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Chemical constituents, antimicrobial and antioxidant properties of the aerial parts of *Coccinia barteri*

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A B S T R A C T

Phytochemical analysis of *n*-hexane, ethyl acetate and methanol extracts of the aerial parts of *Coccinia barteri* was carried out. These extracts exhibited satisfactory inhibitory activities against bacteria and fungi strains, which include; *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhii, Klebsiella pneumoniae, Candida albicans, Aspergillus niger, Penicillium notatum* and *Rhizopus stolonifer.* Methanol extract of *C. barteri* possesses antioxidant activity by scavenging DPPH free radical with IC_{50} of 187.56 µg/mL, using DPPH antioxidant assay. GC-MS analysis of n-hexane, ethyl acetate and methanol extracts of the plant principally revealed the presence of phytol, ethyl hexadecanoate and clionasterol with their corresponding percentage abundance of 57.75%, 18.33% and 9.79%, respectively.

Capsule Summary: Chemical constituents, antimicrobial and antioxidant properties of the aerial parts of *Coccinia barteri*. phytol, ethyl hexadecanoate and clionasterol were recorded up to 57.75%, 18.33% and 9.79%, respectively along with considerable antimicrobial and antioxidant properties.

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INTRODUCTION

Coccinia species are perennial and climbing herbs. They possess unequally bifid tendrils which are used for climbing. They also possess simple one-seed leaves (cotyledons), and have a blunt tip. They usually have stalked and rarely sessile leaves. The leaf sides often bear small nectar-producing glands. *Coccinia,* which is distributed into numerous habitat types, is mainly found in the sub-Saharan Africa. *C. grandis* is the only coccinia species that is spread to the highlands of the Arabian Peninsula and tropical Asia, and is now an invasive weed on the Pacific Islands and in the Neotropics (Jeffrey, 1967). *Coccinia* comprises of 27 species and they are all pollinated by bees, including honeybees (Holstein and Renner, 2011). *Coccinia* is a suitable plant in which niche evolution among close relatives can be studied because of the numerous habitat types occupied by its 27 species (Holstein

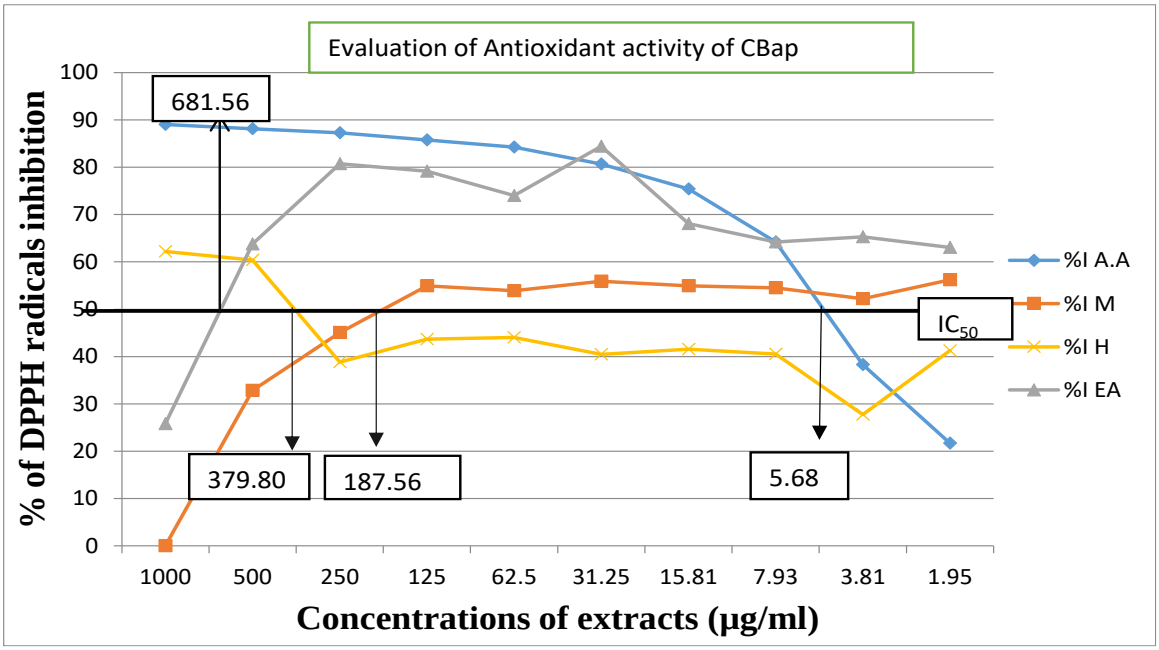


Fig. 1: IC_{50} of antioxidant activities of *n*-hexane, ethyl acetate and methanol extracts of the aerial parts of *C. barteri*
Keywords: H = Hexane extract, % I = Percentage inhibition, A .A = Ascorbic Acid, EA = Ethyl acetate extract, M = Methanol extract, IC = Inhibition Concentration, CBap = *Coccinia barteri*

and Renner, 2011). Coccinia species generally occur in semi-arid habitats, woodland, and forest, vegetation types with contrasting precipitation regimes (Holstein and Renner, 2011). *Coccinia* species produce flowers with only male or only female organs, hence, they are dioecious. They have sepals which are connected and have shaped lobes. The corolla is also connected at the base and has five free lobes. Literature shows that some *Coccinia* species e.g. *Coccinia grandis* otherwise known as Ivy Gourd have antidiarrhoeal activity and the phytochemical analysis of these species revealed the presence of some metabolites such as alkaloids, glycosides and saponins. Therefore, these species are said to be pharmacologically active. Hossain *et al.,* 2014 showed that the plant species are used traditionally as antirheumatic because the ethanol extracts of some of these species possess analgesic effects which support the traditional uses of the plant.

