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Investigations on the anti-ulcerogenic activity of *Sideritis caesarea* H. Duman, Aytaç & Başer

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ABSTRACT

Ethnopharmacological relevance: Aerial parts of *Sideritis caesarea* H. Duman, Aytaç & Başer are used for complaints such as stomach-aches, and intestinal spasms as traditional medicine in Kayseri, Turkey.
Aim of study: To investigate the anti-ulcerogenic activity by using bioassay guided fractionation technique (BAGF) and to identify the compound(s) that are responsible for anti-ulcerogenic activity through ethanol-induced anti-ulcerogenic activity model in vivo.
Materials and methods: Liquid-liquid partition and then different chromatographic techniques were utilized for the BAGF of the ethanol (80%) extract of the aerial parts of *Sideritis caesarea*. Ethanol-induced gastric ulcer method on rats was employed for the determination of the anti-ulcerogenic activity, and the ulcer index was also calculated for anti-ulcerogenic activity detection.
Results: The ethanol (80%) extract of *S. caesarea* showed statistically potent anti-ulcerogenic activity (95.9% ulcer inhibition, $p < 0.001$). Among the liquid-liquid fractions, strongest anti-ulcerogenic activity was observed with the ethyl acetate fraction (91.4% inhibition, $p < 0.001$) and therefore BAGF studies were proceeded with the ethyl acetate fraction. Two anti-ulcerogenic flavonoids {4′-*O*-methylhypolaetin-7-*O*-[6‴-*O*-acetyl-β-D-allopyranosyl-(1→2)]-6″-*O*-acetyl-β-D-glucopyranoside and isoscutellarein-7-*O*-[6‴-*O*-acetyl-β-D-allopyranosyl-(1→2)]-6″-*O*-acetyl-β-D-glucopyranoside} were isolated from this fraction together with a sesquiterpene glycoside [(2*E*,6*E*)-2,6,10-trimethyl-2,6,11-dodecatriene-1,10-diol-1-*O*-β-D-glucopyranoside] and two additional flavonoids {4′-*O*-methylhypolaetin-7-*O*-[6‴-*O*-acetyl-β-D-allopyranosyl-(1→2)]-β-D-glucopyranoside and isoscutellarein-7-*O*-[6‴-*O*-acetyl-β-D-allopyranosyl-(1→2)]-β-D-glucopyranoside}.
Conclusions: Traditional use of *S. caesarea* in the treatment of stomach-aches was supported by this study and four flavonoids were isolated by using BAGF method and two of them were determined to have significant anti-ulcerogenic activity. Additionally, (2*E*,6*E*)-2,6,10-trimethyl-2,6,11-dodecatriene-1,10-diol-1-O-β-D-glucopyranoside was obtained from a *Sideritis* genus for the first time.

This study was conducted as a part of a doctoral thesis [Tuğba Günbatan, "*Sideritis caesarea* H. Duman, Aytaç & Başer bitkisinin antiülserojenik aktivitesi üzerinde araştırmalar", Gazi University Institute of Health Sciences, Ankara, 2017] and was partly presented as posters at The International Gazi Pharma Symposium (Günbatan et al., Two anti-ulcerogenic flavonoid glycosides from *Sideritis caesarea* H. Duman, Aytaç & Başer, Antalya, Turkey, 2015) and The 19th International Congress Phytopharm (Gürbüz et al., In vivo anti-ulcerogenic effect evaluation of *Sideritis caesarea* H. Duman, Aytaç & Başer Lamiaceae, Bonn, Germany, 2015).

1. Introduction

Lamiaceae family, distributed nearly worldwide, includes powerful aromatic herbs which have been in use for culinary, medicinal purposes and especially in making beverages, herbal teas, since ancient times (Fecka and Turek, 2007). Various species of the genera *Salvia, Thymus, Stachys, Lavandula, Origanum, Marrubium, Satureja* and *Sideritis* are commonly used to make herbal teas in Turkey and around (Dirmenci et al., 2018; Gürbüz et al., 2019; Tuzlacı, 2011). Among them, *Sideritis* species stand out with their excellent flavour and fragrance profile and

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a long running history of recreational and medicinal uses. A large number of studies on the members of this genus were conducted due to widespread use of its species especially in circum-Mediterranean countries (Sağır et al., 2017).

Sideritis genus is distributed in a quite wide region with more than 150 species, especially dwell in Mediterranean basin (Duman et al., 2005). According to "Flora of Turkey and the East Aegean Islands" and "Türkiye Bitkileri Listesi/Damarlı Bitkiler (The List of Plants of Turkey/Vascular Plants)", 45 *Sideritis* species grow wild in Turkey (Davis et al., 1988; Güner et al., 2012). Different species of *Sideritis* are known as "Mountain grass"; "Mountain tea", "Ironwort", "Té amarillo", "Te de monte" etc. in some European countries (González-Burgos et al., 2011; Pardo-de-Santayana et al., 2005; Todorova and Trendafilova, 2014). Traditionally, plants of genus *Sideritis* have been reported to be used for disorders like bronchitis, bronchial asthma, pulmonary emphysema, angina pectoris, common cold, flu, hypertension, circulatory disorders, worms in the eyes, eye infections, urinary system infections, gastrointestinal disorders including diarrhoea and peptic ulcer, anaemia, wounds, backache, rheumatism and cataract. Additionally, they are used as appetizer, analgesic, sedative, expectorant, anti-tussive, diuretic, anti-pyretic, aphrodisiac, hypoglycaemic, digestive, carminative, anti-spasmodic, anti-inflammatory, anti-microbial, anti-convulsant, disinfectant, diaphoretic, etc. (Agelet and Valles, 2001; Basile et al., 2006; Belda et al., 2013; González-Burgos et al., 2011; González-Burgos et al., 2009; Hanlidou et al., 2004; Hernandez-Perez et al., 2004; Ivancheva and Stantcheva, 2000; Karousou and Deirmentzoglou, 2011; Lentini, 2000; Leporatti and Impieri, 2007; Pardo-de-Santayana et al., 2005; Pieroni et al., 2005; Raja et al., 1997; Rivera and Obón, 1995; Todorova and Trendafilova, 2014). In Anatolia, *Sideritis* species are generally known as "Adaçayı", "Dağ Çayı" and are used for abdominal pain, digestion problems, stomach disorders, bronchitis, cough, common cold, sore throat and flu among local people (Ahmet Sargın, 2015; Akalın and Alpınar, 1994; Altundağ and Öztürk, 2011; Baytop, 1999; Bulut and Tuzlacı, 2013; Gürdal and Kültür, 2013; Güzel et al., 2015; Kargıoğlu et al., 2008; Kültür, 2007; Özdemir and Alpınar, 2015; Polat and Satıl, 2012; Sargın et al., 2013, 2015a, 2015b; Tetik et al., 2013; Yeşilada et al., 1995).

Since the *Sideritis caesarea* H. Duman, Aytaç & Başer was firstly defined in 1998 and is also an endemic species, ethnobotanical data on this plant is limited. During the ethnobotanical research in the district of Pınarbaşı (Kayseri, Turkey), conducted by our research group, decoction of *S. caesarea* was recorded to be used as a calming herbal tea and a folk remedy for stomach-aches and intestinal spasms (Gençler Özkan and Koyuncu, 2005). Based on this ethnobotanical data, anti-ulcerogenic activity of *S. caesarea* was investigated in our previous screening study, and statistically significant anti-ulcerogenic activity was observed (95.8% ulcer inhibition, $p < 0.001$) by ethanol-induced ulcerogenesis model in rats (Gürbüz et al., 2005).

