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Original Research Article

Annona muricata L., Stem-Bark Exhibit Antidepressant-Like Activity in Sprague-Dawley Rats

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ABSTRACT

Annona muricata L. (Annonaceae) is widely used in the Amazon and Carribean in natural medicine as an antiparasitic, insecticidal, sedative and as an astringent for diarrhea among others. In Nigeria however, *Annona muricata* is among the under-utilized species of plants. This study investigated the antidepressant and ambulatory effect of *A. muricata*, using the open field test and forced swim tests on Sprague-Dawley rats administered ethanol stem-bark extracts of the Nigerian grown species. Rats were administered *A. muricata* stem-bark extract (50, 150 and 300mg/kg) alone and in combination with the drugs imipramine and sertraline (10mg/kg), respectively for 14 days. The administration of the extract was observed to cause a significant reduction in the swimming time and immobility time of the rats in the forced swim test. The ambulatory behavior of the rats was observed to decrease when the extract (150 and 300mg/kg) was administered alone to the rats while a much further decrease was observed in the explorative tendencies of the rats when the extract was combined with the imipramine and sertraline. The results suggested that the ethanol stem-bark extract of *A.muricata*, possess sedative and antidepressant effects

Keywords: Ambulatory, Antidepressant, Forced swim test, Immobility time.

Introduction

Annona muricata is a small upright ever green tree with large glossy dark green leaves. It produces large prickly heart-shaped edible fruits which are green in colour with white fleshy pulp. *Annona muricata* belongs to the annonaceae family and annona genus. Various medicinal uses have been reported ranging from the use of leaves, bark, roots, and fruits to seeds of *A. muricata* in folklore medicine.[1,2,3,4] All parts of the tree are reportedly used in natural medicine in various forms as anti-diarrheal, sedative, heart tonic, anti-tumor, anti-parasitic, anti-hypertensive and as a psychotropic medicine in the Amazon and Caribbean communities.[5,6] The value of medicinal plants and their contributions to modern medicine have been enhanced due to improvement in analysis and quality control along with advances in clinical research. Most rural communities, government and scientific agencies regard medicinal plants as therapeutic agents as well as essential pivot in health care programmes especially in developing countries.[7,8,9] Psychoactive constituents isolated from plants have been shown to have beneficial therapeutic activities and have helped to introduce herbal formulations approved by Food and Drug Administration for the treatment of psychological conditions.[10,11]
Depression is a psychological disorder that affects a person's mood, physical health and behavior, resulting in low self-esteem and a loss of interest or pleasure in normally enjoyable activities.[12] Depression is a major contributor to the global burden of diseases and it is reported as the main symptom of many psychiatric diseases.[13,14]

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Depression is managed by the use of anti-depressant pharmaceutical medications as well as alternate and complimentary medicines.[15,16] A large proportion of Nigerian population still prefers herbal treatment hence the need for a thorough scientific investigation of medicinal plants in order to validate their folklore usage.[17] *A. muricata* is not widely publicized in folklore medicine in Nigeria, making the psychoactive potential health benefits of the plant not to be adequately tapped in the Nigerian grown species.[18] It is therefore important to establish some of the potential health benefit of the different parts of this valuable under-utilized fruit plant. This is a follow-up study on our earlier investigation where the leaf extract of this plant was used.[19] The aim of this study was to investigate the anti-depressant effect of the Nigerian grown *A. muricata*, stem-bark extract using Sprague-Dawley rats.

Materials and Methods

Chemicals and reagents
The standard antidepressant drugs (sertraline and imipramine) used, were obtained from Ranbaxy Ltd, (India and Assos IIac, Istanbul brand). Analytical grade reagents were used.
Plant material: *Annona muricata* stem-bark was collected from Igan Otoko, Ogun state, South-West, Nigeria, in December, 2021. The plant was identified at the Department of Botany University of Lagos, Nigeria and a voucher number (LUH 5252) was deposited in the University herbarium.

