https://doi.org/10.4314/jpb.v17i1.1
Vol. 17 no. 1, pp. 1-7 (March 2020)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Anti-inflammatory and analgesic activities of *Clerodendrum capitatum* (Willd) Schum. & Thonn. (Verbenaceae) leaves

Abdulrahman ADAMU[1*], Abdulmumin Z. ABUBAKAR[1], Adamu A. AMBI[1], Ibrahim M. ALIYU[2], Hamza MOHAMMED[3], Najma ILYAS[1] and Mujtaba S. ABUBAKAR[1]

[1]Department of Pharmacognosy and Drug Development; [2]Department of Pharmacology and Therapeutics; [3]Department of Geography Ahmadu Bello University, Zaria. Nigeria.

Received 9th June 2019; Accepted 26th November 2019

Abstract

Clerodendrum capitatum (Willd) Schumach and Thonn. (Verbenaceae) is a perennial herb, which grows up to 0.5 m to 2 m high. It is widespread over North-East, East, and South Central Africa. It is known as "bambaro or maashayi" by the Hausas of Northern Nigeria. The leaves are used traditionally to treat intercostal pain, headaches and toothaches. The aim of this study is to evaluate the anti-inflammatory and analgesic activities of the aqueous ethanol extract and fractions of *C. capitatum*. The anti-inflammatory activity was investigated using carrageenan induced rat paw oedema model, while acetic acid induced mice writhing model was used to evaluate the anti-nociceptive property. The oral median lethal dose values were found to be greater than 5,000 mg/kg for the aqueous ethanol extract, ethyl acetate and n-butanol fractions, while that of dichloromethane fraction was less than 2000 mg/kg using OECD method. The acetic acid induced writhing was significantly (P<0.05-0.001) reduced particularly for the EF and NF fractions at 200 mg/kg (93.12% and 98.79%). DF showed percentage inhibition of 87.59% and 81.75% at 50 and 100 mg/kg respectively. The extracts and fractions also caused a significant (P<0.05-0.001) dose dependent reduction of inflammation induced by carrageenan when compared with the negative control with the maximum inhibitory effect of 43.69% observed in Ethyl acetate fraction at 50 mg/kg dose at the 3rd hour. It can be concluded that the leaf extracts of *C. capitatum* possess anti-inflammatory and analgesic effects in animal models, which may be mediated through the phytochemical constituents present in the extract and fractions of the plant.

Keywords: *Clerodendrum capitatum;* Anti-inflammatory; Analgesic; *OECD*

INTRODUCTION

Clerodendrum capitatum (Willd) Schumach & Thonn. (Verbenaceae) is a perennial herb, which grows up to 0.5 m to 2 m high, its stems are quadrangular in transverse section and it is covered with soft hair. It is widespread over North-East, East, and South Central Africa [1]. It is known as "bambaro or maashayi" by the Hausas of Northern Nigeria [2]. It is an indigenous tropical African plant. The leaves are used traditionally to treat various ailments such as intercostal pain, headaches and toothaches. In Nigeria, this plant is used to treat diabetes mellitus, obesity, and hypertension [3] and possess various pharmacological activities like antidiarrheal, anti-fungal, immuno-modulatory, anthelmintic, anti-inflammatory, anti-hepatotoxic [4] and antivenom activities [5]. Phytochemical results revealed the

*Correspondence. *E-mail*: ababdulrahman@abu.edu.ng *Tel*: +234-8065539723. ISSN 0189-8442

presence of saponins, flavonoids, alkaloids, tannin, glycosides and reducing sugars in the extract [1,5].

