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FULL PAPER

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γ-Linolenic and Stearidonic Acids from Boraginaceae of Diverse Mediterranean Origin

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Thirty Boraginaceae species from different tribes were evaluated in a search of γ-linolenic (GLA, 18:3*n*-6) and stearidonic acid (SDA, 18:4*n*-3)-rich oils. The high GLA percentages were found in the seed oils of *Symphytum bulbosum* and *S. tuberosum* subsp. *tuberosum* (27.6 and 27.2% of total fatty acids (FA)), which are unusually high values for GLA-oils in the current literature. On the whole seed, noticeable GLA percentages were found in *S. grandiflorum*, *S. tuberosum* subsp. *tuberosum* and *Borago officinalis* (7.43, 4.90, and 4.51 g/100 g, respectively). The main SDA-taxa detected in this study were *Buglossoides arvensis*, *B. incrassata* and *Glandora oleifolia* (21.3, 18.9, and 16.3% of total fatty acids). On total seed weight, *Glandora rosmarinifolia* showed the highest SDA content (3.57 g/100 g). Finally, the higher FA contents were found in *S. grandiflorum* and *Paramoltkia doerfleri* seeds (35.2 and 37.0 g/100 g, respectively). Principal component analysis showed that similarities in FA profiles allow grouping species as botanical criteria for Boraginaceae tribes do, while the FA groupings confirm the metabolic activities of desaturase and elongase enzymes. Data on the FA composition of the seed oils analyzed here suggest their potential use as functional foods and can be considered as novel sources of SDA and GLA.

Keywords: Boraginaceae, seed oil, γ-linolenic acid, stearidonic acid, principal component analysis.

Introduction

Boraginaceae is a sub cosmopolitan plant family with a center of diversity in the northern temperate zone. Taxa belonging to this family occurs worldwide with about 130 genera and 2,300 species.[1] The therapeutic effect of Boraginaceae species is related to the content of several biologically active compounds, including fatty acids (FA), flavonoids, terpenoids and polyphenols.[2] Stearidonic acid (SDA, 18:4*n*−3) and γ-linolenic acid (GLA, 18:3*n*−6) are two polyunsaturated FA (PUFA) that are commonly found in the seed oils of most Boraginaceae species.[3] GLA is a metabolite of linoleic acid (LA, 18:2*n*−6) and the first intermediate compound in the bioconversion of LA into arachidonic acid (ARA, 20:4*n*−6). GLA has been reported to exert multiple health-promoting benefits, such as anti-inflammatory, antimicrobial, and antiplatelet activities.[4,5] In addition, some clinical experiments have shown that the consumption of GLA-containing food supplements could be useful in the treatment of some diseases such as local eczema, diabetes, virus infections and some types of cancer.[6] Regarding SDA, it is a metabolite of α-linolenic acid (ALA, 18:3*n*-3) and also is the biosynthetic precursor of very long-chain PUFA (VLCPUFA), i.e., eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3).[3] EPA gives rise to eicosanoids and these often have differing properties from those of ARA-derived eicosanoids. EPA and DHA give rise to newly discovered resolvins which are anti-inflammatory and inflammation resolving.[7] SDA has been described as a potent inhibitor of cancer cell growth, and its efficiency against skin inflammation and dermatitis has been highlighted.[3] Furthermore, SDA prevents negative

effects on cardiovascular and neurological function in rat models,[8] while the bioconversion of SDA into EPA is more efficient than from ALA. This is because of the reduced activity of the Δ^6-desaturase enzyme in humans, which catalyzes the synthesis of SDA from ALA within the *n*-3 PUFA metabolic pathway.[3] This is the reason why the oils rich in SDA could become a sustainable alternative to marine fish oils and thus the dependence on overexploited marine resources could be lowered.[9]

The commercial sources of GLA are the seed oils of evening primrose (9.6 % GLA of total FA), borage (23 %) and blackcurrant (15–20 %), and some microbiological oils such as those of *Mucor javanicus* (15–18%) and *Spirulina platensis* (21%).[10] The main commercial sources of SDA are the seed oils obtained from some species of Boraginaceae, Grossulariaceae, Caryophyllaceae and Primulaceae,[3] highlighting *Echium* (Boraginaceae, ~10–15% SDA).[3] Recently, *Buglossoides arvensis* seed oil (Ahiflower oil®) is receiving increased demand due to its high SDA content. In this regard, Lefort et al.[11] showed that the consumption of Ahiflower oil® was associated with an anti-inflammatory phenotype in healthy subjects.

New Boraginaceae species have recently been reported as sources of GLA- and/or SDA-rich oils; for instance, *Borago morisiana* (24.4% GLA) and *B. pygmaea* (22.9% GLA).[12,13] Considering that both GLA and SDA provides many health benefits, searching for novel GLA- and SDA-oils is timely. Given that the FA profiles of most Boraginaceae species are still unknown, this work was designed to unravel the FA profiles of several Boraginaceae species collected in Southern Europe, thus contributing to improve the knowledge on potential new sources of bioactive GLA and SDA.

Results and Discussion

The FA profiles (% of total FA) and total FA content of the species analyzed in this work are summarized in *Table 1*. The seeds of *Paramoltkia doerfleri* and *Symphytum grandiflorum* had the highest FA content (37.0 and 35.2%, respectively), whereas most of the studied species showed values below 20%. *Brunnera macrophylla* (Boragineae tribe) and *Aegonychon gastonii* (Lithospermeae tribe) showed the highest proportion of saturated FA (SFA) (43.4 and 37.3%, respectively), and in these species palmitic acid (PA, 16:0) had the highest values (>30%), followed by most species of the Boragineae tribe, whose PA values ranged

Table 1. Fatty acid profiles and total fatty acids of seeds of Boraginaceae species.

