SUPPLEMENTARY MATERIAL

A new irregular monoterpene acetate along with eight known compounds with antifungal potential from the aerial parts of *Artemisia incisa* Pamp (Asteraceae).

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Abstract

A new compound named as santolinylol-3-acetate (4-(2-hydroxypropan-2-yl)-2-methylhexa-1,5-dien-3-yl acetate) (3), along with seven known compounds; linoleic acid (1), benzoic acid (2), santolinylol (4), Ethyl-(E)-p-hydroxy cinnamate (5), scopoletin (6), esculetin (7) isofraxidin (8) and Eupatorin (9), were isolated from the aerial parts (ethanolic extract) of endangered specie: Artemisia incisa Pamp (Asteraceae). The compounds’ structures were determined through modern spectroscopic techniques, and comparison of data (physicochemical constants) with the literature. The relative stereochemistry of santolinylol-3-acetate (3) was determined by comparing its data of NOESY, and specific rotation with its diol analogue; santolinylol (4), isolated from the same plant; A. incisa. The results of the antifungal activity showed that coumarins are as whole less active compounds. Compounds 3 (25 µg/mL and 300 µg/mL), and 4 (12.5 µg/mL and 300 µg/mL), showed good activities against Candida albicans, and Aspergillus flavus, respectively, which justifies A. incisa as a traditional medicine for curing the said fungal infections.

Key words: irregular monoterpene, santolinylol-3-acetate, Candida albicans, Aspergillus flavus.

Experimental

3.1S. Physical data of known compounds (1, 2, 4-9)

Compound 1 (linoleic acid) was obtained as white gummy solid, m.p. 29°C (lit. m.p. 28–29°C). The EIMS showed the molecular ion at m/z 280.24 [M^+] corresponding to C_{18}H_{32}O_{2} (Ulchenko et al., 2005). Compound 2 (benzoic acid) was obtained as colorless crystals having m. p. 122–124 °C (lit. m. p. 121–124°C). The molecular formula of C_{7}H_{6}O_{2} was deduced from EIMS at m/z 122.04 [M^+] (Han et al., 2008). Compounds 4 (santolinylol) was isolated as an off white color gummy solid, with 20[α]D -14.2 (c 1.0, CHCl_3) and UV(MeOH): λ_{max} : 228 nm. The EIMS showed molecular ion peak at m/z 170.12 [M^+] corresponding to the molecular formula of C_{10}H_{18}O_{2} (Susan et al., 2001; Tan et al., 1999). Compound 5 (Ethyl-(E)-p-hydroxy cinnamate) was obtained as white needles like crystals, m.p. 82–83 °C (lit. m.p. 83
°C). The molecular formula of C\textsubscript{11}H\textsubscript{12}O\textsubscript{3} was deduced from LR-EIMS which showed molecular ion peak at m/z: 192.078 [M+] (Pouchert, 1983). Compound 6 (scopoletin) was obtained as white yellow crystals, m.p. 204–205°C (lit. m.p. 205°C). The molecular formula of C\textsubscript{10}H\textsubscript{8}O\textsubscript{4} was obtained from the EI-MS with m/z 192.042 [M+] (Wu et al. 1975). Compound 7 (esculetin) was isolated as needles like crystals of white color, m.p. 269–271°C (lit. m.p. 270°C). The molecular formula was of C\textsubscript{8}H\textsubscript{6}O\textsubscript{4} established from the LR-EIMS showing m/z at m/z 178.027 [M+] (Silvan et al., 1996). Compound 8 (isofraxidin) was obtained as colorless needles like crystals, m.p. 148–149°C (lit. m.p. 148–149°C). The molecular formula of C\textsubscript{11}H\textsubscript{16}O\textsubscript{5} was obtained from HR-ESIMS (positive ion mode) which exhibited a pseudo molecular ion peak at m/z 223.053 [M\textsuperscript{+}+H] (Ahluwalia et al., 1978). Compound 9 (Eupatorin) was obtained as yellow crystals, m.p. 195–196 °C (lit. m.p. 195–196 °C). The molecular formula of C\textsubscript{10}H\textsubscript{16}O\textsubscript{7} was deduced from HR-ESIMS (positive ion mode) which exhibited a pseudo molecular ion peak at m/z 344.088 [M\textsuperscript{+}+H] (Shilin et al., 1989). The complete structure assignments of the known compounds (1, 2, 4-9) (Figure 2) were obtained by comparing their spectral data (1D and 2D NMR, IR and MS and physicochemical constants) with the literature.

Figure S1. Key HMBC (→), COSY (—), NOESY (→) correlations of santolynilol-3-acetate (3)

Figure S2. \textsuperscript{1}H Spectrum of compound 3

Figure S3. \textsuperscript{13}C Spectrum of compound 3

Figure S4. HMBC (\textsuperscript{1}H-\textsuperscript{13}C) Spectrum of compound 3

Figure S5. NOESY (\textsuperscript{1}H-\textsuperscript{1}H) spectrum of compound 3

Table S1. \textsuperscript{1}H-NMR (500 MHz), \textsuperscript{13}C-NMR (100 MHz) and HMBC spectra of (3R,4S)-4-(2-hydroxypropan-2-yl)-2-methylhexa-1,5-dien-3-yl acetate (3) in CDCl\textsubscript{3}.  

