Compounds from *Turraea abyssinica*

KINUTHIA ESTHER WANJIRU[1], MWANGI ELIZABETH MUTHONI[2], CHEPLOGOI PETER KIPLAGAT[3]
[1,2,3] *Chemistry, Egerton University, Kenya*

Abstract- ***Turraea abyssinica belong to the Turraea genus of the Meliaceae family and is used by the Samburus of Kenya as rungus, firewood and as fruits to induce vomiting. No phytochemicals have been reported on the Narok Kenya species. The leaves were collected from Narok Kenya, identified and voucher specimen kept for reference in Biological Department, Egerton University, Kenya. Dry powder of stem bark was successively extracted with hexane, dichloromethane, ethyl acetate and methanol for seventy-two hours. The solvents were evaporated under reduced pressure using a rotary evaporator (Büchi type R-205). With repeated column chromatography using a solvent step gradient of 20% methanol in dichloromethane, 47.5% and 95% dichloromethane in diethylether, three compounds, β-Sitosterol(1), Scopoletein(2) and 2-(1,2-Dihydroxypropyl)tetradecanoic acid (3) were isolated. Identification of pure compounds was achieved by ^{1}H and ^{13}C NMR (500 MHz) spectroscopy***.

Index Terms- ***Compounds, Narok, stembark, Turraea abyssinica,***

I. INTRODUCTION

Plants are extremely important in the lives of people throughout the world and many people depend on them to satisfy basic human needs such as food, clothing, shelter and health care. Historically, plant medicines were discovered by trial and error (Facchini et al., 2000). Turraea abyssinica belong to the Turraea genus of the Meliaceae family that comprises of about 50 genera and 1400 species (Leonardo et al., 2002). It is used by the Samburus of Kenya as rungus, firewood and as fruits to induce vomiting. This family has been known to exhibit a wide variety of biological properties (Amit and Shailendra, 2006). Though not much work has been done on it, its root methanol extract showed some antiplasmodial activity (Ndung'u, 2002). No chemical composition has t been reported on the Kenyan Narok Turraea abyssinica. In the course of this research, three compounds were isolated from the Dichloromethane extract of stembark.

II. IDENTIFY, RESEARCH AND COLLECT IDEA

Turraea abyssinica was collected from Narok Kenya, in June 2014, and a voucher specimen deposited at the Department of Biological Sciences Herbarium Egerton University, Njoro Kenya. 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.

Dry powder of stem bark (1,000 g) 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 dichloromethane leave extract (50 g) was subjected to a solvent step gradient of dichloromethane: methanol. Fractions containing more spots were purified by repeated column chromatography using a solvent step gradient of 20% and 33% ethyl acetate in hexane respectively. The separated components were visualized under UV lamp (254 nm and 365 nm) and then sprayed with anisaldehyde reagent and heated in an oven for one minute at 70^{o}C. The crude extracts were spotted on aluminum 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.

Crude extracts were then fractionated by gravity column chromatography on a 2 cm by 30 cm silica gel column (60-200 mesh Thomas Baker). Further

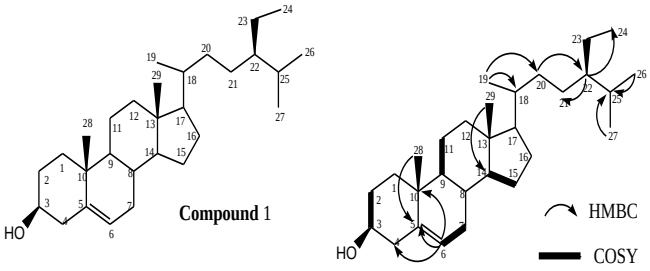
purification was achieved by repeated thin layer chromatography and column chromatography.

