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Antinociception Mechanisms Involved in the Hydroalcoholic Stem Bark Fraction of *Xeroderris stuhlmannii* (Taub.) Mendonça & E.P.Sousa (Fabaceae)

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ABSTRACT

The present study aims to evaluate the antinociceptive activity of the hydroalcoholic fraction from the stem bark of *Xeroderris stuhlmannii* (Taub.) Mendonça & E.P.Sousa ??? and its probable mechanisms. The antinociceptive activity of HAFXS (50,100, and 200 mg/kg) was determined using the acetic acid writhing test (1%), formalin test (1%), tail immersion test (54 ± 1 °C), capsaicin (32 µg/ml), and cinnamaldehyde tests (0.66 %). Possible pathways mediating antinociceptive effects were evaluated using the following antagonists: propranolol, prazosin, yohimbine, atropine, glibenclamide, tetraethylammonium, naloxone, and L-NAME. The HAFXS (200 mg/kg) showed significant (P<0.0001) inhibition of abdominal writhing induced by acetic acid (72.24 %), formalin (in the first phase, 51.89 %), capsaicin (72.37 %), or cinnamaldehyde (56.48 %). HAFXS also significantly increased (p<0.0001) the latency time of tail immersion in hot water with a maximum time of 7.53 seconds. Pre-treatment with propranolol, yohimbine, and atropine did not reverse the antinociceptive activity of HAFXS. However, the previous injection of naloxone, glibenclamide, and prazosin to the animals significantly reduces the analgesic activity of HAFXS, indicating that antinociceptive activity is mediated by the opioid system and α_1-adrenergic receptors. Furthermore, data analysis indicates that the mechanisms underlying HAFXS analgesia could also be linked to its ability to modulate TRPA1 and TRPV1 channels. This study demonstrates the antinociceptive properties of HAFXS, which act through various mechanisms.

Keywords: Antinociception, Hydroalcoholic fraction, Mechanism, *Xeroderris stuhlmannii*

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INTRODUCTION

The revised definition of pain by the International Society for the Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" [1]. Pain is a common symptom of many diseases and can also result from surgery or trauma [2]. Pain is also a pathological condition that negatively affects individuals' lifestyles with enormous financial implications [3]. The notion of pain is multidimensional, involving various aspects such as sensory, physiological, cognitive, affective, behavioral, and spiritual [4]. Nowadays, pain is not just a major global issue. It is also responsible for the growing number of disabilities in the world [5]. Many synthetic analgesics have been developed and used for pain treatment, however, some of them, such as opioids and non-steroidal anti-inflammatory drugs, have associated side effects such as dependence, tolerance, respiratory depression, gastrointestinal disorders, peptic ulcers, nephrotoxicity, leucopenia, and allergic reactions [6, 7]. Accordingly, one of the interesting strategies to overcome these multiple problems is the development of new drugs that lack adverse effects. The traditional pharmacopoeia approach represents an alternative as a source of new pharmaceutical agents. *X. stuhlmannii*(Taub.) Mendonça & E.P.Sousa, a small tree scattered in open forests or wooded savanna [8], is widespread in tropical Africa, from Senegal to Kenya and Zimbabwe. In Cameroon, *X. stuhlmannii* is found in the West region. Different parts of the tree are used in African and Cameroonian medicine for the management of pain-related conditions. Preliminary pharmacological studies have indicated that the hydroalcoholic fraction of *X. stuhlmannii* exhibits greater analgesic activity than the hydroalcoholic extract. The purpose of the present study is to test the anti-nociceptive activity and to elucidate the possible mechanisms of action as well as signaling pathways through which the hydroalcoholic stem bark fraction of *X. stuhlmannii* acts.

MATERIALS AND METHODS

Medicine and Chemicals

Naloxone, Prazosin, Glibenclamide, Yohimbine, Propranolol, Atropine, Tetraethylammonium, Naloxone, Capsaicin, Cinnamaldehyde, Camphor, Ruthenium Red, (Sigma Aldrich, purchased in Germany); N(G)-Nitro-L-arginine methyl ester, acetylsalicylic acid (Sigma Aldrich, purchased in USA); Tramadol, Morphine, Glacial Acetic Acid (VWR CHEMICALS, France).

Animals

Experiments were performed using rodents (Swiss mice between 18 - 25 grams of body weight and Wistar rats weighing 120-200 grams) of both sexes, reared in a standard polypropylene enclosure in the breeding facility of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala. All protocols applied in this study were revised and approved by the national guidelines established by the Institutional Ethics Committee of the University of Douala, Cameroon (N_o 4006CEI-UDo/09/2023/T). The animals were kept in standard ambient conditions under ad libitum access to food and water. Before each experiment, animals were food-deprived for the last 6 hours.

Plant Material

Fresh stem barks of *X. stuhlmannii* were collected from Bitchoua locality, Ndé Department of the West Cameroon region, in January 2020. Herbarium specimen validation was done by comparison with the sample No. 6011/SRF/CAM.

Preparation of Plant Materials

The stem barks of *X. stuhlmannii* were cut, air dried, and powdered. Powder (100 g) was soaked in a solvent mixture of ethanol/water (600 mL, 70/30, v/v), sonicated (10 min), shaken at room temperature (30 min), and filtered. Ethanol was removed from the filtrate under reduced pressure, and the remaining aqueous extract was repeatedly defatted with n-pentane (3 x 300 mL, each), then both fractions were freeze-dried. The yields of the corresponding fractions were between 5-9% (hydroalcoholic) and 2- 4% (pentane).

