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Evaluation of the Analgesic and Anti-Inflammatory Activity of the Decoction and Hydroethanolic Extract of *Ximenia americana* L. (Olacaceae) Bark

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Abstract

In traditional medicine, the decoction of *Ximenia americana* bark is employed for the treatment of various ailments; however, there has been a paucity of research evaluating its pharmacological properties. The objective of this study was to compare the analgesic and anti-inflammatory properties of the decoction (DXA) and a hydroethanol extract (EHEXA) of *Ximenia americana.* The experimental design encompassed assessments of analgesic activity, utilising contortion and formaldehyde tests on laboratory animals, and an evaluation of anti-inflammatory efficacy through a carrageenan test. The results demonstrated that both extracts, at doses ranging from 0.25 to 150 mg/kg, exhibited a dose-dependent inhibition of contortions. The E_{max} obtained was 100% for both extracts, which was identical to the Emax of paracetamol and tramadol. The ED_{50} values for tramadol, DXA, EHEXA and paracetamol were determined to be 2.81, 2.84, 7.94 and 19.05 mg/kg, respectively. In the neurogenic phase of the formaldehyde test, DXA and EHEXA demonstrated significant pain inhibition of 39% to 54% and 38% to 64%, respectively, at doses ranging from 10 to 150 mg/kg. In the inflammatory phase of the formaldehyde test, DXA and EHEXA demonstrated pain inhibition rates of 55% - 71% and 65% - 81%, respectively, at doses ranging from 10 to 150 mg/kg. In the carrageenan test, EHEXA demonstrated transient anti-oedematous activity. This was ob-

served in the 1st hour at doses of 25, 50 and 100 mg/kg, with significant inhibition of 61%, 47% and 46%, respectively. Conversely, DXA manifested delayed anti-oedema activity, with 36% inhibition of oedema observed from the 3rd to the 5th hour, at a dose of 10 mg/kg. It is hypothesised that EHEXA is richer in compounds with anti-oedematous properties. In conclusion, *Ximenia americana* could have a much more pronounced analgesic activity at the same doses as the anti-inflammatory effect.

Keywords

Ximenia americana, Analgesic, Anti-Inflammatory, Potency, Efficacy

1. Introduction

Irrespective of its aetiology, whether somatic, neurological or psychological, the pain remains a subjective experience that necessitates appropriate management, with the global prevalence of chronic pain estimated at 55.2% [1].

Conventionally, pharmaceuticals such as non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and opioids have been utilised to treat pain and inflammation [2] [3]. However, it is important to note that prolonged use and high doses of these drugs can lead to serious side effects, such as gastrointestinal disorders [4] [5], respiratory depression, addiction, constipation [5] and renal dysfunction [2] [3].

According to the World Health Organisation (WHO), the utilisation of plants for medicinal purposes is prevalent among over 80% of the African population [6]. *Ximenia americana* (*Linnaeus*) (Olacaceae) is a notable plant in the traditional pharmacopoeia [7]. It is employed as an anti-inflammatory agent, and studies have indicated substantial anti-inflammatory properties, attributable to bioactive compounds such as flavonoids and tannins [6]. The plant is frequently utilised in traditional medicine for pain relief. Research has demonstrated that *Ximenia americana* extracts possess analgesic effects that are comparable to those of conventional painkillers [8]. Furthermore, *Ximenia americana* has been shown to possess strong antioxidant properties, which may contribute to the prevention of diseases associated with oxidative stress [9]-[12].

A number of studies in the scientific literature have evaluated the anti-inflammatory and analgesic properties of *Ximenia americana L.* [6] [8] [13] [14]. These studies focused on aqueous infusion extracts, hydroethanolic extracts and extract fractions. In these studies, the doses evaluated varied from 25 to 300 mg/kg. However, no study evaluated the effects of extracts at doses lower than 25 mg/kg. Furthermore, no research has been carried out on decoction, despite this being the traditional form of use. The aim of this study is to investigate the potential anti-inflammatory and analgesic effects of *Ximenia americana* decoction (DXA) and hydroethanol extract (EHEXA).

2. Materials and Methods

2.1. Plant Material

The plant material consisted of the trunk bark of *Ximenia americana*, collected in Tiebila, located in the Poro region, 600 km north of Abidjan, Côte d'Ivoire. It was then authenticated at the National Floristic Center (CNF) of Félix Houphouët-Boigny University in Abidjan under herbarium number UCJ013289.

2.2. Animal Equipment

Swiss strain Mus musculus mice, weighing between 20 g and 30 g, and Wistar strain *Rattus norvegicus* rats, weighing between 150 g and 200 g, sourced from the animal facility of the Faculty of Pharmaceutical and Biological Sciences at Félix Houphouët-Boigny University, were used for the tests. The animals were housed under controlled temperature (20˚C - 25˚C) and photoperiod (12/24 hours) conditions, in accordance with OECD good practice guidelines. Prior to experimentation, the animals were fasted for 4 hours with free access to water [15].

Solvents and Chemicals

- Paracetamol 500 mg tablet from SANOFI Laboratory (Doliprane®)
- Tramadol 50 mg capsule from ACINO Laboratory (Trabar™-50)
- Acetic acid 100% from PROLABO Laboratory
- NaCl 0.9% (500 ml) from Pharmivoire Nouvelle Laboratory
- Formaldehyde 1%
- Distilled water
- Carrageenan
- Profenid® 100 mg tablet (Aventis Pharma)
- Aspirin 500 mg from Rhône® (RP-LAB)

2.3. Preparation of Extracts

The bark of *Ximenia americana* L. was sorted, cut, and dried at room temperature, away from light, in the laboratory for 2 weeks. It was then powdered using a blender-grinder (Restsch GM 300 TM). The fine powder obtained, with a particle size of 1 millimeter, was stored in an 8-liter closed plastic container.

