International Journal of Scientific Engineering and Research (IJSER)
ISSN (Online): 2347-3878
Index Copernicus Value (2015): 56.67 | Impact Factor (2017): 5.156

Compounds from Kenyan *Meyna tetraphylla*

Kinuthia E.W.[1], Mwangi E. M.[2]

[1]Pursuing PhD degree program in Chemistry of Natural Products in Egerton University, Kenya

[2]Ssenior Lecturer in Egerton University, Kenya

Abstract: ***One Phaeophytin with a phytol side chain (1), one triterpenoid [α-Amyrin (2)] and Stigmasterol (3) were isolated from the Dichloromethane extract of the leaves of Meyna tetraphylla. Their structures were elucidated using NMR spectroscopic methods.***

Keywords: Kenyan *Meyna tetraphylla*, Phaeophytin, *α*-Amyrin, Stigmasterol, Leaves

1. Introduction

Meyna *tetraphylla* belongs to Rubiaceae family that comprises of about 637 genera and 10,700 species [4]. This family is mostly used to treat malaria, headaches, asthma, epilepsy, sore eyes and as an emetic in many developing countries. The genus consists of about 12 species found in Africa and the Indian Ocean islands to the South East Asia. Many of the members of the closely related genera Keetia, Psydrax and Multidentia have edible fruits [6].They are used to treat malaria, headaches, asthma, epilepsy, sore eyes and emetic in many developing countries [7].

It is called *Tulungwo* in Pokot and *Mutunguru* in *Kikuyu*. The plants are armed with pained spines above the nodes and the leaves appear to be in fours, actually in pairs on very short spurs at each node. The flowers are in short fascicles on these spurs, corolla lobes 4-5 and the fruit is a berry. It is a shrub or tree, which is 5-6 m long. It has white or green flowers and its fruits are bluntly 5-angled, 13-17 by 16-20 mm. The buds are sparsely hairy, pedicels densely hairy [1]. Crushed leaves are put between the infected hooves of goats and camels by the Pokots. It is also used as an animal fodder and the root decoction is given to the pregnant women to alleviate pain [1].

2. Procedure

Meyna tetraphylla leaves were collected from Baringo and Taraka Nthi counties of Kenyain June 2014. The plant was identified and a voucher specimen deposited at the Botany Department, Egerton University. The leaves were cut into small pieces and air-dried under shade to a constant weight. They were then ground to fine powder using a grinder at KALRO, Njoro Kenya and the masses taken using a STANTON electronic balance.

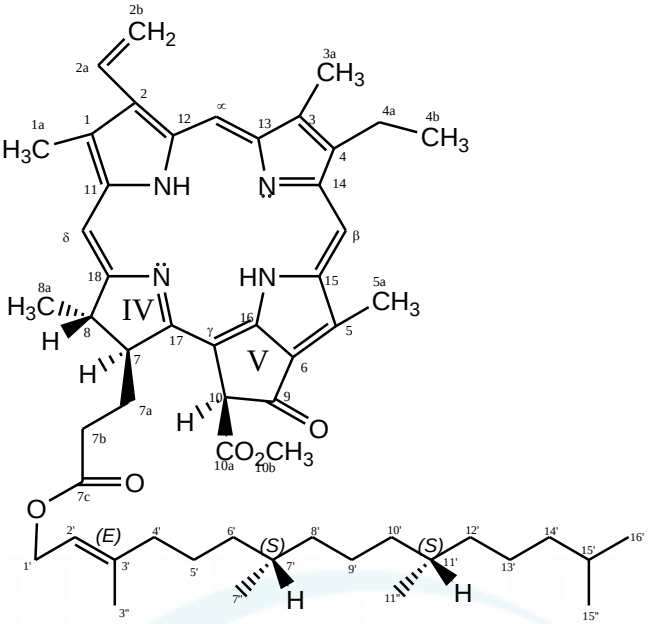
Exactly 1,000 gm of dry powdered leaves was sequentially and exhaustively extracted with 4 L hexane, 4 L dichloromethane, 4 L ethyl acetate and 4 L methanol for seventy two hours each in a 10 L metal tin. The solvents were evaporated under reduced pressure using a rotary evaporator (Büchi type R-205) to give a greenish sticky residue. The crude extracts were spotted on aluminium TLC plates (20x20 cm Macharey Nagel Duren). The mobile phases used were varying ratios of hexane, dichloromethane, ethyl acetate, diethyl ether and methanol (AR, Scharlau). Separations were monitored with inspection under ultraviolet light (UV lamp LF-204-LS, 354 nm and 634 nm) and by spraying the plate with anisaldehyde: sulphuric acid: methanol (1:2:97) mixture. Heating was done in an oven (ELECTROLUX STRUERS) at 70^{o}C for one minute. The plates with the best R_f values were used to determine the best solvent system for the separation. Exactly 50 gm of the crude extract was then fractionated by gravity column chromatography on a 2 cm by 30 cm silica gel column (60-200 mesh Thomas Baker). Further purification was achieved by repeaed thin layer chromatography and column chromatography.