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

III. WRITE DOWN YOUR STUDIES AND FINDINGS

Compound 1 was isolated from dichloromethane stem bark (Narok) as white colourless crystals. The ^{13}C NMR spectrum showed 29 carbon signals. The HMBC spectrum placed ^{13}C resonance δ_C 140.8 and δ_C 121.9 for C_5=C_6 double bond respectively, δ_C 72.0 for C-3 *β*-hydroxyl group, δ_C 12.1 and δ_C 19.0 for angular methyl carbon atoms C_{18} and C_{19} respectively. The ^1H NMR (Appendix 1) spectrum varied between δ_H 0.83 to δ_H 5.36. The spectrum showed presence of six high intensity peaks indicating presence of six methyl groups at δ_H 0.83, δ_H 0.88, δ_H 0.83, δ_H 0.92 and δ_H 1.01. The position corresponding to the 3Hs of a sterol moiety appeared as a triplet of doublet at δ_H 3.52. A ^1H at δ_H 3.38 corresponded to a peak in the form of singlet in the region of the ethylene proton suggesting the presence of one proton.

The correlation between ^1H and ^{13}C was confirmed by HSQC spectrum, COSY and NOESY spectra also confirmed ^1H correlations. All this information (Table 1) confirmed that compound **1** was a *β*-Sitosterol (Chaturvedula and Prakash, 2012). It is usually used for heart disease, hypercholesterolemia, modulating the immune system, prevention of cancer, as well as for rheumatoid arthritis, tuberculosis, cervical cancer, hair loss and benign prostatic hyperplasia (Soodabeh, *et al*.,., 2014). It also possess good anti diabetic activity (Muhammad *et al*., 2017).



HMBC H→C (curved arrows) and ^1H–^1H COSY (bold lines) correlations

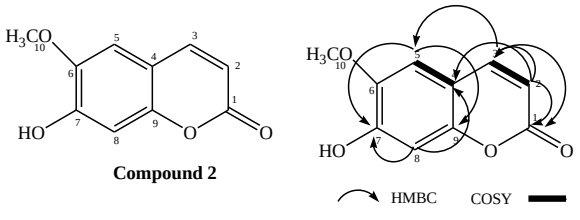
Table 1

No	^1H	^{13}C	DEPT	HMBC	COSY	NOESY
1	1.004, 1.288	34.17	CH_2			
2		31.9	CH_2			10
3	3.521(tdd, 1H)	72.03	CH		6, 19	6, 10
4	2.260, 1.994 (d)	42.54	CH_2			3
5		140.97	C			
6	5.357 (t) 1H, J = 5.2 Hz)	121.94	CH		19	19
7	1.827, 1.994 (t)	31.87	CH_2			6, 9
8	1.432	32.13	CH			
9	1.432	50.35	CH			
10		36.72				
11	1.487 (m)	21.30	CH_2			10, 12, 18, 19
12	1.830, 1.087 (t)	40.00	CH_2			9
13		42.60				
14	1.440	56.28	CH			
15	1.584, 1.052 (m)	24.52	CH_2			
16	1.831, 1.250 (m)	28.46	CH_2			
17	1.005	56.99	CH			
18	0.834 (d, 3H)	12.08	CH_3			
19	0.917 (d, 3H)	19.00	CH_3			12
20	1.640	40.06	CH			
21	1.006 (d, 3H)	19.25	CH_3			17, 24
22	1.250	331	CH_2			
23	1.250	23.3	CH_2			
24	0.834 (t, 3H, J = 7.2 Hz)	46.03	CH			
25	1.827	31.87	CH			
26	0.826 (d, 3H, J = 6.4 Hz)	20.04	CH_3			

27	0.826 (d, 3H, J = 6.4 Hz)	19.61	CH_3			
28	0.917 (s, 3H)	19.00	CH_2			
29	0.877 (s, 3H)	12.20	CH_3			

Compound 2 was isolated from dichloromethane stem bark (Narok) crude extract. It was a brown sticky compound with an R_f of 0.2 in 20% ethyl acetate in hexane. It showed ten carbon resonances in the ^{13}C NMR) spectrum that confirmed compound 2 as a monoterpenoid. It also showed presence of a methoxy group, four methine groups and five quaternary carbons, one being a carbonyl group.

