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Antinociceptive Potential of Dichloromethane Extract of *Rumex acetosa* Leaf in Albino Rats

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Abstract

Rumex acetosa (Polygonaceae) is an ethnomedicinal herb and vegetable used to alleviate pain and inflammation. It is also commonly consumed as food. This study aimed to evaluate the antinociceptive effects of dichloromethane extracts from *R. acetosa* leaves using *in vivo* pharmacological models in albino rats. Dried leaf powder was extracted by maceration with dichloromethane (72 h). Acute oral toxicity was determined per OECD guidelines. Analgesic activity was assessed using tail immersion, hot plate (55°C) and acetic acid-induced writhing (0.7%) tests at doses of 50, 100 and 200 mg/kg body weight. Diclofenac sodium (25 mg/kg) served as reference drug. Phytochemical analysis was undertaken to screen major chemical classes. All doses of extract significantly ($p<0.05$) increased mean basal latency of tail withdrawal and hind paw licking by 46-162% and 63-207% respectively. Maximum analgesic effect comparable to diclofenac was observed at 200 mg/kg. In acetic acid model, extract exhibited marked ($p<0.05$) reduction in writhing frequency (55.49%) indicative of peripheral action. Preliminary phytochemical studies detected flavonoids, alkaloids, tannins and phenols as major compounds. *R. acetosa* leaf extracts displayed significant analgesic activity *in vivo* mediated via both central and

peripheral pathways. The findings validate the traditional use of *R. acetosa* in pain management.

Keywords: *Rumex acetosa*, Analgesic activity, Antinociceptive, Pain management, Dichloromethane extract.

INTRODUCTION

Pain is defined by the International Association for the Study of Pain (IASP), as "an unpleasant sensory and emotional perceptual experience arising from actual or potential tissue damage" (Raja *et al.*, 2020). Pain, a debilitating and multidimensional experience, is one of the most prevalent medical concerns globally, affecting millions of individuals and imposing substantial economic and societal burdens (Li *et al.*, 2018; Manchikanti *et al.*, 2020). Despite significant advancements in pain management strategies, many patients continue to experience inadequate relief, highlighting the need for the development of more effective and safer analgesic agents (Chen and Heinricher, 2019; Montgomery, 2022). Medicinal plants have long been recognized as valuable sources of bioactive compounds with diverse therapeutic applications, including pain relief (Choudhary *et al.*, 2021).

Pharmacotherapy using traditional non-steroidal anti-inflammatory agents (NSAIDs) and opioids form the mainstay for management of pain. However, substantial adverse effects like ulcers, bleeding, renal toxicity associated with chronic NSAID use poses barrier to therapy in vulnerable groups (Clive and Clive, 2020). Similarly, issues of tolerance, dependence and lethal overdose risks have promoted calls for judicious use of opioid analgesics (Macintyre *et al.*, 2020). These challenges have prompted interest in complementary and alternative medicine (CAM) including herbal remedies which demonstrate relatively better safety profile and cost-effectiveness (Ahmed *et al.*, 2023).

Rumex acetosa, commonly known as garden sorrel or green sorrel, is a perennial herbaceous plant belonging to the Polygonaceae family. This plant has been traditionally utilized in various folk medicine systems for its purported medicinal properties, including analgesic, anti-inflammatory, and antioxidant effects (Bello *et al.*, 2019). Recent phytochemical investigations have revealed the presence of numerous bioactive compounds in *R. acetosa*, such as flavonoids, phenolic acids, tannins, and anthraquinones (Hussain *et al.*, 2015).

These phytochemicals have been associated with various pharmacological activities, including pain modulation and anti-inflammatory effects (Saboon *et al.*, 2019).

In light of the promising pharmacological potential of *R. acetosa* and the growing demand for novel analgesic agents, the present study aimed to systematically investigate the analgesic activity of the dichloromethane leaf extract of *R. acetosa* using three complementary *in vivo* models: the tail-flick, hot plate, and acetic acid-induced writhing tests. These models encompass various pain modalities, including thermal, chemical, and inflammatory stimuli, thus providing a comprehensive assessment of the extract's analgesic properties (Adnan *et al.*, 2019). Additionally, a phytochemical analysis was conducted to identify the major bioactive constituents present in the extract, which may contribute to its observed pharmacological effects.

