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Flavonoid glycosides profiling in dwarf elder fruits (*Sambucus ebulus* L.) and evaluation of their antioxidant and anti-herpes simplex activities

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ABSTRACT

Dwarf elder (*Sambucus ebulus* L.) is a popular medicinal plant, used for centuries in the folk medicine of the Balkan Peninsula. *S. ebulus* preparations have shown anti-inflammatory, anti-neoplastic and antimicrobial properties, besides abundant wound healing, antioxidant and anti-ulcerogenic activities. We developed a scheme for isolation of individual compounds utilizing different chromatographic techniques, while the structure elucidation was performed by means of 1D and 2D NMR. Five flavonoid glycosides, *e.g.* quercetin-3-*O*-laminaribioside [**1**], isorhamnetin-3-*O*-laminaribioside [**2**], quercetin-3-*O*-rutinoside [**3**], isorhamnetin-3-*O*-rutinoside [**4**], isorhamnetin-3-*O*-glucoside [**5**], were identified accordingly. Compounds **1** and **2** are reported for the first time in the genus *Sambucus*. Several triterpenes – ursolic, oleanolic and maslinic acid – were tentatively identified by GC–MS. The evaluation of anti-herpes simplex virus type 1 and antioxidant (in $ORAC_{FL}$ and $HORAC_{FL}$) properties suggests that the dwarf elder fruits might serve as a powerful source of valuable molecules for various purposes.
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1. Introduction

Sambucus ebulus (Adoxaceae), commonly known as dwarf elder, is a perennial herbaceous plant, predominantly distributed in Europe, North Africa and Western Asia (cited from Pieri et al., 2009). Dwarf elder is a popular medical plant with a prominent place in the folk medicine of the people from the Western Europe, Balkan Peninsula and Middle East. In the oriental medicine, *S. ebulus* leaves, rhizomes and roots were administered to patients to treat bites, burns, infectious wounds, edema, eczema, urticaria, arthritis and sore-throat (Shokrzadeh and Saeedi Saradi, 2010). More recently, *S. ebulus* preparations have shown anti-inflammatory (Schwaiger et al., 2011), anti-neoplastic activity in colon cancer (CT26 cell line) and hepatocellular carcinoma (HepG2 cell line; Shokrzadeh et al., 2009), antimicrobial (Tosun et al., 2004), incl. anti-*Helicobacter pylori* (Yesilada et al., 1999), properties, besides abundant wound healing (Süntar et al., 2010), antioxidant (Duymus et al., 2014) and anti-ulcerogenic (Yesilada et al., 2014) activities. Dwarf elderberries are known to be rich of sugars and fibers, but also accumulate high value anthocyanins (Duymus et al., 2014), flavonoids (Süntar et al., 2010; Yesilada et al., 2014) and sterols (Bubulica et al., 2012), among others. Overall, the popularity of elderberries is not due only to their nutritional value, but also to the increasing evidence of their health-promoting effects (Vlachojannis et al., 2010).

The natural flavonoids, especially their glycosides, are the most abundant polyphenols in foods and are of great general interest due to their diverse biological properties. Epidemiological and pharmacological data indicate that flavonoids play key roles in the prevention and management of chronic diseases such as cancer, diabetes, cardiovascular and neurodegenerative conditions (Del Rio et al., 2013; Xiao et al., 2014). Flavonoids are, in particular, abundant in commonly consumed fruits and apparently of increasing interest due to the above mentioned diverse beneficial effects on human health (Xiao et al., 2014).

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Reactive oxygen species (ROS), as the superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl anion (HO^-), hydroxyl radicals ($OH^\bullet$) and hypochlorous acid (HOCl), among others, are generated as byproducts of cellular metabolism through the electron transport chain in mitochondria as well as *via* the cytochrome P450 (Mittal et al., 2014). Excessive ROS production leads to pathological disorders like inflammation, neoplasm development, atherosclerosis, and other cardiovascular diseases (Circu and Aw, 2010). To prevent the damaging effects of oxidative stress, cells have evolved an array of antioxidant defense systems that effectively scavenge/remove ROS, which include naturally occurring dietary phytochemicals among battery of cellular constituents (Niture et al., 2014).

