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Oxytocic effect of aquous, ethanolic, n-hexane and chloroform extracts of *Xylopia aethiopica (Anonaceae)* and *Ocimum gratissium (Labiate)* on guinea pig uterus

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ABSTRACT

Xylopia aethiopica and Ocimum gratissium, are commonly used as spices and flavoring agents in Southern Nigeria. Aqueous, ethanolic, N-hexane and chloroform fractions of extracts of both plants were analysed for their phytochemical composition and oxytocic effect on the uterus of female guinea pig. Acute toxicity studies of the extracts in mice gave LD50 values of 1258.92mg/kg b.w for xylopla aethiopica and 1778.3mg/kg b.w for Ocimum gratissium, suggesting low toxicity of the extracts. Both extracts, xylopia aethioplca and Ocimum gratissium showed rich content of alkaloid 0.3% and 1.47%, flavonoid 0.99% and 0..25%, tannins 46mg/100ml and 48mg/100ml, saponins 0.56% and 0.57%, and phenol 0.21% and 0.02% respectively. The N-hexane extracts of both plants showed more oxytocic activity than aqueous, ethanolic and chloroform extracts. The studied revealed that Xylopia aethiopica extracts showed more oxytocic activity on the guinea pig uterus than extracts of ocimum gratissium. The phytochemical substances in the plants may be responsible for the medicinal properties of the spices, which form the basis for their use in herbal medicine and as food additive for breast feeding women in Nigeria.

Key words: *Xylopia aethiopica, ocimum gratissium,* oxytocic bioactive compound and, food additive.

INTRODUCTION

Xylopia aethiopica Dunal A. Rich and *Ocimum grastissimum* (Labiale) are traditional dietary and medicinal herbs in Nigeria. They are mainly consumed as spice, flavourants and stimulants. Modern herbalists value these spices primarily for their anti-inflammatory, antispasmodic, febrifuge and diaphoretic properties. Fruits and seeds of *X.aethiopia* are hot to the taste and are used as stimulants and restorative after childbirth [1]. These spices are alleged to possess medicinal properties and are used to obtain relief from gripping conditions of the stomach after delivery [1].

Xylopia aethiopica and *Ocimum gratissimum* are used to prepare soups, which exhibit hot and spicy taste and are consumed during cold season. Fruits and seeds of *X. aethiopica* are sometime added to food meant for pregnant and nursing mothers as medicinal spices. It is also claimed that these spices and herbs assist in the contraction of the uterus in post-partum women [2]. It is generally assumed that the active dietary constituents contributing to these medicinal properties exhibited by herbs and spices are the phytochemicals, vitamins and minerals [2].

Ocimum gratissimum is a herb and also a spice which grows to about six feet high with an erect stem. The whole herb but most commonly the leaves are used in herbal medicine. Inhaled aroma from hot leaf preparation is believed to heal cold and catarrh. The Juice is used to treat stomach pain and sometimes applied with the leave to piles [3]. *O. gratissimum* has been a valuable source of essential oils. The components include non-cyclic sesquiterpenes, phenols, carbohydrate and lipids [3]. The Isolated compounds in the oil are eugenol, thymol, a-pinene, camphor and tarpinene, [4].

xylopia aethiopica (Dunal) A Rich (Anonaceae) is found in low land rain forest and fringing forest in the savanna zones [5]. The seed has a peppery taste while the fruit is used as a condiment [4]. The main chemical constituents isolated from *X. aethiopica* comprises mainly *xylopic* acid (15β-acetoxy(-) Kauran-16-ene-19-oic acid; three diterpenic alcohols, one of them identified as Kauran-16 a-ol, 4-diterpenic acids, fats and essential oils [4]. Essentials oils enhance the aroma of foods. The various acids are responsible for the hot taste, which characterize *X aethiopica*.

In spite of the various uses of these plants in food and herbal medicine in Nigeria, their phytochemical constituents have not been fully documented. As part of a research programme on antispasmodic and oxytocic plants used in Igbo folk medicine, we report our study on chemical composition and oxytocic activity of the fruits of *xylopia aethiopica* and leaves of *Ocimum gratissimum.*

MATERIALS AND METHODS

Sample collection: The fruits of *xylopia aethiopica* were purchased from Umuahia main market, Local Government Area of Abia State Nigeria while the leaves of *Ocimum gratissimum* were collected from a farmland in Umudike, Abia State. Oxytocin drug was purchased from a reputable Pharmaceutical shop in Abia state, Nigeria.