Species	Relative content (% of total FA)[a,b]														Σ SFA	Σ MUFA	Σ PUFA	*n*-3	*n*-6	g FA/ 100 g seed	g GLA/ 100 g seed	g SDA/ 100 g seed
	16:0	16:1*n*-7	18:0	18:1*n*-9	18:1*n*-7	18:2*n*-6	18:3*n*-6	18:3*n*-3	18:4*n*-3	20:0	20:1*n*-9	22:0	22:1*n*-9	24:1*n*-9								
Tribe Boragineae																						
Anchusella cretica (A)	12.8 ± 0.4^{e}	nd	2.8 ± 0.0^{f}	17.0 ± 0.2^{i}	0.5 ± 0.5^{b}	23.1 ± 1.0^{g}	5.8 ± 0.4^{h}	26.6 ± 0.6^{g}	5.5 ± 0.4^{h}	nd	2.8 ± 0.0^{d}	$0.5\pm0.0^{a,b}$	1.8 ± 0.1^{d}	nd	16.1	22.1	61.0	32.1	28.9	16.4 ± 2.1^{f}	0.95	0.90
A. cretica (B)	10.9 ± 0.2^{e}	nd	2.2 ± 0.4^{f}	17.7 ± 0.1^{i}	0.5 ± 0.5^{b}	$22.8\pm0.5^{g,h}$	5.5 ± 0.4^{h}	28.5 ± 0.7^{f}	5.0 ± 0.3^{h}	0.3 ± 0.0^{d}	$3.2\pm0.0^{c,d}$	0.3 ± 0.0^{c}	2.2 ± 0.0^{c}	0.5 ± 0.1^{d}	13.7	24.1	61.8	33.5	28.3	15.8 ± 1.3^{g}	0.87	0.79
Borago officinalis	10.9 ± 0.3^{e}	0.3 ± 0.1^{a}	$5.0\pm0.3^{b,c}$	$21.6\pm1.2^{f,g}$	nd	$33.7\pm1.1^{b,c}$	18.8 ± 1.3^{c}	1.3 ± 0.1^{o}	0.9 ± 0.2^{j}	0.3 ± 0.1^{d}	4.1 ± 0.3^{b}	0.2 ± 0.1^{c}	$1.6\pm0.2^{d,e}$	1.3 ± 0.1^{c}	16.4	28.9	54.7	2.2	52.5	$24.0\pm1.2^{c,d}$	4.51	0.22
Brunnera macrophylla	37.2 ± 1.7^{a}	nd	6.2 ± 0.4^{a}	10.1 ± 0.8^{n}	nd	$28.1\pm1.3^{d,e}$	9.1 ± 0.5^{f}	9.4 ± 0.7^{l}	nd	nd	nd	nd	nd	nd	43.4	10.1	46.6	9.4	37.2	8.4 ± 0.5^{i}	0.76	nd
Hormuzakia aggregate	11.8 ± 0.9^{e}	0.3 ± 0.1^{a}	4.7 ± 0.2^{c}	34.5 ± 2.0^{b}	nd	37.4 ± 2.2^{a}	$6.3\pm0.4^{g,h}$	0.9 ± 0.2^{p}	0.3 ± 0.0^{k}	0.1 ± 0.0^{f}	2.3 ± 0.3^{d}	nd	1.2 ± 0.3^{e}	0.2 ± 0.0^{e}	16.6	38.5	44.9	1.2	43.7	15.0 ± 1.0^{g}	0.95	0.05
Symphytum bulbosum	13.9 ± 1.0^{d}	0.1 ± 0.0	$1.4\pm0.2^{g,h}$	11.0 ± 1.2^{m}	nd	25.8 ± 1.4^{f}	27.6 ± 1.1^{a}	12.6 ± 0.8^{k}	3.9 ± 0.2^{i}	1.6 ± 0.1a	nd	0.2 ± 0.1^{c}	0.1 ± 0.0^{g}	nd	17.1	11.2	69.9	16.5	53.4	12.9 ± 0.7^{h}	3.56	0.50
S. bulbosum immature	22.2 ± 1.9^{d}	0.3 ± 0.1^{a}	0.4 ± 0.1^{i}	5.3 ± 0.3^{q}	nd	$28.1\pm1.5^{d,e}$	$12.7\pm0.8^{d,e}$	20.9 ± 1.1^{i}	$4.1\pm0.2^{h,i}$	0.5 ± 0.1c	0.4 ± 0.2e	nd	nd	0.8 ± 0.2^{d}	23.1	6.8	65.8	25.0	40.8	8.7 ± 0.8^{i}	1.1.0	0.36
S. grandiflorum	$10.5\pm0.7^{e,f}$	$0.2\pm0.0^{a,b}$	$3.8\pm0.1^{d,e}$	$19.9\pm0.7^{g,h}$	nd	$34.\ 6\pm0.8^{b}$	21.1 ± 0.6^{b}	4.5 ± 0.4^{n}	1.3 ± 0.1^{j}	0.3 ± 0.0d	2.3 ± 0.2d	nd	nd	1.3 ± 0.0^{c}	14.6	23.7	61.5	5.8	55.7	35.2 ± 2.5^{a}	7.43	0.46
S. gussonei immature	26.2 ± 0.8^{c}	nd	2.4 ± 0.2^{f}	4.0 ± 0.0^{q}	nd	27.2 ± 1.4^{e}	$10.2\pm1.0^{e,f}$	25.0 ± 1.2^{g}	5.1 ± 0.3^{h}	nd	nd	nd	nd	nd	28.6	4.0	67.5	30.1	37.4	9.0 ± 0.5^{i}	0.92	0.46
S. ibiricum	$12.4\pm0.5^{d,e}$	$0.2\pm0.0^{a,b}$	$3.7\pm0.2^{d,e}$	$22.3\pm1.3^{e,f}$	nd	32.2 ± 1.0^{c}	18.9 ± 0.7^{c}	4.2 ± 0.2^{n}	1.2 ± 0.0^{j}	0.3 ± 0.0^{d}	2.1 ± 0.0^{d}	0.2 ± 0.1^{c}	0.9 ± 0.0^{e}	1.0 ± 0.1^{c}	16.6	26.5	56.5	5.4	51.1	6.3 ± 0.2^{j}	1.19	0.08
S. tuberosum. subsp. *tuberosum*	11.5 ± 0.7^{e}	nd	3.0 ± 0.3^{e}	$13.2\pm0.8^{k,l}$	0.2 ± 0.1^{e}	$33.7\pm2.0^{b,c}$	27.2 ± 1.0^{a}	6.3 ± 0.6^{m}	2.4 ± 0.2^{i}	nd	nd	nd	0.9 ± 0.2^{e}	1.2 ± 0.1^{c}	14.5	15.5	69.6	8.7	60.9	$18.0\pm0.6^{e,f}$	4.90	0.43
Tribe Eritrichieae																						
Amsinckia tessellate	$10.3\pm0.2^{e,f}$	0.1 ± 0.0^{b}	3.4 ± 0.2^{e}	26.8 ± 0.0^{c}	0.2 ± 0.0^{e}	13.0 ± 0.0^{k}	$8.0\pm0.2^{f,g}$	20.2 ± 0.1^{i}	12.2 ± 0.4^{e}	0.2 ± 0.0e	2.2 ± 0.0^{d}	0.1 ± 0.0^{d}	1.2 ± 0.0^{e}	1.3 ± 0.0^{c}	14.0	31.8	53.4	32.4	21.0	18.8 ± 0.4^{e}	1.50	2.29
A. vernicosa var. *furcate*	11.3 ± 0.1^{e}	$0.2\pm0.1^{a,b}$	$2.7\pm0.9^{e,f}$	31.1 ± 0.8^{c}	$0.3\pm1.3^{d,e}$	$26.4\pm0.7^{e,f}$	21.1 ± 0.0^{b}	1.4 ± 0.1^{o}	1.1 ± 0.3^{j}	0.2 ± 0.1^{e}	3.0 ± 0.0^{c}	nd	nd	1.0 ± 0.0^{c}	14.2	35.6	50.0	2.5	47.5	19.2 ± 2.0^{e}	4.05	0.21
Tribe Lithospermeae																						
Aegonychon gastonii	31.0 ± 1.4^{b}	nd	5.5 ± 0.2^{b}	7.0 ± 0.3^{p}	0.9 ± 0.0^{a}	29.1 ± 1.0^{d}	8.5 ± 1.4^{f}	14.2 ± 0.2^{j}	3.0 ± 0.7^{i}	nd	nd	nd	nd	nd	37.3	7.9	54.8	17.2	37.6	14.3 ± 0.0^{g}	1.22	0.43
A. purpureo-caeruleum	7.5 ± 0.3^{g}	nd	2.2 ± 0.1^{f}	$9.4\pm0.7^{n,l}$	nd	$22.3\pm1.5^{g,h}$	17.5 ± 1.1^{c}	33.9 ± 2.1^{d}	7.2 ± 0.4^{g}	nd	nd	nd	nd	nd	9.7	9.4	80.9	41.1	39.8	10.0 ± 0.3^{i}	1.75	0.72
Buglossoides arvensis (A)	8.9 ± 0.2^{f}	nd	2.3 ± 0.2^{f}	7.6 ± 0.4^{o}	nd	11.5 ± 1.1^{l}	7.4 ± 0.5^{g}	40.9 ± 2.0^{b}	21.3 ± 1.4^{a}	nd	nd	nd	nd	nd	11.2	7.6	81.1	62.2	18.9	15.0 ± 0.4^{g}	1.11	3.20
B. arvensis (B)	7.5 ± 0.1^{g}	nd	$1.9\pm0.5^{f,g}$	8.9 ± 0.3^{l}	0.9 ± 0.1^{a}	13.0 ± 1.6^{k}	5.4 ± 0.2^{h}	45.6 ± 2.8^{a}	16.8 ± 1.0^{c}	nd	nd	nd	nd	nd	9.4	9.8	80.8	62.4	18.4	17.6 ± 1.9^{f}	0.95	2.96