Herpes simplex virus type 1 (HSV-1) is amongst the widespread human pathogens (Xu et al., 2006). Antiviral chemotherapy is nowadays a standard therapy in the management of herpesvirus infections, and *ca.* 11 anti-herpes virus drugs are available on the market (De Clercq et al., 2006). The most commonly used are the nucleoside analog acyclovir and its derivatives. These drugs have been well established for over two decades, however, their excessive use led to the development of resistant strains (Bacon et al., 2003). That makes the search for new effective therapeutic agents imperative, as a special focus is on the compounds of natural origin, incl. complex plant extracts. The advantages of herbal products (over the synthetic drugs) are in general the reduced toxicity and the delayed occurrence of resistance due to complexity of the structures, among others.

The present study deals with the isolation of bioactive flavonoid glycosides from the *S. ebulus* mature fruits (hereafter called dwarf elderberry) and evaluation of their antioxidant capacity and antiviral properties. To our knowledge this is the most comprehensive evaluation to date of the antioxidant potential of extracts, fractions and pure flavonoids isolated from dwarf elderberry. The isolated flavonoid glycosides were characterized by reliable techniques, *e.g.* 1D and 2D NMR spectroscopy.

2. Materials and methods

2.1. Reagents and chemicals

Gallic acid and chlorogenic acid were purchased from Sigma (St. Louis, MO, USA). Fluorescein disodium salt, 2,2′-azobis (2-amidino-propane) dihydrochloride (AAPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Sigma–Aldrich (Steinheim, Germany). Bis(trimethylsilyl)-trifluoroacetamide (BSTFA) was supplied from Merck (Darmstadt, Germany). Dimethylsulfoxide-d_6 (DMSO) was purchased from Deutero-GmbH (Kastellaun, Germany). Polyamide 6 (Fluka) was used as an adsorbent for column chromatography. Low pressure liquid chromatography (LPLC), thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) were carried out on Lobar RP-18 columns, silica gel 60 F_{254} aluminum sheets and pre-coated silica gel 60 F_{254} plates (layer thickness 0.5 mm), respectively (Merck, Darmstadt, Germany). Detection of separation and purification was performed by UV (λ 254 and 366 nm) and spraying with H_2SO_4 (20% v/v CH_3OH solution). All chemicals used apart from those mentioned above were of analytical grade.

2.2. Plant material

The *S. ebulus* fruits (154.85 g) were collected in the region of Bansko, Blagoevgrad province, Bulgaria (946 m above sea level; latitude 41.82°, longitude 23.49°), in October 2012. Collected mature fruits were cleaned (incl. removal of immature fruits), dried at room temperature under shade, powdered and subjected to extraction and isolation.

2.3. Extraction and isolation

The dried and powdered fruits (22.36 g) were mixed with pure methanol (670 mL, plant material/solvent ratio 1:30, w/v), vortexed for 1 min and ultrasonicated (ultrasound frequency of 35 kHz, UCI-50Raypa®; R. Espinar S.L., Barcelona, Spain) for 20 min at room temperature (Fig. 1). The resulting extract, designated fraction SE-CME, was filtered and evaporated to syrup-like residue (9.72 g), then dissolved in water and subjected to column chromatography on polyamide 6 column (extract/sorbent ratio 1:100, w/w), eluted with 600-mL portion of distilled water, 500 mL portions of 25%, 50%, and 75% methanol–water solutions followed by 700 mL pure methanol. Twenty-nine fractions (100 mL each) were obtained accordingly. Seven main fractions were then combined according to TLC chromatograms using $CHCl_3$—CH_3OH—H_2O (60:15:4; 60:22:4 and 61:32:7; v/v/v) as mobile phase. The flavonoid-enriched fractions, collected between 25% and 75% methanol–water mixtures, SE-1E (49 mg), SE-1G (55.8 mg), SE-1I (160 mg), SE-1 K (63.8 mg) and SE-1 N (164 mg) were evaporated *in vacuo* and further purified. Compound **2** (9.9 mg) was isolated from fraction SE-1G by PTLC with $CHCl_3$—CH_3OH—H_2O (61:32:7; v/v/v). Fraction SE-1I was subjected to Lobar column (RP-18, size A, eluted with water–methanol gradient 10–45% CH_3OH, 50 mL) to afford compound **1** (7.9 mg) and additional amount of **2** (9.2 mg). Compounds **3** (7.9 mg) and **4** (11.7 mg) were isolated from fraction SE-1 K by PTLC with $CHCl_3$—CH_3OH—H_2O (60:22:4; v/v/v). Fraction SE-1 N was dissolved in 40% CH_3OH and the soluble part (105 mg) was loaded on Lobar column (RP-18, size A, eluent 40–50% CH_3OH, 50 mL) to afford compound **5** (10.4 mg). Additional amounts of **3** (3.0 mg) and **5** (3.9 mg) were obtained from fraction SE-1E using PTLC ($CHCl_3$—CH_3OH—H_2O, 60:22:4; v/v/v). The experimental scheme for isolation is illustrated in Fig. 1. The isolated quercetin-3-*O*-laminaribioside [**1**], isorhamnetin-3-*O*-laminaribioside [**2**], quercetin-3-*O*-rutinoside [**3**], isorhamnetin-3-*O*-rutinoside [**4**], isorhamnetin-3-*O*-glucoside [**5**] were identified by ^1H NMR (600.13 MHz), ^{13}C NMR (150.92 MHz), 2D HSQC (heteronuclear single quantum coherence) and HMBC (heteronuclear multiple bond correlation) using a Bruker AVII+ 600 spectrometer (Bruker, Karlsruhe, Germany) and comparing the resulting spectra with reported data.

