International Journal of Pharmacy and Pharmaceutical Science
www.pharmacyjournal.org
Online ISSN: 2664-7230, Print ISSN: 2664-7222
Received: 02-02-2022, Accepted: 15-02-2022, Published: 04-03-2022
Volume 7, Issue 1, 2022, Page No. 4-10

Toxicity study of saponin extracted from *Clerodendrum volubile* (P. Beauv.) leaves

Apata Joseph Tosin[1*], Ogunbiyi Oluwagbenga John[2, 3], Babalola Olubunmi Olusegun[1]
[1] Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria
[2] Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria
[3] Biology Unit, Faculty of Science, Air Force Institute of Technology, Kaduna, Nigeria

Abstract
Today, in this era, the entire world is facing the severe challenge of providing permanent solutions to cancer. However, currently available anti-cancer drugs are associated with many expensive side effects and are not safe to control and treat cancer clinically. Therefore, due to its effectiveness and safety, plant saponins have received more attention in combating the proliferation of human cancer cells. This study aimed to study the protective and cytotoxic potential of saponin extracted from *Clerodendrum volubile* leaves. Crude saponin is extracted from powdered plant material. The cytotoxic potential of crude saponin on nauplii of brine shrimp was measured at doses of 10, 100, 250, 500, and 1000 µg/ml, and diclofenac was used as a reference drug. The median lethal dose (LD_{50}) for acute toxicity studies was confirmed using standard procedures in white albino rats at concentrations of 10, 100, 1000, 1600, 2900, and 5000 mg/kg body weight. The crude saponins extracted from *C. voluble* leaves showed significant cytotoxicity to brine shrimp nauplii in a dose-dependent manner at all tested doses from 6 hours to 18 hours, with LC_{50} ranging from 743.35 ± 1.42 µg / ml, 414.027 ± 0.012 µg/ml and 103.913 ± 0.019 µg/ml. At 24 h, compared to standard drugs (46.91 ± 0.018 µg / ml), crude saponins showed excellent cytotoxic potential, with an LC_{50} value of 16.073 ± 0.015 µg / ml. In the acute toxicity test with rats, since there was no death, the median lethal dose is estimated to be above 5000 mg/kg of body weight. The saponins extracted from *C. volubile*leaves are non-toxic and can be used safely for the management and treatment of cancer.

Keywords: saponins, *Clerodendrum volubile*, cytotoxicity, anti-cancer drugs

Introduction

Clerodendrum volubile is a succulent herb that is rarely used in south-western Nigeria but it is specifically prominent in the south-south regions. The dried leaves are used as a spice in cooking. It is popularly referred to as Obenetete in Urhobo and Itsekiri tribes, Niger Delta, South-South, Nigeria. It is locally called "Marugbo" or "Eweta" in the South-West region especially among the Ikale, Ilaje, and Apoi people of Ondo State where it is used as a spice to improve aroma and taste (Ogunwa *et al.*, 2015) [22]. It can be found in the *Verbenaceae* family. It has numerous flowers (1.5 cm long), green and white. It is usually found in deciduous forests and secondary forests, spanning the entire region from Senegal to Portugal (Burkhill, 1985). Macroscopic studies have shown that it has a simple leaf with a reticulated rib, an acuminate top, and a covered base. The leaves are green with a golden smell (Fred-Jaiyesimi and Adekoya, 2012) [15]. In ethno pharmacology, the plant (*C. volubile*) leaves are shown to be used to treat arthritis, rheumatism, edema, and swelling (Erukainure *et al*., 2010) [13]. The leaves are also widely used to preserve, protect and maintain pregnancy in early growth and development (Erukainure *et al*., 2010) [13]. It is used as an analgesic, systemic therapy and sedative (Burkhill, 1985). Reports show that the leaf of *the C. volubile* plant has anti-proliferation, anti-diabetic and anti-hypertensive activities (Stephen and Ganiyu, 2016; Erukainure *et al.*, 2018) [27, 11]. Molehin *et al*. (2017) [21] also reported that *C. voluble* has a protective effect on liver toxicity caused by carbon tetrachloride. *C. volubile* (P. Beauv.) is an example of a plant rich in saponin (Erukainure *et al.*, 2011; Akinpelu *et al.*, 2016) [12, 5]. In previous study, *C. volubile* leaf extract reacted positively to the presence of saponins and showed anti-hyperlipidemia and anti-inflammatory potential (Akinpelu *et al*., 2016; Olarenwaju *et al*., 2018) [5, 23]. In addition to this, our previous study had suggested that the hexane fraction of the leaf of *C. volubile* could serve as a source for bioactive principles which might be employed as ingredients for the formulation of novel drugs. Also, the bioactive compounds identified in the leaf of *C. volubile* have been predicted to be responsible for the ethnomedical or traditional use of the plant in the management and treatment of various diseases (Apata *et al*., 2017).

