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Full Length Research Paper

Investigation of the chemical composition and biological activity of *Xylopia aethiopica* Dunal (Annonacae)

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The phytochemical composition and physicochemical properties of oil extractable from the fruits of *Xylopia aethiopica* were determined. Extracts' effects on cell membrane stability and prostaglandin synthetase activity were also evaluated. *X. aethiopica* oil extracted with chloroform: methanol (2:1, v/v) mixture contained carbohydrates, glycosides, flavonoids, saponins, tannins and phytosterols. The characteristic volatile and sweet smelling nature of *X. aethiopica* was predominantly inherent in this fraction. The sterol content was 64.30 mg/100 ml; with a high degree of unsaturatedness as evident in its high iodine value (85.76). High pressure liquid chromatographic analysis of the lipid extract revealed a fatty acid profile of palmitic acid (19.21%), palmitoleic acid (0.81%), stearic acid (4.54%), oleic acid (39.12%), linoleic acid (25.98%) and linolenic acid (1.10%). Investigation of the effect of the extract on hypotonicity- induced haemolysis of human red blood cells produced by water showed that the methanol extract of *X. aethiopica* (XAME) stabilized the red blood cells against the haemolytic action of distilled water. The lipid extract, on the other hand did not show any protective action against the osmotic shock. *Xylopia aethiopica* fruits may therefore be helpful in the maintenance of the integrity of the cellular membranes. The lipid extract also, *in vitro*, exhibited a prostaglandin synthetase substrate activity, whereas the methanol extract enhanced the synthesis of prostaglandins using *X. aethiopica* oil as substrate. The presence of appreciable quantity of unsaturated fatty acids, stabilization of the cellular membrane integrity, promotion of the biosynthesis of the hormone-like substances, prostaglandins, may be responsible for the usefulness of *X. aethiopica* fruits in the healing of wounds, inflammatory disorders and treatment of post-natal pains.

Key words: *Xylopia*, linoleic acid, membranes, haemolysis, pains.

INTRODUCTION

The aromatic plant *Xylopia aethiopica* Dunal (Annonacae), commonly known as Ethiopia or Negro pepper has been used in Europe, Asia and Africa as pepper substitute and spice in local cooking. Various parts of the plant have been traditionally employed in different therapeutic preparations. Sometimes, a combination of *X. aethiopica* with other plant types or a combination of different parts of *X. aethiopica* is used to achieve the desired effects (Fall *et al*., 2003; Ogunkunle and Ladejobi, 2006). In Nigeria, *X. aethiopica* in combination with the roots of *Strychos inogia*, *Gardenia tennifolia*, *Uvaria chamae*, *and Annona senegalensis*, serves as a remedy for stomach ache and coughs. The sauce is usually given to women after delivery to relieve pains, promote healing and lactation.

Preliminary studies have shown that *X. aethiopica* fruits contain pharmaceutical constituents such as alkaloids, tannins and flavonoids. The essential oil from various parts of *X. aethiopica* has also been well characterized (Kouninki *et al*., 2005; Kouninki *et al*., 2007). Several plant lipids have been reported to enhance healing from diverse ailments due to their antioxidant and anti-inflammatory

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properties (Azeb *et al*., 2004; Motrin, 2005). In the present study, we report the physicochemical analysis and further characterization of the oils of *X. aethiopica*. The effects of this plant oil on membrane stabilization and prostaglandin synthase activity were also studied to provide an insight into its action on the inflammatory response which has been implicated in the pathogenesis of many disorders as well as the healing process.

MATERIALS AND METHODS

Materials

Dried fruits of *X. aethiopica* were collected in July, 2006 from the local market at Nsukka, Enugu State, Nigeria. It was identified and authenticated at the International Centre for Drug Development, Nsukka (INTERCEDD) Human blood samples were obtained by venipuncture from healthy male volunteers who had not taken any drug for one week.

Experimental methods

Dried fruits of *X. aethiopica* (100 g) were soaked in 500 ml of chloroform: methanol mixture (2:1) at 28°C for 24 h. The extract was filtered using Whatman no. 1 filter paper and separated into two fractions on addition of 0.2 vol. of distilled water. The lower chloroform layer containing the lipids was washed severally with distilled water and the oil obtained following evaporation of the solvent *in vacuo* to give a yellowish-brown sweet smelling oily extract (16.3% yield). The upper methanol layer was also concentrated to yield the methanol extract, MEXA (4.8%). The physicochemical properties of the oil were determined immediately by the AOAC methods (1975).

