International Journal of Biochemistry Research & Review

Volume 32, Issue 8, Page 1-12, 2023; Article no.IJBCRR.106327
ISSN: 2231-086X, NLM ID: 101654445

Anti-inflammatory and Antinociceptive Activity of *Terminalia avicennioides* Guill. & Perr. Stem Bark Extract is Distinct from the Antioxidant Effect Mediated by Its Phenolic-rich Fraction

Okwulu O. J. [a], Ezenyi I. C. [a*], Fidelis S. A. [a], Okhale S. E. [b] and Ezike A. C. [a,c]

[a] Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria.
[b] Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria.
[c] Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author EIC designed the study, authors OOJ and FSA conducted the experiments, author OSE performed HPLC analyses, and author EAC supervised the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2023/v32i8829

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/106327

Original Research Article

Received: 25/07/2023
Accepted: 27/09/2023
Published: 06/10/2023

ABSTRACT

Background: *Terminalia avicennioides* stem bark has been used in the management of pain and inflammatory disorders in northern Nigeria. This study evaluated the antinociceptive and anti-inflammatory properties of *T. avicennioides* stem bark extract and its fractions. The correlation of these effects to the presence and concentration of phenolics was also ascertained.

**Corresponding author: E-mail: iphie_odike@yahoo.com;*

Study Design: Experimental Design.
Place and Duration of Study: Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria. The study was conducted from October to December 2022.
Methods: A 70%v/v ethanol extract of *T. avicennioides* stem bark was prepared. Aqueous and ethyl acetate fractions (AF, EF) and sub-fractions (EF1 – EF4) were also prepared. The antioxidant capacity of the extract and fractions were determined using DPPH radical scavenging test, while xylene-induced topical ear edema and formalin test were used to assess antinociceptive and anti-inflammatory activity. The ethyl acetate sub-fractions were further assessed using the egg albumin–induced inflammation. The extract and fractions were characterized by High-Performance Liquid Chromatography (HPLC), and phenolic content was quantified as gallic acid equivalent (GAE)/mg of extract or fraction.
Results: The ethyl acetate and aqueous fractions showed higher antioxidant capacity compared to the parent ethanol extract. *T. avicennioides* ethanol extract significantly inhibited pain (p<0.001) and inflammatory responses (p<0.05) and these effects were more significant compared to those produced by the fractions. Fractions AF and EF exhibited similar activity, although EF produced better inhibition of pain and topical edema. The subfraction EF2 also showed anti-inflammatory activity but this effect was insignificant (p>0.05). The HPLC profiles of the extract and fractions showed peaks corresponding to caffeic acid, chlorogenic acid, and gallic acid. The EF and AF revealed higher peak areas corresponding to gallic acid and rutin respectively. This correlated with a comparatively high gallic acid content and antioxidant effect of the ethyl acetate fraction (GAE: 1.32/mg, IC_{50} = 0.036 mg/ml), relative to the extract (GAE: 0.88/mg, IC_{50} = 0.052 mg/ml).
Conclusion: The constituents responsible for the antinociceptive and anti-inflammatory activity of *T. avicennioides* extract appear distinct from antioxidative principles in phenolics-rich fractions.

Keywords: Anti-inflammatory; anti-nociceptive; antioxidant; phenolics; Terminalia avicennioides.

1. INTRODUCTION

Inflammation, part of the body's defense against injury or infection, can be observed from the classic signs namely pain, heat, redness, swelling and loss of function [1]. It can result from injury, or disease, and can be immunologically mediated. Inflammation can be acute, subacute, or chronic, and is sustained by the release of mediators like histamine, prostaglandins, leukotrienes, bradykinins, lipoxins, nitric oxide, platelet-activation factor, growth factors and cytokines [2]. Chronic inflammatory diseases contribute to more than half of the total deaths worldwide [3,4]. Thus, the need to manage inflammation and pain in any disease condition as part of the treatment is important. Inflammation and oxidative stress are interrelated to the pathophysiological events in numerous diseases [5]. Although there are many available synthetic anti-inflammatory drugs like NSAIDs and corticosteroids, their potency in the alleviation of inflammation and adverse effects is of major concern [6].

Plants have been used in traditional medicine to treat diseases and hence possess a great potential for producing new drugs. Some plants have been reported to possess anti-inflammatory activity [7,8,9]. Within the genus *Terminalia*, species such as *T. chebula, T.arjuna, T. bellirica and T. catappa* were also reported to possess anti-inflammatory activity [10,11,12]. *Terminalia avicennioides* Guill. & Perr. (*Combretaceae*) is a yellowish-brown, hardwood, commonly found in the savanna region of West Africa [10]. Different parts and extracts of the plant have been used traditionally to manage conditions such as gastric ulcers, gastrointestinal disorders (diarrhea), bloody sputum, cough, and gastrointestinal helminth parasites [13]. *Terminalia* species have been used in folkloric medicine to treat malaria [14].

