Research Article

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Phytochemical profiling and bioactivity assessment of underutilized *Symphytum* species in comparison with *Symphytum officinale*

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Abstract

BACKGROUND: *Symphytum* (comfrey) genus, particularly *Symphytum officinale*, has been empirically used in folk medicine mainly for its potent anti-inflammatory properties. In an attempt to shed light on the valorization of less known taxa, the current study evaluated the metabolite profile and antioxidant and enzyme inhibitory effects of nine *Symphytum* species.

RESULTS: Phenolic acids, flavonoids and pyrrolizidine alkaloids were the most representative compounds in all comfrey samples. Hierarchical cluster analysis revealed that, within the roots, *S. grandiflorum* was slightly different from *S. ibericum*, *S. caucasicum* and the remaining species. Within the aerial parts, *S. caucasicum* and *S. asperum* differed from the other samples. All *Symphytum* species showed good antioxidant and enzyme inhibitory activities, as evaluated in DPPH (up to 50.17 mg Trolox equivalents (TE) g^{-1}), ABTS (up to 49.92 mg TE g^{-1}), cupric reducing antioxidant capacity (CUPRAC, up to 92.93 mg TE g^{-1}), ferric reducing antioxidant power (FRAP, up to 53.63 mg TE g^{-1}), acetylcholinesterase (AChE, up to 0.52 mg galanthamine equivalents (GALAE) g^{-1}), butyrylcholinesterase (BChE, up to 0.96 mg GALAE g^{-1}), tyrosinase (up to 13.58 mg kojic acid equivalents g^{-1}) and glucosidase (up to 0.28 mmol acarbose equivalents g^{-1}) tests. Pearson correlation analysis revealed potential links between danshensu and ABTS/FRAP/CUPRAC, quercetin-*O*-hexoside and DPPH/CUPRAC, or rabdosiin and anti-BChE activity.

CONCLUSIONS: By assessing for the first time in a comparative manner the phytochemical–biological profile of a considerably high number of *Symphytum* samples, this study unveils the potential use of less common comfrey species as novel phytopharmaceutical or agricultural raw materials.

Supporting information may be found in the online version of this article.

Keywords: comfrey; metabolite profiling; antioxidant; enzyme inhibitory; Boraginaceae; pyrrolizidine alkaloids

INTRODUCTION

Symphytum L. is a genus of the Boraginaceae family comprising around 40 species that grow spontaneously in the flora of Europe and Asia or have been naturalized throughout North America.[1] The name of the genus, referenced for the first time in Dioscorides' work *De Materia Medica*, comes from the Greek word *symphuo* ('to knit together'), whereas the English name 'comfrey' originates from the Latin word *comfirmare* ('to join').[2] Throughout the Middle Ages, comfrey poultices were applied for fractures, burns and bruises. External (e.g. compresses, pastes, ointments) or internal (e.g., decocts, infusions, tinctures, extracts) preparations obtained from the roots (*Symphyti radix*), leaves (*Symphyti*

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folium) or aerial parts (*Symphyti herba*) are empirically administered in swellings, phlebitis, hematomas, rheumatic pain and contusions and gastrointestinal, respiratory and genitourinary disorders.[3] Due to numerous human trials, comfrey-based topical formulations are clinically prescribed to alleviate the symptomatology (e.g. swelling, pain and inflammation of muscles and joints) related to sprains, contusions, strains or arthritis.[2]

Phytochemically, the *Symphytum* genus is reported to contain four major types of constituents: polysaccharides (up to 30 wt %), purine derivatives (0.6–4.7 wt%), polyphenols and pyrrolizidine alkaloids. Polysaccharides are considered one of the most complex groups of comfrey phytochemicals endowed with hypoglycemic, hypolipidemic, immunomodulatory, antioxidant and anticancer properties.[4,5] Allantoin is a purine derivative acknowledged to exhibit immunomodulatory and wound healing (e.g. extracellular matrix synthetic, fibroblast proliferative) effects.[6,7] Polyphenols (e.g. rosmarinic acid, chlorogenic acid, caffeic acid, globoidnan A, rabdosiin, flavonoids) are the most potent bioactive molecules in comfrey.[8,9] For instance, rosmarinic acid was shown to act as a polypharmacology agent through its antioxidant, anticancer, antimicrobial, anti-allergic and anti-inflammatory properties.[10] Lycopsamine, intermedine, echimidine, symphytine and their *N*-oxides are some of the most notable examples of pyrrolizidine alkaloids. In contrast to the previously mentioned classes of comfrey constituents assumed to have mostly beneficial therapeutic effects, pyrrolizidine alkaloids are implicated in severe toxicity at hepatic, lung, cellular or DNA level.[11] Consequently, the Committee on Herbal Medicinal Products of the European Medicines Agency restricted the intake of pyrrolizidine alkaloids to a maximum limit of 1 μg day^{-1} for a maximum of 3 years, after which it should be decreased to <0.35 μg day^{-1}.[12]

Nonetheless, the phytopharmaceutical and agricultural exploitation of the *Symphytum* genus is currently restricted only to a limited number of species, mainly *S. officinale* L. and *S.* × *uplandicum* Nyman.[6] To expand the industrial use of the genus, research efforts to chemically and biologically prospect unexploited *Symphytum* species are demanded. Recent studies have already shown that species like *S. aintabicum* Hub.-Mor. & Wickens,[13] *S. anatolicum* Boiss.,[14,15] *S. caucasicum* M. Bieb.,[16] *S. asperum* Lepech.[17,18] or *S. ibericum* Steven[19] are rich sources of biomolecules that endow them with interesting pharmacological properties.

In this context, the study reported here aimed to perform the untargeted metabolite profiling of 29 hydroethanolic extracts obtained from the roots and aerial parts of nine *Symphytum* species. In addition, their antioxidant (free radical scavenging, reducing power and chelating activity) and enzyme inhibitory (cholinesterase, tyrosinase, amylase and glucosidase) effects were evaluated by *in vitro* assays. To the best of the authors' knowledge, no previous study has assessed comparatively the phytochemical–biological profile of such a significant number of *Symphytum* species and samples.

MATERIALS AND METHODS

Plant materials and extraction

The aerial (stems, leaves, flowers) and underground (rhizomes, roots, etc.) parts of *Symphytum* spp. were collected in July 2022. The identification data of the acquired samples (e.g. source, international plant exchange number, IPEN, GPS coordinates, voucher numbers, etc.) are provided in Table 1. The plant materials were dried in an acclimatized room (20 ± 2 °C; 55 ± 5% relative humidity) for 1 month. After drying, the aerial parts were separated from the underground parts, pulverized and separately extracted at room temperature in an ultrasound bath (ultrasonic frequency of 35 kHz) for 30 min with 65% ethanol (0.5 g of plant material with 12.5 mL of solvent). The extracts were centrifuged at 13 500 rpm for 5 min, filtered through 0.22 μm pore size filters and analyzed immediately.

Phytochemical characterization

Liquid chromatography with tandem high-resolution mass spectrometry (LC-HRMS/MS) analysis was performed with an Agilent 1200 LC system (Agilent Technologies, Palo Alto, CA, USA) connected to a G6530B accurate-mass quadrupole time-of-flight MS detector. The LC separation was accomplished on a Phenomenex Gemini C18 column (2 × 100 mm, 3 μm) operated at 20 °C. Gradient elution was achieved using 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at a flow rate of 0.2 mL min^{-1}. The gradient program was 5–60% B from 0 to 45 min and 95% B from 46 to 55 min. The samples were injected in a volume of 10 μL. The detection was carried out in negative and positive electrospray ionization modes, with the spectra recorded by MS scanning in the range of *m/z* 50–1700. The ion source parameters were as follows: drying gas (N_2) flow rate, 10 L min^{-1}; drying gas (N_2) temperature, 275 °C; sheath gas (N_2) flow rate, 12 L min^{-1}; sheath gas (N_2) temperature, 325 °C; nebulizer pressure, 35 psi; capillary voltage, 4000 V; nozzle voltage, 1000 V; fragmentor voltage, 140 V; skimmer voltage, 65 V; octapole RF peak voltage, 750 V. The MS/MS analyses were carried out by automated and targeted fragmentation, with the collision dissociation energies set at 10 and 30 V. Data were processed with MassHunter v. B08.00 (Agilent Technologies).

