Journal of Experimental Research

DECEMBER 2023, Vol 11 No 4

Email: editorinchief.erjournal@gmail.com
editorialsecretary.erjournal@gmail.com

Received: April, 2023
Accepted for Publication: Dec., 2023

ANTIDEPRESSANT AND ANXIOLYTIC EFFECTS OF *Tetracarpidium conophorum* SEEDS IN SWISS MICE

Joseph O. Erhabor* and Kenneth C. Ezeugwu

Phytomedicine Unit, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, PMB 1154, Benin City, Nigeria

***Author for Correspondence:** joseph.erhabor@uniben.edu;

ABSTRACT

Naturally, herbs are the principal constituent of herbal or traditional medicine used in managing and treating diverse sicknesses and diseases globally. *Tetracarpidiumconophorum* belongs to the family Euphorbiaceae and is traditionally used as a nerve tonic, amongst other conditions. This study examined the antidepressant and anxiolytic properties of the aqueous extract of *T. conophorum* seed. The antidepressant and anxiolytic indexes at 25, 50 and 100 mg/kg of *T. conophorum* extract and standard drugs (*30 mg/kg of Amitriptyline and 2 mg/kg of diazepam*) were assessed in an animal model using the force swimming, tail suspension and hole board tests. The animals (mice) were randomly placed in five groups of three animals each (n=5). The extract expressed a significant decrease in the immobility time (reduced depression) in the forced swim and tail suspension tests compared to distilled water (negative control). The head-dip frequency was significantly reduced (slightest display of anxiety) in the hole board test for anxiety. The 25 and 50 mg/kg of *T. conophorum* extract presented the best impacts against depression and anxiety. In conclusion, the study affirms the capacity of *T. Conophorum* extract to manage depression and anxiety. The probable mode(s) of action of *T. Conophorum* extract as an antidepressant and an anti-anxiety agent is recommended for future studies.

Keywords:*Tetracarpidiumconophorum*; depression; anxiety; extract; medicinal plant

INTRODUCTION

Depression and anxiety are common diseases responsible for most mental disorders linked to stress-related psychiatric abnormalities and disability worldwide (Whiteford et al. 2013). Major depressive and anxiety disorders are taunted the leading cause of disease burden in both high-income and low- and middle-income countries (Santomauro et al. 2021).

Depression affects 5 % of adults and an estimated 3.8% of the population globally. It is projected that nearly 280 million individuals worldwide suffer from depression (IHME, 2019). Depression is characterized by depressive mood, loss of pleasure or interest, diminished energy, guilt or low self-worth, disrupted sleep, changes in appetite or weight, and poor attention. It often leads to impairment of an individual's ability to function and carry out everyday tasks and responsibilities and could lead to suicide (WHO, 2021). It is also presented with symptoms of anxiety. On the other hand, anxiety is a physiological and psychological state related to cognitive, somatic, emotional and behavioural alteration (Eisenberg *et al*. 1998). It is a condition with intense sympathetic hyperactivity and fear that characterizes motor tension with vigilance syndromes. It interrupts mental power, psychomotor roles, and memory disparity (Pine *et al*. 1999).

Although there are known, effective treatments for mental disorders, more than 75% of people in low- and middle-income countries receive no treatment (Evans-Lacko et al. 2018). Barriers to effective care include a lack of resources, a lack of trained healthcare providers and social stigma associated with mental disorders. In countries of all

income levels, people who experience depression are often not correctly diagnosed, and others who do not have the disorder are too often misdiagnosed and prescribed antidepressants (WHO, 2021).

Nevertheless, Herbal medicines, specifically the use of medicinal plants, have presented alternatives to dealing with a myriad of diseases, including depression and anxiety. It has been asserted that medicinal plants have multipurpose applications that have increased their utilization as herbal drugs or derivation of substances for detecting new therapeutic agents. Natural products from some of these natural resources, like plants, have gained momentous use in pharmaceutical preparations as crude extracts, fractions, pure compounds or analogous compounds from highly active isolated compounds (El-Sayed *et al*. 2012). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the wealthiest bio-resource of drugs of traditional medicines, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs production (Nirmala *et al.* 2011).