This paper focuses on the constituents and antimicrobial property of *Coccinia barteri* extracts, and to account for the free radical scavenging activity of the extracts of aerial parts of the plant.

MATERIAL AND METHODS

Extraction

Coccinia barteri Hook. F. (*Cucurbitaceae*) aerial parts were collected from Ondo town, Ondo state, Nigeria. The plant was identified and authenticated by a taxonomist, Mr. Bolu Ajayi of the Department of Plant Biology, University of Ilorin where voucher specimens (UIH002/1145) was deposited in the herbarium. The aerial parts of *C. barteri* were air dried and crushed into smaller sizes to increase its surface area. The plant sample was weighed and extracted using serial exhaustive 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). The aerial parts of the plant were extracted using standard procedure (Das *et al.,* 2010). The extracts were dried by using rotary evaporator and kept in the refrigerator for further use.

Phytochemical screening

Preliminary phytochemical screening of the crude extracts was carried out using the modified methods as described by Pranshant et al. (2011).

Antimicrobial assay

Microorganisms: Cultures of six human pathogenic bacteria made up of four gram negatives and two gram positives were used for the antibacterial assays. These cultures include; *Salmonella typhii, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonae* which belongs to the gram-negative, and *Bacillus subtilis* and *Staphylococcus aureus* which are gram positive bacteria. Four fungi were also utilized for the Antifungal assays. These are; *Candida albicans, Aspergillus niger, Rhizopus stolon* and *Penicillium notatum.* All the microorganisms used were clinical strains from the Medical Microbiology (University College Hospital, Ibadan) and screened in the Laboratory of Pharmaceutical Microbiology Department, University of Ibadan, Ibadan, Nigeria. Media: Nutrient agar, Sabouraud dextrose agar, nutrient broth and tryptone soya agar were used in this study. Hexane, ethyl acetate and methanol were used in solubilizing the extracts and as negative controls in the assays.

Table 1: Phytochemical screening of the extracts of *C. barteri* aerial parts

Chemical constituents	CBAH	CBAE	CBAM
Saponin	-ve	-ve	+ve
Tannins	-ve	-ve	-ve
Steroids	+ve	+ve	-ve
Glycosides	+ve	+ve	-ve
Alkaloids	-ve	+ve	+ve
Carbohydrates	-ve	-ve	-ve
Flavonoids	+ve	+ve	+ve
Anthraquinone	-ve	-ve	+ve
Fat and Oil	+ve	+ve	+ve
Protein	-ve	-ve	-ve
Terpenoid	+ve	+ve	-ve
Phenol	-ve	+ve	-ve

CBAH: Hexane extract of *Coccinia barteri* aerial parts. CBLE: Ethyl acetate extract of *Coccinia barteri* aerial parts.
CBLM: Methanol extract of *Coccinia barteri* aerial parts, +ve: Present; -ve: Absent

Table 2: Antimicrobial activity of n-hexane extract of *C. barteri*

Extract Conc. (mg/mL)	S. A	E. C	B. S	Ps. A	Sal	Kleb	C. A	A. U	Pen	Rhiz
200	19	19	22	19	19	17	16	15	17	15
100	12	15	15	16	16	14	14	13	14	12
50	14	12	13	13	14	12	12	10	12	10
25	11	10	10	11	12	10	10	--	10	--
12.5	14	13	14	13	12	14	12	12	11	10
6.25	--	--	--	--	--	--	--	--	--	--
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	36	40	38	38	36	26	26	28	26

KEYS: +ve : Gentamycin (10 µg/mL); Tioconazole (0.7 mg/mL), -ve: *n*-hexane

Table 3: Antimicrobial activity of ethyl acetate extract of *C. barteri*

Extract Conc. (mg/mL)	S. A	E. C	B. S	Ps. A	Sal	Kleb	C. A	A. U	Pen	Rhiz
200	23	23	23	21	20	25	20	20	20	20
100	20	20	20	19	21	14	18	18	17	17
50	18	17	17	15	18	15	14	14	12	12
25	15	14	14	13	13	12	12	12	10	10
12.5	12	11	11	13	10	10	10	13	--	--
6.25	10	--	--	--	--	--	--	--	--	--
-ve	-	-	-	-	-	-	-	-	-	-
+ve	40	38	40	38	38	38	28	28	28	28

KEYS: +ve : Gentamycin (10 µg/mL); Tioconazole (0.7 mg/mL), -ve: ethylacetate

Table 4: Antimicrobial activity of methanol extract of *C. barteri*

Extract Conc. (mg/mL)	S. A	E. C	B. S	Ps. A	Sal	Kleb	C. A	A. U	Pen	Rhiz
200	29	27	25	27	25	25	21	20	20	18
100	25	23	22	24	21	21	18	18	18	16
50	21	19	18	20	18	19	16	16	16	14
25	18	16	16	17	15	16	14	14	14	12
12.5	14	13	12	14	13	13	12	12	12	10
6.25	11	11	10	10	10	10	10	10	10	10
-ve	_	_	_	_	_	_	_	_	_	_
+ve	40	40	40	40	38	38	28	28	26	28

KEYS: +ve : Gentamycin (10 µg/mL); Tioconazole (0.7 mg/mL), -ve: methanol

Table 5: Absorbance and percentage inhibition of Ascorbic Acid Standard for DPPH Antioxidant activity of the aerial parts of *C. barteri extract*. Absorbance of control is 1.265

Conc (µg/mL)	A1	A2	A3	AV±SD	%I of A
1000	0.138	0.138	0.14	0.139±0.0012	89.02
500	0.15	0.15	0.15	0.15±0.000	88.14
250	0.161	0.162	0.16	0.161±0.001	87.26
125	0.18	0.18	0.18	0.180±0.000	85.79
62.5	0.193	0.195	0.194	0.194±0.001	84.26
31.25	0.245	0.245	0.245	0.245±0.000	80.67
15.62	0.311	0.311	0.311	0.311±0.000	75.44
7.81	0.453	0.452	0.454	0.453±0.001	64.18
3.9	0.782	0.781	0.78	0.781±0.001	38.26
1.95	0.991	0.991	0.991	0.991±0.000	21.66

