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ISOLATION OF TARAXASTEROL AND STIGMASTEROL FROM THE AERIAL PART OF *Centaurea perrottetii* DC. (Asteraceae)

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ABSTRACT

Centaurea perrottetii is a well-known plant used in African traditional medicine for its wound healing, anti-inflammatory, anti-microbial, anti-oxidant as well as cytotoxic properties. Preliminary phytochemical screenings of the methanol extract of the aerial partof *Centaurea perrottetii* revealed the presence of triterpenes, flavonoids, steroids, tannins, saponins and carbohydrates. Extensive phytochemical investigation of the n-hexane fraction led to the isolation of a pentacyclic triterpenoid (taraxasterol) and a steroid (stigmasterol). The isolated compounds were characterised on the basis of spectral data obtained from IR, 1D,2D ^{1}H- and ^{13}C-NMRspectroscopy and comparison withreported literature data.To the best of our literature search, this is the first report of isolation of taraxasterol and stigmasterol from *Centaurea perrottetii*.

Keywords: Asteraceae, triterpenes, steroids, NMR spectroscopy, taraxasterol, stigmasterol.

INTRODUCTION

The plant *Centaurea perrottetii* belongs to the Asteraceae family-the largest plant family comprising of 1,528 genera and 22,750 species (Heywood *et al*., 2007). The genus Centaurea, of which *C. Perrottetii* belongs, is made up of flowering plants consisting of annual, biennial and perennial herbs and less often shrubs. The Centaurea genus has about 500 species of herbaceous thistle-like flowering plants with wide distribution mostly in Europe and the Mediterranean with few species found in the tropics (Hilpold *et al*., 2014; Koca *et al*., 2009).

Centaurea perrottetii, also called English star thistle, danyi or surandu in Hausa, chaile in Fulfulde and gargam in Wolof, is a prostrate or erect perennial herbthat can reach up to 50 cm high with spiny leaves and flower-heads (Burkill, 1985). The herb is used ethno-medically for the treatment of pain and inflammation, parasitic infections, wound healing, malaria and as a stomachic. It is also used as fodder for cattle (Senosy *et al*., 2018).

Despite the medicinal potentials of *Centaurea perrottetii*, there is dearth of information regarding its chemical constituents. To the best of our literature search, there is no report yet, on the isolation of any compound from *Centaurea perrottetii*. However, several sesquiterpene lactones such as 13-Acetysolstitialin A, Chlorojanerin, cnicin, repin and flavonoids such as Quercetin, Apigenin, Kaempferol and luteolin have been isolated from other species of the centaurea genus (Khammar & Djeddi, 2012).

MATERIALS AND METHODS

Experimental

Extracts were concentrated *in vacuo* on a rotary evaporator (R-II-HB, Switzerland). Column chromatography (CC) was carried out on Kieselgel (60-120 mesh) Merck, Glass columns (75 cm × 3.2 cmand 30 cm × 2 cm), pre-coated thin layer chromatography (TLC) aluminium plates (Silica gel 60 F_{254}) for TLC analysis (Merck Germany), melting point were recorded on gallenkhamp melting point apparatus and were uncorrected. Both 1D,2D ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AVANCE III NMR spectrometer (400 Hz) using TMS as the internal standard.

Plant Material

The aerial part of *Centaurea perrottetii* growing wild in thebushes of Kudingi village, Giwa local government area of Kaduna state, Nigeria, was collected after identification on the field using descriptions in the monograph (Burkill, 1985). The identity of the plant was confirmed and authenticated by Mallam Namadi Sanusi of the herbarium unit,Department of Botany, Ahmadu Bello University, Zaria, by comparison with deposited herbarium specimen of voucher reference number 21923. The aerial part was air dried under shade and pulverized manually to a coarse powder.

Extraction and Partitioning

The powdered aerial part (2kg) was extracted with 15 L of methanol using maceration method with occasional shaking for 72 hours and the marc was re-extracted for 14 days. The extract was concentrated in *vacou* to obtain a green oily mass subsequently referred to as the crude methanol extract (CME).The extract (120g) was suspended in distilled water and filtered using a Whatman No. 1filter paper to obtain water soluble and water insoluble portions. The water insoluble fraction was washed with hexane and chloroform while the water soluble fraction was successively partitioned with hexane, chloroform, ethyl acetate and n-butanol to give the hexane, chloroform, and ethylacetate and n-butanol fractions respectively.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was conducted on the crude extract and partition fractions according to standard procedures described by Trease & Evans (1996).