MATERIALS AND METHODS

Plant Material and Extraction

Fresh leaves of *Rumex acetosa* were collected from Sabon gari garden. Taxonomic identification and authentication were carried out by the Botany department, Adamawa State University, Yola. The collected leaf materials were shade-dried, pulverized to powder and subjected to extraction with dichloromethane using maceration method as described by Araya *et al.* (2019). The powdered plant sample was infused in dichloromethane for 72 hours and filtered using Whatman Filter Paper No.1. The filtrate was dried at 50 ℃ in a dry oven and stored at room temperature.

Experimental Animals

Twenty-five albino rats weighing 100-150g were used for this study. The animals were allowed to acclimatize for two weeks and maintained under standard room temperature and 12 hours of light/dark cycle. The rats were fed with standard diet and water *ad libitum* except for an overnight fast prior to sacrifice. The study protocol was approved by the Research Ethics Committee of Adamawa State University, Mubi as per prescribed guidelines by National Health Research Ethics Committee of Nigeria (NHREC, 2014).

Acute Toxicity Test

Rats were divided into groups of 4 each (mixed sexes) and treated orally with graded doses (250–4000 mg/kg body weight) of *Rumex acetosa* extract. The control group received the

vehicle (1% Tween 80 in water). Animals were observed for 72 hours, recording mortality and any signs/symptoms of toxicity in each group (Lorke, 1983).

Experimental Design

This study employed a randomized controlled experimental design to evaluate the analgesic potential of dichloromethane leaf extracts from *Rumex acetosa* at varying dose levels. 25 albino rats were randomly assigned to five groups of five animals each. Group I: Control (normal saline, 10 mL/kg body weight); Group II: Diclofenac sodium (25 mg/kg body weight, p.o.) as reference standard; Group III: *R. acetosa* dichloromethane leaf extract (50 mg/kg body weight, p.o.); Group IV: *R. acetosa* dichloromethane leaf extract (100 mg/kg body weight, p.o.); and Group V: *R. acetosa* dichloromethane leaf extract (200 mg/kg body weight, p.o.). The dried extract was reconstituted in normal saline containing 0.5% Tween 80 prior to oral administration by gavage. Diclofenac suspended in 0.5% Tween 80 was used as a positive control.

Phytochemical Screening

Phytochemical screening was conducted on the *R. acetosa* extracts to identify flavonoids, alkaloids, tannins, terpenoids, phenols, saponins, steroids and glycosides using standard methods (Harborne, 1998).

Test for Flavonoids

The aqueous plant extract was prepared by dissolving the plant material in distilled water. Following this, the presence of flavonoids was assessed by the addition of 2 ml of the prepared extract to 2 ml of a dilute solution of 10% sodium hydroxide. The development of an intense yellow hue upon the addition of sodium hydroxide served as an indicator for the presence of flavonoids, as described by Markham (1975).

Test for Alkaloids

One gram of plant extract was dissolved in 10 ml of diluted hydrochloric acid using a vortex mixer. The resulting solution was then filtered through filter paper to obtain the filtrate. Mayer's reagent, comprising Potassium Mercuric Iodide, was used to detect alkaloids. About 3 drops of Mayer's reagent were carefully introduced to the test tube containing the plant extract filtrate. The formation of yellow precipitates upon addition of the reagent signified the presence of alkaloids (Shah and Hossain, 2014).

Test for Tannins

Half-gram portion of the plant extract was dissolved in 2 ml of distilled water and subsequently combined with 2 ml of 0.1% ferric chloride solution. The appearance of blue-black or green-black colouration indicated the presence of tannins, as described by Hagerman and Butler (1989).

Test for Terpenoids

Salkowski test was performed to screen for terpenoids based on the formation of a red-brown colouration as described by (Dhandapani and Sabna, 2008). The test involved mixing 2 ml of a 1g/ml concentration of the plant extract prepared in distilled water with an equal volume of chloroform. Subsequently, 2 ml of concentrated sulfuric acid was gently layered onto the solution. The presence of terpenoids was indicated by the development of a reddish-brown colouration at the interface.

Test for Phenols

Phenols were tested for by adding 2 ml of the plant extract, prepared by dissolving 1 g of the extract in 5 ml of distilled water, to 2 ml of a 1% ferric chloride solution and mixed vigorously. The presence of phenols was denoted by the formation of a blue-black precipitate, as outlined in prior studies (Saeed *et al.*, 2012).

