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Original Research Article

Evaluation of the Antioxidant, Antinociceptive and Anti-inflammatory Activities of *Combretum nigricans* Ethanol Leaf Extract

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ABSTRACT

Combretum nigricans is a known medicinal plant employed in Nigerian ethnomedicine for the treatment of pain, inflammation and other ailments. This study aims at evaluating the antioxidant, antinociceptive and anti-inflammatory effects of *C. nigricans* ethanolic leaf extract. The antioxidant activity of *C. nigricans* ethanolic leaf extract was evaluated *in vitro* using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) model, while acetic acid writhing test and formalin nociception test were used in evaluating the antinociceptive activity in mice. Formalin-induced inflammation was used to evaluate the anti-inflammatory effect in mice. HPLC analysis of the extract showed the presence of several compounds including ferulic acid. The extract exhibited good DPPH antioxidant activity. Its IC_{50} value was 0.0003971 mg/mL. Oral acute toxicity test showed the LD_{50} is greater than 2000 mg/kg. In the acetic acid test, all extract test doses (200 – 800 mg/kg) had significant inhibitory effect against abdominal writhes usually evoked by acetic acid. The different extract doses had antinociceptive effect against phases 1 and 2 pain. Also, the extract doses used for the study (200 – 800 mg/kg) had anti-allodynia effect with the 800 mg/kg dose having a better effect of 44.44 and 79.83 % on days 1 and 2 respectively. Furthermore, the extract demonstrated significant anti-inflammatory activity against formalin induced inflammation in the experimental animals. The study outcome shows that *C. nigricans* extract has good antioxidant, anti-nociceptive and anti-inflammatory effects.

Keywords*:Combretum nigricans*, HPLC, antioxidant activity, antinociceptive activity, anti-inflammatory activity

Introduction

Pain is among the major reasons for visit to medical facilities. It reduces the quality of life of patients suffering from it and could contribute to the development of mental health disorders. Some reports have opined that almost everyone have or will experience pain at a point in their life time.[1 - 2] Pain can be described as an unpleasant sensory and emotional experience affiliated with or resembling that affiliated with potential or actual tissue damage.[3] Acute pain is usual a direct consequence of body tissue or organ damage, its intensity however dissipate over a short time period. However, acute pain may also metamorphose into chronic pain. This is usually accompanied with elevation of mediators of inflammation.[4]Inflammation is a pathophysiological response of living mammalian tissues to tissue damage/ injuries. It is a defense mechanism that involves a complex cascade of activities. Pathological conditions such as arthritis, infections, muscle injury and post-surgical procedures could elicit pain and inflammation.[5]The pain cascade could be by peripheral or central stimuli.

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This may also lead to increased oxidative stress if the pain process is associated with tissue damage and inflammation. Opioids and non-steroidal anti-inflammatory drugs (NSAIDs) are the common agents used for the management/treatment of pain and inflammation.[6]The accompanying side effects of these medications such as respiratory depression, ulcer and other associated side effects have been a major concern with regards to their usage. Hence, there is need to develop new agents with fewer side effects. [6]Medical history has demonstrated the role of medicinal plants with regards to maintaining man's health from ancient times till date.[7] Thus in the past few decades, there have been increased research focus on medicinal plants and this is aimed at discovering/developing new efficacious and safer agents for the management of different heath challenges.Scientific evaluation of plants is often as a result of their claims and use in traditional medicine. *Combretum nigricans* is a herbal plant employed in Nigerian ethnomedicine for different therapeutic purposes including for the management of pain, inflammation, hypertension, malaria and central nervous system disorders. [8] However, the plant has not been evaluated scientifically for its analgesic and anti-inflammatory activity. Hence, this study aims at evaluating the antioxidant, antinociceptive and anti-inflammatory effects of *C. nigricans* ethanolic leaf extract.

Materials and Method

Drugs and chemicals

Acetic acid (BDH, poole, UK); formalin (Balaji formalin Pvt. Ltd, India); aspirin (SIGMA chemical company, United States); dihydrocodeine (Accord pharmaceutical, Nepals); indomethacin (Embassy Pharmaceuticals and Chemicals Ltd). All the drugs used

were of high standard grade and solutions from these were freshly prepared daily before use.

Collection and identification of plant materials

Fresh leaves *C. nigricans* leaves were harvested October, 2022 from a botanical garden in northern Abuja, Nigeria. To remove dirt, the harvested leaves were properly rinsed using clean running water. The plant was subjected to identification by Mr. Mallam Muaza, a taxonomist at the Herbarium unit, National Institute for Pharmaceutical Research and Development, Abuja. A Voucher specimen (FHJ223) was prepared and deposited at the herbarium of Federal College of Forestry, Jos after authentication.

Extraction

The plant materials were air-dried for two weeks at room temperature, and then pulverized into fine powder using a mechanical grinder. Using standard procedures previously described by Handa *et al*.,[9] 590.62g of the pulverized leaves was macerated in 70% ethanol at room temperature for 72 h with periodical shaking to ensure complete interaction of the solvent and pulverized plant material. The resultant solution was filtered through a mesh sieve, then through cotton plugged funnel and lastly through a filter paper (Whatmann No. 1). The filtrate was concentrated in vacuo using a rotary evaporator set at 40^0C.

