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Contribution of Phenolic Compounds to the Antioxidant Activity of Leaf and Flower Extracts of *Sinapis pubescens* L. subsp. *pubescens* (*Brassicaceae*)

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Within a study focused on *Sinapis pubescens* subsp. *pubescens* wild from Sicily (Italy), an edible species still unexplored, our earlier published work has demonstrated good *in vitro* antioxidant properties for the flower and leaf hydroalcoholic extracts, exhibiting quite different qualitative-quantitative phenolic profiles. Herein, further research was designed to elucidate the role played by phenolic compounds in the different antioxidant mechanisms highlighted for the extracts. To achieve this goal, the crude extracts were subjected to liquid-liquid partitioning with solvents of increasing polarity; then, the fractions were investigated for their antioxidant properties using different *in vitro* assays. For both flowers and leaves, the ethyl acetate fractions exhibited the best activity in DPPH and reducing power assays, followed by *n*-butanol. The total phenolic content determination indicated these fractions as the phenolic-rich ones, which were characterized by HPLC-PDA/ESI-MS analysis. Conversely, the phenolic-rich fractions did not show any chelating activity, which was highlighted for the more hydrophobic ones.

Introduction

In the last decades, wild plants have attracted substantial interest due to their significant relevance in the agri-food sector, as well as for the treatment of numerous diseases.[1,2] Wild species have great potential as sources of bioactive compounds; to adapt to harsh environments, these plants have been induced to enhance their chemical defense mechanisms by increasing the production of specialized secondary metabolites.[3] Indeed, wild plants are increasingly considered potential resources of natural health products; experimental research based mainly on ethnobotanical investigations and empirical traditional knowledge has demonstrated that they are valuable sources of nutrients and phytochemicals with interesting antioxidant properties.[4–6]

The *Brassicaceae* family includes a large number of species with great economic and nutritional relevance around the world; many of these species are native to the Mediterranean basin.[7,8] For years, several species belonging to this family have been an attractive research topic, due to their chemical composition characterized by many valuable metabolites, which are directly linked to different recognized biological activities.[9,10] For some years now, several species included in the *Brassicaceae* family belonging to the spontaneous flora of Sicily (Italy) have been investigated by our research team to discover novel sources of health-promoting compounds through the chemical and biological characterization of the active secondary metabolites. As part of this line of research, recently a study was started on specific and intraspecific taxa of *Sinapis* L., utilized in traditional medicine and as food. Current investigations have focused on *Sinapis pubescens* L. subsp. *pubescens*, an edible species still unexplored. The earlier published study, carried out on hydroalcoholic extracts (70% MeOH) obtained from different plant parts (flowers, leaves and stems), demonstrated good antioxidant properties for the extracts from leaves and flowers of *S. pubescens* subsp.

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pubescens, suggesting that they could be considered potentially suitable as sources of nutraceuticals.[11] Particularly, the leaf extract has been shown to have higher primary antioxidant properties, as evaluated through DPPH radical scavenging and reducing power assays, which appeared to be related to the higher polyphenol content; on the other hand, the flower extract exhibited greater secondary antioxidant properties in the ferrous ion chelating activity assay.

As far as the analytical techniques are concerned, HPLC coupled with photodiode array and mass spectrometry detection have been by far the most adopted ones; especially in the last decade multi-dimensional liquid chromatography techniques have been proposed.[12–14]

Accordingly, the phytochemical characterization of the phenolic compounds contained in these extracts, carried out by means of spectrophotometric methods and HPLC-PDA/ESI-MS analysis, highlighted a quite different qualitative-quantitative profile.

The interesting results obtained in the experimental study prompted us to continue this research, aiming to define the actual role played by the phenolic compounds in the antioxidant properties of *S. pubescens* subsp. *pubescens* leaf and flower extracts. For such a purpose, the crude extracts were subjected to sequential liquid-liquid partitioning with various solvents of increasing polarity; then, the obtained fractions, *i.e.*, *n*-hexane (*Spp*-HE), dichloromethane (*Spp*-DCM), ethyl acetate (*Spp*-ETAC), *n*-butanol (*Spp*-nBA), and the aqueous residue (*Spp*-AQ) were investigated for their phenolic content and subjected to antioxidant testing using different *in vitro* systems based on diverse mechanisms of action.

Results and Discussion

Phytochemical Investigations

Determination of Total Polyphenol Content

The total polyphenol content of *S. pubescens* subsp. *pubescens* flower and leaf fractions was determined by means of the Folin-Ciocâlteu method. This colorimetric assay, widely used for quantification of phenolics in plant extracts, is based on electron transfer (ET) reactions between Folin-Ciocâlteu reagent and phenolic compounds under basic conditions.[15]

As depicted in Table 1, the colorimetric assay highlighted a predominance of phenolic constituents in the *Spp*-ETAC fraction of both flowers and leaves (310.30 ± 2.07 and 129.18 ± 0.86 mg GAE/g, respectively), followed by *Spp*-nBA (60.03 ± 2.12 and 102.56 ± 2.43 mg GAE/g). Notably, the obtained results highlighted a different distribution of the polyphenol compounds in the polar fractions of *S. pubescens* subsp. *pubescens* leaves and flowers; indeed, they are mainly concentrated in the *Spp*-ETAC fraction for the flowers, whereas they are distributed almost equally between *Spp*-ETAC and *Spp*-nBA for the leaves, highlighting the different phenolic composition of the two organs of the plant. As expected, for both flowers and leaves the non-polar *n*-hexane fractions contain the lowest quantity of polyphenols (*Spp*-HE 17.76 ± 0.69 and 12.03 ± 1.15 mg GAE/g, respectively). Finally, the phenolic content of the *Spp*-DCM and *Spp*-AQ fractions is comparable in both cases, being intermediate between the *Spp*-HE and the *Spp*-nBA fractions. For both flowers and leaves, the total polyphenol content of the fractions and the aqueous residue decreases in the following order: *Spp*-ETAC > *Spp*-nBA > *Spp*-DCM = *Spp*-AQ > *Spp*-HE.

Table 1. Total phenolic content of the fractions from leaves and flowers of *Sinapis pubescens* subsp. *pubescens*.

	Total phenolic content (mg GAE/g fraction)	
	Leaves	Flowers
Spp-HE	12.03 ± 1.15^{a}	17.76 ± 0.69^{A}
Spp-DCM	46.99 ± 2.16^{b,}	33.77 ± 1.69^{B}
Spp-ETAC	129.18 ± 0.86^{c}	310.30 ± 2.07^{C}
Spp-nBA	102.56 ± 2.43^{d}	60.03 ± 2.12^{D}
Spp-AQ	46.04 ± 2.09^{b}	32.61 ± 1.62^{B}

The data shown in the table are the mean ± SD (n = 3). $^{a–d}$Different letters within the same column indicate significant differences between mean values ($P < 0.05$).