Column Chromatography of Hexane Fraction

The hexane fraction (5g) was subjected to chromatographic separation using wet slurry method. Slurry of silica gel was packed into the column and allowed to settle. The sample was adsorbed on silica gel and loaded on to the settled column. The column was eluted by gradient elution with hexane 100% followed by 90:10, 80:20, 70:30 hexane:ethyl acetate mixture up to 100% ethyl acetate and the column was finally washed with methanol.

The different fractions collected were pooled together based on similarity in their TLC profile to give 10 major fractions (A-J). Fraction G which was eluted with 70:30 hexanes: ethylacetate mixture was further chromatographed over silica gel column using 8:2 hexanes: ethyl acetate mixture which led to the isolation of compound G1. The same procedure was repeated for fraction I leading to the isolation of compound I1. Both compounds G1 and I1 showed single spot on TLC plate using

solvent systems of n-hexane: ethyl acetate, 9:1 and 4:1 indicating their level of purity. Compounds G1 and I1 were subjected to IR, 1D and 2D NMR analyses to elucidate their chemical structures.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the methanol aerial part extract and partitioned fractions of *Centaurea perrottetii* revealed the presence of carbohydrates, saponins, flavonoids, cardiac glycosides, tannins, steroids/ terpenes and alkaloids. Compound G1 (R_f values 0.3 and 0.65) and compound I1 ($R_f$values 0.4 and 0.6) gave single spots on TLC using hexane: ethyl acetate 9:1 and 4:1 as solvent system respectively. Both G1 and I1 tested positive to Liebermann-Burchard test for steroids/triterpenes. The uncorrected melting point of compound G1 was found to be between 225-226°C. While that of compound I1 was found to be 139-141°C.

Compound G1: The IR spectrum of G1 shows absorption bands at 3380 cm^{-1} indicative of free OH group, coupled vibrational frequencies at 2937-2855 cm^{-1} typical of asymmetrical and symmetrical C-H stretch in alkanes, absorption bands at 1450, 1386 cm^{-1} for the gem dimethyl groups, absorption band at 1640 cm^{-1} indicates a C=C stretch and C-O stretch was observed at 1040 cm^{-1}. An absorption band was also observed at 879 cm^{-1} indicative of an exocyclic double bond (Mewara, 2015).
^{1}HNMR ($CDCl_3$, 400MH_Z); δ_H3.21(1H, m, H-3), 2.43(1H, m, H-21a),), 2.19 (1H, m, H-21b), 2.09 (1H, m, H-19), 4.60 (1H, *brs*, H-30b), 4.62 (1H, *brs*, H-30a), 0.79 (1H, m, H-5), 1.01(3H, s, H-23), 0.76(3H, s, H-24), 0.92(3H, s, H-25), 1.13(3H, s, H-26), 0.97(3H, s, H-27), 0.85(3H, s, H-28), 1.20 (3H, d, H-29).

The ^{1}HNMR spectrum of compound G1 revealed the presence of seven methyl groups of which six were singlets at δ_H0.76, 0.85, 0.92, 0.97, 1.01, 1.13, and one doublet atδ_H1.20(d, J = 4.0 Hz, 3H), was assigned to H-29 for taraxasterol. The doublet signal at H-29 contrary to a singlet in lupeol is indicative of taraxasterol; this is the major difference between taraxasterol and lupeol. A multiplet signal integrating for one proton at δ_H2.09 is ascribable to H-19 while multiplet signals at δ_H 2.43 and δ_H2.19 are ascribable to H-21a and H-21b respectively. The H-3 proton showed a multiplet signal at δ_H 3.21(1H,m, J = 11.4, 6.2 Hz).The high J value indicates that the proton is in the axial position. A pair of broad singlets at δ_H 4.60 and 4.62 (integrating for 1H each) was indicative of exomethylene double bond at (H-30a and H-30b).These assignments are in good agreement with the data reported for taraxasterol by Mouffok *et al*., 2012; Reynold *et al*., 1986, Yekta and Alavi, 2008 (Table 1).

