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Two new bis-iridoids isolated from *Scabiosa stellata* and their antibacterial, antioxidant, anti-tyrosinase and cytotoxic activities

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ABSTRACT

This study presents the chemical profile investigation of a 70% ethanol extract obtained from *Scabiosa stellata*, a medicinal herbaceous traditionally used to treat heel cracks. A ^{13}C NMR-based dereplication methodology was firstly applied on centrifugal partition chromatography-generated fractions in order to quickly identify the major compounds of the extract. The dereplication process was then completed by semi-preparative high-performance liquid chromatography in order to identify unknown or minor compounds. Two new bis-iridoids, namely 7-*O*-caffeoyl-sylvestroside I (**1**) and 7-*O*-(*p*-coumaroyl)-sylvestroside I (**2**), together with ten known compounds (**3** − **12**) were isolated. Their structures were elucidated by spectroscopic methods including NMR and HR-ESI-MS. The antibacterial, anti-tyrosinase and DPPH radical scavenging activities of the crude extract, fractions, and isolated compounds were evaluated. A significant antibacterial activity was observed for nine isolated compounds, particularly **1** and **2** which yielded MIC values of 31.2 μg/mL against *Enterococcus faecalis* and 62.5 μg/mL against *Staphylococcus epidermidis*. The cytotoxic activity of these new bis-iridoids was evaluated on a fibrosarcoma cell line (HT1080) and only compound **1** exhibited a moderate cytotoxic activity (IC_{50} 35.9 μg/mL).

1. Introduction

The genus *Scabiosa* belongs to the Caprifoliaceae family and comprises about 100 species. The majority of *Scabiosa* species occurs in the Mediterranean region [1] and among them 12 species grows in Algeria [2]. In Catalonia (Spain), the decoction of the aerial part of *S. columbaria* is traditionally used against diphtheria [3]. In Algeria, *S. arvensis* is used in folk medicine against diarrhea, inflammation, microbial infections and skin disorders [4]. In moroccan folk medicine, the leaves and flowers of *S. stellata* are used against heel cracks [5]. Many extracts obtained from *Scabiosa* species have already demonstrated antibacterial activities, such as *S. atropurpurea* [6], *S. hymettia* [7], *S. columbaria* [8], or *S. arenaria* [9]. The extracts of *S. arenaria* and *S. tschiliensis* have shown antioxidant properties [10,11].

Up to date, chemical investigations of *Scabiosa* species have mainly revealed the presence of saponins [12,13], flavonoids, and coumarins [14]. Previous studies have also reported that the genus *Scabiosa* characteristically contains bis- and mono-iridoid glucosides [15,16].

Scabiosa stellata Cav., known with the common name starflower pincushions, is an herbaceous, bristly-hairy annual plant (20–60 cm). The lower leaves are 7–12 cm long, spoon-shaped in outline, tapered at the base, the margins with blunt teeth irregular in size and placement. The outer florets are light gray-blue, irregularly shaped. The central florets are smaller and subtended each by a rounded translucent bract with a green midrib [4]. In this work, we have investigated the chemical profile of *S. stellata* and the antibacterial, tyrosinase inhibitory, DPPH radical scavenging and cytotoxic activities of the crude extract, fractions and isolated compounds.

2. Results and discussion

The antibacterial activity of the 70% EtOH extract obtained from the whole plant *S. stellata* was evaluated against 22 μ-organisms including 17 Gram-positive and Gram-negative bacteria and 5 yeasts. The

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Table 1
Antimicrobial activity of 70% ethanol extract of *S. stellata* and fractions A–E (solid medium).

Micro-organisms	70% EtOH extract MIC (mg/mL)	Fractions MIC (mg/mL)					Positive controls MIC (µg/mL)		
		A	B	C	D	E	G	V	Am
Gram positive bacteria									
Bacillus subtilis	5	> 10	2.5	2.5	10	> 10	0.12	2	NT
Enterococcus faecalis ATCC 1034	> 10	> 10	1.2	2.5	10	> 10	16	> 64	NT
Staphylococcus aureus 8325-4	> 10	> 10	0.6	1.2	10	> 10	0.5	4	NT
Staphylococcus aureus CIP 53.154	> 10	> 10	0.6	1.2	10	> 10	4	> 64	NT
Staphylococcus epidermidis	2.5	10	1.2	1.2	5	5	0.25	4	NT
Micrococcus luteus	2.5	10	1.2	2.5	5	5	0.5	4	NT
Listeria innocua	> 10	> 10	> 10	10	> 10	> 10	0.5	≤ 4	NT
Streptococcus pyogenes	1.2	10	1.2	2.5	5	5	2	1	NT
Gram negative bacteria									
Escherichia coli CIP 54.127	> 10	> 10	> 10	10	> 10	> 10	≤ 4	> 16	NT
Enterobacter cloacae	> 10	> 10	> 10	10	> 10	> 10	≤ 4	> 16	NT
Salmonella enterica	> 10	> 10	> 10	10	> 10	> 10	≤ 4	> 16	NT
Serratia marcescens	> 10	> 10	> 10	10	> 10	> 10	0.5	> 64	NT
Proteus vulgaris	> 10	> 10	> 10	10	> 10	> 10	≤ 4	> 16	NT
Klebsiella pneumoniae	> 10	> 10	> 10	10	> 10	> 10	> 64	> 64	NT
Providencia stuartii	> 10	> 10	> 10	10	> 10	> 10	2	> 64	NT
Pseudomonas aeruginosa ATCC 9027	> 10	> 10	> 10	10	> 10	> 10	8	> 64	NT
Shigella sonnei	> 10	> 10	> 10	10	> 10	> 10	0.5	8	NT
Yeast									
Candida albicans	5	10	0.6	1.5	5	10	> 64	> 64	0.5
Candida glabrata	2.5	10	2.5	2.5	10	10	> 64	> 64	0.25
Candida tropicalis	5	> 10	1.5	1.5	5	10	> 64	> 64	0.25
Candida kefyr	2.5	5	1.2	1.2	5	5	> 64	> 64	0.25
Cryptococcus neoformans	2.5	5	1.2	1.2	5	5	> 64	> 64	0.5

