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Symphytum ibericum Steven: LC–HRMS/MS-based phytochemical profile, in vitro antioxidant and enzyme inhibitory potential

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Abstract

Background: *Symphytum* L. (comfrey, Boraginaceae) has a longstanding use as a remedy to alleviate the clinical symptomatology in arthritis, strains, contusions or sprains. In the recent years, considerable research efforts were put into assessing the chemico-biological profile of unexploited *Symphytum* species, with the aim to extend the medicinal valences of the genus to new pharmacological applications. However, to date there are no previous comprehensive phytochemical characterization and multi-biological evaluation of *S. ibericum* Steven, a perennial Boraginaceae plant distributed in the Northeastern Turkey and Caucasus region.

Results: Total phenolic and flavonoid content of extracts obtained from the leaves and roots of *S. ibericum* varied between 10.53 ± 0.20 to 84.95 ± 0.60 mg gallic acid equivalents/g and between 0.81 ± 0.06 to 20.88 ± 0.29 mg rutin equivalents/g. The liquid chromatography hyphenated with tandem high-resolution mass spectrometry (LC–HRMS/MS)-based phytochemical profiling revealed a number of 29 distinct compounds, such as phenolic acids (e.g., caffeic acid, rosmarinic acid, globoidnan B, rabdosiin, globoidnan A), flavonoids (e.g., quercetin derivatives, luteolin, apigenin), pyrrolizidine alkaloids (e.g., intermedine–*N*-oxide, lycopsamine-*N*-oxide, symphytine-*N*-oxide), organic and oxygenated unsaturated fatty acids. The evaluation of the antioxidant activity showed potent scavenging activity against synthetic radicals, cupric ion reducing (37.60 ± 0.15–436.26 ± 7.12 mg Trolox equivalents/g), ferric ion reducing (21.01 ± 0.74–229.99 ± 3.86 mg Trolox equivalents/g) and chelating capacity; in general, the leaf extracts displayed superior antioxidant effects than the corresponding root extracts. With respect to the inhibitory activity tested on various pharmacologically relevant enzymes, interesting anti-acetylcholinesterase (0.32 ± 0.03–3.32 ± 0.12 mg galanthamine equivalents/g), anti-butyrylcholinesterase (0.88 ± 0.06–5.85 ± 0.16 mg galanthamine equivalents/g) and anti-tyrosinase (21.84 ± 0.21–61.94 ± 2.86 mg kojic acid equivalents/g) properties were noticed. Exploratory multivariate analysis revealed four clusters with respect to phytochemical profile, of which one rich in danshensu, quercetin hexoside, dehydrorabdosiin, dihydrogloboidnan B and quercetin acetylhexoside.

Conclusions: As evidenced through the phytochemical characterization and multi-biological evaluation, *S. ibericum* can be regarded as a prospective source of pharmaceutical or cosmeceutical ingredients with putative uses in the management of chronic conditions linked to oxidative stress, such as Alzheimer's disease or skin pigmentation disorders.

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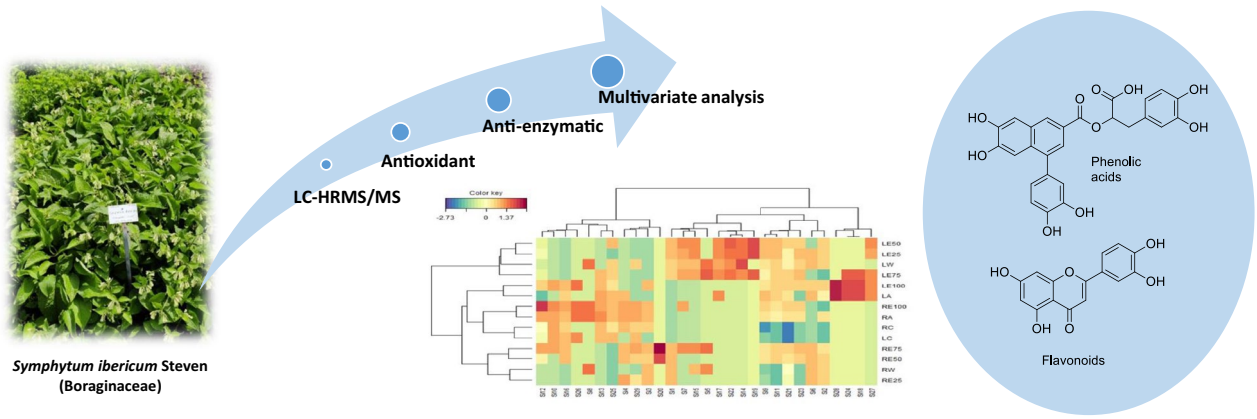
Keywords: *Symphytum ibericum* Steven, LC–HRMS/MS, Antioxidant, Anti-enzymatic, Multivariate analysis

Graphical Abstract



Background

Symphytum officinale L. (comfrey) is one of the most well-known and studied species of the Boraginaceae, a family that comprises around 130 genera and 2300 species distributed around the world, in both tropical and temperate regions [1, 2]. Ethnopharmacologically, different internal (tinctures, infusions, decocts) or external (compresses, ointments) formulations prepared from the roots (*Symphyti radix*), leaves (*Symphyti folium*) or whole aerial parts (*Symphyti herba*) have been empirically used since Ancient times in swellings, bruises, phlebitis, contusions, respiratory, gastro-intestinal and genitourinary disorders [3–6]. Currently, comfrey-based topical applications are administered in the clinical symptomatology (inflammation, pain and swelling of joints and muscles) from arthritis, strains, contusions or sprains [7, 8]. These indications are based on the solid scientific knowledge acquired from numerous cell-free, cell-based, animal and human studies that demonstrated the efficacy (wound healing, antimicrobial, anti-inflammatory and anti-nociception potential), safety and tolerability profile of *S. officinale* preparations [4, 5, 9–16]. Comfrey contains four major classes of constituents: polysaccharides (up to 30% mucilage), purine derivatives (0.6–4.7% allantoin), polyphenols and pyrrolizidine alkaloids (PAs). Polysaccharides are considered one of the most potent biomolecules in comfrey, endowed with antioxidant, immunomodulatory, anticancer, hypoglycemic and hypolipidemic effects [17]. Purine derivatives are degradation products of purine bases and nucleotides; in particular, allantoin was shown to exert wound healing (fibroblastic proliferation, extracellular matrix synthesis) and immunomodulatory activities [18]. Polyphenols (e.g., rosmarinic acid, caffeic acid, chlorogenic acid, lithospermic acid, globoidnan A, globoidnan B, rabdosiin, etc.) are the most diverse and well-represented class of comfrey phytochemicals [6, 8, 19]. Rosmarinic acid is known for its numerous biological properties proven in different experimental models, such as anti-inflammatory, antioxidant, anticancer, antimicrobial and anti-allergic activities [4, 6]. Finally, comfrey PAs are a large group of 1,2-unsaturated necine ring structures (usually retronecine-type) that can occur either as free bases or as their *N*-oxides (PANOs). The most common PAs in *S. officinale* as well as other *Symphytum* species are intermedine, lycopsamine, 7-acetylintermedine, 7-acetyllycopsamine, echimidine, symphytine and their corresponding PANOs [20]. However, since PAs are linked with serious health problems, particularly hepato-, cyto-, pneumo- and geno-toxicity, the use of comfrey-based preparations is partly overshadowed. In addition, the European Medicines Agency [21] restricted the intake of PAs-containing or PAs-contaminated herbal medicinal products to a maximum limit of 1 µg PAs/day.