B. arvensis (C)	$8.3\pm0.5^{f,g}$	nd	2.9 ± 0.3^{e}	6.9 ± 0.4^{p}	nd	$12.1\pm1.1^{k,l}$	$6.5\pm0.3^{g,h}$	43.6 ± 1.9^{a}	18.7 ± 1.1^{b}	nd	nd	nd	nd	nd	11.2	6.9	80.9	62.3	18.6	$16.0\pm0.5^{f,g}$	1.04	2.99
B. incrassate	$10.1\pm0.6^{e,f}$	nd	2.9 ± 0.2^{e}	$8.1\pm0.4^{l,o}$	0.3 ± 0.1^{d}	$12.3\pm0.8^{k,l}$	6.7 ± 0.5^{g}	39.9 ± 2.0^{b}	18.9 ± 1.0^{b}	nd	nd	nd	nd	nd	13.0	8.4	77.8	58.8	19.0	14.0 ± 0.4^{g}	0.94	2.65
B. minima	13.4 ± 1.1^{d}	nd	4.8 ± 0.3^{c}	$8.4\pm0.1^{l,o}$	nd	19.0 ± 0.7^{i}	9.2 ± 0.3^{f}	31.2 ± 1.6^{d}	12.9 ± 0.8^{e}	nd	nd	nd	nd	nd	18.2	8.4	72.3	44.1	28.2	$25.3\pm1.3^{b,c}$	2.33	3.26
Glandora diffusa	9.0 ± 0.4^{f}	0.3 ± 0.1^{a}	$4.6\pm0.3^{c,d}$	$18.4\pm0.6^{h,i}$	0.3 ± 0.1^{d}	19.4 ± 1.1^{i}	$12.8\pm0.9^{d,e}$	27.5 ± 1.3^{f}	5.7 ± 0.4^{h}	0.2 ± 0.1e	1.0 ± 0.2e	nd	0.2 ± 0.0g	nd	13.8	20.2	65.4	33.2	32.2	17.2 ± 1.0^{f}	2.20	0.98
G. oleifolia	13.6 ± 0.4^{d}	0.3 ± 0.1^{a}	2.2 ± 0.2^{f}	$18.2\pm1.0^{h,i}$	$0.4\pm0.1^{c,d}$	16.0 ± 1.0^{j}	7.4 ± 0.4^{g}	25.0 ± 1.3^{g}	$16.3\pm1.1^{c,d}$	nd	nd	nd	nd	0.2 ± 0.0^{e}	15.8	19.1	64.7	41.3	23.4	14.9 ± 0.4^{g}	1.10	2.43
G. rosmarinifolia	12.6 ± 0.8^{d}	nd	$2.5\pm0.5^{e,f}$	17.8 ± 1.3^{i}	$0.4\pm0.0^{c,d}$	17.5 ± 0.9^{j}	7.5 ± 0.2^{g}	25.4 ± 2.4^{g}	15.4 ± 1.0^{d}	nd	nd	nd	0.4 ± 0.0^{f}	0.2 ± 0.0^{e}	15.1	18.8	65.8	40.8	25.0	23.2 ± 0.4^{d}	1.74	3.57
Halacsya sendtneri (A)	5.0 ± 0.2^{h}	0.1 ± 0.1^{b}	3.0 ± 0.1^{e}	11.9 ± 1.1^{m}	$0.4\pm0.1^{c,d}$	$18.9\pm1.1^{i,j}$	$8.6\pm0.5^{f,g}$	43.8 ± 2.4^{a}	7.2 ± 0.5^{g}	0.1 ± 0.1^{f}	0.6 ± 0.1^{e}	0.1 ± 0.1^{d}	0.1 ± 0.0^{g}	0.1 ± 0.0^{e}	8.2	13.2	78.5	51.0	27.5	15.7 ± 0.5^{g}	1.35	1.13
H. sendtneri (B)	5.1 ± 0.3^{h}	0.1 ± 0.1^{b}	2.3 ± 0.4^{f}	15.1 ± 0.9^{j}	0.6 ± 0.1^{b}	19.9 ± 1.4^{i}	8.9 ± 0.5^{f}	39.9 ± 1.7^{b}	7.1 ± 0.4^{g}	0.1 ± 0.0^{f}	0.6 ± 0.2^{e}	0.1 ± 0.0^{d}	0.1 ± 0.0^{g}	nd	7.6	16.5	75.8	47.0	28.8	7.9 ± 0.6^{j}	0.70	0.56
Onosma echioides	5.8 ± 0.2^{h}	nd	1.8 ± 0.1^{g}	17.2 ± 1.0^{i}	$0.2\pm0.0^{d,e}$	23.7 ± 0.2^{g}	9.0 ± 0.2^{f}	32.4 ± 0.6^{d}	8.7 ± 0.5^{f}	nd	0.7 ± 0.0^{e}	nd	0.2 ± 0.0^{g}	nd	7.6	18.3	73.8	41.1	32.7	19.9 ± 0.8^{e}	1.79	1.73
O. polyphyllum	5.6 ± 0.3^{h}	nd	2.9 ± 0.1^{d}	15.3 ± 0.2^{j}	$0.2\pm0.0^{d,e}$	$28.8\pm0.3^{d,e}$	13.6 ± 0.2^{d}	23.5 ± 0.4^{h}	8.9 ± 0.0^{f}	0.2 ± 0.0^{e}	0.9 ± 0.0^{e}	nd	$0.3\pm0.0^{f,g}$	nd	8.7	16.7	74.8	32.4	42.4	26.1 ± 1.1^{b}	3.55	2.32
O. heterophylla (A)	10.3 ± 0.6^{e}	nd	1.8 ± 0.2^{g}	11.4 ± 0.9^{m}	1.0 ± 0.2^{a}	21.4 ± 1.5^{h}	7.3 ± 0.6^{g}	37.4 ± 2.1^{c}	$8.6\pm0.4^{f,g}$	nd	0.8 ± 0.1^{e}	nd	nd	nd	12.1	13.2	74.7	46.0	28.7	22.3 ± 1.4^{d}	1.63	1.92
O. heterophylla (B)	6.6 ± 0.5^{g}	nd	3.0 ± 0.3^{e}	$14.8\pm1.3^{j,k}$	$0.4\pm0.1^{c,d}$	$20.9\pm1.2^{h,i}$	$6.6\pm0.5^{g,h}$	$38.3\pm2.1^{b,c}$	$8.3\pm0.2^{f,g}$	nd	0.7 ± 0.2^{e}	nd	0.2 ± 0.1^{g}	nd	9.6	16.1	74.1	46.6	27.5	10.9 ± 0.3^{i}	0.72	0.90
O. stellulata	$8.1\pm0.6^{f,g}$	nd	2.3 ± 0.2^{f}	$12.4\pm0.9^{l,m}$	$0.5\pm0.1^{b,c}$	$22.8\pm1.6^{g,h}$	11.4 ± 0.8^{e}	31.6 ± 1.8^{d}	9.5 ± 0.4^{f}	nd	0.7 ± 0.1^{e}	$0.2\pm0.1^{c,d}$	$0.3\pm0.0^{f,g}$	nd	10.6	13.9	75.3	41.1	34.2	$9.9\pm0.2^{i,j}$	1.13	0.94
Paramoltkia doerfleri	$6.4\pm0.4^{g,h}$	$0.2\pm0.2^{a,b}$	2.0 ± 0.2^{f}	$22.1\pm1.6^{e,f}$	$0.5\pm0.2^{b,c}$	$18.7\pm1.5^{i,j}$	$10.4\pm0.7^{e,f}$	$29.4\pm1.4^{e,f}$	8.7 ± 0.5^{f}	0.1 ± 0.0^{f}	0.8 ± 0.2^{e}	0.1 ± 0.0^{d}	0.2 ± 0.0^{g}	0.1 ± 0.0^{e}	8.6	23.9	67.2	38.1	29.1	37.0 ± 2.1^{a}	3.85	3.22
Tribe Cynoglosseae																						
Cynoglossum montanum	6.3 ± 0.0^{g}	nd	1.0 ± 0.1^{h}	25.6 ± 0.2^{d}	0.2 ± 0.0^{e}	32.5 ± 0.1^{c}	9.5 ± 0.0^{f}	4.3 ± 0.3^{n}	2.0 ± 0.0^{j}	0.4 ± 0.1^{c}	5.2 ± 0.3^{a}	0.6 ± 0.0^{a}	10.0 ± 0.1^{a}	2.5 ± 0.3^{b}	8.3	43.5	48.3	6.3	42.0	15.8 ± 3.5^{g}	1.50	0.32
Omphalodes cappadocica	13.6 ± 0.6^{d}	nd	$1.8\pm0.2^{f,g}$	22.5 ± 0.1^{e}	nd	$24.2\pm1.4^{f,g}$	8.8 ± 0.4^{f}	9.4 ± 0.7^{l}	3.9 ± 0.2^{i}	0.9 ± 0.1^{b}	5.8 ± 0.3^{a}	nd	3.9 ± 0.3^{b}	3.5 ± 0.3^{a}	16.3	35.7	46.3	13.3	33.0	6.4 ± 0.6^{j}	0.56	0.25
Tribe Myosotideae																						
Myosotis capitate	10.8 ± 0.0^{e}	$0.2\pm0.0^{a,b}$	4.2 ± 0.2^{d}	27.9 ± 0.5^{c}	0.1 ± 0.0^{e}	$20.9\pm0.0^{h,i}$	11.1 ± 0.1^{e}	8.7 ± 0.4^{l}	$6.3\pm0.3^{g,h}$	0.5 ± 0.2^{c}	4.3 ± 0.2^{b}	0.3 ± 0.0^{c}	2.1 ± 0.0^{c}	1.9 ± 0.3^{b}	15.8	36.5	47	15.0	32.0	22.5 ± 1.7^{d}	2.50	1.42
Tribe Hydrophylleae																						
Pholistoma auritum	$12.3\pm0.3^{d,e}$	nd	$1.9\pm0.2^{f,g}$	56.1 ± 0.2^{a}	nd	$26.0\pm0.5^{e,f}$	nd	2.1 ± 0.3^{o}	1.6 ± 0.4^{j}	nd	nd	nd	nd	nd	14.2	56.1	29.7	3.7	26.0	12.8 ± 1.1^{h}	nd	0.20