Medicinal plant toxicity had been investigated on different plant genus and species and most of the investigations had been extensively conducted on crude extract using different extraction techniques and the conclusion had been reached that "medicinal plants have little or no toxicity effect" with a dearth of toxicity findings on pure or partially purified extracts or compounds such as phenols, alkaloids, flavonoids, and saponins precipitated, extracted or isolated from medicinal plants like Moringa plants or *C. volubile* plants. Therefore, the

current study was designed to investigate the protective and cytotoxicity potential of crude saponin extracted from *C. volubile* leaves using brine shrimps lethality test (BSLT) and median lethal dose (LD_{50}) of acute toxicity in white albino rats to determine if it is safe and pharmacologically active to be used as an anti-cancer or antitumor drug candidate in the management and treatment of cancer.

Materials and Methods

PlantMaterial

The fresh leaves of *Clerodendrum volubile* were collected from the village of Fashina (latitude 7° 29` 30" north latitude 4° 28` 30" east longitude), "the Ife Central Local Government in Ile-Ife, Osun State". Mr. Ademoriyo from the Department of Botany of Obafemi Awolowo University in Ile-Ife, Osun State, Nigeria, conducted identification and authentication of plant materials at the Ife Herbarium. The samples are stored in the Ife Herbarium, and the sample is assigned number as IFE 17506.

Preparation of the methanol extract

C. *volubile* (P. Beauv.) plant leaves were collected and dried under shade for two weeks. An electric grinder was used to grind the dried leaves into fine powder form. At room temperature, the finely "powdered plant materials (500 g) were suspended in 3 L of 98.99% (v/v) methanol for 72 h". Whatman Number 1 filter paper was used to filter the suspension repeatedly to obtain a clear solution. The filtrates were combined and concentrated on a rotatory evaporator at 40 °C under reduced pressure to obtain a dark green residue. The crude total methanol extract was stored in the desiccator until further processing is required.

Extraction of saponins from methanol extract of *C. volubile* leaves

"Abdel-Gawad *et al.* (1999) [1] and Wagner *et al.* (1984) [28]" procedures were adopted to extract saponin from *C. volubile* leaves as described by Akinpelu *et al.* (2014) [6]. The methanol extract (10 g) was partitioned twice with chloroform (50 ml x 2) and then partitioned twice with ethyl acetate (50 ml x 2). The residue was dissolved in 20% (v/v) ethanol, and the solution was extracted 3 times with butanol (100 ml x 3) and concentrated to dryness. The resulting residue was dissolved in 50% (v/v) methanol (50 ml), and diethyl ether (100 ml) was added to precipitate the saponin. The upper diethyl ether layer was carefully removed while the residue was dissolved in a little amount (10 ml) of methanol and poured into diethyl ether (200 ml). The upper diethyl ether was removed again, the residue was dissolved as described above and precipitated by adding diethyl ether. The precipitate was further purified by repeated dissolution in methanol and precipitation with diethyl ether until a dark brown precipitate called crude saponin was obtained.

Tests for saponins

Evans (2002) [14] and Sofowora (2008) [25] procedures were adopted to test qualitatively for the presence of saponin with slight modification.

Frothing test

Five milliliters of distilled water (5 ml) was used to dissolve 5 mg of crude saponin in a test tube. The mixture was stirred vigorously. After warming up to 15 mins at 50 °C, the honeycomb foam which was produced persistently represented the presence of saponin.

Lieberman-burchard reaction test

The crude saponin (0.2 g) was dissolved in 10 ml of acetic anhydride in a test tube, and concentrated sulfuric acid (2 ml) was carefully added throughone side of the test tube using a Pasteur pipette. The reddish-brown upper liquid layer at the junction of the two liquid layers is blue-green, which is a positive steroid nuclear test.

