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Research Paper

Anti-ulcerogenic activity and isolation of the active principles from *Sambucus ebulus* L. leaves

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ABSTRACT

Ethnopharmacological relevance: *Sambucus ebulus* L. has a very prominent place in Turkish folk medicine. Leaves of the plant are practiced externally to relieve rheumatic pain, to treat abscess, for wound healing and internally against hemorrhoids and stomachache. In a previous work, aqueous extract of the leaves was shown to possess potent antiulcerogenic activity on water immersion and immobilization-induced stress ulcer model in rats. This study aims to investigate the antiulcerogenic activity profile of the plant on various in vivo peptic ulcer models and gastric biochemical parameters and through bioassay-guided processing to isolate the active constituent (s) and to elucidate its structure.

Materials and methods: Among the subextracts obtained by successive solvent extractions from the MeOH extract of the leaves, the butanol subextract exerted significant antiulcerogenic activity against water-immersion and immobilization-induced stress ulcer model in rats as the bioassay model. This subextract was then subjected to successive chemical separation techniques (precipitation, column chromatography based on ion-exchange, silica gel and sephadex) and the activity of each fraction/subfraction was tested using the same bioassay model. After determination of active principles, further studies were performed on the active subextract by using various in vivo test models (ethanol-, serotonin-, pyloric ligation-induced ulcerogenesis) in rats as well as biochemical methods for the evaluation of antiulcerogenic potential.

Results: Bioassay-guided fractionation procedures yielded two flavonol glycosides as the active antiulcerogenic principles. The structures of these compounds were elucidated as isorhamnetin-3-*O*-monoglycoside and quercetin-3-*O*-monoglycoside by using ^{1}H, ^{13}C-NMR, and FAB-MS techniques.

Conclusion: This study has proven the folkloric use of *Sambucus ebulus* leaves for the treatment of gastric ailments in Turkish folk medicine. The antiulcerogenic activity of the two flavonol glycosides isolated in the present study was not previously reported elsewhere.

1. Introduction

Two species of *Sambucus* (*Sambucus nigra* and *Sambucus ebulus*), a member of Caprifoliaceae family, are grown naturally in Turkey. Particularly, the latter species has a very prominent place in Turkish folk medicine, may be due to the widespread distribution of the plant (Chamberlain, 1997). Generally, leaves of the plant are practiced externally to relieve rheumatic pain (Fujita et al., 1995; Honda et al., 1996; Sezik et al., 1991; Yesilada et al., 1995). Moreover, leaves are used externally to treat abscess, wound, sunstroke, snakebite, edema/inflammations, against piles on foot, common cold, high fever and internally as a purgative, diuretic or diaphoretic as well as against hemorrhoids and for the treatment of stomachache in Turkish folk medicine (Baytop, 1999; Fujita et al., 1995; Kültür, 2007; Sezik et al., 1992; Yesilada et al., 1995, 1999a). Referring to this broad range of applications in healing practice, this plant is also called “hekimana”, which means “motherphysician” in some Anatolian settlements.

In our previous study, significant anti-inflammatory and antirheumatic activities were reported and through in vivo bioassay-guided processing, a caffeic acid derivative was isolated as the active anti-inflammatory principle from the aerial parts of *Sambucus ebulus* (Yesilada, 1992). Other investigations also revealed the antigiardial (Rahimi-Esboei et al., 2013), scolicidal (Gholami et al., 2013), wound healing (Süntar et al., 2010), cytotoxic (Shokrzadeh et al., 2009), antioxidant (Ali et al., 2009) and anti-*Helicobacter pylori* (Yesilada et al., 1999b) activities of *Sambucus ebulus* leaves.

