A natural triglyceride from the methanol root extract of *Cyphostemma adenocaule* (Steud. ex A. Rich.) Wild & R.B.Drumm

Abdulbasit Haliru Yakubu[1,2], Iliya Ibrahim[1], Garba Mohammad Tom[1], Abdulqadir Bukar Bababe[1], Hassan Yesufu Braimah[1].

1: Department of Pharmaceutical Chemistry, Faculty of Pharmacy, PMB 1069, University of Maiduguri, Maiduguri, Nigeria.

2: Department of Pharmaceutical Service, PMP 1414, University of Maiduguri Teaching Hospital, Maiduguri, Nigeria.

Corresponding author: pharmahy071@gmail.com

Abstract

Cyphostemma adenocaule (Steud. ex A. Rich.) is one of the specie plant that belongs to the family *vitaceae*. In this study, Trilinolein was isolated and characterized from the methanol root extract of the plant. Column chromatography over silica gel granules as the stationary phase and eluted with a mobile phase mixture of n-Hex-EtA; EtA-CHCL3 and $CHCL_3$-MeOH with gradient increasing polarity, followed by a second column using saphadex-LH20 and 100% MeOH as stationary and mobile phase vehicle respectively. TLC was developed with EtA 15: CHCL3 8: MeOH 4: H_2O 1 as solvent system; sprayed with 10% H_2SO_4 ,Vanillin-sulphuric acid, and/ or Polyethylene glycol PEG and heat for spot detection and confirmation of bioactive principles. Compound CA1 was obtained and purified with CHCL3 to give a yellow semi-solid compound (0.23g). The ^{1}H-NMR spectra showed 9 different signals; a signal peak of a glycerol (-C**H**$_2$OCOR-) moiety on the first α-C chain and on the third α′-C at 4.143-4.187ppm and 4.296-4.325ppm respectively, while that of a β glycerol (-C**H**COR-) at 5.286ppm. Signals of an allylic methylene group at 2.023-2.035ppm, Olefenic hydrogen group at signal peak of 5.362ppm and a diallylic methylene group at signal 2.790ppm were also observed. In the ^{13}C NMR spectra of compound CA1, 57 carbon atoms where observed, multiple signals overlapping at a range of 14.13-34.21ppm corresponding to the aliphatic CH3 (**C18**), CH2 (**C2, C3, C4, C5, C6, C7, C15, C16, and C17**) and allylic (**C8, C14**) carbon atoms. Signals at 127.90-130.24ppm were assigned to the olefienic C atoms (**C9, C10, C12**, and **C13**) while signal of 172.87ppm and 173.32ppm were assigned to the carbonyl (**C**=O) carbon atoms (**C1** and **C2**) respectively (Table 2).

Analysis with DEPT-135, H-H COSY, HMBC and HSQC assignments of CA1 augments assignment of signals made for CA1 from ^{1}H-NMR and ^{13}C-NMR and corresponded to that of Trilinolein ($C_{57}H_{98}O_6$, MW 879.4 g/mol). The isolated compound was positive for the acrolein test for triglycerides; fat & oil and had an IC_{50} of 46.08µg/ml radical scavenging activity.

Key words: Cyphostemma adenacaule, NMR, Trilinolein

Introduction

Plants had been constantly explored for their bioactive principles known as secondary metabolites. This principles display a wide range of Biological activity and present novel entities for synthesis, combinatorial and chemoinformatics applications in drug design, discovery and development.

The *vitaceae* family is one of the explored plant family and are known for their potent bioactive principles with diverse chemical structures (Murias *et al*., 2005; Piotrowska *et al*., 2012; Rivière *et al*., 2012 and Shen et al., 2013). *Cyphostemma adenocaule* (Steud. ex A. Rich.) Wild & R.B.Drumm. is a wild climbing plant widespread in West Africa countries, from Senegal east to Eritrea and south to Angola, Malawi and Mozambique (Bukill, 1985). Bello and colleagues gave a review on the plant together with its ethnomedicinal, phytochemistry and pharmacological properties (Bello et al., 2019). In this study, we report the isolation and characterization of a natural triglyceride; Trilinolein from the methanol root extract of *C. adenocaule* using standard chromatographic and spectroscopic technique.

Fig 1: *Cyphostemma adenocule* whole plant. (Bello *et al*., 2019)

Fig. 2. *C. adenocaule* vegetative distribution in Africa. (Bello *et al*., 2019)

METHOD

Plant Collection and Identification

Fresh root parts of *C. adenocaule* was collected aseptically in July, 2017 from Shuwarin town, Dutse LGA, Jigawa State, Nigeria, and identified by a Medicinal Botanist (Mal Namadi Sanusi) of the Biology department, ABU Zaria, Nigeria.

Preparation of Plant Extract and Its Fractions

The root parts was size reduce into small pieces and shadow-dried for 14 days under room temperature, then grinded into fine powder using a grinding machine. Half kilograms (0.5kg) of the pulverized sample material was extracted and defatted with Hexane, followed by Methanol using maceration method (Banu and Cathrine, 2015). The maceration method was repeated three times after every 24hrs. The mixture was filtered and allowed to dry under room temperature to obtain the crude extract. The dry methanol extract was weighed, labeled and stored in a dessicator, and used further analysis.

