



## IMMUNOMODULATORY AND ANTIMICROBIAL ACTIVITIES OF METHANOL EXTRACT OF *Uvaria chamae* LEAVES IN RATS

Enabulele, O.I.<sup>1</sup> and Ifeka, L.J.<sup>1</sup>

<sup>1</sup>Department of Microbiology, Faculty of Life Sciences, University of Benin.

### ABSTRACT

**The immunomodulatory and antimicrobial effects of methanol extract air-dried, powdered leaves of methanol extract of *Uvaria chamae* (MEUL) was evaluated in this study using standard methods. Graded doses (50 mg/kg body weight, 100 mg/kg body weight, 200 mg/kg body weight and 400 mg/kg body weight) of the extract were administered to winster rats. The control group was given dimethyl sulphoxide (DMSO). Immunomodulatory effect was assessed by delayed type hypersensitivity (DTH) and haemagglutination. Antimicrobial activity of the extract against some clinical bacteria isolates was also conducted. The phytochemicals, alkaloids, flavonoids, glycosides, saponins, tannins, carbohydrates, steroids and terpenoids were detected. The extract showed dose related increase in cellular immunomodulatory activity as assessed by inflamed foot pad of the experimental animals with mean diameter ranging from 2.60±0.95 to 4.90±1.04 mm as against the control group which was 1.30±0.14 mm, ( $p<0.05$ ). There was significant increase in haemagglutinating antibody titre against sheep red blood cells which ranged from 272±21.27 to 1344±160.64 as against the control group which was 80±10.63 ( $p<0.01$ ). The methanol extract of *Uvaria chamae* leaf showed inhibitory activity against the test bacteria isolates. This work indicated that methanol extract of *Uvaria chamae* leaf has potential immunostimulatory and antimicrobial activity.**

**Keywords:** Immunomodulatory, Antimicrobial, Phytochemicals, *Uvaria chamae*

### INTRODUCTION

The use of plants for treating ailments has long been in existence (Taiwo, 2005). In most rural communities in Nigeria, the first people to consult for the treatment of ailments are the herbalists, and the aim is to cure diseases with herbal plants. *Uvaria chamae* (finger root or bush banana) is a widely distributed rain forest tropical shrub. Various parts of *Uvariachamae* are used for treatment of various ailments such as common cold and injuries (Oliver, 1986). It is also used for treatment of gastroenteritis, diarrhoea, dysentery (Kone *et al.*, 2015), wound, sore throats, inflamed gum and others (Ogbulie *et al.*, 2007). The antimicrobial properties of *U. chamae* have been properly documented. Ogbulie *et al.* (2007) listed *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* among the microorganism that *U. chamae* can significantly inhibit their growth. It is reported to have anti-cancer properties (Mathias, 1982) and as alternative treatment for diabetes (Olumese and Onoagbe, 2017). The methanol extract of *U. chamae* leaves, taken orally is reported to have sedative, analgesic, antispasmodic, cardioprotective and inflammatory

activity (Edet, 1998). It has been suggested that the n-hexane extract may have immunostimulatory potentials (Olumese *et al.*, 2016). The harmful side effect of most of the orthodox immunotherapeutic agents, rising prevalence of resistance to antimicrobial agents and the very little progress in the development of new and effective antimicrobials has led to an increased interest in research into plants as alternative drugs. The aim of this study is to evaluate the immunomodulatory and antimicrobial effects of the methanol extract of *Uvaria chamae*.

### MATERIALS AND METHODS

#### Sample collection and authentication

*Uvaria chamae* leaf samples were obtained from Ewu Monastery, Esan Central L. G. A of Edo State and identified at Department of Plant Biology and Biotechnology, University of Benin and given Voucher Number UBH<sub>0</sub>353. The leaves were washed, air-dried and ground to powder using an attrition miller (model YL112M-4, No.98090057; Viking Exclusive Joncod, Britain). The powdered leaf of the *U. chamae* plant was then stored in an air-tight container.

