IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 15, Issue 7 Ser. I (July 2022), PP 01-06
www.iosrjournals.org

Triterpenes and Sterols from *Ocimum suave*

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Abstract
Ocimum species which belong to the family Lamiaceae have several medicinal uses including antimalarial, antimicrobial, antidiabetic, hepato-protective, anti-inflammatory, anti-carcinogenic, cardio-protective and insect repellent. Ocimum suave Willd is used in traditional medicine to treat ulcers, fever, stomach ache, and bronchopneumonic infections. However, information about bioactive principles from the plant is scanty. This study was conducted to determine the chemical composition of Ocimum suave. Hexane and ethyl acetate extracts from the plant were subjected to chromatographic fractionation using organic solvents. This led to isolation and identification of five compounds namely β-amyrin, betulinic acid, lupeol, β-sitosterol and stigmasterol. The structures of the compounds were determined using spectroscopic method of analysis. Further studies aimed in identification of bioactive compounds from extracts of the plant, especially from polar solvent which are in traditional medicine are needed.

Keywords*: Ocimum suave; solvent extraction; ^{1}H NMR; ^{13}C NMR; Column chromatography.*

Date of Submission: 20-06-2022 Date of Acceptance: 03-07-2022

I. Introduction

Utilization of plants for medicinal benefits has been practiced for several thousands of years. In many developing countries, traditional medicine plays an important role in meeting the primary healthcare needs of the population and native healers are the main health providers for millions of people living in rural areas[1, 2]. For example, the ratio of traditional health practitioners to citizens in Africa is 1:500, whereas the ratio of medical doctors to citizens is 1:40,000[3]. Traditional medicine is often seen as more accessible, more affordable, and more acceptable to local populations and can therefore be a tool to help in achieving the universal health coverage.

Plants produce important metabolites that are used in defense against pests and pathogenic microorganism[4-14]. Search for insecticidal compound from plants through *in-vivo* and in-vitro experiments has led to identification of important compounds which include alkaloids, terpenoids, flavonoids, steroids and quinones[5,6, 15-20]. Such compounds represent an important source of drugs in the process of developing new pharmacologically active compounds. The use of botanical for pests and disease control is preferred because they are environmentally friendly and non-toxic to non-targeted organisms [13, 21-33]. In addition, chances of pests and pathogens developing resistance to botanical pesticides are highly unlikely[34].

Ocimum species have been reported to exhibit antimicrobial, antidiabetic, hepato-protective, anti-inflammatory, anti-carcinogenic, cardio-protective and insect repellent activities[6, 16, 35]. *Ocimum suave* Willd is used in traditional medicine to treat ulcers, fever, stomach ache, and bronchopneumonic infections[36]. Previous studies have shown that extracts from the plant are toxic to many pathogenic microorganisms and insect pests[35, 37]. Despite the wide application of *Ocimum suave* in traditional medicine, reports on the chemical compounds responsible for the plant's medicinal activities are scanty. This study reports the isolation and characterization of five compounds from the plant.

II. Materials and Methods

General

Melting points were determined on a Gallenkamp (Loughborough, UK) melting point apparatus and are uncorrected. IR data were recorded on a PerkinElmer FTIR 600 series spectrophotometer (Waltham, MA, USA) as KBr pellet. The ^{1}H and ^{13}C NMR data were measured in $CDCl_3$ and $CDCl_3$-DMSO-d_6 on a Bruker NMR Ultrashield TM (Darmstadt, Germany) operating at 500 and 125 MHz, respectively. The MS data were obtained on a Varian MAT 8200A instrument (Bremen, Germany).

Collection and Preparation of Plant Materials

Plant materials were collected from Kitambo village in Kenya. Identified of the sample was done at Maseno University Herbarium after comparison with authentic samples. The plant materials were chopped into

small pieces, air dried and ground into fine powder using a mill. Powdered plant material (2 kg) was extracted sequentially with *n*-hexane, ethyl acetate and methanol by soaking the material in the solvent for seven days with occasional shaking. The mixture was filtered and the solvent evaporated using rotary evaporator to yield 49.5, 70.8 and 120 g of *n*-hexane, ethyl acetate and methanol extracts, respectively. The extracts were stored at 4°C in brown glass bottles.