Nevertheless, in the recent years, considerable research efforts were put into assessing the chemico-biological profile of several other unexploited *Symphytum* species. For instance, Zengin et al. [7]. showed that *S. aintabicum* Hub.-Mor. & Wickens, is a rich source of bioactive constituents, such as phenolic acids (vanillic, gallic, ferulic, cinnamic, coumaric, caffeic, syringic, chlorogenic acids), flavonoids (kaempferol-3-*O*-glucoside, quercetin, hyperoside, quercitrin) and secoiridoids (swertiamarin, sweroside). In addition, various methanolic and aqueous aerial part extracts of *S. aintabicum* displayed significant antioxidant activity and acted as cholinesterase and tyrosinase inhibitors [7]. Similarly, the ethyl acetate, methanol

and aqueous aerial part extracts of *S. anatolicum* Boiss. exhibited potent antioxidant properties as well as anti-tyrosinase, anti-amylase and anti-cholinesterase effects, which could be attributed to their complex phytochemical profile, mostly phenolic acids (caffeic, chlorogenic, ferulic, gallic, and rosmarinic acids, salvianolic acids A and C, rabdosiin) and flavonoids (hyperoside, luteolin, quercetin, hesperidin, rutin, isoquercitrin) [2, 22]. Beside the chemosystematics importance, the above-mentioned studies also revealed the potential use of these species as rich sources of pharmaceutical or cosmeceutical ingredients in the management of chronic conditions linked to oxidative stress, such as diabetes, Alzheimer's disease or skin pigmentation disorders. It is, therefore, imperious to thoroughly bio-prospect other poorly studied *Symphytum* species that can be subsequently exploited at industrial, agricultural or pharma-technological scale.

Symphytum ibericum Steven (creeping comfrey, dwarf comfrey, Iberian comfrey) is a perennial Boraginaceae plant distributed in the Northeastern Turkey and Caucasus region. The stems can grow up to only 27–38 cm, with ovate to ovate-lanceolate leaves; the basal leaves are petiolate, whereas the cauline leaves are shortly petiolate; the inflorescences are grouped into cymes, with a hairy calyx of 4–5 mm and a cream corolla [23]. Except for a few botanical (anatomical, morphological) and taxonomical studies [23–26], there are no further investigations on this particular species. Therefore, the aim of our study was to perform a comprehensive liquid chromatography hyphenated with tandem high-resolution mass spectrometry (LC–HRMS/MS) phytochemical profiling of *S. ibericum* leaf and root extracts obtained with solvents with different polarity. In addition, the antioxidant (free radical scavenging, reducing power and chelating) and enzyme inhibitory effects (cholinesterase, tyrosinase, amylase, and glucosidase) were evaluated by in vitro assays. Finally, the chemico-biological differences within the leaf and root extracts of *S. ibericum* were explored via clustered image map (CIM) analysis, principal component analysis (PCA), hierarchical cluster analysis (HCA) and correlation (Corr) analysis.

Methods

Plant material and extraction

The leaves and roots of *Symphytum ibericum* Steven were collected in August 2021 from material cultivated at the Botanical Garden München–Nymphenburg (Munich, Germany, IPEN no. GE-0-M-2012/2393, 48° 09′ 50″ N, 11° 30′ 02″ E and 531 m). The region has a mild climate characterized by annual precipitation of 1000 mm and an average temperature of 8.8 °C. The cultivated plant stems originate from a wild population from Imereti Province in Georgia, between Tskaltubo and Tsageri (42° 34′ 25″ N, 42° 40′ 07″ E and 563 m) (A. Gröger et al. 226–4, 30-VIII-2012).

After drying, 5 g of ground plant material (leaves, roots) were separately extracted with 50 mL of chloroform, acetone, ethanol (25–100%) and water in an ultrasound bath, at room temperature for 30 min. After filtration, fresh solvent was added and the extraction procedure was repeated two more times. The pooled filtrates were evaporated to dryness, yielding leaf extracts of chloroform (**LC**, 0.18 g), acetone (**LA**, 0.06 g), ethanol (**LE100**, 0.11 g), 75% ethanol (**LE75**, 0.52 g), 50% ethanol (**LE50**, 0.35 g), 25% (**LE25**, 0.31 g) and water (**LW**, 0.57 g) extracts as well as root extracts of chloroform (**RC**, 0.08 g), acetone (**RA**, 0.03 g), ethanol (**RE100**, 0.09 g), 75% ethanol (**RE75**, 0.48 g), 50% ethanol (**RE50**, 0.59 g), 25% (**RE25**, 0.51 g) and water (**RW**, 0.54 g).

Phytochemical characterization

Total phenolic content (TPC), and total flavonoid content (TFC) were assessed spectrophotometrically as described in [27, 28], with data expressed as mg gallic acid equivalents (GAE)/g extract, and rutin equivalents (RE)/g extract, respectively. LC–HRMS/MS analysis was performed on an Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump (G1312C), column thermostat (G1316A), autosampler (G1329B) and accurate–mass quadrupole–time-of-flight MS detector (G6530B). The chromatographic separations were performed under the following conditions: column Phenomenex Gemini C18 (2 × 100 mm, 3 µm); column temperature 20 °C; mobile phase 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B); gradient 5–60% B (0–45 min), 95% B (46–50 min); flow rate 0.2 mL/min; injection volume 2 µL. The following MS parameters were used: Agilent dual jet stream (AJS) electrospray ionization source (ESI); full-scan high-resolution accurate–mass acquisition mode; negative and positive mode; *m/z* range 100–1000; gas (N_2) temperature 275 °C; N_2 flow 10 L/min; nebulizer 35 psi; sheath gas temperature 325 °C; sheath gas flow rate 12 L/min; capillary voltage 4000 V; nozzle voltage 1000 V; skimmer 65 V; fragmentor 140 V; fixed collision-induced dissociation energies 10 and 30 V. Data acquisition was achieved with MassHunter Workstation Data Acquisition 8.0, whereas MassHunter Workstation Qualitative Navigator 8.0 was used for data analysis. Peak assignment from the base peak chromatograms (BPC) of the analyzed samples was carried out by comparing the spectro-chromatographic data with previous literature reporting the LC–MS analysis of similar constituents from *Symphytum* [1, 6, 13, 15, 20] or online databases (METLIN, KNApSacK, PubChem, NIST Chemistry WebBook).

Antioxidant and enzyme inhibitory assays

The antioxidant and enzyme inhibitory assays were performed according to methods previously described [27, 28]. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzothiazoline) 6-sulfonic acid (ABTS) radical scavenging activity, cupric ion reducing antioxidant capacity (CUPRAC) and ferric ion reducing antioxidant power (FRAP) were expressed as mg Trolox equivalents (TE)/g extract. The metal chelating ability (MCA) was provided as mg EDTA equivalents (EDTAE)/g extract, whereas the total antioxidant activity (phosphomolybdenum assay, PBD) was given as mmol TE/g extract. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities were expressed as mg galanthamine equivalents (GALAE)/g extract; tyrosinase inhibitory potential was provided as mg kojic acid equivalents (KAE)/g extract; amylase and glucosidase inhibitory effects were presented as mmol acarbose equivalents (ACAE)/g extract.

