Phenolic composition and *in vitro* antiproliferative activity of *Borago* spp. seed extracts on HT-29 cancer cells Running title: Phenolics from *Borago* species

Svetlana Lyashenko[a], Dmitri Fabrikov[b], María José González-Fernández[a], Francisco Gómez-Mercado[c], Rosalía López Ruiz[d], Aleksandr Fedorov[e], Gérard de Bélair[f], Miguel Urrestarazu[g] , Ignacio Rodríguez-García[h], Míriam Álvarez-Corral[h], and José Luis Guil-Guerrero*[a]

[a]Tecnología de Alimentos, CeiA3; [b]Zoología; [c]Botánica-CECOUAL;

[d]Departamento Química-Física, Química Analítica de Contaminantes;

[g] Production Vegetal, [h]Química Orgánica, CeiA3

[a,b,c,d,g,h] Universidad de Almería, 04120 Almería, Spain

[e]Udmurt Federal Research Center of the Ural Branch RAS, Introduction and Plant Establishment Department, 426067, Izhevsk, Russian Federation

[f] Département de Biologie, Université Badji-Mokhtar, BP 12, 23000 Annaba, Algérie

*Corresponding author: jlguil@ual.es; ORCID: 0000-0003-2666-1774

*This is the accepted version of the following article: Food Bioscience 42 (**2021**)* 101043*, which has been published in final form at [* https://doi.org/10.1016/j.fbio.2021.101043*].*

Abstract:

Borage oil contains γ-linolenic acid (GLA, 18:3*n*-6), and significant amounts of tocopherols, sterols and squalene. However, data on phenolics are absent for most *Borago* species, while such compounds are relevant for improving human health. In this work, seeds of all endemic-wild *Borago* species and wild and farmed *B. officinalis* were surveyed for phenolic compound composition and *in vitro* antiproliferative activities. Phenolic analyses were conducted by HPLC-DAD and LC-MS, and antitumor actions were checked by the MTT assay. Besides a flavonol (kaemferol), 13 phenolic acids were identified and quantified. Among *B. officinalis* samples, the highest amounts of total phenolics and rosmarinic acid were found in the warmest and southernmost localities. Rosmarinic acid ranged from 12.21 (*B. officinalis* 'Alba' B_2) to 105.06 mg/100 g seed (*B. longifolia*). Ferulic acid was also found in all *Borago* species, ranging from 1.38 in wild *B. officinalis* to 55.98 mg/100 g seed in *B. longifolia*. The MTT assay showed dose- and time-dependent inhibitory effects of *Borago* extracts on HT29 cancer cells, with GI_{50} values within the 250-300 μg/mL range after 72 h of cell exposure to seeds extracts. Due to the occurrence of some compounds lacking antiproliferative activity in the crude phenolic extract, as evidenced by ^{1}H NMR spectrum, the antiproliferative activity of the phenolics-containing extracts was lower than that obtained due to pure phenolics. This work constitutes the first approach to evaluate the phenolic composition of all *Borago* species and provides arguments for using endemic-wild *Borago* taxa by the pharmaceutical, cosmetic and food industries.

Keywords: *Borago* species; phenolic compounds; rosmarinic acid; LC-mass spectrometry; HPLC-DAD; HT29 cells

Abbreviations:

^{1}H NMR: proton Nuclear Magnetic Resonance; AIF: all-ions fragmentation; ARA: arachidonic acid, 20:4*n*-6; DAD: diode array detector; DGLA: dihommo-gamma linolenic acid, 20:3*n*-6;

DMSO: dimethylsulfoxide; ESI: electrospray ionization; FA: fatty acid; GLA: γ-linolenic acid, 18:3*n*-6; GI_{50}: dose of extract/compound that inhibits the growth of cells by 50%; HCD: higher collisional dissociation; LC-MS: LC-mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; PA: phenolic acid; PUFA: polyunsaturated FA; ROS: reactive oxygen species; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

1. Introduction

Epidemiological studies have shown that the intake of fruits and vegetables is correlated with a reduced risk of chronic diseases (Boeing et al., 2012), while recent works highlight the significant link between phenolics-containing foods and their antioxidant properties (Shahidi and Ambigaipalan, 2015). Phenolics have constrictive action on capillaries and can reduce the damage caused by pro-oxidant processes, thus they are useful for the prevention and treatment of reactive oxygen species (ROS)-induced diseases, such as cancer, diabetes immune dysfunctions, Parkinson's and Alzheimer's, multiple sclerosis, and inflammatory lung disorders (Mao et al., 2017; Tanase et al., 2019). Nowadays, there is a growing demand of natural plant extracts with antioxidant activity because of the increased report on carcinogenic effects of some synthetic antioxidants, which are currently used as food additives, cosmetics and in pharmaceutical formulations (Ivanov et al., 2014). In addition to their antioxidant capacity, plant phenolics act as cytotoxic anti-cancer agents by promoting apoptosis and reducing angiogenesis, growth and differentiation of tumours. For instance, the anticarcinogenic activity of common borage (*Borago officinalis* L.) leaves has been related to their phenolic composition (Lozano-Baena et al., 2016).

B. officinalis is the only species of this genus with traditional applications. It is native to the eastern Mediterranean region and is cultivated in various areas of Europe, Great Britain, and North America. The leaves and flowers are used in salads, and in Europe the leaves are cooked as a vegetable. It has several ethnobotanical applications in the abovementioned areas where it is indigenous and in other areas, where it has been introduced (Ramezani et al., 2020). In EEUU, the seeds, leaves and flowers of *B. officinalis* are ingested to stimulate the ovaries (Ososki et al., 2002), and in India are used for skin rashes (Khanday et al., 2017).

In addition to *B. officinalis*, the genus *Borago* L. includes 4 endemic species: *B. longifolia* Poir. (Algerian-Tunisian endemic species), *B. trabutii* Maire (native to Morocco and Algeria), *B. pygmaea* (DC.) Chater & Greuter (native to Corse, Sardegna, and Capraia), and *B. morisiana*

78 Bigazzi & Ricceri) (native to Sardegna and S. Pietro Islands) (Selvi et al., 2006; Mansion et al.,
79 2009).
80 *B. officinalis* is widespread, growing in the wild or cultivated for seeds production, which are a
81 good source of γ-linolenic acid (GLA, 18:3*n*-6), a bioactive polyunsaturated fatty acid (PUFA).
82 GLA has several healthy benefits; in the body is metabolized to dihomo-γ-linolenic acid (DGLA,
83 20:3*n*-6) and further to arachidonic acid (ARA, 20:4*n*-6), which in turn acts as eicosanoids
84 precursors. All endemic-wild *Borago* species are remarkable GLA-producers, and their seeds
85 contain significant amounts of tocopherols, sterols, and squalene (Guil-Guerrero et al., 2017;
86 Fabrikov et al., 2019); however, knowledge on phenolic compounds of *Borago* species is
87 restricted to *B. officinalis*. The seeds of *B. officinalis* contain high amounts of phenolic
88 compounds (2.45-10.98 mg GAE/g) (Mhamdi et al., 2010). Among these, highlight gallic,
89 chlorogenic, rosmarinic, syringic, synergic, synaptic, *p*-coumaric and trans-cinnamic acids
90 (Mhamdi et al., 2010; Nogala-Kalucka et al., 2010; Zadernowski et al., 2002).
91 There is a gap of knowledge on the phenolic compounds of the seeds of endemic-wild *Borago*
92 species as well as on the bioactive properties of such seeds, while these can become new GLA-
93 rich oils to be used as functional foods (Guil-Guerrero et al., 2017; Guil-Guerrero et al., 2018). .
94 The present study was conducted to investigate the phenolic compounds profiles of endemic
95 *Borago* species and *B. officinalis* of diverse origin and their *in vitro* antiproliferative activity
96 against human colon cancer cells.
97

2. Materials and Methods

2.1 Plant material and chemicals

Borago seeds were collected from their natural habitats in the wild, other seeds were obtained from cultures at the University of Almería (Spain) or taken from several botanical gardens (Table 1). Each wild species was collected from three well-differentiated subpopulations in each collection location. *B. officinalis* F and G seeds collected in the Alpujarra (Spain) were classified as mature, immature or early mature stage by the coloring of the seed. Upon receipt, the seeds were cleaned, labeled and placed in a glass desiccator until analysis. Commercial *B. officinalis* oil was purchased from local markets. Just prior to analysis, seeds were ground to powder with a mortar, then they were immediately analyzed. ~2 g of each sample was used for moisture analysis. This was carried out in a forced air oven at 105 ºC for 8 h, and all results are expressed on dry weight (d.w.) basis. Moisture content for al seeds was ~7.5±0.5%.

