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Metabolomic profiling of ethanolic extracts of the fruit of *Xylopia aethiopica* (Dunal) a. rich using gas chromatography and high-performance liquid chromatography techniques

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Abstract
The study was aimed at identifying the phytochemicals present in the ethanolic extracts of the dried fruit of *Xylopia aethiopica* by GC-MS and HPLC-DAD analyses. A total of 39, 38 and 35 compounds were identified in the ethanol extract (EE), residual fraction (RF) and n-hexane fraction (HF) of ethanolic extracts of *X. aethiopica,* respectively by the GC-MS. The HPLC-DAD analysis revealed the presence of xylopic acid, chlorogenic acid, caffeic acid and ellagic acid in all the samples while apigenin, kaempferol, rutin and quercetin were only present in EE and RF. The results postulated that the ethanolic extracts and the fractions of the extract contain various bioactive compounds which can be used for the treatment and management of various diseases.

Keywords: *Xylopia aethiopica,* HPLC-DAD, GC-MS, fraction

1. Introduction
Xylopia aethiopica (Dunal) A. Rich, commonly called Negro pepper or Ethiopia pepper, has been confirmed to be a source of multiple biologically active compounds, specifically with immunomodulatory, antioxidant, microbicidal, antitumoural, antimalarial, antipyretic and anti-androgenic properties [1-4]. These properties have been linked with the presence of xylopic acid (*5β-Acetoxy-(−)-kaur-16-en-19-oic Acid*), a major components of the fruit and other phytocompounds present. The essential oil from the dried fruits has been characterised and the main constituents include 4-terpineol, β-pinene, α-terpineol, 1,8-cineole, *cis*-α-copaene-8-ol,13-epimanoyl oxide, (+)-spathulenol, L-pinocarveol, myrtenol, o-cymene, eudesma- 1,3-dien-11-ol, eudesma,4-11(13)-dien-2-ol, cumic alcohol, kaur-16-ene, α-pinene, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid [5, 6]. However, there might be degradation and loss of some thermally unstable components by thermal effects connected with the conventional extraction of essential oil [7]. Thus, the present study aimed at investigating the metabolomics profiling of the ethanolic extract and fractions of the ethanolic extract of the dried fruit of *Xylopia athiopica* using gas chromatography and high-performance liquid chromatography techniques.

2. Materials and Methods
2.1 Chemicals
All chemicals used in this study were of analytical grade. The HPLC reference standards xylopic acid, caffeic acid, ellagic acid and chlorogenic acid were purchased from Merck (Darmstadt, Germany).

2.2 Plant Materials
The plant material was collected from farms in the North Central Zone of Kwara and air-dried in dark at room temperature (29±2ºC). The sample was authenticated at the Department of Plant Biology, University of Ilorin with the voucher number UIH001/1089. Dried fruits were ground and stored in tight-seal dark containers until needed. The powdered fruits of *X. aethiopica* was mixed with ethanol for seventy-two hours at ambient temperature and solubilised with the aid of a shaker. The mixture was filtered and filtrate was evaporated to dryness. Partitioning of the dried crude extract was done using distilled water and n-hexane, and each extract was subsequently evaporated to dryness.

2.3 Phytochemical screening
The qualitative phytochemical screening to detect the presence of different phytochemical groups was carried out as described by Harborne [8].

2.4 Identification of constituents of ethanol extracts using gas chromatography coupled with mass spectrophotometer (GC-MS)

The GC-MS analysis was performed using Agilent Technologies GC-MS (Model 7890A) equipped with Agilent 19091S-433HP-5MS 5% Phenyl Methyl Silox column (30 m × 250 µm × film thickness 0.25 µm). The principle is based on separation of mixtures into distinct substances when heated. The carrier gas used was pure helium at a flow rate of 1.5 ml/min. GC-MS analysis resulting in chromatogram (Figures 1 to 3) was compared to complete library using data base of National Institute of Standard and Technology (NIST).

