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Phenolic acids in defatted seeds of borage (*Borago officinalis* L.)

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Abstract

An ethanolic extract of borage meal was fractionated (fractions I–VI) on a Sephadex LH-20 column. All fractions tested positive for phenolics, but were negative for condensed tannins. Silica gel thin-layer chromatography (TLC) of fractions allowed the location of one and two strong antioxidative spots in fractions I and IV, respectively. Other fractions produced spots containing either weak antioxidative compounds or compounds with low concentrations. High performance liquid chromatography of the three major TLC spots in fractions I and IV showed the presence of one phenolic compound in each spot. Ultraviolet, proton nuclear magnetic resonance and proton-proton correlation spectroscopies, as well as electron impact-mass spectrometry, allowed the identification of three phenolic acids, namely rosmarinic acid in fraction I, and syringic and sinapic acids in fraction IV. These three compounds contributed to 3.9% of the dry mass of the crude extract whereas their total contribution in the meal was 0.6% (w/w).

1. Introduction

Phenolic compounds are ubiquitous in oilseeds and numerous studies have shown their antioxidant potential. It has been documented that the antioxidant compounds of sesame are tocopherols, sesamol, sesamolinol, sesaminol, pinoresinol, syringic acid, ferulic acid and sesamol dimer (Fukuda, Osawa, Namiki, & Ozaki, 1985; Osawa, Ramarathnum, Kawakishi, Namiki, & Tashiro, 1985). Shahidi, Wanasundra, and Amarowicz (1994) have reported that ethanolic extracts of mustard flour exhibit antioxidant properties in a β-carotene-linoleate model system. These authors attributed the antioxidant effect of mustard extracts to polyhydroxyphenolics, such as flavones and flavonols, present in them. Wanasundara, Amarowicz, and Shahidi (1994) identified the most active phenolic antioxidant in canola meal as 1-O-β-D-glucopyranosyl sinapate.

The antioxidants present in soybean oil are α-tocopherol, γ-tocopherol and δ-tocopherol (Gyorgy, Murata, & Ikehata, 1964). The main antioxidative compounds in soybean meal have been identified to be isoflavones, glycosides and their derivatives, tocopherols, amino acids, and peptides (Gyorgy et al., 1964; Hayes, Bookwalter, & Bagley, 1977; Naim, Gestetner, Zilkah, Birk, & Bondi, 1973, 1974, 1976). Some of the isoflavone glycosides of soybean meal have been identified as genistein, daidzein, glycitein, prunetin, formononetin and 4′,6′,7′-trihydroxyisoflavone (Rackis, 1972).

An antioxidative flavonol, dihydroquercetin, has been isolated from methanolic extracts of peanut (Pratt & Miller, 1984). Apart from phenolic antioxidants, protein fractions of peanut have been shown to inhibit lipid oxidation in model systems containing metmyoglobin and Fe^{2+}-EDTA as catalysts (Rhee, Ziprin, & Rhee, 1979). Antioxidant activity of protein fractions of peanut in fresh beef homogenates and beef patties has also been reported (Ziprin, Rhee, Carpenter, Hosetler, Terrel, & Rhee, 1981). The major phenolic compounds present in the methanolic extracts of cottonseed were

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quercetin glycosides (Whiltern, Miller, & Pratt, 1984). The main quercetin glycoside of cottonseed was rutin and its antioxidant activity was reported to be inferior to that of quercetin (Yousseff & Rahman, 1985).

Rapeseed and its genetically improved version, canola, contained several phenolic compounds that exist as phenolic acids, their derivatives and condensed tannins (Amarowicz, Shimoyamada, Okub, & Koslowska, 1990). Rapeseed meal contained phenolic acids, namely salicylic, cinnamic, *p*-hydroxybenzoic, veratric, vanillic, gentisic, protocatechuic, syringic, caffeic, sinapic and ferulic acids (Kozlowska, Naczk, Shahidi, & Zadernowski, 1990). The major units of condensed tannins of rapseed meal were cyanidin, pelargonidin and kaempferol and their derivatives.

Borage (*Borago officinalis* L.) has gained importance, due to the occurrence of high levels of γ-linolenic acid in its seed oil. In a recent publication, an extensive investigation on antioxidant properties of extracts of borage meal was reported (Wettasinghe & Shahidi, 1999). Borage extracts demonstrated excellent antioxidant properties and these effects were attributed to their phenolic constituents. The objective of the present study was to identify major phenolic compounds present in borage extracts using appropriate techniques for isolation and structure elucidation of compounds involved.

