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Chemical constituents from the leaf extracts of *Scleria depressa* (C.B. Clarke) Nelmes with its antioxidant and antiinflammatory activity.

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Abstract

Antioxidant and antiinflammatory activity of the extracts of Scleriadepressa leaf part were investigated in this study. The leaf part of Scleriadepressa were dried, ground, weighed, and exhaustively extracted with n-hexane, ethylacetate and methanol. GC-MS analysis of the extracts was carried out to know the compounds present in the extracts as well as their molecular formula. These extracts of the plant were evaluated for antioxidant and antiinflammatory activity using peroxide scavenging, lipoxidase and membrane stabilization. Hexane, Ethylacetate and methanol extracts of the Scleriadepressa leaves exhibited antioxidant activity on peroxide radicals at different concentrations ranging from 10-150 µg/mL, using ascorbic acid as standard antioxidant. Ethylacetate and methanol extracts of the plant's leaves possessed antioxidant activity by exhibiting peroxide free radical scavenging with $IC_{50}$of 106.23 and 148.79µg/mL respectively, using peroxide antioxidant assay. The hexane extract shows inhibition that is more pronounced compared to that of ethylacetate for the anti-inflammatory activity while methanol extract of the plant's leaves shows activity higher than that of hexane and ethylacetate for the anti-inflammatory activity. The GC-MS analysis shows the presence of 13 compounds for hexane extract with Carvomenthol,(an α-Terpenol) and 1,2,3-Trimethylbenzene being the abundant compounds with %abundanceof 23.78% and 19.20% respectively, while ethylacetate extract revealed 19 compounds with 1,2-Benzenedicarboxylic acid,bis(2-methylpropylester), and 4,7-dimethylundecane with corresponding % abundance of 24.56% and 16.83% being the abundant compound also the GC-MS analysis of methanol extract of Scleriadepressa leaves showed the presence of 11 compounds. The compound with highest abundance is Methyl-9-octadecenoate, with %abundance of 69.86 and retention time of 14.714.

Keyword: Antioxidant activity, anti-inflammatory activity, carvomenthol, α-Terpenol, GC-MS.

1. Introduction

Present in the body are some reactive oxygen species commonly called free radicals that cause oxidative stress which could lead to some chronic conditions and diseases such as carcinogenesis, and damaging of cells (Sies, 1997). There is need to convert these unstable free radicals into a normal or fairly stable

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ones, therefore the search for antioxidant drugs becomes a compulsory tool. *Scleria depresa*is a Nigeria native plant that belongs to the family Cyperaceae with the common name sword grass, it is a robust plant with erect stems up to 2m high. The section of the stem is triangular in shape. It has large linear leaves which measures up to 3cm wide, with well-marked ribs. Theses leaves are scabrous on the margin and veins. The inflorescence is a terminal panicle composed of a set of tiered panicles, each one located at the axil of a bract leaf. The male spikelet is long and female spikelet are swollen. The fruit is a compressed achene smooth and shiny, bluish grey colour, which has circular groove around the apex. It has sedge weed type. It reproduces by seeds. It is usually found on lowland, hydromorphic areas, sides of ponds and stream in the savanna. It is frequent and usually found in Nigeria, while rare and not abundant in countries like Benin and Burkina-Faso (Johnson, 1997).

The plant parts can be used for the treatment of cough, irregular menstruation, easing of labour, it can be used to produce new antibiotics as it is from natural source. This research work focuses on the antioxidant and anti-inflammatory activity of the plant extracts.

2. Materials and Methods

Scleria depressa plant was collected at Ajase-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. Ajayi Bolu of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. A voucher specimen [UIH/002/1188] was deposited into the herbarium section of the department. Leaves' 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 1300 g. The plant samples were weighed and extracted using successive extraction method by moving from a non-polar (n-hexane) solvent to a medium polar solvent (ethyl acetate) and then to a polar solvent (methanol).

Antioxidant activity

Hydrogen peroxide scavenging activity

The ability of the samples to scavenge peroxide radicals was assessed following the procedure of Ruch *et al.,* (1989). 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 concentration was 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 IC_{50} values (Inhibition Concentration at 50%) were estimated from the %inhibition versus concentration graph (Aiyelaagbe *et al.*, 2016).