Gallic acid (97.5-102.5%, G7384), proto catechuic acid (≥97.0%, 37580), DL-*p*-hydroxyphenyllactic acid (≥97%, H3253), *p*-hydroxy benzoic acid (≥99%, 240141, chlorogenic acid (≥95%, C3878), 3,4-dihydroxy hydrocinnamic acid (98%, 102601), caffeic acid (≥98.0%, C0625), vanilic acid (97%, H36001), salicylic acid (≥99.0%, 247588), *p*-coumaric acid (≥98.0%, C9008), ferulic acid (99%, 128708), 2-hydroxy-4-methoxy benzoic acid (99%, 173479), rosmarinic acid (≥98%, R4033), kaempherol (≥97.0%, 60010), and all chemicals and solvents were purchased in high purity grade from Sigma- Aldrich Química SA (Madrid, Spain).

2.2 Extraction of phenolics from Borago *seeds*

~0.2 g of dry seed powders were extracted three times with 3 ml of methanolic solution (MeOH:H_2O, 60:40, v/v). The mixture was centrifuged at 1000 xg for 10 min, and the supernatants were collected, combined and vacuum-evaporated at 60 °C to dryness. The obtained residue was dissolved in 1 mL of the previous methanolic solution. Before injection, samples were filtered through a 0.45-mm membrane filter.

2.3 Characterization of phenolic compounds by HPLC-DAD

Phenolics were analyzed using a HPLC Finnigan Surveyor LC Pump Plus Thermo Scientific system equipped with a DAD Thermo Scientific and Hypersil Gold C18 column (250 x 4.6mm, 5 µm, Thermo Electron, Cambridge, UK). A flow rate of 0.65 mL/min was set. The compounds were separated with gradient elution using aqueous acetic acid (acetic acid: H_2O, 1:99, v/v) (A) and methanol (B) as eluents at ambient temperature. The step gradient was as follows: 0–28 min 80% of A; then, it was linearly decreased to 25% in 8 min and remained constant during 8 min. Later, it was increased to 80% in 6 min and remained constant during 5 min. The total running time was 55 min. The column temperature was 25 °C, and the injection was 10 µL. . The mobile phase was filtered through a 0.45 µm membrane filter (Millipore, Durapore®, Ireland), and the injection volume was 10 µL. Peaks were monitored at 240, 280 and 330 nm and identified by retention times in comparison with pure standards. Quantification of the compounds was made using external calibration curves obtained from pure standards (Sigma Aldrich, St. Louis, MO, USA) in the HPLC system (Table 2).

2.4 Characterization of phenolic compounds by LC-MS

The chromatographic separation was performed on a Thermo Fisher Scientific Transcend 600 LC (Thermo Scientific Transcend™, Thermo Fisher Scientific, San Jose, CA, USA) by using a Hypersil Gold C18 column (250 x 4.6mm, 5 µm, Thermo Electron, Cambridge, UK). A flow rate of 0.65 mL/min was set. The compounds were separated with gradient elution using aqueous acetic acid (acetic acid: H_2O, 1:99, v/v) (A) and methanol (B) as eluents at ambient temperature. The step gradient was as follows: 0–26 min 80% of A; then, it was linearly decreased to 25% in 8 min and remained constant during 8 min. Later, it was increased to 80% in 8 min and remained constant during 5 min. The total running time was 55 min. The column temperature was 25 °C, and the injection volume was 10 µL.

147 The LC system was coupled to a single mass spectrometer Orbitrap Thermo Fisher Scientific
148 (Exactive^{TM}, Thermo Fisher Scientific, Bremen, Germany) using electrospray ionization (ESI)
149 (HESI-II, Thermo Fisher Scientific, San Jose, CA, USA) in positive and negative ion mode. ESI
150 parameters were as follows: spray voltage, 4 kV; sheath gas (N_2>95%), 35 (adimensional);
151 auxiliary gas (N_2>95%), 10 (adimensional); skimmer voltage, 18 V; capillary voltage, 35 V; tube
152 lens voltage, 95 V; heater temperature, 305 °C; capillary temperature, 300 °C. The mass spectra
153 were acquired employing two alternating acquisition functions: (1) full MS, ESI+, without
154 fragmentation (higher collisional dissociation (HCD) collision cell was switched off), mass
155 resolving power = 25,000 FWHM; scan time = 0.25 s; (2) all-ions fragmentation (AIF), ESI+,
156 with fragmentation (HCD on, collision energy 30 eV), mass resolving power = 10,000 FWHM;
157 scan time = 0.10 s, (3) full MS, ESI- using the aforementioned settings and (4) AIF, ESI- using
158 the settings explained for (2). Mass range in the full scan experiments was set at *m/z* 50–1000.
159 LC chromatograms were acquired using the external calibration mode and they were processed
160 using Xcalibur^{TM} version 3.0, with Qualbrowser and Trace Finder 4.0 (Thermo Fisher Scientific,
161 Les Ulis, France). Unknown analysis was carried out with Compound Discoverer^{TM} version 2.1
162 (Thermo Scientific, Les Ulis, France).

163 *2.5. Accuracy and validity of the analysis protocols*

164 HPLC-DAD and LC-MS analyses were performed using the experimental set-up described in
165 Table 2. Precision/injection repeatability test (expressed as relative standard deviation, %) was
166 performed by six replicated injections of the standard mixture of phenolic acids (PA) at a
167 concentration of 0.03, 0.1, 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 ppm. The accuracy was also evaluated
168 by the standard addition procedure (% of recovery) with three addition levels (30% of the
169 expected values, in duplicate). Standard mixture was added to the samples, and all the extraction
170 procedures were carried out. Recovery rates were calculated by the following equation:

171 $R\% = [(Cs - Cp)/Ca] \cdot 100$

where R (%) is the percent recovery of added standard; Cs the compound content in spiked sample; Cp the compound content in sample; and Ca the compound standard added.

To determine the limits of detection (LOD) and quantification (LOQ) of the assays, pure phenolic compounds were diluted in the 0.001–20 mg/mL range in methanol in triplicate and quantified by HPLC-DAD. Negative controls made without phenolics addition were also analyzed. The LOD was defined as the minimum concentration at which distinct peaks could be detected above the baseline noise. The LOQ was defined as the lowest concentration of compounds that could be quantified with an accuracy and precision within 15%.

2.6 *Cell assays on cancer and normal cell lines*

The HT29 colon cancer cells line and the CCD18 colonic human myofibroblasts cells line were used to check antiproliferative activities. Cultures were supplied by the Technical Instrumentation Service of University of Granada (Granada, Spain). First, it was checked for the absence of *Mycoplasma* and bacteria. Then, cells were grown at 37 ºC and 5% CO_2 humidified atmosphere in medium RPMI-1640 supplemented with 5% fetal bovine serum, 2 mM L-Glutamine, 1mM sodium pyruvate, 0.125 mg/mL amphotericin and 100 mg/mL penicillin-streptomycin. All cultures were plated in 25 cm^2 plastic tissue culture flasks (Sarstedt, USA). All culture media and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cell culture and cell assay, that is, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test were accomplished as previous described (Ramos-Bueno et al., 2016).

In the MTT assay, cells were divided into 96-well microtiter plates, adjusted of $1x10^4$ cell/well and cultivated in medium at 37 ºC, 5% CO_2 prior to adding the different extracts dissolved in medium. The phenolics-containing extracts of *B. morisiana, B. officinalis* H, *B. pygmaeae, B. longifolia* and *B. trabutii* were supplied to cells dissolved in a mixture of distilled water-methanol (40:60, v/v) and then in the culture medium at designed concentrations (0-300 µg/mL). 48 and 72 h later, 5 mg/mL of an MTT solution was added to the culture medium to determine

197 the viability of cells. The absorbance was recorded at 570 nm on an enzyme-linked
198 immunosorbent assay (ELISA) plate reader (Thermo Electron Corporation, Sant Cugat del
199 Valles, Barcelona, Spain) (Ramos-Bueno et al., 2016). The formazan crystals produced were
200 solubilized using 100 μL dimethylsulfoxide (DMSO). Cells without phenolic extracts were
201 considered as negative controls. Cell survival in exposed cultures relative to unexposed cultures
202 (negative controls) was calculated, and the number of viable cells was calculated using the
203 following equation:
204 Percentage of viable cell (%) = (Absorbance of treated cells/Absorbance of untreated cells) ×
205 100%.
206 The concentrations causing 50% cell growth inhibition (GI_{50}) were calculated from the growth
207 curves. Doxorubicin (98.0-102%, D1515), from Sigma-Aldrich (Madrid, Spain) was used as a
208 positive control, while DMSO and methanol were used as the negative (vehicle) controls.
209 Phenolic extracts, pure phenolics and controls were evaluated in three independent assays.
210 Values presented are mean ± standard error of the mean.
211 *2.7 ^{1}H NMR analysis of the MeOH-H_2O extract of* Borago trabutii
212 The extract (20 mg) was dissolved in CD_3OD (0.5 mL) and ^{1}H NMR was measured (300 MHz)
213 in a Bruker advance system. Nuclear magnetic resonance (NMR) spectra was obtained using a
214 Bruker Avance 300 MHz spectrometer (Bruker BioSpin GmbH, Germany). The filtered seed
215 extract (20 mg) was dissolved in CD_3OD (0.5 mL) and the spectrum acquired at 298 K.
216 Chemical shifts are reported as ppm using residual CH_3OH as internal standard.
217 2.8 *Statistical Analysis*
218 Data on seeds from botanical gardens correspond to the analyses effected to seeds received in a
219 single shipment, which were analyzed three times in triplicate each. Data on cultures from the
220 University of Almería correspond to the average of three samples from different plots, each of
221 them analyzed in triplicate. Seeds from the wild were collected from different species

populations, and each of which was analyzed in triplicate. Borage oil data correspond to the average ± SD obtained from three different commercial packages from the same manufacturer, each of them analyzed in triplicate. All data in tables were analyzed using one-way ANOVA (Statgraphics Centurion XVI.I, Warrenton, VA, USA) and expressed as the average ± SD. Differences among mean values of phenolic compounds and GI_{50} values were tested by Duncan's test at $P<0.05$ (Table 3)..

