Chemical compositions from the leaf extracts of *Funtumiaafricana* (Benth.) stapf with its antioxidant and anti-inflammatory activity.

Hamid, A. A.^{1*}, Zubair, M. F.², Olajide, F. O.¹, Ibrahim, S. O.², Shehu, A.³, Bale, M. I.⁴, Muhammed, S. O.¹, Aina, S. D,¹ and Jimoh, A.¹

 ¹Department of Chemistry, Faculty of Physical Sciences, University of Ilorin, Ilorin, Nigeria
²Department of Industrial Chemistry, Faculty of Physical Sciences, University of Ilorin, Ilorin, Nigeria
³Department of Chemistry, Faculty of Science, Federal University Lokoja, Lokoja, Kogi State,
⁴Department of Bioscience and Biotechnology, Micobiology Unit, Kwara State University, Malete, Nigeria

*Corresponding Author: Tel No: +2347035931646, E-mail: <u>hamidmemo@gmail.com</u>, hamid.aa@unilorin.edu.ng

ABSTRACT

Antioxidant and anti-inflammatory activity of the extracts of Funtunmiaafricana(Benth.) stapfleaves were investigated in this study. The leaf part of Funtunmiaafricanawere dried, weighed, and exhaustively extracted with n-hexane and chloroform. GC-MS analysis of the extracts was carried out to know the number of compounds present in the extract as well as their molecular formula. These extracts of the plant were evaluated for antioxidant and anti-inflammatory activity using peroxide scavenging, lipoxidase and membrane stabilization. Hexane extract showed antioxidant activity with IC_{50} of 194.09 µg/mL. Hexane and chloroform extracts also showed pronounced anti-inflammatory activity. The GC-MS analysis of the plant extracts showed the presence of twenty-eight compounds in chloroform extract, with terpenoids, fatty acids and unsaturated hydrocarbons as its principal components, while seven compounds were revealed in hexane extract of the plant, and its most abundant compound is 1,2-Benzenedicarboxylic acid, bis(2methylpropyl)ester.

Keywords: Antioxidant activity, anti-inflammatory activity, α-bisabolol, 13-methyl pentadecanoic acid, GC-MS.

INTRODUCTION

Medicinal plants include various types of plants used in herbalism and some of these plants have medicinal exertions. Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis¹. *Funtumiaafricana*(Benth.) stapf is a tropical tree up to 30 m tall (usually shorter) with a straight, cylindrical trunk and a narrow tree crown. Bark brown to dark in color, thin and slightly fissured becoming granular on old trees. Slash orange exuding latex copiously². Leaves elliptic or ovate, base round orcuneate, apex acuminate 20 x 9 cm, with approximately 8-14 main lateral veins on each side, leaf margins wavy. Axils on the main lateral veins not pitted.Flowers yellow-white, fragrant in dense cymes.Corolla tube 6-10 mm, lobes 5-7 mm.Fruit grey-brown, fusiform, with an acute or acuminate apex, up to 30 cm long, with hairy wind-borne seeds.The generic epithet is derived from 'funtum', a local Ghanaian (Akan dialect) name of the plant. The specific epithet means 'of Africa³. In Africa this species is used to treat urinary incontinence and burns. The leaf and bark are used as enema. The principle alkaloids of *F. africana* are hypotensive⁴.*F. Africana* is used to treat and manage diverse ailments including fever, inflammation, malaria, cancer and urinary incontinence in Africa.

MATERIALS AND METHODS

F. africana plant was collected at Ajashe-Ipo, Kwara state, November 2017. The plant was firstly identified using its vernacular name by an area hunter, Mr. Sumanu and later identified and authenticated by Mr. AjayiBolu of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. A voucher specimen [UILH/004/337] was deposited into the herbarium section of the department. Leaf part of the plant were washed with water, air dried for more than a week, crushed and grounded into a powdery form. The weight after grinding was 1,660g.The plant samples were weighed and extracted using cold extraction method with two solvents n-hexane and chloroform.

Antioxidant Activity Hydrogen peroxide scavenging activity

The ability of the samples to scavenge peroxide radicals was assessed following the procedure⁵.

