



**CENTRAL NERVOUS SYSTEM DEPRESSANT ACTIVITY OF  
ETHANOL LEAF EXTRACT OF *Globimetula braunii* (Engler)  
(Loranthaceae) GROWING ON *Terminalia catappa* L. (Combretaceae)**

**Suleiman Danladi<sup>1,2</sup>, Musa Aliyu Muhammad<sup>2</sup> and Abdullahi Hamza Yaro<sup>3</sup>**

1. Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Bayero University, Kano, Nigeria
2. Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria
3. Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Bayero University, Kano, Nigeria
4. Corresponding Author: [sdanladi.phc@buk.edu.ng](mailto:sdanladi.phc@buk.edu.ng) +2348062228858

**ABSTRACT**

*This study was carried out to determine phytochemical constituents, median lethal dose (LD<sub>50</sub>) and central nervous system depressant activity of ethanol leaf extract of *Globimetula braunii* using laboratory animal models. The intraperitoneal median lethal dose (LD<sub>50</sub>) of ethanol leaf extract of *Globimetula braunii* was determined in mice according to the method described by Lorke, 1983. The CNS depressant activity of *Globimetula braunii* leaf extract was determined using diazepam induced sleep, hole board test and beam walk assay. The ethanol leaf extract of *Globimetula braunii* revealed the presence of tannins, saponins, steroids, triterpenes, flavonoids and glycoside. The intraperitoneal median lethal dose of the leaf extract of *Globimetula braunii* was estimated to be 2852 mg/kg bodyweight in mice. The ethanol leaf extract of *Globimetula braunii* significantly ( $p < 0.05$ ) prolonged the duration of sleep in mice at the dose of 800 mg/kg. The extract prolonged the time to complete the beam walk, and exhibited significant decrease in number of head dips there by indicating a decrease in the exploratory behaviour of the animal. This study suggests that *Globimetula braunii* possessed sedative property.*

**Keywords:** *Beam walk assay, diazepam induced sleep, hole board test, central nervous system, *Globimetula braunii**

**INTRODUCTION**

*Globimetula braunii* (Engler) Van Tiegh belongs to the family Loranthaceae, it is a hemi-parasitic shrub (Mistletoe) that grows on dicotyledonous trees and attaches itself to the host by modified roots (Aliyu *et al.*, 2014). The plant is widely distributed in tropical countries like Malaysia, India (Okpuzor *et al.*, 2009a), Cameroun, Ghana and Nigeria and it was reported to be widely used in herbal medicines (Burkhill, 1985). In south western Nigeria the plant is used as an analgesic and in the treatment of pulmonary diseases (Okpuzor *et al.*, 2009a). Different parts of the plant such as leaves, fruits and flowers are used to treat hypertension; however, the roots are employed for other therapeutic uses such as ulcer and cancer treatment (Burkhill, 1985). Mistletoe is used in the treatment of insomnia, nervousness and epilepsy (Ogunmefun *et al.*, 2013). Scientific investigations have shown that, *Globimetula*

*braunii* has various pharmacological activities; a study carried out by Okpuzor *et al.*, 2009a has shown that, *Globimetula braunii* possessed antihypertensive activity similar to standard antihypertensive drug. Moreover, *Globimetula braunii* exhibited lipid lowering property in rat (Erukainure *et al.*, 2011; Fred-Jaiyesimi, *et al.*, 2009; Okpuzor *et al.*, 2009a). Additionally, it was found to have antibacterial activity against some selected test organisms (Alonge, and Aliyu, 2015). The ethanol leaf extracts of *Globimetula braunii* demonstrated analgesic, anti-inflammatory and laxative effect (Atiku *et al.*, 2015). The aqueous extract of *Globimetula braunii* was found to exhibit oxytocic activity on the uterine smooth muscle in dose dependent manner (Ie, and Zam, 2008). The ethyl acetate fraction of *Globimetula braunii* leaves extract possesses psychoactive compound that may be useful in the management of petit mal epilepsy (Aliyu *et al.*, 2014).