- **Preparation of the decoction of *Ximenia americana* L.**

The dry decoction of *Ximenia americana* was prepared according to the method described by Boolamou *et al.* [16]. Two hundred (200) grams of stem bark powder from *Ximenia americana* were brought to a boil in two liters of distilled water for ten minutes. The decoction was then filtered twice through absorbent cotton and once through Whatman N˚3 filter paper. The filtrate was dried in an oven at 50˚C ± 5˚C for 72 hours to obtain the aqueous extract (dry decoction). The dry residue or dry macerate was stored in vials at 4˚C for further testing. The extraction yield was 13.79%.

- **Preparation of the hydroethanolic extract of *Ximenia americana* L. trunk bark**

Two hundred (200) grams of dry bark powder were macerated at room tem-

perature for 24 hours in 2 liters of ethanol-water mixture (70:30). The mixture was filtered through absorbent cotton and then through filter paper (WHATMAN). The resulting filtrate (1455 ml) was concentrated to dryness using a controlled temperature oven (50˚C ± 5˚C) for 72 hours. The dry residue or dry macerate was stored in vials at 4˚C for further testing [17]. The extraction yield was 17.63%.

2.4. Phytochemical Studies (Characterization Reactions)

The characterization reactions were performed in test tubes to identify the main chemical groups present in the bark extracts of *Ximenia americana*. Phytochemical screening was carried out using specific reagents tailored to each chemical group [17]-[20].

- ✓ Alkaloids were detected using Bouchardat's reagent (iodine-iodide reagent) and Dragendorff's reagent (potassium iodobismuthate reagent).
- ✓ Flavonoids were identified through the cyanidin reaction.
- ✓ Polyphenols were detected using the ferric chloride ($FeCl_3$) reaction.
- ✓ Catechin tannins were identified using Stiasny's reagent.
- ✓ Quinonic substances were screened using Bornträger's reagent.
- ✓ Sterols and polyterpenes were detected using Liebermann's reaction.
- ✓ Saponins were identified by the formation of persistent foam after agitation.

2.5. Investigation of Pharmacological Activities

2.5.1. Dorso-Abdominal Writhing Test Induced by 1% Acetic Acid

The method used was that described by Zimmermann (1983) [21].

The mice were divided into 23 groups of five (5) mice each:

- **Group 1:** Negative control group treated orally with physiological water.
- **Group 2:** Group treated orally with paracetamol at 100 mg/kg body weight (BW).
- **Group 3:** Group treated orally with tramadol at 25 mg/kg BW.
- **Groups 4 to 13:** Experimental groups receiving *Ximenia americana* decoction, administered orally at doses of 0.25, 1, 2, 5, 10, 25, 50, 75, 100, and 150 mg/kg BW.
- **Groups 14 to 23:** Experimental groups receiving the hydroethanolic extract of *Ximenia americana*, administered orally at doses of 0.25, 1, 2, 5, 10, 25, 50, 75, 100, and 150 mg/kg BW.

Expression of results

For each group of mice, we calculated the mean (M) of writhing responses. The percentage of pain inhibition for each group treated with different doses of the various extracts of *Ximenia americana*, paracetamol, tramadol, and 0.9% NaCl was determined by comparing the mean writhing responses of the treated groups (extracts, paracetamol, and tramadol) with that of the control group treated with 0.9% NaCl. The percentage of inhibition was calculated using the following formula:

$$\%\ \text{Inhibition} = \frac{\left(\text{M. control group writhing} - \text{M. treated group writhing}\right)}{\text{M. control group writhing}} \times 100$$

A significant reduction in the mean number of writhing responses compared to the control group is considered an analgesic response.

2.5.2. Formaldehyde-Induced Paw Irritation Test in Rats

This test evaluates edema induced by formaldehyde, following the method of Santol *et al.* (1997), and by Carolyn J. T., *et al.* (1998) [22] [23].

Expression of results: Percentage of inhibition

The percentage of inflammation inhibition was estimated using the following formula:

$$\%\ \text{Inhibition} = \frac{\left(\text{Licking time control group} - \text{licking time treated group}\right)}{\text{Licking time control group?}} \times 100$$

2.5.3. Carrageenan-Induced Paw Irritation Test in Rats

This test is based on carrageenan-induced edema following the method of Winter *et al.* (1962; 1968) [24] [25]. The increase in paw thickness, *i.e.*, the inflammation (%), was calculated using the equation provided by Delporte *et al.* [26]:

$$\%\ \text{Inhibition} = \frac{\left(T_t - T_o\right)}{T_o} \times 100$$

where:

T_o = initial paw thickness of each animal

T_t = final paw thickness of each animal

Furthermore, the anti-inflammatory effect (% A) was calculated for each group of treated rats compared to the control group using the formula provided by Delporte *et al.* (2005) [26].

2.6. Ethical Considerations

The experimental procedures involving animals in this study were conducted in accordance with the ethical guidelines and regulations set forth by [mention relevant ethical committees or organizations, e.g., the Institutional Animal Care and Use Committee (IACUC), European Union regulations, or national guidelines]. All efforts were made to minimize animal suffering and ensure humane treatment during the experiments. The use of animals was essential for the investigation of the pharmacological effects, and the study was designed to minimize the number of animals used, adhering to the principles of the 3Rs (Replacement, Reduction, and Refinement). Ethical approval for this study was obtained from [name of the institution or ethics committee [27].