[a] Minor FA that contributed with less than 0.1 % of the total FA were ignored; nd: no detected. [b] Data represent the mean of three complete independent experiments ± SD. Within each column, means followed by the same superscript letter are not significantly different from one another by Fisher's LSD Test ($P < 0.05$).

between 10.5 and 22.2%. The highest proportion of monounsaturated FA (MUFA) was obtained in *Pholistoma auritum* (56.1%), due to its high oleic acid (OA, 18:1*n*-9) figures. *Cynoglossum montanum*, *Hormuzakia aggregata* and *Myosotis capitata* also contained high MUFA amounts (43.5, 38.5 and 36.5% of total FA, respectively). OA reached high values in Hydrophylleae, Borageineae and Eritrichieae, and *P. auritum* (56.1%), *H. aggregata* (34.5%) and *Amsinckia vernicosa* var. *furcata* (31.1%) had the highest percentages of total FA, whereas the lowest OA values were achieved in *Buglossoides* and *Aegonychon* genus (Lithospermeae tribe), ranging from 6.9 to 9.4% of total FA.

The PUFA detected in the Boraginaceae species analyzed here were LA, ALA, GLA and SDA. The highest PUFA percentages were found in species belonging to Lithospermeae tribe, highlighting *B. arvensis* (80.8–81.1% of total FA), closely followed by *Aegonychon purpurocaeruleum* (80.9%). Considering the *n*-3 series, *B. arvensis* reached the higher values (62.2–62.4%), while within the *n*-6 series *Symphytum tuberosum* subsp. *tuberosum* had the higher figures (60.9% of total FA). *H. aggregata* (Borageineae tribe) showed the highest LA% (37.4), while among the remaining species, highlights *C. montanum* (Cynoglosseae tribe, 32.5% of total FA). Lithospermeae tribe showed the highest ALA%, and *B. arvensis* showed the highest values of the range (40.9–45.6% of total FA).

The highest relative contents of GLA were found in mature seeds of *Symphytum bulbosum* (27.6%), *S. tuberosum* subsp. *tuberosum* A (27.2%), and *S. grandiflorum* (21.1%) (Borageineae tribe), and *A. vernicosa* var. *furcata* (21.1%, Eritrichieae tribe). Other species having high relative GLA amounts were *B. officinalis* and *S. ibiricum* (18.8 and 18.9%, respectively). Species from Cynoglosseae tribe contain low GLA percentages (8.8–9.5 of total FA), whereas values for other Cynoglosseae species from Morocco and Germany were below 8%,[14,15] thus confirming that the relatively low GLA % is a feature of this tribe. However, poor GLA producers can appear in all the tribes of Boraginaceae, as *Anchusella cretica* and *Hormuzakia aggregata* in Borageineae, and *B. arvensis* and *Onosma heterophylla* in Lithospermeae (*Table 1*). Moreover, *P. auritum* (Hydrophylleae) lacked detectable GLA amounts. On the whole seeds, GLA ranged from 0.56 in *Omphalodes cappadocica* (Cynoglosseae) to 7.43 g/100 g in *S. grandiflorum* (Borageineae) (*Table 1*).

