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UVARIA CHAMAE (P. BEAUV): A PROSPECTIVE SOURCE OF INNOCUOUS ANALGESIC AND ANTI-INFLAMMATORY DRUG LEADS.

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Abstrac

Currently available analgesic and anti-inflammatory drugs such as NSAIDs are inaccessible and cause undesired and serious adverse effects. Therefore, the search for new analgesic drugs with promising pharmacological action and reduced side effects has become an urgent necessity. The plant *Uvaria chamae* (P. Beauv.) belongs to the Uvaria genus which is in the Annonaceae family. It is a climbing large shrub or small tree native to the tropical rain forest of West Africa and Central Africa which is known to be highly therapeutic. The methanol leaf extract of the plant was investigated for its phytochemical composition, toxicological and anti-inflammatory properties on animal models. The toxicological activity of the plant extract was carried out on animal models using the Lorke`s method. Acute toxicity was investigated in mice by

Introduction:

Inflammation is a biological defense mechanism (induced by pathogenic factors including tissue injury, pathogens and toxic compounds) for eradication of noxious stimuli and restoration of tissue structure and function (Sajid *et al.*, 2017). Although inflammation is a defense process that is indispensable to health, uncontrolled acute inflammatory mechanism may cause chronic inflammation, contributing to a host of chronic inflammation-mediated diseases (Zhou *et al.*, 2016). Presently, inflammatory diseases including arthritis, diabetes mellitus,

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administering with the following dosage of the methanol extract of *Uvaria chamae* leaves 10 mgkg−1, 100 mgkg−1 and 1000 mgkg−1 (Phase I) and 1600 mgkg−1, 2900 mgkg−1 and 5000 mgkg−1 of the extract (Phase II). The mice were observed for signs of toxicity within 0 to 24 h and thereafter daily for 14 days. Body and organ weights of rats were also monitored and used as measures of toxicity. Evaluation of toxicity, analgesics and anti-inflammatory activities of the extract were carried out in experimental rats and mice after day 14 of the exposure. Exposed rats showed no signs of toxicity. The median lethal dose of the extract was estimated to be above 5000 mgkg−1. Significant ($p < 0.05$) weight gain in pooled body weights of rats was observed at the end of the experiment but this was significant ($p < 0.05$) in Phase I only. There were no significant ($p > 0.05$) differences in the analgesic and anti-inflammatory activities of the extract in exposed rats and mice. Although the methanol extract of *U. Chamae* leaves was apparently non-toxic and also discovered to be anti-inflammatory to the exposed rats as demonstrated, there is still a need to identify the bioactive compounds that are responsible for the bioactivities reported in this study.

Keywords: *Uvaria chamae*, analgesic, anti-inflammatory, drug lead.

Cardiovascular disease and cancer jointly represent leading causes of debility and death around the world (Bennett *et al.*, 2018).

Common therapeutic remedies used to treat inflammation related diseases include steroidal (glucocorticoids) or non-steroidal (opioids) drugs. However, these medications also have unwanted and fatal side effects including respiratory depression, euphoria, gastrointestinal irritation and renal damage (Kunanusorn *et al.*, 2009). Consequently, the search for novel therapies with promising pharmacological action and reduced side effects has become a crucial inevitability. Earlier reports have confirmed that naturally occurring antioxidant agents from plant extracts play a protective role in managing and preventing oxidative stress and inflammation-mediated disorders (Ferrante *et al.*, 2017; Locatelli *et al.*, 2017). Many plants including *Uvaria chamae* have been used in traditional medicine for long as remedies

for various ailments without published reports confirming their pharmacological effect and mechanism of action.