Identification of pure compounds was achieved by ^{1}H and ^{13}C NMR. NMR spectra were recorded at room temperature on a 500 MHz Bruker AVANCE NMR at the School of Biomedical and Molecular Sciences, University of Surrey at Guildford UK. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS) as internal standard and coupling (J) are given in Hz.

3. Results

All the leaves crude extracts showed almost similar spots with the dichloromethane extracts having more spots on visualizing with a UV lamp and anisaldehyde spraying reagent. **Compound 1** (9.20 mg) was a green sticky solid with a green spot on visualization with anisaldehyde reagent and UV active with an R_f of 0.5 in 5% diethyl ether in dichloromethane. The ^{13}C NMR spectra gave fifty five carbon resonances. Sixteen of the resonances belong to four pyrrole carbons, one methoxy carbons (δ 53.1), eleven methyl carbons ranging between δ 11.2 ppm and δ 23.3 ppm, three carbonyl carbons (δ 172.6 ppm, δ 171.0 ppm, δ 189.8 ppm), sixteen methylene carbons ranging between (δ 20.0 ppm and δ 142.3 ppm), nine methine carbons (δ 28.2 ppm - δ 132.0 ppm) and fifteen quaternary carbon signals. The three carbonyl carbon signals (C-9, C-7c, and C-10a) occurred at the low field of δ 171.0 ppm-δ 189.8 ppm (Table 1). All the carbon resonances were characterized by DEPT experiments.

The ^{1}H resonances at δ 1.81 ppm, δ 3.67 ppm and δ 2.52 ppm showed a characteristic of four methyl groups attached to the pyrrole ring corresponding to the ^{13}C NMR resonance at δ 23.3 ppm, δ 12.3 ppm and δ 11.3 ppm in the HSQC-DEPT spectrum (Fig 1). The ^{1}H and ^{13}C signals at δ 3.9 ppm (δ 53.1 ppm) were characteristic of one methoxy group. In the COSY spectrum there was a correlation between H-8 (δ 4.46 m) and resonance at δ 1.81 d (H-8a) and δ 1.71 t (H-4b). The spectrum further showed a correlation between H-7a (δ 2.32 m) and resonance at δ 4.21 m (H-7). **Compound 1** was identified as Phaeophytin [8]