Phytochemical analysis

Preliminary phytochemical screening was carried-out on *C. nigricans* ethanol leaf extract (ETCNE) to identify the bioactive principles present in the extract using standard methods described by Trease and Evans.[10]

High performance liquid chromatography (HPLC) analysis

The chromatographic analysis of the extract was performed on a high performance liquid chromatography (HPLC) system consisting of ultra-fast LC-20AB prominence, equipped with SIL- 20AC autosampler; DGU-20A3 degasser; SPD-M20A UV-photodiode array (UV-PDA) detector at a wavelength range of 190 – 800 nm; column oven CTO-20AC, system controller CBM- 20 Alite and Windows LC solution software (Shimadzu Corporation, Kyoto Japan). A VP-ODS column, (5µm; 150 x 4.6 mm) was used and the chromatographic conditions included mobile phase solvent A: 0.2% v/v formic acid and solvent B: acetonitrile; under isocratic elution mode (mobile phase solvent A and B in the ratio 80:20); flow rate 0.6 ml/min and column oven temperature of 40°C. The total run time was 15 minutes. An injection volume of 10 µl of 50 mg/ml solution of extract in methanol was used and detection was at 219 nm. Authentic sample of ferulic acid (Sigma-Aldrich, Germany) prepared as 50 µg/ml standard solution in methanol was analyzed separately under the same conditions.

Animals

In-bred Swiss albino mice of both sexes (weight range 20 – 28 g) housed at the animal facility of Pharmacology and Toxicology Unit, National Institute for Pharmaceutical Research and Development were used for the study. The animals were kept in standard mice cages, under recommended laboratory conditions at room temperature and humidity. Access to water and mice feed was ad libitum. Animal studies were performed in conformity with the "NIH revised guidelines for laboratory animal care and use" [11] and the National Institute for Pharmaceutical Research and Development ethical codes and regulations for laboratory animal use.

Evaluation of antioxidant activity (DPPH free radical scavenging activity)

The free radical scavenging potential of *C. nigricans* ethanolic extract (ETCNE) against DPPH (2,2-diphenyl-1-picrylhydrazyl) was evaluated using the method described by Moriasi *et al.*[12] The antioxidant assay was performed using different ETCNE concentrations (0.0625, 0.125, 0.25, 0.5 and 1mg/mL) prepared in dimethyl sulfoxide. The standard antioxidant used was gallic acid. To 50 µl of ETCNE solution, 150 µl of freshly prepared DPPH solution (0.2 mM) in methanol was added and was incubated in the dark for 30 minutes. Absorbance for each ETCNE concentration and standard was taken at 492 nm using a spectrophotometer. The assay was carried-out in triplicates. The IC_{50} (i.e, the concentration that can inhibit DPPH activity by 50 %) was determined using a non-linear regression graph.

Acute toxicity test (LD_{50})

The acute oral toxicity of *C. nigricans* ethanolic extract (ETCNE) was carried-out on Swiss albino mice using the modified Organization for Economic Cooperation and Development (OECD) method.[13] Eight fasted female swiss albino mice divided into two groups was used for the test. Group 1 which served as control was administered water 10 ml/kg orally, while group two was administered the limit dose of ETCNE 2000 mg/kg orally. The animals where closely monitored for signs of toxicity for 6 hours, and then subsequently for 24 hours. They were placed under further surveillance for 14 days and were observed for delayed toxicity.

Acetic acid writhing test

The acetic acid writhing test was carried-out using the method previously described by Woode *et al.*[14] with slight modifications. Thirty Swiss albino mice of either sex were used for the study. The animals were randomly assigned to five groups of six animals each. The animals were pre-treated orally with the vehicle, extract or standard drug. Group 1 received distilled water 10 ml/kg body weight, groups 2 – 4 were administered ETCNE 200, 400 and 800 mg/kg body weight respectively, while group 5 was administered aspirin 150 mg/kg body weight.

One hour after the respective administrations, the animals were administered acetic acid (0.6%, 0.1ml/10g body weight *i.p*). The mice were placed in individual cages after acetic acid administration and the number of writhes was counted for each mouse for 15 minutes after 5 minutes latency period. The percent inhibition of writhes was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{Mean\ number\ of\ writhes\ (control) - Mean\ number\ of\ writhes}{Mean\ number\ of\ writhes\ (control)} \times \frac{100}{1}$$

Formalin nociception test

In the formalin nociception test, the method described by Hunskaar and Hole was adopted [15] with slight modifications. Thirty-six Swiss albino mice of either sex were used for the study. The animals were randomly assigned to six groups of six animals each. The animals were pre-treated orally with the vehicle, extract or standard drug. Group 1 received distilled water 10 ml/kg body weight, groups 2 – 4 were administered ETCNE 200, 400 and 800 mg/kg body weight respectively, group 5 was administered dihydrocodeine 20 mg/kg body weight, while group 6 was treated with Indomethacin 10 mg/kg

Each mouse was administered 20µL of 2.7% (v/v) formalin solution into the dorsal surface of the right hind paw using a micro syringe with 26-guage needle, 1 h after the respective treatment of each group. The nociceptive scores were calculated in 5-minute time blocks as the product of the duration and frequency of biting/licking of the right hind paw. The results were designated as first/neurogenic phase (0 − 10 minutes) and second/inflammatory phase (10 − 60 minutes).