In the present study, by using bioassay guided fractionation technique (BAGF), identification of the fraction(s)/compound(s) of the *S. caesarea* responsible for the anti-ulcerogenic activity was aimed. The quantitative determination of two anti-ulcerogenic flavonoids isolated from the bioactive fraction in the ethanol extract (80%) was carried out by HPLC analysis as well.

2. Methods and materials

2.1. Plant material

Flowering aerial parts of *Sideritis caesarea* H. Duman, Aytaç & Başer (Dağ çayı) were collected from Kayseri, the district of Sarız, Dayıoluk village, the slopes in the east of the village N: 38° 21′ 01″, E: 26° 30′ 57″ July 07, 2014 and were dried under shade at room temperature. Voucher specimens were identified by Prof. Dr. Ayşe Mine GENÇLER ÖZKAN (botanist) and prepared herbarium specimens are stored in Gazi University Faculty of Pharmacy Herbarium (GUE-3232). The plant name was checked with http://www.theplantlist.org.

2.2. Chemicals

Ethanol (99.5%, Baker HPLC analyzed J.T. Baker, Deventer, Holland; 96% Tekkim, Bursa, Turkey), methanol (99.8% J.T. Baker, Deventer, Holland; EMSURE® ACS, ISO, Reag. Ph Eur Sigma, Germany), chloroform (for analysis EMSURE® ACS, ISO, Reag. Ph Eur Merck Darmstadt, Germany), *n*-hexane (ACS Reagent, Reag. Ph. Eur., ≥99% Riedel-de Haën, Germany; for HPLC, ≥95% Sigma, Germany), dichloromethane (for analysis EMSURE® ACS, ISO, Reag. Ph Eur Merck, Darmstadt, Germany; for HPLC, ≥99.8%, Sigma, Germany), ethyl acetate (ACS Reagent, ≥99.5% Riedel-de Haën, Germany; ACS reagent, ≥99.5% Sigma, France), *n*-butanol (EMSURE® ACS, ISO, Reag. Ph Eur Merck, Darmstadt, Germany), acetonitrile (for HPLC, %99.9 Dop, Ankara, Turkey), formaldehyde (ACS, Reag. Ph Eur Merck, Darmstadt, Germany), phosphoric acid (85% Baker Analyzed Reagent J.T. Baker Deventer, Holland), sulphuric acid (%92 Birpa, Ankara, Turkey), silica gel (Merck, Darmstadt, Germany), thin layer chromatography plates (Merck, Darmstadt, Germany), preparative thin layer chromatography plates (Merck, Darmstadt, Germany), polyamide 6 (Fluka, Germany), sephadex LH-20 (Sigma, Germany), carboxymethyl cellulose (Aklar Kimya, Ankara, Turkey), omeprazole (Zydus Cadila), ketamine (Alfamine, Holland, Woerden), xylazine (Alfazyne, Woerden, Holland).

2.3. Chemical experiments

2.3.1. Thin layer chromatography (TLC)

During chemical experiments, obtained fractions were subjected to TLC, when required. Different mobile phase systems [chloroform:methanol:water (61:32:7 and 8:2:0.2), chloroform:methanol (9:1), methanol:water (50:50 and 75:25), upper phase of *n*-butanol:acetic acid:water (4:1:5) etc.] were utilized according to the polarity of analyzed fraction. Spots were examined in daylight and under the ultraviolet lamp (at 254 and 366 nm). Subsequently, TLC plates are revealed by spraying 5% sulphuric acid in methanol after heating at 100°C.

2.3.2. Extraction

Air dried flowering aerial parts of *S. caesarea* (500 g) were coarsely grinded and macerated at room temperature with 4 L 80% ethanol for 48 h while being stirred by a mechanical stirrer. The macerate was filtered, and the plant residue was subjected to the same process for seven times with 2.5 L 80% ethanol. The filtrates were combined and evaporated to dryness under reduced pressure at temperatures not exceeding 45°C.

2.3.3. Fractionation of 80% ethanol extract (SC–EtOH) by solvent-solvent extraction

137 g SC-EtOH was dissolved in 1 L 90% methanol and extracted with 500 mL *n*-hexane, 26 times. Remaining extract was evaporated and dissolved in 800 mL distilled water. Afterwards, this aqueous solution extracted with dichloromethane (400 mL, 34 times), ethyl acetate (1 L, 43 times), *n*-butanol (400 mL, 71 times), respectively. Each fraction was evaporated to dryness under reduced pressure at temperatures not exceeding 45°C, and the remaining aqueous layer was lyophilised. 4.8 g *n*-hexane (SC-Hex), 4.4 g dichloromethane (SC–CH_2Cl_2), 23.2 g ethyl acetate (SC-EtOAc), 48.1 g *n*-butanol (SC-BuOH) and 40.4 g water fraction (SC-Rw) were obtained. Fractionation process was summarized in Fig. 1.

2.3.4. Fractionation of the SC-EtOAc with polyamide column chromatography (SC/PA1)

7 g SC-EtOAc was applied to polyamide 6 column (120 g; 4.5 × 60 cm) and eluted with water:methanol gradient [(100:0), (75:25), (50:50), (25:75), (0:100)]. Total of 329 fractions were

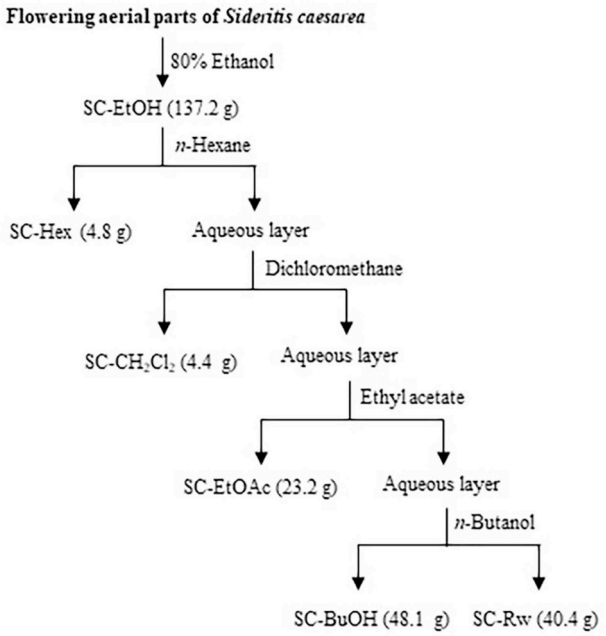


Fig. 1. Fractionation process of *S. caesarea* by solvent-solvent extraction.

collected, which were successively combined into 28 fractions based on their TLC profiles (SC/PA1/Fr.1-2; SC/PA1/Fr.3-8; SC/PA1/Fr.9-11; SC/PA1/Fr.12-27; SC/PA1/Fr.28-36; SC/PA1/Fr.37-47; SC/PA1/Fr.48-64; SC/PA1/Fr.65-75; SC/PA1/Fr.76-91; SC/PA1/Fr.92-101; SC/PA1/Fr.102-110; SC/PA1/Fr.111-117; SC/PA1/Fr.118-132; SC/PA1/Fr.133-143; SC/PA1/Fr.144-180; SC/PA1/Fr.181-229; SC/PA1/Fr.230-239; SC/PA1/Fr.240-250; SC/PA1/Fr.251-262; SC/PA1/Fr.263-264; SC/PA1/Fr.265-268; SC/PA1/Fr.269-272; SC/PA1/Fr.273-278; SC/PA1/Fr.279-287; SC/PA1/Fr.288-297; SC/PA1/Fr.298-303; SC/PA1/Fr.304-316; SC/PA1/Fr.317-329). These 28 fractions were evaluated as 3 major groups in the anti-ulcerogenic activity tests [SC/PA1/Fr.1-75 (852.9 mg), SC/PA1/Fr.76-229 (4184.1 mg) and SC/PA1/Fr.230-329 (1499.3 mg)]. This chromatographic process was later repeated to increase the quantity of fractions.