2. Materials and methods

Borage seeds were obtained from Bioriginal Food Co. Ltd., Saskatoon, SK. All reagents and authentic standards were purchased from Sigma Chemical Co. (St. Louis, MO). Solvents used in this study were of HPLC grade and were obtained from Fisher Scientific Co. (Nepean, ON). Deuterated solvents were obtained from Cambridge Isotope Laboratories (Andover, MA).

2.1. Preparation of borage meal

Borage seeds were ground in an electric coffee grinder (Black & Decker Canada Inc., Brockville, ON) for 15 min and then defatted by blending with hexane (1:5 w/v, 5 min, ×3) in a Waring Blendor (Model 33BL73, Waring Products Division, Dynamics Corporation of America, New Hartford, CT) at ambient temperature. The resulting slurry was suction filtered and the residue (defatted seeds or meal) was air-dried for 12 h. The dried meal was stored in vacuum-packaged polyethylene pouches at −20°C until used.

2.2. Preparation of borage crude extracts

Crude extracts of borage meal were prepared using 52% (v/v) aqueous ethanol. The detailed extraction protocol was the same as that explained previously (Wettasinghe & Shahidi, 1999).

2.3. Qualitative detection of phenolic compounds

Folin-Denis reagent (0.5 ml) was added to a centrifuge tube containing 0.5 mg of crude extract in 0.5 ml of methanol. Contents were mixed and 1 ml of a saturated sodium carbonate solution was added into each tube. Volume was then adjusted to 10 ml by the addition of 8 ml of deionized water and the contents were mixed vigorously. Tubes were allowed to stand at ambient temperature for 25 min until the characteristic blue colour developed (Swain & Hillis, 1959).

2.4. Qualitative detection of vanillin-positive compounds

To 1 mg of crude extracts in 1 ml of methanol, 5 ml of freshly prepared 0.5% (w/v) vanillin solution in 4% (w/v) hydrochloric acid were added, mixed vigorously and allowed to stand for 20 min at 30°C. A positive test was indicated by the appearance of a characteristic pink colour in the solutions (Price & Butler, 1977).

2.5. Column chromatographic fractionation of crude extracts

A 1-g portion of crude extracts was dissolved in 10 ml of HPLC grade methanol and applied to a column (1.5 cm diameter and 77 cm height) packed with Sephadex LH-20 (particle size 25–100 mm, Sigma Chemical Co., Nepean, ON) and eluted with methanol. Methanolic fractions (8 ml each) were collected in test tubes placed in a LKB Bromma 2112 redirac fraction collector (Pharmacia, Uppsala, Sweden) and their absorbance was read at 280 nm. Eluates were then pooled into fractions I–VI. Solvent was evaporated under vacuum at 40°C. Dried fractions were stored in tinted glass bottles at −18°C until used. Presence or absence of phenolic and vanillin-positive compounds in borage fractions was determined using the same methods as explained in Sections 2.3 and 2.4.

2.6. Silica gel thin-layer chromatography (TLC) of fractions

Column chromatographic fractions (I–VI) were loaded onto analytical TLC plates (Silica gel, 60 A mean pore diameter, 2–25 mm mean particle size, 250 mm thickness, Sigma Chemical Co., St. Louis, MO). Chromatograms were developed in a glass chamber (22×22×10 cm, Fisher Scientific Ltd., Nepean, ON) using chloroform–methanol–water (65:35:10, v/v/v) as the mobile phase (Amarowicz, Koslowska, & Shimoyamada, 1992). After drying, bands were located by viewing under short (254 nm) and long (365 nm) UV

radiation (Spectraline, Model ENF-240C, Spectronics Co., Westburg, NY). The following sprays were also used to locate phenolic compounds (spray A) and to examine their antioxidant properties (spray B):

2.6.1. Spray A — ferric chloride-potassium ferricyanide [$FeCl_3$-$K_2Fe(CN)_6$]

Equal volumes of 1% (w/v) aqueous solutions of each salt were freshly mixed (producing an orange-brown coloured reagent). Phenolics produce a blue colour with this reagent (Barton, Evans, & Gardner, 1952).

