ABSTRACT

A new rotenoid named 12-O-methylrotenolol along with five known rotenoid and isoflavone metabolites were isolated from the seeds of *Dalbergia lanceolaria subsp. paniculata*, collected from Egypt. The structures of these compounds were identified by physical and spectroscopic data measurements ([α]D, UV, 1D- and 2D-NMR and MS). The methanol extract of the seeds exhibited strong antioxidant activity with IC50 value 0.7 µg/µl against DPPH radical, in respect to quercetin as antioxidant reference (IC50 1.5 µM), while the tested compounds from this extract showed weak activities with IC50 values ranged from 19.6 to 33.0 µM.

1. Introduction

The species of the genus *Dalbergia* (belong to Fabaceae family) are widely distributed especially in the tropical and subtropical regions. The genus is an abundant source of secondary metabolites mainly isoflavonoids (Nagarajan et al. 2006; Manoj et al. 2007,
Kaennakam et al. 2016; Liu et al. 2018). These metabolites exhibited various pharmacological activities including cancer chemopreventive, antimicrobial, antioxidant, anti-inflammatory, antianalgesic, antipyretic, anti-diarrhoeal, antiulcerogenic, anti-idiopathic and antifertility activities (Vasudeva et al. 2009; Saha et al. 2013). Dalbergia lanceolaria subsp. paniculata (Roxb.) Thoth commonly called Padru pachhali, is distributed in India and in some other tropical areas (Scheffer and Morrell 1998). The leaves and bark of the plant were proved to have antibacterial and anticancer (Amin et al. 2012), and antioxidant and anti-inflammatory properties (Ganga et al. 2012). The previous phytochemistry of the plant resulted in C-glycosyl isoflavanone from the flowers (Adinarayana and Rajasekhara 1975), flavones and isoflavones from the leaves (Radhakrishniah 1973; Rao and Ahamed 1992), isoflavones, and C- and O-glycosylisoflavones from bark and roots (Parthasarathy et al. 1974a, 1974b; Radhakrishniah 1979; Rajulu and Rao 1980; Khalivulla et al. 2007; Saha et al. 2013). A number of isoflavone and rotenoid compounds were reported from the seeds such as dalpanitin, dalpatin, dalpanin, dalpatien, caviunin, caviunin 7-O-rhamnoglucoside, isocaviunin 7-O-glucoside, dalpaniculin, dalpanol O-glucoside dehydro-dalpanol O-glucoside, dalpatein, 6a,12a-dehydrodalpanol, milldurone (Saha et al. 2013); caviunin (Adinarayana et al. 1971); caviunin, isocaviunin, 3’-O-methylorobol, dalpalatin (Rajasekhara and Srinivasa 1990); 6a,12a-dehydroamorphigenin and 6a,12a-dehydrodalpanol (Ramachandraiah et al. 1992).

Isoflavonoids and their subgroup rotenoids are naturally occurring plant metabolites, mainly distributed in Leguminosae family (Deyou et al. 2015). Rotenoids are structurally isoflavonoids having an extra carbon at the C-2 position connected to the C-2’ position through an ether linkage to form a tetracyclic ring system. They have shown potential as anti-inflammatory (Tewtrakul et al. 2009), antiviral (Phrutivorapongkul et al. 2002) and anticancer candidate agents (Deng et al. 2010; Mittraphab et al. 2018). In our continuing search for natural antioxidants, the MeOH extract of the seeds of D. paniculata was found to exhibit strong scavenging DPPH free radicals. The extract was subjected for isolation of the flavonoid constituents as antioxidant candidates. In this paper, we report the isolation and the structure elucidation of 12-O-methylrotenolol as a new rotenoid and five known rotenoid and isoflavone compounds. The isolated compounds were evaluated for their antioxidant compared to its active extract.

