doi.org/10.1002/cbdv.202300309 Research Article CHEMISTRY & BIODIVERSITY

www.cb.wiley.com

Comparative Study on Phenolic Profile and Biological Activities of the Aerial Parts of *Sinapis pubescens* L. subsp. *pubescens* (*Brassicaceae*) Wild from Sicily (Italy)

Paola Arena[+],[a, b] Natalizia Miceli[+],*[a] Andreana Marino,[a] Federica Davì,[a, b] Emilia Cavò,[a, b] Vivienne Spadaro,[c] Francesco Maria Raimondo,[d] Francesco Cacciola,[e] Roberto Laganà Vinci,[a] Luigi Mondello,[a, f, g] and Maria Fernanda Taviano[a]

This work aimed to investigate *Sinapis pubescens* subsp. *pubescens* spontaneously grown in Sicily (Italy) as new potential source of active metabolites; specifically, a comparative study on leaf, flower, and stem hydroalcoholic extracts was performed. Polyphenols were quantitatively determined by spectrophotometric methods and characterized by HPLC-PDA/ESI-MS; a total of 55 polyphenolic compounds were identified, highlighting considerably different qualitative-quantitative profiles. The extracts showed antioxidant activity, evaluated by *in vitro* assays; particularly, the leaf extract displayed the best radical scavenging activity (DPPH test) and reducing power, while the flower extract showed the greatest chelating activity. The antimicrobial properties of the extracts were investigated against bacteria and yeasts by standard methods; no antimicrobial activity was found against the strains tested. The extracts resulted to be non-toxic after preliminary toxicity evaluation by the *Artemia salina* lethality bioassay. The aerial parts of *S. pubescens* subsp. *pubescens* proved to be valuable sources of antioxidants for pharmaceutical and nutraceutical applications.

Introduction

Since ancient times, plants that grow spontaneously have aroused remarkable interest, especially for food purposes and in the treatment of many diseases. In the last decades, they have been extensively studied for both their considerable importance in the agri-food sector and the potential recovery of bioactive compounds with applications in the pharmaceutical and food fields.

In the last years, our team has focused much research on the spontaneous flora of Sicily (Italy), including about 3,000 specific and intraspecific taxa, many of which (141) belong to the *Brassicaceae* family and represent potential resources of bioactive compounds.[1,2] Within this family are also comprised the taxa of the genus *Sinapis* L., herbaceous or suffruticose annual or perennial species originating from the Mediterranean basin and the Middle East.[3,4]

Currently, in the "Flora Europaea" as well as in the "BrassiBase", the main database of the *Brassicaceae*, the genus *Sinapis* includes four taxa, *S. alba* L., *S. arvensis* L., *S. flexuosa* Poir. and *S. pubescens* L.[5,6] In Italy, and thus also in Sicily, the genus is represented by *Sinapis alba* L. subsp. *alba*, *S. alba* subsp. *dissecta* (Lag.)Bonnier, *S. alba* subsp. *mairei* (H.Lindb.) Maire, *S. arvensis* L. subsp. *arvensis* and *S. pubescens* L. subsp. *pubescens*.[7,8]

Both the seeds and the aerial parts of *S. alba* (white mustard) and *S. arvensis* (wild mustard) are used for food purposes, to increase soil fertility, as fodder for livestock and in the traditional medicine of several countries for the anti-inflammatory, anticancer, antiseptic, diuretic, laxative, and digestive properties.[9–15] Several ethnobotanical studies report the use of wild *Sinapis* species as food plants. In many Italian

[a] *P. Arena,[+] N. Miceli,[+] A. Marino, F. Davì, E. Cavò, R. Laganà Vinci, L. Mondello, M. F. Taviano*
Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy
E-mail: nmiceli@unime.it

[b] *P. Arena,[+] F. Davì, E. Cavò*
Foundation "Prof. Antonio Imbesi", University of Messina, Piazza Pugliatti 1, 98122 Messina, Italy

[c] *V. Spadaro*
Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, Section of Botany, Anthropology and Zoology, University of Palermo, Via Archirafi 38, 90123 Palermo, Italy

[d] *F. M. Raimondo*
PLANTA/Research, Documentation and Training Center, Via Serraglio Vecchio 28, 90123 Palermo, Italy

[e] *F. Cacciola*
Department of Biomedical, Dental, Morphological and Functional Imaging Sciences, University of Messina, Via Consolare Valeria, 98125 Messina, Italy

[f] *L. Mondello*
Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Palatucci, 98168 Messina, Italy

[g] *L. Mondello*
Department of Sciences and Technologies for Human and Environment, University Campus Bio-Medico of Rome, via Àlvaro del Portillo 21, 00128 Rome, Italy

[+] *These authors equally contributed to this work.*

regions the aerial parts (tender shoots, leaves and inflorescences) of the spontaneous edible species *Sinapis pubescens* subsp. *pubescens* (hairy mustard), along with those of *S. alba* and *S. arvensis*, are used for the preparation of soups, in salads, boiled or fried; all three taxa are used in traditional Sicilian cuisine.[16–20]

Most of the studies on *Sinapis* spp. have been focused on the seeds of *S. alba*, due to their economic importance in the food industry, and on the aerial parts (flowers, leaves, stems) of *S. arvensis*. As far as *S. pubescens* subsp. *pubescens* is concerned, to the best of our knowledge no similar studies have been conducted on the phytochemical characterization and the evaluation of biological activities; therefore, the study of this species reveals to be of considerable interest for the discovery of new sources of bioactive compounds with potential applications in the pharmaceutical and nutraceutical fields.

Sinapis pubescens subsp. *pubescens* is a perennial, herbaceous plant, with stems generally between 30 and 80 cm tall, pubescent or villous. The leaves are pubescent, the lower are stalked, lyrate-pinnatisect up to 15 cm long, with 5–6 pairs of small segments and rounded terminal segment; the upper leaves are sessile, oblong, less divided or simple. The flowers, gathered in racemes, have yellow-greenish sepals and yellow spatulate petals. The fruit is a siliqua, erect, covered to the apex with long, fine, ascending hairs, with a long curved beak, containing 4–8 seeds per locule.[5,7]

The objective of this research was to carry out a comparative study on the hydroalcoholic extracts obtained from different organs (leaves, flowers and stems) of *S. pubescens* subsp. *pubescens* grown spontaneously in Sicily. Specifically, the qualitative and quantitative profile of the phenolic constituents of the extracts was characterized by means of spectrophotometric methods and high-performance liquid chromatography online coupled to photodiode array and electrospray ionization mass spectrometry detection (HPLC-PDA/ESI-MS). Furthermore, some biological activities were investigated; in particular, the antioxidant activity of the extracts was assessed by means of different *in vitro* methods, and the antimicrobial properties were determined on Gram-positive and Gram-negative bacteria and on yeasts by evaluating the minimum inhibitory concentration (MIC) and the minimum bactericidal and fungicidal concentration (MBC and MFC). Finally, the toxicity of the extracts was evaluated through the *Artemia salina* Leach (brine shrimp) lethality bioassay.

Results and Discussion

Phytochemical Investigations

Determination of total phenolic, flavonoid, and condensed tannin content

Phenolic compounds represent a huge class of metabolites which includes flavonoids, phenolic acids, tannins, lignans, coumarins, and stilbenoids, the former being the most isolated compounds.

Due to their ability to scavenge free radicals and active oxygen species such as singlet oxygen, superoxide free radicals and hydroxyl radicals, phenolic components are often defined as "high level antioxidants". As a consequence, such metabolites have gained considerable attention due to their potential use in nutraceutical and pharmaceutical formulations for their beneficial properties on human health.[21,22] In most cases, the antioxidant activities of plant extracts are explained with good correlation with their total phenolic content; therefore, preliminary investigations to quantify the total phenolics contained in the extracts of *S. pubescens* subsp. *pubescens* were conducted in this study.

