Phytochemical study of *Joannesia princeps* Vell. (Euphorbiaceae) leaves

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ABSTRACT

A phytochemical study of chloroform-methanol and methanol extracts of *Joannesia princeps* Vell. leaves led to the isolation of twenty eight compounds, including two α-ionones (**2**, **5**), three glycosylated monoterpenes (**1**, **3**, **4**), eight phenolic compounds (**6**, **8**, **9**, **12**, **14**, **17**, **18**, **24**), two gallotannins (**10**, **11**), twelve flavonoids (**7**, **15**, **16**, **19**, **20**-**23**, **25**-**28**), and one lignan (**13**). The structural characterization of the isolated compounds was performed by spectroscopic data and comparison with the literature. All compounds were isolated from this species and from the genus *Joannesia* for the first time. The chemotaxonomic importance of these metabolites is therefore summarized.

Keywords: *Joannesia princeps*; Flavonoids; Gallotannins; Phytochemical study; Chemotaxonomy

1. Subject and source

The genus *Joannesia* (Euphorbiaceae) comprises two species, one of which is *J. princeps* Vell., endemic to subtropical and coastal regions of Brazil, but it is also cultivated in Africa and Asia (Araújo et al., 2016). *Joannesia princeps* leaves were collected and identified by Momdouh Shokry at El-Zoharia Botanical Garden, Cairo, Egypt, in June 2013. A voucher specimen has been deposited at the Herbarium Horti Botanici Pisani, Pisa, Italy (n. 4429 *Joannesia princeps* Vell./1, Flora Aegyptiaca).

2. Previous work

Different secondary metabolites have been isolated from *J. princeps*, such as lignans, neolignans, and sesquineolignans from the methanol extract of the seeds (Waibel et al., 2003), while sesquiterpenes, diterpenes, triterpenes, steroids, proanthocyanidins, and phenolic compounds have been obtained from the root bark (Achenbach and Benirschke, 1997; Lange et al., 1997). Only one old phytochemical study on *J. princeps* leaves reported the occurrence of five flavonoid glycosides (Hilal et al., 1982). These compounds were not fully characterized since the interglycosidic bonds among the saccharide moieties remained undefined. *Joannesia princeps* leaves have shown antibacterial, antioxidant (Deepak et al., 2015) and anti-inflammatory activities (Sousa et al., 2009), however to our knowledge, their complete phytochemical characterization was never carried out.

3. Present study

The dried leaves of *J. princeps* (1 kg) were extracted for 72 h with solvents of increasing polarity: *n*-hexane, chloroform, chloroform-methanol (9:1) and methanol by exhaustive maceration (2 L) to give 12.2, 11.4, 11.5, and 35.7 g of residues, respectively. Part of the chloroform-methanol extract (6.7 g) was submitted to passage over Sephadex LH-20 column (5 x 75 cm), flow rate (1.0 mL/min) using methanol as eluent and collecting ten fractions (A-J) grouped by TLC. Fractions D (283.2 mg), E (360.7 mg), and I (177.7 mg) were subjected to RP-HPLC on a C-18 μ-Bondapak column (7.8 x 300 mm, flow rate 2.0 mL/min) with MeOH-H_2O (3:7) as eluent, to give compound **1** (2.5 mg) from fraction D; compounds **2** (3.5 mg), **3** (5.7 mg), **4** (3.6 mg), and **5** (1.8 mg) from fraction E; compounds **6** (2.4 mg), **8** (3.9 mg), and **9** (4.4 mg) from fraction I. Fraction J (615.3 mg) was subjected to RP-HPLC on a C-18 μ-Bondapak column (7.8 x 300 mm, flow rate 2.0 mL/min) with MeOH-H_2O (2:3) as eluent, to give compounds **6** (4.5 mg) and **7** (1.0 mg). The methanol extract was partitioned between *n*-butanol and water to afford a *n*-BuOH residue (6.8 g), that was submitted to passage over Sephadex LH-20 column (5 x 75 cm, flow rate 1.0 mL/ min) using methanol as eluent and collecting seventeen major fractions (A-Q) grouped by TLC. Fractions F (259.2 mg), H (309.3 mg), and M (192.4 mg) were subjected to RP-HPLC on a C-18 μ-Bondapak column (7.8 x 300 mm, flow rate 2.0 mL/min) with MeOH-H_2O (35:65) as eluent to give compounds **12** (4.3 mg) and **13** (2.7 mg) from fraction F; compounds **14** (16.3 mg), **15** (3.5 mg), and **16** (3.1 mg) from fraction H; compounds **24** (1.0 mg), **25** (3.0 mg), and **26** (1.0 mg) from fraction M. Fractions I (648.5 mg), K (250.5 mg), and N (96.5 mg) were purified by RP-HPLC on a C-18 μ-Bondapak column (7.8 x 300 mm, flow rate 2.0 mL/min) with MeOH-H_2O (2:3) as eluent, to give compounds **6** (3.2 mg), **7** (1.4 mg), **17** (6.5 mg), **18** (2.6 mg), **19** (1.9 mg), and **20** (0.7 mg) from fraction I;