2.4. Gas chromatography–mass spectrometry (GC–MS)

Approximately 5 mg of the last fraction (SE-1O) of the polyamide column was mixed with 50 μL of dry pyridine and 75 μL of BSTFA and heated at 80 °C for 20 min. The silylated sample was analyzed by GC–MS.

The GC–MS analysis was performed with a Hewlett-Packard gas chromatograph 5890 Series II Plus linked to a Hewlett-Packard 5972 mass spectrometer system, equipped with 30 m long, 0.25 mm i.d., and 0.5 μm thickness HP5-MS capillary column. The temperature was programmed from 100 to 300 °C at a rate of 5 °C/min. Helium was used as a carrier gas at flow rate of 0.7 mL/min. The injector temperature was 280 °C and the ionization voltage was 70 eV. The GC–MS full scanning was in the mass range m/z 30–700.

The identification was based on comparison of the obtained spectra with those of computer searches in HP Mass Spectral Library NIST98 and reference spectra published by Caligiani et al. (2013), Li et al. (2007) and Popova et al. (2010).

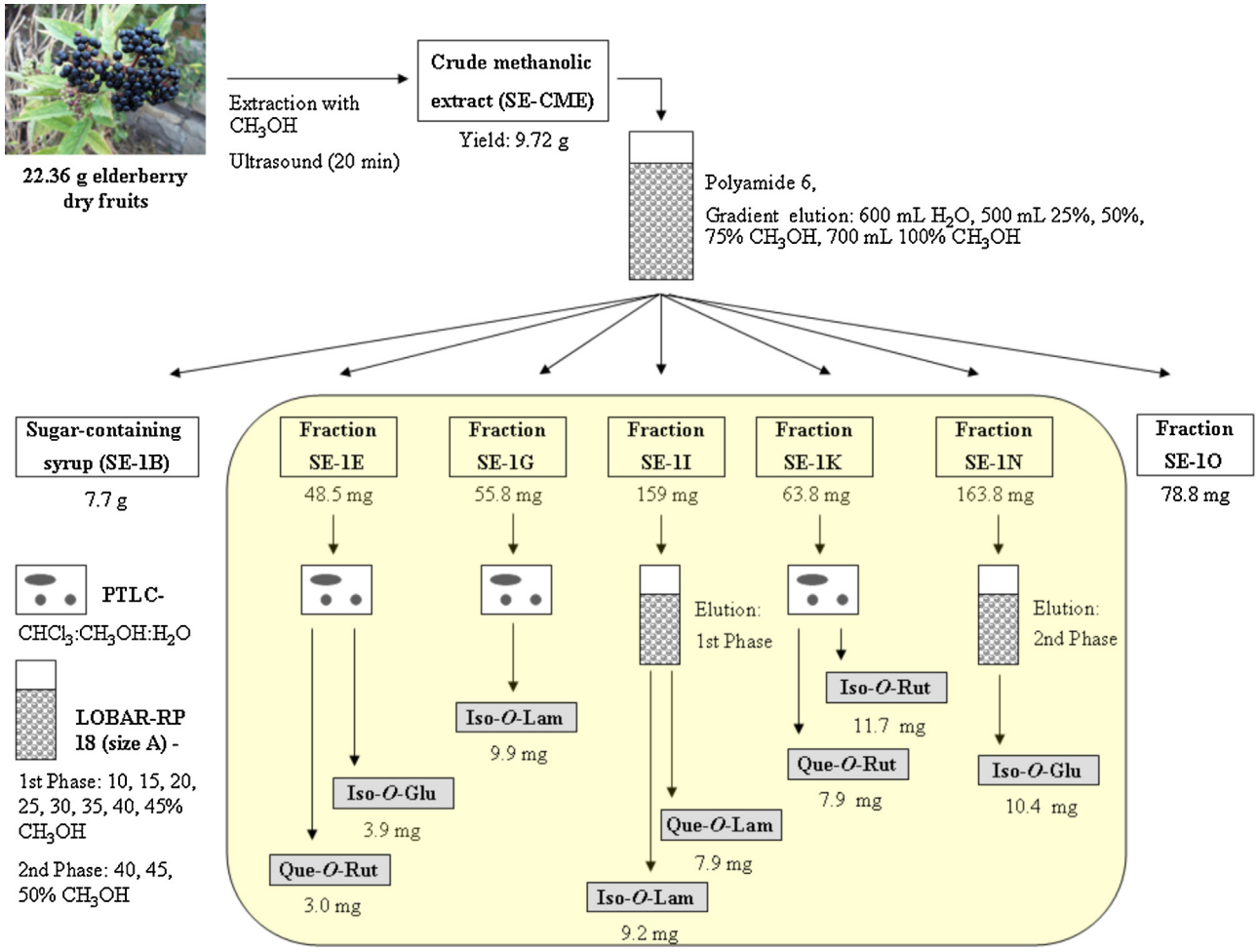


Fig. 1. Extraction and purification scheme for antioxidant activities analyses of *Sambucus ebulus* fruits. SE-CME: crude methanolic extract, Que-*O*-lam: quercetin-3-*O*-laminaribioside [**1**], Iso-*O*-lam: isorhamnetin-3-*O*-laminaribioside [**2**], Que-*O*-rut: quercetin-3-*O*-rutinoside [**3**], Iso-*O*-rut: isorhamnetin-3-*O*-rutinoside [**4**], Iso-*O*-glu: isorhamnetin-3-*O*-glucoside [**5**].

2.5. Antiviral activity against herpes simplex

Herpes simplex virus type 1, strain Vic (HSV-1), was supplied by the National Center of Infectious and Parasitic Disease (NCIPD), Sofia, Bulgaria. The cell line MDBK (Madin-Darby Bovine Kidney) was obtained by the National Cell Culture Bank (Sofia, Bulgaria).