In a preliminary in vivo screening study, some Turkish folk remedies claimed to be effective against stomachache were examined for their antiulcer potential (Yesilada et al., 1993). Among these

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plants the effect of aqueous and methanol extracts from *Sambucus ebulus* leaves were also studied against water immersion and immobilization-induced stress ulcer model in rats. The aqueous extract demonstrated a dose-dependent anti-ulcerogenic activity, resulting in 42.3 to 71.3% inhibitions, while methanol extract was found inactive.

In the present study, *Sambucus ebulus* leaves were subjected to bioassay-guided fractionation and isolation procedures through successive solvent–solvent extraction and chromatographical techniques. Finally, two flavonoids were isolated as the anti-ulcerogenic principles of this medicinal plant. In addition, further studies were carried out by using various in vivo anti-ulcerogenic test models as well as biochemical methods in experimental animals (rats and mice) for the evaluation of anti-ulcerogenic activity mechanism.

2. Materials and methods

2.1. Plant material

The leaves of *Sambucus ebulus* L. were collected in Bolu during flowering period (June, 2011). The plant material was dried under shade and coarsely powdered. Identification of the voucher specimens was fulfilled by one of the authors (E.Y.). Herbarium specimens are stored in the Herbarium of Gazi University, Faculty of Pharmacy.

2.2. Chemical processing

2.2.1. Extraction of leaves

Powdered leaves of *Sambucus ebulus* (3.0 kg) were extracted with MeOH (50 l) using a percolator at room temperature and combined methanolic extracts were evaporated to dryness under reduced pressure to yield 690 g MeOH extract (23%, extraction yield).

2.2.2. Fractionation of MeOH extract through solvent–solvent extraction

MeOH extract (345 g) was redissolved in MeOH/H_2O (9:1) mixture. Then the extract was further extracted with *n*-hexane (15 × 1 l). After removal of MeOH from the residual aqueous methanolic extract, it was diluted with H_2O (1 l) and extracted with $CHCl_3$ (12 × 0.5 l), and *n*-BuOH saturated with H_2O (16 × 0.5 l), successively. Each solvent extract was then evaporated to dryness under reduced pressure to give *n*-hexane subextract (87.5 g), $CHCl_3$ subextract (172.0 g), *n*-BuOH subextract (180.0 g) and remaining aqueous subextract [RH_2O] (186.0 g). Since aqueous extract exerted the highest antiulcerogenic activity in our previous experiments, only the polar subextracts (*n*-BuOH and RH_2O subextracts) were submitted to the anti-ulcerogenic activity testing for the isolation of active ingredient (s).

2.2.3. Fractionation and isolation processes

2.2.3.1. Precipitation of n-BuOH extract. The *n*-BuOH subextract (180.0 g) was dissolved in 300 ml MeOH and this solution was added drop-wise into cold diethylether (1000 ml) by stirring the solution. The precipitate formed was then removed by filtration and dried in a vacuum desiccator overnight to give [*n*-BuOH↓] (105.4 g) fraction and the organic phase (diethylether phase) was evaporated under reduced pressure to dryness to give [*n*-BuOH↑] (64.2 g) fraction.

2.2.3.2. Fractionation of n-BuOH↓. 10.5 g [*n*-BuOH↓] fraction was chromatographed on an Amberlite XAD-2 column and eluated with H_2O, H_2O–MeOH [(8:2); (7:3); (1:1)] and MeOH, successively. Eluents were combined into three major subfractions based on their TLC profiles [solvent system $CHCl_3$:MeOH:H_2O (65:25:4 and 7:4.2:1) and EtOAc:*n*-BuOH:H_2CO:H_2O (5:3:1:1)]. All subfractions were evaporated to dryness under reduced pressure; [XAD/Fr.1-6] (5.8 g); [XAD/Fr.7-20] (1.1 g); [XAD/Fr.21-60] (2.8 g).

