

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

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.

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]. *Terminalia avicennioides* Guill. & Perr. (*Combretaceae*) is a yellowish-brown, hardwood, commonly found in the savanna region of West Africa [12]. 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 [11]. *Terminalia* species have been used in folkloric medicine to treat malaria [13].

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 [14], attributed to the presence of phytochemicals such as tannins, flavonoids, phenolics, and saponins, among others present in the extract [14]; and antibacterial effects of aqueous and ethanol crude extracts against *E.coli* and *S. typhimurium*, clinical and reference isolates from diarrheal patients [15]. 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* [16]; the antimalarial activity in *Plasmodium berghei*-infected mice, [17]; and the antidiabetic potential of the stem bark extract on alloxan-induced diabetic rats [18] 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-ene. The isolated friedelin and arjunolic acid exhibited potent anti-mycobacterial activity against a strain of *Mycobacterium bovis* (BCG) [19]. 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.

2.0 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°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°C in a refrigerator.

2.4 Column fractionation of ethyl acetate portion

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 profile, these 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 $24 \pm 2^\circ\text{C}$) 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

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 reduce DPPH activity by 50% (EC_{50}) 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 [20]. The mice were treated orally with water (10 ml/kg), the extracts at 500 mg/kg each, dihydrocodeine (10 mg/kg) and indomethacin (10 mg/kg). One hour after treatment, the mice were injected with 0.05 ml of 2.5% v/v formalin subcutaneous 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 each 5 min). 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 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 [21]. Adult Swiss albino mice of either sex were randomly divided into 6 groups ($n = 5$) to receive either ethyl acetate or aqueous potion, 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 animals received indomethacin (5 mg/ear). Two hours after induction of inflammation, mice were sacrificed by overdose of 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 control animals using the relation:

$$\text{Edema inhibition (\%)} = 100 [1 - (\text{EET} / \text{EEC})]$$

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

2.9 Egg albumin–induced hind paw inflammation in rats

The anti-inflammatory activity of fractions of the ethyl acetate portion was determined using egg albumin–induced acute inflammation of the rat hind paw. Twenty-five rats were divided into five groups of five rats each. Paw volumes of the 25 rats were recorded as a base-line volume using a digital plethysmometer (LE 7500, Letica Scientific Instruments). Groups II-V were administered 300 mg/kg of fractions EF1 – EF4, respectively, while group I was the control, and group V 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 of Ultra-Fast LC-20AB prominence, 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, (5 μ m; 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 μ l 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 μ g/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 0.25mg/ml solutions of the extract, fractions, and sub-fractions as described by Okpoko *et al.*, [10]. First, 2.5ml of 10% Folin Denis reagent was added to 1ml of each test solution in duplicates. The mixture was allowed to stand for 2 min at room temperature after which 2 ml of Na₂CO₃ 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).

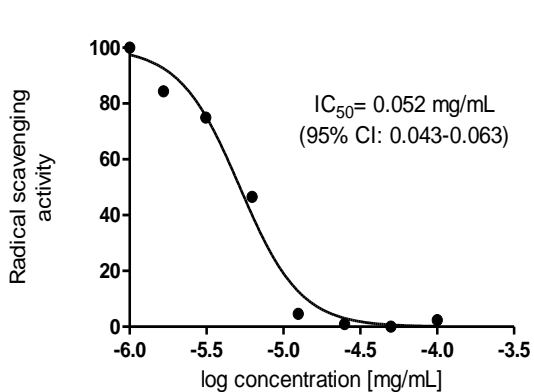
2.12 Statistical Analysis

All values were presented as the mean \pm 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.

