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ELUCIDATION OF BIOACTIVE COMPOUNDS IN METHANOL EXTRACT OF XYLOPIA AETHIOPICA LEAF FROM AN UNCULTIVATED COASTAL LAND IN SUB-SAHARA

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Keywords: *Xylopia Aethiopica Leaf, Bioactive compounds, Methanol extracts.*

Abstract
Xylopia aethiopica (Dunal) A. Rich is known for its numerous traditional uses and pharmacological activities. This study was carried out to elucidate the bioactive components of methanolic leaf extract of Xylopia aethiopica (Dunal) A. Rich. through both qualitative and quantitative analyses of the leaf extract. The qualitative analysis was carried out post methanol extraction through the phytochemical screening of some secondary metabolites such as the tannins, saponins, alkaloids, flavonoids, steroids, terpenoids, and cardiac glycosides. Moreso, quantitative analyses were carried out using Gas chromatography-Mass spectroscopy (GC-MS) technique for the identification and profiling of the different bioactive components present in the aforementioned methanol leaf extract. There was an observable presence of phytochemicals such as the tannins, saponins, alkaloids, flavonoids, and steroids and an absence of terpenoids and cardiac glycosides in the methanolic leaf extract of Xylopia aethiopica (Dunal) A. Rich. Furthermore, the presence of 62 bioactive components was observed with five components including, Bicyclo[3,1,0] hex-2-ene,2 methyl-5-(1-methylethyl) (8.75%), (1S)-2,6,6-Trimethylbicyclo[3,1,1]hept-2-ene-Hexadecanoic acid (11.60%), 2-Octylcyclopropene-1-heptanol (8.43%), Stigmasterol (4.30%) and Octadecanoic acid exhibiting the highest peaks. The presence of these phytocompounds observed in this study thus potentiates its pharmacological uses further making it a good candidate for drug discovery.

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INTRODUCTION

The use of spices as both nutraceuticals and functional foods as a result of their pharmacological activities has gradually picked up momentum as researchers are now gainfully aware of their numerous health benefits (1). Xylopia aethiopica (Dunal) A. Rich, generally known as African guinea pepper is a tall evergreen plant grown in the West African rain forests (2). It belongs to the Annonaceae family and it is well known for its nutritional and medicinal properties (3). Its traditional uses are not far-fetched, In Nigeria for instance, the fruit extract used together with other spices to concoct a spicey soup consumed by pregnant women post-delivery for its belief to be a uterine contractile agent (4). The mature pods are commonly used as spices in most local meals because of their peculiar flavor. In the rural areas of Africa, the herb is decocted and used in traditional medicine to treat different illnesses like ulceration, bronchitis, dysentery, etc. (3,5, 6, 7). In vivo and in vitro studies have shown that X. aethiopica extracts possess anti-bacterial, anti-SARS-CoV, antioxidant (8, 9, 10), anti-inflammatory (11), antibiofilm (12), antiproliferative (9, 13), anti-viral, anti-carcenogenic (14, 15), free-radical scavenging, anti-microbial (16), and anti-depressant (17) properties, which could be a resultant of its function as a potent reservoir of several bioactive components in the plant. Xylopia ethiopicae (Dunal) A Rich. (Annonaceae) plant has been well studied for its biological activities, most especially the seeds/fruits (18, 19, 20). For instance, Previous in vivo and in vitro studies using different experimental models on the antidiabetic effect of various extracts of X. ethiopicae (Dunal) A Rich. (Annonaceae) fruit reported its anti-diabetic activity (21). Mohammed and colleagues in their laboratory, further conducted a bioassay guided isolation of possible bioactive compounds responsible for the antidiabetic action of X. ethiopicae (Dunal) A Rich. (Annonaceae) fruit extract and reported the involvement of oleneanolic acid as a bioactive agent responsible for the antidiabetic effect of X. ethiopicae (Dunal) A Rich. (Annonaceae) through the inhibition of α-amylase and α-glucosidase enzyme activities (2). It has been shown to improve learning and memory in mice (22). Moreso, animal studies carried out to investigate the effect of the anti-inflammatory properties of the hydroethanolic leaf extract of X. ethiopicae (Dunal) A Rich. through the interference with pro-inflammatory cytokines in the THP-1 derived macrophages discovered a significant inhibition (>90%) of TNF-alpha levels with a significant decrease on IL-6 levels at 250 and 500 ug/mL (11). Also, studies on the pharmacological activities of the essential oil obtained from X. ethiopicae (Dunal) A Rich. (Annonaceae) was carried out (23, 24, 25). The anti-inflammatory and anti-bacterial activities of the essential oil from X. ethiopicae (Dunal) A Rich. (Annonaceae) plant obtained from Nigeria and Ghana were carried out using the GC-MS-based metabolomics approach to characterize the quality of essential oil obtained from dried Xylopia ethiopicae (Dunal) A Rich. (Annonaceae) fuit, and was reported to have both anti-bacterial and anti-inflammatory effects which could be attributed to the presence of the 14 different metabolites found in the the X. ethiopicae (Dunal) A Rich. (Annonaceae) fruit extract (26).