### Preparation of concentrations of the methanol extract of *Uvaria chamae* leaf

Seven hundred and twenty grammes (720g) of the powdered plant material was subjected to soxhlet extraction using 1.5 litres of high grade methanol (99.98 %) as extractor. The solvent was removed by evaporation process using rotary evaporator and water bath set at 50 °C. Then the slurry was further dried into a semi-solid extract in an oven set at 50 °C. The percentage yield was calculated using the formula: [(mass of dried extract)/ (mass of powder) ×100% (Gieleri, 2008). The dried extract was stored at 4 °C. Concentration of methanol extract of *U. chamae* leaf was prepared according to the method described by Bhari (2013). Five grams (5 g) of the dried extract was dissolved in 50 ml of DMSO to give a concentration of 100 mg/ml. Two-fold serial dilution preparations (50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml) of the extract were used to determine the minimum inhibitory concentration (MIC) of the extract.

### Qualitative analysis of Phytochemicals of methanol extract of *Uvaria chamae* leaf

#### Alkaloids

Qualitative Phytochemical analysis of the extract was performed according to standard procedures for the detection of Alkaloids, Glycosides, Carbohydrates (Prashant *et al.* (2011); Flavonoids, Saponins, Reducing Sugar, Tannins (Muhammad and Amzad, 2014) and Terpenoids, Resins, Anthraquinone and Anthracene Glycosides (RahmanGul *et al.*, 2017).

### Quantitative analysis of Phytochemicals of methanol extract of *Uvaria chamae* leaf

#### Tannins

One gramme (1 g) of *U. chamae* leaves powder was boiled in 100 ml of distilled water for 1 hour on an electric hot plate, filtered using number 42 Whatman filter paper into a 100 ml volumetric flask and made up to the mark with distilled water. Then 10 ml of the diluted extract was pipetted into 50 ml of distilled water in a conical flask. Commercially available Folin-Denis reagent (5 ml) and 10 cm<sup>3</sup> of saturated Na<sub>2</sub>CO<sub>3</sub> solution added for colour development. The solution was allowed to stand for 30 minutes in a water bath at a temperature of 25°C after thorough agitation. Optical density was measured at 700 nm with the aid of a Spectrum Lab 23A spectrophotometer and compared on a standard tannic acid curve (Ezeonu and Ejikeme, 2016).

Tannic acid (mg/g) =  $(C \times \text{extract volume} \times 100) / (\text{Aliquot volume} \times \text{weight of sample})$

C is concentration of tannic acid read off the graph.

#### Saponins

Ten gramme (10 g) of the powdered *U. chamae* leaf powder was defatted by adding 30 ml of n-hexane. Then 30 ml of methanol was added to the residue and filtered. Then the filtrate was heated to 1/3 of its original concentration. A 10 ml of cold acetone was added and the solution placed in fridge for 50 min. Filter paper was weighed, W<sub>1</sub> and the solution filtered. The residue, with the filter paper, was weighed, W<sub>2</sub> (Ezeonu and Ejikeme, 2016).

Saponins (mg/g) =  $(W_1 - W_2) / W_1$

#### Alkaloids

Ten gramme (10 g) of the powdered *U. chamae* leaf powder was added to 50 ml of 10% acetic acid in ethanol and left to stand for 4 hrs at 28°C. It was filter and concentrated to ¼ of its original volume by evaporation. The filtrate was treated with drop wise addition of concentrated aqueous ammonium hydroxide until alkaloid is precipitated. Weigh a filter paper W<sub>1</sub> was determined. The solution was filtered with a filter paper (12.5 cm), washed with 1% ammonium hydroxide, dried in oven at 80% and weighed again to determine W<sub>2</sub> (Ezeonu and Ejikeme, 2016).

Alkaloid (mg/g) =  $(W_1 - W_2) / W_1$ .

#### Terpenoids

Ten gramme (10 g) of the *U. chamae* leaf extract was soaked in 9 ml of ethanol for 24 hrs. It was filtered and extracted with 10 ml petroleum ether using separating funnel. The extract was separated in five weighted glass vials and heated to a constant weight and weighed again, W<sub>2</sub> (Ezeonu and Ejikeme, 2016).

Terpenoids (mg/g) =  $(W_1 - W_2) / W_1$

#### Flavonoids

Fifty millilitres of 2M HCl (2M, 50 ml) was added to 5g of powdered *U. chamae* leaf sample and boiled for 30 min. Then 5 ml of ethyl acetate was added to 50 ml of *U. chamae* solution in drops until a precipitate was obtained (Ezeonu and Ejikeme, 2016).