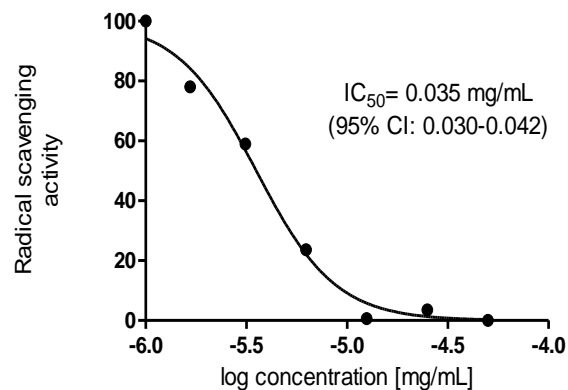
3.0 Results

3.1 Antioxidant activity

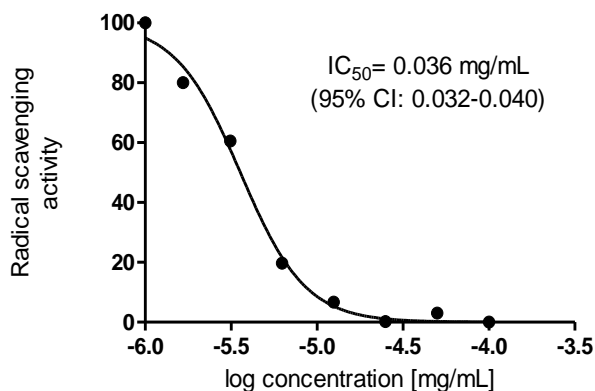
The extract and the fractions exhibited strong antioxidant activity against DPPH. However, the aqueous and ethyl acetate fractions produced better antioxidant activities as observed from their lower IC_{50} values (0.035, 0.036 mg/mL), compared to the parent extract (IC_{50} = 0.052 mg/mL, Figure 1).



i. Ethanol extract



ii. Aqueous fraction



iii. Ethyl acetate fraction

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

3.2 Anti-nociceptive effects of *T. avicennioides* 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.

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

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.

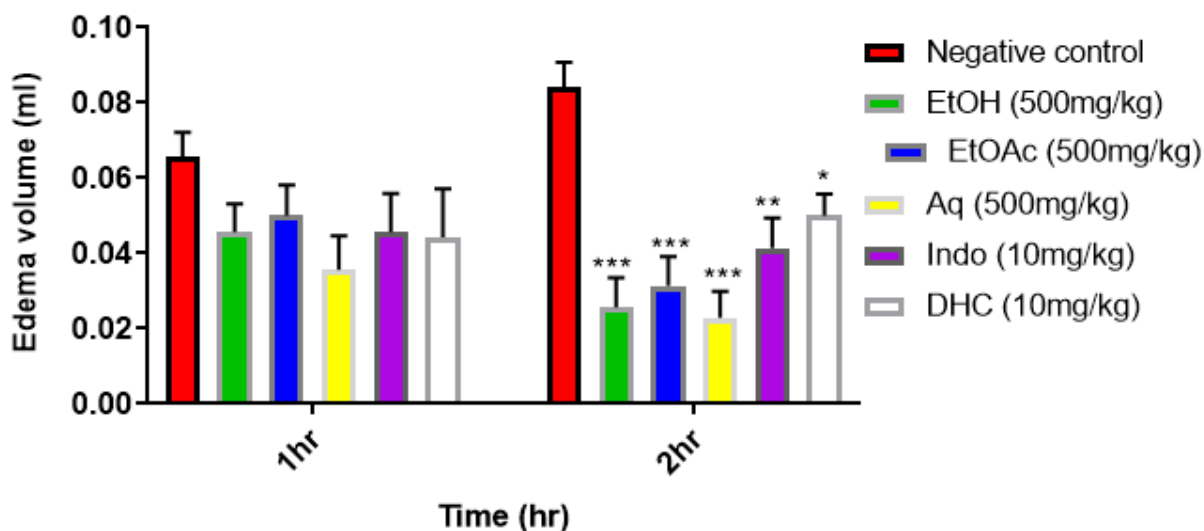


Figure 2: Effect of extract and fractions on formalin-induced paw inflammation

Values are mean \pm S.E.M. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ significantly different from control; (n = 6, per group).

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. However, the percentage of inhibition (27.13%) in the ethyl acetate fraction group was like the positive control group as shown in Table 2

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 the ear (mg) | Inhibition (%) |
|-----------|--------------|--------------------------------------|----------------|
| Control | - | 11.55 \pm 2.91 | - |
| EtOH | 500mg/kg | 8.32 \pm 1.56* | 40.00 |
| EtOAc | 500mg/kg | 10.10 \pm 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).

3.5 Anti-inflammatory effect of sub-fractions of *T. avicennioides* ethyl acetate fraction

The paw volumes in groups treated with 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 (Figure 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.