compounds **21** (1.6 mg), **22** (2.4 mg), and **23** (5.2 mg) from fraction K; compounds **27** (2.3 mg) and **28** (1.6 mg) from fraction N. Fractions P and Q yielded compounds **10** (90.7 mg) and **11** (670.0 mg), respectively.

The structural characterization of compounds **1**-**28** (Fig. 1) was performed by 1D and 2D NMR spectroscopic techniques, mass spectrometry analyses, and comparison with data reported in the literature. Compounds were characterized as 3,7-dimethyl-oct-1-en-3,6,7-triol-6-*O*-β-D-glucopyranoside (**1**) (Abe et al., 2000), icariside B_2 (**2**) (Miyase et al., 1987), (3*S*, 6*R*)-*cis*-linalool-3,7-oxide-β-D-glucopyranoside (**3**) (Jiang et al., 2001), (3*S*,6*S*)-*cis*-linalool-3,6-oxide-β-D-glucopyranoside (**4**) (Jiang et al., 2001), blumenol C glucoside (**5**) (Miyase et al., 1988), methyl gallate (**6**) (Kuroyanagi et al., 1982), isovitexin (**7**) (Agrawal, 1989), *p*-hydroxyacetophenone (**8**) (Aladesanmi, 1988), *p*-hydroxybenzaldehyde (**9**) (Teguchi et al., 1981), 1,3,6-tri-*O*-galloyl-β-D-glucopyranoside (**10**) (Zhang et al., 2004), corilagin (**11**) (Sudjaroen et al., 2012), *cis*-1-*p*-coumaroyl-β-D-glucopyranoside (**12**) (Fons et al., 1998), isolariciresinol-9'-*O*-β-D-xylopyranoside (**13**) (Lee et al., 2001), β-D-glucogallin (**14**) (Haddock et al., 1982), isovitexin 2''-*O*-β-D-xylopyranoside (**15**) (Otsuka and Kijima, 2001), isovitexin 2''-*O*-α-L-rhamnopyranoside (**16**) (Otsuka and Kijima, 2001), gallic acid (**17**) (Zhang et al., 2004), methylbrevifolin carboxylate (**18**) (Sudjaroen et al., 2012), isoorientin 2''-*O*-β-D-xylopyranoside (**19**) (Saleh et al., 2005), myricetin 3-*O*-rutinoside (**20**) (Kazuma et al., 2003), orientin (**21**) (Kato and Morita, 1990), isoorientin (**22**) (Kato and Morita, 1990), quercetin 3-*O*-β-D- galactopyranoside (**23**) (Agrawal, 1989), brevifolin carboxylic acid (**24**) (Nawwar et al., 1994), 2''-*O*-galloylvitexin (**25**) (Latté et al., 2002), quercetin-3-*O*-β-D-glucoside (**26**) (Hassan et al., 2014), 2''-*O*-galloylorientin (**27**) (Latté et al., 2002), 2''-*O*-galloylisovitexin (**28**) (Latté et al., 2002).

4. Chemotaxonomic significance

The genus *Joannesia* belongs to the tribe Jatropheae, together with the genera *Brasilocroton*, *Colobocarpus*, *Croton*, *Eremocarpus*, *Jatropha*, *Julocroton*, *Moacroton*, *Opheltanta*, *Sandwithia*, and *Sawotia* (Wurdack et al., 2005). From a literature survey, diterpenoids are characteristic secondary metabolites of these genera; however, also monoterpenes, sesquiterpenes, and sterols are reported (Liu et al., 2015; Turiel et al., 2013; Zhang et al., 2016). The twenty-eight secondary metabolites obtained from *J. princeps* leaves could be grouped into: gallic acid derivatives (**6**, **14**, **17**, **18**, **24**), gallotannins (**10**, **11**), flavonoids, including *C*-glycosyl flavones (**7**, **15**, **16**, **19**, **21**, **22**), galloylated *C*-glycosyl flavones (**25**, **27**, **28**), and flavonol *O*-glycosides (**20**, **23**, **26**), α-ionones (**2**, **5**), glycosylated monoterpenes (**1**, **3**, **4**), lignan (**13**), and other phenolic derivatives (**8**, **9, 12**). All these compounds were isolated from this species and from the genus *Joannesia* for the first time.