Plant extract preparation
A. muricata stem-bark was air dried for 5 days before being placed in the oven at 40°C to dry until a constant weight was obtained. The dried pieces of the stem-bark were pulverized with Christy-Norris Laboratory Hammer Mill and kept in an air tight container until needed. *A. muricata* ethanol extract was obtained by maceration of six hundred grams (600 g) of the coarsely ground stem-bark in 95% ethanol (5 litres) for 72 hours at room temperature. The stem-bark extract was obtained after evaporation and concentration of the filtrate at controlled temperature (40 – 50°C) with a rotary evaporator in vaco. The extract was stored at 0°C until used.

Animal Handling and Treatment: Female Sprague-Dawley rats, weighing 160.50 – 180.50 ± 7.30 g were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, and maintained under standard conditions (27 ± 2ºC, 12h dark and light cycle). The rats were fed commercial rat pellets (Pfizer Lagos, products) and water *ad- libitum*, and allowed to acclimatize for l4 days. The experiment was performed with the permission of the University's Animal Ethical Committee, and in accordance with approved Institutional and National guidelines for the care and use of laboratory animals.

Twelve (12) groups of 6 rats each were randomly created. Group 1 was the control which was given 5% DMSO orally. Three groups received orally varied concentrations (50,150 and 300 mg/kg body weight) of *A. muricata* stem-bark extract. Another two groups received 20mg/kg body weight of sertraline and imipramine orally respectively, while six other groups received varied doses (50, 150 and 300 mg/kg body weight) of *A. muricata* extract in combination with 10 mg/kg body weight of sertraline or imipramine respectively. Treatments were carried out for 14 days respectively. The animals were weighed weekly and allowed feed and water *ad- libitum* throughout the period of the assay. Experimental doses of 50, 150, and 300 mg/kg body weight were selected for comparative purpose from previous studies.[19] Open-field test and forced swim test were used to evaluate sedative and anti-depressant activity of the extract.

Determination of Ambulatory behaviour

The open-field test of Royce,[20] was used to assess the ambulatory behavior of the rats. The open- field apparatus consisted of a square wooden arena (64cm x 64cm and 40cm high). The floor of the wooden arena was partitioned into 16 equal marked squares with a well demarcated center square of the same dimension. The animals were placed individually in the centre (marked) of the arena and allowed to explore the arena freely. The locomotion frequency (number of squares crossed, latency to mobility and distance covered by the rat) as well as the number of times the animal stood on the hind paws (number of rearings and grooming) were recorded during a test period of 5min. These movements were also recorded with a cam-recorder (DNEwebcam, Porto Algre, Brazil). In order to eliminate possible bias caused by odours left by previous rats, the arena was cleaned between each trial with 10% ethanol solution. The test was carried out at room temperature (27 ± 2^{0}C), in a noise and light controlled room.

Determination of Antidepressant-Like effect

The antidepressant-like effect of the stem-bark extract of *A. muricata* was evaluated using the forced swim test as modified by Slattery and Cryan.[21,22] Rats were forced to swim in a water-filled cylindrical transparent glass vessel (50cm high and 21cm diameter), at room temperature (27±2°C). The water was sufficiently deep to stop the animals from resting their tails or limbs on the bottom of the glass vessel. A 10 min test session was carried out on each rat 24h before the main test. The total duration of immobility (sec) was measured during a 5min. test session. Except the movements necessary to keep the rat's head above the water, the rats were considered immobile when they ceased struggling and remained floating in water. To avoid the influence of water temperature and substances left from the previous session, the water was changed after each rat. The rats' movements were also recorded with a cam-recorder (DNE webcam, Porto Algre, Brazil).

Statistical analysis

Results were analyzed using one-way analysis of variance (ANOVA) with Dunnet's test and presented as mean ± standard error of mean (SEM). Values were considered statistically significance at $p < 0.05$.

Results and Discussion

The oral administration of *A. muricata* stem-bark extract to the rats, resulted in a significant ($p < 0.05$) dose dependent reduction in the distance moved by the animals as indicated by the reduced number of crossings in the open field test compared to the control group as shown in Figure 1A.

