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Inula viscosa (L.) Aiton leaves and flower buds: Effect of extraction solvent/technique on their antioxidant ability, antimicrobial properties and phenolic profile

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ABSTRACT

This study was designed to establish the most effective solvent/technique for extracting antioxidant phytoconstituents from leaves and flower buds of *Inula viscosa* (L.) Aiton (Asteraceae) grown wild in Morocco. Maceration and hot extraction with methanol or water and Soxhlet ethanol extraction were utilized. The antioxidant potential was evaluated *in vitro* by DPPH, reducing power, and ferrous ions chelating activity assays. *I. viscosa* leaf and flower bud extracts displayed the strongest effect in the DPPH test, being the Soxhlet ethanol the most active ones (IC_{50} = 54.24 ± 0.21 μg/mL and 39.77 ± 0.23 μg/mL); thus, they were selected for further investigations. The antimicrobial efficacy of the Soxhlet ethanol extracts against ATCC and food isolates strains was assayed; the leaf extract showed the best activity, and *Candida albicans* was the most sensitive strain (MIC = 125 μg/mL). The extracts resulted non-toxic against *Artemia salina*. Among the phenolics characterised by HPLC-PDA-ESI-MS, hispidulin hexoside, patuletin and spinacetin were identified for the first time.

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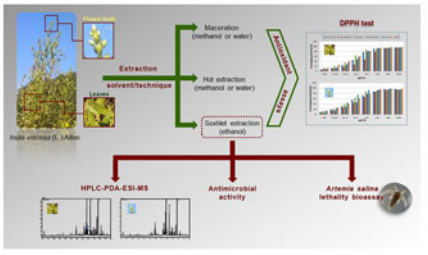

1. Introduction

Morocco is a Mediterranean country having a rich and ancient tradition and historical knowledge of medicinal plants. *Inula viscosa* (L.) Aiton (syn. *Dittrichia viscosa* L.) is an herbaceous perennial species belonging to the Asteraceae family. *I. viscosa* is reported to have many uses in traditional medicine; in Morocco it is utilised as diuretic, anti-anemic and anthielminthic and for the treatment of rheumatic pain, bronchitis, tuberculosis, cardiac disease, hypertension and diabetes mellitus (Seca et al. 2014). Due to the ethnomedicinal uses, various studies on the phytochemical composition and the biological activities of *I. viscosa* have been carried out (Seca et al. 2014; Ben Sassi et al. 2008; Fontana et al. 2007). In recent years, a substantial body of evidence has indicated a key role for free radicals as major contributors to aging, to the pathogenesis of several ailments such as cancer and cardiovascular disease, and in diabetes complications. For this reason, a number of investigations have been focused on the therapeutic potential of medicinal plants as antioxidants in reducing such free radical-induced tissue injury. Most antioxidants isolated from higher plants are polyphenols, which have been shown to exert numerous biological effects such as antibacterial, anti-carcinogenic and anti-inflammatory (Taviano et al. 2018a; Miceli et al. 2017; Taviano et al. 2017; Marino et al. 2016; Srivastava and Mishra 2015).

This work was designed with the main objective of establishing the most effective solvent and technique for extracting antioxidants from leaves and flower buds of *I. viscosa* grown wild in Morocco. For this purpose, maceration and hot extraction with methanol or water (mac-MeOH and mac-H_2O, hot-MeOH and hot-H_2O) and Soxhlet ethanol extraction (Sox-EtOH) were used. The results of our investigation will provide additional information for a feasible use of this species as a source of antioxidants. Besides, the best antioxidant extracts from both leaves and flower buds have been selected for further studies: the antimicrobial potential and the toxicity were evaluated and the phenolic profile was characterised.

