

# Antibacterial diterpenoids from *Jatropha podagrica* Hook

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## Abstract

Japodagrins (1) and japodagrone (2), two macrocyclic diterpenoids possessing lathyrane and jatropane skeletons, respectively, have been isolated from the root of *Jatropha podagrica* Hook. Four other diterpenoids (3–6) were also isolated from this plant. The structures of these compounds were elucidated on the basis of NMR and HRMS analysis, and by spectral comparisons. The compounds displayed antibacterial activity against some gram-positive bacteria.

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**Keywords:** *Jatropha podagrica*; Euphorbiaceae; Antibacterial; Macrocyclic diterpenoid

## 1. Introduction

*Jatropha podagrica* is a multipurpose shrub in the family Euphorbiaceae commonly found in Africa, Asia and Latin America. The plant is known locally in south western Nigeria as *lapalapa funfun*. *Jatropha* species are used in traditional medicine for various diseases such as skin infections, sexually transmitted diseases like gonorrhoea, jaundice and fever (Dalziel, 1937; Burkill, 1994; Chopra et al., 1956; Martinez, 1959). Different pharmacological activities including antibacterial, antitumor and anti insect have been reported for this plant (Aiyelaagbe et al., 1998, 2000; Sanni et al., 1988). Previous phytochemical studies on *Jatropha* species have resulted in isolation of several types of diterpenoids from the roots of these plants (Kupchan et al., 1970; Torrance et al., 1976; Naengchomnong et al., 1986; Villarreal et al., 1988). In continuation of our work on the biological activities of *Jatropha* species grown in Nigeria, we have isolated six diterpenoids from the title plant. We now report the isolation and structure elucidation of the new diterpenoids (1–2), together with the four known diterpenoids (3–6) and their biological activities.

## 2. Results and discussion

The powdered, dried roots of the plant were successively extracted with hexane, CHCl<sub>3</sub>, and MeOH in a soxhlet extractor. The three extracts showed activity against gram-positive bacteria in agar disk diffusion assays, but the hexane extract was most active. Silica gel vacuum liquid chromatography of the hexane extract with hexane, EtOAc, and MeOH gave fractions that were further purified by preparative and semi-preparative reversed-phase HPLC to afford compounds 1–6.

HRFABMS data for 1 gave a mass of 371.1824 (M + Na)<sup>+</sup>, calculated for 371.1834. The mass and <sup>13</sup>C NMR (Table 1) indicated the molecular formula C<sub>20</sub>H<sub>28</sub>O<sub>5</sub> (seven unsaturations). <sup>1</sup>H NMR and DEPT spectra revealed the presence of five methyl groups, three methylene groups, five methines (one olefinic), and two exchangeable (OH) protons. Signals for two ketone groups, one olefin, two oxymethines, and two oxygenated quaternary carbon atoms were present in the <sup>13</sup>C NMR spectrum. The <sup>13</sup>C NMR spectrum accounted for three unsaturations suggesting that compound 1 was tetracyclic. The two oxygenated quaternary carbons (δ 75.6 and 82.3) were attributed to tertiary alcohol groups, while the other two (δ 62.5 and 64.0) were suggestive of an epoxide. These units

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Table 1  
 $^1\text{H}^{\text{a}}$ ,  $^{13}\text{C}^{\text{b}}$  NMR, HMBC<sup>c</sup>, and NOESY<sup>c</sup> Data for Japodagrins (1) in  $\text{CDCl}_3$