A = Absorbance, MA = Mean absorbance, %I of A = % Inhibition

Table 6: Antioxidant activity (DPPH) and % inhibition of *n*-hexane extract of the aerial parts of *C. barteri* with 0.365 as absorbance of control

Conc (µg/mL)	A1	A2	A3	AV±SD	%I of A
1000	0.14	0.139	0.135	0.138±0.0027	62.192
500	0.142	0.146	0.146	0.145±0.0023	60.365
250	0.217	0.223	0.23	0.223±0.0065	38.813
125	0.201	0.21	0.206	0.206±0.0045	43.653
62.5	0.204	0.206	0.203	0.204±0.0015	44.018
31.25	0.216	0.222	0.214	0.217±0.0042	40.457
15.81	0.214	0.213	0.213	0.2133±0.0006	41.553
7.93	0.215	0.22	0.216	0.217±0.0026	40.548
3.81	0.269	0.26	0.262	0.264±0.0047	27.762
1.91	0.215	0.214	0.214	0.214±0.0006	41.279

Explanation as given in Table 5

Table 7: Antioxidant activity (DPPH) and %inhibition of ethyl acetate extract of the aerial parts of *C. barteri* with 0.462 as absorbance of control

Conc (µg/mL)	A1	A2	A3	AV±SD	%I of A
1000	0.334	0.348	0.346	0.343±0.0076	25.829726
500	0.167	0.167	0.168	0.167±0.0006	63.780664
250	0.089	0.089	0.089	0.089±0.0000	80.735931
125	0.096	0.096	0.097	0.096±0.0006	79.148629
62.5	0.12	0.12	0.12	0.120±0.0000	74.025974
31.25	0.072	0.072	0.072	0.072±0.0000	84.415584
15.81	0.148	0.148	0.146	0.147±0.0012	68.109668
7.93	0.165	0.165	0.166	0.165±0.0006	64.213564
3.81	0.16	0.161	0.16	0.160±0.0006	65.295815
1.91	0.171	0.171	0.17	0.171±0.0006	63.059163

Explanation as given in Table 5

Table 8: Antioxidant activity (DPPH) and %inhibition of methanol extract of the aerial parts of *C. barteri* with 0.316 as absorbance of control

Conc (µg/mL)	A1	A2	A3	AV±SD	%I of A
1000	0.312	0.319	0.317	0.316±0.0036	-
500	0.213	0.212	0.212	0.212±0.0006	32.806
250	0.173	0.174	0.174	0.174±0.0006	45.042
125	0.142	0.141	0.144	0.142±0.0015	54.958
62.5	0.147	0.145	0.145	0.146±0.0012	53.903
31.25	0.139	0.14	0.139	0.139±0.0006	55.907
15.81	0.141	0.142	0.144	0.142±0.0015	54.958
7.93	0.144	0.143	0.144	0.144±0.0006	54.536
3.81	0.15	0.151	0.152	0.151±0.0010	52.215
1.91	0.138	0.138	0.139	0.138±0.0006	56.224

Explanation as given in Table 5

Antimicrobial agents used: Gentamycin (10 µg/mL) and Tioconazole (0.7 mg/mL) as antibacterial and antifungal drugs respectively, were employed as standard reference drugs in this study.

Determination of antimicrobial activity

Agar diffusion (Ditch) method (for bacteria): An overnight culture of each organism was prepared by taking two wire-loop of the organism from the stock, each inoculated into 5ml of sterile nutrient broth and incubated for 24 hr at 37°C. 0.1 mL of each organism was taken from overnight culture and put into the 9.9 mL of sterile distilled water to obtain 10^{-2} inoculum concentration of the test organism. 0.2 mL was taken from the diluted test organism (10^{-2}) into the prepared sterile nutrient agar cooled to about 45 °C and then poured into sterile petri dishes which were allowed to solidify for about 60 min. A sterile cork borer of 8mm diameter was used to make 8 wells on the media according to the number of the diluted extracts for the experiment. The graded concentrations (6.25–200 mg/mL) of the extracts were put into each well and separated from the controls. The studies were done in duplicates to ascertain the results obtained. The plates were left on the bench for about 2 hrs to allow the extract diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were incubated for 24 hrs at 37°C (Collins and Lyne, 1970).

Agar diffusion (surface plate) method (fungi): A sterile sabouraud dextrose agar was prepared accordingly and aseptically poured into the sterile plates in triplicates and solidified. 0.2 mL of the 10-2 inoculum concentration of the test organism was spread on the surface of the agar using a sterile Petri-dish to cover all the surface of the agar. Eight wells were bored by using a sterile cork-borer of 8 mm diameter. The graded concentrations of the extracts were put into each well separately with the controls. All the plates were left on the bench for 2hr to allow the extract diffuse properly into the agar i.e. prediffusion. The plates were incubated at 25°C for 72 hrs (Collins and Lyne, 1970).