The plant *Uvaria chamae* (P. Beauv.) belongs to the Uvaria genus in the Annonaceae family. It is a climbing large shrub or small tree that can be up to 4.5 m tall; it is found commonly in the tropical rain forest of West and Central Africa (Okwu and Iroabuchi, 2009). The sweet, aromatic alternate leaves are usually used to heal wounds and cure diseases. The plant derived some of its common names including finger roots and bush banana from its finger-like bunches of edible yellow ripe fruits. *U. chamae* is taken orally to treat catarrhal inflammation of the mucous membranes; gonorrhea and bronchitis (Burkill, 1985). It is also used in the treatment of stomach pain, dysentry, haemorrhoids, epistaxis, haematuria, haematemesis and haemoptysis. The leaf is an excellent remedy for treating malaria (Koudouvo *et al.*, 2011). The leaf and roots macerated together are used internally as a cough mixture, and also as a strong medicine for renal and costal pain. The root bark is used as an astringent, styptic and galactagogue (Burkil, 1985 and Achigan 2009).

In Nigeria*, Uvaria chamae* has been employed in traditional medicine as a therapeutic agent in the effective management of pain and inflammation. However, literature search revealed that there is dearth of information on scientific investigation of analgesic and anti-inflammatory activity of the leaves of the plant. Thus, the aim of this study is to carry out the in vivo screening of the plant extract for analgesic properties and investigate into its anti-inflammatory potentials using different experimental animal models.

Materials and Methods

Material

Fresh leaves of *Uvaria chamae* (Figure 1) were collected from Obobu, Ikachi Ukpa Local Government Area, Benue State, Nigeria. They were authenticated at the Herbarium of the National Institute for Pharmaceutical Research and Development, Idu, Abuja, where a voucher specimen was deposited. The leaves of *Uvaria chamae* collected were air-dried at room temperature and then pulverized. The powdered plant material was weighed, and part of it was used for extraction. The solvents used were of analytical grade by Sigma-Aldrich, Germany. Reagents used for the phytochemical screening were

freshly prepared. Locally bred Swiss albino mice and Wister rats of various body weights and either sex used for the acute toxicity study, analgesic and anti-inflammatory screening were obtained from the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were fed with laboratory diet and water ad libitum and maintained under standard conditions in cages at room temperature.

Figure 1: *Uvaria chamae* (P. Beauv.) African plants-A Photo Guide. www. africanplants.senckenberg.de

Methods

Extraction of plant material

Approximately 1 kg of the powdered plant material was macerated with 8 L of 100% methanol in an aspirator bottle with frequent swirling for 14 days. The extract was filtered using filter paper and the solvent removed using rotary evaporator. The weight of the extract obtained was recorded and the extract stored in a refrigerator.

Preliminary phytochemical screening

The crude extract obtained was subjected to preliminary qualitative phytochemical screening for secondary metabolites. This screening was based on standard procedures and protocol described by Sofowora (1993) and Trease and Evans (2002).

Acute toxicity assay

The method of Lorke (1983) was adopted for the study. This method was carried out in two parts. In the first parts, 3 groups of 3 animals each (mice)

were administered the methanol extract of varying doses (10, 100 and 1000 mg/Kg body weight) orally and observed for the first 4 h and intermittently for 24 h for any sign of toxicity and mortality. In the second phase, three groups with one mouse each were treated with doses of 1,600, 2,900 and 5000 mg/Kg of the extract and observed for another 24 h for signs of toxicity and death. The median lethal dose was calculated as a geometric mean of the highest non-lethal dose (with no death) and the lowest lethal dose.

$$LD_{50} = \sqrt{minimum\ lethal\ dose \times maximum\ tolerated\ dose}$$

Evaluation of Analgesic Activities in Mice

Acetic acid-induced abdominal constrictions in mice

The method described by Koster *et al.,* (1959) was adopted for this study. 25 albino mice were divided into 5 groups of 5 mice each. Groups 1, 2 and 3 were orally administered with 250, 500 and 1000 mg/kg of the methanol leaf extract of Uvaria chamae respectively. While group 4 was administered with Piroxicam 10 mg/kg (positive control) and group 5 was orally administered with 1 mL/Kg of normal saline (negative control). Sixty minutes after oral administration, each mouse was injected intraperitonealy with 10 mL/kg of aqueous solution of acetic acid (0.6%) and placed in observation cage. The number of abdominal constrictions for each mouse was counted 5 minutes after injection of acetic acid for a period of 10 minutes. A reduction in the number of writhes as compared to the negative control was considered as evidence for the presence of analgesia and expressed as percent inhibition of writhes. The percentage inhibition of abdominal constrictions was calculated using the following formula:

$$= \frac{\text{mean number of writhing (control)} - \text{mean number of writhing (test)}}{\text{mean number of writhing (control)}} X100$$

