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Short communication

Phytochemical investigation and antimicrobial assessment of *Bellis sylvestris* leaves

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ABSTRACT

The phytochemical study of *Bellis sylvestris* has been carried out. This study led to the isolation of 28 secondary metabolites belonging to different classes. The structures of these compounds have been elucidated on the basis of extensive 2D-NMR spectroscopic analyses, including COSY, TOCSY, HSQC, CIGAR-HMBC, H2BC and HSQC-TOCSY, along with Q-TOF HRMS^2 analysis. Three of them, two megastimane derivatives and a glycoside of 3-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone, were reported for the first time.

The compounds isolated from *Bellis sylvestris* were tested for their antimicrobial activity against some microorganisms associated with urinary tract infections (*Proteus mirabilis*, *Pseudomonas aeruginosa*, *Streptococcus aureus* and *Candida albicans*). The bacterial strains showed variable degrees of susceptibility to the compounds. Selected compounds were evaluated for their anti-biofilm properties against *Pseudomonas aeruginosa* and *Candida albicans*. Caffeic and rosmarinic acids were the once showing a higher reduction rate of biofilm formation.

1. Introduction

Plants are an overwhelming source of bioactive secondary metabolites. It is no wonder many of them have been used for medicinal purposes since the origin of mankind and the phytochemical research based on ethnopharmacology is now considered an effective approach in the discovery of novel chemicals with potential as drug leads (Brusotti et al., 2013).

The existence of this great and partially unexplored source of bioactive compounds fits well with the increasing demand of new drugs, especially in the field of antibiotics. Indeed, the number of multi-drug resistant bacteria is rising and the treatment of infections caused by these microorganisms is extremely challenging (Wu et al., 2015). Among these problematic infections, those affecting urinary tract (UTI) are common worldwide and vary from asymptomatic bacteriuria, cystitis, and simple pyelonephritis to complicated UTIs (cUTIs) (Chen et al., 2013). Although Enterobacteriaceae, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., and *Proteus* spp., are the most common pathogens involved in these infections, several non-glucose fermentative Gram negative bacilli, such as *Pseudomonas aeruginosa*, as well as several Gram positive cocci, including *Staphylococcus aureus*, contribute to the majority of cases of health-care-associated cUTIs. Candidal UTI usually occurs in patients with urinary catheters, and sometimes bacterial and candidal infections occur simultaneously (Kim et al., 2011).

In the search of new secondary metabolites endowed with antimicrobial activity, the phytochemical study of *Bellis sylvestris* has been carried out. *Bellis sylvestris*, the southern daisy, a stenomediterranean species belonging to this family, is an officinal and edible plant. Its use is highly superimposable to that of another plant of the same genus, *Bellis perennis*. Young leaves are eaten as salad, while leaf and flowers are known for their diuretic, purgative and diaphoretic properties (Karakas et al., 2012). They also have anti-inflammatory and astringent properties and have been used to treat common cold and infections of the upper respiratory tract in traditional medicine (Cakılcıoglu et al., 2010). A previous study

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led to the isolation of triterpenoid saponins (Scognamiglio et al., 2012a).

This study led to the isolation of many secondary metabolites belonging to different classes. The compounds isolated from *Bellis sylvestris* were tested for their antimicrobial activity against some microorganisms associated with UTIs (*Proteus mirabilis*, *Pseudomonas aeruginosa*, *Streptococcus aureus* and *Candida albicans*). They were also assayed for their antibiofilm activity.

2. Results and discussion

2.1. Isolation and determination of structure

The phytochemical study of *B. sylvestris* led to the isolation of 28 compounds (Fig. 1). Compounds **1**–**6** were identified as hydroxycinnamate based on the comparison with NMR data of compounds previously isolated from *Bellis perennis* (Scognamiglio et al., 2012b). In particular, compound **1** was identified as caffeic acid, while compounds **2** and **3** were identified as chlorogenic and neochlorogenic acid, respectively. Compounds **4** and **5** were 4,5-dicaffeoylquinic and 3,5-dicaffeoylquinic acids, respectively and compound **6** was identified as rosmarinic acid.

Compounds **7**–**17** were glycosylated flavonoids, all quercetin or kaempferol derivatives and one luteolin derivative.

Compound **7** was identified as luteolin-7-*O*-β-D-glucuronide (Beninger and Hall, 2005). Compounds **8**, **9** and **10** were quercetin7-*O*-β-D-glucuronide (Kajjout and Rolando 2011), quercetin-3-*O*-β-D-glucopyranoside (Kazuma et al., 2003) and quercetin-3-*O*-β-D-arabinopyranoside (De Almeida et al., 1998), while compound **11** was identified as rutin (Kazuma et al., 2003).

Compounds **12**–**17** were respectively identified as kaempferol-3-*O*-β-D-glucopyranoside (Kazuma et al., 2003), kaempferol-3-*O*-β-D-galattopyranoside (Lin et al., 2011), kaempferol-3-*O*-β-D-arabinopyranoside (De Almeida et al., 1998), nicotiflorin (Kazuma et al., 2003), kaempferol-3-robinobioside (Cardoso et al., 2005) and kaempferol-3-neohesperidoside (Kazuma et al., 2003).

Compound **18** was identified as a new metabolite, the glucoside of 3-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone; data of the aglicone were in accordance with Lin et al. (1994). The sugar was identified based on the chemical shift values as glucose. The site of glycosylation was determined based on long range correlations observed among the sugar anomeric proton at δ 4.78 and the aromatic carbon at δ 135.0 (C-4).