Table 9: GC-MS analysis of *n*-hexane extract of the aerial parts of *Coccinia barteri*

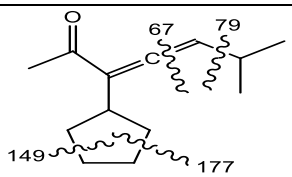
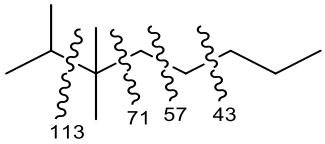
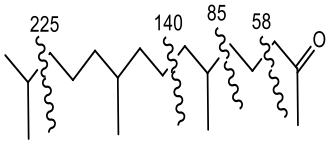
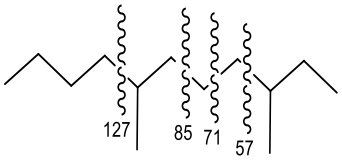
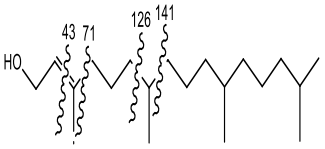
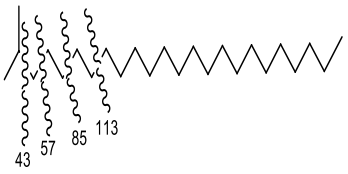
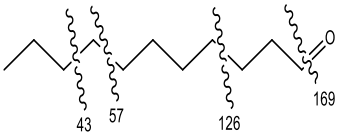
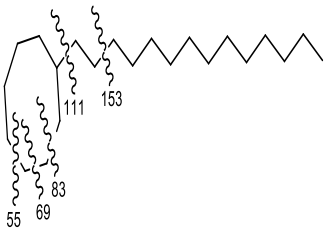
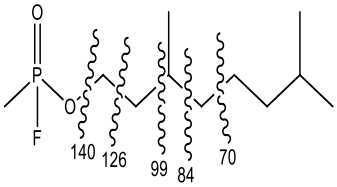
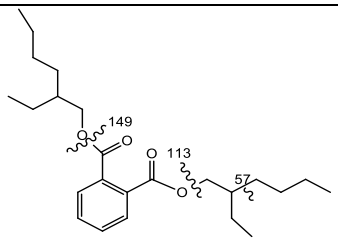
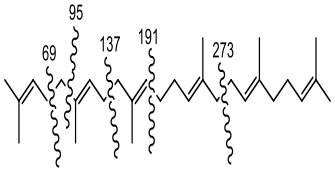
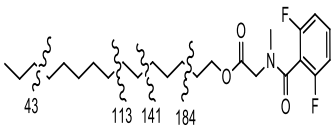
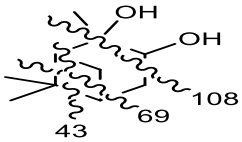
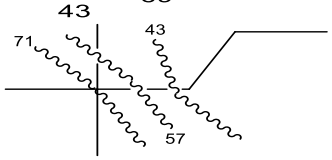
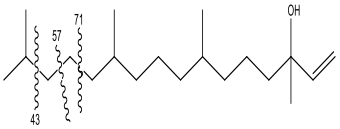
S/N	Compound	Molecular Formula	MW	Peak area%	Retention Time	Mass Spectral fragments	Fragmented structures
1	3-cyclopentyl-6-methyl-3,4-Heptadien-2-one	$C_{15}H_{24}O$	220	1.78	11.256	43, 67, 93, 107, 149, 177, 79	
2	2,3,3-trimethyl Octane	$C_{11}H_{24}$	156	1.35	14.701	43, 55, 71, 85, 99, 113, 57	
3	Hexahydro farnesyl acetone	$C_{18}H_{36}O$	268	17.06	15.265	43, 85, 124, 225, 140, 58	
4	3,7-dimethyl Undecane	$C_{13}H_{28}$	184	1.11	17.265	43, 113, 127, 85, 71, 57	
5	Phytol	$C_{20}H_{40}O$	296	57.75	18.313	43, 57, 95, 141, 126, 71	
6	2-methyl tetracosane	$C_{25}H_{52}$	352	2.01	19.161	43, 71, 85, 99, 113, 57	
7	Undecanal	$C_{11}H_{22}O$	170	1.65	19.306	43, 82, 95, 109, 126,57	
8	Tetradecyl cyclooctane	$C_{22}H_{44}$	308	1.78	20.387	55, 69, 83, 97,153, 111	
9	3,7-dimethyl-1-octyl methylphosphono Fluoridate	$C_{11}H_{24}FO_2P$	238	2.26	20.487	55, 70, 84, 112, 126,99	

Table 9: Continue…

S/N	Compound	Molecular Formula	MW	Peak area%	Retention Time	Mass Spectral fragments	Fragmented structures
10	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	3.37	22.059	43, 57, 71, 84, 113, 149	
11	Squalene	$C_{30}H_{50}$	410	2.88	24.189	69, 81, 95, 137, 273, 69	
12	Sarcosine, N-(2,6-difluorobenzoyl)-, pentadecyl ester	$C_{25}H_{39}F_2NO_3$	439	4.60	26.659	43, 57, 81, 113, 184, 141	
13	2,3- Pinanediol	$C_{10}H_{18}O_2$	170	0.79	10.459	69, 71, 93, 126, 108	
14	2,2-dimethyl Pentane	C_7H_{16}	100	0.54	11.457	43, 71, 85, 57	
15	Isophytol	$C_{20}H_{40}O$	296	0.70	16.542	43, 57, 95, 109, 71	

Antioxidant activity

The free radical scavenging activity of the extracts was carried out using DPPH as the test radical, and was assessed by the standard method adopted with suitable modifications (Sies, 1997)**.** The stock solutions of extracts were prepared in methanol to achieve the 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. DPPH (2,2-diphenyl-1-hydrazine) is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity. The absorbance was measured in triplicate at varying concentrations and the mean absorbance was determined. Parallel to examination of the antioxidant activity of plant extracts, the value for the standard compound (Ascorbic acid) was obtained and compared to the values of the antioxidant activity, the percentage inhibitions of the serial concentrations of the *n*-hexane, ethyl acetate and methanol extracts and that of the standard which was determined at different concentrations using the expression as shown in eq. 1.

$$\%inhibition = \left(\frac{A\,of\,control - A\,of\,sample}{A\,of\,control}\right) \times 100 \qquad (1)$$

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

GC-MS 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 phenyl methyl silox, (length; 30m x 250µm; film thickness 0.25µm). 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.