Hot-plate test

The hot plate test was carried-out using the method described previously by Simões *et al*.[16]Thirty swiss albino mice of either sex were used for the study. The animals were randomly assigned to five groups of six animals each. Baseline reaction time in seconds was determined for each animal using a digital hot plate set at constant temperature of 55 ± 1 ^{0}C. The animals were treated orally with the vehicle, extract or standard drug. Group 1 received distilled water 10 ml/kg body weight, groups 2 – 4 were administered ETCNE 200, 400 and 800 mg/kg body weight respectively, while group 5 was administered dihydrocodeine 20 mg/kg body weight.

After administration of the drugs, the reaction time for each animal was measured 30 minutes, 60 minutes, 90 and 120 minutes after administration. Latency to withdraw paw (in seconds) was recorded using a stopwatch as the time between placing the animal on the hot plate and the appearance of symptoms of discomfort such as licking or

shaking of the hind paws or jumping off from the surface of the hot plate apparatus.

Mechanical allodynia (Von frey test)
Thirty swiss albino mice of either sex were used for the study. The animals were randomly assigned to five groups of six animals each. Baseline force and reaction time in grams and seconds respectively were determined for each animal using Von Frey test apparatus (Ugo Basile Plantar Von Frey Aesthesiometer 57820). The animals were pre-treated orally with the vehicle, extract or standard drug. Group 1 received distilled water 10 ml/kg body weight, groups 2 – 4 were administered ETCNE 200, 400 and 800 mg/kg body weight respectively, while group 5 was administered indomethacin 10 mg/kg body weight.
Each mouse were treated with 20µL of 2.7% (v/v) formalin solution into the dorsal surface of the right hind paw using a micro syringe with 26-guage needle, 1 h after the respective treatment of each group. The ipsilateral reaction time and force in seconds and grams respectively was determined on day 1 (2 h after formalin administration) and day 2 (24 h after formalin administration).

Formalin inflammation
Thirty Swiss albino mice of either sex were used for the study. The animals were randomly assigned to five groups of six animals each. Baseline paw volume was determined for each animal using a digital plethysmometer (Ugo Basile Plethysmometer 37140). The animals were pre-treated orally with the vehicle, extract or standard drug. Group 1 received distilled water 10 ml/kg body weight, groups 2 – 4 were administered ETCNE 200, 400 and 800 mg/kg body weight respectively, while group 5 was administered indomethacin 10 mg/kg body weight.
Each mouse were treated with 20µL of 2.7% (v/v) formalin solution into the dorsal surface of the left hind paw using a micro syringe with 26-guage needle, 1 h after the respective treatment of each group. The paw volume for each animal was taken 1 h after formalin administration and subsequent every 1 h interval five consecutive times, then after 24 h.

Statistical analysis
Data obtained was expressed as mean ± standard error of mean (SEM). One way analysis of variance (ANOVA) and Dunnet's post hoc test were used to test for significance, $P<0.05$ was considered significant. GraphPad Prism for windows (version 8.0), San Diego California USA was the statistical package used for analysis.

