

Evaluation of the analgesic and anti-inflammatory activities of *Annona senegalensis* Pers. (Annonaceae) leaves aqueous extract in rats and mice

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Abstract

Annona Senegalensis Pers. (Annonaceae) is a medicinal plant used to treat many pathologies, including inflammatory diseases and pain. The aim of this study was to investigate the analgesic and anti-inflammatory properties of *Annona Senegalensis* leaves aqueous extract in mice and rats. The analgesic activity was evaluated using the acetic acid (1%) induced writhing test and formalin (1%) test. The anti-inflammatory activity was performed using the carrageenan and the dextran induced hind paw oedema in rats.

The extract induced a significant ($p<0.05$) and dose-dependent decrease in abdominal contortions compared with control mice. The maximum inhibition was 63.36% at the dose of 200 mg/kg body weight. Only the late phase of formalin induced nociception was significantly inhibited by the extract with a maximum inhibition value of 56.96% at the dose of 200 mg/kg body weight. In the anti-inflammatory investigation, the aqueous extract of the leaves of *Annona Senegalensis* produced a significant ($p<0.05$) and dose-dependent decrease in edema induced by carrageenan and dextran. The maximum inhibition was 57.14% obtained at the fifth hour at the dose of 200mg/kg for the carrageenan test. For the dextran test, the maximum inhibition was 59.80% obtained at the second hour at the dose of 200 mg/kg body weight.

Our results show that *Annona Senegalensis* has peripheral analgesic and anti-inflammatory properties. It could therefore be an advantage in alternative medicine.

Keywords: Analgesic, Anti-inflammatory, *Annona Senegalensis*, Rats, Mice

1. INTRODUCTION

Inflammation and pain are usually treated using non-steroidal and steroid anti-inflammatory drugs, opioids or non-narcotic drugs such as synthetic anti-paludics. These drugs, though effective, are very expensive, and also have side-effects when administered over a very long period. Their side effects include gastrointestinal ulceration leading to haemorrhage, and renal disorders^{1,2}. In response to these challenges, alternative solutions are needed and these involve the use of natural resources and particularly medicinal plants. The World Health Organization (WHO) is promoting the study of traditional knowledge in many developing countries, which could lead to new discoveries in the field of medicines, so as to provide local populations with more cost-efficient treatments. A number of traditional herbal drugs have been found to have an anti-inflammatory effect^{3,4}. Other plants have other therapeutic properties^{5,6}. Indeed, *Annona Senegalensis* is a plant widely used in both traditional human and animal medicine. The plant is used as a stimulant, analgesic and to treat dysentery. It also has antioxidant, antimicrobial, antidiarrheal, anti-inflammatory, antiparasitic, anticonvulsant, antimalarial and antinociceptive effects⁷. Although effective, traditional medicines are often criticized for the lack of proof of their efficacy and tolerability. Therefore, scientific research in this field is necessary and essential, in order to enhance and promote traditional medicine and pharmacopoeia⁸.

The aim of this study was to evaluate the analgesic and anti-inflammatory properties of the aqueous extract of the leaves of *Annona Senegalensis* in experimental animals. A phytochemical screening was carried out to assess the main bioactive chemical groups that could confer the properties attributed to the plant.

2. MATERIAL AND METHODS

2.1. Plant

The leaves of *Annona senegalensis* were collected in the Hauts Bassins, a region of western part of Burkina Faso. They were washed and dried under ventilation without sunlight and finely powdered. The plant was identified at the Department of Plant Biology and Ecology, Joseph Ki-ZERBO University, Ouagadougou, where a sample was kept under identification number 18049.

2.2 Animals

Female Wistar rats weighing between 90 and 100 g and female Naval Medical Research Institute (NMRI) mice weighing between 28 and 35 g from Université Joseph KI ZERBO were used. They were bred at an average temperature of $22\pm 3^\circ\text{C}$, a relative humidity of $50\pm 10\%$ and subjected to a 12-hour light cycle. They had free access to food and water.

2.3. Preparation of the aqueous extract

One hundred grams (100 g) of this powder was macerated in 1000 mL of distilled water for 24 hours. The filtrate was centrifuged at 2000 rpm for 10 minutes. The supernatant was freeze-dried. The aqueous extract of *Annona Senegalensis* leaves (AEAS) was thus obtained. The extraction yield was 24.23%.