Chromatographic fractionation

Hexane extract (40 g) was dissolved in small amount of n-hexane and adsorbed onto silica gel for column chromatography. Fractionation of the extract using gradient of n-hexane-ethyl acetate afforded 200 fractions (20 ml each) whose composition were monitored by TLC using solvent systems n-hexane-ethyl acetate 9:1, 4:1 and 2:1. Fractions with similar TLC profiles were combined resulting into four pools (I-IV). Pool II (fractions 24-76, 18 g) contained two major spots and was further purified using medium pressure chromatography (pressure ≈ 1 bar), eluting with n-hexane-ethyl acetate (9:1 and 4:1) to give compound **1** (124 mg) and compound **2** (88 mg). Pool III (fractions 77-143, 12 g) on subjected to repeated fractionation using n-hexane-ethyl acetate (4:1 and 3:1) yielded compound **2** (78 mg), compound **3** (84 mg) and compound **4** (65 mg). Pool IV (fractions 144-200, 7.2 g) gave compound **2** (24 mg) and compound **4** (34 mg).

Ethyl acetate extract (40 g) was pre-adsorbed onto silica gel and chromatographed with n-hexane-ethyl acetate gradient to pure ethyl acetate to afford 133 fractions of 20 ml each. The composition of the fractions was monitored by TLC using hexane-ethyl acetate mixtures 4:1, 3:2 and 1:1. Fractions that exhibited similar TLC profiles were combined to constitute two major pools (V and VI). Pool V (fractions 33-79, 17 g) was further purified by chromatography using n-hexane- ethyl acetate (4:1) followed by the same solvent system in the ratio 3:2 to give compound **3** (53 mg), compound **4** (42 mg) and compound **5** (96 mg). The remaining fractions (pool VI, 6 g) contained one major compound as shown by its TLC profile. The fraction was further purified by chromatography using n-hexane-ethyl acetate (3:2) followed by the same solvent system in the ratio 1:1 to yield compound **5** (26 mg).

Compound 1: White crystals, mp 129-131.IR ν_{max} (KBr, cm^{-1}) 3424, 2921, 1640, 1462, 1380, 1048, 1022, 723. ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 5.39 (1H, d, *J* = 7.2 Hz), 3.50 (m), 1.02 (3H, s), 0.94 (3H, d, *J* = 8.4 Hz), 0.86 (9H, m), 0.70 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MH_Z) δ ppm (Table 1).

Compound 2: White crystals, mp 168-170 °C, ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 5.19 (1H), 5.08 (1H, dd, *J* = 15.3, 8.1 Hz), 5.14 (1H, d, *J* = 15.3 H_Z), 3.53(1H, m) 1.01, 0.91, 0.85, 0.81, 0.77, 0.68; ^{13}C NMR ($CDCl_3$, 125 MH_Z) δ ppm (Table 1).

Compound 3: White crystals mp 181-182 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.21 (t, 1H, *J*= 3.6, H-12), 3.23 (dd, 2H, *J*=10.8, 4.8, H-3), 1.28 (s, 3H, H-27), 1.11 (s, 3H, H-26), 1.01 (s, 3H, H-28), 0.98 (s, 3H, H-25), 0.95 (s, 6H, H-29, 30) 0.80 (s, 3H, H-23), 0.79 (s, 3H, H-24). ^{13}C NMR ($CDCl_3$, 125 MH_Z) δ ppm (Table 1).

Compound 4: White crystals, mp 128-130 °C; IR ν_{max} (KBr, cm^{-1}) 3433, 2942, 2357, 1663, 1564, 1417, 1035 and 889; ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 4.63 (1H, d), 4.56 (1H, d), 3.16 (1H, t), 1.67 (3H, s, H-30), 1.02 (3H, s, H-26), 0.96 (3H, s, H-23), 0.94 (3H, s, H-27), 0.82 (3H, s, H-25), 0.78 (3H, s, H-28) and 0.75 (3H, s, H-24). ^{13}C-NMR ($CDCl_3$, 125 MH_Z) δ ppm (Table 1).

Compound 5: White crystals, mp 279-280°C IR ν_{max} (KBr) cm^{-1}: 3455, 3072, 2942, 2869, 1687, 1642, 1458, 1387; ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm: 4.71 (1H, *br*. s, H-29a), 4.63 (1H, *br*. s, H-29b), 3.20 (1H, dd, *J* = 8.2, 6.0 Hz, H-3), 1.69, 0.97, 0.97, 0.96, 0.83, 0.75 (18H, s, 6 x Me); ^{13}C NMR: ($CDCl_3$, 125 MH_Z) δ ppm (Table 1).

