Original paper

Researches on the Epilobium angustifolium L. ethanol extract, Turkey

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Abstract
In this paper, *Epilobium angustifolium* L. in Turkey was investigated for total phenolics, flavonoids, and anthocyanin amounts. Furthermore, the ethanol extract of the leafs was evaluated for antioxidant activities as ABTS, hydroxyl radical scavenging, superoxide radical scavenging, power of reduction, and bleaching of beta-carotene. The total phenolic and flavonoid contents of the ethanol extract were found to be 468,11±1,53 micrograms of pyrocatechol per milligram of the extract and 103,05 ± 2,36 µg catechin per milligram of the extract, respectively. As the extract concentration increased, the amount of anthocyanins showed a decrease (17.92±8.08 and 13.56±8.22 for the 1 mg/mL and 2 mg/mL concentrations, respectively). In the β-carotene bleaching test, higher activity was observed than BHA (1.540±0.053 and 1, respectively). The results of the reduction power, hydroxyl radical scavenging, and ABTS tests gave either very close values or higher than the standards chosen. Superoxide radical scavenging activity gave a lower value than the Trolox selected as standard. Antioxidant activity of ethanol extract was found to be 94.22±0.05958 (ABTS), 78.77±0.84099 (hydroxyl radical scavenging) and 47.86±0.03915 (superoxide radical scavenging). The experimental data indicate that Turkey Epilobium angustifolium L. ethanol extract can be used pharmacologically.

Keywords *Epilobium angustifolium* L., ethanol extract, antioxidant activities.

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Introduction

Reactive oxygen species (ROS) are widely accepted to be associated with diseases like Alzheimer’s, Parkinson’s, aging, and cancer (Kim et al., 2016 [9]; Liochev, 2013 [14]; Wen et al., 2013 [33]). Antioxidants are considered to prevent oxidative damage by eliminating reactive oxygen species. In particular, natural antioxidants are considered to have more effective and have safety in reducing ROS in excessive amounts (Ndhlala et al., 2010 [17]). Therefore in recent years the number of studies on plants, which have been used for folk medicine for centuries, is increased. The genus Epilobium is one of the widely used medicinal plants in the world. Epilobium angustifolium L. (another name Chamaenerion angustifolium), belonging to the Onagraceae family, is a showy, perennial, commonly self-growing 3-5 ft. tall wild plant. Epilobium plants can be divided into two groups according to their flower’s size: the small ones (E. roseum, E. parviflorum, E. montanum) and the large ones (E. angustifolium, E. hirsutum) (Stolarczyk & al., 2013 [29]). There are more than 200 species of Epilobium plants in the world, and 21 species have been identified in Turkey, especially in the Aegean, Black Sea, and Marmara regions.

As a folk medicine, Epilobium angustifolium is widely used to treat various gastrointestinal diseases such as mouth ulcers, swelling, stomach ulcers, duodenal ulcers, gastritis, colitis, dysentery, diarrhea, prostate or urinary problems (Lebeda et al., 2004 [13]; Kujawksi & al., 2011 [12]; Feshchenko & al., 2019 [7]). It has also been used to treat voiding disorders, prostatic adenoma and benign prostatic hyperplasia (BPH) (Stolarczyk & al., 2013 [29]; Deng & al., 2019 [5]). It has been reported that Epilobium is used as a tea in the treatment of migraine headaches, insomnia, anemia and colds (Bushueva & al., 2016 [3]). There are many studies on the antitumor, anti-inflammatory, analgesic, antivirus and antioxidant activities of Epilobium angustifolium extract in the literature (Tóth & al., 2009 [31]; Kosalec & al., 2013 [11]; Tita & al., 2001 [30]; Webster & al., 2008 [32]). The reason for these activities is that Epilobium species are rich in polyphenols, steroids, triterpenoids, and fatty acids, especially Oenotherin B (a dimeric macrocyclic ellagitanin) (Schepetkin & al., 2009 [24]; Ramstead & al., 2012 [21]; Kiss & al., 2011[10]).

