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Anti-oedematous and antinociceptive activities assessment of stem bark aqueous extract of *Ficus exasperata* Vahl. (Moraceae) in rat and mice.

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Abstract

Background: *Ficus exasperata* Vahl. (Moraceae) is widely used in African traditional medicine for the treatment of various diseases. The present study is undertaken to assess the anti-oedematous and antinociceptive activities of the stem bark aqueous extract of *Ficus exasperata* in mice and rats.

Methods: The anti-oedematous activity was investigated following carrageenan or histamine-induced rat paw oedema models. Antinociceptive activity was evaluated using acetic acid induced writhing test (1 %, 10 ml/kg), capsaicin-induced neurogenic pain (32 µg/mL, 30 µL) and formalin-induced test (1%, 20 µL). Extract was administrated orally at 37.5, 75 and 150 mg/kg.

Results: Pre-treatment of rats with *Ficus exasperata* stem bark aqueous extract exhibited significant inhibition of paw oedema during all the phases of both carrageenan and histamine induced edema in rat. The maximum inhibition percentages were 94.75 % (3 h) and 30.64 % after one hour at the dose of 37.5 mg/kg, respectively, in carrageenan or histamine models. Antinociceptive activity showed that aqueous extract reduced significantly ($p < 0.001$) the pain induced by acetic acid with an inhibition percentage of 70.8% (150 mg/kg). In the formalin-induced test, the extract also reduced significantly ($p < 0.001$) licking time during neurogenic phase and inflammatory phase with inhibition percentages of 44.75% and 52.78% respectively at the dose of 75 and 150 mg/kg. In addition, aqueous extract of *F. exasperata* reduced significantly ($p < 0.001$) neurogenic pain induced by capsaïcin by 71.28 % at the highest dose (150 mg/kg).

Conclusion: This finding suggests that the stem bark aqueous extract of *Ficus exasperata* possess potent anti-oedematous and antinociceptive activities.

Keywords: Aqueous extract; anti-oedematous; antinociceptive; *Ficus exasperata.*

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Background

Painful is one of the leading causes of medical consultation features of many diseases and affects persons of all ages. Also, pain is a symptom associated with several disorders, including inflammation and cancer. Inflammation is a physical response that protects against injury, infection, and other diseases through multiple mechanisms. The pain treatment involves several drug classes including non-steroidal anti-inflammatory drugs, corticosteroids, and narcotics [1 - 3]. The number of new drugs used to treat both pain and inflammation remains insufficient, despite the great attention during these ten years in analgesic and anti-inflammatory research [4]. Also, various drugs which are available remain inaccessible, expensive and have adverse effects. It is becoming urgent and important to find new alternative molecules. Medicinal plants represent a source of new drugs in pharmacy domain.

Ficus exasperata is a tree that grows in evergreen and secondary dry forests, rocky forests and sometimes on undeveloped land [5-6]. It is widely distributed throughout Africa in many countries including Senegal, North-Ethiopia, Djibouti, Angola, Mozambique as well as Cameroon. In these countries, the different parts of the plant have been used in traditional medicine for the treatment of several pathologies as pain, arthritis, hemorrhoids, renal infections, ophtalmique, diarrhea, parasitize infection and also as diuretic, abortive, healing [7-9].

The chemical studies of *F. exasperata* revealed the presence of many classes of secondary metabolites including tannins, saponins, steroids, flavonoids in the roots, leaves and barks [10, 11]. Some of those compounds have been reported to possess anti-inflammatory effect by acting on mediators and/or enzymes of inflammatory [12 - 15].

The present study is undertaken to assess the anti-oedematous and analgesic activities of the stem bark aqueous extract of *Ficus exasperata.*

Methods

Plant material

Stem barks of *Ficus exasperata* were collected in the Douala locality, Littoral region, Cameroon. Botanical identification was performed at the National Herbarium (Yaounde, Cameroon) in comparison with herbarium voucher specimen N° 2347/SRFK.