2.3.5. Fractionation of the SC/PA1/Fr.1-75

SC/PA1/Fr.1-75 was retained in 3 subgroups (SC/PA1/Fr.1-8, SC/PA1/Fr.9-27, SC/PA1/Fr.28-75), and fractionation studies were carried out on those having anti-ulcerogenic activity. The BAGF studies continued on the active fractions (SC/PA1/Fr.9-27 and SC/PA1/Fr.28-75). A preliminary test was performed by applying a small amount of SC/PA1/Fr.28-75 on Sephadex LH-20 column chromatography (SC/LH1). One hundred mg SC/PA1/Fr.28-75 was applied to Sephadex LH-20 column (33 g; 3 × 23 cm) and eluted with methanol. Obtained fractions were combined into 6 groups according to their TLC profiles: SC/LH1/Fr.1-3 (6.2 mg); SC/LH1/Fr.4-8 (27.1 mg); SC/LH1/Fr.9-10 (22.4 mg); SC/LH1/Fr.11-13 (17.4 mg); SC/LH1/Fr.14-15 (4.6 mg); SC/LH1/Fr.16-22 (**2**: 4.0 mg). The obtained fractions were not used in BAGF as there was no satisfactory separation with Sephadex LH-20 column system. Nevertheless, we obtained a pure substance **(2)** and completed the structure determination.

Therefore, 1162.8 mg SC/PA1/Fr.28-75 was fractionated (SC/FL1) over reverse phase column (Redisep 50 g C18 Gold cartridge) by flash chromatography system (Combi flash EZ prep). The flow rate of the mobile phase was 20 mL/min; fraction volume was 5 mL. The fractions obtained with water:methanol gradient elution were combined into 8 groups according to their TLC profiles: SC/FL1/Fr.1-6 (414.4 mg), SC/FL1/Fr.7-8 (107.0 mg), SC/FL1/Fr.9-62 (183.2 mg), SC/FL1/Fr.63-70 (86.5 mg), SC/FL1/Fr.71-81 (68.3 mg), SC/FL1/Fr.82-117 (54.7 mg), SC/FL1/Fr.118-132 (15.9 mg), SC/FL1/Fr.133-171 (17.1 mg). Then 15.9 mg SC/FL1/Fr.118-132 was fractioned (SC/pHPLC) with preparative HPLC (Combi flash EZ prep) by using reverse phase column (Redisep Prep C18 100 A° 5 µm, 250 × 20 mm). The flow rate of the mobile phase was 15 mL/min; fraction volume was 15 mL. Fractions obtained with water:methanol gradient elution were combined into 9 groups according to their TLC profiles: SC/pHPLC/Fr.1-19 (1.7 mg), SC/pHPLC/Fr.20 (0.8 mg), Fr.21 (0.8 mg), SC/pHPLC/Fr.22-23 (1.1 mg), SC/pHPLC/Fr.24 (0.3 mg), SC/pHPLC/Fr.26 (**1**; 6.8 mg), SC/pHPLC/Fr.25 + 27 (1.5 mg), SC/pHPLC/Fr.28-29 (1.6 mg), SC/pHPLC/Fr.30-38 (0.9 mg).

2.3.6. Fractionation of SC/PA1/Fr.76-229

SC/PA1/Fr.76-229 (8885.0 mg) was applied to silica gel column (422 g; 4.5 × 67 cm; Silica gel 60, 70-230 mesh, Merck) (SC/SG1). Elution was performed by chloroform:methanol (100:0, 97:3, 95:5, 90:10, 87.5:12.5, 85:15, 80:20, 70:30, 65:35, 60:40, 50:50, 25:75, 0:100) and methanol:water gradient (50:50, 25:75, 0:100). Obtained fractions were combined into 9 groups according to their TLC profiles: SC/SG1/Fr.1-19 (727.3 mg), SC/SG1/Fr.20-35 (456.3 mg), SC/SG1/Fr.36-74 (**3**: 576.2 mg), SC/SG1/Fr.75-98 (759.6 mg), SC/SG1/Fr.99-294 (4117.6 mg), SC/SG1/Fr.295-374 (2001.4 mg). Fraction SC/PA1/Fr.76-229 (30.0 mg) was also subjected to preparative TLC (SC/pTLC) on pre-coated silica gel 60 glass plates (Merck, 105721) and developed by chloroform:methanol:water (8:3:0.5) solvent system. Same process was repeated for scale-up for three times. One compound was isolated by this method: SC/pTLC/Fr.3 (**4**: 4.4 mg).

2.3.7. Fractionation of SC/PA1/Fr.230-329 (SC/FL2)

SC/PA1/Fr.230-329 (967.0 mg) was fractioned by flash chromatography (Reveleris 120 g silica gel cartridge) with dichloromethane:methanol:water (90:10:1) isocratic system. The flow rate of the mobile phase was 2 mL/min. Obtained fractions were combined into two groups according to their TLC profiles: SC/FL2/Fr.1 **(5**: 387.0 mg**)** and SC/FL2/Fr.2 **(6**: 352.0 mg**)**.

Whole isolation process was summarized in Fig. 2.

2.3.8. Structure elucidation of isolated compounds

LC-MS data were obtained by Waters LCT Premier XE (High sensitive orthogonal acceleration time of flight device)-AQUITY ultra performance liquid chromatography system using Mass Lynx 4.1 software. 1D and 2D (COSY, HMBC, HSQC and NOESY) NMR spectra (400 MHz) were recorded on Varian Oxford AS400 spectrometer with TMS as internal standard at room temperature. Structures of the isolated compounds were given in Fig. 3.

2.3.9. HPLC quantitation of **3** (=5) and **6**

HPLC analysis of SC-EtOH, **3 (=5)** and **6** was carried out on Agilent Technologies 1220 Infinity HPLC system using Lichrospher 100 RP-8 column (250 × 4 mm, 5 µm particles). The elution was monitored in 300 nm. Chromatographic separations were obtained by water (1% phosphoric acid) and acetonitrile gradient elution program: 0-5 min 10%–20% acetonitrile; 5-15 min 20% acetonitrile; 15-25 min 20%–30% acetonitrile; 25-35 min 30%–50% acetonitrile; 35-45 min 50%–70% acetonitrile; 45-53 min 70%–100% acetonitrile. The flow rate was 0.8 mL/min; the temperature was 25°C, and the injection volume was 20 µL. Calibration solutions were prepared by doing serial dilutions with **3 (=5)** and **6** in 10% acetonitrile. Calibration equations were obtained from graphic of peak area vs. concentration.