There are several reported studies on the pharmacological activities of *Terminalia avicennioides*, including*:* the anticancer property of the aqueous extract of the root against Ehrlich Ascites Carcinoma (EAC) cell lines [15], attributed to the presence of phytochemicals such as tannins, flavonoids, phenolics, and saponins, among others present in the extract [15]; and antibacterial effects of aqueous and ethanol crude extracts against *E.coli* and *S. typhimurium,* clinical and reference isolates from diarrheal patients [16]. The antifungal effect of the ethanolic, methanolic, ethylacetate, chloroform, and aqueous root extracts of *T.*

avicennioides on *A. niger, A. fumigatus, Penicillium species, M. audouinii and T. rubrum* [17]; the antimalarial activity in *Plasmodium berghei*-infected mice, [18]; and the antidiabetic potential of the stem bark extract on alloxan-induced diabetic rats [19] have also been reported. Triterpenoids have been isolated from the root bark of *T. avicennioides* and they include friedelin, arjunolic acid, alpha-amyrin, and 2, 3, 23-trihydroxylolean-12-eneas. The isolated friedelin and arjunolic acid exhibited potent anti-mycobacterial activity against a strain of *Mycobacterium bovis* (BCG) [20]. A recent study reported that the ethanol extract of the stem bark exhibited potential anti-inflammatory activity [10].

Considering previous reports and evidence of the antioxidant and anti-inflammatory properties of this plant, the present study was designed to examine the anti-inflammatory, antinociceptive and antioxidant activities of ethanol extract and portions of *T. avicennioides* as related to their phenolic contents.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Folin Denis reagent, DPPH, indomethacin, silica gel (Davisil, grade 643, 200-425 mesh) ethanol and ethyl acetate were sourced from Sigma Aldrich, Germany, through a regional representative. Other chemicals used were analytical grade reagents.

2.2 Plant Material

Terminalia avicennioides stem bark was collected from Suleja, Niger State, Nigeria in January 2022. The plant material was authenticated by a botanist at the herbarium of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research & Development (NIPRD) where a voucher specimen (NIPRD/H/6982) was deposited for future reference.

2.3 Preparation of Plant Extract and Fractions

The collected stem bark was washed with water, cut into smaller pieces, air-dried, and pulverized. The powdered stem bark (200 g) was extracted with 1.2 L of 70%v/v ethanol by cold maceration for 48 h with intermittent agitation. The resulting extract was filtered under gravity using a Whatman filter paper (grade 3, 125 mm), and the filtrate was concentrated under vacuum in a rotary evaporator. The extract was dried over a water bath maintained at 50^{o}C till a constant weight was obtained. The ethanol extract was dissolved in water and partitioned with ethyl acetate to collect the organic ethyl acetate soluble portion, and the polar aqueous portion. The dried extract and solvent portions were transferred into air-tight glass vials and stored at 4^{o}C in a refrigerator.

2.4 Column Fractionation of Ethyl Acetate Fraction

A 7 g quantity of the ethyl acetate fraction was mixed with 4.2 g silica gel and loaded in wet-packed column (silica gel: 40 g, loading solvent: 100% hexane, length: 20.4 cm, internal diameter: 7 cm). Gradient elution under gravity was used to collect 15 fractions. The mobile phase starting composition was 100%v/v hexane, and this was adjusted with increasing gradients of ethyl acetate, then 100%v/v ethyl acetate, and increasing gradients of methanol to a final composition of 100%v/v methanol. Based on thin layer chromatographic profiles, the fractions obtained were pooled to afford four sub fractions.

2.5 Experimental Animals

Swiss mice were sourced from the Animal Facility center of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD). The animals were maintained under standard environmental conditions (temperature 24 ± 2°C) with free access to a standard rodent diet and water. The animals were acclimatized to laboratory conditions for two weeks before the studies. Ethical approval for animal experiments was granted by the Animal Care and Ethics committee, NIPRD (05.03.05-30). All experiments were done according to the National Institute of Health guide for the care and use of laboratory animals (NIH, 2008).

2.6 Antioxidant Activity

An antioxidant assay was performed using 0.0064-1 mg/mL extract solution prepared in dimethyl sulfoxide, while gallic acid was used as a reference antioxidant. Briefly 0.2 mM solution of DPPH was freshly prepared in methanol and 150 µl of this solution was added to 50 µl of extract solution. After 30 min of incubation in the dark, absorbance was read

spectrophotometrically at 492 nm. All assays were performed in duplicate. The concentration required to effectively inhibit DPPH activity by 50% (IC50) was determined graphically by non-linear regression.

2.7 Formalin Test in Mice For Inflammation And Pain

A test of the fractions on acute pain and inflammation was performed in six groups of six mice each (three male, three female), according to a method described previously [21]. The mice were treated orally with water (10 ml/kg), the extract and fractions at 500 mg/kg each, dihydrocodeine (10 mg/kg) and indomethacin (10 mg/kg). One hour after treatment, 0.05 ml of 2.5% v/v formalin was subcutaneously injected into the sub plantar tissue of the left hind paw. Pain severity was scored in two distinct phases for 60 min: Phase 1 (0–10 min, scored five times at 2 min intervals) and Phase 2 (15–60 min, scored at 5 min intervals). Scoring was done using 3 behavioral signs of pain, thus: 0 – normal weight-bearing on the injected paw; 1 – light resting on the paw on the floor; 2 – elevation of the injected paw and 3 –licking, biting, or grooming of the injected paw. The mean of readings in each phase was recorded as the pain severity score. Paw volume was measured with a digital plethysmometer (LE 7500, Letica Scientific Instruments) before formalin injection into the paw, then thereafter at 1 h, and 2 h.