Flavonoids (mg/g) =  $(W_1 - W_2) / W_1$

Where W<sub>1</sub> = weight of filter paper and W<sub>2</sub> = weight of filter paper and precipitate

### Experimental Animals

Albino rats (*Rattus norvegicus*) weighing between 158g and 200g were obtained from Anatomy Department, University of Benin, Benin City and allowed to acclimatize to laboratory condition for two weeks at the animal house of Anatomy Department, University of Benin, Benin City. The animals were fed on standard diet and

water *ad libitum* two for weeks before treatment.

### Preparation of Antigen (SRBC)

Sheep whole blood was obtained from Aduwawa slaughtering house with heparin bottles, centrifuged and plasma decanted to obtain sheep red blood cell (Cluskey, 1974).

### Acute Toxicity Test

Methanol extract of *Uvaria chamae* leaf was tested for acute toxicity using method described by Organization for Economic Co-Operation and Development (OECD, 2000), using a concentration of 2000 mg/kg body weight. Five albino rats (*Rattus norvegicus*), two females and three males, were used for the test while two males and two female served as control. The animals were starved of food but not water over night (12 hours) prior to the test. They were weighed just before feeding them orally by gavage with the extract. First, a dose of 2000 mg/kg body weight of the extract was administered to one animal which was observed every 30 min for the first 24 h and then daily for 14 days for any behavioral changes such as diarrhoea, food intake, sleep pattern, convulsion or mortality. A second animal was administered with the same dose of the extract and observed. Another two animals were administered with the same dose of the extract and observed, following the same pattern. The last animal was then administered with the same those of the extract and observed. The control group of four animals received DMSO. The effect of the extract on the weight of the animals was also tested.

### Delayed Type Hypersensitivity (DTH)

The delayed type hypersensitivity (DTH) response was determined using the footpad swelling test as parameter Gupta *et al.* (2010). Briefly, 30 Albino rats (*Rattus norvegicus*), were divided into five groups (6 rats per group). Group one (control group) received DMSO. Groups 2, 3, 4 and 5 were administered 50 mg/kg body weight, 100 mg/kg body weight, 200 mg/kg body weight and 400 mg/kg body weight of the methanol extract of *Uvaria chamae* (MEUL) dissolved in DMSO respectively. The extract was administered for two consecutive days prior to the sensitization day and two consecutive days after. On the sensitization day, 0.1 ml of 1% of the SRBC was subcutaneously injected into the right footpad of the animals. Seven days later, their immune system was challenged by injecting them with 0.2 ml of 1% of the SRBC through the left hind footpad. The footpad thickness was measured with veneer

calipers 24 h after the challenge for delayed type hypersensitivity reaction and the mean thickness reported in mm

### Humoral Immunity by Haemaagglutination Reaction

Albino rats (*Rattus norvegicus*) were divided into five groups. Group one serve as control while groups 2, 3, 4 and 5 formed the experimental groups. Each group consisted of six rats. The control group received DMSO while groups 2, 3, 4 and 5 received 50 mg/kg body weight, 100 mg/kg body weight, 200 mg/kg body weight and 400 mg/kg body weight of the extract dissolved in DMSO respectively. Administration of MEUL was by oral gavage for two consecutive days before and after immunization 0.1ml of 1% SRBC, (antigen). Two millilitres (2 ml) of 1% of SRBC, was given intraperitoneally to all groups. Blood and serum were obtained from the experimental animals after 10 days. Two fold serial dilution of the serum up to 0.02 uL was made in test tubes. Then 0.1ml of 1% SRBC was added to each tube. The tubes were incubated at 37 °C for 2h after which they were observed virtually for haemagglutination and compared with normal saline, the control. Reciprocal of the highest dilution that showed visible haemagglutination was taken as antibody titre (Gupta *et al.*, 2010).