The results obtained by the Folin Ciocâlteu assay showed that the leaf extract presents the highest phenolic content compared to the others (64.06 ± 1.63 mg GAE/g extract). The stem extract showed the lowest quantity of phenols (31.02 ± 1.45 mg GAE/g extract), about half than that of the leaves and about two-thirds than that of the flowers (46.38 ± 0.74 mg GAE/g extract) (Table 1).

The results of the aluminium chloride assay showed that the leaf extract contained the highest amount of flavonoids (44.94 ± 0.33 mg QE/g), whereas the lowest content of 23.46 ± 0.43 mg QE/g was found in the flower extract, resulting approximately half than that of the leaves. The total amount of flavonoids quantified in the stem extract resulted slightly higher than that of the flowers (27.02 ± 0.77 mg QE/g) (Table 1).

The results obtained by the vanillin method highlighted the highest quantity of condensed tannins for the stem extract (10.41 ± 0.27 mg CE/g extract), which resulted respectively about three and four times higher than that of the flowers (3.20 ± 0.24 mg CE/g extract) and the leaves (2.72 ± 0.24 mg CE/g extract) (Table 1).

As shown in Table 1, significant differences ($p < 0.01$) in total phenolic and flavonoid contents were found among the extracts; as far as condensed tannins are concerned, no statisti-

Table 1. Quantitative determination of total polyphenols (calculated as gallic acid equivalents), total flavonoids (calculated as quercetin equivalents) and condensed tannins (calculated as catechin equivalents) in flower, leaf and stem hydroalcoholic extracts of *Sinapis pubescens* subsp. *pubescens*.

Sinapis pubescens subsp. pubescens extracts	Total polyphenols (mg GAE/g)	Total flavonoids (mg QE/g)	Condensed tannins (mg CE/g)
Flowers	46.38 ± 0.74[a]	23.46 ± 0.43[a]	3.20 ± 0.24[a]
Leaves	64.06 ± 1.63 [b]	44.94 ± 0.33[b]	2.72 ± 0.24[a]
Stems	31.02 ± 1.45[c]	27.02 ± 0.77[c]	10.41 ± 0.27[b]

Values are expressed as the mean ± SD (n = 3). [a–c] Different letters within the same column indicate significant differences between mean values ($p < 0.01$).

cally significant difference was highlighted between the flower and leaf extracts.

Determination of Polyphenolic Compounds by HPLC-PDA/ESI-MS

The qualitative-quantitative profile of the polyphenolic components of *S. pubescens* subsp. *pubescens* aerial parts has been characterized by HPLC-PDA/ESI-MS analysis. Figure 1 shows the HPLC-PDA chromatograms of the flower (A), leaf (B) and stem (C) hydroalcoholic extracts of *S. pubescens* subsp. *pubescens*, extracted at 330 nm. For the identification, information from retention times, absorption and mass spectra were used, further supported by literature data.[23–32] Table 2 shows the 67 detected compounds, together with the retention times, the characteristic maximum of absorption and the $[M-H]^-$ values. Moreover, when observed, the fragment ions deriving from the fragmentation of the precursor ion $[M-H]^-$ have also been reported; in all these cases the ions derived from the loss of the glycosidic portion, allowing the detection of the corresponding aglycones.

As can be seen from Table 2, a total of 55 polyphenolic compounds has been tentatively identified, including 45 flavonoids and 9 phenolic acids. From the comparison of the polyphenols characterized in the extracts, a consistently different qualitative-quantitative profile was highlighted. Flavonoids, all belonging to the class of flavonols, are the main constituents; the leaf extract was found to contain the greatest number of flavonoid compounds (30) compared to those of flowers (26) and stems (11). Among the detected flavonoids, kaempferol derivatives represent the vast majority of compounds, all of them in the glycosylated form (18 in leaf, 9 in flower and 6 in stem extract). The other detected flavonoids are derivatives of quercetin and isorhamnetin. Secoisolariciresinol 9,9′-diglucoside (peak 55), a lignan, has also been identified in the flower extract. The quantitative determination of flavonoids was made by interpolation of the calibration curves obtained with three standards: quercetin-3-*O*-glucopyranoside, kaempferol-3-*O*-glucoside and isorhamnetin-3-*O*-glucoside. The total amount of flavonoids identified in the leaf extract (51.2 mg/g) was found to be approximately double that of the flower extract and 16-fold higher than the stem extract (26.5 mg/g and 3.13 mg/g, respectively).

The main constituent of flowers was isorhamnetin dihexoside isomer (11.23 ± 0.48 mg/g extract, peak 19), followed by quercetin dihexoside isomer (2.26 ± 1.90 mg/g extract, peak 12), while in the leaves predominated kaempferol rutinoside isomer (10.61 ± 0.40 mg/g extract, peak 40) and kaempferol coumaroyl rutinoside isomer (10.04 ± 1.22 mg/g extract, peak 45), followed by quercetin dihexoside isomer (6.11 ± 0.057 mg/g extract, peak 12). Finally, in the stems, the most abundant compounds were: kaempferol rutinoside isomer (1.21 ± 0.01 mg/g extract peak 57) and kaempferol dirhamnoside dihexoside isomer (0.56 ± 0.01 mg/g extract, peak 43).

The greatest number of phenolic acids was found in the leaf extract (9), in particular ferulic, coumaric, and sinapic acid derivatives (peaks 2,3,5,6,10,11,58,60 and 62); *p*-Coumaric acid glucoside (peak 5) and ferulic acid hexoside (peak 11) were detected in the flower extract, whereas no phenolic acids were found in the stem extract. These compounds have been identified but not quantified.

In addition to polyphenols, malic acid (peak 1), N^1,N^{10}-Dicoumaroylspermidine (peak 34) and 4,4′′-bis(N-feruloyl)serotonin isomer (peak 51) were detected in traces; the first in all the hydroalcoholic extracts and the others in the flower extract only.

Antioxidant Activity

The study of the antioxidant properties of plant species has gained considerable interest in the last few years since oxidative stress has been recognized to play a crucial role in the pathogenesis of different diseases. Polyphenols represent the main group of phytochemicals with antioxidant ability; these compounds have drawn increasing attention due to their powerful antioxidant properties and their beneficial effects in the prevention of various oxidative stress-related diseases, such as cancer, neurodegenerative disorders, and diabetes.[33] In the present work, the antioxidant properties of *S. pubescens* subsp. *pubescens* have been investigated. As it is known, antioxidants may act by different mechanisms; therefore, using different analytical methods and substrates may be suitable to evaluate the mechanisms through which the components contained in the phytocomplexes could exert their effect.[34] It has been demonstrated that flavonoids and phenolic acids can act as hydrogen or electron donors, reducing agents, and metal ion chelators.[35] Phytochemicals belonging to both these classes have been detected in *S. pubescens* subsp. *pubescens* aerial parts; therefore, all potential mechanisms were taken into account to investigate the *in vitro* antioxidant properties of the extracts. Specifically, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test, based on the hydrogen atom transfer (HAT) and electron transfer (ET) mechanisms, and the reducing power assay, based on the electron transfer to Fe (III) were used to evaluate the primary antioxidant properties of the extracts.[36] One of the most important mechanisms of action of secondary (preventive) antioxidants is chelation of prooxidant metals. In detail, metal chelators reduce the amount of available metals such as iron, thereby decreasing the extent of hydroxyl radicals generated by Fenton reaction and limiting metal ion-induced lipid oxidation. Therefore, the secondary antioxidant properties of the extracts were investigated by the Ferrous ion chelating activity assay.[36]