Column Chromatography

General column chromatographic technique was employed. The column was placed on a retort stand in a vertical position and a plug of cotton wool was pushed down to the bottom of the column. Silica gel slurry was prepared with methanol and packed (wet packing) gently in to the column (this serves as the stationary phase). Dried silica 1/3 of the extract weight was mixed

with the extract and applied on top of the column. The Eluting solvents (mobile phase) was applied from top through the column, starting with the less polar solvent (Hexane) that elute less polar compounds to the most polar solvent (methanol) which elute polar compounds and also in ratio combination of the two. The component of the mixture run down the column forming different layers. The mobile phase will drip down by gravity, and different components in the mixture having different interactions with the stationary and mobile phases at varying degrees was clearly separated. The solvents from collected fractions (25mls each) was allowed to dry off in a fume cupboard. The column fractions were then be analyzed using TLC to determine quantitatively the chemical composition of each fraction collected. Fractions that show similar chemical composition on TLC plate were combined together (pooled) and subjected to further separation.The separated components were collected sequentially and carefully labeled for further analysis. Where applicable, pooled fractions that showed promising results from TLC analysis, were subjected further in to a second column. In this case, Saphadex LH-20 was employed as the stationary phase and 100% methanol as the mobile phase. Eluded fractions obtained as above, were run on TLC for analysis.

Thin Layer Chromatography

Precoated aluminium sheets (plates) of silica gel were used for TLC analysis. The plates are measured from bottom (baseline) and lines drawn across the plate with a pencil at 1.5cm. Micro capillary tube were used to spot the sample solutions on the baseline. Development of the TLC plate was done inside a chromatographic tank. Various developing solvents were employed e.g Ethyl acetate(15): chloroform(8): Methanol(4): water(1), 100% chloroform, Ethyl acetate(4):Chloroform(2):Formic acid(0.5). The tank was left undisturbed for the TLC to develop. The plate was removed from the chamber when the solvent reaches the front line and allowed to dry and visualized.

Spot detection and visualization

The developed TLC plate were air dried (and heated mildly) and viewed under UV at 254nm and 366nm wavelengths respectively (Wagner and Bladt, 1996).

Spray reagents

Vanillin-sulphuric acid, 10% sulphuric acid and Polyethylene glycol PEG were employed for spot detection and confirmation of bioactive principles (Wagner and Bladt, 1996). Dipping plates into Ammonia fume and heat plate was also be applied.

NMR Analysis

The ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker AV400 spectrophotometer using $CDCl_3$ as solvent and TMS as internal standard. The 1H-NMR had acquisition parameter of; spectral width 8013Hz, number of scans 16, acquisition time of 4.09sec, FID resolution of 0.24Hz and temperature 294.8K, while that of 13C-NMR; spectral width 24038.461 Hz, acquisition time 1.363sec and temperature of 296.4K.

Two dimensional (2D) NMR measurements COSY, HSQC and HMBC, as well as DEPT-135 were similarly used to elucidate the structure of the compound. Both 1D and 2D NMR were run at the NMR unit of the Faculty Pharmacy, Mansoura University, Mansoura, Egypt.

DPPH antioxidant assay

The DPPH method of evaluating free radical scavenging activity described by Brand et al., (1995) was adopted. The percentage antioxidant inhibition (AI) was obtained by the equation:

$$\%\ \mathrm{AI} = \mathrm{Control\ (Abs)} - \mathrm{Sample\ (Abs)} \div \mathrm{Control\ (Abs)} \times 100$$

Inhibition curves were made and regression analysis was deployed to calculate and obtain the IC_{50} from the regression equation using Excel 2016.

RESULTS

The resulting methanol extract was subjected to column chromatography over silica gel granules as the stationary phase and eluted with a mobile phase mixture of n-Hex-EtA; EtA-CHCL3 and CHCL3-MeOH with gradient increasing polarity. A total of 30 fractions of 25mls each was collected, which were combined on the basis of TLC analysis to 5 fraction A-E developed on EtA 15: CHCL3 8: MeOH 4: H_2O 1 as solvent system and sprayed with 10% H_2SO_4 and heat.

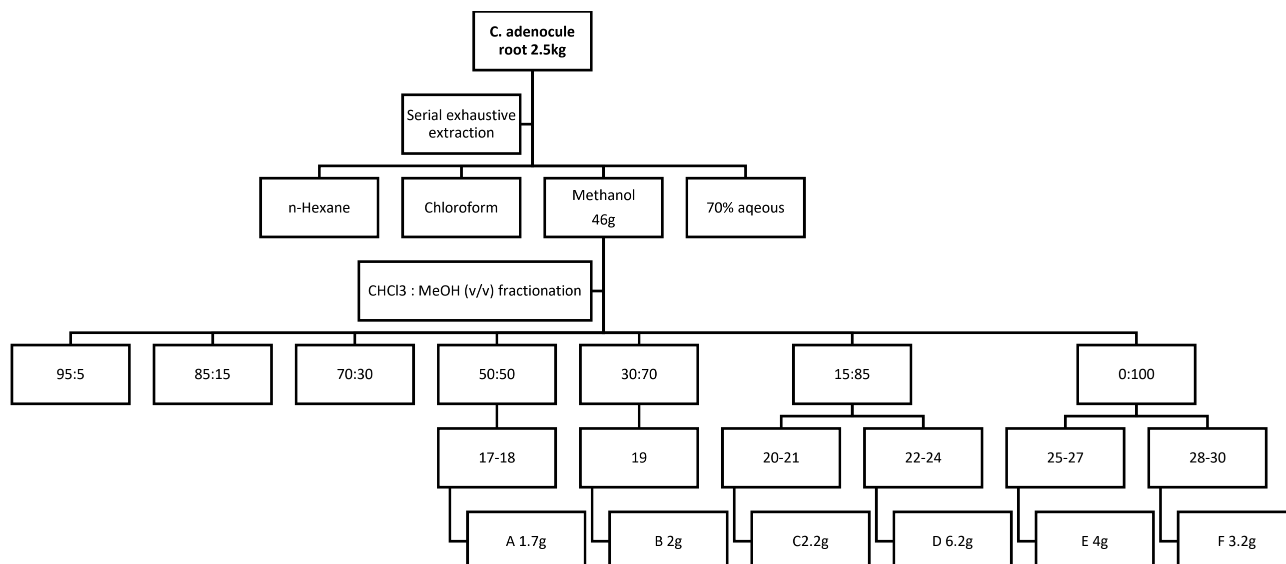

Fig 3: Schematic representation of fractionation of methanol extracts of *C. adenocaule* (silica)

Fractions B, C and D where combined and re-subjected to column chromatography with saphadex LH-20 as the stationary phase and 100% MeOH as mobile phase (fig 4). 15 fractions of 5mls were collected which showed a distinct two spot; virtually in all fractions upon TLC analysis; sprayed with Gibbs reagent and dipped in NH_3 vapour. The fractions were pulled and developed on a preparative TLC developed with CHCL35: EtA 4: FA 1. The two spots where collected and labelled CA1 and CA2. CA1 was purified with CHCL3 to give a yellow semi-solid compound (0.23g).