The administration of the standard drugs (imipramine and sertraline) alone caused a significant ($p < 0.05$) increase in the distance moved by the animals compared to the control group as shown in Figure 1A, while the combination of the extracts with the standard drugs resulted in a further reduction of the ambulatory behavior of the rats compared to the control group as shown in Figure 1B, 2A and 2B. There was a significant ($p < 0.05$) reduction in the distance moved by the rats treated with the extract combined with 10 mg/kg body weight of imipramine at 150 mg/kg body weight as shown in Figure 1B, 2A and 2B in the open field test.

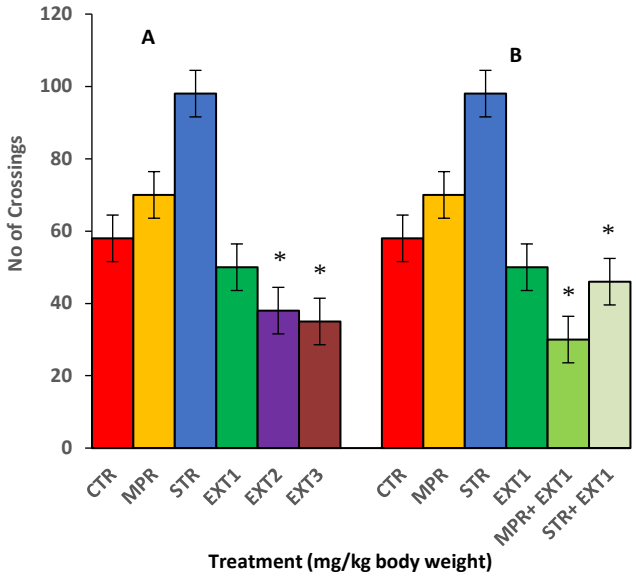

Figure 1: Ambulatory response of rats administered, *A. muricata* stem-bark extracts (A) alone and (B) combination of 50mg/kg b.w extract with imipramine and sertraline (10 mg/kg b.w) respectively. Values represent ± Mean SEM (n = 6). *P (0.05) significant compared to control.

CTR - Vehicle control, MPR and STR - rats administered 20 mg/kg body weight imipramine and sertraline respectively. EXT 1, EXT 2, EXT 3 – rats administered, 50, 150, 300 mg/kg body weight extracts respectively. (B) – (MPR + EXT 1 and STR + EXT 1) – rats administered 50 mg/kg body weight extract in combination with 10 mg/kg body weight imipramine and sertraline respectively.

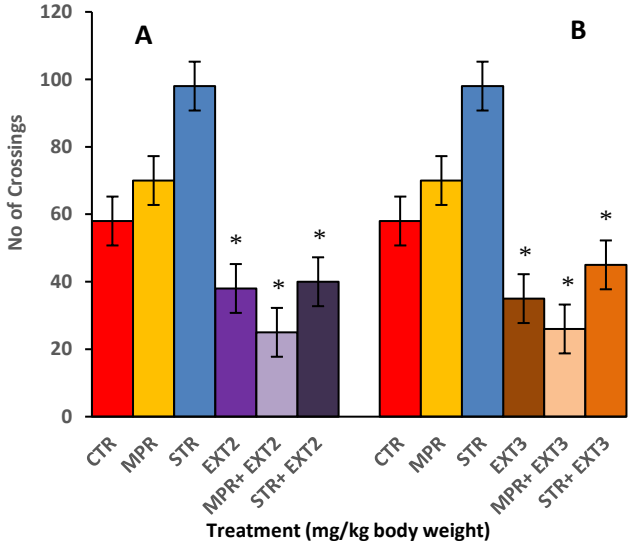

Figure 2: Ambulatory response of rats administered, *A. muricata* stem-bark extracts (150 and 300 mg/kg b.w (A) and (B), and in combination with (10 mg/kg b.w) imipramine and sertraline respectively. Values represent ± Mean SEM (n = 6). *P (0.05) significant compared to control.

