RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 660-668
DOI: https://doi.org/10.31015/jaefs.2023.3.20

Determination of free, esterified, bound bioactive compound contents of *Euphorbia cyparissias* organs and their biological activities

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Citation: Karaaslan, A. (2023). Determination of free, esterified, bound bioactive compound contents of Euphorbia cyparissias organs and their biological activities. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 660-668

Received: August 18, 2023
Revised: September 15, 2023
Accepted: September 17, 2023
Published Online: September 29, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract

Euphorbia plants have long been used as herbs in numerous traditional medicines in Anatolia. They were employed for the treatment of microbial infections, skin wounds and gastrointestinal diseases. *Euphorbia* species are rich sources of phenolic acids, flavonoids and many other natural compounds with antioxidant effects. In the context of this study the phenolic content, antioxidant activity and antidiabetic effect of *Euphorbia cyparissias* (*E. cyparissias*) leaf, flower and stalk extracts were evaluated. Three separate phenolic fractions namely free, esterified and bound extracts were prepared from leaf, stalk, and flower organs. Enzymatic treatment was utilized to remove bound phenolics from the cellular structures. A total of nine different extracts obtained from *E. cyparissias* organs. The highest phenolic fraction was bound phenolics in all three assayed extracts. The highest total phenolic compound (TPC) was found as bound phenolic fraction form in leaf extracts (21.088±0.32 mg GAE/g). Similarly the leaf samples displayed the highest total flavonoid contents (TFC) as bound form (1.798±0.02 mg CE/g). Four different methods were employed to determine the antioxidant potencies of the extracts. In parallel with the TPC and TFC results the bound fraction of leaf extract displayed the highest antioxidant capacities when evaluated with DPPH, ABTS and CUPRAC assays. According to FRAP analysis, free phenolic compounds of the leaves had the highest antioxidant potential. Free, esterified and bound phenolic compound fractions were all displayed inhibitory activity against α-amylase and α-glycosidase enzymes which is associated with their antidiabetic effects. Especially esterified phenolic compounds displayed significant inhibitory activity against α-amylase while bound fractions found in stalks and flowers exhibited stronger α-glycosidase activities.

Keywords: *Euphorbia cyparissias*, Phenolic compounds, Flavonoids, Antioxidant, Antidiabetic

INTRODUCTION

It has long been known that plants can be utilized in medicinal purposes because of their curing and healing abilities originating from their rich phytochemical contents. There are many studies in the literature showing that plant organs confer antimicrobial (Passari et al., 2015), antioxidant (Huang et al., 2011), anticancer (Rajasekar et al., 2012), and anti-diabetic activities (Gad et al., 2006) under *in vitro* and *in vivo* conditions. Currently plants are being used by the nutraceutical and pharmaceutical industries for the production of phyto-tablets, food-supplements and drugs. Recently, plant based diets were suggested by the researchers because of lower risk of cardiac diseases associated with regular daily

consumption of fruits, vegetables and the other plant tissues (Salehin et al., 2023). Therefore, the antioxidant, antimicrobial and biological activities of plant species should be evaluated to provide alternative phyto-chemical sources to the industry.

Anatolia harbors a myriad of different endemic plant species. One of those plants is *Euphorbia* genus. *Euphorbia* is a member of *Euphorbiaceae* family which is generally known as spurge, falls into a diverse genus of perennial flowering plants, and comprises more than 2000 species (Özbilgin et al., 2012). *Euphorbia* exhibits biological activities associated with medicinal plant attributes and has been widely used by the local people because of its curing, healing, protecting and antimicrobial properties. *Euphorbia* is originated from Asia and Arabian Peninsula and distributed up until to Anatolia. Especially in the Southeastern Region of Anatolia local people use this plant while they are producing grape molasses because of its food protecting ability. It was known that use of *Euphorbia* in the production of grape molasses confers better stability and longer shelf life to molasses.