Characterization of the Phenolic-rich Fractions by HPLC-PDA/ESI-MS

The composition of the *S. pubescens* subsp. *pubescens* flower and leaf phenolic-rich fractions was assessed by HPLC-PDA/ESI-MS and the results attained are illustrated in Figure 1. The chromatograms, extracted at 330 nm wavelength, highlight the occurrence of 18 compounds for the leaf *Spp*-ETAC fraction (A), 21 for the leaf *Spp*-nBA (B) and 10 for the flower *Spp*-ETAC fraction (C); for the flower *Spp*-nBA, except for malic acid, no further compounds were detected. Identification, reported in Table 2, was carried out on the basis of the information coming from retention times, UV and mass spectra, along with literature data.[3,11,16–19]

Among the 34 bioactive compounds identified, with the exception of bis-Noryangonin, 14 were phenolic compounds (free or linked to a sugar moiety) and 18 were glycosylated flavonoids.

In the leaf *Spp*-ETAC fraction, 1-*O*-Feruloyl-β-D-glucose, peak n. 13, turned turned out to be the most abundant compound (63.50 ± 0.282 mg/g of dried fraction) followed by Kaempferol (X)-rhamnoside (Y)-glucoside and Quercetin glucoside, peak n. 24 + 25 (47.38 ± 0.143 mg/g of dried fraction); the latter were the most abundant ones in the leaf *Spp*-nBA fraction (42.30 ± 0.016 mg/g of dried fraction). On the other hand, Isorhamnetin glucoside, peak n. 32, turned out to be the most abundant compound (17.87 ± 0.016 mg/g of dried fraction) in the flower *Spp*-ETAC fraction. The structures of all major compounds are illustrated in Figure 2.

For comparison's sake, the total polyphenolic content of the *Spp*-ETAC and *Spp*-nBA leaf fractions well correlated with the

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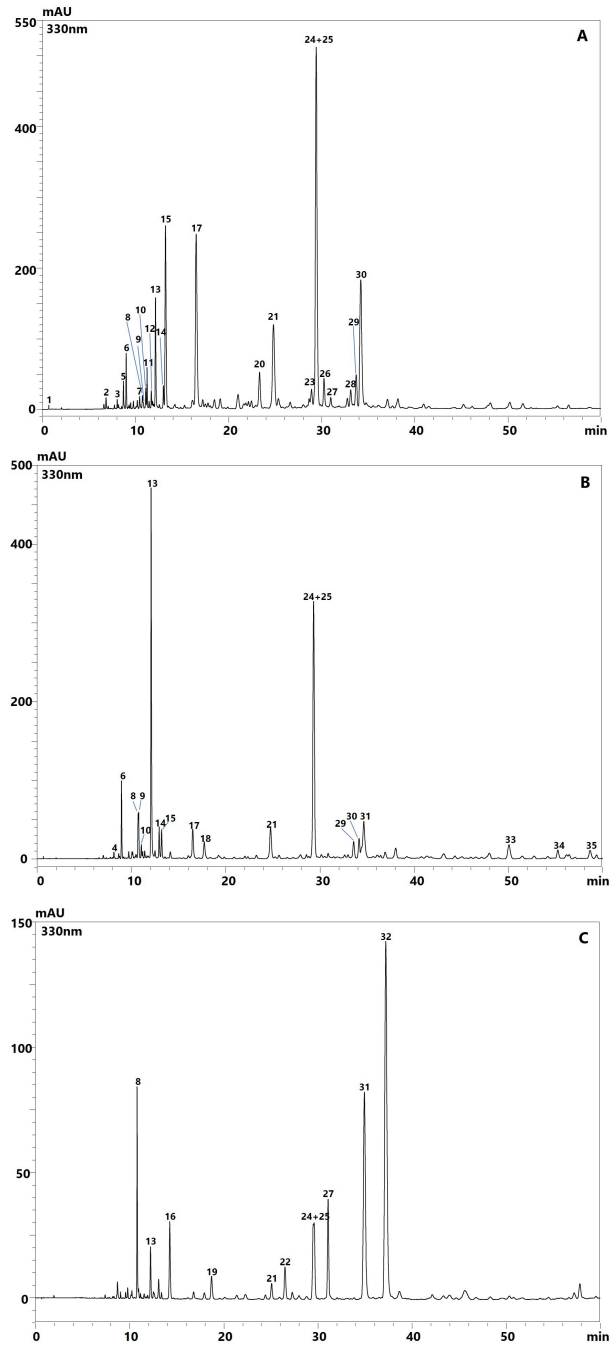


Figure 1. HPLC-PDA analysis of *Spp*-ETAC (A) and *Spp*-nBA (B) fractions from the leaves, and *Spp*-ETAC (C) fraction from the flowers of *Sinapis pubescens* subsp. *pubescens* (chromatograms extracted at 330 nm). Tentative identification is shown in Table 3.

total phenolic content achieved by Folin-Ciocâlteu method (183.01 mg/g of dried fraction *vs.* 129.18 mg GAE/g fraction and 143.58 mg/g of dried fraction *vs.* 102.56 mg GAE/g fraction); on the other hand, higher contents were attained for the Folin-Ciocâlteu method especially with the *Spp*-ETAC flower fraction, with values as high as 310.30 mg GAE/g fraction.

In our previous study, we evaluated the phenolic profile of the crude flower and leaf hydroalcoholic extracts of *S. pubescens* subsp. *pubescens*.[11]

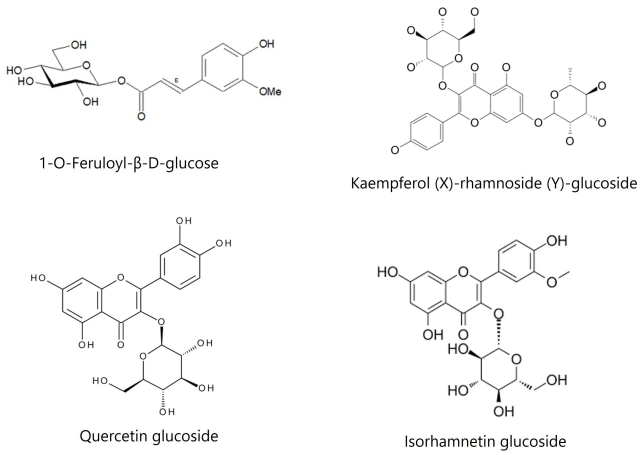


Figure 2. Structures of the most abundant polyphenolic compounds identified in the fractions from the leaves and the flowers of *Sinapis pubescens* subsp. *pubescens*.

Comparing the whole extracts with the phenolic-rich fractions analyzed in the current work, a total of 22 bioactive compounds matched with the ones reported for the aerial parts. Isorhamnetin diglucoside was the most abundant compound in the flower extract (11.23 ± 0.48 mg/g of dried extract) and Kaempferol coumaroyl rutinoside in the leaf extract (10.04 ± 1.22 mg/g of dried extract).