Data analysis

All the experiments were performed in triplicate, with the results given as mean ± standard deviation (SD). One-way analysis of variance with Tukey's post-hoc test was conducted using XLSTAT software; $p < 0.05$ was considered statistically significant. The chemical data sets (base peak areas extracted from the LC–HRMS/MS profiling) were logarithmically transformed, scaled, centered and submitted to CIM analysis. The bioactivities data sets were also scaled, centered and subsequently submitted to the PCA and HCA. For both CIM and HCA, "Ward's rule" and "Euclidean distance" were employed in sample clustering. The relationship between the phytochemical composition and investigated bioactivities was evaluated by Corr analysis; a Pearson's coefficient above 0.7 was considered significant. CIM, PCA, HCA and Corr analysis were performed using R v 4.1.2 software.

Table 1 Extraction yields, total phenolic and flavonoid content of *S. ibericum* leaf and root extracts

Sample	Yield [%]	TPC [mg GAE/g]	TFC [mg RE/g]
LC	3.6	23.60 ± 0.29^{i}	4.03 ± 0.14^{e}
LA	1.2	32.64 ± 0.06^{e}	3.23 ± 0.18^{f}
LE100	2.2	48.45 ± 0.18^{d}	3.14 ± 0.20^{f}
LE75	10.4	75.24 ± 0.50^{c}	4.73 ± 0.06^{d}
LE50	7.0	84.95 ± 0.60^{a}	3.54 ± 0.09^{f}
LE25	6.2	79.26 ± 0.13^{b}	6.19 ± 0.27^{c}
LW	11.4	23.28 ± 0.18^{i}	1.48 ± 0.12^{g}
RC	1.6	13.21 ± 0.39^{l}	5.14 ± 0.10^{d}
RA	0.6	24.45 ± 0.16^{h}	20.88 ± 0.29^{a}
RE100	1.8	26.75 ± 0.10^{g}	13.27 ± 0.16^{b}
RE75	9.6	28.70 ± 0.12^{f}	0.81 ± 0.06^{h}
RE50	11.8	17.72 ± 0.15^{j}	0.91 ± 0.06^{h}
RE25	10.2	10.53 ± 0.20^{m}	1.12 ± 0.07^{gh}
RW	10.8	15.38 ± 0.13^{k}	1.09 ± 0.14^{gh}

Data are presented as mean ± standard deviation (SD) of three determinations; different superscript letters within columns indicate significant differences between the tested extracts ($P < 0.05$)

GAE gallic acid equivalents, *LA* acetone leaf extract, *LC* chloroform leaf extract, *LE100* ethanol leaf extract, *LE25* 25% ethanol leaf extract, *LE50* 50% ethanol leaf extract, *LE75* 75% ethanol leaf extract, *LW* water leaf extract, *RA* acetone root extract, *RC* chloroform root extract, *RE100* ethanol root extract, *RuE* rutin equivalents, *RE25* 25% ethanol root extract, *RE50* 50% ethanol root extract, *RE75* 75% ethanol root extract, *RW* water leaf extract, *TFC* total flavonoid content, *TPC* total phenolic content

Results and discussion

Total phenolic and flavonoid content

To assess the TPC and TFC of *S. ibericum*, the leaves and roots were separately extracted with solvents with different polarities (chloroform, acetone, ethanol 25–100% and water). With respect to the extraction solvents (Table 1), it can be noticed that ethanol 75% and water led to the highest yields among the leaf extracts, whereas ethanol 25–75% and water displayed considerable higher extract masses than the other root extracts.

Overall, the leaf extracts (23.28 ± 0.18–84.95 ± 0.60 mg GAE/g) showed higher TPC than the root extracts (10.53 0.20–28.70 ± 0.12 mg GAE/g) (Table 1). Furthermore, the medium polarity solvents (ethanol 25–100%) allowed the highest recovery of polyphenols from the leaves and roots of *S. ibericum*. This is in agreement with the conventional processing of comfrey, as European Medicines Agency lists in the "Assessment report on *Symphytum officinale* L., radix" only medicinal preparations obtained with ethanol, ethanol 65% or ethanol 60% [21]. In addition, the TPC of *S. ibericum* is comparable with the TPC reported in other *Symphytum* species, such as *S. officinale* leaf and root extracts (5.39–125.50 mg GAE/g), *S. aintabicum* aerial part extracts (35.50–112.25 mg GAE/g) and *S. anatolicum* aerial part extracts (11.45–44.75 mg GAE/g) [1, 2, 7, 22].

From all the leaf extracts, TFC reached its peak in **LE25** (6.19 ± 0.27 mg RE/g), with the remaining values ranging from 1.48 ± 0.12 mg RE/g (**LW**) to 4.72 mg RE/g (**LE75**). In general, the root extract contained lower TFC than the corresponding leaf extracts (0.81 ± 0.06–5.14 ± 0.10 mg/g), with the exception of **RA** (20.88 ± 0.29 mg RE/g) and **RE100** (13.27 ± 0.16 mg RE/g) that showed unusually higher amounts of flavonoids (Table 1). Previous research reported TFC values ranging from 0.19 to 33.89 mg RE/g in *S. officinale*, 2.54 to 25.12 mg RE/g in *S. aintabicum* or 2.74 to 13.30 mg RE/g in *S. anatolicum* [1, 2, 7, 22].

Table 2 LC–HRMS/MS phytochemical profiling of *S. ibericum* leaf and root extracts