Of the medicinal plants explored, *Tetracarpidium. conophorum* has been used to manage several diseases. *Tetracarpidiumconophorum* (Mull. Arg) Hutch & Dalziel Syn. *Plukenetiaconophora,* generally called African Walnut, Black walnut, Nigerian walnut or Conophor, belongs to the family Euphorbiaceae. *T. conophorum*is a perennial climbing shrub native to tropical western and central Africa and can be found in Togo, Congo, Sierra Leone, Nigeria, and Cameroon (Amusa, 2014). The plant is a small tropical flowering plant, about 6 m – 18 m long on the attainment of the reproductive phase. The stem can be up to 16 cm in girth and dark grey when old but is green and glabrous when young (Nwachoko and Jack, 2015). The fruit is a capsule, 6 cm – 10 cm long by 3 cm – 11 cm wide, containing subglobular seeds. The seed is about 2.5 cm in diameter, and the fruit (7 cm across) is light green to brown when ripe. It is a perennial cash crop and an economic tree widely grown for its edible seed nut (Chijoke*et al*. 2017).

Earlier research by Oyenuga (1997) reported the nuts' amino acid and fatty acid components, and its leaf juice's use to treat prolonged and constant hiccups. The plant parts of *T. Conophorum* have been used ethnomedically, including the stem bark, leaves, seeds, and roots. Local people use the bark as a mild laxative (Akomolafe et al. 2017a). When eaten raw, the seed kernel has a bitter taste like the kola nut and is used as an analgesic, nerve tonic and aphrodisiac (Aiyeloja and Bello, 2006). An investigation suggested that ingesting walnut increases fat oxidation and reduces carbohydrate oxidation without affecting total consumption. African Walnut helps treat rheumatism, gout, cold, kidney pain, and heavy menstrual bleeding as a blood cleanser and to expel worms (Ekhuosuehi, 2008). It is believed to halt asthma and is prescribed to be taken between bouts of asthma but not for acute asthma. The undeveloped leaves and shoots are consumable vegetables. The leaf is vital in the treatment of cancers that occurs in the neck region. They are used as a tea for diarrhoea, gastrointestinal system and gums, mouth, and throat inflammation. The root is used for treating piles. The bark serves as a laxative in tea and is chewed for toothache. It helps to prevent and control high blood pressure (Ekhuosuehi, 2008). Drinking water immediately after consuming the edible nut has a bitter taste because of some alkaloid-containing compounds in the plant. Ayoola *et al.* (2011) reported the use of *T. conophorum*in the treatment of stomach disorders, and for controlling high blood pressure. The fruits are edible and used for various purposes, including masticatory, thrush, anti-helminth, syphilis, and antidote against snake bites (Obianime and Uche, 2010). They are said to tonify the kidneys, strengthen the back and knees and moisten the intestines. They are used by the elderly to cure constipation and flatulence (Ayoola *et al*. 2011; Ogundolie *et al*. 2017). Previous research on the biological activities of *T. conophorum* includes Anti-diarrhoea activity (Nwachoko and Jack, 2015), Anti-ulcer and wound healing activities (Ezealisiji *et al*. 2014), Anti-inflammatory activity (Olaniyi*et al*. 2016), Anti-lipidemic activities (Ezealisiji *et al*. 2016; Analike *et al*. (2017), Anti-diabetic activities (Ajilore et al. 2021) and antimicrobial activities (Suara *et al*. 2016). Others include Male fertility-enhancing actions (Chijoke *et al*. 2017), Oxidative stress-induced penile damage (Akomolafe *et al*. 2017b), Anti-malarial activity (Ogundolie *et al*. 2017), and safety assessment (Akomolafe *et al*. 2017a).

