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Phytochemical screening and isolation of fatty acid and fatty acid esters of triterpene from root extract of *Vernonia auriculifera* grown in Sidama zone, Southern Ethiopia

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Abstract
Vernonia auriculifera is well known for its use in traditional medicine in several countries of tropical regions of the world including Ethiopia. Therefore, the aim of the present study was to conduct phytochemical screening tests on extracts of the root of *Vernonia auriculifera* plant species and also to isolate compounds from the extracts. The root of *V. auriculifera* was extracted with three solvent systems (n-exane, dichloromethane/methanol (50:50% by volume) and methanol) using maceration technique and sequential extraction approach. All the extracts were subjected to phytochemical screening tests following standard procedures reported in literatures. The preliminary phytochemical analysis revealed the presence of alkaloids, saponins, flavonoids, phenols, steroids, glycosides, terpenoids and tannins in the crude extracts of dichloromethane and dichloromethane: methanol. The crude extract of n-hexane showed the presence of saponins, flavonoids, phenols, steroids, glycosides, terpenoids and tannins. On the other hand, the crude extract of methanol revealed the presence of all the secondary metabolites listed above except tannins. The dichloromethane/methanol extract was subjected to column chromatographic separation to afford two compounds namely Oleic acid (AY-4) and β-amyrin palmitate (AN-1). The structures of the compounds were elucidated based on spectroscopic (FTIR, ^{1}H-NMR and ^{13}C-NMR) data and comparison with literature reports.

Keywords: *V. auriculifera*, phytochemical screening, spectral analyses, Oleic acid, *β-amyrin palmitate*

1. Introduction
Medicinal plants have been used for centuries by human beings for treatment of human and animal illnesses via traditional medicine/traditional healers. Different morphological parts seeds, leaves, roots, barks and/aerial parts of these plants are used to treat diseases by people of different cultural and economic backgrounds (Singh, 2015; Rungsung *et al*. 2015, Shukla *et al*., 2010) [40, 36, 38]. The medicinal values of these plant could be attributed to the presence of secondary metabolites. These metabolites categorized to different classes such as alkaloids, terpenoids and saponns (Raja and Sreenivasulu, 2015; Rungsung *et al*. 2015) [35, 36]. There are reports that revealed about 80% world population, especially those in the developing world are highly dependent on medicinal plants/traditional healers for several reasons. Some of the reasons (i) modern drugs are costly and some of them faced resistance by disease causing organisms, culturally acceptable (Sani, 2015; Pooja *et al*., 2017; Agyei-Baffour *et al*., 2017) [37, 31, 2]. Nowadays, people (companies) in developed countries are turning their faces towards medicainal plants (Cragg, 1997; Hoareau, 1999; Vieira *et al*., 2014] [10, 17, 46]. There are significant number of modern drugs currently in market are obtained from medicinal plants. Moreover, many more drug candidates in different phases of drug discovery programs of pharma companies have been obtained from medicnal plants (Cragg, 1997; Chakraborty, 2018; Harvey, 2008) [10, 8, 16].
Vernonia auriculifera Hiern (*V. auriculifera*) is one of thousands of the species that belong to genus *Vernonia*. It is distributed in the tropical and subtropical areas of the world, especially Africa and South America and Asia (Silva *et al*., 2013; Costa *et al*., 2008; Dematteis and Pire, 2008) [39, 9, 11]. The plant is well known for its traditional medicinal use in different parts of the world including Ethiopia. Some of the medicinal uses of *V. auriculifera* (or its different parts) include treatment of eye infections, malaria, diabetes, fever, headache, toothache, Amoebiasis (*Mageana*), bacterial infection, wound, venereal diseases, hepatitis, removal of placenta during giving birth, alimentary infection and gastrointestinal problems (Anoka *et al*., 2008; Misra *et al*. 1984; Namukobe *et al*, 2011; Worku, 2015; Sentayehu *et al*., 2011; Abiyu *et al*., 2014, Kusamba, 2001; Muthaura *et al*., 2007) [4, 26, 29, 47, 1, 20, 28].

In Ethiopia, *V. auriculifera* is known by different local names such as *Gengorita* (in Amharic), *Reejii* (in Oromifaa) and *Rejicho* (in Sidamic), and it is widely distributed in Sidama zone (Southern Ethiopia) and West Shewa zone (Central Ethiopia). In the above mentioned areas, its different parts (roots, leaves and stems) are used in traditional medicines for treatment of human diseases. For instance, the leaf of the plant is used for healing wounds by rubbing (as ointment) around the injured areas after soaking the fresh leaf problems, and also used to treat malaria, stomach ache (Asnake *et al.*, 2006; Tausha *et al*., 2018; Doffana, 2017) [5, 12].

There are several reports on phytochemical investigation for secondary metabolites and biological activities of crude extracts (Sobrinho *et al*., 2015; Githua, 2008) [42, 15]. For instance, Bekele *et al*. (2015) [7] reported the presence of tannins, flavonoids, terpenoids and saponins, but absence of anthraquinones, steroids, and alkaloids in the methanol extract of leaves of *V. auriculifera.* The authors also reported high antibacterial activities of methanol and water extracts that could be attributed to the presence of polar constituents such as polyphenols. Kiplimo *et al*. (2011) [19], reported moderate antibacterial activities of hexane, dichloromethane, ethyl acetate and methanol extracts of root bark, stems and leaves of *V. auriculifera* against *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. maltophilia*, *S. aureus*, *B. subtilis*, *E. faecium*, *S. epidermidis* and *S. saprophyticus.* In a recent report, larvicidal activity and phytochemical composition of leaves and root crude extracts derived from *Vernonia* spp (including *V. auriculifera*) were tested against the malaria vector *Anopheles gambiae*. In such a report, it has been reported that hexane, chloroform, ethyl acetate, acetone, methanol and water extracts possess steroids, saponins, flavonoids, terpenoids and cardiac glycosides (Ngeranwa, 2017) [44].