Preparation of extract

The fresh stem barks were cut, air dried and crushed in powder (2.0 kg). One kilogram of this powder was macerated in 10.0 L of distilled water for 48 hours at room temperature. The resulting mixture was filtered using Whatman filter paper N°1. The resulting filtrate was lyophilized to obtain 81.0 g of brown crude extract (yield of 4.05%), which was kept at 3-5°C.

Animals

Animals of both sexes were used for this experiment. Swiss mice (*Mus musculus)* weighting 20 - 30 g and 90 days old were used for antinociceptive activity while anti oedematous activity was performed in *Wistar* rats *(Rattus norvegicus)* weighting 110 - 160 g and 60 to 70 days old. These animals were obtained from the Animal House of the Laboratory of Biology and Physiology of Animals Organisms, Faculty of Science at the University of Douala, Cameroon. They were maintained under constant temperature conditions (23-25°C) in a 12 h light, 12 h dark cycle, provided with standard food and water *ad lib*. All animals were fasted overnight before dosing, tap water being available *ad libitum*. The experiments were conducted in accordance with prior authorization for the use of laboratory animal obtained from the Cameroon National Ethical Committee (Ref N° CEI-2015/0758).

Antinociceptive activity

The antinociceptive effect of the aqueous extract of *F. exasperata* stem bark was tested in mice using acetic acid –induced abdominal contractions, capsaicin test and formalin test induced nociception.

Acetic acid induced abdominal Writhing

The anti-nociceptive effect of the stem bark aqueous extract of *F. exasperata* was investigated in mice using the method described by Koster *et al* [10]. Thirty minutes after receiving oral plant aqueous extract (37.5, 75 and 150 mg/kg), reference substance (acetaminophen, 100 mg/kg) or solvent (distilled water, 10 mL/kg) to group of 7 mice, acetic acid (1%, 10 ml/kg) was injected intraperitoneally to each mouse. Immediately, each animal was individually placed in Plexiglas cage. The number of writhes and stretching of the hind limbs was counted for a period of 30 minutes as previously reported [16, 17]. Antinociceptive activity was expressed as percentage of reduction of the number of writhing and stretching in treated animals with respect to the control as follow:

(Control mean - Treated mean) x 100 / Control mean [18].

Capsaicin- induced nociception

This test was performed as described previously by Mesia-Vela *et al* [19]. The animals received by oral route, 1 hour before intraplanar capsaïcin injection: distilled water (10 mL/kg), plant aqueous extract (37.5, 75 and 150 mg/kg) or reference substance (Tramadol, 20 mg/kg). At the end of 1 hour, 30µl of capsaicin (32 µg/mL) was injected to each animal in sub-plantar region. Animals were observed individually in plexiglass cage. The time spent licking the injected paw was considered as indicative of nociception and recorded from 0 to 5 minutes.

Formalin- induced nociception

This assay was performed as previously described by Tjolsen *et al* [20]. Animals were pretreated orally with *F. exasperata* extract (37. 5, 75 or 150 mg/kg), distilled water (10 mL/kg) or aspirin (200 mg/kg) used as reference analgesic drug. 30 minutes after pre-treatment, 20 *µ*l formalin (1%) was injected in the subplantar the right hindpaw. Mice were individually placed in a transparent plexiglass cage for observation. The total time spent licking the injected paw considered as indicative of pain, was recorded in two different time periods (from 0 to 5 min for the early acute phase and from 20 to 30 min for a late phase). The percentage inhibition of licking was calculated by the following ratio: (C - T)/T x 100
Where C represent the vehicle control group value for each phase and T represent the treated group value for each phase [21].