Formalin – Induced Pain Test in Mice

The method of Dubuisson and Dennis (1977) as modified by Tjølsen *et al.,* (1992) was adopted in this study. Twenty-five mice were used. The mice were divided into 5 groups (n = 5) each containing 5 mice. Groups 1, 2 and 3 were administered with 250, 500 and 1000 mg/Kg of the methanol leaf extract of *Uvaria chamae* respectively. While group 4 was administered with morphine 5 mg/kg (positive control) and group 5 was administered with 10 mL/kg of

normal saline (negative control). Sixty-minute post treatment; 20 µL of freshly prepared 2.5 % solution of formalin in saline was injected subcutaneously under the planter surface of the right hind paw of each mouse. The mice were placed individually in an observation chamber and monitored for 1 hr.

The severity of pain response was recorded for each mouse based on the following scale:

[0] The mouse walked or stood firmly on the injected paw.
[1] The injected paw was favoured or partially elevated.
[2] The injected paw was clearly lifted off the floor.
[3] The mouse licked, chewed or shook the injected paw.

Anti-nociceptive effect was determined in two phases. The early phase (phase 1) was recorded during the first 5 minutes, while the late phase (phase 2) was recorded during the last 45 minutes with a 10-minute lag period in between both phases.

Thermally-induced pain test in mice (Hot plate method)

The methanol leaf extract of *U. chamae* was subjected to test for analgesia using hot plate method in mice as described by Turner (1965). The mice were divided into five groups of five mice each. The first group served as negative control and received distilled water 10 mL/Kg. The second, third and fourth groups were pre-treated with 250, 500 and 1000 mg/Kg of the extract respectively while the fifth group served as positive control and was treated with 5 mg/Kg of morphine. Thirty (30) minutes post treatment, each mouse was gently placed on a hot plate maintained at 50 $\pm$ 1ºC. The basis for selection and grouping of the animals was done after a baseline test to determine the animal's response to electrical heat–induced nociceptive pain stimulus and animals that stayed for than 15 seconds on the hot plate were considered to have impaired nociception and therefore excluded. The time taken by the mouse to lick its paw or jump off the plate was taken as the latency to pain response. To avoid tissue damage, the cut-off time or latency response in control was taken as 30 seconds. The determination of latency of pain response was performed before pre-treatment and repeated at intervals of 30, 60, 90 and 120 minutes after oral administration of the test drug. The

extension of the latency times was interpreted as an analgesic response (percent maximum possible effect {%MPE}.

$$\%MPE = \frac{Test - Basaline}{Cutoff - Baseline} x100$$

Where:

Test = latency to respond after treatment

Baseline = latency to respond prior to treatment and

Cut–off (30 sec) = preset time at which the test was ended in the absence of a response

Evaluation of Anti–inflammatory Activities

Carrageenan-induced paw oedema test in rats

The Carrageenan induced paw oedema in rats was used in the acute anti-inflammatory investigation described by (Winter *et al.,* 1962). Twenty-five rats of either sex weighing between 113 to 181 g were used for this experiment. The rats were divided into five groups each containing 5 rats (n=5). Acute inflammation was induced by injecting 0.1 mL of 1 Carrageenan into sub plantar surface of rat hind paw. The methanol leaf extract (250, 500 and 1000 mg/kg), normal saline (10 mL/kg) and piroxicam (10 mg/kg) as positive control were administrated orally sixty minutes before Carrageenan injection. The paw diameter was measured at 0, 1, 2, 3, 4, 5 and 24 hours, using vennier caliper to measure oedema diameter. The increase in paw diameter (oedema index) before and after carrageenan injection at each time interval was used to determine the oedema index for each rat, while the percent inhibition of oedema, was calculated for each group with respect to negative control group using the following relationship:

$$\frac{mean\ increase\ in\ paw\ volume\ of\ control - mean\ increase\ in\ paw\ volume\ of\ treated}{mean\ increase\ in\ paw\ volume\ of\ control}$$

x 100

Statistical Analysis

The results were expressed as Mean±SEM and the mean values of the control groups were compared with the mean values of the treated groups using one way and Split plot Anova followed by Bonferroni test for multiple comparison. The results obtained were considered statistically significant at ($P < 0.05$).