### Susceptibility of the Test Microorganisms to *Uvaria chamae*

The susceptibility of the test microorganisms to *Uvaria chamae* was tested by agar in well diffusion method as described by Obi (2000). A solution of 500 mg/ml of the extract was prepared. Then 0.2 ml of standardized inoculum ( $10^8$ cfu/ml) of the test microorganisms (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhi* and *Klebsiella pneumoniae*) was inoculated into six plates and using pour plate method, 7 mm diameter well were made on the plates. Then three drops of the methanol extract of *Uvariachamae* leaf solution were placed into the well and a pre-diffusion period of 30 min allowed before incubation at 37 °C for 24 h. The isolates were also screened for susceptibility to standard antibiotics using commercially available discs of ciprofloxacin (5µg), amoxicillin (30µg) and gentamicin (10µg), (Abtek Biological Ltd, Liverpool, UK) by the method of National Committee for Clinical Laboratory Standard, NCCLS (2016). Zones of inhibition (Z.I) were measured in mm and standard chart, NCCLS (2016) used to interpret zones of inhibition as susceptible or resistant (Z.I value > 15 mm = susceptible, 12mm-15mm

= moderate susceptible and < 12 mm = resistant. The minimum inhibitory concentration (MIC) of the extract to the isolates was also conducted using broth dilution method described by Obi (2000). Dilutions used were 50 mg/ml, 25 mg/ml, 12.5000 mg/ml, 6.2500 mg/ml, 3.1250 mg/ml, 1.5625 mg/ml, 0.7813 mg/ml, 0.3906 mg/ml and 0.19573 mg/ml using DMSO as diluent. A 0.2 ml ( $10^6$ cfu/ml) of the overnight culture of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhi* and *Klebsiella pneumoniae* was inoculated into corresponding plates, and 20 ml of nutrient agar poured into each plate and mixed thoroughly. The plates were allowed to set and 7 mm wells made on them with a cork borers. Three drops of the different extract concentrations were dispensed into the wells. The tests were performed in triplicates. A pre-diffusion period of 30 min was allowed before incubating the plates at 37 °C for 24 h. The lowest concentration which did not show visible growth was taken as the minimum inhibitory concentration of the extract.

#### Statistical Analysis

Data obtained were analyzed using one-way ANOVA, followed by Duncan multiple comparison test. P value<0.05 and P value<0.01 were considered statistically significant.

#### RESULTS AND DISCUSSION

Qualitative and quantitative analysis of the methanol soluble phytochemical components revealed the presence of alkaloids (10.2 mg/g), saponins (3.1 mg/g), tannins (784 mg/g), terpenoids (0.6 mg/g), flavonoids (7.2 mg/g), carbohydrates (2.21 mg/g) (Tables 1 and 2). Previous studies had reported phytochemical composition of *Uvaria chamae* to include alkaloids, flavonoids, tannins, saponins, glycosides and methanol has been reported to extract most of both polar and non-polar compounds in plant materials such as leaves (Jimoh, 2000; Okwu and Iroabuchi, 2009; Bart and Pilz, 2011). Acute toxicity testing indicated that methanol extract of *Uvaria chamae* leaf was non-toxic to the experimental animals up to the dose of 2000 mg/kg body weight (Table 3). The result is supported by the work of Olumese *et al.* (2016) who had earlier reported that the LD<sub>50</sub> of *U. chamae* methanol extract was greater than 2000 mg/kg body weight. Characteristics such as mortality and morbidity, food and water intake and body weight change (Adewale *et al.* 2015); change in eye colour, tremour, convulsion, diarrhoea, sleep, coma, hair and skin colour (Ghosh and Ramarishna, 2019) have been listed as parameters for measuring toxicity.

