Available online at www.scholarsresearchlibrary.com

Scholars Research Library

J. Nat. Prod. Plant Resour., 2013, 3 (2):57-60
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Isolation of oleanolic acid from chloroform extract of *Borreria stachydea*[(DC) Hutch. and Dalziel]

[1]Onoja E. and [2]Ndukwe I. G.

[1]Department of Science Laboratory Technology, Federal Polytechnic, KauraNamoda, Zamfara State, Nigeria
[2]Department of Chemistry, Ahmadu Bello University Zaria, Nigeria

---

ABSTRACT

The whole plant of Borreria stachydea[(DC) Hutch. & Dalziel] was collected fresh from Birnin Magaji village, in Zamfara state, Nigeria in November, 2010. Taxonomical identification was done by Mallam Musa Abdullahi at Herbarium unit of Biological Science Department, ABU, Zaria, Nigeria. The whole plant wasair dried under shade, segregated and pulverized. 791.94g of the pulverized plant materials were carefully weighed and loaded into a Soxhlet extractor. It was extracted with Chloroform. 10.0g of the chloroform crude extract was loaded in a column and was developed by gradient elution. The purified sample from the above column chromatography was subjected to preparative TLC. The result gave rise to WIPO 2. WIPO 2 was subjected to spectroscopic analysis for structural elucidation and characterization. The results are shown in Table 1. Gas Chromatography – Mass Spectroscopy (GC-MS) was carried out on WIPO 2 to determine the molecular mass of the compoundsusing (GC-MS)machine. Infrared (IR) spectroscopic analysis was carried out on both WIPO 2 to determine the functional groups present in the compounds using Perkin Elmer Spectrum 100 FTIR Spectrometer.

Keywords: *Borreria stachydea*, isolation, characterization and oleanolic acid

---

INTRODUCTION

Borreria stachydea[(DC) Hutch.&Dalziel] is an erect, hairy and weedy herb, about 1ft in height with mauve flowers. It belongs to the phylum magnoliophyta, and its class is magnoliopsida and a member of the Rubiaceae family. It is found in Nigeria, Ghana, Sudan, Malaysia, India and several other nations of the world. A poultice of the whole plant is used to heal leg ulcers, wounds, urinary tract infections and it was found to be highly anti-oxidative in nature. *Borreria*is a relatively large genus of herbs or half-shrubby plants. This genus consists of about 100 species distributed throughout the tropics [1].

Plant materials contain thousands of chemicals which act against diseases and infections of humans and animals when properly used. Plants contain different types of compounds such as resins, rubbers, gums, waxes, dyes, flavors, fragrances, proteins, amino acids, bioactive peptides, phyto hormones, sugar, flavonoids and bio pesticides [2].
Interest in Natural Products Chemistry can be largely due to the growing awareness that many of the secondary metabolites of living things serve important biological and ecological roles, mainly as chemical messengers and defensive compounds [3].

MATERIALS AND METHODS

Collection, Identification and Preparation of Plant Materials

The whole plant was collected fresh from BirninMagaji village, in Zamfara state Nigeria in November, 2010. Taxonomical identification was done by Mallam Musa Abdullahi at Herbarium unit of Biological Science Department, ABU, Zaria, Nigeria. The whole plantwas sorted, air dried under shade, segregated and pulverized by mechanically pounding them using wooden mortar and pestle. The pulverized plant material was stored away from moisture.

Extraction

791.94g of the pulverized plant materials were carefully weighed and loaded into a Soxhlet extractor. It was extracted with Petroleum ether (60-80^{o}C) by hot continuous percolation method in the Soxhlet apparatus for 72 hours. The marc was subjected to Chloroform, Ethyl acetate (76-78^{o}C) and Methanol and was extracted successively for 72 hours respectively. The extracts were concentrated in vacuo at 40^{o}C using rotary evaporator and subjected to air drying to give dried crude extracts.

Isolation

10.0g of the chloroform crude extract was dissolved in 10 ml chloroform and pre-adsorbed in 10.0g Merck 7731 60 G Silica gel. The chloroform was removed from the slurry under reduced pressure on a rotary evaporator. The dried pre-adsorbed extract was transferred to a mortar and ground to give a fine powder. A cylindrical sintered glass funnel (porosity 3) was filled with 7.0 cm of loose Merck 7731 60 G Silica gel and then tapped to give a level surface of silica gel. The glass funnel was placed in a Buchner flask and vacuum was applied. The silica gel was pressed firmly with a flat rubber stopper to give a flat, well compacted silica gel column of approximately 5.0 cm high. The silica gel at the circumference of the glass funnel was compressed with a clean spatula. The column was checked for voids and channels by pouring 100% petroleum ether onto the surface (protected by filter paper) while applying vacuum. The petroleum ether descended on a horizontal line indicating that the column was well packed. The fine powdered pre-adsorbed extract was added as a thin uniform layer on top of the column and compressed under pressure as above. A filter paper was placed on the surface of the loaded column to protect the surface of the column when the eluting solvent is poured. The column was developed by gradient elution.