2.8 Xylene-Induced Topical Ear Inflammation in Mice

The effect of the extract and fractions on topical inflammation was evaluated [22]. Adult Swiss albino mice of either sex were randomly divided into 6 groups (n = 5) to receive either ethyl acetate fraction, aqueous fraction, or ethanol extract (5 mg/ear) applied on the anterior surface of the right ear. Topical inflammation was instantly induced on the posterior surface of the same ear by application of xylene (0.05 ml). Control mice received indomethacin (5 mg/ear). Two hours after induction of inflammation, mice were euthanised by chloroform anesthesia and both ears removed. Circular sections (6 mm diameter) of both the right (treated) and left (untreated) ears were punched out using a cork borer and weighed. Ear edema in each animal was quantified as the weight difference between the two ear plugs. The anti-inflammatory activity was evaluated as percent edema inhibition in the treated animals relative to untreated control animals using the relation:

Edema inhibition (%) = 100 [1 - (EET / EEC)]

Where,

EET = mean ear edema of treated mice;
EEC = mean ear edema of untreated control mice.

2.9 Egg Albumin–Induced Hind Paw Inflammation in Rats

The anti-inflammatory activity of sub fractions of the ethyl acetate fraction was determined using egg albumin–induced acute inflammation of the rat hind paw. Thirty rats were used for this test. Paw volumes of the 25 rats were recorded as a baseline volume using a digital plethysmometer (LE 7500, Letica Scientific Instruments), before randomization into 6 groups of five rats per group. Groups II-V were administered 300 mg/kg of fractions EF1 – EF4, respectively, while group I was the control, and group VI was given indomethacin (10 mg/kg). Egg albumin (0.05 ml) was injected subcutaneous into the sub plantar tissue of the left hind paw of the rat to induce inflammation at 1 h post-treatment of the experimental groups. Paw volume was measured at 1 h, 2 h, 3 h, 4 h, and 5 h post-induction using the digital plethysmometer and recorded.

2.10 High-Performance Liquid Chromatography (HPLC) Analysis

The chromatographic system comprised of an HPLC system consisting an Ultra-Fast LC-20AB prominence system, equipped with SIL- 20AC auto sampler; DGU-20A3 degasser; SPD-M20A UV diode array detector (UV-DAD, wavelength of 190 – 800 nm); column oven CTO-20AC, system controller CBM- 20 Elite and Windows LC solution software (Shimadzu Corporation, Kyoto Japan). A VP-ODS column, (5µm; 150 x 4.6 mm) was used and chromatographic conditions included mobile phase solvent A: 0.2% v/v formic acid in water and solvent B: acetonitrile; mode: isocratic elution (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 30 min. An injection volume of 10 µl of 10 mg/ml solution of extract or fraction in methanol was used and sample detection was at 254 nm. Standard solutions of gallic acid, chlorogenic acid, caffeic acid, rutin, ferulic acid, and luteolin

(Fluka, Germany) were prepared as 20 µg/ml solutions in methanol and analyzed separately under the same conditions.

2.11 Determination of Phenolic Content of Extract and Fractions

Phenolic content was determined using 0.25 mg/ml solutions of the extract, fractions, and sub-fractions as described by Okpoko et al. [10]. First, 2.5 ml of 10% Folin Denis reagent was added to 1 ml of each test solution in duplicates. The mixture was allowed to stand for 2 min at room temperature after which 2 ml of Na_2CO_3 solution (75 g/L) was added. The resulting mixture was maintained at 50°C for 15 min in a water bath, and afterwards, cooled in ice-cold water for 3 min. Absorbance was read spectrophotometrically at 760 nm (Cary 60, Agilent Technologies). A standard gallic acid plot (0.0016-0.1 mg/ml) was prepared using the same method. Phenolic content of the extract, fractions and sub-fractions was determined from the standard gallic acid plot and expressed as gallic acid equivalent (GAE).

2.12 Statistical Analysis

All values were presented as the mean ± standard error of mean (SEM). Differences between means were assessed by one-way or two-way analysis of variance (ANOVA) followed by Dunnett's post hoc test where appropriate using GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA). A *p* value of 0.05 or lower was accepted as being statistically significant.

3. RESULTS

3.1 Antioxidant Activity

The extract and the fractions exhibited strong antioxidant activity against DPPH. However, the aqueous and ethyl acetate fractions produced better antioxidant activities as observed from their lower IC_{50} values (0.035, 0.036 mg/mL), compared to the parent extract (IC_{50} = 0.052 mg/mL, Fig. 1).