High relative content of GLA was previously found in some seed oils of Boraginaceae, for instance in *Echium callithyrsum* (26.31%),[10] *B. morisiana* (24.6%),[12,13] and *E. gentianoides* (27.4%).[16] The high GLA percentages of total FA in the seed oil of some Boraginaceae species are due to genetic features, although temperature, humidity, soil composition and other factors could marginally influence the FA profiles.[16] In this regard, Özcan[17] reported variations for FA percentage of total FA among several *Symphytum* species, depending on the developmental stages of the seeds, and the need to obtain data on genetic and biochemical variations for molecular delimitation of *Symphytum* was suggested, and also for high-GLA genotypes selection. Because of these facts, genetic and agronomic variables should be optimized for an efficient cultivation of Boraginaceae species. In this regard, Urrestarazu et al.[18] concluded that GLA content in borage seeds tripled by increasing salinity in the nutrient solution. Conversely, Jaffel-Hamza et al.[19] found that salinity in irrigation water induced a decrease in PUFA content when applied to borage plants, whereas the SFA fraction increased. Hafid et al.[20] found that in borage plants the GLA content in seed oil was not affected by nitrogen application rate and GLA content increases as the environmental temperature decreases during seed development. Regarding to genetic variables, De Haro et al.[21] reported that the seeds of white flowered cultivated genotypes of borage plants had higher GLA percentage than blue flowered wild seeds.

As for SDA, the highest percentages of total FA were detected in the tribe Lithospermeae, and *B. arvensis* A (21.3%), *B. arvensis* C (18.7%) and *B. incrassata* subsp. *incrassata* (18.9%) reached the highest values, followed by other Lithospermeae species: *Glandora oleifolia* (16.3%) and G. *rosmarinifolia* (15.4%). Interestingly, *Onosma* species from Albania showed SDA in the 8.3–9.5% range, which was higher than the one reported for other Turkish *Onosma* species (0.08–7.22%).[22] Such differences could be due to genetic characteristics, considering that the data from both studies correspond to different species, although neither climatic factors nor the influence of the analytical methodology used in both works could be ruled out. The remaining seeds had inconspicuous SDA percentages of total FA, and *B. officinalis* (0.9%) and *H. aggregata* (0.3%) had the lowest values, while in *B. macrophylla* it was not detected. Among commercial sources, the seed oil from viper's bugloss (*E. plantagineum*) is characterized by a relatively high content of SDA (14%).[3] Recently, the seed oil from *B. arvensis* (Ahiflower oil®) has begun to commercialize due to its high SDA content.[11] In this regard, SDA and GLA levels in Ahiflower oil® (17.3 and 4.9% of total FA) are lower than the amounts detected in the *Buglos-*

soides species analyzed in this study. Kuhnt et al.[15] reported SDA content (% of total FA) for various species collected in botanic gardens of Germany, and *Omphalodes linifolia* (8.4%) and *Aegonychon purpurocaeruleum* (6.1%) reached the highest values. Interestingly, *A. purpurocaeruleum* from Germany had SDA percentage similar to the one detected here. Moreover, most species from Boragineae tribe showed SDA content lower than 5% of total FA, despite the high relative content of GLA, and this fact agrees with previous reports from Guil-Guerrero et al.[14] Finally, on the whole seeds, *G. rosmarinifolia* reached the highest absolute SDA amount (3.57 g/100 g).

Functional foods are those containing bioactive FA, as well as bioactive components such as tocopherols, phytosterols and phenolics, among others healthy compounds.[23] Thus, the GLA- and SDA-rich species detected in this work could be advantageous functional oils producers. It has been reported that the intake of SDA-oils such as *Echium* oil increases plasma ALA, SDA, EPA and DHA concentrations.[24] In this regard, Lemke et al.[25] concluded that a supplementation with 4.2 g SDA/day significantly increased the omega-3 index and the increase was not significantly different from that obtained after supplementation with 1.0 g EPA/day. Although functional foods have become popular worldwide, the EU market is still underdeveloped and the legal regulations of functional oils is not yet fully developed in many countries.[26,27] Functional food regulation should be extensive and complex, especially when health claims are involved. In this regard, the EU Regulation EC 1924/2006 on nutrition and health claims was stablished on foodstuffs.[28] Health claims are defined as pertaining to relations between food and health either about a function of the body (Article 13 claims), or about reducing a risk factor for a disease (Article 14a claims). Based on this EC Regulation, Tan[29] described virgin avocado oil as a functional oil due to its bioactive compounds, which has positive effects in the management of chronic diseases such as hypertension, diabetes, and fatty liver disease. Similarly, Boraginaceae species represent a resource of pharmacological and nutraceutical valuable compounds, because of their high concentrations of GLA and/or SDA, tocopherols, squalene, and sterols.[30] Thus, some Boraginaceae oils could be considered potentially functional ones, as is the novel food *Echium* oil, which was approved for consumption by 2008/558/EC Regulation.[31] Moreover, different studies have remarked the antioxidant activity of borage extracts (obtained from defatted seeds), especially related to their content in phenolic compounds.[30,32] All these facts have promoted the marketing of functional oils obtained from some species of Boraginaceae, which is continuously growing. Regarding to by-products, Soto et al. concluded that borage seed oil obtainment by cold pressing process using enzymatically-assisted extraction increased solids and phenolic compounds recuperation from defatted meal.[33]

Finally, it is worthy to consider that the high amounts of GLA and SDA contained in the seed oil of some of the species checked in this work makes them candidates to be cultivated. Such culture would be implemented not only for food purposes, but also for the purification of both PUFA according to several procedures.[34]

Principal Component Analysis

The possible correlation between FA profiles and phylogenetic relationships among the Boraginaceae species analyzed in this study was performed through PCA. Similar analysis was performed for *Echium* species from Macaronesia,[10] several European GLA-producer species,[35] and *Ribes* taxa.[23] In all these works, the taxonomic sections defined by morphological or genetic data and PUFA profiles were adequately correlated.

In this study, PCA was performed using all FA data (% of total FA) excepting those of 16:1*n*-7 and 18:1*n*-7, because their small percentages greatly distorted the model. In this PCA, 12 components have been extracted as requested. Together, they explain 100.0% of the variability in the original data. In the present analysis, the first two PC explained 38.7 and 19.8% of the variance, respectively, which represent the 58.5% of the total variance. When considering the three first components, a 70.3% of the total variance was accumulated. However, in the resulting 3D plots, no improvement was obtained in the visualization of the variables nor of the observations with respect to that obtained in the 2D plots, thus, the following discussion is based in the latter plots.