2.6.2. Spray B—β-carotene-linoleate spray

The relative antioxidant activity of band components on the developed TLC plates was determined using the β-carotene–linoleate spray method (Pratt & Miller, 1984). β-carotene (9 mg) was dissolved in 30 ml of chloroform. Two drops of linoleic acid and 60 ml of ethanol were added to the β-carotene–chloroform solution. The plates were sprayed with this solution, followed by their exposure to fluorescent light for 3 h or until the background orange colour disappeared. Bands with persisting orange colour were considered as possessing antioxidant activity. The colour intensity, as judged by the naked eye, was related to their antioxidant strength.

The fractions containing compounds with high antioxidant activity (as determined by β-carotene-linoleate spray) were scraped and extracted into spectral grade methanol. The slurry was centrifuged (3 min at 5000×***g***) and the supernatant evaporated to dryness under a stream of nitrogen. The dried residues of active components were used for further analysis.

2.7. Analytical and preparative high performance liquid chromatography (HPLC)

A Shimadzu HPLC system (Kyoto, Japan), equipped with LC-6A pump, SPD-6A V UV-VIS spectrophotometric detector, SCL-6B system controller and CR 501 Chromatopac, was used for analytical and preparative HPLC analysis of isolated compounds. The column used for preparative HPLC was a Hilber prepacked column RT (10×250 mm) with Lichrosorb RP-18 (7 μm, Merck, Darmstad, Germany). The volume injected was 500 μl. Mobile phase was water-acetonitrile-methanol-acetic acid (79.5:18:2:0.5, v/v/v/v) and introduced to the column at a flow rate of 3 ml/min. Pure compounds, so obtained, were also examined by HPLC separation at ambient temperatures of 20–22°C on an analytical column. An analytical CWSL column (4.5×250 mm) with Spherisorb-ODS-2 (10 μm, Chromatography Sciences Co. Inc., Montreal, PQ) was used for this purpose. Flow rate was 0.8 ml/min and the volume injected was 20 μl. For both preparative and analytical HPLC, the detector was preset at 280 nm (Amarowicz & Shahidi, 1996).

2.8. Ultraviolet (UV) spectroscopy of purified compounds

The UV absorption spectra (200–400 nm) of purified compounds (in methanol) were recorded using a Hewlett Packard diode array spectrophotometer (Model 8452A, Hewlett Packard Co., Mississauga, ON).

2.9. Electron impact-mass spectrometry (EI-MS) of purified compounds

All mass spectra of purified compounds (in methanol) were recorded using an electron impact (EI) mode at 70 eV in a 7070 HS Micromass double focusing mass spectrometer (V.G. Micromass Ltd., Manchester, UK). The source, probe and scanning temperatures, used in this study, were 200, 100–300, and 20–25°C, respectively.

2.10. Proton nuclear magnetic resonance (^1H NMR) spectroscopy

The NMR spectra were recorded using a General Electric 300-NB spectrometer (General Electric, Palo Alto, CA). ^1H NMR (at 300 MHz) and (^1H–^1H) correlation spectroscopic (COSY, at 300 MHz) data were collected at room temperature in deuterated methanol (CD_3OD). Chemical shifts (δ, ppm) were reported relative to tetramethylsilane (TMS) as an internal standard. Data were analyzed using NUTS software (NMR Data Processing Program, Acron NMR Inc., Fremont, CA).

2.11. Quantification of identified compounds

Once the identification of isolated compounds was achieved, a known mass of each of the fractions was chromatographed on preparative silica gel TLC plates. The spots representing the identified compounds were located. They were scraped off, suspended individually in methanol, centrifuged (for 3 min at 5000×***g***) to remove solids and then the supernatant dried under a stream of nitrogen to recover the compounds of interest. The yield of each compound was recorded on a weight basis of the relevant fraction, crude extract and the defatted seed (meal). Equations used in the calculations are given in the footnotes of Table 8.