Experimental animals

The white albino rats procured and used for this study were of different sex and approximately 100 ± 0.2 g in average weight. The rats were acclimatized to environmental conditions for 2 weeks before the experimental procedure. All the rats were fed a standard diet (Ladokun Feeds, Ibadan, Oyo State, Nigeria) in the Department of Biochemistry and Molecular Biology at the Animal House, and drank water *ad libitum*. This study followed the guidelines and procedures of the "Principles of Laboratory Animal Care" (NIH Publication No. 8523) (NIH Publication Revised Edition, 1985).

Acute toxicity study of saponin extracted from *Clerodendrum volubile (P. Beauv.)*

According to the improved method of Lorkes (1983), the acute toxicity of saponin extracted from *C. volubile* was studied in two stages. White albino rats (9) with an average weight of 100 ± 0.2 g were divided into three groups. In the first stage, nine rats were randomly divided into three groups and given a single dose of 10, 100, and 1000 mg/kg of crude saponin. After treatment, the general behavior of the rats was continuously observed for 1 h, and then intermittently observed for 4 h within 24 h for signs of toxicity, death, and death latency. Based on the results obtained from the first phase of the study, the procedure (phase two) was repeated using another group of nine rats, which were randomly divided into three groups and orally administered 1600, 2900, and 5000

mg/kgof body weight respectively. The median lethal dose (LD_{50}) is estimated based on a graph of the percentage of mortality versus the log concentration.

Brine shrimps lethality test (BSLT)

Hatching brine shrimps

McLaughlin *et al.* (1998) [18] brine shrimps hatching method was adopted for this experiment. Artemia cysts (eggs) obtained from the American Aquarium Skartemia Trade Store (American Eagle) were cultured in a rectangular plastic chamber at room temperature in natural seawater for 24 h. The plastic chamber was divided into two unequal compartments (dark and illuminated chamber) with the aid of a perforated divider. Artemia cyst (0.1g) was spread on the surface of seawater in the dark chamber or compartment restricted from the presence of light generated by the electric bulb while the other compartment was exposed to high intensity of light. The incubation period occurred in the dark chamber, and after 24 h, the brine shrimps nauplii (the larva stage of *Artemia salina*) were hatched from their shells. The nauplii were attracted by bright light and migrated from the dark compartment to the illuminated compartment. The light enhanced the visibility, appearance, or identity of the nauplii. Pasteur pipette was used to harvest or pick up the nauplii from the illuminated chamber.

Brine shrimps lethality assay (BSLA)

According to the method of McLaughlin *et al.* (1998) [18], five different concentrations (10, 100, 250, 500, and 1000 µg/ml) of saponin extracted from *C. volubile* leaves were prepared from the stock solution (1 mg/ml) using a serial dilution formula as reported by Jacinta *et al.* (2018) [16] with slight modification. After 24 h of incubation, 10 brine shrimp (nauplii) were removed from the incubator (illuminated chamber) and transferred to a glass petri dish, which contained 10 ml of crude saponin and reference drug (diclofenac) at various concentrations. After treatment, the cytotoxic effect of crude saponins on nauplii was continuously observed for 0 hours (0 h), and then intermittently observed for 6 h within 24 h to determine mortality. The use of magnifying glass was employed to improve the proper visibility and identity of the nauplii in each petri dish. The assay was conducted in triplicate and percentage (%) mortality was determined using the formula showing below:

$$\text{Percentage (\%) Mortality} = \frac{\text{Number of Dead Nauplii}}{\text{Initial Number of Live Nauplii}} \text{ X } 100$$

In the Petri dish, the count and record of the total number of nauplii were taken. The mean percentage mortality rate (%) was plotted against the concentration at which 50% nauplii were killed (LC_{50}). The concentration was determined by a line graph and the regression equation was obtained during extrapolation using a Microsoft Excel computer program. All data were analyzed by one-way analysis of variance (ANOVA) using the statistical software Graph Pad Prism.

Results

The brine shrimps lethality test

The brine shrimps lethality test after 6h

The summary of the percentage mortality and LC_{50} of crude saponin extracted from *C. volubile* leaves within 6 h were shown in Table 1. The crude saponin gave the LC_{50} of 743.35 ± 1.42 µg/ml while the reference drug demonstrated LC_{50} of 1306.97 ± 0.32 µg/ml.