A solution of H_2O_2 (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). The extracts at different concentrations in 3.4 ml phosphate buffer was added to 0.6 mL of H_2O_2 solution (0.6 mL, 43 mM). The absorbance value of the reaction mixture was recorded at 230 nm. H_2O_2 scavenging activity (%) = ($A_0 - A_1$) / $A_0 \times 100$ Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

The absorbance was measured in three folds at different concentrations and the mean absorbance for each concentrationwere determined. Parallel to examination of the antioxidant activity of the plant extracts, the value for the standard compound (Ascorbic acid) was obtained and compared to the values of the antioxidant activity and percentage inhibition of the standard and the extracts was determined using the expression above.The IC₅₀ values (Inhibition Concentration at 50%) were estimated from the %inhibition versus concentration graph⁶.

Anti-Inflammatory Assay of the Crude Extract Anti-Lipoxygenase activity

Anti-Lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme⁷. Test samples were dissolved in 0.25 mL of 2 M borate buffer pH 9.0 and added 0.25ml of lipoxidase enzyme solution (20,000 U/mL) and incubated for 5 min at 25°C. After which, 1.0mL of linoleic acid solution (0.6 mM) was added, mixed well and absorbance was measured at 234nm. Indomethacin was used as reference standard. The percentage inhibition was calculated from the following equation, % inhibition= [{Abs control- Abs sample}/Abs control] x 100, A dose response curve was plotted to determine the IC₅₀ values. IC₅₀ is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged

Membrane stabilization test

Preparation of red blood cells (RBCs) suspension.

Fresh whole human blood (10 mL) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline⁸.

GC-MS analysis of the extracts

GC-MS analysis of the two plants' extracts was performed with Agilent 19091GC plus automatic sampler system coupled with a quadruple Mass Spectrometer 433HP-5MS. Compounds were separated in HP5MS column fused with phenylmethylsilox, (length; 30 m x 250 μ m; film thickness 0.25 μ m). Samples were injected at a temperature of about 25°C with a split ratio of 10:1 with a flow rate of helium 1 mL/min.

RESULTS AND DISCUSSION

Antioxidant activity of Funtumiaafricana

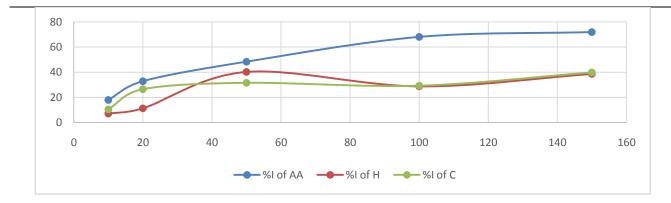
The ability of the plants' extracts (n-hexane and chloroform) against peroxide radical scavenging was analyzed.

Antioxidant Activity (IC_{50} Graph) of F. africana leaf extracts

Hexane extract of *Funtumiaafricana* leaves exhibited antioxidant activity on peroxide radicals at different concentrations, using ascorbic acid as standard antioxidant. Hexane extract of the plant leaves showed inhibition of peroxide radicals at concentrations in the range of 10-150 µg/mL, by scavenging the free radicals with IC₅₀ of 195 µg/mL while chloroform extract of the plant shows inhibition of peroxide radical scavenging with IC₅₀ of 218 µg/mL.

	Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Mean	% Inhibition
	10	0.1903	0.1828	0.2385	0.030226864	17.93755
	20	0.2965	0.2012	0.1974	0.056150601	32.86092
	50	0.5081	0.3608	0.2534	0.127869817	48.35491
	100	0.5844	0.448	0.4298	0.084495917	68.01345
Ascorbic acid	150	0.6802	0.5735	0.5296	0.077451555	71.85589
	10	0.343	0.3368	0.4634	0.3674	7.102227
	20	0.3442	0.3501	0.3977	0.301567	11.2628
	50	0.2432	0.2325	0.2601	0.280733	40.20803
Hexane	100	0.2868	0.2969	0.2936	0.290167	28.70957
extract	150	0.2929	0.2226	0.2402	0.246967	38.59093
Chloroform	10	0.2899	0.2623	0.2799	0.381067	10.43393
extract	20	0.268	0.2635	0.2567	0.364	26.48302
	50	0.2395	0.2211	0.2162	0.245267	31.56184
	100	0.2321	0.2078	0.2079	0.292433	29.26215
	150	0.2015	0.1978	0.2007	0.2519	39.7936

Table 1:Hydrogen Peroxide Radical Scavenging of Hexane and ChloroformExtract of *Funtunmia Africana* Leaves



AA=Ascorbic Acid, H=Hexane extract, C=Chloroform extract.