The reported biological studies show a dearth of information exists on the study of the plant's

potential as an antidepressant and anxiolytic agent, despite its folkloric claim as a nerve tonic. Therefore, the study aimed to investigate the antidepressant and anxiolytic potential of the extract of ***Tetracarpidiumconophorum seed in Swiss mice***

MATERIALS AND METHODS

Plant material Collection of and Extract Preparation

The fresh seeds of ***Tetracarpidiumconophorum*** were obtained from Ugbojiobo market, Benin City, Edo state. It was identified and authenticated by Prof MacDonald Idu of the Department of Plant Biology and Biotechnology, University of Benin, with Voucher specimen number UBH-E17. The chopped seeds were washed, and air-dried for fourteen days. It was further oven-dried at 40 ^{0}C for 4 hours. The powdered seed sample (1550 g) was extracted via maceration with distilled water (2500 ml) for 72 hours with intermittent stirring and shaking. It was sieved, and the filtrate was concentrated using crucibles in a water bath at a regulated temperature of 45^{o}C. The concentrate was weighed, and the percentage yield determined.

Purchase and Handling of the Animals

Swiss mice of 25-30 g of either sex were obtained from the Animal House of the Department of Biochemistry, Faculty of Life sciences, University of Benin, Benin City, Edo state, for the study. The mice were placed into five groups (n=3). The mice were adapted to laboratory conditions for fourteen days (14) before the experiment and had unfettered access to food, and water, *ad libitum*. The fasted overnight animals had unrestricted access to water before the experiment. The experimental animals were handled correctly and used according to the established protocol of CIOMS and ICLAS (2012).

Administration of Extract

The aqueous extract of ***T. conophorum*** seed was prepared in distilled water to get a stock solution of 3.4 mg/mL.The animals (n=3) were randomly placed in five groups and given the appropriate dose of the aqueous extract of *T.**conophorum***. Group 1 (negative control (untreated group) was given 0.2 mL distilled water, Group 2 (positive control) was administered the appropriate standard drug, Group 3 (25 mg/kg of **T. *conophorum*** extract, Group 4 (50 mg/kg of extract) and Group 5 (100 mg/kg of the extract of *T. conophorum).*

The extract at graded doses of 25, 50 and 100 mg/kg was orally administered using an oral gastric tube to the animals. For the antidepressant test, 30 mg/kg of Amitriptyline was used as the standard drug while 2 mg/kg of diazepam was adopted for the anxiolytic study. The standard drugs were prepared in distilled water and orally administered to the mice.

Determination of Antidepressant Activity Forced Swim Test

In assessing the antidepressant activity, the forced swim test (FST) was utilized (Andrews *et al*. 2010). The mice were separately placed in a glass cylinder (20 cm in height, 14 cm in diameter) filled with water up to a height of 10 cm at 25 ± 2 °C. The mice were forced to swim for 5 min, and the immobility period was noted and recorded during the 5 min interval of the test. The duration of immobility was regarded as the time spent by the mice floating in water with no struggle and making only those movements necessary to keep its head above the water. To assess the fitness of each test mice, a pre-test was done 24 h before the FST by exposing each test mice to a 15 min swimming session.

Tail Suspension Test

The Tail suspension test (TST) was done following the earlier described method by Steru *et al*. (1985) and Binder *et al*. (2004). The mice were suspended 58 cm above the floor with an adhesive tape, and placed approximately 1 cm from the tip of the tail. The total time of immobility was noted during the 5 min test period. The mice were considered immobile when they were completely motionless.

Percentage Inhibition of Immobility

The percentage inhibition of immobility for both the Force swim and Tail suspension tests was done. The inhibition of immobility in percentage was calculated by adopting the formula below.

$$\text{Inhibition of Immobility} = \frac{\text{Negative Control} \times \text{duration of immobility (Sec)}}{\text{Negative Control}}$$

Where control = water (0.2 ml/kg)

Duration of immobility = time the animal was immobile in seconds after each dose (25, 50 and 100 mg/kg) cum the standard antidepressant drug were administered.