Table 1: Percentage Mortality and LC_{50} of Crude Saponin Extracted from *C. volubile* Leaves after 6 h

Extracted Compound from Plant/Drug	Concentration of Crude Saponin (µg/ml)	Mortality after 6 h (%)	BSLA, n = 3, LC_{50} (µg/ml)
	10	20	
	100	30	
Crude Saponin	250	30	743.35 ± 1.42
	500	40	
	1000	60	
	10	10	
	100	20	
Diclofenac	250	30	1306.95 ± 0.32
	500	30	
	1000	40	

The brine shrimps lethality test after 12h

Table 2 is the summary of the percentage mortality and LC_{50} of crude saponin extracted from *C. volubile* leaves after 12 h. The result showed that crude saponin gave the LC_{50} of 414.027 ± 0.012 µg/ml while the standard drug exhibited LC_{50} of 886.41 ± 0.007 µg/ml within the same hour.

The brine shrimps lethality test after 18h

Table 3 is the summary of the percentage mortality and LC_{50} of crude saponin extracted from *C. volubile* leaves after 18 h. The result showed that crude saponin gave the LC_{50} of 103.913 ± 0.019 µg/ml while Diclofenac exhibited LC_{50} of 117.183 ± 0.012 µg/ml within the same hour.

The brine shrimps lethality test after 24h

Table 4 is the summary of the percentage mortality and LC_{50} of crude saponin extracted from *C. volubile* leaves after 24 h. The result showed that crude saponin produced a better cytotoxicity effect with the LC_{50} of 16.073 ± 0.015 µg/ml than the standard drug with the LC_{50} of 46.907 ± 0.018 µg/ml within the same hour.

Table 2: Percentage Mortality and LC_{50} of Crude Saponin Extracted from *C. volubile* Leaves after 12 h

Extracted Compound from Plant/ Drug	Concentration of Crude Saponin (µg/ml)	Mortality after 12 h (%)	BSLA, n = 3, LC_{50} (µg/ml)
	10	30	
	100	40	
Crude Saponin	250	40	414.027 ± 0.012
	500	50	
	1000	80	
	10	10	
	100	40	
Diclofenac	250	40	886.407 ± 0.007
	500	40	
	1000	50	

Table 3: Percentage Mortality and LC_{50} of Crude Saponin Extracted from *C. volubile* Leaves after 18 h

Extracted Compound from Plant/ Drug	Concentration of Crude Saponin (µg/ml)	Mortality after 18 h (%)	BSLA, n = 3, LC_{50} (µg/ml)
	10	40	
	100	50	
Crude Saponin	250	60	103.913 ± 0.019
	500	80	
	1000	100	
	10	20	
	100	60	
Diclofenac	250	70	117.183 ± 0.012
	500	80	
	1000	90	

Table 4: Percentage Mortality and LC_{50} of Crude Saponin Extracted from *C. volubile* Leaves after 24 h

Extracted Compound from Plant/ Drug	Concentration of Crude Saponin (µg/ml)	Mortality after 24 h (%)	BSLA, n = 3, LC_{50} (µg/ml)
	10	40	
	100	50	
Crude Saponin	250	70	16.073 ± 0.015
	500	100	
	1000	100	
	10	40	
	100	60	
Diclofenac	250	60	46.907 ± 0.018
	500	80	
	1000	100	

Acute Toxicity Test Result

Oral acute toxicity test results of crude saponin extracted from *C. volubile* leaves are summarized in Table 5. The results showed that all the doses given to the rats did not produce any mortality; all the animals were alive. The median lethal dose was therefore estimated to be higher than 5000 mg/kg body weight. This showed that the crude extract was not toxic and it is safe to be used at all doses.

