University of Benin, Journal of Science and Technology, Vol. 7. No.1, June 2019.- www.ubjst.uniben.edu

QUALITATIVE PHYTOCHEMICAL ANALYSIS, ACUTE TOXICITY AND ANTI-DEPRESSANT ACTIVITY OF *RAUVOLFIA VOMITORIA*

C. A. Unuigbe and O. K. Ogbeide
Department of Chemistry,
Faculty of Physical Sciences,
University of Benin, Benin City, Nigeria.
charles.unuigbe@uniben.edu

ABSTRACT

This research was designed to determine the phytochemical composition, acute toxicity, as well as anti-depressant effect of the ethyl acetate root extract of Rauvolfia vomitoria. The phytochemical analysis of Rauvolfia vomitoria was determined using standard methods, while FST (forced swim test) and TST (tail suspension test) were applied to evaluate the anti-depressant activity. The result indicated that the ethyl acetate root extract of Rauvolfia vomitoria is rich in tannins, phenols, flavonoids, terpenoids, glycosides and alkaloids. The oral administration of ethyl acetate root extract of Rauvolfia vomitoria to Swiss mice shows no mortality at maximum dose of 100 mg/kg after 14 days. Immobility was observed during the 5-minute interval after administering doses of 25 mg/kg, 50 mg/kg and 100 mg/kg of the root extract respectively to Swiss mice. It was observed that Rauvolfia vomitoria has anti-depressant effect at low dose of 25 mg/kg. It has potential detrimental properties when used in high doses. Therefore, the plant should be taken with caution when used for therapeutic purposes.

Keywords: *Rauvolfia vomitoria, phytochemical composition, acute toxicity, force swim test and anti-depressant activity.*

INTRODUCTION

Plants are major sources of therapeutic compounds and are the essential foundation of medicine since prehistoric times. Medicinal plants are important sources of new chemical compounds with potential therapeutic effects (Ogbeide *et al.*, 2020). Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates, and

chemical entities for synthetic drugs. Plants synthesize thousands of chemical compounds possessing different properties like defense against insects, bacteria, fungi, disease and herbivorous mammals (Ogbeide *et al.*, 2022). Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines. Medicinal plants have been discovered and used in traditional medicine practices since prehistoric times. There has been renewed interest in screening higher plants for novel biologically active compounds, particularly those that effectively intervene in human ailments. The development of drug-resistant strains of microorganisms and autoimmune problems make it imperative for a continued search for new drugs from natural products (Edward and Vaitheeswarn, 2014). (Cowan, 1999) reported that plants still represent large untapped sources of structurally novel compounds that might serve as lead for the development of novel drugs. Plants have been used for centuries as a remedy for human diseases because they possess phytochemicals of therapeutic values. However, their general acceptability has been limited by lack of dosage regimen and adequate toxicity data to evaluate their safety (Pousset, 1988). *Rauvolfia vomitoria* is a shrub found mainly in West Africa. The roots, leaves, and stem are used in medicine. It is a small tree or large shrub, growing to 8 m (26 ft) high. In Nigeria, *Rauvolfia vomitoria* has been used over the years for the treatment of hypertension and mental disorder. It is called African serpent wood or swizzle stick. In Yoruba, it is called "asofeyeje", "ira" in Igbo and "Wadda" in Hausa. *Rauvolfia vomitoria* has many alkaloids used mainly as anti-hypertensive agents and sedatives. The plant contains several compounds used by the pharmaceutical industry which include reserpine, reserpinine, deserpidine, ajmalicine and ajmaline. Based on these properties, this work seeks to carry out the phytochemical assessment, acute toxicity and anti-depressant activity of *Rauvolfia vomitoria.* Extensive studies carried out on its chemical properties showed that the plant contains more than 50 active indole alkaloids, each possessing remarkable pharmacological activities (Pousset and Poisson, 1965). A bioactive carboline alkaloid, alstonine, present in the root and leaf were previously shown to have anti-cancer activity (Pettit *et al*., 1994). While the antipyretic effect of the leaf extract has also been demonstrated (Amole and Onabanjo, 1999). The pharmaceutical derivatives are used mainly as antihypertensives and