Anxiolytic study

Hole Board Test

The hole board test (HBT) is commonly utilized to determine the animal's exploratory behaviour and overall activity, which is similar in principle to the open field test (Hall, 1934). The HBT was conducted by adapting the method outlined by Takeda *et al*. (1998). A wooden board of 20 cm by 40 cm with sixteen exactly spaced holes was used for the study. The mice were placed into five groups, of three animals. Thirty minutes after treatment, the animals were positioned singly on the board, and the number of times the mice dipped their head into the hole at the level of their eyes during a five-minute period was counted using a tally counter.

Statistical Analysis of Data

Data were presented as Mean ± standard error of means. The data were analyzed using a one-way analysis of variance to compare the means of different groups. The Duncan multiple range test was used to ascertain the differences among the various means and the interaction between the variables. The computer software package Graph pad prism version 7 was used in analyzing the data. Differences at $P < 0.05$ were considered statistically significant.

RESULTS

Percentage Yield of Extract

The percentage yield showed the quantity of the extract derived from the powdered sample of the plant as 45.9 %.

Antidepressant study

The results obtained from the force swimming test indicated that the standard antidepressant drug-Amitriptyline (5 mg/kg), and the 25 mg/kg of *T. conophorum* extract had the most significant effect on the duration of immobility time (0.00 sec.) when compared with the negative control (0.2 ml/kg distilled water) as presented in Table 1. The extract at 25 mg/kg displayed a remarkable 100 % inhibition of immobility (depression).

Table 1: Effects of aqueous extract of ***Tetracarpidiumconophorum*** seed (TCE) on the duration of immobility in mice.

Treatment	Doses (mg/kg)	Immobility (Sec.)	Inhibition of immobility (%)
Control (H_2O)	0.2 ml/kg	79.50 ± 6.21^{a}	0
Amitriptyline	30	0.00 ± 0.00^{d}	100
TCE	25	0.00 ± 0.00^{d}	100
TCE	50	19.67 ± 3.60^{c}	75.3
TCE	100	72.33 ± 11.33^{a}	9.02

Values are expressed as Mean ± SEM of three animals in each group. Values with different alphabets are significantly different ($p<0.05$) across the column. Sec. (Seconds)

The potential of the extract on the duration of immobility in the tail suspension test in mice is presented in Table 2. The impact of the extract across the tested doses (25, 50 and 100 mg/kg) and Amitriptyline (30 mg/kg) on the duration of immobility was significantly not different ($p<0.05$).

Table 2: Effects of aqueous extract of ***Tetracarpidiumconophorum*** seed (TCE) in Tail suspension test in mice

Treatment	Doses (mg/kg)	Immobility (Sec.)	Percentage Inhibition of immobility
Control (H_2O)	0.2 ml/kg	62.67±7.75[a]	0
Amitriptyline	30	23.00±16.01[b]	63.30
TCE	25	26.67±3.84[b]	57.44
TCE	50	25.67±4.67[b]	59.04
TCE	100	25.67±5.37[b]	59.04

Values are expressed as Mean ± SEM of three animals in each group. values with different alphabets are significantly different (p<0.05) from each other across the column. Sec. (Seconds)

Anxiolytic Study

Table 3 presents the anti-anxiety impact of the extract and the standard drug following the head dip test in mice. Of the extract doses, the 25 mg/kg, had the least display of anxiety (reduced frequency of head-dipping of the mice), while the 50 mg/kg of the *T. conophorum* extract showed the highest frequency of head-dipping by the mice (anxiety). The standard anti-anxiety drug (Diazepam) at 2 mg/kg significantly decreased the number of the mice head dips compared with untreated control (water), which showed a significant increase in the frequency of head dips by the mice.

