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Short Communication

Isolation of Phytochemical Constituents from *Hunteria umbellata* K. Schum

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ABSTRACT

Hunteria umbellata K. Schum has been reported for the treatment of diabetes in Nigeria. In the present study, the ethanolic extract of dried leaves of *Hunteria umbellata* K. Schum was investigated for the chemical principles. The isolated pure compounds were characterized by NMR, IR and Mass spectral studies. Ursolic acid (**1**), oleanolic acid (**2**) and squalene (**3**) were the main constituents isolated from the extract.

Keywords: *Hunteria umbellata*, Ursolic acid, Oleanolic acid, Squalene

Introduction

Herbal medicine has been considered the best source of naturally produced compounds that account for drug discovery that is an expensive, long, and complex process.[1] The polyherbal formulations have been reported for the treatment of type 2 diabetes, a chronic metabolic disorder resulting from either insulin insufficiency or insulin dysfunction.[2, 3] As recently reported, approximately one-third of all stroke patients have diabetes[4], and diabetes mellitus is one of the risk factors associated with increased breast cancer mortality.[6, 7]
The genus Hunteria belongs to the family Apocynaceae. *Hunteria umbellata* K. Schum (Syn: *Carpodinus umbellatus* K. Schum.) has been reported to be used in the treatment of diabetes and obesity in Nigeria.[8] *Hunteria umbellata* K. Schum (*H. umbellata*) has been reported for its aphrodisiac effect,[9] and as phosphodiesterase type 5 (PDE-5) and arginase inhibitor,[10] antioxidant, hypolipidemic,[8] analgesic,[11] antiinflammatory[12] etc. *H. umbellata* has also been reported to decrease hepatic glucose-6-phosphatase activity.[13] Phytochemically the plant *H. umbellata* has been reported to contain saponins, steroids, and alkaloids.[14]
The main aim of this research was to investigate the ethanolic extract of *H. umbellata* for various chemical constituents. As a result of our investigation, ursolic acid (**1**), oleanolic acid (**2**) and squalene (**3**) were isolated and identified. However, compound (**2**) and (**3**) are being reported for the first time from *H. umbellata*.

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Materials and Methods

Instrumentation and chromatography
The IR spectra were recorded on JASCO FT/IR-5300 spectrophotometer. ^{1}H (300 MHz), ^{13}C (300 MHz; 62 MHz), and 2D NMR spectra were recorded using JEOL JNM-A500 and/or Varian INOVA-500 spectrometers. MS spectra were recorded using JEOL JMS-700 instrument. Silica-gel 60 (Merck) column chromatography was employed for isolation and purification, and pre-coated silica gel plates (Merck) were used for TLC study. The spots were visualized by spraying with ceric sulphate followed by heating.

Plant material
Fresh leaves of *H. umbellata* were collected from the Ugbowo Campus of the University of Benin, Nigeria in January 2011. The plant material was authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan and an herbarium specimen with the voucher number FHI-107678 was deposited.

Extraction and fractionation
The fresh leaves of *H. umbellata* were collected, dried in shade and powdered (500 g), macerated with 2L EtOH at room temperature for 32 hours. The crude extract was obtained by evaporating the filtrate solvent under reduced pressure at 40°C with the help of rotary evaporator.. The combined ethanolic extract (82 g) was further fractionated with n-heptane, chloroform, and ethyl acetate. The n-heptane soluble fraction was employed to medium pressure liquid chromatography (MPLC) to get 100% n-heptane and 100% $CHCl_3$ sub fractions.

Phytochemical isolation
The 100% n-heptane sub-fraction was subjected to column chromatography over silica gel eluting with n-heptane (100%), EtOAc:n-heptane (10% to 90%), EtOAc (100%) in increasing order of polarity. The fraction obtained from (100%) EtOAc elution gave ursolic acid (**1**, 110 mg). The 100% $CHCl_3$ sub fraction was subjected

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to CC over silica gel eluting with n-heptane (100%), EtOAc:n-heptane (10% to 90%), EtOAc (100%), MeOH:EtOAc (1% to 10%), MeOH (100%) in increasing order of polarity. The fraction obtained from (60%) EtOAc elution gave oleanolic acid (**2**, 32 mg) and in further elution the fractions obtained from (70% EtOAc:n-heptane to 5% MeOH:EtOAc) were combined and this yielded squalene (**3**, 7.3 mg). The structures of the isolated compounds as shown in figure 1, were determined using a combination of spectral techniques i.e. IR, ^{1}H-NMR, ^{13}C-NMR, and MS, the compound structures were further verified by direct comparison with the literature.