There are also reports on isolation and identification of compounds from different morphological parts of *V. auriculifera*. Some of the compounds even tested for biological activities and showed moderate to high activities against different bacterial strains (Keriko *et al*., 1995; Kiplimo *et al*., 2011; Bekele *et al*., 2015) [18, 7, 19]. The majority of the compounds reported so far are terpenoids. Some of the compounds are lupenyl acetate, α-amyrin, β-amyrin, ß-amyrin acetate, friedelanone, friedelin acetate, oleanolic acid, β-sitosterol, oleanolic acid and β-sisterol from leaves, stem bark and root bark extracts of the same plant grown in Kenya (Kiplimo *et al*., 2011) [19]. Keriko *et al*. (1995) [18] reported isolation of 8-des-Acylvernodalol from methanolic extract of fresh leaves. Isolation of Lupeol and α-taraxerol octanoate have also been reported from root bark extract of Kenyan *V. auriculifera* (Githua, 2008) [15]. To the best of our knowledge, there are no previous reports on phytochemical investigation of on *V. auriculifera*. Thus, this study was initiated to study phytochemicals of roots of *V. auriculifera* grown in Ethiopia.

2. Methods and materials

2.1. Collection and preparation of plant materials

The root of *V. auriculifera* was collected in December, 2017 from the area around Awada Campus, Hawassa University. The plant was authenticated by a botanist Mr. Reta Regassa, Department of Biology at Hawassa College of Teacher Education. It was given voucher number NS/023. The collected plant material (root) was chopped to small pieces. It was then air-dried under shade for 30 days withot exposing it to a direct sun light and moisture. The dried plant material was grounded using mortar and pistil.

2.2. Extraction

The root of *V. auriculifera* (1300g) was sequentially extracted with n-hexane dichloromethane: methanol (50:50% by volume) and methanol by macerating for 72 hrs with continuous shaking. The extracted matter was filtered using Whatmann No.1 filter paper, and the residual solvent in each gradient extract was removed using Rotary evaporator under reduced pressure. The mass of the crude extract of each solvent was stored in refrigerator until used for further analyses.

2.3. Phytochemical screening of extracts

Phytochemical examinations were carried out for all the extracts as per the standard methods reported in the literature. The secondary metabolites considered in the screening were alkaloids, flavonoids, phenols, glycosides, terpinoids, tannins, saponins and steroids (Pradeep *et al*., 2014) [32].

2.4. Chromatographic isolation and structural elucidation of compounds

25.5 g of dichloromethane: methnol (50:50% by volume) crude extract was adsorbed on to silica gel (80 g). The adsorbed sample was then subjected to glass column that was loaded with 120 g silica gel. It was eluted with increasing gradient of ethyl acetate in n-hexane. A total 56 fractions (50ml each) were collected and analyzed by Thin Layer Chromatography (TLC). The Fractions 7-16 were combined and further fractionation was conducted using small column in ethyl acetate in n-hexane solvent system with various proportions (00:100,1:99, 2:98, 3:97, 4:96, 5:95, 10:90, 15:85, 20:80, 25:75, 30:70, 40:60, 50:50, 40:60% by volume). A total of 45 fractions each with volume of 40 ml were collected. The collected fractions were then concentrated using rotary evaporator. The purities of the fractions were monitored using TLC. The spots on the TLC plates were visualized using UV light (at 254 nm and 365 nm) followed by iodine vapor. Fractions showing the same TLC profiles were combined and concentrated. Thus, fractions 6-11 were combined and concentrated to afford 38 mg of white crystal compound (labeled as compound AY4). Similarly, 28 mg, light yellow crystalline solid compound (labeled as compound AN1) was obtained by combining fractions 16-20. The compounds were then subjected to spectroscopic (IR and NMR) analyses. The NMR and IR spectra data were generated using Bruker Advance 400 MHz spectrometer and Perkin Elmer BX infrared spectrometer(400- 4000cm^{-1}), respectively. All spectral analyses were carried out at The Department of Chemistry, Addis Ababa University, Ethiopia.

3. Results and Discussion

3.1. Determination of Extraction Yield

The extraction yield is a measure of the solvent efficiency to extract specific components from the original material. The percentage yield of crude extract in respective solvent was recorded (Table 1). The observation revealed that the yield is higher for polar solvents as compared to the non-polar solvent (n-hexane). This is consistent with several literature reports showing that the yield of extracts to be lower for non-polr solvents (Bekele *et al*., 2015; Fillipo *et al*., 2018) [7].

$$Percent\ Yield = \frac{\text{Weight of the crude extract (g)}}{\text{Grounded plant material used for extraction (g)}}\ x\ 100\%$$

Table 1: The percent yield of crude extracts obtained from roots of *V. auriculifera*

S. No	Solvent used for extraction	Mass of extract (g)	Yield (%)
1	n-hexane	3.50	0.27
2	Dichloromethane: methanol (50:50% by volume)	58.50	4.50
3	Methanol	55.80	4.30