Total phenolic content (TPC), total phenolic acid content (TPAC) and total flavonoid content (TFC) were determined according to previously described methods,[20,21] with the results presented as mg gallic acid equivalents (GAE) g^{-1} d.w. plant material, mg caffeic acid equivalents (CAE) g^{-1} and mg rutin equivalents (RE) g^{-1}, respectively.

Biological evaluation

The antioxidant (2,2-diphenyl-1-picrylhydrazyl (DPPH), radical scavenging; 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), radical scavenging; ferric ion reducing antioxidant power (FRAP); cupric ion reducing antioxidant capacity (CUPRAC); metal chelating ability (MCA); phosphomolybdenum (PBD)) and enzyme (acetylcholinesterase (AChE); butyrylcholinesterase (BChE); tyrosinase; amylase; glucosidase) inhibitory assays were performed according to previously described methods.[20,21] Standard compounds, such as Trolox and ethylenediaminetetraacetic acid (EDTA), were used to quantify the antioxidant activity, whereas galanthamine (for cholinesterases), kojic acid (for tyrosinase) and acarbose (for amylase and glucosidase) were used to express the enzyme inhibitory potential.

Data analysis

Extractions, LC-HRMS/MS analysis and antioxidant and enzyme inhibitory assays were performed in triplicate, with the data expressed as mean ± standard deviation. Statistical analysis (one-way ANOVA with Tukey's *post hoc* test) was performed with XlStat 2016; $P < 0.05$ was considered significant. Hierarchical cluster analysis (HCA) was conducted to assess the phytochemical dissimilarities and similarities between analyzed samples. For this, the LC-HRMS/MS peak areas of all compounds were extracted from the chromatograms and log transformed before the multivariate analysis. Pearson correlation analysis was conducted to find correlations between the phytochemical profile (LC-HRMS/

Table 1. Identification data of *Symphytum* species included in the study

Species	Source	IPEN/GPS	Internal voucher no.	Plant parts	Sample code
S. asperum Lepech.	Botanical Garden Frankfurt (Germany)	IPEN: XX-0-FRT-0000/3681	SA/220706	Roots	SA1-R
	Maria Curie-Skłodowska University Botanical Garden Lublin (Poland)	GPS: 51° 14′ 26″ N, 22° 32′ 16″ E	SA/220731	Roots + aerial parts	SA2-R, SA2-A
	Botanical Garden Poznań (Poland)	GPS: 52° 25′ 15″ N, 16° 52′ 57″ E	SA/220730	Roots + aerial parts	SA3-R, SA3-A
S. bulbosum K.F.Schimp.	Botanical Garden Frankfurt (Germany)	IPEN: XX-0-FRT-0000/3682	SB/220706	Roots	SB-R
S. caucasicum M.Bieb.	Botanical Garden Munich-Nymphenburg (Germany)	GE-0-M-2021/0968	SC/220707	Roots + aerial parts	SCa-R, SCa-A
S. cordatum Willd.	Maria Curie-Skłodowska University Botanical Garden Lublin (Poland)	GPS: 51° 14′ 26″ N, 22° 32′ 16″ E	SC/220731	Roots + aerial parts	SCo1-R, SCo1-A
	Botanical Garden Poznań (Poland)	GPS: 52° 25′ 15″ N, 16° 52′ 57″ E	SC/220730	Roots + aerial parts	SCo2-R, SCo2-A
S. grandiflorum DC.	Botanical Garden Frankfurt (Germany)	IPEN: XX-0-FRT-0000/3684	SG/220706	Roots	SG1-R
	Botanical Garden Poznań (Poland)	GPS: 52° 25′ 15″ N, 16° 52′ 57″ E	SG/220730	Roots + aerial parts	SG2-R, SG2-A
S. ibericum Steven	Botanical Garden Munich-Nymphenburg (Germany)	IPEN: GE-0-M-2012/2393	SI/220707	Roots + aerial parts	SI-R, SI-A
S. officinale L.	Osnabrücker Hügelland, Am Gut Sandfort, Osnabrück (Germany)	GPS: 52° 15′ 05″ N, 8° 6′ 34″ E	SO/220701	Roots + aerial parts	SO1-R, SO1-A
	Botanical Garden Frankfurt (Germany)	IPEN: XX-0-FRT-0000/3685	SO/220706	Roots	SO2-R
	Botanical Garden Munich-Nymphenburg (Germany)	IPEN: RS-0-M-2019/1286	SO/220707	Roots + aerial parts	SO3-R, SO3-A
	Maria Curie-Skłodowska University Botanical Garden Lublin (Poland)	GPS: 51° 14′ 26″ N, 22° 32′ 16″ E	SO/220731	Roots + aerial parts	SO4-R, SO4-A
S. tuberosum L.	Maria Curie-Skłodowska University Botanical Garden Lublin (Poland)	GPS: 51° 14′ 26″ N, 22° 32′ 16″ E	ST/220731	Roots + aerial parts	ST1-R, ST1-A
	Botanical Garden Poznań (Poland)	GPS: 52° 25′ 15″ N, 16° 52′ 57″ E	ST/220730	Roots + aerial parts	ST2-R, ST2-A
S. tuberosum subsp. *nodosum* (Schur) Soó (syn. S. *tuberosum* ssp. *angustifolium* (A.Kern.) Nyman)	Botanical Garden Frankfurt (Germany)	IPEN: XX-0-FRT-2012/570	ST/220706	Roots	SN-R

MS peak areas of identified compounds) and biological activity (antioxidant, enzyme inhibitory). HCA and Pearson correlation analyses were conducted with the R v.4.2.3 statistical program.

RESULTS

LC-HRMS/MS-based phytochemical profiling

The LC-HRMS/MS phytochemical profiling of the extracts obtained from the roots and aerial parts of the nine *Symphytum* species allowed the identification of various compounds (e.g. phenolic acids, flavonoids, pyrrolizidine alkaloids, organic acids and fatty acids). Their labeling was carried out as total annotation (standard injection) or partial annotation (spectro-chromatographic data corroboration with the MS dereplication strategies proposed in previous works – see Table 2 and Data S1, supporting information).

Hydroxybenzoic acid derivatives (**7** and **11**), cinnamic acid derivatives (**8** and **15**) and caffeic acid oligomers (**17**, **21**–**25**) were included in the class of phenolic acids. Some inter-species differences were observed. For instance, hydroxybenzoic acid-*O*-hexoside (**7**) was present in all species except for *S. grandiflorum* and *S. ibericum*, whereas hydroxybenzoic acid (**11**), dihydroglo-boidnan A (**23**), dehydrorabdosiin (**24**) and globoidnan A (**25**) were lacking in *S. bulbosum*. Globoidnan B (**17**) was absent in *S. asperum*, *S. bulbosum*, *S. caucasicum* and *S. tuberosum* subsp. *nodosum*.