2.5 Identification of constituents of ethanol extracts using high performance liquid chromatography coupled with photodiodde array detector (HPLC-DAD)

HPLC-DAD was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system, equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with aCBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1software. The samples (10 mg/ml) were injected by means of a model SIL-20A Shimadzu Autosampler. Separations of the phytochemicals were performed using Phenomenex C_{18}column (4.6 mm x 250 mm x 5 µm particle size). The mobile phase was water with 1% formic acid (v/v) (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 0.6 ml/min and injection volume 50 µl. The composition gradient was prepared in accordance with the method described by Duarte *et al.* [9]. The mobile phase with the sample was filtered using membrane filter (0.45 µm, Millipore). Varying concentrations of the standards (0.025 - 0.300 mg/ml) were prepared in equal volumes of acetonitrile and water. Quantifications were carried out by integration of the peaks using the external standard method, at 210 nm for xylopic acid; 325 nm for caffeic acid, chlorogenic acid and ellagic acid; and 366nm for apigenin, kaempferol, quercetin and rutin. The relationship between the retention time of the samples and those of the standards was used to determine the chromatography peaks by the DAD spectra (200 to 600 nm). Calibration curve for the reference standards are; xylopic acid: Y = 11953x + 1328.7 (r^2= 0.9999); caffeic acid: Y = 10972x + 1401.6 (r^2 = 0.9997); ellagic acid: Y = 12495x + 1185.4 (r^2 = 0.9999); chlorogenic acid: Y = 12358x + 1349.1 (r^2 = 0.9995); quercetin: Y = 14093x + 1176.9 (r^2 = 0.9998); rutin: Y= 11752x + 1407.5 (r^2 = 0.9997), apigenin: Y = 13683x + 1058.3 (r^2 = 0.9996), kaempferol: Y = 12804x + 1249.0 (r^2= 0.9993). The limit of detection (LOD) and limit of quantification (LOQ) were respectively estimated as 3.3 and 10 σ/S (σ = standard deviation of the response, S= slope of the calibration curve), as described by Boligon *et al.* [10].

2.6 Statistical analysis

The data were evaluated using analysis of variance model and Turkey's post hoc test at $p < 0.05$.

3. Results

