OXYTOCIC EFFECTS OF THE AQUEOUS LEAF EXTRACT OF *COSTUS LUCANUSIANUS* – FAMILY COSTACEAE ON ISOLATED NON-PREGNANT RAT UTERUS

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ABSTRACT

Costus lucanusianus J. Braun (Costaceae) is a climbing herb, found mainly in the Niger Delta region of Nigeria. This plant is locally used in situations of pains, inflammation, dysmenorrhoea and in pyrexia. The purpose of this study was to investigate this claim with view to validating scientifically the ethno-medicinal usage. The aqueous extract was subjected to pharmacological testing in vitro on a piece of isolated rat uterus previously pretreated with 1 mg/kg stilbestrol for 24 h. The dose response curves of oxytocin and that of the extract were first obtained. The effects of antagonists like atropine (1 mg) and salbutamol (2 µg) on the dose response curve of the extract were also investigated. Possible synergy was investigated via co-administration of the extract and oxytocin. Finally the proximate analysis of the extract was investigated. The aqueous extract of C. *lucanusianus* and oxytocin both produced a dose dependent contraction of the uterus. An effect of 0.63±0.06 g force of uterine contraction produced by 12.5 mg of the extract was increased to 1.37±0.09 g when 200 mg of the extract was administered. Oxytocin at 0.16 i.u was observed to produce a similar force of contraction with 200 mg of the aqueous extract. Synergy was established as co administration of the extract at 200 mg and oxytocin at 0.08i.u, produced higher contractile effect, significantly higher ($p<0.05$) than when either the extract (200mg) or oxytocin (0.08 i.u) was administered alone. Both atropine and salbutamol significantly ($p<0.0001$) inhibited the contractile effect produced by the extract. The inhibitory effect showed by atropine on the contractile effect of the extract seems to suggest the involvement of muscarinic receptors. The proximate analysis carried out in this study is used to establish the identity of the crude drug sample. A moisture content of 10.047 % was obtained. The total ash is a measure of the non-volatile inorganic constituents remaining after ashing. The values of 3.42 % were obtained.

Keywords: *Costus lucanusianus,* oxytocic effect, rat uterus.

INTRODUCTION

Herbal medicine is the use of plants, plant parts, their water or solvent extracts, essential oils, gums, resins, exudates or other form of advanced products made from plant parts used therapeutically to provide proactive support of various physiological systems; or, in a more conventional medical sense, to treat, cure, or prevent disease in animals or humans (Tyler and Foster, 1999).

In traditional medicine, many plants are claimed to have an oxytocic effect without any scientific basis. The plant *Costus lucanusianus* locally called monkey sugar cane in the Niger delta region of Nigeria is a herb up to 3 m tall. The stem contains some amount of juice. The leaves are light green, with a green stem, base with leaves smaller toward base, larger above; and dark green. The juice from leaves has a wide reputation in folk medicine for the treatment of diarrhoea, vomiting and dysmenorrhoea (Gill, 1992).

Data on the effect of *Costus lucanusianus* on the contraction or relaxation of the uterine smooth muscle is lacking. The uterus contracts rhythmically *insitu* and when excised. These spontaneous contractions and relaxation originate from the uterine smooth muscle itself with the myometrial cells of the fundus as pacemakers (Rang *et al*., 2003)

The frequency and force of contraction vary greatly with different conditions of the sex cycle perhaps due to the complex hormonal changes associated with these conditions (Ganong, 2005). In this study, the aqueous extract of the leaves was screened for possible activity on the isolated uterus of non-pregnant rats based on its use as a remedy for dysmenorrhoea.

MATERIALS AND METHODS

Collection and identification of Plant material

The leaves of *Costus lucanusianus* J. Braun (Costaceae) were collected in Amarata, Yenagoa, Bayelsa State in May, 2007. The botanical identity of the plant and its leaves was authenticated by Dr B.A Ayinde of the

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Department of Pharmacogonosy, Faculty of Pharmacy, University of Benin, Benin City. Botanical authentication was confirmed at the Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria where a voucher specimen (No FHI 107855) was deposited for future reference. Immediately after collection, the leaves were air dried for one week. This was further subjected to another one week of drying in an oven maintained at 40°C.

