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Phytochemical Composition and Biological Activities of *Uvaria chamae* and *Clerodendoron splendens*

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Abstract: *Uvaria chamae* P. Beauv and *Clerodendron splendens* A Cheval are known to have various medicinal and therapeutic properties. Their anti-inflammatory and oxytocic properties were assessed in this study. The extracts and aspirin were found to inhibit carrageenan-induced paw oedema on albino rats and mice with a strong activity in aspirin having (80.43 %) inhibition while *U. chamae* and *C. splendens* have 69.57% and 47.83% inhibition respectively. The plants extract exhibition and uterine contraction activity on guinea pig. Phytochemical studies on the plants revealed the presence of bioactive components comprising flavonoids (0.70 – 5.70 mg. 100 g^{-1}), alkaloids (0.81-5.40 mg. 100 g^{-1}), tannins (0.40 – 3.60 mg. 100 g^{-1}), saponins (0.38 – 2.10 mg. 100 g^{-1}) and phenols (0.08 – 0.10 mg. 100 g^{-1}). These bioactive compounds may be responsible for the medicinal properties of *U. chaemae* and *C. splendens* that form the basis of their use in herbal medicine in Nigeria.

Keywords: *Clerodendron spledens*, *Uvarea chamae*, Bioactive compounds, Oxytocic activities, Anti-inflammatory properties.

Introduction

Clerondendron splendens (A. Cheval) and *Uverea chamae* (P. Beauv) are Nigeria medicinal plants used in phytomedicine to cure diseases and heal injuries. These plants have various effects on living systems. They are sedative, analgesic, cardio-protective, anti-inflammatory, oxytocic, antispasmodic and immune modulators[1].

The utilization of these plants against diseases such as cancer, parasitic infection, rheumatism, wound treatment, tumor growth; stroke, jaundice, typhoid, fibroid, syphilis and gonorrhea have been well documented[2-4]. The health benefits of medicinal plants are attributed in part to their unique phytochemical composition[5,6]. Phytochemicals act as anti-oxidants, stimulate the protective enzymes in the liver or block damage to genetic materials[5]. Phytochemicals prevent the occurrence of oxidative chemical species (OCS), stimulate antioxidant repairing mechanism and scavenging capacity for radicals in the system. Among these plants include *Clerondendron splendens* (A. Cheval) and *Uverea chamae* (P. Beauv).

Clerondendron splendens belongs to the family of Verbenaceae and is very widely distributed in tropical and subtropical regions of the world[7,8]. It is comprised of smell trees, shrubs and herbs[9]. It is a climbing evergreen bush plant with attractive red flowers produced during the dry seasons of the year[9]. *Clerondendron splendens* are cultivated and some are wild. It can be propagated by seeds and by cutting[10]. As a result of its bright, attractive red colored flowers, it is planted around homes as an ornamented plant[9]. The roots and leaf extracts of *C. splendens* have been used for the treatment of rheumatism, asthma and other inflammatory diseases[8]. The leaves and barks are used in traditional medicine to treat coughs, scrofulous infection, buboes problem, venereal infections, and skin diseases and as a vermifuge, febrifuge, ulcers, inflammations and fibroid[8].

Uverea chamae Beauv belongs to the family of Annonaceae. It is a climbing plant predominantly found in the tropical rain forest of West Africa[3,11]. It is an evergreen plant that grows about 3.6 to 4.5 m high, cultivated as well as wild[1,11]. The plant is extensively branched with sweet, aromatic and alternate leaves[1,13] commonly used to cure diseases and heal injuries. *U. chamae* is a plant with both medicinal and nutritional values. It has been reported[12] that extracts of *U. Chamae* have mutagenic effects. The drug benzyl benzoate used in antifungal preparations has a mutagenic compound, chamuvaritin, a benzyl dihydrochalcone that was isolated from *U. Chamae*[12]. Recently, uvarinol, a novel cytotoxic tribenzylated flavanone compound has been isolated from *U. chamae*[14]. The root is used in Nigeria as a purgative[11]. The root bark is used for respiratory catarrh and the root extract is used in phytomedicine for the treatment of piles, menrrhegia, epiostaxis, haematuria and haemalysis[4,11]. A root infusion is used to cure abdominal pains. The juice from the roots, stems or leaves is commonly applied to wounds and sores[11]. The antifungal and antibacterial inhibitory properties of the plant have been reported[3]. In folk medicine, extracts of the roots, barks and leaves are used to treat gastroenteritis, vomiting, diarrhea, dysentery, wounds, sore throats, inflamed gums and a number of other ailments[1].