2.4. Pharmacological experiments

2.4.1. Animals

Male Spraque-Dawley rats (170-280 g) that purchased from "Kobay Experimental Animals Laboratory" (Ankara, Turkey) were used for anti-ulcerogenic activity tests. The animals were left at least seven days for acclimatisation. During this period, animals were kept in a 12-h day-

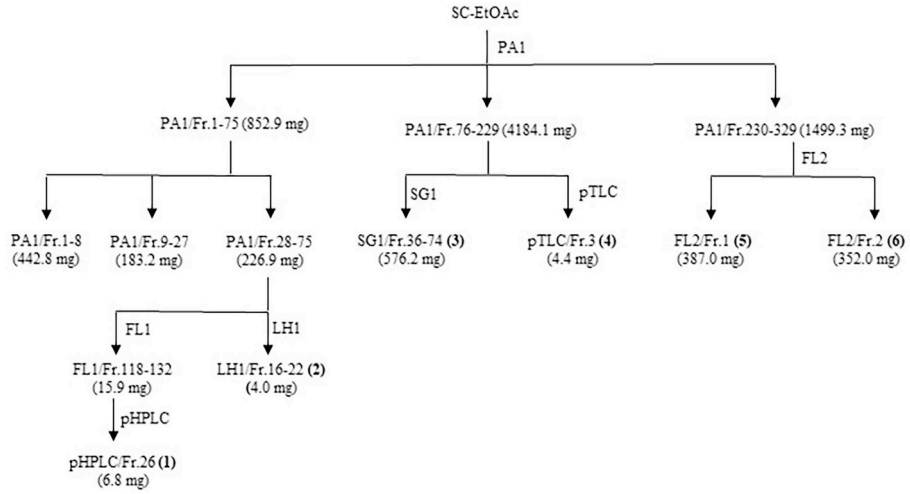
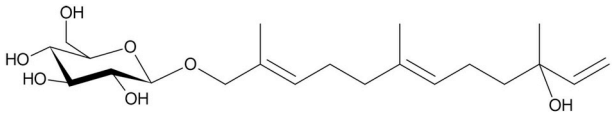
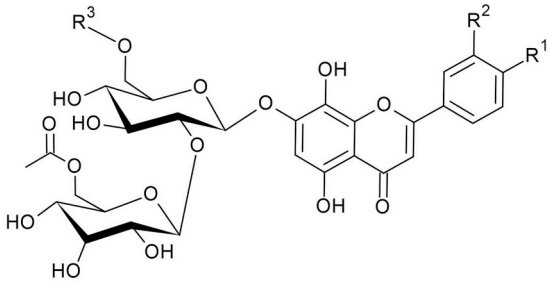


Fig. 2. Isolation process of compounds **1-6**.



Compound 1



Compounds 2-6

2 : R^1=OCH_3 R^2=OH R^3=H
3/5: R^1=OCH_3 R^2=OH R^3=CH_3CO
4 : R^1=OH R^2=H R^3=H
6 : R^1=OH R^2=H R^3=CH_3CO

Fig. 3. Structures of compounds **1-6**.

night period and 25°C room temperature provided laboratory and fed with standard pellet diet and water (ad libitum). Fodder was withdrawn 24 h before experiment; but animals were allowed to drink water until 1 h before the experiment. Rats were kept in the wire-bottomed cages to avoid coprophagy. Each experiment group consisted of 6 animals (Gürbüz et al., 2005; Robert et al., 1979). Authorization for the use of laboratory animals in this study was obtained from the Gazi University Local Ethics Committee for Animal Experiments (protocol number: B.30.2.GÜN.0.05.06.00/15-994).

2.4.2. Preparation of test samples

Required amount of extract/fraction/compounds or omeprazole used as standard anti-ulcerogenic agent were suspended in 0.5% carboxymethyl cellulose (CMC) in distilled water (Gürbüz et al., 2005; Robert et al., 1979).

2.4.3. Ethanol induced gastric ulcer method

Test samples, omeprazole and 0.5% CMC solution (for control) were given orally to test animals by intragastric gavage. 15 min after the application of test samples/omeprazole/CMC, 1 mL 96% ethanol was given orally to all animals by intragastric gavage to form ulcerogenesis. One hour later, animals were euthanized by ketamine (50 mg/kg i.p.)/xylazine (10 mg/kg i.c.) injection. Subsequently, their abdomens were dissected and their stomachs were tied from oesophagus, nearest to cardia, with surgical suture. Their tied stomachs were distended by 10 mL 10% formaldehyde solution injection from duodenum side. Following this procedure, a second knot was tied with another surgical suture from pyloric sphincter to prevent the formaldehyde solution leakage. The distended stomachs were removed by cutting out of the knots and immersed to 10% formaldehyde solution for the fixation of tissues. Then the stomachs were dissected along the greater curvature, washed with tap water to remove the stomach contents or blood clots and preserved in 10% formaldehyde solution until examination (Robert et al., 1979). Although different methods are available for the detection of anti-ulcerogenic activity, ethanol-induced gastric ulcer is one of the most frequently utilized reliable experimental model for assessment of agents with potential anti-ulcer activity by means of simulating several characteristics of the human peptic ulcer condition and allowing rapid results (Arab et al., 2015; Liang et al., 2018; Liu et al., 2012; Santos and Rao, 2001; Sidahmed et al., 2016; Simoes et al., 2019). Although the mechanism of ethanol-induced gastric ulcerogenesis has not been yet fully identified, its pathology occurs through three main pathways: inflammatory response, oxidative stress and apoptosis (Liang et al., 2018; Liu et al., 2012). As the result of decreased mucus secretion, sulfhydryl groups, nitric oxide, blood flow, inhibition of prostaglandin synthesis and severe infiltration of sub-mucosa which support the formation of reactive oxygen species, gastric damage is formed (Rios et al., 2010; Salga et al., 2012; Sidahmed et al., 2016).

In order to apply this method, required permission was provided from Gazi University Local Ethics Committee for Animal Experiments and animals were processed according to international ethical guidelines for the care of laboratory animals as well as the rules of the Gazi University Local Ethics Committee for Animal Experiments.

2.4.4. Examination of stomachs and determination of ulcer index

Stomachs were examined under a dissecting microscope, the size of lesions which occurred in antrum and corpus were measured. Sum of all lesion lengths (mm) for each stomach was evaluated as "Ulcer Index (UI)" and inhibition percentages were calculated by following formula (Robert et al., 1979):

$$\text{Inhibition (\%)} = (UI_{control}\text{-}UI_{test})\text{x}100/UI_{control}$$

$UI_{control}$ = Ulcer index of control
UI_{test} = Ulcer index of tested sample

2.4.5. Statistical evaluation

Results were expressed as mean ± S.E.M. The statistical difference between the mean ulcer index of the treated group and that of the control was performed by using ANOVA and Tukey multiple comparison test. The degree of significance was set at $p < 0.05$.

