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(RESEARCH ARTICLE)

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Assessment of sedative and anxiolytic activities of fruit extract of V*itex doniana* (black plum)

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Abstract

This research study was aimed at investigating the common folkloric use of *Vitex doniana* fruit extract for induction of sleep. Extraction of the fruit extract was carried out with 80 %v/v ethanol. Acute toxicity study and qualitative phytochemical screening of the extract were also done. Twenty-five adult mice divided into five groups (n=5) were used for both the anxiolytic study and the phenobarbital-sleeping time analysis tests. Groups I and II served as negative (distilled water 10 ml/kg) and positive (diazepam 1 mg/kg i.p.) controls, respectively. Groups III, IV and V received the extracts of 250, 500 and 1000 mg/kg p.o., respectively. The effect of extract on time spent in and number of entries into the open arms and closed arms when compared to control were noted. The effect on onset and duration of sleep -were also noted. The extract was found to be relatively safe. Secondary metabolites like tannins and flavonoids were present. There was a dose-related significant ($p < 0.05$) increase in time spent on the open arm and a decrease in time spent on closed arm when compared to the negative control. Dose-related significant ($p < 0.05$) decrease in time for sleep onset and increase in duration of sleep when compared to negative control were also observed. From the results of this study, the folkloric use of this fruit extract for the induction of sleep and relaxation seem to be justified.

Keywords: Sedative; Anxiolytic; Sleeping-time; *Vitex doniana*

1. Introduction

Many important drugs used in medicine today are derived directly or indirectly from plants [1]. Anxiolytics are medications used in the treatment of anxiety and insomnia. They are also used as adjunct in the treatment of mental illness. Anxiolytics are also called tranquilizers, which are often used to treat anxiety disorders, such as generalized anxiety disorder (GAD), panic disorder, social anxiety disorder, and phobias. They work by altering the brain's chemistry; thereby exerting calming effect on the nervous system and reducing the feelings of fear and anxiety (https://en.wikipedia.org).There are several types of anxiolytics, including diazepam, sertraline, and amitryptiline. Anxiolytics are often associated with side effects such as dependence, addiction, and withdrawal symptoms when stopped (https://en.wikipedia.org).

Sedatives are a class of medications that slow down the central nervous system (CNS), leading to relaxation and reduced anxiety. They are often used to treat insomnia, anxiety, seizures, and muscle spasms. Sedatives include benzodiazepines (example is diazepam) and non-benzodiazepines which are barbiturates (example is phenobarbitone). Sedatives can also lead to dependence, addiction and withdrawal symptoms (https://en.wikipedia.org).

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Herbal medicine is becoming a viable alternative treatment over the commercially available synthetic drugs for management and treatment of some diseases [2]. This is premised on its lower cost, perceived effectiveness, availability as well as little or no adverse effects.

Some plants' extracts are used in folkloric medicine to induce anxiolytic and muscle relaxant effect [3]. Some plants have been scientifically proven to possess anxiolytic and sedative effects [4]. With respect to common side effects that are associated with anxiolytics and sedatives, effective plant remedies are more likely to have advantages over conventional drugs like the benzodiazepines due to reduced side effect usually associated with herbal medicine. Moreover, the fact that plants extracts exert their pharmacological effect through multiple mechanisms [5] could be an added advantage.

Vitex doniana is a food plant that is mainly known for its fruit which serves as a delicacy for majority of people, especially in the Eastern part of Nigeria where it is also believed to have the ability to induce sleep. It is of the genus *Verbanacae* and breeds in open woodland and grassland regions of hot Africa. The fruitlets are green when matured and transforms into sweet-smelling, dark brown fruits when completely ripe (Figure 1) [6]. The fruit is commonly known as Black plum (English), Dinya (Hausa), Mbembe (Igbo) and Oriri (Yoruba) by some ethnic groups of Nigeria. In traditional medicine, the leaf, the bark, dried and fresh fruit serve as ingredients in preparations for treatment of conjunctivitis, inflammation, headache and stiffness [7]. From available literature, there is no scientific investigations on the sedative and anxiolytic effects of *Vitex doniana* fruit extract, hence our decision to carry out this study**.**