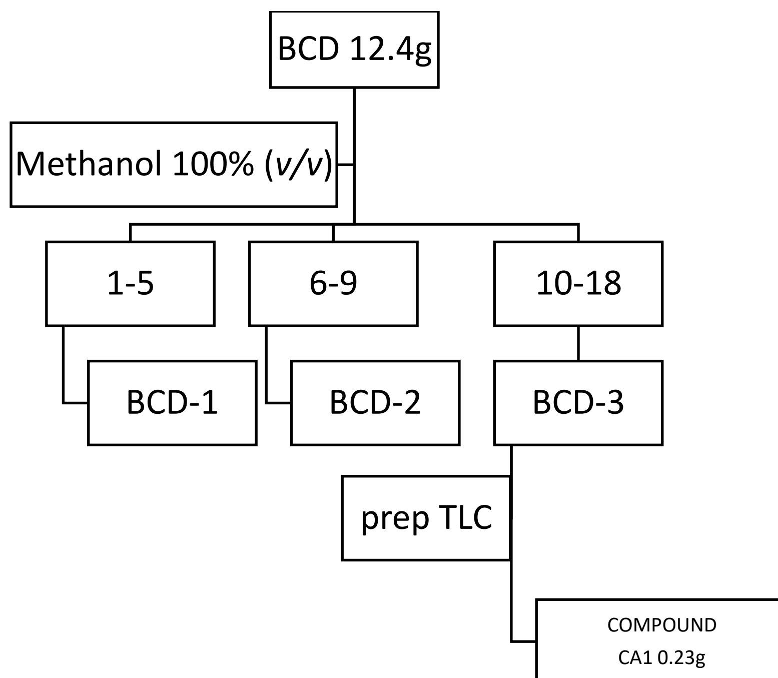

Fig 4: Schematic fractionation of fraction BCD on Saphadex LH-20

Characterization of isolated compound CA1

The 1H-NMR spectra and assignments of CA1 is given below.

