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Phytochemical study and biological evaluation of chemical constituents of *Platanus orientalis* and *Platanus* × *acerifolia* buds

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ABSTRACT

One flavonol glycoside, two *O*-isoprenylated flavonols, one α,α-dimethylallyl flavonol, one dihydrochalcone, two furanocoumarins and one terpenoid previously undescribed, along with 42 known compounds were isolated from the buds of two European Platanaceae, *Platanus orientalis* and *Platanus* × *acerifolia*. Their chemical structures were elucidated on the basis of spectroscopic analysis, including homonuclear and heteronuclear correlation NMR (COSY, NOESY, HSQC, and HMBC) experiments, as well as HRMS data. The estrogen-like and antiestrogen-like activity of dichloromethane and methanol extracts of *P. orientalis* and *P.* × *acerifolia* buds and isolated compounds was evaluated using estrogen-responsive cell lines. The potency of selected estrogen agonists to regulate gene expression through ERα and/or ERβ was compared with their *in vitro* osteoblastogenic activity. Kaempferol and 8-*C*-(1,1-dimethyl-2-propen-1-yl)-5,7-dihydroxyflavonol displayed osteoblastogenic as well as ERα-mediated estrogenic activity similar to estradiol.

1. Introduction

The genus *Platanus* is the unique living member of Platanaceae family and includes seven accepted species and twelve subspecies widespread throughout different regions of the world (The Plant List, 2013). Amongst these, two species, namely *Platanus orientalis* Linneaus and *Platanus* × *acerifolia* (Aiton) Willd are represented in Europe. *P. orientalis*, the most extended species of the genus *Platanus*, is traditionally used in Eastern Europe and Near East to treat various ailments including blepharitis, conjunctivitis, and hemorrhage (Nishanbaev et al., 2004), gastro-intestinal disorders, toothache, skin disease, fever, and body pain (Haq et al., 2011; Murad et al., 2011), kidney stones and itching (Polat and Satıl, 2012). Previous phytochemical and pharmacological reports provided scientific basis for these medicinal uses. Specifically, *P. orientalis* possesses anti-hepatotoxic, anti-oxidant and cytotoxic activities (El-Alfy et al., 2008), and elicits anti-inflammatory and anti-noceptive effects *in vivo* (Haider et al., 2012; Hajhashemi et al., 2011). As regards to chemical composition, tocopherols derivatives, esters of phytol with fatty acids (Abdullaev et al., 1994) and several polyphenols (El-Alfy et al., 2008; Tantry et al., 2012) were isolated from the leaves; kaempferol derivatives and caffeic acid were isolated from the buds (Dimas et al., 2000; Mitrokotsa et al., 1993); and triterpenoids, proanthocyanidins and proanthocyanidin glycosides were also isolated from the barks (Khan et al., 2013; Nishanbaev et al., 2004, 2010, 2005).

Platanus × *acerifolia* (Aiton) Willd is an interspecific hybrid between *Platanus occidentalis* Linneaus and *P. orientalis* growing as a street tree in major cities in North America, Europe, and Asia (Yang et al., 2013). The buds of *P.* × *acerifolia* has been reported to contain a number of dihydrochalcones and oxodihydrochalcones (Kaouadji, 1986, 1989; Kaouadji et al., 1986a, 1986b), *C*- & *O*-isoprenylated

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flavanones (Kaouadji et al., 1986b), flavonols and flavonol glycosides (Barron et al., 1994; Kaouadji, 1990, 2014; Kaouadji et al., 1992, 1993, 1988) and pyrano-flavanones (Kaouadji et al., 2013). 2-styrylchromones and prenylated flavonols were recently isolated from the bark of *P.* × *acerifolia* (Yang et al., 2013) as well as three triterpenoids and two flavonoids (Lin et al., 2013). To the best of our knowledge, no pharmacological studies has been performed related to *P.* × *acerifolia* extracts and there are only two studies dealing with the bio-activities of isolated components thereof (Yang et al., 2013, 2014).