2.4. Analgesic activity

The analgesic properties of the aqueous extract of *Annona Senegalensis* leaves were evaluated using the acetic acid-induced writhing test and the formalin test.

2.4.1. Acetic acid-induced writhing test

This study was carried out using the method described by Collier et al⁹ and modified by Soro et al¹⁰. The plant was tested for protection against abdominal cramps and writhing induced by intraperitoneal injection of 1% acetic acid in mice. Forty (40) mice fasted for 12 hours were divided into eight (8) groups of 5 animals each. The control group (group 1) received distilled water (10 mL/kg), groups 2, 3 and 4 received the extract at doses of 40, 100 and 200 mg/kg body weight respectively. Groups 5 and 6 (reference drug groups) received acetylsalicylic acid (aspirin) and tramadol (trabar*) at doses of 100 mg/kg body weight and 15 mg/kg body weight respectively. The last two groups received naloxone (0.4 mg/kg) beforehand and tramadol (15 mg/kg) or the highest dose of aqueous extract (200 mg/kg) fifteen (15) minutes later. Distilled water, extract, acetylsalicylic acid and tramadol were administered orally, while naloxone was administered intraperitoneally.

One hour after treatment each mouse was given 10 ml/kg of 1% solution of acetic acid injected intraperitoneally to create pain sensation. Each mouse was immediately placed in a plastic transparent observation cage. After a latency period of 5 minutes, the number of abdominal constrictions was counted over the following twenty (20) minutes¹¹. The intensity of inhibition of writhings was calculated, expressed as percentage, using the relation $(N_c - N_t) / N_c * 100\%$, where N_c and N_t are mean numbers of writhings in the control group and treated groups respectively.

2.4.2. Formalin test

This test evaluated the analgesic activity of the aqueous extract of the leaves of *Annona Senegalensis* according to the method described by Hunskaar and Hole¹². Female mice, fasted for 12 hours, were divided into eight (8) groups of five (5).

- Group 1 (control group) received distilled water at 10 mL/kg body weight;

Groups 2,3,4 (test groups) received the aqueous extract of the leaves of *Annona Senegalensis* at doses of 40, 100 and 200 mg/kg body weight;

Groups 5 and 6 (reference drug groups) received acetylsalicylic acid (aspirin) or tramadol (trabar*) at doses of 100 mg/kg and 15 mg/kg body weight respectively.

Groups 7 and 8 were pretreated with naloxone (0.4 mg/kg) before receiving tramadol (15 mg/kg) or the aqueous extract at a dose of 200 mg/kg 15 minutes later.

One hour after each treatment, 0.1 mL of formalin solution (1%) was injected in hind paw of rats. The length of time the animal licked the paw was measured during the first 5 minutes, then between the 15th and 30th minute after formalin injection. The analgesic activity was expressed as percentage using the following relation: $(T_c - T_t) / T_c * 100\%$. T_c and T_t are mean licking time for control group and treated groups respectively.

2.5. Anti-inflammatory activity

The anti-inflammatory effect of the aqueous extract of the leaves of *Annona Senegalensis* was evaluated in two models of acute inflammation: the carrageenan test and the dextran test.

2.5.1. Carrageenan test

This test was performed according to the protocol of Lanher et al¹³. Twenty-five (25) female rats fasted for 12 h were divided into five (5) groups of 5 animals each.

Group 1 (control group) received distilled water (10mL/kg);

Group 2 (reference drug groups) received diclofenac at a dose of 20 mg/kg body weight;

Groups 3, 4 and 5 (test groups) received the aqueous extract of the leaves of *Annona Senegalensis* at doses of 40, 100 and 200 mg/kg body weight respectively. All were given by oral route.

One hour after these treatments, inflammation was produced in the hindlimb by injection

of 0.1 mL solution of carrageenan 1% into the plantar surface of the hind paw after measuring the initial paw volume (V_0). The paw edema was then measured at the intervals of 0.5, 1, 2, 3, 4, 5, and 6 hours after injection (V_t) using the Ugo Basile plethysmometer. Results were expressed as percentage inhibition of edema in the treated groups compared to the control using the following formula: $(V_c - V_t) / V_c * 100\%$, where V_c and V_t are mean increases in the paw volume in the control and test groups respectively.