sedatives. Its sedative property is attributed to its ability to balance body response to stress and anxiety, and to increase oxygen delivery to the brain (Oliver-Bever, 1982).Medicinal uses of the roots are extensive, particularly for their aphrodisiac, emetic, purgative, dysenteric, abortive, and insecticidal properties (Principe, 1989). Decoctions of the leaves of *Rauvolfia vomitoria* have a powerful emetic effect and chopped leaves stewed with animal fat are applied to swellings (Burkill, 1994). The root is also brewed as tea and used on humans to treat snakebite and cholera (Waterman, 1986).

Figure 1: ***Rauvolfia vomitoria*** root

The most prevalent affective illness is depression, which is characterised as a disease of mood rather than mental or cognitive abnormalities. It can range from a very mild state that is verging on normalcy to a severe psychotic depression associated with delusions and hallucinations.Depression is a major contributor to disability and early mortality on a global scale (Rang *et al*., 2008). Intense sorrow and despair are common symptoms of depression, as are mental sluggishness and memory loss, gloomy concern, a lack of enjoyment, self-deprecation, and fluctuating anger or aggression. Additionally, there are physical changes, especially in cases of severe, crucial, or melancholic melancholy. Depression does not have a single identified cause. Instead, a confluence of genetic, physiological, environmental, and psychological elements is probably to blame. Some forms of depression frequently run in families, which points to a hereditary connection. But even those without a history of

sadness in their families might experience depression. With patients responding to therapy in 65 to 80% of cases, antidepressants are now the cornerstone of care for this potentially fatal condition. (Rajput *et al.*, 2011). Understanding the pathophysiology of depression has advanced quickly in recent years. Most of the studies focus on newer and better drug therapy. Most research focuses on the development of new, more effective pharmacological therapies. There are several medications for use in the treatment of depression, however clinical examination of these medications has demonstrated prevalence of relapses, adverse effects, and drug interactions. This has served as the justification for the creation of novel antidepressants, including natural remedies (Rajput *et al.*, 2011).

Material and Methods

Reagents, materials and instruments

All reagents used were of analytical grade and distilled water was used in the preparation of all solutions. These include: Ethyl acetate, dilute ammonia, ethanol, sulphuric acid, formaldehyde, ferric chloride, hydrochloric acid, Potassium ferricyanide, sodium carbonate, folin-ciocalteu reagent, distilled water, ammonium hydroxide, chloroform, acetic anhydride, phosphomolybdic acid, lead acetate, glacial acetic acid, *Rauvolfia vomitoria* roots, crestor high speed milling machine, spectrophotometer, incubator, and rotary evaporator.

Collection of plant material

The fresh roots of *Rauvolfia vomitoria* were obtained from Ugbogiobo market, Benin City, Edo State. It was identified and authenticated by Dr H. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.

Preparation of Extract

The fresh roots of *Rauvolfia vomitoria* were washed, chopped into pieces and air-dried for two weeks. It was further oven dried at 40 ^{o}C for 4 hours. The powdered root sample (1.55 kg) was extracted via maceration with ethyl acetate (2.10 l) for 72 hours with intermittent stirring and shaking. It was filtered, and the filtrate was concentrated using crucibles in a water bath at a regulated temperature of 45 ^{o}C. The weight of the concentrate was taken, and the percentage yield was calculated.

Phytochemical Screening

Qualitative Phytochemical Tests

Determination of Flavonoids: One gram of the test sample was properly macerated with 20 mL of ethyl acetate for three (3) days. The solution was filtered using Whatman filter paper. 5 mL of the filtrate was

pipetted into a test tube and dilute ammonia (5 mL) was added. The upper layer was collected, and the absorbance read at 490 nm.

