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HPLC Profiling and Phytochemical Analysis of Fruit Extracts of Guinea pepper - *Xylopia aethiopica,* (Dunal) A. Richard

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Abstract

The hexane, ethyl acetate and methanol extracts of the fruits of Xylopia aethiopica were subjected to HPLC fingerprinting to establish their profile. The extracts were also screened for classes of secondary metabolites, and the pharmacognostic parameters of the fruit were determined using standard procedures. HPLC analysis of the hexane, ethyl acetate and methanol extracts revealed eleven, nine and nineteen resolved peaks, respectively. The HPLC profiling revealed that the methanol extract contained caffeic acid (8.19%) and ferulic acid (4.91%). The result of the phytochemical screening of the three extracts showed the presence of carbohydrates, balsams, pseudotannins, saponins, tannins, terpenes, steroids and glycosides in the methanol extract while the hexane and ethyl acetate extracts contained less number of classes of secondary metabolites. The three extracts did not test positive for resin, flavonoids, alkaloids and simple sugars. Pharmacognostic determination showed that the fruits had moisture content of 9.17%, alcohol extractive value of 43.77% and water extractive value of 34.97%. The findings of this study established the HPLC, phytochemical and pharmacognostic profile of the commonly used fruit for references purposes, monograph development and samples authentication.

Keywords: *Xylopia aethiopica, fruit extracts, HPLC profile, Caffeic acid, ferulic acid, Phytochemistry, pharmacognosy*

Introduction

Natural compounds have attracted considerable attention as preventive and therapeutic agents against diseases. Indeed 74% of therapeutic compounds are natural products derivatives (Nobili *et al.* 2009). According to the world health organization (WHO, 2004) 80% of the African population (and some Asian countries) still use plant preparations to treat their illnesses, including cancer. The cloves of the plant *Xylopia aethiopica,* a member of the custard apple family, Annonaceae, are used as a spice in various traditional dishes of Western and Central Africa. The plant, *X. aethiopica* is an evergreen aromatic tree, growing up to 20 m high. It is a native to the lowland rain forests and moist fringe forest in the savannah zone and the coastal regions of Africa (Dalziel, 1995), largely located in West and Central Africa where it thrives in wet, swampy soil and in South Africa (Keita, 2003). In Sudan, it is distributed in high rainfall savannah and swampy forests (El-Amin, 1990). The fruit is refer to as Guinean pepper and used as spice and soup condiment, and it is valued for its carminative effect. *Xylopia aethiopica* (Dunal) A. Richard, has a wide variety of application, the very odorous root of the plant are employed in west Africa as tinctures, administered orally to expel worms and other parasitic animals from the intestines, or in teeth-rinsing and mouth-wash extracts against toothaches. The fruits are also used in various forms and exhibit revulsive properties, especially when mashed with grains. These properties are used advantageously in the external treatment of rheumatism. Crushed powder fruits can also be mixed with Shea butter fat and coconut oil and used as creams, cosmetic products, and perfumes (Burkil 1985). The dried fruits are also used as spices in the preparation of two special local soups named obe ata and Isi ewu taken widely in the southwest and southern part of Nigeria. The plant can also be used in decoction to treat dysentery, bronchitis, ulceration, skin infection and female sterility. Several studies have shown that *X. aethiopica* extracts possess antibacterial, cytotoxicity, anticancer (Asekun and Adeniyi 2004), antifungal and anti-plasmodial characteristics (Tatsadjieu *et al.* 2003). *X. aethiopica* possess antioxidants activity (Karioti *et al.*, 2004; Adaramoye *et al.* 2011)

The chemical composition of *Xylopia aethiopica* was first reported by Ekong and Ogan (1968). Since then, several other publications have appeared in literature. Some diterpenes have been reported from the bark, fruit and pericarp of the plant (Faulkner *et al.,* 1985; Rabunmi and Pieeru 1992; Harrigan *et al.*, 1994), and the volatiles of *Xylopia aethiopica* had been reported to consist mainly of mono- and sequiterpenoids with typical constituents being α- and β-pinene, myrcene, p-cymene, limonene, linalool, and 1,8-cineole (Ekundayo 1989; Tairu *et al.*, 1999). Elemol and guaiol and other terpenoids, had been reported from in the essential oil of the fruit from the Republic of Benin (Ayedoun *et al.* 1996; Jirovetz *et al.* (1997).

