in treating a wide range of inflammatory and pain-related conditions.

The findings reinforce the traditional use of *G. senegalensis* as a natural remedy for pain and inflammation. The ethanol extract demonstrated significant analgesic and anti-inflammatory activities, comparable to or exceeding standard drugs in some models. These results highlight its potential as a safe, effective, and accessible therapeutic agent, particularly in regions where reliance on medicinal plants is prevalent. Further studies are necessary to unlock its pharmacological potential fully and elucidate the bioactive compounds' mechanisms of action.

Declarations

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

All data generated or analyzed during this study are included in the manuscript.

Ethics Statement

The study protocol was reviewed and approved by the Faculty of Basic Medical Sciences Research and Ethics Committee at Sa'adu Zungur University Bauchi (Approval No. BASUG/FBMS/REC/VOL.3/0098).

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