African Journal of Pharmaceutical Research & Development

Vol. 7 No.2; pp. 72-76 (2015)

Isolation and Characterization of two Pentacyclic Triterpenoids from *Glossonema boveanum* Decne (Apocynaceae)

Shehu S[1]*, Abubakar MS[1], Musa AM[2], Tajuddeen N[3], Zakariya AM[1]

1. Department of Pharmacognosy and Drug Development, ABU Zaria, Nigeria
2. Department of Pharmaceutical and Medicinal Chemistry, ABU Zaria, Nigeria
3. Department of Chemistry, ABU Zaria, Nigeria

ABSTRACT

Two pentacyclic triterpenes α-amyrin acetate and lupeol were isolated from the n-hexane soluble fraction of the aqueous ethanol extract of the aerial parts of *Glossonema boveanum*, a plant used in traditional medicine for the treatment of epilepsy and stimulation of lactation using a combination of silica gel column chromatography and preparative TLC. The structures of these compounds were elucidated using NMR spectroscopic analysis (1D& 2D) and by comparison with reported data. This is the first report of isolation of these compounds from this plant and its genus.

KEYWORDS: *Glossonema boveanum, α-amyrin acetate, lupeol, NMR spectroscopic analysis*

INTRODUCTION

Glossonema boveanum (Apocynaceae), commonly known as 'Taaringida' in Hausa is a hairy canescent perennial herb that reaches up to 10-30 cm long. It often grows wild and as well cultivated in many places of the Sahel zone, from Mauritania to Northern Nigeria and Western Cameroon, and extending throughout North and North Eastern Africa to Saudi Arabia and North Western India [1, 2].

The plant is edible raw, especially the young flowering top and fruits. It provides a good fodder for all stock, and serves as a famine-food for man. It contains a copious amount of milky sap and is taken by women in Northern Nigeria to increase lactation, hence the Hausa epithet for the plant [1, 2]. The powdered whole plant suspended in local diary milk has been used for the treatment of epilepsy (Mal. Zakir Abdulhamid, Personal communication).

Despite medicinal potentials of *G. boveanum*, there is dearth of information regarding its chemical constituents. The few chemical compounds reported from this plant are flavonol glycosides, which includes; quercetin 3-O-galactoside, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, Kaempferol-3-O-neohesperidoside and Kaempferol-3-O-rutinoside [3]. In this paper, we report the isolation of two pentacyclic triterpenoids; α-amyrin acetate and lupeol.

MATERIALS AND METHODS

Materials/Equipment

Glass columns (75 cm long x 3.2 cm diameter and 20 cm long x 2 cm diameter), Silica gel (60-120 mesh) for column chromatography (Merck Germany), Pre-coated thin layer chromatography (TLC) aluminium plates (Silica gel 60 F254) for TLC analysis (Merck Germany) and Pre-coated thin layer chromatography

***Author for correspondence:** shehus3621@yahoo.com; +2348134924454*

(TLC) glass plates (Silica gel 60 F254, thickness 2mm) for preparative TLC (Merck Germany). Stuart melting point apparatus (model number 5315, Britain), Bruker AVANCE-600 Japan (600MHz) and Rotary evaporator (R-II-HB, Switzerland).

Collection and identification of the plant material

The whole plant of *G. boveanum* was collected from Kauran-Namoda, Zamfara State, Nigeria in January 2014. A sample from the freshly collected plant material was identified by the Taxonomist in the herbarium section of the Department of Biological Sciences Ahmadu Bello University, Zaria, where a voucher number 617 was deposited for future reference. The plant material was then shade dried and ground to a fine powder (0.7 kg).

Preparation of the extract

The powder (0.7 kg) was macerated in a glass jar with 2L of aqueous ethanol (70% v/v) at room temperature for 3 days (72 hrs). The content of the jar was then filtered through a cotton plug and finally through a filter paper (Whatman no.1), the filtrate was then concentrated to dryness using rotary evaporator to afford aqueous ethanol extract (119 g). Thereafter, about 80 g of the extract was suspended in water (400 ml) and then successively partitioned with n-Hexane (2×300 ml), Chloroform (2×300 ml), Ethyl acetate (3×200 ml), and n-butanol (2×200 ml) to afford 15.6 g, 2.08 g, 1.32 g, and 11 g weight fractions respectively.