Determination of Alkaloids: A gram of the test sample was measured, introduced into a beaker, and macerated with 20 mL of ethanol with 20% H_2SO_4 (1:1) and then filtered. 1 mL of the filtrate was pipetted and 5 mL of 60% H_2SO_4 was added. 60% H_2SO_4 containing 0.5% formaldehyde was added after five minutes, shaken thoroughly, and stood for three hours. The absorbance was read at 565 nm.

Determination of Tannins: A gram of the extract was weighed into a beaker, macerated with distilled water (20 mL) and then filtered. Exactly 5 mL of the filtrate was pipetted into a test tube, 0.3 mL of 0.1 N ferric chloride in 0.1 N HCl and 0.3 mL of 0.0008 M potassium ferricyanide were added. The absorbance was then read at 720 nm by a spectrophotometer.

Determination of Phenols: A gram of the test sample to be analyzed was weighed into a beaker and macerated with 80% ethanol (20 mL) and then filtered. 5 mL of the resulting filtrate was then introduced into a test tube using a pipette as 0.5 mL folin-ciocalteu reagent was also added. 20% sodium carbonate (2 mL) was introduced after thirty minutes and absorbance was measured at 650 nm.

Steroids Determination: This was determined by the method described by Edeoga *et al*., 2005 as summarized herein. About 3 g of the sample was added into 100 mL of distilled water and homogenized. The filtrate was eluted with normal ammonium hydroxide solution at pH of 9. The elute (1 mL) was added into test tube and mixed with 1ml of chloroform. Then, 2mL of acetic anhydride and 2 drops of concentrated H_2SO_4 were added to the mixture. The absorbance's of prepared samples and standard were measured in a spectrophotometer at 420nm.

Determination of Terpenoids: A gram of the test substance was measured and macerated with distilled water (50 mL) and then filtered. The filtered solution (1 mL) was transferred into a test tube using a pipette and 5% phosphomolybdic acid solution (1 mL) was added. 1mL of concentrated H_2SO_4 was gradually introduced and allowed to stand for 30 minutes. Finally, two milliliters of ethanol was introduced and the absorbance was measured at 700 nm.

Glycosides Determination: This was done according to the method of Harborne, 1973. The sample (1 g) was macerated with distilled water (20 mL), 15% lead acetate (2.5 mL)

was added and then filtered. 2.5 mL of chloroform was added, and the mixture shaken vigorously. The lower layer was collected and evaporated to dryness. The residue was dissolved with 3ml of glacial acetic acid. 5% ferric chloride (0.1 mL) and concentrated H_2SO_4 (0.25 mL) was added, shaken, and incubated for 2 hours in the dark. The absorbance was read at 530 nm.

Acute toxicity study

The acute toxicity was determined by the method described by Lorke, 1983 and Ogbeide *et al*., 2018. Twelve (12) mice were randomly divided into four (4) groups of three (3) mice each. Extracts were administered in single dose of 25, 50 and 100 mg/kg orally. The acute toxicological signs were observed for 4 to 24 hours and further observed for 14 days for mortality. Observations were focused on parameters such as piloerection, sensitivity to sound and touch, locomotion, aggressiveness, the appearance of faeces, salivation, urinating, convulsing, coma, and death. Median lethal dose (LD_{50}) was evaluated and classified according to the Globally Harmonized System (GHS) for the classification of chemicals (OECD, 1984). The LD_{50} was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

D0 = Highest dose with no mortality,

D100 = Lowest dose with mortality.

Experimental Animals

Thirty (30) Swiss mice weighing between 25-30 g of either sex were procured from the Animal House of the Department of Biochemistry, Faculty of Life sciences, University of Benin, Benin City, Edo State Nigeria. They were randomly divided into 5 groups (n=5). The animals were acclimatized to laboratory condition for fourteen (14) days before the experiment and were allowed free access to food and water. Animals were fasted overnight with access to water prior to the experiment. Proper handling and use of experimental animals were carried out according to the established protocol of (Idu, 2011).