Figure 1 The leaves, fruits of *Vitex doniana*

2. Material and methods

2.1. Plant materials

2.1.1. Sample collection and Identification

The fresh fruits of *Vitex doniana* were purchased from Ekwulobia in Anambra state of Nigeria. The fruit was authenticated by a Taxonomist at the department of Botany, Nnamdi Azikiwe University, Awka, Nigeria, and a voucher specimen number (NAUBT 3218) was assigned to it.

2.1.2. Chemicals, reagents and drugs

Diazepam (Square Pharmaceuticals Ltd., Dhaka, Bangladesh), 96 % v/v ethanol analytical grade (Zigma, India), chloroform (Zigma, India), Elisa reagents and Phenobarbitone (Harman Finochem Limited India).

2.2. Extraction of plant material

Extraction was done using a method described by [8]. The fruits were cleansed to remove sand and other debris. After removing the thin mericarp, the fleshy juicy mesocarp was scraped off from the seeds and dried for a period of two weeks under room temperature. This dried mesocarp was grinded into powder with the help of an electrical grinder. About, 800 g of the powdered material was cold macerated in 80 % ethanol. The mixture was agitated intermittently for three days (72 hours). The filtrate was recovered and concentrated to dryness using water bath at 40^0C. The percentage yield of the extract was calculated and the extract stored in a refrigerator until when needed.

2.3. Qualitative phytochemical analysis

Screening for the presence of secondary metabolites was carried out following the phytochemical tests as demonstrated by [9]. The following secondary metabolites were tested for: tannins, alkaloids, reducing sugars, flavonoids, glycosides, saponins, fats and oils, acidic compounds and proteins.

2.4. Acute toxicity study

The toxicity test was conducted using the up and down procedure (UDP) adopted by [10] and revised by [11]. Using this method, the animals were dosed one at a time and the doses were dependent on the response of the first animal to the initial dose. The second animal receives a lower dose if the first animal dies (the initial dose is decreased by a factor of 3.2) or the second animal receives a higher dose if the first animal survives (the initial dose is increased by a factor of 3.2). Three mice weighing 30-35g were used as starting point. Two mice served as negative control having received 10 ml/kg of distilled water orally while the test animal received a default oral dose of 5000 mg/kg of the extract. The animals were then observed continuously for 4 hours for changes in behavior and for any other obvious signs of toxicity and subsequently daily for a total of 14 days for delayed toxicity.

2.5. Dosage selection

Dosage of extract administered to animals were $1/20^{th}$, $1/10^{th}$ and $1/5^{th}$ of the estimated LD_{50} [12] and the fruit extract was administered orally.

2.6. Experimental design

2.6.1. Animals grouping

Twenty-five adult mice of both sexes (30-35g) obtained from the Animal house of the Department of Pharmacology and Toxicology, Chukwuemeka Odumegwu Ojukwu University (COOU), Anambra State, Nigeria, were used for the study. The mice were housed in clean plastic cages, supplied with clean drinking water and fed with commercial pelleted (Guinea Feed®, Nigeria). Ethical approval number PHACOOU/AREC/2023/032 was assigned to attest the animals were cared for according to the Faculty of Pharmacy (COOU) Animal Research Ethics Committee guidelines (PHACOOUAREC), which are in line with the National Institute of Health (NIH), USA, guidelines for the care and use of laboratory animals. The animals were divided into five groups (n=5) for this study:

- Group 1=Negative control (distilled water 10 ml/kg p.o)
- Group 2=Positive control (diazepam 1mg/kg; i.p.)
- Group 3 = Extract 250 mg/kg
- Group 4= Extract 500 mg/kg
- Group 5= Extract 1000 mg/kg

2.6.2. Elevated plus-maze test

For the elevated plus-maze test, diazepam was administered 15 minutes before the experiments, while the extract and distilled water were given 30 min before the experiments.