2.2.3.3. Fractionation of [XAD/Fr.21-60]. 2.8 g [XAD/Fr.21-60] was rechromatographed on Amberlite XAD_2 column using the same solvent systems as described above. Eluents were combined into two major groups based on their TLC profiles [solvent system $CHCl_3$:MeOH:H_2O (65:25: and 7:4.2:1)]. [Re-XAD/Fr.1] (1520 mg) and [Re-XAD/Fr.2] (290 mg) [**compound 1**=Rutin].

2.2.3.4. Fractionation of [Re-XAD/Fr.1] on vacuum chromatography. 1520 mg [Re-XAD/Fr.1] was further chromatographed on silica gel (Silica gel BW-200, 70 mesh, Merck) column using a solvent system $CHCl_3$–MeOH–H_2O (80:20:2). Eluents were combined into two major groups based on their TLC profiles [solvent system $CHCl_3$:MeOH:H_2O (65:25:4)]; [SG/Fr.1] (350 mg); [SG/Fr.2] (980 mg).

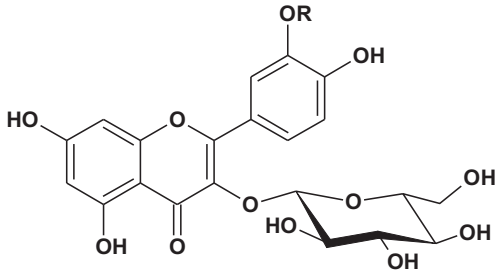
2.2.3.5. Fractionation of [SG/Fr.1] on Sephadex LH-20. A quantity of 350 mg [SG/Fr1] was passed through a Sephadex LH-20 column (700 × 35 mm, i.d.) for further separation and was eluted with MeOH. Eluents were combined into two groups based on their chromatographic profiles [solvent system $CHCl_3$:MeOH:H_2O (65:25:4 and 7:4.2:1); [LH/Fr.1] (110 mg) [**compound 2**=quercetin-3-*O*-monoglucoside] and [LH/Fr.2] (135 mg) [**compound 3**=isorhamnetin-3-*O*-monoglucoside]. Later the same procedures were performed for scale-up.

*2.2.3.6. Structure elucidation of the isolated components **1–3**.* The structure elucidation of the isolated compounds (**1–3**) were carried out by using the spectroscopic techniques i.e. ^1H and ^{13}C-NMR [COSY, NOESY], in conjunction with FAB-MS and by comparing the previously published data for the compounds. NMR analyses were performed by using Bruker NMR instrument [Bruker Bioapex 30es High Resolution FTMS]; for ^1H-NMR (300 MHz), and for ^{13}C-NMR (75 MHz) in DMSO-d_6 was used. Names and chemical formulae of the isolated compounds are given in Fig. 1.

2.3. Pharmacological experiments

2.3.1. Animals

Male Wistar rats (120–150 g) were purchased from Animal Breeding Laboratories of Military Academy of Medicine (Ankara, Turkey). The animals were left at least for 5 days for acclimatization to animal room conditions (24 °C) and were maintained on standard pellet diet and water *ad libidum*. The food was withdrawn 48 h before the experiment, but allowed free access of water. To avoid coprophagy the rats were fasted in wire-bottomed cages. For each group 6 rats were used. Animal experiments were



R: H Isoquercitrin (Quercetin-3-O-glucoside) [compound 2]

R: CH_3 Isorhamnetin-3-O-glucoside [compound 3]

Fig. 1. Chemical formulae of the isolated compounds **2** and **3**.

performed following the current internationally accepted instructions for the care of laboratory animals and the ethical guidelines approved by the Ethics Committee of the Gazi University.

2.3.2. Preparation of test samples for bioassay

The test samples, except in gastric secretion experiments, were given orally or intraperitonally to test animals in a 10 ml/kg body weight dosage after suspending in a mixture of 0.5% sodium carboxymethyl cellulose (Na-CMC) in distilled water. The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with administration of appropriate volumes of the dosing vehicle. H_2-receptor antagonist drug, famotidine (2–20 mg/kg, per os), was used as reference compound.

