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Gas Chromatography-Mass Spectrometric analysis and antioxidant property of leaf extracts of *Chamaecrista rotundifolia*

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ABSTRACT

Chamaerista rotundifolia is a medicinal plant whose leaves are useful for the treatment of many diseases including cancer, diarrohea and pain. This plant is underexploited in the area of extraction and bioassays that will establish its efficacy. The aim of the present study is to determine the antibacterial and antioxidant properties as well as to analyze the chemical compositions of the extracts from the plant leaves through the use of Gas chromatography – Mass Spectrometry (GC- MS) technique. The antibacterial activity of n-hexane, chloroform, ethyl acetate, and methanol extracts of the plant was tested against six (6) strains of bacteria named Pseudominas aeruginosa, Staphylococcus aerus, Klebsiella pneumoniae, Acinetobacter baumannii, Enterococcus faecalis, and Escherichia coli. The extracts of the plant exhibited no significant antibacterial property on the tested organisms. The result of antioxidant activity (using 1,1-diphenyl-2-picryl hydrazy (DPPH) radical scavenging) revealed that Chamaecrista rotundifolia ethyl acetate extract has low antioxidant properties due to its high (>1164.878), and negative (< 0) Inhibition Concentration at 50% values for antioxidant. However, the methanol extract exhibited antioxidant activity with an IC_{50} value of 5 µg/mL, which is comparable to that of ascorbic acid, 2.55 µg/mL. The GC-MS results of this extract showed that it contains some bioactive compounds. Meanwhile, the ethyl acetate extract of the plant contains 75 chemical compounds, 4 of which are principal constituents, 6 are minor and others are trace. The chloroform extract of C. rotundifolia leaf was, however, inactive in inhibiting the growth of the bacteria as the extract also had a high inhibition value when compared with the inhibition concentration at 50%, which shows its low level of antioxidant activity. Our results of antibacterial activity were found not promising while the antioxidant activity on its own showed an interesting result that could be useful in the prevention of oxidative stress side effects and cancer related diseases.

Keywords: Antibacterial, antioxidant, GC-MS, Chamaecrista rotundifolia

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INTRODUCTION

Chamaecrista rotundifolia is a legume which possesses sub-woody, semi-erect stem covered in small hairs which can grow up to 1 metre in height, with a shallow tap-root. Some identifiable physical traits are its small axillary yellow flowers (depending on the season) and the characteristic round bifoliolate leaves for which it is named Round-leaf Cassia [1]. The plant usually grows in the summer, but remains active during the spring and autumn seasons. It is self-fertilizing and produces a good amount of seed on its own through natural reseeding [2]. *C. rotundifolia* serves as a source of feed for livestock and as green fertilizer. It is used for open grazing, hay, and silage using its high dry matter content which contains the necessary nutrients (i.e. protein, fibre, energy, vitamins) for livestock [3]. Integrating leaves of *C. rotundifolia* into feed can result in healthy weight gains in cattle. The plant also has potentials as an antiseptic agent, making it useful in the treatment of skin and other microbial infections. For instance, extracts of *C. rotundifolia* have been proven to possess anticancer and anti-inflammatory activities [4].

This research focuses on the antibacterial and antioxidant properties of the extracts of *C. rotundifolia* as there are very few research reports on the antibacterial and antioxidant properties of the plants' aerial parts to the best of our knowledge. The research also reports the chemical constituents present in the leaf of this plant with the aid of Gas Chromatography-Mass Spectrometry (GC-MS).

MATERIALS AND METHODS

Plant Materials

The aerial parts of *Chamaecrista rotundifolia* were collected from various locations in Ilorin, Kwara State, Nigeria. They were identified by the plant taxonomist, Mr. Bolu Ajayi of the Department of Plant Biology, University of Ilorin, where a voucher specimen of the plant (Herbarium Number: UILH/003/1540/2022) was deposited at the herbarium. The leaf parts of *C. rotundifolia* were air-dried at the room temperature, and pulverized into powdery form at Ipata market, Ojagboro, Ilorin using a locally fabricated grinding machine to increase the surface area of the plant and enhance the efficiency of extraction. The grinder was thoroughly cleaned before grinding the plant material to avoid

impurities and other foreign matter deposited on it. The plant samples were weighed (600 g) and extracted using successive extraction method that is known as merceration in increasing order of polarity, with the volume of (200 mL) n-hexane, chloroform, ethyl acetate and methanol [5].