The results of the preliminary qualitative phytochemical screening revealed the presence of saponin, phenols, steroids and glycosides in the ethanolic extract and its fractions (Table 1). A total of 39, 38 and 35 volatile compounds were identified in the EE, RF and HF, respectively in the GC-MS profiling (Table 2). The identified compounds comprise alcohols, aldehydes, carboxylic acids, esters, hydrocarbons, ketones and oxides. The predominant alcohol identified in the ethanol extract and residual fraction is 1-naphthalenepentanol,decahydro-5-(hydroxylmethyl)-5,8a-dimethyl-y,2-bis(methylene)-,(1α,4aβ,5α,8aα)-, with RF having the highest composition of 23.13% as against 7.88% and 0.63 % by EE and HF, respectively. However, the predominant alcohol in the HF is bicyclo[3.1.0]hexan-2-ol,2-methyl-5-(1-methylethyl)-,(1α,2α,5α)-. Kaur-16-ene is the major carboxylic acid in all the extracts.

Compounds that were identified exclusively in the HF include (-)-spathulenol, (-)-myrtenol, 4-epi-cubedol, androstane-3,11-diol,(3β,5α,11β)-, longipinocarveol, trans-, tricyclo[5.2.2.0(1,6) undecan-3-ol,2-methylene-6,8,8-trimethyl-, n-hexanedecanoic acid, oleic acid, aristolene epoxide, caryophyllene oxide, 1,6-octadien-3-ol,3,7-dimethyl-, 1H-cyclopenta[1,3]cyclopropa[1,2]benzene,octahydro-7-methyl-3-methylene-4-(1-methylethyl)-,[3aS-(3aα,3bβ,4β,7α,7aS*)]-,1-phenanthrenecarboxaldehyde,7-ethenyl-1,2,3,4a,4b,5,6,7,9,10,10a-dodecahydro-1,4a,7-trimethyl-,[1R-(1α,4aβ,4bα,7β,10aα)]-, 2-naphthalenemethanol,decahydro-α,α,4a-trimethyl-8-methylene-,[2R-(2α,4aα,8aβ)]-, androst-2,16-diene, cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1α,2β,4β)]-, naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethy)-,(1α,4aβ,8aα)-, spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane],1-methyl-4-isopropyl-7,8-dihydroxy-,(8S)-and γ-muurolene. isoaromadendrene epoxide, androstan-17-one,3-ethyl-3-hydroxy-,(5α)- and pregn-4-ene-3,20-dione,16,17-epoxy-,(16α)- are major compounds identified exclusively in the RF. These compounds were presumably produced either by isomerization and/or decomposition during partitioning and subsequent evaporation process. Compounds such as 9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)-, fenretinide, cis-5,8,11,14,17-eicosapentaenoic acid, oleic acid eicosyl ester, ethyi iso-allocholate,. psi.,psi.-carotene,11,1',2,2'-tetrahydro-1,1'-dimethoxy, isoaromadendrene epoxide, 2,4,6-decatrienoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopro[e]cyclodecen-6-yl ester, β-copaene, betulin and columbin were not detected in the fractions. The HPLC-DAD identified the phenolics in the extracts (Figures 4 to 6). The HPLC-DAD analysis of the extracts also revealed the presence of xylopic acid (kaur-16-ene) as shown in Table 3. Other compounds quantified are apigenin, chlorogenic acid, caffeic acid, ellagic acid, rutin, kaempferol and quercetin. The absence of flavonoids in the n-hexane fraction substantiates the report of the qualitative phytochemical analysis.