Results

Preliminary Phytochemical Screening

The results of the preliminary phytochemical screening of the extract of the dried powdered leaves of *Uvaria chamae* are shown in Table 1 below.

Table 1: Results of Preliminary Phytochemical Screening of Extract of Uvaria chamae Leaves

Phytochemicals	Result
Carbohydrates	+
Anthraquinones	-
Steroids/Terpenes	+
Glycosides	+
Saponins	+
Tannins	+
Flavonoids	+
Alkaloids	+

KEY: (+) = Present, (-) = Absent

Result of Acute Toxicity Assay

The results of the acute toxicity profile of the successive extraction of the dried powdered leaves of *Uvaria chamae* against mice are shown in Tables 2a–2b below.

Table 2: Results of Phase one (1) of Acute Toxicity Profile of Extract of Uvaria chamae Leaves against Mice

Dose (mg/kg)	Number of mice used	Mortality
10	3	0/3
100	3	0/3
1000	3	0/3

Table 2b: Result of the Second Phase of Acute Toxicity Studies of the Methanol Leaf Extract of *Uvaria chamae*

Dose(mg/kg)	Number of mice used	Mortality
1600	1	0/1
2900	1	0/1

5000	1	0/1

Results of Analgesic Studies

The result of the leaf extract of *Uvaria chamae* on acetic acid-induced writhing in mice is shown in Table 3 and Figure 2 below.

Table 3:The result of the leaf extract of *U. chamae* on acetic acid-induced writhing in mice.

Treatment	Dose (mg/kg)	Mean number of writhing (± SEM)	Inhibition (%)
Normal Saline	10 mL/kg	29.40 ± 3.0	-
MLE	250	15.40 ± 1.8*	47.62
MLE	500	8.20 ± 0.7*	72.12
MLE	1000	6.20 ± 1.0*	78.91
Piroxicam	10	8.20 ± 2.1*	72.12

