The Effects of *Eryngium campestre* Extracts on Glutathione-S-Transferase, Glutathione Peroxidase and Catalase Enzyme Activities

Merve G. GÜNEŞ¹, Belgin S. İŞGÖR², Yasemin G. İŞGÖR³, Naznoosh SHOMALI MOGHADDAM¹, Fatmagul GEVEN¹, Özlem YILDIRIM¹,*

¹Ankara University, Faculty of Science, Department of Biology, 06100 Ankara, TURKEY, ²Atilim University, Faculty of Engineering, Department of Chemical Engineering and Applied Chemistry, 06836 Ankara, TURKEY, ³Ankara University, Department of Medicinal Laboratory Techniques, Vocational School of Health, 06290 Ankara, TURKEY

*Correspondence: E-mail: yildirim@science.ankara.edu.tr; Fax:+90 312 2232395

*Eryngium campestre* L. (Apiaceae) are known in Turkish folk medicine as ‘Bogadikeni’ and wildly distributed in all parts of Turkey. Infusion of different parts of this species are well known as diuretic, appetizer and stimulant. In the present study, methanol extracts from flowers and leaves of *E. campestre* were evaluated for their potential medicinal value in terms of biological targets participate in antioxidant defense such as glutathione-S-transferase (GST), glutathione peroxidase (GPx) and catalase (CAT).

Results obtained show that the methanol extract of flowers were found to have higher amount of phenolic and flavonoid contents than the leaves. The evaluation of extracts against biological target revealed that, the maximum GST enzymes inhibition activities was observed with methanol extract from flowers of *E. campestre* with IC₅₀ value of 363 ng/mL. In this study, none of the extracts evaluated were shown any reasonable GPx and CAT inhibition.

Key words: *Eryngium campestre*, Glutathione-S-transferase, Glutathione peroxidase, Catalase


Anahtar kelimeler: *Eryngium campestre*, Glutatyon-S-transferaz, Glutatyon peroksidaz, Katalaz
INTRODUCTION

The genus *Eryngium* L. belonging to the subfamily Saniculoideae of the family of Apiaceae is represented by 317 taxa worldwide and are known to contain flavonoids, coumarins and triterpene saponins (1, 2). *Eryngium campestre* L. grows mainly in central and southern Europe, northern Africa and most of the Asia (3). In Turkish folk medicine *E. campestre* is known as “Bogadiken” and wildly distributed in all parts of Turkey (4, 5). Infusion of aerial and root parts of this species are used in folk medicine to treat cough, urinary infections, disturbed functions of kidney, increased urine secretion and for regulation of the function of prostate (6). Also, the root of *E. campestre* is known as diuretic, appetizer, stimulant and aphrodisiac (1). Phytochemical studies show that the aerial parts and roots of *E. campestre* contain flavonoids, monoterpen glycosides, coumarin derivatives and saponins (7, 8).

The resistance of human tumor to multiple chemotherapeutic drugs has been recognized as a major reason for the failure of cancer therapy. Multidrug resistance can consist many mechanisms include alternations in drug transport, enhanced DNA repair, alternations in target proteins and alternations in drug metabolism such as; the detoxifying enzymes that are involved the glutathione dependent enzymes, glutathione-S-transferase (GST) and glutathione peroxidase (GPx) (9, 10). The glutathione-S-transferases (GSTs; EC: 2.1.5.18) are major phase II detoxification enzymes and most of the isoenzymes are located in the cytosol. They catalyze the conjugation of glutathione (GSH) to variety of exogenous and endogenous electrophilic compounds (11), serve roles in the development of drug resistance as inhibitor of the mitogen-activated protein (MAP) kinase pathway (12-14). Glutathione peroxidase (GPx; EC: 1.11.1.19) is the important enzyme which functions to protect the cell from oxidative damage by catalyzing the reduction of hydroperoxides, including hydrogen peroxide. Catalase (CAT; EC: 1.11.1.6) is a very important enzymes of living organisms which catalyzes the decomposition of hydrogen peroxide to water and oxygen. The balance between activities and the intracellular levels of antioxidant enzymes are very important for the survival of organisms and their health. Previous studies have shown that, chemotherapy causes several side effects on biological system. During cancer therapy, some chemotherapeutic agents generate free radicals which cause apoptosis of target cells, but antioxidant enzymes may scavenge these radical molecules (15, 16) and may reduce the effectiveness of therapeutic agents. Thus, in this way, antioxidant enzymes inhibition may improve the treatment of proliferative diseases.

The aim of the present study is to evaluate the total amount of the phenolic and flavonoid contents of methanol extract obtained from flowers and leaves of *E. campestre* and to determine their inhibitory effect on the glutathione-S-transferase, glutathione peroxidase and catalase enzymes.