Table 10: GC-MS analysis of ethyl acetate extract of aerial parts of *Coccinia barteri*

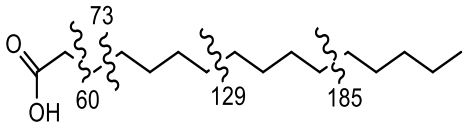
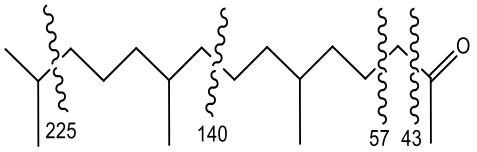
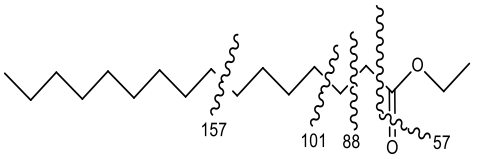
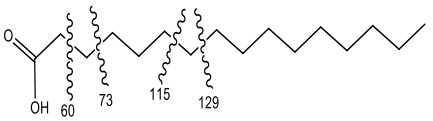
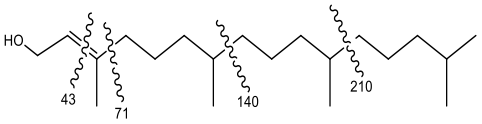
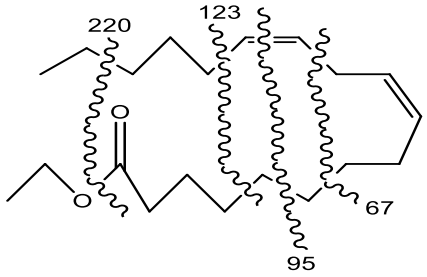
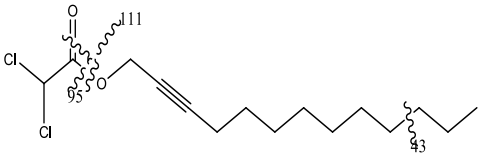
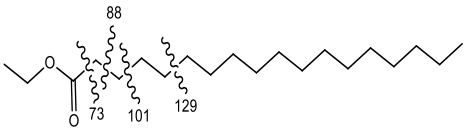
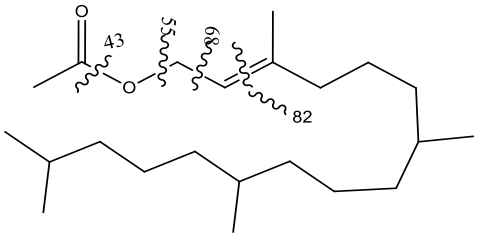
S/N	Compound	Molecular Formula	MW	Peak area %	Retention Time	Mass spectral Fragments	Fragmented structures
1	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.12	14.185	43, 60, 85, 98, 115, 129, 185, 73	
2	6,10,14-trimethyl-2-pentadecanone	$C_{18}H_{36}O$	268	3.17	15.267	43, 58, 71,85, 109, 124, 140, 225, 57	
3	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	18.33	15.523	43, 57, 73, 101, 115, 129, 157, 88	
4	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	12.83	16.900	43, 60, 85, 98, 115, 129, 143, 157, 73	
5	Phytol	$C_{20}H_{40}O$	296	11.30	18.329	43, 57, 95, 111, 123, 140, 210, 71	
6	Linoleic acid, ethyl ester	$C_{20}H_{36}O_2$	308	6.99	18.765	55, 81, 95, 109, 123, 135, 220, 67	
7	Dicholoroacetic acid tridec-2-ynyl ester	$C_{15}H_{24}Cl_2O_2$	306	11.89	18.837	43, 67, 79, 95, 111, 121, 135, 149	
8	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	3.95	19.082	43, 57, 73, 101, 115, 129, 157, 88	
9	Phytol acetate	$C_{22}H_{42}O_2$	338	8.30	19.325	43, 55, 82, 95, 109, 123, 137, 68	