2. Results and discussion

The 70% aqueous MeOH extract of D. paniculata seeds was concentrated and defatted with n-hexane. The residue fraction was separated by polyamide column and preparative PC (3MM) chromatographies, and then purified by Sephadex LH-20 column to afford four rotenoids (1–4) and two isoflavones (5 and 6) (Figure 1). The general features of the NMR data for compounds 1–4 (Table S1) suggested rotenoid structures with a typical 12a-hydroxyrotenoid skeleton (Lin et al. 1992), identified as dalbinol (2) (Kim et al. 2011), cis-12a-hydroxyrot-2-enonic acid (3) (Singhal et al. 1982), and 2-methoxygliricido (4) (Luca et al. 1999), while isoflavone compounds were identified
as pseudobaptigenin (5) (Cook et al. 1978), and dalpatein (6) (Adinaraya and Rao 1972). Compounds 1–5 were isolated for the first time from *D. paniculata*.

Compound 1 was isolated as an amorphous powder with a molecular formula of C_{24}H_{26}O_{7} as determined from HRESI-MS positive spectrum at m/z 449.1761 [M + Na]^{+}, in conjunction with 13C-NMR data. 1D-NMR spectra (1H, 13C and DEPT-135) of 1 with the aid of HSQC experiment displayed resonances for a rotenoid unit including four aromatic methines CH-1 (δ_{C} 111.1; δ_{H} 6.84, s), CH-4 (δ_{C} 100.9; δ_{H} 6.37, s), CH-10 (δ_{C} 101; δ_{H} 6.25, d, J = 8.04 Hz), CH-11 (δ_{C} 130.6; δ_{H} 6.99, d, J = 8.04 Hz); olefinic methylene CH_{2}-7'' (δ_{C} 111.8; δ_{H} 4.98 & 4.84); three sharp singlet signals of methoxy groups at δ_{C} 56.8, 56.4 and 55.7, bearing protons at δ_{H} 3.62, 3.65 and 3.38, respectively. The most downfield singlet assigned for aliphatic 8'-CH_{3} (δ_{C} 17.5; δ_{H} 1.67). The NMR spectra additionally revealed methylene carbons C-6 (δ_{C} 64.6) and C-4' (δ_{C} 31.6) bearing in HSQC spectrum pairs of non equivalent protons, where oxy H_{2}-6 protons resonated at δ_{H} 4.38 (dd, J = 2.4&12 Hz, H-6β) and 4.36 (d, J = 12 Hz, H-6α), while H_{2}-4' protons resonated at δ_{H} 3.09 (dd, J = 9.6 Hz, H-4'α) and 2.77 (d, J = 7.8 Hz, H-4'β). These methylene protons correlated in COSY spectrum with H-6a at δ_{H} 4.43 (d, J = 1.08 Hz), and H-5' at δ_{H} 5.14 (t, J = 9.0), respectively. However, the 13C-NMR spectrum of 1 revealed the carbon resonances of rotenoid derivative as in rotenolone (Magalhaes et al. 1996) with absence of carbonyl carbon (C-12), and instead OCH_{3} and aliphatic oxy-methin (δ_{C} 77.0; HSQC with δ_{H} 4.71, s) were observed, suggested that the carbonyl group at position 12 was reduced to secondary alcoholic OH and then methylated. This further confirmed by the HMBC data (Table S2) through the correlations from H-12 to C-11, C-6a (δ_{C} 70.7), C-12a (δ_{C} 64.4), C-11a (δ_{C} 112.7), C-1a (δ_{C} 112.0) and C-7a (δ_{C} 150.2), while C-12 received correlations from protons 6a, 11 and OCH_{3} (δ_{H} 3.38). Also, the later OCH_{3} protons showed strong cross peak only with C-12. The HMBC correlations (Table S2) additionally confirmed the locations of the two methoxy groups δ_{H} 3.62 and 3.65 at C-2 (δ_{C} 143.3) and C-3 (δ_{C} 150.1), respectively, and the hydroxyl group
(δH 5.43, br) at C-12a. Other HMBC correlations (Figure S1) assigned the quaternary carbons located on the ring junctions and also confirmed the assignments of all proton and carbon resonances in 1. The relative configuration of the new compound was cis-rotenoid structure (cis coupled B/C ring system) deduced from the chemical shift of the epimer proton H-1 at δH 6.97 (cis about δH 7.00), whereas trans-analogues with a trans linked C/D ring unit (trans rotenoids), it resonates at about δH 8.00) (Unai et al. 1973). The chemical shift of H-6a and its small J value (1.08 Hz) coupling with H2-6, suggested its β-configuration as an equatorial proton, correlated in ROESY spectrum with methylene proton (H-6β) at δH 4.38 Moreover, the J value of H-5′ with the upfield 4′-H2 (in the ABX system) and upfield 8′-H3 were determined the configuration of the E-dihydropyran ring and 6αβ/12αβ positions with 5′β configuration in the cis-rotenoid 1 (Kostova and Ognyanov 1986). The ROESY correlation between protons H-12 and H-6a indicated a cis relationship. The absolute configuration of C-6a and C-12a was suggested R,R, in comparison the optical rotation of 1 with those of 12a-hydroxyrotenoids (Wu et al. 2015). Thus, the structure of compound 1 was identified as 12-O-methylrotenolol.