Experimental design

Rauvolfia vomitoria ethyl acetate root extract was reconstituted in

distilled water to make a stock solution (3.4 mg/mL). It was orally administered at graded doses of 25, 50 and 100 mg/kg of the root extract. Standard drugs (5 mg/kg of Amitriptyline and 2 mg/kg diazepam) were prepared in distilled water and administered to the mice orally using an oro-gastric tube. The following are the various groups for this study.

Group 1: Negative control (Untreated group) given 0.2 mL distilled water.

Group 2: Positive control (Reference drug) administered (5 mg/kg of Amitriptyline and 2 mg/kg diazepam)

Group 3: Administered 25 mg/kg of *Rauvolfia vomitoria* root extract

Group 4: Administered 50 mg/kg of *Rauvolfia vomitoria* root extract

Group 5: Administered 100 mg/kg of *Rauvolfia vomitoria* root extract

Determination of Antidepressant Activity

Forced Swim Test

For the determination of antidepressant activity, the forced swim test (FST) was employed (Porsolt *et al*, 1977). During the test, animals were individually 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. All animals were forced to swim for 5 minutes and the duration of immobility was observed and measured during the 5 minutes interval of the test. The immobility period was regarded as the time spent by the rats floating in water with no struggle and making only those movements necessary to keep its head above the water. To check the fitness level of each test animal, a pre-test was carried out 24 h before the FST by subjecting each test animal to a session of 15 minutes swimming.

Formula for % inhibition:

$$\%\ inhibition = \frac{control - treated}{\text{control}}\ x\ 100 \quad \ldots\ldots\ldots\ldots\ (2)$$

Tail suspension test

Tail suspension test was performed based on the method earlier prescribed by Steru, 1985. The mice were suspended 58 cm above the floor by means of an adhesive tape, placed approximately 1cm from the tip of the tail. The total duration of immobility was quantified during a test period of 5 minutes. Mice were considered immobile when they were completely motionless.

Formula for % inhibition:

$$\%\ inhibition = \frac{control - treated}{\text{control}}\ x\ 100 \quad \ldots\ldots\ldots\ldots\ldots\ (3)$$

Statistical Analysis of Data Mean ± standard error of mean and bar chart were used for the representation of data. The software package used was Graph pad prism version 7.

Results and Discussion

Phytochemical Composition

The qualitative phytochemical analysis of *Rauvolfia vomitoria* ethyl acetate root extract, revealed the presence of alkaloids, tannins, flavonoids, terpenoids and glycosides (Table 1).

Table 1: **Phytochemical constituents of *Rauvolfia vomitoria* root**

Classes of secondary metabolites	inferences
Alkaloids	+
Tannins	+
Flavonoids	+
Anthracene derivative	-
Saponin glycosides	+
Cardiac glycosides	+
Cyanogenetic glycosides	-
Terpenoids	+

- = absent; + = present

The results of the phytochemical screening (table 1) agrees with existing literature by (Etim *et al*., 2018; Alagbe, 2021) who documented the presence of saponins, alkaloids, flavonoids, terpenoids and tannins in *Rauvolfia vomitoria* roots. Studies have demonstrated that many phytochemicals such as saponins, alkaloids, triterpenoids, and flavonoids possess antidepressant-like effects (Fajemiroye *et al.*, 2016).