Interestingly, there are ongoing researches on the toxicity study of X. ethiopicae (Dunal) A Rich. (Annonaceae) (27) study carried out by Abarikwu et al. on the effects of the ethanolic extract from the seeds and pods of X.

ethiopicae (Dunal) A Rich. (Annonaceae) on the testicular function of adult male Wistar rats using doses ranging from 50 mg/kg b.w. to 200 mg/ kg b.w reported its anti-androgenic effects on the reproductive organs. They went further to explain that the X. ethiopicae (Dunal) A Rich. (Annonaceae) seed extract had more potent inhibitory action on the testosterone level than the pod extract (28).

The use of medicinal plants as natural compounds is being explored not only for their traditional uses but also, for their pharmacological activities. Furthermore, bioactive compounds present in plants are known to be responsible for eliciting pharmacological actions in the body, and thus are being further elucidated using different techniques (29). This work, therefore, aims to elucidate the bioactive components present in the methanol extract of Xylopia ethiopicae (Dunal) A Rich. (Annonaceae) through both qualitative and quantitative analyses using the Gas chromatography-Mass spectrometry (GC-MS) technique.

MATERIALS AND METHODS

Plant collection

Fresh aerial parts of Xylopia Aethiopica leaves were collected from uncultivated coastal area at Apa village of Badagry area of Lagos State, Southwest Nigeria. Plant sample was identified at the Department of Pharmacognosy, Faculty of Pharmacy, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria.

Preparation of plant extract

The leaves were thoroughly cleaned under running tap water and air-dried on a clean laboratory bench at ambient temperature for 7 days. The dried leaves were separated from stalks and grinded to fine powder using porcelain mortar and pestle. Methanol extract was prepared by weighing 100 g dried powder, which was soaked in 2 L of 80% methanol for 72 h with intermittent mixing. This was thereafter filtered with a muslin cloth followed by Whatman No.1 filter paper. The filtrate was concentrated and evaporated at 40°C under reduced pressure using rotary evaporator to obtain the extract.

QUALITATIVE ANALYSES of Xylopia aethiopica (Dunal) A. Rich leaf.

Phytochemical screening

Phytochemical tests were carried out on the methanol extract of Xylopia Aethiopica leaf to identify the constituents using standard procedures (30, 31, 32).

Tannins test

0.5 g of extract was boiled in 20 ml of water in a test tube and filtered. Few drops of 0.1% ferric chloride were added to the filtrate. The occurrence of blackish green coloration confirmed that the presence of tannins.

Saponin test

2 g of extract was dissolved in 20 mL of distilled water, boiled in a water bath and then filtered. 5 ml of distilled water was thereafter mixed with 10 ml of the filtrate and vigorously shaken to give a consistent froth. 3 drops of olive oil were added to the froth. The presence of emulsion formation indicated the presence of saponin.

Flavonoids test

5 mL of dilute ammonia solution was added to 1 mL of methanol extract with a drop of conc. H_2SO_4. A transient yellow precipitate which disappeared after sometime indicates the presence of flavonoids

Steroids test

0.5 mL of methanol extract was added to 2 mL of acetic anhydride with 2 ml H_2SO_4. The colour change from violet to blue confirmed the presence of steroids.

Cardiac Glycosides test (Keller-Killani test)
5.0 mL of methanol extract was mixed with a solution of 2 mL of glacial acetic acid with a drop of ferric chloride. 1 mL of concentrated H_2SO_4 was slowly and cautiously added. The absence of brown ring at interface demonstrated the absence of glycosides.

Test for terpenoids (Salkowski test):
Five ml of methanolic extract was mixed in 3 mL of concentrated H_2SO_4 was carefully added after 2 mL of chloroform was added. Absence of reddish-brown coloration at the inter face shows negative results for terpenoids.