The cytotoxicity was determined by microscopic examination of the cell morphology of treated/untreated cultures. The maximum concentration, which did not alter the morphology of the cells, was recognized as maximum tolerable concentration (MTC; Montanha et al., 2004). In a second experiment, the cell viability was determined by the ability of the cells to cleave the tetrazolium salt MTT (Sigma–Aldrich, St. Louis, MO, USA) through the mitochondrial enzyme succinate dehydrogenase which gives a formazan blue product, following the procedure described earlier (Mosmann, 1983). The CC_{50} values – indicating the concentrations of samples, which result to 50% reduction of the viable MDBK cells – were then calculated.

Effect on the extracellular virus (*in vitro* virucidal effect). Equal volumes of viral stock containing $10^{5.5}$ $CCID_{50}$/mL and media with MTC of the elderberry extract were mixed and incubated at 37 °C for 5, 10, 15, 30, 60, 120, and 240 min. The samples were then frozen and thawed and the infectious virus titers were calculated after 48 h of culture. The virucidal effect was determined by the reduction of the infectious virus titer of each sample as compared to that of the relevant viral control (equal volumes of viral stock and medium incubated as described above).

2.6. Oxygen radical absorbance capacity ($ORAC_{FL}$) and hydroxyl radical averting capacity ($HORAC_{FL}$) assays

$ORAC_{FL}$ assay examines the capability of tested samples to scavenge peroxyl radicals generated by 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) at 37 °C (Ou et al., 2001), while $HORAC_{FL}$ determines the metal-chelating activity of antioxidants under the conditions of Fenton-like reactions, employing a Co(II) complex and hence the protecting ability against formation of hydroxyl radical (Ou et al., 2002). The protocols for $ORAC_{FL}$ and $HORAC_{FL}$ assays have been thoroughly described elsewhere (Georgiev et al., 2010, 2011). Chlorogenic acid was employed as a positive standard.