Sample preparation: Ripe fruits of *Xylopia aethiopica* and fresh leaves of *Ocimum gratissimum* were each weighed (1kg). The dried plant samples were grounded into uniform powder using Thomas-Wey machine and stored in airtight bottles.

Animal: Guinea pig weighing between 250-100g gotten from animal house of the Department Veterinary Pharmacology, College of Veterinary Medicine Michael Okpara University of Agriculture Umudike Abia state were used. All the animals were kept in the animal house at ambient temperature. They were fed with standard diets (Ptizer feeds Plc Lagos), and water was supplied freely.

Quantitative determination of chemical constituents

Preparation of fat free sample: Two (2g) of the sample was de-fatted with 100ml of diethylene using a soxhlet apparatus for 2 hrs.

Alkaloid determination: Five (5g) of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered to stand for 4 hrs. 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 precipitation was complete. The whole solution was allowed to settle and the decimate was collected and washed with diluted ammonium hydroxide solution and then filtered. The residue which was taken as the crude alkaloid was weighed [6].

Determination of total phenols by Spectrophotometric method

The fat free sample was boiled with 50ml of ether for 15minutes for the extraction of the phenolic component. A 5ml portion of the extract was pipette with 50ml flask, then 10ml of distilled water was added, 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were added. The samples were made up to mark and left for 30mins for color development. The absorbance of the solution was read at 505nm wavelengths using a spectrophotometer [6,7].

Tannin Determination: A 500mg of the sample was weighed into 100ml plastic bottle.A 50ml of distilled water was added and shaken for 2 hrs in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up the mark. Then 5ml of the filtrate was pipette out into a tube and mixed with 3ml of 0.1M $FeCI_2$ in 0.1N HCI and 0.008M potassium ferocyanide. The absorbance was measured in a spectrophotometer at 120nm wavelength within

10mins. A blank sample was prepared and the color also developed and read at the same wavelength. A standard was prepared using tannin acid and obtained 100ppm measurement. [8].

Saponin determination:A 20g of each plant sample were dispersed in 200ml of 20% ethanol. The suspension was healed over a hot water bath for 4hrs with continuous stirring at 55^0C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over the water bath at 90^0C.

Tthe concentrate was transferred into a 250ml separator funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discharged. The purification process was repeated; 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 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 saponin content was calculated in percentage [7].

Flavonoid determination: A 10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper, no 42 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight [9].

Extraction and isolation: Ground dried fruits of *Xylopia aethiopica* and leaves of *Ocimum gratissimum* (4kg) each was refluxed with ethanol in a Soxhlet apparatus for 4 days.

The extract was concentrated to dryness (50g X*xylopia aethiopica* and 50g *O.gratissumum*) and the residue was successively extracted with aqueous, ethanol, N-hexane and chloroform at room temperature. All the extracts were used for the evaluation of the oxytocic activities.

Determination of lethal dose (LD50) (acute toxicity testing)
The acute toxicity of the plants extracts were tested on the mice using the method and calculation proposed by Lorke 1983[10].

Different doses of the plants extracts base on body weight of the anninals were administered intraperitionially into the animal in seven groups, each group received 250, 500, 750, 1200, 1500 and 2000mg/kg body weight respectively. The animal were monitored for the next three hours and then examined after 24 hours for mortality.

Oxytocic assays: Uterus; from young virgin guinea pig was used. The guinea pig was killed with a blow on the head followed by cutting of the carotid artery. A piece of the uterus was set up in the organ bath of 14ml capacity containing tyrodes physiological solution [11] aerated with a mixture of oxygen (95%) and carbon dioxide (5%) maintained at 37^0C. Increasing aliquots of standard solution of 0.0251µml of oxytocin (Syntocinon® Laboratorio Sandoz, Santiago, Chile) were added to the organ bath and was rinsed three time with tyrode solution and the tissues allowed to rest for 3min before suspended in aqueous aliquots of aqueous, ethanol, n-hexane and chloroform extracts of different dose (concentration),

Statistical analysis
This was done by using combination of Statistical procedure. All the valves were represented as mean LSD. Analysis of variana (ANOVA) was used to test for the differences among all the groups at $P<0.05$. Excel graphic plot was used for the bar charts.