2.7. Data Treatment and Analysis

The data were entered using Microsoft Office Excel 2016, and the results were represented as means ± standard deviation. These data were analyzed using GraphPad Prism 9.3.0 software. The comparison of means was performed using

the non-parametric Kruskal-Wallis test, and the observed differences were statistically interpreted with a significance level of $\alpha = 5\%$.

3. Results

3.1. Phytochemical Screening of *Ximenia americana* Stem Bark Extracts

Phytochemical screening allowed the characterization of the main chemical groups that may be responsible for the properties of *Ximenia americana* extracts, as mentioned in Table 1. These include sterols and polyterpenes, flavonoids, alkaloids, polyphenols, and saponins.

Table 1. Phytochemical screening of *Ximenia americana* stem bark extracts.

Extracts		Decoction	Hydroethanolic
Sterols et Polyterpenes		++	++
Polyphenols		+++	+++
Flavonoïds		++	++
Tannins	Catéchic	−	−
	Gallic	−	−
Quinones		−	−
Alkaloïds	B	+	+
	D	++	+
Saponins		+++	−

B = Bouchardat; D = Dragendorff; C = Catechic; G = Gallic. Reaction strongly positive +++, - Reaction positive ++, - Reaction moderately positive +, Negative reaction −.

3.2. Analgesic Activities

3.2.1. Dorso-Abdominal Writhing Test Induced by 1% Acetic Acid

1) Analgesic potential

The following Figure 1 shows the percentage of inhibition of *Ximenia americana* extracts on the writhing induced by 1% acetic acid at 30 minutes.

At 30 minutes, the DXA reduced writhing by 8% at 0.25 mg/kg, reaching 90% at 150 mg/kg. The EHE XA reduced writhing by 10% at 0.25 mg/kg, reaching 94% at 150 mg/kg. Doses ≥ 2 mg/kg of both extracts showed a significant reduction in writhing compared to the control ($p < 0.05$). Both extracts exhibited a dose-dependent analgesic activity and were comparable to reference analgesics at doses ≥ 5 mg/kg (Paracetamol (83%) and Tramadol (98%)). However, the EHEXA stands out with slightly higher efficacy at low doses and a higher maximal inhibition (94% vs 90%).

2) Determination of pharmacodynamic parameters (efficacy-potency)

The following Figure 2 shows the Dose-inhibition percentage curve of *Ximenia americana* extracts on the writhing induced by 1% acetic acid at 30 minutes.

