Effects of Hexane Extract and Phytochemical Content of *Xylopia aethiopica* and *Ocimum gratissimum* on the Uterus of Guinea Pig

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Abstract

Xylopia aethiopica and Ocimum gratissimum, which are commonly used as spices and flavoring agents in Southern Nigeria, were found to have oxytocic properties in guinea pig. Phytochemical studies on the spices revealed the presence of bioactive compounds such as flavonoids (0.22-0.85%), tannins (0.08-0.54%), alkaloids (0.25-1.32%), phenoles (0.02-0.19%) and saponins (0.03-0.52%). These substances may be responsible for the medicinal properties of the spices, which form the basis of their use in herbal medicine, and as food additive for breast-feeding women in Nigeria.

Keywords: *Xylopia aethiopica, Ocimum gratissimum,* Bioactive compounds, Oxytocic, Food additive.

Introduction

Xylopia aethiopica Dunal A. Rich and *Ocimum gratissimum* (Labiate) 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. aethiopica* are hot to the taste and are used as stimulants and restorative after childbirth (Ojimelukwe *et al,* 2000). These spices are alleged to possess medicinal properties and are used to obtain relief from gripping conditions of the stomach after delivery (Ojimelukwe *et al,* 2000).

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 sometimes added to food meant for pregnant and nursing mothers as medicinal spices (Okwu, 1999, 2001a, 2003, 2004). It is also claimed (Okwu, 2001a, 2001b, 2003) that spices and herbs assist in the contraction of the uterus in post-partum women. 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 (Okwu and Ekeke, 2003; Okwu 2004).

Phytocemical constitute one of the most numerous and widely distributed groups of substances in the plant kingdom. Woody plants synthesize and accumulate in their cells a great variety of phytochemicals including low molecular phenolics (hydroxybenzoie and hydroxycinnamic acids, acetophenone, flavonoids, stilbenes and lignans) and oligo and polymeric forms (hydrolysable and condensed tannins and lignins) (Close and McArthur, 2002).

Phytochemicals exhibit a wide range of biological effects as a consequence of their antioxidant properties (Okwu, 2004). It have been reported that fruits are rich in a group of flavonoids particularly proanthocyanidins, or condensed tannins (Uchida, 1987). Researchers have identified about 250 different proanthocyanidins in plants, and as a group, they constitute one of the 12 subcategories of flavonoids. They are even part of a larger class of chemicals known as polyphenols (Uchida, 1987). Along with their common antioxidant properties, flavonoids display some striking medicinal functions. Some inhibit tumor growth by preventing angiogensis (Uchida, 1987). Flavonoids like proanthocyanidins have been used in treating and preventing circulatory disorders (Zafirov, 1990; Delacripis, 1981). These researchers reported that flavonoids strengthened blood vessel's wall and reduced capillary permeability in ways that vitamins C did not.

Research on the circulatory benefits of proanthocynidines is well established. These nutrients strengthen both collagen and elastin, which are necessarily for tissue to hold together (Gabor, 1988). As antioxidants, they also neutralize free radicals involved in the break down of tissue and blood vessel walls (Gabor, 1988). Also, flavonoids and polyphenols, genistein and daidzein appear to reduce the risk of prostate and breast cancers. Elegiac acid is a potent antioxidant and cancer inhibitor (Akagi, 1995). The broad chemical reactivity of phytochemicals particularly flavonoids and phenolic compounds arise from the transformation they undergo in the presence of enzymes under different conditions, (Galati *et al,* 1994; Close and McArthur 2002). Phytochemicals as antioxidants play vital roles in human health (Del-Rio *et al,* 199; Elangovan *et al,* 1994; Close and McArthur, 2000; Okwu 2004).

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 to piles with the leaves (Esvanszhuga, 1986). *O. gratissimum* has been a valuable source of essential oils. The component includes non-cyclic sesquiterpenes, phenols, carbohydrates and lipids (Esvanszhuga, 1986). The isolated compounds in the oil include eugenol,

thymol, α -pinene, camphor and tarpinene, (Oliver-Bever, 1986).

Xylopia aethiopica (Dunal) A Rich (Anonaceae) is found in low land rain forest and fringing forest in the savanna zones (Keay, 1989). The seed has a peppery taste while the fruit is used as a condiment (Oliver-Bever, 1986; Okwu, 2001a, 2004). The main chemical constituents isolated from *X. aethiopica* comprises mainly xylopic acid (15 β-acetoxy (-) Kauran-16-ene-19-oic acid; three diterpenic alcohols, one of them identified as Kauran-16- α -ol, 4-diterpenic acids, fats and essential oils (Oliver-Bever, 1986; Okwu 2001a, 2004). Essentials oils enhance the aroma of foods. The various acids are responsible for the taste or hot taste, which characterize *X. aethiopica* (Okwu, 1999, 2001a, 2004).

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 pharmacological activities of the fruits of *Xylopia aethiopica* and leaves of *Ocimum gratissimum*.

Materials and Method

Sample collection: The fruits of *Xylopia aethiopica* were purchased from Ariam market, Ikwuano Local Government Area of Abia State, Nigeria on 20th April 2003; while the leaves of *Ocimum gratissimum* were collected from a cultivated farmland in Umudike, Abia State. Oxytocin drug was purchased at Awka, Anambra State, Nigeria.

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

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 hrs.

Alkaloid 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 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 the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide solution and then filtered. The residue which was taken as the crude alkaloid was weighed (Harborne, 1973).