In spite of the various uses of *U. chamae* and *C. splendens* in herbal medicine in Nigeria, the phyto-constituents of these plants have not been fully documented. The present study was undertaken to evaluate the secondary metabolites constituents and consequently assess the oxytocic and anti-inflammatory properties of the plants. The aim is to assess their potential usefulness as pharmaceutical raw material for drug formulation.

Experimental

The leaves of *Clerodendron splendens* and roots of *Uvarea chamae* were collected from an uncultivated farmland in Ubakala, Umuahia South, Abia State, Nigeria. The plants were identified and authenticated by Dr. A. Nmeregini of Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture Umudike, Nigeria. The voucher specimens were deposited in the University Herbarium of Forestry Department.

Preparation of plant extracts

The leaves of *Clerodendron splendens* and roots of *Uvarea chamae* were air-dried at the laboratory bench for 10 days and then ground into a uniform powder using a Thomas Wiley mill machine (Model Ed – 5 USA). The powdered materials (850 g for each sample) were stored in airtight bottles for chemical analysis.

Quantitative determination of chemical constituents

Preparation of fat free sample

2 g of the sample was de-fatted with 100 mL of diethyl ether using a soxhlet apparatus for 2 h.

Alkaloids determination

5 g of the sample was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate washed with dilute ammonium hydroxide solution and then filtered. The residue, which was taken as the crude alkaloid was weighed[15].

Total phenol determination

For the extraction of the phenolic constituents, the fat free sample was boiled with 500 mL of ether for 15 min. 5 mL of the extract was pipette into a 50 mL flask, and then 10 mL of distilled water was added. 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for color development. The absorbance of the solution was read using a spectrophotometer at 505 nm wave length[15,16].

Flavonoid determination

10 g of the plant samples were extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over water bath and weighed[17].

Saponins determination

The samples were ground 20 g of each plant samples were dispersed in 200 mL of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55 ^{o}C. The mixture was filtered and the residue re-extracted with another 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90 ^{o}C. The concentrate was transferred into a 250 mL separator funnel and 20 mL of diethyl ether was added and shaken vigorously.

The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 mL of *n*-butanol was added. The combined *n*-butanol extracts were washed twice with 10 mL 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage[16].

Tannin determination

500 mg of the sample was weighed into 100 mL plastic bottle. 50 mL of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 mL of the volumetric flask and made up to mark. Then 5 mL of the filtrate was pipette out into a tube and mixed with 3 mL of 0.1 m $FeCl_3$ in 0.1 N HCl and 0.008 m potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 605 nm wavelength within 10 min. A blank sample was prepared and the color developed and need at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured[18].

Extraction

Each of the powdered plant materials (100 g) was packed into a soxhlet apparatus (2 L) and extracted exhaustively with 500 mL of ethanol for 6 h. The ethanol was evaporated using a water bath and then left at laboratory temperature for two days for evaporation of the remaining ethanol. The extract was stored in the refrigerator for proper preservation until when needed. The yields were 8.20 g for *U. chamae* and 10.80 g for *C. splendens.* The concentration was made in 0.9% saline for the experimental studies[19].

Animal studies.

Mature male Swiss Mice (25) and male Sprague Dawley albino rats (150) of Wister strain were used for the anti-inflammatory studies while a guinea pig was used for the oxytocic studies. They were supplied from the animal house, College of Medicine, Nnamdi Azikwe University Hospital Nnewi, Nigeria. The animals were grouped in polycyclic cages (38 cm X 23 cm X 10 cm) with five animals per cage and maintained under standard laboratory conditions (temperature 25±6 °C). They were allowed free access to standard dry pellet diet and water ad libitum. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment.

Anti-inflammatory activity of carrageenan induced rat paw oedema

Carrageenan pedal inflammatory in rats was induced according to the method described by Onasanwo *et al*[19]. The rats were divided into five groups (n =5). The animals in the test group were treated orally with 150 mg/kg of plant extracts, one hour before carrageenan injection. At the same time, the control group received 0.9% saline and the reference group received 150 mg/kg aspirin. An injection of 0.1 mL of 1% Carrageenan was given into the right hind foot of each rat under the sub plantar aponeurosis. The measurement of the increase in paw size was done immediately before and after 3 h following carrageenan injection. The inhibitory activity after 3 h was taken as a measure of paw oedema[19].

The inhibitory activity was calculated according to the formula

$$\text{Percentage inhibition} = \frac{[C_t - C_o]_{control} - [C_t - C_o]_{text} \times 100\%}{[C_t - C_o]_{control}}$$

Where: $[C_t - C_o]_{control}$ = Change in paw size

Oxytoxic assays

Uterus from young virgin guinea pig was used. The guinea pig was killed with a blow on the head followed by cutting the carotid artery. A piece of the uterus was set up in the organ bath of 14 mL capacity containing tyrodes physiological solution[20], aerated with a mixture of oxygen (95%) and carbon dioxide (5%) maintained at 37 °C.