Acute Toxicity

Table 2: Acute toxicological effects of *Rauvolfia vomitoria* root extract in mice

	Treatment/ Doses mg/kg			
Parameters	**Distilled water**	***Rauvolfia vomitoria*** **25 mg/kg**	***Rauvolfia vomitoria*** **50 mg/kg**	***Rauvolfia vomitoria*** **100 mg/kg**
Number of mortalities	0	0	0	0
% Mortality	0	0	0	0
Adverse effects	Nil	Nil	Nil	Nil

From the results of the acute toxicity test (Table 2), no mortality was observed at 100 mg/kg (highest dose) after fourteen (14) days of the study. Also, no significant toxic sign such as writhing, hyper-respiration, vomiting, stooling blood, restlessness, jerking, salivation, lacrimation, hemorrhage**,** nausea, diarrheal, motor- movement, dizziness, drowsiness, convulsion, cough or coma was observed. The result is in accordance with existing literature by N'Cho1 *et al*., 2021. Hence, *Rauvolfia vomitoria* root extract is safe in the treatment of depression.

Antidepressant Activity

Forced Swim Test

Table 3: Effects of ethyl acetate extract of *Rauvolfia vomitoria* root extract on force swimming antidepressant test.

Treatment	Doses (mg/kg)	Immobility (Sec.)	% Inhibition
Control (H_2O)	0.2	8760.00 ± 37.80^{a}	0.00
Amitriptyline	5.0	3372.00 ± 64.18^{b}	61.50
Rauvolfia vomitoria	25	6796.00 ± 21.43^{c}	22.42
Rauvolfia vomitoria	50	6367.00 ± 72.19^{c}	27.32
Rauvolfia vomitoria	100	6146.00 ± 11.20^{c}	29.84

Values were expressed as Mean ± SEM; values with same alphabetical superscript are non-significantly different across the column ($p<0.05$).

The Forced Swim Test was developed by (Porsolt *et al*, 1977). FST is used to monitor depressive-like behavior and is based on the assumption that immobility reflects a measure of behavioural despair indicating depression (Liao et al., 2013). The percentage inhibitory effect of the force swimming test showed an indication that the reference drug (Amitriptyline) had a significant inhibition of 61.5% compared with the control (water), which had 0% inhibitory effect. Similar effect was obtained for 50 and 100 mg/kg of *Rauvolfia vomitoria* root extract which had a significant inhibition when compared with negative control (0%) as shown in Table 3.

The result of the Force Swim Test illustrated that ethyl acetate extracts of *R. vomitoria* showed good antidepressant activity by decreasing the immobility time in rats especially at 50 and 100 mg/kg. The result agrees with existing literature by Asoro *et al*., 2020. *R. vomitoria* antidepressant like activity may be dose dependent as stated by Asoro *et al*., 2020.

TAIL SUSPENSION TEST

Table 4: Effects of ethyl acetate extract of *Rauvolfia vomitoria* root on Tail suspension antidepressant test.

Treatment	Doses (mg/kg)	Immobility (Sec.)	% Inhibition
Control (H_2O)	0.2	7160.00 ± 32.62 [a]	0.00
Amitriptyline	5.0	1942.67 ± 31.26[c]	72.87
Rauvolfiavomitoria	25	2586.00 ± 57.05[c]	63.88
Rauvolfiavomitoria	50	3599.67 ± 11.20[c]	49.73
Rauvolfiavomitoria	100	4419.67 ± 91.05[b]	38.27

Values *were expressed as Mean* ± SEM; values with same alphabetical superscript are non-significantly different across the column (*p<0.05).*

Table 4 showed the result of the Tail suspension test. The result indicated that the reference drug (Amitriptyline) produced a significant decrease in the level of immobility compared to the control (water), which showed a significant increase in immobility. Similar results were obtained with the test groups across the doses (25, 50 and 100 mg/kg) of *Rauvolfia vomitoria* root extract when compared with the untreated control. The total amount of immobility time (defined as the time during which the animal is hanging passively and motionless) was measured for each animal and considered as an index of "depression-like" behaviour (Steru *et al*., 1985). The percentage inhibitory effect of the tail suspension test showed that the reference drug (Amitriptyline) had a significant increase of 72.87% when compared with the control (water), with no inhibitory (0%) effect on depression. Similar effect was obtained across the doses 25 mg/kg, 50 mg/kg, 100 mg/kg of the root extract with inhibition of 63.88%, 49.73% and 38.27% respectively to the positive control when compared with untreated control (0%), as shown in Figure 3.