Table 1: Qualitative determination of phytochemical constituents of ethanol extract, residual fraction and n-hexane fraction of ethanolic extracts of *X. aethiopica*

Test	EE	RF	HF
Saponin	+	+	+
Alkaloids	-	-	-
Phenols	+	+	-
Tannin	+	+	-
Steroids	+	+	+
Terpenoids	+	-	+
Flavonoids	+	+	-
Glycosides	+	+	+
Phlobatannin	-	-	-

(+)=Present, (-) =Absent, EE=Ethanolic extract, HF=n-Hexane fraction, RF=Residual fraction

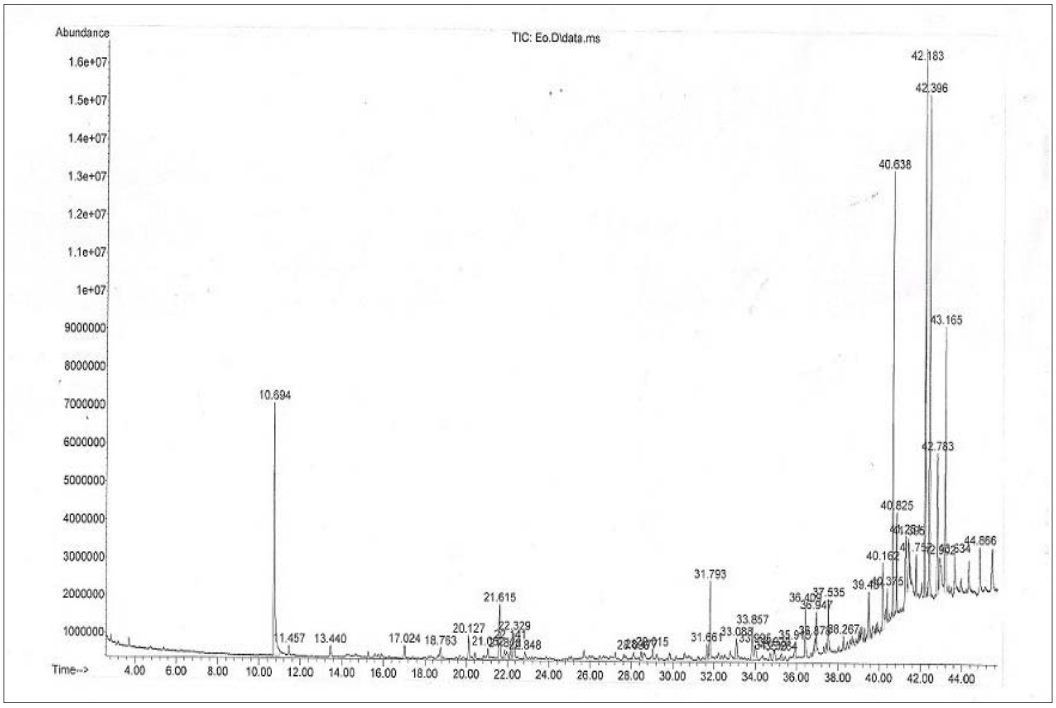

Fig 1: Gas chromatogram of ethanolic extract of *X. aethiopica*

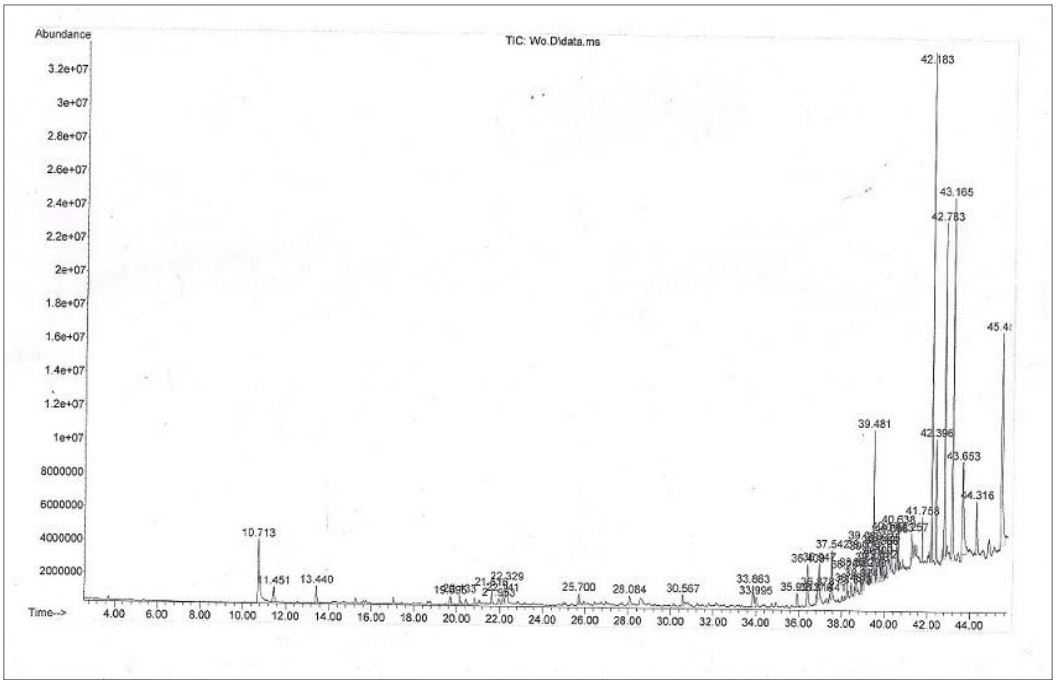

Fig 2: Gas Chromatogram of residual fraction of ethanolic extracts of *X. aethiopica*