3.2. Phytochemical screening

Phytochemical screening tests were carried out on n-hexane, dichloromethane: methanol (50:50% by volume) and methanol root extracts of *V. auriculifera* using standard procedures to identify the class of secondary metabolites. The results revealed that the extracts contain secondary metabolites such as terpenoids, saponins, steroids, flavonoids, tannins, phenols, glycosides, and alkaloids (Table 2). The data observations are in consistent with the report of Tarwish *et al.* (2017) [44] who reported the presence of tannins, saponons, flavonoids, steroids terpenoids and cardiac glycosides in the n-hexane, ethylacetate, chloroform, methanol and water extracts of leaves and root extracts of three vernonia species (*V. auriculifera, V. galamensis and V. lasiopus*) growing in Kenya. The presence of such important classes of compounds in different extracts obtained from different parts of *V. auriculifera* can be used as evidences for the current use of the plant by traditional healers (Bekele *et al.,* 2015) [7] and also its future potential as source of novel drugs for treatment of human illnesses.

Table 2: Phytochemical Analysis of root extract of *V. auriculifera*

Phytochemicals	n-hexane extract	Dichloromethane: methanol (50:50% by volume) extract	Methanol extract
Steriods	+	+	+
Terpeniods	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Tanins	+	+	-
Alkaloids	-	-	+
Phenol	-	+	+
Glycosides	-	+	+

3.3. Structural elucidation of the isolated compounds

Two compounds **AY4, AN1** were characterized, on spectroscopic (NMR and IR) data. The details of structural elucidations of the compounds are discussed in the sub-sections below.

3.3.1. Characterization of compound AY4

The compound was isolated as a white crystalline solid (38 mg) with R_f value of 0.63 (2% ethyl acetate in n-hexane).The IR spectrum of compound AY4 (Appendix 1) showed the absorption bands at 3417 cm^{-1} and 1696 cm^{-1} corresponding to the stretching vibration bands of the hydroxyl (OH) and carbonyl (C=O) groups, respectively. The appearance of bands at 2918 cm^{-1} and 2847 cm^{-1} could be attributed to C-H stretching of methylene and methyl groups of an alkane, respectively. Other characteristic bands of olefinic carbon-carbon double bond and C-O stretching of the carbonyl carbon of carboxylic acid were observed at 1643 cm^{-1} and 1271 cm^{-1} (of medium intensity).

The ^{1}H-NMR ($CDCl_3$, 400 MHz) spectrum of compound AY4 (Appendix 2) showed the peaks at δ1.32 and δ1.62 indicated presence of aliphatic methylene ($-CH_2$) group whereas the peak at δ2.03 could be assigned to vinylic protons or protons of methylene group that is bonded to C=C bond. The peak at δ2.35 indicates presence of protons of a methylene group α to a carbonyl group. The multiplet peaks at δ5.36 could suggest the presence of at least two olefinic protons in the structure whereas. The other peaks indicate additional protons of methylene group (δ1.30 and δ1.32) and methyl protons (δ0.89) (Appendix 2). The ^{13}C-NMR spectrum of compound AY4 (Appendix 3) showed a single peaks at δ180.1 that could be attributed to a quaternary carbon atom (of carbonyl carbon) of carboxylic acid. The peak at δ34.03 indicates most likely a carbon that is α to carbonyl group of acid. The peaks were observed at δ130.1 and 129.9 could be attributed to olefinic carbons or indicated the presence of C=C bond in the compound. The other methylenes of hydrocarbon chain resonated in the range of δ31.91-22.68 while the terminal methyl group showed the signal at δ14.1. Moreover, absence of a peak at δ180.1 in DEPT-135 spectrum (Appendix 4) indicated the presence of quaternary carbons atoms in the chain of fatty acid group.

All the spectral data (Apendices 2-4) obtained from the spectal analyses and also comparison of the data and spectral pattern reported in literature reports suggest that the compound AY4 to be Oleic acid (1) (Table 3, Figure 1) (Mulugeta *et al.* 2013; Malarvizhi *et al.*, 2016; www.magritek.com; Atika *et al.* 2001) [27, 21].

Table 3: Comparison of the ^{13}C-NMR and ^{1}H-NMR spectra of compound **AY4** with reported Oleic acid (Mulugeta *et al.* 2013) [27].

Carbon No	^{13}C-NMR of compound AY4 (ppm)	DEPT-135 of compound AY4	Reported ^{13}C-NMR of Oleic acid (Mulugeta *et al.* 2013) [27]	^{1}H-NMR of ompound AY4 (ppm)	Reported ^{1}H-NMR of Oleic acid (Mulugeta *et al.* 2013) [27]	Nature of carbon
1	180.1	-	180	----	-----	C=O
2	34.2	34.2	34.1	2.35(t, 2H, H 2)	2.36(t, 2H, H 2)	CH_2
3	24.7	24.7	24.7	1.62(m, 2H, H 3)	1.63 (m, 2H, H 3)	CH_2
4	29.1	29.1	29.2	1.30 (m, 2H, H 4)	1.27(m, 2H, H 4)	CH_2
5	29.3	29.3	29.2	1.30 (m, 2H, H 5)	1.27 (m, 2H, H 5)	CH_2
6	29.1	29.1	29.2	1.30 (m, 2H, H 6)	1.27 (m, 2H, H 6)	CH_2
7	29.7	29.7	29.7	1.35 (m, 2H, H 7)	1.32 (m, 2H, H7)	CH_2
8	27.2	27.2	27.2	2.03 (m,2H,H 8),	2.02(m, 2H,H8)	CH_2
8	27.2	27.2	27.2	2.03 (m,2H,H8),	2.02(m, 2H,H11)	CH_2
9	130.1	130.1	130	5.36(d, 1H, H 9)	5.34 (d, 1H, H 9)	CH
10	129.9	129.9	130	5.36(d, 1H, H10)	5.34 (d, 1H,H 10)	CH
11	27.2	27.2	27.2	2.03 (m, 2H, H11)	2.20 (m, 2H, H 11)	CH_2