Antinociceptive Study

Acetic Acid Test

The antinociceptive activity of the hydroalcoholic fraction from the stem bark of *X. stuhlmannii* (HAFXS) was investigated on acetic acid-induced pain in mice, by a procedure previously described by Koster *et al*. [9]. Subjects were grouped (n = 6), group I, a control, received distilled water (10 ml/kg), while group II, a standard, received aspirin and the remaining groups III, IV, and V used as test groups received plant preparation at different doses (50, 100 and 200 mg/kg). Animals were dosed *per os* with HAFXS, acetylsalicylic acid, or distilled water exactly thirty minutes previous pain induced by intraperitoneal injection of acetic acid (1%, 10 mL/kg) to each mouse. After five minutes following acetic acid injection, the total number of abdominal writhings, which indicate pain sensation, were was counted for 30 minutes. Antinociceptive activity was expressed as percentage inhibition of writhes relative to the control group, determined according to the following equation:

$$\text{Inhibition percentage (PI)} = ((N_{control} - N_{treated}) / N_{control})) \times 100$$

Where N is the average number of abdominal writhings of the control group or treated group.

Formalin Test

This test was performed as previously described by Tjolsen *et al*. [10]. Following randomisation of animals in the five groups as described below, they were pretreated orally with *X. stuhlmannii* (50, 100, 200 mg/kg), tramadol (10 mg/kg), or distilled water (10 ml/kg). Thirty minutes later, each mouse underwent aponeurotic injection of formalin (1%, 20 µL) on the left hind paw. The mice were placed separately in a plexiglass vivarium for observation. The time during which the mouse spent licking the paw intensively, indicating formalin-induced pain, could be recorded in two distinct periods: the first period, named the neurogenic phase, occurs between 0 and 5 minutes, and the second period, also called the inflammatory phase, which occurs later between 20 and 30 minutes subsequent to formalin injection. Effect, expressed as the percentage of inhibition, was quantified as the reduction of licking time at each phase using the following equation:

$$\text{Inhibition percentage (PI)} = [(T-T') / T] \times 100$$

Where T represents the value of licking time of the control group at each phase, and T' represents the value of the licking time of the assay group for each phase [11].

Tail Immersion Test

This test was undertaken according to the method described by D'amour and Smith [12]. The rats used were previously acclimatized to heat (hot water, 54 °C) for 5 days. A total of forty-two rats were preselected to conduct the test, based on the time spent in hot water before tail withdrawal. After randomization into 7 groups (n = 6), control group, received distilled water (vehicle, 10 ml/kg), standard group, received tramadol (20 mg/kg) as reference drug, assay groups were treated with the plant preparation (50, 100 and 200 mg/kg) and the two last groups received intraperitoneal injection of naloxone 15 min before tramadol (20 mg/kg) or plant preparation (200 mg/kg) dosing, respectively. Thirty minutes after administering the different substances, the rat's tail was immersed in a water bath (54 °C) to induce pain. The latency time in the water bath before tail withdrawal was recorded before (t = 0), at 30 min, 1, 2, 3, 4, 5, and 6 h after substance administration.

Capsaicin Test

The procedure described by Mesia-Vela *et al*. [13] was carried out. The mice randomized in 5 groups (n = 7) were pre-treated with distilled water (10 ml/kg) for group 1, red ruthenium (5 mg/kg), a reference drug for group II, plant preparation at the doses of 50, 100 and 200 mg/kg for groups III, IV and V respectively. After 30 min, capsaicin solution (30 µl, 32 µg/mL) was injected into the sole of the paw of each mouse, and animals were observed for 5 min inside the cage. The time it took for each mouse to lick its paw following capsaicin injection was calculated and considered a nociceptive indication. The inhibition percentage of paw licking time was expressed by the following equation:

$$\text{Inhibition percentage (PI)} = [(T-T') / T] \times 100$$

Where T represents the value of licking time of the control group and T' represents the value of licking time of the assay group.

Cinnamaldehyde Test

The method was conducted as described by Rodrigues *et al*. [14]. After randomisation into 5 groups of 7 mice each, the mice were previously treated *per os* with distilled water (10 ml/kg) as vehicle, camphor (7.6 mg/kg), as reference drug, or HAFXS (50, 100, 200 mg/kg), as plant preparation to be tested. Thirty minutes after the substance's administration, cinnamaldehyde solution (20 µL, 0.66%) was injected into the sole of the hind paw of animals. Immediately after injection, each mouse was isolated in a plexiglass cage, and the time during which it licked the injected paw was recorded for five minutes. The percentage of inhibition of paw licking time was expressed using the equation described above.

Assessment of the Pharmacological Mechanisms of Antinociceptive Effect of Hydroalcoholic Fraction from the Stem Bark of *X. stuhlmannii*

The possible mechanisms underlying the antinociceptive effect of HAFXS were investigated using methods previously described by Sawada *et al*. [15] and Olorukooba and Odoma [16]. The possible implication of the noradrenergic system in the antinociceptive

effect of HAFXS was undertaken using an acetic acid-induced writhing test. A total of fifty-four mice were preselected and randomized into nine groups of six mice each. Distilled water (10 ml/kg), morphine (10 mg/kg), or extract (200 mg/kg) were administered *per os* to the first, second, and third groups, respectively. Groups four and five were dosed with prazosin (1 mg/kg, *i.p*) or prazosin + plant preparation (200 mg/kg). The sixth and seventh groups were treated with yohimbine (1 mg/kg, *i.p*) or yohimbine + plant preparation (200 mg/kg). The last two groups (eight and nine) were treated with propranolol (10 mg/kg, *i.p*) or propranolol + plant preparation (200 mg/kg).