The elevated plus maze (EPM) test is a widely used model to investigate anxiolytic effects. The apparatus consists of two open arms ($15 \times 5\ cm^2$) and two closed arms ($15 \times 5 \times 5\ cm^3$), extending from a central platform ($5 \times 5\ cm^2$) and raised 50 cm above floor level. Animals were randomly divided into groups that were treated orally with extract 250, 500, and 1000 mg/kg, distilled water, or diazepam (i.p) as stated above. After the desired time as earlier mentioned, each animal was placed at the center of the plus maze facing its head to the closed arms and allowed free exploration for 5 minutes. The number of entries the mouse made into open arms, closed arms, the time it spent in the open arm and the closed arms were recorded with stop watch within the indicated time of 5 minutes [13].

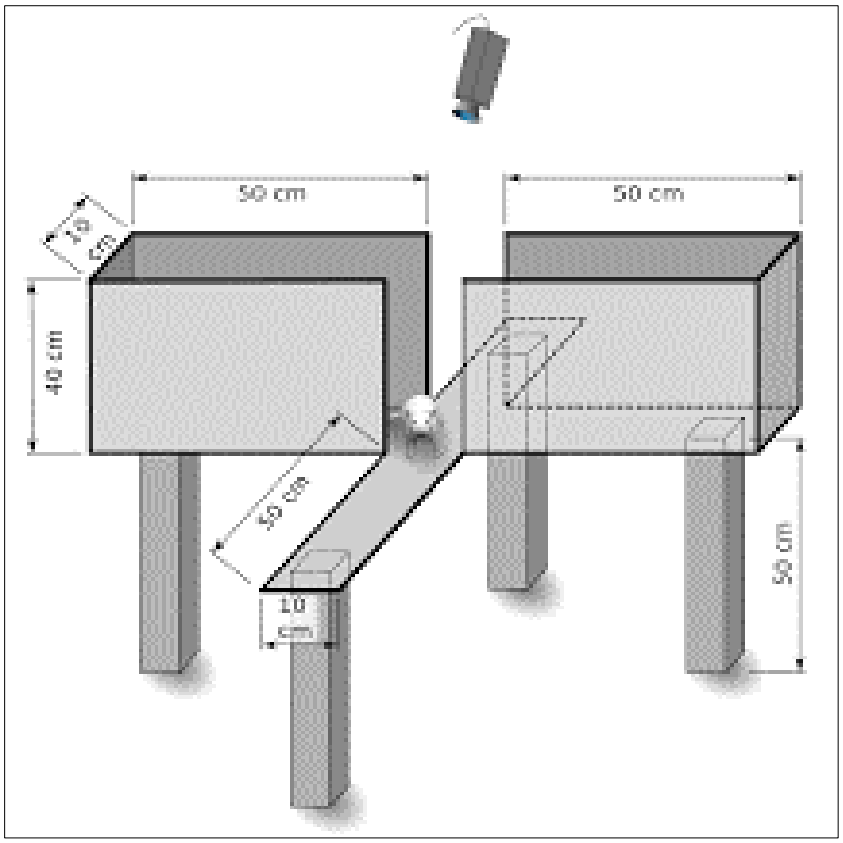


Figure 2 Diagram of the elevated plus maze apparatus

2.7. Effect of extract on Pentobarbital-induced sleeping time

Pentobarbital-induced sleeping time test was performed according to the previously described method by [14]. In this study, the sleep inducer, Pentobarbitone (20 mg/kg) was administered 15 minutes after treatment with diazepam and 30 minutes after treatment with the extract or distilled water. The mice were allowed three weeks after being used for the elevated plus-maze test before commencing with this study. Grouping and treatment were as described previously. After 30 minute of treatment with the extract (or 15 minutes for diazepam), Pentobarbitone (20 mg/kg, i.p.) was administered to each mouse to induce sleep. The total sleeping time was recorded for both controls (positive and negative) as well as for test groups. Hence, animals were observed for the latent period (time between pentobarbitone administration to loss of righting reflex) and duration of sleep (time between the loss of righting reflex and recovery of righting reflex).