Table 10: Continue…

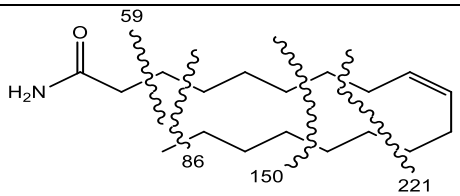
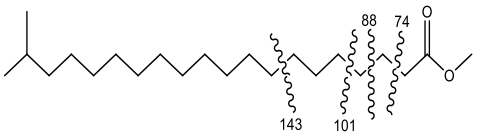
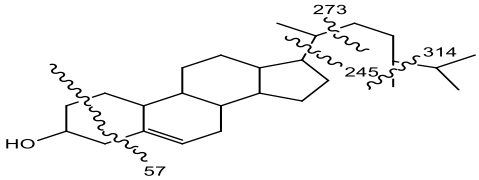
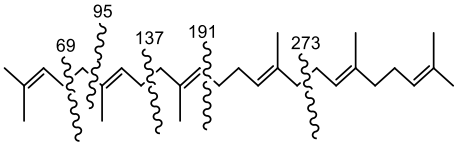
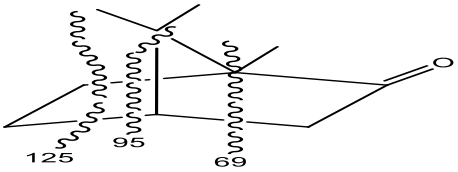
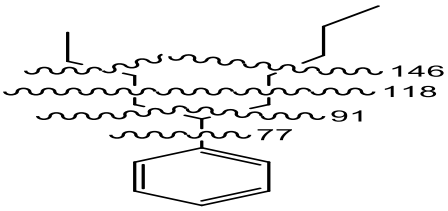
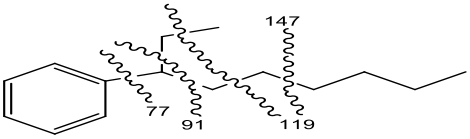
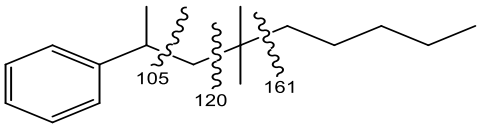
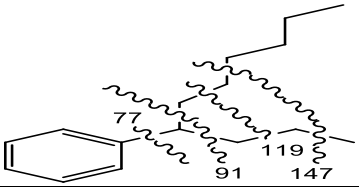
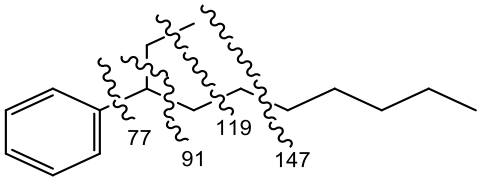
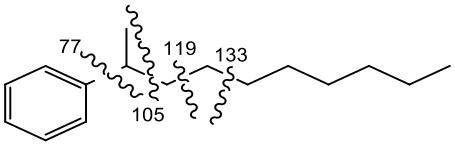
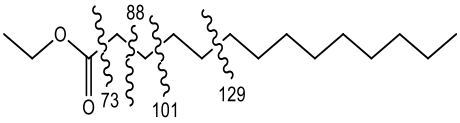
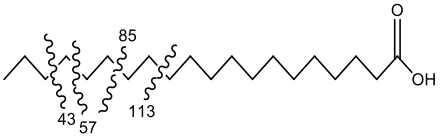
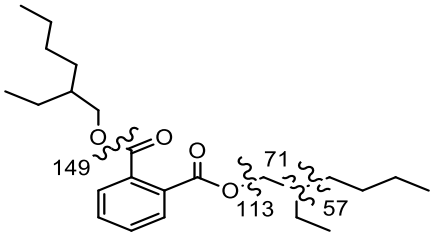
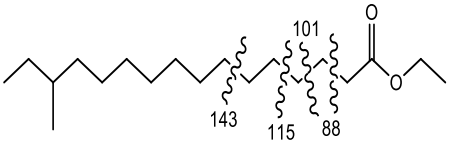
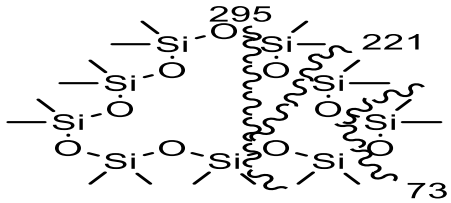
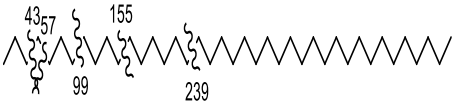
S/N	Compound	Molecular Formula	MW	Peak area %	Retention Time	Mass spectral Fragments	Fragmented structures
10	(Z)-9-octadecanamide	$C_{18}H_{35}NO$	281	2.88	20.542	55, 59, 72, 98, 112, 150, 221, 86	
11	Methyl 19-methyl-eicosanoate	$C_{22}H_{44}O_2$	340	1.37	20.848	55, 74, 101, 115, 129, 143, 157, 88	
12	Gamma-sitosterol	$C_{29}H_{50}O$	414	3.12	22.719	43, 57, 81, 95, 107, 245, 273, 314, 57	
13	Squalene	$C_{30}H_{50}$	410	2.33	24.183	81, 95, 109, 121, 137, 191, 273, 69	
14	(1R,4R)-(+)-Camphor	$C_{10}H_{16}O$	152	0.75	5.316	69, 81, 108, 125, 95	
15	1-butylhexyl-benzene	$C_{16}H_{26}$	218	0.50	10.490	77, 105, 147, 161, 91	
16	1-ethyloctyl-benzene	$C_{16}H_{26}$	218	0.57	10.876	77, 105, 119, 133, 91	
17	1,3,3-trimethylnonyl-benzene	$C_{18}H_{30}$	246	0.60	11.452	57, 71, 85, 120, 105	
18	1-propyloctyl-benzene	$C_{17}H_{28}$	232	0.58	12.252	77, 105, 119, 133, 91	

Table 10: Continue…

S/N	Compound	Molecular Formula	MW	Peak area %	Retention Time	Mass spectral Fragments	Fragmented structures
19	1-ethylnonyl-benzene	$C_{17}H_{28}$	232	0.62	12.616	77, 105, 119, 133, 91	
20	1-methyldecyl-benzene	$C_{17}H_{28}$	232	0.52	13.225	79, 91, 119, 133, 105	
21	Ethyl myristate	$C_{16}H_{32}O_2$	256	0.68	14.574	43, 57, 73, 101, 88	
22	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.88	15.171	43, 73, 85, 98, 57	
23	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	0.88	22.061	57, 71, 113, 167, 149	
24	Ethyl 14-methyl-hexadecanoate	$C_{19}H_{38}O_2$	298	0.54	22.450	55, 70, 101, 115, 88	
25	Octadecamethyl-cyclononasiloxane	$C_{18}H_{54}O_9Si_9$	666	1.49	23.434	147, 207, 221, 281, 73	
26	n-Tetrateracontane	$C_{40}H_{82}$	562	1.32	24.725	55, 71, 85, 99, 57	

Extracts of the leaf parts of *C.barteri* and *A. muricata* were dissolved in the respective solvent (n-hexane, ethyl acetate and methanol) to form solution. After this, the extracts were inserted into GC-MS instruments for chromatographic separation of the respective constituents and mass spectra of these constituents were obtained.

