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Total Phenolic Content and Antioxidant Activities of Invasive Erigeron annuus Pers. (Asteraceae) from Different Localities

Emel H. Yusuf [1,*]

[1]Department of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław University of Environmental and Life Sciences, 37 Chełmońskiego Street, 51-630 Wrocław, Poland

[*]Corresponding Author: emel.hasan.yusuf@upwr.edu.pl

Abstract

Erigeron annuus Pers. is a harmful invasive species to natural flora, although it is used in the treatment of indigestion, hepatitis, lymphadenitis, enteritis, and hematuria in traditional medicine. In this study, *E. annuus* samples were investigated in terms of total phenolic content, antioxidant activities and invasive features. Aerial parts of *E. annuus* were collected from Ayder-Çamlıhemşin (Rize), Pazar (Rize), and Trabzon (Turkey). Total phenolic contents, free radical scavenging characteristics against 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and 2,20-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) radicals (ABTS) were analyzed and compared with the collected localities. The invasive feature helps *E. annuus* to adapt everywhere. However, invasion of the plant is not an issue for medicinal applications except the collecting place of the plant. *E. annuus* is a lead accumulator, and the plant is seen on roadsides. Thus, the collecting region of the species should be chosen carefully to not obtain the side effects of heavy metals.

Keywords: Lead accumulation**,** Ecology, TPC, DPPH, ABTS

Introduction

Plant samples found in Neanderthal tombs show that human-plant relationships go back 50,000 years (Solecki, 1972). Human beings have used plants for various purposes in terms of food and medicine. Moreover, ethnobotanical studies are still applied to find new drug active substances. Owing to the studies carried out with plants and their chemical contents, new applications are being developed each day.

Since Reactive Oxygen Species (ROS) cause uncontrollable diseases. The situation occurs when natural by-products of oxygen cause oxidative stress in the brain (Olanow, 1993). On the other hand, antioxidant activities of herbal natural products increase the quality of applications and help prevent chronic diseases (Yu et al. 2003).

In this paper, *Erigeron annuus* Pers. samples were investigated in terms of TPC and antioxidant properties by comparing their invasive properties. While the species is used in traditional Chinese medicine to treat indigestion, hepatitis, lymphadenitis, enteritis, and hematuria (Jo et al., 2013), and as a hypoglycemic agent in Japanese ethnomedicine (Miyazawa and Kameoka, 1979). Besides, various compounds have been isolated from *E. annuus* in scientific studies (Song, et al., 2016; Nazaruk and Kalemba, 2009; Nam et al., 2008; Lis et al., 2007; El-Razek, 2006; Iijima et al., 2003a,b; Hashidoko, 1995), has aldose reductase inhibitory, antioxidant and neuroprotective (Bakar et. al., 2015; Jeong, et al., 2011; Jang et al., 2010; Kim, et al., 2005), anti-inflammatory effects (Yi et al., 2016; Jo et al., 2013; Sung, et al., 2011), anti-cancer (Nazaruk et al., 2014; Réthy, et al., 2007), anti-tumour (Li et al., 2006), antifungal (Kumar et al., 2014), anti-obesity (Choi et. Al., 2019), and anti-diabetic activities as well (Kim et. al., 2009).

Indeed, *E. annuus* is an invasive species native to North

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America and naturalized to Europe, Asia and Australia with the influence of humans (Frey, 2003). However, *E. annuus* is a medicinal plant and is dangerous for the natural flora of countries. More importantly, the species is on the 150 most widespread weed list in Europe. Besides, the plant is one of the most dangerous species in Serbia, Hungary, and Slovakia with rapid development and phenotypic flexibility of the species (Pacanoski, 2017). Furthermore, *E. annuus* is a lead accumulator (Bi et al., 2005) and might be the other reason for the invasive trait of the plant.

Hence, in the present study, antioxidant activities of *E. annuus* collected from three varied localities were investigated. Antioxidant activities were tested with DPPH and ABTS, while TPC was determined by the Folin-Ciocalteu method. Therefore, the study aims to compare the relationship between the spreading properties, TPC and antioxidant activities of *E. annuus*.

Materials and Methods

Reagents and Standards

Folin–Ciocalteu standard, sodium carbonate, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), L-Ascorbic acid, and sodium persulfate, ethanol and methanol were purchased from Merck (Darmstadt, Germany).

Plant Material

Aerial parts of *E. annuus* were collected in August 2017 from Northeastern Turkey (Table 1). Plant materials were dried in shadows at room temperature. Plant extracts were prepared by methanol in an orbital shaker for 27 hours. After the evaporation process at 40°C, dried extracts were kept at 4°C in a dark place until analyses.