Isolation and purification of the compounds

The hexane fraction (2 g) was subjected to silica gel column chromatography. The column was eluted with hexane 100%, then hexane-chloroform mixture (95:5, 90:10, 85:15, 80:20) successively to afford 80 column fractions (40 ml each). Base on the TLC profiles, these fractions were pooled together to 8 major fractions (F1 to F8). Repeated silica gel column chromatographic separation of Fraction F3 (0.3 g) eluted using hexane (100%), then hexane-ethyl acetate mixture (95:5, 90:10, 80:20) followed by Preparative TLC led to the isolation of a compound coded SAL_1. Similarly, Repeated silica gel column chromatographic separation of fraction F5 (0.4 g) eluted using hexane (100%), then hexane-ethyl acetate mixture (99:1, 98:2, 95:5) followed by Preparative TLC led to the isolation of another compound coded SAL_2.

Spectroscopic Analysis

The spectra of the isolated compounds were recorded on a Bruker AVANCE-600 Japan (600MHz) for HNMR and 150MHz for 13C in deuterated chloroform with TMS as internal standard at the University of Kwazulu Natal, Westville Campus, Durban, South Africa.

RESULTS & DISCUSSION

Compound SAL_1 was obtained as white needles (22 mg), readily soluble in chloroform. It gave a positive reaction with Libermann buchard test and pink coloured spot (R_f 0.52) when sprayed with 10% sulphuric acid suggesting the presence of a steroidal or a triterpenoidal nucleus. It showed a relatively narrow melting point range of 223-227°C and this serves as an indication of its purity. The ^1H NMR spectrum revealed the presence of several signals between 0.85 and 1.92 (Table I) which are due to overlapping methyl, methylene and methine protons typical of steroids and triterpenes. The signal observed at δ_H 5.12 ppm, a triplet proton is typical of an olefeinic proton (H-12). Also observed in the spectrum is a multiplet at δ_H 4.51 ppm corresponding to the oxymethine proton typical of hydrogen at C-3 of steroid and triterpenes. This relatively desheilded signal is indicative of hydroxyl substitution with acetate at C-3. An intense proton signal (a singlet) ascribable to methyl protons of the acetate group was observed at δ_H 2.05 ppm. The de-shielding of this methyl proton was as a result of its proximity to a carbonyl functional group. The spectrum also revealed the presence of eight methyl proton signals at δ_H 0.88, 0.79, 1.02, 1.02, 1.00, 1.13, 0.79 and 0.92 ppm. These assignments are in good agreement with that of alpha-amyrin acetate [4, 5].

The ^{13}C NMR experiment showed thirty two prominent carbon resonances indicative of 32 carbon atoms in the compound. DEPT showed the presence of 9 methyl, 9 methylene, 7 methine and 7 quatenary carbon atoms in the compound (Table I). A highly de-shielded signal at δ_C 170.86 ppm indicates the carbonyl carbon of an ester attached to C-3 of the alpha amyrin moiety [4], while the signal at δ_C 81.66 ppm indicated the oxymethine carbon (C-3) which was slightly desheilded due to its attachment to the acetate group. The signals at δ_C 125.55 ascribed to C-12 and δ_C 139.65 ascribed to C-13 indicated the

olefeinic carbons at C-12 and C-13 respectively. Also, there was an observed carbon signal δ_C 59.4 ppm which corresponds to the methine carbon C-18 of alpha-amyrin moiety. All these assignments conformed to the data obtained from literature for alpha-amyrin acetate [4, 5].