Results and Discussion

Ursolic acid (1)

Pure white solid; 110 mg; IR (ATR-Messung) ν_{max} cm^{-1}: 3400, 2924, 2869, 1687, 1455, 1386, 1028, 996, 661. ^{1}H-NMR (DMSO, 300 MHz) δ: 5.12 (brs, 1H, H-12), 4.26-4.28 (m, 1H, H-3), 3.49-3.37 (m, 1H), 1.09-1.55 (m, 7H), 1.44-1.29 (m, 11H), 0.64-1.03 (m, 27H). ^{13}C-NMR (DMSO-d_6, 300 MHz): See Table 1. HR-ESI-MS (negative ion mode) m/z: 455.3527. HR-EI-MS m/z: 456 (1), 262 (5), 248 (100), 203 (60), 133 (49), 69 (19), 55 (17), 44 (29), 39 (4). Compound (**1**) was identified as ursolic acid.

Oleanolic acid (2)

Colorless solid; 32 mg; IR (ATR-Messung) ν_{max} cm^{-1}: 3400, 2923, 2855, 1683, 1651, 1455, 1028, 996, 661. ^{1}H-NMR ($CDCl_3$/CD_3OH, 300 MHz) δ: 5.01 (brs, 1H, H-12), 4.00-3.98 (m, 1H, H-3), 3.49-3.37 (m, 1H, H-18),2.05-1.68 (m, 7H), 1.44-1.29 (m, 11H), 1.11-0.55 (m, 27H). ^{13}C-NMR ($CDCl_3$, 300 MHz): See Table 1. HR-ESI-MS (-ve ion mode) m/z: 455.3530.

Squalene (3)

Oily substance; 7.3 mg;IR (ATR-Messung, cm^{-1}): 2962, 2919, 2852, 1722, 1666, 1444, 1376, 1106, 834; ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.52 (18H, s, 6CH_3, H-24, H-25, H-26, H-27, H-28, H-29), 1.60 (6H, s, 2CH_3, H-1, H-30), 1.90-2.02 (20H, m, 10CH_2, H-4, H-5, H-8, H-9, H-12, H-13, H-16, H-17, H-20, H-21), 4.99-5.07 (6H, m, 6CH, H-3, H-7, H-11, H-15, H-19, H-22). ^{13}C-NMR ($CDCl_3$, 300 MHz): See Table 2; GC MS m/z (%): 410 (0.97), 341 (1.83), 149 (7.01), 81 (51.10), 69 (100), 41 (29.29); ESI MS (+ve ion mode): 410, 377, 338, 325, 301, 279 (100), 125.

Compound **1** was obtained as pure white solid. Based on HR-ESI-MS (negative ion mode) m/z: 455.3527 and ^{1}H-NMR and ^{13}C-NMR spectral data, the molecular formula of **1** was deduced to be $C_{30}H_{48}O_3$. The ^{1}H-NMR spectrum showed the presence of one broad singlet at δ 5.12. The ^{13}C-NMR spectrum exhibited 30 well resolved signals. The carbonyl carbon for the acidic group was observed at δ 179.1 (C-28), and the two olefinic carbon signals were observed at δ 125.5 (CH, C-12), and 139.0 (C, C-13). The oxygenated methine carbon signal was observed at δ 77.7 (CH, C-3). Similarly seven methyl, nine methylene, seven methine, and seven quaternary carbons were observed (Table 1). These data showed that compound **1** is a triterpene derivative. By comparison with the NMR spectral data in the literature,[15, 16] the structure of the compound **1** was characterized and identified as ursolic acid.

