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Evaluation of the anxiolytic effects of the aqueous and ethanolic extracts of the leaves and bark of *Annona muricata* using the elevated plus maze test

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ABSTRACT

Introduction: Natural products since time immemorial have been the source of traditional medicine. A number of well-known anxiolytic agents currently used have several side effects that limit their use. Among medicinal plants, leaves of *Annona muricata* (AM) are being recommended by traditional healers for the management of anxiety. This study is performed to evaluate the anxiolytic activity of the aqueous and ethanolic extracts of the leaves and bark of *Annona muricata*. **Methodology:** Aqueous and ethanolic extracts of the leaves and bark of *Annona muricata* were prepared and assessed for anxiolytic effect using the elevated plus maze (EPM) model. The antianxiety activity of the extracts were compared to the control (distilled water 10ml/kg) and standard drug Diazepam (5mg/kg). **Results and discussion:** All doses of the aqueous leaf extract of *Annona muricata* exhibited significant increase in mean entries into open arms ($P<0.01$) and mean time spent in open arms ($P<0.05$) compared to the control. 100mg/kg and 400mg/kg of the ethanolic bark extract of *Annona muricata* showed significant increase in open arm entries ($P<0.01$) with 100mg/kg also showing an increase in time spent in open arms which was significant. **Conclusion:** This study demonstrated that both the aqueous and ethanolic extracts of the leaves and bark of *Annona muricata* exert an anxiolytic effect on rats which substantiates its traditional use in the management of anxiety.

Keywords: Anxiolytic, *Annona muricata*, Elevated plus maze model, Rats.

INTRODUCTION

Annona muricata belongs to the Annonaceae family and is known for its many medicinal benefits. It is known as soursop (English), Aluguntugi (Twi, Ghana), Omusitafeli (Uganda), Graviola (Portuguese) [1]. *Annona muricata* is extensively distributed in the tropical areas of Western, Eastern and Central Africa as well as South and Central America [2]. The fruit is edible and green in color with a white and creamy flesh that possess a characteristic aroma and flavor.

All parts of the plant have been employed for the treatment of diverse ailments and diseases in folkloric medicine [3]. Extracts of various parts of the plant have been shown to possess several therapeutic activities including anti-viral [4], anti-inflammatory [5, 6], to manage respiratory illness [1] and gastrointestinal disorders [7]. The leaves are also used by traditional healers in bath for pregnant women before delivery [8]. The plant has gained popularity in recent times for its anti-cancer activity [9-12]. The anxiolytic activity of *Annona muricata* has not been explored considerably, therefore, this research investigates the anxiolytic effect of the ethanolic and aqueous extracts of the bark and leaves of *Annona muricata*.

Mental health disorders like depression and anxiety still make up the main causes of mortality notwithstanding considerable progress made in understanding and managing these disorders [13]. The World Health Organization reported in 2001 that an estimated 450 million individuals have one form of mental or behavioral disorder and just a negligible number receive any form of treatment [14]. One of the most common neurological disorders in the world is anxiety affecting one-eighth of the world's population. Current treatment strategy for anxiety disorder involves the use of synthetic anxiolytic agents which are expensive with several unwanted adverse effects including dependence, amnesia and impaired motor coordination [15,16]. Therefore new, effective and safer agents from medicinal plants are needed.

MATERIALS AND METHODS

Sample Collection and Authentication

Fresh leaves and bark of *Annona muricata* were obtained from Community 18 Spintex in Accra Ghana. The plant samples were authenticated by Professor Kwasi Adomako Ohemeng from The Department of Pharmacognosy and Medicinal Chemistry School of Pharmacy Central University. A specimen of the sample (AM030) was kept at the department for subsequent reference.

Sample Preparation

The leaves and bark of *Annona muricata* were air dried and milled to fine powder and then extracted separately with ethanol and distilled water using cold maceration. Complete extraction with distilled water and alcohol was ensured. The extracts were evaporated to dryness using the rotary evaporator for ethanol solvent and freeze dried for the aqueous solvent. The extracts were stored in a refrigerator at 4°C.

Phytochemical Screening

The ethanolic and aqueous extracts of the bark and leaves of *Annona muricata* were evaluated for the presence of several phytochemical constituents such as: tannins, glycosides, alkaloids, saponins, flavonoids, anthraquinones and phenols using standard procedures outlined by Trease and Evans [17].

Experimental Animals

Adult Swiss Albino rats (90-120g), were obtained from Noguchi Memorial Institute for Medical Research, Ghana. Animals were housed in plastic cages under controlled conditions of 12 hours light and dark cycles with soft wood shavings used as beddings. All animals had unlimited access to standard pellet diet and water and were allowed 10 days for acclimatization. All protocols employed for the handling of experimental animals followed the Organization for Economic Cooperation and Development [18, 19]. This research was performed in the Pharmacology laboratory of Central University, Ghana.

Acute Toxicity Test

Acute toxicity studies were carried out in accordance with guidelines by Organization for Economic Cooperation and Development (18). Swiss Albino rats were fasted for 24 hours with unlimited access to only water. All extracts (aqueous, ethanol) of *Annona muricata* were administered orally at the dose of 2000mg/kg. The animals were observed for the first 7 hours after administration for initial signs of toxicity and mortality and for the subsequent 7 days for delayed manifestation of toxicity.