Antibacterial Assay

The antibacterial activity was determined using the agar diffusion pour-plate method [6]. An overnight culture of each bacteria was prepared appropriately from its stock and inoculated each into the sterile nutrient broth of 5 mL, each inoculated for 18 – 24 hours at 37 ^{0}C. From the overnight culture, 0.1 mL of each organism was taken and put into the 9.9 mL of sterile distilled water to get the inoculum concentration of the bacteria medium [7]. From the diluted organism, 0.2 mL was taken into the prepared sterile nutrient agar cooled to 40 – 45 ^{0}C, then poured into sterile Petri-dishes and allowed to solidify for about 45 – 60 minutes. Using a sterile cork-borer of 8 mm diameter, the wells were made.

the number of the concentration of the extracts for the experiment. The graded concentrations of the extracts were put into the wells accordingly including the control. Dimethyl sulphur oxide was used as negative control and Gentamycin (antibacterial) as positive control. The bacteria plates were incubated at 37^{o}C for 24h. The degree of inhibition was determined by the size of the zone of inhibition measured in mm and was taken as evidence of antimicrobial activity of each of the extracts [8].

The plates were left on the bench for about 2 hours to allow the extract to diffuse properly into the nutrient agar. The plates were incubated for 18 – 24 hours at 37 ^{0}C.

Antioxidant Assay

Since DPPH is widely used to test the ability of compounds to trap free radical or hydrogen donors and to evaluate antioxidant activity, the ability of the plant samples to scavenge DPPH free radicals was assessed by the standard method adopted with suitable modifications [9]. The stock solutions of extracts were prepared in methanol to achieve a concentration of 1 mg/*mL*. Dilutions were made to obtain concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.99 µg/*mL*. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is

reduced. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV/VIS spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA). The mixture of ethanol and sample serve as blank. The control solution was prepared by mixing ethanol and DPPH radical solution. The absorbance was measured in triplicate at varying concentrations and the mean absorbance was determined and reported. Parallel to the examination of the antioxidant activity of the plant extracts, Ascobic acid was used as positive control, the percentage inhibitions of the extracts, and that of the standard which was determined at different concentrations using the expression below:

$$\% \, inhibition = \left(\frac{A \, of \, control - A \, of \, sample}{A \, of \, control}\right) \times 100$$

The IC_{50} values (Inhibition Concentration at 50%) were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm.

GC-MS analysis of the extracts

Active fractions of the extracts of the plant were selected for GC-MS analysis to identify and elucidate the structure of compounds present. GC-MS was performed with Agilent 19091GC plus automatic sampler system coupled with a quadruple Mass Spectrometer, 433HP-5MS. Compounds were separated in the HP5MS column fused with phenylmethylsilox, (length; 30 m x 250 µm; film thickness 0.25 µm). Samples were injected at a temperature of about 250°C with a split ratio of 10:1 with a flow rate of helium 1ml/min. The molecules were eluted at different retention time and were characterized by the mass spectrometer downstream to capture, ionize, accelerate, deflect and detect the ionized molecules separately [10]. The mass spectrometer breaks each molecule down to its fragments and these fragments were detected by their mass to charge (m/z) ratio [11].

RESULTS AND DISCUSSION

Antibacterial Activity

The antibacterial activity of the chloroform extract of the plant (Table 1) was determined using the diffusion-pour plate method. The activity was measured by studying the zone of Inhibition. The extract showed no significant activity against all the test organisms (*Pseudomonas aeruginosa,*

Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Enterococcus faecalis, and *E. coli*) as there was no clear zone of inhibition against the organisms.

Table 1: Antibacterial activity of chloroform extract of *Chamaecrista rotundifolia* leaves

Test Organisms	Chloroform Extract	Control (mm)	Gentamycin (mm)
Acinetobacter baunmanni	_	_	10.00
Entericoccus Faecalis	_	7.50	21.00
Escherichia coli	_	_	10.50
Klepsulla pneumonia	_	_	4.50
Pseudomonas aeruginosa	_	_	11.00
Staphylococcus aureus	_	_	19.00

Antioxidant activity

The ability of the methanol extracts of *Chamaecrista rotundifolia* to scavenge DPPH radicals and reduce their effects was analyzed. The results of this analysis are as shown in the tables and figures below:

