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### Comparative Characterization of Phytomedicinal Constituents of *Xylopia Aethiopica*.

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#### ABSTRACT

Preliminary characterization and isolation of phytomedicinal components of dried black fruits of *xylopia aethiopica* in hydro-methanolic (1:4 v/v), hydro-ethanolic (1:4, v/v), methanolic, ethanolic and aqueous solvents has been compared. Results showed variability and significant differences in phytomedicinal compositions and the potency ranked: hydro-methanolic > hydro-ethanolic > methanolic > ethanolic = aqueous and the percentage difference was 75%, 54%, 45.8%, 29% =29% which perhaps validates the efficacy of the therapeutic potentials of *xylopia aethiopica* for many of the traditional medicinal applications. Anthraquinone and combined anthraquinone were exclusively found in hydro-methanolic and methanolic concentrates and accounted for 44.4 % and 33.3 % respectively. Glycosides (-terpene, sterols and deoxy-sugar) accounted for 100% in hydro-alcoholic concentrates respectively. Alkaloids and the phenolic compounds flavonoids and tannins with the exception of saponins which was negligible or absence in the solvents were also the phytomedicinal constituents. Results suggest that the ability of hydro-alcoholic to enhance the isolation of useful constituents might be attributed to the differential solubility of the combined hydrophobic and hydrophilic components of the phytomedicinal bioactive ingredients in the solvent which, is still subject to further studies. Hydro-alcoholic, specifically hydro-methanolic, therefore is recommended for isolation and characterization of economically important medicinal plants of medical interest.

**Keywords:** *Xylopia aethiopica*, hydro-ethanolic, hydro-methanolic, differential solubility anthraquinone, combined anthraquinone, medicinal plant.

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## INTRODUCTION

According to World Health Organization<sup>1</sup> medicinal plant is any plant which in one or more of its organ contains substances that can be used for therapeutic purposes or which are precursors for synthesis of useful drugs. Hitherto many plants employed for the treatment of diseases in different systems in traditional medicine local to Africa, Nigeria in particular, exist but we directed our scientist research interest towards this economically important medicinal plant *xylopia aethiopica* [(Dunal) A. Rich. (Annonaceae)]. *Xylopia* (in Greek means "bitter wood") *aethiopica* (refers to the origin of the tree) is a tropical evergreen tree native to the Western Africa. It is local to the lowland rainforest and moist fringe forests in the savanna zones of Africa. The dried black fruits of this species commonly known as Guinea pepper, Ethiopian pepper, and Negro pepper and in various local names<sup>2,3,4,5</sup> were once of commercial importance in Europe and Asia as a tangy condiment and drug; in African, Nigeria in particular, is used as spice and traditional medicine. Traditionally, the crude drug is always available easily in abundance, comparatively cheap, and it is often frequently prescribed to patients of all age groups, in particular, women as post partum tonic. It has been employed in different therapeutic preparations<sup>6,7,8,9</sup>. It has been suggested to be effective in reproductive related health care and family planning means<sup>10,11,12,13</sup>. Nonetheless, its side effect is not properly understood<sup>4</sup>. Locally, the medicaments are prepared and dispensed in one of these three: - aqueous, alcoholic and/or hydro-alcoholic preparations. Hitherto, literature is replete that compares and establishes scientifically the phytomedicinal constituents of the extracts in the various solvents. Thus evidence on the phytomedicinal composition and physio-medicinal properties of fluid extractability is needed to provide scientific credence to the folklore use of the traditional medicine before it could be helpful in the development of future medicines and treatments and treatment guidelines. This study therefore, was carried out to elucidate and compare the phytomedicinal screening qualitatively and quantitatively on the aqueous, ethanolic, methanolic, hydro-ethanolic (1:4, v/v) and hydro-methanolic (1:4, v/v) mixtures of this economically important medicinal plant for which has not previously been reported.