n = 5, Data analyzed using One-way ANOVA followed by Bonferoni post-hoc test,

* = P < 0.05 statistical significance versus normal saline

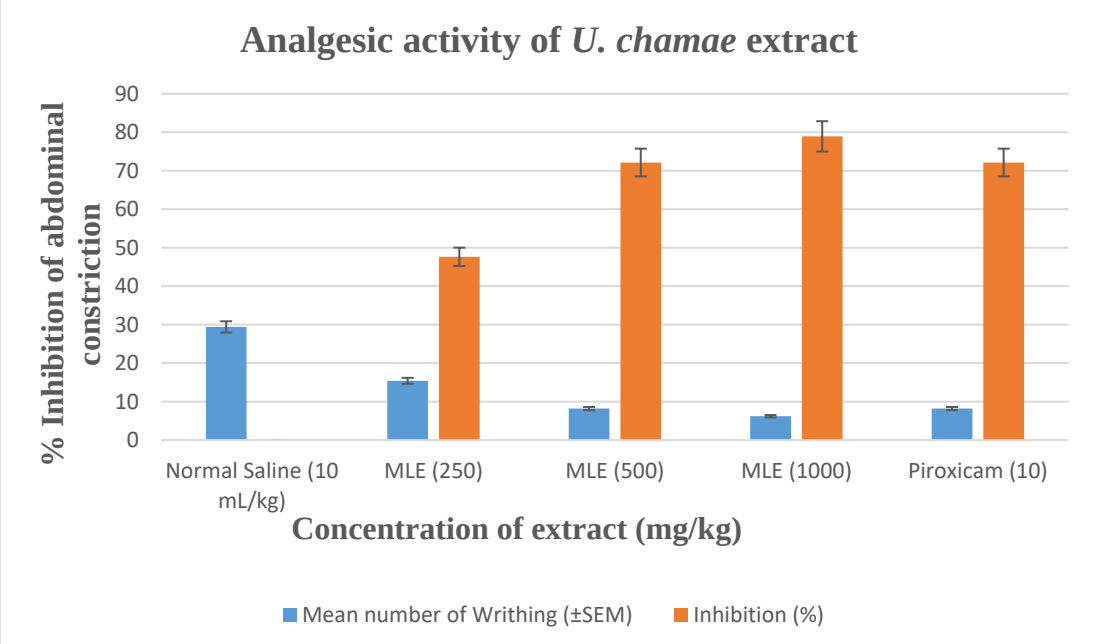

Figure 2:The result of the leaf extract of *U. chamae* on acetic acid-induced writhing in mice.

Table 4: Effect of Methanol Leaf Extract (MLE) of *Uvaria chamae* on Hot Plate Test in Mice

		Mean reaction time (sec) ± SEM				
Treatment	Dose (mg/kg)	0 min	30 min	60 min	90 min	120 min
Normal Saline	10 ml/kg	7.00 ± 1.22	9.00 ± 1.41	11.00 ± 1.00	5.40 ± 1.03	13.40 ± 2.01
MLE	250	12.40 ± 1.29	12.60 ± 1.33	14.60 ± 2.25	15.20 ± 1.77*	11.60 ± 1.86
MLE	500	14.20 ± 2.31	18.60 ± 1.21	15.80 ± 1.66	14.80 ± 2.40*	13.20 ± 2.56
MLE	1000	16.80 ± 3.12	15.00 ± 3.16	14.40 ± 1.60	12.00 ± 1.30	14.40 ± 1.63
Morphine	5.0	20.80 ± 1.71	16.80 ± 3.26	16.60 ± 1.96	17.00 ± 2.98*	16.20 ± 1.36

n = 5, Data analyzed using Split plot ANOVA followed by Bonferoni post-hoc test,
* = P <0.05 statistical significance versus Normal saline,
# = P <0.05 statistical significance versus 0 min

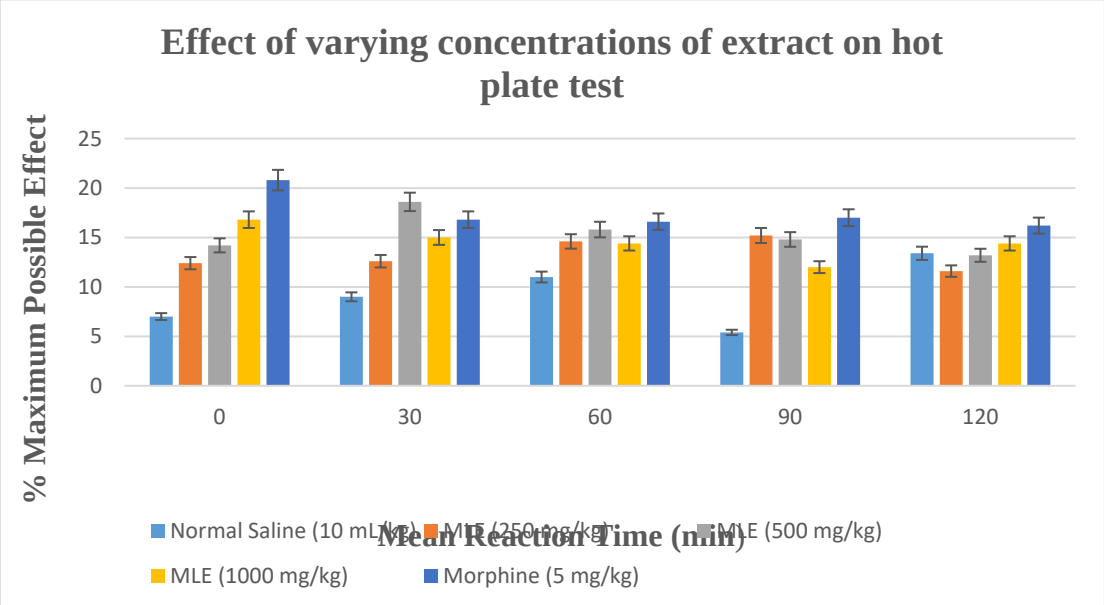

Figure 3: Effect of Methanol Leaf Extract (MLE) of *Uvaria chamae* on Hot Plate Test in Mice