| Position | $\delta^1 \text{H}$ (mult., $J_{\text{HH}}$ ) | $\delta^{13} \text{C}$ (mult.) | HMBCCorrelations [H $\rightarrow$ C] | NOESY [H $\leftrightarrow$ H] |
|----------|-----------------------------------------------|--------------------------------|--------------------------------------|-------------------------------|
| 1        | 3.58 (s)                                      | 64.0 (d)                       | C-3, 4, 15                           | H-5, 16, 20                   |
| 2        | —                                             | 62.5 (s)                       | —                                    | —                             |
| 3        | —                                             | 195.6 (s)                      | —                                    | —                             |
| 4        | —                                             | 134.3 (s)                      | —                                    | —                             |
| 5        | 6.87 (s)                                      | 151.5 (d)                      | C-3, 4, 6, 7, 15, 17                 | OH-6, H-7b, 8a, 17            |
| 6 – OH   | 3.25 (br, s)                                  | 75.6 (s)                       | C-5, 6, 7, 17                        | H-5, 9, 17, OH-15             |
| 7a       | 1.61 (m)                                      | 41.9 (t)                       | C-5, 6, 8, 9, 17                     | H-7b, 8b                      |
| 7b       | 1.91 (m)                                      | —                              | —                                    | H-5, 7a, 8a, 17               |
| 8a       | 0.73 (m)                                      | 19.3 (t)                       | C-6, 7, 9, 10, 11                    | H-7b, 8b                      |
| 8b       | 1.63 (m)                                      | —                              | —                                    | H-7a, 8a                      |
| 9        | 0.38 (ddd)                                    | 26.9 (d)                       | C-7, 18                              | OH-6, H-11, 18                |
| 10       | —                                             | 17.7 (s)                       | —                                    | —                             |
| 11       | 0.60 (m)                                      | 19.2 (d)                       | C-13, 18                             | H-9, 12b, 18, 20              |
| 12a      | 1.40 (m)                                      | 28.7 (t)                       | C-9, 10, 11, 13, 14, 20              | H-8a, 12b, 13, 19             |
| 12b      | 1.41 (m)                                      | —                              | —                                    | H-11, 12a, 20                 |
| 13       | 2.66 (m)                                      | 41.2 (d)                       | C-11, 12, 20                         | H-12a, 20                     |
| 14       | —                                             | 209.2 (s)                      | —                                    | —                             |
| 15 – OH  | 5.15 (br, s)                                  | 82.3 (s)                       | C-1, 4, 14, 15                       | OH-6, H-17                    |
| 16       | 1.65 (s)                                      | 10.1 (q)                       | C-1, 2, 3                            | H-1                           |
| 17       | 1.30 (s)                                      | 28.9 (q)                       | C-5, 6, 7                            | H-5, 7b, OH-6                 |
| 18       | 0.97 (s)                                      | 28.3 (q)                       | C-9, 10, 11, 19                      | H-9, 11, 19                   |
| 19       | 0.65 (s)                                      | 14.9 (q)                       | C-9, 10, 11, 18                      | H-12a, 18                     |
| 20       | 1.20 (d, 6.2)                                 | 17.0 (q)                       | C-12, 13, 14                         | H-1, 11, 12b, 13              |

<sup>a</sup> 360/600 MHz.

<sup>b</sup> 90/150 MHz.

<sup>c</sup> 600 MHz ( $^1\text{H}$  dimension).

accounted for all five oxygen atoms and both exchangeable protons in the molecule. The presence of a 1,1-dimethylcyclopropane ring was evident from the corresponding  $^1\text{H}$  and  $^{13}\text{C}$  NMR shifts (Table 1) and from NMR comparisons with similar compounds (Aiyelaagbe et al., 1998).

Correlations from the  $^1\text{H}$ – $^1\text{H}$  COSY, and long-range correlations obtained from HMBC spectra (Table 1) led to the establishment of the structure for **1**. The methylene protons on positions 7 and 8 showed geminal and vicinal coupling to each other and their neighbours. The spectra further revealed coupling between H-8a and H-9, H-9 and H-11, H-11 and H<sub>2</sub>-12 and between H<sub>2</sub>-12 and H-13. The coupling between H-13 and H<sub>3</sub>-20 confirmed that the methyl group is attached to C-13. Further more, H<sub>3</sub>-20 appeared as a methyl doublet ( $J = 6.72$  Hz) in the  $^1\text{H}$  NMR spectrum while H-13 appeared as a multiplet. Long-range HMBC correlations were also observed from H-7 to C-5, C-6, C-8, C-9 and C-17, and from H-8 to C-6, C-7, C-9, C-10 and C-11. Additional correlations observed were H-5 to C-3, C-4, C-6, C-7, C-15, C-17; OH-6 to C-5, C-6, C-7, C-17; OH-15 to C-1, C-4, C-14, C-15 and H<sub>3</sub>-16 to C-1, C-2 and C-3. The sharp proton singlet at  $\delta$  3.58 ppm (H-1) was assigned to the epoxide ring and this was confirmed by its correlation to C-2, C-4 and C-15. The downfield chemical shift of this proton is also indicative of the presence of neighbouring oxygenated carbon atoms. The correlation of H<sub>3</sub>-16 to C-1 and C-2 further confirmed that the epoxide is trisubstituted. All the methyl groups showed the expected HMBC correlations. Details of these and other correlations are presented in

Table 1. The correlations confirmed the gem-dimethyl cyclopropane ring system and established the epoxycyclopentanone and cycloundecane rings. Major HMBC correlations were shown in Fig. 1.