Table1. Information about the sample area of invasive *E. annuus*

Place	Geographical Location
Pazar	N 41°10'55" E 40°54'11"
Ayder	N 40°57'3" E 41°7'18"
Trabzon	N 40°55'47" E 39°44'1"

Determination Procedure of Total Phenolic Contents (TPC)

TPC of extracts was determined with Folin–Ciocalteu method (Singleton et al., 1999), to 4.0 ml distilled water, 50 µl of the sample, 250 µl Folin–Ciocalteu, and 750 µL Na_2CO_3 was added after 2 h incubation at 25 °C and was measured at 760 nm, the results were calculated as average values of gallic acid equivalent (GAE). Analyses were performed in triplicate.

Analyses of Antioxidant Activities

DPPH·Assay

The samples were estimated on DPPH according to Brand-Williams et al. (1995). DPPH was prepared in 2 mg/25 ml of methanol. Dilutions were implemented with stock solutions of extracts. Diluted plant extracts were mixed with DPPH and put for 30 min at room temperature. The UV absorbance was read at 517 nm. Gallic acid was the control group. The scavenging activity of the DPPH was calculated with the following equation:

DPPH Scavenging Effect % = [(A0 - A1)/A0] x 100

A0 is the control group and A1 is the sample. Analyses were performed in triplicate.

ABTS Assay

ABTS was prepared by 7 mM ABTS and 2.5 mM sodium persulfate and put for 12-16 hours at room temperature in dark. ABTS standard was diluted with ethanol to be absorbance 0.8 to 0.7 at 734 nm in a spectrophotometer. Trolox was used as the antioxidant standard. 10 µL sample was mixed with 990 µL ABTS. The samples were read at 734 nm in spectrophotometer after 30 min incubation at room temperature (Re et al., 1999). Gallic acid and ascorbic acid were used as control groups. Analyses were performed in triplicate.

Statistical analysis

Statistical analyses were applied using SPSS 10.0.1. (SPSS Inc., Chicago, IL). The data were submitted as mean values. Analysis of variance (ANOVA) was implemented by ANOVA procedures.

Results and Discussion

E. annuus samples were collected from the North-Eastern part of Turkey (Rize and Trabzon). The region is specific to temperature differences and precipitation throughout the year (Okcu and Karabulut, 2019). Besides, the average annual temperature of Rize and Trabzon do not increase above 15.2 °C, nor fall below 13 °C (Polat and Sunkar, 2017). However, excessive rainfall causes podzolization in soils of the Eastern Black Sea region. More importantly, Rize and Trabzon provinces demonstrate red-yellow podzolic soil characteristics (Ozyazici et al., 2013). Thus, these soil and climate features are the similarities of studied plant samples.

The TPCs of *E. annuus* samples were shown in Figure 1. In the study, TPC values of the samples were listed as Pazar ≥ Ayder > Trabzon. According to study results, Pazar and Ayder localities were closer to each other than Trabzon. Thus, the study result can be the reason for this feature. Moreover, TPC might change with plant species, applied method, used solvent, ecological conditions, harvesting season and used plant part (Skotti et al., 2014). Nevertheless, in the present study, different TPCs are not influenced by all counted reasons, except ecological factors.

Applications of medicinal plants are the results of their antioxidant activities. Besides, the determination of *in-vitro* antioxidant activities of medicinal plants should be supported with at least two antioxidant activity assays (Schlesier et al.; 2002). Therefore, in the present study, DPPH and ABTS tests were used for *Erigeron* samples. Moreover, Asteraceae members are known for high antioxidant activities (Michel et al., 2020). In the present study, methanol was used to prepare

plant extracts, and the Ayder sample demonstrated the highest activity for DPPH (Figure 2), Trabzon and Pazar samples had close results to each other. Moreover, in the study of Lee and Seo (2006), *E. annuus* has represented potent activities on the peroxynitrite and DPPH radical for DPPH testing with methyl hydrogen peroxide and butanol fractions. In turn, DPPH activities of *E. annuus* samples are elevated. However, the differences in DPPH might be the results of accumulated metals, soil chemicals and stress tolerance of the samples.

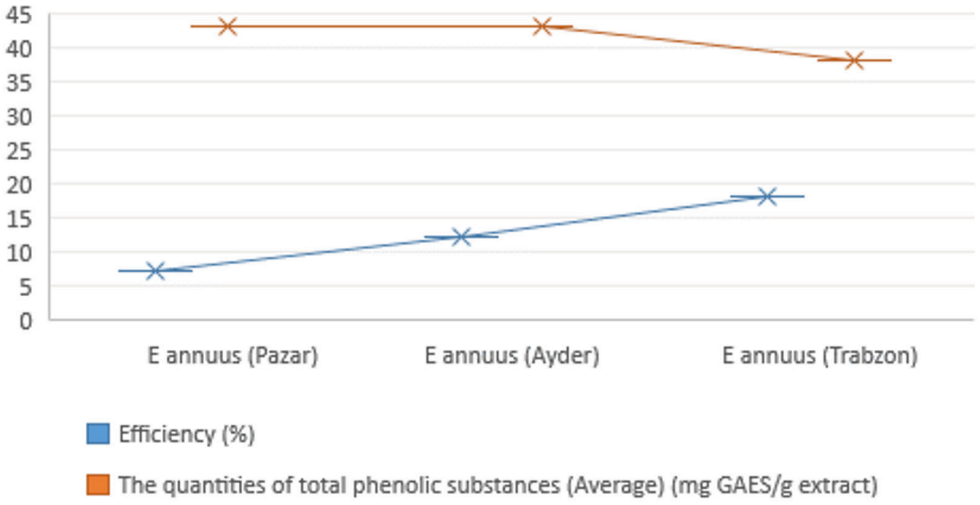

Figure 1. The efficiencies and quantities of total phenolic substances of extracts