Phytochemical screening was carried out according to established procedures by Sofowora (1980) and Cuiled (1982) for the presence of alkaloids, flavonoids, saponins, tannins, glycosides, sterols and carbohydrates.

Quantitative determination of the plant sterol was by Bassie (1975). Fatty acid profile was obtained using a high pressure liquid chromatography.

The effects of the oil extract, XAOE and methanol extract, XAME, on erythrocyte membrane stabilization was investigated using hypotonicity-induced haemolysis of human erythrocytes as a model (Ezekwesili and Nwodo, 2000).

The effect of the *X. aethiopica* oil on prostaglandin synthetase activity was determined *in vitro* by a modification of the method of Yoshimoto *et al*. (1970). In this method, the ability of XAOE to serve as a substrate for the biological synthesis of prostaglandin E_2 was investigated by substituting XAOE (0.10 ml) for the arachidonic acid substrate. The enzyme activity in the presence of XAME and the reference drug, indomethacin (a non-steroidal anti-inflammatory agent known to inhibit prostaglandin synthetase activity) was also evaluated.

Statistical analysis

Statistical evaluation was done using the ANOVA test.

RESULTS

The physicochemical characteristic of the *X. aethiopica* oil, XAOE, are reported in Table 1. The refractive index and relative density were low whereas acid value, iodine value, saponification number, peroxide value, unsaponifiable matter and plant sterols were high. The results of phytochemical analyses are presented in Table 2. XAOE contains carbohydrates, glycosides, cyanogenetic glycosides, flavonoids, saponins, tannins and sterols. Table 3 shows the fatty acid composition of XAOE as given by HPLC. Predominant fatty acids in the extract are oleic acid, linoleic acid and palmitic acid. *X. aethiopica* oil effectively served as a substrate for the synthesis of prostaglandin E_2 by prostaglandin synthetase. Eighty percent (80%) inhibition of prostaglandin synthetase activity was recorded in the presence of indomethacin. On the other hand, XAME enhanced the activity of this enzyme in a dose-dependent fashion (Table 4).

When *X. aethiopica* extracts were tested for stabilization of human erythrocyte membrane, results clearly revealed that the methanol extract, XAME, significantly ($p < 0.001$) protected the red blood cells against osmotic shock. Concentration-dependent increase in percentage prevention of haemolysis from 3.41 to 72.73% was noted.

On the other hand, the oil did not stabilize the intactness of the erythrocyte membrane (Table 5).

DISCUSSION

The fruits of *X. aethiopica* yielded 16.30% of a sweet smelling oil. Phytochemical screening of the oil indicated the presence of plant sterols and the phenolic compounds such as flavonoids, tannins and the saponins. These phenolic substances as well as the alkaloids in plants have been listed as the most important bioactive constituents of natural products (Edeoga *et al*., 2005) which are valuable supplements used for the maintenance of human health (Kumar *et al*., 2005) and sometimes possessing remarkable therapeutic potentials. Phytosterols, on the other hand, include such plant sterols as sitosterol, stigmasterol and campesterol, and these plant sterols have been reported to have lowering effects on blood cholesterol of humans, and experimental animals (Law, 2000; Ostlund, 2002).

Results presented in Table 1 show that *X. aethiopica* fruit oil contains 64.30 mg/100 ml of plant sterols which are, therefore, less atherogenic than animal cholesterol. Further investigation into the physicochemical properties of the oil revealed that it has a high degree of unsaturation in the fatty acid component as is evident in its high iodine value which is a measure of unsaturatedness (Table 1). This observation was confirmed from the fatty acid profile of the oil given by high pressure liquid chromatographic technique (Table 3). The oil contained high percentage of oleic acid (39.12%), linoleic acid (25.98%), with linolenic acid (w-3 fatty acid) accounting for 1.10% of the oil content. The high concentration of

Table 1. Physicochemical properties of *Xylopia aethiopica* oil, XAOE.

State at room temperature	Liquid
Colour	Deep green
Odour	Sweet smell
Refractive index	1.500
Relative density	1.043
Acid Value (mg KOH/g)	15.980
Iodine Value (wijs)	85.760
Saponification Number (mg KOH/g)	198.200
Peroxide value (mEq/kg)	15.800
Unsaponifiable matter (g/kg)	17.800
Sterol content (100 ml^{-1})	64.300

Table 2. Phytochemical constituents of the oil extract of *Xylopia aethiopica*, XAOE.