Table 2: Absorbance of Ascorbic acid standards and methanol leaf extract of *Chamaecrista rotundifolia* for DPPH antioxidant activity

Sample	Concentration (µg/mL)	Absorbance
Ascorbic acid standard	15.62	0.1257
	31.25	0.2514
	62.5	0.1257
	125	0.3758
	250	0.4996
	500	0.687
	1000	0.751
Methanol extract of	15.62	0
Chamaecrista rotundifolia	31.25	0
	62.5	0.003761
	125	0.018308

250	0.0163
500	0.0592
1000	0.1969

Table 3: Absorbance and percentage inhibition of ascorbic acid standard for DPPH antioxidant activity of the ethyl acetate leaf extract of *Chamaecrista rotundifolia*

S/N	Concentration (µg/mL)	absorbance of Sample	absorbance of Sample	% Inhibition of sample	% Inhibition of standard
1.	15.62	0.019681	0.143495	96.08182	71.43241
2	31.25	0.03491	0.159605	93.04798	68.22516
3.	62.5	0.035721	0.174118	92.88851	65.33586
4.	125	0.036914	0.018696	92.65101	96.27788
5.	250	0.064548	0.019974	87.14951	96.02341
6.	500	0.134863	0.209469	73.15091	58.29803
7.	1000	0.238287	0.219011	52.56082	56.39837

Table 4: Absorbance and percentage inhibition of ascorbic acid standard for DPPH antioxidant activity of the ethyl acetate leaf extract of *Chamaecrista rotundifolia*

S/N	Concentration (µg/mL)	Absorbance of Sample	%I CAEA	%I A
1.	15.62	0.02393	95.23591	71.43241
2.	31.25	0.014621	97.08919	68.22516
3.	62.5	0.02036	95.94665	65.33586
4.	125	0.021804	95.65917	96.27788
5.	250	0.064124	87.23392	96.02341
6.	500	0.134392	73.24467	58.29803
7.	1000	0.210349	58.12283	56.39837

Control = 0.5023

Keywords:
%I: Percentage inhibition
%I CAEA: Inhibition concentration of ethyl acetate extract
%I A: Inhibition of ascorbic acid

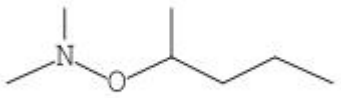
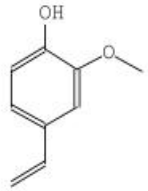
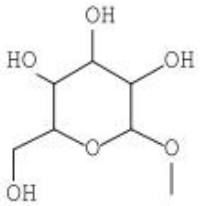
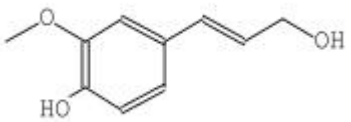
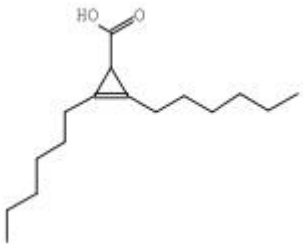
Table 2-4 showed that the various extracts of *C. rotundifolia* leaves exhibit significant antioxidant activities by scavenging DPPH radicals in a dose-dependent manner. The IC_{50} were used to determine the antioxidant capacity of a sample compared to standard, where IC_{50} less than 50 µg/mL indicate highly active, 51 µg/mL - 100 µg/mL

indicate active, 101 µg/mL - 250 µg/mL indicate moderate, 251 µg/mL - 500 µg/mL weak, and more than 500 µg/mL indicate inactive. The result of the methanol extract of *C. rotundifola* indicates moderate antioxidant activity with an IC_{50} value of 81.5 µg/mL while the chloroform extract has an IC_{50} of 1164.878 µg/mL which is higher than 50 µg/mL, hence it as low antioxidant activity.