The ^1H NMR (Appendix 2) spectrum showed four aromatic doublet proton at δ_H 6.26, and δ_H 7.60 both with coupling constant of 9.5 Hz corresponding to ^{13}C resonance at δ_C 113.3, δ_C 144.7. Two singlet at δ_H 6.46 and δ_H 6.78 corresponding to ^{13}C resonance at δ_C 111.8 and δ_C 111.3 were observed. One methoxy group singlet at δ_H 3.75 attached to the benzene ring corresponding to the ^{13}C resonance at δ_C 56.4 was also observed. All this was confirmed by the HMBC, COSY and NOESY experiments. The HMBC spectrum showed correlation between H-2 resonance δ_H 6.26, 6.28 (doublet J = 9.45 Hz) with δ_c 162.5, δ_c 143.3, δ_c 129.3, H- 3 resonance δ_H 7.58, δ_H 7.60 (doublet J = 9.48 Hz) with δ_c 162.5, δ_c 111.8 confirming the position of the carbonyl group. The correlation between H-5 resonance δ_H 6.46 with δ_c 143.3, δ_c 144.6, δ_c 144.0, H-8 resonance δ_H 6.78 with δ_c 129.5, δ_c 150.3 and δ_c 144.6 confirmed the position of the hydroxyl group. The NOESY and COSY correlation between H-2 and H-3, H-5 and H-8 confirmed compound 2 as Scopoletin (Akhmad *et al*, 2012). Scopoletin has significant pharmacological activities, such as antiarthritic, spasmolytic, antitumor, antidepressant-like, antifungal, antihyperglycemic and antioxidative (Zhou *et al.*,.2012). A summary of NMR data is shown in Table 2.



Compound 2

HMBC H→C (curved arrows) and ^1H–^1H COSY (bold lines) correlations

Table 2

S/N	^{13}C	^1H	HMBC (H→C)	COSY	NOESY
1	162.45	-	-	-	-
2	113.42	6.256, 6.275 d (2H) J = (9.45 Hz	1, 3, 4	3	3
3	143.30	7.584, 7.603 d (2H) J = 9.48 Hz	1, 5	2	2
4	129.52	-		-	-
5	111.79	6.462 s (1H)	3, 9, 7	8	
6	150.28	-	-	-	-
7	144.56	-	-	-	-
8	111.30	6.78 s (1H)	4, 7, 6	5	-
9	144.03	-	-	-	-
10	56.43	3.75 s (3H)	-	-	-

Compound **3** was isolated from dichloromethane stem bark (Narok) crude extract as white shinny crystals. The ^{13}C NMR spectrum of the compound showed presence of 17 carbons with eleven methylene groups, three methine groups, two methyl groups and one carboxyl carbon at δ_C 176.1 indicating presence of carboxylic acid. They were confirmed by DEPT-135 and HSQC**)** spectra**.** The ^1H NMR (Appendix 3) spectrum showed one triplet methylene proton at δ_H 0.87, δ_H 0.89 with coupling constant of 6.74 Hz corresponding to ^{13}C resonance at δ_C 14.3. Ten multiplet methylene protons ranging between δ_H 1.26 and δ_H 1.89 corresponding to ^{13}C resonance between δ_C 22.7 and δ_C 29.7 indicated a straight chain compound. Two doublet triplet methine protons, one at δ_H 2.57, δ_H 2.58 with coupling constant of 5.10 Hz, the other one at δ_H 3.84 corresponding to ^{13}C resonance at δ_C 48.9 and δ_C 79.4 respectively were observed. One doublet methyl proton at δ_H 1.45, δ_H 1.46 and a multiplet methine proton at δ_H 4.21, δ_H 4.20 with coupling constants of 6.21 Hz and 6.54 Hz corresponding to ^{13}C resonances at δ_C 18.5 and δ_C 80.0 respectively were also observed.