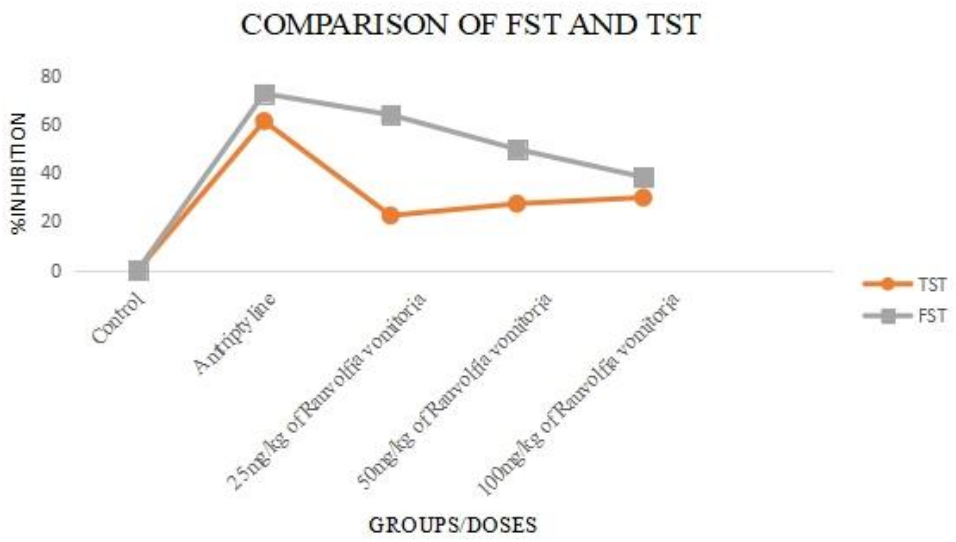

Figure 2: *Comparison of the antidepressant effect of ethyl acetate extract of Rauvolfia vomitoria root on Tail Suspension Test and Forced Swim Test.*

The two tests showed significant difference with respect to the concentration of the root extract given, the root extract had more effect at its lowest dose for the Forced Swim Test, while it had more effect at its highest dose in the Tail Suspension Test, but the effect was still lower than in all doses in the Forced Swim Test.

CONCLUSION

The results obtained from this research indicated that ethyl acetate extract of *Rauvolfia vomitoria* root is rich in phytochemicals (saponins glycosides, alkaloids, flavonoids, terpenoids, tannins, and Cardiac glycosides). Hence, it is used in treating many diseases in traditional medicine by herbalists. The absence of mortality also showed that the plant extract is not toxic and is good enough to be used as an anti-depressant. The present study suggests that the ethyl acetate extract of *Rauvolfia vomitoria* has the ability to reduce immobility time in the forced swimming test as well as in the tail suspension test. Although, the root extract has more effect in its lowest dose for the Forced Swim Test, while it has more effect in its highest dose in the Tail Suspension Test but still lower than all doses in the Forced Swim Test.

REFERENCES

Alagbe J. O. (2021). Dietary Supplementation of Rauvolfia Vomitoria Root Extract as A Phytogenic Feed Additive in Growing Rabbit Diets: Growth Performance and Caecal Microbial Population. *Con Dai & Vet Sci* 4(2).

Amole, O. O., and Onabanjo, A. O. (1999). The effect and uses of *Rauvolfia vomitoria*. *J. Chemother.* 3: 45-47.

Anderson, L. (2013). Can Prescription drugs cause weight gain*? PharmD. New Delhi.*

Andrews, G., Cuijpers, P., Craske, M. G., 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*.