Elevated Plus Maze Test

The elevated plus maze (EPM) test structure comprises of two open arms (50cm by 10cm) and two closed arms (50cm by 10cm by 30cm) all with an open roof elevated 25cm from the floor. This test is employed to assess anxiety responses in rodents and is based on a conflict between the animal's natural inclination for protected or secluded and darker areas and the innate tendency to explore novel environments [20]. Animals were pretreated with distilled water (10ml/kg), 100mg/kg, 200mg/kg, 400mg/kg of *Annona muricata* extract and with 5mg/kg Diazepam as the standard. Each rat was placed on the maze with its head pointing towards the open arm 45minutes after pretreatment and allowed to explore for 5minutes [21, 22]. The frequency of entries into open arms as well as the time spent in open arms were recorded while the average time spent in the open arm was calculated for each animal [13]. Precaution was taken to make sure that there were no extrinsic stimuli that could cause anxiety or fear to the animals during the experiment and also the maze was wiped with cotton wool soaked in 10% ethanol before each observation.

Statistical Analysis

Data obtained were analyzed using GraphPad Prism Version 8.0.2. All results are reported as Mean ± Standard Error of Mean (SEM). Significant differences between groups was determined using one-way analysis of variance (ANOVA) followed by post hoc Tukey test. $P<0.05$ was regarded statistically significant.

RESULTS

Phytochemical Screening

Results of the phytochemical analysis carried out on the solvent extracts of *Annona muricata* indicated the presence of alkaloids, tannins, saponins and other constituents.

Table 1: Showing the phytochemical constituents in each of the extracts of *Annona muricata*

Phytochemical constituent	Ethanol leaves of *Annona muricata*	Ethanol bark of *Annona muricata*	Aqueous leaves of *Annona muricata*	Aqueous bark of *Annona muricata*
Flavonoids	+	+	+	+
Glycosides	+	+	+	+
Saponins glycosides	+	+	+	+
Tannins	+	+	+	+
Alkaloids	+	+	+	+
Anthraquinones	+	+	+	+

Present (+)

Acute toxicity studies

All extracts of *Annona muricata* (aqueous and ethanolic) employed did not cause death at the highest dose of 2000mg/kg. Additionally, the general behavior of the animals remained unchanged when compared to the control.

Table 2: Anxiolytic activity of various extracts of *Annona muricata* using EPM test.

Treatment groups	Dose (mg/kg)	Mean entries in open arms± SEM	Mean time spent in open arms ± SEM (sec)
Distilled water	10	1.0±0.55	8.38±4.22
Diazepam	5	3.2±1.02	104.8±35.92**
	100	5 ±0.89**	122.1±41.09
Aqueous leaves of *Annona muricata*	200	4.4±0.24*	136.4±28.05*
	400	5.2±0.80**	116.7±17.03
	100	4.0±0.84	86.36±37.48
Aqueous bark of *Annona muricata*	200	4.0±0.45	74.10±9.61
	400	2.0±0.63	127.5±44.13
	100	2.2±0.37	49.86±7.22
Ethanol leaves of *Annona muricata*	200	1.0±0.45	24.73±14.03
	400	2.0±0.63	7.00±3.74
	100	4.8±0.58**	164.6±46.64**
Ethanol bark of *Annona muricata*	200	3.2±0.37	38.12±7.95
	400	5.4±0.68**	83.04±16.04

Values are expressed as Mean ± SEM (n=5). *P<0.05, **P<0.01

DISCUSSION

Anxiety disorders have a relatively high prevalence with a lot of morbidity. More than 200million individuals live with anxiety disorders which is 4% of the world population, this translates to approximately 2.5% and 6.5% of people in each country. An estimated 62% of people that suffer from anxiety are females (170 million) as against 105 million which are males [23, 24].

The elevated plus maze (EPM) is presently one of the most commonly used model for evaluating anxiety, and has been substantiated for evaluating sedative and anxiolytic substances in mice as well as rats. EPM model was employed for this research to evaluate the anxiolytic effect of the aqueous and ethanolic extract of the leaves and bark of *Annona muricata*. An increase in open arm activity is an indicator of anti-anxiety effect. The behavior of the rat in the open arms in this model show a struggle between the rat's natural inclination to stay in an enclosed area (closed arm) and interest to survey a new environment. Anxiolytics are shown to promote this exploratory activity of the animals in open arms [20]. Diazepam, an anxiolytic drug, caused an increase in open arm entries and the time spent in open arms substantiating its anxiolytic effect thereby explaining its use as a standard in this study.

The aqueous and ethanol leaf and bark extract of *Annona muricata* were positive for alkaloids, flavonoids, tannins and glycosides (Table 1). Flavonoids are reported to possess anxiolytic activity by activating benzodiazepine receptors [25]. All doses of the *Annona muricata* employed for acute oral toxicity studies did not cause any mortality and the general behavior of the animals remained unchanged with identical observations recorded for the positive and negative controls. The aqueous leaf extract of *Annona muricata* at all doses significantly increased entries into the open arms of the EPM while the ethanol bark extract of *Annona muricata* at 100mg/kg and 400mg/kg also caused a significant increase in open arm entries relative to the control (Table 2). The ethanol leaf and the aqueous bark extracts also caused an increase in open arm entries which was not significant when compared to the control. A significant increase in the time spent in open arms was observed by the standard drug, diazepam, as well as the ethanol bark extract of *Annona muricata*. The anxiolytic effect observed by the aqueous and ethanolic leaf and bark extract of *Annona muricata* could be as a result of some of these phytochemical constituents.

CONCLUSION

The aqueous and ethanolic leaves and bark extract of *Annona muricata* showed significant anxiolytic effect using the elevated plus maze test model. Studies are currently ongoing to isolate compounds from these extracts and to investigate them for anxiolytic activity.

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Conflict of interest

The authors declare no conflict of interests

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