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Table 2. LC-HRMS/MS-based phytochemical profiling of *Symphytum* species

No.	Proposed identity	T_R (min)	HRMS	Exp. (*m/z*)	Calcd (*m/z*)	Δ (ppm)	MF	HRMS/MS (*m/z*)	Ref.
Phenolic acids									
7	Hydroxybenzoic acid-*O*-hexoside	9.3	$[M-H]^-$	299.0773	299.0772	−0.20	$C_{13}H_{16}O_8$	239.0420, 209.0516, 179.0231, 137.0182	22
8	Danshensu	11.2	$[M-H]^-$	197.0458	197.0455	−1.28	$C_9H_{10}O_5$	179.0340, 135.0422	22
11	Hydroxybenzoic acid*	14.5	$[M-H]^-$	137.0247	137.0244	−2.05	$C_7H_6O_3$	111.0023	22
15	Caffeic acid*	19.1	$[M-H]^-$	179.0347	179.0350	1.57	$C_9H_8O_4$	161.0434, 135.0451	22
17	Globoidnan B*	23.4	$[M-H]^-$	537.1048	537.1038	−1.77	$C_{27}H_{22}O_{12}$	493.1173, 339.0554, 295.0652, 229.0106, 197.0461, 179.0337	22
21	Rabdosiin*	26.2	$[M-H]^-$	717.1448	717.1461	1.82	$C_{36}H_{30}O_{16}$	537.0967, 519.0937, 493.0976, 475.1058, 365.0537, 339.0530	22
22	Rosmarinic acid*	26.9	$[M-H]^-$	359.0763	359.0772	2.61	$C_{18}H_{16}O_8$	197.0441, 179.0336, 161.0234, 135.0453	22
23	Dihydrogloboidnan A	28.1	$[M-H]^-$	493.1127	493.1140	2.67	$C_{26}H_{22}O_{10}$	295.0606, 185.0233	22
24	Dehydrorabdosiin	28.9	$[M-H]^-$	715.1308	715.1305	1.62	$C_{36}H_{28}O_{16}$	517.1001, 337.0402	19
25	Globoidnan A*	30.1	$[M-H]^-$	491.0981	491.0984	0.55	$C_{26}H_{20}O_{10}$	311.0538, 293.0186, 267.0618, 135.0399	22
Flavonoids									
19	Quercetin-*O*-hexoside	24.6	$[M-H]^-$	463.0864	463.0882	3.88	$C_{21}H_{20}O_{12}$	301.0262, 271.0229, 255.0284, 151.0013	14
20	Quercetin-*O*-malonylhexoside	26.0	$[M-H]^-$	549.0871	549.0886	2.72	$C_{24}H_{22}O_{15}$	301.0299, 271.0230, 255.0255, 151.0029	14
Pyrrolizidine alkaloids									
3	Echimplatine-*N*-oxide	2.7	$[M+H]^+$	332.1696	332.1704	2.35	$C_{15}H_{25}NO_7$	314.1607, 270.1328, 172.0983, 138.0924	23
4	Intermedine/lycopsamine*	3.8	$[M+H]^+$	300.1789	300.1805	5.19	$C_{15}H_{25}NO_5$	156.106, 138.0951, 120.0850, 112.0679, 108.0700	24
5	Uplandicine-*N*-oxide	6.1	$[M+H]^+$	374.1795	374.1809	3.87	$C_{17}H_{27}NO_8$	356.1712, 312.1457, 298.1302, 70.1335, 214.1093, 180.1032, 154.0847, 137.0848	25
6	Dihydroechinatine	6.9	$[M+H]^+$	302.1970	302.1962	−2.66	$C_{15}H_{27}NO_5$	158.1132, 140.1075, 122.0963	19
9	Intermedine-*N*-oxide/lycopsamine-*N*-oxide*	11.6	$[M+H]^+$	316.1740	316.1755	4.65	$C_{15}H_{25}NO_6$	172.1004, 154.0895, 138.0950, 111.0724	22
12	7-Acetylintermedine/lycopsamine-*N*-oxide*	14.6	$[M+H]^+$	358.1849	358.1860	3.16	$C_{17}H_{27}NO_7$	214.1025, 180.0974, 154.0869, 137.0795, 120.0757, 101.0565	22
14	Heliosupine-*N*-oxide	17.9	$[M+H]^+$	414.2102	414.2122	4.95	$C_{20}H_{31}NO_8$	396.2056, 352.1786, 254.1414, 220.1352, 154.0875, 137.0849, 120.0824	25
16	Symphytine-*N*-oxide/symviridine-*N*-oxide	21.4	$[M+H]^+$	398.2156	398.2173	4.35	$C_{20}H_{31}NO_7$	254.1228, 220.1157, 172.0829, 154.0773, 138.0795, 120.0731	22
18	3′-Acetylsymphytine-*N*-oxide	23.5	$[M+H]^+$	440.2271	440.2279	1.81	$C_{22}H_{33}NO_8$	398.2165, 380.2075, 340.1775, 254.1393, 214.1075, 180.1033, 154.0885, 137.0839	22
Organic and fatty acids									
1	Malic acid	1.8	$[M-H]^-$	133.0145	133.0142	−2.25	$C_4H_6O_5$	115.0045	19
2	Citric acid	2.1	$[M-H]^-$	191.0199	191.0197	−0.91	$C_6H_8O_7$	129.0198, 111.0100	19

Table 2. Continued

No.	Proposed identity	T_R (min)	HRMS	Exp. (*m/z*)	Calcd (*m/z*)	Δ (ppm)	MF	HRMS/MS (*m/z*)	Ref.
10	Viridifloric acid	13.3	$[M-H]^-$	161.0819	161.0819	0.01	$C_7H_{14}O_4$	135.0578, 117.0455	22
13	Trachelantic acid	17.1	$[M-H]^-$	161.0820	161.0819	0.11	$C_7H_{14}O_4$	135.0588, 117.0554	22
26	Trihydroxy octadecenoic acid	34.2	$[M-H]^-$	329.2349	329.2333	−4.71	$C_{18}H_{34}O_5$	229.1432, 211.1382, 199.1159	26

Concerning the pyrrolizidine alkaloids, it must be mentioned that, due to the high degree of stereoisomerism, co-elution chromatographic behavior and similar MS patterns, multiple structural assignments could be theoretically proposed for one peak. However, for simplification purposes, only 1–2 potential structures are suggested in the current work (Table 2). Pyrrolizidine alkaloids were generally more frequent in the roots than in the aerial parts (Data S1, supporting information). Some differences are worth mentioning. For example, echimplatine-*N*-oxide (**3**) was absent in *S. caucasicum*, *S. grandiflorum* and *S. ibericum*. On the other hand, *S. tuberosum* lacked intermedine/lycopsamine (**4**). Uplandicine-*N*-oxide (**5**) was characteristic of *S. asperum*, *S. cordatum*, *S. tuberosum* and *S. tuberosum* subsp. *nodosum*, whereas dihydroechinatine (**6**) was present only in *S. asperum* and *S. grandiflorum*. Furthermore, 3′-acetylsymphytine-*N*-oxide (**18**) was observed in all species except *S. ibericum*.

The two flavonoids quercetin-*O*-hexoside (**19**) and quercetin-*O*-malonylhexoside (**20**) were present only in the aerial parts. However, *S. grandiflorum* did not contain these two compounds. Lastly, a series of organic (**1**, **2**, **10** and **13**) and fatty (**26**) acids were annotated in the *Symphytum* species (Table 2) as non-specific metabolites.

HCA was subsequently performed for a more comprehensive assessment of the phytochemical dissimilarities and similarities between the samples. A high peak area of a molecule is represented by a red color, whereas a low abundance is represented by a blue color (Fig. 1). Based on the HCA, the samples can be divided into two main clusters: one 'root cluster' and one 'aerial part cluster'. Within the 'root cluster', the roots of *S. grandiflorum* formed an individual subcluster (discriminative compound: dihydroechinatine). Two subclusters could also be evidenced within the 'aerial part cluster'. One subcluster contained only aerial parts (e.g. *S. tuberosum*, *S. grandiflorum*, *S. cordatum*, *S. ibericum*), whereas the other included the aerial parts of *S. caucasicum* and *S. asperum*, and additionally the roots of *S. ibericum*, *S. caucasicum* and *S. officinale*.