The plant has been studied for antioxidant activities in China, Poland, Croatia, Serbia, Russia, and many countries (Shikov & al., 2006 [25]; Stajner & al., 2007 [28]; Stolarczyk & al., 2013 [29]; Monschein & al., 2015 [16]; Deng & al., 2018 [6]). There are only two studies about the antioxidant activity of Turkish Epilobium. One of the studies is our previous study which contains the antioxidant activities of the aqueous extract of the plant (Onar & al., 2012 [18]). The other is related to some biological activities of the ethanol and methanol extracts (Sayik & al., 2017 [23]) DPPH and metal chelating activity were investigated for antioxidant activity.

Total phenolics, flavonoids, and anthocyanin amounts were also examined in Epilobium angustifolium L. in Turkey. Furthermore, its ethanolic extract was evaluated for antioxidant activities like ABTS, radical scavenging experiments with hydroxyl and superoxide, power of reduction, and test of beta-carotene bleaching. This study aimed to complete the missing data of Epilobium angustifolium L. plant grown in Turkey.

Materials and methods

Plant collection and extraction

We collected the leaves of E. angustifolium in September, from Canakkale district, Turkey, and identification was performed by Prof. Dr. Kerim Alpınar, a faculty member of Istanbul University, Faculty of Pharmacy. Voucher specimens are being kept in the Herbarium of the Faculty of Pharmacy, Istanbul University with the code (ISTE 83909). Deionized water was used to wash the leaves and they were dried for five days to a week left in the shade, and the medium was at room temperature. After the leaves were dried, manual grinding of them yielded a fine powder.

The powder was extracted in the soxhlet apparatus with absolute ethanol at 75-79°C for 22 h. The extract obtained was evaporated at 45°C, and the remainder was dried and stored in a container that is sufficiently air-tight. The extract’s yield was 11.84 % w/w.

Determination of total phenolic content

With Folin-Ciocalteau reagent, it was possible to measure the total phenolics in the extract, and minor modifications were applied to the method by Sliskard and Singleton (1977) [26]. In brief, plant extract (0.1 mL; 1000 to 1500 μg/mL) was taken to tube and the final volume was made, with distilled water, to 4.6 mL. The Folin-Ciocalteau reagent was diluted three times with distilled water, and 0.1 mL of it along with 0.3 mL of 2% Na₂CO₃ solution were added and, after vortexing the tube, it was allowed to settle 2 h with shaking intermittently. With a spectrophotometer set at 760 nm, the measurement of absorbance was performed. In the extract, the total phenolic compounds were calculated as milligrams of pyrocatechol (with the help of calibration curve) and as milligrams of pyrocatechol equivalents (expressed as milligrams of the extract).

Determination of total flavonoid content

Sakanaka’s method (2005) [22] was employed for total flavonoid content, and catechin was used as a standard flavonoid. Briefly, in a test tube, 1.25 mL of distilled water was added to the extract (0.25 mL, 1000 to 3000 micrograms per milliliter) or a standard solution of (+)-catechin (20 to 100 microgram per milliliter), and then 75 μL of a sodium nitrite solution (5%) was introduced. 6 min later, 150 μL of an aluminum chloride solution (10%) was mixed with the others and the resulting mixture was rested for another 5 minutes and finally, x M NaOH (0.5 mL) was added. The final volume was made to 2.5 mL with distilled water and mixed well. Using a spectrophotometer at 510 nm, the absorbance was immediately measured. From the
calibration curve, the results were calculated as milligram of (+)-catechin equivalents per milligram of the extract.