12	29.7	129.7	29.7	1.30(m, 2H, H12)	1.27(m, 2H, H12)	CH_2
13	29.4	29.4	29.4	1.30(m, 2H, H 13)	1.27 (m, 2H, H 13)	CH_2
14	29.6	29.6	29.6	1.30(m, 2H, H 14)	1.27 (m, 2H, H 14)	CH_2
15	29.4	29.4	29.4	1.30(m, 2H, H 15)	1.32 (m, 2H, H 15)	CH_2
16	31.9	31.9	32.0	1.30(m, 2H, H 16)	1.32 (m, 2H, H 16)	CH_2
17	22.7	22.7	22.4	1.30(m, 2H, H 17)	1.27 (m, 2H, H17)	CH_2
18	14.1	14.1	14.0	0.89(t, 3H,18)	0.80 (t, 3H,H18)	CH_3

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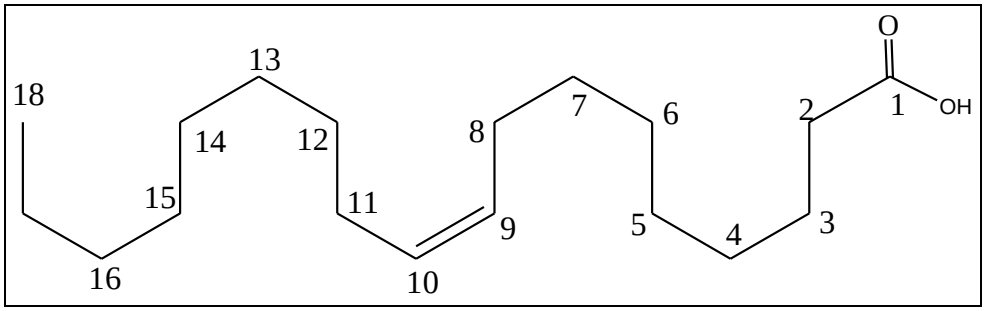


Fig 1: The proposed structure of compound AY4 (Oleic acid, **1**)

3.3.3. Characterization of compound AN1

Compound AN1 was obtained as a light yellow crystal solid (28 mg). Its R_f value was determined to be 0.58 (in n-hexane: ethyl acetate, 96:4% by volume). The IR spectrum of compound AN1 (Appendix 5) showed a strong absorption band at 2920.4 cm^{-1} represents C-H stretch of methylene whereas, the weak band around 2844.7 cm^{-1} indicated the C-H stretching of methyl groups. The band at 1726.6 cm^{-1} indicate the presence of carbonyl the C=O functional group. On the other hand, medium absorption at 1174.7 cm^{-1} indicates C-O stretching of an ester whereas the band at 1469 cm^{-1} could indicate C-H bending of unsaturated alkene. ^{1}H-NMR spectrum (Appendix 6) of compound AN1 showed singlet peaks in the range of δ 0.99-1.69 indicating the presence of protons of methyl (-CH_3) and methylene (CH_2) groups that are slightly different in their electronic environment whereas the peak at δ5.20 the presence of at least one proton bonded to C-C double bond. The ^{13}CNMR spectrum (Appendix 7) of compound AN1 showed single peak at δ173.4 indicated quaternary carbon atom (or carbonyl carbon) of an ester**,** a disubstituted double bond at δ121.7 and 139.7 which showed olefinic carbon of pentacyclic triterpinoids. The observed spectral data of compound AN1 are similar to that of compounds such as 3β,28dihydroxyl olean-12-enyl-palmitate (2), 3β,28dihydroxyl-11-oxo olean-12-enyl-palmitate (3) and β-amyrin palmitaet (4) reported in literature (Barreiros *et al*., 2002; Ragasa *et al*., 2011) [6, 34] (Figure 2). Anlysis of ^{1}H-NMR spectrum revealed that there are no peaks around δ3.25-3.60 suggest that the compound AN1 has no OH group at C-28 positions that are commonly observed in 3β,28dihydroxyl olean-12-enyl-palmitate (2) (Nurhamidah *et al*., 2016) [30]. Moreover, absence of negative peak at δ80.5 in the DEPT-135 spectrum also confirmed that there is no OH group at C-28. This rules out the possibility of compound AN1 from being 3β,28dihydroxyl olean-12-enyl-palmitate (2)(Nurhamidah *et al*., 2016; Barreiros *et al*., 2002) [30, 6]. Similarly, there are no peaks in the ^{13}CNMR of of compound AN1 that correspond to δ2002., 128.1 and 170.6 (characteristic peaks of α,β unsaturated ketone) (Zhou *et al*., 2015) [48]. Moreover, absence peaks at δ3.25 and 3.55 in the ^{1}HNMR spectrum of compound AN1 could indicate that it has no OH group at C-28 of CH_2OH). This argument is furthere reinforced by IR spectrum of compound AN1 (Appendix 5) that has no broad band at 3440 cm^{-1}. Thus, it can not be 3β, 28dihydroxyl-11-oxo olean-12-enyl-palmitate (3). Therefore, comparison of spectral (^{13}CNMR, ^{1}HNMR and DEPT-135, Appendices 6-8) data of compound AN1 with data in literatures indicated that it is β-amyrin palmitaet (4) (Nurhamidah *et al*., 2016; Barreiros *et al*., 2002) [30, 6]. Further evidences that support the identification was observation of peak a characteristic peak at δ4.50 (in the ^{1}HNMR spectrum of compound AN1) that indicate presence of a proton a carbon (C-3) bearing an ester functional group, and peak at 80.50 in the ^{13}C-NMR peak of that could be assigned to a carbon that is bonded to C-3 that is bonded to ester (Figure 2). The spectral data of compound AN1 were found to be in good agreement with reported spectral data of β-amyrin palmitaet (4) (Table 2) (Nurhamidah *et al*., 2016; Sultana *et al*., 2018; Barreiros *et al*., 2002) [30, 43, 6]. It has been reported this compound to show antidiabetic potetntial in the experiment conducted on rats (Nair *et al*., 2014; Maurya *et al*., 2012) [22]. This justifies the traditional use of the plant (its diffetent motphological parts) for treatment of diabetes in traditional healers **(**Bekele *et al*., 2015**)** [7]**.**