There have been several studies and reports on the biological activity of *X. aethiopica* fruit powder and its essential oil. The studies demonstrated that the fruit powder and essential oil can be used against cowpea bruchid beetle (*Callosobruchus maculatus* Fab.) and *Coleopter bruchidae* or maize weevil (*Sithophylus zeamais* motsch) (Okonkwo and Okoye, 1996; Ngamo *et al.* 2001; Kouninki *et al.*

2005; Asawalam *et al.* 2006). *Xylopia aethiopica* had also been reported to be active against the wood termites and bugs (Ladjide *et al.* 1995). The microbial activity of *Xylopia aethiopica* essential oil against *E. coli*, *Staphylococcus aureus* or *Aspergillus flavus*, among other microorganisms, has been well established (Tatsadjieu *et al.* 2003; Asekun and Adeniyi 2004; Konnings *et al.* 2004). Among the compounds that confer biologic properties to *X. aethiopica* are the diterpenes belonging to the kauranes, the trachylobanes and the kolavanes families (Hasan *et al.* 1982; Harrigan *et al.* 1994). Some of the compounds that can also be found in *Xylopia aethiopica* include linalool, vanillin and thymol. The Phytochemical screening of the fruit of *Xylopia aethiopica* according to studies carried out by (John-Dewole *et al.*, 2012) confirms the presence of Saponnin, Saponnin glycoside, Tannin, Balsam, Cardiac glycoside and Volatile oil. According to the research carried out by (Tairu *et al.* 1999), some of the key aroma compounds identified in the dried fruits of *Xylopia aethiopica* include α-thujene, α-pinene, ethyl-2-methylbutanoate, camphene, sabinene, β-pinene, α-terpinene, α-phellandrene, limonene, 1, 8-cineole, trans-β-ocimene, β-phellandrene, p-mentha-3, 8-triene, 3-cerene, myrtenol, linalool, decanal, α-terpinol, terpinen-4-ol, α-fernessene, β-citroneollol, graniol and fenchone. There is no doubt that the medicinal value of this plant lies in some chemical compounds that produce definite physiological actions on the body of human. This paper gives a comparative HPLC analysis of extracts of *Xylopia aethiopica,* with a view toward establishing a reference profile for sample authentication.

Materials and Methods

Materials

All reagents used were of analytical grade. Shimadzu (Japan) HPLC was used for HPLC profiling.

Plant collection and extraction

Dried fruits of *Xylopia aethiopica* were purchased from a spice vendor in Karmo market and the voucher specimen was identified at the Taxonomist at the NIPRD on the 21st of May 2013. The dried bulk samples of the fruits were pulverized using pestle and mortar. 150 g of pulverized plant material was separately extracted with 200 ml of each solvent (hexane, ethyl acetate and methanol) using a Soxhlet apparatus. The three extracts were concentrated using a rotary evaporator and allowed to air-dry before transfer into a clean sealed glass container, and kept in the dark until required.

HPLC analysis

Specific weight of the various extracts were taken and reconstituted into 50 mg/ml, and separately analyzed in a high performance liquid chromatography. The HPLC consisted of Ultra-Fast LC-20AB equipped with SIL-20AC auto-sampler; DGU-20A3 degasser; SPD-M20A UV-diode array detector; column oven CTO-20AC, system controller CBM-20Alite and Windows LC-solution software (Shimadzu Corporation, Kyoto Japan); column, 5µm VP-ODS C18 and dimensions (4.6 x 150 mm). The chromatographic conditions included mobile phase: 0.2% v/v formic acid and acetonitrile (20:80); mode: isocratic; flow rate 0.6 ml/min; injection volume 10 µl of 50 mg/ml solution of extract in methanol; detection UV 254 nm. The HPLC operating conditions were programmed to give solvent B: 20%. Column oven temperature was 40 °C. The total run time was 20 minutes. Flavonoids and phenolic acid standards such as apigenin, rutin, quercetin, caffeic acid, ferulic acid were employed for the identification of the phytoconstituents of extracts by comparing the retention time under similar experimental conditions (Krishna and Manohar, 2014).

Phytochemical and Proximate pharmacognostic analyses

The determination of the presence of secondary metabolites and the proximate parameters were carried out using standard methods (Egharevba *et al.*, 2015).

Results and Discussion

The results of HPLC analyses of hexane, ethyl acetate and methanol extracts of the fruits of *X. aethiopica* are presented in the Figures and Tables 1, 2 and 3, respectively. There were total of 11, 9 and 19 peaks for hexane, ethyl acetate and methanol chromatogram respectively. The HPLC analyses revealed that the profile of methanol extract contained peaks corresponding to caffeic acid (8.19%) and ferulic acid (4.91%) with retention times of 4.821 and 8.777 minutes respectively. Most of the peak were broad and not sharp suggesting that they could be multicomponent peaks. However, the patterns were different for each extraction solvents and reproducible.