## MATERIALS AND METHODS:

### **Preparation of Plant Material:**

Dried black fruits of *xylopia aethiopica* were purchased from Ariaria market in Aba, Abia State of Nigeria, identified and authenticated by taxonomist in the Department of Plant Science & Biotechnology (PSB), Faculty of Science, University of Port Harcourt, Rivers State, Nigeria. The

voucher specimen UUH1819 was deposited at the University of Port Harcourt Herbarium. The carpals of *xylopia aethiopica* were removed from their strands, washed, sun dried cut into smaller pieces and further oven dried for some days at 40°C. After which, 2kg was pulverized with an electric grinding machine into a fine powdery form which 100g was soaked in 300ml of either hydro-methanolic (1:4, v/v), methanolic, hydro-ethanolic (1:4, v/v), ethanolic or aqueous solution at 28°C and shaken intermittently. The mixture was kept for 72 hours after which there were filtered with Whatman No.1 filter paper. The obtained samples were concentrated (dried) using a rotary evaporator at 45°C and then collected and kept at 4°C until analysis. Samples employed for the study were freshly prepared unless otherwise stated.

### **Phytomedicinal Screening of Extracts:**

Phytomedicinal screening was carried out at the Pharmacognosy laboratory, Faculty of Pharmacy, University of Port Harcourt on methanolic, ethanolic, aqueous, hydro-methanol(1:4, v/v) and hydro-methanol (1:4, v/v) extracts of the fruits of *xylopia aethiopica* according to established procedures<sup>14,15</sup> briefly as:.

**Test for tannins:** About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration for a positive test.

**Test for saponin:** About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

**Test for flavonoids:** A few pieces of magnesium metal are added to 5ml of the extract and concentrated Hydrochloric acid. A reddish colouration would indicate a positive test for flavonoids.

**Test for Alkaloids:** About 0.5g extract was stirred with 1 percent aqueous hydrochloric acid on steam bath; 1 ml of the filtrate was treated with a few drops of Mayer's reagent. Precipitation with reagent was taken as preliminary evidence for the presence of alkaloid in the extract evaluated.

### **Test for cardiac glycosides:**

**Lieberman's Test:** 0.5g of the extract was dissolved in 2ml of acetic anhydride and cooled in ice. Concentrated sulphuric acid was then carefully added.

**Keller-Killani Test:** 0.5g of the extract was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric

acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

**Salkowski Test:** 0.5g of the plant extract was dissolved in 2ml of chloroform. Concentrated Sulphuric acid was carefully added to form a lower layer.

#### **Test for Anthraquinones:**

**Free Hydroxy-anthraquinones.** The Borntrager's test for anthraquinones was used. 5mg of the plant extract was shaken with 10ml of benzene, filtered and 5ml of 10% ammonia solution added to the filtrate and the mixture shaken.

**Combined anthraquinones:** 5mg of plant extract was boiled with 10ml aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of benzene, the benzene layer separated and half its own volume of 10% ammonia solution added.

#### **Statistical analysis**

All data collected were summarized in percentage.

#### **RESULTS AND DISCUSSION:**

Phytomedicinal constituents in aqueous, ethanolic, methanolic, hydro-ethanolic (1:4. v/v) and hydro-methanolic (1:4, v/v) fruit extracts of *xylopiya aethiopica* are showed in Table1 and showed predominantly glycosides (-terpene, sterols and deoxy-sugar) which constituted 100% in hydro-alcoholic extracts respectively followed by methanolic (66.7%) then ethanolic (mostly sterols) and aqueous 33.3% respectively. Alkaloids accounted for 66.7% respectively in the hydro-alcoholic extracts and 33.3% respectively in methanolic, ethanolic and aqueous extracts. Anthraquinone and combined anthraquinone were found exclusively in hydro-methanolic and methanolic extracts and collectively accounted for 44.4% and 33.3% respectively. The phenolic compound saponins was negligible or absence in the solvents while flavonoids accounted for 66.7 % respectively in hydro-methanolic, ethanolic and aqueous extracts and 33.3% in hydro-ethanolic and methanolic respectively; while tannins accounted for 66.7% in hydro-methanolic isolate and 33.3% respectively in ethanolic ,aqueous and hydro-ethanolic concentrates. Qualitatively and quantitatively, the ability of hydro-methanol to extract the phytomedicinal constituents of the fruits of *xylopiya aethiopica* were 75% followed by hydro-ethanolic (54%) then methanolic (45.8%) and to a lesser extent ethanolic (29%) and aqueous (29%). *Xylopiya aethiopica* has previously not been subjected to any hydro-alcoholic, more specifically, hydro-methanolic investigation.