GC-MS Results

Methyl β-D-glucopyranoside (17.52%), n-hexadecanoic acid (6.50%), cis-9-hexadecenal (5.39%), 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (3.64%), and 9,10-anthracenedione, 1,8-dihydroxy-3-methyl (3.06%) are the principal constituents (Table 5) identified from the methanol extract of *C. rotundifolia*. The most active of these compounds in the extract is methyl β-D-glucopyranoside (17.52%), which is used as a chemical intermediate in the production of a variety of products including emollients, emulsifiers, humectants, moisturizers, thickening agents, plasticizers, surfactants, varnishes, and resins. While 2-pentadecanone-6,10,14-trimethyl (5.22%), n-hexadecanoic acid (6.93%), heptadecafluoro nonanoic acid (10.92%) and phytol (4.07%) are the major constituents of the ethyl acetate extract of the plant (Table 6), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (14.14%), β-sitosterol (6.50%), n-hexadecanoic acid (2.97%), octadecanoic acid-2-(2-hydroxyethoxyl)ethyl (2.49%), (1S,6R,9S)-5,5,9,10-tetramethyltricyclo (2.53%), stigmast-4-en-3-one (4.30%), 1-pyrroldinebutanoic acid (3.66%), heptadecafluororionanoic acid (undecyl east) (4.26%), phytol acetate (7.26%), vitamin E (2.20%), campesterol (2.12%) and stigmasterol (2.90%), were present in the chloroform extract of *Chamaecrista rotundifolia* as major constituents (Table 7). Other major constituents of the chloroform extract are 9,12-octadecadienoic acid (Z,Z) (1.22%), oxirane (1.24%), spinasterone (1.1.%) etc. While caparratriene (0.31%), tetracontane (0.35%), 2-hydroxy-1,1,10-trimethyl-6,9-epidioxyl (0.37%) and 4,8,13-cyclotetradecatriene-1,3-diol (0.29%) were present in trace quantity.

Table 5: GC-MS results of the methanol extract of leaves of *Chamaecrista rotundifolia*

S/N	R.I (min)	% Area	Name	Molecular Weight (g/mol)	Molecular formulae	Structure
1.	6.530	2.80	N,N-dimethyl-o-(1-methyl-buthyl)-hydroxyl	131	$C_7H_{17}NO$	
2	8.796	2.69	2-methoxy-4-vinylphenol	150	$C_9H_{10}O2$	
3	12.097	17.52	Methyl β-D-glucopyranoside	194	$C_7H_{14}O_6$	
4	12.553	3.64	4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol	180	$C_{10}H_{12}O_3$	
5	12.683	2.77	1,2-dihexylcyclopropene-3-carboxylic acid	252	$C_{16}H_{28}O_2$	

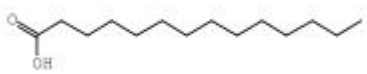
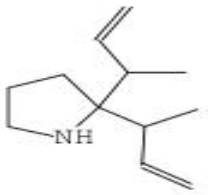
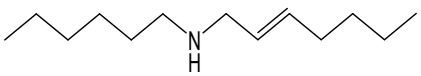
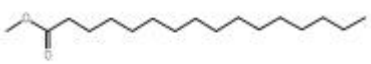
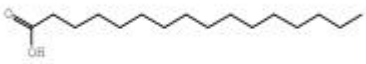
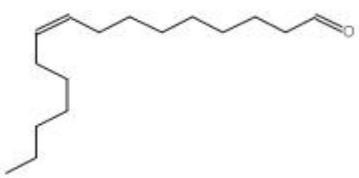
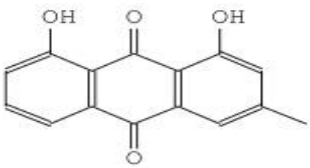
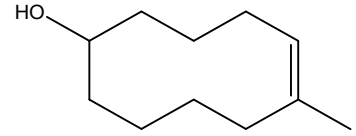
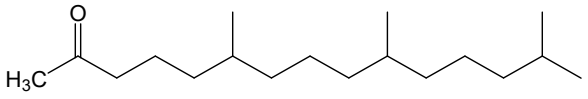
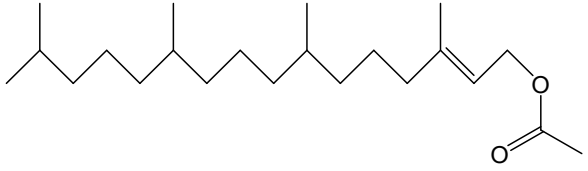
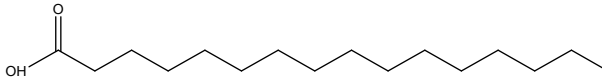
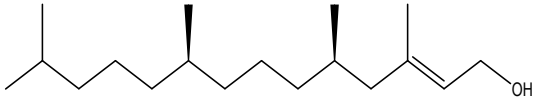
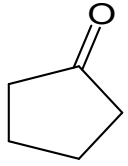
6	12.776	2.48	tetradecanoic acid	228	$C_{14}H_{28}O_2$	
7	12.934	2.41	2,3-Bis(1-methylallyl)pyrrolidine	179	$C_{12}H_{21}N$	
8	13.034	2.14	N-hexyl-2-heptyn-1-amine	195	$C_{13}H_{25}N$	
9	13.991	2.22	methyl hexadecanoate	270	$C_{17}H_{34}O_2$	
10	14.307	6.50	n-hexadecanoic acid	256	$C_{16}H_{32}O_2$	
11	15.476	5.39	*cis*-9-hexadecenal	238	$C_{16}H_{30}O$	
12	16.915	3.06	9,10-anthracenedione, 1,8-dihydroxy-3-methyl	254	$C_{15}H_{10}O_4$	