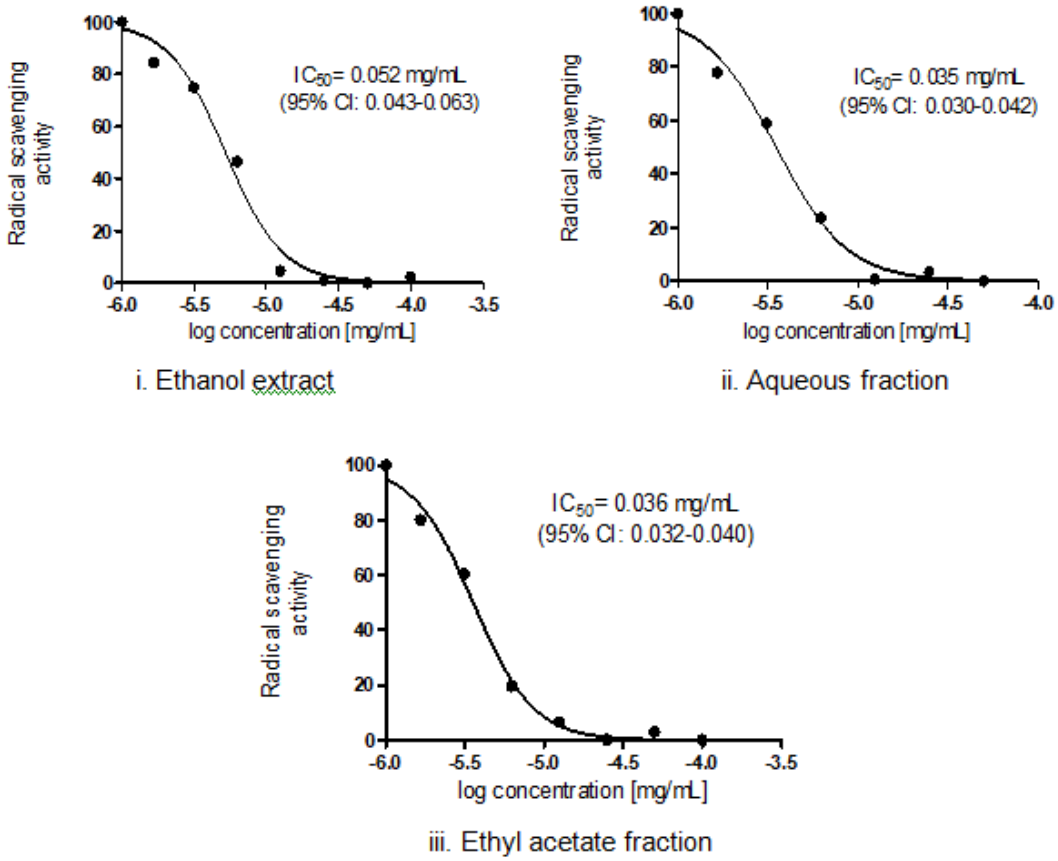

Fig. 1. Antioxidant activities of ethanol extract and the fractions of *T. avicennioides* stem bark

3.2 Anti-nociceptive Effects of *T. Avicennoides* Extract and Fractions

In phase 1 after the induction of pain in the paw, there were reductions in pain severity score in the extract and fraction-treated groups compared with the control group, but the effects were not statistically significant (p>0.05) (Table 1). In phase 2, there was a significance difference (p<0.001) in the anti-nociceptive effect produced by the ethanol extract, and this was higher than the effect produced by dihydrocodeine (DHC). The group treated with the aqueous fraction showed the strongest anti-nociceptive activity in Phase 1 compared to other groups.

3.3 Effects of Extract and Fractions on Formalin-Induced Paw Inflammation

The paw volume showed no significant difference (p>0.05) in the first 1 h of induction in the parent extract and the fractions groups but they indicated significance difference (p<0.001) at 2 h compared to the untreated group. Indomethacin and DHC also showed significant difference (p<0.01) compared to the untreated group.

Table 1. Effect of *T. avicennioides* extract and fractions on formalin-induced pain in mice

	Dose (mg/kg)	Pain severity score	
		Phase 1	Phase 2
Control	-	1.90±0.30	2.09±0.19
Ethanol extract	500	1.23±0.27	0.65±0.14***
Ethyl acetate fraction	500	1.54±0.28	1.40±0.27
Aqueous fraction	500	1.20±0.14	1.89±0.13
Indomethacin	10	1.97±0.08	1.84±0.31
DHC	10	1.40±0.16	1.16±0.17*

*Values are mean ± S.E.M. *p<0.05, **p <0.01, ***p<0.001 significantly different from control (Dunnett's post hoc test); (n = 6, per group); DHC = dihydrocodeine*

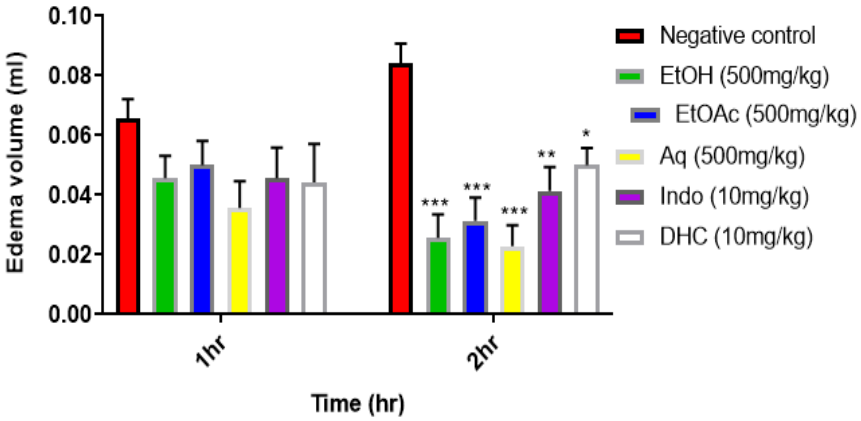

Fig. 2. Effect of extract and fractions on formalin-induced paw inflammation
Values are mean ± S.E.M. *p<0.05, **p<0.01, *** p<0.001 significantly different from control; (n = 6, per group)