2.8. Statistical analyses

Data obtained from the study was analyzed using Statistical Package for Social Sciences (SPSS-25). Results were presented as mean ± Standard error of mean (SEM) of sample replicates. Raw data was subjected to one-way analyses of variance (ANOVA) followed by post hoc turkey's test. $P<0.05$ was considered to be statistically significant.

3. RESULTS

3.1. Phytochemical screening of the extract

The qualitative phytochemical screening of the fruit extract revealed the abundance of alkaloids, tannins, flavonoids, glycosides, saponins, moderate presence of fats and oil, acidic compounds with traces of reducing sugar (Table 1).

3.2. Acute toxicity study (LD50)

Oral administration of the extract up to 5000 mg/kg body weight dose produced no change in behavior, neither was there any mortality in any of the groups. Therefore, the LD_{50} of fruit extract of *Vitex doniana* was above 5000 mg/kg body weight

Table 1 Qualitative phytochemical screening of the extract

Tannin	Flavonoid	Steroid	Saponin	Fats & oil	Acidic Cpds	Gly	Proteins	Alk	Reducing sugar
+++	+++	+++	+++	++	++	+++	++	+++	+

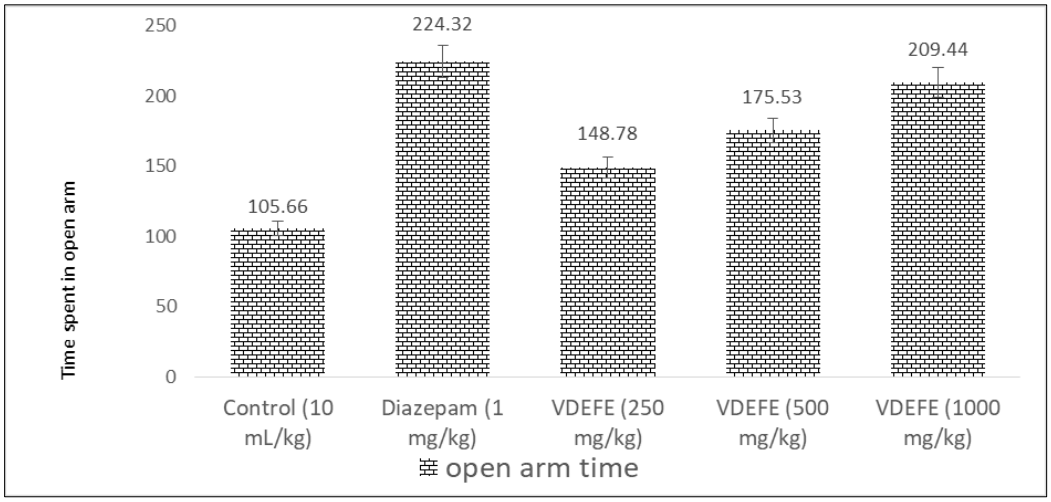
Key: (+)=faintly present; (++)=moderately present; (+++)=abundance. Gly=glycoside; Alk=alkaloids; Cpd=compounds.

3.3. Anxiolytic effect of the extract

The extract caused a dose-related significant ($p < 0.05$) increase and decrease in time spent on the open arm and closed arm respectively when compared to the negative control (Figure 3a and 3b respectively). There were also dose-related significant ($p < 0.05$) increase in entries into open arm and significant ($p < 0.05$) decrease in entries into closed arm when compare to the negative control (Figure 4a and 4b respectively).