The relative stereochemistry was deduced from the NOESY data (Table 1). There were strong NOESY correlations between H-9, H-11 and H<sub>3</sub>-18 which showed that the gem-dimethyl cyclopropane ring was *cis*-fused. NOESY correlations between H-1 and H<sub>3</sub>-16 also proved that the

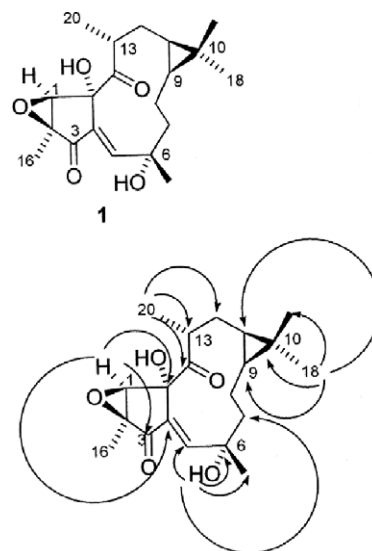


Fig. 1. Selective HMBC Correlations for **1**.

epoxide ring was also *cis*-fused to the cyclopentanone ring. The C-4–C-5 double bond was assigned the E-configuration based on comparison of the NMR data with similar compounds in the literature (Schmeda-Hirschmann et al., 1992). Structure **1** was thus named japodagrins. Japodagrins is 1,2-epoxy-15-*epi*-4E-jatrogrossidentadion. The structure represents the lathyrane ring system which is common in the Euphorbiaceae family, but japodagrins is the first compound to have a trisubstituted epoxide on C-1 and C-2.

Analysis of the  $^1\text{H}$ ,  $^{13}\text{C}$ , and DEPT NMR spectra of compound **2** suggested the molecular formula  $\text{C}_{20}\text{H}_{28}\text{O}_4$  indicative of seven unsaturations. This formula was confirmed by its HREIMS which gave a mass of 332.1981[M<sup>+</sup>], calculated for 332.1987. The  $^1\text{H}$ , HMQC, and DEPT NMR data (Table 2) revealed the presence of five methyl groups, four methylene groups, four methines including vinyl groups, and one exchangeable proton. These data accounted for all 28 protons in the molecule.  $^{13}\text{C}$  NMR, HMQC, and HMBC data (Table 2) indicated the presence of two ketone moieties and two olefin units, suggesting that compound **2** is tricyclic. The overlapping signals in the  $^1\text{H}$  NMR spectrum were resolved with the aid of the HMQC spectrum. Analysis of the HMQC, COSY and HMBC data permitted construction of the structure of compound **2**. HMBC correlations of the olefinic proton,  $\delta$  6.80 (H-1) with C-2, C-3, C-4, C-15 and C-16 assisted in constructing the cyclopentenone ring. Correlation of the olefinic methyl group,  $\delta$  1.88 (H<sub>3</sub>-16) with

C-1, C-2 and C-3 also confirmed that H<sub>3</sub>-16 was attached to C-2 as part of the cyclopentenone ring. Additional long range correlations observed were between H<sub>2</sub>-7 and C-5, C-6, C-8, C-9 and between H<sub>2</sub>-8 and C-6, C-7, C-9 and C-10. These correlations established the tetrahydrofuran ring. The methylene protons displayed geminal and vicinal coupling with each other and all the five methyl groups showed all the expected long-range HMBC correlations. Some of these correlations are shown in Fig. 2. Further correlations as shown in Table 2 completed the structure. The absence of cyclopropane signals was also evident in the spectra which suggested strongly that **2** is not a lathyrane diterpenoid. Based on all these spectra data, the structure was assigned as shown and compound **2** was given the name japodagrone. The relative stereochemistry proposed for japodagrone was deduced from the NOESY data (Table 2). The compound has the jatrophane skeleton which was found in jatrophane (Kupchan et al., 1970).