RESULTS AND DISCUSSION

Phytochemical screening

The preliminary phytochemical analysis of the crude extracts of *C. barteri* aerial parts revealed the presence of phenolic compounds, alkaloids, steroids, glycosides, fats and oils, flavonoids and terpenoids and saponins as shown in Table 1.

Table 11: GC-MS analysis of methanol extract of the aerial parts of *Coccinia barteri*

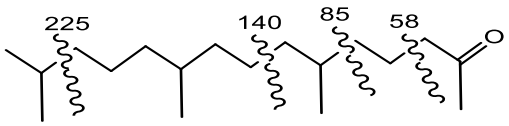
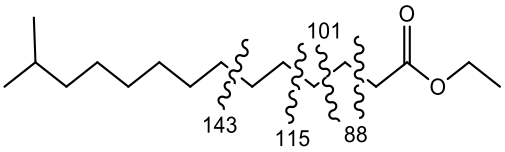
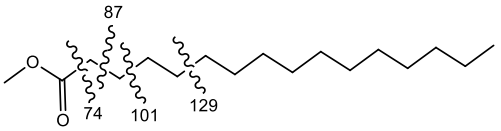
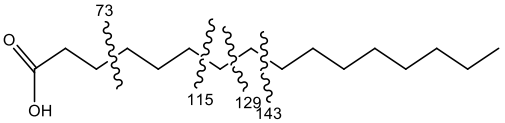
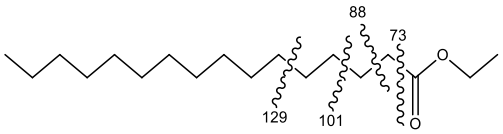
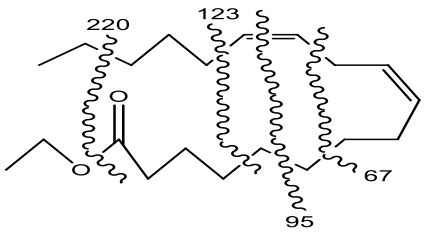
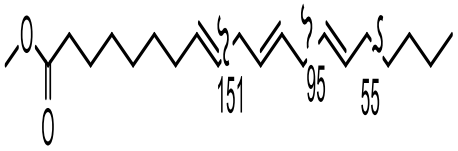
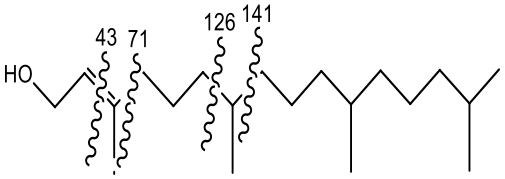
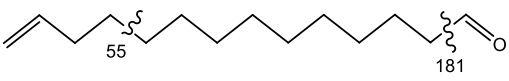
S/N	Compound	Molecular formula	MW	Peak area %	Retention Time (min)	Mass spectral fragments	Fragmented structures
1	6,10,14-trimethyl-2-pentadecanone	$C_{18}H_{36}O$	268	1.97	15.254	43, 71, 85, 109, 58	
2	Ethyl 13-methyl-tetradecanoate	$C_{17}H_{34}O_2$	270	0.71	15.512	55, 70, 101, 115, 88	
3	Methyl ester Palmitic acid	$C_{17}H_{34}O_2$	270	2.19	16.252	43, 57, 87, 101, 74	
4	Palmitic acid	$C_{16}H_{32}O_2$	256	9.55	16.782	43, 60, 85, 98, 73	
5	Hexadecanoic, ethyl ester	$C_{18}H_{36}O_2$	284	10.93	17.048	57,73,101, 115, 88	
6	Methyl ester Linoleic acid	$C_{19}H_{34}O_2$	294	1.00	18.080	55, 81, 95, 109, 67	
7	Methyl ester 8,11,14-eicosatrienoic acid	$C_{21}H_{36}O_2$	320	1.18	18.143	55, 67, 87, 107, 74	
8	Phytol	$C_{20}H_{40}O$	296	7.66	18.304	57, 95, 111, 71	
9	13-tetradecenal	$C_{14}H_{26}O$	210	3.52	18.589	67, 81, 95, 121, 55	