The COSY spectrum of SAL_1 exhibited some cross peaks such as between δ_H 5.12, (H-12) and δ_H 1.92, (H-11) methine and methylene protons respectively. Cross peaks are also observed between δ_H 4.51 an oxymethine proton signal (H-3) and methylene proton signal (δ_H 1.62, H-2). In the HMBC spectrum, methyl proton signal at δ_H 2.05 (H-2') of the acetate showed cross peaks with carbonyl carbon signal (δ_C 170.96, C-1') by J_2 correlation. Also, there is an observed cross peak between oxymethine proton signal δ_H 4.51 (H-3) and carbonyl carbon (δ_C 170.96, C-1') which further supports the positioning of the acetate group at C-3 of the triterpenoidal nucleus. Cross peaks were also observed between δ_H 5.12 methine proton signal (H-12) and methylene carbon signal (δ_C 23.61, C-11) by J_2 correlation, and with another methine carbon signal (δ_C 47.77, C-9) by J_3 correlation. Methyl proton δ_H 1.13, H-27 also showed cross peaks with quaternary carbon signal (δ_C 139.65, C-13) by J_3 correlation. Analysis of the results of 1D and 2D NMR and comparing the spectral data obtained with that in the litereature suggests that; compound SAL_1 is alpha-amyrin acetate, an ursane type pentacyclic triterpenoid. This compound has been previously isolated from *Tylophora hirsuta* of *Asclepiadaceae* sub-family, and was found to have antispasmodic activity on rabbits' jejunum [5].

Compound SAL_2 was obtained as amorphous white powder (26 mg), readily soluble in chloroform. It gave a positive reaction with Libermann buchard test and pink spot (R_f 0.46) when sprayed with 10% sulphuric acid suggesting the presence of a steroidal or a triterpenoidal nucleus. It showed a melting point range of 210-213°C. The relatively narrow melting range could serve as an indication of its purity. The ^{1}H NMR of compound SAL_2 displayed a pair of broad singlet at δ_H 4.56 and δ 4.67 (1H each) an important characteristic of oleifinic protons (H-29 a and b) typical of lupeol [6]. It displayed a multiplet proton signal δ_H 3.19 ascribed to H-3 which was de-shielded because of the hydroxyl group attached to C-3. The spectrum also showed a signal at δ_H 2.36(m) integrating for one proton ascribed to the 19 β-H of lupeol [7]. Multiplet proton signals observed at 0.93 to 1.45 in the spectrum are due to several methylene and methine protons in the compound as shown in table I. The up-field region of the spectrum revealed the presence of seven tertiary methyl proton signals at δ 0.99,0.75,0.80, 1.02.0.91,0.75 and 1.68. The assignments and the data are in good agreement with that of lupeol as obtained in the literature [6, 8].

The ^{13}C NMR experiment showed thirty prominent carbon resonances indicative of 30 carbon atoms in the compound. DEPT spectra revealed the nature of these carbon atoms. It showed 7 methyls, 11 methylenes, 6 methines and 6 quatenary carbon atoms. The signal at δ_C 150.87 ascribed to C-20 and that at δ_C 109.47 assigned to C-29 were strongly de-shielded due to the olefeinic bond between them. Likewise, signal at δ_C 79.29 (C-3) was de-shielded because of the hydroxyl group attached to C-3.

The COSY spectrum of SAL_2 exhibited some cross peaks such as those between δ_H 2.36, H-19 and proton signals at δ_H 1.28, H-21, between proton signal δ_H 3.19, H- 3 and that at δ_H 1.60, H-2. There also exist a cross peak between δ_H 4.67, H-29a and signal at δ_H 4.56, H-29b.