### **Special Conference Edition, November, 2019**

In a study carried out by Fred-Jaiyesimi, *et al.*, 2009, *Globimetula braunii* was found to be safe when administered orally in rats at acute dose. However, the chloroform fraction of *Globimetula braunii* was reported to have an influence on hematologic functions and liver enzyme levels in laboratory rats (Okpuzor, *et al.*, 2009b). This study aimed to investigate central nervous system depressant activity of ethanol leaf extract of *Globimetula braunii*.

### **MATERIALS AND METHODS**

#### **Collection and Identification of Plant Material**

The plant *Globimetula braunii* growing on *Terminalia catappa* was collected in December 2017, from its natural habitat around Aminu Kano Teaching Hospital, Zaria road, Tarauni Local Government Area, Kano State. The plant was presented to the Herbarium Unit of the Department of Botany, Ahmadu Bello University, Zaria for its identification and authentication.

#### **Preparation of Extract**

The leaves of *Globimetula braunii* were washed with water to remove the sand and other foreign matters and then air dried under shade. The dried leaves were grounded into powder with the aid of blender; the powdered sample (2500 g) was extracted by maceration with 20 L 70% v/v ethanol for the period of 72 hrs. The solution was filtered by the use of filter paper (Whatmann No. 1). The filtrate collected was concentrated in rotatory evaporator at 40°C and reduced pressure. The extract was dried at 40°C and kept in a desiccator for further use.

#### **Animals**

The adult Swiss albino mice of either sex (weighing between 18-30g) were used for the determination of LD<sub>50</sub> as well as central nervous system depressant activity evaluation. The mice were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The ethical approval for the experimental procedure was sought from the Ahmadu Bello University Animal Ethics Committee and the mice were maintained in a well-ventilated room in cages with stainless steel wire mesh covers.

#### **Preliminary Qualitative Phytochemical Screening of *Globimetula braunii***

The qualitative evaluation for the presence of phytochemical groups in the extract of *Globimetula braunii* was carried out according to the established procedures described by Evans (2002).

#### **Determination of Acute Toxicity (LD<sub>50</sub>) Profile of ethanol leaf extract of *Globimetula braunii***

The intraperitoneal median lethal dose (LD<sub>50</sub>) was determined in mice according to the method of Lorke, 1983, the study was carried out in two phases; in the first phase, the mice were grouped in to three groups of three mice, each received extract of *Globimetula braunii* at doses of 10, 100 and 1000 mg/kg body weight intraperitoneally and then observed for signs of toxicity including death within 24 hrs. In the second phase, three mice were treated with the extract at doses of 1600, 2900 and 5000 mg/kg body weight. The mice were observed for signs of toxicity and death within 24 hours. The LD<sub>50</sub> value was calculated as the geometric mean of the lowest lethal dose and the highest non-lethal dose.

#### **Determination of depressant activities of extract of *Globimetula braunii*:**

##### **Diazepam Induced sleep in mice:**

Sleep potentiating effect of an extract of *Globimetula braunii* at different doses was determined according to the method described by Rakotonirina *et al.*, (2001). The mice were grouped in to four groups of six animals each, groups 1, 2, and 3 received the extract at doses of 200, 400 and 800 mg/kg body weight intraperitoneally while mice in the fourth group received normal saline 10 ml/kg to serve as negative control. Thirty minutes later, diazepam at a dose of 20 mg/kg was administered intraperitoneally to mice in all the groups to induce sleep. The loss of righting reflex was considered as time of onset of sleep (Miya *et al.*, 1973) and sleeping time was measured as the time between disappearance and recovery of righting reflex (Fujimori and Cobb, 1965).

