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Antioxidant activity and Phytocompounds from *Rogeria adenophylla* J. Gay crude extract

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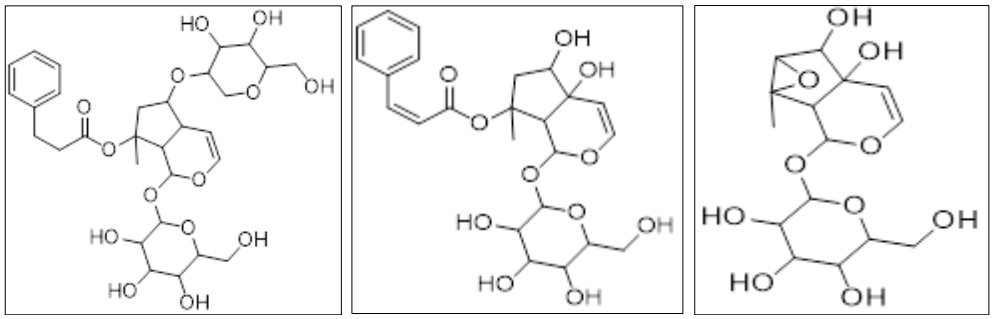
Abstract
Rogeria adenophylla is a medicinal plant widely used in ethnomedicine for the treatment of inflammations, infectious diseases, wounds and as an antinociceptive. Therefore, there is need to investigate its antioxidant property and identify some of its phytocompounds. The crude extract of *R. adenophylla* was obtained using maceration technique. The extraction solvent was removed using rotary evaporator at 45 ^{o}C to yield a brown residue. The antioxidant activities of fractions were assessed using DPPH while isolation of compounds was achieved on open column chromatography and coded A1 and A3. The characterization of all isolated and purified compounds were carried out using IR, NMR (1D and 2D) and GC-MS data. The study revealed that the ethanol leaf extract possessed the highest antioxidant property of about 87.17% while that of ascorbic acid standard was 75.40%. Compounds A1 and A3 were identified as stigmasterol and palmitic acid methyl ester based on the analysis of all spectroscopic data and in comparison with literature. The presence of these phytocompounds and the observed antioxidant activity justify the use of *R.adenophylla* in ethnomedicine.

Keywords: Antioxidant, *R. adenophylla*, Stigmasterol, ester

1. Introduction
Many medicinal plants had been used in the management and treatment of cellular and metabolic disorders such as diabetes, obesity, inflammations and cancer. Several studies have shown that the generation of free radicals inside the body is responsible for numerous cellular changes that produce a disease condition [1]. Studies have also shown that plant derived antioxidant substances can scavenge free radicals and modulate oxidative stress-related degenerative effects such as cancer, atherosclerosis, diabetes, neurodegenerative disorders and aging [2].

Rogeria adenophylla family Pedaliaccae is a herb that grows up to 2.5 m in height. It is commonly found in the dry savannah areas of Nigeria. The Hausa and Fulfulde names are "Babarodoo" and "Kebbe" respectively [3]. Its leaves are used as an antipyretic in ethnomedicine. The seeds are used in the treatment of breast inflammations, wounds, muscular pain and skin infections. The plant also possesses insecticidal properties [4]. Cepleanu *et al.* [5] reported the larvicidal activity of *R. adenophylla* on the larvae of *Aedes aegypti* (the yellow fever mosquito). The stem and roots of the plant were found to exhibit antimicrobial properties [4].

Three harpagide glycosides had been isolated from the aerial part of the *R. adenophylla*.and identified as 8-O-*cis*-cinnamoyl harpagide(1), 8-0-*cis*-cinnamoyl-6-O-β-D-glucosyl harpagide(2) and 6-O-*p*-coumaroyl harpagide(3) [6].



(1) (2) (3)

Fig 1: Some compounds isolated from *R. adenophylla*

In view of the importance of *R. adenophylla* in ethnomedicine and the dearth of literature, there is need for the study of its antioxidant properties and identification of some of its phytocompounds.

2. Materials and Methods

2.1 Sample collection and identification

The leaf and stem samples of *R. adenophylla* were collected from Kalam in Dukku LGA of Gombe State in paper bags. The samples were identified by a Botanist at the Department of Botany, Gombe State University, Mr. Mohammed Chindo. Sample specimen with voucher number; Gsu 250 was deposited in the Departmental herbarium.