Anti -oedematous activity

Carrageenan-induced paw oedema

Carrageenan induced paw inflammation was assessed according to the method described by Winter *et al* [22]. The plant extract (37. 5, 75 or 150 mg/kg), indomethacin (10 mg/kg), or vehicle (distilled water, 10 mL/kg) were given orally 30 min before. Administration of the oedema-inducing agent (0. 1 ml of carrageenan 1%) injected into the plantar surface of the left hind paw of rats. The volumes of injected paw were measured using Ugo Basile 7510 plethysmometer before (volume displacement technic carrageenan injection) and 0.5, 1, 2, 4, 5 and 6 hours after injection of carrageenan. Anti-inflammatory activity was expressed as the percentage reduction in edema in treated rats by comparison with the using following ratio:

$[(v_t - v_o)$ Control - $(v_t - v_o)$ Treated] x 100/ $(v_t - v_o)$ Control

Where v_t is the average volume of each group and v_o is the average volume obtained for each group before any treatment [23].

Histamine- induced paw oedema

This assay was performed as described by Lanhers *et al* [23]. The plant extract following dose (37.5, 75 or 150 mg/kg), pyrilamine maleate (1 mg/kg), or vehicle (distilled water,10 mL/kg) was administrated orally 30 min before injection of 0,1 ml histamine (1 mg/mL). Paw volume was measured with Ugo Basile 7510 plethysmometer before histamine injection, then 30 minutes and 1 hour after injection of histamine.

Statistical analysis

Data were expressed as mean ± standard error of the mean (S.E.M). Comparisons between experimental and control groups were performed by one-way analyze of variance (ANOVA) followed by Dunnett's tests. P value less than 0.05 were considered significant.

Results

Antinociceptive activity

Effect of aqueous extract on abdominal writhing response induced by acetic acid

Aqueous extract of *Ficus exasperata* (37.5, 75 or 150 mg/kg) induced a significant reduction (p< 0.001) in the number of writhing provoked by an intraperitoneal injection of acetic acid in mice. The maximum percentage of reduction was 70.78 % at the dose of 150 mg/kg. The positive control drug, acetaminophen (100 mg/kg), also provoked significant (p< 0.001) protective effect 55.76% against acetic acid-induced pain (Table 1).

Effect of aqueous extract on paw licking response induced by formalin

Injection of 20 *µ*l formalin (1%) into the surface of the right paw generated classical biphasic nociceptive responses (neurogenic and inflammatory pain) in mice. The plant extract (37.5, 75 or 150 mg/kg) significantly reduced licking time in both phases of the formalin test. The maximum percentage of inhibition was 44.75% (p< 0.01) at the early phase at the dose of 75 mg/kg and was 52.78 % (p< 0.001) at the late phase at the dose of 150 mg/kg. A positive control group, tramadol (20 mg/kg) also significantly reduces pain at both phases with the values of 47.49 % (p< 0.001) at the early phase and 32.46 % (p< 0.01) at the late phase (Table 2).

Effect of aqueous extract on paw licking response induced by Capsaïcin

As shown in Figure 1, the administration of *Ficus exasperata* (37.5, 75 or 150 mg/kg) produced significant inhibition of the capsaicin-induced licking in mice by 51.98 %; 55.44 % and 71.28 % respectively. Positive control animals showed the decrease of the licking time by 88.66% (Figure 1).

Anti-inflammatory effect of Ficus exasperata aqueous stem bark extract

Effect of aqueous extract on carrageenan-induced rat paw oedema

As shown in Table 3, a pretreatment of rats with extract aqueous of *Ficus exasperata* significantly decrease paw oedema caused by injection of carrageenan when compared to control group. The smallest doses tested (37.5 and 75 mg/kg) reduced significantly (p<0.001) inflammation at all hours. The maximum percentages of inhibition were 93.20 (2h), 94.75 (3h), 94.60 (4h) and 92.40 % (6 h) at the dose 37.5 mg/kg. Indomethacin, a Non-Steroidal Antiinflammatory drug, also exhibited an anti-inflammatory effect, showing 77.30%, 77.30% and 90.07 % of inhibition at 2, 5 and 6 hours, respectively (Table 3).