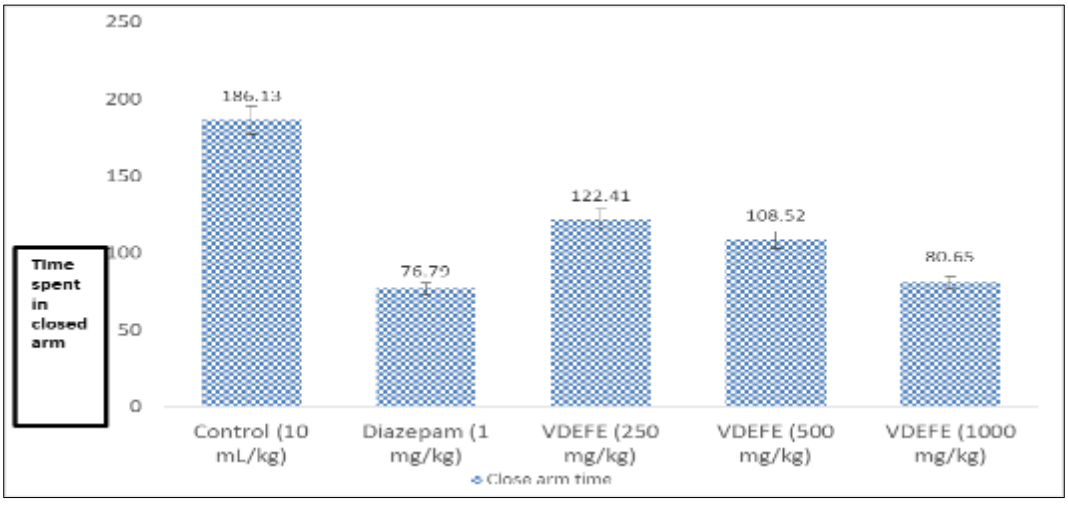
3.4. Effect of extract on Pentobarbitone-induced sleeping time

The fruit extract exhibited a dose-related significant ($p < 0.05$) decrease in onset of sleep as well as a significant ($p < 0.05$) increase in duration of sleep when compared to negative control (Figure 5a and 5b respectively).



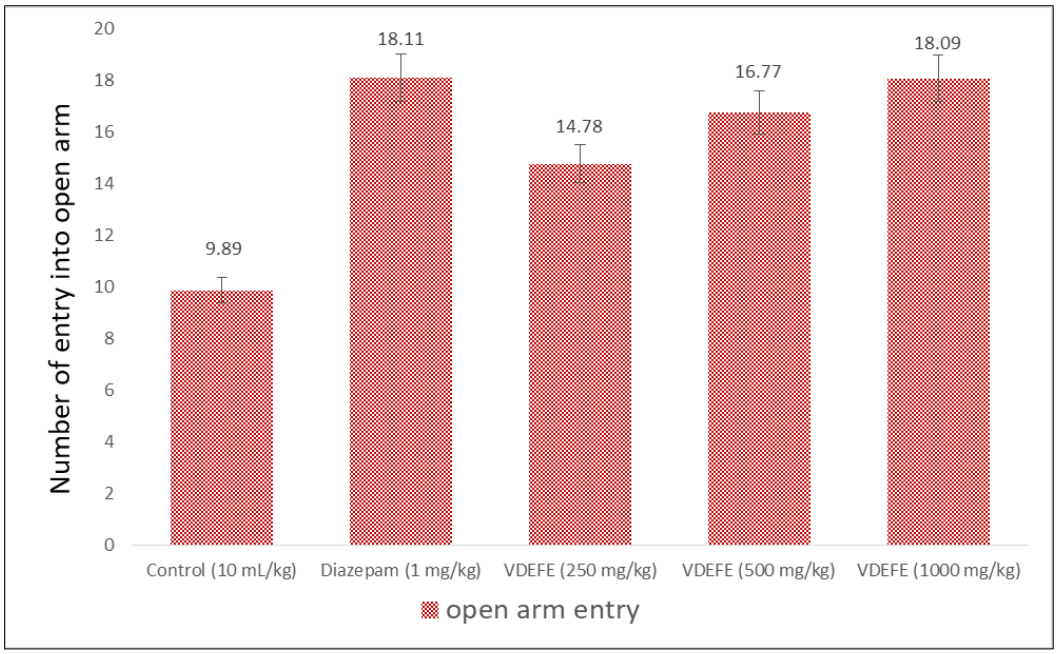
Key: VDEFE=*Vitex doniana* ethanol fruit extract