Table 5: Effect of Methanol Leaf Extract of *U. chamae* on Formalin-Induced Pain in Mice

		Mean pain score (± SEM)	
Treatment	Dose (mg/kg)	Early phase(5 min)	Late phase (45 min)
Normal Saline	10 ml/kg	96.60 ± 2.66	105.00 ± 2.90#
MLE	250	69.20 ± 0.66*	94.20 ± 1.71*#
MLE	500	56.20 ± 1.71*	85.00 ± 1.00*#
MLE	1000	46.60 ± 1.03*	48.40 ± 0.81*#
Morphine	5.0	44.40 ± 0.75*	33.80 ± 1.71*#

n = 5, Data analyzed using Split plot ANOVA followed by Bonferoni post-hoc test,
* = P< 0.05 statistical significance versus Normal saline,
# = P <0.05 statistical significance versus Early phase

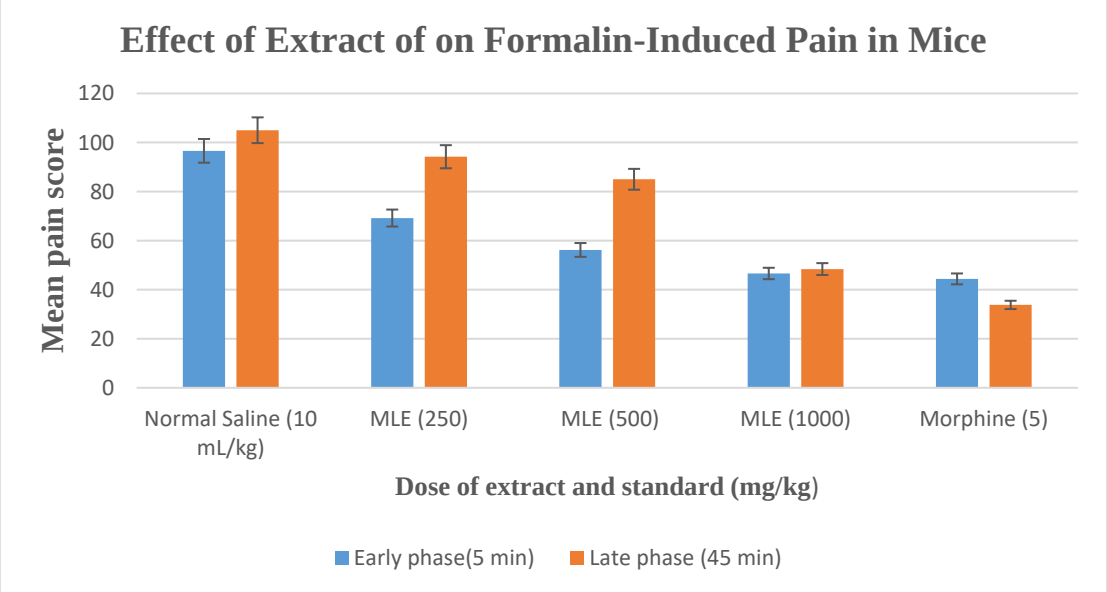

Figure 4:Effect of Methanol Leaf Extract of *U. chamae* on Formalin-Induced Pain in Mice