In the HMBC spectrum, Methine proton signal at δ_H 3.19 (H-3) showed cross peaks with a methyl carbon signal δ_C 28.0, C-23 and δ_C 15.5, C-24 by ^{3}J correlation. The multiplet, methine proton signal at δ_H 2.36 (H-19) showed cross peaks with methylene carbon signals δc 30.0 (C-21) by ^{2}J, oleifinic carbon signal δ_C 109.47 (C-29) by ^{3}J correlation, methine carbon signal δ_C 48.25 (C- 18) by ^{2}J correlation and a methyl carbon signal δ_C 19.44 (C-30) by ^{3}J correlation. The pair of broad singlets of olefinic proton at δ_H 4.67 and 4.56 showed cross peaks with a methylene carbon signal δ_C 48.10 (C-19) and δ_C 19.44 (C-30) by ^{3}J correlation. Careful spectral analysis and comparison with reported data [8, 9] led to the suggestion that; compound SAL_2 is lupeol. This pentacyclic triterpene, have been previously isolated from some members of Asclepiadaceae such as; *Oxystelma esculentum* [10] and *Calotropis gigantean* [11]. Some important pharmacological activities of lupeol includes; anti-imflammatory, anti-microbial, anti-protozoal, anti-proliferative and cholesterol lowering properties [12].

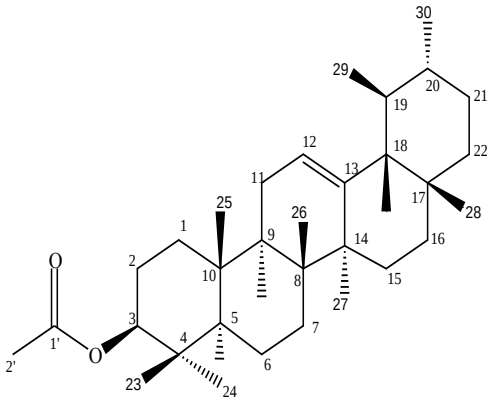

Figure 1. Proposed structure of compound SAL_1

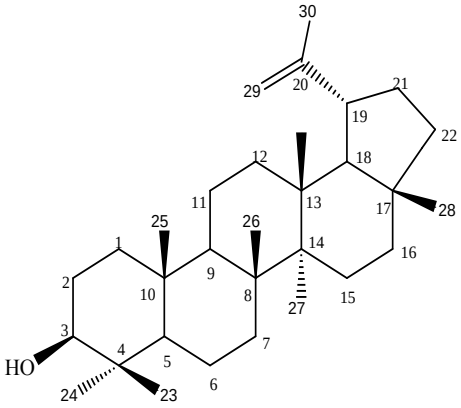

Figure 2. Proposed structure of compound SAL_2

Table I. ^{13}C and ^{1}H Chemical Shift Values of Compound SAL_1 and SAL_2

	Compound SAL_1			Compound SAL_2		
Position	**DEPT**	**δ_C (Hz)**	**δ_H (Hz)**	**DEPT**	**δ_C (Hz)**	**δ_H (Hz)**
1	CH_2	38.6	1.06m,1.67m	CH_2	38.8	0.93 m
2	CH_2	23.6	1.62m	CH_2	27.2	1.60 m
3	CH	81.6	4.51dd	CH	79.3	3.19 dd
4	C	37.8	-	C	39.1	-
5	CH	55.6	0.85m	CH	55.3	0.68 m
6	CH_2	18.2	1.50m	CH_2	18.3	1.40 m, 1.19m
7	CH_2	33.1	0.87	CH_2	34.2	1.38 m
8	C	40.3	-	C	41.1	-
9	CH	47.8	1.53m	CH	50.6	1.28 m
10	C	37.2	-	C	37.2	-
11	CH_2	23.6	1.92m	CH_2	21.1	1.4 m
12	CH	125.6	5.12t	CH_2	25.5	1.45 m, 1.68 m
13	C	139.7	-	CH	38.3	1.63 t
14	C	42.2	-	C	43.2	-
15	CH_2	26.7	1.8, 0.98m	CH_2	28.1	0.97 m, 1.60 m
16	CH_2	28.1	1.98m	CH_2	35.2	1.45 m
17	C	33.9	-	C	43.3	-
18	CH	59.4	1.33m	CH	48.3	1.38 m
19	CH	40.0	1.33m	CH	48.1	2.36 m
20	CH	40.0	1.33m	C	150.9	-
21	CH_2	31.1	1.22, 1.37m	CH_2	30.0	1.28 m
22	CH_2	41.7	1.40m	CH_2	40.5	1.17 m
23	CH_3	15.4	0.88s	CH_3	28.0	0.99 s
24	CH_3	28.9	0.79s	CH_3	15.6	0.75 s
25	CH_3	16.4	1.02s	CH_3	16.2	0.80 s
26	CH_3	16.4	1.02s	CH_3	16.0	1.02 s
27	CH_3	23.1	1.13s	CH_3	14.8	0.91 s
28	CH_3	17.2	0.79s	CH_3	18.2	0.75 s
29	CH_3	17.8	1.00m	CH_2	109.5	4.67 , 4.56 br s
30	CH_3	22.2	0.92m	CH_3	19.4	1.68 s
1'	C	170.9	-			
2'	CH_3	22.2	2.05s			