Figure 1 shows the plot of the two first component weights. In this analysis, all FA have a high influence on the model. All *n*-6 and *n*-9 FA and SFA have positive loads along the PC1, while ALA and SDA have negative loads; for PC2, PA, SA and *n*-6 PUFA have negative loads. As can be noted, the variables ALA and SDA have similar and negative high loads for PC1 and positive for PC2, which allows to both variables to be relatively close in the plot, being highly correlated ($r = 0.7605$; $P = 0.0000$). This situation can be interpreted

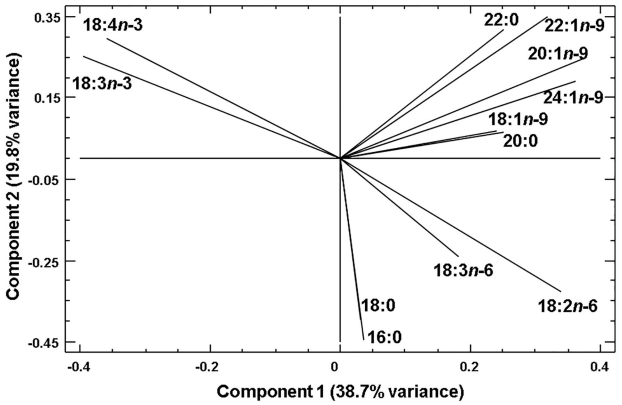

Figure 1. Loading plot analysis for the two first principal components of Boraginaceae species based on percentages of fatty acid of total FA.

considering that SDA constitute the metabolic result of ALA, trough the Δ^6-desaturase enzyme activity. The same situation can be noted for LA and GLA ($r=$ 0.4756; $P=0.0039$), the last being also de metabolic product of the former by the same enzyme. Both *n*-6 PUFA have similar loads and are placed symmetrically opposite to the *n*-3 PUFA. The position in the plot for the two first component weights of *n*-6 and *n*-3 PUFA, suggests that the Δ^6-desaturase enzyme have preferential affinity to desaturate either LA or ALA, which is consistent with the negative correlation between LA and ALA ($r=-0.7809$; $P=0.0000$), and between GLA and SDA ($r=-0.4072$; $P=0.0152$). However, it has been pointed out that the activity of Δ^6-desaturase enzyme might depend on substrate availability rather than substrate specificity in *B. arvensis*.[36]

The Δ^6-desaturase enzyme catalyzes the final step of FA biosynthesis pathway in plants, where high amounts of both LA and/or ALA substrates are present. So, it is unlikely that environmental factors or substrate competition inhibit Δ^6-desaturase activity. Therefore, differential activity of this enzyme in Boraginaceae species can be related to factors as changes in the secondary structure or active sites, deficiencies in substrate binding motifs, and/or weak expression of the enzyme.[37]

PA and stearic acid (SA, 18:0) are closely correlated ($r=0.4076$; $P=0.0151$), and present high and negative loads for PC2. This location is consistent with their simultaneous occurrence in the plastid of cells. From this organelle they are exported together with OA (derived from the SA acyl desaturase activity) to the cytosol were an acyl-CoA pool is obtained. Very long-chain FA (VLCFA) are synthesized by a microsomal FA elongation (FAE) system by the sequential addition of C_2H_4 moieties, which are derived from malonyl-CoA to preexisting SA. The subsequent elongation and desaturation activity in the cell microsome can be noted in the upper right quadrant of *Figure 1*. Elongase enzymes produces VLCFA: 20:0 and 22:0 from SA, while 20:1*n*-9, 22:1*n*-9 and 24:1*n*-9 are biosynthesized by elongation from OA. Although all these FA are closely located in the plot, there is only a statistically significant correlation between OA and 20:1*n*-9 ($r=$ 0.3476, $P=0.0407$). This metabolic scheme on SFA and MFA for Boraginaceae seeds agrees with that described for *Arabidopsis* seeds.[38]

Figure 2 shows the scatterplot of the samples on the first two components, where the species are distributed according to their scores for the different FA. This plot clearly shows that Lithospermeae species have negative scores for PC1, which can be attributed to both their relatively high *n*-3 PUFA content, and to and absence of MUFA and VLCFA. On the other hand, the Borageneae cluster is distinguished mainly by the increased relative levels of LA and GLA compared to species from other tribes, although sample 9, immature *S. gussonei*, was grouped within Lithospermeae cluster. Nevertheless, for this species the FA profile was not conclusive, since the FA profiles are quite different between mature and immature seeds. Finally, tribes Hydrophylleae and Cynoglosseae have positive scores for PC1 and PC2, and their location in the plot is related to their relatively high MUFA and VLCFA contents. However, for the latter tribes is difficult to conclude on taxonomical relationships, because only a representative species of each tribe was analyzed.

As currently and previously argued,[10,35] the PCA analysis based on the FA profile can be successfully used for differentiation of seed oils from Boraginaceae plants. However, the proper classification of unidentified Boraginaceae oils or prediction of the FA profile for unexamined Boraginaceae plants requires to build a more advanced discriminating model, using analyzes of more species from different tribes, as well as other chemometric methods such as a partial least squares discriminant analysis (PLS-DA).

Conclusions

According to our findings, the seeds of *S. grandiflorum* and *P. doerfleri* showed the higher FA content among all studied species in this work (35.2 and 37.0 g/100 g, respectively), whereas the remaining species showed values between 6.3–26.1 g/100 g. Regarding GLA