3. Results and Discussion

3.1 Compounds identification and quantitation

Two LC systems were used for both qualitative and quantitative analysis of phenolics. An HPLC-DAD chromatogram of *B. longifolia* seed extract is shown in Figure 1. All investigated compounds good molar extinction coefficients at 280, 300 and 320 nm, which were used to monitor the chromatographic separations (Table 2). The constituents under investigation were also identified by the recorded absorption spectra of each peak identified in *Borago* seed extracts with those of pure standards. The structures of all identified compounds present in *Borago* seeds extracts were confirmed by LC-MS. Precision/injection repeatability test showed good precision in peak area (standard deviation <1%) and peak retention time (±2%). The results of the regression equation, linearity range, LOD, LOQ and recoveries for each compound is presented in Table 2. Notice that the developed methodology is precise, accurate and sensitive, robust for the simultaneous quantitative evaluation of the investigated phenolic compounds.

3.2 Total phenolics (Table 3) ranged from 55.36 in *B. officinalis* I to 250.86 mg/100 g seeds in *B. longifolia*. In endemic-wild *Borago* species, phenolic compounds ranged from 92.80 in *B. pygmaea* to 250.86 mg/100 g in *B. longifolia* seeds. Total phenolic levels in *Borago* species were similar to those reported in other typical GLA sources: *Ribes nigrum* (108.14 mg/100 g seeds) and *Echium vulgare* (122.60 mg/100 g seeds) (Nogala-Kalucka et al., 2010). Regarding *B. officinalis*, the higher phenolics content was found in wild *B. officinalis* A (204.25 mg/100 g) and the lower amount in cultivated *B. officinalis* I (55.36 mg/100 g). Such data agrees with previous reports data by Nogala-Kalucka et al. (2010) and Mhamdi et al. (2010). *Phenolic acids content*

Rosmarinic acid was found in all analyzed species in high quantities, ranging from 12.21 (*B. officinalis* 'Alba' B_2) to 105.06 mg/100 g seeds (*B. longifolia*) respectively. Rosmarinic acid content in *B. longifolia* seeds was similar to that detected in commercial borage oil (105.06 and

255 103.64 mg/100 g, respectively). Such amounts agree with previous ones obtained by
256 Wettasinghe et al. (2001), who identified rosmarinic acid as the major phenolic compound in
257 borage seeds. According to Petersen et al. (2009), rosmarinic acid accumulation is a rather
258 consistent feature of species within the *Boraginaceae* family.
259 Ferulic acid was found in all analyzed *Borago* species, ranging between 1.38 (*B. officinalis* G_2)
260 and 55.98 mg 100/g seeds (*B. longifolia*). Among the cinnamic acid derivatives, 3,4-
261 dihydroxyhydroxycinnamic acid was identified in all samples, except in *B. pygmaea* and
262 immature seeds of *B. officinalis* G_2, while chlorogenic acid was identified in *B. trabutii* and all *B.*
263 *officinalis* samples. Another PA of the hydroxycinnamic acid group, *p*-coumaric acid, ranged
264 from trace amounts (*B. morisiana* A and B and wild *B. officinalis* G_1) to 8.24 mg/100 g in
265 cultivated *B. officinalis* I. The occurrence of caffeic acid was restricted to *B. officinalis*
266 (excepting wild *B. officinalis* D and G_1), *B. trabutii* and *B.*
267 *longifolia*. The ester of caffeic and quinic acid, namely chlorogenic acid, was detected in a small
268 number of samples. Wild *B. officinalis* D had the highest chlorogenic acid content (2.31 mg/100
269 g seeds), while it was absent in *B. morisiana* and *B. pygmaea*.
270 Gallic acid was present in all *Borago* species, the highest amounts detected in *B. morisiana* B
271 (20.39 mg/100 g seeds). Other compounds belonging to this group, vanillic and salicylic acids,
272 were identified in most samples. The content of salicylic acid varied from trace amounts in *B.*
273 *officinalis* oil to 18.53 mg/100 g in *B. longifolia*. The highest concentrations of protocatechuic
274 acid (3,4-dihydroxybenzoic acid), were identified in wild *B. officinalis* A and *B. longifolia*
275 (21.48 and 20.09 mg/100 g, respectively). Besides the above listed phenolic compounds, 2-
276 hydroxy-4-methoxybenzoic acid was identified in all analyzed *Borago* species except in *B.*
277 *pygmaea,* and the highest content was found in *B. officnalis* D (7.95 mg/100 g seeds). *p*-
278 hydroxybenzoic acid was not detected in most *B. officinalis* samples, and contents ranged from
279 traces to 25.69 mg/100 g^1 *(B. morisiana* A).

Hydroxyphenyllactic acid content ranged from 3.17 (*B. officinalis* F_1) to 49.80 mg 100 g^{-1} (*B. officinalis* A), both data for mature seeds, while it was not detected in cultivated *B. officinalis* I. Among the endemic *Borago* species, *B. morisiana* B contained the highest hydroxyphenyllactic acid amounts (35.87 mg/100 g).

3.3. Other compounds

Two flavonols, galangin and kaempferol, were found in the seeds of some *Borago* species. Kaempferol were detected in *B. trabutii* (1.25 mg/100 g), *B. officinalis* I (0.32 mg 100/g), and *B. officinalis* A and G_1 (traces). In *B. trabutii* were also detected: kaempferol-3-O-hexoside, quercetin, quercetin-3-O-hexoside and quercitrin, while kaempferol-3-O-hexoside was identified also in *B. longifolia*. Galangin was detected in the seeds of *B. trabutii*. In addition, a heterocyclic phenol, 8-hydroxyquinoline, was detected in *B. pygmaea*, *B. trabutii* and *B. longifolia* seeds. Finally, a non-alkaloid nitrogen glucosinolate, gluconapin, which is usually found in *Brassica* species (Rohit et al., 2016), was detected in *B. pygmaea* seeds.

3.4. Factors influencing the concentrations of phenolics in Borago *species*

Phenolic compounds, as well as other secondary metabolites, represent a chemical interface between plants and the environment. The amounts of PA were highly variable among *Borago* taxa, and this can be related to phenological stage, environmental conditions (geographical distribution) and chemotypes, which can modify the constituents of the plants (Amzad and Shah, 2015; Medina-Medrano et al., 2015). Climate features have been cited as a determining factor in the concentration of phenolics in plants. In this way, the maximum amounts of phenolic compounds were found in *B. longifolia* and *B. officinalis* A, closely followed by *B. trabutii*, the three samples from North Africa, where there is a hot-summer Mediterranean climate (Beck et al., 2018). Such climate is characterized by arid summer and low annual rainfall, which is stressful for plants. Seasonal environmental changes activate a multi-gene response that leads to changes in secondary metabolites accumulation. Then, plants synthesize phenolic compounds in

order to adapt to high environmental pressure (Stavroula and Rahul, 2016). Among all the species analyzed, *B. officinalis* is the only one growing in well differentiated climatic areas; therefore, the differences in phenolic profiles among seeds could be related to climate effects, although the existence of chemotypes is not ruled out. Both total phenolic and rosmarinic acid contents were significantly higher in *B. officinalis* A (the southernmost of the wild samples from *B. officinalis*), while the minimum phenolic content was detected in *B. officinalis* I cultivated in Kiev (Ukraine), under a humid continental climate (55.36 mg $100/g^{1}$). These findings agree with those of Wang & Zheng (2001), who indicated that higher growing temperatures increase the flavonol an anthocyanin contents in strawberries.