In Figure 2 A are reported the results of the DPPH assay, carried out to determine the free scavenging activity of the extracts. Compared to the BHT standard, both the flower and leaf extracts of *S. pubescens* subsp. *pubescens* have shown good radical scavenging activity, which increases with increasing concentration. The comparison between the two extracts shows that the leaf extract has a higher activity than that of flowers, reaching an inhibition of about 90% at a concentration of 1.5 mg/mL, close to that of BHT; the same inhibition was highlighted for the flower extract at the highest concentration tested. The stem extract of *S. pubescens* subsp. *pubescens*

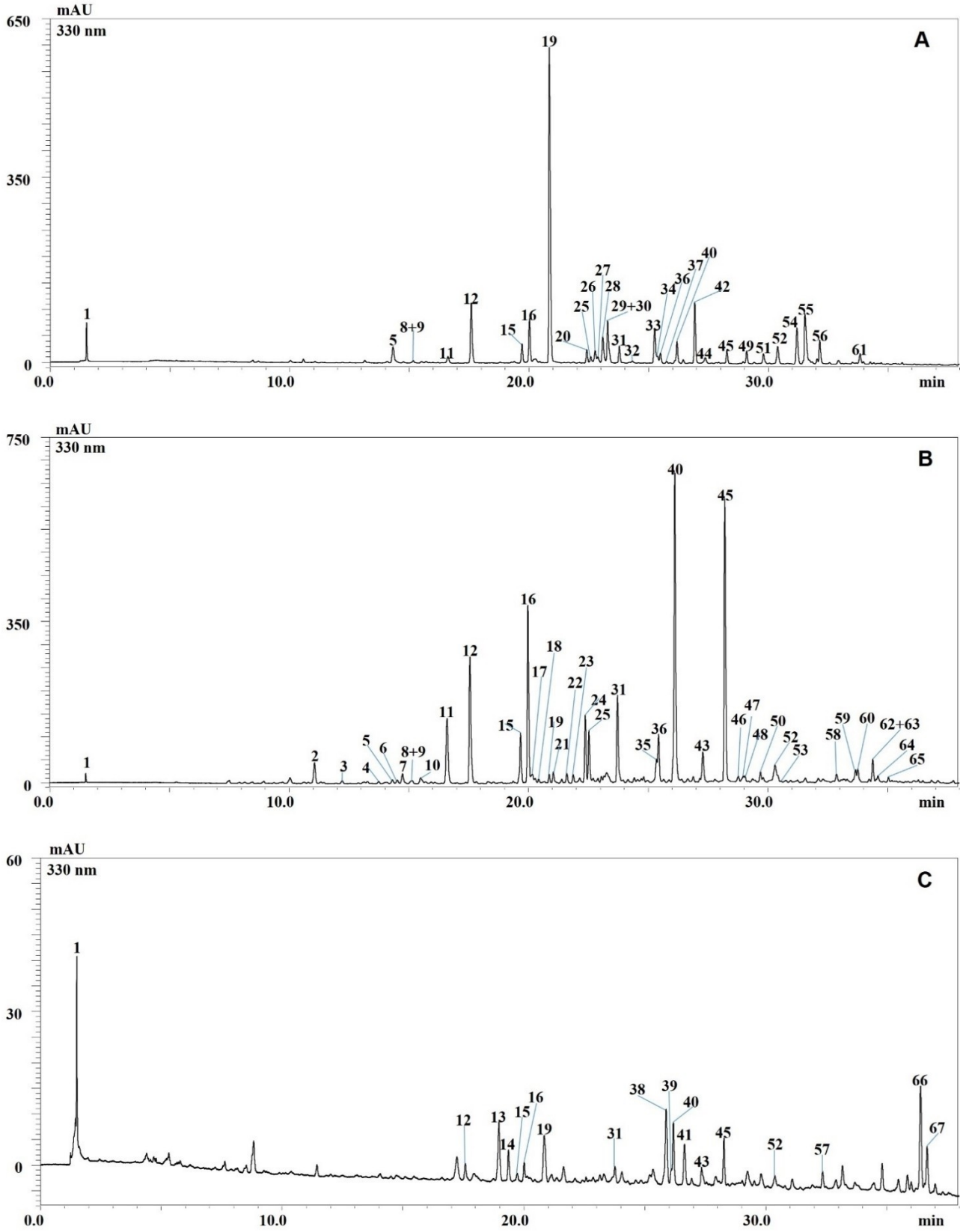

Figure 1. HPLC-PDA analysis of flower (A), leaf (B) and stem (C) hydroalcoholic extracts of *Sinapis pubescens* subsp. *pubescens* (chromatograms extracted at 330 nm). Tentative identification is shown in Table 2.

Table 2. Semi-quantification of phenolic compounds in the flower, leaf and stem hydroalcoholic extracts of *Sinapis pubescens* subsp. *pubescens* through HPLC-PDA/ESI-MS analysis. Quantification of phenolic compounds is reported in mg/g of extract (dw) ± SD (n = 3).