2. Results and discussion

2.1. Antioxidant activity

In order to achieve a comprehensive view of the antioxidant profile of *I. viscosa* extracts, three *in vitro* test systems based on different approaches and mechanisms

were employed. The results of DPPH test showed that the extracts have a strong effect on scavenging free radicals, compared to the standard BHT (IC_{50} = 48.47 ± 0.44 μg/mL). Based on IC_{50} values, the scavenging effect on DPPH radical ranged from 54.24 ± 0.21 μg/mL (Sox-EtOH) to 148.79 ± 0.11 μg/mL (mac-MeOH) for the leaves, and from 39.77 ± 0.23 μg/mL (Sox-EtOH) to 86.06 ± 0.25 μg/mL (mac-MeOH) for flower buds (Table 1, Figure S1). The extracts exhibited reducing power, with ASE/mL values ranging from 5.05 ± 0.17 (hot-MeOH) to 8.20 ± 0.63 (mac-H_2O) for the leaves, and from 4.65 ± 0.45 (hot-H_2O) to 9.03 ± 0.64 (mac-H_2O) for flower buds (Table 1, Figure S2). Previous investigations demonstrated the antioxidant activity of ethanol and ethyl acetate extracts obtained by maceration from *I. viscosa* aerial parts selected from three regions of Morocco (Chahmi et al. 2015); from a comparison of the results, all the extracts tested in our study displayed stronger activity both in the DPPH and reducing power assays. This could depend on the different solvents utilised, as well as on the extraction conditions (time, temperature and solvent-to-solid ratio). In the Fe^{2+} chelating activity assay only mac-H_2O extracts from both leaves and flower buds and hot-H_2O extract from flower buds showed mild activity (Table 1**)**. Our results agree with those previously reported by Orhan et al. (2017), who didn't found any chelating activity in methanol extracts of *I. viscosa* flowers and leaves collected in Turkey. The results of the antioxidant tests showed that *I. viscosa* extracts displayed noticeable radical scavenging activity in the DPPH test, and the Soxhlet ethanol extraction was the most effective technique; thus, the Sox-EtOH extracts of both leaves and flower buds were selected for further investigations.

2.2. Antimicrobial activity

The antimicrobial properties of *I. viscosa* Sox-EtOH extracts were tested against ATCC and isolates from chickens bacterial strains (Aghraz et al. 2018; Benameur et al. 2018; Płuchtová et al. 2018) and the yeast *Candida albicans* ATCC 10231. Both extracts exhibited the best antimicrobial efficacy against *C. albicans*, being the leaves extract more active than flower buds one (MICs = 125 μg/mL and 250 μg/mL, respectively). Among bacteria *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* S20/16 food isolate were sensitive to the leaves extract (MIC = 250 μg/mL). Our results disagree with those of Oskay and Sar (2007), that reported for *I. viscosa* leaf ethanol extract higher antimicrobial efficacy *vs. S. aureus* than *C. albicans* and

Table 1. Free radical scavenging activity (DPPH test), Reducing power, and Fe^{2+} chelating activity of *Inula viscosa* leaf and flower bud extracts.

	DPPH test IC_{50} (μg/mL)		Reducing power ASE/mL		Fe^{2+} chelating activity IC_{50} (μg/mL)	
I. viscosa extracts	Leaves	Flower buds	Leaves	Flower buds	Leaves	Flower buds
mac-MeOH	148.79 ± 0.11	86.06 ± 0.25	7.21 ± 0.19	9.03 ± 0.64	—	—
mac-H_2O	77.48 ± 0.16	54.63 ± 0.85	8.20 ± 0.63	5.51 ± 0.17	450.85 ± 5.23	199.08 ± 2.14
hot-MeOH	75.17 ± 0.60	74.44 ± 0.32	5.05 ± 0.17	5.02 ± 0.12	—	—
hot-H_2O	59.65 ± 0.68	47.45 ± 0.62	5.20 ± 1.27	4.65 ± 0.45	—	549.57 ± 0.31
Sox-EtOH	54.24 ± 0.21	39.77 ± 0.23	7.56 ± 0.72	5.45 ± 0.12	—	—
Standard	BHT: 48.47 ± 0.44		BHT: 1.97 ± 0.08		EDTA: 6.68 ± 0.04	

Values are expressed as the mean ± SD (n = 3).

no effect against *E. coli*, as well as with those of Talib and Mahasneh (2010), that didn't found any activity for *I. viscosa* flower ethanol extract against different strains, including *C. albicans*.