Pain, is as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [6]. It is a fundamental problem in communities around the globe. Its incidence and prevalence have exacted clinical, social, and economic burden in children, adolescents, and adults. Assessments have suggested that 20% of adults suffer from pain worldwide and that 10% are newly diagnosed with chronic pain annually [7]. Pain and inflammation act as a warning of external or internal noxious stimuli to the body. However, they are viewed as sources of discomfort and are commonly suppressed with analgesics and anti-inflammatory medications respectively [8]. The mediators and regulators like leukotrienes, peptides, cytokines, growth factors, prostaglandins are all involved in overbearing of these events. These mediators and cells depends on the cause (chemical or physical injury, pathogen type, auto-immune), stage or type (acute or chronic) of inflammatory process. Many degenerative diseases such as rheumatoid arthritis, shoulder tendonitis, Alzheimer's disease, inflammatory bowel disease, heart disease, gouty arthritis and asthma are often associated with the inflammatory process [7]. The aim of the study is to evaluate the anti-inflammatory and analgesic activities of the leaf extract and fractions of *Clerodendrum capitatum*.

EXPERIMENTAL

Drugs and chemicals used include diclofenac and piroxicam (Pfizer), acetic acid (BDH), carrageenan (Sigma Aldrich, Germany).

Plant collection and identification. The leaves of *Clerodendrum capitatum* (Willd) Schumach & Thonn. were collected in Samaru Zaria, Kaduna State, Nigeria. The plant material was identified and authenticated by Namadi Sunusi of the Department of Biological Sciences, Ahmadu Bello University, Zaria. The plant material was compared with an existing herbarium specimen and a reference specimen (voucher number 301). The leaves were cleaned, air dried and ground to powder using a Tigmax grinding machine TX160 then stored at room temperature in the laboratory until when needed.

Preparation of extract and fractions. The powdered leaves of *C. capitatum* (1 kg) was defatted with 2.5 L of n-hexane. The marc was air-dried and macerated with 2.5 L of 80% v/v aqueous ethanol for three days, after which it was filtered. The filtrate was collected and concentrated *in vacuo* and coded (EE80). The aqueous ethanol extract (EE80) portion (100 g) was suspended in water (400 ml). It was subsequently filtered to remove undissolved particles. This mixture (solution) was transferred into a 1 L separating funnels and then successively partitioned with (3x500 ml) dichloromethane, (5x500 ml) ethyl acetate, (3x500 ml) n-butanol. The ethyl acetate and the n-butanol partitioned fractions were collected and concentrated at room temperature and using rotary evaporator and coded EF and NF respectively. The dichloromethane fraction (DF) was concentrated at room temperature likewise and kept in a desiccator at room temperature for further analysis (modified method [9]).

Experimental animals. Swiss albino mice (17-25 g) and Wistar rats (150-200 g) were housed in clean plastic cages and maintained under standard laboratory conditions (Room temperature and normal light/dark cycles). They were fed with commercial food (Vital Feed PLC, Zaria, Kaduna, Nigeria) and water *ad libitum*. The procedures adopted in this study were in accordance with Guidelines for Care and Use of Laboratory animals approved by the Ahmadu Bello University committee on Animal use and care (ABUCAUC/2019/).

Acute toxicity studies in rats. Healthy Wistar rats were used in this study according to the Organization for Economic Cooperation and Development (OECD) revised up-and-down procedure for acute oral toxicity testing [10]. The extract (EE80), ethyl acetate fraction (EF) and n- butanol fraction (NF) of *C. capitatum* were dissolved in sterilized distilled water and dichloromethane fraction (DF) in Tween 80 in a volume of 10 mL/kg body weight of the experimental animal and administered orally. All the animals were fasted overnight, but with free access to water and they were weighed before the sample administration. Animals were randomly divided in two groups (one for the control group and the second for the acute toxicity group for each extract and fractions; n = 5 per group). The control group was given distilled water and Tween 80 orally while the acute toxicity group was treated as follows: a dose limit of 5 g/kg of EE80, DF, EF, and NF were given to the first animals and were monitored for mortality, signs of acute toxicity and behavioural changes (restlessness, unusual vocalization, somnolence and sedation, tremor, twitch, ataxia, catatonia, paralysis, convulsion, unusual aggressiveness) for the first thirty minutes and the first hour, then hourly for 5 h and, finally periodically until 24 h. Following the survival of the first animal, then two additional animals each were given the same 5 g/kg dose sequentially at 24 h intervals. All the experimental animals were individually observed daily for general behavioural and body weight changes, hazardous symptoms and mortality for a period of 14 days' post treatment. The LD_{50} was predicted to be above 5 g/kg if two or more rats survived.