Figure 3a Effect of the fruit extract on time spent on open arm



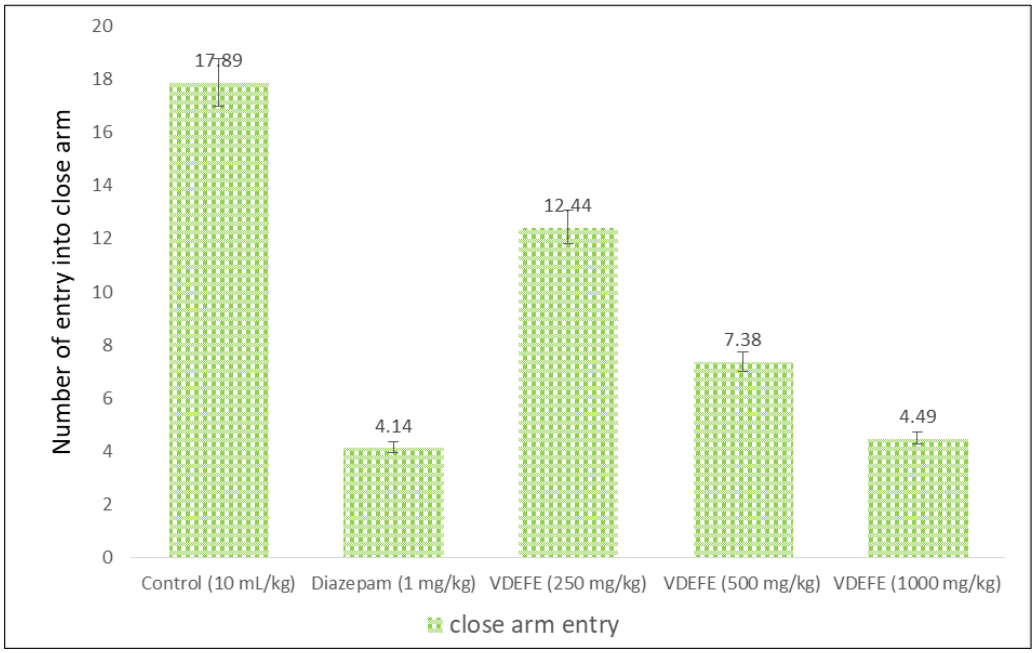
Key: VDEFE=*Vitex doniana* ethanol fruit extract

Figure 3b Effect of the fruit extract on time spent in close arm.



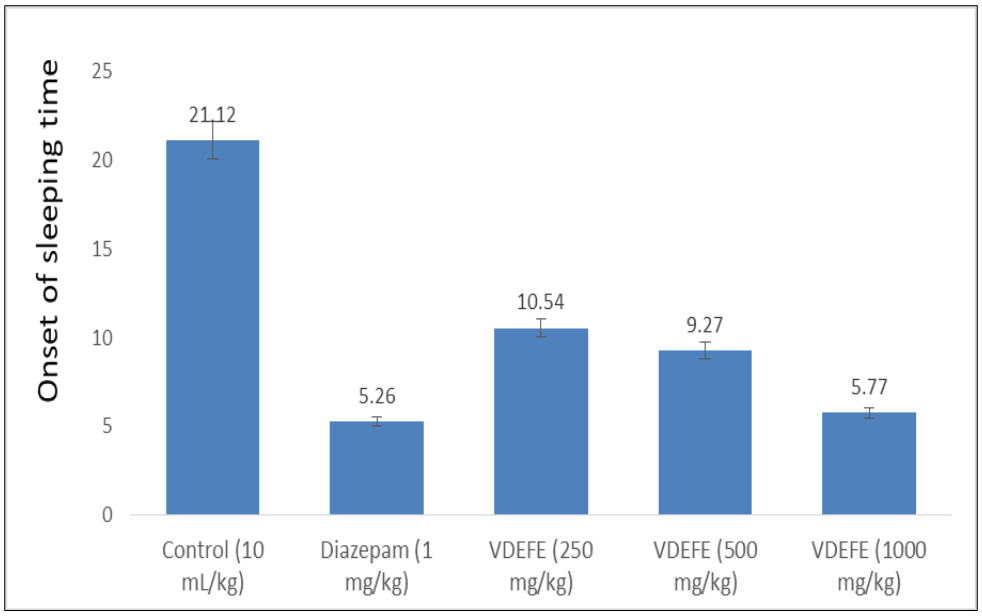
Key: VDEFE=*Vitex doniana* ethanol fruit extract