**Determination of total anthocyanin**

The determination of the content of total anthocyanins for *Epilobium angustifolium* L. was performed according to GIUSTI and WROLSTAD (2001) [8]. The samples are prepared by dissolving the dry plant in methanol and acidifying it with 1% HCl. With a spectrophotometer, absorbances of 1 and 2 mg/mL samples (pH values were at 1.0 and 4.5) were measured at two different wavelengths, namely at 510 and 700 nm. The total absorbance of anthocyanin depended on two different pH values and two different absorption wavelengths and was calculated with the formula as seen below:

$$A = [(A_{510} - A_{700})_{pH \ 1.0} \ - \ (A_{510} - A_{700})_{pH \ 4.5}]$$

**Bleaching test of β-carotene**

To a boiling flask which contains linoleic acid (20 mg) and Tween-40 (200 mg) (Sigma) was added 0.2 mL solution of approximately trans-β-carotene (10 mg) (type 1, synthetic, Sigma, St. Louis, MO, USA), and they were dissolved in chloroform (10 mL). After all chloroform was evaporated, distilled water (50 mL) was introduced to the flask and vigorously shaken. A 5-mL aliquot was taken and mixed with 0.2 mL of the plant extract (20-1000 µg / mL). The contents of the test tubes were heated at 50 °C, on a water bath. Absorbances at 470 nm were recorded after incubation periods of 60 and 120 minutes. Butylated-hydroxy-anisole (BHA) was the positive and the emulsion of linoleic acid and β-carotene was the negative control. The following formula calculates the relative antioxidant activities (RAA):

$$RAA = \frac{Abs \ of \ sample}{Abs \ of \ BHA} \quad (BRUNI \ & \ al., \ 2004[2])$$

**Activity of ABTS radical scavenging**

ABTS** scavenging activity was described by ARNAO & al. (2001) [1]. This procedure was followed to measure the activity of our plant extract. 7.4 millimolar ABTS** solution and 2.6 millimolar K2S2O8 (potassium persulfate) solution were used. These two stock solutions in equal amounts was mixed and kept in reaction for twelve hours at room temperature in the dark, and 60 mL of methanol was used to dilute 1 mL of the ABTS** solution. Every time of essay measurement, a fresh solution of ABTS** was prepared.

The plant extracts (in a volume of 150 µL) were reacted with 2.850 mL of the ABTS** solution for two hours at dark. The spectrophotometer was set to record absorbances at 734 nm. We used the following equation for calculating the ABTS** scavenging activity:

$$ABTS \ radical \ scavenging \ activity \ (\%) = \frac{(A_0 - A_1)}{A_0} \times 100$$

$A_0$ = without ethanolic extract sample (with methanol)

$A_1$ = ethanol extract sample

**Reducing power**

A 100-1000 µg sample of extracts in 1 mL of distilled water was mixed with phosphate buffer (pH 6.6, 0.2 M, 2.5 mL) along with 2.5 mL of 1% [KtFe(CN)6], and incubation of the mixture was performed for 30 min, at 50 °C. Afterward, trichloroacetic acid 10% (2.5 mL), was added to the mixture, followed by centrifugation at 3000 rpm (10 minutes). Finally, a 2.5 mL-supernatant was mixed with distilled water (2.5 mL) and 0.5 mL of FeCl3 (0.1%), and the spectrophotometer was set at 700 nm wavelength, then the absorption was read (OYAZIU, 1986 [19]). Increased absorbance is indicative of increased reducing power.

**Hydroxyl radical scavenging activity**

According to CHUNG & al., 1997 [4], deoxyribose method was used for the affection of hydroxyl radicals by antioxidative effect of the plant extract. In the reaction mixture, there were sodium phosphate buffer (0.2 M, 0.45 mL, pH 7.4.), 2-deoxyribose (10 mM, 0.15 mL), FeSO4-EDTA (10 mM, 0.15 mL), hydrogen peroxide (10 mM, 0.15 mL), distilled water (0.525 mL) and the extract (0.075 mL) were added in a test tube. Hydrogen peroxide initiated the reaction. After incubation for 4 h at 37 °C, by adding 2.8% TCA (0.75 mL) and 1.0% of thiobarbituric acid (0.75 mL), the reaction was stopped. After boiling the mixture for 10 min, the reaction contents were cooled on an ice bath, and the absorbance was measured with a spectrophotometer set at 520 nm. The following equation calculates hydroxyl radical scavenging activity:

Hydroxyl radical scavenging activity (%) = \( \frac{(A_0 - A_1)}{A_0} \times 100 \)

$A_0$ is control sample’s absorbance and $A_1$ is sample’s absorbance.

