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Evaluation of anxiolytic potential of ethanol root extract and fractions of *Pterocarpus mildbraedii* in mice

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Abstract

To evaluate the anxiolytic potential of ethanol root extract and fractions of *Pterocarpus mildbreadii* in mice. Elevated-I-maze model apparatus is a straight wooden passage, divided equally (16cm each) into two enclosed areas (close arms) at both ends of the "maze" and an open area in the centre of two enclosed ends (arms). The ethylacetate fraction of *Pterocarpus mildbraedii* showed significant increase ($p<0.05$) in number of unprotected head dips (uHDIPS) when compared to control group but there was no significant difference when compared with other group. The butanol fraction of *Pterocarpus mildbraedii* showed significant increase in number of unprotected head dips (uHDIPS) at higher dose of 200mg/kg when compared to control group ($p<0.05$), there was no significant difference when compared with diazepam, crude extract, n-hexane, increased dose of butanol fraction (200mg/kg) there was increased significant difference. The crude extract of *Pterocarpus mildbreadii* showed significant increase in number of unprotected head dips (uHDIPs) at the dose of 100mg/kg, it also showed same significant increase in number of unprotected head dips with ethylacetate fraction at the dose of 200mg/kg. Ethylacetate fraction of *Pterocarpus mildbreadii* (200mg/kg) showed significant increase in number of unprotected head dips when compared with the crude extract at the dose of 100mg/kg ($p<0.05$).Diazepam (2mg/kg) showed significant increase in number of unprotected head dips when compared with the control group. Ethylacetate fraction of *Pterocarpus mildbreadii* (100mg/kg) showed significant increase ($p<0.05$) in number of protected head dips when compared with the control group. Increased dose of ethylacetate fraction of *Pterocarpus mildbraedii* (200mg/kg) showed significant increase ($p<0.05$) in number of protected head dipping when compared with the control group. The oral administration of ethylacetate fraction (100mg/kg) and 200mg/kg) to mice showed anti-anxiety effects indicated by increase in number of unprotected head dips and decrease in number of unprotected head dips.. Experimental evidence obtained in the laboratory test model could provide a rational for the traditional use of this plant. The plant can be further screened to evaluate and elucidate the mechanism of action and possibly isolate the active principle.

Keywords: *Pterocarpus mildbraedii; R*oot extract; Fractions, Anxiolytic potential.

1. Introduction *ot*

The application of medicinal plants in the treatment of mental and neurological disorders has been documental over decades. Categorically, medicinal plants comprising secondary metabolites such as alkaloids, taninins, saponnins, flavanoids and sterol are highly associated with anxiolytic activity[1,2,4,8,23].In addition, orthodox medicines available such as benzodiazepines are commonly associated with physical dependence, day time fatigue, tolerance and cognitive

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impairment. As such, there is need to search for medicinal plants that are capable of alleviating mental disorders without many side effects[8, 23, 26.]

Anxiety is defined as 'a state of intense apprehension, uncertainty and fear resulting from the anticipation of a threatening event or situation, often to a degree that normal physical and psychological functioning is disrupted" [2]. Anxiety disorders, nowadays are one of the common causes for frailty. Nearly one fourth of the adult population suffers from psychiatric disorders during the course of their life[18]. Anxiety disorders are among the most prevalent categories of mental illnesses[16,31]. Anxiety disorders are characterized by excessive and unrealistic worry about everyday tasks or events or may be specific to certain objects. Recent epidemiological studies of anxiety disorders provided evidence of their high frequency in the general population worldwide. Anxiety disorders afflicts an estimated 15.7 million people in United States each year[20]

Anxiety is associated with substantial negative effects on childrens social, emotional and academic success[25]..Anxiety disorders are highly prevalent in adults especially females show higher preponderance of 2:1 as compared to males.[3.]

1.1. Description of plant (Pterocarpus *mildbreadii*)

Pterocarpus mildbreadii leaves known as 'Oha' in Eastern Nigeria are used as vegetable in the preparation of soup in Nigeria. *Pterocarpus mildbreadii* is a green leafy vegetable which grows more like a big tree reaching a height of 2m (6.6ft) and having stem diameter of 20m (0.79 inch).*Pterocarpus mildbreadii* has a smooth,gray or pale brown bark, exuding red gum when *Pterocarpus mildbraedii* (Oha)[34.]

2. Material and methods

2.1. Animals

A total of 63 albino mice (weighing between 20-30g) were purchased from the laboratory Animal Facility of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Enugu State. They were housed in clean metal cage, supplied portable water and feed with commercial pellleted feed (Guniea Feed, Nigeria). The animals were acclimatized for two weeks handled in compliance with the National Institute of Health Guidelines for care and use of laboratory animals.