Extraction and preparation of the extract

The leaves were pulverized into a smooth powder using an impact mill. The pulverized material, about 150 g was subjected to maceration using 3 litres of distilled water and left for 3 days. The mixture was stirred at intervals and passed through a Whatman No 3 filter paper. The filtrate was concentrated *in vacuo* in a rotary evaporator at 40°C, giving a yield of 5.53 %. The concentrated extract was stored in universal bottles, labeled and refrigerated at -4^{0}C prior to use.

Drugs and chemicals

The following drugs were used: Diethylstilbestrol (Merck), Oxytocin (Rotex Medica), Salbutamol (Glaxosmithkline), Atropine (Sisbu Xierkang pharm Co Ltd), Stock solutions of the various drugs and the aqueous extract of *C. lucanusianus* were prepared fresh for each experiment.

Animals

Female non-pregnant Wistar rats weighing 155-200g were obtained from the Animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria. The animals were maintained on a standard diet (Ewu feeds, Edo State, Nigeria) and had free access to food and water. Research was conducted in accordance with the internationally accepted principles for laboratory animal use and care.

Proximate analysis of the powdered crude leaves

The following quantitative parameters were carried out using standard methods (African Pharmacopoeia, 1986, British Pharmacopoeia 1988, and AOAC, 2003).

Moisture content/water loss on drying

The powdered drug (2.0 g) was weighed into a clean crucible of known weight. After oven drying at 105°C for 5 hours and cooled, the crucible was weighed again to determine weight loss in the powdered drug. The average percentage weight loss, with reference to the air dried powdered drug was determined for four replicates.

Total ash determination

The crucibles were washed thoroughly, dried in hot oven at 100°C, cooled in desiccators and weighed. A 2.0 g portion of each of the samples were weighed into the crucible and put in the furnace. Heating was started gradually until temperature of 600 ^{O}C was reached. This temperature was maintained for 6 hours. The crucible was then put inside the desiccators and cooled. After cooling the sample was reweighed and the percentage ash calculated.

$$\% \text{ Ash} = \frac{\text{W - Z} \times 100}{\text{N}}$$

W =Weight of the crucible and ash Z=Weight of empty crucible, N = Weight of Sample.

Determination of Extractives

(a) Alcohol soluble extractive value

Powdered leaf drug (5.0 g) was weighed into a 250 ml stopper conical flask. Ethanol 90 % (100 ml) was added to the conical flask and stoppered. The flask was shaken in a mechanical shaker for 6 hours and then allowed to stand for 18 hours. The extract was filtered by suction filtration using a Buckner funnel. The weight of a heated cooled flat bottom porcelain crucible was accurately determined. The filtrate (20 ml) was poured into weighed crucible and evaporated to dryness at 100°C. The residue was dried to constant weight and the final weight noted. The weight of the residue obtained from the extract (20 ml) was determined by subtracting the constant weight of crucible from the residue. The alcohol extractive was then calculated with reference to the initial weight of the powdered drug and expressed as percentage.

(b) Water soluble extractive value

The above experiment was repeated using chloroform: water (400:1). The water soluble extractive value was done for the powdered drug

Pharmacological screening

Female non-pregnant wistar rats were pretreated intraperitoneally with 1 mg/kg of Stilbestrol 24 h prior to the actual experiment (Veale *et al.,* 1999). (n = 5-6). The rats were killed by cervical dislocation and exanguinations. The abdomen was opened and the two horns of the uterus carefully isolated, freed of mesenteric fat and a 1 cm piece was mounted in a 50 ml organ bath containing De Jalon physiological salt solution having the following chemical composition: NaCl, 9 g/l, $NaHCO_3$, 0.5 g/l, D-glucose, 0.5 g/l, KCl, 0.402 g/l, $CaCl_2.2H_20$, 0.08 g/l.

The tissue was aerated with air and temperature maintained at 37°C, with a pH of 7.4. The spontaneous contraction of the uterus was recorded with 7003-B transducer connected to an Ugo Basile recorder (7075). The transducer was previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection (500 mg).