Table 5: The Summary of Acute Toxicity Study of Crude Saponin Extracted from *C. volubile* Leaves

Dose (mg/kg)	Mortality
First Phase	
10	0/3
100	0/3
1000	0/3
Second Phase	
1600	0/3
2600	0/3
5000	0/3

Discussion

Brine shrimp lethality bioassay which is popularly known as cytotoxicity assay is a technique for assessing and screening natural products or indigenous medicinal plants for the presence of phytochemicals or secondary metabolites that are pharmacologically bioactive with the motive to be processed as an active ingredient or drug during drug discovery and development, especially anti-tumor or anti-cancer drugs. Cytotoxicity test results are usually expressed in lethal concentrations (LC_{50} µg/ml). The lower the LC_{50} of a test substance which could be either a crude plant extract or isolated phytochemical compounds, the better their pharmacological activities. This present study investigated the cytotoxicity and median lethal dose of acute toxicity study of crude saponin extracted from *Clerodendrum volubile* using brine shrimps nauplii and albino rats respectively. The results of cytotoxicity activity of crude saponin obtained from *C. volubile* leaves were expressed in LC_{50} (µg/ml) and estimated from 0 h to 24 h. Results showed that there was a marginal decrease in the median lethal concentration (LC_{50}) from 6 h to 24 h after exposure of the Brine shrimps nauplii to crude saponin and the standard drug at various concentrations. The finding of this current study could be corroborated by the work of Abdulwakeel and Abdulwadud (2018) [2] where cell line was used in place of Brine shrimps for the cytotoxicity study. Also, Afolabi *et al*. (2019) [4] investigated the activity of *C. volubile* leaf on cell viability, the result of the study showed a concentration-dependent inhibitory activity on cell growth and viability. This finding was in agreement with the investigation carried out in this present study. Moreover, in this study, the nauplii were subjected to a maximum 24 h time course, and the cytotoxic effect was studied at 6 h intervals commencing from 0 h to 24 h. Results showed that as the time course increases, toxicity also increases with a decrease in LC_{50} value and an increase in mortality rate. This implies that crude saponin extracted from *C. volubile* leaves has a better cytotoxic effect. It could be used as a cytotoxic agent to inhibit the proliferation of cancer cells and cell viability. This observation supported the result obtained by Daniel and Ekam (2016) and Afolabi *et al*. (2019) [4]. Meyer *et al*. (1982) [20] and Clarkson *et al*. (2004) [9] gave a criterion to classify toxicity based on lethal concentration at which extract or drug will cause 50% mortality in microgram per milliliter (µg/ml); LC_{50} value > 1000 µg/ml was regarded as not toxic, LC_{50} ranging from 500 to 1000 µg/ml was classified as low toxic, LC_{50} ranging from 100 to 500 µg/ml was categorized as medium (mild) toxic, while LC_{50} ranging from 0 to 100 µg/ml was categorized as highly toxic. These toxicity criteria were also reported by Mentor *et al.* (2014) [19]. Adekola *et al.* (2020) [3] evaluated the Brine shrimps lethality test of ethanol extract of *Blighia sapida*, the result showed that the median lethal concentration was less than 100 µg/ml (LC_{50}< 100 µg/ml) indicating that the ethanol extract could be categorized in the class of high cytotoxic drug. Sonibare *et al.* (2011) [26] also investigated the varying degree of cytotoxicity or lethality in two species of Blighia plants to provide a clue to their diversity. It was evident from the LC_{50} result obtained that the two plant species could be categorized as mild cytotoxic drugs with LC_{50} in the range of 100 to 500 µg/ml. Also, in the Olufade *et al.* (2018) [24] report, the LC_{50} was in the range of 500 to 1000 µg/ml. This showed that the plant used in this study could be categorized in the class of low or weak cytotoxic drugs. In this present study, the result showed that crude saponin could be classified as "low toxic" at 6 h while at 12 and 18 h, it could be classified as "medium (mild) toxic". At 24 h, the actual time course required for the study, the crude saponins could be classified as highly cytotoxic drugs. Additionally, one of the prominent indices required to assess the cytotoxicity of plant extract and compounds isolated from the plant is mortality. It is usually expressed in percentage (%). In Brine shrimps lethality assay (BSLA) the rate of mortality is usually dependent on the concentration of the extracts/compounds. This determines how effective the plant extracts or compounds obtained from plants are as cytotoxic agents or drugs. In the current study, the result showed no record of mortality at zero hours. On the other hand, there was a marginal increase in the percentage mortality rate in the test medium at 6 h to 24 h with a decrease in the LC_{50} value. This implies that there was a direct relationship of concentration (10, 100, 250, 500, and 1000 µg/ml) used in this study to mortality rate; the higher the concentration the higher the number of the observable death rate of the brine shrimp nauplii in the test medium. The concentration of the crude saponin isolated from *C. volubile* with the highest percentage mortality between 6 h to 24 h was 1000 µg/ml, and the concentration of crude saponin with 100% mortality rate was 500 µg/ml and 1000 µg/ml at 18 h and 24 h respectively (Table 3 and 4).