Test for Alkaloids
5 mL of the extract was treated with Wagner's reagent (iodine crystals and KI). A presence of a yellowish-brown precipitate indicates the presence of alkaloids

Alkaloid determination
5 g of the finely blended sample was weighed into a 500 mL beaker and 200 mL of 20 % acetic acid in ethanol was added and covered to at ambient temperature for 6hrs. This solution was stirred and filtered. The resulting extract was concentrated in a water bath till three quarters of the initial volume was evaporated. The alkaloid was precipitated gradually using dropwise addition of concentrated ammonium hydroxide. The solution was left to settle out and then Filtered using a whatman filter paper, the precipitate was dried and weighed (33, 34).

Saponins Determination
20g of the sample was weighed into a 500 ml beaker and 200 mL of 20 % ethanol was added and gently stirred with a stirrer. The temperature of the water bath was set at 55°C. The mixture was then heated over water bath for 4hrs. The residue was extracted with another 200 mL of 20% ethanol. The water bath was then set to 90°C and the extract evaporated till 40 mL was left. The concentrated extract was transferred into a 250 ml beaker and 20 mL of diethyl ether was added and mixed thoroughly. The aqueous layer was decanted while the ether layer was discarded. This process was repeated two more times followed by the addition of 60 mL of n-butanol. 10 mL of 5 % sodium chloride was used to wash the extract twice. The remaining solution was heated over water bath and the residue dried to stable weight. The saponin content was calculated in percentages (33, 34).

Quantification of Tannins
20g of the sample measured into a 500 mL beaker and 150 ml of water was added. The sample was gently stirred and left to stay for 4hrs. This was then filtered using a whiteman filter paper. The solution was acidified with few drops of conc. HCl, followed by the addition of ethyl acetate. This solution was thoroughly mix and allowed to separate out. The aqueous solution was collected while the ethyl acetates layer was carefully discarded off. The solution was heated to a stable dry weight and tannins was determined.

Quantitative analyses of Xylopia aethiopica (Dunal) A. Rich leaf.
Gas Chromatography-Mass Spectroscopy (GC-MS) profiling of bioactive compounds present in Xylopia aethiopica (Dunal) A. Rich leaf
Gas Chromatography/Mass Spectrometry (GC-MS) analyses was carried out using a Shimadzu QP-5050A (SHIMADZU, JP) instrument. This apparatus is equipped with a PTETM-5 column (30 m, 0.25 mm, 0.25 µm, Supelco, USA), the carrier gas was helium gas. The Helium gas was initiated at 22.3 mL/min with the injector temperature maintained at 230°C. The oven was set at an initial temperature of 80°C for an initial period of 3 minutes and thereafter heated to 300°C at 7°C/min, this temperature

was maintained for 5 min. The split valve was closed during the initial first minute of injection but later opened, at a 1:10 ratio. The mass detector was programmed to scan from 50 to 500 *m/z*, at a rate of 2 scans per second. NIST11 Library software database was employed for the identification of compounds by comparing the mass spectrum of the unknown compounds with the known compounds stored in the software database Library.

RESULTS

Phytochemical compositions of X. **ethiopicae (Dunal) A Rich. (Annonaceae)**

The phytochemical screening of Xylopia aethiopica (Dunal) A. Rich leaf. Revealed the presence of some phytochemicals including tannins, saponins, alkaloids, flavonoids, steroids, and the absence of cardiac glycosides and terpenoids as shown in **Table 1.**

The percentage yield of the different phytochemicals prent in X. ethiopicae (Dunal) A Rich. (Annonaceae)

The percentage yield obtained from the quantitative screening of Xylopia aethiopica (Dunal) A. Rich leaf showed showed the percentage yield of the phytochemicals present in descending order which includes alkaloids (5.8%, saponins (4.6%) tannins (1.2%) as shown in **Table 2.**

Identification and profiling of different bioactive components of X. ethiopicae (Dunal) A Rich. (Annonaceae)

The profiling of Xylopia aethiopica (Dunal) A. Rich leaf using GC-MS technique revealed the presence of sixty-seven (67) bioactive compounds with their corresponding peaks as shown in the chromatographic profile **Fig. 1.** and the GC-MS chromatogram **(Fig. 2).** Five bioactive compounds including included Bicyclo[3,1,0] hex-2-ene,2 methyl-5-(1-methylethyl) (8.75%), (1S)-2,6,6-Trimethylbicyclo[3,1,1]hept-2-ene-Hexadecanoic acid (11.60%), 2-Octylcyclopropene-1-heptanol (8.43%), Stigmasterol (4.30%) and Octadecanoic acid were shown with the highest peak were identified (**Figs. 3-7**).