The position of the three hydroxyl groups and one carboxyl group were confirmed by HMBC**,** COSY and NOESY spectra. The HMBC spectrum showed correlation between H-2 and H-3 resonance δ_H 2.53 and δ_H 1.57 with δ_c 176.1 confirming the position of

the carboxyl group. Also H-2, H-3 and H-3' (δ_H 1.45, δ_H 1.46 doublet, J = 6.21 Hz) with δ_c 79.4, and δ_c 80.0 confirmed the position of the two hydroxyl groups. This was also confirmed by NOESY and COSY correlation between H-2 with H-1', H-3 and H-1' with H-2', H-3'. The ^{1}H NMR and ^{13}C NMR spectra of compound **3** were compared with the spectra of Myristic acid, indicating that the compound was a straight chain acid with a dihydroxy propyl substituent. All this information **(Table 3)** confirmed the structure of the compound as 2-(1,2-Dihydroxypropyl) tetradecanoic acid **(**Biological Magnetic Resonance Data Bank, 2018**).** Essential oil are used by the Eastern Capetraditional healers for the treatment of respiratory tract infections, tuberculosis, dysentery, diabetic mellitus, laryngitis and vaginal infections (Omoruyi *et al*., 2014).

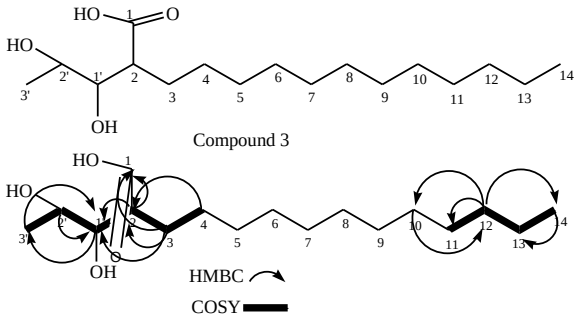
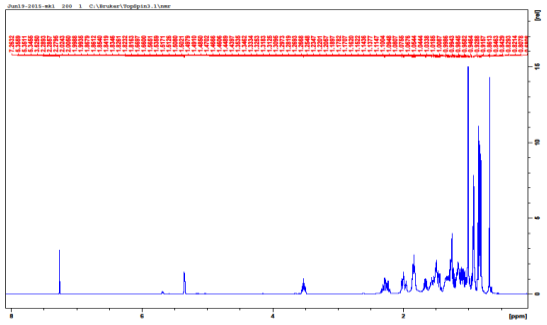


Table 3

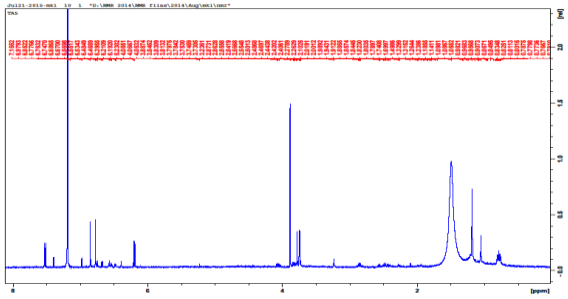
No	^{1}H	^{13}C	DEPT	HMBC	COSY	NOESY
1	2.53, 2.57 (td)	176.1	C			
2	1.57 (m)	48.9	CH	1,1',3, 4	1',3	3,4
3	1.89 (t)	27.0	CH_2	1, 2, 1'	2, 4	2
4	1.26 (m)	28.7	CH_2	1, 2	3	2
5	2.53, 2.57 (td)	29.6	CH_2			
6	1.26 (m)	29.6	CH_2			
7	1.26 (m)	29.8	CH_2			
8	1.26 (m)	29.9	CH_2			
9	1.26 (m)	29.9	CH_2			
10	1.26 (m)	29.9	CH_2	12		
11	1.26 (m)	29.8	CH_2	12		
12	1.26 (m)	32.1	CH_2	10, 11	11, 14	
13	1.31 (s)	22.9	CH_2		14	
14	O.87, 0.89 (t) J = 6.74 Hz	14.3	CH_3	12, 13	12, 13	
1'	3.84 (dt)	79.4	CH	3	2, 2'	3'
2'	4.210, 4.197 (m) J = 6.541Hz	80.0	CH	1'	1' 3'	3'
3'	1.446, 1.459 (d) J = 6.21 Hz	18.5	CH_3	1' 2'	1'	1' 2'