III. Results and Discussion

Repeated chromatographic fractination of *n*-hexane and ethyl acetate extracts from *O. suave* leaves afforded five compounds (Figure 1) which were identified as β-sitosterol [**1**], stigmasterol [**2**], β-amyrin [**3**], lupeol [**4**] and betulinic acid [**5**]. The structures of the compounds were determined using spectroscopic methods. Mass spectrum of compound **1** displayed the molecular ion peak at m/z 437 $[M+Na]^+$ which corresponded to molecular formula of $C_{29}H_{50}O$. The ^{1}H-NMR spectrum of the compound showed a signal at δ 5.39 (1H, d *J*=7.2 Hz), a multiplet at δ 3.50 and six signals at δ 1.02 (3H, s), 0.94 (3H, d, *J* = 8.4 Hz), 0.86 (9H, m) and 0.70 (3H, s) which are characteristic of a sterol[15, 21, 38]. The ^{13}C-NMR and DEPT data of the compound showed the presence of six methyl carbons, 11 methylene carbons, nine methine carbons and three quaternary carbons (Table 1). ^{13}C-NMR data also showed the presence of two olefinic carbons at δ 140.5 and 121.3 ppm which were assigned to C-5 and C-6 carbons respectively, and one oxymethine carbon at δ 71.8 assignable to C-3 carbon. Based on the spectral data which was in agreement with literature data[11] compound **1** was identified to be β-sitosterol.

Table no 1: ^{13}C-NMR DEPT data of Compounds 1-5

	^{13}C-NMR (δ_C multplicity)				
C	Compound **1**	Compound **2**	Compound **3**	Compound **4**	Compound **5**
1	37.3 t	37.3 t	38.8 t	38.7 t	38.9 t
2	31.9 t	31.7 t	27.4 t	27.4 t	27.6 t
3	71.8 d	71.6 d	79.2 d	79.2 d	79.1 d
4	42.3 t	42.2 t	39.0 s	38.9 s	38.5 s
5	140.5 s	140.6 s	55.4 d	55.3 d	55.6 d
6	121.3 d	121.4 d	18.6 t	18.3 t	18.4 t
7	31.7 t	31.9 t	32.9 t	34.3 t	34.5 t
8	33.9 d	31.7 d	40.2 s	40.8 s	40.8 s
9	50.2 d	50.2 d	47.4d	50.4 d	50.6 d
10	36.5 s	36.5 s	37.2 s	37.2 s	37.4 s
11	21.1 t	21.1 t	23.8 t	20.9 t	21.1 t
12	39.5 t	39.7 t	121.8 d	25.1 t	25.6 t
13	42.3 s	42.3 s	145.3 s	38.1 d	38.9 d
14	56.7 d	56.9 d	41.9 s	43.0 s	42.5 s
15	24.3 t	24.4 t	26.4 t	27.4 t	29.7 t
16	28.2 t	28.9 t	27.2 t	35.6 t	32.3 t
17	56.1 d	56.0 d	32.8 s	43.1 s	56.8 s
18	12.1 q	12.1 q	47.9 d	48.3 d	46.9 d
19	19.0 q	19.4 q	47.1 t	48.0 d	49.5 d
20	36.5 d	40.4 d	31.2 s	151.2 s	150.6 s
21	19.3 q	21.0 q	37.3 t	29.8 t	30.6 t
22	31.9 t	138.1 d	34.8 t	40.0 t	37.2 t
23	26.7 t	129.6 d	28.2 q	28.0 q	28.2 q
24	45.9 d	51.2 d	15.7 q	15.4 q	15.2 q
25	29.2 d	31.9 d	15.5 q	16.0 q	16.2 q
26	18.9 q	19.1q	17.2 q	16.0 q	16.2 q
27	19.8 q	21.4 q	26.2 q	14.6 q	14.7 q
28	23.1 t	25.4 t	28.6 q	18.0 q	180.2 s
29	11.7 q	12.8 q	33.6 q	109.1 t	109.5 t
30			23.8 q	19.3 q	19.6 q