ANTI-INFLAMMATORY ASSAY OF THE CRUDE EXTRACT

Anti-Lipoxygenase activity

Anti-Lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme (Shinde *et al.*, 1999). Test samples were dissolved in 0.25 mL of 2M borate buffer pH 9.0 and added 0.25 mL of lipoxidase enzyme solution (20,000 U/ml) and incubated for 5 min at 25 ^oC. After which, 1.0 mL of linoleic acid solution (0.6 mM) was added, mixed well and absorbance was measured at 234 nm. Indomethacin was used as reference standard. The percent 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 IC_{50} values. IC_{50} 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 10 min 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 saline (Sakat *et al.*, 2010).

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; 30m x

250 µm; film thickness 0.25 µm). Samples were injected at a temperature of about 250^{o}C with a split ratio of 10:1 with a flow rate of helium 1*mL*/min.

3. Result and Discussion

Antioxidant activity of *Scleria depressa*

The ability of the plants' extracts (n-hexane, ethyl acetate and methanol) against peroxide radical scavenging was analyzed. The results of this analysis are as shown in the tables and figures below:

Table 1: Hydrogen Peroxide radical scavenging of ascorbic acid, Hexane, Ethyl Acetate and Methanol Extracts of *Scleria depressa* leaves

	Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Mean	%Inhibition
Ascorbic acid $A_{ctr} = 0.4102$	10	0.1903	0.1828	0.2385	0.203867	17.93755
	20	0.2965	0.2012	0.1974	0.2317	32.86092
	50	0.5081	0.3608	0.2534	0.3741	48.35491
	100	0.5844	0.4448	0.4298	0.486333	68.01345
	150	0.6802	0.5735	0.5296	0.59443	71.85589
Hexane extract $A_{ctr} = 0.4386$	10	0.2873	0.2615	0.2384	0.2624	40.17328
	20	0.2585	0.2468	0.2574	0.254233	42.03526
	50	0.2409	0.2292	0.2431	0.237733	45.79723
	100	0.2386	0.2126	0.2611	0.237433	45.86563
	150	0.2383	0.2296	0.2259	0.231367	47.27162
Ethyl acetate extract A_{ctr}=**0.4386**	10	0.2316	0.2492	0.2325	0.237767	45.78963
	20	0.2336	0.2495	0.2237	0.2356	46.28363
	50	0.2377	0.2387	0.2353	0.2349	46.44323
	100	0.2582	0.2076	0.2357	0.23383	46.68643
	150	0.2166	0.2097	0.2152	0.21383	51.24639
Methanol Extract A_{ctrl} =**0.4386**	10	0.2761	0.355	0.383	0.338033	22.92902
	20	0.2433	0.2664	0.281	0.263567	39.90728
	50	0.2189	0.2231	0.2277	0.223233	49.10321
	100	0.2179	0.2065	0.2091	0.211167	51.85439
	150	0.2061	0.2081	0.2084	0.206533	52.91078

Please note that all the cells were deleted to conform with the latest standard and the table above and graph below were separated

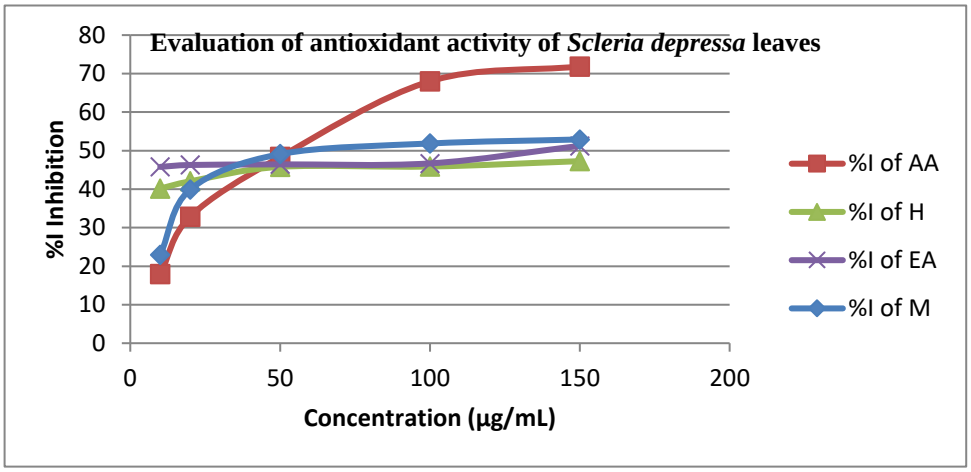

Figure 1: Evaluation of Antioxidant activity of *Scleria depressa* leaves.