No	Proposed identity	Class	T_R [min]	HRMS	Exp. [*m/z*]	Calcd. [*m/z*]	Δ [ppm]	MF	HRMS/MS [*m/z*]
1	Malic acid	Organic acid	2.0	$[M-H]^-$	133.0144	133.0142	− 1.14	$C_4H_6O_5$	115.0038
2	Citric acid	Organic acid	2.5	$[M-H]^-$	191.0191	191.0197	3.26	$C_6H_8O_7$	129.0151, 111.0079
3	Dihydroechinatine	Alkaloid	3.0	$[M+H]^+$	302.1978	302.1962	− 5.29	$C_{15}H_{27}NO_5$	158.1165, 140.1077, 122.0958
4	Intermedine-*N*-oxide*	Alkaloid	3.7	$[M+H]^+$	316.1743	316.1755	3.69	$C_{15}H_{25}NO_6$	172.0951, 138.0895
5	Lycopsamine-*N*-oxide*	Alkaloid	4.4	$[M+H]^+$	316.1755	316.1755	− 0.11	$C_{15}H_{25}NO_6$	172.0963, 138.0887
6	Hydroxybenzoic acid hexoside	Phenolic acid	4.5	$[M-H]^-$	299.0770	299.0772	0.80	$C_{13}H_{16}O_8$	239.0388, 209.0491, 179.0320, 137.0135
7	Danshensu	Phenolic acid	5.7	$[M-H]^-$	197.0469	197.0455	− 6.83	$C_9H_{10}O_5$	179.0348, 135.0456, 123.0455, 107.0472
8	Trachelantic/viridifloric acid	Organic acid	7.2	$[M-H]^-$	161.0820	161.0819	− 0.42	$C_7H_{14}O_4$	135.0577, 117.0545
9	Hydroxybenzoic acid	Phenolic acid	10.0	$[M-H]^-$	137.0243	137.0244	0.85	$C_7H_6O_3$	108.0232
10	7-Sarracinyl-9-trachelantyl-retronecine-*N*-oxide	Alkaloid	13.2	$[M+H]^+$	414.2116	414.2122	1.56	$C_{20}H_{31}NO_8$	396.2040, 352.1821, 270.1355, 254.1373, 220.1315, 172.0893
11	Caffeic acid	Phenolic acid	14.9	$[M-H]^-$	179.0341	179.0350	4.90	$C_9H_8O_4$	161.0434, 135.0443, 107.0445
12	Symphytine-*N*-oxide	Alkaloid	20.9	$[M+H]^+$	398.2183	398.2173	− 2.45	$C_{20}H_{31}NO_7$	254.1228, 220.1157, 172.0929, 154.0773, 138.0795, 122.0731, 106.0575
13	7-Hydroxymetylbutyryl-9-trachelanthylretronecine	Alkaloid	21.9	$[M+H]^+$	400.2324	400.2330	1.45	$C_{20}H_{33}NO_7$	256.1550, 222.1516, 156.0978
14	Dihydrogloboidnan B	Phenolic acid	22.2	$[M-H]^-$	539.1188	539.1195	1.30	$C_{27}H_{24}O_{12}$	495.1237, 359.0748, 341.0532, 315.0886, 255.0606, 197.0429, 179.0315, 161.0220, 135.0424
15	Globoidnan B*	Phenolic acid	23.2	$[M-H]^-$	537.1078	537.1038	− 4.18	$C_{27}H_{22}O_{12}$	493.1154, 339.0517, 295.0601, 197.0475, 179.0356, 135.0433
16	3′-Acetylsymphytine-*N*-oxide	Alkaloid	23.5	$[M+H]^+$	440.2285	440.2279	− 1.38	$C_{22}H_{33}NO_8$	380.2113, 254.1414, 220.1335, 172.0915
17	Quercetin hexoside	Flavonoid	24.1	$[M-H]^-$	463.0855	463.0882	5.82	$C_{21}H_{20}O_{12}$	301.0335, 271.0201, 255.0269, 151.0021
18	Quercetin rhamnoside	Flavonoid	25.3	$[M-H]^-$	447.0915	447.0933	3.98	$C_{21}H_{20}O_{11}$	301.0329, 271.0260, 255.0310, 151.0030
19	Quercetin acetylhexoside	Flavonoid	25.9	$[M-H]^-$	505.9879	505.0988	1.71	$C_{23}H_{22}O_{13}$	463.0787, 300.0248, 255.0295
20	Rabdosiin*	Phenolic acid	26.2	$[M-H]^-$	717.1464	717.1461	− 0.41	$C_{36}H_{30}O_{16}$	537.1127, 519.0977, 475.1069, 339.0477, 197.0434, 135.0406
21	Rosmarinic acid*	Phenolic acid	27.1	$[M-H]^-$	359.0768	359.0772	1.22	$C_{18}H_{16}O_8$	197.0471, 179.0355, 161.0251, 135.0461
22	Dehydrorabdosiin	Phenolic acid	29.2	$[M-H]^-$	715.1327	715.1305	− 3.13	$C_{36}H_{28}O_{16}$	517.0868, 473.0954, 337.0380, 197.0425,
23	Globoidnan A*	Phenolic acid	30.3	$[M-H]^-$	491.0997	491.0984	− 2.70	$C_{26}H_{20}O_{10}$	311.0579, 267.0656, 135.0454
24	Luteolin*	Flavonoid	31.3	$[M-H]^-$	285.0412	285.0405	− 2.58	$C_{15}H_{10}O_6$	199.0378, 175.0387, 151.0028, 133.0279
25	Trihydroxyoctadecadi-enoic I	Fatty acid	32.5	$[M-H]^-$	327.2188	327.2177	− 2.44	$C_{18}H_{32}O_5$	291.2021, 209.1199
26	Trihydroxyoctadecadi-enoic II	Fatty acid	33.2	$[M-H]^-$	327.2185	327.2177	3.95	$C_{18}H_{32}O_5$	291.1941, 229.1452, 211.1345
27	Cirsimaritin	Flavonoid	33.5	$[M-H]^-$	313.0720	313.0718	− 0.76	$C_{17}H_{14}O_6$	161.0245, 151.0404
28	Apigenin*	Flavonoid	33.8	$[M-H]^-$	269.0452	269.0455	1.28	$C_{15}H_{10}O_5$	183.0469, 159.0472, 133.0304
29	Trihydroxyoctadecenoic acid	Fatty acid	34.5	$[M-H]^-$	329.2331	329.2333	0.75	$C_{18}H_{34}O_5$	229.1405, 211.1332, 183.1351

calcd. Calculated, *exp*. Experimental, *HRMS* high resolution mass spectra, *MF* molecular formula, T_R retention time

Δ, mass error; *confirmed by standard

LC–HRMS/MS phytochemical profiling

The spectro-chromatographic results (retention time, molecular formula, *m/z* of the molecular and fragment ions) of the LC–HRMS/MS profiling are provided in Table 2, while the base peak chromatograms are given in Additional file 1: Figures S1 and S2. A total number of

29 specialized metabolites belonging to different phytochemical classes (phenolic acids, flavonoids, fatty acids and PAs) were assigned in the leaf and root extracts of *S. ibericum*. Of these, the highest number of constituents (17–18) were found in **LE50**, **LE100**, **LW** and **RE75**, followed by 15 phytochemicals in **RA**, **RE100**, **LA**, **LE50** and **LE25**, 13 in **RE50**, 10 in **RW**, nine in **RC** and **RE25** and eight in **LC**.

Phenolic acids were the most abundant group, represented by danshensu (**Si7**), hydroxybenzoic acid (**Si10**) and its hexoside (**Si6**), caffeic acid (**Si11**), dihydrogloboidnan B (**Si14**), globoidnan B (**Si15**), rabdosiin (**Si20**), rosmarinic acid (**Si21**), dehydrorabdosiin (**Si22**) and globoidnan A (**Si23**). Rosmarinic acid is a ubiquitous constituent of *Symphytum* genus, while rabdosiin, globoidnans A and B were only recently isolated from *S. officinale* [4, 15] and proposed as phytochemical markers in comfrey roots obtained from the spontaneous European flora or experimental and commercial crops [3, 20]. The fragmentation patterns of the rather unusual derivatives, such as dihydrogloboidnan B (**Si14**) and dehydrorabdosiin (**Si22**), are proposed in Additional file 1: Figure S3. Overall, the LC–HRMS/MS fingerprinting of the phenolic acid profile of *S. ibericum* revealed a significant overlapping with *S. officinale* [1, 4, 6, 13, 15, 20] and in a lesser extent with *S. aintabicum* [7] and *S. anatolicum* [2, 22].

As a member of the Boraginaceae family, *S. ibericum* contained several PAs, putatively labeled as dihydroechinatine (**Si3**), intermedine-*N*-oxide (**Si4**), lycopsamine-*N*-oxide (**Si5**), 7-sarracinyl-9-trachelantylretronecine-*N*-oxide (**Si10**), symphytine-*N*-oxide (**Si12**), 7-hydroxmethylbutyryl-9-trachelanthylretronecine (**Si13**) and 3'-acetylsymphytine-*N*-oxide (**Si16**). Compounds **Si4**, **Si5**, **Si10**, **Si12** and **Si16** (or their stereoisomers) were repeatedly reported in *S. officinale* [1, 3]. Furthermore, the presence of echimidine, lycopsamine and symphytine was previously shown by thin layer chromatographic analyses in *S. ibericum* [25]. Nevertheless, since dihydroechinatine, 7-sarracinyl-9-trachelantylretronecine-*N*-oxide, 7-hydroxymethylbutyryl-9-trachelanthylretronecine and 3'-acetylsymphytine-*N*-oxide have unusual structures, their HRMS/MS fragmentation patterns were proposed in Additional file 1: Figure S4.