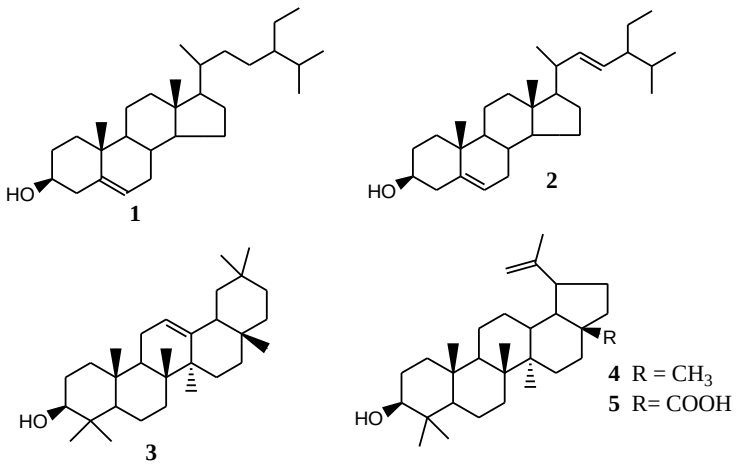

Figure no 1. Structures of compounds isolated from *Ocimum suave*

Mass spectrum of compound **2** gave the molecular ion peak at m/z 412 that corresponded to molecular formula $C_{29}H_{48}O$. The mass spectrum further gave fragment ions peaks at m/z 369 $[M\text{-}C_3H_7]^+$, 300 $[M\text{-}C_8H_{17}]^+$ and 271 $[M\text{-}C_{10}H_{21}]^+$ which are associated with sterols[13, 16, 38]. The ^{13}C NMR and DEPT data showed presence of twenty nine carbon atoms consisting of six methyl, nine methylene, eleven methine and three quaternary carbon atoms. ^{13}C NMR further showed presence of four olefinic carbons at δ 121.4, 129.6, 138.1 and 140.6 ppm which were assigned to C-6, C-23, C-22 and C-5 respectively; one oxymethine carbon at δ 71.6 ppm which was assigned to C-3; and six methyl carbon at δ 12.1, 12.8, 19.1, 19.4, 21.0 and 21.4 ppm which were assigned to C-18, C-29, C-26, C-19, C-21 and C-27 respectively. The ^{1}H NMR spectrum of the compound showed the presence of three olefinic protons at δ 5.19 m, 5.14 d (J = 15.3 Hz) and 5.08 dd (J = 15.3, 8.1 Hz);

one oxymethine proton at δ 3.53 m and six methyl carbons at δ 1.01, 0.91, 0.85, 0.81, 0.77 and 0.68 corresponding to two tertiary, three secondary and one primary carbons[10, 39]. Based on the spectral data as well at comparison with literature information, compound **2** was identified to be stigmasterol.

ESI-MS spectrum of compound **3** gave a molecular ion peak at *m/z* 449 $[M+Na]^+$ which corresponded to a molecular mass of 426 and a molecular formula of $C_{30}H_{50}O$. The ^{1}H NMR spectrum gave olefinic peak at δ 5.21 (t, *J* = 3.6 Hz) which was assigned to H-12 and an oxymethine peak at δ 3.23 (dd, *J* = 10.8, 4.8 Hz) which was assigned to H-3. The large coupling constant (*J* = 10.8 Hz) observed for H-3 showed the OH to be in equatorial position[40]. The ^{1}H NMR spectrum also showed the presence of eight methyl groups at δ 1.28 (s, 3H, H-27), 1.11 (s, 3H, H-26), 1.01 (s, 3H, H-28), 0.98 (s, 3H, H-25), 0.95 (s, 6H, H-29, 30) 0.80 (s, 3H, H-23) and 0.79 (s, 3H, H-24). ^{13}C NMR and DEPT data (Table 1) exhibited 30 carbon peaks corresponding to eight methyl carbons, nine methylene carbons, seven methine carbons and six quaternary carbon atoms. The ^{13}C NMR data showed the presence of two double bond carbons at δ 145.3 and 121.8 ppm which were assigned to C-13 and C-12 respectively, one oxymethine peak at δ 79.2 ppm, and eight tertiary methyl carbon peaks at δ 28.2 (C-23), 15.7 (C-24), 15.5 (C-25), 7.2 (C-26), 26.2 (C-27), 28.6 (C-28), 33.6 (C-29) and 23.8 (C-30). Based on the spectral the data as well as comparison with literature data[40, 41], compound **3** was identified as β-amyrin.