Phytochemical test	Result
Alkaloids	-ve
Carbohydrates	+ve
Anthracene glycosides	-ve
Cardiotonic glycosides	-ve
Cyanogenetic glycosides	+ve
Flavonoids	+ve
Saponins	+ve
Tannins	+ve
Sterols	+ve

+ = Present; - = absent or not detected.

Table 3. Fatty acid profile of *Xylopia aethiopica* oil as given by HPLC.

Fatty acid	Percentage by weight
C_{16},0 Palmitic acid	19.21
C_{16},1 Palmitoleic acid	0.81
C_{18},0 Stearic acid	4.54
C_{18}, 1 Oleic acid	39.12
C_{18},2 Linoleic acid	25.98
C_{18},3 Linolenic acid	1.10

monounsaturated fatty acids in *X. aethiopica* oil may be beneficial for the heart, as these compounds have been found to inhibit the heart- damaging oxidation of low-density-lipoprotein (LDL) cholesterol. In addition, detection of high level of w-6 fatty acid is a significant finding in this study since both omega-3 and omega-6 fatty acids are important not just in lowering the triacylglycerol level in human system but are essential precursors for the biological synthesis of the prostaglandins in a reaction catalyzed by prostaglandin synthetase (Luisa, 2007). These hormone-like substances possess a variety of physiological and pharmacological properties which include smooth muscle contraction, a biochemical event implicated in the expulsion of the placental debris after delivery in women and healing of wounds.

When the effects of the *X. aethiopica* extracts on prosta-

Table 4. Effect of dried *X. aethopica* fruit extracts on prostaglandin synthetase activity.

Sample	Mean absorbance at 278 nm	Enzyme activity (u/g)	% Change in enzyme activity
XAOE, (0.1 ml)	0.60	0.05 ± 0.000	-
Indomethacin (4.0 µg/ml)	0.16	0.01 ± 0.000	- 80
XAME (0.25 ml)	1.19	0.10 ± 0.012	+ 100
XAME (0.50 ml)	2.90	0.23 ± 0.010	+ 360

XAOE = *Xylopia aethiopica* oil; XAME =*Xylopia aethiopica* methanol extract, % Enzyme activity = 100 x ($Act_2 - Act_1$)/ Act_1; -= Decrease in activity; + = increase in activity. Values are means ± SEM.

Table 5. Effect of *Xylopia aethiopica* extracts on erythrocyte membrane stabilization.

Sample	Concentration (ml) percentage	Prevention of haemolysis
Normal saline	-	100.00
Water	-	0.00
Methanol extract	0.10	3.41[a]
Methanol extract	0.20	26.71[b]
Methanol extract	0.30	72.70[b]
Oil extract	0.10	5.68
Oil extract	0.20	2.27
Oil extract	0.30	- 1.42

a = $p < 0.05$; b = $p < 0.001$; - = increase in haemolysis. % Prevention of haemolysis = 100x [OD_1, - OD_2/OD_1], where OD_1 and OD_2 are absorbance of water and extract respectively.

glandin synthetase activity were evaluated *in vitro*, the oil extract was found to have prostaglandin synthesis substrate activity (Table 4). The methanol extract, unlike indomethacin which is a standard drug that inhibits the activity of prostaglandin synthtase, enhanced the synthesis of prostaglandins using *X. aethiopica* oil as substrate.

The presence of appreciable quantity of these fatty acids in *X. aethiopica* fruits may therefore be responsible for many of the tradomedical applications of this plant.

Results of the test for red blood cell membrane stabilization showed that the methanol extract prevented haemolysis suggesting that it maintains the integrity of the erythrocytes and possibly acts by enhancing active transport across the membrane of the erythrocytes as opposed to osmosis or by reducing the permeability of the erythrocyte membrane to water.

During inflammatory reactions, such as pains, the lysosomes lyse thereby releasing the chemical mediators-histamine, serotonins and kinins. Since similarities exist between human erythrocyte and lysosomal membranes (Varadarasou *et al*., 2007), stabilization of erythrocyte membrane by methanol extract of *X. aethiopica* may be extrapolated to stabilization of lysosomal membrane which is usually taken as an indication of anti-inflammatory potentials of drugs.