A number of six flavonoids were tentatively annotated in *S. ibericum*, namely, hexoside (**Si17**), rhamnoside (**Si18**) and acetylhexoside (**Si19**) of quercetin as well as the aglycons luteolin (**Si24**), cirsimaritin (**Si27**) and apigenin (**Si28**). Interestingly, all these phytochemicals were found only in the leaf extracts, suggesting that the underground parts do not accumulate/biosynthesize flavonoids. A similar trend was noticed for *S. officinale* and *Anchusa ochroleuca* M. Bieb., another member of the Boraginaceae family [1]. Quercetin hexoside (such as hyperoside, quercetrin, or isoquercitrin), quercetin

Table 3 Antioxidant activity of *S. ibericum* leaf and root extracts

Sample	DPPH [mg TE/g]	ABTS [mg TE/g]	CUPRAC [mg TE/g]	FRAP [mg TE/g]	MCA [mg EDTAE/g]	PBD [mmol TE/g]
LC	13.37 ± 0.26^{j}	21.29 ± 0.25^{i}	83.96 ± 3.48^{g}	28.29 ± 0.82^{h}	19.02 ± 0.66^{bc}	1.49 ± 0.10^{d}
LA	28.29 ± 0.21^{g}	39.32 ± 0.50^{g}	123.65 ± 3.58^{f}	45.82 ± 0.67^{fg}	15.71 ± 0.46^{d}	2.22 ± 0.08^{b}
LE100	42.99 ± 0.12^{d}	78.27 ± 0.49^{d}	199.79 ± 4.58^{d}	81.75 ± 1.09^{d}	18.16 ± 0.27^{c}	2.69 ± 0.04^{a}
LE75	48.13 ± 0.04^{c}	106.83 ± 0.01^{b}	287.31 ± 2.66^{b}	173.76 ± 3.76^{b}	10.68 ± 0.42^{fg}	1.72 ± 0.17^{cd}
LE50	184.19 ± 0.19^{a}	293.97 ± 1.97^{a}	436.26 ± 7.12^{a}	229.99 ± 3.86^{a}	22.54 ± 0.13^{a}	1.67 ± 0.06^{cd}
LE25	43.02 ± 0.03^{d}	106.14 ± 0.14^{b}	273.27 ± 2.55^{c}	146.91 ± 0.67^{c}	22.24 ± 0.16^{a}	1.79 ± 0.02^{c}
LW	72.75 ± 1.25^{b}	71.99 ± 0.81^{e}	116.24 ± 0.33^{f}	63.92 ± 0.28^{e}	20.35 ± 0.43^{b}	0.60 ± 0.02^{g}
RC	15.98 ± 0.15^{i}	5.95 ± 0.61^{i}	44.99 ± 1.55^{i}	21.87 ± 0.34^{i}	22.65 ± 1.61^{a}	1.17 ± 0.05^{e}
RA	26.29 ± 0.15^{h}	26.08 ± 5.30^{h}	84.84 ± 0.51^{g}	31.91 ± 1.19^{h}	15.16 ± 0.30^{de}	1.65 ± 0.12^{cd}
RE100	32.98 ± 0.01^{f}	46.81 ± 1.85^{f}	117.30 ± 0.98^{f}	49.01 ± 0.65^{f}	9.12 ± 0.95^{g}	2.06 ± 0.12^{b}
RE75	48.24 ± 0.04^{c}	90.99 ± 0.63^{c}	151.63 ± 3.29^{e}	80.02 ± 0.30^{d}	11.11 ± 0.28^{f}	1.11 ± 0.02^{e}
RE50	38.49 ± 0.22^{e}	49.85 ± 0.18^{f}	77.18 ± 0.54^{g}	41.54 ± 0.41^{g}	13.62 ± 0.59^{e}	0.98 ± 0.03^{ef}
RE25	27.97 ± 0.21^{g}	26.86 ± 0.72^{h}	37.60 ± 0.15^{i}	21.01 ± 0.74^{i}	16.28 ± 0.28^{d}	0.99 ± 0.06^{ef}
RW	28.64 ± 0.72^{g}	47.55 ± 0.55^{f}	56.96 ± 0.91^{h}	32.29 ± 0.19^{h}	19.36 ± 0.24^{bc}	0.81 ± 0.02^{fg}

Data are presented as mean ± standard deviation (SD) of three determinations; different superscript letters within columns indicate significant differences between the tested extracts ($P < 0.05$)

ABTS 2,2′-azino-bis(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, *LA* acetone leaf extract, *LC* chloroform leaf extract, *LE100* ethanol leaf extract, *LE25* 25% ethanol leaf extract, *LE50* 50% ethanol leaf extract, *LE75* 75% ethanol leaf extract, *LW* water leaf extract, *MCA* metal chelating activity, *PBD* phosphomolybdenum assay, *RA* acetone root extract, *RC* chloroform root extract, *RE100* ethanol root extract, *RE25* 25% ethanol root extract, *RE50* 50% ethanol root extract, *RE75* 75% ethanol root extract, *RW* water leaf extract, *TE* trolox equivalents

acetylhexoside and luteolin were previously reported in *S. officinale*, *S. aintabicum* or *S. anatolicum* [2, 7, 22].

Finally, three organic acids, such as malic (**1**), citric (**2**) and trachelantic/viridifloric acids (**8**) as well as three oxygenated unsaturated fatty acids, such as trihydroxy-octadecadienoic acid isomers (**25**, **26**) and trihydroxy-octadecenoic acid (**29**), were assigned as non-specific metabolites in the leaf and root extracts of *S. ibericum*. As expected, the hydrophilic organic acids were mostly distributed in the polar extracts (**RE25–75**, **RW**, **LE75–L25**, **LW**), whereas the hydrophobic fatty acids were majorly found in the non-polar extracts (**RC**, **RA**, **RE**, **LC**, **LA**, **LE**). The occurrence of organic and fatty acids in *Symphytum* genus has been recently brought into attention through different LC–MS analyses [1, 20, 29].

Antioxidant activity

The antioxidant potential of the 14 extracts of *S. ibericum* (roots and aerial parts) was investigated through a series of complementary assays, such as radical scavenging, reducing and chelating tests (Table 3). The radical scavenging activity varied between 13.37 ± 0.26 mg TE/g (**LC**) and 184.19 ± 0.19 mg TE/g (**LE50**) in the DPPH assay and between 5.95 ± 0.61 mg TE/g (**RC**) and 293.97 mg TE/g (**LE50**) in the ABTS assay. **RE75** showed the most significant radical scavenging effects (48.24 ± 0.04 mg TE/g in DPPH assay and 90.99 ± 0.63 mg TE/g in ABTS assay) in the roots; nevertheless, the activity of the root extracts was inferior to that displayed by the corresponding leaf extracts. Previously, various extracts of *S. officinale* (roots and aerial parts) showed comparable DPPH and ABTS radical scavenging effects (7.94–189.87 mg TE/g in DPPH assay and 0.51–257.57 mg TE/g in ABTS assay) [1]. Similarly, the aerial part extracts of *S. anatolicum* exhibited 24.57–95.76 mg TE/g in DPPH test and 53.95–197.00 mg/g in ABTS test [2, 22], whereas the aerial part extracts of *S. aintabicum* displayed values of 0.56–232.34 mg TE/g (DPPH) and 109.98–389.96 mg TE/g (ABTS) [7]. When assessing the reducing power of the 14 extracts of *S. ibericum*, a very strong potency was noticed in **LE50** (436.26 ± 7.12 mg TE/g in CUPRAC and 229.99 ± 3.86 mg TE/g FRAP), whereas the root extracts were significantly less active than the corresponding leaf extracts (Table 3). Our values are comparable with those reported in the aerial part and root extracts of *S. officinale* (15.78–553.32 mg TE/g in CUPRAC and 6.74–299.86 mg TE/g in FRAP), aerial part extracts of *S. anatolicum* (95.92–217.52 mg TE/g in CUPRAC and 68.22–162.22 mg TE/g in FRAP) and aerial part extracts of *S. aintabicum* (106.20–575.69 mg TE/g in CUPRAC and 47.89–379.90 mg TE/g in FRAP) [1, 2, 7, 22].