The purified sample from the above column chromatography was subjected to preparative TLC. The different layers were scrapped out and eluted with chloroform to release the compounds. The result gave rise to WIPO 2.

Melting Point Determination:

The melting point of the compound was determined using Ernst LeitzWetzlar melting point apparatus.

Spectroscopic Analysis:

The isolated compound WIPO 2 was subjected to spectroscopic analysis for structural elucidation and characterization.

The NMR spectroscopic analyses One-Dimensional (1D) and Two-Dimensional (2D) were performed on WIPO 2 using Bruker Advanced FT-NMR 400MHz spectrometer. The analyses were carried out in $CDCl_3$. The results are shown in Table 1.Gas Chromatography – Mass Spectroscopy (GC-MS) was carried out on WIPO 2 to determine the molecular mass of the compoundsusing (GC-MS)machine. The results are as shown in Table 1. Infrared (IR) spectroscopic analysis was carried out on both WIPO 2 to determine the functional groups present in the compounds using Perkin Elmer Spectrum 100 FTIR Spectrometer.

RESULTS AND DISCUSSION

Chromatographic works on the chloroform extract of *Borreria stachydea* afforded WIPO 2. WIPO 2 was a light yellowish powder with melting point that ranges between 296-298^{o}C.

The structure of WIPO 2 was elucidated using ^{1}H NMR, ^{13}C NMR, Distortionless Enhancement Polarization Transfer (DEPT–135 and DEPT–90), 2D Correlation Spectroscopy (COSY), Nuclear Overhauser Enhancement Spectroscopy (NOESY), Heteronuclear Multiple Bond Coherence (HMBC), Heteronuclear Single Quantum Correlation (HSQC), Electron Impact Mass Spectroscopy (EI-MS) and Infrared (IR) Spectroscopy.

The ^{1}H NMR showed 48 proton signals. The signals between δ-0.70 and δ-1.97, may be due to the presence of triterpene skeleton while a signal at δ-3.20 may be due to a proton attached to oxygen. The ^{13}C NMR consists of 30 carbons peaks. The peak at δ-182.38 may be due to the presence of carbonyl group. The two peaks at δ-122.66 and δ-143.58 may be due to the presence of a pair of sp^2 hybridized carbons. The peaks at δ-28.11, 15.55, 15.33, 17.11, 25.92, 33.07 and 23.58 are likely due to methyl substituent. This is in agreement with the seven methyl (CH_3 positive) carbon atoms seen in the DEPT 135 experiment. Also DEPT–90, showed five methine (CH) carbon atoms with signals at δ-79.04, 55.22, 47.63, 122.66, and 41.04 respectively, while the DEPT 135 experiment showed ten methylene (CH_2 negative) groups whose signals are at δ-38.41, 27.20, 18.31, 32.65, 23.00, 27.69, 23.40, 45.89, 33.81 and 32.44 respectively. The COSY displayed a correlation between δ 1.60 and δ 3.20 while δ 1.06 also correlates with δ 1.88. The NOESY shows correlation of coupled protons in space. The HMBC displayed some important correlations between ^{1}H signals and ^{13}C signals. Some of these correlations include the cross peak between methyl proton peaks at δ-0.98 with carbon signals at δ-79.02, 38.41, 55.22 and 15.55 respectively. There were also correlations between methyl proton peaks at δ-0.77 with carbon signals at δ-79.02, 38.41, 55.22 and 28.11 respectively. Other correlations existed between methyl proton peaks at δ-0.92, with carbon signals at δ-45.89 and 33.81 respectively. The HMBC also shows a correlation between methyl proton peak at δ- 0.75 with carbon signals at δ-47.63 and 41.63 respectively.The HSQC correlates the ^{13}C in 1D with the ^{1}H shifts via one-bond CH coupling (J_{CH}) as shown in Table 1. The Electron Impact Mass Spectroscopy had a possible molecular ion [M+33] peak at m/z 489, which may correspond to a molecular mass of 456 and may be due to formula mass $C_{30}H_{48}O_3$. The Infrared (IR) showed a peak at 3346cm^{-1} which may be due to the presence of hydroxyl group, while the peak at 1687cm^{-1} may be due to the presence of carbonyl group. These two peaks may likely be due to the presence of carboxylic acid functional group (COOH). The peak at 1387cm^{-1} may be due to the presence of tri-substituted olefinic group, while the peak at 1456cm^{-1} may be due to the presence of $–CH_3$ and $–CH_2$- signals. The peak at 2937cm^{-1} may be due to the presence of C-H stretch for alkanes. This signal suggests that the molecule may be highly saturated.