Antioxidant Activity

Oxidative stress represents a common factor of several diseases characterized by multiple mechanisms through which reactive oxygen species (ROS) cause cellular damage, including cancer, neurodegenerative, metabolic and cardiovascular diseases, and other disorders. Numerous studies have shown that natural antioxidants play an essential role in preventing and treating several diseases, due to their ability to reduce oxidative stress, thus contributing to the maintenance of human health.[20–22] Most of the natural antioxidants are derived from plant matrices, such as herbs, spices, seeds, fruits, and vegetables; indeed, in recent years, a considerable number of studies have focused on plant antioxidants, giving evidence of their health-promoting effects.[23,24] Considering the health benefits provided by antioxidants, increasing knowledge of their content in alternative plant matrices may represent a valuable objective.

The antioxidant activity of the fractions from flower and leaf extracts of *S. pubescens* subsp. *pubescens* was determined through the same *in vitro* assays used in our previous study for the crude extracts.[11] The selected methods are based on different mechanisms of action, to consider the diverse mechanisms involved in the inhibitory effect of the oxidation process through which the antioxidant compounds contained in the phytocomplex could act. Therefore, to evaluate the primary antioxidant properties two assays were performed, namely the DPPH test, based on the dual mechanism of hydrogen atom transfer (HAT) and electron transfer (ET), and the reducing power assay, based on electron transfer (ET); the

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Table 2. Semi-quantification of phenolic compounds in the fractions from leaves and flowers of *Sinapis pubescens* subsp. *pubescens* through LC-PDA/ESI-MS analysis.

Peak	Compound	t_R (min)	UV max (nm)	$[M-H]^-$	*Sinapis pubescens* subsp. *pubescens*				Ref.
					Leaves		Flowers		
					Spp-ETAC	*Spp*-nBA	*Spp*-ETAC	*Spp*-nBA	
1	Malic acid	1.02	274	133	–	x	–	x	[11]
2	bis-Noryangonin	6.82	319	229, 211	–	x	–	–	[16]
3	Caffeoyl-sucrose	8.02	323	503, 179	–	0.28 ± 0.006	–	–	–
4	Caffeoyl-glucose	8.15	325	341, 179	0.35 ± 0.009	-	–	–	[11]
5	Caffeoyl-sophorose	8.70	320	503, 179	–	1.47 ± 0.024	–	–	–
6	Caffeoyl-glucose	8.99	329	341, 179	4.99 ± 0.140	3.46 ± 0.006	–	–	[11]
7	Feruloyl-dihexose	10.44	324	517, 193	–	0.55 ± 0.030	–	–	[11]
8	p-Coumaroyl-glucose	10.72	315	325, 163	4.99 ± 0.140	1.11 ± 0.011	4.24 ± 0.026	–	[11]
9	Caffeic acid	10.83	321	179	5.75 ± 0.129	0.20 ± 0.007	–	–	Std.
10	Feruloyl-dihexose	11.09	326	517, 193	1.28 ± 0.020	1.11 ± 0.006	–	–	–
11	Feruloyl-dihexose	11.24	328	517, 193	–	2.43 ± 0.012	–	–	[11]
12	Unknown	11.69	274, 332	547, 345, 227	–	x	–	–	–
13	1-*O*-Feruloyl-β-D-glucose	12.15	329	355, 193	63.50 ± 0.282	10.23 ± 0.001	1.12 ± 0.004	–	[17]
14	1-*O*-Sinapoyl-β-D-glucose	12.99	330	385, 223	5.14 ± 0.028	1.91 ± 0.059	–	–	[17]
15	Quercetin 3-*O*-sophoroside	13.21	255, 352	625, 463, 301	2.86 ± 0.013	14.70 ± 0.003	–	–	[17]
16	p-Coumaric acid	14.27	307	163	–	–	2.81 ± 0.001	–	Std.
17	Kaempferol 3-O-sophoroside	16.52	264, 345	609, 447, 285	4.46 ± 0.027	20.29 ± 0.047	–	–	[18]
18	Ferulic acid	17.74	322	193	5.67 ± 0.195	–	–	–	Std.
19	Isorhamnetin diglucoside	18.72	252, 350	639, 477, 315	–	–	0.58 ± 0.002	–	[11]
20	Kaempferol (X)-coumaroylglucoside (Y)-glucoside	23.31	264, 341	755, 593, 447, 285	–	3.39 ± 0.010	–	–	–
21	Quercetin rhamnosyl-glucoside	24.82	255, 352	609, 447, 301	7.56 ± 0.021	15.01 ± 0.008	0.39 ± 0.001	–	–
22	Kaempferol (X)-glucoside (Y)-glucoside	26.51	265, 347	609, 447, 285	–	–	0.77 ± 0.000	< LOQ	–
23	Kaempferol (X)-coumaroyl-glucoside (Y)-glucoside	28.90	265, 345	755, 593, 447, 285	–	1.42 ± 0.003	–	–	–
24 + 25	Kaempferol (X)-rhamnoside (Y)-glucoside + Quercetin glucoside	29.40	265, 345	593, 447, 285 + 463, 301	47.38 ± 0.143	42.30 ± 0.016	3.54 ± 0.008	< LOQ	–
26	Quercetin dicoumaroyl-glucoside	30.23	255, 348	755, 593, 447, 301	–	2.44 ± 0.005	–	–	–
27	Isorhamnetin rhamnosyl-glucoside	30.96	254sh, 346	623, 461, 315	–	0.67 ± 0.043	2.55 ± 0.056	< LOQ	–
28	Kaempferol (X)-coumaroyl-glucosyl-rhamnoside (X)-glucoside	33.11	264, 341	901, 739, 593, 431, 285	–	1.34 ± 0.008	–	–	–
29	Kaempferol glucosyl-malonyl-rhamnoside	33.69	265, 346	679, 635, 431, 285	2.64 ± 0.004	2.92 ± 0.025	–	–	–
30	Kaempferol dicoumaroyl-glucoside	34.18	264, 342	739, 593, 431, 285	2.95 ± 0.004	16.35 ± 0.056	–	–	[11]
31	Kaempferol glucoside	34.65	264, 347	447, 285	9.66 ± 0.208	–	7.79 ± 0.000	–	[19]
32	Isorhamnetin glucoside	37.19	254, 352	477, 315	–	–	17.87 ± 0.016	–	[11]
33	1,2-diferuloyl gentiobioside	50.06	328	693, 499, 717 [M + Na]	8.14 ± 0.020	–	–	–	[3]