The profiles of the secondary metabolites in the extracts are presented in Table 4. The result of the phytochemical screening of the three extract showed the presence of, saponins, balsams, saponin glycosides, steroids and terpenes in the hexane extract, tannins, pseudotannins, balsams, glycosides and steroids in the ethyl acetate extract, and carbohydrates, tannins, pseudotannins, balsams, steroids and terpenes in the methanol extract. Pharmacognostic determination showed that the fruits had a moisture content of 9.17%, alcohol extractive value of 43.77% and the water extractive value of 34.97% (Table 5). The moisture content is within the limit for vegetable drug and therefore suggests that the dried fruit could be stored under conducive environment without concerns of microbial spoilage (Egharevba *et al.*, 2015). The high alcohol and water extractive values suggest that the fruit contain high amount of extractable mater and alcohol is a better solvent.

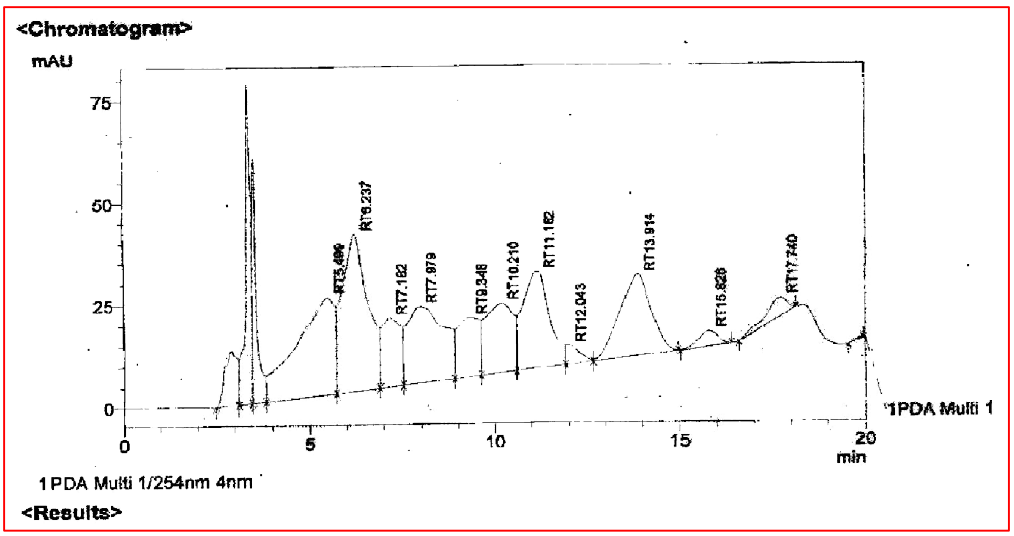

Fig 1: HPLC Chromatogram of Hexane extract of *X. aethiopica* fruit

Table 1: Results of HPLC analysis of the Hexane extract *X. aethiopica* fruit

Peak No.	Retention time (min)	Area %
1	5.499	17.380
2	6.237	19.219
3	7.182	6.100
4	7.979	13.153
5	9.348	6.092
6	10.210	9.146
7	11.182	12.304
8	12.043	1.171
9	13.914	12.140
10	15.826	1.401
11	17.740	1.893
Total:		100

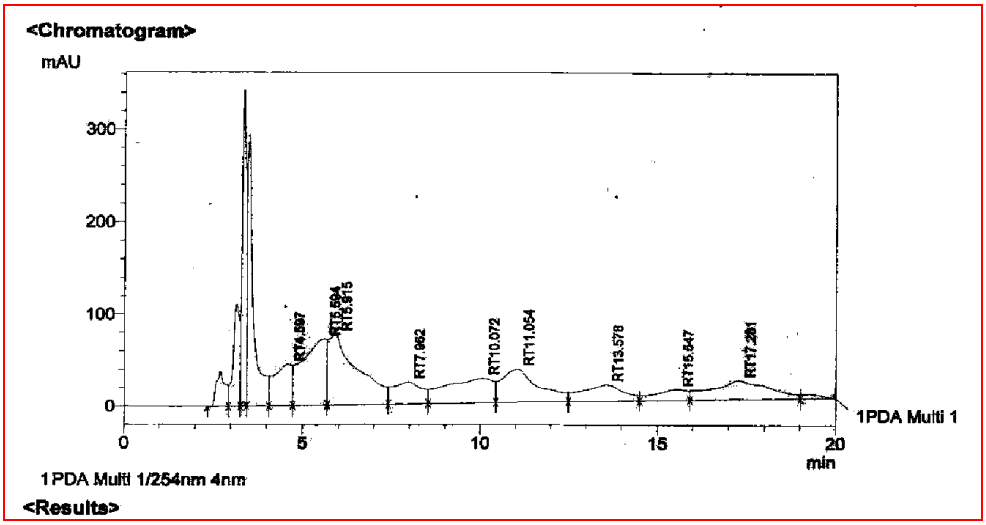

Fig 2: HPLC Chromatogram of Ethyl acetate extract of *X. aethiopica* fruit

Table 2: Results of HPLC analysis of the Ethyl acetate extract *X. aethiopica* fruit