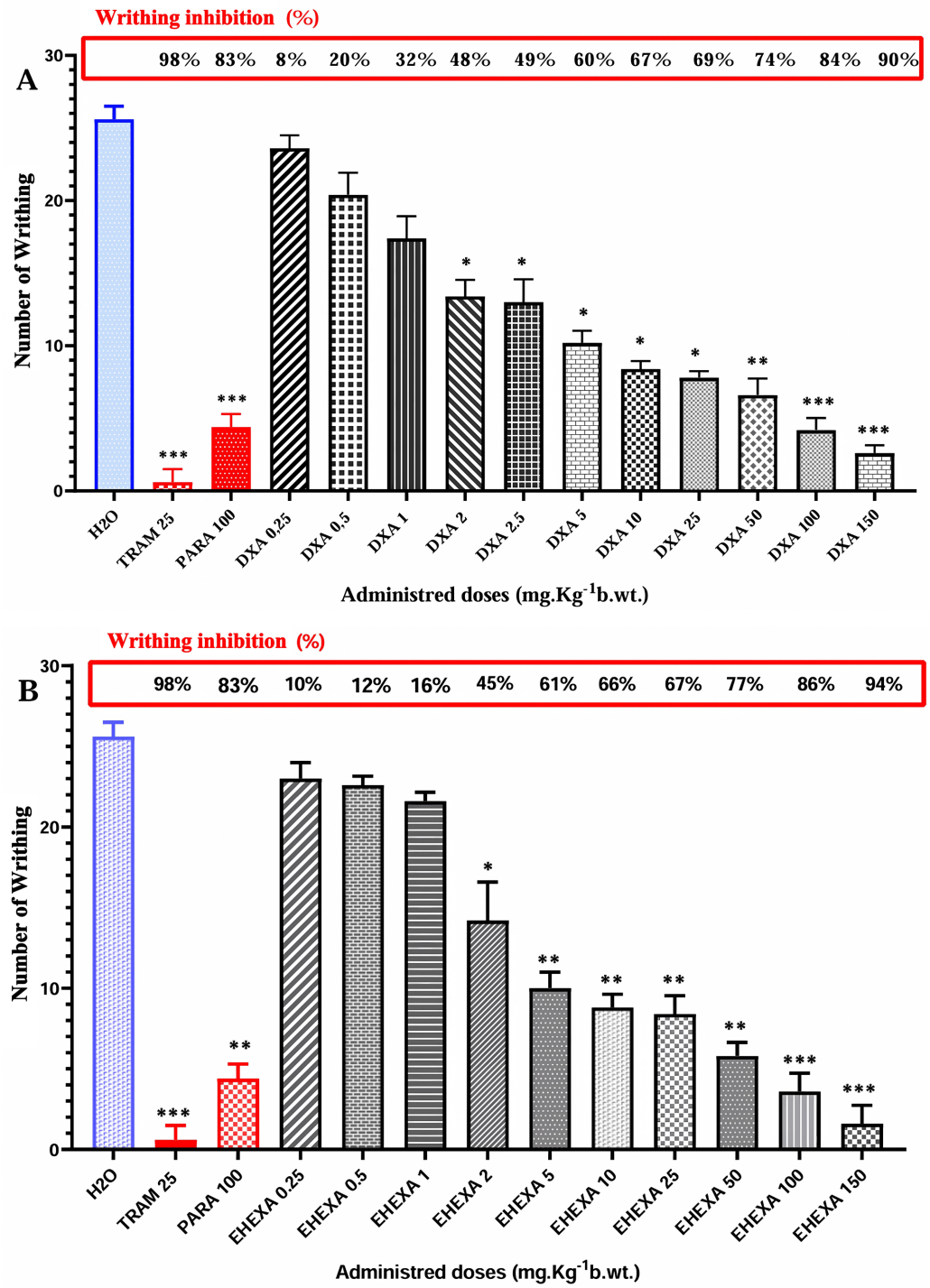


Figure 1. Effect of Ximenia americana stem bark extracts on acetic acid-induced abdominal writhing at T 30 minutes. Kruskal-Wallis Test: Values expressed as mean ± standard deviation; significance level α = 5%; Significant difference *p < 0.05; **p < 0.01; *** or ****p < 0.001; of extract compared to negative control group distilled water.

The pharmacological efficacy of *Ximenia americana* extracts (DXA and EHEXA), paracetamol and tramadol was found to be equivalent, with a maximum effect value (Emax) of 100%.

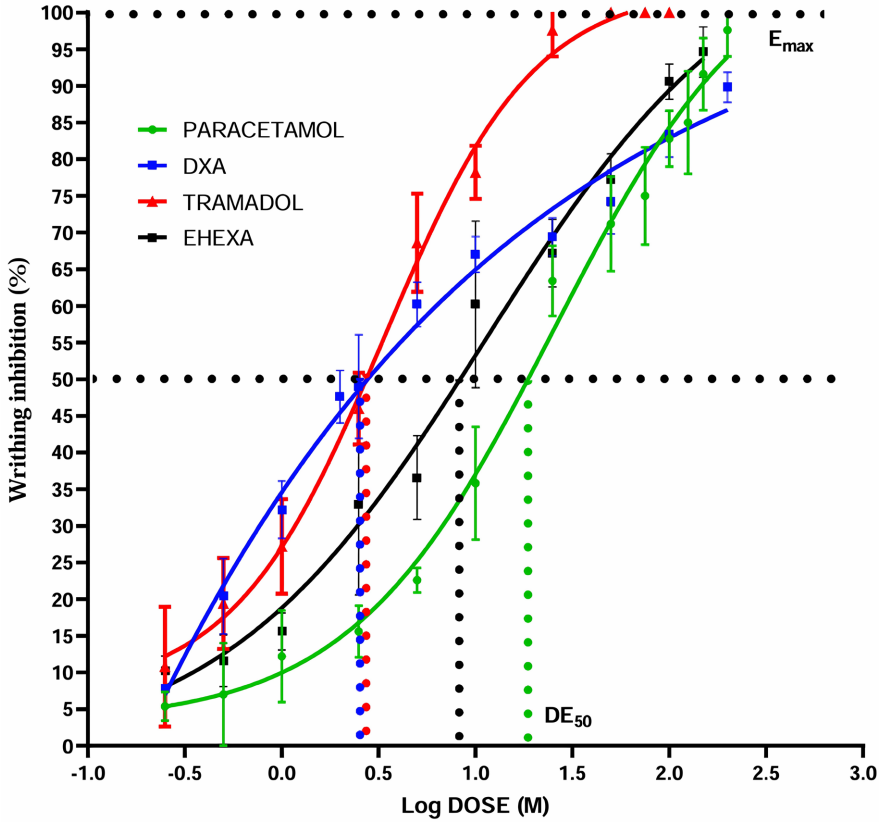


Figure 2. Dose-percentage curve for inhibition of Ximenia americana extracts at T 30 minutes. Norlin-fit test: Contortion inhibition rate; Values expressed as mean deviation. Significant difference in inhibition of extracts (DXA (p = 0.001) and EHEXA (p = 0.01) and tramadol (p = 0.001) compared with paracetamol. Significant difference between tramadol-EHEXA (p = 0.001) and tramadol-DXA (p = 0.129).

The pharmacological potency, as determined by the 50% effective dose (ED_{50}), was 2.81, 2.84, 7.94 and 19.05 mg/kg, respectively, for tramadol, DXA, EHEXA and paracetamol. The ED50 of paracetamol (19.05 mg/kg) is twice that of EHEXA (ED_{50} = 7.94 mg/kg); seven times that of DXA (2.84 mg/kg); and approximately six times that of tramadol (2.81 mg/kg). The ED_{50} of tramadol is approximately three times lower than that of EHEXA, and is broadly similar to that of DXA (ED_{50} = 2.84 mg/kg). The differences between the ED_{50} of DXA (2.84 mg/kg), EHEXA (7.94 mg/kg) and paracetamol (ED_{50} = 19.05 mg/kg) were statistically significant, with respective P values of p = 0.01 and p = 0.001. The ED_{50} of tramadol (2.81 mg/kg) was found to be statistically different from that of EHEXA (p = 0.01) and paracetamol (p = 0.001), but not statistically different from the ED_{50} of DXA (p = 0.129).

The pharmacological potency of DXA was found to be similar to that of tramadol, but greater than that of EHEXA and paracetamol at T30 minutes.

3.2.2. Formaldehyde-Induced Paw Irritation Test in Rats

1) Neurogenic phase

The following **Figure 3** presents the anti-inflammatory activity of the decoction

(DXA) and the hydroethanolic extract (EHEXA) of *Ximenia americana* compared to the control during the first phase (neurogenic phase) after the induction of inflammation by the injection of 2.5% formaldehyde.