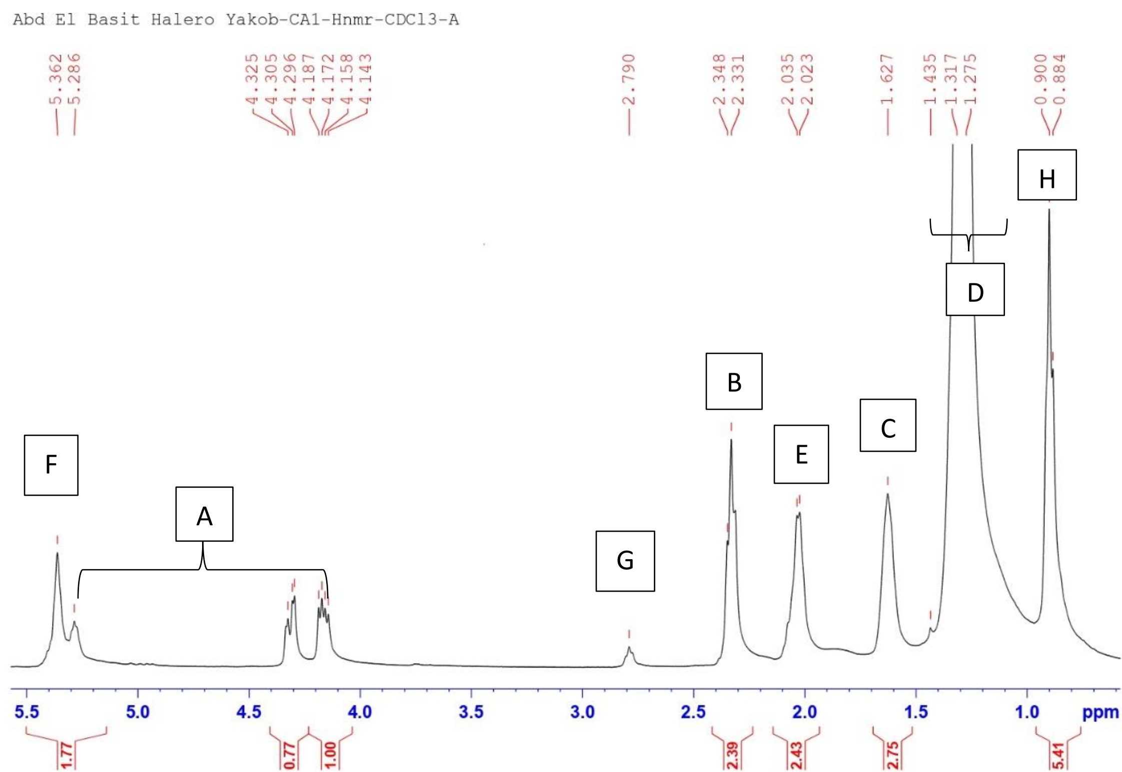

Fig 5: ^{1}H-NMR spectra of compound CA1

Table1: ${}^{1}H$-NMR assignment for compound CA1

Signal	Position	Chemical shift (ppm)	Reference	Proton
A	**1** **1'** **1''**	4.143-4.1879(m) 4.296-4.325 (dd) 5.286 (m)	4.095[a] 4.340[a] 5.343[a]	-C**H2**OCOR -C**H2** OCOR -C**H**OCOR
B	**2**	2.331-2.348 (t)	2.34[b]	-O-CO-C**H**2-CH2
C	**3**	1.627 (m)	1.65[b]	-O-CO- CH2- C**H**2
D	**4,5,6,7** **15,16,17**	1.275-1.317 (m*) 1.435 (m*)	1.19-1.42[c] 1.19-1.42[c]	-(C**H2**)n- -(C**H2**)n-
E	**8,14**	2.023-2.035 (m*)	2.08[b]	- C**H2**-CH=CH
F	**9,10,12,13**	5.362 (m)	5.38[b]	-C**H**=C**H**-
G	**11**	2.790 (t)	2.80[b]	-CH=CH-C**H2**-CH=CH
H	**18**	0.88-0.900 (t)	0.88[b]	-C**H**3

Key:

dd: double doublet

m: multiplet

m*: multiplet with overlapping signal

t: triplet

a: Xi *et al*., 2016.

b: Dos Santos *et al*., 2017.

c: Nieva-Echevarría *et al*., 2014.

The ^{13}C NMR spectra of compound CA1 (fig 6) and Assignments of Carbon atom position of compound CA1, together with a literature assignment (Alemany, 2002) is given in Table 2

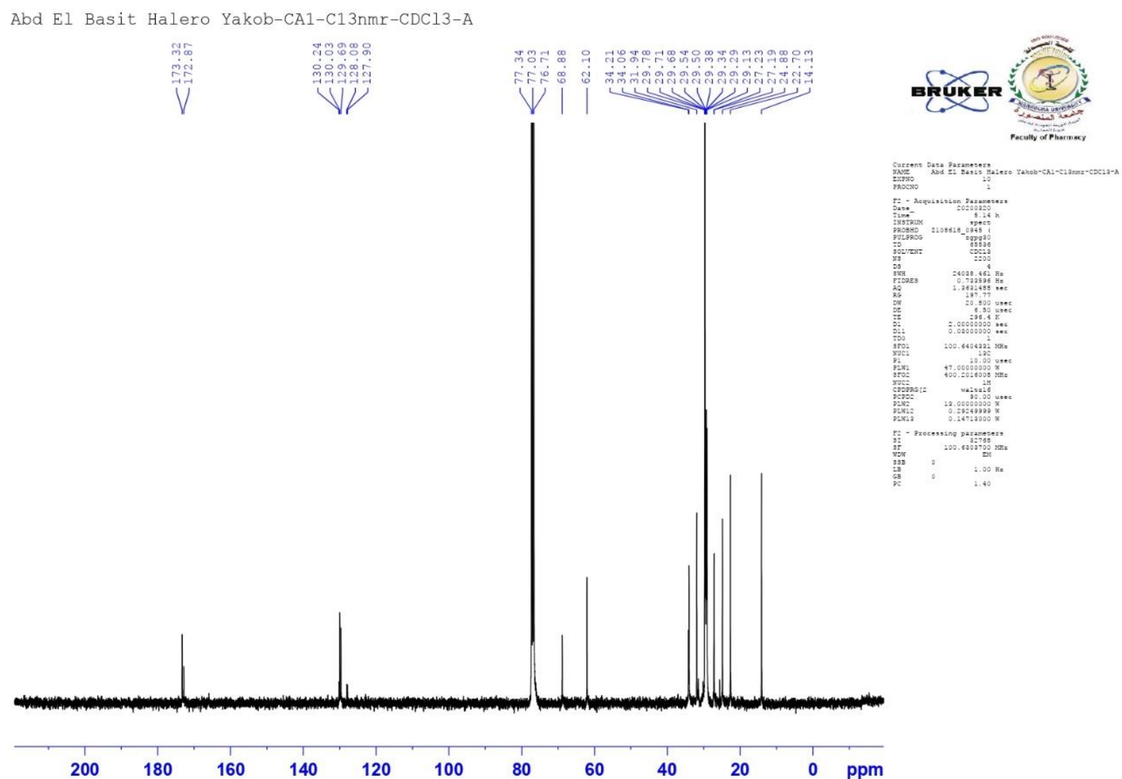

Fig 6: ^{13}C-NMR spectra of compound CA1

Table 2: ^{13}C-NMR experimental values and assignment for compound CA1 in position1, 3 (Sn1, 3) and 2 (Sn2).

Position		**CA1**	**Ref**	**Position**		**CA1**	**Ref**
C1	Sn1,3	173.32	173.23	**C11**	Sn1,3	29.71	25.64
	2	172.87	172.82		2	29.78	25.64
C2	Sn1,3	34.06	34.03	**C12**	Sn1,3	127.90	127.91
	2	34.21	34.19		2	127.90	127.90
C3	Sn1,3	24.88	24.85	**C13**	Sn1,3	130.24	130.20
	2	24.88	24.89		2	130.24	130.21
C4	Sn1,3	29.10	29.09	**C14**	Sn1,3	27.19	27.21
	2	29.04	29.05		2	27.19	27.21
C5	Sn1,3	29.29	29.19	**C15**	Sn1,3	29.38	29.36
	2	29.34	29.21		2	29.38	29.36
C6	Sn1,3	29.14	29.13	**C16**	Sn1,3	31.94	31.54
	2	29.20	29.14		2	31.94	31.54
C7	Sn1,3	29.68	29.62	**C17**	Sn1,3	22.70	22.59
	2	29.65	29.63		2	22.70	22.59
C8	Sn1,3	27.23	27.20	**C18**	Sn1,3	14.13	14.08
	2	27.23	27.20		2	14.13	14.08
C9	Sn1,3	130.03	129.99	**Glycerol CHO (1'')**		68.88	62.91
	2	129.69	129.97	**Glycerol CH_2O 1, 1'**		62.10	62.11
C10	S1,3	128.08	128.08				
	2	128.08	128.10				

