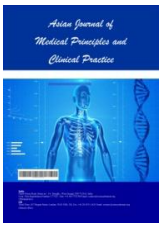

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Qualitative Determination of the Phytochemical Composition of Ethanolic Extract of *Xylopia aethiopica* Fruit

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Authors' contributions

This work was carried out in collaboration among all authors. Author INN conceptualized the study. Author PCU designed the study. Author EOO managed the literature searches and managed the analyses of the study. Author UO wrote the protocol while author AIA performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study is to qualitatively determine the phytochemical compositions of ethanolic extract of *Xylopia aethiopica* fruit.
Methodology: The fruits of *Xylopia aethiopica* were obtained from new market in Aba, Abia State and were identified and authenticated by Prof. (Mrs) Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo with the voucher number UU/PH/4e. The plant was deposited in the Herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Akwa-Ibom State. Extraction was done using Soxhlet apparatus using ethanol as the solvent. Qualitative analysis of the phytochemical composition was carried out using standard methods.

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Results: The result showed that extract of *X. aethiopica* fruit contained different phytochemicals such as alkaloids, cardiac glycoside, flavonoid, oxalate, phytate, phenol, saponin, steroid, tannins etc. Lignin and trypsin inhibitor were absent.
Conclusion: The presence of varieties of phytochemicals in *X. aethiopica* fruit suggests that it will be a potent medicinal plant and support its use in folklore medicine

Keywords: Ethanolic extract; phytochemical composition; medicinal value; Xylopia aethiopica.

1. INTRODUCTION

Africa, since ancient times is blessed with so many medicinal plants whose extract can be used in the treatment and management of diseases. Factors such as poverty and illiteracy still militate against availability and accessibility of conventional medical services. Large number of these tropical plants have shown beneficial therapeutic effects such as contraceptives, fertility enhancing capacities, antioxidant, anti-inflammatory, anticancer and antimicrobial potentials [1]. Amongst these plants with great therapeutic potential is *Xylopia aethiopica* which is commonly referred to as 'African guinea pepper' or 'Ethiopian pepper' or locally known as 'Uda' in the south eastern part of Nigeria. It is an angiosperm of the Annonaceae family and grows up to 20metres in height, bearing aromatic seeds, predominantly in humid forest zones of West Africa [2]. The tree has a straight stem and smooth back, and remains ever green with a constituent aroma [3]. It is found all over the lowland rainforest and savannah zones of Nigeria [4].

Xylopia aethiopica possesses great nutritional and medicinal values in traditional medicine [5]. The seeds have been reported to contain chemical constituents like alkaloids, glycosides, saponins, tannins, sterols, carbohydrates, proteins, free fatty acids, mucilages and acid compounds. These phytochemicals contain antioxidant and play vital roles in human health [6,7]. According to Okeke et al. [8], the fruit serves as spice, while its aqueous decoctions are used for its antiseptic properties. The powdered root is employed as a dressing and in local treatment of cancer [9]. Its antihypertensive and diuretic effects have been reported [10]. Extracts of the fruit are used in the treatment of cough, biliousness, bronchitis, rheumatism, dysentery, malaria, uterine fibroid and amennorrhoea [11,12].

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients [13]. They protect plants from disease and damage and contribute to the plant's colour, aroma and flavour. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called phytochemicals [14,15]. It has been discovered that these compound play important roles in human health when ingested into the body. Dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices [15]. Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, raspberries, beans, legumes, and soy foods are common sources [16]. Phytochemicals can be found in different parts of the plants, such the leaves, flowers, roots, stems, seeds and fruits. Phytochemical concentration varies from plant to plant depending on the variety, growth conditions etc. These compounds are plants secondary metabolites. Plants produce these chemicals to protect themselves but it has been discovered that these compounds can protect humans against diseases. Depending on their role in plant metabolism, phytochemicals are classified as either primary or secondary constituents. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll etc. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignins, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides [17]. Phenolics have been reported to be the most abundant and structurally diverse plant phytochemicals [18]. This study is aimed at determining the phytochemicals present in *X. aethiopica.*

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Materials

The fruits of *Xylopia aethiopica* were obtained from new market in Aba, Abia State and were identified and authenticated by Prof. (Mrs)

Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo with the voucher number UU/PH/4e. The plant was deposited in the Herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Akwa-Ibom State, Nigeria.