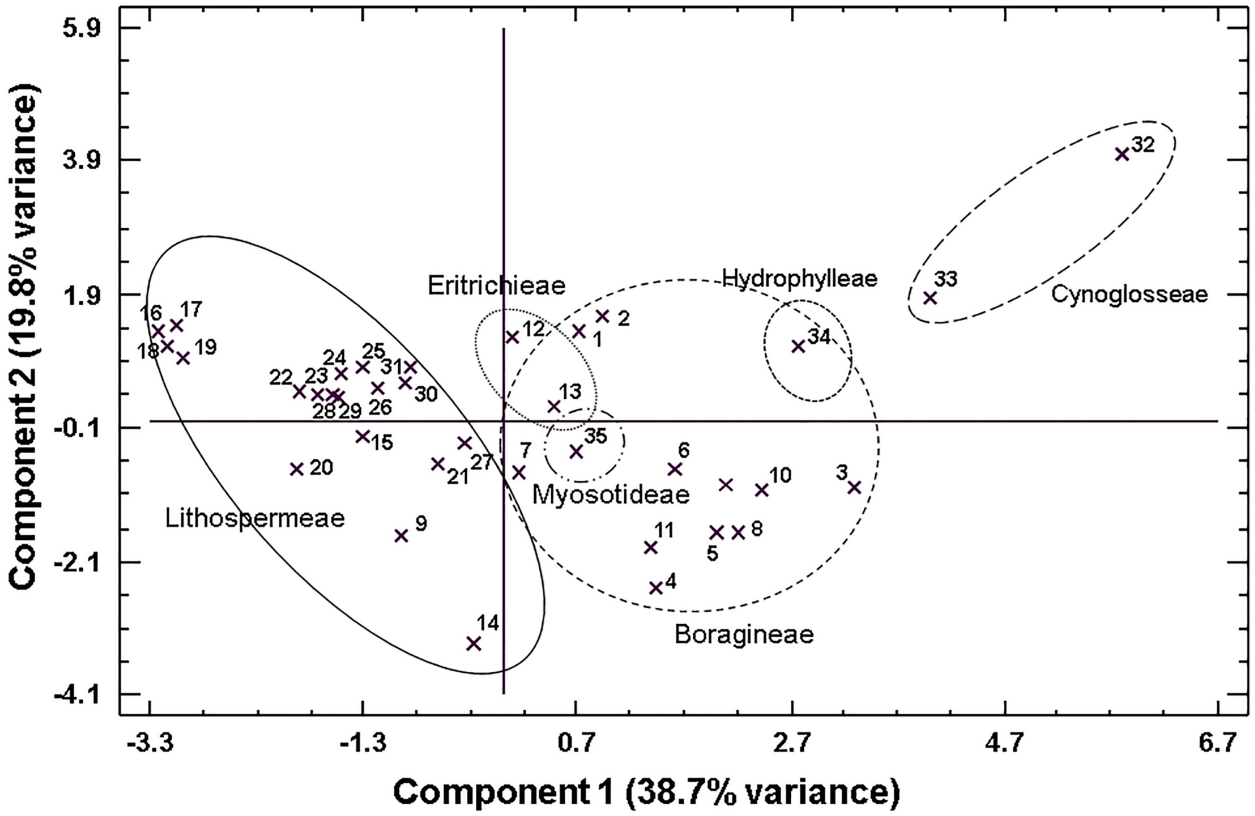

Figure 2. Scores plot analysis for the two first principal components of Boraginaceae species based on percentages of fatty acid of total FA. The numbers represent the species as listed in *Table 2*.

content (% of total FA), *S. bulbosum* (27.6%), *S. tuberosum* subsp. *tuberosum* A (27.2%) and *Amsinckia vernicosa* var. *furcata* (21.1%) may be considered as new sources of GLA, while the species containing the highest proportion of SDA was *B. arvensis* A (21.3%). Additionally, we corroborate that the FA profiles of Boraginaceae seed oils have utility as taxonomical markers. In this regard, PCA showed that both *n*-6 and *n*-3 PUFA were the more discriminant FA for species clustering. Some of the species analyzed here show advantageous FA profiles with respect to other Boraginaceae species currently cultivated for obtaining functional oils. However, before considering any of these oils as candidate to be marketed as functional food, the oil extraction from seeds should be checked by cold press extraction and food-grade solvents, such as ethyl acetate or hexane. Then, the various quality index of the oils, such as the peroxide value and free FA, should be determined. Also, the current cultured chemotype of *B. arvensis* used for SDA-rich oils production might be exchanged by other chemotypes of the same species detected in this work, which are better SDA-producers. Future actions should be devoted to study the health benefits associated with Boraginaceae seed oils intake, including *in vivo* studies, while genetic and agronomic variables should be explored to allow an efficient cultivation of the various Boraginaceae species as potential sources of GLA- and/or SDA-rich oils.

Experimental Section

Abbreviations

ALA: α-linolenic acid; ARA: arachidonic acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; FA: fatty acid; FAME: Fatty acid methyl ester; GLA: γ-linolenic acid; LA: linoleic acid; LOD: limit of detection; LOQ: limit of quantification; MUFA: monounsaturated FA; OA: oleic acid; PA: palmitic acid; PCA: Principal Component Analysis; PUFA: polyunsaturated fatty acid; SA: stearic acid; SDA: stearidonic acid; SFA: saturated FA; VLCFA: very long-chain FA; VLCPUFA: very long-chain PUFA.

Sample Collection

Sample collection is detailed in *Table 2* where all species are taxonomically grouped by tribes. Some ripe seeds were collected from their natural habitats. In this case, the herbarium code (HUAL: Herbarium of the University of Almería), location, geographical coordinates and collection date of each sample are displayed. Taxa identification was carried out by authors using specific flora monographs. For instance, those of Cecchi and Selvi,[39,40] and Pignatti.[41] The remaining seeds were purchased from B&T World Seeds® (Olonzac, France). Prior to analysis, seeds were freeze-dried and ground to powder with a mortar, and then, they were immediately analyzed.

Oil Transesterification and FA Analyses

FA determination was carried out after direct derivatization to FA methyl esters (FAME) through the transesterification according to Lepage and Roy methodology,[42] and modifications proposed by Rodríguez-Ruiz et al.[43] FAME were analyzed in a Focus GC (Thermo Electron, Cambridge, UK) equipped with flame ionization detector and an Omegawax 250 capillary column (30 m × 0.25 mm i. d. × 0.25 μm film thickness) (Supelco, Bellefonte, PA, USA). The temperature of the oven was: 90 °C (1 min), 10 °C/min to 100 °C (3 min), 6 °C/min to 260 °C (5 min). The injector temperature was set at 250 °C with a split ratio 50 : 1 and an injection volume of 4 μL. The detector temperature was set at 260 °C and the carrier gas flow was 1 mL/min.[44] The FA profiles were reported as percentages of total FA, and pentadecanoic acid (15:0, 98% purity) supplied by Sigma-Aldrich (Barcelona, Spain) was used as internal standard to quantify the mass of every FA in the resulting chromatograms. Peaks were identified by their retention times obtained for known FAME standards (PUFA 1, product number 47033, Sigma-Aldrich).

Quality Control

Previous protocols were used for the quality control of FA analyses. Pure OA and LA were transmethylated and diluted in toluene (0.001 – 20 mg/mL) and analyzed by triplicate and quantified by GC-FID in order to determine the limits of detection (LOD) and quantification (LOQ).[45,46] Negative controls, were conducted using samples lacking the transmethylation procedure. The LOD and LOQ are defined as the minimal concentration at which different peaks over baseline noise could be detected and quantified, respectively. The estimated LOD for OA and LA were in the range of 0.8 – 0.9 mg/mL, whereas LOQ were in the range of 2.4 – 3.8 mg/mL. Recoveries were determined applying the following formula 1:

$$R = \frac{Css - Cus}{Cstd} \times 100 \quad (1)$$

being R the recovery percentage of the added standard (OA or LA); Css the concentration of the standard found in the spiked sample; Cus the concentration of the standard found in the unspiked sample; and $Cstd$ the concentration of the spiked standard. Recoveries for OA and LA were in the range of 94.4 – 100.3 % and 97.3 – 102.0 %, respectively. As a GC quality control, a blank sample (hexane) was run in every batch of analyses to check the GC performance.

Statistical Analysis

Data on seeds from botanical gardens correspond to the analyses effected to seeds received in a single shipment, which were analyzed three times in triplicate each. Seeds from the wild were collected from three different species populations and each of which was analyzed in triplicate. All data in tables are expressed as the average ± SD. The significance of differences among mean values was assessed by one-way ANOVA coupled with Fisher's LSD test at $P < 0.05$. Pearson product moment correlation (r) and statistical significance (P) were obtained for each pair of variables (the FA). $P < 0.05$ was regarded as significant. Correlation and Principal Component Analysis (PCA) was also performed. All statistical analyses were carried out using Statgraphics© centurion XVI (StatPoint Technologies, Warrenton-Virginia, USA).

Acknowledgements

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Table 2. Data on collected Boraginaceae plants.