Total phenolic, phenolic acid and flavonoid content

In roots, TPC (Table 3) varied from 2.32 mg GAE g^{-1} in *S. bulbosum* (**SB-R**) and 15.53 mg GAE g^{-1} in one sample of *S. tuberosum* (**ST1-R**). Three *S. officinale* samples (**SO1-R**, **SO2-R** and **SO4-R**) had a very similar content (9.09–9.76 mg GAE g^{-1}), whereas the fourth sample (**SO3-R**) displayed a slightly higher value (12.58 mg GAE g^{-1}). TPC in the aerial parts ranged from 3.42 mg GAE g^{-1} in **ST2-A** to the maximum of 21.10 mg GAE g^{-1} in **SO1-A**. TPAC showed a similar trend. Among the roots, *S. bulbosum* had the lowest TPAC (0.54 mg CE g^{-1}), while the maximum value was attained in **ST1-R** (7.05 mg CE g^{-1}). TPAC varied from 1.04 mg CE g^{-1} (**ST2-A**) to 8.89 mg CE g^{-1} (**SO1-A**) within the aerial parts. Regarding TFC, the roots were characterized by low values (between 0.13 mg RE g^{-1} in **SG1-R** and 0.80 mg RE g^{-1} in **ST1-R**). In contrast, higher levels of flavonoids were noticed in the aerial parts of most samples; for instance, one *S. asperum* sample (**SA2-A**) showed a concentration of 3.89 mg CE g^{-1}.

Antioxidant activity

All *Symphytum* species and samples displayed good antioxidant effects, as evaluated in radical scavenging, metal reducing, metal chelating and PBD assays. Within the same species, the aerial parts were generally more active than the roots (Table 4). Concerning the roots, *S. bulbosum* (**SB-R**) showed the lowest antiradical activity (2.98 and 3.54 mg TE g^{-1} in DPPH and ABTS assays, respectively), while the highest scavenging activity against DPPH (31.64 mg TE g^{-1}) and ABTS (36.78 mg TE g^{-1}) was exhibited by *S. tuberosum* (**ST1-R**). Within the aerial parts, **ST2-A** was the least active sample (4.40 and 6.48 mg TE g^{-1} in DPPH and ABTS assays, respectively), whereas **SO1-A** was the most active (50.17 and 49.92 mg TE g^{-1} in DPPH and ABTS assays, respectively). In the roots, the maximum CUPRAC (49.43 mg TE g^{-1}) and FRAP (33.66 mg TE g^{-1}) values were reached in **ST1-R**. In the aerial parts, **SO1-A** showed significantly higher values (92.93 and 53.63 mg TE g^{-1} in FRAP and CUPRAC assays, respectively). Compared with the previous tests, a slightly different activity order was evidenced in the PBD assay for the *Symphytum* roots (Table 4). The lowest activity was noticed for *S. grandiflorum* (**SG1-R**; 0.09 mmol TE g^{-1}), while the highest effects were produced by *S. officinale* (**SO2-R** and **SO4-R**; 0.41 and 0.43 mmol TE g^{-1}, respectively). However, for the aerial parts, **ST2-A** was the least active (0.08 mmol TE g^{-1}), whereas **SO1-A** was the most potent sample (0.47 mmol TE g^{-1}). Lastly, the chelating ability of *Symphytum* species was evaluated, revealing that *S. tuberosum* subsp. *nodosum* (**SN-R**; 5.56 mg EDTAE g^{-1}) was the most active sample within the roots, whereas *S. officinale* (**SO1-A**, **SO3-A** and **SO4-A**) was the most active species within the aerial parts (3.39–3.74 mg EDTAE g^{-1}).

Overall, it can be concluded that all samples displayed good antioxidant activity, as evaluated by radical scavenging, metal reducing and metal chelating. However, inter-organ, inter-species and intra-species differences were noticed (Table 4).

Next, the relation between the antioxidant activity and phytochemical composition was assessed, as illustrated in Fig. 2. Generally, a significant positive correlation ($r > 0.7$) was found between the antioxidant activities (FRAP, CUPRAC, DPPH, ABTS and PBD) and TPC, TFC and TPAC. Regarding individual compounds, there was a slight positive correlation ($0.5 < r < 0.7$) between danshensu (**8**) and ABTS, FRAP and CUPRAC. In addition, DPPH and CUPRAC were linked to quercetin-*O*-hexoside (**19**). In contrast, MCA activity was associated with malic (**1**) and citric (**2**) acids.

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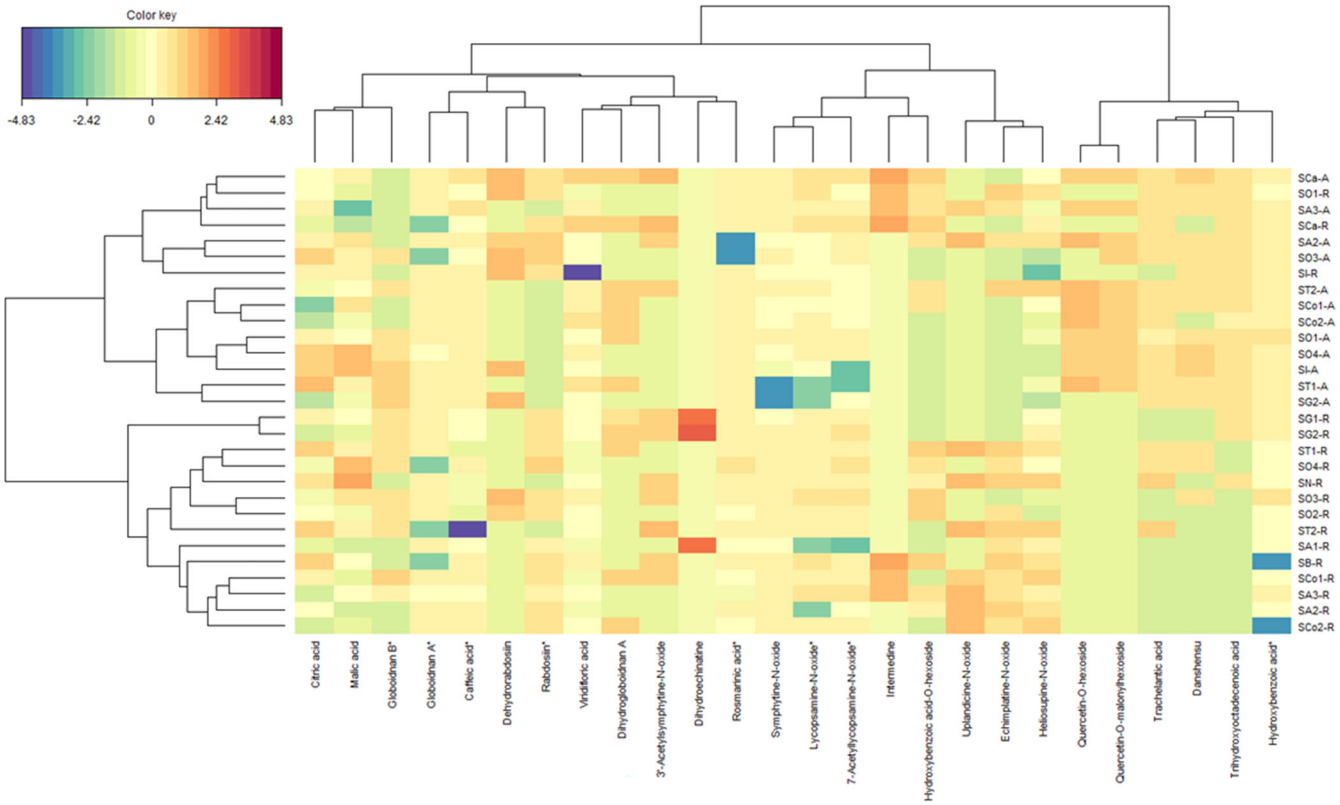

Figure 1. HCA of *Symphytum* samples; sample codes as in Table 1.

