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Phytochemical study of the trunk bark of *Citharexylum spinosum* L. growing in Tunisia: Isolation and structure elucidation of iridoid glycosides

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ABSTRACT

A phytochemical investigation of the trunk bark ethyl acetate extract of *Citharexylum spinosum* L. has led to the isolation of four previously undescribed iridoid glycosides, tunispinosides A-D, and five known phenylethanoid glycosides, verbascoside, leucosceptoside A, martynoside, isoverbascoside and plantainoside C, together with 4-hydroxy-2,6-dimethoxyphenyl 6′-*O*-vanilloyl-*β*-D-glucopyranoside, two 8,3′-neolignan glycosides, plucheosides D_1-D_2, coniferyl aldehyde, vanillic acid, syringic acid, ferulic acid and tyrosol. All compounds were isolated for the first time from *C. spinosum*. Their isolation was carried out using silica gel column and high performance liquid chromatography (HPLC). Structures were established by spectroscopic means including 1D and 2D NMR experiments, and spectrometric ESI-HRMS analysis.

1. Introduction

Plants are always sources of special and original chemical structures. The investigation of previously undescribed natural compounds increases the choice to finding more drugs and dietary supplements. These products are widely known as specialized metabolites such as alkaloids, iridoids, phenolics, lignans, flavonoids, tannins and saponins, generally synthesized by plants thanks to their defense effects, in particular against microorganisms. Furthermore, they are in most cases the responsible for the therapeutic properties of medicinal plants as active principles (Baxter et al., 1998).

Citharexylum spinosum L. also called *Citharexylum quadrangulare* Jacq., is a tree belonging to the Verbenaceae family (Wagner et al., 1999). This tree possesses medicinal properties and is traditionally useful in the treatment of dysmenorrhea (Cordero, 1978) and menstrual irregularities (Mañon Rossi, 1983). Organic extracts of various organs of this plant exhibit antifungal (El Ayeb-Zakhama and Harzallah-Skhiri, 2015), allelopathic (El Ayeb-Zakhama et al., 2015), antiulcer, antihypertensive and hepatoprotective effects (Khalifa et al., 2002). Two iridoid glycosides, spinoside and

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durantoside I, were identified in *C. spinosum* flowers (El Ayeb-Zakhama et al., 2015). From leaves of this plant, five iridoid glycosides, phlomiol, 5-deoxypulchelloside I, lamiide, durantoside I and lamidoside (Khalifa et al., 2002) were isolated, also, other iridoid glucosides, 7-*β*-*O*-acetyl lamiide, duranterectoside C, 8-*epi*-loganin and one lignan glycoside (+)-lyonirenisol-3a-*O*-*β*-D-glucopyranoside, were purified from the aerial parts (Balázs et al., 2006).

Iridoids derive from monoterpenes, structurally they are cyclopentano[c]pyran monoterpenoids, usually as glycosides. They are specialized metabolites of terrestrial and marine flora (Dinda et al., 2007), appearing in about 50 families of plants (Wink, 2003). They were considered a good taxonomic marker in Lamiales (Kooiman, 1972). They act as deterrents against herbivores and insects (Baden et al., 2011). Iridoid glycosides are pharmacologically active compounds, whose they inhibit the formation of prostaglandins and leukotrienes which are important mediators in animals (Wink, 2003). Iridoid glycosides exhibit a wide range of bioactivity: allelopathic (El Ayeb-Zakhama et al., 2015), anticancer (Gu et al., 2013), neuroprotective (Xu et al., 2011), anti-inflammatory, cardiovascular, antihepatotoxicity, antispasmolytic, choleretic, antiviral, hypoglycemic, antitumor, purgative activities (Ghisalberti, 1998).