Table 1: Phytochemical screening of the methanolic leaf extract of Xylopia ethiopicae (Dunal) A Rich. (Annonaceae)

Phytochemicals	Inference
Tannins	+
Saponins	+
Flavonoids	+
Steroids	+
Alkaloids	+
Terpenoids	-
Cardiac glycoside	-

+ means Presence; - means Absence

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Table 2: Percentage yield of some phytochemicals obtained from the phytochemical screening of the methanolic leaf extract of Xylopia ethiopicae (Dunal) A Rich. (Annonaceae)

Phytochemicals	Percentage Yield
Tannins	1.2
Saponins	4.6
Alkaloids	5.8

Table 3: Major bioactive compounds identified in the methanolic leaf extract of Xylopia ethiopicae (Dunal) A Rich. (Annonaceae)

Compound name	Molecular weight	Chemical formula	Percentage composition (%)
Oleic Acid	282	$C_{18}H_{34}O_2$	14.76
9,12-Octadecadienoic Acid	280	$C_{18}H_{32}O_2$	11.25
Hexadecanoic Acid	256	$C_{16}H_{32}O_2$	10.92
Octadecanoic acid	284	$C_{18}H_{36}O_2$	9.21
Squalene	410	$C_{30}H_{50}$	8.87

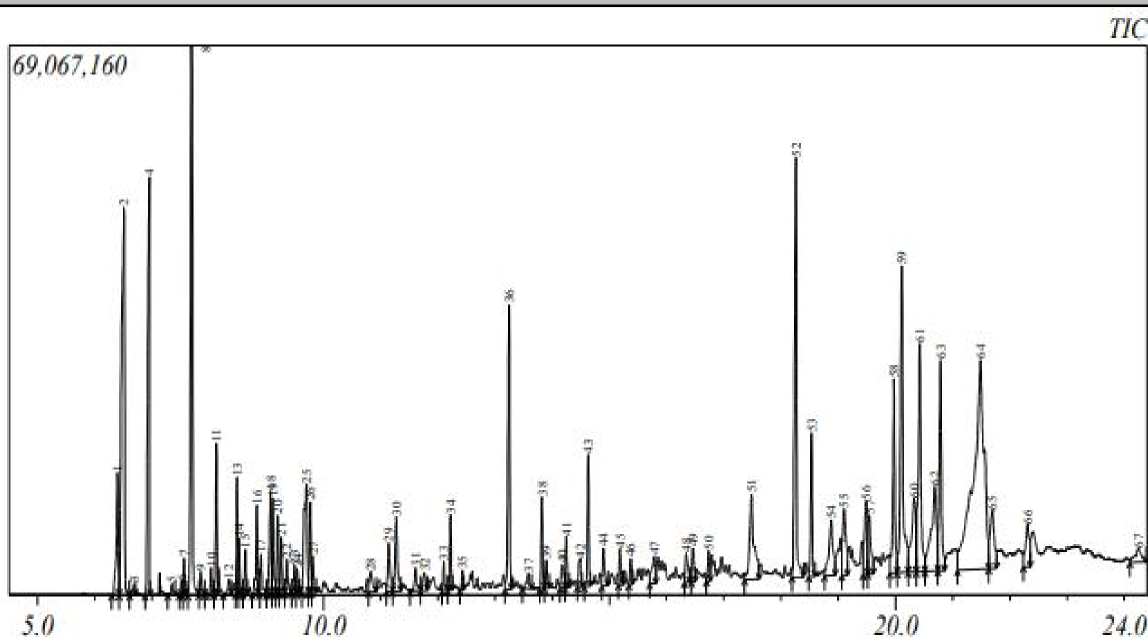

Figure 1. GC-MS chromatogram of Methanol extract of Xylopia Aethiopica, showing different peaks of the phytochemical components identified.