Peak No	Retention time (min)	Area %
1	4.597	7.955
2	5.594	17.357
3	5.915	22.889
4	7.962	6.881
5	10.072	12.945
6	11.054	13.548
7	13.578	7.528
8	15.547	3.957
9	17.281	6.940
Total		100

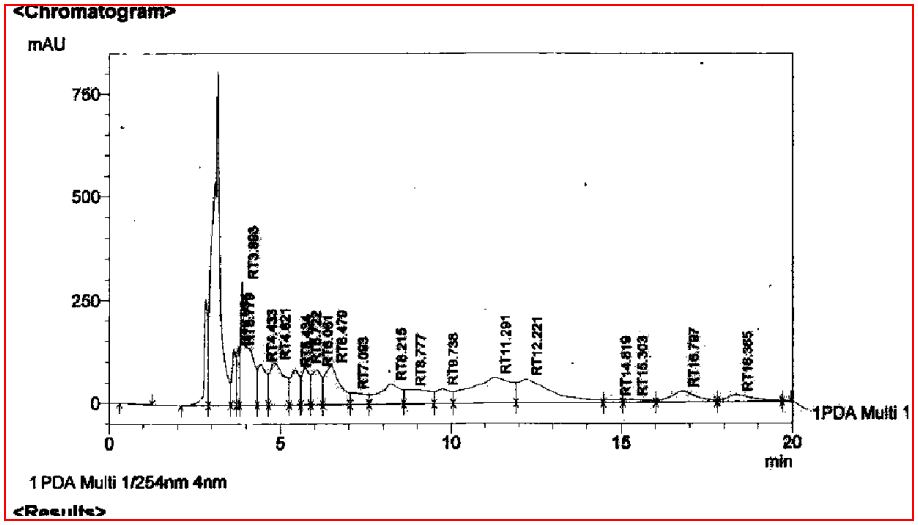

Fig 3: HPLC Chromatogram of Methanol extract of *X. aethiopica* fruit

Table 3: Results of HPLC analysis of the Methanol extract *X. aethiopica* fruit

Peak No.	Retention time	Area %
1	3.654	2.990
2	3.779	2.070
3	3.893	12.654
4	4.433	4.654
5	4.821	8.190
6	5.434	4.226
7	5.722	3.859
8	6.061	4.503
9	6.479	8.090
10	7.093	2.375
11	8.215	5.884
12	8.777	4.912
13	9.738	2.927
14	11.291	14.141
15	12.221	11.050
16	14.819	0.553
17	15.303	0.859
18	16.797	3.709
19	18.365	2.356
Total:		100

Table 4: Results of Phytochemical studies of extracts of *X. aethiopica* fruits

Sn	Parameters	Remarks		
		Hexane Extract	Ethyl Acetate Extract	Methanol Extract
1.	Carbohydrates	-	-	+
2.	Sugars	-	-	-
3.	Tannins	-	+	+
4.	Pseudo tannins	-	+	+
5.	Saponins	+	-	+
6.	Saponin glycoside	+	+	-
7.	Resins	-	-	-
8.	Balsams	+	+	+
9.	Alkaloids	-	-	-
10.	Sterols	+	+	+
11.	Flavonoids	-	-	-
12.	Terpenes	+	-	+

Table 5: Results of Proximate pharmacognostic studies of *X. aethiopica* fruits

SN.	Parameters	% composition
1	Moisture content	9.17
2	Alcohol extractive value	43.77
3	Water extractive value	34.97

Conclusion

This study was able to establish the HPLC profile of the hexane, ethyl acetate and methanol extracts of the fruits of *X. aethiopica*. The HPLC analyses also revealed that the methanol extract may contain caffeic acid (8.19%) and ferulic acid (4.91%). The phytochemical screening of the extracts revealed the presence of carbohydrates, tannins, pseudotannins, saponins, balsams, glycosides, steroids and terpenes, in the methanol extract, which contained more of the classes of the secondary metabolites. None of the extracts tested positive for simple sugars, resin, alkaloids and flavonoids. The HPLC profile and the profile of secondary metabolite in the extracts could be relevant in sample authentication.

Recommendations

Further work is recommended on isolation and characterization of active chemical compounds responsible for the antimicrobial/antibacterial properties of the plant. Some of the isolated compounds may serve as markers for the species.

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