2.3. Artemia salina Leach lethality bioassay

Artemia salina Leach lethality bioassay is one of the most valuable tests available for the preliminary assessment of toxicity, because of the rapidity, low cost and easiness, and also less ethically challenging than murine model. Both *I. viscosa* Sox-EtOH extracts did not display any toxicity against brine shrimps ($LC_{50} > 1000\,\mu g/mL$). From a pharmaceutical point of view, it is an advantage when antibacterial drugs are selectively toxic to the microbe but non-toxic to eukaryotic cells.

2.4. Characterisation of phenolic compounds by HPLC-PDA-ESI-MS analysis

The determination of the phenolic profile of *I. viscosa* Sox-EtOH extracts was carried out by HPLC-PDA-ESI-MS. A total of 31 and 28 different compounds were positively identified in the leaf and flower bud extracts, respectively (Table 2, Figure S3). Among them, 9 were phenolic acid derivatives while the rest was composed of flavonoids and one lactone (helenin). Most of bioactives identified are consistent with previous studies on *I. viscosa* leaves and aerial parts (Kheyar-Kraouche et al. 2018; Orhan et al. 2017; Mahmoudi et al. 2016; Seca et al. 2014). Interestingly, among the identified compounds three polyphenols are reported here for the first time as constituents of *I. viscosa* leaves and flower buds, namely hispidulin hexoside, patuletin and spinacetin. From a quantitative point of view, *I. viscosa* leaf extract presented the highest amount in terms of bioactive compounds (258.66 mg/g), more than double with respect to the flower bud one (118.99 mg/g), and 3-O-acetylpadmatin was the main compound in both extracts (60.45 mg/g and 17.5 mg/g, respectively). Flavonoids and phenolic acids represent the largest classes of plant phenolics; phytochemicals from these classes were found to have excellent antioxidant activity and antimicrobial efficacy against a wide array of microorganisms (Taviano et al. 2018b). Thus, it can be assumed that the effects observed for *I. viscosa* extracts could depend, almost in part, on these compounds.

3. Conclusions

The results obtained from the comparative study indicate that Soxhlet extraction with ethanol represents the most efficient method of extracting antioxidant components from *I. viscosa* leaves and flower buds. The Sox-EtOH extracts exhibited antimicrobial activity and were non-toxic against *A. salina*; besides, three flavonoids were detected for the first time. Our findings contribute to an increase in knowledge about the phytochemical composition of *Inula viscosa* and support the popular use of this species as a folk remedy, also indicating that the Sox-EtOH extracts from leaves and flower buds could represent a valuable safe source of effective antioxidant and antimicrobial agents.

Table 2. HPLC-PDA-ESI-MS phenolic fingerprint of *Inula viscosa* leaf and flower bud Sox-EtOH extracts. Column: Ascentis Express C18. 15 cm × 4.6 mm. 2.7 μm d.p. (ESI: negative ionization mode).