Key: Sn: strictly numbered

Ref: Alemany, 2002

Abd El Basit Halero Yakob-CA1-Dept 135-CDCl3-A

Fig 7: DEPTH-135 spectra of compound CA1

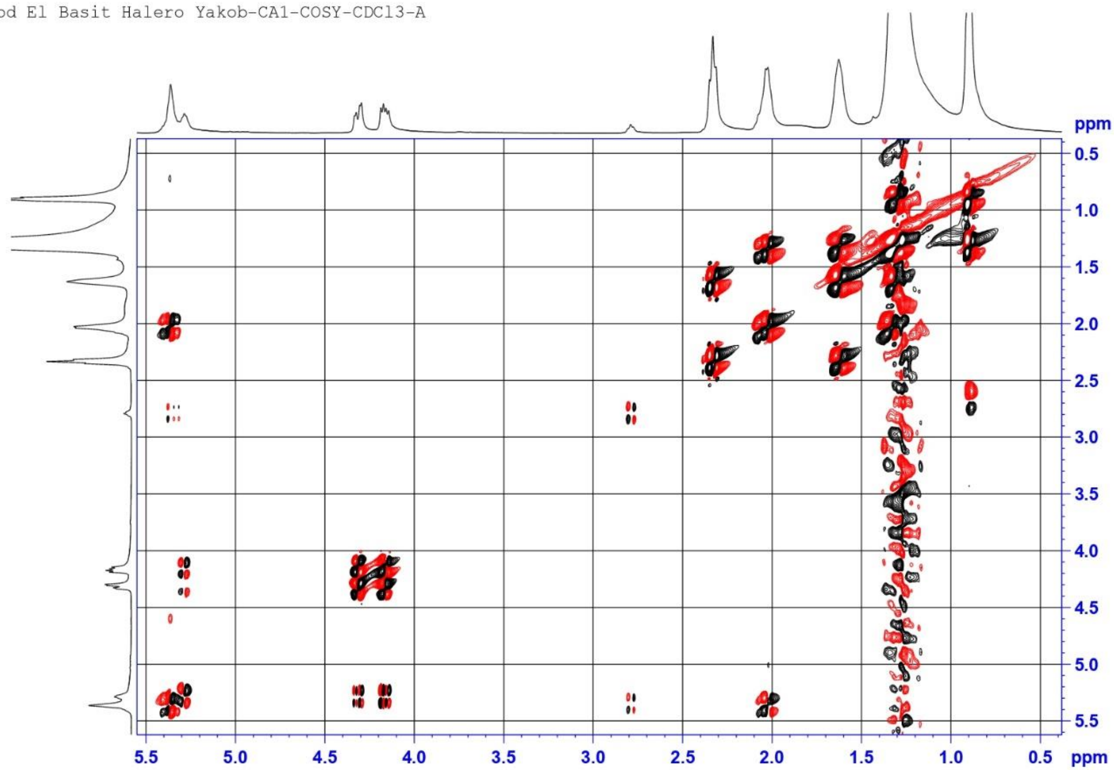

Fig 8: HCOSY spectra of compound CA1

Abd El Basit Halero Yakob-CA1-HMBC-CDCl3-A

Fig 9: HMBC spectra of compound CA1

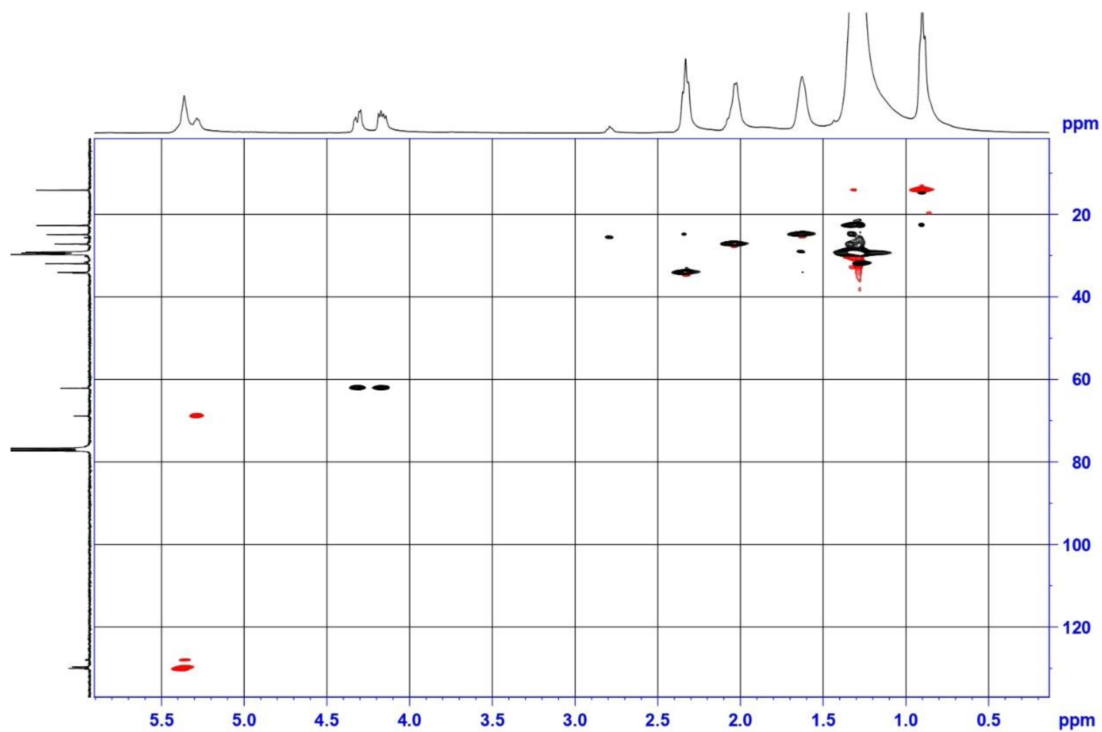

Fig 10: HSQC spectra of compound CA1

Table 3: Chemical Test on the Isolated Compound CA1

Isolated Compound	Test	Result	Inference
CA1	Acrolein ($KHSO_4$) test	+	Triglyceride
	Solubility (CHCL3) test	+	Fat & oil

Key: + = present.