2.2 Extraction of Extract

The extraction was carried out in the Post-graduate Laboratory of Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Akwa-Ibom State, Nigeria. The fruits were washed under running tap water to remove contaminant and air-dried. This plant material was then pulverized using laboratory blender (Moulinex) to provide a greater surface area. The pulverized plant material was macerated in 250 mL of 99.8% ethanol (Sigma Aldrich) contained in round bottom flask, which was then attached to a Soxhlet extractor coupled with condenser and heating mantle (Isomantle). It was then loaded into the thimble, which is placed inside the Soxhlet extractor. The side arm is lagged with glass wool. The mixture was heated using the heating mantle (Isomantle) at 60°C and as the temperature increases it begins to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. This continues until it is exhaustively extracted. The process runs for a total of 13 hours. Once it was set up, it was left to run without interruption as long as water and power supply were not interrupted. The equipment was turned on and off and overnight running was not permitted, and the time split over a number of days. The extract was poured into 1000 mL beaker and concentrated to dryness in water bath (A3672- Graffin Student Water Bath) at 35°C. The total weight of the marc (residue) and the concentrated extract were recorded, these processes took several days. The dried extract was preserved in the refrigerator at 4°C for further analysis.

2.3 Qualitative Analyses of Phyto-chemical Composition of *Xylopia aethiopica* Fruits

The qualitative determination of the phytochemical compositions of *X. aethiopica* fruit was determined according to the methods described in Airaodion et al. [19,20].

3. RESULTS AND DISCUSSION

Preliminary investigation of ethanolic extract of *X. aethiopica* fruit showed that it contained different phytochemicals such as alkaloids, cardiac glycoside, flavonoid, oxalate, phytate, phenol, saponin, steroid, tannins etc. This is presented in Table 1.

Table 1. Phytochemical composition of ethanolic extract of *xylopia aethiopica* fruit

S/N	Phytochemicals	Inference
1.	Alkaloid	+
2.	Anthocyanins	+
3.	Cardiac Glycoside	+
4.	Coumarin	+
5.	Cyanogenic Glycoside	+
6.	Flavonoid	+
7.	Lignin	–
8.	Oxalate	+
9.	Phenol	+
10.	Phlobatannins	+
11.	Phytate	+
12.	Phytosterol	+
13.	Saponin	+
14.	Steroid	+
15.	Tannin	+
16.	Terpenes	+
17.	Trypsin Inhibitor	–

+ means present while – means absent

3.1 Discussion

In this study, preliminary qualitative phytochemical test revealed the presence of alkaloid, cardiac glycosides, saponins, tannins, flavonoids, polyphenols etc. (Table 1) in ethanolic extract of *X. aethiopica* fruit, ascertaining previous reports by Stahls and Sies, [21] and Sato and Yamada, [22]. These bioactive components are known to be bactericidal, pesticidal or fungicidal in nature [23]. It has also been reported that these compounds are mostly secondary metabolites which are capable of producing definite physiological actions in the body [24] and are the most important bioactive constituents of natural products [25]. The use of *X. aethiopica* for treatment of gastrointestinal diseases and other health problems may be due in part to a composite effect of all the bioactive agents or specific constituents in the plants [26]. It has been reported that the fruit and seed oil exhibit antimicrobial effect [9] which may be useful in dysenteric state. Phytochemicals include all plant compounds both plant chemicals that are beneficial and those that are toxic. Some

phytochemicals possess incredible health benefits while others are toxic to health [19,20].

Research carried out on populations consuming plant diet rich in phytates has shown lower incidenceof cancer, which suggests that phytate, has an anticarcinogen effect [27]. The metal binding characteristics of phytate endowed it an antioxidant function, inhibiting the production of hydroxyl radicals that normalize cell homeostasis and it also serves as a natural food antioxidant [19]. Therefore, *X. aethiopica* might have anticarcinogenic properties. Thompson [28] also suggested that dietary phytate may also be beneficial for diabetic patients because it lowers the blood glucose response by reducing the rate of starch digestion and slowing gastric emptying. Phytate has also been shown to regulate insulin secretion. It is believed that phytate decreases blood clots, cholesterol and triglycerides and thus prevents heart diseases [20]. The presence of phytate in *X. aethiopica* fruit might be suggestive that it possesses the propensity of being a natural remedy for the treatment of diabetes mellitus. It has also been reported that phytic acid prevents renal stone development. Wise [29] reported that it has the ability as a complexing agent to remove traces of heavy metal ions from the kidney. It prevents calcium oxalate precipitation in the kidney and reduces oxalate excretion in renal stone patients. Calcium oxalate crystal deposition *in vitro* urothelium is prevented by phytic acid by protecting the membrane from free radical-mediated damage [29]. This might makes *X. aethiopica* fruit potent in prevent ingrenal stone and removing traces of metal ions.

Alkaloids are natural products that contain heterocyclic nitrogen atoms. They are basic in character [19]. Alkaloids are known for different biological activities and each activity has its own specific mechanism of action. D-tubocurarine is one such example of alkaloids that possesses the antiparalytic activity due to its ability to obstruct the acetycholine receptor spots which enable the muscles to unwind at neuromuscular intersections [30]. *X. aethiopica* fruit might possess antiparalytic activity. Alkaloids also possess antioxidant property and anticancer activity due to their ability to act as scavenger of free radicals, metal chelating activity or electron or hydrogen donation ability. These alkaloids have also been reported to exert chemo preventive effect against tumour cells by terminating or causing depolymerisation of protein microtubules that forms the mitotic spindle in cell division. This results in hindrance in the process of division and separation of tumour cells and reduces the incidences of cancer. This is in support of the research carried out by Moura et al. [31] who reported the ROS scavenging ability, antimutagenic and antigenotoxic activities of betacarboline alkaloids, found in medicinal plant and variety of foods. *X. aethiopica* fruit might therefore have potential of having chemo preventive effect.