3. Results and discussion

3.1. Sephadex LH-20 column chromatography of crude extracts of borage meal

A portion of the crude extracts of borage meal was subjected to column chromatography as a first step towards the identification of its active components. Sephadex LH-20 column chromatography has been used extensively to fractionate various plant extracts

(Amarowicz, Koslowska, & Shimoyamada, 1992; Amarowicz & Shahidi, 1996; Amarowicz, Wanasundara, & Shahidi, 1993). Sephadex LH-20 is probably one of the best stationary phases available for separation of phenolics because of the faster, yet satisfactory, separation of phenolics on the column (Wanasundara et al., 1994). Fig. 1 shows the fraction profile of borage crude extracts. Even though the profile was continuous, six major fractions, labelled I–VI, were clearly identifiable. All fractions of borage crude extracts contained phenolic compounds, as they produced the characteristic blue colour with the Folin-Denis reagent. Also, the absence of the flavanoid group of compounds in all borage fractions was confirmed by a negative vanillin test.

3.2. Silica gel TLC and HPLC

Table 1 shows the results of TLC analysis of borage fractions and standard phenolic compounds. When a developed plate was sprayed with a solution of 1% (w/v) ferric chloride-potassium ferricyanide [$FeCl_3$-$K_2Fe(CN)_6$], several pale blue and intense blue colour spots were visualized for each of the fractions; their R_f values are given in Table 1. When sprayed with 1% (w/v) $FeCl_3$-$K_2Fe(CN)_6$ solution, an intense blue colour was produced by the spot at R_f of 0.51 of fraction I. When a second plate (developed in the same solvent system) was sprayed with a β-carotene-linoleate emulsion, the spot at R_f of 0.51 of fraction I showed an intense yellow colour, even after 3 h exposure to fluorescent light. This was an indication of the presence of one or more phenolic compounds with moderate antioxidant activity. The spot with R_f of 0.56 of fraction II gave a pale blue colour with $FeCl_3$-$K_2Fe(CN)_6$ and produced a yellow colour with β-carotene-linoleate emulsion. The other two spots of fraction II had weak antioxidant activities as they lost most of the β-carotene during exposure to fluorescent light. Among four spots observed for fraction III, the spot with R_f of 0.56 showed moderate antioxidant activity and this spot gave a pale blue colour with a 1% (w/v) $FeCl_3$-$K_2Fe(CN)_6$ solution. Other spots of fraction III exhibited weak antioxidant activities. The spot with R_f of 0.82 of fraction IV showed strong antioxidant activity as its colour was an intense orange, even after 3 h exposure to fluorescent light. The other spot with R_f of 0.57, of fraction IV, showed moderate antioxidant activity. These two spots produced a highly intense blue colour upon spraying the spaying them with a 1% (w/v) $FeCl_3$-$K_2Fe(CN)_6$ solution. Fraction V also contained a spot ($R_f = 0.84$) with high antioxidant activity. The other three spots had moderate to weak antioxidant activities. However, none of the spots of fraction V contained sufficient phenolics (as judged by the intensity of blue colour) to be considered for further analysis. Spots of fraction VI showed weak antioxidant activities, as their colour faded rapidly under fluorescent light. As indicated by R_f values of standards, absence of (+)catechin

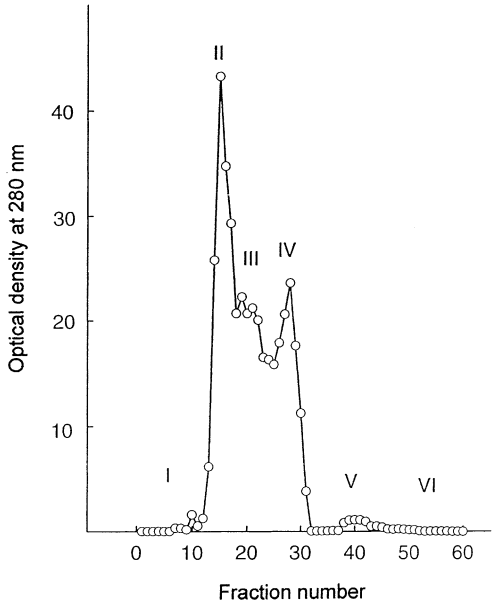

Fig. 1. Column chromatographic fraction profile of crude extracts of borage.