To assess the involvement of the cholinergic system in the antinociceptive mechanism of *X. stuhlmannii*, thirty mice were divided into five groups (n = 6). Distilled water (10 ml/kg), morphine (10 mg/kg), or extract (200 mg/kg) by oral route to the first, second, and third groups, respectively. The fourth and fifth groups were injected with atropine (1 mg/kg, *i.p*). Fifteen minutes later, the fifth group also received HAFXS (200 mg/kg) orally.

To evaluate the potassium channel pathway in the antinociceptive effects of the hydroalcoholic fraction of *X. stuhlmannii*, a sample of forty-two animals was divided into seven groups of six mice each. The first three groups received *per os distilled* water (10 ml/kg), morphine (10 mg/kg), or the HAFXS (200 mg/kg), respectively. The fourth and fifth groups were treated with glibenclamide (5 mg/kg, *i.p*), an ATP-K^+ channel inhibitor, or glibenclamide + HAFXS (200 mg/kg), respectively. The sixth and seventh groups received tetraethylammonium (4 mg/kg, *i.p*), a voltage-dependent K^+ channel antagonist, or tetraethylammonium + HAFXS (200 mg/kg), respectively. Thirty minutes after substance post-treatment, the acetic acid-induced writhing test was performed. The role of the opioid system in the antinociceptive effect of hydroalcoholic fraction of *X. stuhlmannii* was assessed in forty-two animals grouped (n = 6) as follows: Groups I, II, and III were treated orally with distilled water (10 ml/kg), morphine (10 mg/kg), or HAFXS (200 mg/kg). Groups IV and V were prior injected with naloxone (10 mg/kg, *i.p*), 15 min after Group V received HAFXS (200 mg/kg) orally. After 30 minutes following treatment, the animals received an injection of formalin (1%) and the time spent licking the paw was evaluated for 30 min in two periods as previously described in the formalin test.

To evaluate the involvement of the NO pathway in the antinociceptive effects of HAFXS, L-NAME (10 mg/kg, i.p.), a NOS inhibitor, was administered to mice 30 min before HAFXS (200 mg/kg, p.o). 15 minutes after HAFXS treatment, the animals received an injection of formalin (1%) and the time spent licking the paw was evaluated for 30 min in two phases as previously described in formalin test.

Statistical Analysis

The determination of the means and the illustration graphs were carried out using Microsoft Excel. The values were expressed in the form of means ± ESM. The Ordered Analysis of variance (ANOVA) applied using the GraphPad Prism version 5.03 software, followed by Tukey or Bonferroni post-tests, was used to compare the means of the various parameters studied. Statistical significance was assigned at a *p*-value of less than 0.05.

RESULTS

Antinociceptive Effect

Effect of Hydroalcoholic Stem Bark Fraction of *X. stuhlmannii* on Acetic Acid-induced Pain

HAFXS (50, 100, and 200 mg/kg) dose-dependently diminished (P<0.0001) the number of writhes triggered by peritoneal injection of acetic acid (Fig. 1). The maximum percentage of inhibition of writhes quantified was 72.24 % at the dose of 200 mg/kg of body weight, while acetylsalicylic acid, used as a reference drug, reduced writhes by only 45.05 % (10 mg/kg) compared to the distilled water control.

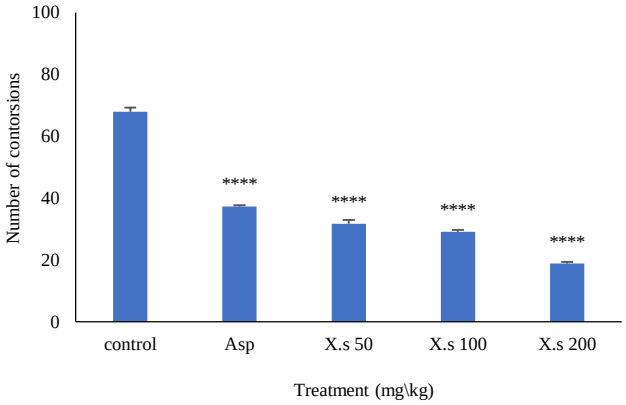

Fig. 1 Effects of different treatments on the number of abdominal writhings in acetic acid-induced pain. Each bar presents the mean of abdominal contortion ± ESM; n=6; ****p<0.0001; ***p<0.001; significant difference compared to the distilled water control; One-way ANOVA followed by Tukey post-test. Asp: acetylsalicylic acid.

Effect of Hydroalcoholic Fraction of the Stem Bark of *X. stuhlmannii* on Formalin-Induced Pain

The biphasic pain characteristic, induced in mice by a formalin injection, is licking of the injected paw. Administration of HAFXS at a dose of 50 mg/kg, noteworthily (P<0.0001) reduced the licking time in mice with an inhibition percentage of 51.89 % during the neurogenic pain (early phase) and 36.94% during the inflammatory pain (late phase). Tramadol (standard) showed 47.78 % (P<0.001) and 32.74 % (P<0.0001) of inhibition during the early phase and late phase of formalin-induced pain, respectively (Fig. 2).