Results and Discussion

This study evaluated *C. nigricans* ethanolic leaf extract for antioxidant activity *in vitro* as well as the antinociceptive and anti-inflammatory effects using *in vivo* mice models. Preliminary phytochemical screening revealed the presence of several bioactive constituents including alkaloids, saponins, phenols, tannins and terpenoids in the extract (Table 1). Previous studies have shown that phenols, alkaloids, flavonoids and saponins found in medicinal plants have the potential to elicit both antinociceptive and anti-inflammatory effects.[17 - 19]Figure 1 and Table 2 shows the result of *C. nigricans* extract HPLC analysis plot at 219 nm. The chromatogram revealed the presence of eleven peaks with different retention time and compositions (Figure 1). These peaks indicate the presence of ferulic acid (which peaked at 7.371 min) and other compounds. Previous reports on ferulic acid suggest that the phenolic compound may poses potent bioactive potentials including antioxidant, analgesic and anti-inflammatory effects.[20 – 22] Thus, it may be among the bioactive culprits responsible for the pharmacological activities demonstrated by ETCNE during the study. Antioxidants inhibit the activity of free radicals hence protects the body against several pathological conditions. Agents that demonstrate good antioxidant activity have been shown to also elicit potent analgesic and anti-inflammatory activities.[23] The DPPH method is a sensitive model used in evaluating the antioxidant effect of potential agents. The principle behind the method lies on DPPH scavenging radical species addition/ decolourization of the DPPH solution by potent antioxidants. In this study, ETCNE demonstrated good DPPH antioxidant activity. Its antioxidant activity was better than that elicited by the standard used (gallic acid), as the extract IC_{50} value was 0.0003971 mg/mL which was lower than that of gallic acid with IC_{50} value of 0.001484 mg/mL (Figure 2a – 2b). This thus indicates that the extract may possess potent analgesic and anti-inflammatory property.
The acetic acid-induced writhing test is a sensitive but non-selective animal model used for screening potential analgesic agents.[24] This model has both peripheral and central input in its nociception pathway. In this model, intraperitoneal administration of acetic acid to mice evokes writhing (a nociceptive behaviour characterized by abdominal constrictions, hind limb stretching and pelvic rotation). Result from the acetic acid-induced writhing test showed that pre-treatment with 200, 400 and 800 mg/kg extract doses one hour before the administration of 0.6 % acetic acid had significant ($P<0.05$, $P<0.01$ and $P<0.001$) inhibitory effect against the characteristic abdominal writhes usually evoked by i.p administration of acetic acid (Table 4). Compared with the control group, ETCNE inhibited the writhes by 33.5, 52.3 and 57.6.1 % at 200, 400 and 800 mg/kg doses respectively. Similarly, the standard drug aspirin inhibited acetic acid induced writhes by 58.1 % at a dose of 150 mg/kg (Table 4).A possible antinociceptive mechanism by ETCNE could be through inhibiting the peripheral nociceptive pathway by blocking the activity of endogenous pro-nociceptive substances involved in modulation of nociception, or may also elicit its effect by inhibiting the central pathway.
The formalin model was used to evaluate its effect on neurogenic and inflammatory pain. Administration of formalin into mice hind paw elicits characteristic behavioral nociceptive response such as licking and biting of the ipsilataral hind paw. The response is biphasic with the first phase being acute neurogenic pain and usually responsive to centrally acting analgesic agents like opioid analgesics. The second phase represents inflammatory pain and is responsive to both centrally acting analgesics and NSAIDs.[25] The nociceptive responses in both phases of pain in this model is usually in terms of duration and frequency.

Table 1: Preliminary Phytochemical Screening

Phytochemical	Inference
Alkaloids	+
Saponins	+
Terpenes	+
Tannins	+
Steroids	+
Terpenoids	–
Phenols	+
Cardiac glycosides	+
Carbohydrates	+

+ = Present; - = Not Detected

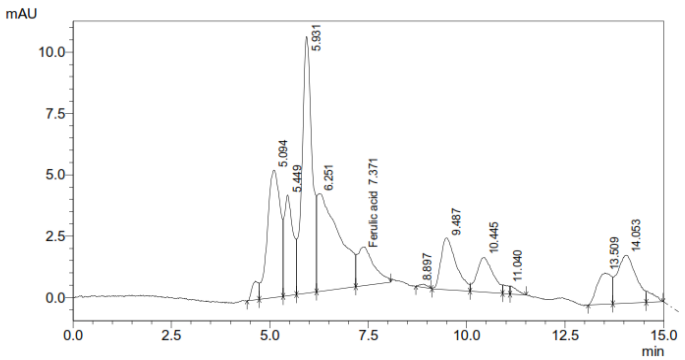

Figure 1: HPLC chromatogram of *Combretum nigricans* ethanolic leaf extract

Result from the study showed that ETCNE test doses of 200 and 800 mg/kg produced significant ($P < 0.05$ and $P < 0.001$) antinociceptive effect against phases 1 and 2 pain, while 400 mg/kg ETCNE dose had a significant ($P < 0.05$) activity only against phase 2 pain. The standard drugs dihydrocodeine and indomethacin both had significant ($P < 0.05$) antinociceptive effect against phases 1 and 2 pain however, dihydroceine had a higher effect against nociception at both phases (Table 5).The result revealed that ETCNE had significant antinociceptive effect against neurogenic and inflammatory pain. Therefore, this suggests that the mechanism by which ETCNE elicits its antinociceptive effect stems from central interaction and synergic anti-inflammatory activity.

To further evaluate the involvement of the central pathway in the antinociceptive activity of ETCNE, the hot plate test was carried-out. This model is very sensitive and suitable for evaluating potential analgesic agents with suspected central analgesic effect.[26]In the hot plate model, all doses of ETCNE used for the study had significant ($P<0.05$) antinociceptive effect. All doses of ETCNE (200 – 800 mg/kg) significantly increased the reaction time of the experimental animals compared with control. Similarly, the standard drug dihydrocodeine had ($P<0.05$) antinociceptive effect throughout the study however, the highest ETCNE test dose (800 mg/kg) had a better antinociceptive activity with the highest effect observed at 120 min (29.02 s) (Table 6).