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Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	6.400	6.300	6.430	35086749	1.45	15416284	1.96	2.28		.alpha.-Phellandrene
2	6.511	6.430	6.605	174264977	7.21	48768190	6.19	3.57	V	(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-e
3	6.686	6.605	6.710	3775708	0.16	1365946	0.17	2.76	V	Camphene
4	6.958	6.880	6.970	122535808	5.07	52563295	6.67	2.33	MI	(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-e
5	7.348	7.280	7.410	3626112	0.15	1396225	0.18	2.60	V	.beta.-Pinene
6	7.516	7.480	7.535	3203402	0.13	1804617	0.23	1.78	V	Cyclohexene, 4-methyl-3-(1-methylethylide
7	7.559	7.535	7.600	8594902	0.36	4680265	0.59	1.84	V	Benzene, 1-methyl-3-(1-methylethyl)-
8	7.695	7.635	7.820	152501899	6.31	68997338	8.75	2.21	V	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-m
9	7.851	7.820	7.925	6229400	0.26	2936248	0.37	2.12	V	.beta.-Ocimene
10	8.033	7.925	8.085	7088780	0.29	3385804	0.43	2.09	V	.gamma.-Terpinene
11	8.130	8.085	8.155	30882032	1.28	18990836	2.41	1.63	V	Cyclohexanol, 1-methyl-4-(1-methylethenyl
12	8.349	8.250	8.390	5021664	0.21	1941441	0.25	2.59	V	.alpha.-Methyl-.alpha.-[4-methyl-3-penteny
13	8.490	8.455	8.510	24172258	1.00	14657168	1.86	1.65	V	1,6-Octadien-3-ol, 3,7-dimethyl-
14	8.522	8.510	8.595	11393750	0.47	7081698	0.90	1.61	V	Cyclohexanol, 1-methyl-4-(1-methylethenyl
15	8.633	8.595	8.675	9568600	0.40	5615763	0.71	1.70	V	cis-Verbenol
16	8.831	8.755	8.855	21812826	0.90	11129387	1.41	1.96	V	1-Cyclohexene-1-carboxaldehyde, 4-(1-met
17	8.902	8.885	9.010	10602159	0.44	4977921	0.63	2.13	V	Cyclopropane, trimethyl(2-methyl-1-proper
18	9.072	9.010	9.095	27445182	1.14	12945746	1.64	2.12	V	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-
19	9.116	9.095	9.170	22595750	0.94	12012685	1.52	1.88	V	cis-Verbenol
20	9.198	9.170	9.230	17109416	0.71	9822434	1.25	1.74	V	1-Cyclohexene-1-carboxaldehyde, 4-(1-met
21	9.259	9.230	9.315	11821302	0.49	7058353	0.90	1.67	V	Pinocarvone
22	9.361	9.315	9.440	10268576	0.43	4326169	0.55	2.37	V	Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1
23	9.492	9.440	9.520	7736122	0.32	3581078	0.45	2.16	V	4-Hydroxyadamantan-2-one
24	9.541	9.520	9.585	7090879	0.29	3146537	0.40	2.25	V	Cyclohexanol, 1-methyl-4-(1-methylethenyl
25	9.699	9.620	9.735	51115925	2.12	13626126	1.73	3.75	V	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-d