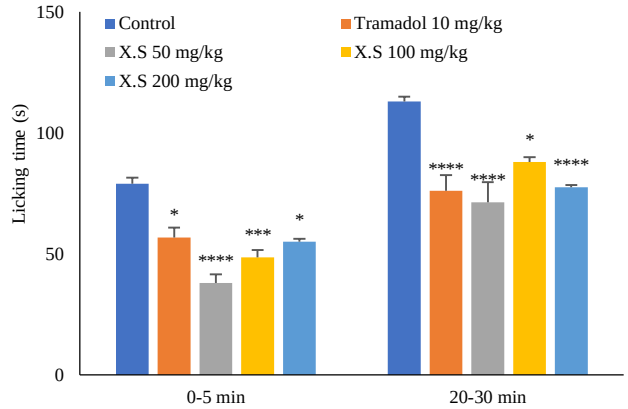

Fig. 2 Effects of different treatments on paw licking time in formalin–induced pain. Each bar presents the mean of licking time of the injected paw ± ESM; n=6; ****p<0.0001; ***p<0.001; *p<0.05 significant difference compared to the control; Two-way ANOVA followed by Bonferroni post-test. X.s: *X. stuhlmanni*.

Effect of Hydroalcoholic Stem Bark Extract of *X. stuhlmannii* on Latency Time in Tail Immersion Test

The administration of HAFXS noticeably (p <0.0001) enhanced the latency time of rat tail immersion from the first to the fourth hour of the test compared to the control (Table 1). The highest immersion times of the tail in hot water maintained at 54 °C were 6.90 seconds and 7.53 seconds at the second hour at the doses of 100 and 200 mg/kg, respectively, compared to negative control animals at the same time (1.80 seconds). Tramadol, used as

standard, also significantly increased the tail immersion latency time at the fourth hour ($p<0.0001$), reaching a maximum of 7.31 seconds compared to 2.05 seconds in the control group. In contrast, we observed that pre-treatment of rats with naloxone has remarkably decreased the withdrawal time of the tail of animals that received tramadol or HAFXS in addition.

Table 1 Effect of HAFXS on latency time of tail withdrawal of different groups following pain induced by hot water

Treatment	Dose	Duration of the test (h)							
		0 h	1/2 h	1 h Latency time (s)	2 h	3 h	4 h	5 h	6 h
Control		1.97 ± 0.08	2.94 ± 0.13	2.35 ± 0.29	1.80 ± 0.16	1.19 ± 0.19	2.05 ± 1.17	1.66 ± 0.14	1.81 ± 0.13
Tramadol	20 mg/kg	2.26 ± 0.31	4.13 ± 0.18	5.62 ± 0.3 ****	5.91 ± 0.2 ****	6.97 ± 0.15 ****	7.31 ± 0.36 ****	5.90 ± 0.65 ****	4.88 ± 0.77 ****
HAFXS	50 mg/kg	2.50 ± 0.29	3.01 ± 0.18	3.29 ± 0.45	3.94 ± 0.45 **	3.65 ± 0.26 *	2.71 ± 0.16	2.22 ± 0.14	1.93 ± 0.10
	100 mg/kg	2.69 ± 0.39	3.96 ± 0.17	5.80 ± 0.30 ****	6.90 ± 0.35 ****	6.30 ± 0.16 ****	5.85 ± 0.43 ****	5.33 ± 0.47 ****	4.66 ± 0.23 ****
	200 mg/kg	2.72 ± 0.29	3.42 ± 0.39	4.90 ± 0.27 ****	7.53 ± 0.52 ****	5.67 ± 0.28 ****	4.93 ± 0.12 ****	4.14 ± 0.17 ****	3.86 ± 0.25 **
Naloxone + HAFXS	5+200 mg/kg	2.18 ± 0.21	2.64 ± 0.16	3.24 ± 0.26	4.07 ± 0.19 ***	3.43 ± 0.38	3.07 ± 0.20	2.74 ± 0.33	2.49 ± 0.22
Naloxone + Tr	5+20 mg/kg	2.52 ± 0.15	3.44 ± 0.26	3.55 ± 0.18	3.05 ± 0.26	3.76 ± 0.41 *	3.99 ± 0.14 **	4.66 ± 0.35 ****	5.02 ± 0.16 ****

****p< 0.0001; ***p< 0.001; **p< 0.01; *p< 0.05 significant difference compared to the control; Two-way ANOVA followed by Bonferroni posttest; Tr=Tramadol.

Effect of Hydroalcoholic Fraction Stem Bark of *X. stuhlmannii* on Paw Licking Time in Capsaïcin and Cinnamaldehyde-induced Pain

As shown in Figure 3 below, HAFXS administration was markedly reduced at all lab-tested doses, the nociception induced after intraplantar injection of capsaïcin (Fig. 3a) or cinnamaldehyde (Fig. 3b). HAFXS (200 mg/kg) considerably decreased the licking time by 72.37 % (p<0.0001) and 56.48% (p<0.0001) in the presence of capsaïcin or cinnamaldehyde, respectively, compared to the distilled water control group. Ruthenium red and camphor, used as reference substances, also markedly (p<0.0001) reduced the licking time by 72.53 % and 37.77 % compared to the distilled water control.