Phenylethanoid glycosides are an interesting class of phenolic products (Wu et al., 2013). Structurally, they are characterised by cinnamic acid (C_6-C_3) and hydroxyphenylethyl (C_6-C_2) derivatives, which are attached to a *β*-glucopyranose (rhamnose, apiose, galactose ...) *via* a glycosidic linkage. They are naturally water-soluble compounds that are widely exist in many medicinal plants (Dembitsky, 2005). In recent years, phenylethanoid glycosides have shown an intersting role in the prevention and treatment of various human diseases. These compounds possess broad beneficial biological activities, including antioxidant, antineoplastic, neuroprotective, anti-inflammatory (Alipieva et al., 2014), antimicrobial (Liu et al., 2013) and cytoprotective (Gu et al., 2013) properties.

Lignans are a family of natural products as specialized metabolites (Kumar et al., 2014) among the most ubiquitous in terrestrial plants. From a chemical point of view lignans and neolignans have a great structural diversity, their molecular skeleton is derived from dimerization of two phenylpropanoid units (C_6-C_3) (Saleem et al., 2005). In addition, the difference between lignans and neolignans is described as follows, lignans are constructed by C2-C2′ linkage through carbons of propyl chains, but the other C-C linkages are known as neolignans (Kumar et al., 2014). They are compounds that have an important role in the development of drugs (Apers et al., 2003). Plentiful types of biological activities have been reported for this group of natural products such as antimitotic, antiangiogenic, antileishmanial, antifungal, antirheumatic, antitumoural, antiviral (including HIV), cytotoxic, hypolipidemic activities, as well as selective inhibitors of phosphodiesterases IV and V, and 5-lipoxygenase (Apers et al., 2003).

In the present study, we clarify the isolation and the structure elucidation of a series of compounds from the trunk bark ethyl acetate extract of *C. spinosum*. Structures were established by spectroscopic means including 1D and 2D NMR experiments, and spectrometric ESI-HRMS analysis, as four previously undescribed iridoid glycosides, tunispinosides A-D **(1–4)**, and five known phenylethanoid glycosides, verbascoside **(5)**, leucosceptoside A **(6)**, martynoside **(7)**, isoverbascoside **(8)** and plantainoside C **(9)**, together with 4-hydroxy-2,6-dimethoxyphenyl 6′-*O*-vanilloyl-*β*-D-glucopyranoside **(10)**, two 8,3′-neolignan glycosides, plucheosides D_1-D_2 **(11–12)**, coniferyl aldehyde **(13)**, vanillic acid **(14)**, syringic acid **(15)**, ferulic acid **(16)** and tyrosol **(17)**. Compounds **(5–17)** were isolated for the first time from *C. spinosum*. Their isolation was carried out using silica gel column (CC) and high performance liquid chromatography (HPLC).

2. Results and discussion

2.1. Structure elucidation of compounds

The ethyl acetate extract of the trunk bark of *C. spinosum* L. was fractionated by successive column chromatography and purified on preparative HPLC to afford four previously undescribed iridoid glycosides **(1–4)** (Fig. 1), together with thirteen known compounds **(5–17)** not previously identified in *C. spinosum*.