Enzyme inhibitory activity

In this section, the cholinesterase, tyrosinase, amylase and glucosidase inhibitory potential of the 29 samples of *Symphytum* is presented. The roots were generally more active than the aerial parts of the same species (Table 5). The strongest AChE and BChE inhibition (0.51 and 0.96 mg GALAE g^{-1}, respectively) was exhibited by *S. officinale* (**SO2-R**), whereas the roots of *S. grandiflorum* (**SG1-R**) were the weakest inhibitors (0.11 and 0.19 mg GALAE g^{-1} in AChE and BChE assays, respectively). In general, the aerial parts displayed lower anti-cholinesterase properties. Among aerial parts, the highest inhibitory effects (0.42 and 0.35 mg GALAE g^{-1} in AChE and BChE assays, respectively) were noticed in *S. officinale* (**SO1-A**). The tyrosinase activity ranged from 2.90 mg KAE g^{-1} (**SG1-R**) to 13.58 mg KAE g^{-1} (**SCa-R**) in the roots and from 2.75 mg KAE g^{-1} (**SA3-A**) to 6.69 mg KAE g^{-1} (**SCa-A**) in the aerial parts. The roots of *S. caucasicum* (**SCa-R**) and *S. officinale* (**SO2-R**) were the most active samples against amylase (both 0.11 mmol ACAE g^{-1}). *S. grandiflorum* (**SG1-R**) and *S. tuberosum* (**ST2-A**) displayed the lowest amylase and glucosidase effects within roots and aerial parts, respectively. Among the aerial parts, *S. officinale* (**SO1-A**) showed the most potent anti-amylase (0.09 mmol ACAE g^{-1}) and anti-glucosidase (0.24 mmol ACAE g^{-1}) effects. Overall, it can be stated that all samples presented to different extents enzyme inhibitory properties. However, inter-organ, inter-species and intra-species differences were noticed (Table 5).

Next, the enzyme inhibitory activity of *Symphytum* species was correlated with the phytochemical composition (Fig. 3). TPC, TFC and TPAC did not associate significantly with the enzymatic effects. However, the anti-BChE activity was linked to rabdosiin (**21**), whereas AChE, BChE and amylase inhibitory effects were linked to some extent to hydroxybenzoic acid-*O*-hexoside (**7**).

DISCUSSION

Plants from the *Symphytum* genus have been traditionally used since ancient times, primarily for their anti-inflammatory and analgesic properties. Nevertheless, the agricultural and phytopharmaceutical utilization of the genus remains predominantly limited to a few species, with *S. officinale* being a prominent example. To shed light on the phytochemical complexity and biological potential of other less known taxa, the current study presented the LC-HRMS/MS-based metabolite profile and antioxidant and enzyme inhibitory properties of 29 hydroethanolic extracts obtained from the roots and aerial parts of nine *Symphytum* species.

The phytochemical profiling revealed the presence of 10 phenolic acids (notably, danshensu, caffeic acid, globoidnan B, rabdosiin, rosmarinic acid and globoidnan A), two flavonoids (hexoside and malonylhexoside of quercetin), nine pyrrolizidine alkaloids (notably, intermedine, acetylintermedine-*N*-oxide and symphytine-*N*-oxide), four organic acids and one fatty acid. These classes are listed as specific metabolites in the *Symphytum* genus. For instance, the chemical composition of *S. officinale* was intensively studied,[9,26,27] with compounds such as allantoin, hydroxybenzoic, caffeic, salvianolic and fatty acids often reported. *S. ibericum* was only recently phytochemically characterized by our group; pyrrolizidine alkaloids, organic acids and oxygenated unsaturated fatty acids were retrieved.[19] Amiranashvili *et al.*[28] annotated caffeic, rosmarinic, chlorogenic and salvianolic acids in the roots and aerial parts of *S. asperum*. Furthermore,

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Table 3. Total phenolic, phenolic acid and flavonoid content of *Symphytum* species

Part	Sample	TPC (mg GAE g^{-1})	TPAC (mg CE g^{-1})	TFC (mg RE g^{-1})
Roots	SA1-R	3.92 ± 0.03^{no}	1.19 ± 0.03^{opq}	0.21 ± 0.01^{pqr}
	SA2-R	7.80 ± 0.16^{k}	2.75 ± 0.08^{lm}	0.47 ± 0.01^{lm}
	SA3-R	11.24 ± 0.08^{e}	4.47 ± 0.14^{f}	0.57 ± 0.01^{kl}
	SB-R	2.32 ± 0.07^{q}	0.54 ± 0.01^{r}	0.14 ± 0.01^{r}
	SCa-R	5.41 ± 0.10^{m}	1.46 ± 0.07^{o}	0.30 ± 0.02^{op}
	SCo1-R	10.46 ± 0.43^{fg}	4.39 ± 0.16^{f}	0.53 ± 0.01^{kl}
	SCo2-R	6.70 ± 0.03^{l}	3.10 ± 0.07^{jkl}	0.33 ± 0.01^{no}
	SG1-R	2.47 ± 0.03^{q}	1.03 ± 0.01^{pq}	0.13 ± 0.01^{r}
	SG2-R	7.47 ± 0.13^{k}	3.33 ± 0.10^{ij}	0.38 ± 0.01^{mno}
	SI-R	3.93 ± 0.09^{no}	1.33 ± 0.05^{op}	0.19 ± 0.01^{pqr}
	SO1-R	9.09 ± 0.04^{i}	3.49 ± 0.18^{hij}	0.54 ± 0.01^{kl}
	SO2-R	9.16 ± 0.06^{i}	2.87 ± 0.06^{klm}	0.41 ± 0.01^{mn}
	SO3-R	12.58 ± 0.33^{d}	5.37 ± 0.11^{de}	0.62 ± 0.01^{jk}
	SO4-R	9.76 ± 0.11^{h}	4.07 ± 0.11^{fg}	0.57 ± 0.01^{kl}
	ST1-R	15.53 ± 0.21^{b}	7.05 ± 0.06^{b}	0.80 ± 0.01^{i}
	ST2-R	3.25 ± 0.02^{p}	0.82 ± 0.02^{qr}	0.16 ± 0.01^{qr}
	SN-R	4.37 ± 0.06^{n}	1.23 ± 0.03^{op}	0.21 ± 0.01^{pqr}
Aerial parts	SA2-A	13.39 ± 0.03^{c}	5.99 ± 0.18^{c}	3.89 ± 0.03^{a}
	SA3-A	8.70 ± 0.15^{ij}	3.83 ± 0.08^{gh}	1.30 ± 0.01^{f}
	SCa-A	10.74 ± 0.37^{ef}	3.38 ± 0.03^{ij}	0.93 ± 0.04^{h}
	SCo1-A	13.30 ± 0.02^{c}	4.97 ± 0.11^{e}	2.45 ± 0.15^{c}
	SCo2-A	8.33 ± 0.15^{j}	2.80 ± 0.14^{lm}	2.52 ± 0.04^{c}
	SG2-A	10.16 ± 0.14^{gh}	3.58 ± 0.20^{hi}	0.72 ± 0.03^{ij}
	SI-A	10.76 ± 0.07^{ef}	5.39 ± 0.17^{d}	1.18 ± 0.02^{g}
	SO1-A	21.10 ± 0.32^{a}	8.89 ± 0.30^{a}	2.76 ± 0.06^{b}
	SO3-A	6.71 ± 0.09^{l}	2.27 ± 0.12^{n}	0.27 ± 0.01^{opq}
	SO4-A	8.87 ± 0.12^{i}	3.23 ± 0.06^{ijk}	1.71 ± 0.07^{d}
	ST1-A	7.80 ± 0.03^{k}	2.52 ± 0.27^{mn}	1.46 ± 0.02^{e}
	ST2-A	3.42 ± 0.03^{op}	1.04 ± 0.08^{pq}	0.53 ± 0.01^{kl}

CE, caffeic acid equivalents; GAE, gallic acid equivalents; RE, rutin equivalents; TFC, total flavonoid content; TPAC, total phenolic acid content; TPC, total phenolic content. Data are presented as mean ± standard deviation of three determinations. Different superscript letters within columns indicate significant differences ($P < 0.05$). Sample codes as in Table 1.