Compound **2** was obtained as colourless solid. Based on HR-ESI-MS (negative ion mode) m/z: 455.3530 and ^{1}H-NMR and ^{13}C-NMR spectral data, the molecular formula of **2** was deduced to be $C_{30}H_{48}O_3$. The ^{1}H-NMR spectrum indicated the presence of one broad singlet at δ 5.01 (1H, H-12). The ^{13}C-NMR spectrum exhibited 30 carbon signals. The carbonyl carbon for the acidic group was observed at δ 180.2 (C-28), and the two olefinic carbon signals were observed at δ 125.1 (CH, C-12), and 137.8 (C, C-13). The oxygenated methine carbon signal was observed at δ 78.3 (CH, C-3). Similarly seven methyl, ten methylene, five methine, and eight quaternary carbons were observed (Table 1). The spectral data showed that compound **2** is a triterpene derivative. By comparison with the previous NMR spectral data in the literature,[15, 16] the structure of the compound **2** was characterized and identified as oleanolic acid.

Compound **3** was obtained as oily substance. Based on GC-MS m/z: 410 and ^{1}H-NMR and ^{13}C-NMR spectral data, the molecular formula of **3** was deduced to be $C_{30}H_{50}$. The ^{1}H-NMR spectrum indicated the presence of one singlet at δ 1.52 for six methyl protons (18H, H-24, H-25, H-26, H-27, H-28, H-29). Another singlet, for two methyl protons, was observed at δ 1.60 (6H, H-1, H-30). Similarly two multiplet signals were observed at δ 1.90-2.02, and 4.99-5.07 for ten methylene protons (20H; H-4, H-5, H-8, H-9, H-12, H-13, H-16, H-17, H-20, H-21) and six methine protons (6H; H-3, H-7, H-11, H-15, H-19, H-22), respectively. The ^{13}C-NMR spectrum exhibited 15 carbon signals. A signal at δ 15.9 was assigned to two methyl carbons (C-27, C-28), and another signal at δ 16.0 was assigned to two other methyl carbons (C-26, C-29). Two methyl carbons (C-25, C-30) were observed at δ 17.6, and methyl carbons (C-1, C-24) were observed at δ 25.6. The methylene carbons were observed at δ 26.6 (C-8, C-17), 26.7 (C-4, C-21), 28.2 (C-12, C-13), 39.7 (C-9, C-16), and 39.7 (C-5, C-20). While the methine carbons were resonating at δ124.2 (C-7, C-18), 124.2 (C-11, C-14), and 124.3 (C-3, C-22). The quaternary carbons were observed at δ 131.2 (C-2, C-23), 134.8 (C-6, C-19), and 135.0 (C-10, C-15). By comparison with the previous NMR spectral data in literature,[17] the structure of the compound **3** was characterized and identified as squalene.

H. umbellata has been investigated for preliminary analysis of saponins and its glycosides, alkaloids, steroid, tannins, oils, etc.[14] Other available reports[18-20] have focused on the phytochemical analysis of *H. umbellata* for the isolation of alkaloids. The triterpenoid saponin derivatives i.e. ursolic acid (**1**) and oleanolic acid (**2**) have already exhibited antimicrobial properties and its mechanism is established.[21, 22]Oleanolic acid has also exhibited anti-tubercular activity.[23] The derivatives of oleanolic acid have been reported for the treatment of pancreatic cancer,[24] and oleanolic acid saponins have shown significant antimicrobial properties,[25] and the combination of oleanolic acid with hypocrellin A has been reported for the treatment of hepatocellular carcinoma diseases,[26] and oleanolic acid derivative SZC014 has exhibited anticancer activity.[27]Oleanolic acid has shown antidiabetic,[28, 29] and hepatoprotective activities,[30,31] which may establish the use of *H. umbellata* as antidiabetic by the local inhabitants in Nigeria.

Conclusion

The present study reveals that the plant extract contains certain bioactive compounds. The triterpenoids including ursolic acid and oleanolic acid isolated from this plant have previously been shown to have hepatoprotective and antiinflammatory potentials.