Determination of total phenols by Spectrophotometric method

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 mins. 5 ml of the extract was pipette with 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol was added. The samples were made up to mark and left to react for 30 mins for color development. The absorbance of the solution was read at 505 nm-wavelengths using a spectrophotometer (Harborne 1973; Obadoni and Ochuko, 2001).

Tannin determination: 500 mg of the sample was weighed into 100 ml plastic bottle. 50 ml of distilled water was added and shaken for 2 hrs in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out into a tube and mixed with 3 ml of 0.1M $FeCl_2$ in 0.1-N HCl and 0.008M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120 nm wavelength within 10 mins. 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 100 ppm measurement (Van-Burden and Robinson, (1981).

Saponin determination: 20 g of each plant sample were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 hrs with continuous stirring at about 55°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°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 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 (Obadoni and Ochuko, 2001).

Flavonoid determination: 10 g of the plant sample was 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 a water bath and weighed to a constant weight (Boham and Kocipia, 1974).

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

Table 1: Phytochemical content of *Xylopia aethiopica* and *Ocimum gratissimum* on dry weight basis (%)

Phytochemical	*O. gratissimum*	*X. aethiopica*
Tannins %	0.54 ± 0.02	0.08 ± 0.10
Alkaloids %	1.32 ± 0.01	0.25 ± 0.10
Flavonoids %	0.22 ± 0.01	0.85 ± 0.20
Phenols %	0.02 ± 0.20	0.19 ± 0.20
Saponins %	0.52 ± 0.10	0.03 ± 0.10

Data are means of triplicate determination ± standard deviations.

Table 2: Effects of *Xylopia aethiopica, Ocimum gratissimum* and oxytocin on the uterus of guinea pig

Concentration µl/ml	*Xylopia aethiopica* Height of uterine contraction. (cm)	*Ocimum gratissimum* Height of uterine contraction (cm)	Oxytocin drug Height of uterine contraction (cm)
20	2.0	0.01	3.8
40	3.10	0.04	5.0
60	5.60	0.6	8.6
80	7.20	0.09	11.7
100	7.80	0.04	13.2
200	11.20	1.90	15.00

The extract was concentrated to dryness (525g *X. aethiopica* and 135 g *O. gratissimum*) and the residue was successively extracted with hexane to yield 58 g *X. aethiopica* and 46. 1 g *O. gratissimum*). The hexane extract was dried and dissolved in ethanol at room temperature. Both ethanol and indissoluble hexane extract were used for the evaluation of the oxytocic activities.

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 14 ml capacity containing tyrodes physiological solution (Laurence *et al*, 1964) aerated with a mixture of oxygen (95%) and carbon dioxide (5%) maintained at 37°C. Increasing aliquots of standard solution of 0.0251 µl/ml of oxytocin (Syntocinon ® Laboratorio Sandoz, Santiago, Chile) were added to the organ bath and contraction changes were weighed on a kymograph. (Erazo *et al*, 2002). The organ bath was rinsed three times with tyrode and the tissues allowed to rest for 3 min before suspended aqueous aliquots of dried hexane extracts 15 mg/100 ml were added.

Results

Xylopia aethiopica and *Ocimum gratissimum* contain phytonutrients, which have significant positive effects on human metabolisms (Table 1). The concentration of flavonoids was higher in *X. aethiopica* (0.85%) than in *O. gratissimum* which contains 0.22% of flavonoid. *Ocimum gratissimum* contained more Saponins (0.52%) and tannins (0.54%) than *X aethiopica* which contained 0.03% of saponins and 0.08% of tannins. The concentration of phenols was higher in *X. aethiopica* (0.19%) than *O. gratissimum* which contained 0.02% of phenol.

The hexane extract of *Xylopia aethiopica* and *Ocimum gratissimum* showed uterine contraction activity. *X. aethiopica* had activity comparable to that of oxytocin (Table 2). *Xylopia aethiopica* extract exhibited more uterine contraction in the guinea pig than *Ocimum gratissimum*. The uterine contraction increases as the concentration of both oxytocin and spices increases.

Discussion

As indicated in Table 1, 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 (Farquar, 1996 and Okwu, 2004). 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 (Farquar, 1996). These flavonoids significantly inhibit lysosomal enzyme secretion and arachidonic acid release from membranes by inhibiting lipoxygenase, cycloxygenase and phospolipase A_2 (Del-Rio *et al* 1997).

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 (Gabor, 1986). Such an effect confirms the decrease in histamine which is known to act in the first stage of the inflammatory process (Middleton, 1986).

Prostaglandins can act to regulate menstruation, prevent conception, induce child birth or abortion, lower blood clothing and possibly even act as decongestants (Reuseh, 1977). 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 (Del-Rio *et al*, 1997). As a result of the availability of flavonoids 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-clotting, antioxidant, immune enhancers and hormone modulators. Phenols have been the subject of extensive research as disease preventives (Duke, 1992). 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 (Duke 1992).

Some alkaloids contained in the plant is used as spasmolytic, anticholinergic and anaesthetic agents (Amokaha *et al*, 2002).

The hexane extract of *O. gratissimum* 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 (Roger, 2002). 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 extract exhibited more uterine contraction on the guinea pig than *Ocimum gratissimum*. This may be due to high flavonoids and phenolic content of *X. aethiopica*. 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.

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.

It is believe that they strengthen and heal the wall of the uterus (Okwu, 2003). These plants contribute to the hormonal balance of the female body thus improving the mental state and providing a sense of well being.

This study demonstrates that *Xylopia aethiopica* and *Ocimum gratissimum* possesses oxytocic and anti-inflammatory activities. These findings justify the traditional use of these plants to induce uterine contraction in traditional medical practice.

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