One $ORAC_{FL}$ and $HORAC_{FL}$ unit was assigned to the net protection area provided by a 1 μM solution of Trolox and 1 μM of gallic acid, respectively. The activity of the tested samples is expressed as μM Trolox equivalents (TE) per gram dry sample ($ORAC_{FL}$ assay) and μM gallic acid equivalents (GAE) per gram dry sample ($HORAC_{FL}$ assay).

Table 1
Antioxidant capacity of extracts, fractions and pure compounds of *Sambucus ebulus*.[a]

	$ORAC_{FL}$, μM TE/g	SD	$HORAC_{FL}$, μM GAE/g	SD
Crude methanolic extract (SE-CME)	849.6	±49.7	471.0	±13.8
Flavonoid fraction (SE-1E)	5460.3	±280.4	2198.0	±136.1
Flavonoid fraction (SE-1G)	5660.0	±289.8	4805.5	±308.2
Flavonoid fraction (SE-1I)	8415.0	±273.9	6443.8	±381.0
Flavonoid fraction (SE-1K)	1953.0	±91.2	1169.0	±55.8
Flavonoid fraction (SE-1N)	3205.3	±266.7	1620.1	±149.5
Quercetin-3-*O*-laminaribioside [**1**]	2560.1	±77.8	887.3	±6.8
Isorhamnetin-3-*O*-laminaribioside [**2**]	5742.1	±387.3	4924.7	±104.3
Quercetin-3-*O*-rutinoside [**3**]	4531.2	±293.8	3157.9	±36.3
Isorhamnetin-3-*O*-rutinoside [**4**]	5450.4	±321.1	4116.0	±209.9
Isorhamnetin-3-*O*-glucoside [**5**]	8678.3	±425.5	6967.3	±420.8
Chlorogenic acid	9648.9	±189.2	4036.8	±202.3

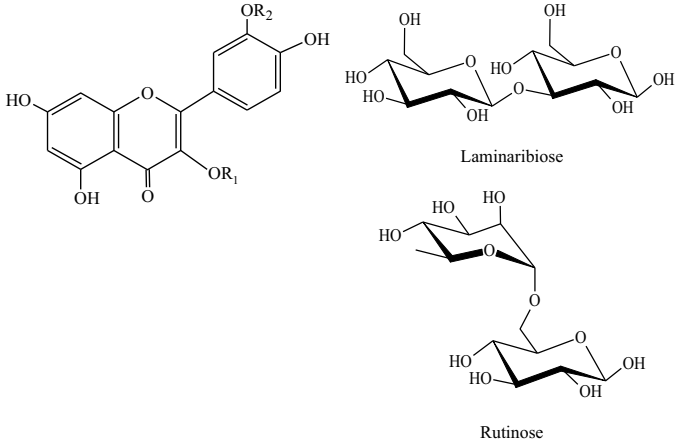
[a] Means ± SD (n = 6).

3. Results and discussion

3.1. Isolation and purification of bioactive constituents from dwarf elderberry fruits

In the present work we have shown that several bioactive compounds accumulate in the mature fruits of *S. ebulus*. To evaluate the antiviral and antioxidant (Table 1) activity of dwarf elderberry constituents we developed a scheme for isolation of individual compounds utilizing different chromatographic techniques, involving low pressure liquid chromatography (Fig. 1). First, metabolites were extracted from the dried and powdered fruits using methanol, which yielded crude extract (SE-CME). The CME was then fractionated by applying it to a polyamide column and eluting with a step water–methanol gradient (Fig. 1). In order to isolate the individual compounds the collected flavonoid fractions (SE-1E, SE-1G, SE-1I, SE-1K, and SE-1N) were further subjected to PTLC and Merck Lobar (RP-18) columns. Five quercetin and isorhamnetin glycosides – quercetin-3-*O*-laminaribioside [**1**], isorhamnetin-3-*O*-laminaribioside [**2**], quercetin-3-*O*-rutinoside (=rutin) [**3**], isorhamnetin-3-*O*-rutinoside (=narcissin) [**4**], isorhamnetin-3-*O*-glucoside [**5**] – were identified (Fig. 2). The structures of the isolated compounds were verified by ^{1}H, ^{13}C NMR, 2D HSQC and HMBC and compared with the reported data (Es-Safi et al., 2005; Imperato, 1995; Liu et al., 2008; Manguro et al., 2004; Park et al., 2007). Compounds **1** and **2** are reported for the first time in the genus *Sambucus*, while compound **5** appeared to be new for *S. ebulus* fruits. It has been recently reported that isorhamnetin-3-*O*-glucoside, isolated from *S. ebulus* leaves, possesses abundant anti-ulcerogenic properties (Yesilada et al., 2014). The ^{1}H NMR spectra of **1** and **2** with assignment of the characteristic protons are presented in Fig. 3, *e.g.* both flavonoids could be easily distinguished, based on the presence or absence of the signal for the $-OCH_3$ group at 3.84 ppm.