During our ongoing search for novel bioactive metabolites from *Platanus* species, the dichloromethane and methanol extracts of the buds of *P orientalis* and *P.* × *acerifolia* were selected to be investigated due to their interesting phytochemical and biological profile. Both extracts displayed a rich content in secondary metabolites as revealed by their HPLC-DAD profile as well as strong potential to promote *in vitro* differentiation of preosteoblasts to mature osteoblasts. In-depth investigation of these extracts was carried out using various chromatographic techniques including HSCCC (high-speed counter current chromatography), MPLC, and column chromatography. This work resulted in the isolation of 50 compounds, including 8 novel compounds and specifically 1 flavonol glycoside, 2 *O*-isoprenylated flavonols, 1 α,α-dimethylallyl flavonol, 1 dihydrochalcone, 2 furanocoumarins and 1 terpenoid. Their structures were elucidated by direct interpretation of their spectral data, using high resolution mass spectrometry (HRMS), 1D and 2D NMR (COSY, NOESY, HSQC, and HMBC). In addition, in view of the above reports that flavonoids are major constituents of *P. orientalis* and *P.* × *acerifolia* buds and of recent findings that flavonoids induce osteoblast differentiation via estrogen receptor (ER) signaling (Guo et al., 2012), the estrogen-/antiestrogen-like and ER-regulatory activities of bud extracts and isolated compounds were evaluated and active components were assayed for effects on the differentiation of MC3T3-E1 preosteoblasts to osteoblasts.

2. Results and discussion

The dichloromethane (CH_2Cl_2) extracts of the buds of *P.* × *acerifolia* and *P. orientalis*, as well as the methanol (CH_3OH) extract of the buds of *P. orientalis* were subjected to various chromatographic techniques, including either HSCCC or MPLC fractionation, column chromatography including silica gel or Sephadex LH-20, followed by preparative TLC purification. The study of these extracts afforded 50 compounds including 8 previously undescribed (**1**–**8**), and 42 (**9**–**50**) previously reported compounds. The previously undescribed compounds were unambiguously identified using spectroscopic (NMR) and spectrometric (HRMS) techniques while comparison with literature data (Supplementary information) confirmed the structures of known compounds as described in Fig. 1.

Compound **1** was isolated as yellow crystals, and displayed a UV spectrum characteristic of a flavonol with a maxima at 261 nm and 273 nm. Its molecular weight was determined based on its deprotonated molecular ion at *m/z* 383.1124 $[M - H]^-$ corresponding to $C_{21}H_{20}O_7$ molecular formula based on ESI(−)-HRMS analysis; and this was also supported by the NMR spectroscopic data (Table 1). The ^{1}H NMR spectrum displayed four aromatic signals. First, an ABX system consisting of a singlet (H-2′) at 7.67 ppm and two doublets (H-5′, $J = 8.2$ Hz) and (H-6′, $J = 8.2$ Hz) at 7.07 and 7.69 ppm successively was noticed. The carbon atoms of the aforementioned protons were found to resonate at 110.7 ppm (C-2′), 114.8 ppm (C-5′), and 122.6 ppm (C-6′), respectively. An additional aromatic signal was observed in the ^{1}H NMR spectrum at 6.30 ppm (H-6), and C-6 resonated at 101.1 ppm. Also, the ^{1}H NMR spectrum provided a signal at 12.15 ppm, characteristic singlet of an aromatic hydroxyl group closed to a carbonyl. The methoxyl protons were observed as a singlet at 3.97 ppm and the corresponding carbon atom at 56.3 ppm. The HMBC spectrum revealed a correlation of the hydroxyl group with C-5 (δ 159.6), and the methoxyl with C-3′ (δ 146.6) confirming their position. The 1,1-dimethylallyl side chain was established by the appearance of a methine group at 6.49 ppm (H-2″, dd, $J = 10.5, 17.5$ Hz), which displayed a ^{1}H-^{1}H COSY correlation to the α-proton of the downfield exomethylene at 5.42 ppm (H-3″α, $J = 10.5$ Hz), and HMBC correlation with the two methyl groups at 1.72 ppm (H-4″ and H-5″). Added to this, the HMBC spectrum revealed that all the protons of this side chain correlated with C-8, specifically H-2″→C-8, H-3″→C-8, H-4″→C-8 and H-5″→C-8, thus indicating the position of this chain on the basic skeleton (Table 1). Accordingly, the structure of **1** was determinated as 5,7,4′-trihydroxy-8-(1,1-dimethylallyl)-3′-methoxyflavonol.