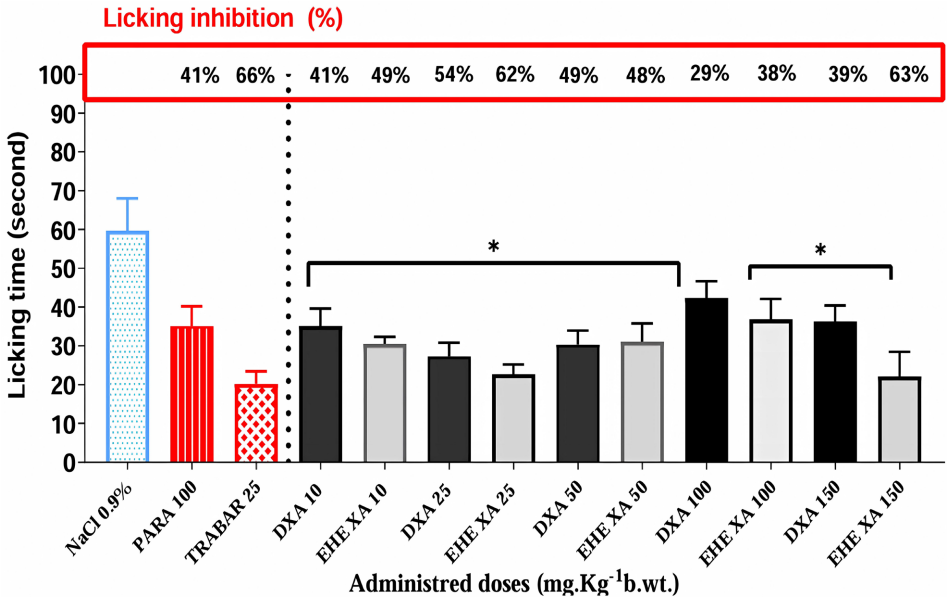


Figure 3. Effect of Ximenia americana stem bark extracts on neurogenic pain induced by 2.5% formaldehyde injection during the early phase. Kruskal-Wallis Test: Values expressed as mean ± standard deviation; significance level $\alpha = 5\%$; Significant difference *p < 0.05; **p < 0.01; *** or ****p < 0.001; tramadol compared to negative control group (NaCl 0.9%).

The DXA showed a significant reduction in neurogenic pain from the pain process induced by formaldehyde at doses of 25 mg/kg (54%) and 50 mg/kg (49%). In contrast, the EHEXA showed a significant reduction in neurogenic pain at doses of 10 mg/kg (49%), 25 mg/kg (62%), and 150 mg/kg (63%). Both extracts reached inhibition levels comparable to those of tramadol (66%) and paracetamol (41%) at specific doses of 25 mg/kg (54%) and 50 mg/kg (49%) for DXA, and 10 mg/kg (49%), 25 mg/kg (62%), and 150 mg/kg (63%) for EHEXA. No significant difference was observed between the extracts and the references at these doses. The EHEXA stands out with higher efficacy at a lower dose (10 mg/kg) and greater inhibition at 25 mg/kg (62% vs. 54% for the decoction). The effect of both extracts decreases over time.

2) Late phase

The following Figure 4 presents the anti-inflammatory activity of DXA and EHEXA compared to the NaCl 0.9% control during the second phase (inflammatory phase) after the induction of inflammation by the injection of 2.5% formaldehyde.

The DXA showed a significant reduction in inflammatory pain from the pain process induced by formaldehyde at doses of 10, 25, 50, 100, and 150 mg/kg, with respective inhibition percentages of 61%, 55%, 67%, 63%, and 77%. In contrast, the EHEXA showed a significant reduction in inflammatory pain at doses of 10,

25, 50, 100, and 150 mg/kg, with respective inhibition percentages of 66%, 79%, 71%, 63%, and 81%. The EHEXA demonstrated slightly higher inhibition rates than the decoction at almost all doses (for example, 79% at 25 mg/kg vs. 55% for the decoction). Both extracts reached inhibition levels comparable to those of ketoprofen (69%) and acetylsalicylic acid (57%).

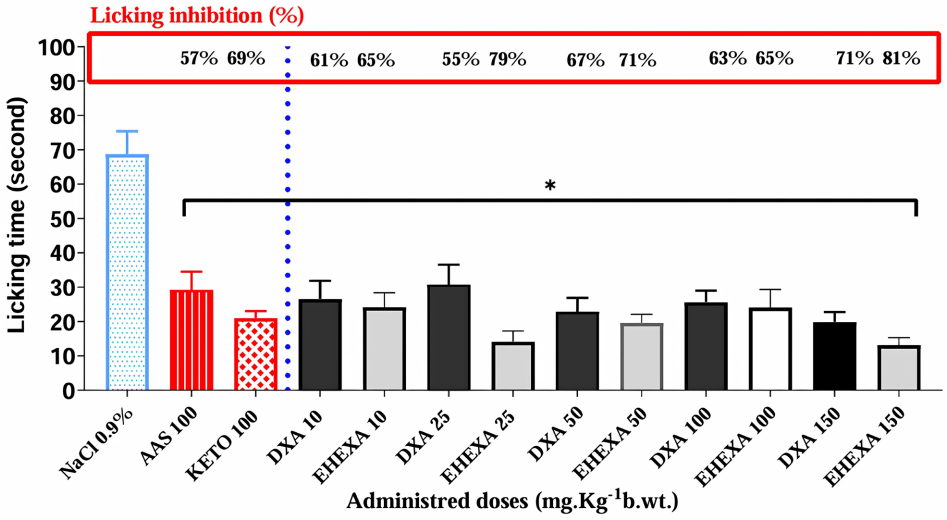
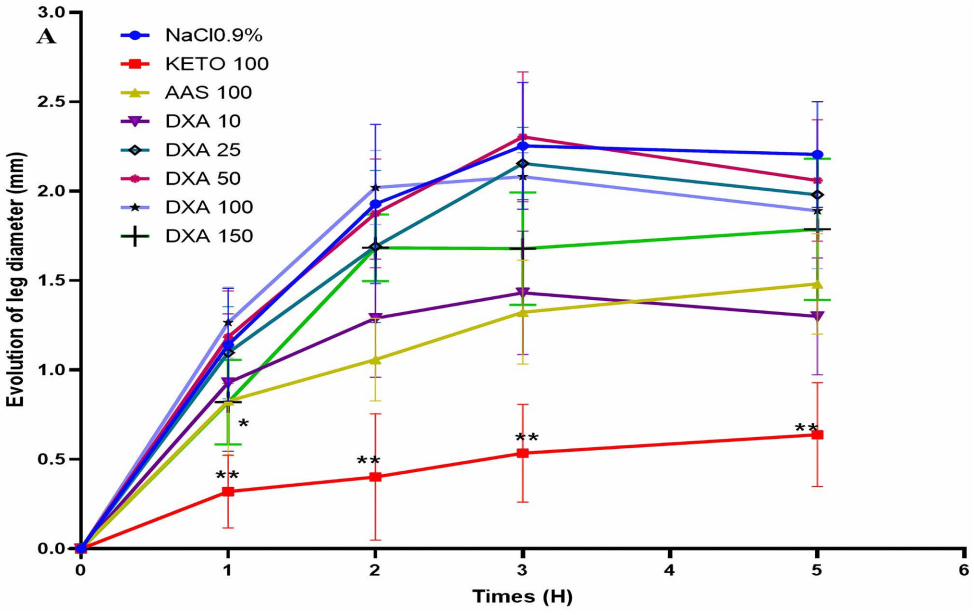