Table 1: NMR Spectra Data for WIPO 2

Carbon position	^{13}C Shift (ppm) Experimental	Type	HSQC (J_{CH})
1	38.41	CH_2	0.97, 1.60
2	27.20	CH_2	1.60
3	79.04	CH	3.20
4	38.41	C_q	-
5	55.22	CH	0.70
6	18.31	CH_2	1.52
7	32.65	CH_2	1.30, 1.43
8	39.27	C_q	-
9	47.63	CH	1.53
10	37.09	C_q	-
11	22.96	CH_2	1.60, 1.64
12	122.66	CH	5.23
13	143.60	C_q	-
14	41.63	C_q	-
15	27.69	CH_2	1.06
16	23.40	CH_2	1.08, 1.88
17	46.51	C_q	-
18	41.04	CH	
19	45.89	CH_2	1.14
20	30.68	C_q	-
21	33.81	CH_2	1.30, 1.43
22	32.44	CH_2	1.76
23	28.11	CH_3	0.97
24	15.55	CH_3	0.76
25	15.33	CH_3	0.91
26	17.11	CH_3	0.75
27	25.92	CH_3	1.15
28	182.38	COOH	-
29	33.07	CH_3	0.88
30	23.58	CH_3	0.92

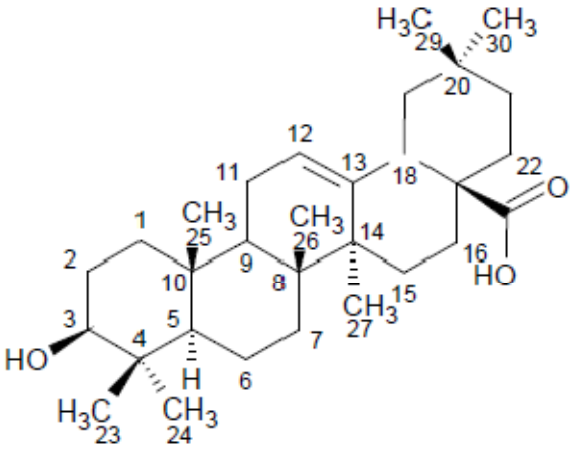
Based on the spectral data available, WIPO 2 is likely an oleanolic acid. From literature, it can be inferred that;

i. The ^{13}C signal at δ-182.38 indicated the presence of carbonyl group assigned to C-28. The two peaks at δ-122.66 and δ-143.58 represent the presence of a pair of sp^2 hybridized carbon atoms assigned to C-12 and C-13 while the

seven peaks at δ-28.11, 15.55, 15.33, 17.11, 25.92, 33.07 and 23.58 are attributable to the seven methyl groups which are assigned to C-23, C-24, C-25, C-26, C-27, C-29 and C-30 respectively. DEPT–90 shows five methine (CH) groups at peaks δ-79.04, 55.22, 47.63, 122.66, and 41.04 which are attributable to C-3, C-5, C-9, C-12 and C-18 respectively. DEPT 135 shows ten methylene (CH_2 negative) groups with signals at δ-38.41, 27.20, 18.31, 32.65, 23.00, 27.69, 23.40, 45.89, 33.81 and 32.44 which are attributed to C-1, C-2, C-6, C-7, C-11, C-15, C-16, C-19, C-21 and C-22 respectively [1].

ii. HMBCshows correlations between methyl proton peaks at δ-0.98 with carbon signals at δ-79.02, 38.41, 55.22 and 15.55 which is attributable to the correlation between H-23 and C-3, C-4, C-5 and C-24 respectively. There were also correlations between methyl proton peaks at δ-0.77 with carbon signals at δ-79.02, 38.41, 55.22 and 28.11 which are also attributable to the correlation between H-24 and C-3, C-4, C-5 and C-23 respectively. The correlation between methyl proton peaks at δ-0.92, with carbon signals at δ-45.89 and 33.81 corresponds to the correlation of H-30 and C-19, C-21 respectively. Also the correlation between methyl proton peak at δ- 0.75 with carbon signals at δ-47.63 and 41.63 corresponds to the correlation between H-26 and C-9, C-14 carbons respectively [4].

The physical and spectra data for WIPO 2 were in complete agreement with those reported in literature for oleanolic acid [5,6]. WIPO 2 was tentatively put as Oleanolic acid.



Oleanolic Acid

REFERENCES

[1] Neoh Bee Keat, RahayuUtami Umar, Nordin Haji Lajis, Tai Yuen Chen, Tu Yen Li, MawardiRahmani, Mohd. AspollahSukari (**2010**): *The Malaysian Journal of Analytical Sciences*. 14(1); 6-11.
[2] Muhammad Gulfraz, Abdul Waheed, SajidMehmood and MunazzaIhtisham (**2006**): *Ethnobotanical Leaflets* 10:13-23.
[3] Wink, M. (**1999**): Function of Secondary Metabolites, in Functions of Plant Secondary Metabolites and Their Exploitation in Biotechnology (Wink, M., ed.), Annual Plant Reviews, vol. 3, Sheffield Academic Press, Sheffield, UK, 1–16.
[4] Mahato, S.B. and Kundu, A.P. (**1994**): *Phytochemistry*, 37(6); 1517- 1575
[5] Nisar, Ullah (**1999**): Phytochemical Investigation of *Daphne oleoides*. Ph.DDissertation, International Centre for Chemical Sciences H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270
[6] Habila, J.D. (**2010**): The Synthesis of New Anti-Malaria and Muti-Targeted Bioactive Drugs. Unpublished Ph.D Dissertation. Ahmadu Bello University, Zaria, Nigeria.