Interestingly, *B. officinalis* B_1 and *B. officinalis* 'Alba' B_2, both cultivated in the University of Almería, present also strong differences regarding 3,4-dihydroxyhydroxycinnamic acid amounts. The levels of this compound in *B. officinalis* B_2 (9.94 mg/100 g^{-}) was much lower than that detected in *B. officinalis* B_1 (40.76 mg/100 g seeds), which suggests the existence of different chemotypes (Hernandez et al., 2009). Strong differences regarding 3,4-dihydroxyhydroxycinnamic, hydroxyphenyllactic, salicylic and rosmarinic acids amounts were found between *B. morisiana* A and B (Table 3). The highest values were found in *B. morisiana* A, growing close to the Mediterranean Sea, while *B. morisiana* B seeds, which were collected in a locality placed at 700 m asl, showed lower values. Simultaneously, *B. morisiana* A and B showed similar ferulic acid contents (28.01 and 30.18 mg/100 g, respectively). In addition to environmental parameters, the chemotype and the degree of development of the seed also affects phenolic compounds amounts. So, mature and immature seeds showed different ferulic acid amounts, which in mature seeds were much higher than in immature seeds (8.89 and 5.49 in *B. officinalis* F, and 19.62 and 1.38 mg/100 g in *B. officinalis* G, respectively). These results were in good agreement with those of Mhamdi (2010). The effect of seeds development was also checked in rosmarinic acid content. Mature *B. officinalis* seeds collected in the Alpujarra region

(*B. officinalis* F_1 and G_1) had rosmarinic acid at 30.82 and 31.28 mg/100 g, respectively, amounts significantly higher than those found in immature seeds: 22.82 and 19.46 mg/100 g seeds, respectively. The amounts of individual phenolic compounds in commercial *Borago* oil were significantly lower than those detected in *Borago* seeds samples, except for rosmarinic and *p*-hydroxibenzoic acids contents. This fact could be related to the industrial methodology used for borage seed oil extraction. Most seed oils are traditionally pre-pressed, while the residual cake is extracted with solvents, and then both crude oils are combined and refined together. In the technical refining, polar phenolic compounds totally disappear, due to their higher affinity for the water phase (soapstock). Moreover, during physical refining, a partial distillation into the deodorizer distillate takes place, and due to the various volatilities of the phenolic compounds, some of them disappear from the oil (Van Hoed, 2010). Conventional pretreatment of oily seeds may include among other procedures thermal treatment (cooking) for the purpose of debilitating cell coats, liquify the oil in the plant cells and preparation of the matter for optimal oil extraction (Soto et al., 2004). Moreover, the cooking of the seeds can increase *p*-hydroxibenzoic acid content, as detected in rapeseed oil (Gawrysiak-Witulska et al., 2018). This fact could explain the high *p*-hydroxibenzoic acid content in commercial borage oil in comparison with other amounts detected in seed samples (10.35 vs ~1 mg/100 g seeds).

Among the procedures used in the industry to obtain oils from seeds, as solvent extraction and cold-pressing are, in order to preserve phenolic compounds content of the various *Borago* oils cold-pressing should be selected, because cold-pressed oils retain higher levels of natural antioxidants (Siger et al., 2008).

3.5. Antiproliferative activity of the water:methanol extracts on cancer cells

After 48 and 72 h of treatment, the MTT assay revealed concentration- and time-dependent inhibitory effects on HT29 cells for all assayed extracts. The effects of the seed extracts of *Borago* species on HT29 and CCD18 cells viability after 72 h of treatment are shown in Figure

2A. Cell viability at 48 h at the maximum concentration tested (300 μg/mL) and for the different species, was 20-30% lower than that obtained at 72 h (data not shown). Note that for CCD18 cells the *Borago* extract lacked significant effects on cell viability. After 72 h culture, cells growth inhibition was exercised much better by *B. longifolia* and *B. trabutii*. The doses of extracts that inhibits the growth of cells by 50% (GI_{50}), as well as those of some pure phenolics are depicted in Figure 2B. GI_{50} values *B. trabutii*, *B. longifolia*, *B. morisiana*, *B. officinalis* were 249, 239, 298, and 293 μg/mL. However, the extracts of *B. pygmaea* showed an undetermined GI_{50} value higher than 300 μg/mL.

In order to have a better understanding of the composition of the extract that caused the inhibition of the proliferation of HT29 cells, we carried out an ^{1}H NMR analysis of the MeOH-H_2O extract of *B. trabutii*, which is depicted in Figure 3. The analysis of the spectrum reveals that the main constituents of the extract are, in order of decreasing abundance: carbohydrates, phenolic compounds, lipids and nitrogen compounds. Detailed examination of the olefinic and aromatic signals in the spectrum (6.0-7.5 ppm) reveals the majoritarian presence of rosmarinic acid among the phenolic compounds. The zone of the spectrum in which the signals due to nitrogen compounds usually appear reveals a complex mixture of substances. Although the presence of pyrrolizidine alkaloids cannot be discarded, the lack of representative signals of those compounds, like the olefinic hydrogen at 5.70 ppm of amabiline and analogs, or the oxygenated methylene (4.78 ppm for amabiline, 4.47 and 4.27 ppm for thesinine), points to a very low concentration, if they exist at all. Thus, the *in vitro* antiproliferative activity of *Borago* extracts should be related to to rosmarinic acid content, which exercised the higher cell growth inhibition (GI_{50} of 37 μg/mL). This compound was previously found to exert strong anticarcinogenic activity on several cancer cell lines, as in the human acute promyelocytic leukemia cell line HL-60. In this model, no viable cells were detected when rosmarinic acid was added to the cell medium at concentrations over 0.55 mM. The *in vivo* anticarcinogenic activity

of rosmarinic acid has been related to the ability of this compound in inhibiting inflammation and scavenging reactive oxygen species (Lozano-Baena et al., 2016). Moreover, the antiproliferative activity of rosmarinic acid has been linked to the inhibition of extracellular signal-regulated kinase (ERK) signaling pathways, which play critical roles in cell proliferation and survival, which are frequently activated in colorectal cancer pathway (Xavier et al., 2009).

We also checked the antiproliferative activity against HT29 cells of other phenolics present at high concentrations in the various *Borago* spp. extracts: 3,4-dihydroxyhydrocinnamic acid and hydroxyphenyllactic acid, which showed GI_{50} values of 83 and 140, respectively, thus these activities were lower than that showed by rosmarinic acid. In addition to rosmarinic acid, antiproliferative activity was described for gallic, caffeic and ferulic acids (Anantharaju et al., 2016); however, these compounds are present in the various *Borago* extracts at lower amounts.

Given the occurrence of other compounds lacking any antiproliferative activity in the crude phenolic extract, such as carbohydrates, the antiproliferative activity displayed by the water:methanol extract was lower than pure phenolic compounds values.. Moreover, we cannot attribute the antiproliferative effects of the extracts to specific compounds, and it is likely that interactions between the various phenolic components of the extract contribute to the overall reported effects.

The antiproliferative activity against HT-29 cells achieved by the phenolic fractions of the different *Borago* species is of interest, since these cells are not very sensitive to phenolic compounds. For instance, the polyphenols from defatted seeds of evening primrose (*Oenothera paradoxa*), another typical GLA-source, were found to induce apoptosis in the human colon cancer Caco-2 cells, whereas HT-29 and IEC-6 cells were affected to a lesser extent (Gorlach et al., 2011).

5. Conclusions

All endemic and farmed *Borago* species analyzed here showed very similar phenolic compounds profiles, in which highlights rosmarinic acid. The concentrations of all identified phenolics in *B. officinalis* samples were highly variable, which could be related to genetic features, phenological stages and environmental conditions. Among *B. officinalis* samples, the highest values of total phenolic and rosmarinic acid were found in the warmest and southernmost localities. Mature and immature *B. officinalis* seeds had significant differences in rosmarinic, hydroxyphenyllactic and hydroxycinnamic acids contents. All checked *Borago* species showed dose- and time-dependent inhibition exercised on the human colorectal cancer cell line HT29. The antiproliferative activity against HT29 cells achieved by the phenolic-containing extracts of the various *Borago* species is of interest, since such cells have been described as not very sensitive to phenolic compounds. Given the occurrence of some compounds lacking on antiproliferative activity in the crude phenolic extracts, as evidenced by the ^{1}H NMR spectrum, the antiproliferative activity was lower for the phenolics-containing water:methanol extracts than that obtained for pure phenolic compounds. Further studies on biological activities of the seeds of the various *Borago* species will be welcomed; for instance, antioxidant, antimicrobial and anti-inflammatory properties. Moreover, works involving separation, purification and one to one bioassays of phenolic fractions may reveal the true nature and extent of their potency for *in vitro* antiproliferative activities. From a nutritional, pharmaceutical and cosmetic point of view, all *Borago* species reported here have interesting phenolic compounds profiles, which is an added value to the previously reported contents of GLA, tocopherols, squalene and sterols..

