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## Original Research Article

# Analgesic, anti-inflammatory and antipyretic activities of ethanol extract of *Annona senegalensis* leaves in experimental animal models

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## ABSTRACT

**Background:** This study was carried out to establish the analgesic, anti-inflammatory and antipyretic effects of the ethanol extract of *Annona senegalensis* leaves in experimental animals.

**Methods:** The analgesic activity was measured using the abdominal constriction and tail flick tests. The anti-inflammatory activity was performed using xylene and egg-albumen paw induced tests, while the antipyretic activity was measured using brewer's yeast and 2, 4 dinitrophenol induced pyrexia tests, respectively.

**Results:** The leaf extract at all doses used exhibited significant ( $p < 0.05$ ) analgesic, anti-inflammatory and antipyretic activities.

**Conclusions:** Results show that ethanol leaf extract of *Annona senegalensis* possess therapeutic potential against pains and feverish conditions, supporting the claims of its this plant as remedy for similar ailments.

**Keywords:** *Annona senegalensis*, Analgesic, Anti-inflammatory, Antipyretic

## INTRODUCTION

Herbs have proven to be of high importance for prevention and treatment of diseases, and of high value to immunological provocation against much pathologic conditions.<sup>1</sup> Almost all plant extracts are virtually used for treatment and prevention of diseases. Many of these plant extracts boost humoral cell mediating immunity against bacteria, virus, fungi, protozoa, cancer and even haematological cells and parameters.<sup>2,3</sup> *Annona senegalensis* pers. (Annonaceae), commonly known as

Africa custard – apple, and wild sour sop, is a specie of flowering plant in the custard apple family.<sup>4</sup> The plant is a perennial shrub which is widely grown in Nigeria. *A. senegalensis* is locally known as “Gwander daji” in Housa, “Abo” in Yoruba, “Uburu ocha” in Igbo, and “Ikpoko” among the Idoma speaking people in the middle Belt region of Nigeria.<sup>5</sup> The leaves, flowers and fruits are edible, and are used by local population in treating many health conditions, ranging from dizziness and indigestion, to chest pain, colds and venereal diseases. The bark can be processed to produce yellow- brown dye used locally

as insecticide and pesticide.<sup>6</sup> In Nigeria, particularly, the roots are used to treat pains.<sup>7</sup> The leaves and stem are used for the treatment of intestinal problems.<sup>8,9</sup> Cancer treatment, diarrhoeal and dysentery, Antimicrobial and anticonvulsant, Trypanosomiasis.<sup>4,5,10-12</sup> Other applications of the leaf extract include neurological disorder and testicular function indices.<sup>13,14</sup> However, our interest in this plant was justified by its important medicinal value in the treatment of pains and feverish conditions.

Therefore, the objective of this study was to evaluate the analgesic, anti-inflammatory and antipyretic properties of the ethanol extract of *A. senegalensis* leaves in experimental animals.

## METHODS

### *Plant collection and identification*

Fresh leaves of *Annona senegalensis* were collected from Suleja, Niger State, Nigeria. The plant was identified by a taxonomist in the department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

### *Extraction procedure*

The leaves were washed, shade dried at room temperature in the Department of Optometry, School of Health Technology, Federal University of Technology, Owerri and subjected to size reduction to a coarse powder by using mortar and pestle. Four-hundred grams (400 g) of the powder was macerated in 2.5 l of ethanol for 24 hours with frequent agitation until the soluble matter has dissolved. The mixture was strained, the marc pressed and the filtrate concentrated to dryness by gentle heating over a water bath set at low temperature (40°C) to obtain a semi solid brownish extract. The extract was stored at 4°C in a refrigerator till when needed for the assay.

### *Experimental animals*

Adult Wistar rats (200-250 g) and albino mice (20-25 g) of either sex were used in this study. The animals were sourced and maintained at the Animal house of Federal University of Technology, Owerri, Imo State. The animals were acclimatized for 2 weeks prior to the tests. They were fed with standard diet and had access to clean water ad libitum. They were maintained under standard conditions of humidity, temperature and 12 hours light and 12 hours darkness cycle. The study protocol was carried out as per the rules and regulations of the Institutional Animal Ethical Committee, School of Health Technology, Federal University Technology, Owerri (FUTO/SOHT/REC/02/0101) as well as the international guidelines on the Use and Handling of Experimental Animals.<sup>15</sup>

### *Acute toxicity test*

Oral acute toxicity test was performed using the organization of economic cooperation and development (OECD) guideline for testing of chemicals 401.<sup>16</sup> Male and female rats weighing 180-200 g were used for this study, and were conducted in two phases. Three groups of 3 rats (male separated from female) in each cage were administered 100, 600 and 1000 mg/kg of the *Annona senegalensis* leaf extract orally. The rats were observed for signs of toxicity and mortality for 24 hrs with special attention given to the first 4hrs. This was followed by administration of the extract (2000, 3000 and 5000 mg/kg) to the next three groups of 1 rat and equally observed as earlier stated, and daily for 7 days for any signs of toxicity which include salivation, paw-licking, writhing, change in body weight and mortality. The number of deaths in each group was recorded and the final LD50 values were calculated.