RESULTS

The results of phytochemical confirm the presence of alkaloids, flavonoids, tannins, saponins and phenol in both *Xylopia aethiopica* and *Ocimum gratissium* (Table 1).

The concentration of flavonoids was higher in *Xylopia aethiopica* (0.99%) than in *Ocimum* gratissium which contains 0.25% of flavonoid. *Ocimum gratissium* contained more saponin (0.57%) and tannins (48.0mg/100mg) than *Xylopia aethiopica* which contained 56% of saponin and 46mg/100ml of tannins. Phenol was higher in *Xylopia*

aethiopica (0.21%) than *Ocimum gratissium* which contained 0.02% of phenol. Alkaloid was found to be higher in *Ocimum gratissium* (1.4%) than *Xylopia aethiopica* which contained 0.36% of alkaloid. (Table 2).

Table1: Result of Phytochemical Analysis Qualitative Analysis of the Phytochemical content of the extracts

Phytochemical	*Xylopia aethiopica*	*Ocimum gratissimum*
Alkaloid	++	+
Tannins	+	+
Flavonoids	+	+
Phenol	+	+
Saponin	+	+

Where + = Present

Table 2: Quantitative Analysis of the Phytochemical Content of the Extracts

Phytochemical content	*Xylopia aethiopica*	Ocimum gratissimum
Alkaloid	0.36 ± 0.007 **%**	1.4 ± 0.07**%**
Tannins	46.08 ± 0.28mg/100lm	48 ± 0.28mg/100ml
Flavonoids	0.99 ± 0.01**%**	0.25 ± 0.01 **%**
Phenol	02.21 ± 0.01 **%**	0.02 ± .02 **%**
Saponin	0.56 ± 0.03 **%**	0.57 ± 0.01**%**

Table 3: Effect of Oxytocin* on the Guinea Pig uterus

Dose of Oxytocin drug (mg / ml)	Log (dose)	Response (cm)	Response (%)
5	0.7	1.20 ± 0.07	12.6
10	1.0	3.50 ± 28	36.8
20	1.3	4.60 ± .14	48.4
40	1.6	6.80 ± 1.4	71.6
60	1.7	8.0 ±. .32	84.2
80	1.9	9.40 ± 60	98.9
100	2.0	9.41 ± 0.07	99.1
120	2.1	9.50 ± .31	100

Table 4: Contraction Amplitude (CM) of various extracts of *Xylopia aethiopica*

Concentration of extract mg x ml	Aqueous extract	Ethanolic extracts	N-hexane extracts	Chloroform extracts
100	2.6 ± 0.06	2.9 ± 0.07	3.20 ± .14	2.10 ± .14
200	3.9 ± 0.15	4.0 ± 0.05	4.6 ± .07	3.30 ± .07
300	5.6 ± 0.20	5.6 ± 0.15	6.4 ± .15	4.9 ± .07
400	10.10 ± 0.30	10.30 ± 0.6	11.30 ± .12	9.8 ± .28
500	12.4 ± .12	13.10 ± .27	12.8 ± .28	11.10 ± .61
600	13.0 ± .12	13.10 ± .27	13.40 ± .28	11.80 ± .07
700	13.8 ± 0.07	13.8 ± .61	13.80± .61	11.90 ± .28
800	14.0 ± 0.07	14.2 ± .07	14.0 ± .07	11.90 ± .28

Table 5: Contraction Amplitude (CM) of Various Extracts of *Ocimum gratissimum*

Concentration of extract mg x ml	Aqueous extract	Ethanolic extracts	N-hexane extracts	Chloroform extracts
100	0.5 ± 0.1	0.5 ± .20	0.8 ± .07	0.3 ± 15
200	0.9 ± 0.1	1.10 ± .10	1.6 ± .15	1.80 ± .07
300	2.2 ± .28	2.4 ± .14	2.6 ± .12	1.80 ± .07
400	3.8 ± 0.7	4.10 ± 0.16	4.40 ± .28	3.0 ± .07
500	4.10 ± 0.14	4.6 ± .12	4.50 ± .31	3.8 ± .12
600	4.4 ± 0.1	4.8 ± .07	4.60 ± .60	4.0 ± .07
700	4.8 ± 0.15	5.6 ±.07	4.70 ± .28	4.10 ± .01
800	5.6 ± .21	14.2 ± .07	5.4 ± .07	4.11 ± .21