EXPERIMENTAL

Chemicals

In this study, 4-aminoantipyrine (4-AP), hydrogen peroxide (H₂O₂) and sodium azide (NaN₃) were purchased from Acros, USA. Ethylenediaminetetraacetic acid (EDTA), Folin-Ciocalteu reagent, reduced glutathione (GSH), glutathione reductase (GR), horseraddish peroxidase (HRP), catalase (CAT), gallic acid and quercetin hydrate were supplied from Sigma Aldrich, Germany. Nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Gerbu, Germany. All other chemicals used were analytical grade and purchased from Sigma Aldrich, Germany.

Plant material

Plant samples of *Eryngium campestre* L. were collected in July 2010 from Ankara-Turkey at their flowering season. The plant specimens were botanically identified by Associated Professor Dr. Fatmagul Geven, in Department of Biology, Ankara University. The plant specimens with their localities and the necessary field records were written and enumerated as voucher specimen number, FG 2010-18. Voucher specimens were deposited.
in herbarium department at Ankara University.

**Extraction of plant**

Different parts of plant samples (flowers and leaves) were washed with tap water then dried at room temperature. For extraction, the previously reported procedure was used with some modifications (17). Basically, the plant samples were ground with liquid nitrogen, to obtain fine powder of samples. Then, these samples were extracted with methanol for 24 hour at 4°C, with sample to solvent ratio of 1:10 (w/v). On the following day, the solvent was evaporated at 40°C until dryness, the obtained product dissolved in DMSO and kept at dark (4°C) until they are used.

**Total polyphenol content**

The total concentration of phenolic content of extracts were determined by employing the method described previously Slinkard & Singleton (18) with use of 2% (w/v) sodium carbonate solution and Folin-Ciocalteu’s reagent (1:10 diluted with distilled water). The absorbance changes were spectrophotometrically measured as endpoints at 750 nm using multimode microplate reader (SpectraMax M2e, USA). The total polyphenol content (TPC) of extract was expressed as milligrams of gallic acid equivalent (GAE)/L of plant extract (mg GAE/L) where the standard curve used were prepared with 0-250 mg/L of gallic acid (GA) in DMSO.

**Total flavonoid content**

The total concentration of flavonoids of extracts were determined by employing the aluminum chloride colorimetric method with use of 95 % ethanol, 10 % aluminum chloride, 1M sodium acetate and DMSO as described previously (19). Then the absorbance of the reaction mixture was measured spectrophotometrically at 415 nm. The total flavonoid content of the extract was expressed milligrams of quercetin equivalent (QE)/L of plant extract (mg QE/L), where the standard curve used was prepared with 0-100 mg/L of quercetin(Q) in DMSO.

**Isolation of cytosol from bovine liver**

The bovine liver were obtained from slaughterhouse of Kazan, Ankara, Turkey. The liver samples were homogenized in 10mM potassium phosphate buffer (pH 7.0), containing 0.15M KCl, 1mM EDTA, and 1mM of DTT, by using a glass teflon homogenizer and centrifuged at 10,000 x g for 20 min. The supernatant was filtered through cheesecloth and the filtrate was centrifuged at 30,000 x g for 60 min. The collected supernatants were filtered again and the resultant filtrate was referred as cytosol (17). The prepared homogenates, containing 46.41mg protein/mL, were kept in Hettich ultralow freezer (-80 °C) until used. The total protein content was determined by the Lowry method (20).

**Inhibition of glutathione-S-transferase (GST)**

The change in total GST activities was measured against the substrate, 1-chloro-2,4-dinitro -benzene (CDNB), by monitoring the thioether (GSH-CDNB conjugate) formation at 340 nm (21). The measurements were performed with cytosol (0.982 mg protein/mL) and plant extracts (7 - 476 ng/mL) or control (DMSO alone), in 100 mM potassium phosphate buffer at pH 6.5 with 2.4 mM CDNB and 3.2 mM GSH, using optimized microplate application protocol (22).

**Inhibition of glutathione peroxidase (GPx)**

Glutathione peroxidase activity was measured by previously reported method (23, 24) after some modifications for microplate applications (25). The GPx activity was measured against the substrate, tertiary butyl hydroperoxides (t-BuOOH), and the decrease in nicotinamide adenine dinucleotide phosphate (NADPH) was monitored at 340 nm. The GPx activity changes were measured by using purified GPx (37.5 × 10–3 U/ml) and plant extracts (7 - 476 ng/mL) or control (DMSO alone), with 2 mM GSH, 0.25 mM NADPH, GSH-reductase (GR, 0.5 unit/ml) and 0.3 mM t-BuOOH, in 50 mMTrisHCl (pH 8.0). The reaction was initiated by adding GPx, and the change in absorbance was recorded at 340 nm for 5 min by using multimode microplate reader.