Allodynia is a state where naturally non-noxious stimuli becomes noxious and activate the pain pathway. It can be experienced when the normally non-noxious stimulus is applied to an already inflamed area or where there is already tissue damage.[27] In this study, a baseline assessment was carried-out to establish the threshold mechanical stimuli and the baseline reaction time. The sub-threshold stimuli are hence non-noxious normally. Administration of formalin into the hind paw produced allodynia to mechanical stimuli. The effect was marked on day 1 of the study as there was decrease in reaction time and threshold force compared to baseline. Control group had decreased reaction time by 3.60 and 2.33s on days 1 and 2 respectively compared to day 0 (baseline). Also, there was decrease in the threshold force by 3.45 and 2.19 g respectively. All doses of ETCNE used for the study (200 – 800 mg/kg) had anti-allodynia effect with the 800 mg/kg dose having a better effect of 44.44 and 79.83 % respectively on days 1 and 2 (Table 7a). ETCNE 200 mg/kg dose had 29.17 and 78.11 %, while ETCNE 200 mg/kg dose had 37.50 and 54.08 % effect on days 1 and 2 respectively. Similarly, the standard drug used indomethacin also exhibited anti-allodynia activity on days 1 and 2 eliciting 42.78 and 32.42 % effect respectively (Table 7b).This thus suggests that ETCNE may also find some use in the management of neuropathic pain characterized by allodynia.

Among the models used for screening prospective anti-inflammatory agents is the formalin model. This model was employed in evaluating the anti-inflammatory activity of the extract. Administration of 2.7 % formalin 0.02 mL into the right hind paw caused inflammation of the experimental animal hind paw which was characterized by an increase in paw volume. The ETCNE doses used for the study (200 – 800 mg/kg) demonstrated significant ($P < 0.05$, $P < 0.01$ and $P<0.001$) anti-inflammatory activity against formalin induced inflammation in the experimental animals (Table 8). Similarly, the standard drug indomethacin which is a non-steroidal anti-inflammatory drug exhibited significant ($P < 0.01$ and $P < 0.001$) anti-inflammatory effect against formalin induced inflammation during the study.

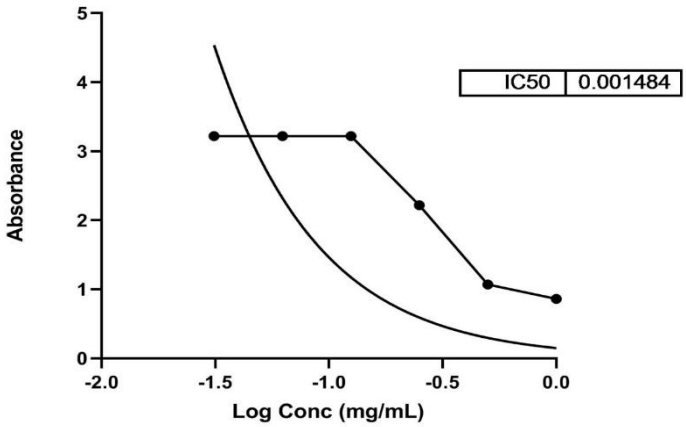

Figure 2a: Graph of gallic acid antioxidant activity

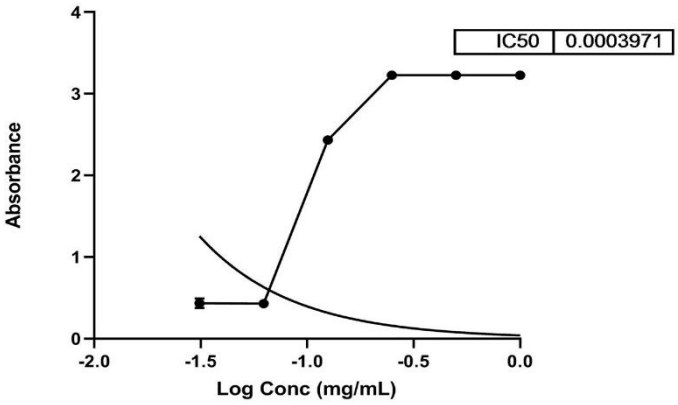

Figure 2b: Graph of *Combretum nigricans* ethanolic leaf extract antioxidant activity

Table 2: Peaks Representing Various Constituents in *Combretum nigricans* Ethanolic Extract

Peak No.	Name	Retention Time (min)	Peak Area	% Composition
1	N/A	5.094	119412	15.57
2	N/A	5.449	63340	8.26
3	N/A	5.931	185234	24.15
4	N/A	6.251	153823	20.06
5	Ferulic acid	7.371	45233	5.90
6	N/A	8.897	1661	0.22
7	N/A	9.487	56898	7.42
8	N/A	10.445	40176	5.24
9	N/A	11.040	3783	0.49
10	N/A	13.509	32326	4.22
11	N/A	14.053	65026	8.48

N/A = Not available

The study outcome supports the result from the formalin induced nociception test which suggests that an input from anti-inflammatory activity may be part of the mechanisms for the extracts antinociceptive effect. Result from oral acute toxicity assessment revealed the absence of mortality and other signs of toxicity during the first 24 h and 14 days of post-treatment surveillance. The oral median lethal dose (LD_{50}) of the extract in mice was thus inferred to be above 2000 mg/kg body weight (Table 3). This may be an indication of a high therapeutic index.