##### **Hole board test**

The influence of the ethanol leaf extract of *Globimetula braunii* on exploratory activity of mice was determined according to the method described by Files and Wardill (1975). Mice were randomly divided into five groups of six mice each. Mice in group 1, 2 and 3 received the extract at doses of 200, 400 and 800 mg/kg body weight intraperitoneally respectively. The animal in the fourth group received diazepam 0.25 mg/kg intraperitoneally while group 5 received normal saline 10 ml/kg intraperitoneally. Thirty minutes post treatment each mouse was placed at one corner of the board and then allowed to move about and dipped its head into the holes to indicate exploratory behaviour. The number of head dips in 5 minutes was recorded (Wolfman *et al.*, 1994).

### Beam walk assay

Mice were trained to walk from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by a wooden support to a goal box. Each mouse was tried three times, and this was designed in such a way that the mice tested would be aware that there was a goal box that could be reached. A ruler was used so that the mouse will find it easy cross; moreover, it will induce minimum anxiety. The mice that successfully walked along the ruler were grouped into five groups, each group having six mice. The first group received normal saline at the dose of 10 ml/kg i.p. An extract of *Globimetula braunii* at doses of 200, 400 and 800 mg/kg was administered intraperitoneally to the second, third and fourth groups respectively, while the fifth group received diazepam (0.25 mg/kg, i.p.). Thirty minutes post-treatment, each mouse was placed on the beam at one end and allowed to walk to the goal box. Mice that fell were returned to the position they fell from, with a maximum time of 60 seconds allowed on the beam. The time to complete the task; the number of foot slips (one or both fore and hind limbs slipped from the beam) and the number of falls were recorded (Stanley *et al.*, 2005).

### Statistical Analysis

Data was expressed as mean  $\pm$  standard error of mean (Mean  $\pm$  SEM.). The means Difference was analyzed by one-way ANOVA followed by Dunnett post hoc test. The values were considered to be significantly different at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Identification of Plant Material

The plant *Globimetula braunii* was identified and authenticated by Namadi Sunusi, Herbarium Unit of the Department of Botany, Ahmadu Bello University, Zaria by comparing with voucher specimen number 2839.

### Percentage Yield of Extract

The extraction of 2.5 kg of *Globimetula braunii* leaves provided the yield of 180 g (7.2%) of the crude extract. The extract was found to be blue black in color.

### Preliminary Phytochemical Screening

The Preliminary phytochemical screening of ethanol leaf extract of *Globimetula braunii* showed the presence of tannins, saponins, steroids, triterpenes, flavonoids and glycoside. However, alkaloid and anthraquinones were found to be absent (Table 1.0).

Table 1.0: Preliminary phytochemical constituents of ethanol leaf extract of *Globimetula braunii*

Test	Inference
Alkaloids	
• Mayer's Test	-
• Dragendoff's Test	-
Tannins	+
Saponins	+
Steroids	+
Triterpenes	+
Antbraquinones	-
Flavonoids (NaOH Test)	+
Glycoside	+

Key: - = absent, + = present

The presence of these secondary metabolite is in agreement with the finding of Aliyu, *et al.*, (2014) which revealed that, ethanol leaf extract of *Globimetula braunii* (growing on *Piliostigma thonningii*) contains saponins, flavonoids, tannins and steroids.

Moreover, this is in line with the finding of Atiku *et al.*, (2015) which reported that, ethanolic leaf extract of *Globimetula braunii* (growing on *Terminalia catappa*) contained steroids, triterpenes, tannins, saponins, alkaloids and flavonoids. However, this finding is contrary to the finding of Adediwura *et al.*, (2008) which reported the absence of flavonoids in

*Globimetula braunii*. The presence of various classes of phytochemical compounds in this plant gives an indication that the plant might possess important bioactive compound which may be used for the treatment of different diseases.