The MCA revealed that **LE50** (22.54 ± 0.13 mg EDTAE/g), **LE25** (22.24 ± 0.16 mg EDTAE/g) and **RC** (22.65 ± 1.61 mg EDTAE/g) were the most potent extracts. Thus, the MCA of *S. ibericum* is similar with the MCA of the aerial part and root extracts of *S. officinale*

Table 4 Enzyme inhibitory activity of *S. ibericum* leaf and root extracts

Sample	AChE [mg GALAE/g]	BChE [mg GALAE/g]	Tyrosinase [mg KAE/g]	Amylase [mmol ACAE/g]	Glucosidase [mmol ACAE/g]
LC	2.76 ± 0.17^{b}	2.58 ± 0.19^{e}	31.79 ± 1.81^{ef}	0.49 ± 0.01^{bc}	0.77 ± 0.04^{e}
LA	2.41 ± 0.25^{c}	3.76 ± 0.49^{cd}	33.60 ± 1.76^{de}	0.51 ± 0.01^{ab}	0.77 ± 0.02^{e}
LE100	3.32 ± 0.12^{a}	5.85 ± 0.16^{b}	51.05 ± 3.96^{b}	0.53 ± 0.02^{a}	0.85 ± 0.01^{d}
LE75	1.95 ± 0.01^{d}	1.16 ± 0.12^{gh}	23.19 ± 1.42^{h}	0.29 ± 0.02^{gh}	0.39 ± 0.02^{f}
LE50	2.07 ± 0.07^{d}	1.48 ± 0.03^{gh}	26.92 ± 0.30^{fgh}	0.32 ± 0.01^{g}	0.97 ± 0.00^{a}
LE25	1.43 ± 0.05^{e}	2.36 ± 0.11^{e}	24.78 ± 1.72^{gh}	0.44 ± 0.00^{de}	n.a
LW	n.a	n.a	n.a	0.29 ± 0.00^{gh}	0.97 ± 0.00^{ab}
RC	2.22 ± 0.14^{cd}	4.42 ± 0.35^{c}	45.26 ± 0.48^{c}	0.42 ± 0.01^{e}	0.93 ± 0.00^{bc}
RA	0.82 ± 0.05^{f}	0.88 ± 0.06^{h}	38.58 ± 3.83^{d}	0.43 ± 0.02^{e}	0.91 ± 0.00^{c}
RE100	2.77 ± 0.09^{b}	7.51 ± 0.42^{a}	61.94 ± 2.86^{a}	0.47 ± 0.01^{cd}	0.81 ± 0.01^{de}
RE75	3.05 ± 0.05^{ab}	3.24 ± 0.17^{d}	30.36 ± 0.34^{efg}	0.27 ± 0.02^{h}	n.a
RE50	1.97 ± 0.03^{d}	2.31 ± 0.12^{ef}	21.84 ± 0.21^{h}	0.29 ± 0.01^{gh}	n.a
RE25	0.32 ± 0.03^{g}	1.65 ± 0.13^{fg}	n.a	0.36 ± 0.01^{f}	0.97 ± 0.00^{a}
RW	n.a	n.a	n.a	0.30 ± 0.00^{gh}	0.97 ± 0.00^{ab}

Data are presented as mean ± standard deviation (SD) of three determinations; different superscript letters within columns indicate significant differences between the tested extracts ($P < 0.05$)

ACAE acarbose equivalents, *AChE* acetylcholinesterase, *BChE* butyrylcholinesterase, *GALAE* galanthamine equivalents, *KAE* kojic acid equivalents, *LA* acetone leaf extract, *LC* chloroform leaf extract, *LE100* ethanol leaf extract, *LE25* 25% ethanol leaf extract, *LE50* 50% ethanol leaf extract, *LE75* 75% ethanol leaf extract, *LW* water leaf extract, *n.a.* not active, *RA* acetone root extract, *RC* chloroform root extract, *RE100* ethanol root extract, *RE25* 25% ethanol root extract, *RE50* 50% ethanol root extract, *RE75* 75% ethanol root extract, *RW* water leaf extract

(1.09–32.25 mg EDTAE/g) and aerial part extracts of *S. anatolicum* (2.98–24.12 mg EDTAE/g) and *S. aintabicum* (6.77–17.99 mg EDTAE/g) [1, 2, 7, 22]. Finally, with respect to the PBD assay, the activity of all samples varied from 0.60 ± 0.02 mmol TE/g (**LW**) to 2.69 ± 0.04 mmol TE/g (**LE100**). Similar decreasing activity orders were noticed for the leaf (**LE100** > **LA** > **LE25** > **LE75** > **LE50** > **LC** > **LW**) and root extracts (**RE100** > **RA** > **RC** > **R75** > **R25** > **R50** > **RW**). The total antioxidant activity of different extracts of *S. officinale* (0.30–2.68 mmol TE/g), *S. anatolicum* (1.02–2.98 mmol TE/g) and *S. aintabicum* (0.82–2.79 mmol TE/g) was comparable to our data [1, 2, 7, 22].

Enzyme inhibitory activity

In this section, the inhibitory activity of the root and aerial part extracts of *S. ibericum* against several key enzymes involved in the management of Alzheimer's disease (AChE, BChE), skin disorders (tyrosinase) and type 2 diabetes mellitus (amylase, glucosidase) was evaluated (Table 4). In general, all extracts acted as cholinesterase inhibitors, except for the **LW** and **RW**; the anti-AChE activity values ranged between 0.32 ± 0.03–2.77 ± 0.09 mg GALAE/g in the root samples and 1.43 ± 0.05–3.32 ± 0.12 mg GALAE/g in the leaf samples. Nevertheless, a significantly higher BChE inhibitory potential was noticed in the leaf and root extracts, with the maximum activity achieved in **RE100** (7.51 ± 0.42 mg GALAE/g) followed by **LE100** (5.85 ± 0.16 mg GALAE/g). Our data is in agreement with the anti-cholinesterase properties reported previously for extracts of *S. officinale* (1.79–2.40 and 1.23–2.35 mg GALAE/g in AChE and BChE assays, respectively), *S. aintabicum* (2.50–2.68 and 4.32–6.04 mg GALAE/g in AChE and BChE assays, respectively) or *S. anatolicum* (2.24 and 1.44 mg GALAE/g in AChE and BChE assays, respectively) [1, 2, 7].