Species	Herbarium code	Sample locations	Geographical coordinates	Collection date
Tribe Boragineae Reichenbach, 1831				
1 *Anchusella cretica* (Mill.) Bigazzi, E. Nardi & Selvi (A)	HUAL 26414	Italy (Sicily), Mesina, Cesarò: Nebrodes, torrente Torti	37.89 N, 14.65 E	16/05/2019
2 *A. cretica* (Mill.) Bigazzi, E. Nardi & Selvi (B)	HUAL 26415	Italia, Reggio Calabria, Sinopoli: Aspromonte, Puente Vasi	38.22 N, 15.88 E	16/05/2019
3 *Borago officinalis* L.	–	Italy (Sicily), Catania, Vizzini: close to SS194	37.17 N, 14.76 E	15/05/2019
4 *Brunnera macrophylla* (Adams) I. M. Johnst	–	Spain, Madrid. Cultivated species	40.41 N, 3.69 W	24/04/2019
5 *Hormuzakia aggregate* (Lehm.) Guşul.	HUAL 26416	Italy (Sicily), Caltanisetta, Gela: Torre di Manfria	37.09 N, 14.13 E	15/05/2019
6 *Symphytum bulbosum* K. F. Schimp.	HUAL 26417	Italy, Reggio Calabria, Sinopoli: Aspromonte, Puente Vasi	38.22 N, 15.88 E	16/05/2019
7 *Symphytum bulbosum* K. F. Schimp. (immature seeds)	HUAL 26418	Italy, Reggio Calabria, Sinopoli: Aspromonte, Puente Vasi	38.22 N, 15.88 E	16/05/2019
8 *S. grandiflorum* DC.	–	Cultivated species. B&T World Seeds, Olonzaz, France		
9 *S. gussonei* F. W. Schult (immature seeds)	HUAL 26419	Italy (Sicilia), Mesina, Cesarò: Nebrodes, torrente Torti	37.89 N, 14.65 E	16/05/2019
10 *S. ibiricum* Steven	–	Spain, Madrid. Cultivated species	40.41 N, 3.69 W	24/04/2019
11 *S. tuberosum* L. subsp. *tuberosum*	HUAL 26420	Spain, Navarra: Baztán, from Elizondo to Beartzum	43.14 N, 1.50 W	12/04/2019
Tribe Eritrichieae Gürke in Engl. & Prantl, 1893				
12 *Amsinckia tessellata* A.Gray	–	Cultivated species. B&T World Seeds, Olonzaz, France		
13 *A. vernicosa* Hook. & Arn. var. *furcata* (Suksd.) Hoover ex Jeps.	–	Cultivated species. B&T World Seeds, Olonzaz, France		
Tribe Lithospermeae Dumortier, 1827				
14 *Aegonychon gastonii* (Benth.) Holub	HUAL 26337	France, Pyrenées Atlantiques, Lées-Athas: Anie	42.95 N, 0.73 W	07/07/2017
15 *A. purpurocaeruleum* (L.) Holub	HUAL 26349	Spain, Gerona, Montagut, Riera de Sant Aniol	42.29 N, 2.58 E	13/06/2018
16 *Buglossoides arvensis* (L.) I. M. Johnst. (A)	HUAL 4396	Spain, Almería, Laujar, Sierra de Gádor: rambla de Ojancos	36.97 N, 2.92 W	01/05/1999
17 *B. arvensis* (L.) I. M. Johnst. (B)	HUAL 4419	Spain, Granada, Caniles: estación de Hijate.	37.40 N, 2.62 W	07/05/1992
18 *B. arvensis* (L.) I. M. Johnst. (C)	HUAL 26345	Spain, Almería, Enix: Marchal de Antón López	36.89 N, 2.625 W	22/04/2018
19 *B. incrassata* (Guss.) I. M. Johnst. subsp. *incrassata*	HUAL 26334	Spain, Almería, Dalías: Cortijo Clavero	36.84 N, 2.83 W	22/04/2018
20 *B. minima* (Moris) R. Fern.	HUAL 26347	Italy (Sicily), Palermo, Petralia Sottana, Parco delle Madonie: Piano Battaglia	37.87 N, 14.02 E	11/05/2018
21 *Glandora diffusa* (Lag.) D. C. Thomas	HUAL 26333	Spain, León, Oseja de Sajambre, Picos de Europa: La Cotorra	43.17 N, 5.00 W	08/07/2018
22 *G. oleifolia* (Lapeyr.) D. C. Thomas	HUAL 26348	Spain, Gerona, Montagut, cerca de Sant Aniol	42.32 N, 2.58 E	13/06/2018
23 *G. rosmarinifolia* (Ten.) D. C. Thomas	HUAL 26346	Italy (Sicily), Trápani, San Vito lo Capo, Riserva dello Zingaro	38.09 N, 12.79 E	10/05/2018
24 *Halacsya sendtneri* (Boiss.) Dorfl. (A)	HUAL 26338	Albania, Shkodër, near to lake of Vau i Dejës	42.01 N, 16.67 E	29/03/2016
25 *H. sendtneri* (Boiss.) Dorfl. (B)	–	Albania, Has, Pastrik mountain	42.20 N, 20.45 E	30/06/2016
26 *Onosma echioides* (L.) L.	–	Cultivated species. B&T World Seeds, Olonzaz, France		

Table 2. (cont.)

Species	Herbarium code	Sample locations	Geographical coordinates	Collection date
27 *O. polyphyllum* Ledeb.	–	Cultivated species. B&T World Seeds, Olonzaz, France		
28 *O. heterophylla* Griseb. (A)	HUAL 26344	Albania, Shkodër, near to lake of Vau i Dejës	42.01 N, 16.67 E	29/03/2016
29 *O. heterophylla* Griseb. (B)	HUAL 26343	Albania, Has, Tobli, near the lake	42.10 N, 20.34 E	30/06/2016
30 *O. stellulata* Waldst. & Kit.	HUAL 26341	Albania, Has, Pastrik mountain	42.20 N, 20.45 E	30/06/2016
31 *Paramoltkia doerfleri* (Wettst.) Greuter and Burdet	HUAL 26342	Albania, Has, Pastrik mountain	42.20 N, 20.44 E	30/06/2016
Tribe Cynoglosseae W. D. J. Koch, 1837				
32 *Cynoglossum montanum* L.	–	Cultivated species. B&T World Seeds, Olonzaz, France		
33 *Omphalodes cappadocica* (Willd.) DC.	–	Cultivated species. B&T World Seeds, Olonzaz, France		
Tribe Myosotideae Rchb. f. in Reichenbach, 1843				
34 *Myosotis capitata* Hook.	–	Cultivated species. B&T World Seeds, Olonzaz, France		
Tribe Hydrophylleae Reichenbach, 1831				
35 *Pholistoma auritum* (Lindl.) Lilja ex Lindbl.	–	Cultivated species. B&T World Seeds, Olonzaz, France		

Author Contribution Statement

M. J. González-Fernández, S. Lyashenko, D. Fabrikov, M. Á. Rincón-Cervera, F. Gómez-Mercado, M. Urrestarazu, and J. L. Guil-Guerrero performed the experiments. M. J. González-Fernández, F. Gómez-Mercado, and J. L. Guil-Guerrero analyzed the data and wrote the article. M. J. González-Fernández, S. Lyashenko, F. Gómez-Mercado, M. Urrestarazu, D. Fabrikov, M. Á. Rincón-Cervera, and J. L. Guil-Guerrero contributed to the samples/reagents/materials/analysis tools and analyzed the data. S. Lyashenko, F. Gómez-Mercado, and J. L. Guil-Guerrero conceived and designed the experiments.

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