Table S2. Result of the antimicrobial screening of the selected compounds (3-9) against the two fungal strains.

Table S3. Minimum inhibitory concentrations (MICs) of the selected compounds (3, 4, 5, 8-9) against the two fungal strains.
Figure S1. Key HMBC (→), COSY (→), NOESY (↔) correlations of santolynilol-3-acetate (3)
Figure S2. $^1$H Spectrum of compound 3
Figure S3. $^{13}$C Spectrum of compound 3
Figure S4. HMBC (\(^1\text{H}-^{13}\text{C}\)) Spectrum of compound 3
Figure S5. NOESY \((^1\text{H}-^1\text{H})\) spectrum of compound 3
Table S1. $^1$H-NMR (500 MHz), $^{13}$C-NMR (100 MHz) and HMBC spectra of (3R,4S)-4-(2-hydroxypropan-2-yl)-2-methylhexa-1,5-dien-3-yl acetate (3) in CDCl$_3$.

| C. No. | $^{13}$C-NMR (δ) | Multiplicity | $^1$H-NMR (δ) ($^{1}J_{HH}$ Hz) | HMBC Correlation |
|--------|------------------|--------------|-------------------------------|----------------|
| 1      | 116.41 CH$_2$    |              | 4.95 (1H, br. s, Ha)          | C-2, C-3       |
|        |                  |              | 5.04 (1H, br. s, Hb)          |                |
| 2      | 143.46 C         |              | ---                           | ---            |
| 3      | 81.54 CH         |              | 5.36 (1H, d, $^{1}J = 9.6$)   | C-1, C-2, C-4, C-1’ |
| 4      | 53.45 CH         |              | 2.53 (1H, dd, $^{1}J = 9.6$, 6.8) | C-3, C-5, C-6, C-9 |
| 5      | 135.76 CH        |              | 5.50 (1H, ddd,               | C-4, C-6, C-3, C-9 |
|        |                  |              | $^{1}J = 15.6$, 6.2, 2.0)     |                |
| 6      | 120.23 CH$_2$    |              | 5.16 (1H, dd, $^{1}J = 17.0$, 2.0, Ha) | C-4, C-5       |
|        |                  |              | 5.13 (1H, dd, $^{1}J = 12.0$, 1.8, Hb) |                |
| 7      | 18.23 CH$_3$     |              | 1.76 (3H, s)                 | C-1, C-2, C-3  |
| 8      | 22.41 CH$_3$     |              | 1.21 (3H, s)                 | C-4, C-9, C-10 |
| 9      | 76.77 C          |              | ---                           | ---            |
| 10     | 27.05 CH$_3$     |              | 1.24 (3H, s)                 | C-4, C-8, C-9  |
| 1’     | 170.22 C         |              | ---                           | ---            |
| 2’     | 29.05 CH$_3$     |              | 2.09 (3H, s)                 | C-1’            |

*) The assignments were based on DEPT, COSY, HSQC, HMBC, and NOESY experiments, with multiplicities and coupling constants in parentheses. Overlapped signals were reported without multiplicities.
Table S2. Result of the antimicrobial screening of the selected compounds (3-9) against the two fungal strains.

| Compound                        | Test organism       | Candida albicans | Aspergillus flavus |
|--------------------------------|---------------------|------------------|---------------------|
|                                | Zone of inhibition in mm at 1 mg/mL |                  |                     |
| Santolinylol-3-acetate (3)     |                     | 14               | 12                  |
| Santolinylol (4)               |                     | 18               | 12                  |
| trans-Ethyl cinnamate (5)      |                     | 10               | 10                  |
| Scopoletin (6)                 |                     | NA               | NA                  |
| Esculetin (7)                  |                     | NA               | NA                  |
| Isofraxidin (8)                |                     | 12               | 10                  |
| Eupotorin (9)                  |                     | 10               | 12                  |
| Amphotericin B 1mg/mL          |                     | 16               | 16                  |
| Fluconazole 1mg/mL             |                     | 20               | 14                  |

NA = Not Applicable

Table S3. Minimum inhibitory concentrations (MICs) of the selected compounds (3, 4, 5, 8-9) against the two fungal strains.

| Compound                        | Test organism       | Candida albicans | Aspergillus flavus |
|--------------------------------|---------------------|------------------|---------------------|
|                                | MIC = µg/mL         |                  |                     |
| Santolinylol-3-acetate (3)     |                     | 25 µg/mL         | 300 µg/mL           |
| Santolinylol (4)               |                     | 12.5 µg/mL       | 300 µg/mL           |
| trans-Ethyl cinnamate (5)      |                     | 700 µg/mL        | 500 µg/mL           |
| Isofraxidin (8)                |                     | 300 µg/mL        | 400 µg/mL           |
| Eupotorin (9)                  |                     | 500 µg/mL        | 1000 µg/mL          |
| Amphotericin B 1mg/mL          |                     | 0.48 µg/mL       | 0.48 µg/mL          |
| Fluconazole 1mg/mL             |                     | 0.24 µg/mL       | 25 µg/mL            |

NA = Not Applicable