Peak No.	Compound	t_R (min)	UV max (nm)	$[M-H]^-$	*Sinapis pubescens* subsp. *pubescens* extracts (mg/g)			Ref.
					Flowers	leaves	stems	
1	Malic acid	1.51	272	133	X	x	x	[23]
2	Caffeic acid glucoside	11.06	330	341, 179, 161, 135		x		[24]
3	Hydroxyferuloyl-hexoside	12.21	323	371, 209		x		[25]
4	Quercetin trihexoside isomer	13.73	253sh, 345	787, 625, 463		0.10 ± 0.04		[23]
5	*p*-Coumaric acid glucoside	14.32	315	325	X	x		[24]
6	Ferulic acid dihexoside isomer	14.51	325	517, 193		x		[25]
7	Unknown	14.74	326	357		x		–
8 + 9	Quercetin dihexoside rhamnoside isomer + Kaempferol trihexoside isomer	15.17	266, 343	771, 609, 447	X	x		[23]
10	*p*-Coumaric acid hexoside derivative	15.49	325	325		x		–
11	Ferulic acid hexoside	16.62	330	355, 193	X	x		[25]
12	Quercetin dihexoside isomer	17.59	254, 352	625, 463, 301	2.26 ± 1.90	6.11 ± 0.06	0.06 ± 0.01	[23]
13	Unknown	18.97	295, 318	323			x	–
14	Unknown	19.37	291, 312	434			x	–
15	Quercetin malonyl dihexoside isomer	19.72	254, 352	711, 301	0.68 ± 0.02	2.47 ± 0.07	0.05 ± 0.01	–
16	Kaempferol dihexoside isomer	20.03	265, 345	609, 447, 285	1.00 ± 0.05	4.94 ± 0.02	0.07 ± 0.01	[26]
17	Quercetin-3,4′-diglucoside-3′-(6-*p*-coumaroyl-glucoside)	20.18	252, 339	933, 609, 447, 301		0.46 ± 0.02		[27]
18	Quercetin hexoside rutinoside isomer	20.44	254, 342	771, 463, 301		0.21 ± 0.01		[28]
19	Isorhamnetin dihexoside isomer	20.85	254, 353	639, 477, 315	11.23 ± 0.48	0.38 ± 0.00	x	[23]
20	Kaempferol dihexoside derivative	22.43	264, 345	695, 447, 285		0.39 ± 0.03		–
21	Kaempferol coumaroyl dihexoside isomer	21.06	265, 339	755, 593, 431, 285		0.31 ± 0.04		[28]
22	Kaempferol 3-triglucoside-7-rhamnosyl-caffeoyl isomer	21.63	267, 332	933, 771		0.27 ± 0.03		[28]
23	Kaempferol-3-*O*-coumaroyl-sophoroside-7-*O*-D-glucoside	21.90	267, 331	917, 431, 285		1.88 ± 0.04		[26]
24	Kaempferol dihexoside derivative	22.40	265, 346	695, 447, 285	0.31 ± 0.02			–
25	Kaempferol coumaroyl dihexoside isomer	22.59	262, 341	755, 593, 447, 285	0.16 ± 0.03	1.37 ± 0.14		–
26	Quercetin dihexoside isomer	22.77	255, 350	625, 463, 301	0.46 ± 0.12			[23]
27	Quercetin dihexoside isomer	22.89	257, 343	625, 463, 301	0.16 ± 0.00			[23]
28	Kaempferol dihexoside isomer	23.10	265, 337	609, 447, 285	0.52 ± 0.01			[26]
29 + 30	Isorhamnetin hexoside derivative + Isorhamnetin rutinoside isomer	23.30	254, 353	725, 681, 519 + 623, 477, 315	1.58 ± 0.05			[25]
31	Rutin	23.79	255, 353	609, 447, 301	0.69 ± 0.18	4.84 ± 0.64	x	[29]
32	Isorhamnetin dihexoside isomer	24.33	257, 342	639, 477, 315	0.13 ± 0.05			[23]
33	Kaempferol dihexoside isomer	25.26	265, 346	609, 447, 285	0.99 ± 0.12			[26]
34	N^1,N^{10}-Dicoumaroylspermidine	25.37	297	436, 316, 273	X			[24]
35	Kaempferol 3-*p*-coumaroylsophoroside-7-glucoside isomer	25.38	265, 345	917, 755, 593, 447, 285		0.77 ± 0.32		[30]
36	Quercetin derivative	25.50	254, 354	695, 651, 505, 301	X	2.48 ± 0.48		–
37	Isorhamnetin dihexoside derivative	25.77	267sh, 342	939, 725,639, 477, 315	0.66 ± 0.07			–
38	Unknown	25.88	322	451, 213			x	–
39	Quercetin dirhamnosyl hexoside isomer	26.10	265, 341	755, 593, 447, 301			0.24 ± 0.10	–
40	Kaempferol rutinoside isomer	26.19	265, 345	593, 447, 285	1.62 ± 0.02	10.61 ± 0.40	0.15 ± 0.01	–
41	Unknown	26.65	289, 322	695			x	–
42	Isorhamnetin rutinoside isomer	26.94	254, 353	623, 477, 315	0.30 ± 0.13			[25]
43	Kaempferol dirhamnoside dihexoside isomer	27.32	264, 344	901, 739, 593, 431, 285		1.03 ± 0.09	0.56 ± 0.01	–
44	Quercetin hexoside	27.38	255sh, 352	463, 301	0.37 ± 0.01			[31]
45	Kaempferol coumaroyl rutinoside isomer	28.28	265, 345	739, 593, 431, 285	0.38 ± 0.04	10.04 ± 1.22	0.35 ± 0.02	–
46	Kaempferol rutinoside derivative isomer	28.79	265, 339	679, 431, 285		0.24 ± 0.01		–
47	Kaempferol dirhamnoside	29.00	264sh, 331	577, 431, 285		0.21 ± 0.10		–
48	Kaempferol pentosyl dirhamnoside	29.07	265sh, 331	709, 577, 431, 285		0.17 ± 0.13		–
49	Isorhamnetin rutinoside isomer derivative	29.11	254, 353	709, 665, 519, 315	0.09 ± 0.04			–
50	Unknown	29.73	330	709, 635, 485		x		–
51	4,4″-bis(N-feruloyl)serotonin isomer	29.81	312	701, 349	X			–
52	Kaempferol hexoside isomer	30.40	264, 344	447, 285	1.19 ± 0.02	0.90 ± 0.05	0.44 ± 0.02	[32]

Table 2. continued

Peak No.	Compound	t_R (min)	UV max (nm)	$[M-H]^-$	*Sinapis pubescens* subsp. *pubescens* extracts (mg/g)			Ref.
					Flowers	leaves	stems	
53	Quercetin trihexoside isomer	30.45	262, 334	787, 625, 301		0.11 ± 0.03		[23]
54	Isorhamnetin hexoside isomer	31.21	254, 352	477, 315	0.34 ± 0.03			[23]
55	Secoisolariciresinol 9,9′-diglucoside	31.54	309	685, 342	X			–
56	Unknown (maybe some dimer)	32.16	313	715, 357	X			–
57	Kaempferol rutinoside isomer	32.35	263, 333	593, 285			1.21 ± 0.01	–
58	Disinapoyl gentiobioside	32.90	327	753, 529		x		[23]
59	Quercetin feruloyl dihexoside isomer	33.69	267sh, 331	801, 639, 463, 301		0.47 ± 0.05		[31]
60	Sinapoyl-feruloyl-gentiobiose	33.79	326	723, 529, 499		x		[23]
61	Isorhamnetin hexoside derivative	33.85	267sh, 321	699, 563, 315	1.40 ± 0.27			–
62 + 63	Diferuloyldiglucoside + Kaempferol dihexoside dirhamnoside isomer	34.41	327	693, 499 + 901, 739, 577, 431, 285		x		[23]
64	Kaempferol pentosyl rhamnosyl dihexoside isomer	34.62	267sh, 331	887, 725, 447, 285		0.28 ± 0.32		–
65	Kaempferol feruloyl dihexoside isomer	35.06	267sh, 326	785, 623, 285		0.16 ± 0.07		[26]
66	Unknown	36.40	326	508			x	–
67	Unknown	36.68	318	903, 451			x	–

x: detected, but not quantified; sh: wavelength shoulder.

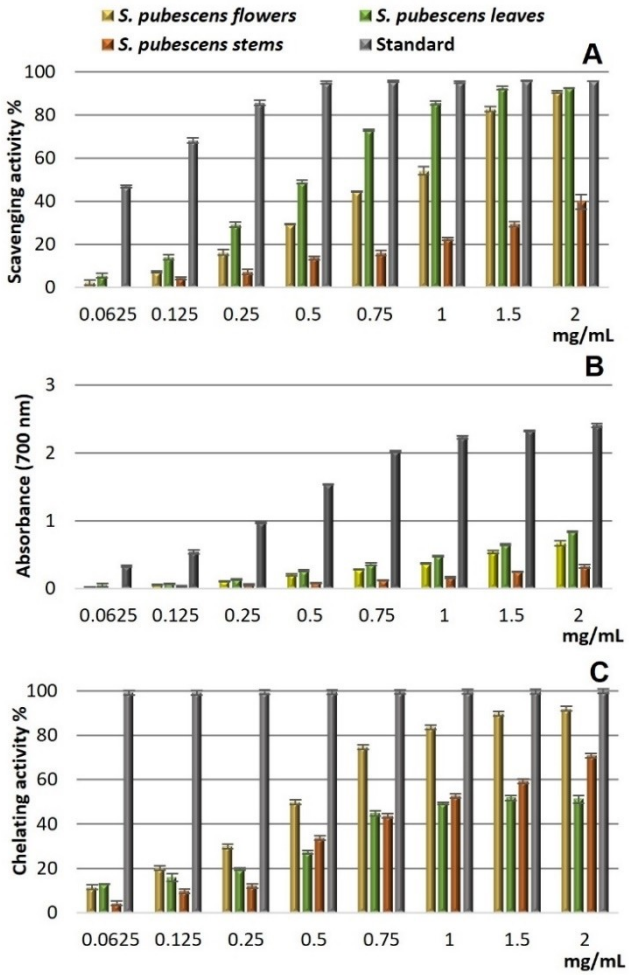

Figure 2. Free radical scavenging activity (DPPH assay) (A), reducing power (B), and ferrous ion chelating activity (C) of the flower, leaf and stem hydroalcoholic extracts of *Sinapis pubescens* subsp. *pubescens*. Reference standard: BHT (A, B), EDTA (C). Values are expressed as the mean ± SD (n = 3).

exhibited much lower activity than the others, reaching an inhibition of about 40% at the concentration of 2 mg/mL. The stronger radical scavenging activity of the leaf extract was indicated also by IC_{50} value, equal to 0.53 ± 0.02 mg/mL, approximately two-fold lower than that of the flowers (0.90 ± 0.04 mg/mL) and five-fold lower than that of the stems (2.67 ± 0.07 mg/mL) (Table 3).