2.2 Preparation and Extraction of plant material

The collected samples of *R. adenophylla* were dried under shade in a well-ventilated room. The samples were then ground to powder and stored in an air tight container. Powdered leaves and stems (900 g each) of the *R. adenophylla* were socked separately in 2 litres of n-hexane for three days. The extract was filtered using Whatmann no.1 filter paper and concentrated on a Büchi rotary evaporator at 45 ºC. The extract was fractionated using n-hexane and ethyl acetate [7]. The mass of each fraction obtained were recorded and their percentage yield was calculated.

2.3 Determination of antioxidant activity

The free radical scavenging activity of the crude extract was carried out using the DPPH (1, 1-diphenyl-2 picrylhydrazyl) assay as described by Nour *et al.,* [8] with slight modification. Various concentrations of 0.25, 0.5, 1.00, 1.5 µgml^{-1} of sample extract in methanol were prepared. DPPH (0.3 mM, 1.0 cm^3) in methanol was added to 2.5 cm^3 solution of the extract or standard, and allowed to stand at room temperature in a dark chamber for 30 minutes. For the negative control, 1ml of 0.3 mM of DPPH in 1.0cm^3 methanol was used. Ascorbic acid was used as standard (Positive control). The change in colour from deep violet to light yellow was then measured at 518 nm on a spectrophotometer. The decrease in absorbance was then converted to percentage antioxidant activity (% AA) using the formula:

AA%=100 - {[(Abssample-Absblank) x100]/Abscontrol}

2.4 Isolation of compounnds from *R. adenophylla*

The glass column was packed using silica gel 60, mesh size 70-230, slurry according to the method described by Kwaji *et al.* [9] with slight modification. The column was allowed to settle down uniformly under gravity. An n-hexane soluble fraction (9.35 gram) was adsorbed onto silica gel and loaded on the column filled with silica gel 60. The column was first eluted with 100% petroleum ether, then followed by gradual increase in polarity with petroleum ether-dichloromethane (9:1), (8:2), (7:3), (6:4), (5:5), (4:6),…….(0:10), dichloromethane/ethylacetate (9:1), (8:2), (7:3), (6:4), (5:5), (4:6),…….(0:10). Aliquots of 500 mL solvent combinations were used for the gradient elution and 100 mL fractions were collected glass conical flasks. The collected fractions were concentrated using rotary evaporator at 45ºC. Thin layer chromatography was used to combine fractions with similar composition. The isolates are then washed and recrystallized. Compounds A1 and A3 were isolated as white crystalline solids from the leaf of *R. adenophylla*.

FT-IR analysis of the samples was obtained and recorded on Perkin Elmer Universal ATR spectrum 100 FT- IR spectrometer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on 400 MHz Bruker Avance II Ultra shield with samples all dissolved in deuterated chloroform ($CDCl_3$). The GC-MS analysis was carried out on Shimadzu GC-MS- QP-2010-SE. All sample analysis was achieved at KwaZulu-Natal University, South Africa. Department of Chemistry.

3. Results and Discussions

Table 1 shows the free radical scavenging activity of the extracts of *R. adenophylla* and is concentration dependent. The ethanol leaf extract gave the highest antioxidant activity of 87.17%. The extracts were found to have higher free radical scavenging activity compared to standard ascorbic acid. Literature reports showed that the reduction mechanism of DPPH is principally due to the presence of hydroxyl groups on the antioxidant molecules [7]. It was also reported by Kar, [10] that extracts containing saponins and alkaloids demonstrated strong antioxidant properties. Sandhya *et al.* [11] reported that *Sesamum indicum* L, a plant belonging to the Pedaliaccae family as *R. adenophylla* possess strong antioxidant activity. This shows that *R. adenophylla* may be quite useful in the treatment and mitigation of disorders associated with free radicals.