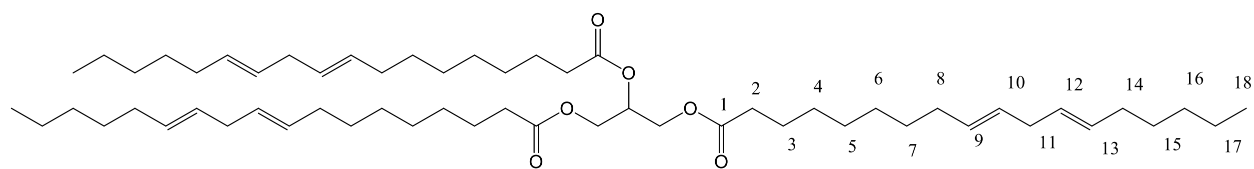

Fig 11: chemical structure of Compound CA1

Table 4: *Invitro* antioxidant activity (DPPH assay) of *C. adenocaule* extract and compound CA1.

Concentration	MeOH	CA1
6.25	32.8	30.9
12.5	55.2	44.09
25	67.2	58.35
50	79.3	67.88
100	97.2	88.55
IC_{50}	10.87	46.08

In the application of chromatographic procedures in the analysis of the MeOH root extract of *C. adenocaule*, this study buttress the use of silica and Saphadex-LH20 as suitable stationary phase for column chromatography as evident in other studies. Also, solvent system, EtA 15: CHCL3 8: MeOH 4: H_2O 1 offers a good option for TLC analysis whereas, 10% H2SO4, Gibbs reagent, NH4 vapour and heat application to be considered for visualization of TLC analysis for MeOH root extract of *C. adenocaule*.

Nuclear magnetic resonance spectroscopy is used for the study of the interaction of radio frequency (RF) of the electromagnetic radiation with unpaired nuclear spins in an external magnetic field to extract structural information about a given sample (Hailemichael, 2005). It is used to study chemical structure of simple molecules as well as more complicated molecules using one dimensional technique (1D-NMR) and two dimensional techniques (2D-NMR) respectively (Hailemichael, 2005, Abraham *et al*., 1988).

In the ^{1}H NMR spectra of compound CA1; the assignments of the signals were made taking into consideration previous published literatures. The spectra showed 9 different signals; signals A-H (fig5). Band A reflects a signal peak of a glycerol (-C**H**$_2$OCOR-) moiety on the first α-C chain at 4.143-4.187ppm (1H; **H-1**) and another on the third α′-C at 4.296-4.325ppm (1H; **H-1'**) while Band B showed a signal peak of a β glycerol (-C**H**COR-) at 5.286ppm (1H; **H-1''**). A peak of methylene group α to the acyl group at 2.331-2.348ppm (1H, **H-2**) is observed in band C, while at band D, signal peak was identified as a methylene group β to the acyl group at 1.627ppm (1H; **H-3**). Band E gives signal peak of an acyl group at 1.275-1.317ppm (1H; **H-4, H-5, H-6, H-7, H-15, H-16,** and **H-17**). Band F signals that of an allylic methylene group at 2.023-2.035ppm (2H, **H-8** and **H-14**) while, Olefenic hydrogen group at signal peak of 5.362ppm (2H, **H-9, H-10, H-12,** and **H-13**) was identified with band G. Also identified, a diallylic methylene group at signal 2.790ppm (1H; **H-11**) and a methyl group at signal of 0.88-0.900ppm (1H; **H-18**) corresponding to band H and I respectively (Table 1). To verify the ^{1}H-NMR assignments of CA1 above; a H-H correlation spectroscopy (COSY) experiment was performed (fig 8), which further buttress the assignments made earlier from the ^{1}H NMR.

In the ^{13}C NMR spectra of compound CA1, 57 carbon atoms where observed (fig 6). As expected, multiple signals overlapping at high field are seen, and at a range of 14.13-34.21ppm; corresponding to the aliphatic CH3 (**C18**), CH2 (**C2, C3, C4, C5, C6, C7, C15, C16, and C17**) and allylic (**C8, C14**) carbon atoms. Diallylic CH (**C11**) signals was identified at 29.71-

29.78ppm. Signals at 62.10ppm and 68.88ppm were assigned to the glycerol **C**H2 and **C**H triester C atoms respectively. Signals at 127.90-130.24ppm were assigned to the olefienic C atoms (**C9, C10, C12**, and **C13**) while signal of 172.87ppm and 173.32ppm were assigned to the carbonyl (**C**=O) carbon atoms (**C1** and **C2**) respectively (Table 2).

The DEPT-135 experiment revealed the presence of 1 terminal methylene C (total=3) 1 diallylic C (total=3), 2 allylic C (total= 6), 4 vinylic C (total= 12) and 3 triester C atoms (fig 7).

The ^{1}H-^{13}C Heteronuclear Multiple Bond Correlation Spectroscopy (HMBC); which shows the correlation between H and C separated by multiple bond. The HMBC of CA1 revealed correlation of H-11 with C9, C10, C12 and C13. There's also a correlation of C1 and C2 to the protons of α glycerol (H-1 and H-1'), likewise a correlation of C1 and C2 with H-2 and H-3 (fig 9). This buttress the assignments made based on 1H and COSY spectrum.