Table 1
R_f values and antioxidant activities of various borage phenolics and authentic standards resolved on thin-layer chromatographic (TLC) plates

Fraction	Spray A	Spray B		
	Blue[a]	Orange[b]	Yellow[b]	Pale yellow[b]
I	0.33, 0.51, 0.57, 0.68, 0.76	–	0.51	–
II	0.44, 0.56, 0.78	–	0.56	0.44, 0.78
III	0.44, 0.56, 0.82, 0.90	–	0.56	0.44, 0.82, 0.90
IV	0.57, 0.82	0.82	0.57	–
V	0.30, 0.44, 0.56, 0.84	0.84	0.56	0.30, 0.44
VI	0.32, 0.44	–	–	0.32, 0.44
Syringic acid[c]	0.57	0.57	–	–
Sinapic acid[c]	0.82	0.82	–	–

[a] Colour produced when Spray A [1%$FeCl_3$-$K_2Fe(CN)_6$] was sprayed.
[b] Intensity of colour of β-carotene after 3 h from spraying of Spray B (β-carotene-linoleate emulsion).
[c] Concentration of authentic standard solutions was 1 mg/ml.

and (−)epicatechin (data not given in Table 1) in any of the fractions was evident. Absence of the flavonoid class of compounds in borage crude extract was also confirmed by a negative vanillin test. The R_f values of spots of fraction IV matched with those of syringic (0.57) and sinapic (0.82) acids. The spot with R_f of 0.51 in fraction I did not correspond with R_f of any of the standards used, but the intense blue colour of the spot indicated that it contained a major phenolic compound(s). After isolating the spots on a preparative TLC plate, they were subjected to preparative HPLC. A sharp single peak produced by analytical HPLC of each of the spots indicated that a single compound was involved in each case. Purified borage compounds were labelled A, B and C and subjected to instrumental analysis in order to elucidate their chemical structures.

3.3. Structural analysis of active components of borage crude extracts

As described previously, tentative identities of compounds B and C were established as syringic acid and sinapic acid, respectively. Tentative identity of compound A was not established. The following sections will focus on the structure elucidation of these compounds by means of UV, NMR and EI-MS.

3.4. UV spectral data and EI-MS fragmentation pattern of compound A

As shown in Fig. 2, compound A had a UV absorbance maximum at 330 nm and a shoulder at 294 nm. This could be due to a phenolic acid with two aromatic rings (Rice-Evans, Miller, & Pagana, 1996). The mass fragment profile (Table 2) showed a fragment ion with m/z of 299, which could conceivably be produced via losses of CO_2 and OH from the molecular radical ion ($M^{+\bullet}$) of the compound A (structure given in Fig. 3). As expected, the molecular radical ion ($M^{+\bullet}$) at m/z of 360 was not present as it decomposed in the ionization chamber. The fragment ion with m/z of 181 was due to the loss of a $C_9H_7O_4$ moiety from the $M^{+\bullet}$ and further loss of one H from the fragment resulted in an ion with m/z of 180. The fragment ion with m/z of 179 indicated loss of a molecule of hydrogen from the fragment ion at m/z of 181. The loss of OH, H_2O and H_3O, respectively, from the fragment ion at m/z of 181 resulted in three fragment ions with m/z of 164, 163 and 162. However, it is evident that the loss of water or dehydration was the major factor in the fragmentaion process because the intensity of fragment with m/z of 163 was 70%. The loss of CO_2 from the fragment ion at m/z of 181 produced a fragment ion with m/z of 137. The fragment ion at m/z of 136 was formed by loss of both H and CO_2 or an acid group from the fragment ion at m/z of 181. The loss of

Table 2
Electron impact mass (EI-MC) fragmentation for compound A

m/z	%Intensity	Fragment ion
360.00	ND[a]	$C_{18}H_{16}O_8^+$
299.04	1.00	$C_{17}H_{15}O_5^+$
181.01	11.00	$C_9H_9O_4^+$
179.96	38.50	$C_9H_8O_4^+$
178.99	4.25	$C_9H_8O_3^+$
164.06	17.25	$C_9H_8O_3^+$
163.03	70.00	$C_9H_7O_3^+$
162.00	23.00	$C_9H_6O_3^+$
137.06	8.00	$C_8H_9O_2^+$
136.07	25.75	$C_8H_8O_2^+$
135.05	14.50	$C_8H_6O_2^+$
124.08	23.00	$C_7H_8O_2^+$
123.06	87.00	$C_7H_7O_2^+$
109.93	34.00	$C_6H_5H_2^+$
44.02	100	CO_2^+

[a] ND, not detected.