**Table 1. Comparison of Phytochemical Constituents of *XylopiA AethiopiCA*.**

S/No	Test	Hydro-Methanolic (1:4)	Hydro-ethanolic (1:4)	Methanolic	Ethanolic	Aqueous
1	Alkaloids	++	++	+	+	+
2	Saponins	-	-	-	-	-
3	Flavonoids	++	+	+	++	++
4	Tannins	++	+	+	+	+
5	Borntrager's					
I	Anthraquinone	+	-	+	-	-
Ii	Combined Anthraquinone	++	-	+	-	-
6	Glycosides					
I	Lieberman's (Terpene)	+++	+++	++	+	+
Ii	Salkowski's	+++	++ +	+++	++ (sterols)	+
iii	Keller Kiliani's	+++	+++ (Deoxy-Sugar)	+	-	+
Iv	Cyanogenetic Glycoside	-	-	-	-	-

(+ 33.3%; ++ 66.7% and +++ 100%)

This research is aimed at providing scientific knowledge of clinical relevance of the comparative phytochemical composition of the bioactive ingredients of *xylopiA aethiopiCA* in various solvents of local interest. Upon comparing compositions of bioactive constituents, there was variability and significant differences in phytochemical compositions and the potency of extractability are ranked: hydro-methanolic > hydro-ethanolic = methanolic > ethanolic = aqueous and the ratio was 3:2:2:1:1. Overall, results revealed predominantly glycosides (terpene, sterols and deoxy-sugar) which accounted for 100% in hydro-alcoholic extracts respectively and to a lesser extent in methanolic (66.7%) than in aqueous (33.3%) and ethanolic (mostly sterols, 33.3%). Significant variations were also found among alkaloids and accounted for 66.7% in hydro-alcoholic extracts and 33.3% in methanolic, ethanolic and aqueous extracts respectively. Similarly, significances were also found for the phenolic compounds (flavonoids and tannins) collectively accounted for 66% in hydro-methanolic, 50 % in ethanolic and aqueous extracts respectively and 33.3% in hydro-ethanolic and methanolic extracts respectively. The phenolic compound saponins were negligible or absence in the solvents which perhaps may justify the use of the preparations by traditional physicians, specifically, in arrest of bleeding after childbirth. Saponins have been associated with haemolytic activities on erythrocyte membranes<sup>16</sup>. This is in contrast to a previous study where phytochemical screening of *xylopiA aethiopiCA* oil extracted with chloroform: methanol (2:1, v/v) mixture revealed the presence of saponins together with other phytochemical constituents<sup>17</sup> which might suggest solvent-solute pharmacokinetics which

needed further elucidation. The present study also revealed the presence of anthraquinone and combined anthraquinone which were found exclusively in hydro-methanolic and methanolic extracts and collectively accounted for 50 % and 33.3% in the concentrates respectively.

#### CONCLUSION:

Results reported here suggest more importantly that hydro-methanolic highly potentiated the isolation of the key compounds in the samples which perhaps might be due to differential solubility of the combined hydrophobic or fat –soluble and hydrophilic or water –soluble part in hydro-methanolic and/or due to some yet unidentified mechanisms which however, needed further work to validate the present findings. Results obtained may justify claims of the locals of the uses of the various preparations by traditional physicians; and more importantly the use of hydro-methanolic for isolation, characterization or dispense of economically important medicinal plants of Physico-medical interest.

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