Table 1: Antioxidant activity of the leaf and stem extracts of *R. adenophylla*

Concentration (µg/mL)	% Antioxidant activity				
	LHE	LEE	SHE	SEE	AA
0.25	41.03±0.5	67.03±0.5	38.23±1.4	38.17±0.9	44.30±0.2
0.50	50.73±0.1	70.13±0.6	37.53±0.7	55.00±0.4	65.67±0.1
1.00	58.40±0.9	82.17±1.5	46.33±0.8	60.70±0.4	68.20±0.7
1.50	58.83±1.2	87.17±1.0	53.57±1.3	67.93±0.6	75.40±0.6

Key: LHE= leaf hexane extract, LEE= leaf ethanol extract, SHE= stem hexane extract, SEE= stem ethanol extract, AA= ascorbic acid standard

3.1 Spectroscopic Data

The IR spectrum of A1 in Table 2 showed an intense broad band absorption peak at 3362.51 cm^{-1} for the O-H bond vibrations of hydroxyl group and the absorption band at 2932.24 cm^{-1} and 2865.12 cm^{-1} stands for the aliphatic C-H stretching of CH_3 and CH_2 respectively, and at 1040 cm^{-1} a weak C-O stretch was observed. These assignments were in good agreement with reported values by Chaturvedula and Prakash [12].

Table 2: Infrared Result of Compound A1

S. No.	Frequency (cm^{-1})	Type of vibration
1	3362. 51	O-H stretching
2	2932.24	C-H due to CH_3
3	2865.74	C-H due to alkane -CH_2-
4	1680.50	C=C stretching vibration
5	1462.92	CH_2 bending vibrations
6	1379.00	CH_3 bending vibrations
7	1052.41	C-O stretching

From Table 3, the downfield signal in the 1H-NMR spectrum at δ 5.35 (1H, triplet) was due to an olefinic proton at C6 and a methine proton at C3 was represented by a signal at δ 3.53 (1H, m). The presence of a pair of doublets at δ 5.01 ppm and δ 5.15 ppm was due to the sp2 methine protons at C22 and C23 in the molecule. The existence of six methyl signals was also noted at δ 0.68 (H3-C18), 1.00 (H3-C19), 0.92 (H3-C21), 0.83 (H3-C26), 0.83 (H3-C27) [13].

The 13C NMR revealed signals at 140.78 and 121.90 ppm due to C5 and C6 double bonded carbon atoms while at δ 71.83 ppm is due to a C-3 hydroxy group. The signals at δ19.39 ppm and δ11.98 ppm correspond to carbon atoms at C-18 and C-19 respectively. DEPT experiment revealed six methyl carbons at C-18, C-19, C-21, C-26, C-28, and C-29; nine methylene carbons at C-1, C-2, C-4, C-7, C-11, C-12, C-15, C-16 and C-25; eleven methine carbons at C-3, C-6, C-8, C-9, C-14, C-17, C-20, C-22, C-23, C-24 and C-27 and three quaternary carbons; C5, C10 and C13. The de-shielded signal at δ71.8 ppm was due to C-3 attached to a hydroxyl group. Based on the IR, ^{1}H-NMR, and ^{13}C-NMR data and in comparison with literatures, suggests the presence of stigmasterol [12, 13].

Table 3: ^{1}H and ^{13}C NMR of Compound A1

Position	Group	δC Literature	δC	δH Literature	δH
1	CH_2	37.6	37.28	1.84	1.30 (2H, m)
2	CH_2	32.1	33.00	1.50	1.50 (2H, quartet)
3	CH	72.1	71.83	3.50	3.53 (1H, m)
4	CH_2	42.4	42.33	2.27	2.25 (2H, doublet)
5	C	140.78	140.78		
6	CH	121.8	121.71	5.38	5.35 (1H, dd)
7	CH_2	30.8	30. 49	1.89	1.99 (2H, triplet)
8	CH	31.8	31. 94	1.70	1.70 (1H, m)
9	CH	50.2	50.18	0.92	0.90 (1H, doublet)
10	C	36.6	36.53		
11	CH_2	21.5	21.11	1.50	1.50 (2H, triplet)
12	CH_2	39.9	39.81	2.01	2.00 (2H, triplet)
13	C	42.4	46.0		
14	CH	56.8	56.83	1.10	1.00 (1H, triplet)
15	CH_2	24.4	24.31	1.70	1.9 (2H, quartet)
16	CH_2	29.3	29.22	1.70	1.70 (2H, quartet)
17	CH	56.2	56.11	1.09	1.20 (1H, m)
18	CH_3	12.0	11.98	0.68	1.27 (3H, singlet)
19	CH_3	11.2	11.68	1.00	1.00 (3H, singlet)
20	CH	40.6	42.33	1.28	1.60 (1H, m)
21	CH_3	19.6	19.80	0.92	1.25 (3H, dd)
22	CH	138.5	138.29	5.01	5.01 (1H, dd)
23	CH	129.4	129.33	5.16	5.15 (1H, triplet)
24	CH	51.4	50.18	1.50	1.20 (1H, quartet)
25	CH_2	24.5	29.69	1.20	1.91 (2H, m)
26	CH_3	12.3	19.80	0.83	1.0 (3H, triplet)
27	CH	21.3	19.80	1.90	0.81 (1H m)
28	CH_3	18.9	18.79	0.83	0.68 (6H, doublet)
29	CH_3	18.9	18.79	0.83	0.68 (6H, doublet)