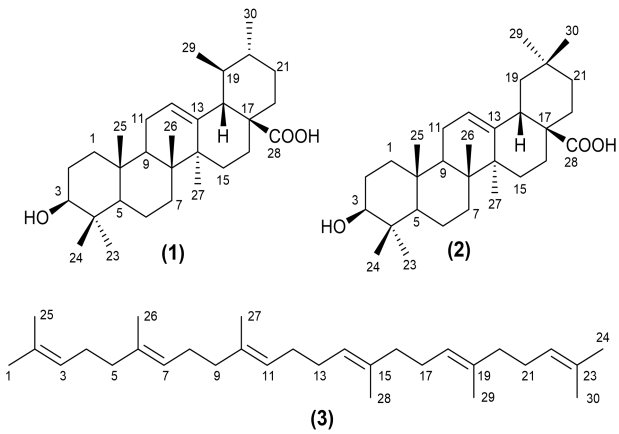

Figure 1: Molecular structures of chemical constituents isolated from *H. umbellata*.

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Table 1: ^{13}C-NMR chemical shifts (δ, ppm) of Compounds (**1**) in DMSO-d_6, 300 MHz and (**2**) in $CDCl_3$, 300 MHz.

C No	Compound (1) δ C		Literature [15]	Literature [16]	Compound (2) δ C		Literature [15]	Literature [16]
1	39.1	CH_2	39.2	40.3	38.3	CH_2	39.0	38.7
2	27.9	CH_2	28.2	29.4	27.6	CH_2	28.1	27.8
3	77.7	CH	78.2	79.4	78.3	CH	78.2	77.8
4	39.3	C	39.6	40.6	39.0	C	39.4	39.1
5	55.7	CH	55.9	57.1	54.9	CH	55.9	55.5
6	18.9	CH_2	18.8	19.9	17.9	CH_2	18.8	18.7
7	33.6	CH_2	33.7	34.8	32.6	CH_2	33.4	33.0
8	40.0	C	40.1	41.2	38.2	C	39.8	39.5
9	47.7	CH	48.1	49.3	52.4	CH	48.2	49.4
10	37.2	C	37.5	38.5	36.5	C	37.4	37.1
11	23.7	CH_2	23.7	24.9	23.8	CH_2	23.8	23.6
12	125.5	CH	125.7	126.9	125.1	CH	122.6	122.3
13	139.0	C	139.3	140.5	137.8	C	144.8	144.6
14	40.4	C	42.6	43.7	41.6	C	42.2	41.9
15	28.4	CH_2	28.8	29.9	29.2	CH_2	28.4	28.1
16	24.7	CH_2	25.0	26.2	22.8	CH_2	23.8	23.6
17	42.5	C	48.1	49.3	47.3	C	46.7	47.9
18	53.2	CH	53.6	54.8	38.7	CH	42.1	41.8
19	39.4	CH	39.5	40.7	36.4	CH_2	46.6	46.2
20	39.2	CH	39.4	40.6	29.2	C	31.0	30.7
21	31.1	CH_2	31.1	32.3	30.2	CH_2	34.3	34.0
22	37.4	CH_2	37.4	38.7	26.3	CH_2	33.2	32.9
23	29.1	CH_3	28.8	30.1	27.5	CH_3	28.8	28.5
24	16.9	CH_3	16.5	17.5	16.4	CH_3	16.5	16.6
25	16.1	CH_3	15.7	16.4	14.9	CH_3	15.6	15.4
26	17.8	CH_3	17.5	18.5	16.5	CH_3	17.5	17.7
27	24.1	CH_3	24.0	25.2	20.5	CH_3	26.2	25.9
28	179.1	C	179.7	181.6	180.2	C	180.0	180.0
29	17.9	CH_3	17.5	18.6	15.1	CH_3	33.4	33.0
30	21.9	CH_3	21.4	22.3	23.0	CH_3	23.8	23.6

Table 2: ^{13}C-NMR chemical shifts (δ, ppm) of Compound (**3**) in $CDCl_3$, 300 MHz.

C No.	Compound (3) δ C		Literature[17]
1, 24	25.67	CH_3	25.67
2, 23	131.22	C	131.22
3, 22	124.39	CH	124.40
4, 21	26.76	CH_2	26.77
5, 20	39.74	CH_2	39.75
6, 19	134.87	C	134.88
7, 18	124.26	CH	124.20
8, 17	26.65	CH_2	26.66
9, 16	39.72	CH_2	39.72
10, 15	135.08	C	135.08
11, 14	124.29	CH	124.30
12, 13	28.26	CH_2	28.27
25, 30	17.66	CH_3	17.65
26, 29	16.02	CH_3	16.02
27, 28	15.98	CH_3	15.98

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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