Figure 4. Effect of Ximenia americana stem bark extracts on inflammatory pain induced by 2.5% formaldehyde injection during the late phase. Kruskal-Wallis Test: Values expressed as mean ± standard deviation; significance level $\alpha = 5\%$; Significant difference *p < 0.05; **p < 0.01; *** or ****p < 0.001; tramadol compared to negative control group (NaCl 0.9%).

3.2.3. Carrageenan-Induced Paw Irritation Test in Rats

The following **Figure 5** presents the anti-inflammatory activity of the decoction compared to the control during inflammation induced by the injection of 1% carrageenan.



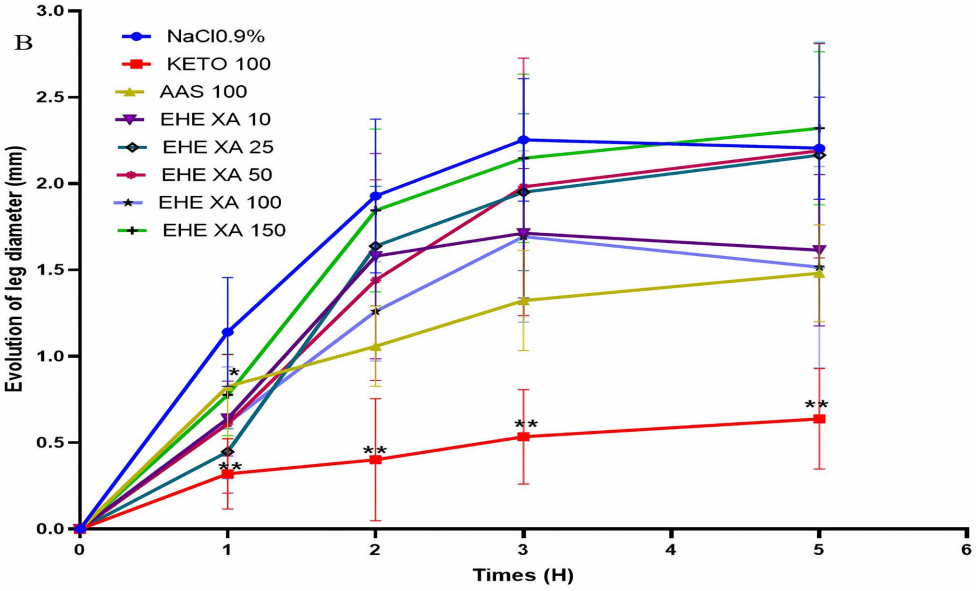


Figure 5. Effect of Ximenia americana bark extracts (decoction and hydroethanolic extract) on inflammatory edema induced by the 1% carrageenan injection from T1h to T5h. Kruskal-Wallis test: Values expressed as mean ± standard deviation; α = 5% risk; Significant difference *p < 0.05; **p < 0.01; *** or ****p < 0.001; tramadol compared to negative control group (NaCl 0.9%).

With DXA at T3h and T5h, only the 10 mg/kg dose showed a significant reduction (36%) in the carrageenan-induced inflammatory edema. This was favorably compared to ketoprofen (76%) and acetylsalicylic acid (41%). On the other hand, EHEXA at T1h significantly reduced inflammatory edema at doses of 25, 50, and 100 mg/kg (61%, 47%, 46%) with effectiveness comparable to ketoprofen (72%).

4. Discussion

The decoction of *Ximenia americana* bark is utilised in traditional medicine for a variety of ailments; however, there is a paucity of studies that have evaluated its pharmacological properties. The aim of this study was to investigate the analgesic and anti-inflammatory activity of the decoction and hydroethanol extract of *Ximenia americana.* The analgesic activity of the extracts was determined by the Writhing test coupled with the formaldehyde test (neurogenic phase), while the anti-inflammatory effect was determined by the formaldehyde test in its second phase (inflammatory phase) combined with the carrageenan test [24] [28]-[30].