**Superoxide radical scavenging activity (SOD)**

Superoxide anion scavenging activity of the aqueous extract of the plant was determined by LIU & al.’s method (1997) [15]. A phenazine methosulfate (PMS)-NADH system that is non-enzymatic generates superoxide radicals by the oxidation of NADH (nicotinamide adenine dinucleotide) and quantified by NBT’s reduction (nitroblue tetrazolium). The mixture contained Tris-HCl (16 mM, 3 mL, pH 8), NBT (50 mM, 1 mL), and NADH (78 mM, 1 mL) and diluted samples (1 mL). By adding phenazine methosulfate (PMS, 10 mM, 1 mL), the reaction was initiated. The tubes were incubated at 25 °C for 5 minutes and the absorbances were recorded with a spectrophotometer set at 560 nm against blank. If the samples’ absorbances are low, it means that the superoxide scavenging activity is higher. The formula below calculates the percentage inhibition of superoxide anion:

Superoxide anion radical scavenging activity (%) = \( \frac{(A_0 - A_1)}{A_0} \times 100 \)

$A_0$ is the control’s absorbance. $A_1$ is the samples’s absorbance of the sample.

**Results and discussions**

In this work, *Epilobium angustifolium* L. plant collected from Çanakkale, Turkey was used. The plant was identified by University of Istanbul (Herbarium code ISTE 83909). The yield of the ethanol extract is 11.84 % (w/w ) and was kept at -20 °C.
Total phenolic and flavonoid content

The total phenolic group in the compound and the number of potentially oxidizable groups (total phenolic content) were determined by the Folin-Ciocalteu reagent using the Slinkard and Singleton method (SLINKARD and SINGLETON, 1977 [26]). This method includes an electron transfer between the antioxidants and molybdenum ion, and the formed blue-colored products from the reaction can be measured in a spectrophotometric manner. This reaction is valid in an alkaline environment and does not work in any other medium (pH ≈ 10). The amount of total phenolics in the plant extract was found from the drawn calibration curve as milligram equivalent of pyrocatechol. Total flavonoid content was determined as catechin equivalent as a standard flavonoid compound (SAKANAKA & al., 2005 [22]). Reporting of the results were as the milligram amount of (+)-catechin equivalents per milligram amount of the extract from the drawn calibration curve. Flavonoidal and phenolic compounds are responsible for the biological activities of the plant extracts (SOOBRATTEE & al., 2005 [27]). In the ethanolic extract, the total phenol and flavonoidal contents were 468.11± 1.53 micrograms per milligram of extract (in 1500 micrograms per milliliter plant concentration) and 103,05 ± 2,36 micrograms of catechin per milligram of extract (in 3000 micrograms per milliliter plant concentration), respectively, which was presented in Table 1.

Table 1. Total phenolic compounds and total flavonoids in ethanol extract from Epilobium angustifolium L. leaves

| Concentration (µg/ml) | Total phenolic compound (µg pyrocatechol/mg extract)* | Total flavonoid (µg catechin/mg extract)* |
|-----------------------|-------------------------------------------------------|------------------------------------------|
| 1000                  | 174,68±19,97                                          | 24,16±0,78                               |
| 1250                  | 360,52±6,14                                           | 24,16±0,78                               |
| 1500                  | 468,11±1,53                                           | 57,96±1,06                               |
| 2000                  | 468,11±1,53                                           | 103,05±2,36                              |
| 3000                  | 468,11±1,53                                           | 103,05±2,36                              |

* Values are means of independent analyses of the extract±SD in triplicate, SD: Standard deviation

Anthocyanins

Anthocyanins (Ac), which responsible for colorization (blue, purple, and red) in many plants, are water-soluble flavonoids. Not only as food colorants but also as antioxidants, anthocyanins are becoming densely important. In our study, when the concentration of sample increased, the number of anthocyanins showed a decrease. The total anthocyanin amount is represented in Table 2 (GIUSTI and WROLSTAD, 2001 [8]).