Compound 1

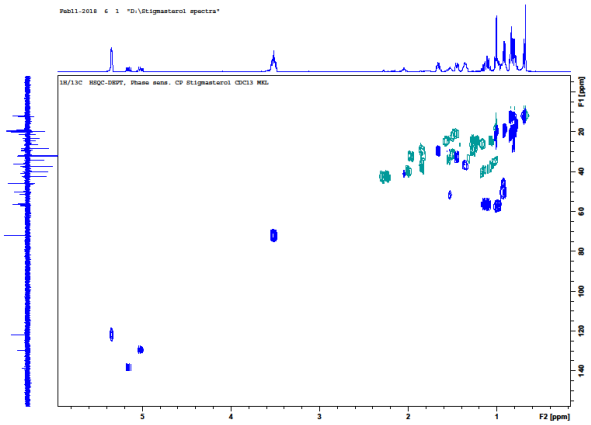


Figure 1: HSQC-DEPT for compound 1

Table 1: NMR data for Phaeophytin and compound1

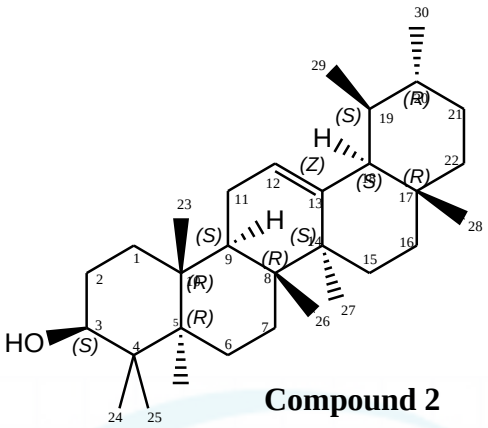
S/N	^{1}H	^{1}H NMR ($CDCl_3$, 300 MHz, Sianne *et,al*, 1998) δ ppm	δ ^{13}C (ppm) $CDCl_3$	^{13}C NMR ($CDCl_3$, 300 MHz, Sianne *et,al*, 1998) δ ppm	COSY
1	-		132.1	131.8 (C)	-
2	-		136.5	136.5 (C)	-
3	-		136.4	136.1 (C)	-
4	-		145.5	145.2 (C)	-
5	-		129.3	129.1 (C)	-
6	-		129.2	129.0 (C)	-
7	4.21 m	4.21 ddd	51.4	51.1 (CH)	7a
8	4.46 m	4.46 m	50.3	50.1 (CH)	8a
9	-		186.9	189.6 (C)	-
10	6.26 s	6.26 s	64.9	64.7 (CH)	-
11	-		143.1	142.9 (C)	
12	-		136.4	136.2 (C)	
13	-		155.9	155.5 (C)	
14	-		151.2	151.0 (C)	
15	-		138.2	137.9 (C)	
16	-		149.9	150.0 (C)	
17	-		161.5	161.3 (C)	
18	-		172.4	172.2 (C)	
α	9.36 s	9.36 s	97.8	97.5 (CH)	
β	9.51 s	9.50 s	104.7	104.4 (CH)	
γ	-		105.5	105.2 (C)	
δ	8.56 s	8.55 s	93.3	93.1 (CH)	
1a	3.67 s	3.39 s	12.3	12.1(CH_3)	
2a	8.10 dd (5.0 Hz)	7.98 dd	129.3	129.0 (CH)	2b
2b	7.26 d 6.19 d (11.5 Hz)	6.17 dd, 6.28 dd	123.0	122.8 (CH_2)	2a
3a	2.52 s	3.21 s	11.5	11.2 (CH_3)	
4a	3.64 m	3.66 m	19.9	19.7 (CH_2)	
4b	1.71 t (7.5)	1.68 t	16.5	16.3 (CH_3)	
5a	3.67 s	3.88 s	12.3	12.2 (CH_3)	
7a	2.32 m		29.9	29.8 (CH_2)	7
7b	3.20 t (6.5 Hz)		31.4	31.2 (CH_2)	
7c	-		173.2	173.0 (C)	
8a	1.81 d (7.5 Hz)	1.80 d	22.8	22.7 (CH_3)	8
10a	-		169.8	173.0 (C)	
10b	3.88 s		53.1.	53.0 (CH_3)	
1’	4.50 d	4.35 d	61.7	61.0 (CH_2)	
2’	5.30 t	5.10 t	118.0	118.0 (CH_2)	
3’			142.3	142.0 (CH_2)	
4’	1.81 t	1.96 t	39.6	39.4 (CH_2)	
5’	1.13 m	1.33 m	25.2	25.0 (CH_2)	
6’	1.13 m	1.25 m	37.6	37.8 (CH_2)	
7’	1.60 m	1.65 m	33.0	33.3 (CH)	
8’	1.13 m	1.25 m	37.5	37.7 (CH_2)	
9’	1.55 m	1.29 m	24.6	24.7 (CH_2)	
10’	1.12 m	1.25 m	37.5	37.7 (CH_2)	
11’	1.70 m	1.65 m	33.0	33.2 (CH)	
12’	1.13 m	1.25 m	37.6	37.7 (CH_2)	
13’	1.13 m	1.29 m	24.6	24.4 (CH_2)	
14’	1.13 m	1.25 m	40.0	39.9 (CH_2)	
15’	1.81 m	1.83 m	28.2	28.2 (CH)	
16’	1.02 m	1.01 m	23.3	23.2 (CH_3)	
3”	1.70 s	1.71 s	17.6	17.1 (CH_3)	
7”	1.11 d	1.06 d	22.8	21.0 (CH_3)	
11”	1.11 d	1.06 d	19.9	21.0 (CH_3)	
15”	1.01 d	1.01 d	23.3	23.2 (CH_3)	