Table 11: Continue…

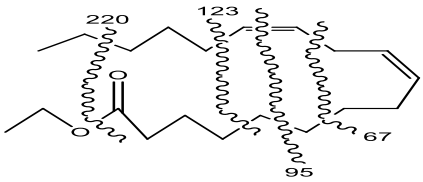
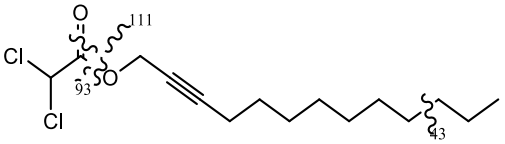
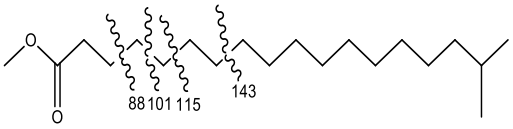
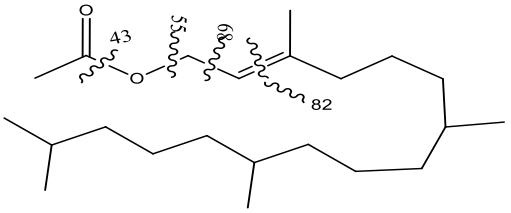
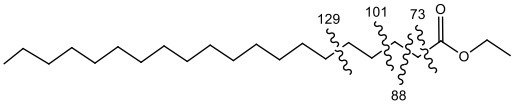
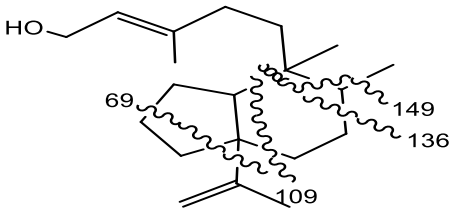
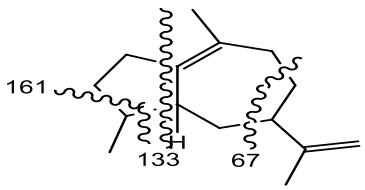
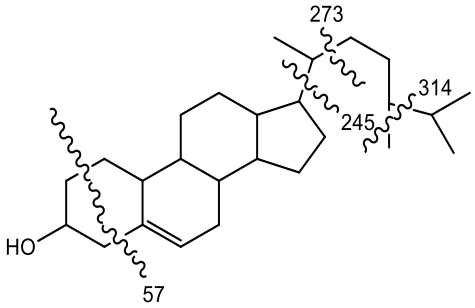
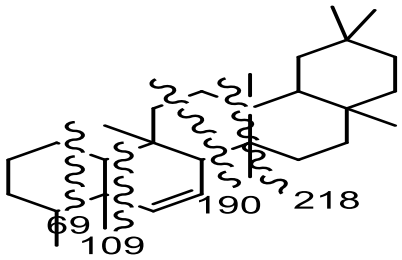
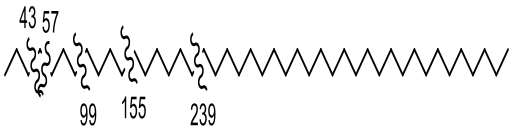
S/N	Compound	Molecular formula	MW	Peak area %	Retention Time (min)	Mass spectral fragments	Fragmented structures
10	n-propyl linoleate	$C_{20}H_{36}O_2$	308	3.13	18.749	55, 81, 95, 109, 67	
11	Ethyl Oleate	$C_{20}H_{38}O_2$	310	5.60	18.811	69, 81, 88, 101, 55	
12	Methyl 17-methyloctadecanoate	$C_{20}H_{40}O_2$	312	2.08	19.065	55, 70, 101, 115, 88	
13	Phytol acetate	$C_{22}H_{42}O_2$	338	6.94	19.305	43, 82, 95, 123, 68	
14	Ethyl icosanoate	$C_{22}H_{44}O_2$	340	0.85	20.834	57, 73, 101, 115, 88	
15	5-(7a-Isopropenyl -4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol	$C_{20}H_{34}O$	290	4.46	22.049	81, 95, 109, 123, 149	
16	Guaia-1(10), 11-diene	$C_{15}H_{24}$	204	10.77	23.180	79, 93, 107, 119, 161	

Table 11: Continue…

S/N	Compound	Molecular formula	MW	Peak area %	Retention Time (min)	Mass spectral fragments	Fragmented structures
17	Clionasterol	$C_{29}H_{50}O$	414	9.79	23.764	81, 95, 107, 119, 57	
18	D:A-Friedoolean	$C_{30}H_{50}$	278	2.85	25.107	81, 95, 109, 121, 218	
19	n-Petatriacontane	$C_{35}H_{72}$	492	1.63	26.638	43, 71, 85, 99, 57	

The presence of these bioactive compounds especially, flavonoids, is an indication that this plant possesses pharmacological activity.

Antimicrobial activity

The three crude extracts *C. barteri* gave a clear zone of inhibition against the growth of the test bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomona aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*) at moderate concentrations of the hexane (12.5 mg/mL), ethyl acetate (25 mg/mL) and methanol extracts (12.5 mg/mL) of the aerial parts of *C. barteri*, as well as test fungi (*Candida albicans*, *Aspergillus niger*, *Penicillium notatum* and *Rhizopus stolonifer*) at corresponding concentrations (Table 2-4). The activities of the hexane, ethyl acetate and methanol extracts of *C.barteri* against microorganisms may be ascribed to the existence of bioactive compounds such as alkaloids, terpenoids and flavonoids in the extracts (Table 1) which have been reported to exhibit antimicrobial activity.

Antioxidant activity

Antioxidant activities of *n*-hexane, ethyl acetate and methanol extracts of the aerial parts of *C. barteri* and that of standard control, Ascorbic acid were shown in Table 5–11. Hexane extract of the plant revealed low free-radical scavenging activity with IC_{50} of 379.80 µg/mL, ethyl acetate extract of the plant revealed very low free radical scavenging activity with IC_{50} of 681.59 µg/mL, while methanol extract of the aerial parts of *C. barteri* showed moderate antioxidant activity at IC_{50} of 187.56 µg/mL (Figure 1).

GC-MS analyses

GC-MS analysis of *n*-hexane extract of C. *barteri* aerial parts showed a total number of fifteen (15) chemical constituents with phytol and hexahydrofarnesylacetone being highly abundant compounds constituting 57.75 and 17.06% respectively. Ethyl acetate extract of the plant revealed twenty six (26) compounds with two abundant compounds: ethyl hexadecanoate (18.33%) and hexadecanoic acid (12.83%), while methanol extract afforded nineteen (19) compounds with ethyl hexadecanoate (10.93%) and clionasterol (9.79%) being the abundant compounds.

CONCLUSIONS

The aerial parts of *Coccinia barteri* have been investigated in this research and the preliminary phytochemistry of the crude extracts of the plant revealed the presence of bioactive

compounds such as phenolic compounds, alkaloids, steroids, glycosides, fats and oils, flavonoids and terpenoids. Antimicrobial activity of crude extracts from the plant against all the test bacteria and fungi was found to be very interesting and encouraging at moderate to high concentrations of the extracts, which accounts for the uses of the plant in traditional treatment as antirheumatic. The GC-MS revealed various peaks of bioactive compounds of which the activity of the plant as antioxidant, and against bacteria and fungi may be attributed to the prominent compounds in synergistic effect with all the other compounds present in smaller quantities in the extracts.

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