CONCLUSION

Chromatographic and spectroscopic analysis on the n-Hexane fraction of the aqueous ethanol extract of *G. boveanum*, led to the isolation and characterization of two compounds; alpha-amyrin acetate and lupeol and these are reported from this plant and its genus

REFERENCES

1. Burkill H.M. (1985).The useful plants of West Tropical Africa. 2nd Edition. Volume 1, Families A–D. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 960 pp.
2. Neuwinger, H.D (2000). African traditional medicine: a dictionary of plant use and applications. Medpharm Scientific, Stuttgart, Germany. Pp 589.
3. Heneidak S., Renee J. G., Geoffrey C. K. and Monique S.J S., (2006).Flavonoid glycosides from Egyptian Species of the tribe Asclepiadeae (Apocynaceae, Sub-family Asclepiadoideae). Biochemical Systematics and Ecology. *Elsevier*. 34(7) 575- 584.
4. Rhourri-Frih B, Isabelle R, Patrick C, Patrice A. and Gaetan H, Michel L. (2013). Pentacyclic triterpenes from Manilkara bidentata resin. Isolation, identification and Biological properties. *Fitoterapia* Elsevier. 88: 101-108.
5. Niaz Ali (2013). Brine shrimp cytotoxicity of crude methanol extract and antispasmodic activity of α-amyrin acetate from Tylophora hirsuta Wall. BMC Complementary and Alternative Medicine. 13:135. Retrieved from: http://www. biomedcentral.com/1472-6882/13/135.
6. Abdullahi, S.M, Musa, A.M, Abdullahi, M.I., Sule M.I and Sani, Y.M.(2013). Isolation of Lupeol from the Stem-bark of Lonchocarpus sericeus (Papilionaceae). Scholars Academic Journal of Biosciences (SAJB). 1(1):18-19.
7. Jain P.S. and Bari S.B., (2010). Isolation of Lupeol, Stigmasterol and Campesterol from Petroleum ther Extract of Woody Stem of Wrightia tinctoria. Asian Journal of Plant Sciences, 9: 163-167.
8. Soumia M., Hamada H., Catherine L., Christophe L., and Mohammed B. (2012). Chemical constituents of Centaurea omphalotricha Coss. And Durieu ex Batt. & Trab. Rec. Nat. Prod . 6:3 292-295.
9. Thanakijcharoenpath W. and Theanphong O. (2007). Triterpenoids from the Stem of Diospyros glandulosa. Thai J. Pharm. Sci. Vol. (31) Pp 4.
10. Pandya D. J and Anand I.S., (2011). Isolation and High Performance thin layer Chromatographic Estimation of Lupeol from Oxystelma esculentum. Pharm. Methods . 2 (2). 99-105.
11. Saratha V., Pillai S. L., and Subramanian S. (2011). Isolation and Characterization of Lupeol, a Triterpenoid from Calotropis gigantean latex. *International Journal of Pharmaceutical Sciences Review and Research* 10(2): 54-56.
12. Siddique H. R, Saleem M. (2011). Beneficial Health Effect of Lupeol Triterpene: a review of Preclinical Studies. *Life Science* 88 (7-8).