Figure 2 B shows the results of the reducing power assay performed to assess the Fe^{3+}–Fe^{2+} transformation ability of the extracts. As can be seen, the extracts of the aerial parts of *S. pubescens* subsp. *pubescens* exhibited mild-moderate reducing power compared to the BHT standard. Similarly to what observed in the DPPH test, the leaf extract showed greater activity than the flowers; however, the ASE/mL values calculated for the extracts overlapped (Table 3). The reducing activity of the stem extract was considerably lower than the other two extracts, as also confirmed by the ASE/mL value (Table 3).

In Figure 2 C are presented the results of the Ferrous ion chelating activity assay, used to determine the effectiveness of

Table 3. Free radical scavenging activity (DPPH assay), reducing power, and ferrous ion chelating activity of the flower, leaf and stem hydroalcoholic extracts of *Sinapis pubescens* subsp. *pubescens*.

Sinapis pubescens subsp. pubescens extracts	DPPH scavenging activity IC_{50} (mg/mL)	Reducing power ASE/mL	Chelating activity IC_{50} (mg/mL)
Flowers	0.90 ± 0.04^{a}	15.14 ± 0.34^{a}	0.49 ± 0.02^{a}
Leaves	0.53 ± 0.02^{b}	15.11 ± 0.14^{a}	1.02 ± 0.07^{b}
Stems	2.67 ± 0.07^{c}	35.19 ± 0.38^{b}	0.91 ± 0.03^{c}
Standards	BHT 0.07 ± 0.01^{d}	BHT 1.44 ± 0.02^{c}	EDTA 0.007 ± 0.001^{d}

Values are expressed as the mean ± SD (n = 3). [a–d] Different letters within the same column indicate significant differences between mean values ($p < 0.01$).

S. pubescens subsp. *pubescens* extracts to compete for ferrous ions with ferrozine, a compound capable of forming stable magenta-colored complexes with Fe^{2+}.[36] All the extracts showed chelating activity; unlike the other antioxidant activity tests, the flower extract was much more active than the others, followed by the stem extract. As can be seen from the graph, the activity of the flower extract increases with increasing concentration, reaching about 90% at the maximum concentration tested, in any case lower than the reference standard (EDTA). The activity of the stem extract, concentration-dependent, was about 70% at the concentration of 2 mg/mL. These results were confirmed also by the IC_{50} values, being that of the flowers approximately half of that of the stems (0.49 ± 0.02 mg/mL and 0.91 ± 0.03 mg/mL, respectively). The activity of the leaf extract showed a different trend; indeed, the extract reached its maximum chelating activity at the concentration of 1 mg/mL, equal to about 50%; this activity does not vary at the highest concentrations tested. Notably, the IC_{50} value calculated for the leaf extract (1.02 ± 0.07 mg/mL) was close to that of the stems.

As shown in Table 3, significant differences ($p < 0.01$) in both DPPH scavenging and chelating activities were found among the extracts; concerning the reducing power, no statistically significant difference was highlighted between the flower and leaf extracts.

Previous literature data indicated another wild *Sinapis* species, namely *S. arvensis* (wild mustard) as potential source of antioxidant compounds.[37,38] The studies carried out by Tabaraki et al. (2013) and Başyiğit et al. (2020) on extracts obtained from different parts of *S. arvensis* by various extraction procedures, showed radical scavenging and reducing properties stronger for the flower hydroalcoholic extracts than those of leaves and stems; the flower extracts were also found to contain higher amounts of total polyphenols than the others. Differently, the results of our investigations highlighted stronger primary antioxidant ability for *S. pubescens* subsp. *pubescens* leaf extract, whereas the flower extract exhibited the best secondary antioxidant properties. Notably, the amounts of total polyphenols detected in all the *S. pubescens* subsp. *pubescens* extracts are much higher than those reported for *S. arvensis*.

A positive correlation between the total polyphenol content and both the radical scavenging ability and the reducing power of the extracts was highlighted, which resulted strong in the first case ($R^2 = 0.8449$ and $R^2 = 0.7154$, respectively); no correlation with the flavonoid and condensed tannin contents was found. Differently, a positive correlation was found between the chelating properties of the extracts and the total flavonoid content ($R^2 = 0.6108$).

The results described above pave the way for future studies, which will be necessary to clarify the effective role of polyphenol compounds in the antioxidant properties of *S. pubescens* subsp. *pubescens* aerial parts, particularly of flavonoids, identified numerous and in large quantities in the extracts, and to evaluate their potential effects in oxidative stress-related diseases.

Antimicrobial Activity

Considering the use of *Sinapis* species in traditional medicine for their antiseptic properties, it seemed interesting to evaluate the antimicrobial ability of the flower, leaf and stem hydroalcoholic extracts of *S. pubescens* subsp. *pubescens* on different bacterial strains and yeasts.

The results of the screening showed lack of activity against all the strains tested, except for a fungistatic effect highlighted for the flower extract on *Candida glabrata* 33 clinical isolate at a concentration equal to 500 µg/mL.

In the article recently published by Başyiğit et al. (2020), the antimicrobial activity of a flower extract of *S. arvensis*, evaluated against several bacteria and yeasts by disk diffusion method, was highlighted against Gram-positive bacterial strains, namely *Enterococcus faecalis*, *Staphylococcus aureus*, *S. haemolyticus*, and *S. epidermidis*, whereas no activity against the tested strains of *Candida* spp. was found.[38] Notably, the antimicrobial activity of *S. arvensis* flower extract was evidenced at concentration levels in the range 5–100 mg/mL, far superior to those utilized in our experiments (maximum concentration tested 1 mg/mL).

Artemia salina Lethality Bioassay

Artemia salina Leach (brine shrimp) lethality bioassay is commonly employed for the preliminary toxicity assessment of plant extracts. The convenience of using such method is due the rapidity, ease of execution, adaptability to different testing conditions and reduced costs.[39]

The obtained results of the bioassay carried out for flower, leaf and stem extracts of *S. pubescens* subsp. *pubescens* showed

the absence of toxicity against brine shrimp larvae for all the extracts on the basis of the Clarkson's toxicity criterion applied for the assessment of the degree of toxicity ($LC_{50} > 1000$ μg/mL).[40] In fact, after 24 h of exposure to the extracts, the larvae were found to be all alive even at the maximum tested concentration of 1000 μg/mL.