The antioxidant activities of the crude extract of D. paniculata seeds and the isolated rotenoid and isoflavone compounds (1–6) were evaluated using DPPH free radical in vitro assay (Abou El-Kassem et al. 2012). With this method, the radical scavenging activity of the total extract exhibited a strong antioxidant with IC50 value 0.7 μg/ml compared to quercetin as antioxidant reference (IC50 value 0.45 μg/ml). However, the tested compounds here lack hydroxyl groups and therefore displayed weak activities with IC50 values ranged from 19.6 to 33.0 μM (Table 1), in respect to the potent antioxidant activity of their crude extract (Rice-Evans et al. 1996). The activity of the extract may be attributed to a possibly synergistic effect of these compounds or may be due to the presence of unidentified compounds in the extract.

### Table 1. Antioxidant activities of D. paniculata extract and isolated compounds (1–6).

| Sample                          | IC50 (μM) |
|---------------------------------|-----------|
| Crude extract                   | 0.7 ± 0.22 (μg/ml) |
| 12-O-methylrotenolol (1)        | 33.8 ± 0.66 |
| Dalbinol (2)                    | 19.5 ± 0.78 |
| cis-12a-Hydroxyrot-2-enonic acid (3) | 26.8 ± 0.50 |
| 2-Methoxygliricido (4)          | 27.6 ± 0.39 |
| Pseudobaptigenin (5)            | 22.4 ± 0.31 |
| Dalpatein (6)                   | 20.7 ± 0.43 |
| Quercetin (antioxidant)         | 1.5 ± 0.02 (0.45 μg/ml) |

3. Experimental

#### 3.1. General

Column chromatography as polyamide 6S (Merck) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden) were used. Paper chromatography (PC, Whatman No. 1 and 3 MM, Kent, England) was used for spotting and preparative separation, respectively, using eluent: H2O; H2O/HOAc (85:15, v/v) and BAW (n-BuOH/HOAc/H2O, 4:1:5, v/v/v), upper
layer). NMR spectra were obtained on Bruker 600 MHz NMR spectrometer with standard pulse sequences operating at 600 MHz for $^1$H and 150 MHz for $^{13}$C NMR, respectively. DMSO was used as solvent. UV-visible absorption spectra were taken on UV-2550 UV-vis spectrometer (Shimadzu, Kyoto, Japan). Optical rotations were detected by Jasco P-1010 Polarimeter. UV spectra were obtained using Shimadzu UV 240 spectrometer (Tokyo, Japan). ESI-MS (Electron Spray Ionization mass spectrometry) spectra were recorded on LC-MS TOF (Bruker, Germany).

3.2. Plant material

The seeds of *Dalbergia lanceolaria subsp. paniculata* (Roxb.) Thoth were collected from Zoo Garden, Giza, Egypt in October 2014. The recent accepted denomination of the species is *Dalbergia lanceolaria subsp. paniculata* (Roxb.) Thoth with a synonym *Dalbergia paniculata* Roxb. The plant was identified by Dr. Mohammed El-Gibaly, Consultant of Plant Taxonomy, Cairo University, and the respective voucher specimen (No. 23-4-2015) has been deposited in Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