Previously, total phenolic compounds (TPC), total flavonoid compounds (TFC), and antioxidant activities of three different *Euphorbia* species were evaluated and the results showed their high phyto-chemical contents and antimicrobial activity against *Staphylococcus aureus* (Budhathoki et al., 2016). The reported data also indicated that *Euphorbia* extracts contain a remarkable amount of phenolic acids, flavonoids, tannins and glycosidic compounds which indicate the high potential of *Euphorbia* extracts for the food and drug applications. In another study, it was determined that *Euphorbia tirucalli* contains a significant amount of bioactive compounds including alkaloids and phenolic derivatives while extracted with ethyl acetate (Le et al., 2021). Ethyl acetate fraction of the extract displayed antimicrobial activity against *Xanthomonas axonopodis*. In a similar fashion, methanolic extract of *Euphorbia* plant conferred significant antiproliferative activity against MiaPaCa-2 cell line of pancreatic cancer (Munro et al., 2015).

There are similar reports in the literature regarding the bioactive compound content of *Euphorbia* and its functional properties. However these studies were focused on the free phenolic content of the *Euphorbia* plant. Recently it was shown that besides the free fraction of phenolic compounds; esterified and bound fraction of the phenolic compounds also display functional properties and those two fractions should be measured to determine the exact amount of TPC (Acosta-Estrada et al., 2014).

Phenolic compounds draw significant attention because of their free radical scavenging activity and health promoting effects. It was shown that phenolic compounds are related to protecting human health, inhibition of tumor formation, and DNA protecting ability (Bernatoniene et al., 2023). Flavonoids and tannins also display strong biological activities such as inhibition of cancer cell proliferation, antioxidant activity and anti-diabetic activity (Maphetu et al., 2022). However to get a complete profile of the phenolic compounds and assess their health promoting potential three fractions of the phenolic compounds should be isolated and their biological activities determined separately.

In general, phenolic compounds fall into three separate classes according to their chemistry and existing forms in plant organs such as free phenolics, esterified phenolic compounds and insoluble ones which covalently bonded to the cellular structural components (Wu et al., 2021). In general, aqueous, methanolic, ethanolic and most of the solvent extracts of plant organs contain free phenolic compounds rather than the bound ones. Bound phenolic compounds are covalently linked to the cellulose micro-fibrils or pectin matrix existing in the cell wall of plant cells. Thermal treatments, alkalization or enzymatic treatments could be employed to remove such compounds from their existing compartments after the removal of free phenolic compound fraction and esterified phenolics (Acosta-Estrada et al., 2014; Shahidi et al., 2016). High blood glucose levels, type-2 diabetes, and insulin resistance are the common diseases among people which are associated with uncontrolled depolymerization of carbohydrates. α-amylase and α-glucosidase are the two common enzymes that control the digestion of carbohydrates in the human body and thus increase the blood glucose level. Therefore it is necessary to explore drugs or natural compounds to inhibit the activity of these enzymes. Plant extracts display significant enzyme inhibitory activities that can be used in the remedy of diabetes (Başyiğit et al., 2020). *E. cyparissias* plant should also be evaluated to assess its antidiabetic activity.

In the context of this study *E. cyparissias* plants grown in the Sanliurfa region were collected and leaf, stem and stalk organs were used as the bioactive compound sources. Three different phenolic compound fractions such as free, esterified and bound have been isolated and their biological activities were determined. TPC, TFC, hydrolysable tannin content, antioxidant and antidiabetic activities of each phenolic compound fraction were determined.

MATERIALS AND METHODS

Materials

Euphorbia plant employed in this study was obtained from a local market in Şanlıurfa. The plants were transported to the laboratory under suitable conditions and stored in a refrigerator until the analyses. The organs underwent a thorough washing process, being rinsed three times with distilled water, succeeded by manual

cleaning. The drying trials were conducted openly, without exposure to sunlight, and at room temperature. Post-drying, the organs were fragmented using a waring commercial lab blender (Conair, Stamford, CT, USA). For the analytical processes, the enzymes Vegazym HC, Fructazym MA-LG, and EnerZyme P7 were procured from Erbslöh (Geisenheim, Germany). The necessary chemicals and standards were sourced from Sigma or Merck (Darmstadt, Germany), unless exceptions are explicitly mentioned. Throughout the procedures, solvents of standard analytical quality were employed.