Table 2. Effect of extract and fractions of *T. avicennioides* on xylene-induced ear edema in mice

Treatment	Dose (mg/kg)	Difference in weight of ear tissue (mg)	Inhibition (%)
Control	-	11.55±2.91	-
EtOH	500mg/kg	8.32±1.56*	40.00
EtOAc	500mg/kg	10.10±1.69	27.13
Aq	500mg/kg	11.48±3.62	17.15
Indo	10mg/kg	10.10±0.98	27.13

*Values are mean ± S.E.M. *P<0.05, significantly different from control; One-way ANOVA followed by Dunnett's test (n = 6, per group)*

Table 3. HPLC profiles of *T. avicennioides* ethanol extract, ethyl acetate, and aqueous portions

		Ethanol extract		Ethyl acetate fraction		Aqueous fraction	
Peak number	**Reference Phenolics**	**Retention time (min)**	**Relative composition (%) per 100 µg**	**Retention time (min)**	**Relative composition (%) per 100 µg**	**Retention time (min)**	**Relative composition (%) per 100 µg**
1	Gallic acid	3.063	9.06	3.002	18.97	2.995	12.86
2		3.608	10.13	3.701	5.61	3.682	5.63
3	Cholorogenic acid	3.966	16.15	4.044	8.10	3.996	8.78
4	Caffeic acid	4.474	7.05	4.565	6.65	4.483	7.63
5		5.505	6.84	5.291	8.72	5.171	10.46
6		5.986	6.41	5.667	8.53	5.527	12.26
7				7.162	13.06	5.976	4.45
8				8.437	4.67		
9				12.361	6.75		
10				22.019	18.97		
11	Rutin	6.92	11.09			6.916	13.18
12	Ferulic acid	7.994	4.98			8.072	4.70
13		11.644	4.15			9.775	1.38
14		12.252	1.91			11.773	5.61
15		14.332	0.89			19.479	5.46
16		15.313	0.77			24.755	7.61
17		17.328	0.89				
18		18.953	2.05				
19		23.494	3.75				
20	Luteolin	26.115	13.89				