These parameters were normal on the test animals when compared to the control group after two weeks of observation. The rats quickly returned to normal position when placed on their back. They fed well. There was no significant weight loss. The nontoxicity of *Uvaria chamae* leaves, as demonstrated in this study, may be the reason for its use in a wide range of purposes ranging from treatment of various ailments to dietary purposes where it is either consumed raw or used as spices. The cellular and humoral immune responses to treatment with MEUL possess some immunostimulatory activity. The footpad thickness ranged from 2.6±0.5 mm in rats administered 50 mg/kg weight through 3.3±0.91 mm for those given 100 mg/kg body weight to 4.9±1.04 mm for those given 400 mg/kg body weight (Table 4). Increased footpad thickness (oedema) has been used to provide a functional in vivo assessment of cell mediated immunity (Jayapal, 2007). It provides a means of assessment of inflammatory skin response to intradermal inoculation of an antigen which is dependent on antigen specific memory T-cells, and the oedema is due to recruitment of mononuclear cells and neutrophils at the site of the infection. Many Arthurs have linked increase in footpad thickness to inflammatory processes. Nfambi *et al.* (2015) reported that induced increased vasodilation increases vascular permeability, producing inflammation that increases the footpad in previously immunized animal. Jayapal (2007) stated that oedema results from Delayed Type Hypersensitivity (DTH) reaction mediated by immune cells at sites of infection. Secondary plant metabolites such as alkaloids, saponins, tannin, terpenoids, flavonoids, carbohydrates, glycosides and steroids have been reported to possess immunomodulatory activities. Garcia *et al.* (2013) reported that glycosides and tannins enhanced proliferation of lymphocytes. Puncture *et al.* (2005) indicated that saponins, flavonoids and alkaloids increased phagocytic index and total white blood-cell count. The presence of these phytochemicals (alkaloids, saponins, flavonoids and tanins) in *U. chamae* leaves, as indicated in this study and supported by other research works (Olumese and Onoagbe 2017; Ogbulie *et al.*, 2007; Donatus and Friday, 2005) may be responsible for the cellular immunostimulatory activities of MEUL observed in this paper. The extract showed statistically significant increase in antibody titre from dose levels of 100 mg/kg body to dose level of 400 mg/kg body weight, indicating that *U. chamae* leaves possess humoral immunostimulatory activities. The result is in line with the report of Olumese *et al.* (2016) that *Uvaria chamae*

extracts may have immunostimulatory properties. Indeed *Uvaria chamae* leaves contain many phytochemicals that have been linked to factors that enhance antibody proliferation. Terpenoids enhance interleukins and total antibody production (Venkatalashmi, 2016). Glycosides enhance secretion of interferon gamma (Punture *et al.*, 2005) which enhances B cell proliferation and class switching (Jayapal, 2007).

Furthermore, Brindha (2016) and Garcia *et al.* (2013) reported that the phytochemicals, terpenoids, alkaloids, glycosides, flavonoids and tannins had potential immunostimulatory activity. The immunostimulatory potential of MEUL, as indicated by this study, could be

exploited to boost immune system for disease prevention and treatment of ailments involving immunodeficiency. The methanol extract of *Uvaria chamae* leaf also inhibited the growth of some of clinical bacterial isolates (table 6). The result is in agreement with the works of Ogbulie *et al.* (2007) and Okwu and Iloabuchi (2009). Phytochemical such as alkaloids, saponins, flavonoids and tanins have been reported to possess antimicrobial activities (Monte *et al.* 2014). The antimicrobial activities of these chemical groups, alkaloids, flavonoids, tannins, saponins and glycosides present in *Uvaria chamae* may be responsible for the high antimicrobial activity of the methanol extract of *Uvaria chamae* leaf as recorded in this study.

**Table 1: Extract Yield.**

Mass of powder of <i>U. chamae</i> (g)	Dried extract (g)	Yield (%)
720	48.60	6.8

**Table 2: Quantitative Analysis of Methanol extract of *Uvaria chamae* leaves**

Phytochemicals	Tannins	Saponins	Alkaloids	Terpenoids	Flavonoids
Quantity (mg/g)	784	3.1	10.2	0.60	7.2

**Table 3: The Effect of Methanol extract of *Uvaria chamae* leaves on the Weight of Albino Rats at Dose Levels 2000 mg/kg body weight.**

Group	Treatment	Body weight (g)	
		Before treatment	14 days after treatment
Control	DMSO	183.29±23.13	161.24±31.00 <sup>a</sup>
Test group	2000 mg/kg of the extract	177.67±10.12	157.43±1.10 <sup>a</sup>

**Table 4: Effect of methanol extract of *U. chamae* leaves on Cellular and humoral Immunity response (DTH)**

Dosage of MEUL (mg/kg wt)	Food pad thickness (mm) (Mean±SEM)	Haemagglutinating antibody titre (Mean±SEM)
Control	1.3±0.14	80±10.63
50	2.6±0.95	272±21.27
100	3.3±0.91*	448±67*
200	4.1±1.59*	832±94.21**
400	4.9±1.04*	1344±160.64**

Values with single superscript has significance difference at p<0.5

Values with double superscript has significance difference at p<0.01

**Table 5: Minimum Inhibitory Concentration of Methanol Extract of *Uvaria chamae* Leaf on Bacterial Isolates measured by zone of inhibition (mm)**