In previous study on acute toxicity, the results showed no record of mortality when a single dose of ethanol extract of *C. volubile* leaves was orally administered and in addition to this, the sign of toxicity such as loss of appetite and body weaknesses were observed (Akinpelu *et al.,* 2016; Olarewaju *et al.,* 2018). This present study determined the acute toxicity using saponin extracted from the same plant. The results showed that at 1 h oral

single administration of the crude saponin obtained from *C. volubile* leaves, there was no sign of toxicity while at 4 h, a sign of toxicity such as sluggishness, weakness, and dullness was observed. However, the animals returned to their normal life after 6 h and no mortality was recorded. The median lethal dose (LD_{50}) was estimated to be greater than 5000 mg/kg body weight. This showed that crude saponin extracted from *C. volubile* leaves was safe at the tested doses.

Conclusion

It could be concluded from this study that crude saponin extracted from the leaf of *Clerodendrum volubile* (P. Beauv) exhibited a high cytotoxic effect and was safe to be used in the management and treatment of cancer.

Reference

1. Abdel-Gawad MM, El-sayed M, Abdel-Hameed ES. Molluscicidal Steroidal Saponins and Lipid Content of *Agave decipiens. Fitoterapia*,1999:70:371-381.
2. Abdulwakeel AA, Abdulwadud AA. *Clerodendrum volubile* P. Beauv (Lamiaceae), An Underutilized Indigenous Vegetable of Utmost Nutritive and Pharmacological Importance. Beni-Suef University Journal of Basic and Applied Sciences,2018:7(4):606-611.
3. Adekola MB, Areola JO, Oriyomi OV, Apata JT, Apalowo OE, Adesina AF *et al*. Brine Shrimps Lethality Test of Ethanol Extract and Gas Chromatography-mass Spectrometry Analysis of Ethyl Acetate Fraction of Blighiasapida, Asian Journal of Pharmaceutical Clinical Research,2020:13(8):208-212.
4. Afolabi S, Olorundare O, Gyebi G, Syed DN, Mukhtar H, Albrecht R. Cytotoxic Potentials of Clerodendrum Volubile against Prostate Cancer Cells and Its Possible Proteomic Targets. Journal of Clinical Nutrition and Food Science,2019:2(2):046-053.
5. Akinpelu BA, Apata JT, Iwalewa EO, Oyedapo OO. Evaluation of Anti-hyperlipidemic Potential of Ethanolic Leaf Extract of *Clerodendruni volubile P. Beauv*. Ife Journal of Science,2016:18(3):789-800.
6. Akinpelu BA, Igbeneghu OA, Awotunde AI, Iwalewa EO, Oyedapo OO. Antioxidant and Antibacterial Activities of Saponin Fractions of *Erythropheleumsuaveo* Lens (Gull. and Perri.) Stem Bark Extract. Academic Journal,2014:9(18):826-833.
7. Apata JT, Ogunleye SG, Ogunbiyi OJ, Babalola OO. GC-MS Analysis and Phytochemical Screening of n-Hexane Fraction Constituents from the Leaf of *Clerodendrum volubile* P. Beauv. International Journal of BioSciences and Technology, 10(11), 80-88.
8. Burkhill H. The Useful Plants of West Tropical Africa, Entry for *Peristrophebicalyculata* (Retz) Nees [Family Acanthaceae], 1958, 1.
9. Clarkson C, Maharaj VJ, Crouch NR, Grace OM, Pillay P, Matsabisa MG *et al*. *In vitro* Antiplasmodial Activity of Medicinal Plants Native to or Naturalized in South Africa. Journal of Ethnopharmacology,2004:92:177-191.
10. Daniel IE, Ekam US. Preliminary Phytochemical Screening and Toxic Effect of Melantherascandens Leaf Extracts Using Brine Shrimp (*Artemia salina*) Test. Journal of Complementary and Alternative Medical Research, 1(3), 1-7.
11. Erukainure OL, Hafizur RM, Kabir N, Choudhary MI, Atolani O, Banerjee P *et al*. Suppressive Effects of *Clerodendrum volubile* P Beauv. [Labiatae] Methanolic Extract and Its Fractions on Type 2 Diabetes and Its Complications. Frontier Pharmacology, 2018, 9(8).
12. Erukainure OL, Oke OV, Ajiboye AJ, Okafor OY. Nutritional Qualities and Phytochemical Constituents of *Clerodendrum volubile*, a Tropical Non-conventional Vegetable. International Food Research Journal,2011:18(4):1393-1399.
13. Erukainure OL, Oke OV, Owolabi FO, Adenekan SO. Antioxidant Nutrient Properties and Antioxidant Activities of Obenetete (*Clerodendrum volubile*). African Journal of Food Agriculture, Nutrition and Development,2010:10:4156-4167.
14. Evans WC. Saponins, Cardioactive Drug, and other Steroids. In: Trease and Evans Pharmacognosy, 15th Edition, 2002.
15. Fred-Jaiyesimi A, Adekoya Y. Pharmacognostic Studies and Anti-inflammatory Activities of *Clerodendrum volubile* (P Beauv) Leaf. International Journal of Phytomedicine,2012:4:414-418.
16. Jacinta NO, Aruh OA, John AO. Brine Shrimps Lethality and Acute Toxicity Tests of Different Hydro-methanol Extracts of *Anacardiumoccidentale* Using *In vitro* and *In vivo* Models: A Preliminary Study. Clinical Pathology, 2018, 1-5.
17. Lorke DA. A New Approach to Practical Acute Toxicity Testing. Archives of Toxicology,1983:54:275-287.
18. McLaughlin JL, Rogers LL, Anderson JE. The Use of Biological Assays to Evaluate Botanicals. Drug Information Journal,1998:32:513524.
19. Mentor R, Hamidi BJ, Tatjana KP. Toxicological Evaluation of the Plant Products Using Brine Shrimp (*Artemia salina L.*) model. Macedonian Pharmaceutical Bulletin,2014:60(1):9-18.
20. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. PlantaMedica,1982:45:31-34.
21. Molehin OR, Oloyede OI, Idowu KA, Adeyanju AA, Olowoyeye AO, Tubi OI *et al*. White Butterfly (*Clerodendrum volubile*) Leaf Extract Protects against Carbon Tetrachloride-induced Hepatotoxicity in Rats. Biomedicine Pharmacotherapy,2017:69:924-929.