3.3. Extraction and isolation

The air-dried seeds of *D. paniculata* (1 kg) were exhaustively extracted with methanol over a period of 5 days at room temperature. Removal of solvent under reduced pressure provided MeOH crude extract (45 g). The resulting total extract was defatted by $n$-hexane and the residue (15 g) was further fractionated by column chromatography over polyamide 6S (Merck) eluted with water followed by percentage increasing MeOH (up to 100%). All fractions obtained were combined according to their PC (1 MM in BAW eluent) analysis to give 7 fractions. Each of these fractions was subjected to preparative PC, where the compounds appeared as separated bands under UV. Each paper band was cut and eluted with 70% aqueous MeOH to extract the absorbed compound. Finally, column Sephadex LH-20 eluted with aqueous MeOH was used to obtain pure compounds 1 (7 mg), 2 (36 mg; $R_f$: 0.82; $[\alpha]_D^{20} - 12.9$, c 0.1, MeOH), 3 (11 mg; $R_f$: 0.84; $[\alpha]_D^{20} - 0.75$, c 0.01, MeOH), 4 (9 mg, $R_f$: 0.81), 5 (4 mg, $R_f$: 0.92) and 6 (5 mg, $R_f$: 0.90).

12-O-Rotenolol;(6a$\beta$,12a$\beta$)-12a-Hydroxy-2,3,12-trimethoxy-4',5'-tetrahydro-5'-isopropenylfuran-[2',3':9,8]-6H-rotoxen-12-ol (1):

Amorphous white solid; mp 137–141 °C; $R_f$: 0.87 (BAW); $[\alpha]_D^{20} - 0.95$ (c. 0.01, MeOH). UV $\lambda_{max}$ (MeOH): 286, 261, 254; ESI-MS $m/z$ 449.2 [M + Na]$^+$ and 875.4 [2M + Na]$^+$; HRESI-MS $m/z$ 449.1761 [M + Na]$^+$, (calcd for C$_{24}$H$_{26}$O$_7$Na 449.1678), and 875.3640 [2M + Na]$^+$. $^1$H-NMR: $\delta$ 6.99 (1H, d, $J$ = 8.04, H-11), 6.84 (1H, s, H-1), 6.37 (1H, s, H-4), 6.25 (1H, d, $J$ = 8.04, H-10), 5.43 (1H, br, 12a-OH), 5.14 (1H, t, $J$ = 9.0, H-5$^0$), 4.98/4.84 (2H, d, $J$ = 0.9 and br, respectively, H$_2$-7$^0$), 4.71 (1H, s, H-12), 4.43 (1H, d, $J$ = 1.08, H-6a), 4.38 (1H, dd, $J$ = 2.4 & 12, H-6$^\beta$), 4.36 (1H, d, $J$ = 12, H-6$^\alpha$), 3.65 (3H, s, 3-OCH$_3$), 3.62 (3H, s, 2-OCH$_3$), 3.38 (3H, s, 12-OCH$_3$), 3.09 (1H, dd, $J$ = 9.7 Hz, H-4$'$), 2.77 (1H, dd, $J$ = 7.8 Hz, H-4$'$), 1.67 (3H, s, H-8$^\beta$). $^{13}$C-NMR: $\delta$ 161.5 (C-9), 150.2 (C-7a), 150.1 (C-3), 148.7 (C-4a), 144.3 (C-6$'$) 143.3 (C-2), 130.6 (C-11), 113.7 (C-8), 112.0 (C-1a), 111.8...
Antioxidant assay

The antioxidant activity of MeOH extract and isolated compounds 1–6 against 1,1-diphenyl-2-pycrylhydrazyl (DPPH) free radicals was carried out using modified method of (Abou El-Kassem et al. 2012). Briefly, 190 µl DPPH (80 µg/ml in EtOH) was added to a 10 µl sample solution (100 µg/ml in DMSO) in a 96-well plate and mixed by shaking. After incubation for 30 min at room temperature the absorbance was recorded using an ELISA reader at 517 nm. The inhibition percentage (%) of the radical scavenging activity was calculated using the following equation:

\[
\text{Inhibition \%} = \frac{A_0 - A_s}{A_0} \times 100
\]

Where \(A_0\) is the absorbance of the control and \(A_s\) is absorbance of the sample at 517 nm. The experiment was carried out in triplicate, using quercetin as an antioxidant reference.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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