Conclusion

The study outcome showed that the *C. nigricans* extract has good antioxidant, anti-nociceptive and anti-inflammatory effects. The study may thus give scientific credence to the ethnomedicinal use of the plant for pain management. It also revealed that the mechanism of its activity may involve both peripheral and central pathways.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 3: Result of Acute oral Toxicity of *C. nigricans* Ethanolic Leaf Extract

	Mortality After 24 Hours	Mortality After 14 Days
Control (Distilled H_2O 10ml/kg)	0/4	0/4
ETCNE 2000 mg/kg	0/4	0/4
Percent Survival	100 %	100 %

n = 4; ETCNE = *Combretum nigricans* ethanolic extract

Table 4: Effect of *C. nigricans* Ethanolic Extract on Acetic Acid Writhing Test

Treatment	Dose (mg/Kg Body Weight)	Writhes	Percent Inhibition
Distilled H_2O	10 ml/Kg	34.60 ± 3.04	-
	200	23.00 ± 2.82*	33.5
ETCNE	400	16.50 ± 2.77**	52.3
	800	14.67 ± 5.23***	57.6
ASA	150	14.50 ± 2.38***	58.1

Values expressed as Mean ± SEM, where n=6, *significant at $P<0.05$, **significant at $P<0.01$, ***significant at $P<0.001$, ETCNE = *Combretum nigricans* ethanolic extract, ASA = Aspirin

Table 5: Effect of *C. nigricans* ethanolic Extract on Formalin Nociception Test

Treatment	Dose (mg/kg Body Weight)	Nociception Duration (Seconds)		Nociception Frequency		Percent Antinociception	
		Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2
Distilled H_2O	10 ml/Kg	85.33 ± 8.24	404.30 ± 35.70	10.00 ± 1.16	48.50 ± 3.87	-	-
	200	52.48 ± 6.68*	236.50 ± 59.89*	7.83 ± 0.75	38.67 ± 6.38	38.50	41.50
ETCNE	400	60.08 ± 12.79	230.10 ± 48.35*	6.17 ± 1.17	26.33 ± 5.18**	29.60	43.09
	800	25.77 ± 6.76***	139.70 ± 25.22***	3.67 ± 1.31**	24.17 ± 4.34**	69.80	65.45
INDO	10	52.61 ± 3.07*	175.70 ± 31.02**	6.33 ± 0.33	19.00 ± 2.32***	38.35	56.54
DF118	20	17.10 ± 7.59***	102.40 ± 38.68***	5.33 ± 2.01*	24.83 ± 4.19**	79.96	74.67

Values expressed as Mean ± SEM, where n=6, *significant at $P<0.05$, **significant at $P<0.01$, ***significant at $P<0.001$, ETCNE = *Combretum nigricans* ethanolic extract, INDO = Indomethacin, DF118 = Dihydrocodeine

Table 6: Effect of *C. nigricans* Ethanolic Extract on Hot Plate Test

Treatment	Dose (mg/kg Body Weight)	Reaction Time (Seconds)				
		Baseline	30 min	60 min	90 min	120 min
Distilled H_2O	10ml/Kg	8.23 ± 1.07	9.62 ± 0.56	7.90 ±1.45	10.00 ± 0.81	9.98 ± 0.56
	200	9.57 ± 1.84	13.73 ± 2.27	14.92 ± 2.10	24.40 ± 2.84***	26.55 ± 2.18***
ETCNE	400	7.85 ± 0.45	17.42 ± 0.96***	20.47 ± 2.72***	28.43 ± 1.56***	27.02 ± 1.95***
	800	11.95 ± 0.85	17.33 ± 1.14***	26.57 ± 1.36***	25.40 ± 2.47***	29.02 ± 0.63***
DF118	20	9.45 ± 0.41	13.85 ± 0.85*	18.70 ± 1.55***	21.42 ± 1.23***	19.30 ± 1.33***

Values expressed as Mean ± SEM, where n=6, *significant at $P<0.05$, **significant at $P<0.01$, ***significant at $P<0.001$, ETCNE = *Combretum nigricans* ethanolic extract, DF118 = Dihydrocodeine

Table 7a: Effect of *C. nigricans* Ethanolic Extract on Mechanical Allodynia (von Frey Test)

Treatment	Dose (mg/kg Body Weight)	Day 0		Day 1		Day 2	
		Reaction Time (s)	Force (g)	Reaction Time (s)	Force (g)	Reaction Time (s)	Force (g)
Distilled H_2O	10ml/Kg	7.69 ± 0.84	7.94 ±0.85	4.09 ± 0.57	4.49 ± 0.57	5.36 ± 0.54	5.75 ± 0.54
	200	7.44 ± 0.50	7.75 ± 0.49	4.89 ± 0.58	5.27 ± 0.57	6.93 ± 0.70	7.30 ± 0.70
ETCNE	400	8.68 ± 1.01	9.05 ± 1.01	6.43 ± 0.65	6.81 ± 0.65	7.61 ± 1.02	8.02 ± 1.02
	800	7.52 ±0.25	7.92± 0.25	5.23 ± 0.50	5.64 ± 0.49	7.05 ± 0.70	7.44 ± 0.70
INDO	10	8.52 ± 0.49	8.87 ± 0.50	6.46 ± 0.25	6.89 ± 0.25	7.16 ± 0.79	7.39 ± 0.80