Keywords: AA=Ascorbic Acid, H=Hexane, EA=Ethylacetate, M=Methanol.

Ethyl acetate extract of the *Scleria depressa* leaves exhibited antioxidant activity on peroxide radicals at different concentration, using ascorbic acid as standard antioxidant. Ethyl acetate and methanol extracts of the plant showed significant inhibition of peroxide radicals at concentrations in the range of 10-150 µg/mL, by scavenging the free radicals with IC_{50} of 148.79 and 106.23 µg/mL respectively. Hexane extract of *Scleria depressa* showed low inhibition of peroxide radical with IC_{50} of 198.41 µg/mL.

Table 2: Antilipoxygenases activity of indomethacin, Hexane and Ethylacetate extract of *Scleria Depressa* leaves

	Concentration (µg/ml)	Absorbance	Absorbance	Absorbance	Mean	%Inhibition
	10	0.03685	0.094	0.0327	0.054517	47.19987
Indomethacin $A_{control}$ =0.10325	20	0.046325	0.041125	0.027575	0.038342	62.86557
	50	0.041725	0.02355	0.033575	0.03295	68.08748
	100	0.031425	0.0258	0.037075	0.031433	69.55639
	150	0.020075	0.017225	0.021478	0.019593	81.02424
	10	0.078875	0.063523	0.090525	0.077608	24.836
Hexane extract A_{contr} =0.103251	20	0.0644	0.061739	0.062064	0.062734	39.241
	50	0.0071034	0.0572214	0.051982	0.060077	41.815
	100	0.037845	0.037025	0.039675	0.038182	63.021
	150	0.0375	0.035125	0.038775	0.037133	64.036
	10	0.096275	0.0776275	0.091634	0.088511	14.276
Ethyl acetae extract A_{contro}= 0.103251	20	0.076775	0.076775	0.071845	0.075515	26.863
	50	0.041875	0.041875	0.030275	0.038475	62.736
	100	0.043725	0.041425	0.032121	0.03909	62.14
	150	0.011275	0.0333275	0.028423	0.024324	76.442
Methanol A_{ctrl}=0.10325	10	0.06435	0.05575	0.060375	0.060158	41.73583
	20	0.047875	0.061425	0.042458	0.050586	51.00677
	50	0.043275	0.043108	0.040425	0.042269	59.06157
	100	0.041599	0.041425	0.039211	0.040745	60.53791
	150	0.03890	0.031475	0.035325	0.035233	65.87604

Table 3: Membrane stabilization activity of Indomathacin, Hexane, Ethylacetate and Methanol extracts of *Scleria Depressa* leaves.

	Concentration (µg/ml)	Absorbane	Absorbance	Absorbance	Mean	%Inhibition
Indome-thacin $A_{control}$ =1.103	10	0.4661	0.4394	0.3971	0.688967	37.53702
	20	0.6528	0.6972	0.5596	0.637567	42.19704
	50	0.6662	0.6993	0.5315	0.632333	42.6715
	100	0.6537	0.6582	0.6008	0.636533	42.29072
	150	0.7193	0.7212	0.6264	0.4342	60.63463
Hexane extract $A_{contr=1.103}$	10	0.6853	0.681	0.9762	0.7808	29.20822
	20	0.6715	0.6505	0.8727	0.7316	33.67483
	50	0.6287	0.6471	0.8325	0.7028	36.28589
	100	0.6514	0.6659	0.7554	0.6909	37.36174
	150	0.6089	0.6150	0.6913	0.6387	42.09731
Ethyl acetate extract $A_{contr=1.103}$	10	0.6039	0.5184	0.5403	0.5549	49.695
	20	0.5276	0.5183	0.5616	0.5358	51.42
	50	0.5796	0.5173	0.5268	0.5212	52.744
	100	0.5077	0.5027	0.5132	0.5059	54.137
	150	0.4807	0.4992	0.5109	0.4969	54.947
Methanol Extract A_{cotr}=1.103	10	0.6744	0.5442	0.6219	0.6135	44.37897
	20	0.6267	0.561	0.4946	0.560767	49.15987
	50	0.6097	0.5382	0 .5203	0.556067	49.58598
	100	0.6233	0.5487	0.4891	0.5537	49.80054
	150	0.6171	0.5448	0.4762	0.546033	50.49562