The last fraction (SE-1O) with the lowest polarity (Fig. 1), which yielded 78.8 mg, was further subjected to GC–MS analysis. In total 15 compounds (Table 2) were tentatively identified as the most abundant appeared ursolic acid, β-sitosterol and oleic acid. The presence of triterpenes ($C_{30}H_{48}$), such as ursolic acid, oleanolic acid and maslinic acid, in the dwarf elderberry is worth noting, as the mentioned pentacyclic triterpenes were shown to possess strong anti-neoplastic activity in human HT-29 colon cancer cells (oleanolic and maslinic acid; Juan et al., 2008) and in breast cancer cells MCF-7 and MDA-MB-231 (ursolic and oleanolic acid; Chakravarti et al., 2012), among others.



1 Quercetin-3-*O*-laminaribioside R_1 = Laminaribiose R_2 = H
2 Isorhamnetin-3-*O*-laminaribioside R_1 = Laminaribiose R_2 = CH_3
3 Quercetin-3-*O*-rutinoside R_1 = Rutinose R_2 = H
4 Isorhamnetin-3-*O*-rutinoside R_1 = Rutinose R_2 = CH_3
5 Isorhamnetin-3-*O*-glucoside R_1 = Glucose R_2 = CH_3

Fig. 2. Chemical structures of major flavonoid glycosides found in *Sambucus ebulus* fruits.

3.2. Evaluation of antiviral properties against herpex simplex

In order to evaluate the bioactive potential of dwarf elderberry we first started with the determination of its cytotoxicity in MDBK cell line. As shown in Fig. 4, the crude methanolic extract of *S. ebulus* was applied at concentration ranging between 400 μg/mL and

Table 2
Compounds identified in fraction SE-1O from elderberry by GC–MS (TMS derivatives).

Compound	R.T. (min)	m/z [$M^{\bullet+}$]	% of total ion current
p-Hydroxybenzoic acid	18.91	282	0.2
Palmitinic acid	27.62	328	5.2
9,12-Octadecadienoic acid	30.63	352	10.3
Oleic acid	30.76	354	10.9
11-Octadecenoic acid	30.82	354	0.8
Octadecanoic acid	31.14	356	0.8
Dehydroabietic acid	33.54	372	0.4
Campesterol	45.63	472	1.8
Stigmasterol	45.92	484	0.2
β-Sitosterol	46.72	486	12.9
β-Amyrin	46.96	498	1.2
α-Amyrin	47.45	498	1.1
Oleanolic acid	49.24	600	2.4
Ursolic acid	49.94	600	19.0
Maslinic acid	51.60	688	0.8