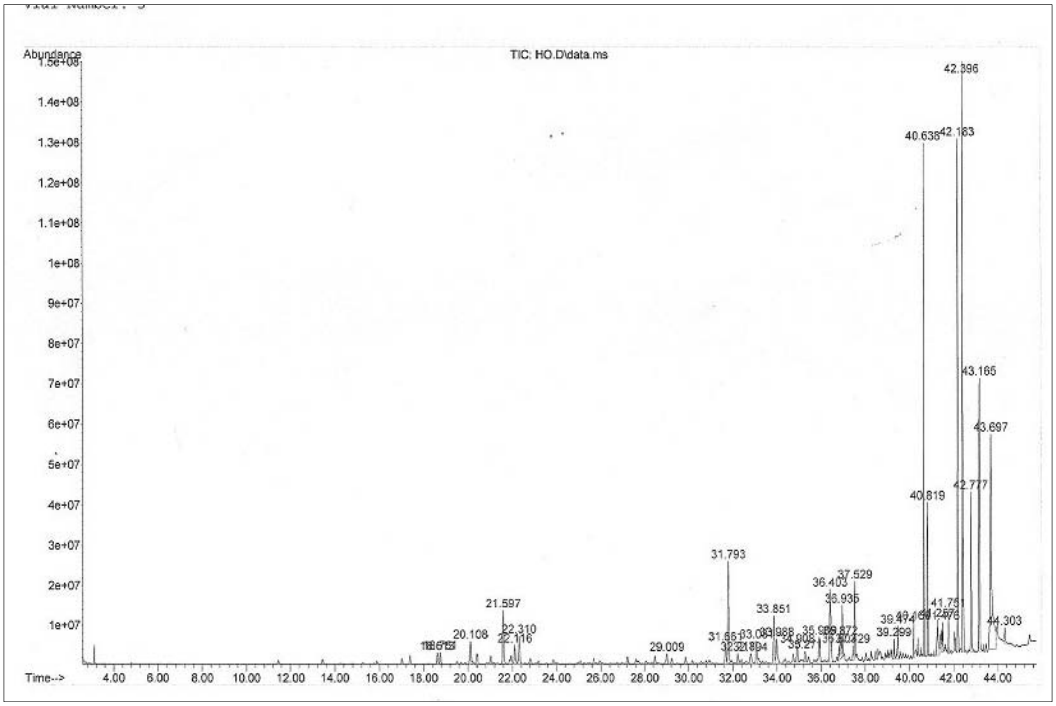

Fig 3: Gas chromatogram of n-hexane fraction of ethanolic extracts of *X. aethiopica*

Table 2: Chemical composition of ethanol extract, residual fraction and n-hexane fraction of ethanolic extracts of *X. aethiopica*

Compounds	EE %	RF %	HF %
Alcohol			
(-)-Myrtenol	-	-	10.56
(-)-Spathulenol	-	-	30.12
1-Naphthalenepentanol,decahydro-5-(hydroxylmethyl)-5,8a-dimethyl-y,2-bis(methylene)-,(1α,4aβ,5α,8aα)-	7.88	23.13	70.63
2-(4a,8-Dimethy-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol		1.44	10.82
4-epi-cubedol	-	-	00.49
5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol	-	0.73	-
6-epi-shyobunol	-	0.65	-
9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)-	1.13	-	-
Androstane-3,11-diol,(3β,5α,11β)-	-	-	00.63
Bicyclo[3.1.0]hexan-2-ol,2-methyl-5-(1-methylethyl)-,(1α,2α,5α)-	-	0.66	1.54
Bicyclo[3.1.1]heptan-3-ol,6,6-dimethyl-2-methylene-,[1S-(1α,3α,5α)]-	1.07	1.79	-
Cubedol	0.44	-	-
Estra-1,3,5(10)-trien-17β-ol	-	1.21	-
Longipinocarveol, trans-	-	-	10.30
L-α-Terpineol	0.87	-	-
p-Cymen-7-ol	-	0.66	-
Platambin	-	4.49	00.95
Terpinen-4-ol	2.44	0.74	20.20
Tricyclo[5.2.2.0(1,6)undecan-3-ol,2-methylene-6,8,8-trimethyl-	-	-	10.28
α-Acorenol	0.49	0.45	-
α-Terpinenol	-	0.63	00.79
Aldehyde			
2-[4-methyl-6-(2,6,6-trimethycyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	0.46	4.06	-
3-Cyclohexen-1-carboxaldehyde,1,3,4-trimethyl-	-	0.38	-
Fenretinide	1.32	-	-
Carboxylic acid			
5-Benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3,6-dimethyl-α-methylene-2-oxo-,methyl ester	-	0.39	-
Cis-5,8,11,14,17-Eicosapentaenoic acid	1.65	-	-
Doconexent	1.06	0.42	
Kaur-16-ene	14.54	11.77	14.61
n-Hexanedecanoic acid	-	-	00.99
Oleic acid	-	-	10.73
Oleic acid, eicosyl ester	2.18	-	-
β-Pimaric acid	-	5.52	13.96