The tissue was allowed to equilibrate for 30 minutes before the commencement of the experiment (Endalk *et al*., 2005). Thereafter the effect of distilled water (which served as the control) on the isolated uterus was also observed (Enriquez *et al*., 2006).

The dose-response curves of oxytocin (0.02-0.64 i.u.) induced contractions was first obtained, the effect of the aqueous extract (12.5-200 mg/ml) on the uterus was also determined. The possibility of synergy between the extract and oxytocin was explored via combined administration of both the extract and oxytocin (Veale, 1999).

Finally the effects of two positive controls (salbutamol and atropine) on the dose response curve of the extract were also investigated.

STATISTICAL ANALYSIS

All results are expressed as the mean of four experiments ± SEM. Where applicable, the data were analysed statistically by Student's t-test using Graph pad instant version 2.05a. The level of significance was $P < 0.05$. (n = 5-6).

RESULTS

Proximate analysis

The proximate analysis is as shown in table 1. A moisture content of 10.047 % was obtained. The total ash which is a measure of the non-volatile inorganic constituents remaining after ashing was 3.42 %.

Table 1: Percentage (%) values of proximate analysis of the leaves of *Costus lucanusianus*

Parameter	Values (%)
Moisture content	10.047
Total ash	3.42
Alcohol extractive	5.024
Water extractive	13.18

Pharmacological Screening

Oxytocin produced a dose-dependent contraction of the uterus. The highest response was produced by 0.64 i.u, giving a response of 1.67 $\pm$ 0.07 g. For the extract, a dose dependent contraction of the uterus was also noted, with the least dose of 12.5 mg producing a mean force of contraction of 0.63 $\pm$ 0.06 g, while the highest dose of 200 mg produced a corresponding maximum uterine force of contraction of 1.37 $\pm$ 0.09 g (table 2).

Table 2: The contractile effects of oxytocin and the aqueous extract of *Costus lucanusianus* on the rat uterus.

Treatment (mg/ml)		Mean uterine contractions ± SEM (g)
Oxytocin	0.02	0.98 ± 0.14
	0.04	1.32 ± 0.12
	0.08	1.28 ± 0.09
	0.16	1.37 ± 0.11
	0.32	1.54 ± 0.10
	0.64	1.66 ± 0.07
C.L	12.5	0.63 ± 0.06
	25.0	1.01 ± 0.10
	50.0	1.16 ± 0.15
	100.0	1.31 ± 0.15
	200.0	1.37 ± 0.09

C.L : Aqueous extract of *Costus lucansianus.*
Oxytocin is in i.u and not mg /ml

Co-administration of 0.08 i.u of oxytocin and 200 mg/ml of the extract produced contractions of the uterus significantly ($p<0.05$) higher than when either oxytocin or the extract was administered alone (table 3).

Table 3: The effects of co-administration of oxytocin and the aqueous extract of *Costus lucanusianus* on the rat uterus.

Treatment (mg /ml)		Mean contractions ± SEM (g)
Oxytocin	0.08	1.28± 0.09
C.L	100	1.31± 0.15
	200	1.37 ± 0.09
Oxy 0.08/C.L	100	1.51 ± 0.13
Oxy 0.08/C.L	200	1.67 ± 0.08*

*$p<0.05$.
C.L : Aqueous extract of *Costus lucansianus.*
Oxytocin is in i.u and not mg /ml

The responses obtained on co-administration was significantly ($p<0.05$) higher than responses obtained when either the extract (200mg) or oxytocin (0.08 i.u) were given alone.

Pretreatment with atropine (1mg/ml) in the presence of the extract significantly inhibited the contractile activity of the extract, ($p<0.0001$), decreasing the contraction of 1.01 $\pm$ 0.10 g produced by the 25 mg of the aqueous extract alone to 0.50 $\pm$ 0.03 g in the presence of atropine (table 4).

Salbutamol (2 µg/ml) was also observed to show significant inhibition of uterine contraction induced by the extract ($p<0.0001$). The contractile effect of 100 mg of

the extract was reduced to 0.13±0.05 g in the presence of salbutamol (table 4).

Table 4: Inhibitory effects of atropine and salbutamol on the uterine contraction induced by the aqueous extract of *Costus lucanusianus*.