Intraperitoneal injection of 1% acetic acid has been demonstrated to induce a pain syndrome, the aetiology of which is attributed to the release of numerous chemical mediators that are involved in the process of pain, including histamine, prostaglandins (PGE2 and PGEα), serotonin and bradykinin [31]. The administration of a substance that possesses analgesic, anti-inflammatory and/or muscle relaxant properties has been shown to be efficacious in the counteraction of the pain induced by acetic acid.

In the acetic acid-induced pain model, both *Ximenia americana* extracts (DXA and EHEXA) demonstrated a dose-dependent analgesic effect, with a range of 8%

to 90% for DXA and 10% to 94% for EHEXA, at doses ranging from 0.25 to 150 mg/kg. Conversely, at low doses ranging from 0.25 to 1 mg/kg, no significant contortion inhibitory activity was observed for either extract compared with the control. A subsequent comparison of the different doses for each extract revealed that the doses of 5, 10 and 25 mg/kg showed no significant difference between them, and the same was true for doses of 100 and 150 mg/kg. Conversely, a significant difference was observed between 25 and 50 mg/kg, and also between 50 and 100 mg/kg.

The analgesic effect of *Ximenia americana* bark has been the subject of several studies, yet none have attempted to quantify this effect. A comparison of the pharmacodynamic parameters (E_{max} and ED_{50}) of the activity of the two extracts suggests that they possess equivalent pharmacological efficacy, with the aqueous DXA extract being more potent than the hydroethanol extract (EHEXA).

In fact, based on the analysis of dose-response curves, it can be deduced that the two extracts would have similar pharmacological efficacy, comparable to that of paracetamol and tramadol, since they all have efficacy levels (E_{max}) approaching 100%.

However, while exhibiting equivalent efficacy, the potency of the DXA extract (ED_{50} = 2.84 mg/kg) surpasses that of EHEXA (ED_{50} = 7.94 mg/kg) and is analogous to that of tramadol (ED_{50} = 2.81 mg/kg).

In the present study, a comparison was made between the results obtained and those of Soro *et al.* [13]. The latter researchers found that doses of 25, 50 and 100 mg/kg of *Ximenia americana* decoction, administered intraperitoneally, induced 40.34%, 53.3% and 61.1% of significant acetic acid-induced contortion inhibition at 10 minutes. In contrast, in our study, *Ximenia americana* decoction, administered at equivalent doses of 25, 50 and 100 mg/kg orally, resulted in a pronounced enhancement in contortions, with inhibition rates of 69%, 74% and 84%, respectively. This observation suggests that the active constituents of the decoction might undergo a significant activation process in response to the application of heat [32] [33]. Alternatively, the active constituents could undergo metabolic processes during intestinal absorption and hepatic passage, thereby enhancing their biological activity [34]-[37].

In the research conducted by Silva *et al.*, the oral administration of a dose of 100 mg/kg of the total polysaccharide fraction of *Ximenia americana* resulted in a 44% inhibition of abdominal contortions. In comparison with the results obtained at the same dose of 100 mg/kg, the decoction exhibited a substantially higher inhibitory effect, reaching 84% inhibition of contortions [8].

In a similar manner, Konaté *et al.* in Burkina Faso also demonstrated that oral administration of the polyphenol-rich fractions of *Ximenia americana* at high doses of 200, 250 and 300 mg/kg, was observed to result in a significant analgesic effect against contortions induced by 0.7% acetic acid, with a percentage of protection of 51.2%, 60.4%, and 73.14%, respectively [14]. Consistent with the findings of Konate *et al.*, the present study also observed such levels of inhibition, albeit at

lower doses. Specifically, at doses ranging from 5 to 50 mg/kg, which correspond to 50- and 6-times lower concentrations than those employed by Konate *et al.*, the study recorded inhibition rates ranging from 60 to 74% [14].

In a recent study, Pessoa *et al.* demonstrated that a hydroethanol extract (50:50) of *Ximenia americana*, administered at doses of 50, 100 and 200 mg/kg, resulted in a significant reduction in abdominal contortions of 84.99%, 87.99% and 89.49%, respectively, compared with the control group ($p < 0.0001$) [38].

Phytochemical characterisation of *Ximenia americana* extracts revealed the presence of several classes of bioactive compounds, including total polyphenols, flavonoids, alkaloids, sterols, polyterpenes and saponins (in the decoction). These phytochemical compounds are thought to play a synergistic role in analgesic activity [39] [40].

The results of the present study are consistent with those obtained by Sharief *et al.*, and Soro *et al.*, in their research on aqueous extracts of *Ximenia americana*, which also identified the presence of sterols, polyterpenes, polyphenols, flavonoids, alkaloids, saponins and catechic tannins [13] [41].

As the Writhing test is a non-specific test for analgesic activity, the formaldehyde test was carried out to confirm this potential, as this experimental model is specific for assessing the analgesic activity of substances. In fact, the two extracts DXA and EHEXA, by significantly reducing the licking time during the neurogenic phase (T0 to T 5min), show that they have analgesic activity. The effects observed between 10 and 150 mg/kg were not found to be dose-dependent, in contrast to the outcomes observed with the Writhing test.