2.2. Collection and authentication of plant materials

Fresh root of *Pterocarpus mildbraedii* were collected from Umuezeala, Ujah Village, Amaifeke, Orlu, lmo State. The plant was authenticated by a Taxonomist, Dr. Babiana Aziagba of the department of Botany, Nnamdi Azikiwe University, Awka. A voucher number (PCG/UNIZIK/162B) was given to the plant after identification.

2.3. Extraction of plant material

The fresh root of *Pterocarpus mildbraedii* were washed in a running tap to remove dust and other debris, and air dried for two weeks. Dried root of *Pterocarpus mildbraedii* were pulverized with electrical blender and kept in clean air tight amber bottle. 750gram of the powdered material was cold macerated I in 80% ethanol. The mixture was agitated continually for two days (48 hours). The filtrate was recovered and concentrated to dryness using water bath at 40^0C. The extract was stored in a refrigerator before use. The percentage yield of the extract was calculated using the following formula.

$$\%\text{Yield} = \frac{\text{Mass of Dry extract}}{\text{Weight after extraction}} \times 100$$

2.4. Fractionation of ethanol crude extract

Fractionation was carried out using N-hexane, Ethyl acetate and butanol following the method described by.[14.] One hundred grames of crude extract was dispersed in 500ml of distilled water the poured inside a separating funnel n-hexane 500ml was added to funnel and shake thoroughly to mix. The mixture was allowed to separate into two distinct layers. The n-hexane portion, (Upper layer) was separated and the other portion was subjected to fresh n-hexane until the n-hexane portion was clean completely. After the n-hexane phase, the other portion was subjected to ethyl acetate and butanol successively using the same process as described for n-hexane. The various fractions were filtered and concentrated to dryness using water bath set at 40^0c. The fractions were stored in a refrigerator before use.

2.5. LD_{50} Determination

Acute toxicity, LD_{50} test was carried out using the method of [22]. A total of 13 mice, weighing 25-30g were used in the two phases.

In the first stage, the animal were divided into 3 groups of 3 mice each and the extract was administered at three dose level (10, 100 and 1000mg/kg) body weight. The animals were then monitored for 24 hours. Absence of deaths in the first phase led to the use of (1,600, 2,900 and 5,000mg/kg dose) of extract for 4 groups of 1 animal each. Animals were examined again for another 24 hours[22]. The number of death(s) was noted for each group and the LD_{50} calculated as follows;

$$LD50 = \sqrt{(D_0 \times D_{100})}$$

Where = D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality

2.6. Phytochemical screening

Phytochemical screening were performed using standard procedures[33].

2.7. Model for anxiolytic activity

2.7.1. Elevated I-maze model apparatus

The apparatus is a straight wooden passage, resembling the English letter 'I". It consists of a 48cm × 5cm, straight passage, divided equally (16 cm each) into two enclosed areas (Close arms) at both ends of the 'Maze' and an open area in the centre of two enclosed ends (arms).Height of the walls of enclosed areas is 12cm high walls at both ends. The entire maze is elevated to the height of 25cm.

2.8. Plasma corticosterone estimation

The quantitative estimation of plasma corticosterone level was performed by the method of [9].

2.9. Experimental protocol

Each experiment animal group consisted of five mice each group. For behavioral characterization, vehicle (Distilled water), diazepam (2mg/kg ,ip) were administered in separate groups of mice, 30 minutes before subjecting them to behavioral testing. After 30 minutes of vehicle or drug treatments, animals were observed for total 5 minutes duration with the help of a video tracking system and behaviors were analyzed and documented from these recordings. In all experiments, i.e before placing each animal on the maze, the test apparatus (Maze) was cleaned with 5% ethanol and thoroughly dried between each test period. All the experiments were carried out between 06:00am and 06:00pm. The following behavior parameters were studied in the mice.

2.9.1. Unprotected head dips (uHDIPS)

It denotes scanning by animal over the sides of the maze downward towards the floor from unprotected area i.e uncovered open arm uHDIPS are counted as the number of head dips from open arm.

2.9.2. Head dipping from close arm (pHDIPS)

It denotes scanning by animal over the sides of the Maze downward towards the floor from protected area i.e. covered close.

2.9.3. Statistical analysis

All the results were analyzed using graph pad prism

3. Results

3.1. Evaluation of anxiolytic potential of ethanol extract and its fraction

In the I-maze model, a significant increase in number of unprotected head dips (uHDIPS) and a significant decrease in protected head dips (pHDIPS) indicates an anit-anxiety-like-behavior. On the other hand, a significant decrease in plasma cortisol as a result of decrease in level of plasma cortisol also indicates anti-anxiety-like -behaviour.

3.2. Effects of ethylacetate fraction

The ethylacetate fraction of *Pterocarpus mildbraedii* showed significant increase ($P<0.05$) in number of unprotected head dips (uHDIPS) when compared to control group but there was no significant difference when compared with other group. Ethylacetate fraction of *Pterocarpus mildbraedii* at the dose of 100mg/kg showed significant increase in level of plasma cortisol when compared with diazepam, control,crude extract, butanol and n-hexane fractions ($P<0.05$).