Treatment (mg/ml)		Mean contractions ± SEM (g)
C.L	25	1.01 ± 0.10
	100	1.31 ± 0.15
	200	1.37 ± 0.09
Atropine	1/ C.L 25	0.50 ± 0.03*
	1/C.L 100	0.57 ± 0.05*
	1/ C.L 200	0.69 ± 0.01*
Salbutamol	2/ C.L 25	0.15 ± 0.04*
	2/ C.L 100	0.13 ± 0.05*
	2/ C.L 200	0.19 ± 0.06*

The force of contraction was significantly *p<0.0001 reduced on pretreatment with atropine and salbutamol.

C.L : Aqueous extract of *Costus lucansianus.*
Salbutamol is in µg /ml and not mg /ml

DISCUSSION

The proximate analysis carried out in this study is used to establish the identity of the crude drug sample. The moisture content shows the susceptibility of crude drug samples to microbial attack especially fungi, and also to degradation due to hydrolysis of the crude powdered drug. A moisture content of 10.047% obtained from this study is indicative of the storage quality for some time without microbial degradation or hydrolytic break down of the chemical constituents. The maximum range is between 6-8% in African Pharmacopoeia (1986).

The total ash is a measure of the non-volatile inorganic constituents remaining after ashing. It is made up of two parts, the physiological and the non physiological ash. The physiological ash consists of carbonates, phosphates, nitrates, sulphates, chlorides and silicates of metals which the plant took up when it was growing. The non-physiological ash represents ash from extraneous matter (AOAC, 2003). The values of 3.42% were obtained (for both the physiological and non-physiological ash)

Oxytocin and the extract both produced a dose-dependent increase in the contraction of the isolated uterus. The 0.64 i.u of oxytocin and 200 mg/ml of the extract gave the highest responses of 1.67±0.07 g and 1.37±0.09 g respectively (table 2). The contractile effect produced by 200 mg/ml of the extract was noted to be similar to that of 0.16 i.u oxytocin.

The differences in the force of contractions after each dose (for the extract) was noted not to be significant except for the first two doses ($p<0.05$) of the extract.

Co-administration of the extract and oxytocin suggests the possibility of synergy between both, as a higher contraction of the uterus was observed, than when either was administered alone.

The effect of atropine and salbutamol on the dose response curve of the extract, shows significant inhibitory effect on the contractile activity of the extract ($p<0.0001$).

Medicinal plants are used to facilitate labour. Their effects may be mediated via stimulation of muscarinic receptors in the uterine tissue. Their effects could also be through the synthesis and release of prostaglandins, which are known to be myometrial stimulants reported to mediate the activity of most drugs that stimulate contraction of the uterus (Sollof, 1953).

The possible involvement of muscarinic receptors in the extract's effect on uterine contraction is validated by the significant inhibitory effect observed when the uterus was treated with atropine before the extract. Atropine is a well known muscarinic receptor antagonist.

The uterine contractile effects of the aqueous extract of *C. lucanusianus* were also inhibited by salbutamol. This inhibitory effect was noted to be highly significant. Salbutamol displayed a higher inhibitory effect on the dose response curve of the extract in comparison with atropine. This point to a higher concentration of salbutamol-sensitive receptors. Salbutamol is known to be a selective β2 – agonist which is known to induce marked relaxation of the uterus even in dysmenorrheic women (Lalos and Joelsson, 1987).

The results suggest that the aqueous extract of *Costus lucanusianus* leaves has a potential oxytocic effect that can be explored for therapeutic advantage as an alternative treatment for retained placenta and for the induction of labour. On the basis of its acclaimed folkloric use in the treatment of threatened abortion and dysmenorrhoea, a relaxant or tocolytic like effect on the uterus was expected. However the results points to a contractile rather than a relaxant effect (Gill, 1992).

This work has thus provided a scientific basis for folkloric oxytocic effect of the aqueous extract of *C. lucanusianus* leaves.

Being a traditional herbal remedy, additional research is needed to define the constituents and bioactivity of this product (Newal *et al*., 1986).

Therefore, further studies are needed for the isolation and characterization of the active constituents in order to elucidate its mechanism(s) of contracting the uterus.

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