Table 2. Total anthocyanins in Epilobium angustifolium L. dry leaves

| Sample | 2h          | 24h          |
|--------|-------------|--------------|
| 1 mg/mL| 17,92 ± 8,08| 22,64 ± 14,29|
| 2 mg/mL| 13,56 ± 8,22| 16,55 ± 8,22 |

Values are means of independent analyses of the extract±SD in triplicate, SD: Standard deviation

β-carotene bleaching test

β-carotene bleaching test yielded that ethanolic plant extracts gave the best results, with RAA (relative antioxidant activity) values. It was observed to have higher activity than butylated-hydroxy-anisole (BHA), which was taken as a positive control, especially at concentrations of 500 and 1000 µg / mL (Table 3).

Table 3. β-carotene bleaching test

| Sample   | Concentration (µg/mL) | RAA (60 min)       | RAA (120 min)       |
|----------|-----------------------|--------------------|--------------------|
| Ethanol  | 20                    | 0.738 ± 0.053      | 0.686 ± 0.019      |
|          | 100                   | 0.966 ± 0.053      | 0.853 ± 0.074      |
|          | 250                   | 0.983 ± 0.021      | 0.903 ± 0.027      |
|          | 500                   | 1.050 ± 0.054      | 0.964 ± 0.092      |
|          | 1000                  | 1.540 ± 0.053      | 1.030 ± 0.005      |
| BHA      | 1                     | 0.406 ± 0.014      | 0.406 ± 0.014      |
|          | 100                   | 0.435 ± 0.010      | 0.435 ± 0.010      |
|          | 250                   | 0.444 ± 0.017      | 0.444 ± 0.017      |
|          | 500                   | 0.451 ± 0.004      | 0.451 ± 0.004      |
|          | 1000                  | 0.456 ± 0.019      | 0.456 ± 0.019      |

*aNegative control:Linoleic acid and β-carotene emulsion

Values are means of independent analyses of the extract±SD in triplicate, SD: Standard deviation
ABTS activity

The ABTS•+ (ABTS cation radical) is formed with a loss of an electron from the nitrogen atom of ABTS the chemical name of which is 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, absorbing at 743 nm (giving a color of bluish-green). When Trolox or another hydrogen-donating antioxidant is present, the nitrogen atom quenches the hydrogen atom, yielding decolorization of the solution (PISOSCHI and NEGULESCU, 2011 [20]). Potassium persulfate or manganese dioxide can be used to oxidize ABTS, and ABTS•+ has a loss of absorbance at 743 nm in the presence of Trolox, which is the standard antioxidant. The method was applied, as based on the reduction of absorbance of the cation radical, to the content determination of antioxidants in plant extracts. Hydrogen-donating antioxidants and chain-breaking antioxidants are determined by the ABTS method, which could be applied to both lipophilic and hydrophilic compounds (ARNAO & al., 2001 [1]).

As a result of the trial, it was found that the ethanolic plant extract showed an activity equivalent to Trolox, which was chosen as the witness antioxidant. Higher levels of anthocyanins, flavonoids, and total phenolic compounds are attributed to the highest ABTS scavenging activity.