Asoro, I. I., Ebuehi, A. T, and Igwo-Ezikpe, M. N. (2020). Role of Monoaminergic Neurotransmission on the Antidepressant Effect of Leaf and Root Extracts of *Rauwolfia vomitoria* on the Locomotion and Immobility of Sprague-Dawley Rats. *EJMP, 31(14): 45-55.*

Borsini, F, and Meli, A. (1988). Is the forced swimming test a suitable model for revealing antidepressant activity. Psychopharmacology. **94**:147–160.

Burkill, H. M. (1994). The Useful Plants of West Tropical Africa. Volume 2, Families EI, Royal Botanic Gardens.

CMAJ . (1926).On the importance of body weight. *Can Med Assoc J*. 16(1):64-6.

Cowan, M. M. (1999). Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews, 12: 564-582

Edeoga, H. O., Okwu, D. E., Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants, *Afr. J. Biotechnol*. 4(7): 685-688.

Edward, D. S, and Vaitheeswaran, M. (2014). FT-IR and Gc-Ms Determination of Bioactive Constituents of Aloe Barbadensis Miller (Liliacae).*World Journal of Pharmacy and Pharmaceutical Sciences. 3(5): 749– 758.*

Etim, E. I., Johnson1, E. C., Bassey, U. S., and Nwafor, P. A. (2018). Phytochemical and

aphrodisiac studies of ethanol root extract of *Rauwolfia vomitoria* Afzel (Apocynaceae) *J. Pharmacy & Bioresources. 15(2): 160-165.*

Fajemiroye, J. O., DaSilva, D. M., DeOliveira, D. R., Costa, I. A. (2016). Treatment of anxiety and depression: medicinal plants in retrospect, *Fundam. Clin. Pharmacol. 30 198–215.*

Gidal, B. E. (2003). Issues in neurotherapeutics: drug treatment and changes in body weight. *Adv Study Med*. 3 (6A): S489-93

Harborne, I. B. (1973). Phytochemical methods: A guide to modern techniques of plant analysis. 2nd Edition, *Chapman and Hall, New York*. 88-185.

IDU. (2011). perspectives on the design and operation of North America's first medically supervised injection facility. 46(5):561-8.

Iwu, M.M, and Court, W.E. (1982). Stem bark alkaloids of *Rauwolfia vomitoria*. 45(2): 105-111

JuttaBergler-Klein, Ursula Klaar, Maria Heger, Raphael Rosenhek, Gerald Mundigler, Harald Gabriel, Thomas Binder, Richard Pacher, Gerald Maurer, Helmut Baumgartner (2004). 109(19):2302-2308

Lorke. Determination of Acute Toxicity. Arch. Toxicol. 1983; 53; 275.

Liao, J., Tsai, J., Liu, C., Huang, H., Wu, L., Peng, W. (2013). Antidepressant-like activity of turmerone in behavioral despair tests in mice. *BMC Complement Altern Med.;13:299.*

N'Cho1, R. p., Koné, M., Gbogbo, M., Oussou, N. J., and Yapo, A. P. (2021). Chronic Toxicity Study of Aqueous Roots Extract of *Rauvolfia vomitoria* AFZEL on Haematological Parameters in Wistar Rats ***Int.J.Curr.Microbiol.App.Sci. 10*(09): 567-577.**

Nelson, M.L., Busboom, J. R., Cronrath, J. D., Falen, L., and Blankenbaker, A. (2000). Effects of graded levels of potato by-products in barley and corn-based beef feedlot diets: feedlot performance, carcass traits, meat composition, and appearance. *Journal of*

animal science,78 (7): 1829-1836.