The mass spectrum of compound **4** gave a molecular ion peak at m/z 426 $[M^+]$ which corresponded to a molecular formula of $C^{30}H_{50}O$. The spectrum also showed fragment ion peaks at m/z 411 $[M\text{-}Me]^+$, 355 $[M\text{-}Me\text{-}3H_2O]^+$, 220 $[M\text{-}C_{15}H_{26}]^+$, 207 $[M\text{-}C_{16}H_{27}]^+$, 189 and $[M\text{-}C_{16}H_{29}O]^+$. The ^{1}H-NMR spectrum gave two singlets at δ 4.63 and 4.56 ppm which were assigned to the terminal olefinic protons at H-29. The spectrum also gave a multiplet peak at δ 3.16 ppm which indicated the presence of oxymethine group and was assigned to H-3. Seven singlet peaks attributed to methyl groups of a pentacyclic terpene were observed at δ 1.67, 1.02, 0.96, 0.94, 0.82, 0.78 and 0.75 ppm [38, 42]. The ^{13}C-NMR data and DEPT experiment showed the presence of 30 carbon atoms which further showed the compound to be a triterpenene. Signals at δ 151.2 and 109.1 ppm were assigned to the double bond carbons at C-20 and C-29 while the peak at δ 79.3 ppm was assigned to the oxymethine carbon atom at C-3. The ^{13}C-NMR spectrum also showed seven methyl signals at δ_C 14.6, 15.4, 16.0, 16.1, 18.0, 19.3 and 28.0 ppm which were assigned to C-23-C-28 and C-30. Based on the spectral data as well as comparison with literature data[38, 42] compound **4** was identified as lupeol.

The EIMS spectrum of compound **5** afforded a molecular ion peak at *m/z* 456 which corresponded to a molecular formula of $C_{30}H_{48}O_3$. EIMS further showed fragment peaks at *m/z* 438 $[M\text{-}H_2O]^+$, 423 $[M\text{-}Me\text{-}H_2O]^+$ and 411 $[M\text{-}45]^+$. The ^{1}H NMR spectrum showed the presence of two singlets at δ 4.71 and 4.63 ppm corresponding to two protons of a terminal vinyl methylene group, a vinyl methyl group centered at δ 1.69 ppm and oxymethine peak at δ 3.20 ppm assignable to H-3. The^{13}C NMR spectrum of the compound exhibited 30 carbon peaks consisting of a carbonyl peak at δ 180.2 ppm, a quaternary vinyl carbon at δ 150.6 ppm, a terminal methylene carbon at δ 109.5 ppm and an oxymethine carbon peak at δ 79.1 ppm. The ^{13}C NMR data also showed the presence of: five quaternary carbons at δ 37.4, 38.5, 40.8, 42.5 and 56.8ppm corresponding to C-10, C-5, C-8, C-14 and C-17 respectively; ten methylene carbons at δ 18.4, 21.1, 25.6, 27.6, 29.7, 30.6, 32.3, 34.5, 37.2 and 38.9 ppm assigned to C-6, C-11, C-12, C-2, C-15, C-21, C-16, C-7, C-22 and C-1 respectively; five methine carbons at δ 55.6, 50.6, 49.5, 46.9 and 38.9, ppm assigned to C-5, C-9, C-19, C-18 and C-13 respectively; and six methyl carbons at δ 14.7, 15.2, 16.2, 16.2, 19.6 and 28.2 ppm assigned to C-27, C-24, C-25, C-26, C-30 and C-23 respectively (Table 1). Based on these spectral data and literature data[43], the compound was identified to be betulinic acid.

IV. Conclusion

Previous studies show that Ocimum suave has numerous bioactivities but the information about the bioactive principles from the plant is not available. This study reports the isolation and identification of five compounds from hexane and ethyl acetate extracts from the plant. Further studies aimed in identification of bioactive compounds from extracts of the plant, especially from polar solvent which are in traditional medicine are needed.

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Sylvia A. Opiyo. "Triterpenes and Sterols from Ocimum suave." *IOSR Journal of Applied Chemistry (IOSR-JAC)*, 15(07), (2022): pp 01-06.