The anti-tyrosinase effects of *S. ibericum* reached the highest peaks in **RE100** (61.94 ± 2.86 mg KAE/g) and **LE100** (51.05 ± 3.96 mg KAE/g), whereas the activity of the remaining samples varied from 21.84 ± 0.21 mg KAE/g (**RE50**) and 45.26 ± 0.48 mg KAE/g (**RC**); three extracts (**LW**, **RE25** and **RW**) were inactive (Table 4). The previous literature data also revealed significant anti-tyrosinase effects of the extracts obtained from *S. officinale* (18.15–43.89 mg KAE/g), *S. aintabicum* (9.52–13.72 mg KAE/g) or *S. anatolicum* (58.40–98.60 mg KAE/g) [1, 7, 22]. A considerable number of extracts (**LC**, **LA**, **LE100**, **LE25**, **RC**, **RA**, **RE100**) displayed an amylase inhibitory activity higher than 0.40 mmol ACAE/g, whereas the anti-glucosidase potential was slightly better, with four samples (**LE50**, **LW**, **RE25** and **RW**) showing a value of 0.97 ± 0.00 mmol ACAE/g. As compared to the extracts of *S. officinale* (0.24–0.79 and 6.86–11.62 mmol ACAE/g in amylase and glucosidase assays, respectively), *S. anatolicum* (0.13–0.61

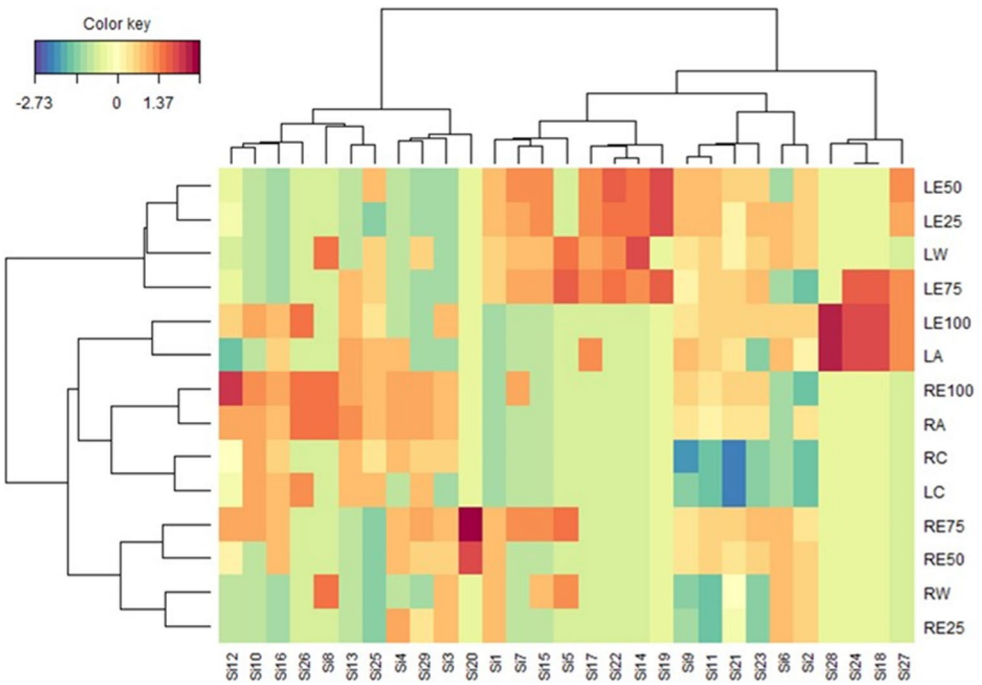


Fig. 1 Clustered image map analysis of *S. ibericum* leaf and root extracts (red color: high content. blue color: low content)

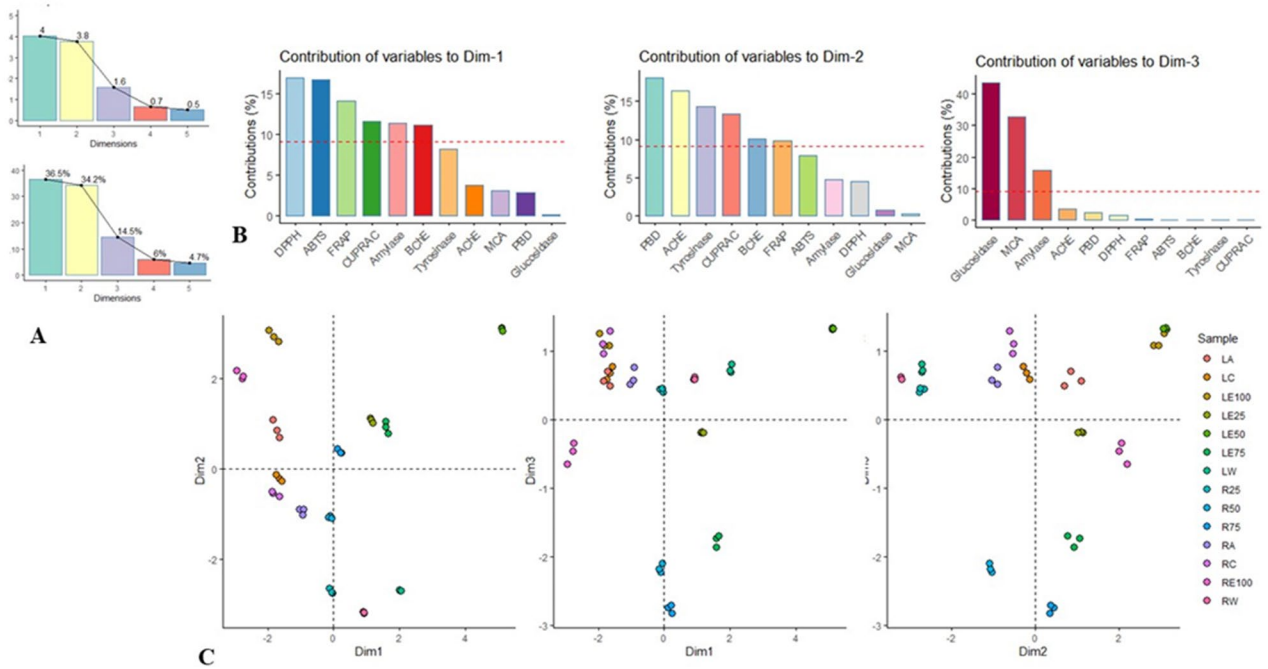


Fig. 2 Exploratory principal component analysis (PCA) of *S. ibericum* leaf and root extracts. **A** Eigenvalues and percentages of explained variances of each dimension. **B** Contribution of variables to the dimensions of PCA. **C** Scatter plot showing the distribution of the samples in the three retained dimensions

and 2.36 mmol ACAE/g in amylase and glucosidase assays, respectively) or *S. aintabicum* (0.41–0.65 mmol ACAE/g in amylase assay), *S. ibericum* exhibited slightly lower anti-amylase and anti-glucosidase properties [1, 2, 7, 22].

Exploratory multivariate analysis

To further explore the chemico-biological variability and interactions of the *S. ibericum* leaf and root extracts, multivariate analysis was performed, including CIM, PCA, HCA and Corr. By examining the resulting heatmap from the CIM analysis of the phytochemical composition, four clusters were retained (Fig. 1). Overall, the samples of cluster A (**LE50, LE25, LW** and **LE75**) were substantially rich in danshensu (**Si7**), quercetin hexoside (**Si17**), dehydrorabdosiin (**Si22**), dihydrogloboidnan B (**Si14**) and quercetin acetylhexoside (**Si19**), while the samples of cluster B (**LE100** and **LA**) were significantly rich in apigenin (**Si28**), luteolin (**Si24**) and quercetin rhamnoside (**Si18**).