Values expressed as Mean ± SEM, where n=6, ETCNE = *Combretum nigricans* ethanolic extract, INDO = Indomethacin

Table 7b: Percent Effect of *C. nigricans* Ethanolic Extract on Mechanical Allodynia (von Frey Test)

Treatment	Dose (mg/kg Body Weight)	Day 1			Day 2		
		Δ Reaction Time (S)	Δ Force (g)	% Analgesia	Δ Reaction Time (S)	Δ Force (g)	% Analgesia
Distilled H_2O	10ml/Kg	-3.60	-3.45	-	-2.33	-2.19	-
	200	-2.55	-2.48	29.17	-0.51	-0.45	78.11
ETCNE	400	-2.25	-2.24	37.50	-1.07	-1.03	54.08
	800	-2.29	-2.28	44.44	-0.47	-0.48	79.83
INDO	10	-2.06	-1.98	42.78	-1.36	-1.48	32.42

Values expressed as Mean ± SEM, where n=6, ETCNE = *Combretum nigricans* ethanolic extract, INDO = Indomethacin

Table 8: Effect of *C. nigricans* Ethanolic Extract on Formalin Inflammation Test

Treatment	Dose (mg/kg Body Weight)	Paw Volume (mL)						
		Baseline	1hr	2hr	3hr	4hr	5hr	24hr
Distilled H_2O	10 ml/Kg	0.167 ± 0.011	0.255 ± 0.014	0.227 ± 0.016	0.213 ± 0.008	0.197 ± 0.003	0.197 ± 0.003	0.197 ± 0.003
	200	0.158 ± 0.008	0.200 ± 0.011**	0.203 ± 0.028	0.143 ± 0.006***	0.167 ± 0.011	0.145 ± 0.009**	0.137 ± 0.009**
ETCNE	400	0.142 ± 0.015	0.183 ± 0.010***	0.170 ± 0.009**	0.163 ± 0.009*	0.167 ± 0.011	0.138 ± 0.013**	0.170 ± 0.011
	800	0.133 ± 0.011	0.182 ± 0.010***	0.165 ± 0.009**	0.182 ± 0.010	0.185 ± 0.008	0.132± 0.016***	0.137 ± 0.016**
INDO	10	0.117 ± 0.011	0.190 ± 0.004***	0.165 ± 0.010**	0.157 ± 0.008**	0.117± 0.011***	0.140 ± 0.019**	0.130± 0.013***

Values expressed as Mean ± SEM, where n=6, *significant at $P<0.05$, **significant at $P<0.01$, ***significant at $P<0.001$, ETCNE = *Combretum nigricans* ethanolic extract, INDO = Indomethacin

References

1. Pandelani FF, Nyalunga SLN, Mogotsi MM, Mkhatshwa VB. Chronic pain: its impact on the quality of life and gender. Front. Pain Res. 2023, 4:1253460.
2. Tompkins DA, Hobelmann JG, Compton P. Providing chronic pain management in the "fifth vital sign" era: historical and treatment perspectives on a modern-day medical dilemma. J Drug Alc Dep 2017;173(1):S11–21.
3. Castroman P, Quiroga O, Mayoral Rojals V, Gómez M, Moka E, Pergolizzi JJ, Varrassi G. Reimagining How We Treat Acute Pain: A Narrative Review. Cureus. 2022;14(4):e23992.
4. Megwas AU, Akuodor GC, Chukwu LC, Aja DO, Okorie EM, Ogbuagu EC, Eke DO, Chukwumobi AN. Analgesic, anti-inflammatory and antipyretic activities of ethanol extract of *Annona senegalensis* leaves in experimental animal models. Int J Basic Clin Pharmacol 2020;9(10):1477 – 1484.
5. Sofidiya MO, Ikechukwu JU, Nnah VE, Olaleye OO, Basheeru K, Sowemimo AA, Ajayi AM. Anti-inflammatory and antinociceptive activities of Daniellia oliveri (Fabaceae) stem bark extract. J Ethnopharmacol 2023;2(309):116337.
6. Gias ZT, Afsana F, Debnath MP, Alam S, Ena TN, Hossain HM, Jain P, Reza MH. A mechanistic approach to HPLC analysis, antinociceptive, anti-inflammatory and postoperative analgesic activities of *Panch phoron* in mice. BMC Complement Med Ther 2020;20(102):1-9
7. Chinedu E, Okhale SE. Ethnomedicinal, phytochemical and pharmacological review of *Asclepidiaceae*. J Prev Diagnos Treat Strateg Med 2023;2:3-18.
8. Chinedu E, Akah PA, Ofili CC, Agatemor UM, Ameh SF, Jacob DL,Onah AI. Evidence supporting the use of *Combrertum nigricans* as an antimalarial agent in ethnomedicine. Int J Current Resear Physio Pharmacol 2021;5(2):13-20.
9. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction technologies for medicinal and aromatic plants. International Centre for Science and High Technology ICS-UNIDO, Trieste, Italy; 2008.
10. Evans WC. Trease and Evans Pharmacognosy, (14th ed), WB Saunders Company Limited, London;2005:357-358.
11. NIH. Guide for the care and use of Laboratory animal (Revised). Washington: NIH Publication; 1985:83-123.

