SUPPLEMENTARY MATERIAL

Natural Antifungal Compounds from the Peels of *Ipomoea batatas* Lam

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Abstract

Three antifungal compounds have been isolated for the first time from the peels of *Ipomoea batatas* Lam. Their structures were established on the basis of 1D and 2D NMR spectra data as well as ESI-MS and IR analysis. Urs-13(18)-ene-3 β -yl acetate was found to possess a weak activity against *Sporothrix schenckii* and *Trichophyton metagrophytes* fungi with an MIC value of 50 μ g/mL each. Stigmasterol and 3-Friedelanol were equally active against *Trichophyton metagrophytes*.

Keywords: Peels, *Ipomoea batatas*, stigmasterol, 3-friedelanol, *Sporothrix schenckii*, *Trichophyton metagrophytes*

Experimental

General experimental procedures: Melting points were determined on a Stuart Melting point Apparatus SMP30 and are uncorrected. IR spectra were measured on an Agilent Cary 630 FTIR Spectrometer.

The ^1H and ^{13}C NMR spectra were obtained on a 400 MHz Bruker (Switzerland) and chemical shifts are given in δ (ppm) with TMS as reference. ESI-MS were obtained on Waters UPLC-TQD Mass spectrometer. Column chromatography (CC) was performed on silica gel (100-200 mesh), thin layer chromatography was performed on a pre-coated silica gel GF₂₅₄ 0.25mm Mark W. Germany.

Plant Material

White skinned variety of sweet potato (*Ipomoea batatas* Lam) was obtained from a local market in Ilorin, Nigeria and identified by a taxonomist at the herbarium of Biological Sciences Department of the University of Ilorin, Ilorin, Nigeria where a voucher specimen number UIH 001/486 was obtained. The peels were carefully removed and air-dried at room temperature. The peels unavoidably contained a little of the sweet potato tissue.

Extraction and isolation

The dried peels (1.7Kg) of the white skinned variety of *Ipomoea batatas* Lam was percolated with 95% (5 x 5L) at room temperature. The extract was concentrated each time with the aid of a rotary evaporator at about 50°C. The ethanolic extract was sonicated in n-hexane to obtain the hexane fraction. The solution was concentrated to give a residue (36.6g). This was separated over silica gel into 3 fractions F002 a, b and c. F002a (10.57g) was separated by silica gel cc using n-hexane/EtOAc (1:0 $\rightarrow$ 0:1, v/v), as eluent affording 9 fraction combinations. Fractions 9-25 afforded compound **I** (30mg), fractions 66-73 yielded compound **III** (4.5mg) while repeated column chromatography of fractions 79-113 (1.855g) yielded compound **II** (15mg).

Urs-13(18)-ene-3 β -yl acetate (I)

Mp: 239-241°C IR (KBr) $\nu_{\max}$: 3019.29, 2957.25, 1723.41, 1523.21 cm^{-1} ESI-MS: 492.3 (M+Na)⁺ HR-ESIMS: 469.3378; HNMR (CDCl₃): 0.7845 (3H, s, H-28), 0.8546 (3H, s, H-23), 0.8644 (3H, s, H-24), 0.8866 - 0.9032 (3H, d, H-29, J=6Hz), 0.9310 - 0.9475 (3H, d, J=6 Hz, H-30), 0.9520 (3H, s, H-25), 0.9973 (3H, s, H-26), 1.0540 (3H, s, H-27), 4.4813-4.5234 (1H, dd, J=5.4, 11.5Hz, H-3). For the Carbon-13 spectra, see table S1.

Stigmasterol (II)

Mp: 134-135°C IR (KBr) $\nu_{\max}$: 3399.86, 3019.69, 1650, 1215.69, 1068.84 cm^{-1} ; ESI-MS: 413 (M+H)⁺, HR-ESIMS: 413.2731 (M+H)⁺; HNMR (CDCl₃): 0.7057 (3H, s, H-29), 0.7239 (3H, s, H-18), 0.8304 (3H, s, H-27), 0.8634 (3H, s, H-26), 1.0348 (3H, s, H-19), 1.0549 (3H, d, J=8.0 Hz, H-21), 3.5481 (1H, m, J= 4.4, 6.5, 4.7Hz, H-3), 5.0129-5.0346 (1H, dd, J=8.5, 6.6Hz, H-23), 5.1504-5.2097 (1H, dd, J=8.5, 6.6Hz, H-22), 5.3690-5.3819 (1H, dd, J=5.1Hz, H-6). For carbon 13 spectra data, see table S1.