Among the Euphorbiaceae species, gallotannins appears to be typical secondary metabolites of the genus *Alchornea* (Banzouzi et al., 2002; Ogungbamila and Samuelsson, 1990) and *Euphorbia* (Minozzo et al., 2016; Ryu et al., 2016). However, the presence of the ellagic acid derivative corilagin (**11**) in the tribe Jatropheae has been reported only once in *Jatropha curcas* L. (Manpong et al., 2009). Since this class of compounds were found in different Euphorbiaceae species (Cui and Tan, 2004) they might represent useful chemotaxonomic markers.

To our knowledge, this is the first report of isolariciresinol-9'-*O*-β-D-xylopyranoside (**13**) in the tribe Jatropheae. Within Euphorbiaceae, this compound has been reported only once in *Euphorbia soongarica* Boiss. (Shi et al., 2009).

Methylbrevifolin carboxylate (**18**) and brevifolin carboxylic acid (**24**) are uncommon gallic acid derivatives. This is the first time that these type of compounds are isolated in a plant

of the tribe Jatropheae. In Euphorbiaceae, these polyphenols were found in different species, such as plants of the genus *Euphorbia* (Lee et al., 1990; Ryu et al., 2016), *Macaranga* (Lin, 1994; Matsunami et al., 2006), and *Mallotus* (Saijo et al., 1989) and in the species *Sapium insigne* (Royle) Benth. & Hook. f. (Prasad et al., 2010),

Herein, a complete characterization of glycosylated flavonoids from *J. princeps* leaves is reported. Notably, plants of Euphorbiaceae frequently have been classified according to their flavonoid pattern (Noori et al., 2009). Compounds **7**, **21**, and **22** have been found in several plants of the genus *Croton* (Subramanian et al., 1971; Wagner et al., 1970) and *Jatropha* (Félix-Silva et al., 2014; Subramanian et al., 1971; Xavier and D'Angelo, 1995), while flavone *C*-glycosides **15**, **16**, and **19** were never characterized before in Euphorbiaceae. Previously, these last compounds have been detected in Fabaceae (Kwon and Bae, 2009), Passifloraceae (Costa et al., 2015; Ulubelen and Mabry, 1980), and Poaceae (Sun et al., 2013). Few references are present in the literature for the galloylated *C*-glycosyl flavones **25**, **27**, and **28**: to date, 2''-*O*-galloylvitexin (**25**) was isolated in two plants of Euphorbiaceae, *Conceveiba guaianensis* Aublet (Braca et al., 2004) and *Cladogynos orientalis* Zipp. ex Span. (Kanchanapoom, 2007), while 2''-*O*-galloylisovitexin (**28**) only in this last species (Kanchanapoom, 2007). On the contrary, the isolation of 2''-*O*-galloylorientin (**27**) is reported now for the first time in a plant of the tribe Jatropheae, and generally in Euphorbiaceae, since it was identified before only in *Pelargonium* (Geraniaceae) genus (Goedecke et al., 2005; Latté et al., 2002). Finally, the flavonol *O*-glycoside myricetin 3-*O*-rutinoside (**20**) has been reported in many different plant species, such as *Clitoria ternatea* L. (Fabaceae) (Kazuma et al., 2003), *Triclisia sacleuxii* (Pierre) Diels (Menispermaceae) (Samita et al., 2016), and *Camellia sinensis* (L.) Kuntze (Theaceae) (Wu et al., 2016); however **20** is reported now for the first time in Euphorbiaceae.

In conclusion, the results of this study suggested close chemotaxonomic relationships between *J. princeps* Vell. and other species of the tribe Jatropheae.