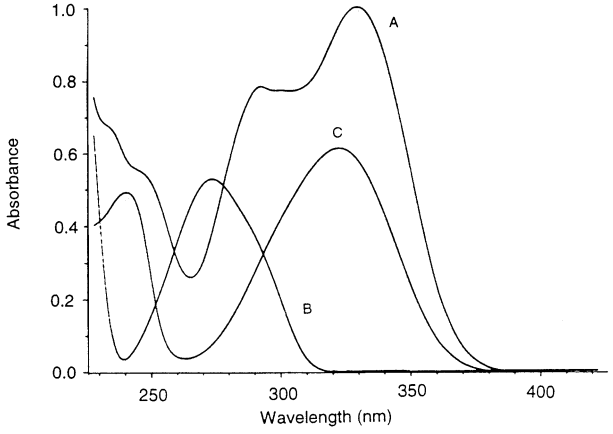

Fig. 2. Ultraviolet (UV) spectra of compounds A, B and C.

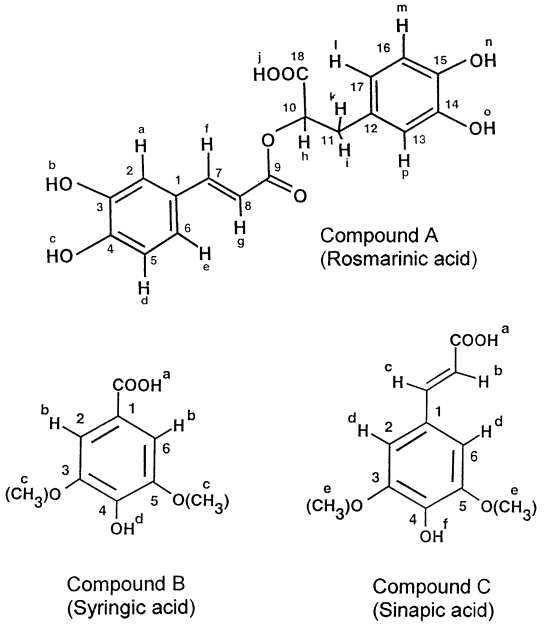

Fig. 3. Chemical structures of compounds A, B and C.

H_2 and CO_2 or H and COOH from the fragment ion at m/z of 180 gave an ion at m/z of 135. This pattern suggests the presence of a carboxylic acid group in the molecule. Two major moieties of the molecule produced a fragment ion ($C_7H_8O_2^+$) at m/z of 124. Further loss of a hydrogen atom, from one of the two OH groups of the $C_7H_8O_2^+$, produced an ion at m/z of 123 and its intensity was 87%. The fragment ion at m/z of 110 represented the dihydroxybenzene ion produced from the two major moieties of the molecule. The loss of CO_2 from the molecule was evident by the presence of a fragment with m/z of 44. This ion had a 100% intensity and suggested possible presence of acid group(s) in the molecule (Pretsch, Clere, Seibl, & Simon, 1983).

3.5. ^1H NMR assignments of compound A

Proton labels used for compound A are referring to those given in Fig. 3 and proton assignments are shown in Table 3. Signal for proton ***f*** appeared at 7.50 ppm and it was split by the magnetic field of the proton ***g***. Proton ***a*** also produced a doublet at 7.09 ppm due to coupling with proton ***e***. Coupling of proton ***e*** with protons ***a*** and ***d*** produced a doublet at 6.79 ppm. Proton ***d*** produced a doublet at 6.79 ppm due to its coupling with proton ***e***. A doublet signal (6.69 ppm) for proton ***p*** also appeared very close to the signal of proton ***d***. The signal for proton ***m*** split, due to its coupling with proton ***l***, while coupling of proton **i** with protons ***m*** and ***p*** produced a doublet of doublet. A doublet for proton ***g*** appeared at 6.23 ppm while proton ***h*** produced a doublet of doublet at 5.17 ppm. Protons ***l*** and ***k*** resolved as quartets at 3.03 ppm. Proton-proton couplings were confirmed by a COSY–NMR analysis. Coupling constants (J) of interacting protons and chemical shifts (ppm) were well in agreement with those reported by Kelly, Mahajan, Brooko, Neubert, Breneman, and Carmack (1975) who reported ^{1}H NMR data for lithospermic acid and rosmarinic acid, isolated from *Lithospermum ruderale*, a plant belonging to the *Boraginaceae* family.