As far as we know, these two compounds are being reported for the first time.

Four other diterpenoids, 4Z-jatrogrossidentadion (**3**), 15-*epi*-4Z-jatrogrossidentadion (**4**), 2-hydroxyisojatrogrossidentadion (**5**), and 2-*epi*hydroxyisojatrogrossidentadion (**6**) were also isolated from the plant. These compounds were purified by reversed-phase HPLC and identified by analysis of their MS and NMR data in comparison with literature data (Schmeda-Hirschmann et al., 1992). Compounds **3–6** were originally isolated from the roots of *Jatropha gross-*

Table 2  
 $^1\text{H}^a$ ,  $^{13}\text{C}^b$  NMR, HMBC<sup>c</sup>, and NOESY<sup>c</sup> Data for Japodagrone (**2**) in  $\text{CDCl}_3$

| Position | $\delta^1 \text{H}$ (mult., $J_{\text{HH}}$ ) | $\delta^{13} \text{C}$ (mult.) | HMBC Correlations [H $\rightarrow$ C] | NOESY [H $\leftrightarrow$ H] |
|----------|-----------------------------------------------|--------------------------------|---------------------------------------|-------------------------------|
| 1        | 6.80 (s)                                      | 153.8 (d)                      | C-2, 3, 4, 15, 16                     | H-16, 20, OH-15               |
| 2        | –                                             | 143.9 (s)                      | –                                     | –                             |
| 3        | –                                             | 196.7 (s)                      | –                                     | –                             |
| 4        | –                                             | 136.0 (s)                      | –                                     | –                             |
| 5        | 6.80 (s)                                      | 139.5 (d)                      | C-3, 4, 7, 15                         | –                             |
| 6        | –                                             | 82.8 (s)                       | –                                     | –                             |
| 7a       | 1.87 (m)                                      | 37.6 (t)                       | C-6, 8                                | H-8b                          |
| 7b       | 2.12 (m)                                      | –                              | C-5, 6, 8, 9                          | H-8b                          |
| 8a       | 1.66 (m)                                      | 25.6 (t)                       | C-6, 7                                | H-7b, 9                       |
| 8b       | 1.89 (m)                                      | –                              | C-6, 7, 9, 10                         | H-8a, 9                       |
| 9        | 3.70 (t, 5.8, 6.1)                            | 88.5 (d)                       | C-7, 11, 18, 19                       | H-7a, 11a, 17, 18             |
| 10       | –                                             | 36.0 (s)                       | –                                     | –                             |
| 11a      | 1.54 (m)                                      | 34.5 (t)                       | C-9, 10, 12, 13, 18, 19               | H-18                          |
| 11b      | 1.56 (m)                                      | –                              | Ditto                                 | H-8b, 9                       |
| 12a      | 1.38 (m)                                      | 31.3                           | C-10, 20                              | H-12b, 130                    |
| 12b      | 2.04 (m)                                      | –                              | C-10, 13, 14                          | H-12a, 19, 20                 |
| 13       | 2.88(m)                                       | 42.0 (d)                       | C-11, 12, 14, 20                      | H-18, 20                      |
| 14       | –                                             | 211.8 (s)                      | –                                     | –                             |
| 15 – OH  | 4.86 (s)                                      | 82.4 (s)                       | C-1, 4, 14, 15                        | H-1, 7b, 8b, 12b, 19          |
| 16       | 1.88 (s)                                      | 10.6 (q)                       | C-1, 2, 3                             | –                             |
| 17       | 1.35 (s)                                      | 24.2 (q)                       | C-5, 6, 7                             | H-9, 12b                      |
| 18       | 0.72 (s)                                      | 24.1 (q)                       | C-9, 10, 11, 19                       | H-9, 13                       |
| 19       | 0.88 (s)                                      | 28.4 (q)                       | C-9, 10, 11, 18                       | H-12b, OH-15                  |
| 20       | 0.90 (d, 7.1)                                 | 20.6 (q)                       | C-12, 13, 14                          | H-1, 13                       |

<sup>a</sup> 360/600 MHz.