PCA of the antioxidant and anti-enzymatic activities was subsequently performed; the scree plot of the eigenvalues and percentages of the explained variances were explored to select the smallest number of dimensions synthesizing most of the variation in data. Based on Kaiser' criterion [30], the first three dimensions, manifesting a variance of 36.5%, 34.2% and 14.5%, respectively, were kept (Fig. 2A). Next, the barplots were graphed to investigate the contribution of the bioactivities on the three

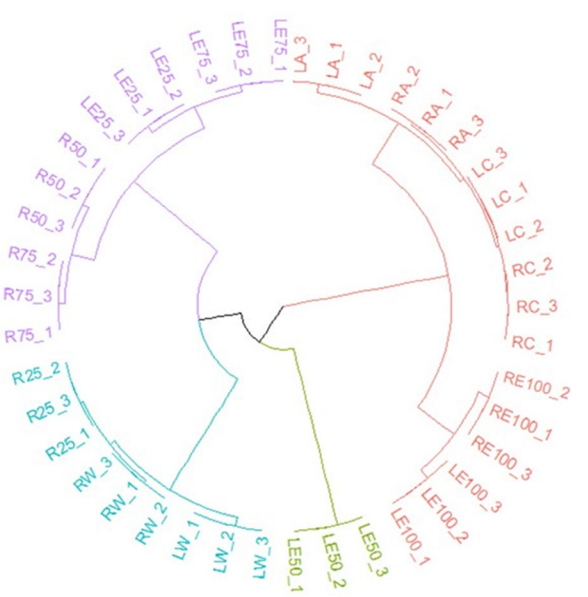


Fig. 3 Dendogram obtained from the hierarchical cluster analysis (HCA) on bioactivities of *S. ibericum* leaf and root extracts

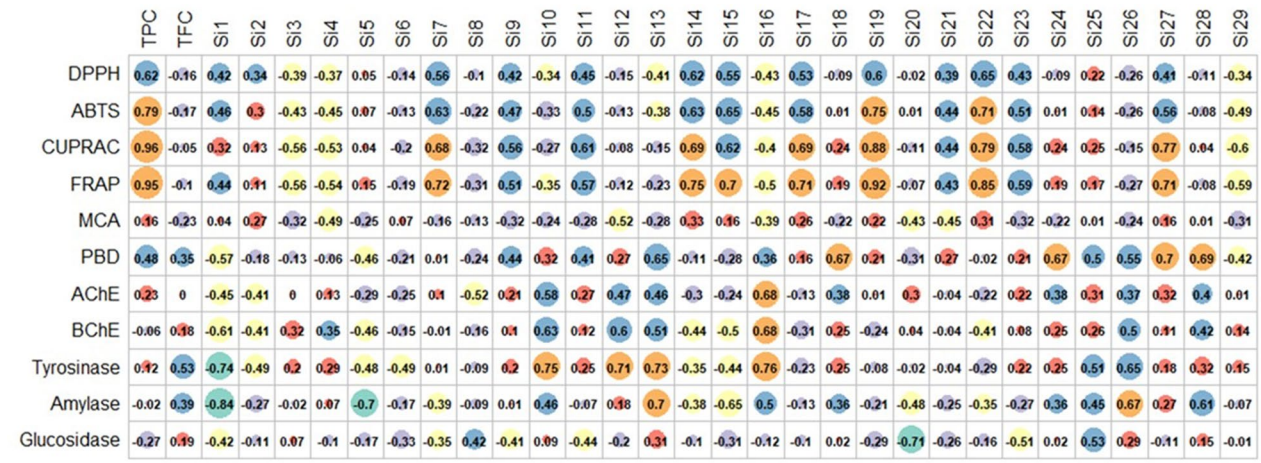


Fig. 4 Pearson's correlation analysis between the bioactivities and total phenolic (TPC), total flavonoid (TFC) and individual chemical constituents of *S. ibericum* leaf and root extracts

retained dimensions. As observed in Fig. 2B, the first dimension was predominantly linked to three bioactivities (DPPH, ABTS and FRAP), the second dimension was significantly bound to four (PBD, AChE, tyrosinase and CUPRAC), while the third dimension was essentially linked to two bioactivities (glucosidase and MCA). The scatter plots (Dim1 vs. Dim2, Dim1 vs. Dim3 and Dim2 vs. Dim3) were then examined to determine the different clusters. Despite the considerable variability that can be observed among the samples, it was not possible to identify accurately different clusters (Fig. 2C). To that end, only HCA allowed to obtain a clearer picture of the clusters (Fig. 3). As observed in the dendogram (Fig. 3), *S. ibericum* samples could be split into four major clusters. Remarkably, **LE50** stood out from the other samples, which might be related to its highest radical scavenging and reducing power activities.

Finally, to explore the relationship between the phytochemical profile of *S. ibericum* and investigated bioactivities, Corr analysis was performed. Based on the calculated Pearson's coefficients (Fig. 4), it was first noticed that the radical scavenging and reducing power activities were positively linked to the TPC. Second, several individual constituents seemed to contribute to different bioactivities; for instance, danshensu (**Si7**), dihydrogloboidnan B (**Si14**), quercetin hexoside (**Si17**), quercetin acetylhexoside (**Si19**), dehydrorabdosiin (**Si22**), cirsimaritin (**Si27**) were positively correlated with both FRAP and CUPRAC, while quercetin acetylhexoside (**Si19**) and dehydrorabdosiin (**Si22**) might be significantly involved to the ABTS radical scavenging activity. With respect to the anti-enzymatic activity, no important correlations were noticed. In essence, the different bioactivities exhibited by the extracts of *S. ibericum* can be attributed to the synergetic or additive action of multiple compounds, as previously noticed by other groups [31].

Conclusions

To the best of our knowledge, this is the first study to report the phytochemical composition and multi-biological potential of *S. ibericum*. The LC–HRMS/MS-based profiling of the 14 extracts obtained with solvents with different polarity from the roots and leaves evidenced a complex metabolite composition, with numerous phenolic acids, flavonoids, PAs, organic and oxygenated unsaturated fatty acids. Overall, the antioxidant activity, as evaluated through radical scavenging (DPPH, ABTS), reducing (CUPRAC, FRAP) and chelating assays, revealed a higher efficacy of the leaf extracts than the corresponding root extracts as well as more potent effects of the polar extracts than the non-polar extracts. With respect to the inhibitory activity against several key enzymes involved in the management of Alzheimer's disease, skin disorders and type 2 diabetes mellitus, good anti-AChE, anti-BChE and anti-tyrosinase effects were observed for some extracts of *S. ibericum*. Exploratory multivariate analysis (CIM, PCA, HCA, Corr) revealed four clusters with respect to the phytochemical profile, with one cluster rich in danshensu, quercetin hexoside, dehydrorabdosiin, dihydrogloboidnan B and quercetin acetylhexoside and four clusters with respect to the biological activities, among which sample LE50 represented an individual group. Aside from the chemosystematics importance, our study could represent a starting point toward the subsequent large-scale exploitation of this previously

uninvestigated *Symphytum* species as agricultural commodity or bio-functional ingredient for nutraceutical, pharmaceutical or cosmeceutical industries. Furthermore, to overcome the well-known toxicological issues raised by the presence of the PAs, implementing technologies that allow obtaining PAs-depleted extracts or using active formulas that contain purified fractions could be explored. Finally, further preclinical and clinical studies are imperious to confirm whether *S. ibericum* can be indeed included in the management of chronic conditions linked to oxidative stress, such as Alzheimer's disease or skin pigmentation disorders.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-022-00308-0.

Additional file 1: Figure S1. LC–HRMS/MS chromatograms (base peak chromatograms) of *Symphytum ibericum* LEAF extracts. **Figure S2.** LC–HRMS/MS chromatograms (base peak chromatograms) of *Symphytum ibericum* ROOT extracts. **Figure S3**. Fragmentation pathways proposed for rare phenolic acids **Figure S4.** Fragmentation pathways proposed for rare pyrrolizidine alkaloids

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Author contributions
AT, GZ, KIS and SVL designed the study, conducted the experiments, compiled the data, and wrote the manuscript, whereas KSW and MM approved the data and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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