Extraction conditions

Free phenolics

Free phenolic extraction was conducted according to the method described by Ambigaipalan et al (2016) with slight changes. 1 g finely grounded plant material was dipped into 10 mL ethanol–water mixture (42–58; v/v). This mixture was subjected to extraction in a shaker at room temperature for a duration of 30 min. Following this, the mixture was centrifuged at 4000 rpm for 5 min, leading to the collection of the resulting supernatant. The solid residue that remained was then subjected to an oven-drying process at 40 °C for 48 h, and this material was reserved for subsequent bound phenolic extraction. This entire procedure was repeated three times, after which the resulting mixture was acidified to a pH of 1.5–2 using 6 M HCl. The final solution underwent three extraction cycles with an equivalent volume of diethyl ether–ethyl acetate (1:1, v/v) using a separator funnel. The organic fractions derived from this process were filtered through sodium sulfate and then evaporated at 40 °C until complete dryness was achieved. Obtained phenolic compounds were dissolved in 10 mL methanol and incubated in a deep freezer at –20 °C.

Esterified phenolics

Esterified phenolics were isolated according to the method reported by Ambigaipalan et al (2016) with minor modifications. The water phase remaining after free phenolic extraction was combined with an equivalent amount of 4 M NaOH. This mixture was then exposed to nitrogen in a shaker at room temperature for 4 h to liberate the esterified phenolic compounds. Subsequently, the mixture was acidified to a pH of 1.5–2 using 6 M HCl. After centrifugation at 4000 rpm for 5 min, the esterified phenolic compounds were subjected to three successive extractions using a 1:1 mixture of diethyl ether and ethyl acetate. This process was conducted as previously described. The organic phase was evaporated, and phenolic compounds were dissolved in 10 mL methanol and incubated in a deep freezer at –20 °C.

Bound phenolics

The approach utilized in this study followed the procedure outlined by Cuevas Montilla et al (2011) albeit with certain modifications. The residual solids obtained after the extraction of free and esterified phenolic compounds were subjected to oven-drying at 40 °C for a duration of 48 h. These dried solids were then combined with 10 mL water at 50 °C, and the pH of the mixture was adjusted to 4.5. For the process of cellulosic and pectolytic hydrolysis, a mixture of 2 µL vegazym and 1 µL fructazym was introduced to the mixture. The hydrolysis took place in a shaker at 55 °C for a period of 1 h. In the case of proteolytic hydrolysis, 2 µL EnerZyme P7 was added to the mixture under conditions of pH 6.5 and 55 °C, again for a duration of 1 h. It's worth noting that the specified conditions for each enzyme were determined based on their respective specifications. Subsequent to centrifugation at 4000 rpm for 5 min, the resulting supernatant was collected and preserved at a temperature of –20 °C in a freezer for subsequent analyses. Meanwhile, the solid residue derived from the centrifugation step underwent oven-drying at 90 °C for 1 h in order to deactivate the enzymes. Subsequently, this residue was allowed to dry for 48 h at 40 °C. The conducted experimental protocols for the extraction steps were summarized by Figure 1.

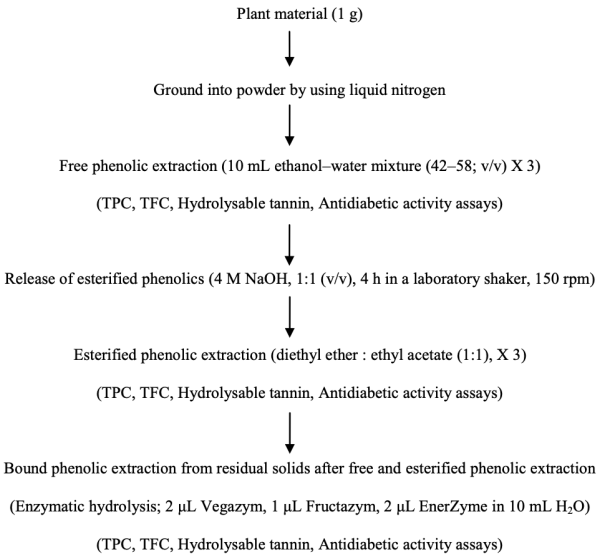

Figure 1. Free, esterified, and bound phenolic fractions' extraction from *E. cyparissias* organs.