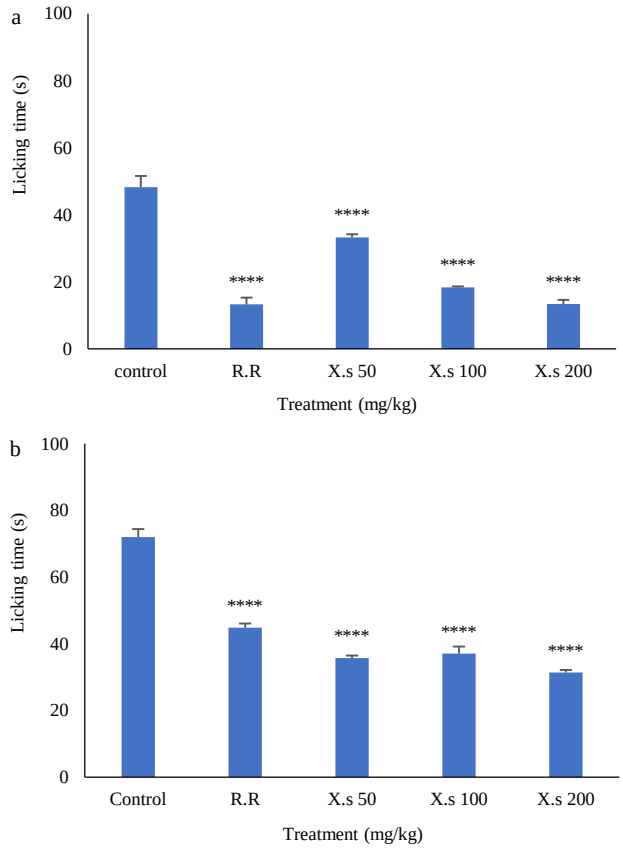

Fig. 3 Effects of different treatments on paw licking time in capsaicin (a) or cinnamaldehyde (b) induced pain. Each bar presents the mean of licking time of the injected paw ± ESM; n=6; ****p<0.0001; ***p<0.001 significant difference compared to the distilled water control; One-way ANOVA followed by Tukey post-test. R.R: Ruthenium Red.

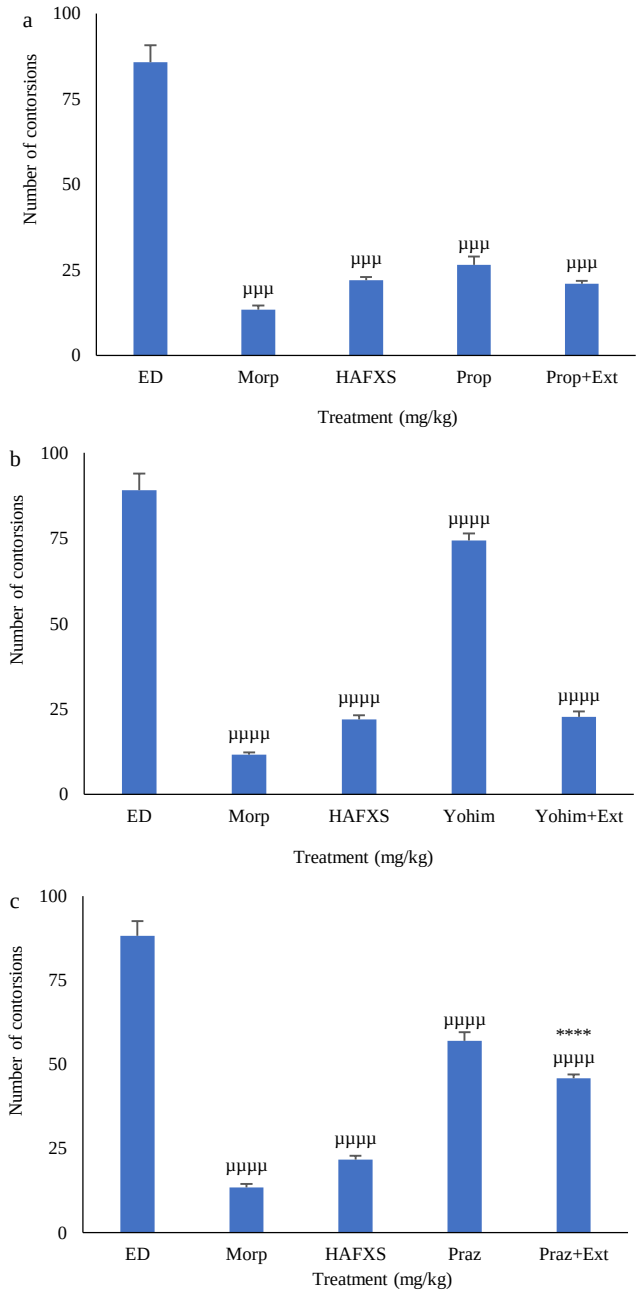

Fig. 4 Effect of propranolol (a), yohimbine (b), and prazosin (c) injection on the anti-nociceptive activity of *X. stuhlmannii*. Each bar presents the mean of contortion ± ESM; n=6; μμμμp<0.0001; μμμp<0.001, significant difference compared to the control; ****p<0.0001 significant difference compared to the extract. One-way ANOVA followed by Tukey post-test. ED: distilled water; Prop: propranolol; Yohim: yohimbine; Praz: prazosin; Morp: morphine, HAFXS: hydroalcoholic fraction of *X. stuhlmannii*.

Hydroalcoholic Fraction Stem Barks of *X. Stuhlmannii* Effect on Nociception Pathways

The results presented in Figure 4 show the potential adrenergic pathways mediating the antinociceptive effects of *X. stuhlmannii*. It appears that the antinociceptive effect of X. *stuhlmannii* did not fail after pretreatment of mice with yohimbine (Fig. 4b) and prazosin (Fig. 4c), while pretreatment with propranolol prevented the antinociceptive effect of HAFXS compared to prazosin administered alone (Fig. 4a).