2.5.2. Dextran test

The test was carried out according to the method described by Gupta et al¹⁴. Twenty-five (25) rats were divided into five groups of five animals each. Group 1 served as control and received distilled water (10 mL/kg). Groups 2, 3 and 4 received the aqueous extract of the leaves of *Annona Senegalensis* at doses of 40, 100 and 200 mg/kg body weight respectively. Group 5 was treated using cyproheptadine (2 mg/kg) as reference drug. Treatments were administered orally. One hour after these treatments, edema was induced by injection under the plantar pad of the right hind paw of 0.1 mL solution of dextran¹⁵ after measuring the initial paw volume (V_0). The paw edema was then measured at the intervals of 0.5, 1, and 2 hours after injection (V_t). The percentage inhibition of edema was determined as above.

2.6. Phytochemical analysis

Phytochemical screening of the aqueous extract of *Annona Senegalensis* was carried out according to the method described by Ciulei¹⁶. Tests were carried out to detect the presence or absence of certain constituents thought to be bioactive in *Annona Senegalensis* leaf extract. Flavonoids were characterized by the Shibata test, tannins by the Ferric chloride test, sterols and triterpenes by the Lieberman and Büchard test. Saponosides were characterized using a test based on the appearance of foam after agitation. The characterization of coumarins was made by the ammonia test based on the observation of fluorescence under UV lamps at 366 nm. Dragendorff, Mayer and Bouchardat reagents were used to detect alkaloids.

2.7. Statistical analysis

The Excel 2019 spreadsheet was used for data entry. Values were expressed as means \pm standard error. Graph Pad Prism version 8.4.3 was used to generate graphs and perform statistical tests. Differences between more than two means were determined by ANOVA followed by Dunnett's test for multiple comparison among groups. Difference in the mean $p < 0.05$ was statistically considered significant.

3. RESULTS

3.1. Analgesic activity

3.1.1. Acetic acid test

Figure 1 shows the effect of the aqueous extract of the leaves of *Annona Senegalensis* on acetic acid-induced pain. The extract at doses of 200 mg/kg and 100 mg/kg significantly ($p<0.05$) decreased the number of abdominal contortions compared

with control mice, with inhibition of 63.36% at 200 mg/kg and 37.14% at 100 mg/kg.

Aspirin at 100 mg/kg and tramadol at 15 mg/kg inhibited 78.38% and 85.21% respectively (Table I). The decrease in the number of abdominal contortions by tramadol in the presence of naloxone was non-significant, with 13.20% inhibition, while aqueous extract at 200 mg/kg still significantly reduced the number of contortions with 61.83% inhibition.