S. caucasicum and *S. asperum* are also documented to contain a typical caffeic acid-derived polyether.[16,17] Dresler *et al.*[29] showed the occurrence of allantoin, rosmarinic acid and chlorogenic acid in *S. cordatum*. However, a detailed comprehensive phytochemical profiling of the remaining taxa with state-of-the-art LC-HRMS/MS platforms has not been previously performed. Nonetheless, pyrrolizidine alkaloids, such as intermedine, echimidine, symphytine, heliosupine and their *N*-oxides, were detected by thin-layer chromatography or gas chromatography in all investigated species.[3,24,30-32]

Considering the relatively high number of samples and species tested, the current study can also be of chemotaxonomic importance. For instance, based on the HCA, it can be stated that the roots of *S. grandiflorum* showed a slightly different phytochemical profile compared with the roots of *S. ibericum* and *S. caucasicum* or the roots of the remaining species. Within the aerial parts, *S. caucasicum* and *S. asperum* differentiated from the other aerial parts. However, *S. officinale* (roots and aerial parts) shared numerous phytochemical features with all investigated *Symphytum* species. Previous studies used only pyrrolizidine alkaloids as the main marker compounds to distinguish species within the *Symphytum* genus. Huizing *et al.*[33] showed that lycopsamine, acetyllycopsamine and symphytine (or their isomers) were generally found in *S. officinale*, whereas echimidine and symphytine in *S. asperum*. However, this was later invalidated by Jaarsma *et al.*,[34] who showed that *S. officinale* also contains significant amounts of echimidine. Furthermore, the same group indicated that *S. officinale* and *S. officinale* subsp. *uliginosum* (A. Kern.) Nyman are similar taxa because they presented a common pyrrolizidine alkaloid profile.[34] In addition, *S. tuberosum* and *S. tuberosum* subsp. *nodosum* showed an almost identical alkaloidal pattern, whereas no apparent discriminatory features were revealed for *S. grandiflorum* and *S. ibericum*.[32]

The pharmacological data from the current work can represent a starting point toward expanding the medicinal valences of the *Symphytum* genus beyond its currently limited applications. For instance, the promising antioxidant, anti-cholinesterase, anti-tyrosinase, anti-amylase and anti-glucosidase effects proven for the nine species might find utilization, after further research, in the management of pathological conditions linked to oxidative stress, such as Alzheimer's disease, skin pigmentation diseases or diabetes. Previously, the antioxidant (reduction of reactive oxygen species in human neutrophils, DPPH radical and superoxide anion scavenging) potential of *S. asperum* and *S. caucasicum* has been documented.[16,17,35] Additionally, the radical scavenging, metal reducing and chelating, anti-cholinesterase, anti-tyrosinase,

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Table 4. Antioxidant activity of *Symphytum* species

Part	Sample	DPPH (mg TE g^{-1})	ABTS (mg TE g^{-1})	CUPRAC (mg TE g^{-1})	FRAP (mg TE g^{-1})	MCA (mg EDTAE g^{-1})	PBD (mmol TE g^{-1})
Roots	SA1-R	6.90 ± 0.01^{lm}	8.24 ± 0.21^{m}	13.41 ± 0.42^{q}	6.77 ± 0.07^{rs}	0.95 ± 0.03^{kl}	0.17 ± 0.04^{jklm}
	SA2-R	11.58 ± 0.02^{i}	15.85 ± 0.18^{i}	27.54 ± 0.16^{lmn}	14.98 ± 0.19^{lmn}	2.23 ± 0.06^{ghi}	0.30 ± 0.02^{defg}
	SA3-R	10.29 ± 0.01^{ij}	15.97 ± 0.01^{hi}	39.67 ± 1.35^{fg}	18.05 ± 0.70^{ij}	0.68 ± 0.05^{l}	0.36 ± 0.03^{bcd}
	SB-R	2.98 ± 0.07^{p}	3.54 ± 0.09^{q}	7.06 ± 0.22^{t}	3.95 ± 0.03^{u}	3.35 ± 0.09^{d}	0.17 ± 0.01^{jkl}
	SCa-R	9.00 ± 0.13^{jk}	9.97 ± 0.09^{l}	16.61 ± 0.22^{p}	9.57 ± 0.17^{q}	2.42 ± 0.02^{fgh}	0.34 ± 0.03^{cde}
	SCo1-R	8.30 ± 0.01^{kl}	12.79 ± 0.01^{jk}	31.97 ± 1.14^{ij}	16.67 ± 0.43^{jk}	1.19 ± 0.07^{jk}	0.28 ± 0.01^{defgh}
	SCo2-R	9.11 ± 0.01^{jk}	12.08 ± 0.47^{k}	21.34 ± 0.49^{o}	12.07 ± 0.40^{p}	1.20 ± 0.11^{jk}	0.28 ± 0.01^{defgh}
	SG1-R	3.11 ± 0.01^{p}	4.53 ± 0.14^{pq}	8.30 ± 0.59st	4.64 ± 0.08^{tu}	0.75 ± 0.02^{l}	0.09 ± 0.01^{mn}
	SG2-R	17.67 ± 0.61^{g}	13.91 ± 0.53^{j}	26.80 ± 0.47^{mn}	15.69 ± 0.21^{klm}	0.32 ± 0.02^{m}	0.18 ± 0.01^{ijkl}
	SI-R	6.54 ± 0.01^{mn}	7.88 ± 0.15^{mn}	13.04 ± 0.21^{q}	6.66 ± 0.16^{rs}	2.64 ± 0.04^{ef}	0.18 ± 0.03^{ijkl}
	SO1-R	10.33 ± 0.03^{ij}	15.76 ± 0.28^{i}	29.87 ± 0.63^{jkl}	15.95 ± 0.57^{kl}	1.35 ± 0.07^{j}	0.31 ± 0.02^{def}
	SO2-R	13.74 ± 0.27^{h}	18.48 ± 0.28^{fg}	26.68 ± 0.79^{mn}	14.42 ± 0.27^{mno}	1.96 ± 0.12^{i}	0.41 ± 0.07^{abc}
	SO3-R	11.20 ± 0.02^{i}	17.52 ± 0.01^{hg}	42.08 ± 1.56^{ef}	23.08 ± 0.81^{f}	1.97 ± 0.09^{i}	0.35 ± 0.03^{bcde}
	SO4-R	13.69 ± 0.03^{h}	18.25 ± 0.10^{fg}	33.54 ± 1.04^{hi}	18.61 ± 0.73^{hi}	3.75 ± 0.17^{bc}	0.43 ± 0.07^{ab}
	ST1-R	31.64 ± 1.78^{c}	36.78 ± 1.95^{b}	49.43 ± 0.49^{d}	33.66 ± 0.58^{c}	2.87 ± 0.15^{e}	0.41 ± 0.01^{abc}
	ST2-R	5.31 ± 0.14^{no}	5.26 ± 0.14^{op}	10.26 ± 0.10^{rs}	5.95 ± 0.17st	3.93 ± 0.18^{b}	0.22 ± 0.03^{ghijkl}
	SN-R	6.56 ± 0.29^{mn}	7.19 ± 0.07^{mn}	13.37 ± 0.11^{q}	7.89 ± 0.15^{r}	5.56 ± 0.14^{a}	0.25 ± 0.03^{fghij}
Aerial parts	SA2-A	37.15 ± 0.23^{b}	36.10 ± 0.69^{b}	64.10 ± 0.56^{b}	35.62 ± 0.14^{b}	2.52 ± 0.05^{fg}	0.27 ± 0.03^{efgh}
	SA3-A	21.77 ± 0.24^{e}	19.82 ± 0.65^{ef}	42.24 ± 0.83^{ef}	23.19 ± 0.89^{f}	1.21 ± 0.15^{jk}	0.18 ± 0.01^{ijkl}
	SCa-A	19.98 ± 0.80^{f}	21.08 ± 0.41^{e}	38.83 ± 0.51^{g}	20.70 ± 0.15^{g}	3.37 ± 0.27^{d}	0.23 ± 0.01^{fghijk}
	SCo1-A	32.89 ± 0.83^{c}	34.02 ± 0.31^{c}	60.03 ± 1.05^{c}	33.05 ± 0.69^{c}	2.13 ± 0.11^{hi}	0.30 ± 0.02^{defg}
	SCo2-A	18.71 ± 0.97^{fg}	19.66 ± 0.77^{ef}	34.95 ± 0.63^{h}	19.83 ± 0.25^{gh}	0.76 ± 0.07^{l}	0.17 ± 0.02^{jkl}
	SG2-A	24.21 ± 0.74^{d}	25.20 ± 0.37^{d}	42.53 ± 1.15^{e}	25.99 ± 0.16^{e}	1.91 ± 0.05^{i}	0.21 ± 0.01^{hijkl}
	SI-A	32.67 ± 0.27^{c}	33.34 ± 0.51^{c}	59.88 ± 0.66^{c}	31.27 ± 0.89^{d}	2.48 ± 0.04^{fg}	0.26 ± 0.01^{fghi}
	SO1-A	50.17 ± 0.27^{a}	49.92 ± 0.83^{a}	92.93 ± 2.48^{a}	53.63 ± 0.42^{a}	3.39 ± 0.07^{d}	0.47 ± 0.01^{a}
	SO3-A	14.10 ± 0.23^{h}	13.37 ± 0.43^{jk}	25.22 ± 0.39^{n}	13.50 ± 0.29^{o}	3.74 ± 0.09^{bc}	0.14 ± 0.01^{lmn}
	SO4-A	4.77 ± 0.01^{o}	7.82 ± 0.01^{mn}	28.65 ± 0.42^{klm}	13.79 ± 0.15^{no}	3.43 ± 0.06^{cd}	0.16 ± 0.01^{klmn}
	ST1-A	14.22 ± 0.47^{h}	17.04 ± 0.41^{ghi}	30.31 ± 0.75^{jk}	16.66 ± 0.25^{k}	1.98 ± 0.03^{i}	0.16 ± 0.02^{klmn}
	ST2-A	4.10 ± 0.01^{op}	6.48 ± 0.01^{no}	11.27 ± 0.20^{qr}	5.65 ± 0.07st	0.66 ± 0.04^{l}	0.08 ± 0.01^{n}