Table 3
Proton assignments for compound A[a]

ppm	Multiplicity	J(Hz)	Integration	Assignment
7.50	d	$J_{fg}=16$	1H	H_f
7.09	d	$J_{ae}=2$	1H	H_a
6.96	dd	$J_{ae}=2$, $J_{de}=8$	1H	H_g
6.79	d	$J_{de}=8$	1H	H_d
6.79	d	$J_{pl}=2$	1H	H_p
6.69	d	$J_{lm}=8$	1H	H_m
6.60	dd	$J_{pl}=2$, $J_{lm}=8$	1H	H_l
6.23	d	$J_{fg}=16$	1H	H_g
5.17	dd	$J_{hk}=5$, $J_{hi}=8$	1H	H_h
3.03	2t	$J_{hk}=5$, $J_{hi}=8$	1H	H_i, H_k

[a] Assignment of coupling interactions (J) confirmes by a COSY experiment. Proton labels refer to Fig. 3.

3.6. UV spectral data and EI-MS fragmentation pattern of compound B

The UV spectrum of compound B is shown in Fig. 2. This compound has an absorbance maximum at 272 nm and also an identical UV spectrum to the authentic syringic acid. The EI-MS fragment profile of compound B (Table 4) showed the presence of molecular radical ion ($M^{+\bullet}$) at m/z of 198 and suggested that the compound could be syringic acid (Fig. 3). A fragment ion at m/z of 184 was formed from the molecular radical ion due to the loss of $CH_2^{\bullet}$. Another fragment ion at m/z of 183 indicated loss of $CH_3^{\bullet}$ from the molecular radical ion. This demonstrated the presence of methyl groups in the molecule. A fragment, at m/z of 181, appeared due to the loss of $OH^{\bullet}$ from the molecular radical ion and suggested that the compound contained OH group(s). The aromaticity of the compound B was confirmed by the formation of fragments at m/z of 79, 67, 51 and 39 (Pretsch et al., 1983). A similar mass fragmentation pattern was observed for the authentic syringic acid.

3.7. ^1H-NMR assignments of compound B

The structure of compound B is shown in Fig. 3 and the proton assignments are given in Table 5. A singlet at 7.32 ppm shows the presence of two aromatic protons (Pretsch et al., 1983). A sharp singlet at 3.87 ppm is characteristic of hydrolysis of methoxy (–O–CH_3) groups and the integration of this peak confirmed the presence of six protons, thus two methoxy groups in the molecule. The proton of the carboxylic acid group did not resolve because it exchanged with deuterium. A

Table 4
Electron impact mass (EI-MS) fragmentation pattern for compound B

m/z	% Intensity	Fragmentation
198.05	100	$C_9H_{10}O_5^+$
184.04	2.82	$C_8H_8O_5^+$
183.03	29.12	$C_8H_7O_5^+$
181.01	7.40	$C_9H_9O_4^+$
78.96	8.53	$C_6H_7^+$
67.06	10.84	$C_5H_7^+$
50.97	11.45	$C_4H_3^+$
38.97	17.98	$C_3H_3^+$

Table 5
Proton assignments for compound B[a]

ppm	Multiplicity	J(Hz)	Integration	Assignment
7.32	S	–	2H	H_b
4.86	S	–	3H	Solvent
3.87	S	–	6H	CH_3O
3.30	S	–	1H	Solvent

[a] Proton labels refer to Fig. 3.

similar ^{1}H NMR spectrum was observed for the authentic syringic acid sample.

3.8. UV spectral data and EI-MS fragmentation pattern of compound C

As evidenced by the UV spectrum of compound C (Fig. 2), the absorbance maximum of this compound was at 322 nm. The shape of the UV spectrum and the absorbance maximum of compound C were identical to those of the authentic sinapic acid. Thus, it was suspected that this compound could well be sinapic acid (Fig. 3). As evidenced by the EI-MS fragment profile (Table 6), the molecular radical ion ($M^{+\bullet}$) appeared at m/z of 224. A fragment ion at m/z of 209 was formed from the loss of a $CH_3^{\bullet}$ from $M^{+\bullet}$. The loss of both CO_2 and $CH_3^{\bullet}$ from $M^{+\bullet}$ produced a fragment ion at m/z of 163. Another fragment ion at m/z of 121 suggested the formation of a quinone structure during the fragmentation process (Pretsch et al., 1983). The aromaticity of the compound was confirmed by the presence of fragments at m/z of 79, 65, 53, 51 and 39 (Pretsch et al., 1983). The mass fragmentation pattern of compound C was similar to that observed for authentic sinapic acid under the same EI-MS conditions.