Table 3: Effects of aqueous extract of ***Tetracarpidiumconophorum*** seed (TCE) in the head dip test in mice

Treatment	Doses (mg/kg)	Head dip count
Control (H_2O)	0.2 ml/kg	26.67±4.67 [a]
Diazepam	2	6.67±1.45[c]
TCE	25	9.33±1.33[c]
TCE	50	16.67±3.33[b]
TCE	100	13.00±3.00[b]

Values are expressed as Mean ± SEM of three animals in each group. values with different alphabets are significantly different (p<0.05) from each other across the column. Sec. (Seconds)

DISCUSSION

In the current study, the antidepressant and anxiolytic impact of the extracts was assessed by exploring the behaviour and general activity of the animal. The results demonstrated that the extracts had antidepressant and anxiolytic properties, as revealed in the FST, TST and HBT. The FST and TST are two widely explored animal models for antidepressant screening and are sensitive and relatively specific to all major classes of antidepressants (Porsolt *et al*. 1977). In TST, immobility mirrors a condition of despair that numerous therapeutically active agents can decrease human depression. Similarly, in the FST, mice are obligated to swim in a constrained space from which they cannot escape. It has been adjudged that TST is less stressful and probably has higher pharmacological sensitivity than FST (Thierry *et al*. 1986).

The aqueous extract of *T. Conophorum* nut displayed a dose-dependent effect on the duration

of immobility and reduced the exploratory activity of the mice. This is similar to an earlier study on the aqueous extract of *T. conophorum* (50 – 200mg/kg p.o.) by Aladeokin and Umukoro (2011). Our results showed that the 25 and 50 mg/kg of the extract shortened the duration of immobility (0.00 and 19.67 seconds) in the force swim test compared to the negative control (79.50 seconds) (Table 1). This indicates the mice were non-tired, less fatigue and had higher stamina with a possibility of an elevated mood. These signs above are reflective of a non-depressed individual. *Results from the Tail suspension test following the administration of T. Conophorum* nut aqueous extract in mice showed a significant decrease in the duration of immobility across the treatment groups (25, 50 and 100) mg/kg of the extract corresponding to 26.67, 25.67 and 25.67 seconds when compared to the negative control (62.67 seconds) as shown in Table 2. The results suggest the extract may have a soothing property.

In another vein, the extracts of *T. conophorum*, showed a good effect of anxiolytic properties in the mice across the doses compared to the negative control (distilled water, 0.2 ml/kg) (Table 3). The extract at 25 mg/kg displayed the best effect against anxiety (reduced frequency of head-dip) and was not significantly different ($p<0.05$) from the standard anti-anxiety drug (diazepam at 2mg/kg). Anxiety is a common mental disorder usually experienced regularly, manifesting in fear and worries commonly associated with normal human feelings. This state of mind, when sustained for a longer period, negatively impacts the individual's mental and physical condition, resulting in hyperactivity and nervousness (Aiwonegbe et al. 2022). A previous study by Takeda and colleagues established a direct proportionality relationship between anxiolytic properties and head-dipping in experimental animals (Takeda et al. 1998). The extracts across the doses, expressly at the best dose (25 mg/kg), significantly reduced the restlessness of the treated mice. Similarly, the frequency of head-dip was also considerably reduced, suggesting the plant had a sedative potential.

CONCLUSIONS

The study revealed that the aqueous extract of ***Tetracarpidiumconophorum*** at the least dose (25 mg/kg) caused marked antidepressant and anxiolytic activities, as observed in the behavioural mouse model. This exploratory behavioural animal model revealed positive therapeutic signals for the antidepressant and anxiolytic actions of T. conophorum. Further studies are required to understand how the extract can cause antidepressant and anxiolytic effects and the possible bioactive compound responsible for the biological activities.

REFERENCES

Aiwonegbe AE, Omoruyi U, Ogbeide OK, Imoukhuede BO, Gabriel BO. (2022). Anxiolytic Effects, Antioxidant and Anti-inflammatory Activities of the Methanol Extract of Jatropha tanjorensis Leaf. Tanzania Journal of Science. 48(3): 596-606.

Aiyeloja AA, Bello OA. (2006). 'Ethnobotanical potentials of common herbs in Nigeria: A case study of Enugu State', Educational Research and Review. 1(1): 16–22.

Ajilore BS, Olorunnisola OS, Owoade AO.(2021). Tetracarpidiumconophorum seed extract reduces intestinal absorption, and increases cellular trapping of glucose. Bulletin of the National Research Centre. 45: 1-10.