3. Results and discussion

S. caesarea was revealed to be used as folk medicine in the treatment of stomach-aches and intestinal spasms in Pınarbaşı-Kayseri, Turkey (Gençler Özkan and Koyuncu, 2005). Previous anti-ulcerogenic activity screening studies, have noted the potent anti-ulcerogenic activity of decoction of the aerial parts of *S. caesarea* at 960 mg/kg (the extract obtained 5 g aerial parts/kg) dose in ethanol-induced ulcerogenesis on rats (Gürbüz et al., 2005). In this study, it was attempted to determine the substance(s) or fraction(s) responsible for the anti-ulcerogenic activity of the aerial parts of *S. caesarea*.

According to general bioactivity screening methods, it is generally considered useful to test the activity of both alcoholic and aqueous extracts of the material to be investigated. Although the anti-ulcerogenic activity of the aqueous extract of *S. caesarea* on ethanol induced gastric ulcers in rats was determined previously (Gürbüz et al., 2005), the anti-ulcerogenic activity of alcoholic extracts has not yet been investigated. For this reason, it was planned to determine the anti-ulcerogenic activity of alcoholic extract prepared from aerial parts of *S. caesarea* by following the same method as previously described in our earlier work with the aqueous extract. Firstly, 925.0 mg 80% ethanol extract (SC–EtOH) was obtained from 5 g aerial parts, similar to the previous anti-ulcerogenic activity screening study, and given to the animals at 925.0 mg/kg and 462.5 mg/kg (half dose) doses. As a result, higher anti-ulcerogenic activity was determined at 925 mg/kg dose (95.9 ulcer inhibition, $p < 0.001$), and the obtained data was almost the same as the previously recorded one (960 mg/kg dose; 95.8% ulcer inhibition, $p < 0.001$) (Gürbüz et al., 2005) (Table 1). The results of this investigation showed that there was no statistically significant difference in the anti-ulcerogenic activity of both alcoholic and aqueous extracts on gastric ulcer areas of ethanol-induced ulceration in rats. However, the ethanol extract was preferred in subsequent BAGF studies since it showed equivalent activity at relatively lower doses, also has the advantage in terms of subsequent phytochemical processes.

In the next step, anti-ulcerogenic activities of the fractions obtained by solvent-solvent fractionation were determined. Due to scarcity of quantities, SC-Hex and SC-CH_2Cl_2 fractions were administered together. In this experiment, SC-BuOH and SC-Rw fractions showed a rather weak activity (Table 2). However, SC-EtOAc and SC-Hex + SC-CH_2Cl_2 fractions showed a moderate activity which was not statistically significant. It is thought that dose reduction during fractionation process could result in activity decrease. For this reason, the bioactivity experiment was repeated by increasing the dose (x2) of the extracts. However, in order to avoid excess use of animal subjects, anti-ulcerogenic activity experiment of the SC-EtOAc and SC-Hex + SC-CH_2Cl_2 fractions possessing stronger activity was repeated. At studied (x2) doses, both SC-EtOAc and SC-Hex + SC-CH_2Cl_2 extracts showed potent anti-ulcerogenic activity (91.4% and 75.0% ulcer inhibition, $p < 0.001$ and $p < 0.01$, respectively). When the experimental groups were evaluated, due to higher ulcer inhibition, ulcer prevention ratio and statistical significance, further BAGF experiments were decided to be carried out with the ethyl acetate fraction (Table 2).

Table 1
Anti-ulcerogenic activity of the SC-EtOH extract at two different doses.

Group	Dose (mg/kg)	Ulcer index (Mean ± S.E.M)	Prevention from ulcer[a]	Inhibition (%)
Control	–	137.8 ± 13.7	–	–
SC-EtOH	462.5	60.7 ± 24.9*	1/6	55.9
SC-EtOH	925.0	5.7 ± 2.6 ***	2/6	95.9
Omeprazole	20.0	9.3 ± 23.9*	2/6	64.2

*: $p < 0.05$; ***: $p < 0.001$, compared to control by using ANOVA and Tukey multiple comparison test.
S.E.M.: Standard error of the mean.
[a] Number of individuals without ulcer.

Subsequently, SC-EtOH was fractionated with polyamide column chromatography and 28 subfractions were obtained. According to their TLC profiles these subfractions were evaluated in bioactivity tests in three groups (SC/PA1/Fr.1-75; SC/PA1/Fr.76-229 and SC/PA1/Fr.230-329). As understood from Table 3, in all three groups significant anti-ulcerogenic activity was observed (inhibitions ranging between 65.6% and 78.9%, $p < 0.01$) and all of the subfractions (SC/PA1/Fr.1-75; SC/PA1/Fr.76-229 and SC/PA1/Fr.230-329) were decided to be evaluated in BAGF studies.

SC/PA1/Fr.1-75 had three subgroups (SC/PA1/Fr.1-8, SC/PA1/Fr.9-27, SC/PA1/Fr.28-75). For this reason, the anti-ulcerogenic activities of these subgroups were examined by the same in vivo technique before any isolation technique was applied (Table 4). Despite the observation of high anti-ulcerogenic activity with SC/PA1/Fr.9-27 and SC/PA1/Fr.28-75, BAGF studies could not be continued due to insufficient amount of test materials for animal experiments. On the other hand, phytochemical studies were performed on the aforementioned fractions. For this purpose SC/PA1/Fr.28-75 was applied on Sephadex LH-20 column chromatography and 4.6 mg of **2** (SC/LH1/Fr.16-22) was isolated. In vivo experiments were not performed on **2** due to its scarce amount. However, the structure of **2** was further studied and established as 4′-*O*-methylhypolaetin-7-*O*-[6‴-*O*-acetyl-β-D-allopyranosyl-(1→2)]-β-D-glucopyranoside with NMR spectroscopy (Halfon et al., 2013; Lenherr and Mabry, 1987; Rodriguez-Lyon et al., 2000).

The rest of SC/PA1/Fr.28-75 was submitted to reverse phase flash chromatography. One of the obtained fractions (SC/FL1/Fr.118-132, 15.9 mg) was further chromatographed on preparative HPLC. SC/pHPLC/Fr.26 was obtained as a pure compound (**1**) and its structure was determined as (2*E*,6*E*)-2,6,10-trimethyl-2,6,11-dodecatriene-1,10-diol-1-*O*-β-D-glucopyranoside (Xi et al., 2014).