6. References

Amzad Hossain, M., & Shah, M.D. (2015). A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant *Merremia borneensis*. *Arabian Journal of Chemistry,* 8, 66–71.

Anantharaju, P.G., Gowda, P.C., Vimalambike, M.G., & Madhunapantula, S.V. (2016). An overview on the role of dietary phenolics for the treatment of cancers. *Nutrition Journal,* 15, 99.

Beck, H., Zimmermann, N., McVicar, T., McVicar, T.R., Vergopolan, N., Berg, A., & Wood, E. (2018). Present and future Köppen-Geiger climate classification maps at 1-km resolution. *Scientific Data*, 5, 180214.

Boeing, H., Bechthold, A., Bub, A., Ellinger, S., Haller, D., Kroke, A., Leschik-Bonnet, E., Müller, M.J., Oberritter, H., Schulze, M., Stehle, P., & Watzl, B. (2012). Critical review: vegetables and fruit in the prevention of chronic diseases. *European Journal of Nutrition,* 51, 637–663.

Fabrikov, D., Guil-Guerrero, J.L., González-Fernández, M.J., Rodríguez-García, I., Gómez-Mercado, F., Urrestarazu, M., Lao, M., Rincón Cervera, M.Á., Álvaro, J.E., & Lyashenko, S. (2019). Borage oil: tocopherols, sterols and squalene in farmed and endemic-wild *Borago* species. *Journal of Food Composition and Analysis*, 103299.

Gawrysiak-Witulska, M., Siger, A., Rudzińska, M., Stuper-Szablewska, K., & Rusinek, R. (2018). Effect of self-heating on the processing quality of rapeseed. *International Agrophysics*, 32, 313–323.

Gorlach, S., Wagner, W., Podsedek, A., Sosnowska, D., Dastych, J., & Koziołkiewicz, M. (2011). Polyphenols from evening primrose (*Oenothera paradoxa*) defatted seeds induce apoptosis in human colon cancer Caco-2 cells. *Journal of Agricultural and Food Chemistry*, 59, 6985-6997.

Guil-Guerrero, J.L., Gómez-Mercado, F., Ramos-Bueno, R.P., González-Fernández, M.J., Urrestarazu, M., & Rincón-Cervera, M.Á. (2017). Sardinian *Boraginaceae* are new potential sources of gamma-linolenic acid. *Food Chemistry*, 218, 435–439.

Guil-Guerrero, J.L., Gómez-Mercado, F., Ramos-Bueno, R.P., González-Fernández, M.J., Urrestarazu, M., Jiménez-Becker, S., & de Bélair, G. (2018). Fatty acid profiles and *sn*-2 fatty acid distribution of γ-linolenic acid-rich *Borago* species. *Journal of Food Composition and Analysis*, 66, 74-80.

Hernandez, T., Canales, M., & Duran, A. (2009). Variation of the hexanic extract composition of *Lippia graveolans* in an arid zone from Mexico: environmental influence or true chemotypes? *The Open Plant Science Journal*, 3, 29–34.

Ivanov, I.G., Vrancheva, R.Z., Marchev, A.S., Petkova, N.T., Aneva, I.Y., Denev, P.P., Georgiev, V.G., & Pavlov, A.I. (2014). Antioxidant activities and phenolic compounds in Bulgarian *Fumaria* species. *International Journal of Current Microbiology and Applied Sciences*, 3, 296-306.

Khanday, Z.H., & Singh, S. (2017). Ethnomedicinal Plants used for curing various Skin diseases in Shopian district of Jammu and Kashmir. *Journal of Phytology* 9:5-6.

Lozano-Baena, M.D., Tasset, I., Muñoz-Serrano, A., Alonso-Moraga, Á., & de Haro-Bailón, A. (2016). Cancer prevention and health benefices of traditionally consumed *Borago officinalis* plants. *Nutrients*, 8, 48.

Malabade, R.H., Taranalli, A.D., Rasal, V., & Kamlesh, K. (2016). Molecular docking study of *Cassia tora*, *Brassica campestris* and *Calotropis procera* as acetylcholinesterase inhibitor. *Indian Journal of Pharmaceutical Education and Research*, 50, 116 – 122.

Mansion, G., Selvi, F., Guggisberg, A., & Conti, E. (2009). Origin of Mediterranean insular endemics in the Boraginales: integrative evidence from molecular dating and ancestral area reconstruction. *Journal of Biogeography*, 36, 1282-1296.

Mao, X., Gu, C., Chen, D., Yu, B., & He, J. (2017). Oxidative stress-induced diseases and tea polyphenols. *Oncotarget*, 8, 81649-81661.

Medina-Medrano, J.R., Almaraz-Abarca, N., González-Elizondo, M.S., Uribe-Soto, J.N., González-Valdez, L.S., & Herrera-Arrieta, Y. (2015). Phenolic constituents and antioxidant properties of five wild species of *Physalis* (*Solanaceae*). *Botanical Studies*, 56, 24.

Mhamdi, B., Aidi Wannes, W., Sriti, J., Jellali, I., Ksouri, R., & Marzouk, B. (2010). Effect of harvesting time on phenolic compounds and antiradical scavenging activity of *Borago officinalis* seed extracts. *Industrial Crops and Products*, 31, 1–4.

Nogala-Kalucka, M., Rudzinska, M., Zadernowski, R., Siger, A., & Krzyzostaniak, I. (2010). Phytochemical content and antioxidant properties of seeds of unconventional oil plants. *Journal of the American Oil Chemists' Society*, 87, 1481–1487.

Ososki, A.L., Lohr, P., Reiff, M., Balick, M.J., Kronenberg, F., Fugh-Berman, A., & O'Connor, B. (2002). Ethnobotanical literature survey of medicinal plants in the Dominican Republic used for women's health conditions. *Journal of ethnopharmacology*, 79, 285-298.

Petersen, M., Abdullah, Y., Benner, J., Eberle, D., Gehlen, K., Hücherig, S., Janiak, V., Hee Kim, K., Sander, M., Weitzel, C., & Wolters, S. (2009). Evolution of rosmarinic acid biosynthesis. *Phytochemistry*, 70, 1663–1679.

Ramos-Bueno, R.P., González-Fernández, M.J., & Guil-Guerrero, J.L. (2016). Various acylglycerols from common oils exert different antitumor activities on colorectal cancer cells. *Nutrition and Cancer*, 68, 518–529.

Rohit M, Ashok T, Vijaykumar R, & Kashniyal K (2016) Molecular docking study of Cassia tora, Brassica campestris and Calotropis procera as acetylcholinesterase inhibitor. Indian *Journal of Pharmaceitical Education and Research*, 50, 116–122.

503 Selvi, F., Coppi, A., & Bigazzi, M. (2006). Karyotype variation, evolution and phylogeny in
504 *Borago (Boraginaceae)*, with emphasis on subgenus *Buglossites* in the Corso-Sardinian
505 system. *Annals of Botany*, 98, 857–868.
506 Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and
507 spices: antioxidant activity and health effects – a review. *Journal of Functional Foods*, 18,
508 820–897.
509 Siger, A., Nogala-Kalucka, M., & Lampart-Szczapa, E. (2008). The content and antioxidant
510 activity of phenolic compounds in cold-pressed plant oils. *Journal of Food Lipids*, 15, 137–
511 149.
512 Soto, C.G., Chamy, R., & Zúñiga, M.E. (2004). Effect of enzymatic application on borage
513 (*Borago officinalis*) oil extraction by cold pressing. Journal of Chemical Engineering of
514 Japan, 37, 326–331.
515 Stavroula, M., & Rahul, J. (2016). Mediterranean climate affects the biosynthesis of
516 secondary metabolites in common medicinal plants. *International Journal* of Business and
517 Systems Research, 6, 17–28.
518 Tanase, C., Coșarcă, S., & Muntean, D.L. (2019). A critical review of phenolic compounds
519 extracted from the bark of woody vascular plants and their potential biological activity.
520 *Molecules*, 24, 1182.
521 Van Hoed, V. (2010). Phenolic compounds in seed oils. *Lipid Technology*, 22(11), 247–249.
522 Wang, S.Y., & Zheng, W. (2001). Effect of plant growth temperature on antioxidant capacity
523 in strawberry. *Journal of Agricultural and Food Chemistry*, 49, 4977–4982.
524 Wettasinghe, M., Shahidi, F., Amarowicz, R., & Abou-Zaid, M.M. (2001). Phenolic acids in
525 defatted seeds of borage (*Borago officinalis* L.). *Food Chemistry*, 75, 49–56.
526 Xavier, C.P., Lima, C.F., Fernandes-Ferreira, M., & Pereira-Wilson, C. (2009). *Salvia*
527 *fruticosa*, *Salvia officinalis*, and rosmarinic acid induce apoptosis and inhibit proliferation of

human colorectal cell lines: the role in MAPK/ERK pathway. *Nutrition and cancer*, 61, 564-571.