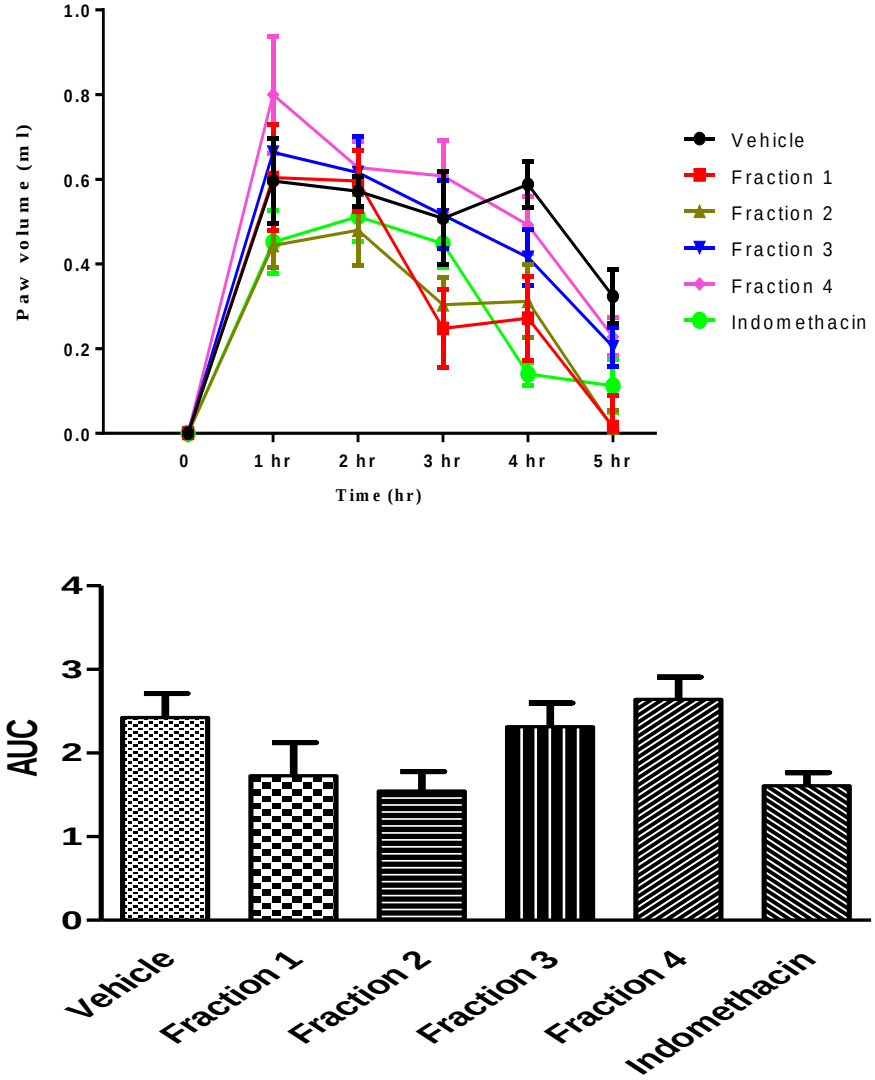

Fig 3. Activity of column fractions on egg albumin – induced paw inflammation

3.4 Effect of Extract and Fractions on Xylene–Induced Topical Edema

The extract-treated group showed a significant decrease (p<0.05) in the weight of ear tissue compared to the control group. The percentage of inhibition (27.13%) in the ethyl acetate fraction group was like the positive control group as shown in Table 2.

3.5 Anti-inflammatory Effect of Sub-Fractions of *T. avicennioides* Ethyl Acetate Fraction

The paw volumes in groups treated with sub fractions 1 and 2 were high at 0.7ml and 0.5ml respectively within the first two hours and reduced to 0.3ml and 0.35ml respectively at the third hour (Fig. 3). In fractions 3 and 4 treated groups, the reduction in paw volume was less than the effects produced in fractions 1 and 2 treated groups.

3.6 High-Performance Liquid Chromatography

The HPLC analysis indicates peaks corresponding to the reference standards in the extract and fractions. Gallic acid, cholorogenic acid, and caffeic acid are detected in the ethyl acetate fraction; gallic acid, cholorogenic acid, caffeic acid, rutin, and ferrulic were identified in the aqueous fraction, and all the standards were identified in the ethanol extract as shown in Table 1. The quantity of gallic acid was higher in the ethyl acetate fraction compared to the ethanol extract and aqueous fraction.

Table 4. Gallic acid Equivalent of *T. avicennioides* Extract and Fractions

Extracts/Fractions	Gallic acid equivalent (GAE)/mg
Ethanol extract	0.8784± 0.126
Aqueous fraction	0.7519±0.057
Ethyl acetate fraction (EF)	1.3180±0.056
EF1	0.8695±0.026
EF2	0.4014±0.004
EF3	0.5052±0.002
EF4	0.0928±0.002

**EF = Ethyl acetate fractions*

3.7 Phenolic Content of Extract, Fractions, and Sub-Fractions

The results showed that the plant phenolics were enriched in the ethyl acetate fraction and its FI sub-fractions, with high gallic acid equivalent (GAE) compared to the ethanol extract, aqueous fraction, and sub-fractions as indicated in Table 4.

4. DISCUSSION

This study evaluated the anti-nociceptive and anti-inflammatory effects of *T. avicennioides* stem bark extract and its fractions, as correlated with phenolic content. In a previous study by Okpoko et al. [10] the plant extract was reported to possess these pharmacological effects. In the present study, the ethyl acetate and aqueous fractions demonstrated higher antioxidant activities compared to the parent extract. Other species of *Terminalia* such as *T. chebula, T.belirica, T. catappa, T. arjuna and T. prunioides* have been reported to have antioxidant activities [23,24]. *Terminalia* species are rich in polyphenols, exemplified by shikimic acid, gallic acid, corilagin, ellagic acid, vixetin, and rutin [24]. Although these compounds contribute significantly to the antioxidant effects of *Terminalia spp*., our current study reveals that other compounds in *Terminalia avicennioides* extract may mediate its anti-inflammatory and anti-nociceptive activity. In formalin-induced pain, there was a reduction in the severity of the pain in extract and fraction-treated groups but the extract ameliorated pain severity more effectively in both phases. In formalin-induced pain, a biphasic response is observed, and these phases are evoked by different mechanisms, characterized by the release of histamine, serotonin, and substance P leading to edema. This study showed that ethanol extract and its fractions can inhibit the release of these substances by blocking the stimulation of prostaglandin either by selectively or non-selectively inhibiting cyclooxygenase [10,24]. This finding is corroborated by the studies of Huang et al. [25], whose study reported that *T. avicennioides* has anti-nociceptive effects at the peripheral level.

The aqueous fractions showed comparable effect with the extract in inhibiting the peripheral inflammatory response to formalin, and this could be hinged upon the suppression of the inflammatory cascade involving various cytokines and chemokines. This finding is supported by a previous report which indicated that other *Terminalia* species display good anti-inflammatory activity [24]. This may be due to the presence of bioactive constituents present in *T. species* which block the release of prostaglandin E_2 and macrophages [26].

The anti-inflammatory effect of *T. avicennioides* extract and fractions was also observed in the xylene-induced topical edema, in which the extract produced a higher effect compared to the standard drug, indomethacin. Indomethacin is a non-selective inhibitor of cyclooxygenases which catalyze the production of prostaglandin which stimulates the release of inflammatory factors like serotonin, cytokines, and others. The extract may elicit its effect via this mechanism, or by reducing nitrous oxide, as shown in a previous study in which the ethanol extract of *T. avicennioides* demonstrated anti-inflammatory activity and reduced nitrous oxide (NO), a reactive species that can cause oxidative stress and drive inflammation [10].