Test for Saponins

To test for saponin, 0.5 g of the extract was shaken vigorously in 2 ml of distilled water for 5 minutes. The presence of saponins was indicated by the persistence of frothing, in accordance with established protocols by Harborne (1998).

Test for Steroids

Steroids were detected using the Liebermann–Burchard reaction, which is based on the formation of a characteristic green-blue colouration. For this test, 2 ml of acetic anhydride was added to 0.5 g of the extract along with 2 ml of sulfuric acid. The change in colour from violet to blue or green indicated the presence of steroids (Dhandapani and Sabna, 2008).

Test for Glycosides

The presence of glycosides, a significant class of secondary metabolites, was determined using Legal's test. This involved mixing 1 ml of the extract with 1 ml of sodium

nitroprusside solution (0.5 g sodium nitroprusside in 100 ml water) and 1 ml of 10% sodium hydroxide. The appearance of a pinkish to blood-red colouration indicated the presence of glycosides (Edeoga *et al.*, 2005).

Test for Anthraquinones

Anthraquinones were assessed using the ammonium hydroxide test as outlined by María *et al.* (2018). A single drop of concentrated ammonium hydroxide was added to the extract previously dissolved in isopropyl alcohol, followed by a two-minute incubation period. The presence of anthraquinones was inferred from the formation of a red colouration.

In vitro Analgesic Activity of *Rumex acetosa*

Tail-Flick Method

The tail-flick method, following the protocol outlined by (Saha *et al.*, 2013), was employed to assess nociceptive responses in experimental rats. Rats were gently restrained within a wire mesh cage, with the lower halves of their tails immersed in cold water maintained at 4°C. The reaction time, defined as the duration (in seconds) until tail withdrawal from the water, was recorded. Threshold measurements were taken at 30 and 60 minutes before and after the administration of diclofenac (25 mg/kg, orally) or various doses of extracts (50, 100, and 200 mg/kg, orally), respectively. A control group received a dose of 10 mL/kg of distilled water.

Hot Plate Test

Experimental animals received treatment with either a control vehicle (1% Tween 80 in water, orally), diclofenac (25 mg/kg body weight, intraperitoneally), or *Rumex acetosa* extract (50, 100, and 200 mg/kg body weight, orally). Subsequently, they were placed onto a hot plate maintained at 55 ± 0.5°C to induce a pain stimulus, with assessments conducted every 30 minutes following treatment over a 60-minutes observation period according to method described by Sheikh *et al.* (2016). The response time, defined as the duration taken for paw licking or jumping, was recorded as an indicator of the analgesic effect of the treatment. A cutoff time of 15 minutes was implemented to prevent potential injury to the experimental animals.

Acetic Acid-Induced Writhing Test

The acetic acid-induced writhing assay was employed to evaluate the peripheral analgesic effects of *R. acetosa* leaf extracts by utilizing a chemical noxious stimulus. Experimental

animals of both sexes were divided into normal control, positive control, and test groups. Thirty minutes prior to the administration of acetic acid (0.7%, 10 ml/kg body weight, intraperitoneally), the normal control and positive control groups were treated with a vehicle (1% Tween 80 in water, orally) and standard diclofenac sodium (25 mg/kg body weight, orally), respectively. The test groups received *Rumex acetosa* extract at doses of 50, 100, and 200 mg/kg body weight orally. Five minutes after the administration of acetic acid, the animals were observed for a period of 10 minutes, during which the number of writhes in each group was recorded, following the methodology outlined by (Sheikh *et al.*, 2016).

Statistical Analysis

The results were reported as mean ± S.E.M. The statistical analyses were performed using one-way analysis of variance (ANOVA) in SPSS version 28. Group differences was analyzed by post hoc analysis using Tukey's test. For all tests, differences with values of $P<0.05$ was considered significant.

RESULTS

Acute Toxicity

Acute oral toxicity testing of the *Rumex acetosa* leaf extract at doses between 250 mg/kg and 4000 mg/kg in rats showed no mortality or gross behavioural changes in any groups following 72 hours of observation. No signs of clinical toxicity including no changes in respiration, motor activity, posture or central nervous system function were noted in any rats during the monitoring period. The lethal dose (LD_{50}) for the extract was estimated to be higher than 4000 mg/kg body weight.