Peak	Compound	t_R (min)	Molecular Formula	$[M-H]^-$	PDA (nm)	Leaves (mg/g ± %RSD)	Flower buds (mg/g ± %RSD)
1	Caffeic acid-*O*-hexoside	13.5	–	341, 179	325	<LoQ	<LoQ
2	Caffeic acid	15.6	$C_9H_8O_4$	179	325	0.90 ± 3.10	0.56 ± 2.52
3	p-Coumaric acid	18.4	$C_9H_8O_3$	163	310	0.88 ± 3.21	–
4	Taxifolin hexoside	25.2	$C_{21}H_{22}O_{12}$	465	337	0.16 ± 2.81	0.15 ± 3.21
5	Hydroxybenzoic acid hexoside	25.4	$C_{13}H_{16}O_8$	299	255	0.97 ± 3.25	0.48 ± 2.9
6	Isorhamnetin-*O*-hexoside	25.7	$C_{22}H_{22}O_{12}$	477	355	0.19 ± 3.57	<LoQ
7	3.4-Dicaffeoylquinic acid	29.5	$C_{22}H_{24}O_{12}$	515, 353	325	1.45 ± 2.59	3.11 ± 2.21
8	3.5-Dicaffeoylquinic acid	29.8	$C_{22}H_{24}O_{12}$	515, 353	325	10.46 ± 1.87	8.50 ± 0.96
9	4.5-Dicaffeoylquinic acid	30.1	$C_{22}H_{24}O_{12}$	515, 353	325	3.73 ± 2.18	5.12 ± 1.25
10	Coumaryl caffeoylquinic acid	32.1	–	499, 353	325	2.28 ± 1.94	0.79 ± 1.56
11	Caffeic acid-*O*-hexoside dimer	33.4	–	683, 179	325	1.68 ± 2.55	0.65 ± 2.54
12	Luteolin	35.0	$C_{15}H_{10}O_6$	285	351	0.69 ± 2.71	–
13	Isorhamnetin-3-O-(6-O-feruloyl)-glucoside	36.7	–	653	355	11.24 ± 0.64	5.43 ± 1.26
14	Hispidulin hexoside	37.6	$C_{22}H_{22}O_{11}$	461	335	1.15 ± 1.65	8.33 ± 1.56
15	Patuletin	38.1	$C_{16}H_{12}O_8$	331	370	2.88 ± 1.68	2.00 ± 2.36
16	7-O-Methylaromadendrin	38.5	$C_{16}H_{14}O_6$	301	365	1.34 ± 2.31	10.24 ± 1.11
17	Padmatin	38.9	$C_{16}H_{14}O_7$	317	355	38.82 ± 0.32	12.61 ± 1.32
18	3-O-Acetylaromadendrin	39.4	–	329	365	4.14 ± 0.68	–
19	Spinacetin	39.7	$C_{16}H_{12}O_8$	345	370	4.21 ± 0.72	3.10 ± 1.26
20	Apigenin	39.9	$C_{15}H_{10}O_5$	269	335	2.14 ± 1.23	0.34 ± 3.21
21	Hispidulin	42.1	$C_{16}H_{12}O_6$	299	335	46.48 ± 0.54	11.38 ± 1.23
22	3.3′ Di-O-methylquercetin	43.1	$C_{17}H_{14}O_7$	329	370	1.59 ± 1.35	1.91 ± 1.25
23	3-O-methylquercetin	43.5	$C_{16}H_{12}O_7$	315	370	1.04 ± 1.33	1.12 ± 1.26
24	Rhamnocitrin	44.9	$C_{16}H_{12}O_6$	299	365	10.00 ± 0.65	7.81 ± 0.98
25	Isorhamnetin	45.3	$C_{16}H_{12}O_7$	315	355	11.84 ± 0.72	8.66 ± 0.99
26	Helenin	46.4	$C_{15}H_{20}O_2$	231	288	n.q.	n.q.
27	Quercetin dihydrate	47.5	$C_{15}H_{14}O_9$	337	370	0.69 ± 1.58	0.39 ± 3.12
28	Nepetin	47.8	$C_{16}H_{12}O_7$	315	351	19.01 ± 0.72	6.04 ± 0.99
29	3-O-Acetylpadmatin	49.0	$C_{18}H_{16}O_8$	359	355	60.45 ± 0.23	17.50 ± 1.25
30	Sakuranetin	51.7	$C_{16}H_{14}O_5$	285	355	16.80 ± 0.83	2.29 ± 1.12
31	Genkwanin	53.8	$C_{16}H_{12}O_5$	283	335	1.45 ± 1.35	0.48 ± 2.65

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Conflict of interest

The authors declare no potential conflict of interest, including any financial interest.

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