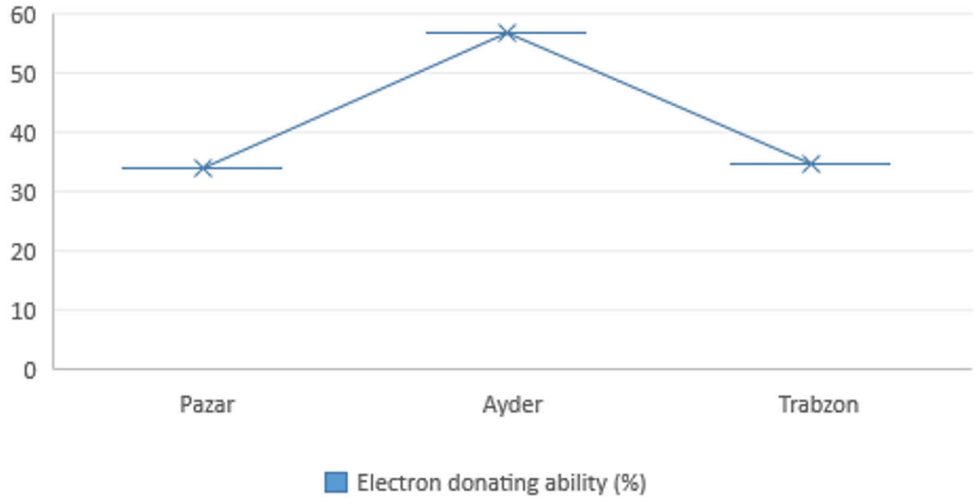

Figure 2. DPPH radical scavenging activities of *E. annuus* crude extracts

According to the ABTS test, three *E. annuus* samples showed the same results equivalent to 3 mM Trolox (Table 2). Moreover, in the study of Jeong et al. (2011), the butanol fraction of *E. annuus* has represented the highest antioxidant activity for the ABTS test. Following the DPPH results, radical scavenging activities of the ABTS experiment might be similar to environmental factors, or different antioxidant activity results may be associated with heavy metal accumulation. Therefore, *E. annuus* demonstrates lead accumulation properties (Bi et al., 2005). Besides, the metal uptake of plants is affected by the metal concentrations of the soil, the cation exchange capacity, the pH of the soil, the plant's organic content and the age of the plant (Annan et al., 2013). Hence, environmental factors and genetic inheritance interact together to adjust the chemical content of plants (Li et al., 2010).

Table 2. ABTS activities of *E. annuus* samples

Extract	Antioxidant radical scavenging activity equivalent to Trolox (mM)
Pazar	3
Ayder	3
Trabzon	3

On the other hand, high phenolic content is associated with high antioxidant activity (Soobrattee et al., 2005). Similar results were determined in the present study, the highest TPCs was observed in Ayder and Pazar samples, but DPPH was the highest in the Ayder sample only, and for the ABTS test all three samples demonstrated similar results. Thus, as similar to the study of Yu et al. (2003), these results reveal accumulation of heavy metal content, soil chemicals and other environmental factors affecting antioxidant properties. However, phenolic contents protect plants against UV radiation (Zhou et al., 2016); and altitude may be associated with the phenolic activity (Guo et al., 2011). Interestingly, in the present study, Ayder samples were collected from the highest altitude, and they ranked first place for TPC and DPPH tests.

Indeed, during the field works, *Erigeron* samples were seen mainly on the roadsides. Besides, road traffic causes a high amount of heavy metal accumulation. Hence, *E. annuus* accumulates heavy metals. Nevertheless, according to the World Health Organization (WHO, 1998), before collecting plants for food or medicinal applications, the localities of plant species should be chosen carefully to not accumulate heavy metals in our body as well.

Conclusion

Lead accumulation feature helps invasion of *E. annuus*, besides, the ecological adaptation of the species supports invasive characteristics for long distances as well. Moreover, *E. annuus* is utilised as a medicinal plant for different applications. Therefore, the invasive feature is not an issue for the medicinal approach, however, local people should be careful about collecting places of the herb. Importantly, the plant should not be collected from heavy traffic regions to not cause the side effects of the species such as heavy metal toxicity. Moreover, the species needs further studies regarding heavy metal accumulation and medicinal applications.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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