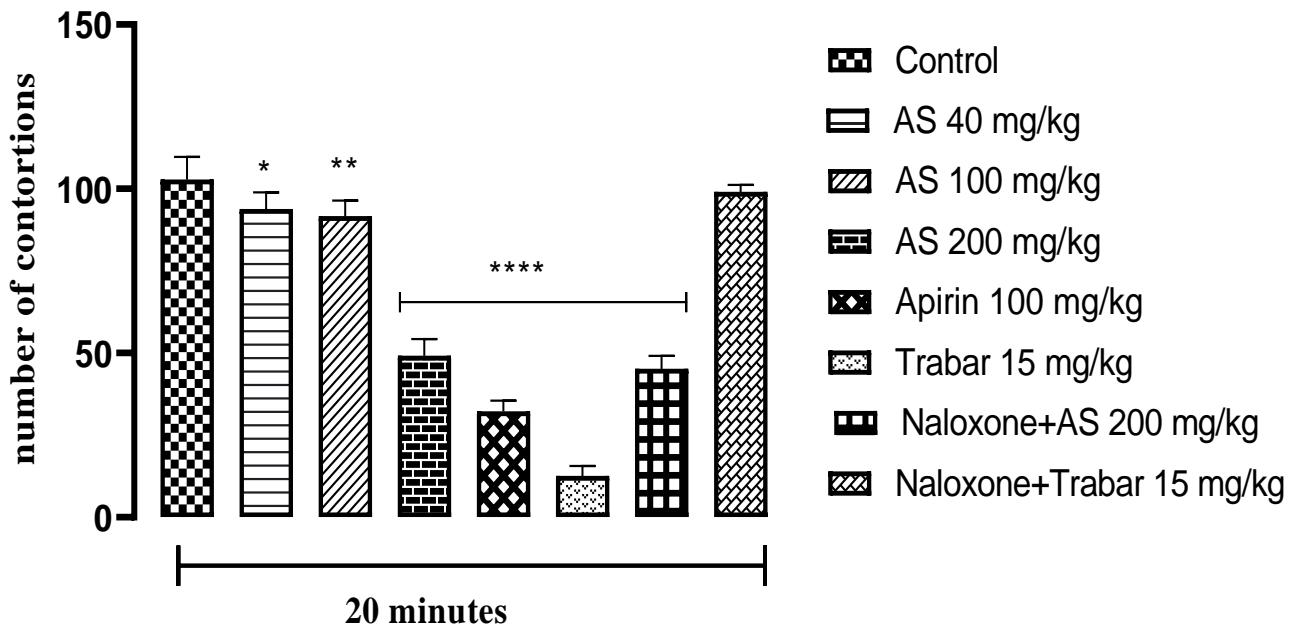


Figure 1: Effects of the aqueous extract of the leaves of *Annona Senegalensis* on acetic acid-induced abdominal contortions. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ significant differences from control. AS: *Annona Senegalensis*.

Table I. Inhibition of acetic acid-induced abdominal contortions by the aqueous extract of the leaves of *Annona Senegalensis*.

	AS 40 mg/kg	AS 100 mg/kg	AS 200 mg/kg	ASP 100 mg/kg	Trabar mg/kg	15 N + AS 200 mg/kg	N+T
Inhibition (%)	15,95*	37,14**	63,36****	78,38****	85,21****	61,83****	13,20

AS : *Annona senegalensis* ; ASP : Aspirine ; N : Naloxone ; T : Trabar

3.1.2. Formalin test

Figure 2 shows the effect of the aqueous extract of the leaves of *Annona Senegalensis* on formalin-induced pain. Extract at doses of 100 and 200 mg/kg and aspirin significantly ($p<0.05$) inhibited the inflammatory phase of pain only. The inhibition of pain was 63.79% for aspirin, 56.96% and 25.14% for extract doses of 200 and 100 mg/kg respectively. Tramadol

significantly decreased paw licking time during the first and second phases, with 62.68% and 74.31% inhibition respectively. In the presence of naloxone, tramadol did not significantly decrease paw licking time during both pain phases. In contrast, aqueous extract at a dose of 200 mg/kg in the same conditions significantly inhibited the inflammatory phase, with 57.21% inhibition (Table II).