Compound **2** was obtained as an amorphous, yellow solid with UV maxima at 297 nm. This compound was established to have a quasi-molecular formula of $C_{21}H_{19}O_7$ based on the ESI(−)-HRMS (*m/z* 383.1145 $[M - H]^-$). The NMR data (Table 1) of **2** were typical of a flavonol trisubstituted in the B ring. In the ^{1}H NMR spectrum were signals corresponding to the H-6 (δ 6.19, d, $J = 1.7$ Hz) and H-8 (δ 6.28, d, $J = 1.7$ Hz) protons of the A ring, the signals of the H-2′ (δ 7.03, s) and H-5′ (δ 6.76, s) protons of the B ring, and signals of an isoprenyl moiety 5.15 (H-2″, t, $J = 7.0$ Hz), 3.27 (H-1″, d, $J = 7.0$ Hz), 1.59 (H-4″, s) and 1.51 ppm (H-5″, s). Moreover, the methoxyl group protons were evident at 3.86 ppm, and the position of this group was revealed from the HMBC spectrum by the correlation with C-3′. The HMBC spectrum also helped determine the position of the isoprenyl moiety at C-6′, with correlations of H-1″ with C-1′, C-5′, and C-6′ observed. Hence, the structure of **2** was assigned as 5,7,4′-trihydroxy-6′-prenyl-3′-methoxyflavonol.

Compound **3** was also obtained as an amorphous, yellow solid. The UV spectrum was alike to that of compound **2** (λmax 294 nm). Its ESI(−)-HRMS (*m/z* 383.1146 $[M - H]^-$), corresponding to $C_{21}H_{20}O_7$ molecular formula was identical to **2** indicating structural isomers. NMR spectra of compounds **2** and **3** were also similar (Table 1), though a difference resided in the position of the isoprenyl group at the B ring. The cross-peaks COSY correlation between H-5′ and H-6′ as well as the HMBC correlations of H-1″ with C-1′ (^3J), C-2′ (^2J), and C-3′ (^3J) determined the position of the side-chain on B-ring. Therefore, the structure of **3** was established as 5,7,4′-trihydroxy-2′-prenyl-3′-methoxyflavonol.

Compound **4** was obtained as amorphous, yellow solid. Its molecular weight was deduced based on its deprotonated molecular ion at *m/z* 577.1354 $[M - H]^-$, which corresponded to the molecular formula $C_{30}H_{26}O_{12}$ as provided by the ESI(−)-HRMS analysis. According to ^{1}H and ^{13}C NMR data (Table 2), **4** was structurally close to **22**, which was previously isolated from the buds of *P.* × *acerifolia* (Kaouadji and Morand, 1993). The kaempferol moiety was indicated by two meta-related signals δ 6.40 (1H, s, H-8) and δ 6.21 (1H, s, H-6); and two groups of ortho-coupled protons δ 7.84 (2H, d, $J = 8.5$ Hz, H-2′, 6′) and δ 6.97 (2H, d, $J = 8.5$ Hz, H-3′, 5′). The *p*-coumaric acid with E configuration was deduced from the two groups of doublets at δ 7.48 (2H, d, $J = 8.5$ Hz, H-2‴, 6‴) and δ 6.82 (2H, d, $J = 8.5$ Hz, H-3‴, 5‴) for the aromatic ring, and at δ 7.73 (1H, d, $J = 15.8$ Hz, H-7‴) and δ 6.43 (1H, d, $J = 15.8$ Hz, H-8‴) for the side chain. The rhamnose moiety was identified by cross-peaks of the anomeric proton to δ 5.47 (1H, d, $J = 1.4$ Hz, H-1″), δ 5.13 (1H, dd, $J = 3.2, 9.9$ Hz, H-3″), δ 4.45 (1H, dd, $J = 1.4, 2.9$ Hz, H-2″), δ 3.62 (1H, t, $J = 9.9$ Hz, H-4″), δ 3.45 (1H, m, H-5″), δ 0.98 (3H, d, $J = 6.4$ Hz, H-6″) in the ^{1}H-^{1}H COSY spectrum. Two main differences were observed while comparing the ^{1}H NMR data of **4** and **22**, H-2″ was shifted up-field (Δ − 1.09 ppm) and H-3″ downfield (Δ + 1.18 ppm) in **4**. Added to this, a HMBC correlation was noticed between H-3″-

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