**Inhibition of catalase (CAT)**

Catalase (CAT) inhibition was determined by monitoring a red quinoneimine dye
remaining hydrogen peroxide (26, 27). The assay was miniaturized for microplate application (28) and contained plant extraction solutions with final concentration of hydrogen peroxide (26, 27). The reaction was stopped by NaN₃ and incubated at room temperature for 5 min, followed by the incubation with chromogen at room temperature for 40 min. Then, the absorbance was read at 520 nm, enzyme activity was calculated with respect to hydrogen peroxide remaining which was determined by calibration curve constructed in the range of 9.61 - 307.6 µM hydrogen peroxide.

Data analysis
The data analysis was performed using Graphpad Prism 6.0. The inhibitory activities of extracts against enzyme targets were calculated as 50 % inhibitory concentration, or IC₅₀ values, and obtained from dose-response curves constructed. The enzyme calibration and the dose response curves were constructed using 2-3 independent experiments in 96 well microplates, each in duplicates or triplicates, using Multimode Microplate Reader (SpectraMax M2e, MDC, Sunnyvale, CA, USA).

RESULTS AND DISCUSSION

Each extract was prepared by dissolving 2g of dry samples in 20mL methanol. The methanol extraction of the flowers and leaves of E. campestre yielded 5.27% and 6.72% of dry weight, respectively.

The total phenolic contents were determined by using the Folin-Ciocalteu’s method. According to the method, the total phenolic contents of extracts were calculated using the equation obtained from the standard curve of gallic acid graphic (y = 0.0749x + 0.0866, R² = 0.9898). The amount of total phenolic compounds found in the methanol extract from flowers of E. campestre was 116.69 mg GAE/L of plant extract. The total phenolic content in the leaves was 109.62 mg GAE/L of plant extract. The total concentration of flavonoids in extracts were determined by employing the aluminum chloride colorimetric method. The total flavonoid contents of extracts were calculated using the equation got from the standard curve of quercetin graphic (y = 0.0429x + 0.153, R² = 0.998). Total amount of the flavonoid contents found in the methanol extracts from flowers and leaves of E. campestre were 110.58 and 108.37 mg QE/L of plant extract, respectively.

GSTs activity was determined against the substrate, 1-chloro-2,4-dinitrobenzene (CDNB), by monitoring the thioether formation at 340 nm. In order to calculate the percent inhibition of GST activity and IC₅₀ values, the final concentration of plant extracts used in the assay, was within the range of 7- 476 ng/mL. In this study, the inhibitory effect of the extracts obtained from E. campsetre flowers and leaves was investigated and as it can be seen in the Figure 1, the flowers extract show better inhibitory effect on GST enzyme with IC₅₀ value of 363 ng/mL than the leaves. In this study none of the extracts evaluated were shown any reasonable GPx and CAT inhibition.

In this study, we were concerned by the evaluation of phenolic and flavonoid contents of methanolic extracts of different parts of E. campestre to determine their inhibitory potential on the glutathione-S-transferase, glutathione peroxidase and catalase enzymes activities. Phenolic compound are having at least one or more aromatic rings with one or more hydroxyl groups attached (29). Many phenolic compounds have been reported to have potentials of antioxidant, anticancer, anti-atherosclerotic, antibacterial, antiviral and anti-inflammatory activities (30). The literature data show that plant extracts with high amount of phenolic compounds are known to have important inhibitory potential on glutathione-S-transferase (17,31,32). Thus, it can be play an important role in developing multi-drug resistance to chemotherapy in tumor cells. In present study, E. campestre flower extract has higher phenolic contents and exhibited expressive effect on the GST inhibitory activity.

Nebija et al. (33) studied mineral composition and in vitro antioxidant activity of the aqueous and ethanol extracts of aerial
RESULTS AND DISCUSSION

In this study, we were concerned by the biological potential of *E. campestre* on the glutathione-S-transferase, glutathione peroxidase and catalase inhibitory activities. In this study we found that the flowers extract of *E. campestre* having higher phenolic content, were also more effective GST inhibitors with an IC₅₀ of 363 ng/mL.

ACKNOWLEDGEMENTS

This study was supported by the grant from The Coordination of Scientific Research Projects of Ankara University awarded to
REFERENCES


16. Simone CB, Simone NL, Simone V, Simone CB, Antioxidants and other nutrients do not interfere with chemotherapy or radiation therapy and can increase kill and increase survival, Alter Therap Health Med 13(1), 22-28, 2007.


Received: 06.03.2014
Accepted: 08.05.2014