Simultaneously, BAGF studies on the other active fractions of polyamide column (SC/PA1/Fr.76-229 and SC/PA1/Fr.230-329) were also continued. SC/PA1/Fr.76-229 was chromatographed by silica gel column chromatography and obtained fractions were given to animals as six groups (SC/SG1/Fr.1-19, SC/SG1/Fr.20-35, SC/SG1/Fr.36-74, SC/SG1/Fr.75-98, SC/SG1/Fr.99-294, and SC/SG1/Fr.295-374). As seen in Table 5, significant gastroprotective activity was observed with SC/SG1/Fr.1-19, SC/SG1/Fr.20-35, SC/SG1/Fr.36-74 and SC/SG1/Fr.295-374 (53.5%, 68.6%, 62.2% and 65.4% ulcer inhibition, respectively). Considering the active doses, SC/SG1/Fr.20-35 and SC/SG1/Fr.36-74 drew our attention. The TLC profile of SC/SG1/Fr.36-74 was revealed a pure compound (**3**), and the same compound was realized to be one of the two compounds in the other active fraction, namely SC/SG1/Fr.20-35 (68.6% ulcer inhibition). SC/SG1/Fr.75-98 had lesser amount of **3** than SC/SG1/Fr.36-74 and SC/SG1/Fr.20-35; therefore, the lower anti-ulcerogenic activity of SC/SG1/Fr.75-98 (45.8% ulcer inhibition) compared to SC/SG1/Fr.36-74 and SC/SG1/Fr.20-35 was noteworthy. On the basis of this data, compound **3**, which was identified as 4′-*O*-methylhypolaetin-7-*O*-[6‴-*O*-acetyl-β-D-allopyranosyl-(1→2)]-6″-*O*-acetyl-β-D-glucopyranoside could be proposed as one of the major contributors of anti-ulcerogenic activity in SC/SG1/Fr.20-98 fraction.

Although preparative TLC is not generally suitable for BAGF studies performed on animals, the other part of SC/PA1/Fr.76-229 was applied to the preparative TLC in order to contribute to the phytochemical profiling of the plant. By this method, **4** was purified and identified as isoscutellarein-7-*O*-[6‴-*O*-acetyl-β-D-allopyranosyl-(1→2)]-β-D-glucopyranoside.

SC/PA1/Fr.230-329, the other active fraction obtained from

Table 2
Anti-ulcerogenic activity of the fractions obtained by solvent-solvent fractionation from the SC-EtOH.

Group	Dose (mg/kg)	Ulcer index (Mean ± S.E.M)	Prevention from ulcer[a]	Inhibition (%)
Control	–	138.7 ± 29.4	0/6	–
SC-Hex + SC-CH_2Cl_2	141.4	74.5 ± 21.9	0/6	46.2
SC-EtOAc	354.5	69.9 ± 15.5	0/6	49.6
SC-BuOH	736.4	92.3 ± 12.1	0/6	33.4
SC-Rw	617.8	112.8 ± 33.9	1/6	18.6
Control	–	196.1 ± 43.0	0/6	–
SC-EtOAc	709.0	16.9 ± 7.8***	2/6	91.4
SC-Hex + SC-CH_2Cl_2	282.9	49.0 ± 21.6**	0/6	75.0

**: $p < 0.01$; ***: $p < 0.001$, compared to control by using ANOVA and Tukey multiple comparison test.
S.E.M.: Standard error of the mean.
[a] Number of individuals without ulcer.

Table 3
Anti-ulcerogenic activity of the fractions obtained from polyamide column chromatography.

Group	Dose (mg/kg)	Ulcer index (Mean ± S.E.M)	Prevention from ulcer[a]	Inhibition (%)
Control	–	174.0 ± 33.1	0/6	–
SC/PA1Fr.1-75	185	36.6 ± 15.3**	1/6	78.9
SC/PA1/Fr.76-229	908	57.3 ± 7.7 **	0/6	67.1
SC/PA1/Fr.230-329	325	59.9 ± 16.9**	0/6	65.6

**: $p < 0.01$, compared to control by using ANOVA and Tukey multiple comparison test.
S.E.M.: Standard error of the mean.
[a] Number of individuals without ulcer.

Table 4
Anti-ulcerogenic activity of SC/PA1/Fr.1-8, SC/PA1/Fr.9-27 and SC/PA1/Fr.28-75.

Group	Dose (mg/kg)	Ulcer index (Mean ± S.E.M)	Prevention from ulcer[a]	Inhibition (%)
Control	–	230.3 ± 31.4	0/6	–
SC/PA1/Fr.1-8	192	190.0 ± 38.5	0/6	17.5
SC/PA1/Fr.9-27	80	84.7 ± 12.1**	0/6	63.2
SC/PA1/Fr.28-75	98	93.4 ± 14.6**	0/6	59.4

**: $p < 0.01$, compared to control by using ANOVA and Tukey multiple comparison test.
S.E.M.: Standard error of the mean.
[a] Number of individuals without ulcer.

polyamide column, was chromatographed by flash chromatography and two compounds were isolated (**5** and **6**). Their structures were elucidated by spectroscopic techniques and identified as 4′-*O*-methyl-hypolaetin-7-*O*-[6‴-*O*-acetyl-β-D-allopyranosyl-(1→2)]-6″-*O*-acetyl-β-D-glucopyranoside (**5,** same as **3**) and isoscutellarein-7-*O*-[6‴-O-acetyl-β-D-allopyranosyl-(1→2)]-6″-*O*-acetyl-β-D-glucopyranoside (**6**). Anti-ulcerogenic activity of these two compounds was determined with ethanol-induced ulcerogenesis model in rats. Due to the presence of both compounds in the mixture with the same ratio and considering the dose calculations in previous steps, **5** and **6** were administered to the animals at a the dose of 250.0 mg/kg. As shown in Table 6, both compounds were found to have equivalent and significant gastro-protective activity (70.0% and 69.0% ulcer inhibition, $p < 0.05$), and the stomachs of two of six rats were protected from ethanol induced gastric ulcerogenesis. Before completing the structure elucidation, **3** was taken into bioactivity tests. Compound **3** had 62.2% ulcer inhibition at 121.0 mg/kg dose, which was quite similar with the data observed for **5** (70% inhibition at 250 mg/kg). The slight activity increase was most probably due to the dose difference.

The purified compounds (except **1**) were previously isolated by other researchers from different *Sideritis* species (*S. argyrea* P.H. Davis, *S. brevibracteata* P. H. Davis, *S. condensata* Boiss. & Heldr., *S. congesta* P.

Table 5
Anti-ulcerogenic activity of the fractions obtained with silica gel column chromatography of SC/PA1/Fr.76-229.

Group	Dose (mg/kg)	Ulcer index (Mean ± S.E.M)	Prevention from ulcer[a]	Inhibition (%)
Control	–	224.3 ± 18.2	0/6	–
SC/SG1/Fr.1-19	151	104.2 ± 19.6*	0/6	53.5
SC/SG1/Fr.20-35	96	70.4 ± 14.2**	0/6	68.6
SC/SG1/Fr.36-74 (**3**)	121	84.7 ± 21.9*	0/6	62.2
SC/SG1/Fr.75-98	160	121.5 ± 32.0	0/6	45.8
SC/SG1/Fr.99-294	866	132.3 ± 36.9	0/6	40.9
SC/SG1/Fr.295-374	421	77.5 ± 29.5**	0/6	65.4

*: $p < 0.05$; **: $p < 0.01$, compared to control by using ANOVA and Tukey multiple comparison test.
S.E.M.: Standard error of the mean.
[a] Number of individuals without ulcer.

Table 6
Anti-ulcerogenic activity of the compounds [SC/FL2/Fr.1 (**5**) and SC/FL2/Fr.2 (**6**)] isolated from SC/PA1/Fr.230-329 by flash chromatography.