MIC: minimum inhibitory concentration, NT: not tested. Positive controls: G: **G**entamicin, V: **V**ancomycin and Am: **Am**photericin B.

Table 2
DPPH radical scavenging and mushroom tyrosinase inhibition of 70% MeOH extract, fractions A–E and compounds isolated from *S. stellata*.

	DPPH radical scavenging activity IC_{50} (µg/mL)	Mushroom tyrosinase inhibition IC_{50} (µg/mL)
70% MeOH extract	86.0 ± 1.8	(40%)[a]
Fraction A	133 ± 2.6	(45%)[a]
Fraction B	48.7 ± 1.1	(15%)[a]
Fraction C	25.0 ± 0.8	1330 ± 23
Fraction D	64.3 ± 1.5	1000 ± 19
Fraction E	> 200	1330 ± 22
6	7.1 ± 0.4	> 665
7	7.2 ± 0.4	> 665
8	8.5 ± 0.5	> 665
10	16.0 ± 0.6	> 665
Ascorbic acid[b]	6.3 ± 0.1	
Kojic acid[b]		6.8 ± 0.1

[a] % Inhibition at 1330 µg/mL.
[b] The positive control.

MIC determination method in solid media was used [17]. The results are presented in Table 1. The highest antimicrobial activity was observed against *Streptococcus pyogenes* (MIC 1.2 mg/mL), whereas a moderate to low antimicrobial activity was observed against *Staphylococcus epidermidis* or *Micrococcus luteus* (MIC 2.5 mg/mL) and *Bacillus subtilis* (MIC 10 mg/mL), Regarding antifungal activity, the crude extract showed a moderate activity (MIC 2.5 to 5 mg/mL). A moderate DPPH radical scavenging activity was also observed for this extract (IC_{50} 86.0 µg/mL), as well as a moderate tyrosinase inhibitory activity when tested on an *in vitro* mushroom tyrosinase assay (40% inhibition at 1.33 mg/mL) (Table 2).

In order to isolate potentially active compounds, a bioassay-guided fractionation strategy was applied throughout the separation procedure. The 70% ethanol extract of the whole plant *S. stellata* was subjected to a Diaion HP-20 column, eluting with 0%, 25%, 50%, 75% and 100% MeOH, yielding five fractions (A-E, respectively). These fractions were evaluated for their antimicrobial, antioxidant and anti-tyrosinase activities (Tables 1 and 2). Fraction C exhibited the best antimicrobial activity against the 22 microorganisms (MIC 1.2 to 10 mg/mL). Whereas fraction B was active only against the gram-positive bacteria (except against *Listeria innocua*) and yeasts (MIC 0.6 to 2.5 mg/mL) (Table 1). Fractions B and C showed also the best DPPH radical scavenging activity (IC_{50} 48.7 and 25 µg/mL, respectively) (Table 2). Fraction C showed also a significant anti-tyrosinase activity (IC_{50} 1.33 mg/mL) and was slightly less active than fraction D (IC_{50} 1 mg/mL) (Table 2). Therefore, the chemical profiles of fractions B and C were investigated in order to determine which compounds were responsible for these activities (Fig. 1). Fractions B (2 g) and C (3 g) were subjected separately to CPC fractionation. The biphasic solvent system M*t*BE/CH_3CN/water (3/3/4, v/v/v) was selected to recover moderately polar compounds. After pooling the collected fractions on the basis of TLC profile similarities, adjacent sub-fractions B_1-B_{23} and C_1-C_{21} containing simplified mixtures or even pure compounds were obtained. Fractions B_1-B_{23} and C_1-C_{21} were all analyzed by ^{13}C NMR for dereplication [18]. Automatic peak picking and binning of ^{13}C signals across spectra resulted in two tables (one table for each CPC separation of B and C) which were independently submitted to Hierarchical Clustering Analysis (HCA) for pattern recognition. In this way, statistical correlations between ^{13}C NMR signals belonging to individual structures within the fraction series were visualized as “chemical shift clusters” on the resulting two-dimensional HCA correlation heat maps in front of the corresponding dendrograms. As illustrated in Fig. 2, several well-defined clusters were intensely colored in yellow. After entering the chemical shift values of cluster 1 located in sub-fractions $B_{4–8}$ and $C_{7–14}$ into the database, the molecular structure of isoorientin (**8**) [19] was proposed. By means of the same database search strategy, cluster 2 present in sub-fractions $B_{2–4}$ and $C_{3–6}$ was identified as hyperin (**10**) [20], cluster 3 present in sub-fractions $B_{20–23}$ and $C_{11–14}$ was identified as eustomoruside (**6**) [21], cluster 4 present in sub-fractions $C_{8–9}$ corresponded to a bis-iridoid structure containing loganic acid and caffeic