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Table 2. continued

Peak	Compound	t_R (min)	UV max (nm)	$[M-H]^-$	*Sinapis pubescens* subsp. *pubescens*				Ref.
					Leaves		Flowers		
					Spp-ETAC	*Spp*-nBA	*Spp*-ETAC	*Spp*-nBA	
34	Kaempferol (X)-coumaroyl-digluco-side (X)-rhamnoside	55.24	264, 328	901, 431, 285	2.95 ± 0.019	–	–	–	–
35	Kaempferol rhamnoside	58.67	264, 364	431, 285	2.74 ± 0.050	–	–	–	–

Quantification of phenolic compounds is reported in mg/g of dried fraction ± SD (n = 2). x: detected, but not quantified; sh: wavelength shoulder.

secondary antioxidant properties were assessed through the ferrous ion chelating activity assay.[25,26]

The DPPH test was used to evaluate the radical scavenging activity of the fractions. The 2,2-diphenyl-1-picrylhydrazyl ($DPPH^•$) is a stable radical, due to the delocalization of the unpaired electron of the nitrogen atom over the molecule, with a deep purple color and strong absorption at 517 nm.[27] The radical is neutralized by accepting either a hydrogen atom or an electron from an antioxidant, so that $DPPH^•$ is reduced to the corresponding hydrazine; consequently, the purple color gradually turns into bright yellow.[25]

As can be seen from Figure 3, the *Spp*-ETAC fraction resulted the most active for both flowers and leaves; nonetheless, a very different trend was highlighted in the range of concentrations tested. Indeed, the fraction from the flowers determined about 97% inhibition of the DPPH radical at the concentration of 0.25 mg/mL and total inhibition at higher concentrations, resulting more active than the BHT standard in the concentration range of 0.25–2 mg/mL. Differently, the DPPH radical scavenging activity of the *Spp*-ETAC fraction from the leaves increases with increasing concentration, resulting in approximately 90% inhibition at the maximum concentration tested, slightly lower than that of the standard.

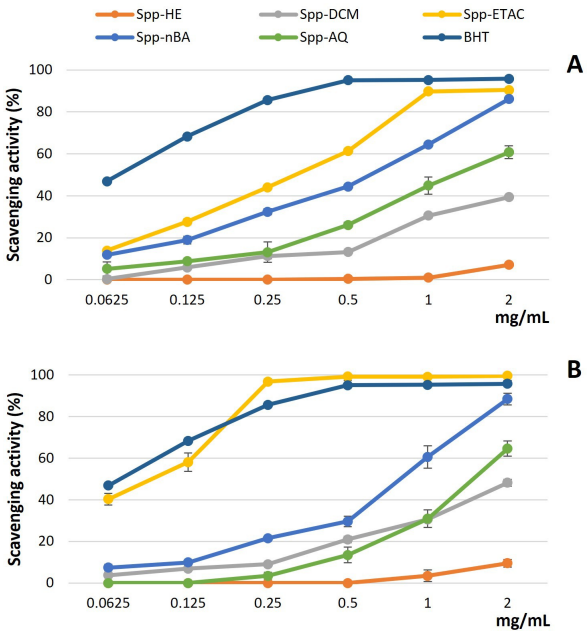


Figure 3. DPPH radical scavenging activity (%) of the fractions from leaves (A) and flowers (B) of *Sinapis pubescens* subsp. *pubescens*. The results are expressed as the mean ± SD (n = 3).

The stronger activity of the *Spp*-ETAC fractions, higher for that of the flowers, is also confirmed by the calculated IC_{50} values, equal to 0.09 ± 0.02 mg/mL and 0.38 ± 0.01 mg/mL, respectively for flowers and leaves (Table 3). For both flowers and leaves, *Spp*-DCM and *Spp*-AQ fractions displayed moderate activity, whereas weak activity was observed for *Spp*-HE fractions (IC_{50} not determined).

Based on the IC_{50} values, the radical scavenging activity of the fractions, the aqueous residue, and the standard decreases in the following order: BHT = *Spp*-ETAC > *Spp*-nBA > *Spp*-AQ > *Spp*-DC > *Spp*-HE. for the flowers and BHT > *Spp*-ETAC > *Spp*-nBA > *Spp*-AQ > *Spp*-DCM > *Spp*-HE for the leaves.

The ability of the fractions to stabilize radicals by donating electrons (ET mechanism) was evaluated by the potassium ferricyanide method, based on the reduction of Fe (III) to Fe (II).[25] As illustrated in the graphs shown in Figure 4, for both flowers and leaves the *Spp*-ETAC fractions exhibited the best reducing power, similarly to what observed in the DPPH test; furthermore, the activity resulted higher for that of the flowers, with an absorbance value equal to approximately 1.35 at the maximum concentration tested. Anyway, in this test the activity of the fractions was lower than that of the standard BHT.

The ASE/mL values calculated for the fractions, shown in Table 3, confirmed this result (ASE/mL = 4.13 ± 0.13 and 7.70 ± 0.30, for flower and leaf *Spp*-ETAC, respectively).

Compared to the standard, the *Spp*-nBA and *Spp*-AQ fractions showed moderate reducing power, whereas weak activity was highlighted for the *Spp*-DCM and *Spp*-HE ones.

Based on the ASE/mL values, the reducing power of the fractions, the aqueous residue, and the standard decreases in the following order: BHT = *Spp*-ETAC > *Spp*-nBA = *Spp*-AQ > *Spp*-DCM = *Spp*-HE for the flowers and BHT > *Spp*-ETAC = *Spp*-nBA = *Spp*-AQ > *Spp*-DCM > *Spp*-HE for the leaves.

The ability to chelate transition metals is another important mechanism to consider for estimating the antioxidant activity of an extract or compound. Indeed, it has recently been demonstrated that transition metals such as Fe^{2+} and Cu^{2+} are implicated in the pathogenesis of some diseases, including

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Table 3. DPPH radical scavenging activity, Reducing power, and Ferrous ion chelating activity of the fractions from leaves and flowers of *Sinapis pubescens* subsp. *pubescens*.

	DPPH IC_{50} (mg/mL)		Reducing power (ASE/mL)		Chelating activity IC_{50} (mg/mL)	
	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers
Spp-HE	N.D.	N.D.	N.D.	26.91 ± 3.02^{A}	N.A.	0.72 ± 0.07^{A}
Spp-DCM	2.38 ± 0.01^{a}	2.00 ± 0.01^{A}	29.12 ± 0.80^{a}	23.68 ± 0.18^{A}	1.17 ± 0.03^{a}	0.16 ± 0.01^{B}
Spp-ETAC	0.36 ± 0.01^{b}	0.09 ± 0.02^{B}	7.70 ± 0.30^{b}	4.13 ± 0.13^{B}	N.A.	N.A.
Spp-nBA	0.68 ± 0.01^{c}	0.65 ± 0.17^{C}	8.49 ± 1.10^{b}	10.14 ± 4.23^{C}	N.A.	N.A.
Spp-AQ	1.46 ± 0.11^{d}	1.56 ± 0.17^{D}	8.91 ± 1.06^{b}	10.69 ± 3.42^{C}	0.78 ± 0.01^{b}	0.19 ± 0.01^{B}
Standard	BHT $0.07 \pm 0.01^{e,B}$		BHT $1.44 \pm 0.02^{c,B}$		EDTA $0.02 \pm 6{,}84E\text{-}05^{c,C}$	

The data shown in the table are the mean ± SD (n = 3); DPPH: 2,2-diphenyl-1-picrylhydrazyl; IC_{50}: half-maximal inhibitory concentration; ASE: ascorbic acid equivalent. N.D.: not determined; N.A.: not active. $^{a-e}$Different letters within the same column indicate significant differences between mean values ($P < 0.05$).