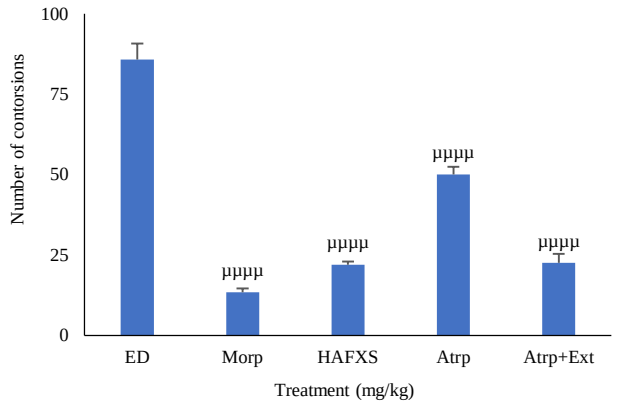

Fig. 5 Effect of HAFXS following atropine injection on the nociceptive pathways. Each bar presents the mean of contortion ± ESM; n = 6; $^{\mu\mu\mu\mu}p<0.0001$significant difference compared to the control; One-way ANOVA followed by Tukey post-test. ED: distilled water; Morp: morphine, HAFXS: Hydroalcoholic fraction of *X. stuhlmannii*; Atrp: atropine

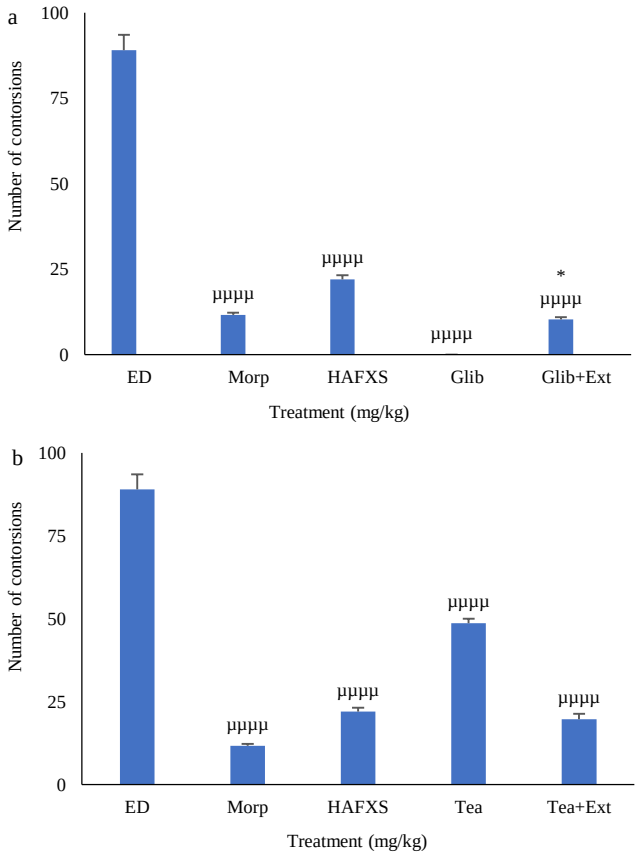

Fig. 6 Effect of HAFXS on potassium channels pathways following glibenclamide (a) and tetraethylammonium (b) injection. Each bar presents the mean of contortion ± ESM; n=6; $^{\mu\mu\mu\mu}p<0.0001$ significant difference compared to the control; $^{*}p<0.01$, significant difference compared to the extract. One-way ANOVA followed by Tukey post-test. ED: distilled water; Morp: morphine; HAFXS: Hydroalcoholic fraction of *X. stuhlmannii*; Glib: glibenclamide; Tea: tetraethylammonium.

As shown in Figure 5 presented below, the pre-injection of mice with atropine (1 mg/kg) prior to HAFXS administration has not considerably ($p\leq0.05$) changed the anti-nociceptive activity of HAFXS compared to the group that received atropine only. The involvement of potassium channels (ATP-sensitive K^+ channels and voltage-gated K^+ channels) was assessed by the acetic acid test. As shown in Figure 6a, previous injection of glibenclamide before the extract has significantly reduced the number of abdominal writhings compared to the group treated with the HAFXS only. The percentage of inhibition of HAFXS in animals pretreated with glibenclamide was 88.34 % versus 74.35 % in the presence of extract. TEA injection to animals did not affect antinociceptive activity when compared to the group treated with HAFXS only (Fig. 6b). The percentage of inhibition was 77.15 % for (HAFXS) versus 74.35 % (TEA+ HAFXS) (Fig. 6b).

L-NAME was used to perform the nitric oxide-mediated pathway through recording the licking time during the formalin test. The results presented in Figure 7 have shown that previous intraperitoneal injection of L-NAME prevented, at least in part, the anti-nociceptive activity of the extract in both phases of formalin-induced pain. The percentages of inhibition values have decreased from 44.18 % to 31.61 % in the early phase and from 46.18 % to 39.63 % in the-late phase of the formalin test.

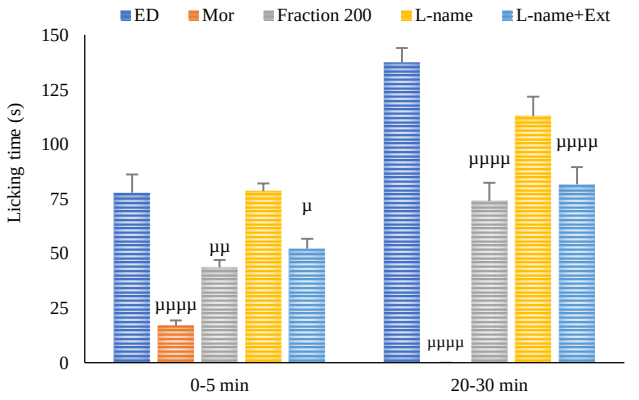

Fig. 7 Antinociceptive Effect of HAFXS following L-NAME injection in animals. Each bar presents the mean of licking time of the injected paw ± ESM; n=6; $^{\mu\mu\mu\mu}p<0.0001$; $^{\mu\mu}p<0.01$; $^{\mu}p<0.05$ significant difference compared to the control; Two-way ANOVA followed by Bonferroni post-test.