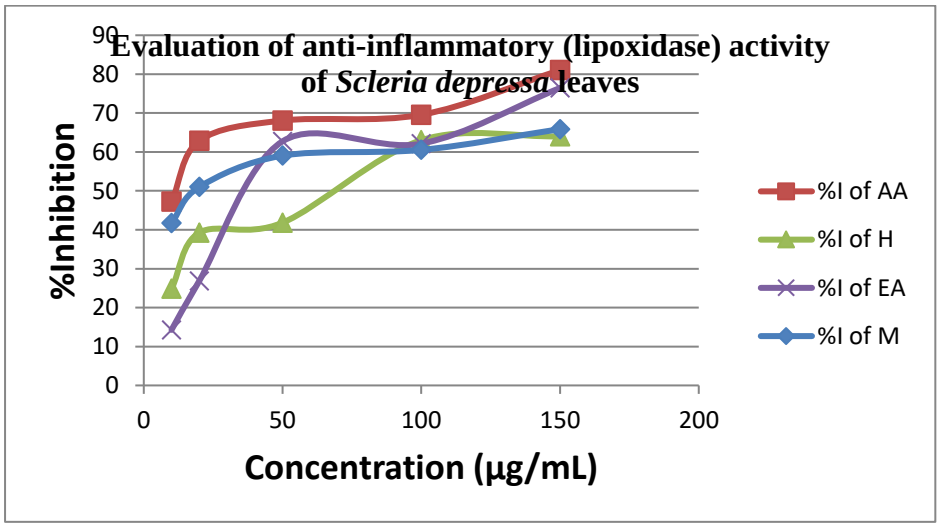

Figure 2a

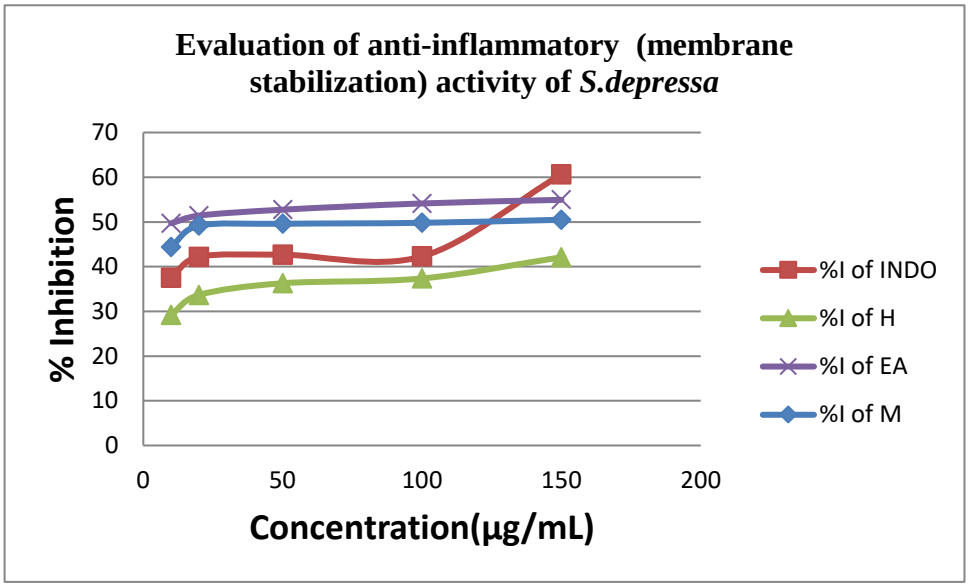

Figure 2b

Figures 2a&2b: Show the graph of %inhibition against concentration for lipoxidase and membrane stabilization test repectively.

KEYWORDS*:* INDO=Indomethacin, H=Hexane, EA=Ethylacetate, M=Methanol, AA=Indomethacin(fig2a).

Lipoxidase: Hexane, ethylacetate and methanol extracts of *Scleria depressa* leaves exhibited anti-inflammatory activity with an IC_{50} values of 78.98, 63.52 and 26.64 µg/mL respectively which are very much comparable with the IC50 value of the indomethacin standard, 18.86 µg/mL. methanol extract out of the three extracts is more anti-inflammatory active than the two other extracts because of its closer value to the value of the standard.