Figure 4a Effect of the fruit extract on number of entries made into the open arm.



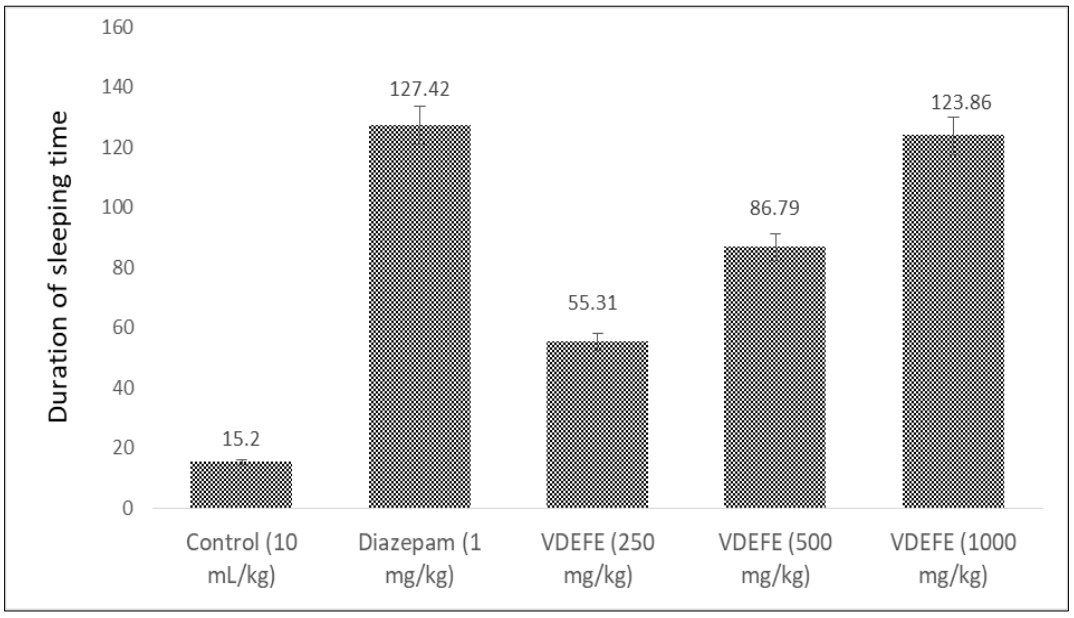
Key: VDEFE=*Vitex doniana* ethanol fruit extract

Figure 4b Effect of the fruit extract on number of entries made into close arm



Key: VDEFE=*Vitex doniana* ethanol fruit extract

Figure 5a Effect of V*itex doniana* on sleep onset



Key: VDEFE=*Vitex doniana* ethanol fruit extract

Figure 5b Effect of *Vitex doniana* on sleeping time

4. Discussion

The beneficial effects of medicinal plant typically result from the combinations of secondary metabolites present in them, through their additive or synergistic actions at single or multiple target sites associated with a physiological process [15]. Medicinal actions of plants are unique to particular plant, and secondary metabolites in a particular plant are often taxonomically distinct [16]. According to [17] some plants' extracts exert actions that are similar to those of endogenous metabolites like ligands, hormones, signal transduction molecules, or neurotransmitters thereby exhibiting medicinal effects on human in their potential target sites (e.g. central nervous system, endocrine system, etc.). The qualitative phytochemical screening of the fruit extract of *Vitex donina* revealed the abundance of alkaloids, tannins, flavonoids, glycosides, saponins with moderate presence of fats and oil, acidic compounds and traces of reducing sugars. These secondary metabolites, individually or in combination, account for the observed pharmacological effects of this fruit extract. Therefore, the observed anxiolytic and sedative activities of the fruit extract of V. *doniana* could be due to the presence