3.3. Effect of butanol fraction

The butanol fraction of *Pterocarpus mildbraedii* showed significant increase in numbers of unprotected head dips (uHDIPS) at higher dose of 200mg/kg. When compared to control group ($P<0.05$) but there was no significant difference when compared with diazepam,crude extract, and n-hexane. However, at increased dose of butanol fraction (200mg/kg) there was significant increase in difference.

3.4. Effect of crude extract

The crude extract of *Pterocarpus mildbraedii* showed significant increase in number of unprotected head dips (uHDIPS) at the dose of 100mg/kg. It also showed some significant increase in number of unprotected head dips with ethylacetate fraction at the dose of 200mg/kg.

3.5. Effect of diazepam

Diazepam (2mg/kg) showed significant increase in number of unprotected head dips when compared with the control group. At 2mg/kg diazepam showed significant increase in number of protected head dips when compared with the control group ($P<0.01$).

3.6. Effect of n-hexane fractions

N-hexane fraction showed significant increase ($P<0.05$) in protected head dips at increased dose of 200mg/kg. When compared with the diazepam (2mg/kg)

Table 1 The number of protected and unprotected Head-dips in mice

Groups	Protected head dips	Unprotected Head dips
Control	4.00±1.30	4.00±0.84
Standard	28.00±2.07	25.00±3.54
Crude Extract (100mg/kg)	20.00±2.07	18.00±1.92
Crude Extract (200mg/kg)	22.00±4.34	23.00±2.77
Ethylacetate Fraction (100mg/kg)	26.00±2.30	23.00±3.96
Ethylacetate Fraction (200mg/kg)	27.00±2.86	31.00±3.51
Butanol Fraction (100mg/kg)	24.00±4.18	20.00±4.21
Butanol Fraction (200mg/kg)	24.00±2.59	28.00±4.06
n-Hexane Fraction (100mg/kg)	20.00±3.27	17.00±2.92
n-Hexane Fraction (200mg/kg)	18.00±3.90	19.00±1.58
Kruskal-wallis statistics	33.32	39.70

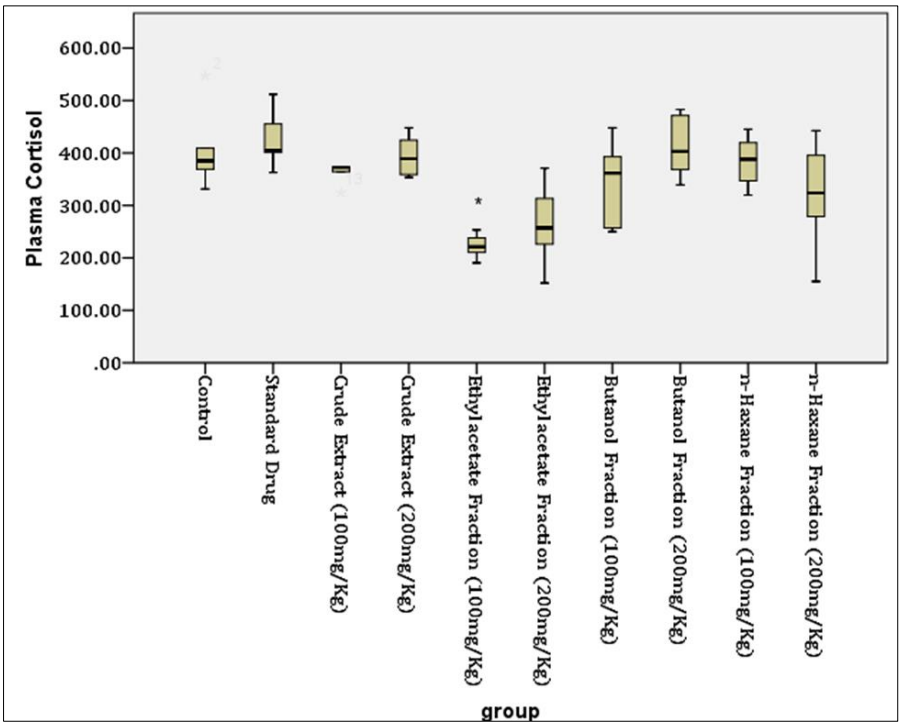

Figure 1 Axiolytic activity measured by plasma cortisol levels.