Akomolafe SF, Oboh G, Oyeleye SI, Olasehinde TA. (2017a), 'Toxicological effects of aqueous extract from African walnut (Tetracarpidiumconophorum) leaves in rats', Journal of Evidence-Based Complementary and Alternative Medicine. 22(4): 919–925.

Akomolafe S, Oboh G, Olaseinde T, Oyeleye S, Ogunsuyi O, (2017b), 'Modulatory effects of Aqueous extracts T. conophorum leaves on key enzymes linked to erectile dysfunction and oxidative stress induced penile lipid peroxidation in penile and testicular tissues'. Journal of Applied Pharmaceutical Science. 7(1): 51–56

Aladeokin AC, Umukoro S. (2011). Psychopharmacological properties of an aqueous extract of Tetracarpidiumconophorum Hutch. & Dalziel in mice. Journal of Natural Medicines. 65: 411-416.

Amusa TO, Jimoh SO, Azeez IO, Awodoin RO, Kareem I (2014). "Stock density and fruit yield of African walnut, Plukenetiaconophora Mull-Arg (Syn. Tetracarpidiumconophorum) in tropical lowland rainforests of southwest Nigeria". Journal of Tropical Forestry and Environment. 4 (2): 73–81.

Analike R, Ahaneku JE, Njoku MC, Ahaneku, GI, Ezeugwunne IP, Ogbodo EC. (2017). 'Effects of Tetracarpidiumconophorum-"Nigerian Walnuts" on blood lipids, lipoproteins and glucose values in adult Nigerians', International Journal of Innovation Research and Advanced Studies. 4(7): 67–71.

Andrews G, Cuijpers P, Craske MG, McEvoy P, Titov N. (2010). Computer therapy for the anxiety and depressive disorders is effective, acceptable and practical health care: a meta-analysis. PLoS One. 5(10):e13196.

Ayoola PB, Onawumi OO, Faboya OOP. (2011). Chemical evaluation and nutritive values of Tetracarpidiumconophorum [Nigerian walnut] seeds. Journal of Pharmaceutical and Biomedical Sciences 11(15): 1 – 5.

Binder EB, Salyakina D, Lichtner P, Wochnik GM, Ising M, Putz B. (2004). Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. Nature Genetics. 36(12):1319-1325.

Chijoke OC, Assumpta AC, Lawrence E, Sunday U. (2017). 'Effect of black walnut (Tetracarpidiumconophorum) leaf extract on the reproductive organ of male albino rats'. International. Journal of Homeopathy and Natural Medicine. 3(2): 9–14.

CIOMS, ICLAS. (2012). International guiding principles for biomedical research involving animals. Council for International Organization of Medical Sciences (CIOMS) and International Council for Laboratory Animal Science (ICLAS). [Online-Available at: https://media-01.imu.nl/storage/iclas.org/5196/cioms-iclas-principles-final.pdf] [Accessed 24th November 2022]. 4p.

Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, Kessler RC. (1998). Trends in alternative medicine use in the United States, 1990–1997: results of a follow-up national survey. JAMA.280 (18):1569-1575.

Evans-Lacko SAGS, Aguilar-Gaxiola S, Al-Hamzawi A, Alonso J, Benjet C et al. (2018). Socio-economic variations in the mental health treatment gap for people with anxiety, mood, and substance use disorders: results from the WHO World Mental Health (WMH) surveys. Psychological Medicine. 48(9):1560-1571.

Ekhuosuehi, A. (2008). Properties of Walnut plant in culture. The Nigerian Observer Online edition www.nigerianobservernews.com/19072010/..../features3.html. 12/10/2012. 2.20pm. Accessed 10th June, 2016.

El-Sayed SA, Salih AB, Mohamed MS, Mortada ME, Eman AE. (2012). Phytochemical Studies and Evaluation of Antioxidant, Anticancer and Antimicrobial Properties of Conocarpus erectus L. growing in Taif, Saudi Arabia. European Journal of Medicinal Plants. 2(2): 93-112

Ezealisiji KM, Ijeomah SC. Agbo MO, (2014). 'Anti-ulcer activity of African walnut "Tetracarpidiumconophorum" nuts against gastric ulcers in rats', Asian Pacific Journal of Tropical Diseases. 4(1): 670–673.