Ethyiso-allocholate	1.45	-	-
Epoxide			
.psi.,psi.-Carotene,11,1',2,2'-tetrahydro-1,1'-dimethoxy	0.91	-	-
Aristolene epoxide	-	-	00.60
Aromadendrene oxide-(2)	-	1.30	00.52
Caryophyllene oxide	-	-	00.83
Isoaromadendrene epoxide	-	2.82	-
Ester			
2,4,6-Decatrienoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopro[e]cyclodecen-6-yl ester	1.84	-	-
2,5-Octadecadiynoic acid, methyl ester	0.95	-	-
9,12,15-Octadecatrienoic acid,2,3-bis[(trimethylsilyl)oxy]propyl ester,(Z,Z,Z)-	-	0.99	-
9-Octadecen-12-ynoic acid, methyl ester	0.53	-	-
Docosahexaenoic acid,1,2,3-propanetriyl ester	0.40	-	-
Hexadecanoic acid, ethyl ester	0.68	-	-
Hexadecanoic acid,1-(hydromethyl)-1,2-ethanediyl ester	2.37	1.05	-
Hydrocarbon			
1,6-Octadien-3-ol,3,7-dimethyl-	-	-	00.46
1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one,1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihdroxy-1,4-bis(hydroxymethyl)-	0.43	2.07	-
1H-Cyclopenta[1,3]cyclopropa[1,2]benzene,octahydro-7-methyl-3-methylene-4-(1-methylethyl)-,[3aS-(3aα,3bβ,4β,7α,7aS*)]-	-	-	40.41
1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol,1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl	-	0.97	-
1H-Naphtho[2,1-b]pyran,3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-,[3R-(3α,4aβ,6aα,10aβ,10bα]-	7.30	0.78	80.65
1-Naphthalenemethanol,decahydro-5-(hydroxy-3-methyl-3-pentyl)-1,4a-dimethyl-6-methylene-,[1S-[1α,4aα,5α(E),8aβ]-	-	9.68	40.47
1-Phenanthrenecarboxaldehyde,7-ethenyl-1,2,3,4a,4b,5,6,7,9,10,10a-dodecahydro-1,4a,7-trimethyl-,[1R-(1α,4aβ,4bα,7β,10aα)]-	-	-	00.56
2-Naphthalenemethanol,decahydro-α,α,4a-trimethyl-8-methylene-,[2R-(2α,4aα,8aβ)]-	-	-	00.72
4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane	2.32	2.18	-
7R,8R-8-Hydroxy-4-isopropylidene-7-methylbicyclo[5.3.1]undec-1-ene	-	1.59	20.03
Androst-2,16-diene	-	-	00.88
Bicyclo[4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene-	0.69	-	-
Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1α,2β,4β)]-	-	-	00.53
Cyclohexanemethanol,4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-,[1R-(1α,3α,4β)]-	1.78	1.18	20.16
Ethanol,2-butoxy-	13.08	4.61	-
Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethy)-,(1α,4aβ,8aα)-	-	-	00.62
Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethy)-,(1S-(1α,4aβ,8aα)]-	0.92	-	1.00
Naphthalene,1,2,3,4a,5,8a-hexahydro-4,7-dimethyl-1-(1-methylethy)-,[1S-(1α,4aβ,8aα)]-	-	-	-
Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane],1-methyl-4-isopropyl-7,8-dihydroxy-,(8S)-	-	-	00.48
Tricyclo[5.2.2.0(1,6)undecan-3-ol,2-methylene-6,8,8-trimethyl-	2.37	1.70	-
β-copaene	3.49	-	-
β-Gualene	-	0.87	
β-Phellandrene	-	0.84	-
γ-Muurolene	-	-	00.86
Betulin	4.22	-	-
Ketones			
5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one,4,9,9a-tris(acetyloxy)-3-[9acetyloxy)methyl]-1,1a,1b,4,4a,7a,7b,8,9,9a-decahy...	0.41	-	-
Androstan-17-one,3-ethyl-3-hydroxy-,(5α)-	-	2.65	-
Hydrocortisone Acetate	1.63	-	-
Podocarp-7en-one,13β-methyl-13-vinyl-	13.05	-	15.14
Pregn-4-ene-3,20-dione,16,17-epoxy-,(16α)-	-	4.42	-
Spirost-8-en-11-one,3-hydroxy-,(3β,5α,14β,20β,22β,25R)-	0.42	-	-
Triamcinolone Acetonide	0.82	-	-
Miscellaneous			
3-Oxo-androsta-1,4-dien-17β-spiro-2'-3'-oxo-oxetane	0.55	-	-
Ascaridole	-	0.62	-
Columbin	1.41	-	-
Ledene oxide-(II)	-	0.41	-
Murolan-3,9(11)-diene-10-peroxy	-	1.14	00.50
2,7-Diphenyl-1,6-dioxopyridazino[4,5:2'3']pyrrolo[4',5'-d]pyridazine	0.48	-	-

Each value is a relative percentage of the chemical constituents expressed as percentage by peak area normalization. EE=Ethanol Extract, RF=Residual fraction, HF=n-hexane fraction, (-) =Absent.