22. Ogunwa TH, Ajiboye SA, Sholanke DR. Nutritional Evaluation of *Clerodendrum volubile* (Marugbo) Leaves. Asian Journal of Plants Science Research,2015:5(11):26-31.
23. Olarenwaju O, Apata JT, Aninpelu BA, Oyemitan AI, Asaolu FT, Ologe MO *et al*. Anti-inflammatory Potentials, Membrane Stabilizing, Xanthine Oxidase Inhibitory Activities of *Clerodendrum volubile* Ethanolic Leaf Extract on Carragenaan-Induced in Rats. Journal of Pharmacology and Toxicology,2018:6(1):7-11.
24. Olufade II, Asimi T, Owonibi KS, Olaoluwa TD, Adegbola VM, Owolabi AS. Lethality Investigation of Toxicity of Extracts from Natural Plants: A Case Study of *Blighiasapida* Leaves. International Conference Journal of Sciences, Engineering and Environmental Technology,2018:3:190-193.
25. Sofowora A. Screening Plants for Bioactive Agents. In: Medicinal Plants and Traditional Medicine in Africa. Third Edition, Spectrum Books Limited, Ibadan, Nigeria, 2008, 181-207.
26. Sonibare MA, Oloyede GK, Adaramola TF. Antioxidant and Cytotoxicity Evaluations of Two Species of Blighia Providing Clues to Species Diversity. Electronic Journal of Environmental Agricultural and Food Chemistry,2011(10):2960-2971.
27. Stephen AA, Ganiyu O. Antioxidant and Inhibitory properties of *Clerodendrum volubile* Leaf Extracts on Key Enzymes Relevant to Non-insulin Dependent Diabetes Mellitus and Hypertension. Journal of Taibah University for Science, 2016, 21-533.
28. Wagner HH, Nickel H. Aynehchi Y. Molluscicidal Saponins from Gundellatournefortii. Phytochemistry,1984:23(11):2505-2508.