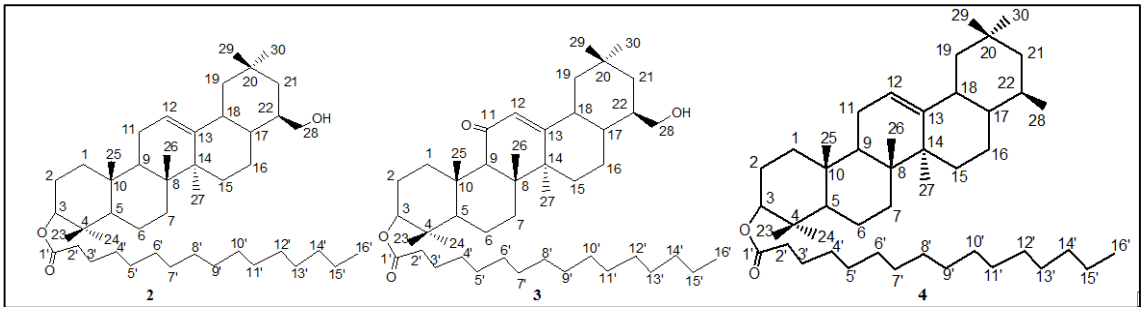
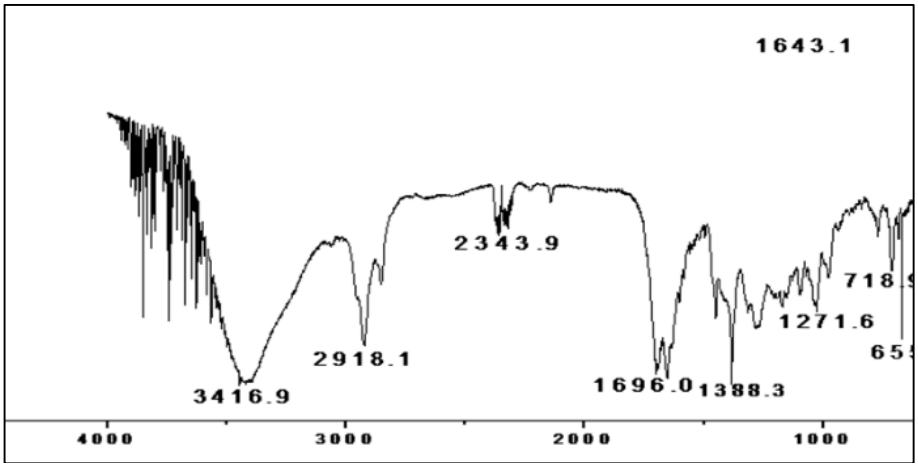


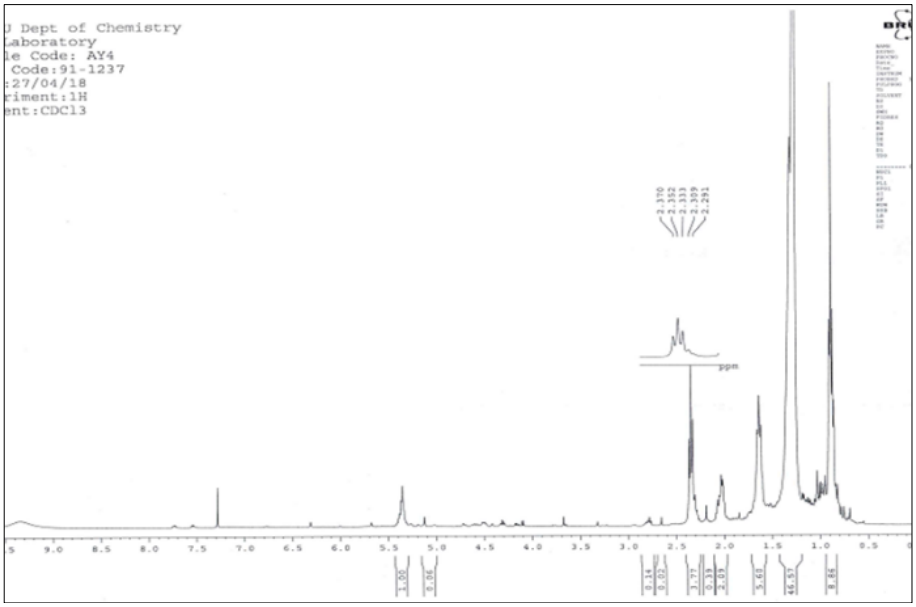
Fig 2: The chemical structures of 3β, 28 dihydroxyl olean-12-enyl-palmitate (2), 3β, 28 dihydroxyl-11-oxo olean-12-enyl-palmitate (3) and β-amyrin palmitaet (4)