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26	9.768	9.735	9.795	21495716	0.89	11476664	1.46	1.87	V	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-d
27	9.812	9.795	9.875	9366613	0.39	4652713	0.59	2.01	V	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimet
28	10.819	10.790	10.920	9973192	0.41	2493086	0.32	4.00	V	3,5-Heptadienal, 2-ethylidene-6-methyl-
29	11.141	11.095	11.210	18965267	0.79	6085513	0.77	3.12	V	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylet
30	11.275	11.210	11.355	28178744	1.17	9355426	1.19	3.01	V	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylet
31	11.611	11.510	11.700	8233874	0.34	2968739	0.38	2.77	V	1,3,6-Heptatriene, 2,5,6-trimethyl-
32	11.763	11.700	11.820	7664523	0.32	2305563	0.29	3.32	V	Cedrene
33	12.105	12.075	12.175	6374027	0.26	3650643	0.46	1.75	V	Copaene
34	12.219	12.175	12.255	15584179	0.65	9478557	1.20	1.64	V	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4
35	12.429	12.385	12.455	4290015	0.18	2530506	0.32	1.70	V	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b
36	13.244	13.170	13.285	70969684	2.94	35780134	4.54	1.98	V	.beta.-copaene
37	13.578	13.480	13.635	6537738	0.27	1938003	0.25	3.37	V	10-12-Pentacosadiynoic acid
38	13.821	13.780	13.860	20602288	0.85	11517230	1.46	1.79	V	Cyclohexanemethanol, 4-ethenyl-.alpha.,.al
39	13.895	13.860	13.950	7624887	0.32	3588512	0.46	2.12	V	Aromadendrene oxide-(2)
40	14.170	14.130	14.220	9361906	0.39	2959404	0.38	3.16	V	Kauran-18-al, 17-(acetyloxy)-, (4.beta.)-
41	14.249	14.220	14.300	11773543	0.49	6437923	0.82	1.83	V	Caryophyllene oxide
42	14.487	14.435	14.520	10465843	0.43	3477288	0.44	3.01	V	cis-Z-.alpha.-Bisabolene epoxide
43	14.626	14.555	14.675	30895936	1.28	16540863	2.10	1.87	V	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1
44	14.896	14.865	14.975	12117846	0.50	4767739	0.60	2.54	V	Aromadendrene oxide-(2)
45	15.184	15.155	15.235	8955160	0.37	4532237	0.57	1.98	V	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8
46	15.373	15.335	15.410	6899385	0.29	3259011	0.41	2.12	V	Kauren-18-ol, acetate, (4.beta.)-
47	15.784	15.700	15.815	15001592	0.62	3509636	0.45	4.27	V	Aromadendrene oxide-(2)
48	16.339	16.305	16.395	12028196	0.50	3612655	0.46	3.33	V	Cyclohexanemethanol, 4-ethenyl-.alpha.,.al
49	16.462	16.430	16.500	10317353	0.43	4203283	0.53	2.45	V	cis-Z-.alpha.-Bisabolene epoxide
50	16.733	16.695	16.760	8883455	0.37	3784792	0.48	2.35	V	Isoaromadendrene epoxide
51	17.485	17.365	17.615	56709505	2.35	10741930	1.36	5.28	V	n-Hexadecanoic acid
52	18.259	18.190	18.390	136564953	5.65	52696544	6.68	2.59	V	1H-Naphtho[2,1-b]pyran, 3-ethenyldodecah
53	18.532	18.485	18.590	35891457	1.49	18027862	2.29	1.99	V	Kaur-16-ene
54	18.875	18.760	18.945	34496743	1.43	7007862	0.89	4.92	V	2-Methyl-Z,Z-3,13-octadecadienol
55	19.104	19.055	19.165	35707901	1.48	8362000	1.06	4.27	V	Heptacosane, 1-chloro-
56	19.484	19.435	19.515	33206874	1.37	9163440	1.16	3.62	V	.beta.-Sitosterol
57	19.541	19.515	19.615	26512467	1.10	7388636	0.94	3.59	V	.beta.-Sitosterol
58	19.974	19.895	20.035	66079351	2.74	24365879	3.09	2.71	V	Kauran-18-al, 17-(acetyloxy)-, (4.beta.)-
59	20.112	20.035	20.225	119838792	4.96	38523029	4.89	3.11	V	Andrographolide
60	20.326	20.225	20.360	47764791	1.98	9253098	1.17	5.16	V	5H-3,5a-Epoxynaphth[2,1-c]oxepin, dodeca
61	20.427	20.360	20.520	101317574	4.19	28649131	3.63	3.54	V	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tet
62	20.686	20.520	20.740	71189297	2.95	10549036	1.34	6.75	V	Lupeol
63	20.787	20.740	20.865	74028524	3.06	26477101	3.36	2.80	V	Kauran-18-al, 17-(acetyloxy)-, (4.beta.)-
64	21.486	21.080	21.625	341948058	14.15	26059827	3.31	13.12	V	Lupeol
65	21.691	21.625	21.775	39209522	1.62	7200530	0.91	5.45	V	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-or
66	22.310	22.235	22.355	24269450	1.00	5195210	0.66	4.67	V	1-Heptatriacotanol
67	24.244	24.095	24.385	12933698	0.54	1617246	0.21	8.00	V	Ethyl iso-allocholate
				2415774097	100.00	788412035	100.00			

Figure 2: Phytochemical compounds identified by GC-MS in Methanol extract of Xylopia Aethiopica