Further analysis was made using the 1H-13C Heteronuclear Single Quantum Coherence Spectroscopy (HSQC); which shows which H are directly attached to which C atom. The HSQC analysis of CA1 (fig 10) augments the previous assignments of signals made for CA1 from the results of 1H & 13C NMR, DEPT-135 and COSY.

Analysis of the NMR, DEPT 135, H-H COSY, HMBC and HSQC assignments of CA1 corresponded to that of Trilinolein (fig 11). The signals assignments are in range and agrees with previous literatures for characterization of Trilinolein (Ken Jie and Lam, 1995, Mannina *et al*., 1999, Mannina 2000, Alemany 2002, Mckenzie and Koch, 2004, Nieva-Echevarría *et al*., 2014 and Xia *et al*., 2016). According to literature, the first assignment of all 13C for Trilinolein was in 1995 (Alemeny 2002). However, assigning signals for Trilinolien was difficult especially for the C5 and C6 in a 2:1; which are separated by 0.06ppm and that of C8 and C14 signals; separated by only 0.01ppm, thus making it difficult to assign positions and leading to disarray in experimental values even though working under similar operating condition. (Alemeny 2002). The difference in the signals assignment of this study and that of the quoted literature might be due to difference in the operating parameters & solvent used in the NMR analysis and purity of test compound.

Further chemical test to identify the chemical class of compound CA1 was done. When subjected to the Acrolein test; where a pungent irritating odour occurs when fat and oil are heated with potassium bisulphide $KHSO_4$; indicating the presence of triglyceride (Table 3).

A query search of Trilinolein in the chemical repository; PubChem revealed its properties. Trilinolein is a Glyceryl trilinoleate, with a chemical formula of $C_{57}H_{98}O_6$, MW 879.4 g/mol and IUPAC name of 2,3-bis[[(9Z,12*Z*)-octadeca-9,12-dienoyl]oxy]propyl (9Z,12*Z*)-octadeca-9,12-dienoate (www. https://pubchem.ncbi.nlm.nih.gov/compound/5322095#section=Names-and-Identifiers).

In this study, the MeOH extract showed potential scavenging activity (10.87µg/ml) compared to the isolated compound showed weak radical scavenging activity (IC_{50} 46.08µg/ml). Feyisao and colleagues, reported a DPPH scavenging activity of the ethanol root extract of *Cissus adenocule* (IC_{50} 38.42µg/ml) (Feyisayo *et al*., 2015).

Chang and colleagues demonstrated Trilinolein as a potent antioxidant agent where it alters the activity of superoxide dismutase in a model to investigate its myocardial protective properties *in vivo* (Chan *et al*., 1997). In another study using enhanced Chemiluminescence, which is used to measure OFR (Oxgen-derived Free radicals), trilinolein showed a dose-dependent potent antioxidant activity with a maximal mean reduction of OFR of -48.0% (chan *et al*., 1996). The disarray in the comperative results of the antioxidant activity of Trilinolien in this study and those reported above, might be due to the difference and sensitivity of the test compound to the two models; method employed in this study is an *invitro* assay while the others are *invivo.* Other biological activity of Trilinolein include, inhibition of platelet aggregation, Nitric oxide mediated effects, reducing thrombogenecity, increasing erythrocyte deformability, anti-ischemic, antiarrythmatic (Chan and Tomlinson, 2000). In a non-small cell lung carcinoma (NSCLC) study, trilinolein showed ability in growth inhibition and induction of apoptosis via the Bcl-2 family and Capase-3, which are associated with cytochrome C release and dephosphorylation on Akt signaling pathway (Chou *et al*., 2011).

CONCLUSION

The present study undertook the isolation and characterization of trilinolein from the methanol root extract of *C. adenocaule*, as well as, determination of its antioxidant activity *invitro*. Utilization of chromatographic techniques and plant drug analysis, coupled with NMR spectroscopy, identified Trilinolein as one of the compounds present in the root of *C. adenocaule.* The DPPH antioxidant assay revealed the free radical scavenging activity; MeOH extract with an IC_{50} of 10.87µg/ml while compound CA1 expressed 46.08µg/ml.

The results from this study adds to the existing literature on *C. adenocaule* and also reports for the first time, the presence and identification of a natural triglyceride, Trilinolein from the plant.

REFERENCE

Abraham, R. J., Fisher, J., Loftus, P. (1988). Introduction to NMR spectroscopy. John Wiley and Sons, In: Garba, M. T. (2014). Development of Herbal Medicinal Products in Nigeria. 1sted. Pyla-mak publishers Ltd. pp 109-111.

Banu, K. (2015). General Techniques Involved in Phytochemical Analysis. *International Journal of Advanced Research in Chemical Science, 2*, 25-32.

Bello, O.M., Jagaba, S.M., Bello, O.E , Ogbesejana, A.B , Dada, O.A , Adetunji, C.O, and Abubakar, S.A. (2019). Phytochemistry, pharmacology and perceived health uses of non-cultivated vegetable *Cyphostemma adenocaule* (Steud. ex A. Rich.) Desc. ex Wild and R.B. Drumm: A review. *Scientific African* 2 e00053.

Brand W. W., Cuvelier, M. E., Berset C., (1995). Use of a Free Radical Method to Evaluate Antioxidant Activity. *Lebensmittel-Wissenschaft und-Technologie/Food Science and Technology*. 28: 25-30.

Burkill H.M. (1985). *The Useful Plants of West Tropical Africa.* Vol 5, Families S–Z, Addenda Kew, United Kingdom, Royal Botanic Gardens, Richmond.

Carvalho Dos Santos, C, R., Alves de Melo Filho, A., Alves Chagas, E., Takahashi, J.A., Fernández Montero, I., Chaves Holanda, L., Estevam Ribeiro, P.R., Franco dos Santos, G., Gonçalves Reis de Melo, A.C. (2017). Chemical Characterization of Oils and Fats from Amazonian Fruits by 1H NMR. *Preprints*, 2017050078 (doi: 10.20944/preprints201705.0078.v1).