	Concentrati on (µg/mL)	Absorbanc e 1	Absorbanc e 2	Absorbance 3	Mean	%Inhibition
	10	0.03685	0.094	0.0327	0.034256471	47.19987
	20	0.046325	0.041125	0.027575	0.009679919	62.86557
	50	0.041725	0.02355	0.033575	0.009103605	68.08748
T 1 (1	100	0.031425	0.0258	0.037075	0.005637505	69.55639
Indomethac	150		0.017225	0.021478	0.002167138	
inA _{control} =0.103251		0.020075				81.02424
	10	0.043681	0.03035	0.03425	0.006854066	66.78805
	20	0.039665	0.031475	0.032675	0.004422974	67.10282
	50	0.047587	0.02085	0.028249	0.01385619	75.35972
Hexane	100	O.038992	0.029225	0.024828	0.0031099149	76.06157
extract	150	0.029098	0.021454	0.025775		78.35243
					0.03832843	
Chloroform	10	0.047	0.041625	0.042319	0.002923583	57.69403
extract	20	0.040075	0.041425	0.037495	0.001996822	61.58391
	50	0.040161	0.042175	0.060425	0.011163545	53.91134
	100	0.037225	0.041875	0.037875	0.002518101	62.23604
	150	0.02042	0.039225	0.02765	0.009485792	71.81787

Table 2: Anti-inflammatory activity of hexane and chloroform extracts of F. africanaleaves (Lipoxidase).

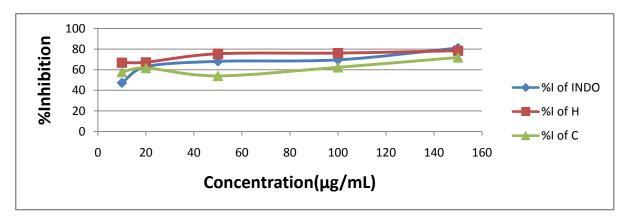
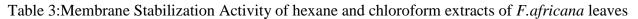


Figure 2a: AntiinflammatoryActivity of hexane and chloroform extracts of *F.africana* leaves (lipoxidase)

	Concentrati on (µg/ml)	Absorbanc e	Absorbanc e	Absorbance	Mean	%Inhibition
Indomethac in A _{control =1.103}	10 20 50 100 150	0.4661 0.6528 0.6528 0.6537 0.7193	0.4394 0.6972 0.6993 0.6582 0.7212	0.3971 0.5596 0.5315 0.6008 0.6264	0.034792672 0.07022744 0.086634077 0.0319202265 0.05419265	37.53702 42.19704 42.6715 42.29072 60.63463
Hexane extract	10 20 50	0.7328 0.7151 0.5977	0.5844 0.5525 0.5704	0.604 0.5486 0.4877	0.08061608 0.095022997 0.057277948	41.94016 45.11333 49.96071
	100 150 10	0.5261 0.5192 0.6187	0.5338 0.5087 0.6456	0.5184 0.5138 0.6151	0.0077 0.005250714 0.016667433	52.30281 53.40888 43.20338
Ethyl acetate extract	20 50	0.6491	0.6146	0.6098	0.021438983	43.38169 44.13116
	100 150	0.6016 0.574	0.5882 0.5907	0.5868 0.6106	0.008170679 0.0183233	46.31006 46.34935



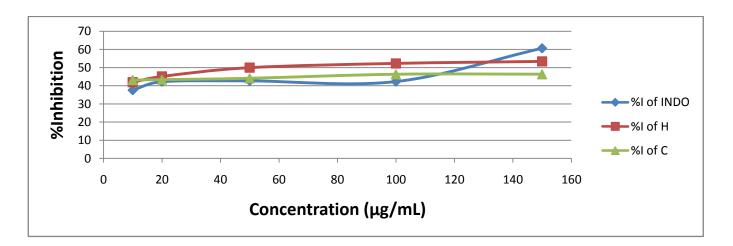


Figure 2b: AntiinflammatoryActivity of hexane and chloroform extracts of *F. africana* leaves (membrane stabilization) INDO=Indomethacin, H=Hexane, C=Chloroform.