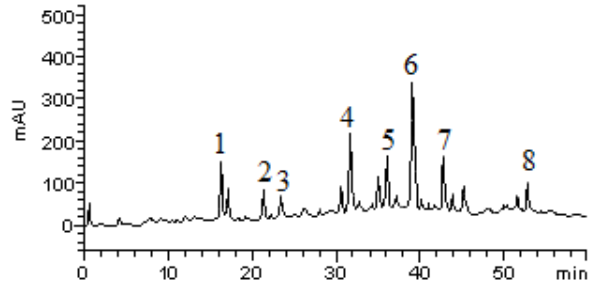

Fig 4: Representative high performance liquid chromatography profile of ethanolic extracts of *X. aethiopica.* Xylopic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), ellagic acid (peak 4), rutin (peak 5), quercetin (peak 6), kaempferol (peak 7) and apigenin (peak 8).

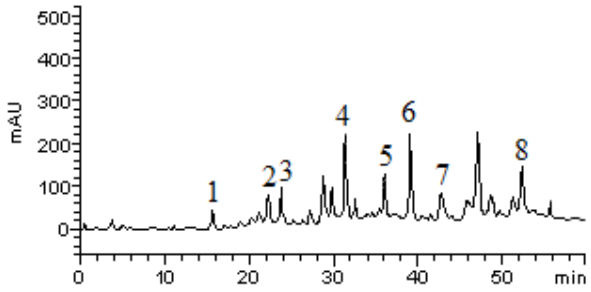

Fig 5: Representative high performance liquid chromatography profile of residual fractionof ethanolic extracts of *X. aethiopica.* Xylopic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), ellagic acid (peak 4), rutin (peak 5), quercetin (peak 6), kaempferol (peak 7) and apigenin (peak 8).

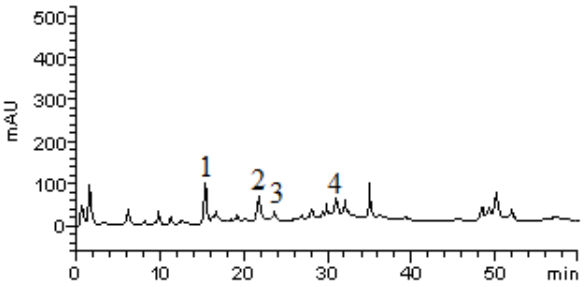

Fig 6: Representative high performance liquid chromatography profile of n-hexane fraction of ethanolic extracts of *X. aethiopica.* Xylopic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), ellagic acid (peak 4), rutin (peak 5), quercetin (peak 6), kaempferol (peak 7) and apigenin (peak 8).

Table 3: Components of ethanol extract, residual fraction and n-hexane fraction of ethanol extracts of *Xylopia aethiopica*

Compounds	EE (mg/g)	RF (mg/g)	HF (mg/g)	LOD (µg/ml)	LOQ (µg/ml)
Xylopic acid	2.65 ± 0.03 [a]	0.94 ± 0.02 [a]	2.26 ± 0.01 [a]	0.013	0.042
Chlorogenic acid	1.23 ± 0.01 [b]	1.16 ± 0.04 [b]	1.34 ± 0.01 [b]	0.007	0.023
Caffeic acid	0.96 ± 0.01 [c]	1.20 ± 0.01 [b]	0.45 ± 0.03 [c]	0.018	0.059
Ellagic acid	3.18 ± 0.04 [d]	3.49 ± 0.01 [c]	0.97 ± 0.02 [d]	0.025	0.083
Rutin	2.61 ± 0.02 [a]	2.63 ± 0.03 [d]	-	0.009	0.029
Quercetin	5.27 ± 0.01 [e]	3.54 ± 0.01 [c]	-	0.021	0.070
Kaempferol	2.64 ± 0.03 [a]	1.21 ± 0.02 [b]	-	0.014	0.046
Apigenin	1.20 ± 0.02 [b]	2.58 ± 0.03 [d]	-	0.023	0.075

Results are expressed as mean ± standard deviations (SD) of three determinations. EE=Ethanol Extract, RF=Residual fraction, HF=n-hexane fraction, (-) =Absent.