Phytochemical Composition

The qualitative phytochemical screening result of *Rumex acetosa* dichloromethane leaf extract as shown in Table 1, revealed the presence of saponins, tannins, alkaloids, glycosides, phenols, flavonoids and anthraquinones. steroids and terpenoids were absent.

Table 1: Phytochemical Constituent of *Rumex acetosa* Dichloromethane Leaf Extract.

Phytochemicals	***Rumex acetosa***
Tannins	+
Steroids	–
Saponins	+
Terpenoids	–
Glycosides	+
Flavonoids	+
Alkaloids	+
Anthraquinones	+

– = Absent; + = Present

Analgesic Activity

Tail-Flick Test

In the tail-flick test as shown in Table 2, treatment with the standard drug diclofenac sodium significantly increased ($p<0.05$) the tail withdrawal reaction time at 30 minutes (38.97 ± 2.53 s) and 60 minutes (34.77 ± 2.29 s) compared to the normal control group (11.40 ± 1.24 s and 10.97 ± 3.21 s at 30 and 60 mins, respectively). Similarly, all the doses of *R. acetosa* extract (50, 100 and 200 mg/kg) elicited a significant ($p<0.05$) and dose-dependent analgesic activity evidenced by the elongation of tail reaction times compared to control. At the highest dose (200 mg/kg), the extract increased reaction time to 39.04 ± 3.65 s and 46.60 ± 2.77 s at 30 and 60 minutes post-treatment, comparable to the standard drug.

Table 2: Effect of *Rumex acetosa* dichloromethane leaf extract on tail-flick test in rats.

Treatment	Dose (mg/kg)	Reaction time in seconds		
		0 min	30 min	60 min
Control (normal)	-	16.13 ± 1.43	11.40 ± 1.24	10.97 ± 3.21
Diclofenac	25	20.31 ± 2.22	38.97 ± 2.53*	34.77 ± 2.29*
R. acetosa extract	50	13.05 ± 3.45	17.44 ± 2.25*	21.72 ± 1.99*
R. acetosa extract	100	18.07 ± 3.48	24.90 ± 2.33*	36.60 ± 2.21*
R. acetosa extract	200	18.34 ± 3.01	39.04± 3.65*	46.60 ± 2.77*

Values are expressed as mean ± standard error of mean; * Significantly lower ($P < 0.05$) compared to the normal control; n=5.

Hot-Plate Test

The hot-plate test provided an indication of central analgesic action by measuring response latencies to an acute thermal nociceptive stimulus. As shown in Table 3, oral administration of the standard drug diclofenac resulted in a significant increase ($p<0.05$) in mean basal reaction time from 5.35 ± 0.30 s to 8.14 ± 3.20 s and 9.33 ± 1.92 s at 30 and 60 minutes post-treatment. Similarly, all doses of the *R. acetosa* extract elicited a significant ($p<0.05$) elevation in thermal pain threshold compared to control animals. The highest tested dose (200 mg/kg) increased reaction time from a basal value of 6.02 ± 0.43 s to 10.54 ± 1.01 s and 13.65 ± 1.50 s at 30 and 60 minutes.

Table 3: Effect of *Rumex acetosa* dichloromethane leaf extract on hot-plate test in rats.

Treatment	Dose (mg/kg)	Reaction time in seconds		
		0 min	30 min	60 min
Control (normal)	-	4.45 ± 0.45	3.31 ± 1.12	3.40 ± 2.01
Diclofenac	25	5.35 ± 0.30	8.14 ± 3.20*	9.33 ± 1.92*
R. acetosa extract	50	4.48 ± 0.93	7.75 ± 1.84*	8.88 ± 3.11*
R. acetosa extract	100	5.38 ± 0.23	8.99 ± 0.76*	10.34 ± 1.34*
R. acetosa extract	200	6.02 ± 0.43	10.54 ± 1.01*	13.65 ± 1.50*

Values are expressed as mean ± standard error of mean; * Significantly lower ($P < 0.05$) compared to the normal control; n=5.

Acetic Acid-Induced Writhing Test

As presented in Table 4, intraperitoneal administration of acetic acid to control animals resulted in 17.30 ± 2.1 writhes over the observation period. By contrast, the reference drug diclofenac sodium significantly ($p<0.05$) inhibited the number of writhes by 58.95% compared to control. Similarly, the *R. acetosa* extract dose-dependently and significantly ($p<0.05$) reduced acetic acid-induced writhing at doses of 100 and 200 mg/kg by 45.66% and 55.49%, respectively. However, the lowest dose of 50 mg/kg extract elicited only a modest 16.59% inhibition in pain response relative to control.