Compounds **19–22** were megastigmane derivatives. Compounds **19** and **20** were identified as byzantionoside B and gusanlungionoside D, respectively, based on comparison of NMR data with the literature (Matsunami et al., 2010; Yu et al., 2011).

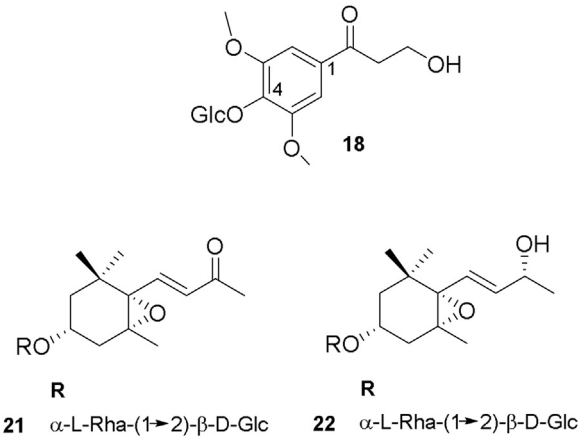

Fig. 1. Structures of new metabolites isolated from *B. sylvestris*.

The new compound **21** showed molecular formula $C_{25}H_{40}O_{12}$ in accordance with the MS spectrum acquired in positive mode, showing the pseudomolecolar peak at $[M+Na]^+$ 555.1653. The ^1H NMR spectrum (Table 1) suggested the presence of a neohesperidoside moiety, confirmed by ^{13}C and 2D NMR data. Besides the sugars signals, the remaining proton and carbon signals (Table 1), attributed based on 1D and 2D NMR analysis, were in accordance with the data reported for the aglycone moiety glucosilated at C-3 by Yamamoto et al. (2008). The presence of an oxyrane ring was confirmed by NMR and MS data and the stereochemistry of the aglicone was determined by comparison of ^{13}C NMR data with those reported in the literature (Yamamoto et al., 2008 and references therein). The neohesperidoside moiety was linked at the C-3 position, based on the long-range correlation occurring among the glucose anomeric proton and the C-3 carbon.

Compound **22**, also reported for the first time, showed molecular formula $C_{25}H_{42}O_{12}$ in accordance with the MS spectrum acquired in positive mode, showing the pseudomolecolar $[M+Na]^+$ peak at m/z 557.1696. The ^{13}C NMR spectrum (Table 1) revealed the presence of signal belonging to a neohesperidoside moiety (confirmed by comparison of 1D and 2D NMR with compound **21**). The remaining 13 carbons were again attributable to a megastigmane with most of the ^1H and ^{13}C NMR data superimposable to those of compound **21** (Table 1). A doublet methyl was detected at δ 1.23 (H-10), which showed COSY correlation with a proton at δ 4.28 (H-9), in turn correlated to an olefinic proton resonating as doublet of doublet at δ 5.63 (H-8, J = 16.0; 5.7), and further correlated to a proton at δ 5.87 (H-7; J = 16.0). These two protons were correlated in the HSQC spectrum with carbons at δ 125.9 and 139.0 respectively, confirming the presence of a double bond. The coupling constants of the protons were compatible with a *trans* geometry. These evidences bear to the conclusion that the main difference with compound **21** was the presence of a hydroxyl group instead of the keto group at C-9. Analogously to compound **21**, the glycosylation site was determined by the long range

Table 1
NMR data of compounds **21** and **22** in CD_3OD.

	21		**22**	
	^{13}C	^1H	^{13}C	^1H
1	36.1	–	36.0	–
2	45.7	1.41 (dd 12.6, 3.6) 1.74 (dd 12.6, 1.8)	46.1	1.36 (dd 12.3, 3.6) 1.74 (dd 12.3, 1.8)
3	72.4	3.91 ov	72.2	3.92 ov
4	38.2	1.77 (dd 14.1, 9.0) 2.48 (dd 14.1, 6.0)	38.4	1.75 (dd 14.1, 9.0) 2.47 (dd 14.1, 4.5)
5	68.7	–	67.9	–
6	71.1	–	71.3	–
7	145.4	7.16 (d 15.9)	125.9	5.87 (d 16.0)
8	133.8	6.18 (d 15.9)	139.0	5.63 (dd 16.0, 5.7)
9	200.3	–	68.7	4.28 (m)
10	27.4	2.29 (s)	23.4	1.23 (d 6.3)
11	29.6	1.19 (s)	24.0	0.97 (s)
12	25.3	0.96 (s)	29.6	1.18 (s)
13	20.1	1.19 (s)	19.8	1.20 (s)
1′ (Glc)	101.4	4.43 (d 7.8)	101.3	4.43 (d 7.8)
2′	78.6	3.33 (m)	78.2	3.34 (m)
3′	79.4	3.45 (m)	79.6	3.47 (m)
4′	71.8	3.27 (m)	72.0	3.27 (m)
5′	77.9	3.25 (m)	77.6	3.24 (m)
6′	62.7	3.66 (m) 3.84 (m)	62.9	3.67 (m) 3.84 (m)
1” (Rha)	101.9	5.24 (s)	101.9	5.23 (s)
2”	72.2	3.90 (m)	72.3	3.91 (m)
3”	72.4	3.66 (m)	72.4	3.64 (m)
4”	74.0	3.40 (m)	74.0	3.36 (m)
5”	69.7	4.04 (m)	69.6	4.04 (m)
6”	18.1	1.22 (d 6.3)	18.1	1.19 (d 6.3)

s = singlet, d = doublet, dd = doublet of doublet, m = multiplet; J values (Hz) are reported in brackets.