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Lipoxidase

Hexane and chloroform extracts of *F*. *africana*leaves exhibited antiinflammatory activity with an IC₅₀values of 211 and 62.5 μ g/mL respectively with IC₅₀ value of the indomethacin standard being 18.86 μ g/mL. chloroform extract out of the two extracts is more anti-inflammatory active than the other extract because of its closer value to the value of the standard.

Membrane Stabilization

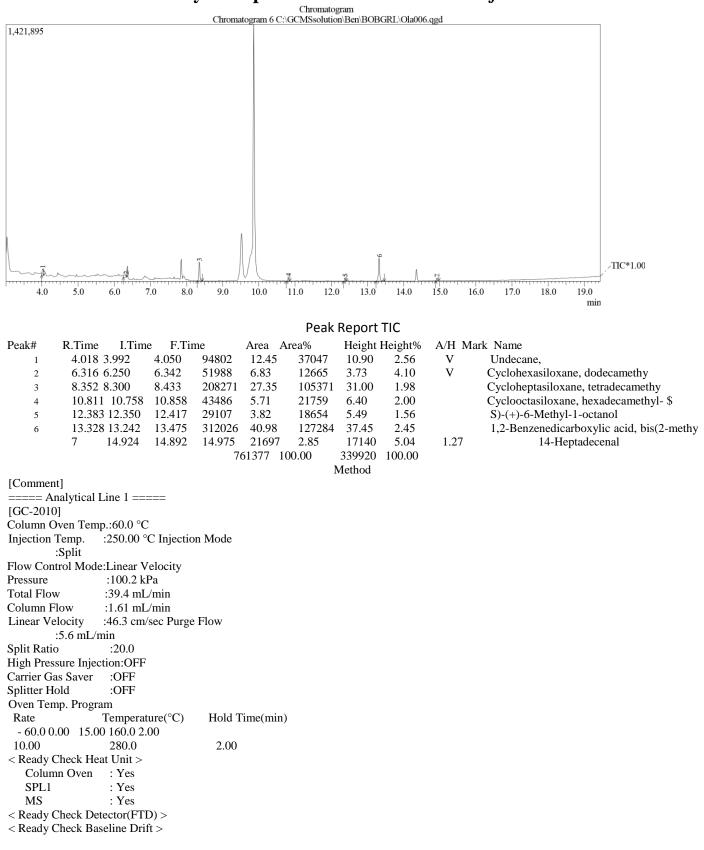
Hexane and chloroform extracts of *Funtumia africana* leaves exhibited anti-inflammatory activity with an IC₅₀ values of 86.27 and 280μ g/mL respectively while the value of indomethacin is 103.97μ g/mL. Hexane extract out of the two extracts shows a more pronounced anti-inflammatory activity than the other extract.

GC-MS Results of Hexane and chlorofoam extracts of F. africana leaves

The GC-MS analysis hexane extract of F.africanarevealed the presence of seven vertical peaks, the comparison of the peak with the pherobase library indicated the presence of seven shown above. 1.2compounds as Benzenedicarboxylic acid, bis (2 methylpropyl)ester is the most abundant with % abundance of 40.98, molecular formula $C_{16}H_{22}O_4$ and retention time of 13.328.

The GC-MS analysis of choroform extract of *F.africana*leaves showed the presence of twentyeight compounds.The principal compounds terpenoids, fatty acids and unsaturated hydrocarbons as its principal components include α -Bisabolol, Carophyllene,1-Nonadecene, Phytol and 13-Methyl pentadecanoic acid, with their corresponding % of abundance of 20.30, 15.71, 6.71, 4.36 and 2.92 respectively.