<sup>b</sup> 90/150 MHz.

<sup>c</sup> 600 MHz ( $^1\text{H}$  dimension).

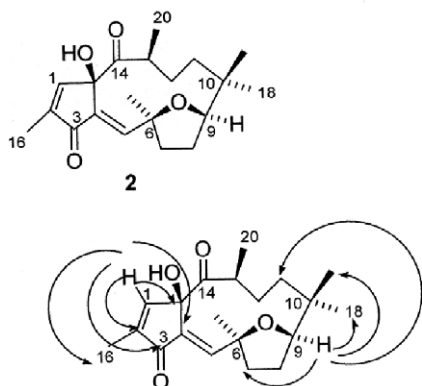
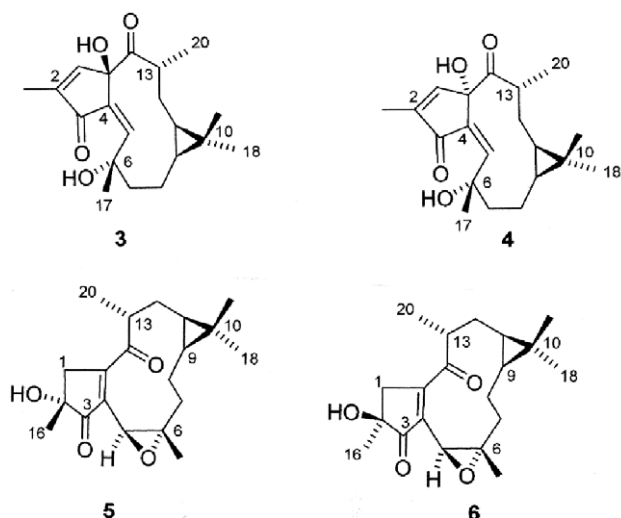


Fig. 2. Selective HMBC Correlations for 2.

*identata* and have also been found in *J. weddelina* (Schmeda-Hirschmann et al., 1992; Brum et al., 2001).



Japodagrins (1) was active in standard disk assays against *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 25923), affording inhibitory zones of 16 and 12 mm, respectively, at 20 µg/disk. Japodagrone (2) only showed activity against *B. subtilis* (ATCC 6051), giving a zone of 12 mm at 20 µg/disk. At 20 µg/disk, compounds 3–6 displayed activity against *B. subtilis* showing zones of inhibition of 20, 17, 31 and 35 mm, respectively, as well as *S. aureus* (10, 9, 21 and 26 mm, respectively). These compounds could be responsible for some of the antibacterial activity exhibited by extracts of this plant. All the compounds were inactive in disk assays against *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Chloroform and methanol were used as solvents for the assays. Streptomycin and gentamycin (20 µg/disk) were used as positive controls. The zones of inhibition for streptomycin were 35, 26, 20 and 14 mm, respectively against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*; while the values for gentamycin were 34, 28, 19 and 10 mm, respectively.

*Japrophia* spp. have afforded many bioactive diterpenoids, but only three has been previously reported from *J. podagrica* (Aiyelaagbe et al., 1998). This paper, thus reports the isolation of six other bioactive diterpenoids from this plant, two of which are new.

### 3. Experimental

#### 3.1. General experimental procedures

$^1\text{H}$  and  $^{13}\text{C}$  NMR data were recorded on Bruker WM-360 and/or AMX-600 spectrometers. NMR spectra were recorded using  $\text{CDCl}_3$  solution and were referenced to the corresponding solvent signals. HMQC and HMBC experiments were optimized for  $^1J_{\text{CH}} = 150$  Hz and  $^nJ_{\text{CH}} = 8$  Hz, respectively. MS data were recorded on VG Trio1 and VG ZAB-HF mass spectrometers. UV spectra were recorded using the cuvette cell compartment of a Beckman model 168 photodiode array detector. Optical rotations were determined in  $\text{CHCl}_3$  using a Jasco DIP 1000 digital polarimeter. Melting points were measured on a Fisher–Johns micro melting point apparatus, and are uncorrected.