Reducing power activity

Electron-donating activity is often attributed to Fe3+ reduction, and this is an important phenolic antioxidant mechanism. With the other antioxidant properties, there is a strong correlation. In this assay, the presence of reductants, referred to as the presence of antioxidants in the samples, leads to a reduction of the Fe3+/ferricyanide complex to the Fe2+ form. At 700 nm, the concentration of Fe2+ complex could be measured from the formation of the Perl’s Prussian Blue complex. If a compound has a good reducing capacity, it implies that its antioxidant potential has been significant (OYAIZU, 1986 [19]).

In Figure 2., it can be seen that E. angustifolium L. ethanolic extract and standards behave as reducing entities in the dose-response curves. The reduction power of the ethanolic extract is almost equal BHT’s performance and better than of tocopherol.

The reductive order of the power of the ethanolic extract and standards showed this increase: Tocopherol < BHT < Ethanol extract < BHA. It means that the ethanolic extract of E. angustifolium did behave as an electron donor, having the capacity to react with free radicals and convert them into products that are more stable. The outcome of reduction terminates the radical chain reactions, so very damaging results are prevented. One could attribute this activity to the presence of phenolic compounds like E. Angustifolium, having natural antioxidants.

Hydroxyl radical scavenging activity

There is a multitude of ways to be sure about the ability of the formation of hydroxyl radicals, and deoxyribose test is one of them. This method uses iron(III) chloride (FeCl3) and ethylenediaminetetraacetic acid (EDTA) complex, and in the presence of ascorbic acid, the complex is reduced to Fe2+-EDTA, and ascorbic acid is oxidized. Upon adding hydrogen peroxide (H2O2), Fe2+-EDTA complex is oxidized to Fe3+-EDTA complex again and HO· is produced. This is referred to as the Fenton reaction, generating the highly reactive hydroxyl radical (Fe2+ + H2O2 → Fe3+ + HO· + HO·) (CHUNG & al., 1997 [4]).

Hydroxyl radicals, which are not terminated by one of the components of the mixture, attack deoxyribose and cause the degradation of it into many small fragments. Some of these could react with thiobarbituric acid after heating (ensuring that the pH is acidic), which yields a pink-colored product the quantification of which can be performed by spectrophotometry. Chromagen formation is inhibited by the compounds which could scavenge hydroxyl radicals. Without ascorbic acid, the deoxyribose method can be performed. If the samples have pro-oxidant ability or not could be determined, because the compounds that are substituted for ascorbic acid may react in Fenton’s reaction or not at all. Some of them cause inhibition of chromagen, and hydroxyl radicals are not involved, but after stable metal chelates are formed, which block the formation of hydroxyl radicals. When identifying chelates
with iron compounds, EDTA is not recommended for use. If a chelate is not present, iron ions are found to bind to deoxyribose and cause damage to hydroxyl radicals specific to the site. If there is a species which is capable of forming iron complexes, there will be less hydroxyl radical present, and the characteristic pink color is reduced.

Hydroxyl radical scavenging activity of Epilobium angustifolium L. ethanol extract is higher than that of ascorbic acid and of gallic acid (Figure 3).

**Statistical analysis**

Results were expressed in triplicate as mean ± standard deviation (SD) analyses.

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**Conflict of Interest**

The authors have no conflict of interest to declare.