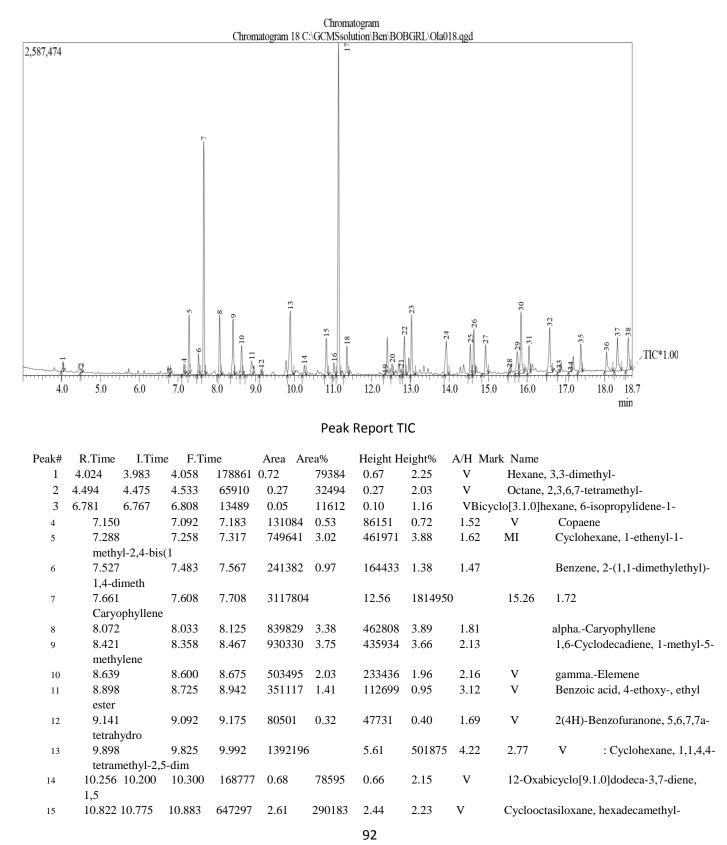
GC-MS Analysis Report Hexane Extract of F. Africana Leaves



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S/N	Compounds	% Abundance	Molecular formula	Retention time
1	5,7-dimethylundecane	12.45	$C_{13}H_{28}$	4.018
2	Dodecamethylcyclohexasiloxane	6.83	$C_{12}H_{36}O_6Si_6$	6.316
3	Tetradecamethylcycloheptasiloxane	27.35	$C_{14}H_{42}O_7Si_7$	8.552
4	Hexadecamethylcyclooctasiloxane	5.71	$C_{16}H_{48}O_8Si_8$	10.811
5	(S)-(+)-6-methyl-1 octanol	3.82	$C_9H_{20}0$	12.383
6	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	40.98	$C_{16}H_{22}O_4$	13.328
7	Cyclohexanepropanol	2.85	$C_9H_{18}O$	14.924