#### 3.2. Plant material

Whole plants of *J. podagrica* Hook were collected from the campus of the University of Ibadan, and premises of St. Stephen Anglican Church, Inalende, Ibadan, Nigeria; between April and June 1995. The plants were authenticated at the Herbarium of the Forestry Research Institute, Ibadan, Nigeria (Herbarium Voucher Number FHI 93265).

#### 3.3. Extraction and isolation

The dried, ground root (200 g) was extracted sequentially using a soxhlet apparatus with hexane,  $\text{CHCl}_3$ , and MeOH. Each resulting solution was concentrated in vacuo, affording hexane- (yellow solid; 4.2 g),  $\text{CHCl}_3$ - (yellow solid; 4.7 g), and MeOH- (maroon solid; 16.0 g) extracts.

The hexane extract was subjected to silica gel VLC over a prepacked column bed. The column was eluted using a stepwise gradient of EtOAc 0–100% (v/v) in hexane and MeOH 10–100% (v/v) in EtOAc. A total of twenty 50 ml fractions were collected. Fractions of similar composition as determined by TLC were pooled together resulting in eight combined fractions. Three fractions that displayed antibacterial activity were purified by HPLC to isolate the constituents. Fraction 3 (125 mg), which was eluted with 30% EtOAc in hexane, was purified on semi-preparative RP HPLC using a gradient of 70–100% MeCN/ $\text{H}_2\text{O}$  over 30 min (Beckman Ultrasphere  $\text{C}_{18}$  column,  $1.0 \times 25$  cm, 2.0 ml/min, UV detection at 254 nm) to afford japodagrins (1; 8 mg,  $t_{\text{R}}$  10 min). Fraction 5 (92 mg) which was eluted with 40% EtOAc in hexane as yellow oil was purified on semi-preparative RP HPLC using a gradient of 60 to 100% MeOH/ $\text{H}_2\text{O}$  over 40 min (Alltech BDS  $\text{C}_{18}$  column,  $1.0 \times 25$  cm, 2.0 ml/

min, UV detection at 254 nm), affording compounds **3** (14 mg,  $t_R$  21.5 min), and **4** (22 mg,  $t_R$  13.2 min).

Fraction 7 (186 mg) which was eluted with 60–100% EtOAc in hexane was purified by semi-preparative reversed-phase HPLC using a gradient of 70–100% MeOH/H<sub>2</sub>O over 30 min (Alltech BDS C18 column, 1.0 × 25 cm, 2.0 ml/min, UV detection at 254 nm), affording compounds **2** (8 mg,  $t_R$  20.5 min), **5** (12 mg,  $t_R$  18.4 min) and **6** (22 mg,  $t_R$  16.7 min).

Compound **2** was further purified by semi-preparative RP HPLC using a gradient of 70–100% MeCN/H<sub>2</sub>O over 30 min (Alltech BDS C18 column, 1.0 × 25 cm, 2.0 ml/min, UV detection at 254 nm), to give **2** (4 mg,  $t_R$  15.0 min) as the major peak.

### 3.4. Japodagrins (1)

White needles from methanol; m.p. 188–190 °C;  $[\alpha]_D^{22} - 129^\circ$  (CHCl<sub>3</sub>;  $c$ 0.001); UV (MeOH)  $\lambda_{max}$  nm (log  $\epsilon$ ) 248 (3.20); <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; LRFABMS (3-NBA matrix)  $m/z$  371 ([M + Na]<sup>+</sup>, rel. int. 17), 349 ([M + H]<sup>+</sup>), 333 (12), 331 (50), 315 (19); HRFABMS (3-NBA matrix) obsd [M + Na]<sup>+</sup> at  $m/z$  371.1824, (calcd. for C<sub>20</sub>H<sub>28</sub>O<sub>5</sub>Na, 371.1834).

### 3.5. Japodagrone (2)

White solid; m.p. 152–154 °C;  $[\alpha]_D^{22} - 261^\circ$  (CHCl<sub>3</sub>;  $c$ 0.001); UV (MeOH)  $\lambda_{max}$  nm (log  $\epsilon$ ) 256 (3.53); <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 2; EIMS (probe) 70 eV  $m/z$  (rel. int.): 332 [M]<sup>+</sup> (5), 314 [M – H<sub>2</sub>O]<sup>+</sup> (2), 304 (3), 223 (7), 176 (14), 163 (16). HREIMS obsd  $m/z$  332.1981 (M<sup>+</sup>), (calcd. for C<sub>20</sub>H<sub>28</sub>O<sub>4</sub>, 332.1987).