### Median lethal dose

The intraperitoneal median lethal dose of the ethanol leaf extract of *Globimetula braunii* was estimated to be 2852.09 mg/kg weight in mice. The finding of this is in line with the finding of Fred-Jaiyesimi, *et al.*, (2009) which showed that, *Globimetula braunii* is relatively safe when administered orally in rats at acute dose.

**Special Conference Edition, November, 2019**

However, when administered orally the median lethal dose (LD<sub>50</sub>) of ethanol extract of *Globimetula braunii* growing on *Terminalia catappa* is greater than 5000 mg/kg (Atiku *et al.*, 2015), this suggests that *Globimetula braunii* is safer when administered orally than intraperitoneally.

**Diazepam Induced Sleep**

Diazepam induce sleep test was carried on ethanol leaf extract of *Globimetula braunii* to determine its sedative property. The extract showed significant sedative property in a dose dependent manner. Sedative property demonstrated by *Globimetula braunii* is significantly higher than the normal saline ( $p = 0.039$ ). Dunnett t-test was carried out and it showed that, *Globimetula braunii* at highest dose (800 mg/kg) demonstrated significant ( $p =$

0.039) sedative activity compares to normal saline. Moreover, ethanol leaf extract *Globimetula braunii* decreased the onset in a dose dependent (Figure 1.0). Although the mean onset of sleep is lower when compared with normal saline but there is no significant difference between *Globimetular braunii* and normal saline ( $p = 0.252$ ).

The finding of this study showed that *Globimetula braunii* possesses sleep induced property. The sedation property of *Globimetula braunii* might be due to the presence of steroids as reported in literature by Gyermek *et al.*, (1967) that, some pregnane-type steroids produced some behavioral changes in experimental animals; the effect is higher than the commonly short acting barbiturates in hypnotic potency.

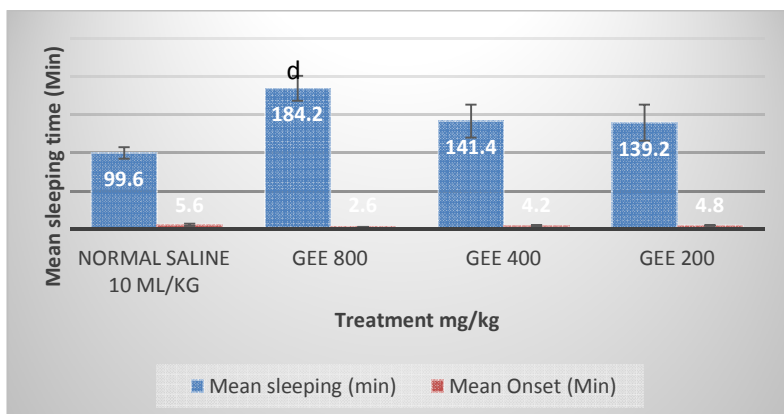


Figure 1.0: Effect of ethanol leaf extract of *Globimetula braunii* on diazepam-induced sleep in mice. Data are expressed as mean ± SEM (n = 6); means were compared by Dunnett test ( $P < 0.05$ ). The mean sleeping /mean onset time with identical alphabets show no significant difference ( $p < 0.05$ ).

**Hole Board Assay**

In the head dip test, the ethanol leaf extract of *Globimetula braunii* decreased the number of head dips which is not dose dependent. This finding shows that Diazepam 0.25 mg/kg, highest dose as well as median dose of *Globimetula braunii* (800 and 400 mg/kg

respectively) significantly ( $p = 0.0001$ ) decreased exploration in mice compared to normal saline (Figure 2.0). Increase in the head dip indicates anxiolytic property where as decrease in the number of head dip is an indication of sedative property.