Saponins are naturally occurring surface-active glycosides with a distinctive foaming characteristic. They are mainly produced by plants. Saponin has been reported by Airaodion et al. [32] to have effect in hemolysis. The hemolytic action of saponins is believed to be the result of the affinity of the aglycone moiety for the phospholipids present in the cell membrane with which they form in soluble complexes. Saponins have alytic action on erythrocyte membranes. This can either be beneficial or of negative effect. Prior to hemolysis, erythrocytes may enter suicidal cell death (apoptosis), thus leading to clearance of defective erythrocytes prior to release of hemoglobin [19]. According to Bissinger et al. [33] exposure of human erythrocytes to saponin stimulates Ca^{2+} entry with subsequent triggering of cell membrane scrambling and thus suicidal death of human erythrocytes. The effect is paralleled by hemolysis. This in turn leads to anemia and thrombosis. The presence of saponin in *X. aethiopica* fruit might make it to have the propensity to make erythrocytes available. Saponin has also been reported to have effect in cholesterol metabolism as it lowers serum cholesterol levels. Large mixed micelles formed by the interaction of saponins with bile acids account for their increased excretion. The resulting accelerated metabolism of cholesterol in the liver causes its serum levels to go down [19]. This might make *X. aethiopica* fruit a natural remedy for disease conditions such as obesity, cardiovascular diseases and other cholesterol related diseases. Saponin has also been reported to possess hypolipidaemic activity. The mechanism involved in the hypolipidemic activity is that saponin has high fiber content. The fiber significantly binds to cholesterol hence aiding its excretion [20]. It has anti-inflammatory properties. The significant ameliorative activity of the saponins may be due to inhibition of the mediators of inflammation such as histamine, serotonin and prostaglandin along with its antioxidant property which inhibits the formation of ROS which also plays a major role in

inflammation [19]. *X. aethiopica* fruit might have a high potential in hypolipidaemic and anti-inflammatory activities. The negative effect of saponins on animal reproduction has long been reported and has been ascribed to their abortifacient, antizygotic and anti-implantation properties [34,35]. Saponins are found to be extremely strong stimulators of luteinising hormone release from cultured hypophysial cells [30]. The saponins show antimicrobial activity by inhibiting the growth of Gram positive and Gram negative microorganisms [36,37]. Some saponins are not effective against Gram negative microorganisms because they are unable to penetrate into the cell membranes of the microorganisms [38]. This might make *X. aethiopica* fruit to have the potential of antimicrobial activity.

As natural antioxidants, flavonoids play an important role in scavenging free radicals and preventing degenerative diseases such as cardiovascular diseases [39]. However, they are also involved in the antiproliferation of carcinogenic cells, in cell cycle regulation and in the induction of apoptosis [40]. They can act to inhibit free-radical mediated cytotoxicity and lipid peroxidation, as anti-proliferative agents to inhibit tumor growth or as weak estrogen agonists or antagonists to modulate endogenous hormone activity [19]. In these ways, they may confer protection against chronic diseases such as atherosclerosis and cancer and assist in the management of menopausal symptoms. They contain conjugated ring structures and hydroxyl groups that have the potential to function as antioxidants *in vitro* or cell free systems by scavenging superoxide anion, singlet oxygen, lipid peroxyradicals, and stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species [19]. Also, coumestrol (a phytoestrogen, mimicking the physiological actions of estrogens and estradiol), daidzein, and genistein showed the strongest protective associations. Several studies have reported the potential of some plants extracts to prevent peptic ulcer due to the presence of flavonoid [41,42,43]. *X. aethiopica* fruit might therefore be a natural remedy for treatment of diseases such as cardiovascular diseases, cancer and atherosclerosis as well as prevention of peptic ulcer.

Tannins and their derivatives are phenolic compounds considered to be primaryanti oxidants or free radical scavengers [6]. Tannins possess wound healing activity by its ability to increase the collagen content, which is one of the factors for promotion of wound healing [44]. This might make *X. aethiopica* fruit potent in wound healing process. Tannin is a non-toxic compound and they can generate physiological responses in animals that consume them [45]. Tannin can be toxic to filamentous fungi, yeast and bacterial. The presence of tannin in *X. aethiopica* fruit might suggest its ability to play key roles as antifungal, antibacterial, antidiarrheal, antioxidant and antihemorrhoidal agent [46].

4. CONCLUSION

The presence of varieties of phytochemicals in *X. aethiopica* fruit suggests that it will be a potent medicinal plant and support its use in folklore medicine.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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