The ^{13} C NMR (100 MHz, $CDCl_3$)spectrum of G1 revealed signals at δ_C38.9 (C-1, CH_2), 27.5 (C-2, CH_2), 79.1 (C-3, CH), 38.9 (C-4, C), 55.5 (C-5, CH), 18.4 (C-6, CH), 34.2 (C-7, CH_2), 41.0 (C-8, C), 50.6 (C-9, CH), 37.2 (C-10, C), 21.6 (C-11, CH_2), 26.3 (C-12, CH_2), 39.3 (C-13, CH), 42.2 (C-14, C), 26.8 (C-15, CH_2), 38.4 (C-16, CH_2), 34.6 (C-17, C), 48.8 (C-18, CH), 39.5 (C-19, CH), 154.7 (C-20, C), 25.6 (C-21, CH_2), 39.0 (C-22, CH_2), 28.1 (C-23, CH_3), 15.4 (C-24, CH_3), 16.4 (C-25, CH_3), 16.0 (C-26, CH_3), 14.9 (C-27, CH_3), 19.6 (C-28, CH_3), 25.7 (C-29, CH_3), 107.2 (C-30, CH_2). All multiplicities were assigned with the aid of the DEPT experiment which showed the presence of 7 methyl, 11 methylene, 6 methine and6 quaternary carbons. The signals due to exomethylene group at δ_c 107.2 (C-30)and a highly deshielded quaternary carbon at δ_c

154.7 (C-20) together with the deshielded signal at δ_c 79.1 due to the hydroxyl group at C-3, were also assigned with the aid of the DEPT experiment. All these assignments conform to data obtained from literature for taraxasterol (Mouffok *et al*., 2012; Yekta and Alavi, 2008).

In the HMBC spectrum, methylene proton signal at δ_H 4.61 (H-30) showed J_3 correlation with the methine carbon signal at δ_C 39.5 (C-19) and a methylene carbon signal atδ_C 25.6 (C-21) supporting the positioning of the exomethylene double bond at C-30. A J_2 correlation observed between methylene proton at δ_H 2.43 (H-21) and a quaternary carbon at δ_C 154.7 (C-20), and a J_3 correlation observed between methine proton at δ_H 2.09 (H-19) and a methylene carbon at δ_C 25.6 (C-21) confirms the position of the quaternary carbon at C-20. A J_3 correlation was observed between methyl proton at δ_H 0.76 (H-24) and a methine carbon at δ_C 55.38 (C-5) and another methine carbon at δ_C 79.1 (C-3) supporting the position of the gem dimethyl groups at C-4. In addition, a J_3 correlation was also observed between methyl proton at δ_H 1.01 (H-23) and methine carbon at δ_C 79.1 (C-3), a methyl carbon at δ_C 15.4 (C-24) and a quaternary carbon at δ_C 38.9 (C-4) confirming the positioning of gem dimethyl groups at C-4. The forgoing spectral analyses and comparison with data obtained from literature has led to the proposed structure of compound G1 as taraxasterol (Figure 1), a pentacyclic triterpene alcohol.

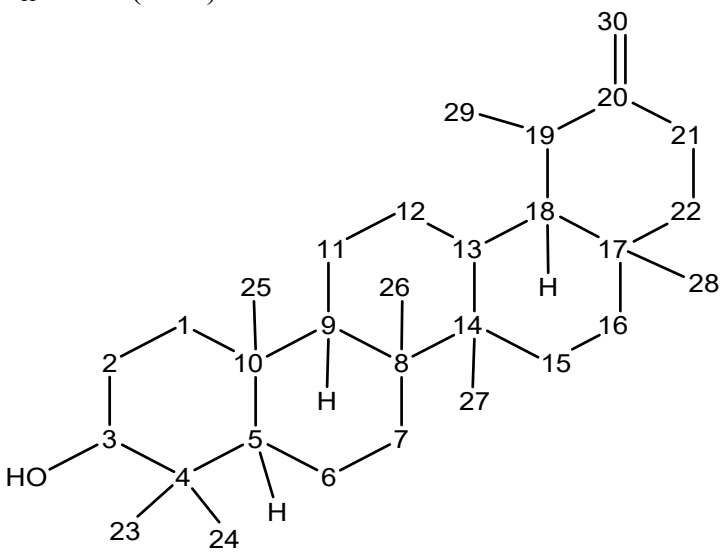


Figure 1: Proposed structure of compound G1

Table 1: Comparison of G1 with Taraxasterol isolated byYekta and Alavi. 2008.