The sub-fractions from the ethyl acetate fraction also exhibited anti-inflammatory activities, although at this level, polyphenolic content appeared to be linked with anti-inflammatory effects although it still less effective than the parent extract in this regard. This implies that although the extract, fractions, and sub-fractions

have a significant concentration of polyphenolics, these do not directly correlate with their anti-inflammatory and antinociceptive effectiveness.

The HPLC analysis of the extract and fractions showed the presence of phenolics and other phytochemicals that may be responsible for the pharmacological activities observed in the plant [27]. Phenolics have been identified in other *Terminalia spp*., which support our findings [24]. The aqueous extract of *Terminalia chebula* Retz.'s (Combretaceae) galls has been reported to have gallic acid, punicalagin, isoterchebulin, 1,3,6-tri-O-galloyl-D-glucopyranose, chebulagic acid, and chebulinic acid as some of the phenolics that were isolated and identified [28]. Other reported constituents of *Terminalia* spp. include apigenin, luteolin, quercetin, epicatechin, ellagic acid, and 1-O-galloyl glucose, phenolic carboxylic acids, seven hydrolyzable tannins, eight triterpenoids, including four oleanane-type triterpene acids, and four of their glucosides. Ellagic acid, tannic acid, ethyl gallate, corilagin, mannitol, ascorbic acid, among others [24,27,29]. Plants contain large amounts of polyphenols, which function as metal chelators and free radical scavengers. Recently, plant-derived bioflavonoids and polyphenols have been widely employed to prevent membrane lipid peroxidation and scavenge free radicals [30].

The presence of diverse bioactive compounds including phenolics in *Terminalia avicennioides* is responsible for the antioxidant, anti-inflammatory, antinociceptive, and other pharmacological activities demonstrated by the plant and as shown in this study. The phenolic content in the ethyl acetate fraction was high compared to the parent ethanol extract but the ethanol extract demonstrated better anti-inflammatory and antinociceptive properties than the ethyl acetate extract. This may be due to the presence of other phytochemicals like luteolin in the ethanol extract which are less concentrated in the fractions.

5. CONCLUSION

The present study demonstrated the effectiveness of *T. avicennioides* in different anti-inflammatory and antinociceptive tests. One major finding was that although extract separation may afford fractions with higher concentrations of phenolics, this may impact its antinociceptive and anti-inflammatory effect. Arguably, drug discovery and development need not always conform to separation to fractions or compounds but may also be focused on the standardization of highly active extracts to avoid reducing pharmacological effectiveness.

ACKNOWLEDGEMENT

Miss Ogochukwu Okeke-Uzowulu and Mr Sunday Dzarma are acknowledged for providing research assistance and technical support.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experimental protocols were examined and approved by the Animal Care and Ethics Committee, Department of Pharmacology and Toxicology, NIPRD (05.03.05-30).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Carullo G, Cappello AR, Frattaruolo L, Badolato M, Armentano B, Aiello F. Quercetin and derivatives: useful tools in inflammation and pain management. Future Medicinal Chemistry. 2017;9(1): 79-93.
2. Matsuda M, Huh Y, Ji RR. Roles of inflammation, neurogenic inflammation, and neuroinflammation in pain. Journal of Anesthesia. 2019; 33:131-9.
3. Verma S. Molecular plants with anti-inflammatory activity. Journal of Phytopharmacology. 2016, 5(4):157-159.
4. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S. Chronic Inflammation in the Etiology of Disease across the Life Span. Nature Medicine. 2019;25(12): 1822–1832.
5. Didangelos A, Puglia M, Iberl M, Sanchez-Bellot C, Roschitzki B, Bradbury EJ. High-throughput proteomics reveal alarmins as amplifiers of tissue pathology and inflammation after spinal cord injury. Sci Rep. 2016, 6:21607.
6. Ilavarasan R, Mallika M, Venkataraman S. Anti-inflammatory and antioxidant activities of *Cassia fistula* Linn bark extracts. Afr. J. Trad. CAM. 2005, 1:70-85.