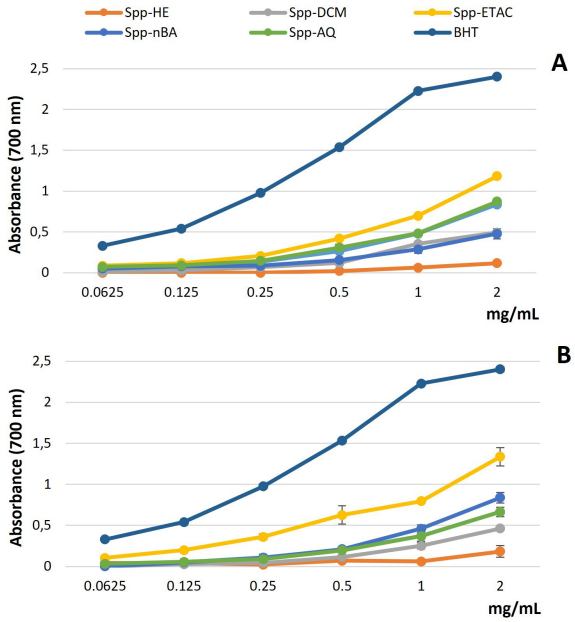


Figure 4. Reducing power (Abs 700 nm) of the fractions from leaves (A) and flowers (B) of *Sinapis pubescens* subsp. *pubescens*. The results are expressed as the mean ± SD (n = 3).

neurodegenerative disorders, such as Alzheimer's and Parkinson's, and cardiovascular diseases.[26] Herein, the ability of the fractions to chelate Fe^{2+} was assessed by the measurement of the Fe^{2+}-ferrozine complex, using the colorimetric method reported by Kumar et al.[28]

As depicted in the graphs shown in Figure 5, the fractions from the flowers were generally more active than those from the leaves. Contrary to what observed in the other tests, the *Spp*-ETAC and *Spp*-nBA fractions from both flowers and leaves did not show any chelating ability; on the other hand, good chelating properties were highlighted for the less polar fractions and the aqueous residue.

Among the flower fractions, *Spp*-DCM and *Spp*-AQ showed the best Fe^{2+} chelating activity, equal to approximately 90% at the highest concentration tested, followed by *Spp*-HE. This is also confirmed by the IC_{50} values (0.16 ± 0.01 mg/mL, 0.19 ± 0.01 mg/mL and 0.72 ± 0.07 mg/mL, respectively) (Table 3). The chelating activity of these fractions was however lower than that of the standard EDTA. As regards the leaf fractions, it was highlighted that *Spp*-DCM displayed the best chelating activity at the maximum concentration tested, equal to approximately 86%, followed by *Spp*-AQ (approximately 75%); however, the IC_{50} value was found to be lower in the latter (1.17 ± 0.03 and 0.78 ± 0.01 mg/mL, respectively). It should also be underlined that the *Spp*-HE fraction from the leaves did not show any chelating activity.

Based on the IC_{50} values, the order of activity of the fractions, the aqueous residue, and the standard was found to be: EDTA > *Spp*-DCM = *Spp*-AQ > *Spp*-HE for the flowers and EDTA > *Spp*-AQ > *Spp*-DCM for the leaves.

Among the classes of plant secondary metabolites, phenolic compounds have gained considerable attention owing to their remarkable antioxidant properties, which play a substantial role in the prevention of oxidative stress and several pathological conditions.[29] The antioxidant ability of polyphenols is linked to their capacity to act as efficient free radical and reactive oxygen species (ROS) scavengers, as well as metal-chelating agents.[30] Indeed, in most cases, the antioxidant properties of plant extracts are associated to a good correlation with their total phenolic content.[31]

The results from the different *in vitro* antioxidant activity assays demonstrated that the phenolic-rich fractions from both flowers and leaves possess the best primary antioxidant properties in terms of radical scavenging activity and reducing power, which was found to be higher for the flower *Spp*-ETAC fraction. This could be explained by considering the differences in the qualitative-quantitative profile of the phenolic compounds contained in the *Spp*-ETAC fractions, characterized by HPLC-PDA/ESI-MS analysis; in particular, 1-*O*-Feruloyl-β-D-glucose turned out to be the most abundant compound in the leaf *Spp*-ETAC fraction, whereas Isorhamnetin glucoside resulted the main phenolic component of the flower *Spp*-ETAC fraction.

The greatest activity highlighted for the flower *Spp*-ETAC fraction could be related to the presence of Isorhamnetin

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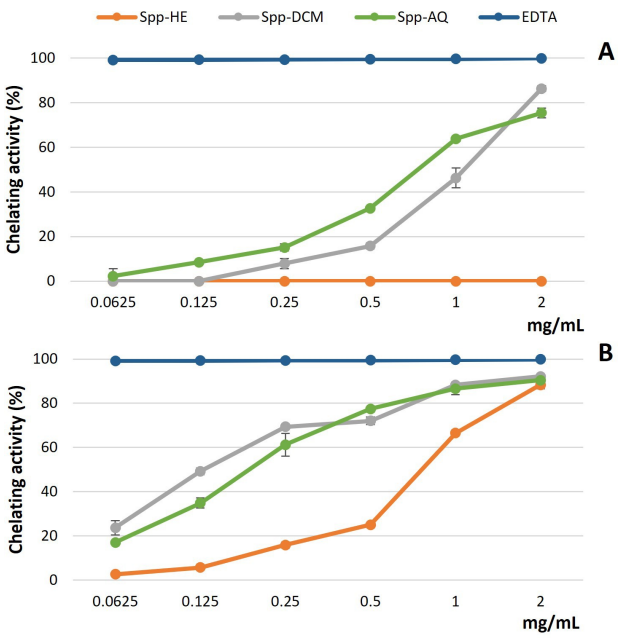


Figure 5. Ferrous ion chelating activity (%) of the fractions from leaves (A) and flowers (B) of *Sinapis pubescens* subsp. *pubescens*. The results are expressed as the mean ± SD (n = 3).