Increasing aliquots of standard solution of 0.0251 m/mL of oxytocin (Syntocinon® Laboratoria Sandoz, Santiago, Chile) were added to the organ and contraction changes were on a kymograph[7]. The organ bath was rinsed three times with tyrodes and the tissues allowed to rest for 3 minutes before suspended aqueous aliquots of dried ethanol extracts 15 mg/100 mL were added.

Statistical analysis

All measurements were replicated three times and standard deviation determined. The student's-test at $P < 0.05$ was applied to assess the difference between the means[21].

Results and Discussion

The phytochemical content of *U. chamae* and *C. splendens* is shown in Table 1. The flavonoids content was very high in *U. chamae* at 5.70 mg 100 g^{-1} while *C. splendens* contained 0.70 mg 100 g^{-1} of flavonoids. The biological functions of flavonoids include protection against allergies, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors[20,22]. Flavonoids reduce the risk of estrogen-induced cancers by interfering with the enzymes that produce estrogen. For example, flavonoids inhibit estrogen synthesase, an enzyme that binds estrogen to receptors in several organs. Flavonoids significantly inhibit lysosomal enzyme secretion and arachidonic acid release from membranes by inhibiting lipoxygenase, cycloxygenase and phospolipase A_2[23]. The inhibition of arachidonic acid release in the inflamed cells would provide less arachidonic substrate for the lipoxygenase and cycloxygenase pathways. This however, leads to a lesser quantity of endoperoxides, prostaglandins, prostacyline and thromboxanes as well as hydroperoxy, hydroxycicosatrienoic acids and leucotrienes[24]. Such an effect confirms the decrease in histamine, which is known to act in the first stage of the inflammatory process[22,25]. Prostaglandins can act to regulate menstruation, prevent conception, and induce child birth or abortion, lower blood clothing and possibly even act as decongestants[20,26]. However, some flavonoids behave as a powerful protective agent against inflammatory disorders. They reduce oedma formation and inhibit the synthesis of prostaglandin E_2, prostaglandin F_2 and thromboxane B_2[20,23].

Table 1. Phytochemical composition of *Clerodendron splendens* leaves and *Uvarea chamae* roots on dry weight basis (mg 100^{-1})

Phytochemical	*Clerndendron splendens* leaves	*Uvarea chamae* roots
Alkaloids	5.40 ± 0.03 ^{a}	0.81 ± 0.02 ^{b}
Flavonoids	0.70 ± 0.04 ^{b}	5.70 ± 0.03 ^{a}
Tannins	3.60 ± 0.02 ^{a}	0.40 ± 0.03 ^{b}
Saponins	2.10 ± 0.01 ^{a}	0.38 ± 0.01 ^{b}
Phenols	0.08 ± 0.01^{cl}	0.10 ± 0.30^{c}

Data are means ± standard deviation of triplicate determinations on dry weight basis. Values with superscript that are the same are not significantly different at P < 0. 0.05).

As a result of the availability of flavonoids in *U. chamae* and *C. splendens,* the extracts prevent platelet stickiness and hence platelet aggregation. Moreover, *U.chamae* and *C. splendens* extracts appears to protect the vascular system and strengthen the tiny capillaries that carry oxygen and essential nutrients to all cells. 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[23,27].

High quantity of alkaloids was found in *C. splendens, which* contained 5.40 mg 100 g^{-1} of alkaloids. Some alkaloids present in the plant function as spasmolytic, anti-cholinergic and anesthetic agents[28].

Tannins content was more in *C. splendens* containing 3.60 mg.100 g^{-1} while *U, chamae* contained 0.40 mg 100 g^{-1} of tannins. The value of saponins in *C. splendens was* 2.10 mg 100 g^{-1} and *U. chamae* contained 0.38 mg 100 g^{-1} of saponins. The identification of the abundant presence of saponins and tannins in the leaves of *C. splendens* and roots of *U. chamae* may be responsible for the haemostatic activity of these plants where they arrest bleeding from damaged or injured vessels by precipitating proteins to form vascular plugs. *U. chamae* contained 0.10 mg 100 g^{-1} of phenol while *C. splendens* contained 0.08 mg 100 g^{-1} phenol.

The presence of phenols in these plants indicates that they could act as anti-clothing agents, antioxidants, immune enhancers and hormone modulators. Phenols have been the subjects of extensive research as bioactive compounds used in disease prevention[29]. Phenols have been responsible in having the ability to block specific enzymes that causes inflammatory disorders. They also modify the prostaglandin pathways, and thereby protect platelets from clumping[29].