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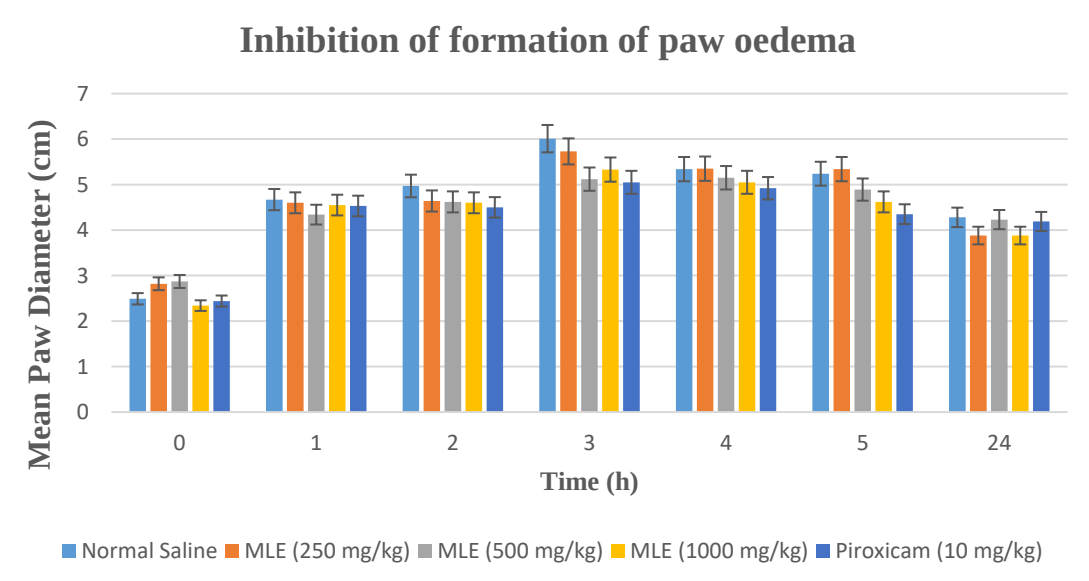
Results of Anti-Inflammatory Activities

Table 6: Effect of Methanol Leaf Extract of *U. chamae* on Carrageenan Induced Paw Oedema in Rats

		Mean paw diameter (cm) ± SEM						
Treatment	**Dose (mg/kg)**	**0 hr**	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**	**24 hr**
Normal Saline	10	2.49± 0.85	4.67± 0.27	4.97± 0.20	6.01± 0.10	5.34± 0.12	5.24± 0.34	4.28± 0.31
MLE	250	2.82± 0.27	4.60± 0.27	4.64± 0.38*	5.73± 0.39*	5.35± 0.39	5.34± 0.31	3.88± 0.22*
MLE	500	2.87± 0.11	4.34± 0.15*	4.62± 0.30*	5.12± 0.41*	5.15± 0.39*	4.89± 0.34*	4.23± 0.19
MLE	1000	2.34± 0.16	4.55± 0.23	4.60± 0.28*	5.33± 0.46*	5.05± 0.49*	4.62± 0.38*	3.88± 0.26*
Piroxicam	10	2.44± 0.31	4.53± 0.36	4.50± 0.27*	5.05± 0.22*	4.92± 0.24*	4.35± 0.23*	4.19± 0.12



Discussion

The results of the preliminary phytochemical screening of the methanol leaf extract of Uvaria chamae revealed the presence of carbohydrates, steroids, glycosides, saponins, tannins, flavonoids and alkaloids. Anthraquinones were absent. These phytochemical constituent have been reported to have varying degree of pharmacological activity (Cowan, 1999; Badam *et al*., 2002; Gupta and Tandon, 2004). The results are presented in Table 1. These secondary metabolites reported from this investigation are known for their broad spectrum of pharmacological and physiological properties in medicinal applications (Ezekiel *et al*., 2010).

The bioactivity of most carbohydrates is mostly dependent on resistance to digestion in the upper gastrointestinal tract (GIT), the stomach, and small intestine. Trehalose can reduce insulin resistance and improve glucose management in addition to having anti-inflammatory properties. Soluble polysaccharides increase viscosity of the upper GIT content, which enhances trapping of bile acids, preventing cholesterol re-absorption and promoting their removal through the feces (Aluko, 2012).

Steroids are perhaps one of the most widely used groups of drugs in present day. Beside the established utilization as immunosuppressive, anti-inflammatory, anti-rheumatic, diuretic, sedative, anabolic and contraceptive agents, recent applications of steroid compounds include the treatment of some forms of cancer and osteoporosis. The microbial biotransformation of steroids has yielded several novel metabolites, exhibiting different activities.

Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis (Brito-Arias, 2007), which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications.