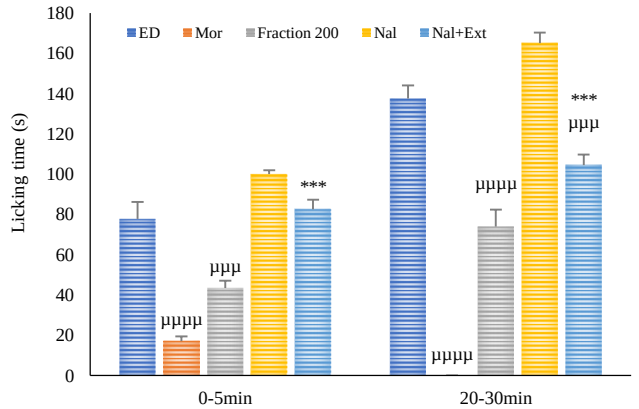

Fig. 8 Antinociceptive effect of HAFXS following naloxone injection in animals. Each bar presents the mean of licking time of the injected paw ± ESM; n=6; $^{\mu\mu\mu\mu}p<0.0001$; $^{\mu\mu\mu}p<0.001$; $^{\mu\mu}p<0.01$; $^{\mu}p<0.05$ significant difference compared to the control; $^{****}p<0.0001$; $^{***}p<0.001$ significant difference compared to the HAFXS. Two-way ANOVA followed by Bonferroni post-test. HAFXS: Hydroalcoholic fraction of *X. stuhlmannii*.

The effect of HAFXS on the opioid system is presented in Figure 8. Plant extract or morphine significantly prevented paw licking in animals. Intraperitoneal administration of naloxone prior to extract treatment significantly reduced the antinociceptive effect of *X. stulhmannii* in both phases of the formalin test.

DISCUSSION

The abdominal writhing triggered in animals throughout acetic acid injection in the peritonea is well representative of one example of inflammatory pain, currently used as a prototype to test the new analgesic as well as anti-inflammatory new drugs [14]. The injection of acetic acid solution inside the peritonea provokes pain in mice which appear as writhing and stretching of the dorso-abdominal musculature [11]. Once injected, acetic acid produces peritoneal inflammation associated throughout elaboration of endogenous inflammatory mediators such as 5-hydroxytryptamine, histamine, bradykinin, and also by means of arachidonic acid metabolites such as prostaglandins which stimulate peripheral nociceptive neurons, causing pain sensation at the level of the abdomen [17]. Our results provide evidence that the antinociceptive activity of *X. stuhlmannii* could be attributed partly to inhibition of the secretion of inflammatory mediators or blocking of peripheral cyclooxygenase activity.

Although claimed to be a highly sensitive and useful example for analgesic drug screening, this visceral pain model is not a specific pain test and does not give the mechanism of action.

The paw licking test has been accepted as a trustworthy model of sustained nociception and has the advantage of discriminating between peripheral (beginning phase) and central (final phase) components of pain [18]. The early phase, which is triggered in the surrounding by the activation of nociceptive neurons through the direct action of formalin, is associated with neurogenic pain, while the late phase, which may appear by the activation of anterior neurons of the spinal cord and the release of inflammatory mediators, is associated with inflammatory pain [19]. Our study showed that administration of *X. stuhlmannii* fraction produced significant protection in both the beginning and final phases of licking responses at all accessed doses.