The number of times the animals stood on their hind limbs and cleaned their paws (rearing and grooming) was non- significantly ($p \geq 0.05$) reduced in the rats administered the extract but was significantly increased ($p \leq 0.05$) in the groups administered the imipramine and sertraline alone compared to the control group as shown in Figure 3A. There was a significant ($p < 0.05$) reduction in the number of rearing and grooming when the extract was administered in combination with imipramine at 150 mg/kg body weight as shown in Figure 3B, 4A and 4B in the open field test.

The open field test provides a way to assess the animal's exploration of the environment, general locomotion activity as well as an initial screening for anxiety-related behaviour in rodents.[23,24] Changes in the measurements of ambulation, explorative tendencies, normal rearing and grooming behaviors in animals are used to assess sedative or stimulant effects of pharmacological agents.[25]

Therefore, the reduction in the ambulatory behavior of the rats observed after the administration of *A. muricata* stem-bark extract may be due to sedative effect of the extract. This result is in agreement with similar effects demonstrated in an earlier study by *A. muricata* leaf extract.[19] The result also suggested that care should be taken in the use of standard drugs along with herbal preparations since such combinations could produce different results with different drugs.[26]

In the forced swim test, a significant dose dependent decrease in the immobility time of the animals (corresponding to the increase swimming or struggling time), administered *A. muricata* stem bark extract was observed compared to the control group as shown in Figure 5. The animals administered the extract exhibited increased swimming (struggling) tendencies. A further decrease in the immobility time was observed in the rats administered the extract in combination with the standard drugs when compared to the control and the extract alone administration as shown in Figure 5A and B. The decrease in the immobility period of the animals in the forced swim test was much more prominent in the groups administered a combination of the extract with 10 mg/kg body weight of sertraline as shown in figure 5B.

The forced swim test (FST) in rats is a pre-clinical test employed to evaluate drugs being screened for antidepressant activity.[27]

Immobility has been interpreted as a behavior which results from a negative mood and represents a kind of hopelessness in the animal. It has been shown that rodents given antidepressants swim harder and longer than controls.[22,28] The reduction in immobility time and increased swimming (struggling) time observed by the administration of *A. muricata* stem-bark extract is an antidepressant-like effect. The results are in agreement with similar effects demonstrated by *A. muricata* leaf extract.[19]

The precise mechanisms by which the extract produce antidepressant-like effect is not completely understood, however the present study demonstrated that the antidepressant-like effect of the extracts is not related to a psycho stimulant activity as demonstrated by the control groups and observed from the open field test.

Conclusion

In conclusion, the administration of *A. muricata* ethanol stem-bark extract caused a reduction in the ambulatory behavior of the rats in the open field test while the extract caused a significant ($p < 0.05$) reduction in immobility time resulting in subsequent increase in swimming or struggling time in the force swim test.

The results of this study indicated that *A. muricata* stem-bark extract produced both sedative effect and anti-depressant- like effect in rats, which corroborate the use of *A. muricata* stem-bark in folklore medicine as a sedative and an antidepressant.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