Table 4: The ^{1}H NMR and ^{13}C NMR spectra data of compound **AN1** and literature reported data of β-amyrin palmitate

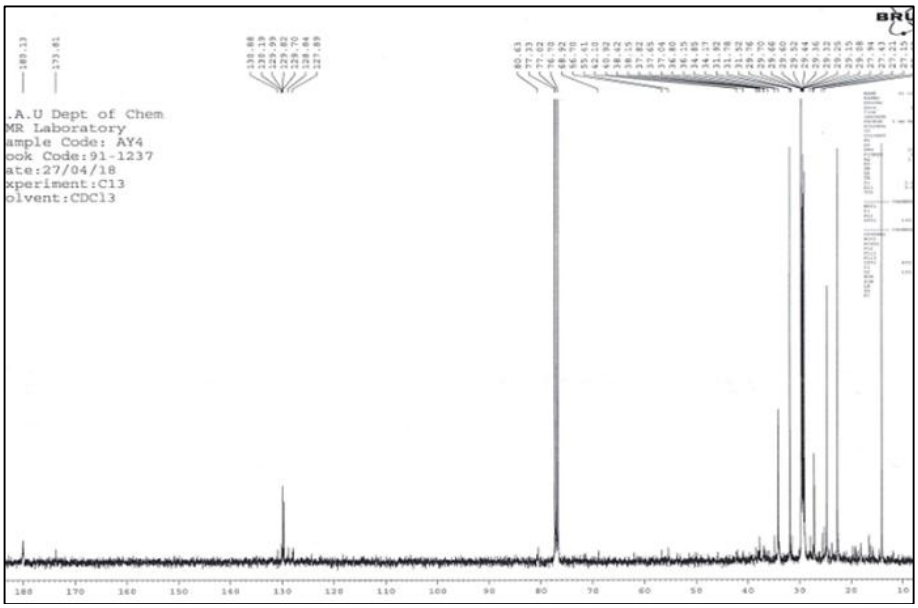
Carbon atom	^{13}C-NMR data of compound AN1 (ppm)	β-amyrin palmitate (Nurhamidah *et al*., 2016)	^{1}H-NMR data of compound AN1(ppm)	β-amyrin palmitate (Nurhamidah *et al*., 2016) [30]	β-amyrin palmitate (Barreiros *et al*., 2002) [6]	3β,28dihydroxy-olean-12-enyl palmitate (Barreiros *et al*., 2002) [6]	3β,28dihydroxy-olean-12-enyl palmitate (Barreiros *et al*., 2002)	Nature of carbon
C-1	31.9	38.45	1.64		38.2	38.1		CH_2
C-2	28.1	23.80	1.64		22.70	23.4		CH_2
C-3	80.5	80.78	4.50 (1H, 3-H)*	4.50 (1H)	80.60	80.4	4.50	C
C-4	39.8	37.96	----		37.7	37.6		C
C-5	47.5	55.44	1.29		55.2	55.1		CH
C-6	18.2	18.46	1.64		18.2	18.1		CH_2
C-7	33.9	32.8	----		32.8	32.4		CH_2
C-8	39.8	40.02	---		39.7	39.7		C
C-9	47.2	47.75	1.10		47.1	47.3		CH
C-10	37.2	37.75	----		36.9	36.8		C
C-11	23.2	23.74	1.64		23.5	23.4		CH_2
C-12	121.7	121.45	5.20 (1H, 12-H)*	5.18 (1H)	121.6	122.1	5.18 (1H)	CH
C-13	139.7	145.51	----		145.0	144.1		C
C-14	42.3	41.93	----		41.6	41.6		C
C-15	31.9	27.11	1.29		26.8	25.4		CH_2
C-16	26.6	26.34	1.29		26.0	25.8		CH_2
C-17	46.7	32.69	---		32.3	31.8		CH
C-18	37.2	47.74	-----		47.8	42.2		CH
C-19	50.3	46.99	1.10		47.4	46.3		CH_2
C-20	29.2	31.28	1.29		31.1	34		C
C-21	42.1	35.07	---		35.6	30.9		CH_2
C-22	36.3	37.34	1.29		37.1	38.1		CH
C-23	22.5	28.59	0.90	0.87	28.4	27.9		CH_3
C-24	16.3	16.96	0.90	0.86	16.6	16.6		CH_3
C-25	16.1	15.74	1.04	0.97	15.6	15.4		CH_3
C-26	18.3	17.01	1.04	0.95	16.8	16.6		CH_3
C-27	26.1	16.15	1.11	1.12	25.9	25.8		CH_3
C-28	28.3	28.26	0.90	0.87	28.0	69.7	3.55, 3.21	CH
C-29	31.1	33.52	0.90	0.87	33.4	33.1		CH_3
C-30	32.4	23.89	0.90	0.86	23.6	23.5		CH_3
1`	173.4	173.90	-----	--	173.6	173.6		C
2’	33.3	34.93	2.30 (2H, 2’-H)*	2.29	29.7			CH_2
3’	29.7	25.38	1.64	1.56,1.25	29.7			CH_2
4’	28.4	4’-13’=29.37-29.90	1.29		29.7			CH_2
5’	25.9		1.29		29.7			CH_2
6’	23.7		1.29		29.7			CH_2
7’	22.5		1.29		29.7			CH_2
8’	22.5		1.29		29.7			CH_2
13’	29.6		1.29		29.7			CH_2
14’	29.7	32.12	1.29	1.25	29.7			
15’	22.7	22.89	1.29	1.25	29.7			CH_2
16’	14.4	14.3	0.90	0.89	14.3			CH_3