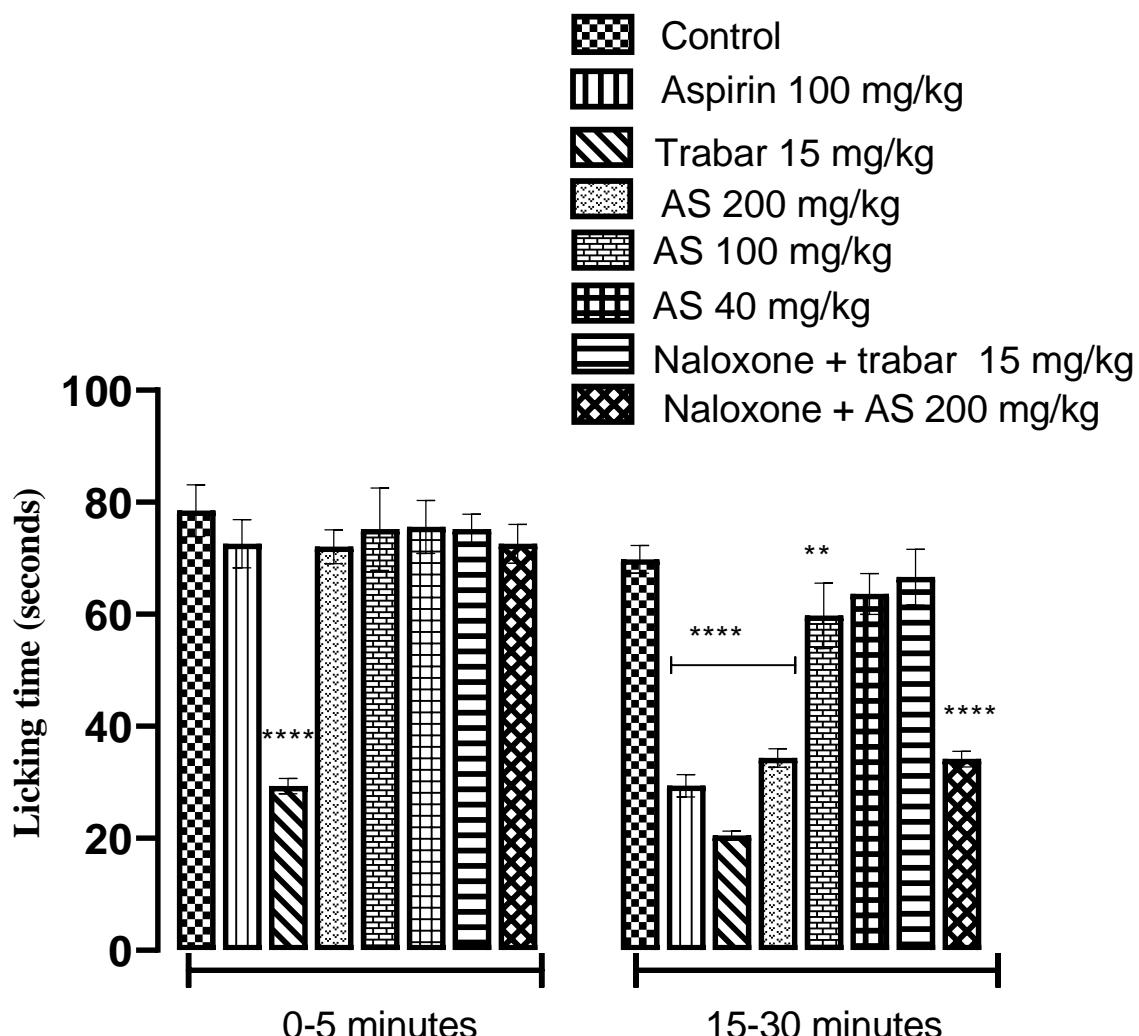


Figure 2: Effect of the aqueous extract of the leaves of *Annona Senegalensis* on formalin-induced pain. n=5, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant differences from control.

Table II. Inhibition percentage of aqueous extract of *Annona Senegalensis* leaves on formalin-induced pain.

	Inhibition (%)						
	Aspirine	Trabar	AS	AS	AS	Naloxone + Trabar 15 mg/kg	Naloxone + AS 200 mg/kg
	100 mg/kg	15 mg/kg	200 mg/kg	100 mg/kg	40 mg/kg		
0-5 mn	7,57	62,68****	8,21	4,23	3,72	4,23	7,53
15-30 mn	63,19****	74,31****	56,96****	25,14***	20,25	16,49	57,21****

3.2. Anti-inflammatory activity

3.2.1. Carrageenan-induced edema test

Table III shows the effect of the aqueous extract of the leaves of *Annona Senegalensis* on carrageenan-induced edema. The extract significantly (p<0.05) reduced edema volume at all doses. The inhibiting effect of the extract was dose-dependent.

The maximum inhibition was obtained at the fifth hour with 57.14%, 52.86% and 34.29% at doses of 200, 100 and 40 mg/kg respectively. The standard molecule, diclofenac, significantly inhibited edema volume over the six-hour period, with a maximal inhibition percentage of 72.28% at the third hour.

Table III: Effects of the aqueous extract of the leaves of *Annona Senegalensis* on carrageenan-induced oedema.

Time (h)	Mean increases in the paw volume (ml) resulting from edema and Percentage of inhibition (%)				
	Control	AS 40 mg/kg	AS 100 mg/kg	AS 200 mg/kg	Diclofénac 20mg/kg
0,5	0,59± 0,03 (5,17)	0,55± 0,03 (5,17)	0,54± 0,02 (6,90)	0,51± 0,04* (12,07)	0,35± 0,03**** (39,66)
1	0,64± 0,03 (5,51)	0,60± 0,04 (5,51)	0,57± 0,03* (10,24)	0,36± 0,04**** (43,31)	0,30± 0,02**** (52,76)
2	0,67±0,02 (15,24)	0,57±0,04** (15,24)	0,51±0,03**** (24,16)	0,34±0,03**** (49,44)	0,30±0,03**** (55,39)
3	1,01±0,04 (33,66)	0,67± 0,03 (33,66)	0,52 ±0,02 (48,51)	0,45± 0,03**** (55,45)	0,28±0,03**** (72,28)
4	0,80±0,02 (33,75)	0,53±0,03**** (33,75)	0,394±0,03*** (50,00)	0,36±0,03**** (55,00)	0,23±0,02**** (71,25)
5	0,70 ±0,03 (34,29)	0,46±0,03**** (34,29)	0,33±0,03**** (52,86)	0,30±0,03**** (57,14)	0,20±0,02**** (71,43)
6	0,68±0,03 (32,35)	0,46±0,05*** (32,35)	0,35±0,04**** (48,53)	0,31±0,03**** (54,41)	0,28±0,03**** (58,82)