2.3.3. Effects on water immersion and immobilization-induced stress ulcer in rats

The method described by Takagi and Okabe (1968) was employed with some modifications (Yesilada et al., 1993). A group of rats weighing 120–150 g was used for each treatment. The animals were placed individually in each compartment of a stress cage (4.5 × 4.5 × 18 cm) and immersed vertically up to the xyphoid level in a water-bath (17–19 °C) and kept in that position for 7 h to induce stress ulcer. Test samples were administered orally or i.p. to rats just before immobilization. Seven hours after immersion, rats were sacrificed under anesthesia. Then the abdomen of each was cut open and stomachs were removed and inflated with 10 ml of a 10% formalin solution and immersed in 10% formalin solution 30 min to fix the outer layer of the stomach. Afterwards each stomach was opened along the greater curvature, rinsed with physiological saline to remove gastric contents and blood clots. Lesion areas were examined under a dissecting microscope (20 × 6.3) to assess the formation of ulcers. The sum of length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the inhibition percentage was calculated by the formula:

$$\text{Inhibition } (\%) = [(\text{UI control} - \text{UI treated})/\text{UI control})] \times 100.$$

2.3.4. Effects on the ulceration induced by ethanol in rats (Robert et al., 1979)

Test sample was administered orally 15 min before the oral application of absolute EtOH (1 ml) to a group of six rats. 60 min later, the animals were sacrificed under anaesthesia. The stomachs were removed and processed as described above, except that the stomach was inflated with 10 ml of formalin solution. The length of each lesion in the glandular portion was measured likewise.

2.3.5. Effects on serotonin-induced ulcerogenesis in rats (Okabe et al., 1976)

Rats were given a single subcutaneous injection of serotonin creatinin sulphate (5-HT; Merck) in physiological serum (0.5 ml, 50 mg/kg) to induce glandular lesions. Test sample was administered orally 30 min prior 5-HT injection. Six hours later the animals were killed and the stomachs were examined and ulcer index was determined as described above.

2.3.6. Effects on pyloric ligation-induced ulcerogenesis in rats (Shay et al., 1945)

Stomachs obtained in the "gastric acid secretion study" as mentioned below were also evaluated for ulcer index by using the scoring method as follows: 0.5, for hemorrhages or lesions or small ulcer less then 3 mm; 1, for each marked ulcer over 3 mm; 5, for perforated ulcer.

2.3.7. Measurement of gastric acid secretion parameters in pylorus-ligated rats

Under anesthesia, the pyloric junction was ligated surgically, as described by Shay et al. (1945), and the incision was immediately closed. Test samples was administered orally (3 ml/kg) immediately following the pyloric ligation. The animals were then placed in cages without food and water. At the end of 4 h, the animals were sacrificed, and their stomachs were removed by clamping of the esophagus at the cervical portion. A small incision was made in the stomach with a fine pair of scissors and the gastric content was allowed to transfer into a centrifuge tube. The content was centrifuged at 2500 rpm for 20 min at 4 °C. The volume of the supernatant (ml) and pH value were measured. Volume was expressed as ml/100 g b.w. An aliquot of 1 ml was taken from each sample, and titrated against 0.01 N NaOH using phenolphthalein reagent as indicator. Acid concentration was expressed as μ-equivalents per ml and for acid output as μ-equivalents per 4 h. The peptic activity was determined by using bovine serum albumin as a substrate and expressed in terms of the amount of liberated μmol L-tyrosine according to the method described by Prino et al. (1971). Briefly, the mixture of gastric (0.1 ml) and 0.5% of bovine serum albumin (Sigma Co.) in 0.01 N HCl (1 ml) was incubated at 37 °C for 20 min the reaction was stopped by adding 10% trichloroacetic acid (2 ml). After the denaturation of protein by heating in a boiling water bath for 5 min, the precipitate was removed by centrifugation (9000 rpm). Then, 1 ml of supernatant was mixed with 0.4 ml of 2.5 N NaOH and 0.1 ml of Folin–Ciocalteau reagent (Sigma-Aldrich Co.) and the volume was adjusted to 10 ml with distilled water. The absorbance value was measured at 700 nm. A control for each sample (gastric juice blank) which did not contain albumin was carried out simultaneously.