Effect of aqueous extract on histamine-induced rat paw oedema

The aqueous stem bark extract of *F. exasperata* significantly inhibited (p< 0.05) histamine-induced oedema with maximum inhibition percentage of 30.64 and 29.48 % at 1h respectively at the doses of 37.5 and 150 mg/kg (Figure 2). Maleate pyrilamine used as a positive control significantly (p< 0.001) inhibited histamine-induced oedema with a maximum inhibition of 52.60 % at the first hour.

Discussion

The objective of the present study was to evaluate the antinociceptive and anti-inflammatory effects of the stem barks aqueous extract of *F. exasperata*. The pain was induced by acetic acid, capsaicin or formalin and the inflammation was induced by carrageenan and histamine.

Intra-peritoneal injection of acetic acid induce pain manifested by abdominal writhing responses in mice. This pain is induced either directly through the stimulation of chemo-sensitive nociceptors [24] or indirectly by irritation of the visceral surface, resulting in the release of algogenic substances such as histamine, serotonin, bradykinin and prostaglandins [25]. These mediators would raise awareness of cholinergic and peritoneal histamine chemo-nociceptors, creating pain [26]. The result demonstrate that the aqueous extract of *F. exasperata* stem bark reduced the number of abdominal contractions induced by acetic acid in mice significantly (p< 0.001) at all doses tested. The analgesic effect of the extract on this type of pain could be due either to an inhibitory action on acid-sensitive nociceptors or to the inhibition of the

production of the algics substances mentioned above. Acetic acid - induced abdominal constriction is an easy and sensitive method commonly employed to measure peripheral analgesic effect [27].

Acetaminophen used here like standard, significantly reduced the number of abdominal constrictions ($p<0.001$) during the experience. It's known as a prostaglandins synthesis inhibitor by fixation on the peroxydase site of the enzyme prostaglandin H_2 synthase [28]. It is also used as cyclooxygenase inhibitor and classified as analsegesic drug according to WHO [27, 29]

According to Le Bars *et al* [27] all analgesic substances possess inhibitory properties on the type of pain induced by acetic acid, it's a typical model used to verified analgesic properties of drugs but not specific. To elucidate the site and mechanism of action, two other tests named capsaïcin-induced pain and the formalin-induced pain were performed. Sub-plantar injection of capsaïcin causes two periods of pain: neurogenic pain at the first phase and inflammatory pain at the late phase. Both phases are characterized by licking the injected paw. This phenomenon is due to the stimulation of ionotropic channel-type vanilloïd receptors TRPV 1 which, when opened, allow the calcium influx and other cations, thus triggering the cellular excitation processes leading to the perception of the painful stimulation [30-32]. Our results obtained showed that, aqueous extract of *F. exasperata* stem bark significantly inhibited ($p< 0.001$) pain induced by capsaïcin at all doses. Substances that are active on capsaïcin-induced pain model, are characterized as central analgesic [33]. Tramadol, a reference standard used, is classified as an analgesic of second stage [29] and recommended for nociceptive and neuropathic pain [34]. It is a morphinic derivative, a central analgesic of moderate to severe pain, and a partial agonist of opiate receptors, because saturates these receptors involved in pain perception. In addition, its mechanism is associated with an inhibitory action of neuronal reuptake of serotonin and noradrenaline [35]. Treatment of animals with tramadol significantly ($p < 0.001$) inhibited pain.

Formalin induces pain model which produce a distinct biphasic response: the first phase (neurogenic pain) that began immediately 3 minutes after injection of the substance; the second phase (inflammatory pain), occurs 20 minutes after formalin injection [27]. The neurogenic pain is characterized by the release of substance P after stimulation of C- fibers and also by direct activation of the TRPA 1 and TRPV 1 channels expressed on nociceptors [36-38]. The inflammatory pain is characterized by the release of chemical mediators such as histamine, serotonin, kinins and prostaglandins [36]. The treatment of animals with aqueous extract of *F. exasperata* significantly reduced pain during both phases at all doses tested. The highest percentage of inhibition was observed in the second phase. Tramadol, whose mechanism of action has been described above, used as a reference substance, significantly inhibited pain ($p< 0.001$) during both phases. The inhibition of the inflammatory phase of formalin-induced pain by the aqueous extract of *F. exasperata* suggests the presence of active substances with anti-inflammatory activities in the barks of *F. exasperata.*