Total phenolic content

Two mL Folin–Ciocalteu's phenol reagent after tenfold dilution and 0.4 mL of either diluted extract or gallic acid solution (5–100 mg/L) were mixed in a flask. To this mixture, 1.6 mL of a 7.5% (w/v) sodium carbonate solution was added. Following the addition, the solution was kept at room temperature during 1 h time period. Subsequently, the absorbance of the samples was determined at 765 nm by utilizing a UV–Vis spectrophotometer (Model UV-1280, Shimadzu Corp) (Singleton et al., 1965).

Total flavonoid content

The method described for determining the TFC was employed as described in a previous study (Zhishen et al., 1999). In this procedure, a 10 mL volumetric flask was utilized. To the flask, 1 mL of diluted extract or catechin in the range of 50 to 250 mg/L, was added. Subsequently, 4 mL distilled water and 0.3 mL sodium nitrite (5%, w/v) were introduced. The flask was then left at room temperature for a duration of 5 min. Following the incubation period, 0.3 mL aluminum chloride (10%, w/v) was incorporated into the flask. After an additional 6 min, a mixture consisting of 2 mL of 1 M sodium hydroxide and 2.4 mL of distilled water was added to the solution. The final solution's absorbance was spectrophotometrically determined at 510 nm.

Total hydrolysable tannin content

The determination of the overall hydrolysable tannin content (HTC) was carried out using a spectroscopic approach (Willis, 1998). A combination of 1 mL of diluted extract or tannic acid (ranging from 125 to 2500 mg/L) with 5 mL of potassium iodate solution (2.5%, w/v) was prepared. This mixture was kept at room temperature for 6 min. The solution's absorbance was spectrophotometrically determined at 550 nm. The obtained results were expressed as tannic acid equivalent (TAE).

Antioxidant activity

In the DDPH (2,2-Diphenyl-1-picrylhydrazyl) assay, a series of extracts dilutions, as well as trolox (ranging from 20 to 1000 µmol/L), were combined with a solution of DPPH (25 mg/L). This mixture was then allowed to incubate at room temperature for a duration of 30 min. Subsequently, the absorbance of the incubated solution was gauged at a wavelength of 515 nm using a UV–Vis spectrophotometer. A trolox curve was employed to measure the antioxidant capacity (Çam et al., 2009).

For the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay, a solution of 0.96 mg ABTS was prepared in a 25 mL volumetric flask by adding 10 mL distilled water and 5 mL potassium persulfate (2.45 mM). The mixture was then brought to a final volume of 25 mL with distilled water. This solution was incubated in darkness at room temperature for 16 h. To adjust the absorbance of the radical solution, 0.2 M sodium phosphate buffer (pH 7.4) was employed to attain a target absorbance of 0.700 ± 0.02 at 734 nm. For subsequent steps, a stock solution (2000 µL) was prepared, and the diluted extract or trolox (ranging from 0.1 to 2 mM) was mixed with it. This mixture was then incubated at room temperature for a period of 6 min. Following incubation, the solution's absorbance was spectrophotometrically determined at 734 nm. A trolox curve was employed to measure the antioxidant capacity (Çam et al., 2009).

For the FRAP (ferric reducing antioxidant power) assay, a volumetric flask was used to combine the following components: 25 mL 30 mM acetate solution, 2.5 mL 10 mM solution of 2,4,6-Tris(2-pyridyl)-s-triazine, and 2.5 mL 20 mM solution of iron (II) chloride. Subsequently, this mixture was blended with 150 µL serially diluted extracts or trolox (ranging from 40 to 300 µmol/L). The resulting solution was then incubated at room temperature for a duration of 30 min. Following incubation, the absorbance of the solution was measured at 593 nm using a UV–Vis spectrophotometer (Benzie et al., 1996).

0.4 mL serial dilution of extracts put into flasks was used for the CUPRAC (cupric reducing antioxidant capacity) assay. To this flask, 1 mL 0.01 M copper (II) chloride, 1 mL 7.5×10^{-3} ethanolic neocuproine solution, and 1 mL 1 M ammonium acetate solution at pH 7 were added. Subsequently, distilled water was used to bring the total volume to 4.1 mL. This mixture was then incubated at room temperature for a duration of 30 min. The absorbance of the solution after incubation was measured at 450 nm using a UV–Vis spectrophotometer. To determine the antioxidant activity of the samples, the regression equation derived from the trolox curve was applied (Apak et al., 2007).