Group	Dose (mg/kg)	Ulcer index (Mean ± S.E.M)	Prevention from ulcer[a]	Inhibition (%)
Control	–	154.0 ± 26.0	0/6	–
SC/FL2/Fr.1 **(5)**	250	46.8 ± 18.0*	2/6	70.0
SC/FL2/Fr.2 **(6)**	250	47.8 ± 19.0*	2/6	69.0

*: $p < 0.05$, compared to control by using ANOVA and Tukey multiple comparison test.
S.E.M.: Standard error of the mean.
[a] Number of individuals without ulcer.

H. Davis & Hub.-Mor., *S. hololeuca* Boiss. & Heldr., *S. hyssopifolia* L., *S. javalambrensis* Pau, *S. libanotica* Labill., *S. perfoliata* L., *S.raeseri* Boiss. & Heldr., *S. scardica* Griseb., *S. stricta* Benth., *S. syriaca* L., *S. trojana* Bornm.) and some other species from Lamiaceae family *(Saccocalyx satureioides* Coss. et Dur, *Stachys anisochlia* Vis. & Pancic, *Stachys candida* Bory & Chaub., *Stachys recta* L.) or *Veronica* species (*V. intercedens* Bornm., *V. multifida* L., *V. orientalis* Mill). Compounds **2-6** were previously isolated from *S. caesarea* as well (Albach et al., 2003; Charami et al., 2008; Güvenç et al., 2010; Halfon et al., 2013; Kırmızıbekmez et al., 2012; Küpeli et al., 2007; Lenherr et al., 1984; Lenherr and Mabry, 1987; Menković et al., 2013; Mohamadi et al., 2015; Özipek et al., 2002; Petreska et al., 2011; Rios et al., 1992; Rodriguez-Lyon et al., 2000; Sattar et al., 1995; Stanoeva et al., 2015; Şahin et al., 2006; Yılmaz, 2013). But (2*E*,6*E*)-2,6,10-trimethyl-2,6,11-dodecatriene-1,10-diol-1-O-β-D-glucopyranoside (**1**) was isolated from *S. caesarea* for the first time.

To the best of our knowledge, anti-ulcerogenic activity of **3 (=5)** and **6** was not previously investigated and these compounds were reported to have such activity for the first time. On the other hand, the other three compounds, that were isolated from the fractions exhibiting significant anti-ulcerogenic activity during BAGF studies, could not be assayed for anti-ulcerogenic activity due to their scarce amounts (**1**, **2** and **4**). Therefore, one could speculate that these compounds might also be contributors of high anti-ulcerogenic activity.

Compound **1** was firstly isolated from *Eclipta prostrata* (L.) L. and determined to have mild anti-inflammatory activity (Xi et al., 2014). As far as it was ascertained, this compound was encountered for the first time in *Sideritis* species in the present study. In a study conducted on different *Eclipta* species, decoction (150 and 300 mg/kg), 50% ethanol extract (150 and 300 mg/kg), and hydrolysed water extract (30 mg/kg) of *E. alba* (Linn.) Hassk were determined to have significant anti-ulcerogenic activity (ulcer inhibitions was in the range of 57.11%–79.78) by using cold restraint stress induced ulcer method (Thakur and Mengi, 2005). But in this research, only the extracts were investigated, compound(s) responsible for anti-ulcerogenic activity were not determined. The results we obtained suggest that **1**, which is found in the fraction with higher anti-ulcerogenic activity, could possibly occur in *E. alba* and be one of the ingredients responsible for anti-ulcerogenic activity.

Previously, anti-ulcerogenic activities of eight *Sideritis* taxa were investigated. In Zarzuleo et al. (1993), anti-ulcerogenic activity of decoction of four *Sideritis* taxa [*S. incana* L. var. *virgata*, *S. funkiana* Willk. subsp. *funkiana*, *S. funkiana* Willk. subsp. *talaverana* Socorro, L.Cano & Espinar and *S. hirsuta L.*] was investigated by stress and indomethacin induced ulcer methods. The studied extracts (except *S. funkiana* subsp. *talaverana*) were found to have anti-ulcerogenic activity in both ulcer methods. Besides, *S. funkiana* subsp. *funkiana* and *S. incana* var. *virgata* were found to be more effective against indomethacin induced ulcer, while *S. hirsuta* were found to be more active against stress ulcer (Zarzuelo et al., 1993). However, in the study, only decoctions of the plants were studied and determination of the effective ingredients was not intended. In another research carried out by Tadic et al., anti-ulcerogenic activity of 70% ethanol extract of *S. scardica* and its diethyl ether, ethyl acetate and *n*-butanol fractions were studied with ethanol induced stress ulcer assay, and dose dependent anti-ulcerogenic activity was observed with all the extract or fractions (50-200 mg/kg). The highest activity, even higher than ranitidine, was obtained with the *n*-butanol fraction at 100 mg/kg dose (Tadic et al., 2012). The petroleum ether and ethanol extracts of *S. taurica* and the dichloromethane and *n*-butanol fractions of the ethanol extract were found to have significant anti-ulcerogenic activity with doses between 200-450 mg/kg, while the highest activity was observed with the *n*-butanol fraction. This research was also conducted with only extracts/fractions but not active substances (Aboutabl et al., 2002).

Hypolaetin 8-glucoside was isolated from *S. mugronensis* Borja and its anti-ulcerogenic activity was evaluated with cold-restraint ulcer method on rats. It was demonstrated to prevent gastric ulcerogenesis by 34.7%, 45.6% ($p < 0.05$) and 67.7% ($p < 0.05$) at doses of 30, 60 and 90 mg/kg, respectively (Villar et al., 1984). In a later study, same compound (hypolaetin 8-glucoside) was isolated from *S. leucantha* Cav and its anti-ulcerogenic activity was evaluated with ethanol and acetylsalicylic acid induced gastric ulcerogenesis. As a result, hypolaetin 8-glucoside was determined to have anti-ulcerogenic activity by increasing mucus secretion. Moreover, hypolaetin 8-glucoside was found to cause decrease in gastric acidity and peptic activity without affecting gastric secretion volume by pyloric ligation ulcerogenesis. According to these findings, the activity of this compound was stated to depend on the cytoprotective effect caused by endogenous prostaglandin and induction in gastric mucus production, but no stimulation on gastric mucosa cell proliferation (Alcaraz and Tordera, 1988). The data in the literature presented so far can be regarded as an indication of the prevalence of anti-ulcerogenic activity in *Sideritis* species.

As described above, there is no comprehensive study concerning anti-ulcerogenic activity of *Sideritis* species. The abovementioned studies deal with a few species other than *S. caesarea,* and generally were conducted with the extracts (Aboutabl et al., 2002; Zarzuelo et al., 1993). On the other hand, hypolaetin 8-glucoside, subject to two investigations, is not a secondary metabolite obtained by BAGF studies; hence it is not clear whether hypolaetin 8-glucoside is the principle compound responsible for activity (Alcaraz and Tordera, 1988; Villar et al., 1984). To the best of our knowledge, the only anti-ulcerogenic activity research that conducted on *S. caesarea* was a general screening study performed by our group, and in this screening study, activity of the main extract was evaluated (Gürbüz et al., 2005). In the present work, compound **3**, a different hypolaetin glycoside, was identified to be responsible for anti-ulcerogenic activity by BAGF studies. In addition, the other substance possessing hypolaetin framework as an aglycon was isolated from the other active fraction as well. These findings signify that the obtained results by the present study are compatible with the reported data.