OECD (1984). Guideline for testing of chemicals, Paris Guideline 202: Daphnia sp. Acuteimmobilisation test and Reproduction Test

Ogbeide, O. K., Aghedo, O. N., Uadia, J. O. (2022). Anti-inflammatory and Analgesic Investigations of Methanol Extract of Ganodermalucidum. *Trop J Phytochem Pharm Sci. 1(1):17-22.*

Ogbeide, O. K., Akhigbe, I. U., Unuigbe, C. A., Erharuyi, O., Oseghale, I., Imieje, V., Iheanacho, C., Ikeke, K., Ayeni, B., Irabor, E., Owolabi, B.J., Falodun, A. (2020). Isolation, characterisation and in vivo anti-malarial investigation of pulcherrimin A from Caesalpiniapulcherrimastem bark. *GSC Biological and Pharmaceutical Sciences. 12(02): 056-063.*

Ogbeide, O. K., Dickson, V. O., Jebba, R. D., Owhiroro, D. A., Olaoluwa, M. O, Imieje V. O., Erharuyi, O., Owolabi B. J., Fasinu P., Falodun, A. (2018). Antiplasmodial and acute toxicity studies of fractions and cassane-type diterpenoids from the stem bark of Caesalpiniapulcherrima (L.) Sw. *Trop J Nat Prod Res. 2(4):179-184.*

Oliver-Bever. (1982). Medicinal plants in Tropical West Africa. I. Plants acting on the cardiovascular system; 5(1):1-72.

Peng, W.-H., Lo, K.-L., Lee, Y.-H., Hung, T.-H., & Lin, Y.-C. (2007). *Berberine produces antidepressant-like effects in the forced swim test and in the tail suspension test in mice. Life Sciences, 81(11), 933–938.* doi:10.1016/j.lfs.2007.08.003

Pettit, G. S., Dodge, K. A., & Brown, M. M. (1994). Early family experience, social problem-solving patterns, and children's social competence. Child Development. 59:107-120.

Porsolt R. D., Bertin, A., Jalfre, M. (1977). Behavioral despair in mice: A primary screening test for antidepressants. *Arch IntPharmacodynTher*. 229:327-36.

Pousset J. L, and Poisson, J. (1965). Rauvolfia alkaloids: new alkaloids from the leaves of *Rauvolfiavomitoria*Afz. *Ann.*

Pharm. Fr. 23(12): 733-738.

Pousset, J. L. (1988). Medicinal Plants Used As A Source of Raw Material for the Pharmaceutical Industry, Problems and Prospects in, "Drug Production from Natural Products", Adesina SK (ed.) Proceedings of the Seventh International Symposium, DRPU, OAU, Ife. Nigeria. pp. 37-41.

Principe, P. (1989). The economic significance of plants and their constituents as drugs. In: Wagner, H., Hikino, H. and Farnsworth, N.R., Eds., Economic and Medicinal Plants Research, Vol. 3, Academic Press, Orlando.

Rajput, M. S., Sinha, S., Mathur, V., Agrawal, P. (2011). Herbal antidepressants. *IJPFR, 1(1) :159-69.*

Rang, H. P., Dale M. M., Ritter J. M., Flower R. J. (2008). Pharmacology. London: Churchill LivingstoneElsevier.

Savithramma, N. P., Venkateswarlu, D., Suhrulatha, S. K., Basha, M, And Venkataramanadevi C. H. (2010).Studies of Boswelliaovalifoliolata Bal. andHerny-An endemic and endangered medicinal plant.*The Biosc*, 5: 359-362.

Sofowora, A. (1993). Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa Edition. Spectrum Books Ltd., Nigeria. 150-156

Steru, L., Thierry B. C., Simon, P. (1985). The tail suspension test: a new method for screening anti-depressant in mice. *Psychopharmacol.* 85: 367-70.

Vinoth,S.,RajeshK.P.,GurusaravananP.,Jayabalan,N.(2011).Evaluationofphytochemical,antimicrobialandGC-MSanalysisofextractsofIndigoferatritaL.F. spp.Subulata(Vahlexpoir).*Int .J.Agric.Res*. 6(4):358-367.

Waterman, P. G. (1986). A Phytochemist in the African Rain Forest. *Phytochemistry.* 25(1): 3-17.

WHO (2020). World Health Organization supports scientifically proven traditional medicine.