Table 6: GC-MS Analysis of ethyl acetate extract of the leaves of *Chamaecrista rotundifolia*

S/N	RT (min)	% of abundance	Compound name	MF	MW(g/mol)	Structure
1.	12.652	3.11	6-methylcyclodec-5-enol	$C_{11}H_{20}O$	168	
2.	13.433	5.22	6,10,14-trimethyl-2-pentadecanone	$C_{18}H_{36}O$	268	
3.	13.469	3.03	phytol acetate	$C_{22}H_{42}O_2$	338	
4.	14.281	6.93	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	
5.	15.324	4.07	Phytol	$C_{20}H_{40}O$	296	
6.	15.499	2.46	cyclopentanone	$C_5H_{18}O$	84	

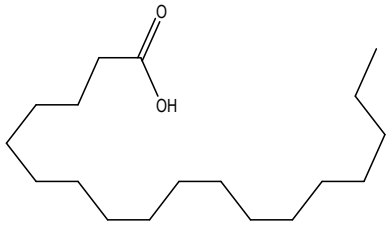
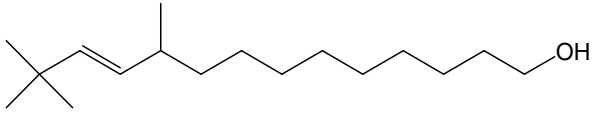
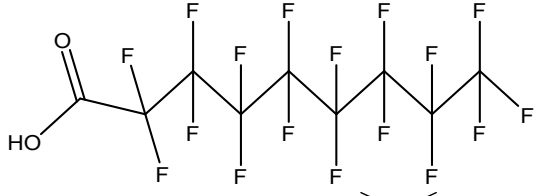
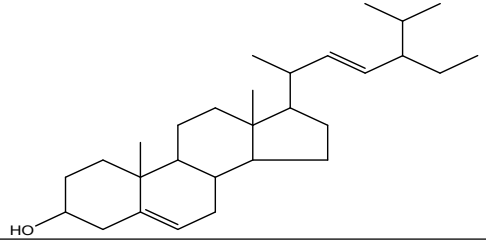
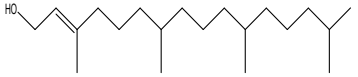
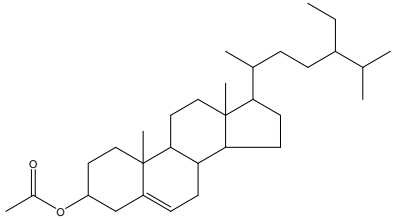
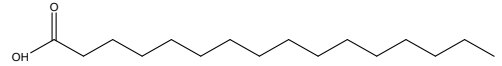
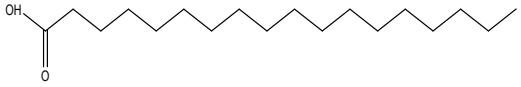
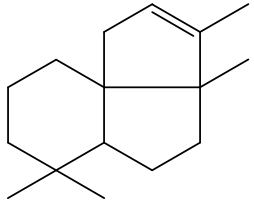
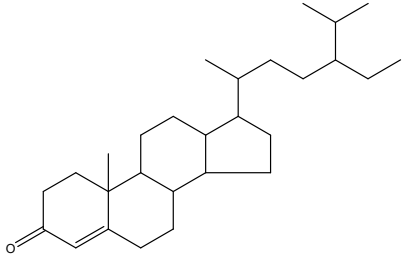
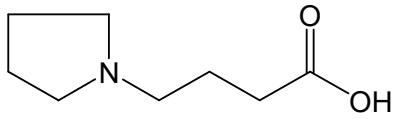
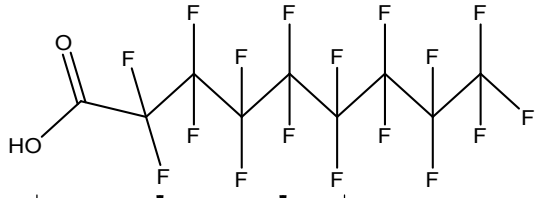
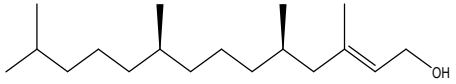
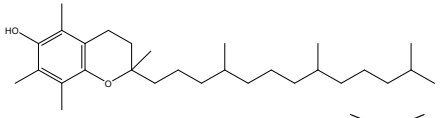
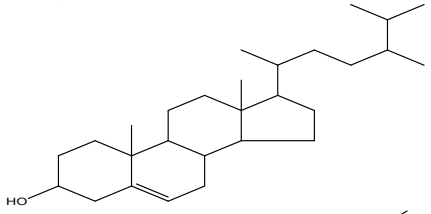
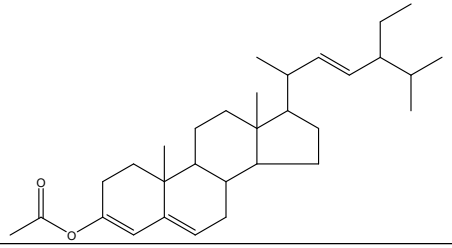
7.	15.612	2.40	octadecanoic acid	$C_{18}H_{36}O_2$	284	
8.	16.294	3.49	E-10,13,13-trimethyl-11-tetradecen-1-ol	$C_{17}H_{34}O$	254	
9.	21.437	10.92	heptadecafluorononanoic acid	$C_9HF_{17}O_2$	294	
10.	25.378	3.04	stigmasterol	$C_{29}H_{48}O$	412	