Evaluation of analgesic activity. The method of acetic acid-induced writhing in mice, described by Koster et al. [11] was employed to evaluate 80% aqueous ethanol extract and fractions (DF, EF and NF) of *C. capitatum*. The mice were divided into 5 groups of 6 mice each. Group I (Negative control) were administered distilled water (10 ml/kg) and 1% Tween 80, while groups II, III and IV (treatment) received graded doses of the extract and fractions (200, 100 and 50 mg/kg respectively) and group V received piroxicam (20 mg/kg), all per oral (p.o.) route. Sixty minutes post treatment, all the animals received 0.6% v/v acetic acid intraperitoneal (10 ml/kg body weight). Five minutes after acetic acid injection, the mice were placed in individual observation cages and the number of abdominal writhing was recorded for each mouse for a period of 10 minutes. A reduction in the number of writhes as compared to the control animals was considered as evidence for the presence of analgesia and expressed as percent inhibition of writhes using the formula:

$$\% \text{ Inhibition} = \{[\text{Mean number of writhes (control)} - \text{Mean number of writhes (test)}] \div \text{Mean number writhes (control)}\} \times 100$$

Evaluation of anti-inflammatory activity. Carrageenan-induced rat paw edema: Evaluation of the anti-inflammatory activity was carried out as described by Winter et al. [12]. The anti-inflammatory activity of *C. capitatum* extract and fractions were evaluated using Carrageenan (0.l ml, 1% w/v in normal saline) as the inflammation inducing agent and diclofenac as the reference drug. The paw diameters of the rats were measured in mm using a SDC041 digital Vanier calipers (Xuzhou Smile Trading Company Ltd., Jiangsu, China) in the Department of Pharmacology and therapeutics ABU Zaria. The animals were divided into five groups consisting of six rats each. The control group (I) received 1 ml/kg distilled water and 1% Tween 80 (DF). The test groups (II, III and IV) received graded doses of *C. capitatum* extract and fractions (200, 100 and 50 mg/kg, p.o.) respectively while Group V rats received diclofenac (20 mg/kg), a standard anti-inflammatory drug and was used as a positive control. The

diameters of the paws were recorded before inflammation induction. The inflammation-inducing agent was then injected into the sub-plantar tissue of left hind paw every hour after administration of the treatments. Paw diameters were then measured hourly after induction of inflammation up to the fifth hour. Paw diameters measured prior to the carrageenan injection was compared with the diameter of the same paw after carrageenan injection by calculating the percentage inhibition and percentage change using the formula:

% inflammation inhibition = (Ct − Tt /Ct) × 100
Where, Ct = Paw diameter at 1 hour after Carrageenan administration
Tt = Paw diameter after Treatment

Statistical analysis. Results obtained were expressed as Mean ± Standard Error of the Mean (S.E.M.). Data obtained for acetic acid-induced writhes was analysed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. While data of oedema index was analysed using repeated measure ANOVA followed by Bonferroni post hoc tests respectively. Values of $p \leq 0.05$ were considered significant. All statistical analysis was performed using GraphPad Prism 5.0 for Windows (GraphPad Software, San Diego, California, USA).

RESULTS

Acute toxicity. A single oral administration of the extract (5 g/kg) did not produce any sign of toxicity and no mortality occurred within 24 h after administration of 80% aqueous ethanol extract, ethyl acetate (EF) and n-butanol (NF) fractions while there was death in dichloromethane fraction (DF). There was also death recorded for (DF) when the dose was reduced to 2 g/kg. The oral Median lethal dose was found to be greater than 5,000 mg/kg for the ethanol extract, ethyl acetate and n butanol fractions while it was less than 2,000 mg/kg for DF (Table 1).