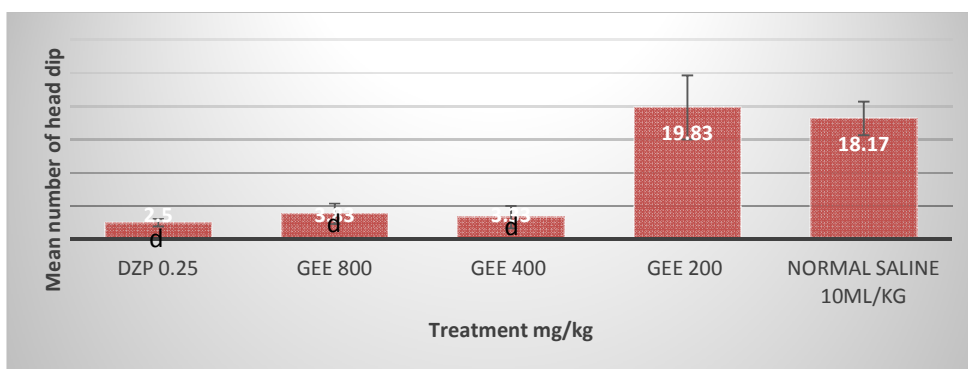


Figure 2.0: Effect of ethanol leaf extract of *Globimetula braunii* on exploratory behaviour in mice. Data are expressed as mean ± SEM (n = 6); means were compared by Dunnett test ( $P < 0.05$ ). The mean number of head dip with identical alphabets show no significant difference ( $p < 0.05$ ).

**Beam Walk**

Ethanol leaf extract of *Globimetula braunii* significantly ( $p = 0.002$ ) and dose dependently delayed the time to reach the goal box compared to normal saline. However, there is no

significant difference between diazepam 0.25mg/kg and different doses of ethanol extract of *Globimetula braunii* (800, 400 and 200 mg/kg) (Figure 3.1)

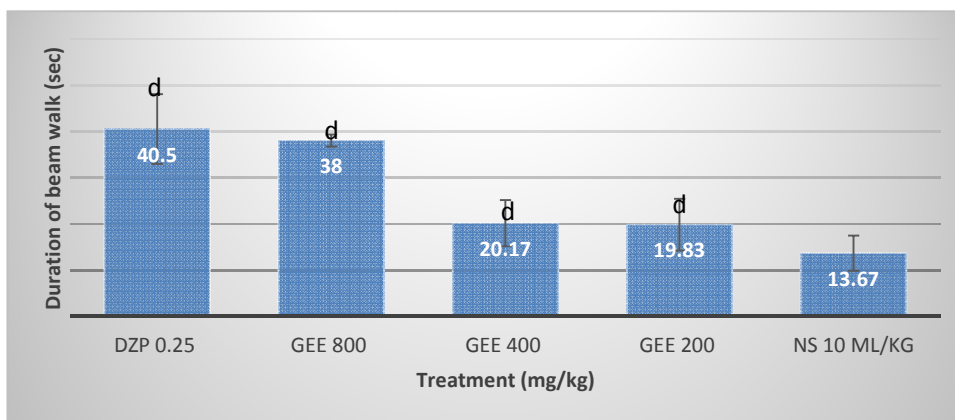


Figure 3.1: Effect of ethanol leaf extract of *Globimetula braunii* on motor coordination in mice. Data are expressed as mean  $\pm$  SEM ( $n = 6$ ); means were compared by Dunnett test ( $P < 0.05$ ). The mean duration of beam walk with identical alphabets show no significant difference ( $p < 0.05$ ).

However, Diazepam (0.25 mg/kg), showed a significant ( $p = 0.0001$ ) increase in the number of foot slips when compared with doses of the extract of *Globimetula braunii* and normal saline. In this study, *Globimetula braunii* does not produced significant increase in the number of

foot slips. The number of foot slips is a good indicator for determination of doses that will produce clinical sedation. There is no significant difference between ethanol leaf extract of *Globimetula braunii* and normal saline (Figure 3.2).