Table 7: GC-MS results of the chloroform extract of the leaves of *Chamaecrista rotundifolia*

S/N	RT (min)	% of abundance	Compound name	Formula	Molecular weight (g/mol)	Structure
1	13.468	14.14	3,7.11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	
2	14.042	6.50	β.-sitosterol	$C_{29}H_{50}O$	414	
3	14.252	2.97	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	
4	15.611	2.49	octadecanoic acid	$C_{18}H_{34}O_2$	372	
5	17.415	2.53	(1S,6R,9S)-5,5,9,10-tetramethyltricyclo[7.3.0.0(1,6)]dodec-10(11)-ene	$C_{16}H_{26}$	218	
6	19.702	4.30	stigmast-4-en-3-one	$C_{29}H_{48}O$	412	

7	21.427	3.66	1-Pyrrolidine butanoic acid	$C_8H_{15}NO_2$	157	
8	21.607	4.26	heptadecafluoron onanoic acid	$C_9HF_{17}O_2$	294	
9	22.586	7.26	Phytol	$C_{22}H_{42}O_2$	338	
10	23.811	2.20	Vitamin E	$C_{29}H_{50}O_2$	430	
11	24.987	2.12	Campesterol	$C_{28}H_{48}O$	400	
12	25.358	2.90	Stigmasterol	$C_{29}H_{48}O$	412	

CONCLUSION AND RECOMMENDATION

The leaf parts of *Chamaecrista rotundifolia* have been investigated in this research. Antibacterial activity of crude extracts from the plants against all the test bacteria was found to be inactive at moderate to high concentration which is unable to justify the ethnomedicinal uses of the plant for treating some diseases attributed to bacteria. Hence, not all the test organisms (bacteria) were inhibited by the plant's extracts at these concentrations. The GC-MS reveals various peaks of different compounds of which culminating to the activities of the plant's extracts against bacteria, as well as their activities against free radicals. The most prominent compound with probable synergistic effect with all other compounds present in smaller quantities in the extracts proffer an explanation that the extracts possess different components of natural products in different varieties. Structural determination of the principal constituent by using Nuclear Magnetic Resonance (NMR) such as carbon ^{13}NMR, proton ^{1}H NMR, and 2D (2-Dimensional) analysis is recommended. This is necessary for structural elucidation of these principal compounds.

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