Conclusions

In the present research, the aerial parts (leaves, flowers and stems) of *S. pubescens* subsp. *pubescens* spontaneously grown in Sicily (Italy) were investigated for their polyphenolic profile and biological properties. The comparison of the polyphenolic compounds characterized in the flowers, leaves and stems highlighted a consistently different qualitative-quantitative profile. The aerial parts of *S. pubescens* subsp. *pubescens* were found to be a valuable source of antioxidant compounds; in detail, the leaf extract showed higher primary antioxidant properties than the others, namely radical scavenging activity and reducing power, which appear to be related to their polyphenol content, while the flower extract showed greater secondary antioxidant properties, namely chelating activity, which seems to be related to the flavonoid content. The lack of toxicity against brine shrimp larvae indicates these extracts as potentially safe and suggests a possible recovery of antioxidant molecules with beneficial properties for human health and well-being. On the other hand, none of the extracts showed antimicrobial properties against the strains tested.

This study substantially improves the knowledge of *S. pubescens* subsp. *pubescens*, an edible plant hitherto unexplored, indicating a promising use of wild species included in the genus *Sinapis* as new sources of active metabolites which could provide health benefits.

Experimental Section

Chemicals and Reagents

LC/MS-grade water (H_2O), acetonitrile (ACN), quercetin-3-*O*-glucopyranoside, kaempferol-3-*O*-glucoside and isorhamnetin-3-*O*-glucoside, Folin-Ciocâlteu reagent, sodium carbonate, gallic acid, aluminum chloride hexahydrate, potassium acetate, quercetin, vanillin, (+)-Catechin, hydrochloric acid 37%, 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), ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), dimethyl sulfoxide (DMSO), tetracycline, and fluconazole were obtained from Merck Life Science (Merck KGaA, Darmstadt, Germany). LC/MS-grade formic acid was purchased from Riedel-de Haën (Seelze, Germany). Methanol (MeOH) was purchased from Carlo Erba (Milan, Italy). Mueller-Hinton Broth (MHB), Mueller-Hinton Agar (MHA), Sabouraud Dextrose Broth (DSB), and Sabouraud Dextrose Agar (SDA) were purchased from Oxoid (Milan, Italy).

Plant Material and Extraction Procedure

The aerial parts of *Sinapis pubescens* subsp. *pubescens* were collected in May 2022 in Sicily (Italy), specifically in the locality of Floresta (Messina), on the outskirts of the town, about 1250 m 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, and 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).

After harvesting, the aerial parts of *S. pubescens* subsp. *pubescens* were immediately grounded and frozen. Then, the plant material was lyophilized, powdered in a mortar and subjected to a preventive maceration with MeOH 70% (1:10 *w/v*) at 25 °C for 1 h. The extraction was performed with 70% MeOH (1:10 *w/v*) in an ultrasonic bath at 50 °C for 15 min; the extraction procedure was repeated four times, then the filtrates were pooled and evaporated to dryness by a rotavapor. The yields of the extracts, referred to 100 g of lyophilized plant material, were 42.8%, 24.6% and 4.60% for flowers, leaves and stems, respectively.

Phytochemical Investigations

Determination of total polyphenol, flavonoid, and condensed tannin content

The quantitative estimation of the total polyphenols, flavonoids, and condensed tannins contained in the flower, leaf and stem hydroalcoholic extracts of *S. pubescens* subsp. *pubescens* was attained using colorimetric methods.

The Folin Ciocâlteu method was employed for the determination of the total phenolic content.[41] Briefly, 100 μL of the sample solution was mixed with 200 μL of Folin Ciocâlteu reagent, 2 mL of distilled water, and 1 mL of 15% sodium carbonate. After incubation at room temperature (2h), the absorbance was measured at 765 nm by a UV-1601 spectrophotometer (Shimadzu, Milan, Italy).

The aluminium chloride colorimetric assay was performed to determine of the total flavonoid content.[42] Briefly, 500 μL of each sample solution was mixed with 1.5 mL of MeOH, 100 μL of 10% aluminium chloride, 100 μL of 1 M potassium acetate and 2.8 mL of distilled water. The reaction mixtures were incubated for 30 min at room temperature, and the absorbance was measured at 415 nm by spectrophotometer.

The vanillin method was used for the determination of the condensed tannin content.[43] Briefly, 50 μL of each sample solution was mixed with 1.5 mL of 4% vanillin in MeOH and 750 μL of concentrated HCl. Then, the reaction mixture was incubated at room temperature, in the dark, for 20 min and the absorbance was measured at 500 nm by spectrophotometer.

For the quantitative estimation of total polyphenols, flavonoids and condensed tannins, calibration curves of the standards gallic acid, quercetin and (+)-catechin, respectively, were constructed. The total polyphenols were expressed as mg gallic acid equivalents (GAE)/g extract (dw) ± standard deviation (SD); the total flavonoids were expressed as mg quercetin equivalents (QE)/g extract (dw) ± SD.; condensed tannins were expressed as mg catechin equivalents (CE)/g extract (dw) ± SD. The results were obtained from the average of three independent experiments.

HPLC-PDA/ESI-MS analysis

The flower, leaf and stem hydroalcoholic extracts of *S. pubescens* subsp. *pubescens* were analyzed by high-performance liquid chromatography coupled to a photodiode array and electrospray ionization mass spectrometry HPLC-PDA/ESI-MS.[44–46]

Sample preparation: The dried extracts were redissolved in 70% MeOH. For the chromatographic separation, an injection volume of 5 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 (150×4.6 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 1 mL/min, under gradient elution 0–5 min, 0–5% B, 10 min, 15% B, 20 min, 30% B, 60 min, 50% B, 70 min, 100% B, 75 min, 100% B. Photodiode array detection (PDA) was applied in the range of 200–400 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: −4.5 kV.

Standards employed: Calibration curves of three standards (quercetin-3-*O*-glucopyranoside, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside) were employed for the quantification of the flavonoid content in sample extracts. Each analysis was performed in 6 repetitions. Data acquisition was performed by Shimadzu LabSolution software ver. 5.99. Quercetin-3-*O*-glucopyranoside (0.1, 1, 10, 50, 100 ppm), $y = 13424x + 898.59$, $R^2 = 0.9939$, LOD = 0.018, LOQ = 0.061; kaempferol-3-*O*-glucoside (0.1, 1, 10, 50, 100 ppm), $y = 17660x–10681$, $R^2 = 0.9963$, LOD = 0.018, LOQ = 0.061; isorhamnetin-3-*O*-glucoside (0.1, 1, 10, 50, 100 ppm), $y = 14948x–2966.9$, $R^2 = 0.9963$, LOD = 0.010, LOQ = 0.033.

Antioxidant Activity

DPPH Assay

The free radical scavenging activity of *S. pubescens* subsp. *pubescens* extracts was determined by of the DPPH assay.[47] The tested concentrations were in the range 0.0625–2 mg/mL, employing butylated hydroxytoluene (BHT) as reference standard. Briefly, 3 mL of DPPH methanol solution (0.1 mM) were added to a 0.5 mL aliquot of each sample solution. The mixture was left in the dark for 20 min, at room temperature; at this time point the absorbance was measured at 517 nm using a model UV-1601 spectrophotometer (Shimadzu). The results were obtained from three independent experiments, and are reported as mean radical scavenging activity (%) ± SD and mean 50% inhibitory concentration (IC_{50}) ± SD.

Reducing Power Assay

The Fe^{3+}-Fe^{2+} transformation method was used to assess the reducing power of *S. pubescens* subsp. *pubescens* extracts.[48] The tested concentrations were in the range of 0.0625–2 mg/mL employing BHT and ascorbic acid as reference standards. Briefly, 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide were mixed with an aliquot of 1 mL of each sample solution. The mixture was incubated at 50 °C for 20 min and rapidly cooled; then 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged for 10 min (3000 rpm, 4 °C). Finally, 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride were mixed with 2.5 mL of the supernatant and the reaction mixture was incubated in the dark for 10 min at room temperature. The optical density change 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/mL (ASE/mL) ± SD.