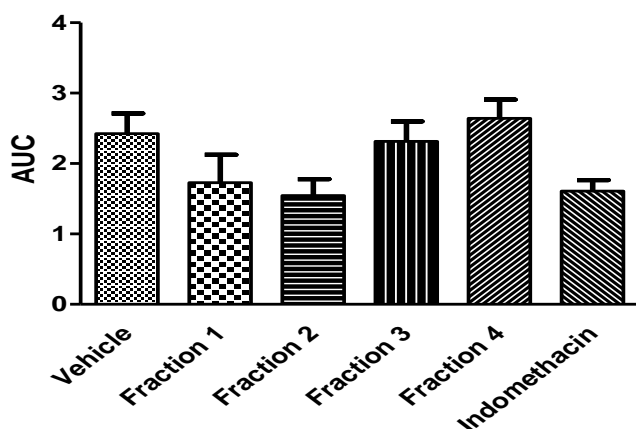
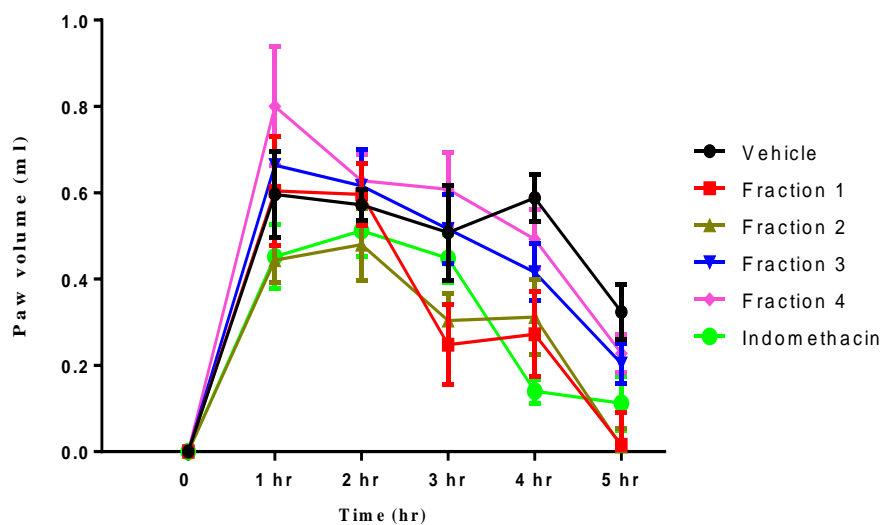


Fig 3: Activity of column fractions on egg albumin – induced paw inflammation

3.6 High-Performance Liquid Chromatography

The HPLC analysis indicates peaks corresponding to the reference standards in the extract and fractions. Gallic acid, chlorogenic acid, and caffeic acid are detected in the ethyl acetate portion; gallic acid, chlorogenic acid, caffeic acid, rutin, and ferulic were identified in the aqueous portion, 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 portion compared to others.

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

| Peak number | Reference Phenolics | Ethanol extract | | Ethyl acetate fraction | | Aqueous fraction | |
|-------------|---------------------|----------------------|-------------------------------------|------------------------|-------------------------------------|----------------------|-------------------------------------|
| | | 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 | Chlorogenic 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 | | | | |

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 other fractions and sub-fractions as indicated in Table 4.

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

4.0 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 (ETA) and aqueous (AQ) 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 [22]. *Terminalia* species are rich in polyphenols, exemplified by shikimic acid, gallic acid, corilagin, ellagic acid, vixetin, and rutin [23]. 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 both the extract and the fractions 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 fraction can inhibit the release of these substances

by blocking the stimulation of prostaglandin either by selectively or non-selectively inhibiting cyclooxygenase [24]. This finding is corroborated by the studies of Huang *et al.* [25], which 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 correlated with a previous study which indicated that *Terminalia* species such as *T. sericea* and *T. chebula* have anti-inflammatory effects [10]. This may be due to the presence of phytochemicals with several antioxidant activities present in *T. species* especially β -sitosterol which block the release of prostacyclin (PGI₂) and prostaglandin E₂ 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 [27]. This relates to previous findings that the ethanol extract of *T. avicennioides* has anti-inflammatory activities and reduced nitrogen oxide (NO), a reactive species that can cause oxidative stress [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. 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 [28]. Phenolics have been identified in other *Terminalia* spp., which support our findings. 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 are the six phenolic chemicals that were isolated and identified [29]. The active ingredients gallic acid, apigenin, luteolin, quercetin, epicatechin, ellagic acid, and 1-O-galloyl glucose were extracted and identified using several spectroscopic methods [30]. The methanol extract of the gall of *Terminalia chebula* Retz has also been shown to have phenolic components, such as two phenolic carboxylic acids, seven hydrolyzable tannins, eight triterpenoids, including four oleanane-type triterpene acids, and four of their glucosides, [31], ellagic acid, tannic acid, ethyl gallate, corilagin, mannitol, ascorbic acid (vitamin C), and other substances were also found in *T. chebula* after a phytochemical investigation. 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 [32].

The presence of these phytochemicals and phenols in *Terminalia* species 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 rutin and ferulic acid in the ethanol extract which are not present in the ethyl acetate fraction.

5.0 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.

Conflict of Interest Declaration

There is no competing interest among the authors.

Declaration of the source of funding

The authors did not receive any specific funding support for this study.

Declaration of the role played by each author

Ezenyi I.C. designed the study, Okwulu O.J. and Fidelis S.A. conducted the experiments, Okhale S.E. performed HPLC analyses, and Ezike A.C. supervised the study. All authors read and approved the final manuscript.

Acknowledgment

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).

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