### *Phytochemical analysis*

The ethanol extract of *Annona senegalensis* leaf was screened for the presence of active constituents using the methods described by slight modifications.<sup>17,18</sup>

### *Analgesic activity*

#### *Acetic acid-induced writhing test*

This test was done using the method described by Akuodor et al adult albino mice of both sexes, weighing between 20-25g were used for the study.<sup>19</sup> The animals were divided into five groups of 6 each and pre-treated as follows. Group 1, which serves as control, received 20 ml/kg of normal saline. Group 2, 3 and 4 received 100, 200 and 400 mg/kg of *A. Senegalensis* extract, respectively. Group 5 received acetylsalicylic acid (ASA) 150 mg/kg. All the treatments were administered orally. Thirty minutes after drug administration, each mouse was intraperitoneally injected 20 ml/kg 0.7% solution of acetic acid in distilled water to create pain sensation. Each mouse was then placed in transparent Perspex observation box, and the number of abdominal constrictions for each mouse was counted for 30 min, commencing 5 min after the administration of acetic acid.

#### *Tail immersion test*

This test was carried out using the method described by Akuodor et al with slight modification. Thirty mice were randomized into five groups of 6 in each cage.<sup>20</sup> The mice were fasted for 24 h but were allowed access to clean water ad libitum. Group 1, which received 20 ml/kg of normal saline and served as control. Groups 2, 3 and 4 were given 100, 200 and 400 mg/kg respectively of the *A. senegalensis* extract. Group 5 was given Morphine 10 mg/kg (standard drug). All were administered orally. Thirty min later, each mouse was restrained in a horizontal cylinder with the tail hanging freely in a water

bath set at  $52 \pm 1^{\circ}\text{C}$  and the time it takes for each mouse to withdraw its tail out of the water was recorded. The latency was evaluated at 30, 60, 90 and 120 min. The initial reading was taken immediately before administration test agents.

### Anti-inflammatory study

#### Xylene- induced ear oedema test in mice

Adult male and female mice were divided into five groups of 6 mice in each cage were used in this study following the method as reported by Jumping et al Group 1 served as control and was administered with distilled water (20 ml/kg).<sup>21</sup> *A. senegalensis* leaf extract (100, 200 and 400 mg/kg, p. o) was given to groups 2, 3 and 4 respectively, while the standard drug, dexamethasone (4 mg/kg), was administered to group 5. One-hour post drug administration, oedema was induced in each mouse by applying a drop of xylene at the inner surface of the right ear. Three hours afterwards, the animals were sacrificed under light anaesthesia and both ears were cut off to equal size and weighed. Inflammation was taken as mean difference between the right and left ear for each group.

#### Egg albumin- induced paw oedema in rats

Adult Wistar rats of both male and female employed in this study were randomized into five groups of 6 rats per cage. The control group (group 1) received normal saline 20 ml/kg, while the extract groups (2-4) were given 100 mg/kg, 200 mg/kg and 400 mg/kg of *A. senegalensis* respectively. The standard drug aspirin 150 mg/kg was given to group 5. All administrations were through oral cannulation. An hour after, oedema was induced in rats by injection of 0.1 ml of fresh egg- albumin into the sub plantar of the right hind paw Essien et al The paw volumes were measured at 0, 20, 40, 60, 80, 100 and 120 min, respectively by using plethysmometer.<sup>22</sup>

### Antipyretic screening

#### Brewer's yeast-induced pyrexia

The method described by Akuodor et al was adopted in this study with slight modification. Rats recruited for this experiment were divided into five groups of 6 rats each with their basal temperature measured using digital

thermometer. The animal was subcutaneously injected with 20 ml/kg of 15% yeast suspended in 0.5% methylcellulose solution to induce pyrexia. Twenty-four hours after yeast injection, the rectal temperature of each rat was again measured and rats showing an increase of  $0.6^{\circ}\text{C}$  were employed for the study. Thereafter, normal saline 20 ml/kg was administered to rats in group 1 (control), while graded doses of *A. senegalensis* ethanol leaf extract 100, 200 and 400 mg/kg were orally administered to rats in groups 2, 3, and 4 respectively. The reference drug group (5) received 20 ml/kg of paracetamol. The rectal temperature of each rat was again taken at 1-hour interval for the next 5 hours.<sup>23</sup>