7. Ji R-R, Chamessian A, Zhang Y-Q. Pain regulation by non-neuronal cells and inflammation. Science. 2016,354(6312): 572-7.
8. Chandrashekar KS, Thakur A, Prasanna KS. Anti-inflammatory activity of *Moringa oleifera* stem bark extracts against carrageenen induced rat paw edema. J Chem Pharm Res. 2010;2:179-181.
9. Londonkar R, Kamble Reddy VC. Anti-inflammatory activity of *Pandanus odoratissmus* extract. International Journal of Pharmacology. 2010;6(3):311-314.
10. Okpoko C, Ezenyi I, Adzu B, Salawu O. Evaluation of two medicinal plants used in northern Nigeria with focus on *Terminalia avicennioides* Guill. &Perr. and its mechanism of action. Scientific African. 2020;8:e00357;
11. Dulce L, Ambriz-Perez, Nayely L, Erick P, Guterrez G. Phenolic compounds: Natural alternative in inflammation treatment. A review. Cogent Food & Agriculture. 2016;2(1):1131412.
12. Zhang XJ, He LJ, Lu Q, Li DY. Pharmacological activity of *Terminalia chebula*. Zhongguo Zhong Yao Za Zhi. 2016;41(4):619-623.
13. Mann A, Ajiboso OSO, Ajeigbe S, Gbate M, Isaiah S. Evaluation of the wound healing activity of the ethanol extract of *Terminalia avicennioides* root bark on two wound models in rats. International Journal of Medicinal Aromatic Plants. 2011; 1(2):95-100.
14. Titanji VPK, Zofou D, Ngemenga MN. The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine. Afr. J. Trad. CAM. 2008;5:302-321.
15. Salau AK, Yakubu MT, Oladiji AT. Cytotoxic activity of aqueous extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* root barks against Ehrlich Ascites Carcinoma cells. Indian Journal of Pharmacology. 2013;45(4):381–385.
16. Musa FM, Ameh JB, Ado SA, Olonitola OS. Evaluation of phytochemical and antibacterial properties of *Terminalia avicennioides* crude extract against selected bacteria from diarrhoeic patients. Bayero Journal of Pure and Applied Sciences. 2016;9(1):129 – 137.
17. Mann A, Banso A, Clifford LC. An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. Tanzania Journal of Health Research. 2008; 10(1):34-38.
18. Omonkhua AA, Cyril-Olutayo CM, Akanbi OM, Adebayo OA. Antimalarial, hematological and antioxidant effect of methanolic extract of *Terminalia avicennioides* in *Plasmodium berghei*-infected mice. Parasitol Res. 2013; 112:3497-3503.
19. Yahaya SF, Suleiman MM, Mohammed A, Ibrahim NDG. Anti-diabetic potential of stem bark extracts of *Terminalia avicennioides* on alloxan-induced diabetic rats. Sokoto Journal of Veterinary Sciences. 2019;17(2):33-44.
20. Ogbadayi EO, Mann A. Isolation and antimycobacterial activity of the four triterpenoids from *Terminalia avicennioides* root bark. Biochemistry and Pharmacology. 2013;2(4):236.
21. Adzu B, Amizan MB, Okhale SE. Evaluation of antinociceptive and anti-inflammatory activities of standardised rootbark extract of *Xeromphis nilotica,* J. Ethnopharmacol. 2014;158:271–275.
22. Ezike AC, Akah PA, Okoli CO, Okoye TC, Ogbu OM, Okonkwo I, Uwakwe G, Ibe S, Ezema V, Studies on the anti-inflammatory, antinociceptive and antimicrobial activities of *Combretum calobotrys* (Combretaceae) leaf. Journal of Medicinal Plant Research. 2013;7(21): 1568-1576.
23. Sheng, Z, Zhao, J, Muhammad, I, Zhang Y. Optimization of total phenolic content from *Terminalia chebula* Retz. fruits using response surface methodology and evaluation of their antioxidant activities. PloS one. 2018;13(8): e0202368.
24. Zhang XR, Kaunda JS, Zhu HT, Wang D, Yang CR, Zhang YJ. The Genus *Terminalia* (Combretaceae): An Ethnopharmacological, Phytochemical and Pharmacological Review. Nat Prod Bioprospect. 2019;9(6):357-392.
25. Huang YH, Wu PY, Kuo-Ching Wen, Chien-Yih Lin, Hsiu-Mei Chiang. Protective effects and mechanism of *Terminalia catappa*. Methanolic extract on hydrogen-peroxide-induced oxidative stress in human skin fibrolast. BMC Complementary and Alternative Medicine. 2018;18(1)1-9.
26. Awad AB., Toczek J., Fink CS. Prostaglandins, Leukotrienes and Essential fatty acid. 2004;70(6):511-520.
27. Meena DK, Sahoo AK, Srivastava PP, Sahu NP, Jadhav M, Gandhi M, Swain HS,

Borah S, Das BK On valorization of solvent extracts of *Terminalia arjuna* (arjuna) upon DNA scission and free radical scavenging improves coupling responses and cognitive functions under *In vitro* conditions. Sci Rep. 2021;11(1):10656.

28. Kesharwani A, Polachira SK, Nair R, Agarwal A, Mishra NN, Gupta SK. Anti-HSV-2 activity of *Terminalia chebula* Retz. extract and its constituents, chebulagic and chebulinic acids. BMC Complement Altern Med. 2017;17(1):110.

29. Bushra, Rehman KU, Khan D, Almehizia AA, Naglah AM, Al-Wasidi AS, Refat MS, El-Sayed MY, Ullah H, Khan S. Isolation and Bioassay of a New Terminalone A from *Terminalia arjuna* Molecules. 2023; 28(3):1015.

30. Beyhan O, Elmastas M, Gedikli F. Total phenolic compounds and antioxidant capacity of leaf, dry fruit and fresh fruit of feijoa (*Acca sellowiana*, Myrtaceae) J. Med. Plants Res. 2010;4201010651072.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/106327