3.7. Results of Level of Plasma Cortisol in Mice

Table 2 Phytochemical constituents of root extract of *Pterocarpus mildbreadii.*

Qualitative (Phytochemicals)	*Pterocarpus mildbreadii*
Flavonoids	***
Alkaloids	*
Saponins	*
Phenols	***
Cardiac glycosides	**
Tanins	*
Terpenoids	**
Carbohydrates	*

Key words: * Represents little quantity; ** Represents moderate quantity; *** Represnts high quantity

4. Discussion

An experimental model of anxiety should be analogous to the human disorder in symptoms. A model is required to produce a behavioral change that (a) can be monitored, (b) should respond to standard clinical treatments and (c) should exhibit reproducibility in animal behavior.[14.] An animal model should at least display three kinds of validity namely (a) face validity i.e. it should produce anxiety-like symptoms in animal, (b) construct validity i.e. its physical design should produce similar biochemical changes as observed in clinical anxiety and (c) predictive validity i.e. animal behavior on the maze should respond to standard therapeutic treatments[10.]

The etiology of most anxiety disorders are not fully understood, but various studies has shown the involvement of GABAergic, Serotonergic neurotransmission in etiology, expression and treatment of anxiety[11,12].The adrenergic and dopaminergic systems have also been shown to play a role in anxiety[5.] Despite the widespread traditional use of *Pterocarpus mildraedii* root for treating various disorders there are no reports of scientific evaluation of its anxiolytic

activity. The conventional elevated I maze is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the $GABA_A$ benzodiazepine complex[7]. This animal model is considered one of the most widely validated tests for assaying anxiolytic substances.

Pterocarpus mildbraedii ethanol root extract was evaluated for anxiolytic effect in order to scientifically validate the traditional claim by using behavioral models namely elevated-I-maze. The oral administration of ethylacetate fraction (100 mg/kg and 200 mg/kg) to mice showed anti-anxiety effects indicated by increase in number of unprotected head dips and decrease in number of unprotected head dips. Phytochemical screening of ethanol root extract showed the presence of flavonoids along with other phytochemical groups. The chemical separation of the active extract allowed isolation of an active fraction (ethylacetate fraction) that exhibited significant anxiolytic properties.

The neurobiology of anxiety disorders is not full known[21]. Low level of GABA in CNS is most frequently associated with anxiety disorders.[30]. In addition to GABA, 5-HT plays an important role in the development and the persistence of anxiety disorders.[30] Many studies have shown that patients with anxiety disorders have genetic polymorphisms in the 5-HT transporter [17]

Anxiolytic activity of diazepam is due to its GABA facilitatory action through GABA-A receptors[19]. Various studies have shown presence of flavonoids in phytochemical screening of *Pterocarpus mildbraedii* root, flavonoids are responsible for anxiolytic effect of *Pterocarpus mildbreadii* root, through benzodiazepine receptors [28,29].Therefore, flavonoids present in the *Pterocarpus mildbraedii* root may be responsible for the anti-anxiety activity in present study. Further studies are required to know the exact mechanism responsible for antianxiety activity .The chemical separation of the active extract allowed isolation of an active fraction (ethylacetate fraction) that exhibited significant anxiolytic properties.

Various *in vivo* studies have recognized flavonoids as novel type of ligand with anti-anxiety outcomes. Behavioral tests in rodents have explored anxiolytic effects of different flavones (e.g., chrysin and apigenin) obtained from medicinal plants. The biological effect produced by these compounds is due to the modulation of GABA (γ-amino butyric acid) ergic system[13]. Neuroprotective manifestation of flavonoids has been attributed to their general bioavailability and *in vivo* occurrence in the brain [6]. Quercetin, one of the flavonol found in large number of herbals, act as a monoamineoxidase A and B (MAO A and B) inhibitor [15]. Research has revealed that substances acting as MAO modulators elicit behavioral alterations in rodents by modifying monoamine level in brain, and consequently display an anxiolytic effect [13]. Recent findings have proposed that MAO-inhibition and enhancement in GABAergic activity may be the fundamental mechanism responsible for anxiolytic activity of quercetin liposomes administered via nasal route.

5. Conclusion

Root extract and fractions of *Pterocarpus mildbraedii* was used for determination of anxiolytic activity (.Elevated-I-maze test), this pharmacological model have been employed in the evaluation of medicinal plant *P.mildbraedii* for neuro-pharmacological activities towards the identification of phytochemical constituent with beneficial effects in the treatment of anxiety as diverse CNS disorder. On the basis of above results, Ethanol root extract and fractions of *Pterocarpus mildbraedii* has potent anxiolytic activity, due to the anxiolytic activity potential exhibited; therefore, it will be useful for the treatment of anxiety disorder. In the end, it can be concluded that the experimental evidence obtained in the laboratory test model could provide a rational for the traditional use of this plant.

Recommendation

The plant can be further screened to evaluate and elicidate the mechanism of action and possibly isolate the active principle.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflict of interest in the choice of publication.

Statement of ethical approval

Approval was obtained from the ethics committee with reference number: NAUTH/CS/66/VOL.10/220/2017/133.

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