The Gas Chromatography Mass Spectrometry (Table 4) for compound A1 gave a molecular peak at m/z 412. Other fragmentations are; loss of water which indicated the presence hydroxyl group is the fragment ion at m/z 394. The subsequent fragment at m/z 379 might be due to the loss of a methyl group. The signal at m/z 271 is the characteristic of stigmasterol fragmentation due to loss of the side chain followed by the loss of two hydrogen atoms (Fig.2). The mass fragmentation pattern is consistent with previous reports [12, 14].

Table 4: Gas Chromatography Mass Spectrometry (GC-MS) Result

Molecular ion mass (m/z)	Masses of fragment ions m/z (% abundance)
412	412(34), 397(12), 394(10), 351(20), 300(20), 271(27), 255(29), 159(33), 145(37), 133(43), 105(35), 95(37), 83(75), 69(58), 55(100)

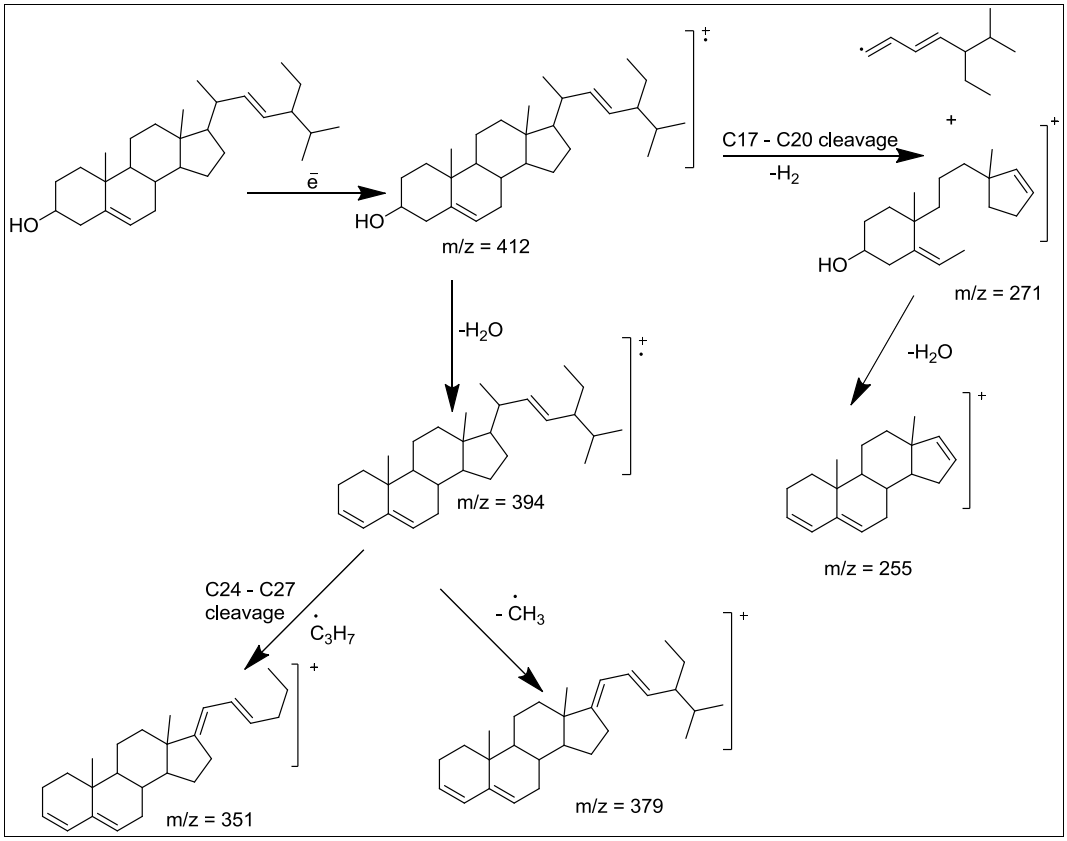


Fig 2: Structure of the Fragment ion.

Based on the spectroscopic information, the compound isolated is identified as stigmasterol and the structure of the compound is shown in figure 3 below. Panda *et al.* [15] reported that the stigmasterol isolated from the stem bark of *Burta monosperma* showed antioxidant activity; also stigmasterol isolated from *Sideritis foetus* was found to exhibit anti-inflammatory activities [16]. These biological activities of stigmasterol support the use of *R.adenophylla* in traditional medicine.