The acute toxicity showed that *Xylopia aethiopica* had an LD_{50} of about 1258-9mg/kg body weight and *Ocimum gratissium* had an LD_{50} of about 1778.30mg/kg b.w . Table 4 and 5 show that the N-hexane extracts of both *Xylopia aethiopica* and *Ocimum gratissium* exhibit more oxytocic activity than aqueous, ethanolic and chloroform extracts respectively.These activities were compared with the result obtained from standard drug (oxytocin)-Table 3.

In all the extracts, *Xylopia aethiopica* showed more Oxytocic activity than ocimum gratissium extracts (Table 4 and 5). The result shows that the uterine contraction increases with increased in the concentration of the extracts.

DISCUSSION

As indicated in Table 1 and 2, the spices are rich in phytonutrients such as flavonoids, phenolic compounds, tannins, saponins and alkaloids. The biological functions of flavonoids include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors (12). Flavonoids reduce the risk of estrogen-induced cancers by interfering with the enzymes that produce estrogen, for example, flavonoids inhibit estrogen synthetase, an enzyme that binds estrogen receptors in several organs (12). These flavonoids significantly inhibit lysosomal secretion and arachidonic acid release from membranes by ssinhibiting lipoxygenase, cyclooxygenase and phospholipase A_2 [13].

The inhibition of arachidonic acid release in the inflamed cells would provide less arachidonic substrate for the lipoxygenase and cyclooxygenase pathways. This however leads to a lesser quantity of endoperoxides, prostaglandins, prostacyline and thromboxanes as well as hydroperoxy, hydroxycicosatrienoic acids and leucotrienes [16]. Such an effect confirms the decrease in histamine which is known to act in the first stage of the inflammatory process [14].

Prostaglandins can act to regulate menstruation, prevent conception, induce child birth or abortion, lower blood clothing and possible even act as decongestants [15]. However, some flavonoids behave as a powerful protective agent against inflammatory disorders. They reduce edema formation and inhibit the synthesis of prostaglandin E_2. Prostaglandin F_2 and thromboxane B_2 [13,16]. As a result of the availability of flavonoid in these spices, they prevent platelet stickiness and hence platelet aggregation. Moreover, the spices protect the vascular system and strengthen the tiny capillaries that carry oxygen and essential nutrients to all cells.

The presence of phenol indicates that the spices could act as anti-inflammatory, anti-clothing, antioxidant, immune enhancers and hormone modulators. Phenols have been the subject of extensive research as disease preventives [17]. Phenols have been responsible in having the ability to block specific enzymes that cause inflammation. They also modify the prostaglandin pathways and thereby protect platelets from clumping [16].

Some alkaloids contained in the plants- *X aethiopical* and *O gratissium* are used a spasmolytic, anticholinergic and anaesthetic agents [18]. The hexane aqueous extract and chloroform extracts of *O. gratissium* and *X. aethiopica* showed uterine contraction activity. These plants produce contractions 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 labour[19]. Therefore, these plants are used in herbal medicine to accelerate labour in south eastern Nigeria. However, if used during the first months of pregnancy, they could have abortifacient properties.

Xylopia aethiopica extracts exhibited more uterine contraction on the guinea pig than *Ocimum gratissimum.* This may be due to high flavonoids and phenolic content of *X. aethiopic*. If these spices are administered in high does, they prepare the uterus and ensures that fatigue disappears, producing strong, regular contraction to facilitate labour during the last month of pregnancy[20]. From these findings, pregnant women should avoid the regular consumption of these spices in the first trimester as their consumption may likely result in uterine contraction and consequently miscarriage. However, the use of these spices in preparing food for pregnant women ready for birth as well as nursing mothers should be encouraged.