Membrane Stabilization: Hexane, ethylacetate and methanol extracts of *Scleria depressa* leaves exhibited anti-inflammatory activity with an IC_{50} values of 257.2, 10.61 and 115 µg/mL respectively while the value of indomethacin is 103.97 µg/mL. Ethylacetate extract out of the three extracts shows a more pronunced anti-inflammatory activity than the two other extracts and interestingly more active than the standard.

GC-MS Results of Hexane, Ethylacetate and Methanol extracts of *Scleria depressa* leaves

GC-MS analysis of hexane extract of *S. depressa* leaves reveals the presence of 13 compounds.5-methyl-2-(1-methylethyl) Cyclohexanol, that is, Carvomentholand 1,2,3-Trimethylbenzene are the most abundant (23.78% and 19.20%) compounds with molecular formula $C_{10}H_{20}O$ and $C_{19}H_{12}$ and retention time 5.172 and 3.494. The most abundant and other

compounds have fragmented ions has shown in the given table 3. The trace compound below 1.0 is 2,2-Dimethyl-propyl-2,2-dimetyl-propanesulfinylsulfone with retention time of 6.544.

GC-MS analysis of ethylacetate extract of *S. depressa* leaves reveals the presence of 19 compounds, two compounds 4,7-Dimethylundecane and 2,2-Dimethylbutane appeared twice each with different retention time and their %area were added up to give single compound each thus reducing the 19 compounds in the library to 17compounds. 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, 4,7-dimetylundecane, 1,2-Diisopropenylcyclobutane are the most abundant (24.56%, 16.83% and 9.47%) compounds with retention time 13.338, 6.298 and 3.819. while the trace compounds below 1.0 are alpha-methylnaphthalene, 1-sec-butoxybutane and 2,2-Dimethylbutane with %area 0.22, 0.52 and 0.74 and retention time 6.500, 6.749 and 7.011.

GC-MS analysis of methanol extract of *Scleriadepressa*leaves showed the presence of 11 compounds. The compound with highest abundance is Methyl-9-octadecenoate with % abundance of 69.86 and retention time of 14.714.

Table 4: Interpretation of GC-MS analysis of hexane extract of *Scleria depressa* leaves

Compound	Formulae	Molecular mass	% Area	% Height	Retention time	Mass Fragmentation
2-Phenyleicosane	$C_{26}H_{46}$	358	7.04	4.80	3.203	27,41,147,161
1,2,3-Trimethylbenzene	$C_{19}H_{12}$	120	19.20	16.72	3.494	14,39,91,105
2-Oxo-2-phenylethyl-3-methylbenzoate	$C_{16}H_{14}O_3$	254	6.06	6.07	3.769	39,51,105,119
3,3-Dimethylhexane	C_8H_{18}	114	9.54	10.11	4.026	27,41,85,99
1,2,4-Butanetriol	$C_4H_{10}O_3$	106	2.71	3.59	4.969	12,45,588
5-methyl-2-(1-methylethyl)Cyclohexanol	$C_{10}H_{20}O$	156	23.78	30.07	5.177	27,41,123,138
1,2,3-Trimethyldiaziridine	$C_4H_{10}N_2$	86	1.25	2.03	6.127	28,71,85
2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone	$C_{10}H_{22}O_3S_2$	254	0.65	1.09	6.544	45,57,71
2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan-2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane	$C_{16}H_{48}O_{10}Si_9$	652	3.09	3.53	8.407	41,57,517,575
N-[(pentafluorophenyl)methylene]-.beta.,3,4-tris[(trimethylsilyl)oxy]	$C_{24}H_{34}F_5N0_3Si_3$	563	2.46	3.13	10.826	45,59,473, 548
1,2-Benzenedicarboxylic acid	$C_{16}H_{20}O_4$	278	12.96	10.83	13.336	27,41,205 ,223
9-Azabicyclo[3.3.1]nona-2,6-diene-9-carboxaldehyde	$C_9H_{11}NO$	149	8.24	6.17	14.366	27,53,120,134
1,3,3-trimethyl Bicyclo[2.2.1]heptan-2-one	$C_{10}H_{16}O$	152	3.03	1.86	17.827	27,41,137,152
9-Azabicyclo[3.3.1]nona-2,6-diene-9-carboxaldehyde	$C_9H_{11}NO$	149	8.24	6.17	14.366	28,54,120,134