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

The Fe^{2+} chelating activity of the hydroalcoholic extracts of *S. pubescens* subsp. *pubescens* aerial parts was determined by the spectrophotometric measurement of the Fe^{2+}-ferrozine complex.[49] The tested concentrations were in the range of 0.0625–2 mg/mL and ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) was used as reference standard. For the test, 0.05 mL of 2 mM ferrous chloride was added to 1 mL of sample solution and 0.5 mL of methanol. Then, 0.2 mL of 5 mM ferrozine solution was added, followed by vigorous shaking and incubation at room temperature in the dark for 10 min. Finally, the optical density change was measured spectrophotometrically at 562 nm. The results were obtained from the average of three independent experiments and are reported as mean inhibition of the ferrozine-(Fe^{2+}) complex formation (%) ± SD and IC_{50} ± SD.

Antimicrobial Activity

For the antimicrobial susceptibility testing the following bacteria and yeasts were used: *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 43300, *Pseudomonas aeruginosa* ATCC 9027, *Candida auris* DSM 21092, *Candida albicans* ATCC 10231, *C. albicans* n° 12 (clinical isolate), *Candida glabrata* n° 33 (clinical isolate), and *C. glabrata* n° 9 (clinical isolate). The strains, obtained from the private collection of the Department of Chemical, Biological, Pharmaceutical and Environmental Sciences (University of Messina, Italy), were stored at −70 °C in Microbanks™ (Pro-lab Diagnostics, Neston, UK). The minimum inhibitory concentration (MIC) and minimum bactericidal and fungicidal concentration (MBC and MFC) values of the flower, leaf and stem hydroalcoholic extracts of *S. pubescens* subsp. *pubescens* were determined using the broth microdilution method according to the protocols recommended by the Clinical and Laboratory Standards Institute (CLSI, 2018) and CLSI (2008) for bacteria and yeasts, respectively, with few modifications.[50,51]

Determination of MIC: Overnight cultures of bacteria and yeasts were grown at 37 °C in MHB or SDB, respectively. The methanol extracts were dissolved in dimethyl sulfoxide (DMSO) and further diluted using MHB or SDB to obtain a final concentration of 2 mg/mL. Two-fold serial dilutions were prepared in a 96-well plate. The tested concentrations ranged from 1000 to 7.8 µg/mL. Working strains cultures were adjusted to the required inoculum of 1×10^5 CFU/mL and 1×10^3 CFU/mL for bacteria and yeasts, respectively. Positive controls (medium with inocula, but without the extracts) and vehicle controls (medium with inocula and DMSO) were included. The concentration of solvent (DMSO) did not exceed 1%. Tetracycline was tested against all bacteria. Fluconazole was tested against all yeasts. MIC was defined as the lowest concentration of the extracts that completely inhibited growth compared to the growth controls.

Determination of MBC and MFC: Aliquots (10 μL) from each well from susceptibility testing assays were plated onto MHA or SDA, for bacteria or yeasts, respectively. The cultures were incubated for 24–48 h at 37 °C. MBC and MFC were defined as the lowest concentration of extracts that did not allow visible growth when the aliquots of the well contents were plated onto agar media and grown for 24–48 h at 37 °C. All experiments were repeated thrice in duplicate.

Artemia salina Leach Lethality Bioassay

The brine shrimp (*Artemia salina* Leach) lethality bioassay was performed to establish the acute toxicity of the *S. pubescens* subsp. *pubescens* extracts.[52] Brine shrimp eggs were placed in a hatchery dish containing artificial seawater (32 g sea salt/L) and incubated for hatching under continuous lighting by means of a 60 W lamp and at a temperature in the range 24–26 °C. At 24 h after hatching, active nauplii free from eggshells were collected from the brighter portion of the hatchery dish and used for the assay. Specifically, ten brine shrimp larvae were incubated for 24 h at 24–26 °C in artificial seawater mixed with different amounts of the extracts dissolved in DMSO, to obtain final concentrations in the range 10–1000 μg/mL in a total volume of 5 mL. Subsequently, the survived larvae were counted, and the median lethal concentration (LC_{50}) values were calculated. Control groups were prepared for seawater and seawater with 2% of DMSO, *i.e.*, the maximum volume of solvent used in the assay, which in preliminary experiments was found to be non-toxic to brine shrimp larvae. The test was carried out in triplicate. The toxicity level of the extracts was assessed according to the Clarkson's toxicity criterion.[40]

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 Software for Science). *p*-values < 0.01 were considered to be statistically significant.

Author Contributions

N. Miceli and M. F. Taviano conceived and designed the study; V. Spadaro and F. M. Raimondo collected and identified the plant material; F. Cacciola, R. Laganà Vinci and L. Mondello, carried out the phytochemical studies; P. Arena, N. Miceli, A. Marino, F. Davì, E. Cavò, and M. F. Taviano performed the biological investigations and analysed the data., P. Arena, N. Miceli, F. Cacciola, A. Marino, and M. F. Taviano wrote the original draft of the manuscript. N. Miceli, V. Spadaro, F. M. Raimondo, L. Mondello and M. F. Taviano revised the article. All authors gave approval of the final version.

Acknowledgements

Paola Arena thanks the "Prof. Antonio Imbesi" Foundation for the fellowship. The authors are grateful to Shimadzu and Merck Life Science Corporations for the continuous support.

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: antimicrobial activity · antioxidants · phytochemistry · sicilian vascular flora · toxicity assessment