Compound 2 (5.65 mg) was isolated as a white power with a brown spot, UV inactive and an R_f of 0.3 in 5% diethyl ether in dichloromethane.

The 1H NMR spectrum showed presence of eight methyl singlets, one olefenic proton at δ 5.26 ppm triplet (J = 3.6 Hz) corresponding to 13C NMR resonance at δ 126.1 ppm. An oxygenated protonated δ 3.22 ppm doublet of doublet corresponding to13C δ 79.3 ppm was also observed all suggesting an olealane type triterpenoid. The thirty carbon resonances observed in the 13C NMR spectrum were characterized by DEPT experiment. This indicated that compound 2 was a triterpenoid with eight methyl groups, nine methylene groups,

seven methine groups (one attached to a hydroxyl and one to a double bond) and six quaternary groups.

In the HMBC spectrum (Fig 2) the correlations between 1H and ^{13}C confirmed the position of the hydroxyl group and the double bond thus confirming that it was an Olealane. Further confirmation was done using COSY and NOESY spectral. **Compound 2** was identified as α-Amyrin [5, 3]. The summary of NMR data is shown in **Table 2**.



Compound 2

Table 2: NMR data for alpha Amyrin and compound 2

S/N	^{13}C NMR ($CDCl_3$, 500 MHz) ppm	^{13}C NMR ($CDCL_3$, 125 MHz) (Nkeoma *et al*., 2014; Liliana *et al*., 2012) ppm	1H NMR (500 MHz) ppm	1H NMR ($CDCl_3$, 500 MHz)(Nkeoma et al., 2014; Liliana *et a*l., 2012) ppm
1	38.8	38.8	0.99, 1.63 t	1.55, 1.49
2	28.2	28.7	1.14, 1.87 m	1.52, 1.55
3	79.3	79.6	3.33 7 t	3.16 (dd, J = 5.1, 11.2)
4	39.0	38.7		
5	55.4	55.1	0.74 t	0.71
6	18.5	18.4	1.55, 1.37 m	1.53, 1.30
7	33.2	32.2	1.48, 1.32 t	
8	39.7	40.7		
9	47.7	47.7	1.46 t	1.95
10	37.2	36.6		
11	23.5	23.3	1.09, 1.91 dd	1.84
12	126.1	124.4	5.26 t	5.06 (t, J = 3.2)
13	138.2	139.5		
14	42.2	42.0		
15	27.4	27.2	1.61 t	1.94 (td, J = 4.5, 13.5 Hβ)
16	24.4	26.6	1.66, 2.02 t	1.76 (td, J = 5.0, 13.5 Hβ)
17	37.2	33.7		
18	52.9	59.0	2.18 d	1.98
19	39.3	39.6	1.34 t	1.38m
20	39.0	39.6	1.34 m	
21	29.9	31.2	1.46, 1.32 m	
22	36.9	41.5	1.73 t	1.85 (dt, J = 3.0, 7.0)
23	28.3	28.1	1.09 s	0.93 s
24	15.7	15.6	0.79 s	0.74 s
25	15.8	15.6	0.78 s	0.73 s
26	17.2	16.8	0.93 s	0.89 s
27	23.8	23.2	1.09 s	1.02 s
28	28.3	28.1	1.09 s	0.94 s
29	17.3	17.4	0.79 d	0.85 (d, J = 6.0)
30	21.4	21.4	0.10 d	0.73 (d, J = 7.0)