4. Discussion

The ethanolic extraction was anticipated to preserve the natural properties of the phytochemicals since the extraction was done at ambient temperature. Moreover, as a polar protic solvent, ethanol would facilitate the extraction of both polar and nonpolar molecules due to its hydroxyl group with high electronegativity and non-polar ethyl group, respectively. The qualitative identifications of the components of the crude extract and the fractions revealed the presence phenolic acids, flavonoids, saponins, steroids and terpennoids and theses classes of phytochemicals have been shown through various studies to mediate biological functions. Terpenoids and steroids were the main phytochemicals in the n-hexane fraction while phenolics, flavonoids, and glycosides were more in the crude ethanol extract and the residual fraction. The biological actions of these phytochemicals have been documented and suggested to probably act via transcription factors such as nuclear factor –kappa B (NFκB) and activator protein 1 (AP-1) that play vial roles in the mediation of biological functions such as the maintenance of the cellular redox status, the immune functions, enhancement of cell-cell recognition, cell proliferation and apoptosis [11-14]. The GC-MS identified volatile and semi-volatile compounds in the samples (Table 2). The identified compounds comprise alcohols, aldehydes, carboxylic acids, esters, hydrocarbons, ketones and oxides. Some of these compounds influence biological properties. Ferentinide and betulin, for instance, promote cell death through apoptosis and or necrosis [15, 16]. The presence of carboxylic acids such as oleic acid, ethyi iso-allocholate, n-hexanedecanoic acid, β-pimaric acid and xylopic acid (kaur-16-ene) indicate that the consumption of the fruit might have beneficial effects on the cardiovascular system. Kaur-16-ene (xylopic acid) is the major compound in all the extracts. It should be noted that there is no registered biological activity for some of the identified compounds such as p-cymen-7-ol, α-acorenol, β-copaeneetc at the moment. Some of the compounds produced during the partitioning and the subsequent evaporation process are suspected to be isomerization and/or decomposition products. Expoxides and hydroperoxides are found mainly in the n-hexane extract

indicating the possibility of epoxidation and hydroperoxidations of unsaturated compounds during partitioning. HPLC-DAD quantification of the phenolic content of the extracts (Table 3) showed the presence of phenolic acids and flavonoids of established antioxidant and anti-inflamamtoory properties such as apigenin, caffeic acid, chlorogenic acid, ellagic acid, kaempferol, rutin and quercetin. These phytochemicals have been demonstrated to mediate antioxidant and inflammatory properties via their interactions with inducers, receptors, messengers and effectors of biosignaling cascades [14, 17-19]. Majority of the phytochemicals identified in the extracts through GC-MS and HPLC-DAD have also been shown through various studies to have modulating effects transcription factors, inhibition of cytokines, down-regulation of cytokine receptors, regulation of signal transductions etc. For instance, xylopic acid (kaurenes diterpenes) has been shown to inhibit iNOS, COX-2 and TNF- α, major mediators of inflammatory response, betulinic acid induces apoptosis through the mitochondrial permeability transition, terpinen-4-ol moderates inflammatory mediator usually produced by activated human monocytes [15, 20, 21]. The results obtained from gas chromatographic separation of EE identified the presence of hydrocortisone, a potentent anti-inflammatory agent. Kowalski *et al.* [14] reported the suppressive effect of apigenin, kaempferol and resveratrol on the expression of interleukin1-β and tumour necrosis factor-α genes in J774.2 macrophages. Caffeic acid and its conjugates such as caftaric acids and chlorogenic have been confirmed to be potent antioxidants in a number of different systems [22]. Quercetin also has been shown to inhibit lipopolysaccharide-induced cytokine production, such as TNF-α production in macrophages and interleukin- 8 production in lung cells considerably endorsing the anti-inflammatory activities of quercetin in diets [23, 24].

5. Conclusion

The results of the study clearly revealed the nature of active principles reputed to be accountable for the reported pharmacological activities of the fruits of *X.aethiopia.* Further study is however suggested to isolate the identified bioactive compounds.

6. References

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