[1] F. M. Raimondo, G. Domina, V. Spadaro, *Quad. Bot. Amb. Appl.* **2010**, *21*, 189–252.
[2] S. Pasta, A. La Rosa, G. Garfì, C. Marcenò, A. S. Gristina, F. Carimi, R. Guarino, *Front. Plant Sci.* **2020**, *11*, 388.
[3] K. Kubitzki, C. Bayer, *'Flowering plants. Dicotyledons - Malvales, Capparales and non-betalain Caryophyllales'*, Springer, Berlin Heidelberg, 2003, p. 159.
[4] A. Mikić, *Genet. Res.* **2016**, *63*, 1–6.
[5] T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, D. A. Webb, '*Flora Europea*', Cambridge University Press, Cambridge,1964, Vol. 1, p. 339.
[6] https://brassibase.cos.uni-heidelberg.de/; Accessed January 27, 2023.
[7] S. Pignatti, *Flora d'Italia*, Edagricole, Bologna, 2° Ed., **2017**, Vol. 2, pp. 1028–1029.
[8] F. Bartolucci, L. Peruzzi, G. Galasso, A. Albano, A. Alessandrini, N. M. G. Ardenghi, G. Astuti, G. Bacchetta, S. Ballelli, E. Banfi, G. Barberis, L. Bernardo, D. Bouvet, M. Bovio, L. Cecchi, R. Di Pietro, G. Domina, S. Fascetti, G. Fenu, F. Festi, B. Foggi, L. Gallo, G. Gottschlich, L. Gubellini, D. Iamonico, M. Iberite, P. Jiménez-Mejías, E. Lattanzi, D. Marchetti, E. Martinetto, R. R. Masin, P. Medagli, N. G. Passalacqua, S. Peccenini, R. Pennesi, B. Pierini, L. Poldini, F. Prosser, F. M. Raimondo, F. Roma-Marzio, L. Rosati, A. Santangelo, A. Scoppola, S. Scortegagna, A. Selvaggi, F. Selvi, A. Soldano, A. Stinca, R. P. Wagensommer, T. Wilhalm, F. Conti, *Plant Biosyst.* **2018**, *152*, 179–303.
[9] M. L. Leporatti, S. Ivancheva, *J. Ethnopharmacol.* **2003**, *87*, 123–142.
[10] A. Alachkar, A. Jaddouh, M. S. Elsheikh, A. R. Bilia, F. F. Vincieri, *Nat. Prod. Commun.* **2011**, *6*, 79–84.
[11] R. Polat, F. Satıl, *J. Ethnopharmacol.* **2012**, *139*, 626–641.
[12] T. Tuttolomondo, M. Licata, C. Leto, G. Bonsangue, M. L. Gargano, G. Venturella, S. La Bella, *J. Ethnopharmacol.* **2014**, *157*, 21–37.
[13] Y. F. Xian, Z. Hu, S. P. Ip, J. N. Chen, Z. R. Su, X. P. Lai, Z. X. Lin, *Phytomedicine* **2018**, *50*, 196–204.
[14] J. Lietzow, *Food* **2021**, *10*, 2089.
[15] F. E. Amrati, M. Bourhia, M. Slighoua, A. Mohammad Salamatullah, A. Alzahrani, R. Ullah, A. Bari, D. Bousta, *Saudi Pharm. J.* **2021**, *29*, 1185–1204.
[16] F. Lentini, F. Venza, *J. Ethnobiol. Ethnomed.* **2007**, *3*, 15.
[17] D. Romano, A. Tribulato, S. Toscano, D. Scuderi, *Acta Hortic.* **2013**, *1005*, 197–204.
[18] R. Di Novella, N. Di Novella, L. De Martino, E. Mancini, V. De Feo, *J. Ethnopharmacol.* **2013**, *145*, 328–342.
[19] N. Biscotti, A. Pieroni, *Acta Soc. Bot. Pol.* **2015**, *84*, 327–338.
[20] G. Mattalia, P. Corvo, A. Pieroni, *Ethnobot. Res. Appl.* **2020**, *19*, 38.
[21] R. Jayabalan, P. Subathradevi, S. Marimuthu, M. Sathishkumar, K. Swaminathan, *Food Chem.* **2008**, *109*, 227–234.
[22] M. Soto-Hernandez, M. Palma-Tenango, M. Garcia-Mateos, IntechOpen, London, 2017.
[23] 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*, 5421.
[24] Y.-Q. Wang, L.-P. Hu, G.-M. Liu, D.-S. Zhang, H.-J. He, *Molecules* **2017**, *22*, 1262.
[25] M. F. Taviano, E. Cavò, V. Spadaro, F. M. Raimondo, V. Musolino, F. Cacciola, Y. O. El Majdoub, L. Mondello, C. Condurso, F. Cincotta, A. Verzera, N. Miceli, *Molecules* **2021**, *26*, 4114.

[26] E. Cavò, M. F. Taviano, F. Davì, F. Cacciola, Y. Oulad El Majdoub, L. Mondello, M. Ragusa, C. Condurso, M. Merlino, A. Verzera, N. Miceli, *Molecules* **2022**, *27*, 2768.
[27] A. Martínez-Sánchez, R. Llorach, M. I. Gil, F. Ferreres, *J. Agric. Food Chem.* **2007**, *55*, 1356–1363.
[28] A. Martínez-Sánchez, A. Gil-Izquierdo, M. I. Gil, F. Ferreres, *J. Agric. Food Chem.* **2008**, *56*, 2330–2340.
[29] N. Miceli, A. Filocamo, S. Ragusa, F. Cacciola, P. Dugo, L. Mondello, M. Celano, V. Maggisano, M. F. Taviano, *Chem. Biodiversity* **2017**, *14*, e1700073.
[30] J. Sun, Z. Xiao, L. Lin, G. E. Lester, Q. Wang, J. M. Harnly, P. Chen, *J. Agric. Food Chem.* **2013**, *61*, 10960–10970.
[31] S. Schmidt, M. Zietz, M. Schreiner, S. Rohn, L. W. Kroh, A. Krumbein, *Rapid Commun. Mass Spectrom.* **2010**, *24*, 2009–2022.
[32] N. Miceli, E. Cavò, M. Ragusa, F. Cacciola, L. Mondello, L. Dugo, R. Acquaviva, G. A. Malfa, A. Marino, M. D'Arrigo, M. F. Taviano, *Molecules* **2020**, *25*, 1461.
[33] M. Rudrapal, S. J. Khairnar, J. Khan, A. B. Dukhyil, M. A. Ansari, M. N. Alomary, R. Devi, *Front. Pharmacol.* **2022**, *13*, 283.
[34] S. Bounatirou, S. Smiti, M. G. Miguel, L. Faleiro, M. N. Rejeb, M. Neffati, M. M. Costa, A. C. Figueiredo, J. G. Barroso, L. G. Pedro, *Food Chem.* **2007**, *105*, 146–155.
[35] J. Dai, R. J. Mumper, *Molecules* **2010**, *15*, 7313–7352.
[36] I. D. Nwachukwu, R. A. Sarteshnizi, C. C. Udenigwe, R. E. Aluko, *Molecules* **2021**, *26*, 4865.
[37] R. Tabaraki, F. Ghadiri, *Int. Food Res. J.* **2013**, *20*, 2111–2115.
[38] B. Başyiğit, H. Alaşalvar, N. Doğan, C. Doğan, S. Berktaş, M. Çam, *J. Food Meas. Charact.* **2020**, *14*, 1671–1681.
[39] N. E. Ntungwe, E. M. Domínguez-Martín, A. Roberto, J. Tavares, V. M. S. Isca, P. Pereira, M.-J. Cebola, P. Rijo, *Curr. Pharm. Des.* **2020**, *26*, 2892–2908.
[40] M. R. Hamidi, B. Jovanova, T. K. Panovska, *Maced. Pharm. Bull.* **2014**, *60*, 9–18.
[41] X. Gao, M. Ohlander, N. Jeppsson, L. Björk, V. Trajkovski, *J. Agric. Food Chem.* **2000**, *48*, 1485–1490.
[42] M.-Y. Chang, C.-M. Liu, D.-E. Shieh, C.-Y. Chen, *Biomed. Res.* **2017**, *28*, 6431–6434.
[43] R. Julkunen-Titto, *J. Agric. Food Chem.* **1985**, *33*, 213–217.
[44] K. Arena, E. Trovato, F. Cacciola, L. Spagnuolo, E. Pannucci, P. Guarnaccia, L. Santi, P. Dugo, L. Mondello, L. Dugo, *Molecules* **2022**, *27*, 1727.
[45] 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.
[46] 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.
[47] M. Ohnishi, H. Morishita, H. Iwahashi, T. Shitzuo, S. Yoshiaki, M. Kimura, R. Kido, *Phytochemistry* **1994**, *36*, 579–583.
[48] M. Oyaizu, *Jpn. J. Nutr. Diet.* **1986**, *44*, 307–315.
[49] T. S. Kumar, S. Shanmugam, T. Palvannan, V. M. B. Kumar, *Iran. J. Pharm. Res.* **2008**, *7*, 211–215.
[50] Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. CLSI, Wayne, PA, USA: **2018**.
[51] Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved Standard M27-A3, CLSI, Wayne, PA, USA, **2008**.
[52] B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobson, D. E. Nichols, J. L. McLaughlin, *Planta Med.* **1982**, *45*, 31–34.

Manuscript received: March 1, 2023
Accepted manuscript online: May 24, 2023
Version of record online: June 6, 2023