Chan, P., Cheng, J.T, Tsao, C.W., Niu, C.S., Hong, C.Y. (1996). The in vitro antioxidant activity of trilinolein and other lipid-related natural substances as measured by enhanced chemiluminescence. *Life Sci*. 59(24):2067-73. doi: 10.1016/s0024-3205(96)00560-7. PMID: 8950309.

Chan, P., Hong, C.Y., Tomlinson, B., Chang, N.C., Chen, J.P., Lee, S.T, Cheng, J.T. (1997). Myocardial protective effect of trilinolein: an antioxidant isolated from the medicinal

plant *Panax pseudoginseng*. *Life Sci.* 61(20):1999-2006. doi: 10.1016/s0024-3205(97)00858-8. PMID: 9366507.

Chan, P., and Tomlinson, B. (2000). Antioxidant effects of Chinese traditional medicine: focus on trilinolein isolated from the Chinese herb sanchi (*Panax pseudoginseng*). *J Clin Pharmacol.* 40(5):457-61. doi: 10.1177/00912700022009215. PMID: 10806597.

Chou, P.Y., Huang, G.J., Pan, C.H., Chien, Y.C, Chen, Y.Y., Wu, C.H., Sheu, M.J., Cheng, H.C. (2011). Trilinolein inhibits proliferation of human non-small cell lung carcinoma A549 through the modulation of PI3K/Akt pathway. *Am J Chin Med*. 39(4):803-15. doi:10.1142/S0192415X11009214. PMID: 21721158.

Feyisayo, A.K., Oluwafemi, A.V., and Oluokun, O.O. (2015). Evaluation of antioxidant capacity and membrane stabilizing potential of stem and root of *Cyphospenna adenocaulis* (Steud). *Africa J. Biotechnol.* 14 (21) 1820–1827. doi: 10.5897/AJB2015.14490 .

Hailemichael, T. (2005). Isolation and structure Elucidation of Natural Products from plants. Ph.D. Thesis, Institute of Organic Chemistry, University of Hamburg. pp. 20-29

Halliwell, B. and Gutteridge, J.M.C. (2006). *Free Radicals in Biology and Medicine.* (4 ed). Oxford; Clarendon Press.

.

Ken Jie, M.L. and Lam, C.C. (1995). 13C-Nuclear magnetic resonance spectroscopic studies of triacylglycerols of type AAA containing (Z)- and (E)-monoethylenic acyl groups. *Chemistry and Physics of Lipids*, 78(1),15-27. https://doi.org/10.1016/0009-3084(95)02479-3.

Mannina, L., Luchinat, C., Emanuele, M.C, and Segre, A. (1999). Acyl positional distribution of glycerol tri-esters in vegetable oils: a 13C NMR study. *Chemistry and Physics of Lipids*, 103 (1–2), 47-55. https://doi.org/10.1016/S0009-3084(99)00092-4.

Mannina, L. Luchinat, C., and Maurizio, P., & Maria, E., Enrico, R., Annalaura, S. (2000). Concentration dependence of 13C NMR spectra of triglycerides: Implications for the NMR analysis of olive oils. *Magnetic Resonance in Chemistry.* 38. 886 - 890. 10.1002/1097-458X(200010)38:10<886::AID-MRC738>3.0.CO;2-J.

McKenzie, J.M. and Koch, K.R. (2004). South African Journal of Science - Rapid analysis of major components and potential authentication of South African olive oils by

quantitative ^{13}C nuclear magnetic resonance spectroscopy. *South African Journal of Science*, 100 (7-8), 349 – 354

Murias, M., Jäger, W., Handler, N., Erker, T., Horvath, Z., Szekeres, T., Nohl, H., and Gille, L. (2005). Antioxidant, prooxidant and cytotoxic activity of hydroxylated resveratrol analogues: Structure-activity relationship. *Biochem. Pharmacol.*, 69, 903–912.

Newman, D.J., Cragg, G.M. and Snader, K.M. (2003). Natural Products as Sources of New Drugs over the Period 1981–2002. *J. Nat. Prod.*, *66*, 1022–1037.

Nieva-Echevarría, B., Goicoechea, E., Manzanos, M.J., Guillén, M.D. (2014). A method based on 1H NMR spectral data useful to evaluate the hydrolysis level in complex lipid mixtures. *Food Research International*,Volume 66,379-387https://doi.org/10.1016/j.foodres.2014.09.031.

Piotrowska, H., Kucinska, M., and Murias, M. (2012). Biological activity of piceatannol: Leaving the shadow of resveratrol. *Mutat. Res.*, 750, 60–82.

.

Rivière, C., Pawlus, A. D., and Mérillon, J. M. (2012). Natural stilbenoids: Distribution in the plant kingdom and chemotaxonomic interest in *vitaceae*. *Natural Product Rep*, 29, 1317–1333.

Shen, T., Xie, C.F., Wang, X.N., & Lou, H.X. (2013). Stilbenoids. *Natural Products, 1901–1949.* doi:10.1007/978-3-642-22144-6_63

Wagner, H., & Bladt, S. (1996). *Plant drug analysis: A thin layer chromatography atlas*. (6th ed.) Berlin: Springer.

Xia S. M. Budge and Mike D. Lumsden (2016). ^{1}H-NMR Characterization of Epoxides Derived from Polyunsaturated Fatty Acids. *Journal of the American Oil Chemist's Society* 9(4) 467-478.

Xiao, K, Zhang, H, Xuan L, Zhang, J, Xu Y, and Bai D. (2008). Stilbenoids: chemistry and bioactivities. In: Atta-ur-Rahman (Ed). *Studies in natural products chemistry*. (p. 453-646). Elsevier.