The anti-inflammatory property of *U. chamae* roots and *C. splendens* leaf extracts on carrageenan-induced paw oedema in rats is shown in Table 2. The extracts and aspirin were found to inhibit paw oedema in rats with a strong activity in aspirin having (80.43%) inhibition. The extracts reduced the incidence of paw oedema from (0.92±0.30) cm to (0.28±0.10) cm in *U. chamae* and (0.48±0.10) cm in *C. splendens,* that is 69.57% and 47.83% inhibition in *U. chamae* and *C. splendens* respectively.

Table 2. Effects of crude extracts of *Uvarea chamae* roots and *Clerodendron splendens* leaf and aspirin on carrageenan induced paw oedema rats.

Group	Dose mg/kg	Change in paw size, cm	Inhibition of paw thickening, %
Normal saline	10 mL/kg	092 ± 0.30	0.00
Uvarea chamae	150 mg/kg	0.28 ± 0.10	69.57
Clerodendron splendens	150 mg/kg	0.48 ± 0.10	47.83
Aspirin	150 mg/kg	018 ± 0.20	80.43

Data are means ± standard deviation of triplicate determinations. n = 5 rats.

The ethanolic extract of the roots of *U. chamae* produced a marked anti-inflammatory activity. It reduced the size of pedal swelling induced by carrageenan as compared with the control group. The anti-inflammatory effects of plants may be due to the presence of bioactive compounds such as flavonoids, saponins and phenolic compounds. This agreed with the findings of Somazundram and Sadique[26] and Shrivastava and Patel[28] that flavonoid glycosides of *C. splendens* showed modulation in calcium transport in isolated inflamed rat liver and thereby showed reduction in inflammation.

The ethanolic extracts of *U. chamae* and *C. splendens* showed uterine contraction activity. *U. chamae* had activity comparable to that of oxytocin (Table 3). *U. chamae* extract exhibited more uterine contraction in the guinea pig than *C. splendens.* The uterine contraction increases as the concentration of both oxytocin and plant extracts increases. These plants produced contraction in the uterus. They have similar action to that of oxytocin, though not so intense. Oxytocin is a hormone, which makes the uterus experience strong contractions, thus producing labor[30]. These plants are used in herbal medicine to accelerate labor in South Eastern Nigeria. However, if used during the first months of pregnancy, they could have abortifacient properties[20]. *U. chamae* extracts exhibited more uterine contraction on the guinea pig than *C. splendens.* This may be due to high flavonoids and phenolic content of *U. chamae.* If the extracts from these plants are administrated in high dose, they

prepare the uterus and ensures that fatigue disappears, producing strong, regular contraction to facilitate labor during the last month of pregnancy. Organic extracts of *U. chamae* showed strong uterine stimulating activity. This study demonstrates that *U. chamae* and *C. splendens* possesses oxytocic and anti-inflammatory activities. These findings, justify the traditional use of these plants to induce uterine contraction in traditional medical practice.

Table 3. Effects of *Uaerea chamae, Clerodendron splendens* and oxytocin on the uterus of Guinea Pig.

Concentration m/mL	*Uvarea chamae*	*Clerondendron splenden.*	Oxytocin drug
	Height of uterine contraction, cm	Height of uterine contraction, cm	Height of uterine contraction, cm
50	3.7 ± 0.02	3.20 ± 0.02	5.6 ± 0.03
100	8.1 ± 0.30	5.80 ± 0.10	10.6 ± 0.13
150	11.8 ± 0.12	7.0 ± 0.02	14.10 ± 0.15
200	15 ± 0.11	7.5 ± 0.13	17.80 ± 0.12
250	16.9 ± 0.10	10.60 ± 0.10	20.10 ± 0.02

Data are means ± standard deviation of three determinations values at the same.

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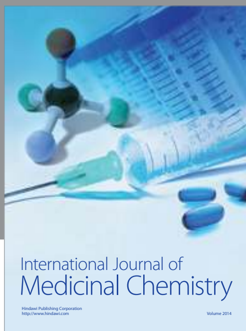

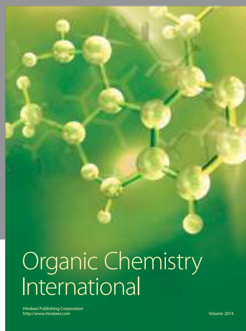

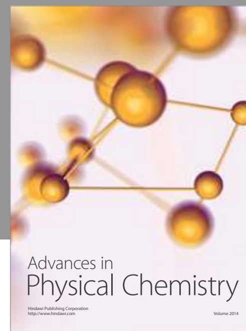

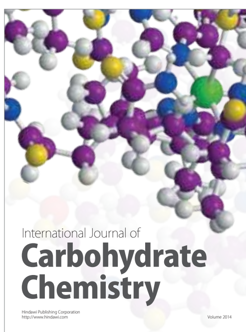

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