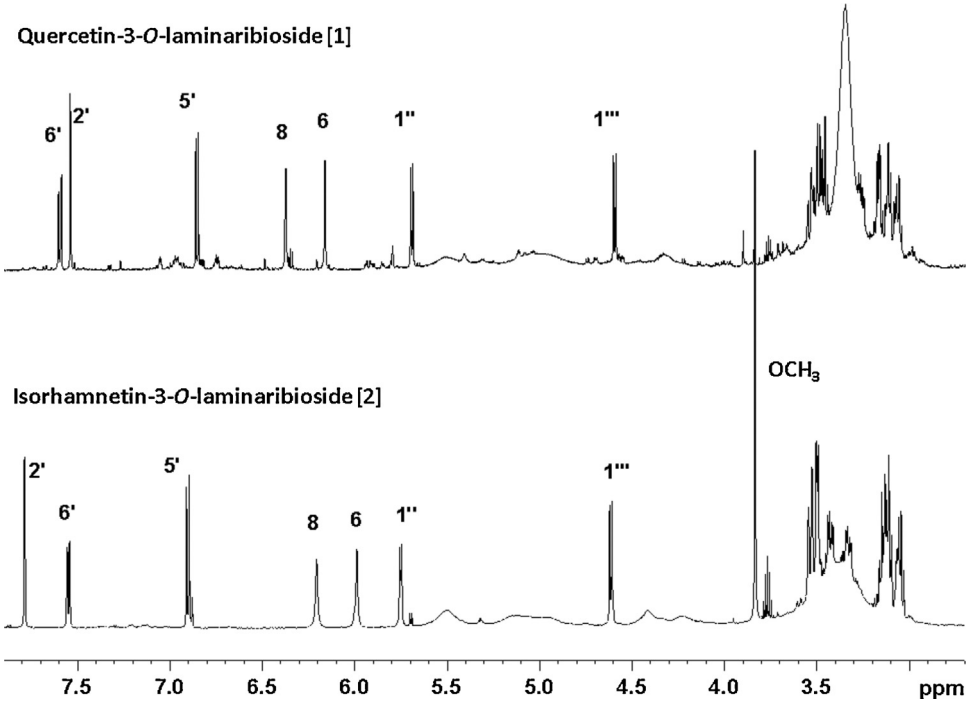


Fig. 3. ^{1}H NMR (600 MHz) spectra of quercetin-3-*O*-laminaribioside and isorhamnetin-3-*O*-laminaribioside in DMSO-d_6 at room temperature.

4.0 mg/mL, and both MTC and CC_{50} were simultaneously determined. The data suggest that the extract slightly altered the cell morphology, as the MTC value of the extract was 1.5 mg/mL. The low toxicity obtained by the MTC assay was further confirmed by the MTT assay. The calculated CC_{50} value of the crude methanolic extract was 3.44 mg/mL (Fig. 4, upper panel).

Virucidal effect of dwarf elderberry methanolic extract (SE-CME) on HSV-1 (Fig. 4, down panel). The data suggest significant inactivation more than 77% after 5 min of contact of HSV-1 virions with the extract applied in maximal nontoxic concentration. The effect was steadily kept over the duration of incubation (Fig. 4 down panel), as the viral inactivation ranges from 62% (Δlog = 0.42) to 85% (Δlog = 0.84). The data from CPE reduction assay showed no inhibitory activity of the dwarf elderberry crude methanolic extract on the replication of HSV-1 *in vitro*. Overall, the data suggest significant inactivation of extracellular HSV-1 by the *S. ebulus* crude methanolic extract (applied at maximal nontoxic concentration). Vlachojannis et al. (2010) summarized that the aqueous elderberry extract Sambucol® (based on *S. nigra*) appears useful for the treatment of viral infections, based on available data of several *in vitro* and *in vivo* human studies. Moreover, some flavonoids are reported to possess abundant anti HSV properties (Hayashi et al., 2012; Yarmolinsky et al., 2012). Therefore, further detailed studies on the mechanism of action of *S. ebulus* extracts, preparation and isolated pure compounds are required.

3.3. Evaluation of antioxidant activities and comparative ranking of the elderberry extracts and bioactive constituents

Due to the rather complex and sophisticated function of the antioxidant defense system and involvement of many different types of ROS (as mentioned above) in the living cells, a single antioxidant assay might not be able to provide a complete overview of the antioxidant capacity of a given sample (Schauss et al., 2006). Thus, we evaluated the antioxidant activities of extracts, fractions and five isolated flavonoid glycosides of dwarf elderberry fruits in both $ORAC_{FL}$ and $HORAC_{FL}$ assays, and compared to pure chlorogenic acid as a reference (Table 1).