Antidiabetic activity

For the α-glucosidase measurement, a mixture of 50 µL diluted extract, 1250 µL potassium phosphate buffer with a pH of 6.8, and 50 µL α-glycosidase enzyme solution was incubated at 37 °C. Following a 5-min incubation period, the enzymatic reaction was initiated by introducing 125 µL 10 mM 4-nitrophenyl α-D-glucopyranoside to the prepared blend. After allowing the reaction to proceed for 20 min, 2000 µL 0.1 M sodium carbonate was introduced to halt the reaction process. The resulting solution's absorbance was then measured at a wavelength of 400 nm (McDougall et al., 2005).

For the α-amylase assay, a mixture of 1 mL diluted extract, 1 mL starch solution (1% w/v), and 1 mL 20 mM sodium phosphate buffer (pH 6.9) was incubated at a temperature of 37 °C. After an initial 5-min incubation period, the enzymatic reaction was initiated by introducing 1 mL the α-amylase solution. Following a 30-min incubation, the reaction was halted by adding 1 mL color reagent. This color reagent was composed of a solution containing 5.31 M sodium potassium tartrate, which was prepared using 2 M NaOH and 96 mM 3,5-dinitrosalicylic acid. The reaction mixture was subsequently boiled for 5 min, and the absorbance of the resulting solution was measured at a wavelength of 540 nm. For the corresponding enzymatic control and blank samples, the preparation involved excluding the extract and the enzyme, respectively (McDougall et al., 2005).

Statistical analysis

Extractions and analyses were carried out in a minimum of two separate instances. Data underwent examination

utilizing the one-way ANOVA within the SPSS 22.0 statistical software package for Windows (SPSS, Inc., Chicago, USA). Distinctions between means were assessed through Tukey's HSD test at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Free, esterified and bound phytochemical content of Euphorbia cyparissias organs

Three different parts of *Euphorbia* plants namely stalk, flower and leaf were used as plant material to extract free, esterified, and bound phenolic fractions. Water/ethanol mixtures were utilized to extract the free phenolic compounds and enzymatic extraction techniques were employed for the further extraction of esterified and bound phenolics remaining in the plant tissues after the removal of free phenolic compounds. Similar extraction procedure was also employed for the isolation of flavonoids and hydrolysable tannins from the *E. cyparissias* tissues as well with specific extraction buffers. In general, *E. cyparissias* leaves contained the highest amount of free, esterified and bound phenolic compounds compared to euphorbia stalks and flowers. While leaf tissues contain 14.22±0.07 mg GAE/g, *E. cyparissias* flowers contain 8.654±0.04 mg GAE/g, the stalks only contain 6.013±0.8 mg GAE/g free phenolic compounds (Table 1). According to the obtained results, *E. cyparissias* leaf is a rich source of free phenolic compounds compared to flower and stalk organs of the plant. The phenolic content *of E. tirucalli* L. was determined as 0.305 mg/g gallic acid equivalent (GAE) by de Araujo et al (de Araújo et al., 2014)). The free phenolic content of *E. cyparissias* was significantly higher than the free phenolic content of *E.tirucalli L.* Yener et al. examined the chemical and biological profile of the *Euphorbia* species grown in different regions of Anatolia and determined a statistically significant difference in TPC (ranging from 63.03 to 372.27 µg pyrocatechol equivalents/mg extract) of the examined samples (Yener et al., 2018). The difference between phenolic contents could be attributed to the difference in extraction methods, species type, soil structure and the climatic conditions in which the plant grows (Yener et al., 2018). The leaf, flower and stalk organs were also assessed in terms of their esterified phenolic compound content. It should be noted that, the esterified phenolic concentrations of the leaf (1.731±0.2 mg GAE/g) and stalk (1.756±0.35 mg GAE/g) are higher than that of flower organs (1.182±0.08 mg GAE/g) (Table 1). An enzymatic treatment was applied for the removal of bound phenolic compounds from the E. *cyparissias* organs and to determine the bound phenolic content of the samples. In a similar fashion the leaf of the E. *cyparissias* contain the highest amount of bound phenolic compounds (21.088±0.32 mg GAE/g) which was followed by flower (16.09±0.2 mg GAE/g) and stalk (8.159±0.94 mg GAE/g). In general the assayed leaf samples had the highest amount of free, esterified and bound phenolic compounds in comparison to the other two organs; the stalk was the second phenolic-rich organ which was followed by flower. The free, esterified and bound flavonoid content of the *E. cyparissias* organs were also investigated (Table 1). The highest amount of free flavonoids was detected in the leaf samples (1.471±0.06 mg CE/g) and followed by flower (1.399±0.37 mg CE/g) and stalk organs (0.614±0.09 mg CE/g). The highest amount of bound flavonoids were detected in leaf samples. According to the obtained results the leaf and flower contain higher amounts of flavonids than that of found in stalks. In general the flavonoid content of the *E. cyparissias* was higher compared to the other nine *Euphorbia* species analyzed in a previous study. The difference between the results could be species-specific or stemmed from the variation in employed phytochemical extraction techniques (Yener et al., 2018). There are at least four flavonoids in the *Euphorbia* species such as naringenin, aromadendrin, apigenin and luteolin (Soliman et al., 2021) which are known for their positive impact on human health. The obtained results indicated that *E. cyparissias* organs could be employed for the preparation of flavonoid-rich plant extracts to be used in the food and health industry. Hydrolysable tannin is an important group of plant natural compounds and include gallic acid, ellagitannins, and gallotannins which exert positive impact on human health due to their anti-ulcerative, anti-microbial, and anti-tumor activities (Orabi et al., 2015). Interestingly the flowers of *E. cyparissias* contain the highest amount of free and esterified hydrolysable tannin (Table 1). The free, and esterified hydrolysable tannin fractions were detected at the highest amount in flower compared to leaf and stalk organs. It should be noted that bound hydrolysable tannins were found at the highest amount in leaves (Table 1).