Zadernowski, R., Naczk, M., & Nowak-Polakowska, H. (2002). Phenolic acids of borage (*Borago officinalis* L.) and evening primrose (*Oenothera biennis* L.). *Journal of the American Oil Chemists' Society*, 79, 335–338.

Conflicts of interest: The authors declare that they have no conflict of interest.

Acknowledgement

The authors acknowledged the botanical gardens listed in Table 1 for providing plant material. Also, the authors acknowledge to the Campus of International Agrifood Excellence (ceiA3) for funding this research.

Figures legend:

Fig. 1 280 nm-HPLC chromatogram of the phenolic-containing water:methanol extract of *B. longifolia* seeds. 1. Gallic acid; 2. Protocatechuic acid; 3. Hydroxyphenyllactic acid; 4. Chlorogenic acid; 5. 3,4-Dihydroxyhydrocinnamic acid; 6. Caffeic acid; 7. Vanilic acid; 8. Salicylic acid; 9. *p*-Coumaric acid; 10. Ferulic acid; 11. Rosmarinic acid; 12. 2-Hydroxy-4-methoxybenzoic acid.

Fig. 2 MTT assay. A) Concentration–response plot for HT29 and CCD18 cells after exposure to *B. longifolia*, *B. morisiana*, *B. officinalis*, *B. pygmaea* and *B. trabutii* extracts for 72 h. Data on CCD18 cells are plotted only for *B. trabutii*, given a close similarity for effects of the various *Borago* extracts on these cells. B) GI_{50} after HT19 cells exposure for 72 h to *B. longifolia*, *B. morisiana*, *B. pygmaea*, and *B. trabutii* extracts, as well as to 3,4-dihydroxyhydrocinnamic acid, hydroxyphenyl lactic acid, rosmarinic acid and doxorubicin. Data represent the mean of three complete independent experiments ± SD (error bars). In a bar, means followed by different letter are significantly different at $P<0.05$.

Fig. 3 ^{1}H NMR of the MeOH-H_2O extract of *B. trabutii seeds*. The expansion from 6.2 to 7.6 ppm shows the aromatic and olefinic signals due to rosmarinic acid.

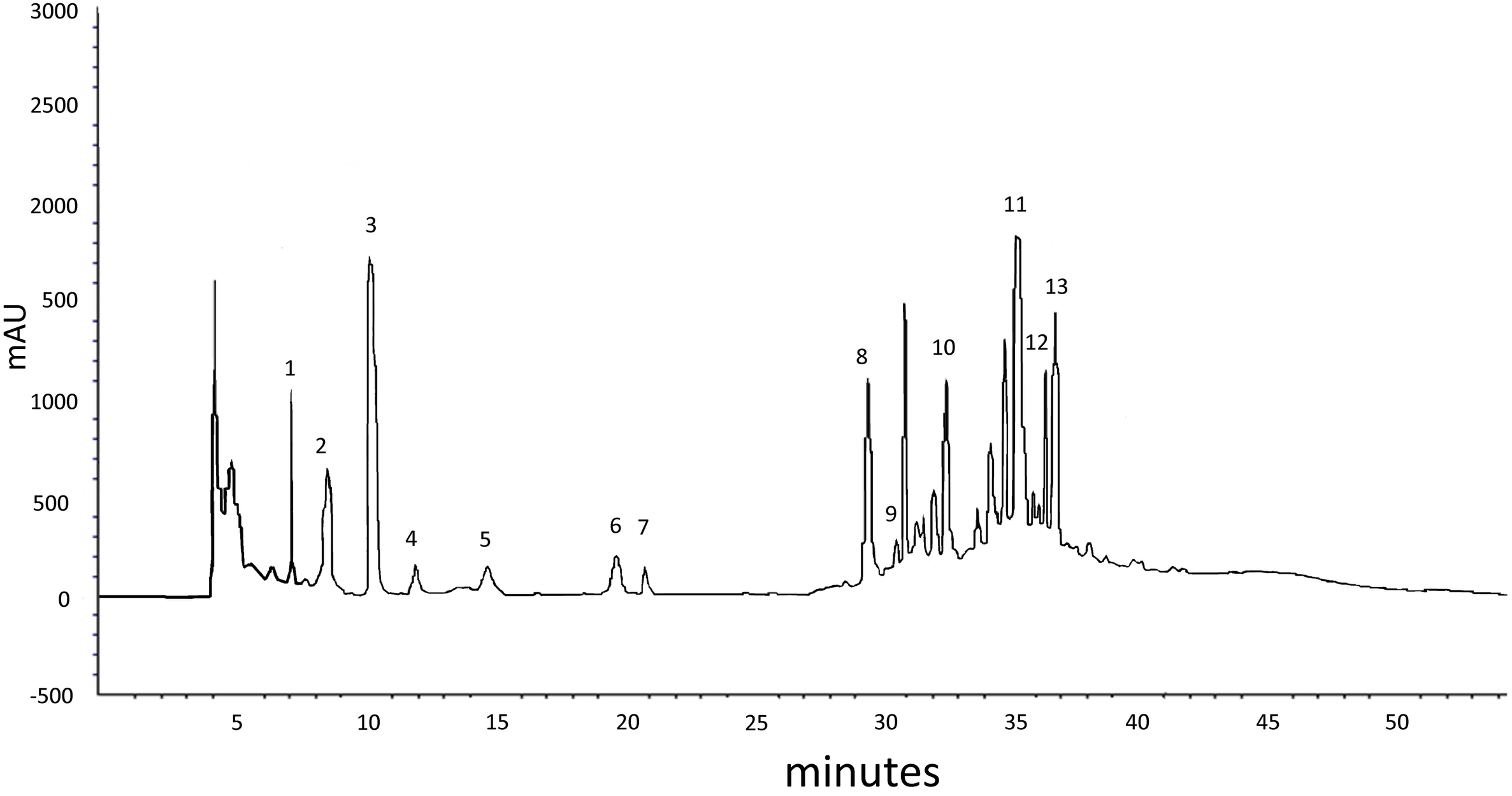
Table 1. Data collection of *Borago* species

Label	Samples	Number of subpopulations studied	Species status	Sample location	Geographical coordinates	Collection year
1	*B. officinalis* A	3	Wild	Tunisia, Béja: Ouechtata	36.951 N 8.999 E	2016
2	*B. officinalis* B_1	1	Cultivated	Spain, Almería: University of Almería	36.829 N 2.405 W	2019
3	*B. officinalis* 'Alba' B_2	1	Cultivated	Spain, Almería: University of Almería	36.829 N 2.405 W	2019
4	*B. officinalis* C	1	Cultivated	Russia, Ekaterinburg: Russian Academy of Sciences, Ural Branch: Institute Botanic Garden	56.813 N 60.604 E	2018
5	*B. officinalis* D	1	Wild	Russia, Udmurt Republic, Igrinskaya district: Zura	57.611 N 53.397 E	2017
6	*B. officinalis* E	1	Cultivated	Kyrgyz Republic, Bishkek: Gareev Botanical Garden of NAS KR	42.835 N 74.636 E	2017
7	*B. officinalis* F_1	3	Wild	Spain, Granada: Bubión, Alpujarra, mature seeds	36.949 N 3.355 W	2019
8	*B. officinalis* F_2	2	Wild	Spain, Granada: Bubión, Alpujarra, immature seeds	36.949 N 3.355 W	2019
9	*B. officinalis* G_1	2	Wild	Spain, Granada: Mecina Bombarón, Alpujarra, mature seeds	36.982 N 3.156 W	2019
10	*B. officinalis* G_2	2	Wild	Spain, Granada: Mecina Bombarón, Alpujarra, immature seeds	36.982 N 3.156 W	2019
11	*B. officinalis* H	1	Cultivated	Russia, Moscow: Botanical garden of All-Russian Scientific Research Institute of Medicinal and Aromatic Plants	55.567 N 37.569 E	2017
12	*B. officinalis* I	1	Cultivated	Ukraine, Kiev: M.M. Glushko National Botanical Garden, National Academy of Sciences of Ukraine	50.245 N 30.334 E	2019
13	*B. officinalis* oil	1	-	Spain, Casa Santiveri SL		2019
14	*B. pygmaea*	2	Wild	Italy, Ogliastra: Gairo, Montarbu di Seui	39.839 N 9.455 E	2016
15	*B. longifolia*.	2	Wild	Algeria, Skikda: Garaet, Sidi Lakhdar	36.910 N 7.191 E	2016
16	*B. morisiana* A	2	Wild	Italy, Oristano: Laconi, Tanca de Cuccuru	39.874 N 9.091 E	2016
17	*B. morisiana* B	2	Wild	Italy, Carbonia Iglesias: Carloforte, Calavinagra	39.164 N 8.242 E	2016
18	*B. trabutii*	3	Wild	Morocco, Marrakech-Tensift-Al Hauz: Anfli, Ourika valley	31.201 N 7.739 W	2018