Values represent mean of edema volume ± ESM. n=5, values in parentheses indicate the inhibition percentages. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant differences from control. AS: *Annona Senegalensis*.

3.2.2. Dextran test

Figure 3 shows the effect of the aqueous extract of the leaves of *Annona Senegalensis* on dextran-induced edema volume. The extract at a dose of 200 mg/kg significantly (p<0.05) inhibited edema volume at 30 minutes, 1 and 2 hours, with 37.94%, 45.68% and 59.80% inhibition respectively. The 100 mg/kg

dose significantly inhibited edema at the first and second hour, with 26.62% and 33.33% respectively. At the dose of 40 mg/kg, edema was significantly inhibited only at the second hour, at 18.95%. Prometazine (20mg/kg) used as a standard drug significantly inhibited edema for both hours, with a maximum inhibition percentage of 79.08% at the second hour (Table IV).

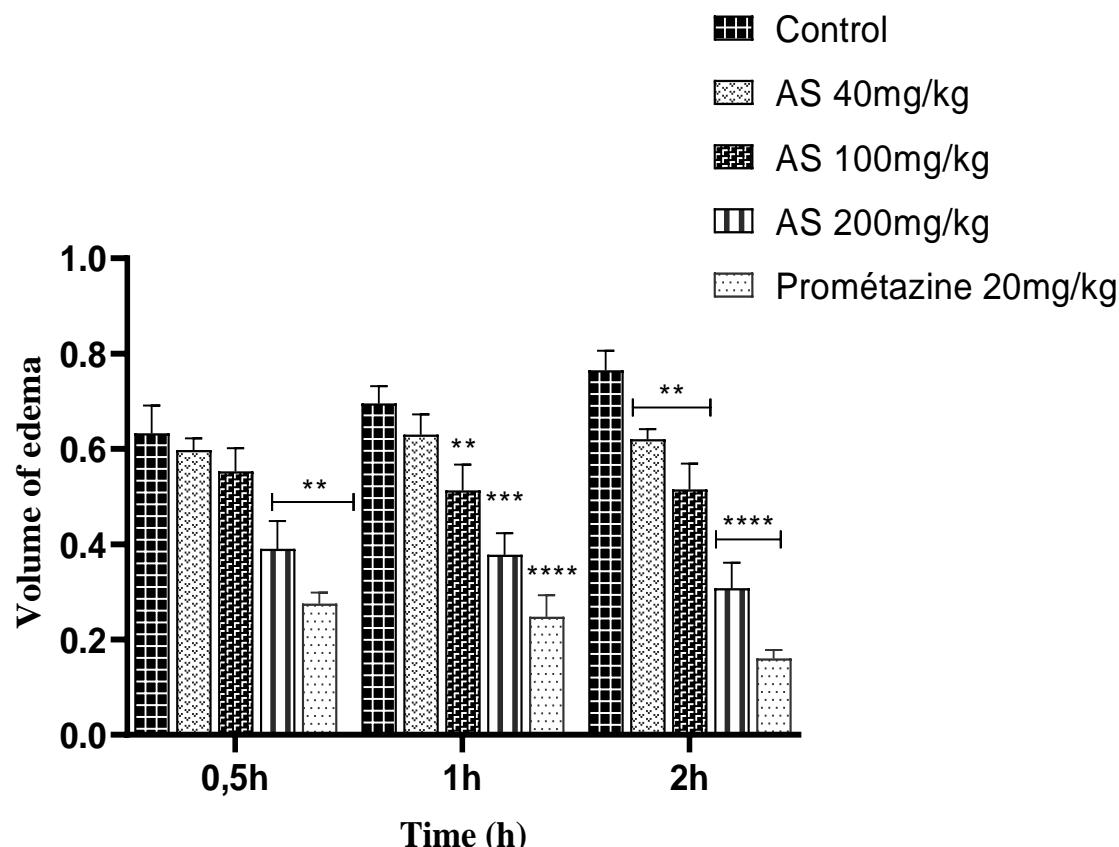


Figure 3: Effects of the aqueous extract of the leaves of *Annona Senegalensis* on dextran-induced oedema. n=5, p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 significant differences from control significant differences from control. AS: *Annona Senegalensis*.