Antioxidant activities of free, esterified and bound phytochemical fractions extracted from Euphorbia cyparissias organs

There are different subgroups of phenolic compounds existing in plant tissues and thus different antioxidant capacity assays should be employed to assess the actual radical scavenging activity and reducing power (Arnao et al., 1999). In the context of this study four different antioxidant activity assays were employed to determine their potential antioxidant activities, namely DPPH, $ABTS^{+}$, FRAP and CUPRAC. In terms of the effect of phenolic fraction type on antioxidant activity; the bound fraction had the highest radical scavenging potency followed by free fraction and finally esterified fraction according to DPPH assay (Table 2). Similar results were also obtained in the $ABTS^{+}$ antioxidant capacity determination assay as well. Bound fractions were displayed better at radical scavenging potency according to $ABTS^{+}$ assay which is an electron-transfer based system and CUPRAC reducing power assay. However in FRAP assay the free fractions

Table 1. The free, esterified and bound phenolic fractions of phenolics, flavonoids and hydrolysable tannin content of *E. cyparissias* organs. Different letters in the same column indicate statistically significant difference (p<0.05).

Sample Name	Phenolic Fraction	Phenolic (mg GAE/g)	Flavonoid (mg CE/g)	Hydrolysable tannin (mg TAE/g)
***E. cyparissias* Stalk**	*Free*	6.013±0.8^{c}	0.614±0.09^{c}	15.591±0.7^{d}
	Esterified	1.756±0.35^{b}	0.094±0.00^{a}	2.062±0.03^{a}
	Bound	8.159±0.94^{d}	1.017±0.02^{d}	ND
***E. cyparissias* Leaf**	*Free*	14.22±0.07^{e}	1.471±0.06^{g}	18.614±1.03^{e}
	Esterified	1.731±0.2^{b}	0.263±0.01^{b}	5.408±0.08^{b}
	Bound	21.088±0.32^{g}	1.798±0.02^{h}	15.797±0.9^{d}
***E. cyparissias* Flower**	*Free*	8.654±0.04^{d}	1.399±0.37^{f}	32.642±1.13^{g}
	Esterified	1.182±0.08^{a}	0.569±0.03^{c}	25.570±1.8^{f}
	Bound	16.09±0.2^{f}	1.082±0.21^{e}	9.839±0.74^{c}

Table 2. The antioxidant activities of free, esterified and bound phenolic fractions extracted from *E. cyparissias* stalk, leaf and flower organs. Different letters in the same column indicate statistically significant difference (p<0.05).