#### 2, 4 – Dinitrophenol (DNP) – induced pyrexia.

Adult Wistar rats were allotted to five groups of 6 animals in each cage. Their basal rectal temperatures were initially taken and 10 mg/kg of DNP was intraperitoneally injected into the rats. Hyperthermia developed 30 minutes after DNP administration. Group 1 control rats received 20 ml/kg of normal saline. The leaf extract at doses of 100, 200 and 400 mg/kg were administered to the rats in groups 2, 3 and 4 respectively, while group 5 received Paracetamol 20 mg/kg. All were given by oral route. Rectal temperature of each rat was taken at an hour interval for 5 hrs.<sup>24,25</sup>

### Statistical analysis

Results were expressed as means $\pm$ SEM and analyzed with statistical package for social sciences (SPSS version 20) by using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Difference in the mean  $p < 0.05$  was statistically considered significant.

## RESULTS

### Phytochemical analysis

It is important to know the chemical nature of plant products when their pharmacological responses are evaluated. Phytochemical evaluation of *A. senegalensis* ethanol leaf extract showed the presence of these secondary metabolites; alkaloids, saponins, tannins, flavonoids, terpenoids, and anthraquinone, while phlobatannins and steroids were not detected. These constituents have been reported to possess important biological activities.<sup>26</sup>

**Table 1: The effect of ethanol leaf extract of *A. senegalensis* on acetic acid- induced writhing in mice.**

Drug	Dose (mg/kg)	Writhes	%inhibition
<b>Control</b>	20	19.00 $\pm$ 1.26	-
<i>A. senegalensis</i>	100	12.67 $\pm$ 1.4	33
	200	5.83 $\pm$ 0.87	67*
	400	3.50 $\pm$ 1.23	83*
<b>Aspirin</b>	150	2.50 $\pm$ 1.59	87*

Results are mean  $\pm$ SEM; (n=6) \*significant at  $p < 0.05$  when compared to control

**Table 2: The effect of the ethanol leaf extract of *A. senegalensis* on tail immersion in mice time Interval (mins).**

Drug	Dose (mg/kg)	0	30	60	90	120
<b>Control</b>	20	7.17±0.87	8.17±0.04	9.67±1.65	9.83±1.62	11.67±0.71
<b><i>A. senegalensis</i></b>	100	7.67±0.49	10.00±0.45	12.33±1.17	13.17±1.10	15.00±0.86*
	200	8.17±0.06	12.67±1.09	13.5±0.72	15.17±1.30	16.83±1.05*
	400	8.49±0.87	14.34±0.23	16.90±0.36	18.05±0.74	20.95±0.37*
<b>Morphine</b>	10	8.67±1.05	17.67±1.33	19.67±1.70	20.67±1.26	23.67±1.09*

Results are mean±SEM; (n=6) \*significant at p<0.05 when compared to control

### Acute toxicity tests

There were no lethality or toxic reactions observed at any of the doses administered. All the rats were healthy and active during and after the period of study. Hence, oral acute toxicity result was greater than 5000 mg/kg in rats.

### Analgesic effects

#### Acetic acid induced writhing in mice

*A. senegalensis* leaf extract showed significant (p<0.05) dose dependent reduction of abdominal contraction in mice. The effect of the herbal extract at 400 mg/kg was comparable to standard drug, aspirin (Table 1).

#### Tail immersion test in mice

The leaf extract of *A. Senegalensis* significantly (p<0.05) protected the animals from thermal stimulus. However, the standard drug, morphine, produced a greater percentage of protection than the extract (Table 2).

### Anti-inflammatory effect

#### Xylene – induced ear oedema in mice

The extract exerted significantly (p<0.05) dose dependent anti-inflammatory activity in xylene- induced ear oedema in mice. The extract compared favourably with the standard drug, Dexamethasone 4 mg/kg (Table 3).

### Egg albumin – induced paw oedema in rats

The ethanol leaf extract of *A. senegalensis* exhibited significant (p<0.05) dose dependent anti-inflammatory activity in egg- albumin- induced paw oedema when compared to control. The effect of the extract was comparable to aspirin, the standard drug (Table 4).