CONCLUSION

In conclusion, it is believed that they strengthen and heal the wall of the uterus [21,22]. These plants contribute to the hormonal balance of the female body thus improving the mental state and providing a sense of well being

[23,24,25]. This study demonstrates that *Xylopia aethiopica* and *Ocimum gratissimum* possesses oxytocic activities. These findings justify the traditional use of these plants to induce uterine contraction in traditional medical practice.

REFERENCES

[1] Ojimelukwe, P.C.; Okoro O. and Madubuike, F.N. **2000** Bactericidal, fungicidal and insecticidal Properties of four Nigeria spices in food and fiber production in Nigeria. L.C. Nwigbo, H.C. Ukpabi and A. Anene Edins, Proceedings. College of Agric Vet medicine Abia State University Uturu, Pp 415-416.
[2] Okwu, D. E. and Ekeke O. **2003** Phytochemical composition of chewing sticks in South Eastern Nigeria. Global *Journal of Pure and Applied Sciences* 9: 235-238.
[3] Esvanszhuga, G. **1986** The chemical constituents of Ocimum gratissimum. Doki Tskha 24(2): 72-77.
[4] Oliver-Bever B. **1986** Medicinal Plants in Tropical West Africa Cambridge University Press, Cambridge, Pp 123-125.
[5] Keay, R.W. J **1980.** Trees of Nigeria. Pp. 19-30.
[6] Harborne, J.B. **1973** Phytochemical Methods. Chapman and Hall London Pp 110-113.
[7] Obadori, B.O. and Ochuko P.O. **2001** *Global Journal of Pure and Applied Sciences* 8:203-208.
[8] Van-Burden, T.P and Robinson W.C**. 1981**. *J. Agric Food Chem.* 1: 77-82.
[9] Boham, B.A. and Kocipia, A.C. **1974** *Pacific Science* 48: 458-463.
[10]L0rke, D **1983**, *Arch.Toxicol*. 54: 275-287.
[11] Elangovar, V Sekar N., Gorindasamy S. **1994** *Cancer Letters*: 87 107-113.
[12] Farquar, J.N **1996** Plant sterols; their biological effects in humans. Handbook of lipids in Human Nutrition. BOCA Rotan FL) CRC Press Pp. 101-105.
[13] Del-Rio A; Obduilo, B.G. Catillo, J. Marin, F.P and Ortuno A. **1997**, *J. Agric Food Chem*. 45: 4505-4515.
[14] Middleton, E. Drzewiwck and Krishnarao D. **1981**, *J. immunol* 127 : 546-550.
[15] Reusch W.H. **1977** An Introduction to Organic Chemistry. Holden Dan Inc. San Francisco Pp. 299-318.
[16] Gabor, M **1988**. Anti-inflammatory and anti-allergic properties of flavonoids in plant flavonoids in biology and medicine: Biochemical, pharmacological and structure activity relationship E. Harborne J.B. Beretz A Eds progress in Clinical and Biological Research 213: Alan, R. Liss Inc, New York Pp. 471-480.
[17] Duke, J. **1992** Handbook of Biological active phtochemicals and their activities. BOCA Raton (FL); CRC press Pp. 99, 131.
[18] Amokaha, R.A., Ubwa, S.T. Otokpa, Mamd Shenge. G. **2002**. *J Chem Soc. Nigeria* 27: 105-106.
[19] Roger P.G.D **2002**. Encyclopedia of Medicinal Plants: Education and health Lobrany, editoral SAPELIZ, Spain Pp 101-102.
[20] Galati, E. M. Monforte M.T. Kirijavainem, S. Forestier A.M. **1994** *IL Farmaco* 40:709-712.
[21]Okwu, D.E. **2003**. *Agric J.* 34:143-148.
[22] Uchida S. **1987.** *Med. Sci. Res:* 15:851-832.
[23] Close, D.C. and McArthur, C. **2002.** Rethinking the role of many plants phenolics protection From photodamage, Oikos 99: 166-172.
[24] Akagi, K. **1995**. *Cancer letters* 94: 113-121.
[25] Erazo S., Mumoz O. Garcfa R. Lemns I and Backhouse W. **2002** Constituents and Biological activities from muchlenbeckia ha. N. Naturforsch 57C: 801-804.