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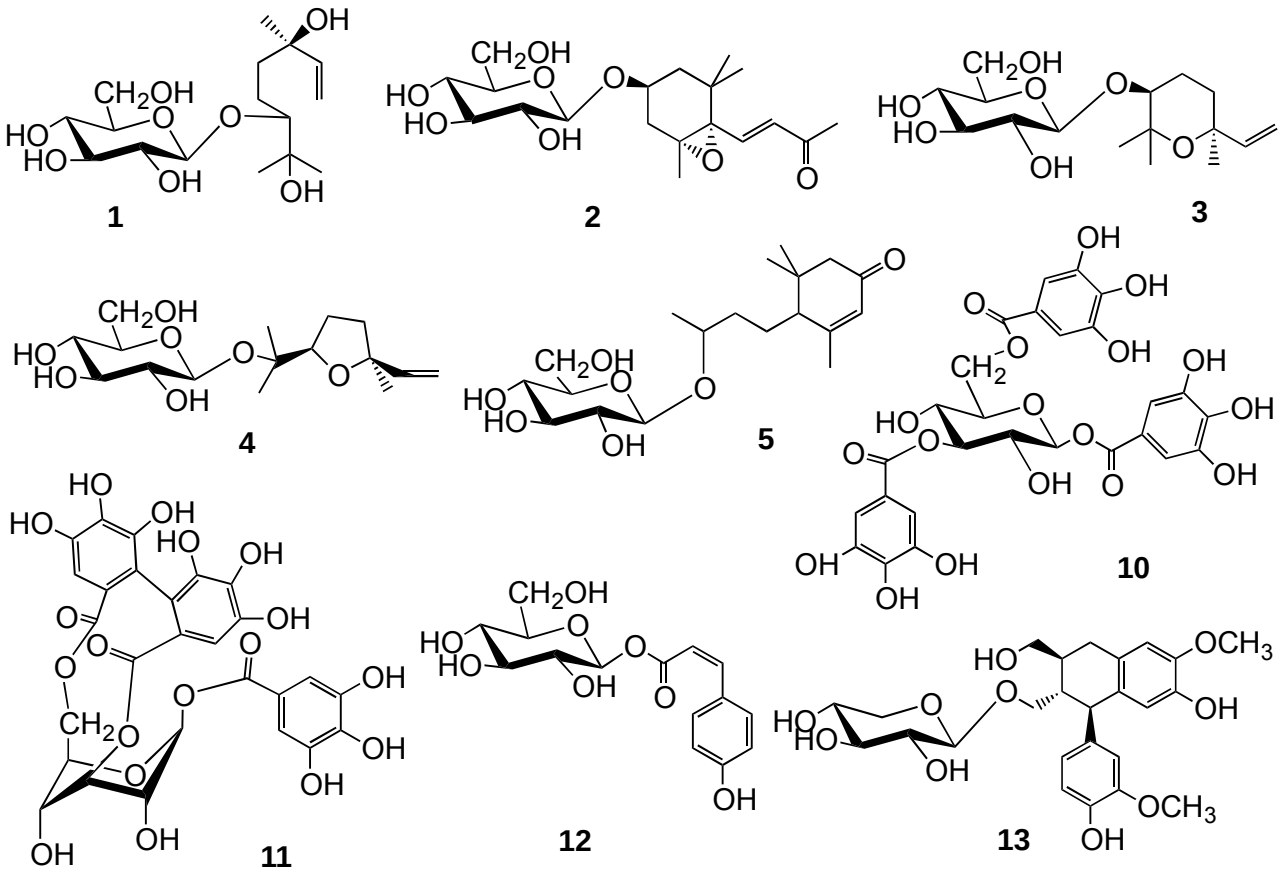
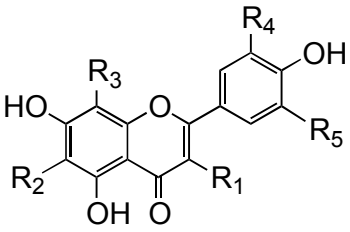
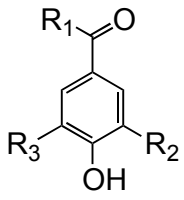
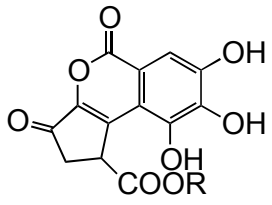




	R_1	R_2	R_3	R_4	R_5
7	H	glc	H	H	H
15	H	glc(1->2)xyl	H	H	H
16	H	glc(1->2)rha	H	H	H
19	H	glc(1->2)xyl	H	OH	H
20	*O*-rut	H	H	OH	OH
21	H	H	glc	OH	H
22	H	glc	H	OH	H
23	*O*-gal	H	H	OH	H
25	H	H	2-*O*-galloyl-glc	H	H
26	*O*-glc	H	H	OH	H
27	H	H	2-*O*-galloyl-glc	OH	H
28	H	2-*O*-galloyl-glc	H	H	H



	R_1	R_2	R_3
6	OCH_3	OH	OH
8	CH_3	H	H
9	H	H	H
14	O-glc	OH	OH
17	OH	OH	OH



	R
18	CH_3
24	H

Fig. 1. Structures of compounds **1**-**28** isolated from *J. princeps* leaves.

glc = glucopyranose, gal = galactopyranose, xyl = xylopyranose, rut = rutinose