Table 6
Electron impact mass (EI-MS) fragmentation pattern for compound C

m/z	% Intensity	Fragment ion
224.08	100	$C_{11}H_{12}O_5^+$
209.08	13.11	$C_{10}H_9O_5^+$
163.03	6.59	$C_{10}H_{11}O_3^+$
120.97	10.48	$C_7H_5O_2^+$
78.97	3.40	$C_7H_7^+$
65.11	12.38	$C_5H_5^+$
53.07	8.70	$C_4H_5^+$
50.97	10.19	$C_4H_3^+$
38.97	10.64	$C_3H_3^+$

Table 7
Proton assignments for compund C[a]

ppm	Mulitplicity	J(Hz)	Integration	Assigment
7.576	d	J_{bc}	1H	H_c
6.878	s	–	2H	H_d
6.322	d	J_{bc}	1H	H_b
4.859	s	–	3H	Solvent
3.863	s	–	6H	CH_3O
3.290	s	–	1H	Solvent

[a] Assignment of coupling interactions (J) confirmed by a COSY experiment. Proton labels refer to Fig. 3.

3.9. ^{1}H NMR assignments of compound C

The structure of compound C and its proton assignments are shown in Fig. 3 and Table 7, respectively. A doublet at 7.576 ppm appeared due to coupling interactions of proton ***c*** with the adjacent proton ***b***. The integration data for this doublet showed the presence of a single proton. Since proton ***c*** was more deshielded than proton ***b***, it resolved in the low field while proton ***b*** resolved as a doublet at 6.322 ppm. Aromatic protons (***d***) resolved at 6.878 ppm and integration showed the presence of two chemically equivalent protons. A sharp peak was evident at 3.863 ppm due to methoxy-substituted benzene ring; the integration of this peak showed the presence of two methoxy groups. All proton–proton couplings were confirmed by a COSY–NMR analysis. Protons of the authentic sinapic acid also had chemical shifts (δ ppm) and coupling constants (J) similar to compound C.

3.10. Silica gel TLC quantification of isolated compounds

Table 8 shows the results for TLC quantification of isolated compounds. Rosmarinic acid, syringic acid and sinapic acid contributed to approximately 3.9% (w/w) of the borage crude extracts. The total content of rosmarinic acid, syringic acid and sinapic acid in borage meal was approximately 0.6% (w/w).

Table 8
Content of isolated crude components in fractions, crude extract and meal[a]

Compound	% (w/w) in each fraction[b]	% (w/w) in crude extract[c]	% (w/w) in meal[d]
Rosmarinic acid (I)[e]	15.2	2.55	0.383
Syringic acid (IV)	10.2	0.633	0.113
Sinapic acid (IV)	11.0	0.715	0.121

[a] Results are averages of two runs. Twenty milligrams of each fraction were chromatographed.

[b] %, (w/w in fraction = (weight of isolated compound in mg/weight of fraction used for TLC in mg)×100.

[c] %, w/w in extract = [(weight of isolated compound in mg×fraction yield in mg)/(weight of fraction used for TLC in mg×weight of extract used for column chromatography)]×100.

[d] %, w/w in meal = [(weight of isolated compound in mg×fraction yield in mg×extract yield in mg)/(weight of fraction used for TLC in mg×weight of extract used for column chromatography×weight of meal used to prepare extract in mg)]×100.

[e] Fraction number.

4. Conclusions

Rosmarinic acid, syringic acid and sinapic acid are the major phenolic compounds present in the ethanolic extract of borage meal. These compounds account for approximately 4 and 0.6% of the dry mass of the crude extracts and the defatted seeds, respectively. Rosmarinic acid is a main component found in rosemary extracts that are widely used by the food processing industry. On the other hand, syringic and sinapic acids are among major phenolic antioxidants of canola and rapeseed. Therefore, potential exists for the extraction and use of borage antioxidants in functional food formulations and in skin care products as the compounds identified are excellent UV absorbers.

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