ABTS, 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid; CUPRAC, cupric ion reducing antioxidant capacity; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EDTAE, EDTA equivalents; FRAP, ferric ion reducing antioxidant power; PBD, phosphomolybdenum assay; TE, Trolox equivalents. Data are presented as mean ± standard deviation of three determinations. Different superscript letters within columns indicate significant differences ($P < 0.05$). Sample codes as in Table 1.

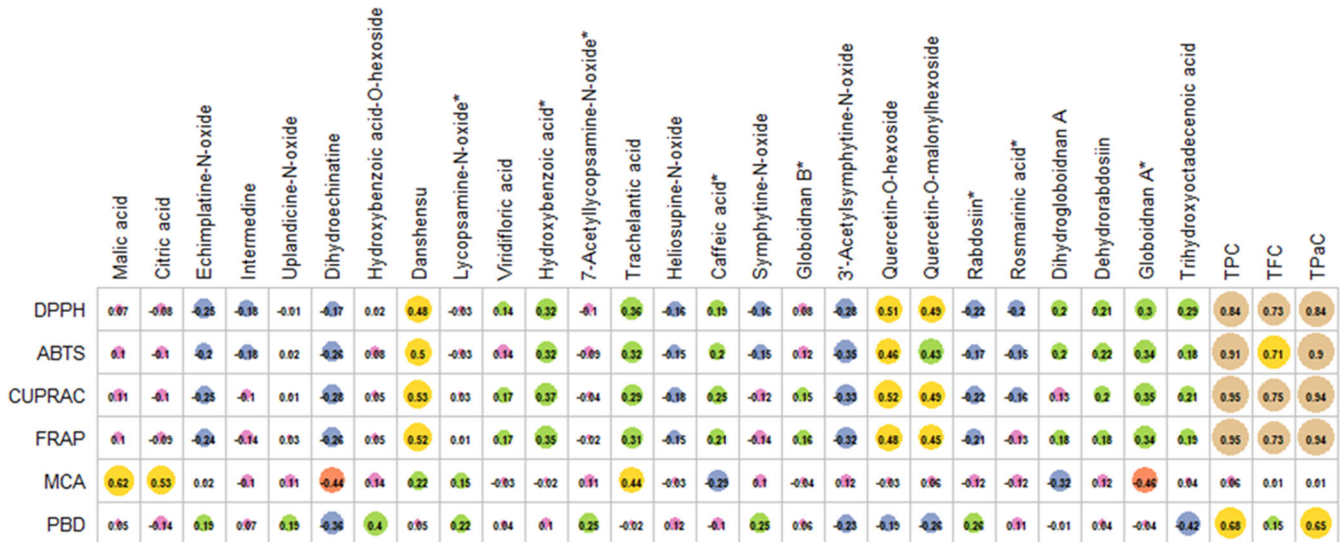

Figure 2. Correlations between the phytochemical composition and antioxidant activity of *Symphytum* samples.