### Antipyretic effect

#### Yeast – induced pyrexia in rats.

Treatment of the animals with the ethanol extract of *A. senegalensis*, at the dose of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight, significantly (p<0.05) decreased the rectal temperature of the test rats in dose dependent manner. The antipyretic effect started from the first 30 min and was maintained for 120 min after extract administration. The result obtained from both the standard drug and the extract – treated rats were statistically compared with control group and a significant reduction in temperature was observed (Table 5).

#### Dinitrophenol – induced pyrexia in rats

Administration of different doses *A. senegalensis* leaf extract caused a significant (p<0.05) reduction in temperature of the herbal treated rats when compared with the control. The antipyretic effect of the extract was comparable to that of the standard drug, aspirin (Table 6)

**Table 3: The effect of ethanol leaf extract of *A. senegalensis* on xylene-induced ear oedema in mice**

Drug	Dose (mg/kg)	Weight of right ear	Weight of left ear	Increase in ear weight	% inhibition
<b>Control</b>	20	0.073±0.01	0.031±0.00	0.042±0.00	-
<b><i>A. senegalensis</i></b>	100	0.050±0.00	0.029±0.00	0.20±0.00	50*
	200	0.038±0.00	0.025±0.00	0.013±0.00	70*
	400	0.032±0.01	0.021±0.00	0.010±0.00	78*
<b>Dexamethasone</b>	4	0.029±0.00	0.020±0.00	0.009±0.00	80*

Results are mean ±SEM; (n=6) \* significant at p<0.05 when compared to control.

**Table 4: The effect of ethanol leaf extract of *A. senegalensis* on Egg-albumin-induced paws oedema in mice.**

Drug	Dose (mg/kg)	0	20	40	60	80	100	120
<b>Control</b>	20	1.24±0.02	1.64±0.01	1.72±0.02	1.79±0.02	1.86±0.02	1.93±0.02	2.05±0.02
<b>A. senegalensis</b>	100	1.22±0.04	1.60±0.02	1.53±0.03	1.46±0.02	1.38±0.03	1.31±0.02	1.20±0.03*
	200	1.24±0.02	1.63±0.01	1.56±0.02	1.48±0.02	1.30±0.02	1.24±0.02	1.8±0.02*
	400	1.19±0.02	1.57±0.03	1.49±0.03	1.38±0.02	1.30±0.02	1.21±0.03	1.15±0.03*
<b>Aspirin</b>	150	1.17±0.04	1.61±0.02	1.49±0.02	1.39±0.03	1.30±0.03	1.22±0.03	1.12±0.03*

Results are mean ±SEM; (n=6) \*significantly at p<0.05 when compared to control.

**Table 5: The effect of ethanol leaf extract of *A. senegalensis* on yeast-induced pyrexia in rat time interval (hr).**

Drug	Dose (mg/kg)	0	0.5	1	2	3	4	5
<b>Control</b>	20	36.59±0.12	37.57±0.14	37.68±0.13	37.76±0.12	37.82±0.11	38.13±0.08	36.13±0.00
<b>A. senegalensis</b>	100	35.63±0.17	37.42±0.12	37.14±0.04	36.18±0.06	36.71±0.08	36.39±0.08	35.72±0.06*
	200	35.46±0.19	37.38±0.08	36.76±0.08	36.57±0.09	36.28±0.13	35.84±0.16	35.24±0.17*
	400	35.50±0.13	37.46±0.13	36.73±0.09	36.32±0.09	35.99±0.09	35.64±0.07	35.35±0.05*
<b>Aspirin</b>	150	36.67±0.04	37.67±0.13	37.38±0.06	36.86±0.04	36.68±0.05	36.22±0.15	35.10±0.30*

Results are mean±SEM; (n=6) \* significant at p<0.05 compared to control

**Table 6: The effect of ethanol leaf extract of *A. senegalensis* on 2, 4 Dinitrophenol – induced pyrexia in rat Time interval (hr).**

Drug	Dose (mg/kg)	0	0.5	1	2	3	4	5
<b>Control</b>	20	35.39±0.06	37.75±0.04	37.88±0.03	37.69±0.03	37.51±0.03	37.32±0.02	36.72±0.02
<b>A. senegalensis</b>	100	35.27±0.03	37.32±0.06	36.99±0.11	36.45±0.03	36.18±0.01	35.54±0.05	35.24±0.02*
	200	35.29±0.03	37.33±0.02	36.55±0.02	36.23±0.01	35.68±0.04	35.45±0.04	35.19±0.02
	400	35.25±0.02	37.35±0.03	36.49±0.05	36.24±0.04	35.55±0.03	35.32±0.02	35.17±0.01*
<b>paracetamol</b>	20	35.24±0.03	37.25±0.03	36.48±0.03	36.20±0.02	35.67±0.02	35.41±0.02	35.15±0.02*