2.3.8. Measurement of gastric hexosamine content

Each group of rats (180–200 g) fasted for 48 h on water prior to the experimentation. After giving the test sample orally, animals were exposed to water immersion and immobilization-induced stress ulcer as described before. But the stomachs were then removed without inflating and cut open at the greater curvature and soaked in a mixture of EtOH-acetone (1:1 v/v) for 24 h to remove fat from tissues. The glandular stomach was then divided into the corpus and the antrum, each of which was dried in a desiccator under reduced pressure overnight and weighed. Accurately weighed portions of either corpus or antrum (35–45 mg) were digested with 1 ml of 2 N HCl in a stoppered test tube in a sand bath (100 °C) for 15 h. After cooling, the digested material was filtered through glass wool into a Dowex 50 column and 3 ml of the eluate was used to measure the content of hexosamine by Narumi's method (Narumi and Kanno, 1972) spectrophotometrically at 530 nm.

2.4. Statistical analysis of data

Results were expressed as mean ± S.E. The statistical difference between the mean ulcer index of the treated group and that of the control was calculated by using ANOVA and Student-Neuman–Keuls multiple comparison tests or Student's test. The degree of significance was set at $p < 0.05$.

3. Results and discussion

For the evaluation of antiulcerogenic activity of *Sambucus ebulus* leaves, the methanolic extract of the material was submitted to successive chemical fractionation processing; i.e. solvent–solvent extraction, precipitation and column chromatography based on ion-exchange, silica gel and sephadex. In a previous study (Yesilada et al., 1993), higher activity was observed with the aqueous extract

Table 1
Effects of subextract and fractions on water immersion and immobilization-induced stress ulcer model in rats.

Material	Dose (mg/kg)	Ulcer Index (Mean ± S.E.M.)	Prevention from ulcer[a]	Inhibition (%)
Control	–	3.7 ± 1.5	–	–
n-BuOH↓ fraction	816	0.1 ± 0.1*	5/6	97.3
n-BuOH↑ fraction	540	8.8 ± 0.6	0/6	–
Remaining H_2O subextract	843	1.4 ± 0.8	2/6	62.2

* $p < 0.05$ significant from the control.
[a] Number of rats in which the bleeding of the stomach was prevented.

Table 2
Effects of fractions from Amberlite XAD_2 column on water immersion and immobilization-induced stress ulcer model in rats (per os).

Material	Dose (mg/kg)	Ulcer Index (Mean ± S.E.M.)	Prevention from ulcer[a]	Inhibition (%)
Control	–	7.8 ± 1.4	–	–
[XAD/Fr.1-6]	1014	3.5 ± 1.9	0/6	55.1
[XAD/Fr.7-20]	195	2.9 ± 1.2*	0/6	62.8
[XAD/Fr.21-60]	495	0.8 ± 0.2***	1/6	89.7
Compound **1** (Rutin)	115	6.8 ± 3.4	0/6	12.8
Famotidine	2	2.3 ± 0.4*	0/6	70.5

* $p < 0.05$.
*** $p < 0.001$ significant from the control.
[a] Number of rats in which the bleeding of the stomach was prevented.

of the leaves, and therefore only more polar subextracts, i.e. *n*-butanol and remaining aqueous subextracts (R-H_2O), were subjected to the further bioassay tests using water immersion and immobilization-induced stress ulcer model in rats. As shown in Table 1, precipitated part of the *n*-butanolic subextract [*n*-BuOH↓] presented a potent antiulcerogenic activity (97.3% inhibition). Then this fraction was used for further experimentation.