Compound 1 was obtained as a white amorphous powder. It was determined to have the molecular formula $C_{25}H_{32}O_{14}$ from the deprotonated ion $[M\text{-}H]^-$ at *m/z* 555.17126 (0.7 ppm) based on ESI-HRMS. The ^{1}H-NMR data of **1** (Table 1) displayed diagnostic signals at δ_H 7.39 (1H, *d*, J = 1.1 Hz) and 5.32 (1H, *d*, J = 3.4 Hz) due to H-3 and H-1 protons, respectively, which were characteristic of iridoids (El Ayeb-Zakhama et al., 2015). The presence of other signals at δ_H 3.86 (1H, *dd*, J = 4.4, 2.5 Hz), 3.47 (1H, *dd*, J = 8.7, 4.4 Hz), 2.87 (1H, *ddd*, J = 9.1, 2.5, 1.1 Hz), 2.68 (1H, *td*, J = 9.1, 3.4 Hz), 2.19 (1H, *m*) and 0.96 (3H, *d*, J = 7.3 Hz), which were attributable to H-6, H-7, H-5, H-9, H-8 and H-10 protons, respectively, suggested the presence of the 5-deoxypulchelloside I skeleton (Ayers and Sneden, 2002), previously identified in leaves of this tree (Khalifa et al., 2002). This result was propped by the ^{1}H-^{1}H COSY correlation crosspeaks H-1/H-9, H-9/H-8, H-8/H-10, H-8/H-7, H-7/H-6, H-6/H-5, H-5/H-3 and H-5/H-9 (Fig. 2). A singlet at δ_H 3.71 ppm (12-OCH_3) observed in ^{1}H-NMR spectrum and its corresponding carbon atom C-12 (δ_C 50.3) deduced from the HSQC spectrum, together with a HMBC correlation crosspeak H-12 (OCH_3)/C-11 (δ_C 167.8) were in agreement with the presence of a methyl ester function attached at C-4 (δ_C 109.7), revealed by the correlation crosspeak of H-3/C-11 supported from the same HMBC spectrum (Fig. 3). Furthermore, the ^{1}H-NMR spectrum showed a signal at δ_H 4.63 (1H, *d*, J = 7.9 Hz) due to anomeric proton H-1′. ^{1}H-^{1}H COSY correlation crosspeaks H-1′/H-2′ (δ_H 3.21), H-2′/H-3′ (δ_H 3.40), H-4′ (δ_H 3.40)/H-5′ (δ_H 3.62) and H-5′/Ha,b-6′ (δ_H 4.49, 4.60) (Fig. 2), together with HSQC spectrum and HMBC correlations of H-1 (δ_H 5.32)/C-1′ (δ_C 98.3) and H-1′ (δ_H 4.63)/C-1 (δ_C 94.5) both reporting that the glucopyranosyl moiety was attached at C-1. In addition to this, four new proton signals at δ_H 7.57 (1H, *m*, H-6″), 7.56 (1H, *m*, H-2″), 6.83 (1H, *d*, J = 8.1 Hz, H-5″) and 3.90 (3H, *s*, 8″-OCH_3), together with carbon signals deduced from the HSQC and HMBC spectra at δ_C 166.6 (C-7″), 152.3 (C-4″), 147.6 (C-3″), 123.8 (C-6″), 120.7 (C-1″), 114.8 (C-5″), 112.4 (C-2″) and 55.0 (8″-OCH_3), suggested the presence of a 1,3,4-trisubstituted benzene ring. Moreover, the HMBC correlations between H-6″ (δ_H 7.57)/C-7″ (δ_C 166.6), C-4″ (δ_C 152.3) and C-2″ (δ_C 112.4), H-5″ (δ_H 6.83)/C-4″ (δ_C 152.3), C-3″ (δ_C 147.6) and C-1″ (δ_C 120.7), and that at 8″-OCH_3 (δ_H 3.90)/C-3″ (δ_C 147.6) (Fig. 3), revealed the presence of vanilloyl moiety. The linkage of the vanilloyl to the 5-deoxypulchelloside I was established at C-6′ by the evidence of the downfield shifts of Ha,b-6′ (δ_H 4.49, 4.60), as well as the HMBC correlation between H-6′/C-7″ (δ_C 166.6).

The comparison of coupling constants and chemical shifts to literature data, jointly to the analysis of the ROESY spectrum allowed determining the stereochemistry of **1**. Therewith, the configuration of the 1-glucose, H-5, and H-9 is *β* in the most of iridoid compounds (Dinda et al., 2007). H-5 and H-9 was decided to be in *cis* configuration from the coupling constant (9.1 Hz), which is larger in *trans* configuration (12–13 Hz) (Krull and Stermitz, 1998; Dinda et al., 2007). Moreover, ROESY correlation of H-8/H-9 (Fig. 3) indicates that the three protons (H-5, H-8 and H-9) have the same spatial orientation in the structure of **1**. Therewith, comparison of carbon chemical shifts to literature data confirms that the configuration of the latter protons and the 1-glucose is *β*, so that when the configuration of them is *α*, the carbon shifts of C-1, C-9 and C-1′ are further in offset downfield at average of 5–6 ppm (Takeda et al.,