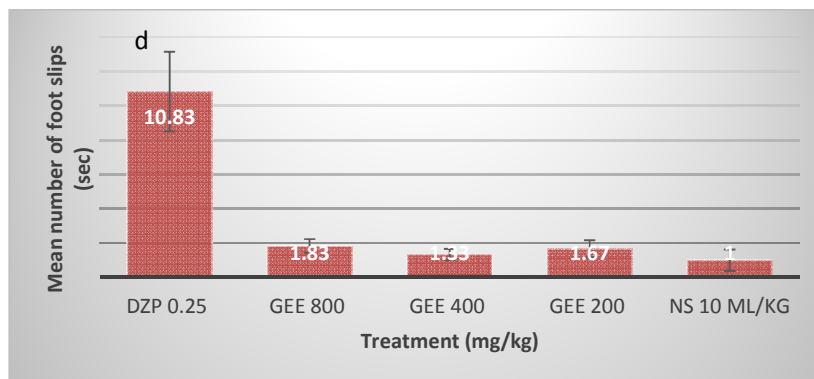


Figure 3.2: Effect of ethanol leaf extract of *Globimetula braunii* on motor coordination in mice. Data are expressed as mean  $\pm$  SEM ( $n = 6$ ); means were compared by Dunnett test ( $P < 0.05$ ). The mean number of foot slips with identical alphabets show no significant difference ( $p < 0.05$ ).

The central nervous system depressant activity exhibited by *Globimetula braunii* may be due the fact that the plant contains some secondary metabolite such as flavonoids, steroids and triterpenes. The finding of this study supported the finding of Meckes *et al.*, (1996), in which Sesquiterpenes isolated from *Psidium guajava* demonstrated central nervous system depressant activity. Additionally, Cardenolides isolated from methanol leaf extract of *Nerium*

*oleander* were reported to possessed central nervous system depressant activity in mice (Siddiqui *et al.*, 1997).

**CONCLUSION**

The ethanol leaf extract of *Globimetula braunii* possessed central nervous system depressant activity and the effect was found to be comparable to standard benzodiazepine (Diazepam).

## RECOMMENDATION

The ethanol leaf extract of *Globimetula braunii* should be fractionated and bioactive compounds responsible for central nervous system depressant activity should be isolated and characterized.

## REFERENCES

- Adediwura, F. J., Temitope, O., & Oluwakemi, A. (2008). Phytochemical and laxative studies of *Globimetula braunii* (Engle) van Tiegh growing on *Cola acuminata* (Schott & Endl). *African Journal of Traditional, Complementary, and Alternative Medicines*, 5(4), 419-420.
- Aliyu, M.M., Musa, A.I.I., Kamal, M.J.A., & Mohammed, M.G. (2014). Phytochemical screening and anticonvulsant studies of ethyl acetate fraction of *Globimetula braunii* on laboratory animals. *Asian Pacific Journal of Tropical Biomedicine*, 4(4), 285-289.
- Alonge, S. O., & Aliyu, R. E. (2015). Antibacterial activity of *Globimetula braunii* sourced from five different host trees in Samaru, Zaria, Nigeria. *Int J Curr Sci*, 18: E117-123.
- Atiku, I., Sule, M. I., Pateh, U. U., Musa, A. M., Ya'u, J., Sani, Y. M., Hanwa U. A., Abdullahi S. M., Adamu S. A., Lawal E. A., & Abdulrahman, H. (2015). Phytochemical, Anti-inflammatory and Analgesic Studies of the Crude Ethanol Leaf Extract of *Globimetula braunii* Van Tieghem (Family: Loranthaceae). *Natural Products Chemistry & Research*, 3(193), 2.
- Burkhill H. M. (1985). Useful plants of West Africa, second edition, volume 1, Families A-D. *Royal Botanic Gardens Kew*, Australia.
- Erukainure, O. L., Abovwe, J. A., Adefegha, A. S., Egwuche, R. U., & Fafunso, M. A. (2011). Antilipemic and hypocholesteremic activities of *Globimetula braunii* in rats. *Experimental and toxicologic pathology*, 63(7), 657-661.
- Evans, W. C. (2002). "Trease and Evans pharmacognosy", 15<sup>th</sup> edition, W.B. Saunders Ltd., London, 191-393.
- Files S., Wardill A.G. (1975) Validity of head-dipping as a measure of exploring a modified hole-board. *Psychopharmacology*, 44:53-9.