Analgesic studies. The oral injection of acetic acid elicited writhing syndrome in control mice with 27.40±4.501 writhes counted in 10 minutes. Both the extract and fractions produced a significant dose dependent ($p < 0.05$ and $p < 0.01$) reduction in the number of writhes compared to control (Table 2). The peak effect of 98.79% inhibition was recorded by n-butanol fraction at 200 mg/kg. The inhibition rates of the number of writhing for 80% aqueous ethanol extract, at 50, 100 and 200 mg/kg, were 54.02, 57.66 and 72.99%, respectively. The percentage inhibition of writhes by the fractions EF at the dose of 200 mg/kg; NF at a dose 200 mg/kg and DF at a dose of 50 and 100 mg/kg were higher than that of piroxicam (81.02%). The percentage inhibition, which was dose dependent, was increasing with increase in dose for the extract, EF and NF but decreased in DF with increase in dose.

Anti-inflammatory studies. The 80% (aq) ethanol extract at tested dose of 50-200 mg/kg was able to reduce the size of the paw oedema significantly ($p< 0.05$ – $p< 0.001$) in a dose dependent manner from 1-5 hours. The maximum inhibitory effect of the extract was recorded at the 200 mg/kg dose (40.00%) at 4th hour (Table 3).The fractions of EF, NF and DF showed significant ($p < 0.05$ – $p< 0.001$) dose dependent change in paw circumference at the tested dose of 50-200 mg/kg from 1-5 hours. The highest inhibitory effect for ethyl acetate fraction (EF) were observed at the 50 mg/kg dose (43.69%) at the 3rd hour and (43.29%) at the 4th hour. N-butanol fractions (NF) were recorded at the 50 mg/kg dose (40.71%), 100 mg/kg dose (40.47%) and 200 mg/kg (40.00%) all at the 4th hour. The maximum inhibitory effect of Dichloromethane fraction (DF) was recorded at 200 mg/kg dose (41.99%) at the 5th hour and (41.65%) at the 4th hour. Diclofenac at 20 mg/kg, a prototype NSAID, inhibited significantly ($p < 0.01$ – $p < 0.001$) paw swelling due to carrageenan injection after 1-

5 hours with (29.49%, 33.25%, 43.46%, 42.82% and 41.02%) inhibitions respectively (Table 3).

DISCUSSION

The overall work was a bioassay-guided isolation to which extraction, fractionation and chromatography are key. In this current study, 80% (aq) ethanol extract and its fractions (DF, EF, and NF) were evaluated for its acute toxicity, analgesic and anti-inflammatory activity. The range of doses or estimate of therapeutic index of drugs could be determined or estimated by the toxicity test [12]. The oral Median lethal dose was found to be greater than 5,000 mg/kg for the EE80, EF, NF and less than 2,000 mg/kg for DF suggesting that the extract and fractions of EF and NF may be relatively safe, while that of DF may not be [10]. The limit test is required only to estimate whether the LD_{50} is greater or less than a certain value, i.e., the limit. The animals were dosed at the limit (5000 mg/kg and 2000 mg/kg), death was observed for DF at 5000 mg/kg and the dose adjusted down to 2000 mg/kg for DF. The decision was made as to whether the $LD_{50}$is greater or less than a certain value. This is the new limit test, which is designed to classify at either 2000 mg/kg or 5000 mg/kg [14]. The analgesic potential of an extract or its fraction could be deduced from the ability to inhibit acetic acid induced writhing. This is a sensitive procedure to evaluate peripheral acting analgesics where there is liberation of endogenous substances that cause pain. Thus, the analgesic activity of the ethanol extract and its fractions may be peripherally mediated via the inhibition of synthesis and release of prostaglandins and other endogenous substances [13].