12. Moriasi G, Ireri A, Ngugi MP. In Vitro Antioxidant Activities of the Aqueous and Methanolic Stem Bark Extracts of *Piliostigma thonningii* (Schum). J Evid Based Integr Med. 2020; 25: 2515690X20937988. doi:10.1177/2515690X20937988.
13. Organization for Economic Co-operation and Development OECD guidelines for the testing of chemicals Organization for Economic Co-operation and Development, Paris (2002) [Online] Available from: http://www.oecd.org/chemicalsafety/testing/2741541.pdf [Accessed on 27th November, 2023]
14. Woode E, Kukuia KKE, Ameyaw EO, Mante PK, Adongo DW. Screening of Central Effects of the Leaves of *Ananas comosus* (Geiseler) Mull. Arg. in Mice. Pharmacologia 2012;3(12): 683-692.
15. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory Pain. 1987;30(1):103–14.
16. Simões RR Kraus IS, Rosso R, Bridi A, Casoti R, Dahmer J, Morel FA, dos Santos SRA, Zanchet ME. Root bark of Discaria americana attenuates pain: A pharmacological evidence of interaction with opioidergic system and TRP/ASIC channels. J Ethnopharmacol 2018; 227: 258-267.
17. Xuanbin Wang, Yan Ma, Qihe Xu, Alexander N. Shikov, Olga N. Pozharitskaya, Elena V. Flisyuk, Meifeng Liu, Hongliang Li, Liliana Vargas-Murga, Pierre Duez, Flavonoids and saponins: What have we got or missed? Phytomed 2023;109:1-10.
18. Chetehouna S, Derouiche S, Réggami Y, Boulaares I, Frahtia A. Gas Chromatography Analysis, Mineral Contents and Anti-inflammatory Activity of *Sonchus maritimus*. Trop J Nat Prod Res. 2024;8(4):6787-98.
19. Christianty FM, Dewi AS, Khasanah F, Holidah D, Dewi IP, Fajrin FA. Drug-Piperaceae Herb Interaction Potency Through Analgesic, Anxiolytic, and Anti-Inflammatory Activity Studies. Trop J Nat Prod Res. 2024; 8(4):6781-6786
20. Bektas N, Arslan R.The Centrally-Mediated Mechanisms of Action of Ferulic Acid–InducedAntinociception. Marmara Pharm J 2016; 20: 303-310.
21. Zduńska K, Dana A, Kolodziejczak A, Rotsztejn H. Antioxidant Properties of Ferulic Acid and Its Possible Application. Skin Pharmacol Physiol. 2018;31(6):332-336.
22. Liu Y, Shi L, Qiu W, Shi Y. Ferulic acid exhibits anti-inflammatory effects by inducing autophagy and blocking NLRP3 inflammasome activation. Mol Cell Toxicol. 2022;18(4):509-519.
23. Rodwattanagul S, Nimlamool W, Okonogi S. Antioxidant, antiglycation, and anti-inflammatory activities of *Caesalpinia mimosoides*. Drug Discov Ther 2023;17(2):114-123.
24. Mothana AAR, Al-Said SM, Al-Rehaily JA, Thabet MT,. Awad AN, Lalk M, Lindequist U. Anti-inflammatory, antinociceptive, antipyretic and antioxidant activities and phenolic constituents from *Loranthus regularis* Steud. ex Sprague. Food Chem 2012;130:344–349.
25. Woode E. *Albizia zygia* (DC.) J.F. Macbr. (Leguminosae-Mimosoideae) root extract exhibits anti-nociceptive and antipyretic activities in murine models. J Ethnopharmacol 2017; 6(199):183-193.
26. Kou J, Ni Y, Li N, Wang J, Liu L, Jiang ZH. Analgesic and anti-inflammatory activities of total extract and individual fractions of Chinese medicinal plant *Polyrhachis lamellidens*. Bio Pharmaceut Bulletin 2005;28:176–180.
27. Ameyaw EO, Woode E, Boakye- Gyasi E, Abotsi WK, Kyekyeku JO, Adosraku RK. Anti-allodynic and Anti-hyperalgesic effects of an ethanolic extract and xylopic acid from the fruits of *Xylopia aethiopica* in murine models of neuropathic pain. Phcog Res 2014;6:172- 9.