GC-MS Analysis Report Chloroform Extract of F. Africana Leaves



16	11.023	10.975	11.075	190881	0.77	98107	0.82	1.95		Aroma	dendrene oxid	de-(2)
17	11.138 11.075	11.200	5113550	C	20.60	2584588	3	21.72	1.98	V	alphaBis	abolol
18	11.352 11.275	11.408	465613	1.88	227376	1.91	2.05	V	2,5-D	imethoxy-	4-ethylamphe	
19	12.333 12.300	12.350	12047	0.05	5604	0.05	2.15			nadecene	• •	
20	12.521 12.492	12.567	180635	0.73	87062	0.73	2.07	V	2,5-D	imethoxy-	4-ethylamphe	tamine
21	12.751 12.725	12.792	95458	0.38	47517	0.40	2.01	V	Hexad			
22	12.835 12.792	12.892	576918	2.32	304711	2.56	1.89	V	Cycloi	nonasiloxa	nne, octadecan	nethyl-
23	13.025 12.992	13.125	905183	3.65	468569	3.94	1.93	V	2-Pen	tadecanon	e, 6,10,14-trin	nethyl-
24	13.915 13.858	13.983	735803	2.96	265020	2.23	2.78	V	Penta	decanoic a	cid, 13-methy	l-, methyl es
25	14.541 14.47	75 14.	.592	489810	1.97	237	606	2.00	2.06	V	Cyclodeca	siloxane,
eicos	amethyl-											
26	14.630	14.592		14.667		687235	2.7	77 3.	52012	2.96	1.95	V
	1-Nonadecene											
27	14.927 14.833	15.000	570836	2.30	227751	1.91	2.51	V	Octade	ecanal		
28	15.544 15.508	15.558	92635	0.37	40041	0.34	2.31	V	2-Hyd	droxy-1,1,	10-trimethyl-6	,9-epidioxyd
29	15.750 15.692	15.792	506561	2.04	170516	1.43	2.97	V	11,14	,17-Eicosa	atrienoic acid,	methyl ester
30	15.844 15.792	15.925	1099272	2	4.43	480380	4.04	2.29	V	Phyto	1\$	
31	16.047	16.000	16.100	491543	1.98	219089	1.84	2.24	V	Cyclo	octasiloxane,	
	hexadecamethyl	-										
32	32 16.581 16.5	08 16.667	989349	3.99 353	470 2.97	2.80 V	1-Nona	adecene				
33	16.826 16.783	16.850	49398	0.20	20700	0.17	2.39	V	Oxira	ne, hexade	ecyl-	
34	17.118 17.067	17.150	15186	0.06	5916	0.05	2.57		3,7,11	,15-Tetra	methyl-2-hexa	decen-1-o
35	17.387 17.308	17.433 48	5166 1.9	5 217493	1.83 2.2	3 V Cyo	clonona	siloxane, oc	ctadecam	ethyl-		
36	36 18.048 18.0	00 18.217	409264	1.65 151	968 1.28	2.69 V	4,8,12	,16-Tetrame	ethylhept	adecan-4-	olide	
37	18.332 18.250	18.433	674537	2.72	262973	2.21	2.57	V	1-Hep	otacosanol		
38	18.607 18.542	18.675	572655	2.31	254455	2.14	2.25		Cyclo	decasiloxa	ne, eicosametl	nyl- \$
			248	21248 10	0.00 11	1897180	100.00					
					Ν	Method						

[Comment]									
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Injection Temp.	:250.00 °C								
Injection Mode	:Split								
Flow Control Mode	:Linear Velocity								
Pressure	:100.2 kPa								
Total Flow	:39.4 mL/min								
Column Flow	:1.61 mL/min								
Linear Velocity	:46.3 cm/sec								
Purge Flow	:5.6 mL/min								
Split Ratio	:20.0								
High Pressure	:OFF								
Injection									
Carrier Gas Saver	:OFF								
Splitter Hold	:OFF								
Oven Temp. Program	n								
Rate	Temperature(°C)	Hold							
		Time(min)							
-	60.0	0.00							
15.00	160.0	2.00							
10.00	280.0	2.00							
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Column Oven	: Yes								
SPL1	: Yes								
MS	: Yes								

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Interpretation of GC-MS analysis of chloroform extract of F. africanaleaves