Position	^{1}H	$^{1}H_{Ref}$	^{13}C	$13C_{Ref}$	DEPT
1			38.9	38.9	CH_2
2			27.5	27.3	CH_2
3	3.21 dd(J= 11.4, 6.2 Hz)	3.22 dd (J=6.3, 13.2 Hz)	79.1	79.0	CH
4			38.9	38.7	C
5	0.79(m)	0.77(m)	55.5	55.2	CH
6			18.4	18.4	CH
7			34.2	34.0	CH_2
8			41.0	40.8	C
9			50.6	50.6	CH
10			37.2	37.0	C
11			21.6	21.5	CH_2
12	1.06(m)	1.03(m)	26.3	26.1	CH_2
13			39.3	39.2	CH
14			42.2	42.1	C
15	0.97(m)	0.93(m)	26.8	26.5	CH_2
16			38.4	38.4	CH_2
17			34.6	34.4	C
18			48.8	48.7	CH
19			39.5	39.4	CH
20			154.7	154.6	C
21			25.6	25.5	CH_2
22			39.0	38.9	CH_2
23	1.01(s)	0.98(s)	28.1	28.0	CH_3
24	0.76(s)	0.85(s)	15.4	15.4	CH_3
25	0.92(s)	0.86(s)	16.4	16.9	CH_3
26	1.13(s)	1.02(s)	16.0	16.0	CH_3
27	0.97(s)	0.93(s)	14.9	14.9	CH_3
28	0.85(s)	0.85(s)	19.6	19.4	CH_3
29	1.20(d1.20 (d, *J* = 4.0 Hz, 0H),)	1.02(d)	25.7	25.5	CH_3
30	4.61	4.60brs, 4.62brs	107.2	107.2	CH_2

Compound I1: The IR spectrum of compound I1 showed absorption bands at 3362 cm^{-1}corresponding to a free hydroxyl group, coupling peaks at 2937 and 2866 cm^{-1} typical of symmetric and asymmetric C – H stretching vibrations in alkane and 1043 cm^{-1}, a band at 1655cm^{-1}which is indicative of C=C stretch and C – O stretch at 1044 cm^{-1} (Noor- Aziiraa *et al*., 2017).

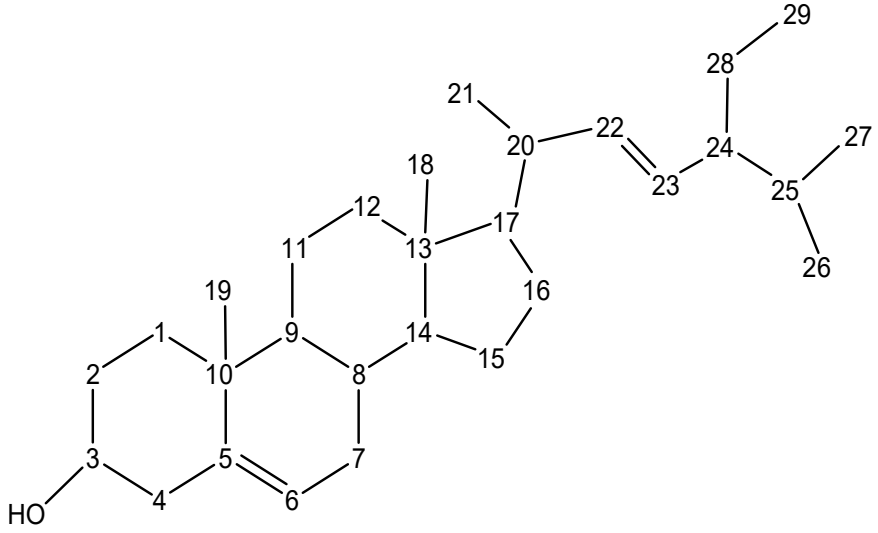
^{1}H-NMR ($CDCl_3$, 400MH_Z);δ_H3.52 (1H, m, H-3), 5.28 (1H, t, H-6), 0.94 (3H, s, H-18), 0.69 (3H, s, H-19), 1.02 (3H, d, H-21), 4.95 (1H, m, H-22), 5.08 (1H, m, H-23), 0.82 (3H,

d, H-26), 0.86 (3H, d, H-27), 0.90 (3H, t, H-29).