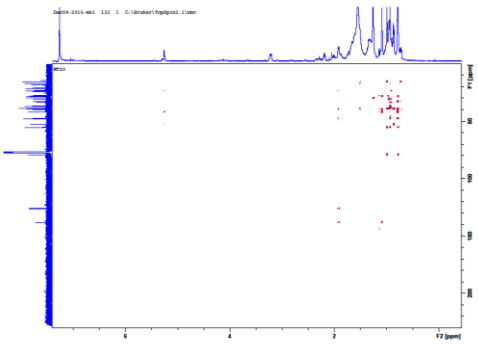
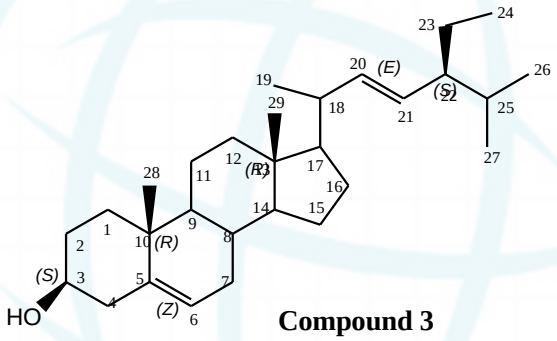


Figure 2: HMBC for compound 2

Compound 3 (5.83 mg) was isolated as white crystals with a brown spot, UV inactive, with an R_f of 0.4 in 5% diethyl ether in dichloromethane. The ^{1}H NMR spectrum showed the presence of two methyl singlets at δ 1.26 ppm and δ 1.16 ppm, three methyl doublets at δ 1.16 ppm, δ 1.01 ppm and δ 1.01 ppm, and a methyl triplet at δ 0.98 ppm. It also showed protons at δ 5.01 ppm, δ 5.48 ppm, and δ 5.37 ppm suggesting the presence of three protons corresponding to that of a trisubstituted and a disubstituted olefinic bond. The proton corresponding to the H-3 of a sterol moiety appeared as a triplet of doublet of doublets at δ 3.25 ppm. The 1H NMR and 13C NMR values for all the protons and carbons were assigned on the basis of DEPT, HSQC-DEPT (Fig 3) and HMBC correlations. The data (Table 3) supported the presence of sterol skeleton having a hydroxyl group at C-3 position with two double bonds at C-5/C-6 and C-20/C-21 with six methyl groups supported by the HMBC correlations. Thus, the structure of compound 3 was assigned as stigmasterol [2].



Compound 3

Table 3: NMR data for Stigmasterol and compound 3

S/N	^{13}C NMR (500 MHz in $CDCl_2$)	^{13}C NMR (600 MHz in $CDCl_3$) (Chaturvedula and Prakash, 2012)	1H NMR (500 MHz in $CDCl_2$)	^{1}H NMR (600 MHz in $CDCl_3$) (Chaturvedula and Prakash, 2012)
1	34.2	37.6	1.38, 1.13 (t, 2H)	
2	31.9	32.1	1.57, 1.32 (td, 2H)	
3	72.0	72.1	3.25 (tdd,1H)	3.51(tdd, 1H. J = 4.5, 4.2, 3.8 Hz)
4	42.5	42.4	2.23, 1.98 (d, 2H)	
5	141.0	141.1		
6	121.9	121.8	5.37 (t, 1H, J = 5.2 Hz)	5.31 (t, 1H, J = 6.1 Hz)
7	31.9	31.8	2.04, 1.79 (dd, 2H)	
8	32.1	31.8	1.45 (tdd, 1H)	
9	50.4	50.2	1.44 (td, 1H)	
10	36.7	36.6		
11	21.3	21.5	1.52, 1.27 (td, 2H)	
12	40.0	39.9	1.49, 1.24 (t, 2H)	
13	42.6	42.4		
14	56.3	56.8	1.40 (td, 1H)	
15	24.5	24.4	1.60, 1.35 (td, 2H)	
16	28.5	29.3	1.60, 1.35 (td, 2H)	
17	57.0	56.2	1.51 (td, 1H)	
18	41.4	40.6	2.33 (s, 1H)	
19	19.0	21.7	1.16 (d, 3H)	0.91 (d, 3H, J = 6.2 Hz)
20	140.1	138.7	5.01 (dd, 1H)	4.98 (m, 1H)
21	128.9	129.6	5.48 (dd, 1H)	5.14 (m, 1H)
22	45.7	46.1	1.15 (td, 2H)	
23	23.3	25.4	1.33 (m, 2H)	
24	11.9	12.1	0.96 (t, 3H)	0.83 (t, 3H, J = 7.1 Hz)