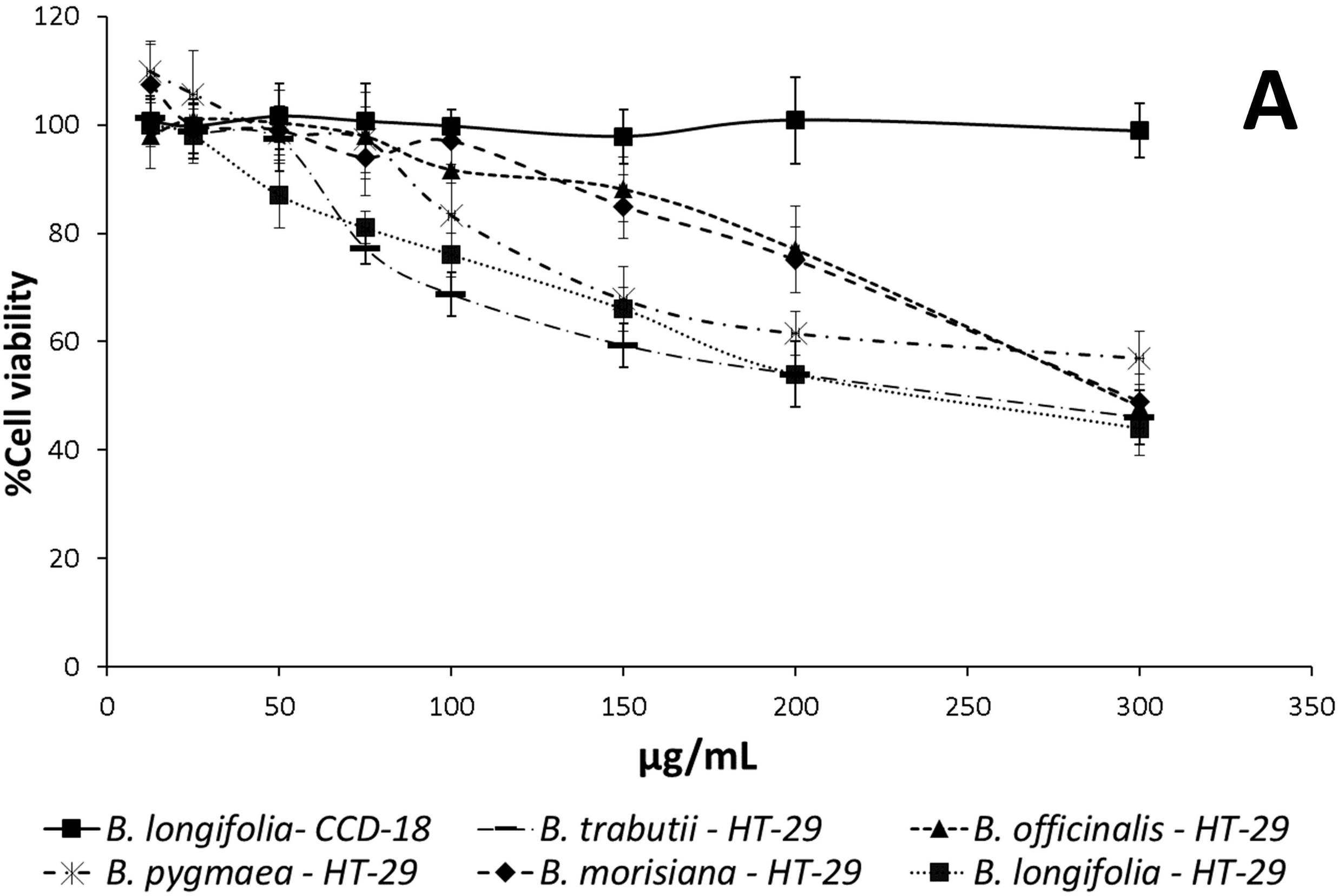
Table 2. HPLC-DAD and LC-MS parameters for analysis of phenolic-enriched extracts of *Borago* seeds

			LC-MS parameters			HPLC-DAD parameters				
N[a]	Rt	Phenolic compound	m/z precursor ion[a]	m/z fragment ion[b]	Ionization mode	Detection wavelength (nm)	Correlation coefficients (R^2)	LOD (μg/mL)	LOQ (μg/mL)	Recovery (%)
		Compounds identified by Rt and m/z ions								
1	6.8	Gallic acid	169.01425	125.02319	Negative	280	0.988	0.01	0.03	99.72
2	9.4	Protocatechuic acid	153.01868	109.02970	Negative	280	0.998	0.20	0.60	87.82
3	10.8	DL-p-Hydroxyphenyllactic acid	181.05063	95.01244	Negative	280	0.979	0.30	0.80	100.02
4	13.1	p-Hydroxybenzoic acid	137.02442	93.03325	Negative	280	0.989	0.20	0.40	98.93
5	15.1	Chlorogenic acid	355.10236	163.03566	Positive	280	0.991	0.30	0.50	98.99
6	15.9	3,4-Dihydroxyhydrocinnamic acid	163.04007	93.03316	Negative	280	0.988	0.20	0.50	101.04
7	21.9	Caffeic acid	179.03498	135.04429	Negative	320	0.979	0.40	1.00	65.99
8	23.7	Vanilic acid	167.03498	108.02023	Negative	280	0.988	0.20	0.70	89.98
9	31.0	Salicylic acid	139.03909	93.03460	Positive	300	0.991	0.20	0.60	89.93
10	31.5	p-Coumaric acid	163.04007	119.04881	Negative	280	0.996	0.30	0.60	93.15
11	32.3	Ferulic acid	193.05063	134.03643	Negative	280	0.978	0.30	0.50	100.22
12	34.6	Rutin	609.14611	301.03474	Negative	320	0.997	0.20	0.50	98.88
13	34.9	Naringenin	271.06120	119.04879	Negative	280	0.995	0.40	0.50	99.89
14	35.2	Rosmarinic acid	359.07724	109.02650	Negative	320	0.994	0.40	0.60	97.89
15	38.2	2-Hydroxy-4-methoxybenzoic acid	167.03498	108.02023	Negative	280	0.969	0.20	0.50	97.96
16	48.1	Kaempferol	287.05501	153.01760	Positive	320	0.973	0.50	0.40	100.01
		Compounds identified by m/z ions[c]								
16		8-Hydroxyquinoline	146.06012	117.056080	Positive		-	-	-	-
17		Gluconapin	432.06398	96.95920	Positive		-	-	-	-
18		Quercetin	303.04993	178.99749	Positive		-	-	-	-
19		Quercetin-3-O-hexoside	463.08820	302.03696	Negative		-	-	-	-
20		Kaempferol-3-O-hexoside	447.09328	255.02924	Negative		-	-	-	-
21		Quercitrin	447.09328	230.98517	Negative		-	-	-	-
22		Galangin	269.04555	223.04036	Negative		-	-	-	-