The ^{1}H-NMR spectrum showed 6 distinct peaks upfield at δ 0.69, 0.82, 0.86, 0.90, 0.94 and 1.02 indicating the presence of six methyl groups; amultiplet at δ 3.52 is ascribable to the H-3 of a sterol moiety, signals for olefinic protons at δ 5.28, 4.95, 5.08 corresponding to H-6, H-22 and H-23 respectively (Pierre & Moses, 2015).

^{13}C NMR (100 MHz, $CDCl_3$); δ_C 36.4 (C-1, CH_2), 32.0 (C-2, CH_2), 73.0 (C-3, CH), 42.2 (C-4, CH_2), 142.7 (C-5, C), 122.0 (C-6, CH), 30.4 (C-7, CH_2), 31.5 (C-8, CH), 50.0 (C-9, CH), 36.2 (C-10, C), 24.6 (C-11, CH_2), 40.1 (C-12, CH_2), 41.9 (C-13, C), 55.0 (C-14, CH), 24.0 (C-15, CH_2), 29.0 (C-16, CH_2), 55.5 (C-17, CH), 12.0 (C-18, CH_3), 19.0 (C-19, CH_3), 40.0 (C-20, CH), 21.0 (C-21, CH_3), 139.0 (C-22, CH), 125.0 (C-23, CH), 55.0 (C-24, CH), 34.0 (C-25, CH), 22.0 (C-26, CH_3), 23.0 (C-27, CH_3), 26.0 (C-28, CH_2), 12.1 (C-29, CH_3).

The^{13}C NMR spectrum revealed 29 carbon signals including six methyl, nine methylene, eleven methine and three quaternary carbons. The signals at δ_C 142.7 and 122.0 were assigned to the C-5 and C-6 double bonds respectively, the signals at δ_C 139.0 and 125.0 were assigned to the C-22 and C-23 double bonds, and the signal at δ_C 73.0 is typical of oxymethine carbon at C-3. Signals at δ_C 12.0 and 19.0 correspond to angular methyl carbon at C-18 and C-19 respectively (Pierre & Moses, 2015). The information obtained from the ^{1}H NMR and ^{13}C NMR of compound I1 and its comparison with data obtained from literature (Table 2) has led to the proposed structure of compound I1 as stigmasterol, a steroid.



CONCLUSION

Chromatographic and spectroscopic analyses on the n-hexane fraction of the aqueous methanol aerial part of *Centaurea perrottetii*, led to the isolation and characterisation of taraxasterol and stigmasterol. This is the first report on the isolation of these compounds from *Centaurea perrottetii*.

Table 2: Comparison of I1 with Stigmasterol isolated by Pierre and Moses, 2015.

Position	^{1}H	$^{1}H_{Ref}$	^{13}C	$^{13}C_{Ref}$	DEPT
1			36.4	37.1	CH_2
2			32.0	31.5	CH_2
3	3.50(m)	3.53(m)	73.0	71.9	CH
4			42.2	42.3	CH_2
5			142.7	140.9	C
6	5.28(m)	5.38(t)	122.0	121.6	CH
7			30.4	31.7	CH_2
8			31.5	31.8	CH
9			50.0	50.0	CH
10			36.2	36.1	C
11			24.6	24.3	CH_2
12			40.1	39.8	CH_2
13			41.9	42.1	C
14			55.0	56.9	CH
15			24.0	24.3	CH_2
16			29.0	28.9	CH_2
17			55.5	55.8	CH
18	0.94(s)	1.03(s)	12.0	12.0	CH_3
19	0.69(s)	0.71(s)	19.0	19.8	CH_3
20			40.0	40.4	CH
21	1.02(d)	0.91(d)	21.0	20.9	CH_3
22	4.95(m)	4.98(m)	139.0	138.4	CH
23	5.08(m)	5.14(m)	125.0	129.3	CH
24			55.0	51.26	CH
25			34.0	34.0	CH
26	0.82(d)	0.80(d)	22.0	21.1	CH_3
27	0.86(d)	0.82(d)	23.0	22.8	CH_3
28			26.0	25.3	CH_2
29	0.90(t)	0.83(t)	12.1	12.0	CH_3

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