**References**

1. ARNAO MB, CANO A, ACOSTA M. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.* 2001; 73: 239-244.
2. BRUNIA R, MUZZOLIA M, BALLEROB M, LOIB MC, FANTINC G. Tocopherols, fatty acids and sterols in seeds of four Sardinian wild *Euphorbia* species. *Fitoterapia* 2004; 75: 50–61. doi: 10.1016/j.fitote.2003.07.009
3. BUSHUEVA GR, SYROESHKIN AV, MAKSIMOVA TV, SKALNY AV. Chamaenerion angustifolium—a promising source of biologically active compounds. *Mikroelementy and Meditsine* (Moscow, Russian Federation). 2016; 17/2: 15-23.
4. CHUNG SK, OSAWA T, KAWAKISHI S. Hydroxyl radical scavenging effects of species and scavengers from brown mustard (*Brassica nigra*). *Biosci. Biotech. Biochem.* 1997; 61: 118-123.
5. DENG L, ZONG W, TAO X, LIU S, FENG Z. Evaluation of the therapeutic effect against benign prostatic hyperplasia and the active constituents from *Epilobium angustifolium* L. *Journal of Ethnopharmacology*. 2019; 232: 1–10.
6. DENGAL Q, ZHOUA SY, MAOAJX, LIUA S, LANB XZ. HPLC-ESI-MS/MS analysis of phenolics and in vitro antioxidant activity of *Epilobium angustifolium* L. *Natural Product Research* 2018; 32/12: 1432–1435. doi: 10.1080/14786419.2017.1344659
7. FESHCHENKO HI, OLESHCHUK OM, MARCHYSHYN SM, NAKONECHNA SS. Experimental study of antiulcerogenic action of Chamaenerion angustifolium the aspirin induced gastric ulcer modeling the rats. *Pharma Innovation* 2019; 8/5-1: 535- 539.
8. GIUSTI MM, WROLOSTAD RE. Anthocyanins. Characterization and measurement with UV-visible spectroscopy. In: Wrolstad RE, editor. Current Protocols in Food Analytical Chemistry. Unit F1.2. 1-13. New York: John Wiley.2001.
9. KIM H, LEE GR, KIM J, BAEK JY, JO YJ. Sulfiredoxin inhibitor induces preferential death of cancer cells through reactive oxygen species-mediated mitochondrial damage. *Free Radical Biol Med.* 2016; 91: 264–274. doi: 10.1016/j.freeradbiomed.2015.12.023.

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**Figure 3.** Hydroxyl radical scavenging activity of *Epilobium angustifolium* L. ethanol extract.

**Figure 4.** Superoxide radical scavenging activity of *Epilobium angustifolium* L. ethanol extract.
10. KISS AK, BAZYLKO A, FILIPEK A, GRANICA S, JASZEWSKA E. Oenothein B’s contribution to the anti-inflammatory and antioxidant activity of Epilobium sp. Phytomedicine 2011; 18: 557–560. doi: 10.1016/j.phymed.2010.10.016

11. KOSALEC I, KOPJAR N, KREMER D. Antimicrobial activity of willowherb (Epilobium angustifolium L.) leaves and flowers. Curr Drug Targets. 2013; 14: 986–991. doi:10.2174/1389450131113149990177

12. KUJAWSKI R, BARTKOWIAK-WIECZOREK J, OZAROWSKI M. Current knowledge on phytochemical profile of Epilobium sp. Raw materials and extracts. Potential benefits in nutrition and phytotherapy of age-related diseases. Herba Pol. 2011; 57: 33–44.

13. LEBEDA AF, JURENKO NI, ISAIKINA AP, SOKO VG. Medicinal Plants: The Fullest Encyclopedia. AST Press Book: Moscow. 2004; 320–323.

14. LIOCHEV SI. Reactive oxygen species and the free radical theory of aging. Free Radical Biol Med. 2013; 60: 1–4. doi: 10.1016/j.freeradbiomed.2013.02.011

15. LIU F, OCI VFC, CHANG ST. Free radical scavenging activity of mushroom polysaccharides. Life Sci. 1997; 60: 763-771.

16. MONSCEIN M, JAINDL K, BUZIMKICˇ S, BUCAR F. Content of phenolic compounds in wild populations of Epilobium angustifolium growing at different altitudes. Pharm Biol. 2015; 53 (11): 1576–1582.