Table 1: OECD Revised Up-and-Down Procedure for acute toxicity testing of extract and fractions of *C. capitatum*

S/N	Extract/	Dose (mg/kg)			Decision
	Fractions	5000	5000	2000	
1	80% EE	Survived	Survived		LD_{50}> 5000
2	EF	Survived	Survived		LD_{50}> 5000
3	NF	Survived	Survived		LD_{50}> 5000
4	DF		Died	Died	LD_{50}< 2000

Table 2: Effects of *C. capitatum* leaf extract and fractions on acetic acid induced writhing in mice.

Treatments	Dose mg/kg)	Mean ± SEM of Writhes	Percentage Inhibition (%)
Control (H_2O)		27.40±4.501	-
80% EtOH	50	12.60± 1.860**	54.02
	100	11.60±1.939**	57.66
	200	7.40±2.379**	72.99
EF	50	10.80± 3.625**	60.58
	100	10.00± 1.140**	63.50
	200	4.40± 1.536**	93.12
NF	50	6.40±1.939**	76.64
	100	5.80± 1.934**	78.83
	200	3.00± 0.949**	98.79
DF	50	3.40± 1.122**	87.59
	100	5.00± 1.517**	81.75
	200	8.20± 1.020**	70.07
Piroxicam	20	5.20± 2.059**	81.02

Values represent Mean ± SEM, ** = p< 0.01 compared to Negative control group. One-way ANOVA followed by Dunnett's post hoc test; 80% EtOH= 80% aqueous Ethanol Extract, EF= Ethyl acetate fraction, NF= n-butanol fraction, and DF= Dichloromethane fraction, n=5

Table 3: Effect of *C. capitatum* on carrageenan induced inflammation in rats

Treatment	Dose mg/kg	Change in paw diameter (mm) and % Inhibition				
		1 h	2 h	3h	4h	5h
Control		3.56 ± 0.128	3.91 ± 0.374	4.28± 0.305	4.25 ± 0.468	4.12 ± 0.246
80% EtOH	50	3.27 ± 0.092*** (8.15%)	3.38 ± 0.368 (13.55%)	2.91 ± 0.200 ^{b**} (32.01%)	2.79 ± 0.092 ^{c***} (34.35%)	3.17 ± 0.155^{a} (23.06%)
	100	3.26 ± 0.091* (8.43%)	3.25 ± 0.235 (16.88%)	2.80 ± 0.201 ^{b**} (34.57%)	2.69 ± 0.164 ^{c**} (36.71%)	3.17 ± 0.224^{a} (23.06%)
	200	3.18 ± 0.209 (10.67%)	3.05 ± 0.229 (21.99%)	2.77 ± 0.195 ^{a*} (35.28%)	2.55 ± 0.198 ^{c**} (40.00%)	2.52 ± 0.186 ^{c***} (38.83%)
EF	50	3.13 ± 0.116 ^{c***} (12.08%)	2.86 ± 0.126 (26.85%)	2.41 ± 0.164 ^{b*} (43.69%)	2.41 ± 0.051 ^{c***} (43.29%)	2.49 ± 0.154 ^{b} (39.56%)
	100	3.19 ± 0.105 ^{b***} (10.39%)	3.12 ± 0.101 ^{a**} (20.20%)	2.64 ± 0.041 ^{c***} (38.31%)	2.69 ± 0.191 ^{c***} (36.71%)	2.89 ± 0.099 ^{c***} (29.85%)
	200	3.60 ± 0.140*** (-1.12%)	3.15 ± 0.128 (19.44%)	3.02 ± 0.214 ^{c***} (29.43%)	2.74 ± 0.075 ^{c***} (35.53%)	3.19 ± 0.251 ^{c**} (22.57%)
NF	50	2.84 ± 0.135* (20.22%)	3.07 ± 0.168 (21.48%)	2.68 ± 0.199* (37.38%)	2.52 ± 0.064 ^{c**} (40.71%)	2.54±0.148** (38.35%)
	100	3.05 ± 0.216 (14.33%)	3.10 ± 0.148 (20.72%)	2.68 ± 0.149* (37.38%)	2.53±0.026 ^{c**} (40.47%)	2.56 ± 0.082^{a} (37.86%)
	200	3.24 ± 0.411** (8.99%)	3.22 ± 0.537 (17.65%)	2.74 ± 0.168* (35.98%)	2.55 ± 0.234 ^{c**} (40.00%)	2.89 ± 0.285 ^{c**} (29.85%)
DF	50	3.36 ± 0.174 (5.62%)	3.09 ± 0.131 ^{c***} (27.29%)	2.97 ± 0.146 ^{c***} (30.61%)	2.96 ± 0.098 ^{c**} (30.35%)	3.16 ± 0.149 ^{c*} (23.30%)
	100	3.30 ± 0.071 (7.30%)	2.89 ± 0.111 ^{b***} (26.09%)	2.64 ± 0.118 ^{c***} (38.31%)	2.73 ± 0.126 ^{c***} (30.18%)	2.91 ± 0.091 ^{c***} (29.37%)
	200	2.79 ± 0.105*** (21.63%)	2.72 ± 0.088 ^{c***} (30.43%)	2.59 ± 0.071 ^{c***} (39.48%)	2.48 ± 0.058 ^{c***} (41.65%)	2.39 ± 0.080 ^{c***} (41.99%)
Diclofenac	20	2.51 ± 0.118^{c***} (29.49%)	2.61 ± 0.122^{c***} (33.25%)	2.42 ± 0.082^{c***} (43.46%)	2.43 ± 0.138^{c***} (42.82%)	2.43 ± 0.090^{c***} (41.02%)