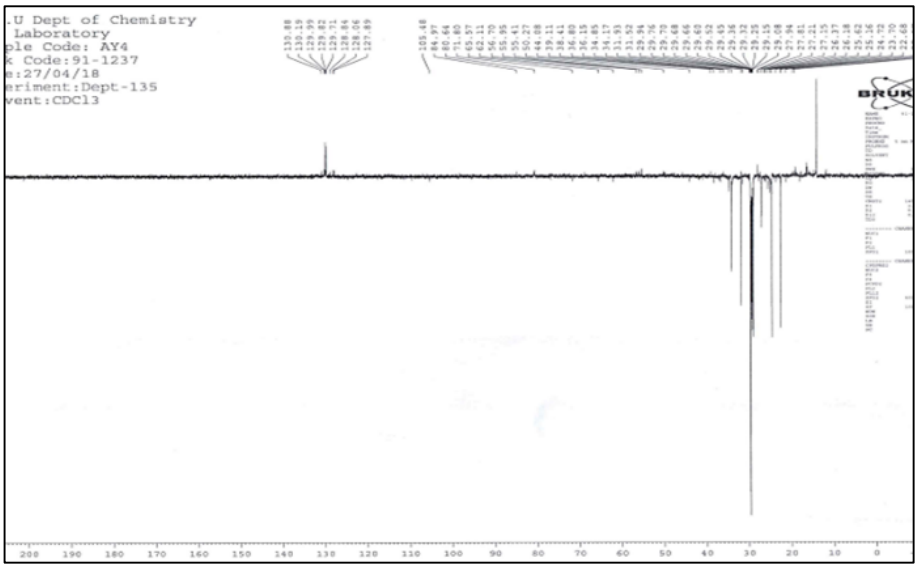
Appendix 1: The FTIR spectrum of compound AY4



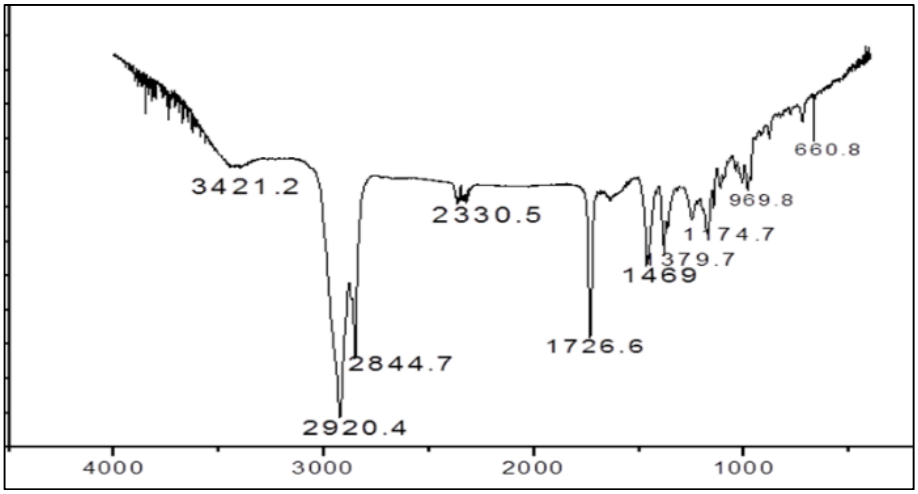
Appendix 2: The [1]HNMR spectrum of compound AY4



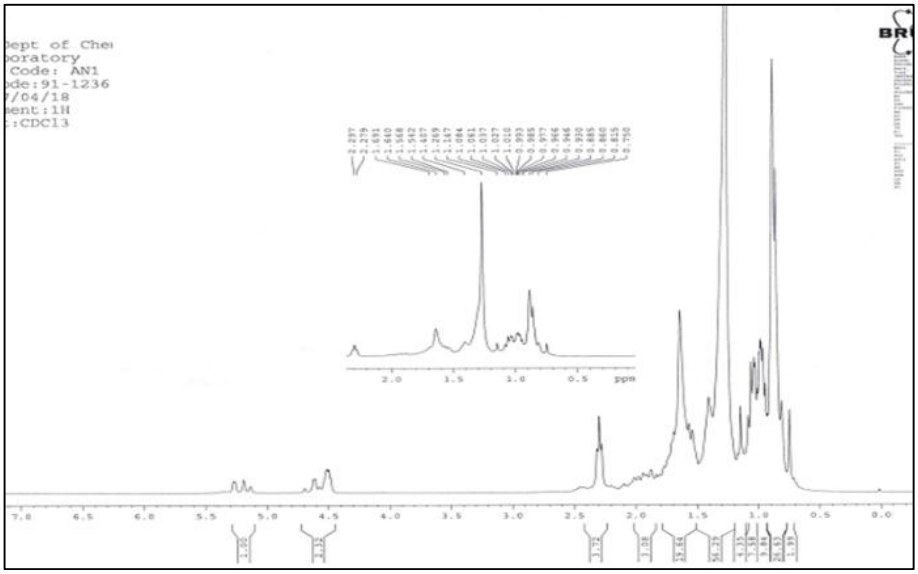
Appendix 3: The [13]CNMR spectrum of of compound AY4