### 3.6. 4Z-Jatrogrossidentadion (3)

Yellow oil;  $[\alpha]_D^{22} + 2.0^\circ$  (CHCl<sub>3</sub>;  $c$ 0.0012); UV (MeOH)  $\lambda_{max}$  nm (log  $\epsilon$ ) 250 (3.25); <sup>1</sup>H and <sup>13</sup>C NMR data (Schmeda-Hirschmann et al., 1992), EIMS (probe) 70 eV  $m/z$  (rel. int.): 314 [M – H<sub>2</sub>O]<sup>+</sup> (2), 271 (15), 163 (33), 95 (53), 69 (64), 43 (100).

### 3.7. 15-Epi-4Z-jatrogrossidentadion (4)

White solid; mp 54–56 °C;  $[\alpha]_D^{22} - 175^\circ$  (CHCl<sub>3</sub>;  $c$ 0.0026); UV (MeOH)  $\lambda_{max}$  nm (log  $\epsilon$ ) 262 (3.45); <sup>1</sup>H and <sup>13</sup>C NMR data (Schmeda-Hirschmann et al., 1992), EIMS (probe) 70 eV  $m/z$  (rel. int.): 314 [M – H<sub>2</sub>O]<sup>+</sup> (2), 299 (2), 271 (11), 161 (34), 109 (26), 67 (50), 55 (59), 43 (100).

### 3.8. 2-Hydroxyisojatrogrossidion (5)

Yellow oil;  $[\alpha]_D^{22} - 15.0^\circ$  (CHCl<sub>3</sub>;  $c$ 0.002); UV (MeOH)  $\lambda_{max}$  nm (log  $\epsilon$ ) 252 (3.26); <sup>1</sup>H and <sup>13</sup>C NMR data (Schmeda-Hirschmann et al., 1992), EIMS (probe) 70 eV  $m/z$  (rel. int.): 332 [M]<sup>+</sup> (1), 314 [M – H<sub>2</sub>O]<sup>+</sup> (1), 299 (2), 271 (9), 161 (6), 123 (7), 109 (11), 67 (20), 55 (29), 43 (100).

### 3.9. 2-Epihydroxyisojatrogrossidion (6)

Yellow oil;  $[\alpha]_D^{22} - 7.0^\circ$  (CHCl<sub>3</sub>;  $c$ 0.001); UV (MeOH)  $\lambda_{max}$  nm (log  $\epsilon$ ) 253 (3.29); <sup>1</sup>H and <sup>13</sup>C NMR data (Schmeda-Hirschmann et al., 1992), EIMS (probe) 70 eV  $m/z$  (rel. int.): 332 [M]<sup>+</sup> (1), 314 [M – H<sub>2</sub>O]<sup>+</sup> (1), 299 (2), 271 (6), 161 (7), 95 (16), 81 (16) 55 (32) 43 (100).

### 3.10. Antibacterial assay

The microorganisms employed in the assay are: *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853).

Agar disk diffusion method was employed and filter paper disks (6.25 mm in diameter) were used. 20 µl of the test solvents (chloroform and methanol) were applied to each disk using a micropipette to sterilize the paper disks. The inoculated plates (15 × 100 mm) were removed from the refrigerator and labelled accordingly. Once pure solvent has evaporated from the disk, 20 µl of the sample solution containing 20 µg of the compounds were applied to the disks. Negative and positive controls were also prepared with 20 µl of pure solvent and standard antimicrobial agents (streptomycin and gentamycin). The impregnated disks were left to dry and the dry disks were placed on the surface of the bacteria seeded plates. The plates were sealed with parafilm to prevent contamination and incubated overnight (24/48 h). The plates of *B. subtilis* and *S. aureus* were incubated at room temperature, while the plates of *E. coli*, and *P. aeruginosa* were incubated at 37 °C. The compound was considered to be active if after 24 h, a clear zone extended around the impregnated filter paper disk in which no growth of the test organism was observed. The activity was reported as the diameter of the zone of growth inhibition and recorded in mm. The assays were carried out in duplicates.

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