Results are mean±SEM; (n=6)\*significant at p<0.05 compared to control

## DISCUSSION

The present study was carried out on *Annona senegalensis* leaf extract to validate its use in traditional medicine as an analgesic, anti-inflammatory and antipyretic agents. The leaf extract was found to possess analgesic activity when assessed using abdominal constriction and tail immersion tests. The response which was concentration dependent show the involvement of peripheral and central analgesic mechanisms. More so, the obtained results also indicated that *A. senegalensis* is effective in inhibiting the analgesic activity caused by the chemical stimulus in the tail immersion method. The acetic acid abdominal constriction test which is highly sensitive to the point that it is able to detect the pain relieving effect of agents and dose levels that may not be active in tail immersion method is however, believed to involve the stimulation of peripheral receptor system and is linked in part to the

local peritoneal receptors on the surface of the cells lining the peritoneal cavity.<sup>27,28</sup> The abdominal constriction test is normally used to assay the peripheral analgesic effects of medicinal agents.<sup>20,29</sup> The observed abdominal constrictions in this study are probably due to irritation of the peritoneal cavity induced by administration of acetic acid.<sup>30</sup> Prolonged irritation leads to an increase in the concentration of peritoneal prostaglandins PGE2 and PGF2x.<sup>31</sup> This increase in prostaglandin levels within the peritoneal cavity then causes inflammatory pain by increasing capillary permeability.<sup>32</sup> Furthermore, *A. senegalensis* ethanol leaf extract reasonably reduced the duration of writing in each mouse which result to the effect on arachidonate release and metabolism.<sup>33</sup> The analgesic activity of the extract which was dose dependent may indicate the involvement of peripheral pathway.

To further confirm the analgesic effect of the *A. senegalensis* extract, tail immersion test was carried out. It has been reported that centrally acting agents like morphine, elevate the pain threshold of mice towards heat and pressure, whereas peripherally acting drugs like aspirin, have been reported to possess analgesic effect in writhing activity only.<sup>34,35</sup> The leaf extract of *A. senegalensis* significantly protected the animals from the thermal stimuli which confirmed its analgesic effect. This goes further to suggest a central mechanism of action of the leaf extract.

Xylene is known to cause irritation in mouse ear which leads to accumulation of fluid and oedema, characteristic of acute inflammatory response. Suppression of this response may indicate antiphlogistic effect.<sup>20</sup> The leaf extract of *A. senegalensis* exhibited a significant inhibition of ear oedema caused by xylene. This activity suggests the inhibition of phospholipase A2 which is involved in the pathophysiology of inflammation due to xylene.<sup>36</sup>

*A. senegalensis* leaf extract also caused significant inhibition of Egg- albumin induced hind paw oedema in rats. Albumin-induced oedema has a biphasic response and the early phase is mediated through the release of histamine, serotonin and kinins. The late phase is related to the release of prostaglandins which is mediated by bradykinin, leucotrienes and polymorphonuclear produced by tissue macrophages.<sup>21,37</sup> These results indicate that the extract acts on both early and late phases of inflammation. The mechanism of anti-inflammatory activity of *A. senegalensis* could be speculated to be through the inhibition of one or more of the released inflammatory mediators.

The leaf extract of *A. senegalensis* also possesses antipyretic activity. This effect was assessed using brewer's yeast and 2, 4 dinitrophenol induced pyrexia tests. It is already established that pyretic activity involves stimulation of the region in the hypothalamus that controls body temperature through prostaglandins synthesized within the central nervous system, and the blood- brain barrier prevents drug molecules or other chemicals from entering the central nervous system.<sup>38</sup> The regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained.<sup>39</sup> The ability to cross the blood brain barrier may be one of the factors contributing to the observed antipyretic activity of *A. senegalensis* leaf extract.

The importance of medicinal plants is mostly a linked to the combination of the secondary metabolites. Different isolated flavonoids from medicinal plants have shown to possess remarkable analgesic, anti-inflammatory and antipyretic activities.<sup>40</sup> It is not surprising however to

have observed these activities in *A. senegalensis* leaf extract, since it contains flavonoids.

## CONCLUSION

The results of this study show that the ethanol leaf extract of *A. senegalensis* possesses both peripheral and central analgesic activity, anti-inflammatory and antipyretic effects which thus supports the folklore uses of the leaf extract for the treatment of various diseases.

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