Values represent Mean ± S.E.M., a = p< 0.05, b = p< 0.01, c = p< 0.001 compared to Negative control group; * = p< 0.05, ** = p< 0.01, *** = p< 0.001 compared to the time 3hr using repeated measure ANOVA followed by Bonferroni post hoc tests. Values in parenthesis are % inhibition, 80% EtOH= 80% aqueous Ethanol Extract, EF= Ethyl acetate fraction, NF= n-butanol fraction, and DF= Dichloromethane fraction

The injection of 1% carrageenan into the sub plantar of the hind paw of the negative control rats produced a local oedema reaching its maximum at the 3rd hour. The 80% (aq) ethanol extract and its fractions (DF, EF, and NF) at graded doses (50-200 mg/kg) were observed to exert significant effect at different stages of inflammation. This inflammatory response is probably biphasic with the first phase attributed to the release of histamines, serotonins and kinins from 1- 2 hours and the 2nd phase attributed to the release of prostaglandins and lysosomal enzymes in the 2-3 hours [15]. This process is characterised by accumulation of fluid, mediator release, a complex array of enzyme activation, cell migration and tissue breakdown [13]. The EF showed the overall highest inhibitory effect of 43.69% at the 3rd hour, which is higher than the extract and even the standard drug diclofenac. This shows that there may be constituents that are concentrated in the ethyl acetate fraction that possess strong anti-inflammatory activity when compared to other fractions or the extract alone.

Conclusion. The Median lethal dose was found to be relatively safe with LD_{50} greater than 5,000 mg/kg for 80% aqueous ethanol extract; ethyl acetate fraction; n-butanol fractions and less than 2,000 mg/kg for Dichloromethane fraction. The leaf extract and fractions possess anti-inflammatory and analgesic effects, which may be mediated through the phytochemical constituents,

present in them. The results support the traditional use of *C. capitatum* in the treatment of various disease associated with pain and inflammation.

REFERENCES

1. Adeneye, A.A., T.I. Adeleke and A.K. Adeneye (2008): Hypoglycemic and hypolipidemic effects of the aqueous fresh leaves extract of Clerodendrum capitatum (Willd) Schumach & Thonn. in Wistar rats. *J. Ethnopharmacol*., 116: 7-10. DOI: 10.1016/j.jep.2007.10.029.