of flavonoids, alkaloids, and terpenoids, since these secondary metabolites have been previously reported to be responsible for anxiolytic and sedative effects observed in some plant extracts [18].

Nevertheless, some herbal medicines have been implicated in organ damage and fatal events [19]. The toxicity study of this extract showed absence of toxic signs and deaths up to the dose of 5000 mg/kg in the animals. Absence of toxic signs and deaths in acute toxicity study are signs of relative safety.

Rodents consistently spend greater time in the closed arms when placed in mazes comprising of open and closed arms [20]. Avoidance of the open arm is due to manifestation of fear and anxiety [20]. Based on these assertions, the elevated plus-maze tests are reliable means of identifying selective anxiolytic effect of drugs. Again, [21] reported rodents naturally avoid the open arms and the avoidance open arm was reduced by diazepam (anxiolytic agent) and enhanced by picrotoxin (anxiogenic agent).

In this study, there was a dose–related significant ($p<0.05$) increase in the time the mice spent in the open arm as well as a dose–related significant ($p<0.05$) increase in entries into the open arm of the plus-maze when compared to the negative control. This result attests the anxiolytic effect of the fruit extract.

Likewise, a dose–related significant ($p<0.05$) reduction in the time spent in the closed arm as well as a dose–related significant ($p<0.05$) decrease in entries into the closed arm of the plus-maze was observed for the fruit extract when compared to the negative control. This again confirms the anxiolytic effect of the fruit extract. However, this anxiolytic effect caused by the extract was less than that produced by diazepam (1 mg/kg).

The open arm–closed arm approach for screening anxiolytic effect has worked well in identifying the anxiolytic potential of benzodiazepine/GABAA receptor-related agents. In this context, the effectiveness of the extract in relieving anxiety in this model suggest a possible positive modulation of the GABAA – chloride channel receptor complex.

For the effect of the extract on phenobarbitone sleeping time, the extract exhibited a dose-related significant ($p < 0.05$) decrease in time for onset of sleep as well as a significant ($p < 0.05$) increase in duration of sleep when compared to negative control. However, this positive effect on duration and onset of sleep was less than that produced by diazepam (1 mg/kg). Central nervous system (CNS) depressants prolong barbiturate sleeping time [22]. Hence this observed effect of the extract on pentobarbitone-sleeping time goes further to confirm the anxiolytic and sedative effect of *Vitex doniana* fruit extract. The folkloric use of the fruit extract of *Vitex doniana* in the induction of sleep and relaxation could therefore be justified.

5. Conclusion

This result therefore provides scientific validation for the use of this plant in traditional medicine for induction of sleep and relaxation. It could therefore be a lead to a pharmaceutical product with lesser side effect and better tolerability. However further studies are needed with the aims of isolation of the bioactive compounds and the demonstration of the precise molecular mechanisms responsible for the observed pharmacological activity of the *vitex doniana* fruit extract.

Compliance with ethical standards

Disclosure of conflict of interest

The authors wish to confirm that there is no known conflict of interests associated with this paper and there has been no significant financial support for this work that could have influenced its outcome.

Statement of ethical approval

Ethical approval number PHACOOU/AREC/2023/032 was assigned to attest the animals were cared for according to the Faculty of Pharmacy (COOU) Animal Research Ethics Committee guidelines (PHACOOUAREC), which are in line with the National Institute of Health (NIH), USA, guidelines for the care and use of laboratory animals.

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