Table 5. Enzyme inhibitory activity of *Symphytum* species

Part	Sample	AChE (mg GALAE g^{-1})	BChE (mg GALAE g^{-1})	Tyrosinase (mg KAE g^{-1})	Amylase (mmol ACAE g^{-1})	Glucosidase (mmol ACAE g^{-1})
Roots	SA1-R	0.25 ± 0.01^{h}	0.43 ± 0.01^{gh}	6.57 ± 0.04^{i}	0.06 ± 0.01^{fg}	0.19 ± 0.01^{fg}
	SA2-R	0.41 ± 0.01^{c}	0.72 ± 0.02^{b}	9.54 ± 0.22^{b}	0.09 ± 0.01^{bc}	0.32 ± 0.01^{b}
	SA3-R	0.39 ± 0.01^{cd}	0.62 ± 0.01^{cd}	9.16 ± 0.09^{cd}	0.07 ± 0.01^{de}	0.30 ± 0.01^{bc}
	SB-R	0.26 ± 0.01^{gh}	0.47 ± 0.02^{fg}	7.33 ± 0.09^{h}	0.06 ± 0.01^{efg}	0.10 ± 0.01^{klm}
	SCa-R	0.51 ± 0.01^{a}	0.90 ± 0.05^{a}	13.58 ± 0.24^{a}	0.11 ± 0.01^{a}	0.31 ± 0.02^{bc}
	SCo1-R	0.28 ± 0.01^{fg}	0.38 ± 0.01^{hi}	6.72 ± 0.10^{i}	0.06 ± 0.01^{g}	0.15 ± 0.01^{ghij}
	SCo2-R	0.32 ± 0.01^{e}	0.56 ± 0.05^{de}	8.34 ± 0.06^{e}	0.06 ± 0.01^{ghi}	0.23 ± 0.01^{e}
	SG1-R	0.11 ± 0.00^{l}	0.19 ± 0.01^{lmno}	2.90 ± 0.04^{p}	0.02 ± 0.01^{k}	0.07 ± 0.01^{m}
	SG2-R	0.21 ± 0.00^{ij}	0.32 ± 0.01^{ij}	5.27 ± 0.02^{k}	0.04 ± 0.01^{j}	0.16 ± 0.01^{ghij}
	SI-R	0.26 ± 0.01^{gh}	0.43 ± 0.01^{gh}	6.67 ± 0.06^{i}	0.04 ± 0.01^{ij}	0.18 ± 0.01^{fgh}
	SO1-R	0.37 ± 0.01^{d}	0.67 ± 0.03^{bc}	9.13 ± 0.01^{cd}	0.08 ± 0.01^{bcd}	0.28 ± 0.01^{cd}
	SO2-R	0.52 ± 0.01^{a}	0.96 ± 0.07^{a}	13.30 ± 0.03^{a}	0.11 ± 0.00^{a}	0.37 ± 0.01^{a}
	SO3-R	0.40 ± 0.01^{c}	0.52 ± 0.07^{ef}	8.06 ± 0.11^{ef}	0.08 ± 0.01^{cd}	0.34 ± 0.01^{ab}
	SO4-R	0.46 ± 0.02^{b}	0.50 ± 0.03^{efg}	9.43 ± 0.21^{bc}	0.08 ± 0.01^{bcd}	0.37 ± 0.05^{a}
	ST1-R	0.41 ± 0.03^{c}	0.31 ± 0.01^{ijk}	7.77 ± 0.15^{fg}	0.07 ± 0.01^{def}	0.21 ± 0.01^{ef}
	ST2-R	0.29 ± 0.01^{fg}	0.23 ± 0.01^{klm}	7.62 ± 0.07^{gh}	0.06 ± 0.01^{g}	0.14 ± 0.01^{ijk}
	SN-R	0.34 ± 0.01^{e}	0.25 ± 0.01^{jkl}	8.84 ± 0.14^{d}	0.06 ± 0.01^{efg}	0.15 ± 0.02^{hij}
Aerial parts	SA2-A	0.27 ± 0.01^{gh}	0.24 ± 0.01^{klm}	3.34 ± 0.12^{o}	0.06 ± 0.01^{gh}	0.16 ± 0.02^{ghij}
	SA3-A	0.18 ± 0.01^{jk}	0.16 ± 0.01^{mno}	2.75 ± 0.05^{p}	0.04 ± 0.01^{j}	0.10 ± 0.01^{klm}
	SCa-A	0.31 ± 0.01^{ef}	0.30 ± 0.01^{ijk}	6.69 ± 0.06^{i}	0.06 ± 0.01^{efg}	0.15 ± 0.01^{hij}
	SCo1-A	0.28 ± 0.01^{gh}	0.23 ± 0.02^{klm}	6.10 ± 0.07^{j}	0.06 ± 0.01^{g}	0.18 ± 0.01^{fghi}
	SCo2-A	0.18 ± 0.01^{ijk}	0.13 ± 0.01^{o}	4.95 ± 0.05^{kl}	0.04 ± 0.01^{j}	0.12 ± 0.01^{jkl}
	SG2-A	0.21 ± 0.01^{i}	0.21 ± 0.01^{lmno}	5.03 ± 0.01^{kl}	0.05 ± 0.01^{hij}	0.12 ± 0.01^{jkl}
	SI-A	0.21 ± 0.01^{i}	0.21 ± 0.01^{lmn}	3.65 ± 0.04^{no}	0.04 ± 0.01^{j}	0.14 ± 0.01^{ijk}
	SO1-A	0.42 ± 0.01^{c}	0.35 ± 0.03^{hi}	6.48 ± 0.20^{i}	0.09 ± 0.01^{b}	0.24 ± 0.01^{de}
	SO3-A	0.21 ± 0.01^{i}	0.21 ± 0.01^{lmno}	4.35 ± 0.06^{m}	0.04 ± 0.01^{j}	0.11 ± 0.01^{klm}
	SO4-A	0.19 ± 0.01^{ijk}	0.18 ± 0.01^{lmno}	2.96 ± 0.08^{p}	0.04 ± 0.01^{j}	0.12 ± 0.01^{jkl}
	ST1-A	0.18 ± 0.01^{ijk}	0.14 ± 0.01^{no}	3.92 ± 0.03^{n}	0.04 ± 0.01^{j}	0.11 ± 0.01^{klm}
	ST2-A	0.17 ± 0.01^{k}	0.19 ± 0.01^{lmno}	4.81 ± 0.04^{l}	0.03 ± 0.01^{jk}	0.09 ± 0.01^{lm}

ACAE, acarbose equivalents; AChE, acetylcholinesterase; BChE, butyrylcholinesterase; GALAE, galanthamine equivalents; KAE, kojic acid equivalents; n.a., not active. Data are presented as mean ± standard deviation of three determinations. Different superscript letters within columns indicate significant differences ($P < 0.05$). Sample codes as in Table 1.

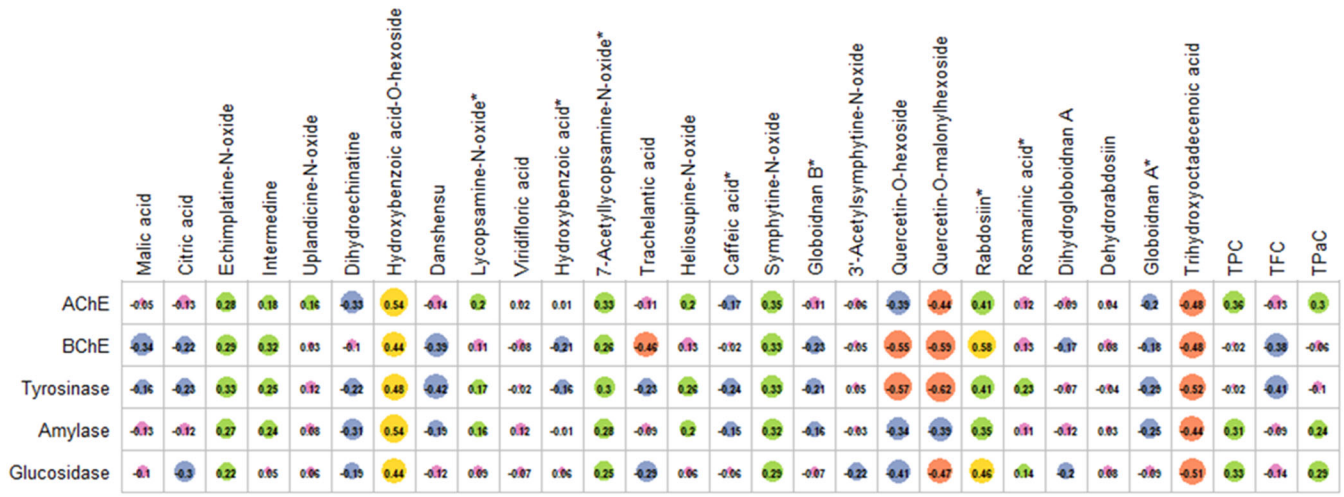

Figure 3. Correlations between the phytochemical composition and enzyme inhibitory activity of *Symphytum* samples.

anti-amylase and anti-glucosidase effects of *S. officinale* and *S. ibericum* have been reported.[19,27] Nonetheless, to the authors' knowledge, the antioxidant and enzyme inhibitory properties of *S. bulbosum*, *S. cordatum*, *S. grandiflorum*, *S. tuberosum* and *S. tuberosum* subsp. *nodosum* are discussed herein for the first time. The correlations found between the bioactivity of comfrey

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samples and their phytochemical composition are particularly worth mentioning, as they can indicate some of the biologically relevant molecules. For instance, some correlations, such as those between danshensu and ABTS, FRAP and CUPRAC; quercetin-*O*-hexoside and DPPH and CUPRAC; malic and citric acids and MCA; rabdosiin and anti-BChE activity; or hydroxybenzoic acid-*O*-hexoside and anti-AChE, anti-BChE and anti-amylase, were noted.

CONCLUSIONS

By assessing for the first time in a comparative manner the phytochemical profile and biological activity of a considerably high number of *Symphytum* samples, this study can open perspectives for the phytopharmaceutical and agricultural utilization of less common comfrey species. In light of the high degree of inter-species chemical similarities revealed by the current study, any of the studied taxa could be an alternative source of active ingredients with similar bio-functional properties to those reported for *S. officinale*, the agro-industrially used species. Nonetheless, since pyrrolizidine alkaloids were reported in all samples, their quantification by adequate analytical methods as well as their removal (e.g. by ionic exchange resin depletion) would be indicated. Concerning the pharmacological properties of the investigated species, evaluating their anti-inflammatory potential (e.g. modulation of pro-inflammatory cytokine profile) in cell-based or animal models would constitute a further step prior to developing cosmeceutical, pharmaceutical or nutraceutical applications.

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CONFLICT OF INTEREST STATEMENT

This research does not have any conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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