[*n*-BuOH↓] fraction was initially fractionated by ion-exchange column chromatography on Amberlite XAD_2 and eluents were combined into three subfractions. All subfractions showed antiulcerogenic activity in different potencies (55.1 to 89.7% inhibition), but activity of the subfraction [XAD-Fr.21-60] was the most prominent. On TLC examination, this fraction was found to be rich in flavonoids and the three main flavonoids were then isolated and encoded as compound-**1**, -**2** and -**3,** respectively. Compound-**1** was identified as rutin. Rutin was also a component of subfraction [XAD-Fr.7-20] which possesses a significant inhibition (62.8% inhibition); however, as shown in Table 2, rutin was found to be almost ineffective on stress-induced ulcer model.

The active subfraction [XAD-Fr.21-60] demonstrated a significant activity not only on oral administration, but also a remarkable inhibition (76.2% inhibition) was observed intraperitoneally (Table 4) against the same experimental ulcer model. Moreover, this fraction exhibited a potent inhibition against ethanol-induced ulcerogenesis in per os administration (98.3% inhibition). Therefore, it has been suggested that the antiulcerogenic effect of this active subfraction would possibly be due to a cytoprotective mechanism. On the other hand, the active subfraction [XAD-Fr.21-60] was found to possess a weak activity against serotonin- and pyloric ligation-induced ulcerogenesis, as well as on some of gastric biochemical parameters (Tables 5 and 6). These results also demonstrated the partial contribution of the antiacid potential as

Table 3
Effects of quercetin-3-*O*-monoglucoside and isorhamnetin-3-*O*-monoglucoside isolated from *Sambucus ebulus* on EtOH-induced experimental gastric ulcer model in rats.

Material	Dose (mg/kg)	Ulcer Index (Mean ± S.E.M.)	Prevention from ulcer[a]	Inhibition (%)
Control	–	94.5 ± 15.0	–	–
Compound **2** (quercetin-3-*O*-monoglucoside)	990	38.7 ± 17.1*	2/6	59.1
Compound **3** (isorhamnetin-3-*O*-monoglucoside)	990	4.3 ± 4.3***	5/6	95.5

* $p < 0.05$.
*** $p < 0.001$ significant from the control.
[a] Number of rats in which the bleeding of the stomach was prevented.

Table 4
Effects of active subfraction (XAD/Fr.21-60) on various ulcer models in rats.

Test samples	Dose (mg/kg)	Ulcer Index (Mean ± S.E.M.)	Prevention from ulcer[a]	Inhibition (%)
***Water immersion and immobilization-induced stress ulcer* (*intraperitoneal administration*)**				
Control	–	12.6 ± 2.5	–	–
[XAD/Fr.21-60]	495	3.0 ± 1.3**	1/6	76.2
Famotidine	5	1.25 ± 0.3**	0/6	90.1
***EtOH-induced ulcerogenesis* (*per os*)**				
Control	–	29.1 ± 8.9	–	–
[XAD/Fr.21-60]	495	0.5 ± 0.5 **	5/6	98.3
Famotidine	2	3.0 ± 0.5 *	0/6	89.7
***Serotonin-induced ulcerogenesis* (*per os*)**				
Control	–	32.1 ± 25.3	–	–
[XAD/Fr.21-60]	495	23.8 ± 7.0	0/6	25.9
Famotidine	2	9.3 ± 3.4*	6/6	71.0
Pyloric ligation-induced ulcerogenesis in per os				
Control	–	6.6 ± 1.7	–	–
[XAD/Fr.21-60]	495	3.0 ± 1.8	1/6	54.6
Famotidine	20	0.9 ± 0.7*	0/6	86.4

* $p < 0.05$.
** $p < 0.01$ significant from the control.
[a] Number of rats in which the bleeding of the stomach was prevented.

Table 5
Effects of the active subfraction on hexosamine content in the glandular stomach strips of rats exposed to stress ulcer model.