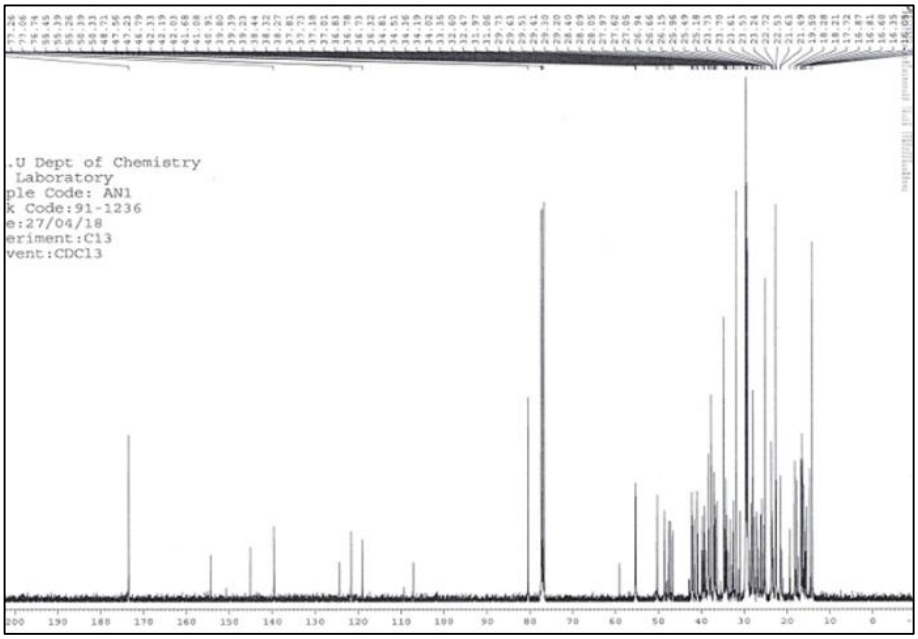
Appendix 4: The DET-135 spectrum of compound AY4



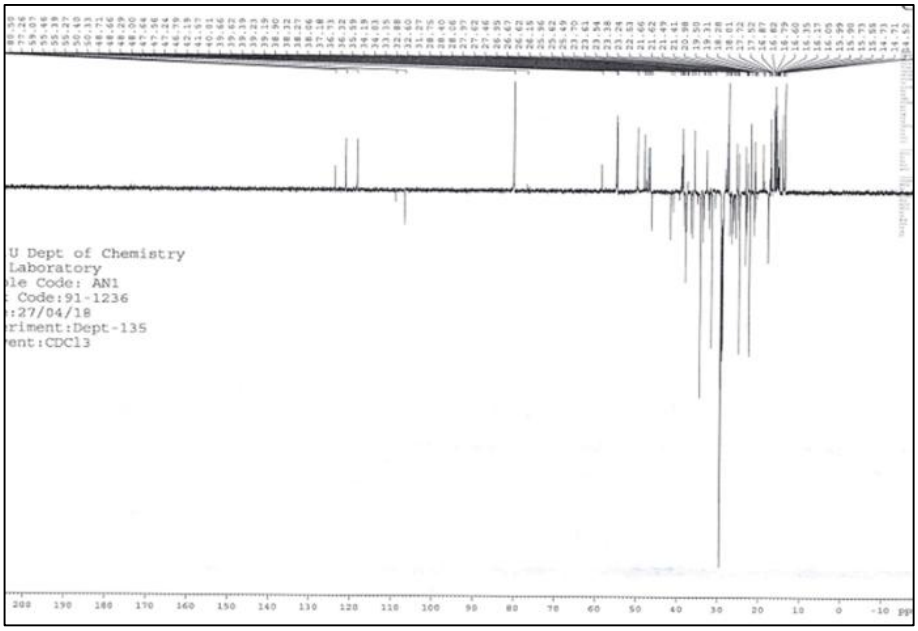
Appendix 5: The FTIR spectrum of compound AN1



Appendix 6: The [1]HNMR spectrum of compound AN1



Appendix 7: The [13]C NMR spectrum of compound AN1



Appendix 8: The DEPT-135 spectrum of compound AN1

4. Conclusion

The preliminary phytochemical studies revealed the seconday metabolites namely Steriods, Terpeniods, Saponins, Flavonoids, Tanins, Alkaloids, Phenol and Glycosides in the extracts of the root. Chromatographic separation led toisolation of two compounds from the dichloromethane: methanol (50:50% by volume) extract. The compounds were long chain fatty acid and fatty acid ester of triterpenoid. Based on the spectral data and comparison with literature reports the two compounds were characterized as oleic acid and β-amyrin palmitate, respectively. This is the first report of isolation of the two compounds from *V. auriculifera*.

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6. References

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