17. NDHLALA AR, MOYO M, VAN STADEN J. Natural antioxidants: fascinating or mythical biomolecules. Molecules 2010;15:6905–6930. doi:10.3390/molecules15106905

18. ONAR HC, YUSUFOGLU A, TURKER G, YANARDAG R (2012). Elastase, tyrosinase and browning reaction prepared from glucosamine. J Ethnopharmacol. 2008; 115: 140–146. doi:10.1016/j.jep.2007.09.014

19. OYAIZU M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 1986; 44: 307-315. doi:10.5364/jjnp.11.127

20. OYAIZU M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 1986; 44: 307-315. doi:10.5364/jjnp.11.127

21. PIPOSCHI AM, NEGULESCU GP. Methods for Total Antioxidant Activity Determination: A Review. Biochem and Anal Biochem. 2011; 1: 106 – 116. doi:10.4172/2161-1009.1000106

22. RAMSTED AG, SCHEPETKIN IA, QUINN MT, JUTILA MA. Oenothelin B, a cyclic dimeric ellagitannin isolated from Epilobium angustifolium, enhances IFNγ production by lymphocytes. PLoS One. 2012, 7 / 11: e50546. doi:10.1371/journal.pone.0050546

23. SAYIK A, YUSUFOGLU AS, ACIK L, TURKER G, AYDIN B. DNA-Binding, Biological Activities, and Chemical Composition of Wild Growing Epilobium angustifolium L. Extracts from Canakkale, Turkey. JOTCSA. 2017; 4/3: 811-840. doi:10.18596/jotcsa.319789

24. SCHEPETKIN IA, KIRPOTINA LN, JAKIW L, KHELENIKOV AI, BLASKOVICH CL. Immunomodulatory activity of oenothein B isolated from Epilobium angustifolium. J Immunol. 2009; 183:754–766. doi:10.1049/jimmunol.0901827

25. SHIKOV AN, POLTANOV EA, DORMAN HJ, MAKAROV VG, TIKHONOVOV VP. Chemical Composition and in Vitro Antioxidant Evaluation of Commercial Water-Soluble Willow Herb (Epilobium angustifolium L.). Extracts. J. Agric. Food Chem. 2006; 54: 3617-3624. doi: 10.1021/jf0526060

26. SLINKARD K, SINGLETON VL. Total phenols analysis: automation and comparison with manual methods. Am. J. Enol. Viticult. 1977; 28: 49-55.

27. SOOBRATTEE MA, NEERGHEEN VS, LUXIMON-RAMMA A, ARUOMA OI, BAHORUN T. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat Res. 2005; 579: 200-213. doi:10.1016/j.mrfmm.2005.03.023

28. STAJNER D, POPOVIC BM, BOZA P. Evaluation of Willow Herb’s (Epilobium angustifolium L.) Antioxidant and Radical Scavenging Capacities. Phytother. Res. 2007; 21: 1242–1245. doi:10.1002/ptr.2244

29. STOLARZYK M, NARUSZEWICZ M, KISS AK. Extracts from Epilobium sp. herbs induce apoptosis in human hormone-dependent prostate cancer cells by activating the mitochondrial pathway. Journal of Pharmacy and Pharmacology. 2013; 65 /7:1044-1054. doi:10.1111/jphp.12063

30. TITA B, ABDEL-HAQ H, VITALONE A, MAZZANTI G, SASO L. Analgesic properties of Epilobium angustifolium, evaluated by the hot plate test and the writhing test. Il Farmaco. 2001; 56: 341–343.

31. TÖTH BH, BLAZICS B, KÉRY Á. Polyphenol composition and antioxidant capacity of Epilobium species. J Pharm Biomed Anal. 2009; 49: 26–31.

32. WEBSTER D, TASCHEREAU P, BELLAND RJ, SAND C, RENNIE RP. Antifungal activity of medicinal plant extracts: preliminary screening studies. J Ethnopharmacol. 2008; 115: 140–146. doi:10.1016/j.jep.2007.09.014

33. WEN X, WU J, WANG F, LIU B, HUANG C. Deconvoluting the role of reactive oxygen species and autophagy in human diseases. Free Radical Biol Med. 2013; 65: 402–410. doi:10.1016/j.freeradbiomed.2013.07.013.