derivatives, particularly of Isorhamnetin glucoside, not detected in the other characterized fractions; indeed, several *in vitro* and *in vivo* investigations have demonstrated the strong antioxidant and radical-scavenging properties of Isorhamnetin glucosides, supporting their use as phytonutrients.[32] In a previous study conducted by Jia and colleagues, 1-*O*-Feruloyl-β-D-glucose was shown to exhibit weak DPPH radical scavenging ability and moderate reducing power; this could explain the lower antioxidant properties of the leaf *Spp*-ETAC fraction, despite the high quantity of 1-*O*-Feruloyl-β-D-glucose, equal to about one-third of the total content of phenolic compounds detected in the fraction.[33]

On the other hand, phenolic-rich fractions did not display any ferrous ion chelating activity, which instead was highlighted for the more hydrophobic ones. These results suggest a limited contribution of phenolic compounds to the secondary antioxidant properties of the hydroalcoholic flower and leaf extracts of *S. pubescens* subsp. *pubescens*, which instead seem to be determined by secondary metabolites belonging to different chemical classes.

It's worth noting that the results of the studies carried out on the fractions have indicated flowers as the best source of natural antioxidants, both primary and secondary; differently, the results of antioxidant activity tests previously performed on the crude extracts of *S. pubescens* subsp. *pubescens* had shown for the flower extract best secondary antioxidant properties, whereas the primary antioxidant properties were found to be higher for the leaf extract.[11]

Conclusions

The research conducted on the fractions obtained from the leaf and flower extracts of *S. pubescens* subsp. *pubescens* provided a deeper understanding of their antioxidant potential in relation to the phytochemical profile.

The results of the phytochemical investigations highlighted the predominance of phenolic compounds in the most polar fractions, which were characterized by HPLC-PDA/ESI-MS analysis. Notably, the phenolic-rich fractions were found to possess strong primary antioxidant properties, whereas they did not display any chelating activity, which was observed for the less polar ones. Thus, it could be assumed that the phenolic compounds contained in the leaf and flower extracts of *S. pubescens* subsp. *pubescens* act mainly as primary antioxidants, whereas the chelating properties can be traced back to other classes of compounds.

The obtained results give further insight into *S. pubescens* subsp. *pubescens*, indicating this edible species that grows spontaneously in Sicily as novel resource of natural antioxidants, which could be suited for the formulation of nutraceuticals and dietary supplements able to provide beneficial effects on human health.

Experimental Section

Chemicals and Reagents

LC/MS-grade water (H_2O), acetonitrile (ACN), caffeic acid and quercetin 3-*O*-rhamnoside, Folin-Ciocâlteu reagent, sodium carbonate, gallic acid, 2,2-diphenyl-1-picrylhydrazyl, butylated hydroxytoluene (BHT), potassium hexacyanoferrate (III), iron (III) chloride hexahydrate, L-ascorbic acid, sodium phosphate monobasic monohydrate, potassium phosphate dibasic, trichloroacetic acid, iron (II) chloride, 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt (ferrozine), and ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), were obtained from Merck Life Science (Merck KGaA, Darmstadt, Germany). Methanol (MeOH)., *n*-hexane (HEX), dichloromethane (DCM), ethyl acetate (ETAC), and *n*-butanol (nBA) were purchased from Omniascientific snc (Messina, Italy).

Plant Material and Extraction Procedure

The aerial parts of *Sinapis pubescens* subsp. *pubescens* were harvested in May 2022 in the locality of Floresta (Messina, Sicily, Italy), on the outskirts, at about 1250 m above sea level (a.s.l.). The taxonomic identification of the plant was confirmed by Prof. F. M. Raimondo, PLANTA/Center for Research, Documentation and Training (Palermo), and Prof. V. Spadaro, University of Palermo. A voucher specimen has been deposited to the Herbarium Mediterraneum of the University of Palermo (Italy) (PAL-Gr) (voucher number Raimondo & Spadaro n.01/22). The leaf and flower hydroalcoholic extracts (70% MeOH) were obtained according to the procedure described in our previous work.[11] The crude extracts were suspended in distilled water (1:10 *w/v*) and fractionated by liquid-liquid extraction with organic solvents of increasing polarity (1:1) in a separating funnel: *n*-hexane (*Spp*-HE), dichloromethane (*Spp*-DCM), ethyl acetate (*Spp*-ETAC), and *n*-butanol (*Spp*-nBA). To achieve exhaustive extraction, several extraction cycles were

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performed with each solvent; then, the different organic phases were pooled and evaporated to dryness by rotavapor. The yield of the fractions and the aqueous residue (*Spp*-AQ), referred to 100 g of crude extract, are given in Table 4.

Phytochemical Investigations

Determination of Total Polyphenol Content

The assessment of total polyphenol content of the fractions obtained from *S. pubescens* subsp. *pubescens* leaf and flower hydroalcoholic extracts was attained using the Folin Ciocâlteu colorimetric method.[34] For the quantitative estimation of total polyphenols, calibration curve of gallic acid, used as standard, was constructed. A 100 μL aliquot of each sample solution, appropriately diluted, was mixed with 200 μL of Folin Ciocâlteu reagent, 2 mL of distilled water, and 1 mL of 15% sodium carbonate. The reaction mixture was incubated for 2h at room temperature; at the timepoint, the absorbance was measured at 765 nm using a UV-1601 spectrophotometer (Shimadzu, Milan, Italy). The results were obtained from the average of three independent experiments, and the total polyphenols are expressed as mg gallic acid equivalents (GAE)/g fraction (dw) ± standard deviation (SD).

Characterization of the Phenolic-Rrich Fractions by HPLC-PDA/ESI-MS

The phenolic-rich fractions obtained from *S. pubescens* subsp. *pubescens* flower and leaf extracts, namely *Spp*-ETAC and *Spp*-nBA, were analyzed by high-performance liquid chromatography coupled to a photodiode array and electrospray ionization mass spectrometry HPLC-PDA/ESI-MS.[35–37]

Sample preparation: The dried fractions were redissolved in 70% MeOH (10 mg/mL). For the chromatographic separation, an injection volume of 2 μL was employed, and the analysis was performed in triplicate.