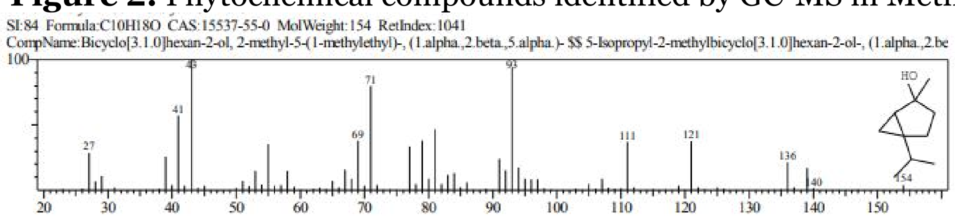

Figure 3: GC-MS mass spectrum and molecular structure of Bicyclo[3.1.0]hexan-2-ol, 2 methyl-5-(1-methylethyl)

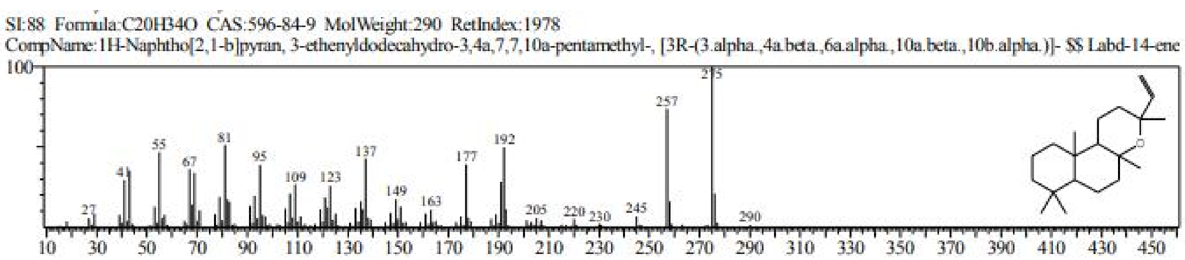

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Figure 4: GC-MS mass spectrum and molecular structure of 1H-Naphthol[2,1b]pyran, 3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-,[3R-(3,alpha,4a,beta,6a,alpha,10a,beta10b,alpha)]

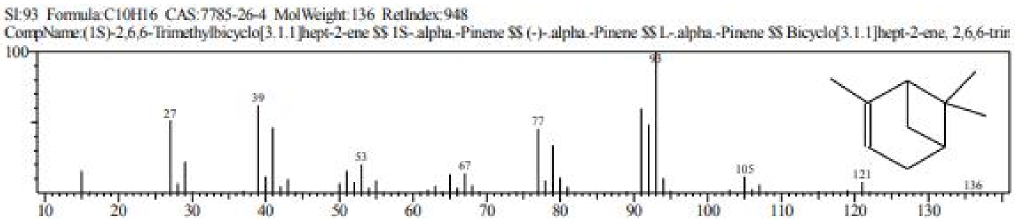

Figure 5: GC-MS mass spectrum and molecular structure of (1S)- 2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene

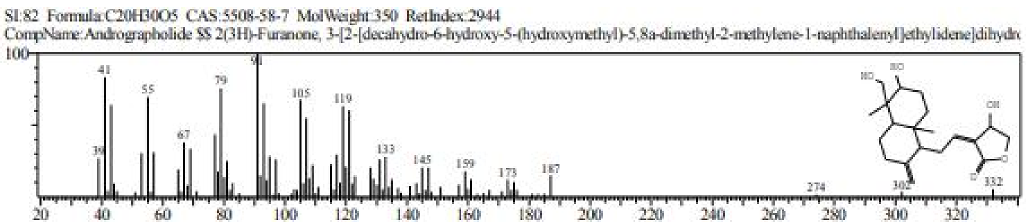

Figure 6. GC-MS mass spectrum and molecular structure of Andrographolide

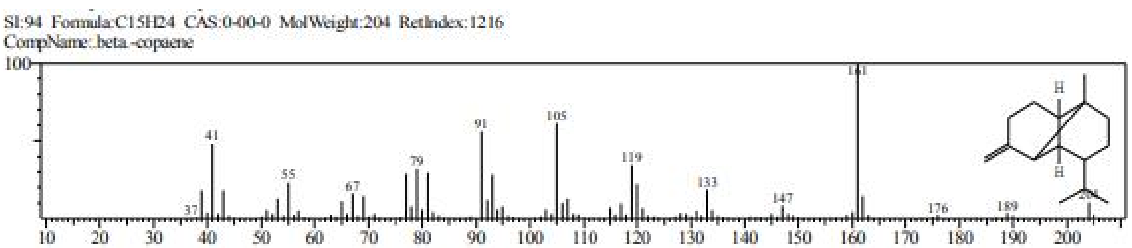

Figure 7: GC-MS mass spectrum and molecular structure of Beta-copaene

DISCUSSION

The leaf extract of Xylopia ethiopicae (Dunal) A Rich. (Annonaceae) investigated in this study is abundantly rich in phytochemicals as observed in the result obtained. The phytochemical screening of X. ethiopicae (Dunal) A Rich. (Annonaceae) methanolic leaf extract exhibited the presence of tannins, saponins, alkaloids, flavonoids, and steroids with an absence of terpenoids and cardiac glycosides. These secondary metabolites present in X. ethiopicae (Dunal) A Rich. (Annonaceae) leaf is a contributory factor to its various pharmacological activities (35). For instance, the presence of flavonoid in X. ethiopica provides it with anti-inflammatory property, thus preventing oxidative damage. Alkaloids which are abundant most especially Anonecaine, are responsible for its anti-pyretic effect, while saponns provide the plant with anti-tumor and alkso, anti-inflammatory properties. Phytochemical profiling of Xylopia ethiopicae has been carried out on the different parts of Xylopia ethiopicae (Dunal) A Rich.

(Annonaceae) plant (21, 36, 37, 38, 39, 40, 41, 42). For instance, Ehiglator et al carried out a Phytochemical screening of ethanolic stem bark extract of Xylopia ethiopicae (Dunal) A Rich. (Annonaceae) and its effect on fertility indices of male rats and noticed that the ethanolic stem bark possesses a robust concentration of alkaloids, saponins, and tannins, with moderate concentrations of steroids and cardiac glycosides (26). Also, a recent study on the suppressive potential of Xylopia ethiopica ethanol seed extract on cadmium chloride-induced ovary and gonadotropins toxicity in adult female Wistar rats by Godam et. al. was reported, attributing the toxicity-suppressive effect to the antioxidant properties present in the Xylopia ethiopica ethanol seed extract (43).

The GC-MS data obtained from the GC-MS chromatogram peak areas showed the presence of eighty-seven (87) compounds with their relative percentage taking into consideration the sum of all eluted peaks as one hundred percent (100%) (Table 3). The major compounds identified were the andrographolide, Bicyclo[3.1.0]hexan-2-ol, 2 methyl-5-(1-methylethyl), 1H-Naphthol[2,1b]pyran ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-,[3R-(3,alpha, 4a,beta, 6a alpha, 10a beta,10b alpha)], (1S)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene, and Beta-copaene as shown in Table 4 with their individual molecular weights. Phenolic profiling of Xylopia ethiopicae (Dunal) A Rich. (Annonaceae) leaf extract by earlier studies led to the identification and quantification of eight constituents namely, caffeoylquinic acid, mono-O-glycosylated flavonols, and the mono-O-glycosyl flavone luteolin-7-O-glucoside of which kaempferol-3-O-rutinoside was discovered to be a contributing factor to its anti-inflammatory activities (39). More so, previous studies carried out on the structural analysis of Xylopia ethiopicae (Dunal) A Rich. (Annonaceae) extract using different techniques showed that this plant contains chemical components which includes volatile oils, essential oils, resins, rutheroside, bitter principles, alkaloids, glycosides, saponins, tannins, sterols, carbohydrate, protein, free fatty acid, and mucilage which is thought to be a contributory factor to its numerous pharmacological effects (42). Some of these phytochemicals were observed in the methanolic leaf extract Xylopia ethiopicae (Dunal) A Rich. (Annonaceae) carried out in our study and as such it could be deduced that the leaf extract of Xylopia ethiopicae (Dunal) A Rich. (Annonaceae) could elicit the aforementioned pharmacological effect in the body.

Thus, the methanolic leaf extract of Xylopia ethiopicae (Dunal) A Rich. (Annonaceae) which have been found to be a reservoir of robust phytocompounds as evidenced in the result obtained in our study and should be incorporated in the development of nutraceuticals which could be useful in the prevention and management of different disease conditions.

CONCLUSION

The findings from our study showed that the methanol extract of Xylopia Aethiopica Leaf contained significant pharmacological properties which are beneficial in the treatment of various diseases. The presence of these phytocompounds observed in this study thus potentiates its pharmacological uses further making it a good candidate for drug discovery.

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