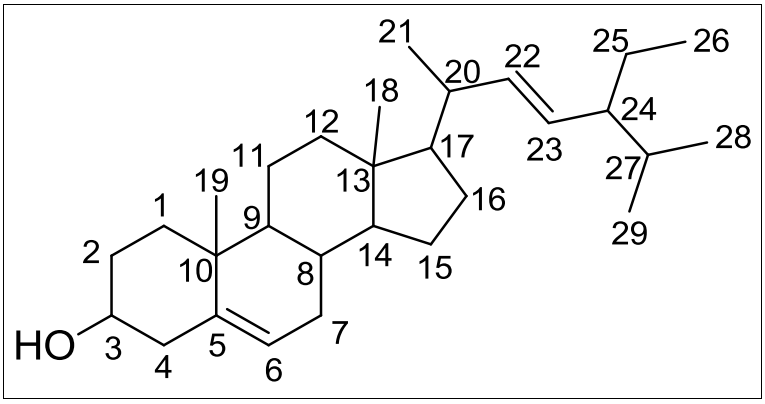


Fig 3: Structure of Compound A1

Table 5: IR Peaks of Compound A3 Spectrum

S. No.	Frequency (cm^{-1})	Type of vibration
1	2916.54	C-H stretching due to $-CH_3$
2	2849.00	C-H stretching due to $-CH_2-$
3	1701.31	C=O Carbonyl stretching
4	1098.08	C-O

Table 6: ^{1}H and ^{13}C NMR chemical shifts of Compound A3 and literature Comparison

Position	Group	δC Literature	δC	δH Literature	δH
1	OCH_3	39.90	39.40	3.64	(3H, singlet)
1	C=O	174.62	178.96		-
2	CH_2	33.93	34.40	2.30	2.23 (2H, m)
3	CH_2	26.01	25.96	1.56	1.56 (2H, m)
4	CH_2	29.79	29.70	1.29	1.29 (2H, m)
5	CH_2	29.64	29.69	1.29	1.29 (2H, m)
6	CH_2	29.59	29.66	1.29	1.29 (2H, m)
7	CH_2	29.59	29.60	1.29	1.29 (2H, m)
8	CH_2	29.45	29.58	1.29	1.29 (2H, m)
9	CH_2	29.45	29.45	1.29	1.29 (2H, m)
10	CH_2	29.45	29.36	1.29	1.29 (2H, m)
11	CH_2	29.36	29.25	1.29	1.29 (2H, m)
12	CH_2	29.35	29.08	1.29	1.29 (2H, m)
13	CH_2	29.15	29.15	1.29	1.29 (2H, m)
14	CH_2	31.79	31.94	2.27	1.29 (2H, m)
15	CH_2	22.54	22.70	1.64	1.33 (2H, sextet)
16	CH_3	14.00	14.11	0.87	0.96 (3H, triplet)

Table 7: GC-MS fragmentation data of compound A3

Molecular ion mass (m/z)	Masses of fragment ions m/z (% abundance)
270	270(m,17), 239(12), 227(10), 129(20), 115(5), 87(65), 74(100), 55(24),