These observations may be supportive of the use of *X. aethiopica* fruits in alleviating post-natal pains in women, and also corroborate reports by several researchers that various herbal remedies are endued with the potency of stabilizing erythrocyte membrane (Olugbenga *et al*., 2005; Varadarasou *et al*., 2007; Omale and Okafor, 2008).

From our findings we may, in conclusion, state that *X. aethiopica* fruit is a good source of unsaturated fatty acids. The presence of therapeutically active phyto-constituents; its ability to promote prostaglandin synthetase activity and stabilization of the red blood cell membrane integrity may be associated with its usefulness in Nigerian traditional medicine for the healing of wounds, inflammatory disorders and treatment of post-natal pains. However, further research is required to elucidate the actual mechanisms of actions.

REFERENCES

Azeb T, Felipe S, Shamnon EJ (2004). Stabilization of red blood cell membranes by thalidomide *in vitro*. Immunopharm. Immunotoxicol. 26(4): 501-509.

Bassie O (1975). Handbook of practical Biochemistry. University Press Ibadan, Nigeria. pp. 38-39.

Cuiled I (1982). Methodology for analysis of vegetable drugs. In: practical manuals of the industrial utilization of medicinal and aromatic plants. Bucharest Romania: p. 67.

Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants, Afr. J. Biotechnol. 4(7): 685-688.

Ezekwesili CN, Nwodo OFC (2000). Anti-inflammatory properties of

Vigna unguiculata seed extract, J. Med. Lab. Sci. 9: 141-145.
Fall D, Badiane M, Ba D, Loiseau P, Bories C, Gleye C, Laurens A, Hocquemiller R (2003). Antiparasitic activities of Senegalese Annonacae used in traditional medicine. Dakar Med. 48(2): 112-116.
Kouninki H, Haubruge E, Noudjou FE, Lognay G, Malaisse F, Ngassoum MB, Goudoum A, Mapongmetsem PM, Ngamo LS, Hance T (2005). Potential use of essential oils from Cameroun applied as fumigant or contact insecticides against *Sitophilus zeamais* Motsch. (*Coleotera: Curcu lionidae*). Commun. Agric. Appl. Biol. Sci. 70(4): 787-792.
Kouninki H, Ngamo LST, Hance T, Ngassoum MB (2007). Potential use of essential oils from Cameroun plants for the control of red flour weevil *Tribulium castaneum* (Harbst.) (*Coleoptera: Tenebrionidae*), Afr. J. Food Agric. Nutr. Dev. 7(5): 1-15.
Kumar RS, Sivakuma T, Sunderem RS, Gupta M, Murugesh K, Rajeshwa Y, Kumar MS, Kumar KA (2005). Antioxidant and antimicrobial activities of *Bauhinia recemosa* L. stem bark. Braz. J. Med. Biol. Res. 38: 1015-1024.
Law M (2000). Plant Sterol and stanol margarines and health. Br. Med. J. 320: 72-83.
Luisa D (2007). Evaluation of the effect of Neptune Krill oil on chronic inflammation and arthritic symptoms, J. Am. Col. Nutr. 26(1): 39-48.
Motrin A (2005). Antinflammatory effects of oil. Nature, 437: 45-46.
Official methods of analysis of the Association of Official Analytical Chemists. 12th ed. AOAC (1975) Washington DC.
Ogunkunle ATS, Ladejobi TA (2006). Ethnobotanical and phytochemical studies on some species of *Senna* in Nigeria. Afr. J. Biotechnol. 5(21): 2020-2023
Olugbenga M, Fatunso MA, Makinde JM (2005): Membrane stabilizing activity: a possible mechanism of action for the anti-inflammatory property of *Gongronema latifolium* leaves, Int. J. Biomed. Health Sci. 1(1): 1-4.
Omale J, Okafor PN (2008). Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*, Afr. J. Biotechnol. 3, 7(17): 3129-3133.
Ostlund RE Jr. (2002). Phytosterols in human nutrition, Ann. Rev. Nutr. 22: 533-549.
Sofowora ER (1980). Guidelines for research promotion and development in traditional medicine, Nig. J. Pharm. 11: 117-118.
Varadarasou MM, Subramanian K, Vaithilingam B, Sabarimuthu DQ (2007).Evaluation of antiinflammatory and membrane stabilizing properties of ethanol extract of *Cansjera rheedii* J. Gmelin (*Opiliaceae)*, Iran. J. Pharmacol. Ther. 6(2): 235-237.