Ezealisiji KM, Stanley CN, Ekanem ES. (2016). 'Evaluation of anti-cholesterol activity of ethyl acetate and n-hexane extracts of Tetracarpidiumconophorum (Mull. Arg.) Hutch and Dalziel (African walnut) towards hypercholesterolemic rats'. International Journal of Pharmacognosy Phytochemical Research. 8(8): 1372–1376.

Hall CS. (1934). Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. Journal of Comparative Psychology. 18: 385–403.

IHME (Institute of Health Metrics and Evaluation) (2019). Global Health Data Exchange (GHDx). http://ghdx.healthdata.org/gbd-results-tool<params=gbd-api-2019permalink/d780dffbe8a381b25e1416884959e88b (Accessed 11 March 2023).

Nirmala MJ, Samundeeswari A, Sankar PD. (2011). Natural plant resources in anticancer therapy-A review. Research in Plant Biology.1: 1-14.

NwachokoN, Jack IR (2015). Phytochemical screening and antidiarrhoea activities of Tetracarpidiumconophoruminduce in albino rats. Sky Journal of Biochemistry Research. 4(4):21-24.

Obianime AW, Uche FI. (2010). The effects of aqueous extracts of Tetracarpidumconophorumseeds on the hormonal parameters of male guinea pigs. Asian Pacific Journal of Tropical Medicine. 2(1) : 21 – 24.

Ogundolie OO, Dada EO, Osho IB, Oloruntola DA. (2017). 'Effects of raw ethanolic seed extract of Tetracarpidiumconophorum on haematological and histopathological parameters in Swiss albino mice infected with Plasmodium Berghei'. Journal of Applied Life Sciences International. 12(2): 1–14.

Olaniyi FE, Bambidele IO, Omokehinde AO, Ayodeji AA. (2016). 'Anti-inflammatory activities of the chloroform extract of the fruit of Tetracarpidiumconophorum (Mull. Arg.) (Nigeria Walnuts)'. Journal of Advance in Medical and Pharmaceutical Sciences. 6(1): 1–7.

Oyenuga VA. (1997). Nigeria food and feeding stuff, University Press Ibadan, Ibadan, pp. 11–12.

Pine DS, Wasserman GA, Workman SB. (1999). Memory and anxiety in prepubertal boys at risk for delinquency. Journal of the American Academy of Child and Adolescent Psychiatry. 38:1024– 31.

Porsolt RD, Bertin A, Jalfre M. (1977). Behavioral despair in

mice: a primary screening test for antidepressants. Archives Internationales de Pharmacodynamie et de Therapie. 229:327–336.

Santomauro DF, Herrera AMM, Shadid J, Zheng P, Ashbaugh C, Pigott DM, et al. (2021). Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. The Lancet. 398(10312): 1700-1712.

Steru L, Chermat R, Thierry B, Simon B. (1985).The tail suspension test: method for screening antidepressants in mice. Psychopharmacology. 85:42-54.

Suara K., Azubuike CP, Okubanjo OO, Igwilo C 2016, 'Neutraceuticals and antibacterial properties of methanol extract of (Plukenetiaconophora Müll.-Arg.) family Euphorbiaceae leaves and physical properties of its cream formulations', Nigerian Journal of Pharmaceutical and Applied Science Research 5(1):91–98.

Takeda H, Tsuji M, Matsumiya, T. (1998). Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. European Journal of Pharmacology. 350:21–29.

Thierry B, Steru L, Simon P, Porsolt RD. (1986). The tail suspension test: ethical considerations. Psychopharmacology. 90:284–285.

Whiteford HA, Degenhardt L, Rehmetal J. (2013). Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. Lancet. 382: 1575–1586.

WHO, (2021). Depression. https://www.who.int/news-room/fact-sheets/detail/depression. (Accessed 11 March, 2023)