In both assays ($ORAC_{FL}$ and $HORAC_{FL}$) used the radical scavenging/averting activity of the isolated pure flavonoid glycosides increased in the following order: Iso-*O*-glu > Iso-*O*-lam > Iso-*O*-rut > Que-*O*-rut > Que-*O*-lam. The peroxyl radical (utilized by the $ORAC_{FL}$ assay) has high reactivity with aromatic compounds and molecules that do not act as electron donors. Therefore, the pure antioxidants with the lower number of hydroxyl groups in their aglycone (isorhamnetin-type flavonoids) displayed the highest activity toward peroxyl radicals. It should be outlined that the $HORAC_{FL}$ values of isorhamnetin-3-*O*-glucoside and isorhamnetin-3-*O*-laminaribioside are 73% and 22%, respectively, higher than those of pure chlorogenic acid (Table 1). Crude methanolic extract (SE-CME) displayed no significant antioxidant activity although the above mentioned flavonoid glycosides were present in it. Therefore, enrichment of the bioactive substances in the extracts is essential if they are to be used as antioxidants (Georgiev et al., 2010). Although the flavonoid-enriched fractions (SE-1E, SE-1G, SE-1I, SE-1K, and SE-1N), did not display the highest activity, they do have prominent potential, and their use would have economic advantages, since only single-step column separation would be required. In both assays the flavonoid-enriched SE-1I fraction displayed significantly higher antioxidant capacity than the rest four fractions, indicating that some minor constituents, present in SE-1E, might possess potent antioxidant activity. The presence of anthocyanins, which were recently shown to possess antioxidant properties (Duymus et al., 2014), in the fractions, should be also considered.

A correlation between $ORAC_{FL}$ and $HORAC_{FL}$ values of all measured samples was observed, though both assays measure two different, but equally important, aspects of antioxidative process – radical chain breaking ($ORAC_{FL}$ case) and radical formation prevention ($HORAC_{FL}$ case; Georgiev et al., 2011).

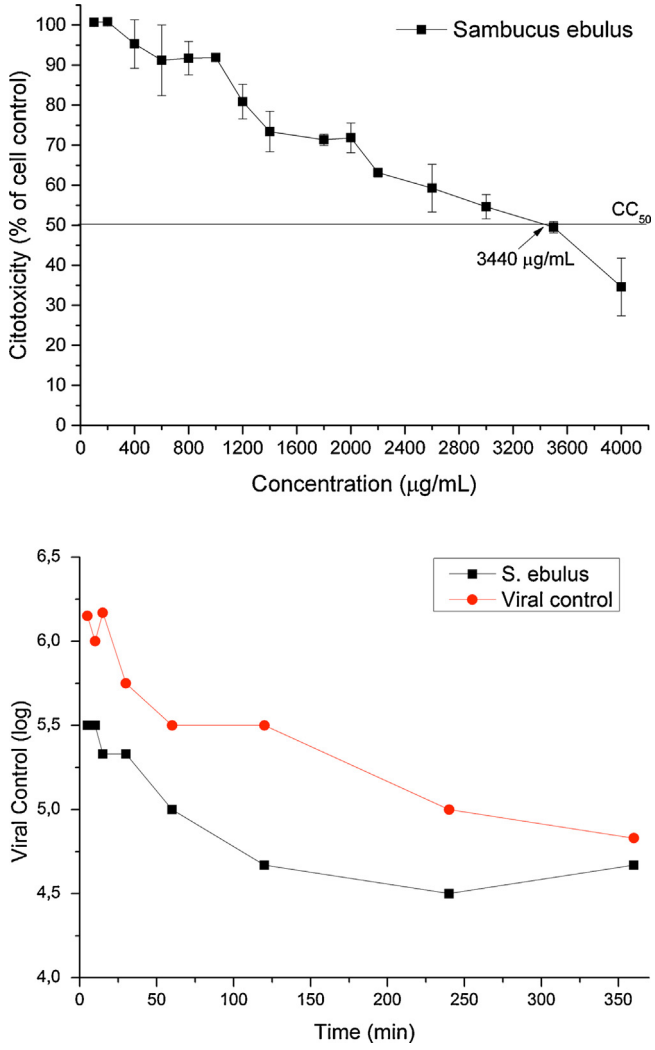


Fig. 4. Viability of MDBK cells (top) and inactivation on the extracellular HSV-1, strain Vic, (bottom) treated with dwarf elderberry crude methanolic extract.

4. Conclusions

Several bioactive constituents of dwarf elderberry were isolated, as some – quercetin-3-*O*-laminaribioside and isorhamnetin-3-*O*-laminaribioside – are reported for the first time within *Sambucus* genus. Isorhamnetin-3-*O*-glucoside and isorhamnetin-3-*O*-laminaribioside proved to be effective as radical scavengers and in prevention of radical formation, thus being potentially attractive antioxidants. The flavonoid-enriched fractions, obtained by applying a one-step chromatographic separation, also exhibited potent antioxidant activity, while crude methanolic extract displayed anti-herpes simplex virus (HSV-1) activity. Therefore, dwarf elderberry could serve as attractive mines of powerful bioactive molecules for various purposes.

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