Material	Dose (mg/kg)	Mucosa weight (mg) (Mean ± S.E.M.).	Hexosamine content (μg/100 mg dry strip)	
			Corpus	Antrum
Control	–	135.8 ± 5.5	561.0 ± 19.6	1140.9 ± 165.2
[XAD/Fr.21-60]	495	131.0 ± 4.5	420.0 ± 24.3	675.3 ± 98.1

Table 6
Effects of the active subfraction (495 mg/kg) on gastric biochemical parameters in pylorus-ligated rats.

	Gastric secretion (ml/100 g b.w.)	Gastric pH value	Titratable acidity (μEq. H^+/l)	Titratable acid output (μEq.)	Peptic activity (μ moles L-tyrosine/4 h)
Control	2.3 ± 0.4	1.81 ± 0.03	112.6 ± 11.4	540.6 ± 149.6	12.77 ± 4.3
[XAD/Fr.21-60]	2.2 ± 0.4	2.30 ± 0.33	114.3 ± 12.6	528.6 ± 100.6	5.91 ± 1.5

well as repairing effect of the active subfraction on the gastric mucosal layer by increasing the muscular blood circulation of stomach (Gürbüz and Yesilada, 2007).

Isolation of two flavonoids, compounds **2** and **3**, from the subfraction [XAD/Fr.21-60] was carried out first by vacuum chromatography on silicagel and following by Sephadex LH-20 column chromatography. The anti-ulcerogenic activity of these flavonoids was examined by using EtOH-induced ulcer model and both were found significantly active (Table 3). The structures of these flavonoids (Fig. 1) were elucidated by ^{1}H, ^{13}C-NMR, and FAB-MS techniques and also by comparing with the previously reported data (Güvenalp and Demirezer, 2005; Wang et al., 2012).

Certain flavonoids have previously been reported to possess antiulcerogenic activity. Especially flavonol derivatives with ortho-dihydroxy groups were reported to possess prominent biological activities. Among these, quercetin derivatives were previously reported as the active antiulcerogenic constituent of several folk remedies by several authors and their possible mechanism of action were investigated (Lewis and Hanson, 1991). Previously, quercetin 3-methyl ether was obtained from *Cistus laurifolius* L. demonstrated to have a high anti-*Helicobacter pylori* activity (Üstün et al., 2006). Another quercetin derivative, quercetin-3-*O*-β-D-glucuronopyranoside, from *Rumex aquaticus* was also reported to prevent the reflux esophagitis and gastritis in rats (Min et al., 2009). However, the active flavonoids, quercetin-3-*O*-glucoside [=isoquercitrin] (compound-**2**) and isorhamnetin-3-*O*-glucoside (compound-**3**), which were isolated in the present study have not been reported for their antiulcerogenic potential. Previously isoquercitrin was determined as one of the components in the active antiulcerogenic fraction of *Byrsonima sericea* leaves, a Brazilian folk remedy, it was not isolated as the active constituent (Rodrigues et al., 2012).

On the other hand, isorhamnetin-3-*O*-glucoside was reported to inhibit inflammatory pathway and suggested in treating and preventing inflammatory diseases (Lee et al., 2010) and also to possess antioxidant activity (Devi et al., 2010). The other active component, quercetin-3-*O*-glucoside, was also shown to have radical scavenging activity (Yesilada et al., 2000). Since both the inflammatory and oxidative damages were known to play key role in the etiology of ulcerogenesis (Gürbüz and Yesilada, 2007), such contributory effects exerted by these flavonoid glycosides would also be beneficial in the healing process of ulcers.

As conclusion, the folkloric use of *Sambucus ebulus* leaves for the treatment of gastric ailments in Turkish folk medicine has been experimentally proven. Two flavonol glycosides isolated in the present study as the active principles were not previously reported for their antiulcerogenic potential elsewhere.

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