Intra-plantar injection of carrageenan in rats causes a triphasic response, the first is mainly due to the release of histamine and serotonin [39]. The second phase, due to the release of kinins including bradykinin, and the third phase, is characterized by the release of prostaglandins and leukotrienes [40]. In our experiments, the oedematous response was significantly suppressed in rats pre-treated with aqueous extract (37.5 mg/kg) on the first phase (85.20%), second phase (93.20%) and on the third phase (94.75%) suggesting an inhibitory effect on the liberation of all mediators if inflammation. Indomethacin used as a reference drug also gave a maximum inhibition percentage of 90.07% at the sixth hour. It is a nonsteroidal anti-inflammatory (NSAI) and known as inhibitor of cyclooxygenase 2 and/or mediators such as histamine, serotonin and kinins. It is known that NSAI significantly inhibits the synthesis of second phase mediators by blocking kallicrein which cause biconversion of liver kininogens in kinins [41]. These results are similar to the work of Nguemfo *et al* [18] who showed an inhibition of rat paw edema by the methylene chloride fraction of *Allablackia monticola* STANNER L.C.

Histamine-induced test confirmed the effect of the extract on the first phase of inflammation. Injection of histamine causes dilation of blood vessels and an increase in the permeability of its vascular walls which represent a typical oedema response of the first phase [42, 43]. The aqueous extract of the stem barks of *F. exasperata* significantly ($p< 0.05$) inhibited histamine-induced inflammation with a maximum inhibition percentage of 30.64% (1h) at 37.5 mg/kg. Pyrilamine maleate, a reference antihistamine drug, significantly ($p< 0.001$) inhibited oedema by 52.60% one hour after histamine injection. Its acts as an antagonist on histaminic H_1 receptors by inhibiting capillary permeability, capture of catecholamines and competitive reduction of histaminic receptors [44].

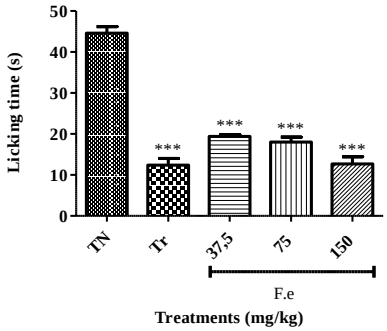

Figure 1. Anti-nociceptive effect of the aqueous extract of the stem bark of *Ficus exasperata* on capsaicin test.
*Each bar represents a mean ± S.E.M of five mice for each group; ***p< 0.001; statistically significant from control group. TN: Negative control; Tr: Tramadol; F.e: Ficus exasperata.*

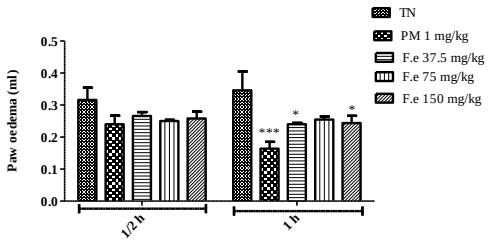

Figure 2. Effect of the aqueous extract of the stem bark of *F. exasperata* on paw oedema induced by histamine.
*Each bar represents a mean ± S.E.M. of five rats for each group. ***P< 0.001; ***P< 0.05; statistically significant from control group. TN: negative control; PM: Pyrilamine Maléate; F.e: Ficus exasperata.*

Table 1: Anti-nociceptive effect of aqueous extract of the stem bark of *Ficus exasperata* on acetic acid-induced writhing

Treatment	Dose (mg/kg)	Number of writhing	Inhibition (%)
Control		74.6 ± 3	-
Acetaminophen	100	33.0 ± 6***	55.8
F. exasperata	37.5	38.0 ± 2***	49.1
F. exasperata	75	36.6 ± 4***	50.9
F. exasperata	150	21.8 ± 4***	70.8