25	31.87	29.6	1.86 (m, 1H)	
26	20.04	20.2	1.01 (d, 3H, J = 6.4 Hz)	0.82 (d, 3H, J = 6.6 Hz)
27	19.61	19.8	1.01 (d, 3H)	0.82 (d, 3H, J = 6.6 Hz)
28	19.00	18.9	1.26 (s, 3H)	0.71 (s, 3H)
29	12.20	12.2	1.16 (s, 3H)	1.03 (s, 3H)

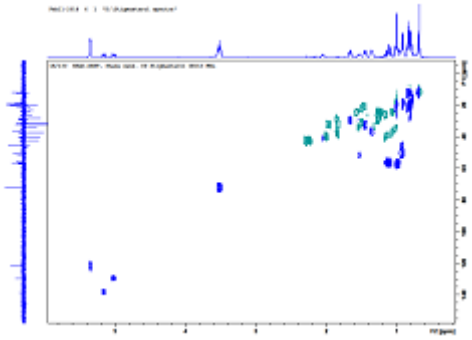


Figure 3: HSQC-DEPT for compound 3

4. Conclusion

In this research, *Meyna tetraphylla* dichloromethane leaves crude extract gave three compounds, **1** (Phaeophytin), **2** (α-Amyrin) and **3** (Stigmasterol).

5. Acknowledgment

The authors wish to thank Dr. Mwangi E.M., Prof Cheplogoi P.K., Dr. Njue A.W., Dr. Lang'at M.K. and Prof. Mulholland, D.A. This work was supported in part by a grant from National Commission for Science, Technology and Innovation (NACOSTI) of Kenya.

References

[1] Beentje, H.J. (1994). *Kenya Trees, Shrubs and Lianas. National Museums of Kenya*, Nairobi, Kenya, page 515-521.
[2] Chaturvedula and Prakash (2012). Isolation of Stigmasterol and *β*-Sitosterol from the dichloromethane extract of Rubus suavissimus,International Current *Pharmaceutical Journal* **9**: 239-242
[3] Liliana, H.V., Javier, P. and Arturo, N.O. (2012). The Pentacyclic Triterpenoid *α,β*-amyrins: A Review of sources and Biological activities. *Phytochemicals* **23**: 488-502.
[4] Mongrand, S., Badox, A., Patouille, B., Lacomblez C., Chavent, M. and Bessoule, J.J. (2005).Chemotaxonomy of the Rubiaceae family based on leaf fatty acid composition.*Phytochemistry* **66**: 549-559.
[5] Nkeoma, N.O., Ajaghaku, D.L., Okeke, H.N., Ilodigwe, E.E., Chekwuemeka, S.N. and Okeye, F.B.C. (2014). Beta-Amyrin and Alpha-amyrin acetate isolated from stem bark of Alstonia boonei display profound anti-inflammatory activity. *Pharmaceutical Biology* **52**: 1478-1486.
[6] Patrick, M. and Bo, T. (2005). *Useful trees and shrubs for Kenya*. World Agroforestry Centre, page 249-308.
[7] Sebastien, M., Alain, B., Brigitte, P., Chantal, L., Marie, C. and Jean, J.B. (2005).Chemotaxonomy of the Rubiaceae family based on leaf fatty acid composition.*Phytochemistry* **66**: 549-559.
[8] Sianne L.S.,, Mulholland, D.A. and Hutchings, A. (1998). Phaeophytins from Tapura fischeri.*Phytochemistry* 49: 2391-2394