Experimental evidence indicates that narcotic pain alleviator inhibits both surrounding and stem mechanisms of pain, while non-steroidal anti-inflammatory drugs block pain only at the peripheral level. Our plant extract inhibits pain by both pathways, suggesting that *X. stuhlmannii* hydroalcoholic fraction may act as an opiate-like substance. Similarly, tramadol, used in this study as a reference drug, produced significant inhibition of the early (28.16%) and late (32.74%) phases of formalin-induced pain. According to Monassier [20], centrally acting drugs like tramadol block both pathways of the formalin test, while peripherally acting drugs like non-steroidal anti-inflammatory drugs only inhibit the final phase. The results provide evidence that the extract could act through peripheral to central mechanisms. To determine the mechanisms of antinociceptive activity of HAFXS, further investigations were carried out. Thus, the formalin-induced licking test was also used to assess not only the contribution of the nitric oxide-mediated pathway but also that of the opioid system in the anti-nociceptive activity of HAFXS in peripheral and/or central levels. It is well established that the L-arginine/NO/cGMP pathway plays a role in the mechanism of nociception at different levels of the sensory system [21]. This pathway may modulate nociceptive and antinociceptive responses according to the dose and site of administration of NO inhibitors or donors [22]. It has been reported that high dose NO induces pain while at low dose NO triggers an antinociceptive effect [23]. L-NAME administered alone induced a slight antinociception at the late pain of the formalin test. Pretreatment of animals with L-NAME did not affect the antinociceptive response related to the extract compared to the animal group that received only the extract. The fact that L-NAME does not enhance the antinociceptive activity in the presence of *X. stuhlmannii*, but maintains it, may suggest that the sufficient level of NO reduced by L-NAME at the peripheral level inactivates several, but not all, NO-associated nociceptive pathway (such as COX, glutamatergic or TRPV1 systems). Moreover, the extract may directly activate pathways not associate NO [22]. Furthermore, effect of *X. stuhlmannii* on opioid receptor-mediated nociceptive response was carried out using formalin induced pain. The administration of naloxone, a non-selective opioid receptor antagonist prior the plant extract treatment significantly decreased the antinociceptive of *X. stulhmannii* in both phases of the formalin test. Based on the above findings, it could be deduced that *X. stuhlmannii* hydroalcoholic fraction mediates its antinociceptive effect through interaction with opioid receptors and/or regulation of endogenous opioid agonists. This result is furthermore supported by the hydroalcoholic fraction of *X. stuhlmannii* ability to inhibit thermal nociception in the tail immersion test also performed in this work. Nociception induced by tail immersion is known to be mediated by μ-opioïd receptors [24], it is a selective model to screen the potential substances acting through opioid receptors [25]. Naloxone used in this test as μ-opioïd antagonists failed to reverse the antinociceptive effect of the extract, indicating that other mechanisms are also involved in the activity of *X. stuhlmannii*. It is well reported that Transient Receptor Potential (TRP) channel activation is widely implicated in essential pain transduction. In the present study, the antinociceptive activity of the hydroalcoholic fraction of *X. stuhlmannii* is conducted through inhibition of Potential vanilloïd 1 (TRPV1) receptor or Transient Receptor Potential ankyrine 1 (TRPA1) mechanism. Indeed, subcutaneous injection of capsaicin (TRPV1) or cinnamaldehyde (TRPA1) induces pain, which is manifested by licking of the injected paw. This phenomenon, which end within approximately five minutes later results in the stimulation of the Transient Receptor Potential vanilloïd 1 (TRPV1) receptor or Transient Receptor Potential ankyrine 1 (TRPA1) receptor of the inotropic channel which, once opened, leads to an influx of calcium and other cations activating cellular excitation leading to the perception of the painful stimulus [26]. Based on the above results obtained in this experiment, it is clearly shown that the oral administration of the *X. stuhlmannii* stem bark fraction (200 mg/kg) has significantly reduced the nociceptive response caused by the injection of capsaicin or by the injection of cinnamaldehyde. Furthermore, pretreatment of the animals with TRP channel antagonists such as ruthenium red, a TRPV1 receptor antagonist, or camphor, a TRPA1 receptor antagonist significantly reduced the pain response. In part, the antinociceptive action of *X. stuhlmannii* may occur either by regulation or extract inhibition of the TRPV1 and TRPA1 receptors, which in turn reduces neurogenic pain.

Various receptor systems also regulate pain mechanisms in the central and peripheral nervous systems [27]. These include numerous adrenergic and cholinergic receptors [28]. To characterize further mechanisms responsible for the analgesic potential of the hydroalcoholic fraction of *X. stuhlmannii* in mice, the capacity of receptor antagonists such as propranolol (non-selective β-adrenoceptor blocking agent), prazosin (α_1 selective-adrenoceptor blocking agent), yohimbine (an α_2-presynaptic adrenoceptor blocking agent), and atropine (cholinergic antagonist)

was used in an attempt to block the analgesic action of *X. stuhlmannii* in acetic acid-induced pain. Only pretreatment with prazosin significantly attenuated the antinociceptive effect induced by *X. stuhlmannii*. This result indicates that α_1-adrenergic receptor may be involved in its antinociceptive mechanism of X. *stuhlmannii* hydroalcoholic fraction [29]. Pretreatment of animals with glibenclamide (an inhibitor of K_{ATP} channels) or TEA (an inhibitor of voltage-dependent K^+ channels) could not reverse the antinociceptive effect of *X. stuhlmannii* hydroalcoholic fraction, indicating that *X. stuhlmannii* at the dose tested may not interfere with K^+ channels.

Preliminary phytochemical analysis of the hydroalcoholic fraction of *X. stuhlmannii* indicated the presence of a number of metabolites belonging mainly to the isoflavonoid and pterocarpan families, which are characteristic of the Fabaceae family (studies not yet published). Some of these metabolites have already been isolated in our previous studies on the leaf extract of *X. stuhlmannii* [30]. The antinociceptive activity of the plant extract could be due to the presence of the isoflavonoid present in *X. stuhlmannii* [31].

CONCLUSION

The results of the present study show that the hydroalcoholic fraction of *Xeroderris stuhlmannii* possesses peripheral and central analgesic potentials in pain models induced by acetic acid, formalin, capsaicin, cinnamaldehyde, and hot water. The possible mechanisms of anti-nociception of this extract involve the activation of opioid receptors and as well as non-opioid systems, namely: alpha1, adrenergic receptors, and nitrergic pathway. Our data also suggest that the antinociceptive effect is related to its modulatory influence on TRPA1 and TRPV1 receptors.

Author's contribution

Conceptualized and designed experiments: Russelle Camelie Nguemnang Tchatchouang (RCNT), Edwige Laure Nguemfo (ELN), Calvin Bogning Zangueu (CBZ), Jacquy Joyce Kojom Wanche (JJKW), William Yousseu Nana (WYN), Gisèle Etame-Loé (GEL), and Alain Bertrand Dongmo (ABD). Performed the experiments: RCNT, ELN, GEL; wrote, reviewed, and edited the manuscript: RCNT, CBZ, JJKW, WYN; GEL; Performed formal analysis: RCNT, CBZ, and ABD

Ethical Approval

All experimental procedures were carried out according to the approved protocols set by the Institutional Ethics Committee, University of Douala, under the approval number N° 4006CEI-UDo/09/2023/T.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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