HPLC/MS analytical condition: Chromatographic analysis was accomplished by means of a Shimadzu HPLC system (Kyoto, Japan) equipped with a CBM-20 A controller, two LC-20AD dual-plunger parallel-flow pumps, a DGU20 A5R degasser, a CTO-20AC column oven, a SIL-30AC autosampler, an SPD-M20 A photodiode array detector, and an LCMS-2020 single quadrupole mass spectrometer, with the employment of ESI source operated in negative and positive ionization modes. Chromatographic separations were carried out on an Ascentis Express RP C18 columns (150×2.1 mm; 2.7 μm) (Merck Life Science, Merck KGaA, Darmstadt, Germany). The employed mobile phase was composed of two solvents: water (solvent A) and ACN (solvent B) both acidified with 0.1% of formic acid *v/v*. The flow rate was set at 0.5 mL/min, under gradient elution 0 min–0% B, 10 min–10% B, 20 min–11% B, 30 min–15% B, 50 min–18% B, 65 min–23% B, 70 min–100% B, 75 min–100% B. Photodiode array detection (PDA) was applied in the range of 190–600 nm and monitored at a wavelength of 330 nm (sampling frequency: 40, time constant: 0.080 s). MS conditions were as follows: scan range and the scan speed were set at a mass-to-charge ratio (*m/z*) 100–1600 and 7500 amu/s, respectively; event time: 0.3 s, nebulizing gas (N_2) flow rate: 1.5 L/min, drying gas (N_2) flow rate: 15 L/min, interface temperature: 350 °C, heat block temperature: 300 °C, DL temperature: 300 °C, DL voltage: 1 V, interface voltage: M- 4.5 kV.

Standards: Calibration curves of two standards (caffeic acid and quercetin 3-*O*-rhamnoside) were employed for the quantification of the phenolic content in sample extracts. Each analysis was performed in 6 repetitions. Data acquisition was performed by Shimadzu LabSolution software ver. 5.97. Caffeic acid (0.1, 1, 10, 50, 100 ppm), $y = 4068.7x + 19686$, $R^2 = 0.9996$, LOD = 0.053, LOQ = 0.161; quercetin-3-*O*- rhamnoside (0.1, 1, 10, 50, 100 ppm), $y = 8054x + 27465$, $R^2 = 0.9997$, LOD = 0.034, LOQ = 0.103.

Table 4. Yield of the fractions obtained from *Sinapis pubescens* subsp. *pubescens* leaf and flower hydroalcoholic extracts.

Fractions	Yield (%)	
	Leaves	Flowers
Spp-HE	0.78	2.63
Spp-DCM	1.63	2.35
Spp-ETAC	2.70	1.98
Spp-nBA	34.12	33.81
Spp-AQ	40.28	51.19

The yields are referred to 100 g of extract (dw).

Antioxidant Activity

DPPH Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was carried out to estimate the free radical scavenging activity of the of the fractions obtained from *S. pubescens* subsp. *pubescens* leaf and flower hydroalcoholic extracts, using the method reported by Ohnishi et al.[38] The fractions were tested in the range of 0.0625–2 mg/mL, using butylated hydroxytoluene (BHT) as reference standard. A 0.5 mL aliquot of each sample solution was added to 3 mL of freshly prepared DPPH MeOH solution (0.1 mM). The reaction mixture was incubated for 20 min at room temperature in the dark; at the timepoint, absorbance was measured at 517 nm against the corresponding blank using a model UV-1601 spectrophotometer (Shimadzu). The results were obtained from the average of three independent experiments, and are reported as mean radical scavenging activity percentage (%) ± SD and mean 50% inhibitory concentration (IC_{50}) ± SD.

Reducing Power Assay

The reducing power of the fractions was determined through the evaluation Fe^{3+}-Fe^{2+} transformation by the potassium ferricyanide method.[39] The fractions were tested in the range of 0.0625–2 mg/mL, using BHT and ascorbic acid as reference standards. A 1.0 mL aliquot of each sample solution was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The reaction mixture was incubated for 20 min at 50 °C and rapidly cooled; then, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged (3000 rpm, 10 min). A volume of 2.5 mL of the supernatant was taken and mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. After incubation for 10 min at room temperature and in the dark, the optical density change of the samples was measured at 700 nm. The results were obtained from the average of three independent experiments, and are expressed as mean absorbance values ± SD and ascorbic acid equivalent (ASE/mL) ± SD.

Ferrous Ion (Fe^{2+}) Chelating Activity Assay

The Fe^{2+} chelating activity of the fractions was assessed by measurement of the Fe^{2+}-ferrozine complex, using the colorimetric

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method reported by Kumar et al.[28] The fractions were tested in the range of 0.0625–2 mg/mL, and ethylenediaminetetraacetic acid (EDTA) was used as reference standard. A 0.05 mL aliquot of 2 mM ferrous chloride was added to 1.0 mL of each sample solution and 0.5 mL of MeOH; then, 0.1 ml of 5 mM ferrozine solution was added to start the reaction. The mixture was vigorously shaken and left to incubate for 10 min at room temperature in the dark; thereafter, the optical density change was measured at 562 nm. The results were obtained from the average of three independent experiments and are reported as mean inhibition of the Fe^{2+}-ferrozine complex formation (%) ± SD and IC_{50} ± SD.

Statistical Analysis

Statistical comparison of the data was performed using the one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test (GraphPAD Prism 10 Software for Science). *P* values <0.05 were considered to be statistically significant.

Author Contributions

M. F. Taviano and N. Miceli conceived and designed the study; V. Spadaro and F. M. Raimondo collected and identified the plant material; M. F. Taviano, P. Arena, F. Davì, and E. Cavò performed the fractionation of the extracts and the antioxidant investigations; F. Cacciola, R. Laganà Vinci, and L. Mondello carried out the phenolic characterization; M. F. Taviano, P. Arena, F. Cacciola, and N. Miceli wrote the original draft of the manuscript. M. F. Taviano, V. Spadaro, F. M. Raimondo, L. Mondello and N. Miceli revised the article. All authors approved the final version of the manuscript.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Antioxidants · edible wild plants · polyphenols · phytochemistry · Sicilian vascular flora