*The values are expressed as mean ± S.E.M. of seven mice for each group. Statistically significant from control group. ***p< 0.001;*

Table 2. Anti-nociceptive effect of the aqueous extract of the stem bark of *Ficus exasperata* on formalin-induced test

Treatment	Dose (mg/kg)	Licking time (sec)		Inhibition (%)	
		0-5 min	20-30 min	Early phase	Late phase
Control		52.6 ± 9.5	61 ± 4.7	-	-
Tramadol	20	20.6 ±2.9***	29.6 ±8.1**	47. 49	32.46
F. exasperata	37.5	30.4 ± 1.9*	29.2±2.2**	30.59	52.13
F. exasperata	75	24.2 ± 3.3**	33.2 ±2.1***	44.75	45.57
F. exasperata	150	31.2 ± 2.1*	28.8 ±1**	28.77	52.78

*The values are expressed as mean ± S.E.M. of seven mice for each group. Statistically significant from control group. *p< 0.05; **p< 0.01; ***p< 0.001.*

Table 3. Effect of the aqueous extract of the stem bark of *Ficus exasperata* on paw oedema induced by carrageenan.

Treatments	Dose (mg/kg)	oedema (ΔV in mL)						
		0.5 H	**1 H**	**2 H**	**3 H**	**4 H**	**5 H**	**6 H**
Control		0.44±0.07	0.76±0.12	1.46±0.15	1.48±0.22	1.27±0.18	0.93+-±0.17	0.80±0.15
Indometacin	10	0.29±0.02* **(34.18)**	0.38±0.07*** **(49.70)**	0.33±0.07*** **(77.30)**	0.55±0.03*** **(63.31)**	0.6±0.0*** **(53.40)**	0.21±0.04*** **(77.30)**	0.08±0.02*** **(90.07)**
F. exasperata	37.5	0.12±0.02 **(72.69)**	0.11±0.01*** **(85.20)**	0.10±0.02*** **(93.20)**	0.08±0.02*** **(94.75)**	0.10±0.0*** **(94.60)**	0.08±0.02*** **(91.40)**	0.06±0.01*** **(92.30)**
F. exasperata	75	(0.09±0.02) *** **(79.40)**	0.24±0.04*** **(68.30)**	0.68±0.09*** **(53.30)**	0.50±0.10*** **(54.50)**	0.68±0.09*** **(63.0)**	0.29±0.12*** **(68.50)**	0.12±0.08*** **(84.11)**
F. exasperata	150	0.27±0.01 **(38.63)**	0.50±0.05 **(33.90)**	1.11±0.07 **(23.90)**	1.15±0.05 **(22.58)**	1.00±0.10 **(24.10)**	0.65±0,13 **(29.90)**	0.46±0.07 **(42.05)**

*The values are expressed as mean ± S.E.M of seven rats for each; each value in parenthesis indicates the percentage inhibition rate. Statistically significant from control group. *P< 0.05; ***p< 0.001.*

Conclusion

The aqueous extract of *F. exasperata* has significant peripheral and central analgesic activity on pain models induced by acetic acid, formalin, capsaicin, and a significant anti-inflammatory activity on acute inflammation induced by the carrageenan. Our results support the therapeutic activities of *F. exasperata* proclaimed by naturopath and justifies its use in traditional medicine.

Abbreviations

NSIA: NonSteroidal Anti-inflammatory
WHO: World Health Organization
TRP V1: Transient Receptor potential Vanilloid subtype 1
TRP A1: Transient Receptor potential Ankyrine1

Authors' Contribution

KNBJ, carried out all the experiment; NEL, the coordinator, had managed student in technical experiment, had written and read the manuscript; BZC, student lab group: they help to realize the experiment; MFAL student lab group: they help to realize the experiment; DAB the supervisor and chief of laboratory: had revised, corrected, and read the final manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

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