## ACKNOWLEDGEMENT

The authors wish to thank Faculty of Pharmaceutical Sciences, Bayero University Kano for providing the facilities used in this research work.

## CONFLICT OF INTEREST

No

- Fred-Jaiyesimi, A., Onabanjo, T., & Jaiyesimi, A. (2009). Biochemical and Toxicological Effects of *Globimetula braunii* (Loranthaceae) Leaf. *African Journal of Traditional, Complementary and Alternative medicines*, 6, 410-411.
- Fujimori H., Cobb D. (1965) Central Nervous System depressant activity of MA 1337, 3-(3,4-M-Chlorophenyl-1-piperazyl prophy) 1-2-4 (1H, 3H) quinozotidione hydrochloride. *Journal of Pharmacology and Experimental Therapeutics*, 148:151-7
- Gyermek L., Genthizr G. & Fleming N. (1967). Some effects of progesterone and related steroids on the central nervous system. *Int. J. Neuropharmacol.* 6; 191-198.
- Ie, O., & Zam, N. (2008). Oxytocic properties of the aqueous extract of *Globimetula braunii* (loranthaceae). *Pak J Pharm Sci*, 21(4), 356-360.
- Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of toxicology*, 54(4), 275-287.
- Meckes, M., Calzada, F., Tortoriello, J., González, J. L., & Martínez, M. (1996). Terpenoids isolated from *Psidium guajava* hexane extract with depressant activity on central nervous system. *Phytotherapy Research*, 10(7), 600-603.
- Miya T.S., Holck H.G.O., Yui G.K.W., Spratto G.R. (1973) Laboratory guide in Pharmacology. Minneapolis MN: Burgess Publishing Company.
- Ogunmefun, O. T., Fasola, T. R., Saba, A. B., & Oridupa, O. A. (2013). The ethnobotanical, phytochemical and mineral analyses of *Phragmanthera incana* (Klotzsch), a species of mistletoe growing on three plant hosts in South-Western Nigeria. *International journal of biomedical science: IJBS*, 9(1), 33.
- Okpuzor, J., Kareem, G., & Ejikeme, C. (2009)a. Lipid Lowering Activity of *Globimetula braunii*. *Research Journal of Medicinal Plant*, 3(2), 45-51.
- Okpuzor, J., Ogbunugafor, H. A., & Kareem, G. K. (2009)b. Hepatic and hematologic effects of fractions of *Globimetula*

**Special Conference Edition, November, 2019**

- braunii* in normal albino rats. *Excli Journal*, 8, 182-9.
- Rakotonirina S.V., Ngo B.E., Rakotonirina A., Bopelet M. (2001) Sedative Properties of the decoction of the rhizome of *Cyperus articulatus*. *Fitoterapia*, 72:91-5.
- Siddiqui SB, Sultana R, Begum S, Zia A. & Suria A. (1997) Cardenolides from the Methanolic Extract of *Nerium oleander* Leaves Possessing Central Nervous System Depressant Activity in Mice. *J. Nat. Prod.* 60; 540-544
- Stanley J.L., Lincoln R.J., Brown T.A., McDonald L.M., Dawson G.R., Reynolds, D.S. (2005). The mouse beam walking assays offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepines. *Journal of Psychopharmacology*, 19(3): 221-7.
- Wolfman, C., Viola, H., Paladini, A.C., Dajas, D., & Medina, J.H. (1994). Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coarctata*. *Pharmacology Biochemistry and Behavior*, 47:1-4