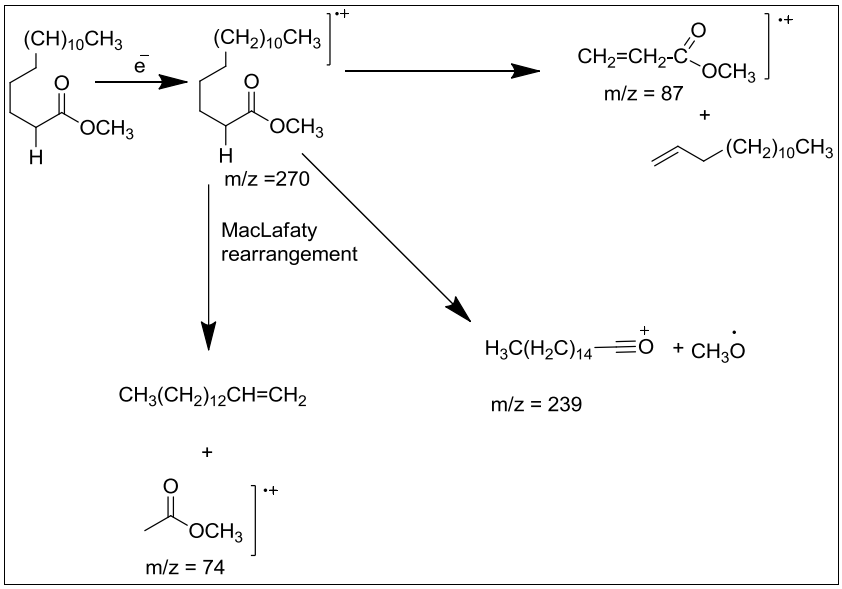


Fig 4: Structure and molecular fragments of Compound A3

The IR spectroscopic analysis (Table 5) shows absorption bands at 2916.5 cm^{-1} and 2849 cm^{-1} are due to C-H vibrations of $–CH_3$- and $–CH_2$- respectively. While vibration peaks at 1701.31 cm^{-1} and 1098.08 cm^{-1} are due to C=O and C-O respectively. . The absorption frequencies are consistent with those published by Bulama *et al.* [17].The ^{13}C NMR chemical shifts (Table 6) showed recognizable signal at δ178.96 confirming the presence of acidic carbonyl group on carbon one (C_1). The signal at δ14.11 shows the presence of a terminal methyl group at C_{16}. The close range signals from δ29.08-29.70 indicates the methylene groups from C_4-C_{13}.The mass spectral data (Table 7 and fig. 4) of the compound gave the molecular ion $[M]^+$ peak at m/z 270 $[M]^+$, other peaks are: 270, 239, 227, 129, 101, 87, 74 and 55. The base peak has m/z 74 which suggested the presence of $[CH_2CO_2CH_3]^+$.

Based on the spectroscopic information, the compound isolated is identified as palmitic acid methyl ester and the structure of the compound is shown in figure 5. Palmitic acid methyl ester is known to exhibit antibacterial and antifungal properties [18]. The reported pharmacological activities of palmitic acid methyl ester support the traditional use of *R. adenophylla* in the treatment diseases and infections caused by microorganisms such as *Salmonella typhi*, *Escherichia coli* and *Aspergillus Niger*. Abay *et al.* [19] and Khan and Usman [20] reported the insecticidal activity of palmtic acid methyl ester. The presence of palmitic acid methyl ester and other related phytocompounds may be responsible for the larvicidal activity of *R. adenophylla* on the larvae of *Aedes aegypti* and cytotoxicity to brine shrimp.

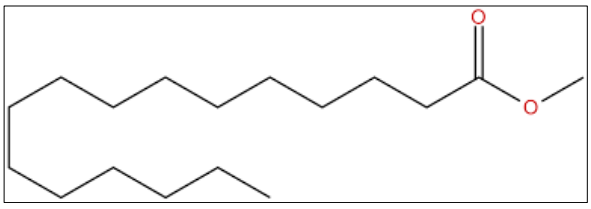


Fig 5: Proposed Structure of Compound A3 ($C_{16}H_{32}O_2$).

Conflict of Interest

Authors declare that conflict of interest does not exist.

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