Saponins have considerable potential as pharmaceutical and/or nutraceuticals agents in natural or synthetic form. Saponins, from a variety of sources have been shown to have hypocholesterolemic, anti-coagulant, anti-carcinogenic, hepatoprotective, hypoglycemic, immunomodulatory, neuroprotective, anti-inflammatory and antioxidant activity (Rao and Gurfinkel, 2000).

Tannins are known to be responsible for the haemostatic activity of plants where they arrest bleeding from damaged or injured vessels by precipitating proteins to form vascular plugs. A2 (Okwu and Iroabuchi, 2009).

Flavonoids are known for their biological functions which include protection against allergies, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors. They reduce the risk of estrogen-induced cancers by interfering with the enzymes that produce estrogen. Flavonoids significantly inhibit lysosomal enzyme secretion and arachidonic acid release from membranes by inhibiting lipoxygenase, cycloxygenase and phospolipase A2 (Okwu and Iroabuchi, 2009).

Alkaloids mainly derived from plant sources, are a large group of secondary metabolites containing usually basic nitrogen in a heterocycle (Achilonu and Umesiobi, 2015). Despite the toxicity of some alkaloids which is widely recognized, they are a source of many biologically active phytochemicals with great potential for medicinal and agricultural uses. Many alkaloids have attractive pharmacological effects and are used as medications, such as recreational drugs, or in entheogenic rituals (Achilonu and Umesiobi, 2015).

The results of the acute toxicity assay showed that all the animals survived at all the concentrations ranging from 10 mg/kg to 5000 mg/kg. Physical and behavioural observation of the experimental mice revealed no visible clinical sign of acute toxicity. All animals survived after 14 days of observation, implying that the LD50 is greater than 5000 mg / kg suggesting that the plant is experimentally non-toxic and safe for human consumption (Lorke, 1983). It is important to note that the failure of the plant extracts to demonstrate an acute in vivo cytotoxicity activity during the general screening process does not necessarily imply a total absence of inherent medicinal value. The possible presence of antagonistic interactions between the different plant constituents in crude extract may be responsible for the low toxicity of the crude. Also, the extract may show acute toxicity behaviour in vitro.

The result of the acetic acid induced writhing analgesic studies of extract of Uvaria chamae reported in Table 3a showed the highest percentage suppression of 78.91 at 1000 mg/kg similar to that of Piroxicam with percentage suppression of 72.12, though at a lower concentration (10 mg/kg). Table 3b presented the results of the effect of the extract on hot plate

test on mice. the highest effect was recorded at the concentration of 14.40 ± 1.63 at 120 min. This activity is both time and concentration dependent. The Mean pain score of the extract on formalin-induced pain in mice was 48.40 ± 0.81 at 1000 mg/kg, a little above the value of morphine reported to be 33.80 ± 1.71 at the concentration of 5 mg/kg as reported in Table 3c.

The methanol extract of the leaves of Uvaria chamae produced a remarkable anti-inflammatory activity. The extract significantly inhibited the formation of paw-oedema induced by formalin. The extract was investigated at various doses (250, 500 and 1000mg/kg). The highest activity resides at the 1000 mg/kg with the mean paw diameter of 2.34±0.16 at 0 hr and 3.88±0.22 at 24 hr followed by 500 mg/kg with mean paw diameter of 3.88±0.26 at 24 hr making the anti-inflammatory activity dose dependent. The anti-inflammatory effects may be due to the presence of bioactive compounds such as flavonoids and saponins present in the extract. Flavonoids such as quercetin have demonstrated significant anti-inflammatory activity because of direct inhibition of several initial process of inflammation. For example, it inhibits both the production of histamine and other allergic inflammatory mediators (Dubuisson and Dennis, 1977; Ahmadiani, 1998).

Conclusion

The results of chemical and biological evaluations carried out on the methanol extract of leaves of *Uvaria chamae* showed that the leaf of the plant is rich in phytochemicals and have high analgesic and anti-inflammatory activities with low toxicity. It might therefore prove to be an auspicious candidate for development of analgesic and anti-inflammatory drugs. However, there is need for further research for isolation and characterization of the bioactive compounds that are responsible for the bioactivities reported in this study.

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