Table IV. Inhibition of dextran-induced edema by the aqueous extract of the leaves of *Annona Senegalensis*.

Inhibition (%)				
	Prometazine 20 mg/kg	AS 200 mg/kg	AS 100 mg/kg	AS 40 mg/kg
0,5 h	58,10 **	37,94 **	13,04	5,14
1 h	64,03****	45,68***	26,62**	9,35
2 h	79,08****	59,80 ****	32,03**	18,95**

3.3 Phytochemical analysis

Phytochemical analysis revealed the presence of tannins, flavonoids, sterols and triterpenes, saponosides and leucoanthocyanins in the aqueous extract of the leaves of *Annona Senegalensis*.

Table V. Chemical composition of the aqueous extract of the leaves of *Annona Senegalensis*

Chemical compounds	Revelation in the extract
Coumarins	-
Flavonoids	+++
Alcaloids	-
Saponosides	+
Tanins	+++
Sterols and triterpenes	++
Anthocyanins	-
Leuco anthocyanins	+++

+ = presence; ++ = large presence; +++ = very large presence; - = absence

4. DISCUSSION

The present study revealed both analgesic and anti-inflammatory properties of the aqueous extract of the leaves of *Annona Senegalensis* and characterized the chemical groups contained in the extract. The analgesic activity of the extract was evaluated on acetic acid- and formalin-induced pain models.

Anti-inflammatory activity was assessed on acute oedema induced by carrageenan and dextran.

The acetic acid test has long been used to assess the analgesic effects of bioactive substances on rodents^{9,17}. Acetic acid causes pain by inducing the release of several chemical mediators involved in pain, such as histamine, substance P, prostaglandins (PGE2 and PGE α), serotonin and bradykinin. These chemical mediators stimulate peripheral nociceptive neurons and increase vascular permeability^{18,19}. The aqueous extract of the leaves of *Annona Senegalensis*, like aspirin and tramadol (Trabar*), significantly reduced the number of abdominal contortions dose-dependently, compared with the control group. The extract therefore has analgesic properties.

The analgesic effect of the extract may be due to its action on acetic acid-sensitive visceral receptors, either inhibiting the production of algesic substances or inhibiting the transmission of painful messages²⁰. To characterize the analgesic effect of the aqueous extract of the leaves of *Annona Senegalensis*, the acetic acid test was carried out again with tramadol and the extract at the dose of 200 mg/kg body weight in presence of naloxone, a morphine antagonist. Naloxone is a non-specific opioid receptor antagonist²¹. Tramadol is an opiate that prevents the upward movement of impulses generated at the peripheral ends of C and A δ fibers by acting on ascending pain

pathways²². It therefore acts via a central mechanism. In the presence of naloxone, the analgesic activity of tramadol was reduced, while that of the extract at 200 mg/kg body weight remained unchanged. The analgesic activity of the aqueous extract of the leaves of *Annona Senegalensis* demonstrated in this test therefore results from a peripheral action, like aspirin²³. The analgesic activity of aspirin results from the suppression of pain mediator formation in peripheral tissues by inhibition of cyclooxygenases COX-1 and COX-2 activity²⁴. The aqueous extract of the leaves of *Annona Senegalensis* would therefore possess chemical compounds able to inhibit prostaglandin synthesis, probably by interfering with the cyclooxygenase and/or lipoxygenase pathways. Similar results were obtained by Megwas et al²⁵ with the ethanolic extract of the leaves of *Annona Senegalensis*.