APPENDIX

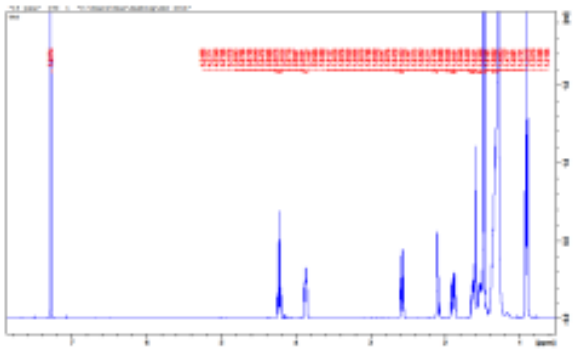
Appendix 1



Appendix 2



Appendix 3



ACKNOWLEDGMENT

The author wish to thank Dr. Mwangi E.M., Prof. Cheplogoi P.K., Dr. Njue A.W. and Dr. Lang'at M.K. This work was partly supported by Natiomal Commission for Science, Technology and Innovation (NACOSTI) of Kenya.

REFERENCES

[1] A.Z. Muhammad, U.K. Saadat, U.R. Taj, and S.S.S. Muhammad (2017). β-sitosterol and stigmasterol from the roots of Indigofera heterantha. Pharmacy & Pharmacology International Journal **5:** 203-207.

[2] Biological Magnetic Resonance Data Bank. .A Repository for Data from NMR Spectroscopy on Proteins, Peptides, Nucleic Acids, and other Biomolecules.

[3] Chaturvedula and Prakash (2012). Isolation of Stigmasterol and β-Sitosterol from the dichloromethane extract of Rubus suavissimus,International Current Pharmaceutical Journal **9:** 239-242

[4] C. Leonardo, S. de C. Rosabel, M. Eduardo and S.C. Jose (2002). Oleanane Triterpenoids from Cedrela montana (Meliaceae). Z. Naturforsch **57:**575-578.

[5] D. Akhmad, K. Soleh, B.S.K. Leonardus and M.S. Yana, M (2012). Scopoletin, a coumarin derivative compound isolated from Macaranga gigantifolia Merr. Applied Pharmaceutical Science **2:** 175-177.

[6] E.B. Omoruyi, A.J. Afolayan and G. Bradley (2014). Chemical composition profiling and antifungal activity of the Essential oil and plant extracts of Mesembryanthemum edule (L) bolus leaves. Traditional Comlementary and Alternative Medicines **11:** 19-30.

[7] J. Zhou, L.Wang, L. Wei, Y. Zheng, H. Zhang, Y. Wang, P.Cao, A. Niu, J. Wang and Y. Dai (2012). Synthesis and Antitumor Activity of Scopoletin derivertives. Letters in Drug Design & Discovery **9:** 397-401.

[8] M.W. Ndung'u (2002). Isolation and characterization of anti-mosquito compounds from selected East African meliaceae plants. PhD Thesis.Jomo Kenyatta University of Agriculture and Technology, Kenya.

[9] P.J. Facchini, K.L. Huber-Allanach and L.W. Tari (2000). Plant aromatic l-amino acid decarboxylases: evolution, biochemistry, regulation, and metabolic engineering applications. Phytochemistry **54:** 21-138.

[10] R. Amit and S. Shailendra (2006). Limonoids: Overview of significant Bioactive Triterpenes Distributed in Plant Kingdom. Biolgical Pharmaceutical Bulletin **29:** 191-201.

[11] S. Soodabeh, M. Azadeh, R.G. Ahmad and A. Mohammad (2014). The story of Beta Sitosterol-A review. European Journal of Medicinal Plants 4: 590-609.