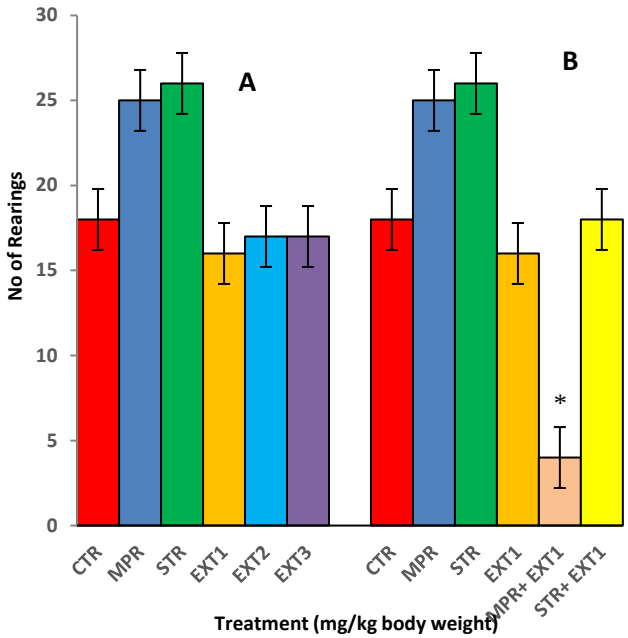

Figure 3: Ambulatory response (grooming and rearings) of rats administered, *A.* muricata stem-bark extracts (50, 150and 300 mg/kg b.w) alone (A) and 50 mg/kg body weight extract (B), in combination with (10 mg/kg b.w) imipramine and sertraline respectively. Values represent ± Mean SEM (n = 6). *P (0.05) significant compared to control

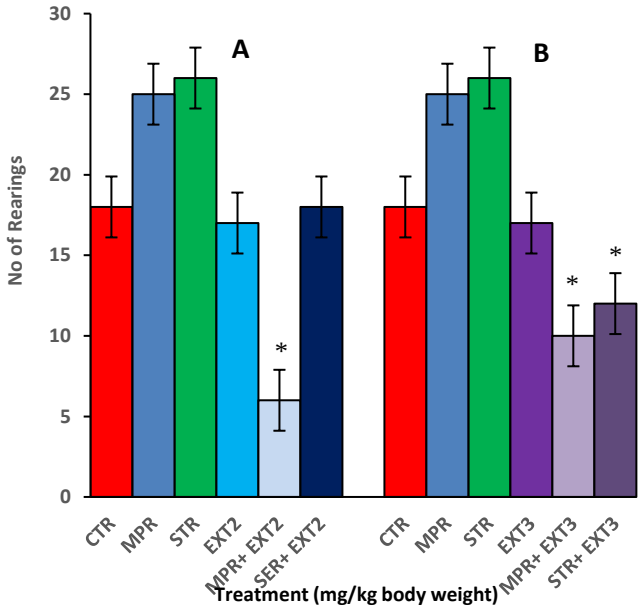

Figure 4: Ambulatory response (grooming and rearings) of rats administered, *A.* muricata stem-bark extracts (150 and 300 mg/kg b.w (A) and (B) respectively, and in combination with (10 mg/kg b.w) imipramine and sertraline respectively. Values represent ± Mean SEM (n = 6). *P (0.05) significant compared to control.

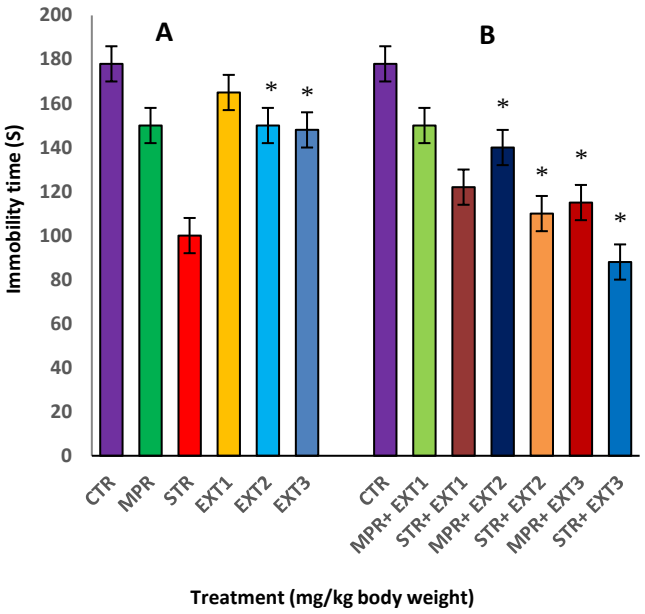

Figure 5: Immobility periods of rats administered *A.* muricata stem-bark extracts alone (A) and in combination with (10 mg/kg b.w.) imipramine and sertraline respectively (B) in the forced swim test. Values represent ± Mean SEM (n = 6). *P (0.05) significant compared to control.

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