Test organism	MIC (mg/ml)	ZI (mm)	Control (DMSO)
<i>E. coli</i>	9±0.37	9±0.37	0.00
<i>S. aureus</i>	9±0.33	9±0.33	0.00
<i>P. aeruginosa</i>	14±0.82	10±0.82	0.00
<i>B. subtilis</i>	8±0.84	8±0.81	0.00

The values are means of the measurements of the different zones of inhibition (ZI) on triplicate culture at the same concentration and standard deviation (mean±SEM)

**CONCLUSION**

The methanol extract of *Uvaria chamae* leaf showed immunostimulatory activity in Albino rats. The extract also showed some antimicrobial activity against some clinical bacteria isolates. It

is therefore recommended that further research work be directed towards isolating the pharmaceutically active components present in the leaf of *Uvaria chamae* with a view to formulating them into therapeutic agents.

## REFERENCES

- Bart, H.J. and Pilz, S. (2011). *Industrial Scale Natural Products Extraction*. Wiley-VCH Verlage & Co., Germany. 56pp.
- Brindha, P. (2016). Role of phytochemicals as immunostimulatory agents: A Review. *International Journal of Green Pharmacy* 10(1): 122 - 128.
- Cluskey, R. T. (1974). *The Inflammatory Process*, (3<sup>rd</sup> Edition) Academic Press, New York. 85p.
- Edet, M. U. (1998). *Nigerian Medical plants for Immunomodulation*, (1<sup>st</sup> Edition). Hebron Press, Florida, USA. 301pp.
- Garcia, M.P., Lack, G.R., Renz, H.Y., Saloga, L.J., Bradley, K. and Loader, J.T. (2013). Lellingng DYM: Effect of Interferon on IgE production. *Journal of Clinical Investigation* 77(152): 2546-2554.
- Gieleri, I. (2008). Practical Manual on the Industrial Utilization of Medical and Aromatic Plants, 1<sup>st</sup> edition. University of Bucharest Press, Boston. 201pp.
- Gupta, A., Guatam, M.K., Singh, R.K., Kumar, M.V., Rao, C.V. and Anupuba, R.K. (2010). Immunomodulatory Effect of *Moringa oleifera* Lam on Cyclophosphamide Induced Toxicity in Mice. *Journal of Experimental Biology* 13 (48): 1157-1160.
- Jimoh, A. A. (2000). *The Useful Plants of West Tropical African*, (3rd Edition) CRC Press, Boca Eaton, USA. 210pp.
- Kone, M., Towe, A., Quattwa, K. and Adama, C. (2015). Phytochemical compopsition, antioxidant and antibacterial activities of of *Uvaria chamae* P. Beauv., (Annonaceae) used in treatment of dyscentry in North Cote d'Ivoire. *International Journal of Phrmacognosy and Phytochemical Research* 7(1): 1047-1053
- Monte, N.T., Nathan, C.F. and Hibbs, J.B. Jr. (2014). Role of phytochemicals in macrophage antimicrobial activity. *Current Opinions in Immunology* 90: 339-346.
- Mathias, E.A. (1982). The State of Plant Research in Nigeria. *Society of Biologica* 59(2): 371-377.
- Ogbulie, J.N., Ogueke, C.C. and Nwnebu, F.C. (2007). Antibacterial properties of *U. chamae*, *Gongronema latifolium*, *Garcinia kola*, *Vemonia amygdaline* and *Aframomium melagueta*. *Journal of Biotechnology* 6(13): 1549-1553.
- Okwu, D.E. and Iroabuchi, F. (2009). Phytochemical composition and biological activities of *Uvaria chamae* and *Clerodendoron splendens*. *E Journal of Chemistry* 6(2): 553-560.
- Oliver, B.B. (1986). *Medicinal Plants in Tropical West Africa*. Cambridge University Press, 125pp.
- Olumese, F.E., Onoagbe, I.O., Ene, G.I. and Omoruyi, F.O. (2016). Safety assessment of *Uvaria chamae* root extract: acute and subchronic toxicity studies. *Journal of African Association of Physiological Science* 4(1): 53-60.
- Olumese, F.E. and Onoagbe I. O. (2017). Proximate analysis and phytochemical composition of *Uvaria chamae* root. *NISB Journal of African Association of Physiological Science* 17(1): 76-81
- Taiwo, W.R. (2005). *Alternative to orthodox medicine*. 1<sup>st</sup> edition, Boston M.C. Corporation, 357p.