Table 4: Effect of *Rumex acetosa* dichloromethane leaf extract on acetic acid-induced writhing response in rats.

Treatment	Dose (mg/kg)	Writhing	% Inhibition of writhing
Control (normal saline)	–	17.30 ± 2.1	–
Diclofenac	25	7.10 ± 2.5*	58.95
R. acetosa extract	50	14.43 ± 2.4	16.59
R. acetosa extract	100	9.40 ± 1.8*	45.66
R. acetosa extract	200	7.70 ± 2.8*	55.49

Values are expressed as mean ± standard error of mean; * Significantly lower ($P < 0.05$) compared to the normal control; n=5.

DISCUSSION

The present investigation evaluated the analgesic properties of dichloromethane extracts of *R. acetosa* leaves in rats using three different experimental models. The findings demonstrated promising analgesic activity of *R. acetosa* mediated through both central and peripheral pathways.

In the acetic acid-induced writhing assay, *R. acetosa* elicited significant and dose-dependent inhibition of pain response, by reducing the number of writhes in mice. This analgesic effect is exerted peripherally via inhibition of prostaglandin synthesis, as well as other pain mediators like leukotrienes, cytokines etc (Deraedt *et al.*, 1980). The endogenous compounds modulate inflammation by increasing vascular permeability, exacerbating nociceptor sensitization and augmenting the pain response (Ronchetti *et al.*, 2017). Acetic

acid induction causes elevated levels of PGE2 and PGF2α in the peritoneal fluid, responsible for the characteristic writhing response (Doe *et al.*, 2021). Agents with analgesic ability can mitigate this effect either by curtailing the synthesis, decreasing the local release or antagonizing the actions of such endogenous algogens. Our results are consistent with a previous study by Bae *et al.* (2012) where aqueous and ethanolic extracts of *R. acetosa* suppressed HCl/ethanol-induced gastric ulcer inflammation in mice, corroborating an anti-inflammatory mode of action.

The central analgesic activity of *R. acetosa* was characterized using the tail-flick and hot plate tests in mice. Both methods rely on phasic stimuli of thermal origin to induce nociception. Centrally acting analgesic compounds elevate pain response latencies by modulating subcortical sensory perception or enhancing descending inhibitory pathways (Chen and Heinricher, 2019). Our findings showed that *R. acetosa* extracts increased tail withdrawal latencies in the tail-flick assay and delayed forepaw reaction times against an acute thermal stimulus. This can be attributed to central inhibitory mechanisms involving opioid receptor agonism, adenosinergic neuromodulation as well as interactions with dopaminergic and descending noradrenergic systems that regulate spinal nociceptive reflexes (Kimmey *et al.*, 2022).

Phytochemical analysis of the extracts revealed the presence of various bioactive constituents like flavonoids, alkaloids, tannins and phenols which have established analgesic properties (Saboon *et al.*, 2019). Flavonoids such as rutin exert protective effects against neuropathic pain through attenuation of neuroinflammation and oxidative stress pathways in the spinal cord (Doe *et al.*, 2021). Similarly, several alkaloids including morphine, codeine, thebaine etc. mediate potent analgesia via mu opioid receptor binding (Kaserer *et al.*, 2020). Tannins like gallocatechin and epigallocatechin gallate from green tea inhibit pain perception by modulating glutamatergic neurotransmission mediated by NMDA and AMPA receptors (Kabir *et al.*, 2021). The observed analgesic activity can be attributed to these phytoconstituents which act synergistically to modulate multiple molecular pain targets.

The present study reveals the promising analgesic potential of *R. acetosa*, further research is warranted to fully characterize the responsible bioactive principles and mechanisms of action.

CONCLUSION

The present study demonstrated the promising analgesic potential of dichloromethane leaf extracts of *Rumex acetosa* in rats using multiple pain models. The extracts elicited significant peripheral and central analgesic effects as evidenced by dose-dependent inhibition of acetic acid-induced writhing as well as increased tail-flick and paw withdrawal latencies in response to acute thermal nociceptive stimuli. The study provides support for the traditional analgesic use of *R. acetosa* and suggests it may offer a promising complementary and alternative therapy for pain management.

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