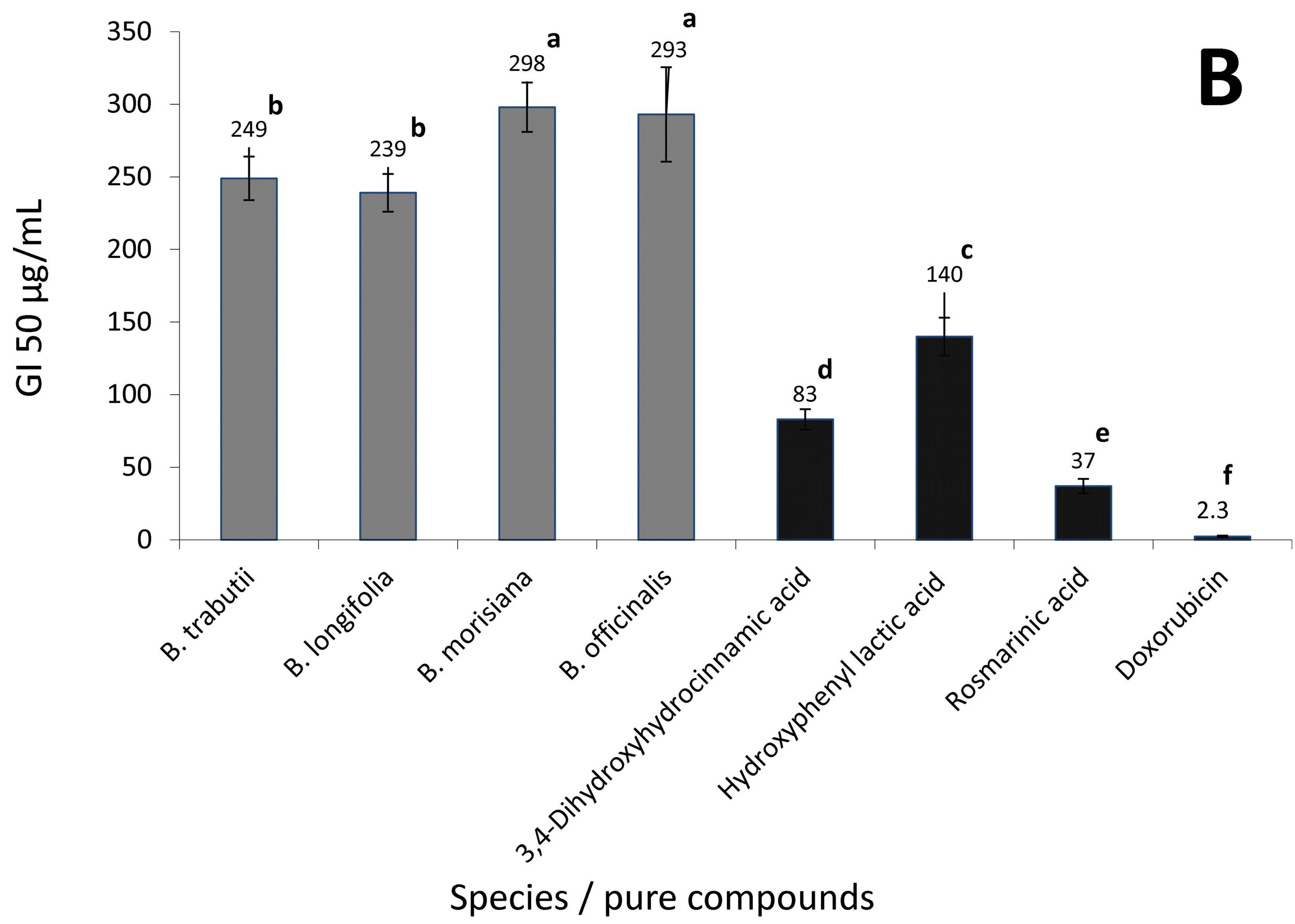
[a] Mass error lower than 5 ppm
[b] Mass error lower than 10 ppm
[c] Phenolic compounds identified by LC-MS

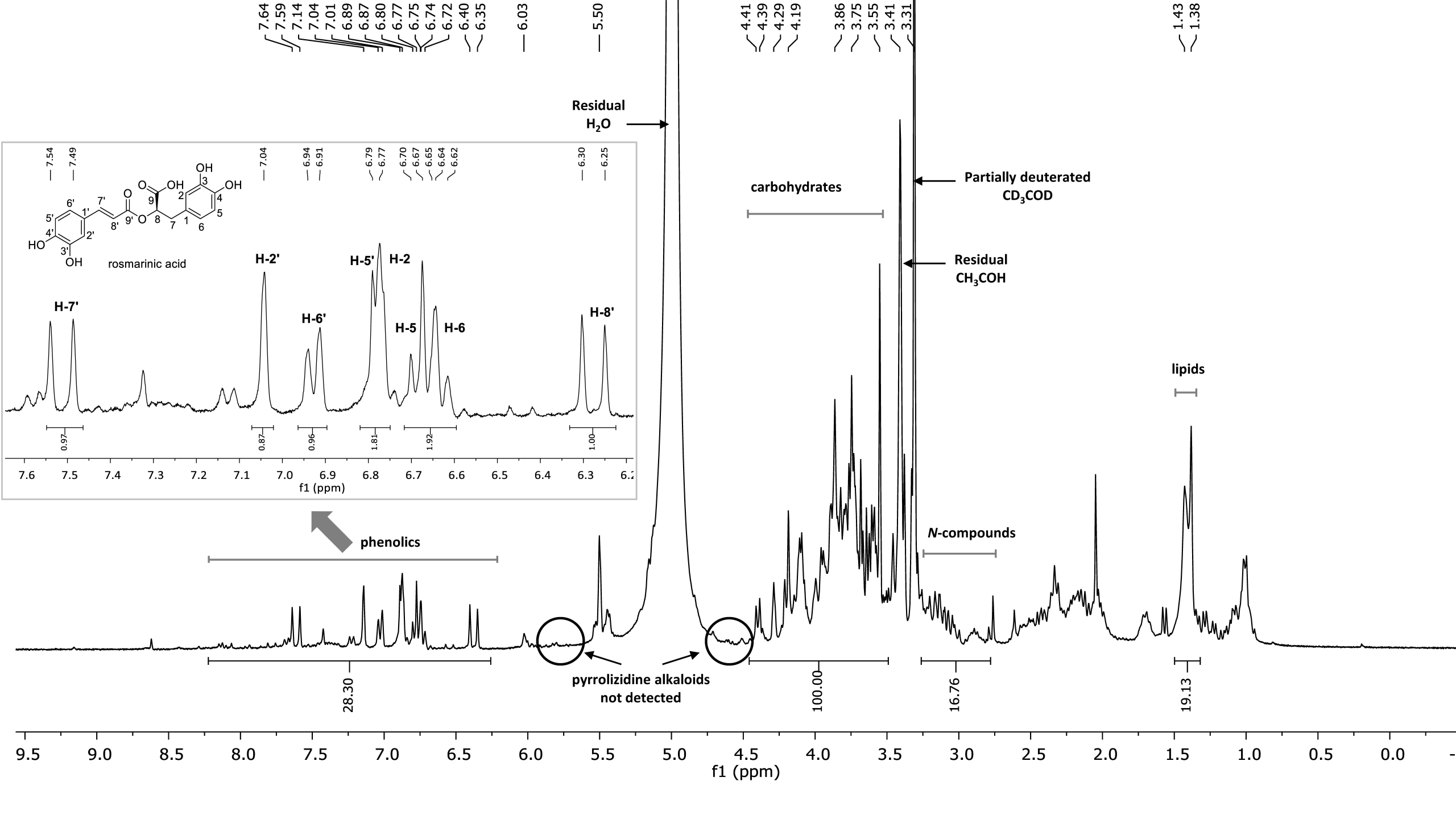

3000
2500
2000
500
1000
500
0
-500
mAU
1
2
3
4
5
6
7
8
9
10
11
12
13
5
10
15
20
25
30
35
40
45
50
minutes

A


120
100
80
60
40
20
0
%Cell viability
0
50
100
150
200
250
300
350
µg/mL
B. longifolia- CCD-18
B. trabutii - HT-29
B. officinalis - HT-29
B. pygmaea - HT-29
B. morisiana - HT-29
B. longifolia - HT-29

B

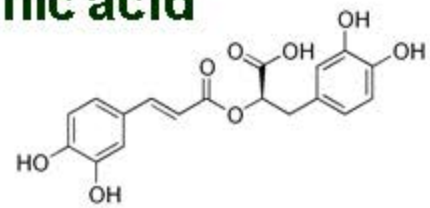

350
300
250
200
150
100
50
0
GI 50 µg/mL
249
b
239
b
298
a
293
a
83
d
140
c
37
e
2.3
f
B. trabutii
B. longifolia
B. morisiana
B. officinalis
3,4-Dihydroxyhydrocinnamic acid
Hydroxyphenyl lactic acid
Rosmarinic acid
Doxorubicin
Species / pure compounds

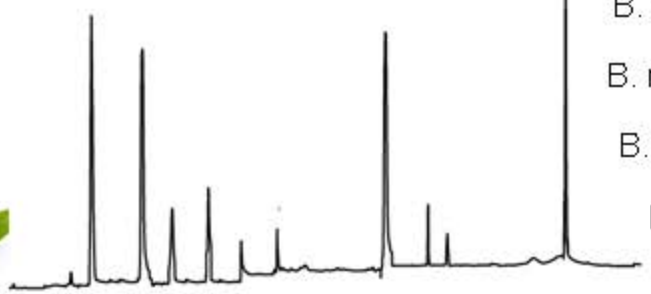

7.64
7.59
7.14
7.04
7.01
6.89
6.87
6.80
6.77
6.75
6.74
6.72
6.40
6.35
6.03
5.50
4.41
4.39
4.29
4.19
3.86
3.75
3.55
3.41
3.31
1.43
1.38
Residual
H2O
carbohydrates
Partially deuterated
CD3COD
Residual
CH3COH
lipids
phenolics
N-compounds
pyrrolizidine alkaloids
not detected
28.30
100.00
16.76
19.13
f1 (ppm)
7.54
7.49
7.04
6.94
6.91
6.79
6.77
6.70
6.67
6.65
6.64
6.62
6.30
6.25
rosmarinic acid
H-7'
H-2'
H-6'
H-5'
H-2
H-5
H-6
H-8'
0.97
0.87
0.96
1.81
1.92
1.00
f1 (ppm)

Endemic-wild and farmed *Borago* species

PHENOLICS

Rosmarinic acid





Antiproliferative activity

HPLC-DAD and LC-MS

GI_{50} on HT29 cancer cells (µg/mL)