Table 5: Interpretation of GC-MS analysis of ethyl acetate extract of *Scleria depressa* leaves

Compound	Formulae	Molecular mass	% Area	% Height	Retention time	Mass Fragmentation
1,2-Diisopropenylcyclobutane	$C_{10}H_{16}$	136	9.47	7.05	3.819	45,5,107,121
Methanecarboxamide	C_2H_5NO	59	2.05	1.99	3.925	13,15,44,59
4-(1,1,3,3-Tetramethylbutyl)phenyl trimethylsilyl ether	$C_{17}H_{30}OSi$	278	2.05	1.65	4.03	42,57,263,278
2,2-Dimethylbutane	C_6H_4	86	4.89	5.64	15.454	14,27,41,43,57,71
1,1,3-Trimethylcyclopentane	C_8H_{16}	112	5.47	6.81	5.293	14,27,41,83,97
3,7-Dimethyldecane	$C_{12}H_{26}$	170	6.53	6.96	5.367	27,41,141,155
Bis(N,N-dimethylamino) pentachlorophenyl phosphate	$C_{22}H_{15}F_3O_5$	416	1.06	1.37	6.232	50,64,145,173
4,7-Dimethylundecane	$C_{13}H_{28}$	184	16.83	20.81	13.538	27,41,141,155
1H-Indene-1-methanol, .alpha.-methyl-, acetate	$C_{13}H_{14}O_2$	202	2.63	3.29	13.620	42,68,142,158
1-Methylnaphthalene	$C_{11}H_{10}$	142	0.22	0.05	6.500	27,39,98,115
1-Sec-butoxybutane	$C_8H_{18}O$	130	0.52	0.93	6.749	27,41,115,130
alpha.-Tridecene	$C_{13}H_{26}$	182	8.04	9.84	7.161	27,41,140,154
5-ethyl-5-isopropyl Barbituric acid	$C_9H_{14}N_20_3$	198	0.81	1.02	7.626	53,69,156,169
1-Hexadecanol	$C_{16}H_{34}O$	242	8.79	7.68	12.397	27,41,168,196
1,2-Benzenedicarboxylic acid-bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	24.56	19.34	13.338	27,41,205,223
1,7-Dimethyl-4-(1-methylethyl)cyclodecane	$C_{15}H_{30}$	210	3.81	4.13	14.636	27,41,151,168

Table 6: Interpretation of GC-MS analysis of methanol extract of *Scleria depressa* leaves

Compound	Formulae	Molecular mass	% Area	% Height	Retention time	Mass Fragmentation
Diethylphthalate	$C_{12}H_{14}0_4$	222	2.38	2.39	8.499	45,50,177
Neodol	$C_{15}H_{32}O$	228	4.13	4.19	10.388	27,41,182,210
Methylmyristate	$C_{15}H_{30}O_2$	242	0.89	1.27	11.312	27,41,199,211
Methylpentadecanoate	C16H32O2	256	0.22	0.28	11.462	27,41,213,225
Methylhexadec-9-enoate	C17H34O2	268	3.14	4.91	13.333	29,41,194,236
Hexadecanoicacid,methylester	C17H34O2	270	2.08	3.56	13.613	27,41,153,167
Heptacosan-1-ol	C27H56O	396	7.63	12.10	13.881	29,41,227,239
Nonadecanol	C19H40O	284	2.05	3.40	14.324	27,41,180,222
9-Octadecenoic,methylester	C19H36O2	296	69.86	56.22	14.714	29,41,180,22
7-Octadecenoic,methylester	C19H36O2	296	3.16	4.60	15.531	27,41,180,222
Strearic acid	C19H38O2	298	2.60	4.24	15.708	27,41,255,267

4. Conclusion

This study has shown that the leaf extracts of *S. depressa* have active ingredients or bioactive compounds which are able to scavenge free radicals and also are antiinflammatory active. The observed antioxidant and antiinflammatory potency of this medicinal plant may be attributed to the presence of the most abundant bioactive compounds present in synergy with all other compounds present in relatively small amounts. This justifies the ethnomedicinal uses of the plant and the plant may be a potential source of novel antioxidant and antiinflammatory drugs.

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