These results are not consistent with those of Soro *et al.*, who demonstrated that the *Ximenia americana* infusion did not significantly reduce pain intensity during the neurogenic phase of the formaldehyde test, with 6.66% inhibition [13]. In contrast, the present results are consistent with those of Pessoa *et al.*, who demonstrated that the hydroethanol extract of *Ximenia americana* at doses of 50, 100 and 200 mg/kg induced a significant inhibition of neurogenic pain of 77.63%, 59.37% and 58.44%, respectively, compared with the negative control group. Furthermore, during the inflammatory phase (T15 - T30 min) of the formaldehyde test, doses of 10 to 150 mg/kg of *Ximenia americana* extracts significantly reduced inflammatory pain, with inhibition rates ranging from 55% to 81%. However, the difference in activity between doses was not significant, with an effect comparable to those of ketoprofen (69%) and acetylsalicylic acid (57%), also revealing that they have anti-inflammatory potential. These results corroborate those of Soro *et al.*, who showed that the inhibition of inflammation by *Ximenia americana* infused at a dose of 100 mg/kg was around 83.33%. Furthermore, these results are consistent with those of Pessoa *et al.*, who concluded that the hydroethanol extract (50:50) of *Ximenia americana*, at doses of 50, 100 and 200 mg/kg, induced a significant inhibition of 56.74%, 69.43% and 54.02% of the inflammatory pain of the formaldehyde test compared with the control group, respectively. [39] In conclusion, both DXA and EHEXA appear to have an inhibitory effect on both phases of

pain, with a more pronounced effect on inflammatory pain.

It is imperative to acknowledge the complexity of inflammation as a physiological response orchestrated by the immune system in response to various forms of aggression, including infection, injury, and chemical irritation. This response is characterised by a specific set of clinical manifestations, known as the inflammation tetrad, which encompasses redness, warmth, swelling (oedema), and pain [16]. Given oedema's inherent nature as a component of the inflammatory process, it was imperative to assess the anti-oedematous potential of *Ximenia americana* extracts. To this end, the carrageenan test was performed. This test involves the injection of carrageenan, which has been shown to induce the production of preformed (e.g., histamine, serotonin, bradykinin) and neoformed (e.g., cytokines, TNF-α, IL-1, IL-6, prostaglandins, leukotrienes) mediators [42]. The resulting effects of this process include vasodilatation, increased vascular permeability and the formation of oedema, which in turn can cause hyperalgesia [42]. The anti-inflammatory properties of DXA were evident in a dose-dependent manner, with a significant reduction in oedema observed from the 3rd to the 5th hour at a dose of 10 mg/kg. This finding suggests that the extract possesses an anti-oedematous effect, with no effect observed at either 1 or 2 hours. These results indicate that the anti-oedematous active ingredients require a considerable time to take effect. In contrast, no such effect was observed at doses exceeding 10 mg/kg, suggesting that the anti-oedematous properties of the extract may be concentration-dependent. This observation aligns with the hypothesis that the active constituents of the extract require time to disengage from the complexes formed through interactions with the plant extracts. It is postulated that the solubility of the extract influences the release of these active ingredients, with more dilute solutions facilitating their release and subsequent activity [43]-[45]. This phenomenon was also observed in the work of Da Silva *et al.* [46].

Concerning EHEXA, all doses showed a fleeting anti-oedematous activity that appeared as early as the 1st hour, showing that EHEXA is therefore richer in compounds with anti-oedematous properties. Conversely, no effect was observed from the 2nd to the 5th hour. The fleeting anti-oedematous effects which appeared at the 1st hour at doses of 10 to 150 mg/kg with EHEXA differ from those observed by the team of Kimondo *et al.* In this study, a hydromethanolic extract (20:80] at a dose of 400 mg/kg, the effect began as early as the 1st hour. This discrepancy may be attributable to the higher dosage employed in the study by Kimondo *et al.*, as well as the nature of the extract [47]. The extract utilised in the present study is a hydroethanolic extract, subjected to 24 hours of maceration, whereas the extract employed in the study by Kimondo *et al.*, is a hydromethanolic extract, subjected to 72 hours of maceration. The secondary metabolites of plants include compounds of varying polarity (flavonoids, alkaloids, terpenoids, etc.). Because of its high polarity, methanol can be used to extract a wide range of polar compounds (e.g., flavonoids, phenolic acids, tannins). Ethanol, on the other hand, is less polar and more selective, favouring the extraction mainly of compounds of moderate

polarity (e.g. alkaloids, certain flavonoids) [48]-[50].

The findings of Da Silva *et al.*, conducted at doses of 50, 100 and 250 mg/kg, further substantiated the anti-oedematous efficacy of EHEXA, evident from the initial hour up to the fifth hour, with respective percentages of inhibition recorded at 50.13%. To 68.96% at the 50 mg/kg dose, 35.10% to 61.05% at the 100 mg/kg dose and 14.41% to 25.20% at the 250 mg/kg dose [46]. The discrepancy in the anti-oedematous activity observed between the present study and that of Da Silva *et al.*, may be attributed to the methodology employed in the preparation of the extracts. The extract utilised by Da Silva *et al.*, was obtained through a hydroethanol extraction process (50:50), whereas the hydroethanol extraction employed in the present study was conducted with a different proportion (30:70), utilising a lower amount of ethanol. Furthermore, the duration of the maceration process differed, with the study by Da Silva *et al.*, lasting 72 hours, whereas our study spanned 24 hours. This suggests that the active anti-oedematous constituents are more readily soluble in alcohol.

5. Conclusion

The analysis of the tests utilised to evaluate the analgesic and anti-inflammatory activity of the decoction and the hydroethanol extract of *Ximenia americana* demonstrated that they exhibited significant analgesic activity. Furthermore, it was observed that only the hydroethanol extract (EHEXA) demonstrated significant anti-oedematous activity from the 1st hour, though this effect was transient at the doses employed. Consequently, *Ximenia americana* extract was found to be more efficacious in its analgesic properties than in its anti-inflammatory capacity. The findings of this study indicate that *Ximenia americana* extracts have the potential to serve as effective natural alternatives to synthetic analgesics, exhibiting pharmacological efficacy comparable to that of paracetamol and tramadol.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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