To investigate the potential involvement of central mechanisms in the analgesic effect of the aqueous extract of the leaves of *Annona Senegalensis*, the formalin test was performed. The formalin test is an experimental model for assessing the central and peripheral analgesic properties of a substance. Formalin-induced pain occurs in two phases: The first or neurogenic phase begins immediately after formalin injection (0-5 min). This early phase results from direct stimulation of nerve fibers, with release of substance p, facilitating transmission of the nociceptive message to the central level²⁶. The second phase of pain, which occurs around 15 minutes after formalin injection and lasts for around 30 minutes, is due to the release of inflammatory mediators such as histamine, serotonin, prostaglandins, leukotrienes and bradykinins. Agents acting primarily via central mechanisms inhibit both phases, while peripherally acting agents inhibit the late phase²⁷. The aqueous extract of the leaves of *Annona Senegalensis* significantly inhibited only the late phase of formalin-induced pain, unlike

Tramadol (trabar*) which inhibited both phases. These results confirm that the effect of the aqueous extract of the leaves of *Annona Senegalensis* on pain is mainly peripheral. Similar results were reported by Adzu et al²⁸ with the metanolic extract of the bark of the roots of *Annona Senegalensis*.

The peripheral analgesic effect observed in these analgesic tests suggests that the aqueous extract of the leaves of *Annona Senegalensis* is able to control inflammation.

The anti-inflammatory properties of the extract were evaluated in acute inflammation induced in rats by injection of carrageenan and dextran. The carrageenan-induced edema test is a suitable test for assessing the anti-inflammatory effects of natural compounds²⁹. Injection of carrageenan triggers a triphasic inflammatory response resulting in the release of several chemical mediators responsible for the inflammatory process. The first phase, which lasts around one to two hours, is caused by histamine and serotonin release from mast cells³⁰. The second phase, which extends from the second to the third hour, is due to the release of kinins accelerating edema formation. The third phase begins three hours after carrageenan injection and is attributable to the synthesis of prostaglandins and leukotrienes³¹. The aqueous extract of the leaves of *Annona Senegalensis* like diclofenac significantly inhibited edema dose-dependently and at all phases. However, the maximum inhibition of edema by the extract was in the third phase. The aqueous extract of the leaves of *Annona Senegalensis* contains secondary compounds able to inhibit the release of inflammatory mediators and the biosynthesis of prostaglandins. Non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac inhibit COX-2 involved in prostaglandin synthesis³². The significant inhibition of edema observed with the extract at the fifth hour suggests that the anti-inflammatory effect of the extract is exerted more on cyclooxygenases. Yougbaré-Ziébrou et al³³ reported similar results on the anti-inflammatory effects of the aqueous extract of the leafy stems of *Saba Senegalensis*.

The dextran oedema induction test confirmed the anti-inflammatory properties of the aqueous extract of the leaves of *Annona Senegalensis* during the first phase of inflammation. In this model of acute inflammation, the oedema induced is mediated by the release of histamine and serotonin from mast cells³⁴. The extract significantly inhibited edema from the first hour like cyproheptadine, an anti-histamine and anti-serotonergic³⁵ used as a standard reference drug.

The aqueous extract of the leaves of *Annona Senegalensis* would therefore possess compounds able to reduce inflammation by acting like cyproheptadine and therefore by competitively inhibiting histamine H1 and serotonin 5-HT2 receptors^{36,37}.

Phytochemical analysis showed the presence of tannins, flavonoids, sterols and triterpenes, saponosides and leucoanthocyanins in the extract. Flavonoids and tannins have the capacity to inhibit the production of pro-inflammatory mediators such as histamine, serotonin, leukotrienes and prostaglandins^{38,39}. Flavonoids' influence on blood vessels is an important factor in their anti-inflammatory and anti-irritant activities. They reduce tissue congestion and exhibit powerful anti-edematous activity⁴⁰. Wynn and Fougère⁴¹ have also demonstrated the anti-inflammatory properties of many saponins. The presence of these secondary compounds may justify the anti-inflammatory and analgesic effects of the aqueous extract of the leaves of *Annona Senegalensis*.

5. CONCLUSION

The present study showed that the aqueous extract of the leaves of *Annona Senegalensis* possesses both analgesic and anti-inflammatory properties similar to non-steroidal anti-inflammatory drugs (NSAIDs). The results constitute a

scientific basis justifying the traditional use of *Annona Senegalensis* in the treatment of pathologies involving inflammation. This pharmacological knowledge could therefore make this plant a major contributor to the health of humans and animals, particularly in the treatment of pain and inflammation such as mastitis in dairy production.

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Conflict of interest statement

We declare that we have no conflict of interest.

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