[1] P. M. de Medeiros, K. F. Figueiredo, P. H. S. Gonçalves, R. de Almeida Caetano, É. M. da Costa Santos, G. M. Cota dos Santos, D. Monteiro Barbosa, M. de Paula, A. M. Mapeli, *J. Ethnobiol. Ethnomed.* **2021**, *17*, 37.
[2] F. Serio, *Int. J. Environ. Res. Public Health* **2022**, *19*(14), 8801.
[3] V. Picchi, R. Lo Scalzo, A. Tava, F. Doria, S. Argento, S. Toscano, S. Treccarichi, F. Branca, *Molecules* **2020**, *25*(15), 3495.
[4] P. Vanzani, M. Rossetto, V. De Marco, L. E. Sacchetti, M. G. Paoletti, A. Rigo, *J. Food Sci.* **2011**, *76*(1), C46–51.
[5] L. Barros, P. Morales, A. M. Carvalho, I. C. Ferreira, *Mediterranean wild edible plants: ethnobotany and food composition tables*, Springer Science + Business Media, New York, **2016**, pp. 209–232.
[6] R. Motti, *Plants (Basel)* **2022**, *11*(3), 344.
[7] S. Toscano, D. Scuderi, A. Tribulato, D. Romano, *Acta Hortic.* **2013**, *1005*, 205–212.
[8] D. Šamec, B. Salopek-Sondi, *Nonvitamin and Nonmineral Nutritional Supplements*, Academic Press, Cambridge, **2019**, pp. 195–202.
[9] M. Jahangir, H. K. Kim, Y. H. Choi, R. Verpoorte, *Compr. Rev. Food Sci. Food Saf.* **2009**, *8*(2), 31–43.
[10] K. M. Favela-González, A. Y. Hernández-Almanza, N. M. De la Fuente-Salcido, *J. Food Biochem.* **2020**, *44*(10), e13414.
[11] P. Arena, N. Miceli, A. Marino, F. Davì, E. Cavò, V. Spadaro, F. M. Raimondo, F. Cacciola, R. Laganà Vinci, L. Mondello, M. F. Taviano, *Chem. Biodiversity* **2023**, *20*(6), e202300309.
[12] T. Hajek, V. Škeříková, P. Česla, K. Vyňuchalová, P. Jandera, *J. Sep. Sci.* **2008**, *31*, 3309–3328.
[13] F. Cacciola, K. Arena, F. Mandolfino, D. Donnarumma, P. Dugo, L. Mondello, *J. Chromatogr. A* **2021**, *1645*, 462129.
[14] F. Cacciola, P. Donato, D. Sciarrone, P. Dugo, L. Mondello, *Anal. Chem.* **2017**, *89*, 414–429.
[15] J. C. Sánchez-Rangel, J. Benavides, J. B. Heredia, L. Cisneros-Zevallos, D. A. Jacobo-Velázquez, *Anal. Methods* **2013**, *5*, 5990–5999.
[16] Plant Metabolics Network (PMN), www.plantcyc.org; Accessed November 22, 2023.
[17] Y.-Q. Wang, L.-P. Hu, G.-M. Liu, D.-S. Zhang, H.-J. He, *Molecules* **2017**, *22*(8), 1262.
[18] A. Martínez-Sánchez, A. Gil-Izquierdo, M. I. Gil, F. A. Ferreres, *J. Agric. Food Chem.* **2008**, *56*(7), 2330–2340.
[19] Y. Oulad El Majdoub, F. Alibrando, F. Cacciola, K. Arena, E. Pagnotta, R. Matteo, G. Micalizzi, L. Dugo, P. Dugo, L. Mondello, *Molecules* **2020**, *25*(22), 5421.
[20] K. Neha, M. R. Haider, A. Pathak, M. S. Yar, *Eur. J. Med. Chem.* **2019**, *178*, 687–704.
[21] M. Sharifi-Rad, N. V. Anil Kumar, P. Zucca, E. M. Varoni, L. Dini, E. Panzarini, J. Rajkovic, P. V. Tsouh Fokou, E. Azzini, I. Peluso, A. P. Mishra, M. Nigam, Y. El Rayess, M. El Beyrouthy, L. Polito, M. Iriti, N. Martins, M. Martorell, A. O. Docea, W. N. Setzer, D. Calina, W. C. Cho, J. Sharifi-Rad, *Front. Plant Physiol.* **2020**, *11*, 694.
[22] K. Jomova, R. Raptova, S. Y. Alomar, S. H. Alwasel, E. Nepovimova, K. Kuca, M. Valko, *Arch. Toxicol.* **2023**, *97*(10), 2499–2574.
[23] R. Szymanska, P. Pospíšil, J. Kruk, *Oxid. Met.* **2018**, *2018*, 1–2.
[24] B. Salehi, E. Azzini, P. Zucca, E. Maria Varoni, N. V. Anil Kumar, L. Dini, E. Panzarini, J. Rajkovic, P. V. Tsouh Fokou, I. Peluso, A. P. Mishra, M. Nigam, Y. El Rayess, M. El Beyrouthy, W. N. Setzer, L. Polito, M. Iriti, A. Sureda, M. M. Quetglas-Llabrés, M. Martorell, N. Martins, M. Sharifi-Rad, L. M. Estevinho, J. Sharifi-Rad, *Appl. Sci.* **2020**, *10*(3), 947.
[25] N. Bibi Sadeer, D. Montesano, S. Albrizio, G. Zengin, M. F. Mahomoodally, *Antioxidants* **2020**, *9*(8), 709.
[26] J. S. Santos, V. R. Alvarenga Brizola, D. Granato, *Food Chem.* **2017**, *214*, 515–522.
[27] İ. Gulcin, S. H. Alwasel, *Processes* **2023**, *11*(8), 2248.
[28] T. S. Kumar, S. Shanmugam, T. Palvannan, V. M. B. Kumar, *Iran. J. Pharm. Res.* **2008**, *7*, 211–215.
[29] U. Bhuyan, J. G. Handique, *Bioactive Natural Products - Studies in Natural Products Chemistry*, Elsevier Science Ltd, Oxford, **2022**, Vol. 75, pp. 243–266.
[30] M. Rudrapal, S. J. Khairnar, J. Khan, A. B. Dukhyil, M. A. Ansari, M. N. Alomary, F. M. Alshabrmi, S. Palai, P. K. Deb, R. Devi, *Front. Pharmacol.* **2022**, *13*, 283.
[31] İ. Gulcin, *Arch. Toxicol.* **2020**, *94*(3), 651–715.
[32] H. Wang, L. Chen, B. Yang, J. Du, L. Chen, Y. Li, F. Guo, *Nutrients* **2023**, *15*, 1947.
[33] X. Jia, D. Yang, H. Xie, Y. Jiang, X. Wei, *J. Funct. Foods* **2017**, *32*, 419–425.
[34] X. Gao, L. Björk, V. Trajkovski, M. Uggla, *J. Agric. Food Chem.* **2000**, *80*, 2021–2027.

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[35] H. El Cadi, H. El Bouzidi, G. Selama, B. Ramdan, Y. Oulad El Majdoub, F. Alibrando, K. Arena, M. Palma Lovillo, J. Brigui, L. Mondello, F. Cacciola, T. M. G. Salerno, *Molecules* **2021**, *26*, 2710.
[36] K. Arena, E. Trovato, F. Cacciola, L. Spagnuolo, E. Pannucci, P. Guarnaccia, L. Santi, P. Dugo, L. Mondello, L. Dugo, *Molecules* **2022**, *27*, 1727.
[37] T. Lechhab, F. Salmoun, W. Lechhab, Y. Oulad El Majdoub, M. Russo, M. R. Testa Camillo, E. Trovato, P. Dugo, L. Mondello, F. Cacciola, *Eur. Food Res. Technol.* **2022**, *247*, 2993–3012.
[38] M. Ohnishi, H. Morishita, H. Iwahashi, T. Shizuo, S. Yoshiaki, M. Rimura, R. Kido, *Phytochemistry* **1994**, *36*, 579–583.
[39] M. Oyaizu, *Jpn. J. Nutr.* **1986**, *44*, 307–315.

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