Compound		% Abundance	Molecular formula	Retention time
Copaene		0.52	$C_{15}H_{24}$	7.150
1-ethenyl-1-methyl-2,4 b	is(1-	2.98	$C_{15}H_{24}$	7.288
methylethenyl) cyclohexane				
2-(1,1,dimethyl)-1,4-dimethoxy Benzer	ne	0.96	$C_{12}H_{18}O_2$	7.527
Caryophyllene		15.71	$C_{15}H_{24}$	7.661
1-methyl-5-methylene-8-(1-methylethy	·l)-	3.69	$C_{15}H_{24}$	8.421
1,6-cyclodecadiene				
Gamma-elemene		2.00	$C_{15}H_{24}$	8.639
4-ethoxy ethyl Benzoate		1.39	$C_{11}H_{14}O_3$	8.898
2(4H)-Benzofuranone,5,6,7,7a-		0.32	$C_{11}H_{16}O_2$	9.141
trimethyl(R)				
1,1,4,4-tetramethyl-2,5 dimethy	lene	5.53	$C_{11}H_2O$	9.898
	Copaene 1-ethenyl-1-methyl-2,4 b methylethenyl) cyclohexane 2-(1,1,dimethyl)-1,4-dimethoxy Benzer Caryophyllene 1-methyl-5-methylene-8-(1-methylethy 1,6-cyclodecadiene Gamma-elemene 4-ethoxy ethyl Benzoate 2(4H)-Benzofuranone,5,6,7,7a- trimethyl(R)	Copaene 1-ethenyl-1-methyl-2,4 bis(1- methylethenyl) cyclohexane 2-(1,1,dimethyl)-1,4-dimethoxy Benzene Caryophyllene 1-methyl-5-methylene-8-(1-methylethyl)- 1,6-cyclodecadiene Gamma-elemene 4-ethoxy ethyl Benzoate 2(4H)-Benzofuranone,5,6,7,7a- trimethyl(R)	Abundance Copaene 0.52 1-ethenyl-1-methyl-2,4 bis(1- 2.98 2.98 methylethenyl) cyclohexane 0.96 2-(1,1,dimethyl)-1,4-dimethoxy Benzene 0.96 Caryophyllene 15.71 1-methyl-5-methylene-8-(1-methyl-thyl)- 3.69 1,6-cyclodecadiene 2.00 4-ethoxy ethyl Benzoate 1.39 2(4H)-Benzofuranone,5,6,7,7a- 0.32 trimethyl(R)	Abundane formula Copaene 0.52 C15H24 1-ethenyl-1-methyl-2,4 bis(1) 2.98 C15H24 methylethenyl) cyclohexane C12H18O2 2-(1,1,dimethyl)-1,4-dimethoxy Benzene 0.96 C12H18O2 Caryophyllene 15.71 C15H24 1-methyl-5-methylene-8-(1-methyl-thyl)- 3.69 C15H24 1,6-cyclodecadiene C15H24 Gamma-elemene 2.000 C15H24 4-ethoxy ethyl Benzoate 1.39 C11H14O3 2(4H)-Benzofuranone,5,6,7,7a- 0.32 C11H16O2

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cyclohexanol

		cyclonexunor			
1	0	12-oxabicyclo[9.1.0]dodeca-	0.67	$C_{15}H_{24}O$	10.256
		3,7diene,1,5,5,8 tetramethyl			
1	1	Hexadecamethylcyclooctasiloxane,	4.52	$C_{16}H_{48}O_8Si_8$	10.822
1	2	Aromedendrene oxide-(2)	6.76	$C_{15}H_{24}O$	11.023
1	3	Alpha- Bisabolol	20.30	$C_{15}H_{26}O$	11.138
1	4	2,5-Dimethoxy-4-ethylamphetamine	2.57	$C_{13}H_{21}NO_2$	12.521
1	5	Hexadecanal	0.38	$C_{15}H_{30}O$	12.751
1	6	Octadecamethylcyclononasioxane	4.22	$C_{18}H_{54}O_9Si_9$	12.835
1	7	6,10,14-trimethyl-2-Pentadecanone	3.59	$C_{18}H_{36}0$	13.025
1	8	13-methyl Pentadecanoic acid	2.92	$C_{17}H_{34}O_2$	13.915
1	9	Eicosamethylcyclodecasiloxane,	4.01	$C_{20}H_{60}O_{10}Si_{10}$	14.541
2	20	1-Nonadecene	6.71	$C_{19}H_{38}$	14.630
2	21	Octadecene	2.27	$C_{18}H_{36}$	14.927
2	22	2-Hydroxyl-1,1,10-trimethyl 1,6,9-	0.37	$C_{13}H_{22}O_3$	15.544
		epidioxydecalin			
2	23	Methyl-11,14,17-eicosatrienoate	2.01	$C_{21}H_{36}O_2$	15.750
2	24	Phytol \$	4.36	$C_{20}H_{40}O$	15.844
2	25	Hexadecyl oxirane,	0.20	$C_{18}H_{36}O$	16.826
2	26	3,7,11,15-tetramethyl-2-hexadecene-1-ol	0.60	$C_{20}H_{40}O$	17.118
2	27	4,8,12,16-tetramethyl heptadecane-4-olide	1.62	$C_{21}H_{40}O_2$	18.048
2	28	1-Heptacosanol	2.62	C ₂₇ H ₅₆ O	18.332

CONCLUSION

Hexane and chloroform extracts of *Funtumiaafricana*leaves showed high antioxidant and antiinflammatory activity. This may be due to the presence of active compounds in the plant extracts.

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