In parallel to BAGF studies, standardisation of the active extract was also aimed. For this, quantity of **5** (=**3**) and **6** in the SC-EtOH was determined by HPLC analysis. The chromatogram of SC-EtOH (at 300 nm wavelength) in Fig. 4, revealed the retention times of compound**s 5** (**3**) a**nd 6** as 31.9 and 31.5 min, respectively. Based on the obtained calibration equations (Figs. 5 and 6), the amount of **5** (**3**) and **6** in 1 mg of the extract was found to be 50.73 μg (5.073% w/w) and 13.85 μg (1.385% w/w), respectively. Considering the obtained extraction yield, the ratios of these compounds in plant were calculated as

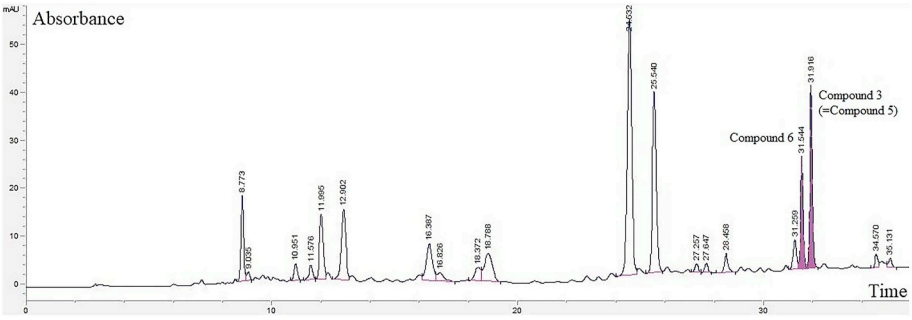


Fig. 4. The HPLC Chromatogram of SC-EtOH (at 300 nm wavelength).

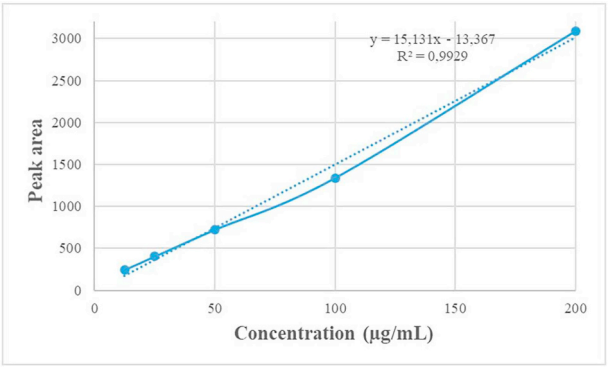


Fig. 5. Calibration curve of **3** (=**5**).

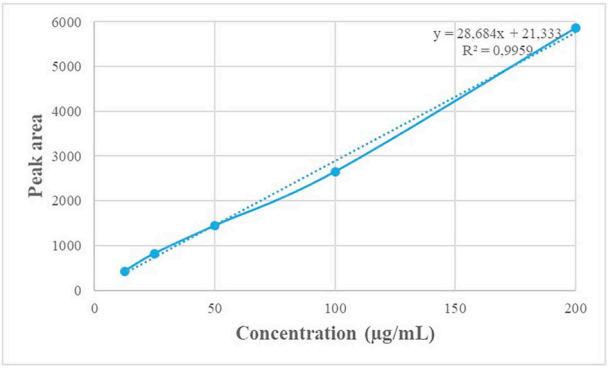


Fig. 6. Calibration curve of **6**.

1.3900% (w/w) and 0.3794% (w/w).

In a previous investigation, **6** was determined to have anti-oxidant activity by DPPH assay, but not to show any inhibitory effect on protein dependent lipid degradation, while it inhibited soybean lipoxygenase (Charami et al., 2008). In another study published by Güvenç et al. (2010), anti-inflammatory and anti-nociceptive activities of **6** and **4** were investigated. At a dose of 100 mg/kg, 14.8% and 13.9% inhibition for carrageenan-induced oedema and 25.2% and 18.8% decrease for *p*-benzoquinone induced abdominal contraction were observed with **4** and **6**, respectively. At 0.5 mg/ear dose, the compounds inhibited 12-*O*-tetradecanoylphorbol 13-acetate induced ear oedema by 13.2% and 18.6%, respectively. Both compounds showed significant anti-oxidant activity in DPPH and lipid peroxidation in liposome assays (Güvenç et al., 2010). On the other hand, *p*-benzoquinone induced abdominal contraction were determined to be inhibited by 24.7% and 19.7% with **6** and **4** (50 mg/kg), respectively; while 29.3% inhibition was observed with the mixture of **4** and **6** (100 mg/kg). At the same doses, the compounds prevent carrageenan induced hind paw oedema by 16.9%, 14.6% and 24.6% (at minute 360), respectively (Küpeli et al., 2007). Anti-oxidant activity of **6** was evaluated with DPPH, on-line HPLC-ABTS and ORAC assays by Mohamadi et al. Anti-oxidant activity close to vitamin C (IC_{50}: 8.36 µg/mL) was observed in DPPH assay; while 11.66 µg trolox equivalent/mL and 551.31 µMol trolox equivalent/mg anti-oxidant activity were determined in on-line HPLC-ABTS and ORAC assays, respectively (Mohamadi et al., 2015). Compound **4** was found to have 52.1 and 25.7 IC_{50} values in $FeSO_4$/cysteine-induced microsomal lipid peroxidation and nitroblue tetrazolium tests, respectively (Rios et al., 1992). Accompanying anti-oxidant and anti-inflammatory activities of **4** and **6** with anti-ulcerogenic effect is important to understand the mechanism. These findings also support the idea that anti-inflammatory compounds that are not harmful to stomach might have gastroprotective effects. Furthermore, it is understood from aforementioned studies that different hypolaetin and isoscutellarein derivatives also have anti-inflammatory and anti-nociceptive activity (Güvenç et al., 2010; Küpeli et al., 2007). In the light of these findings, it is thought that new compounds as drug candidates with much stronger anti-ulcerogenic, anti-inflammatory and analgesic activities would be developed by molecular docking and bioactivity investigations using hypolaetin and isoscutellarein as starting frameworks.

4. Conclusion

In this study, four flavonoids were isolated from *S. caesarea,* a folk medicine, by using BAGF method, and two of the compounds were determined to have significant anti-ulcerogenic activity. Although these compounds are not new substances; their anti-ulcerogenic activities have been determined for the first time in this study, whereas the other flavonoids are also obtained from the fractions possessing anti-ulcerogenic activity. (2*E*,6*E*)-2,6,10-trimethyl-2,6,11-dodecatriene-1,10-diol-1-O-β-D-glucopyranoside, which was only isolated from *E. prostrata,* is obtained from a *Sideritis* species for the first time. It needs to be emphasized that this compound could be one of the active substances as it is present in one of the fractions exhibiting anti-ulcerogenic activity. Gastric cytoprotection could have important role in anti-ulcerogenic activity of the plant; however, in order to determine mechanism of action, mechanistic studies on all factors playing roles on ulcer pathogenesis are needed. Besides, further investigations such as dose-response, structure-activity, toxicity etc. are warranted for the isolated anti-ulcerogenic compounds to suggest them as potent anti-ulcerogenic drug candidate(s).

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