2. Burkill, H. M. (2000). Useful Plants of West Africa. 2nd ed. Vol. 5. Royal Botanic Gardens, Kew, 248 – 249.

3. Abdel Wahab, S. I., Abdel Wahab H. M., Osama Y. M., Manal, M. E., Ahmad, B. A. and Mahjoub O., SyamMohan, M. I., Noroodin, M. R., and Khalid, M. A. (2012): Erectogenic Effects of *Clerodendrum capitatum*: Involvement of Phosphodiesterase Type-5 Inhibition, *Hindawi Publishing Corporation, Evidence-Based Complementary and Alternative Medicine*.doi:10.1155/2012/137386

4. Shirvastava, N. and Patel, T. (2007). *Clerodendrum* and Health Care, an overview. *Journal of Medicinal and Aromatic Plant Science and Biotechnology*, Global Science Book.

5. Adamu, A., Abubakar, A.Z., Abubakar, A. Abubakar M.S., and Musa, K.Y. (2014): Methanol Leave extract of *clerodendrum capitatum* (Willd) Schum and Thonn. (Verbenaceae) confers resistance against *Naja nigricollis* venom. *Journal of Pharmacology and tropical therapeutics,* ISSN: 2408-7483 pp23-28.

6. Claudia Velázquez-González, Raquel Cariño-Cortés, Juan A Gayosso de Lucio1, Mario IOrtiz, Minarda De la O Arciniega1, Diana A Altamirano-Báez, Luis Jiménez- Ángeles and Mirandeli Bautista (2014): Antinociceptive and anti-inflammatory activities of Geranium bellum and its isolated compounds. *BMC Complementary and Alternative Medicine* 2014, 14:506.

7.Nicholas Henschke, Steven J. Kamper, Chris G. Maher (2015) The Epidemiology and Economic Consequences of Pain, *Mayo Clinic proceedings*, Volume 90, Issue 1, Pages 139–147.

8. Shah, B. and Seth, A. (2010). *Textbook of pharmacognosy and phytochemistry* Elsevier Health Science, 1st edition, pp 1 – 40.

9. Emmy Tuenter, Karen Segers, Kyo Bin Kang, Johan Viaene, Sang Hyun Sung, Paul Cos, Louis Maes, Yvan Vander Heyden and Luc Pieters, (2017): Antiplasmodial Activity, Cytotoxicity and Structure-Activity Relationship Study of Cyclopeptide Alkaloids, *Molecules*, 22, 224; DOI: 10.3390/molecules22020224.

10. OECD [Organisation for Economic Co-operation and Development]. 2001a. OECD Guidelines for the Testing of Chemicals. Guideline 425 Acute Oral Toxicity—Up-and-Down Procedure. Paris: OECD.

11. Koster, R., Anderson, M. and Debeer, E.J. (1959); Acetic acid for analgesic screening. *Federation Proceedings*, 18, 412.

12. Winter, C., Risley, E., Nuss, O., 1962. Carrageenin-induced inflammation in the hind limb of the rat. Fed. Proc. 46, 118-126.

13. Odoma, S., Zezi, AU., Danjuma, N.M., Ahmed, A. (2014): Analgesic and Anti-inflammatory properties of methanol leaf extract of *Olax subscorpioidea* Oliv. (olacaceae) in mice and rats. *Journal of Pharmacology and tropical therapeutics*, ISSN: 2408-7483 pp29-35.

14. ICCVAM [Interagency Coordinating Committee on the Validation of Alternative Methods]. 2001. The Revised Up-and-Down Procedure: A Test Method for Determining the Acute Oral Toxicity of Chemicals. Final Report (NIH publication no. 02-4501). Research Triangle Park: NIH/NIEHS. Available at the ICCVAM web site http.7/iccvam.niehs.nih.gov/methods/udpdocs/udpfin/vol_l. and 2.pdf).

15. Jude, E.O., E.U., Awanga, G.F. Samuel, and U.A. Louis, Anti-inflammatory and analgesic activities of *Melantherascandens Asian Pacific Journal of Tropical Biomedicine,* 2, 2 (2012) 144 – 148.