3-Friedelanol (III)

Mp: 276°C IR (KBr) $\nu_{\max}$: 3398.97, 3019.73, 1215.77, 1088.78 cm^{-1} ESI-MS: [M-H₂O+H]⁺ at 411 m/z HR ESIMS: 429.1858; HNMR (CDCl₃): 0.8564 (3H, s, H-27), 0.9250 (3H, s, H-28), 0.9425 (3H, s, H-26), 0.9618 (3H, s, H-24), 0.9881 (3H, s, H-25), 0.9919 (3H, s, H-29), 0.9919-1.0030 (3H, d, J=4.4Hz, H-23), 1.1672 (3H, s, H-30), 1.9 (2H, m, H-2), 3.72 (1H, m, H-3). For carbon 13 spectra data, see table S1.

Antimicrobial Activity

Fungi were tested by NCCLS method in RPMI 1640 medium (2002) and bacteria in Mueller Hinton Broth against 1. *E. coli* (ATCC 9637), 2. *Pseudomonas aeruginosa* (ATCC BAA-427), 3. *Staphylococcus aureus* (ATCC 25923), 4. *Klebsiella pneumoniae* (ATCC 27736), 5. *Candida albicans* 6. *Cryptococcus neoformans* 7. *Sporothrix schenckii*, 8. *Trichophyton mentagrophytes*, 9. *Aspergillus fumigatus* 10. *Candida parapsilosis* (ATCC-22019).

Carbon	I	II	III
1	30.76	37.27	35.34
2	25.72	28.92	35.56
3	81.48	71.82	72.76
4	37.43	42.33	53.2
5	48.59	140.77	39.68
6	19.22	121.72	41.73
7	35.23	31.92	17.55
8	43.17	31.69	49.18
9	46.69	50.16	37.84
10	38.58	36.52	61.36
11	23.01	24.37	15.79
12	27.97	23.09	30.64
13	131.49	39.7	38.38
14	41.77	56.78	37.11
15	38.87	25.41	36.09
16	33.3	28.25	32.34
17	42.92	55.98	29.71
18	142.23	12.25	42.83
19	59.46	12.06	32.82
20	30.2	40.48	30.03
21	26.85	19.4	32.34
22	26.79	138.31	39.28
23	17.56	129.29	18.64
24	29.33	51.24	16.39
25	23.28	29.12	20.12
26	26.06	19.82	11.62
27	27.09	19.04	18.24
28	18.29	21.09	35.19
29	23.45	11.87	31.79
30	23.33		32.09
<u>C</u> OO	171.51		
<u>C</u> H ₃ COO	21.69		

Table S1: ¹³C Assignments for Compounds I-III

Figure S1: Biosynthetic Pathway for Compounds I-III (Jenner, 2005; Jacinda and Ian, 2009)

Code No.	Minimum inhibitory conc. (MIC) in $\mu\text{g/ml}$ against									
	BACTERIA				FUNGI					
	1	2	3	4	5	6	7	8	9	10
Compound I	>50	>50	>50	>50	>50	>50	50	50	>50	>50
Compound II	>50	>50	>50	>50	>50	>50	>50	50	>50	>50
Compound III	>50	>50	>50	>50	>50	>50	>50	50	>50	>50
Gentamycin	3.12	0.78	0.78	1.56	ND	ND	ND	ND	ND	ND
Norfloracin	0.048	1.56	0.78	0.19	ND	ND	ND	ND	ND	ND
Fluconazole	ND	ND	ND	ND	1	2	2	>32	>32	2
Amphotericin-B	ND	ND	ND	ND	0.016	0.125	0.25	0.25	0.5	0.016

Table S2: Antifungal Activity of Compounds I-III

S1: HNMR Spectra of Compound I (Urs-13(18)-ene-3 β -yl acetate)

S2: COSY Spectra of Compound I (Urs-13(18)-ene-3 β -yl acetate)

IB-K3

S3: HSQC Spectra of Compound 1

IB-K3 HSQC

S4: HMBC Spectra of Compound I

S5: TOCSY Spectra of Compound I

S6: HNMR Spectra of Compound II (Stigmasterol)

S7: COSY spectra of Compound II (Stigmasterol)

S8: HNMR Spectra of Compound III (3-Friedelanol)

S9: COSY Spectra of Compound III (3-Friedelanol)