Sample Name	Phenolic Fraction	DPPH (µmol Trolox/g)	ABTS (µmol Trolox/g)	FRAP (µmol Trolox/g)	CUPRAC (µmol Trolox/g)
***E. cyparissias* Stalk**	*Free*	182.012±2.02^{d}	135.052±1.03^{d}	212.085±1.8^{e}	163.598±1.79^{d}
	Esterified	71.364±1.23^{b}	19.645±0.4^{a}	77.872±0.8^{a}	63.915±0.9^{a}
	Bound	191.114±1.09^{e}	155.702±1.21^{e}	193.081±0.913^{d}	170.018±1.86^{e}
***E. cyparissias* Leaf**	*Free*	174.032±1.5^{c}	266.255±1.72^{g}	302.514±1.17	307.336±1.7^{g}
	Esterified	69.378±0.3^{b}	40.78±0.8^{b}	89.33±0.76^{c}	104.67±0.5^{c}
	Bound	269.853±1.7^{h}	421.130±2.05	263.439±1.52^{f}	372.682±1.43
***E. cyparissias* Flower**	*Free*	227.335±1.63^{f}	239.495±1.8^{f}	298.689±1.86^{h}	254.63±1.33^{f}
	Esterified	60.060±0.58^{a}	45.734±0.7^{c}	83.953±0.81^{b}	84.44±0.135^{b}
	Bound	233.773±2.02^{g}	356.601±1.6^{h}	286.853±2.18^{g}	321.534±1.147^{h}

exhibited stronger reducing power compared to other two fractions (Table 2). In general, it was obvious that leaf samples had stronger antioxidant activity compared to stalk and flower samples. The antioxidant activity of plant extracts in general is correlated with their phyto-chemical contents (Mohammed Fazil Ahmed et al., 2012); the higher phytochemical content refers to higher radical scavenging strength. The high phenolic content of the leaf samples could be attributed to their high antioxidant power. The antioxidant activities of *Euphorbia* species were reported in previous studies. Remarkable antioxidant potencies were also detected in the *Euphorbia* species collected from different Anatolian regions (Yener et al., 2018). Four different plant organs (leaves,stems, flowers and roots) obtained from *E. hirta* were assayed to determine the antioxidant activities of the samples. Besides, cyanoferrate method was employed to assess the reducing power of the tissues. Similar to the results obtained in this study they also concluded that the extract of leaves displayed a maximum DPPH scavenging and reducing power activities (Özbilgin et al., 2012).

Antidiabetic activities of free, esterified and bound phytochemical fractions extracted from Euphorbia cyparissias organs

Diabetes mellitus is one of the most common chronic disease types affecting the life quality of human beings. The main cause of this disease could be related to inadequate insulin activity or disturbed secretion of insulin hormone into the bloodstream (Petersmann et al., 2019). The pharmaceutical industry developed many drugs to cure the patients with diabetes mellitus. One of the most common drugs to be used against type 2 diabetes mellitus (T2DM) is metformin (MET). MET displays elongated anti-hyperglycemic effects, does not interfere with cardiac health, a decreased possibility of hypoglycemia risk with affordable price (Lee et al., 2021). However, some of the patients developed intolerance against the use of MET to control the negative effects of T2DM (McCreight et al., 2016). Therefore it is necessary to investigate alternative medicines or natural treatment methods to overcome such side-effects of current drugs. There are many studies in the literature describing the antidiabetic activity of plant extracts (Temiz, 2021; Keskin et al., 2022). There are investigations depicting that pomegranate (Alsataf et al., 2021) and *Quercus infectoria* (Başyiğit et al., 2020) display antidiabetic activity. Flower extracts of *E. hirta* exhibited antidiabetic effect when orally administered to alloxan diabetic mice and also showed in vitro antioxidant activity (Başyiğit et al., 2020). In a similar fashion, Subramanian et al. (Subramanian

Table 3. The enzyme inhibitory activities of free, esterified and bound phenolic fractions extracted from *E. cyparissias* stalk, leaf and flower organs. Different letters in the same column indicate statistically significant difference ($p<0.05$).

Sample Name	Phenolic Fraction	α-amylase IC_{50} (mg/mL extract)	α-glycosidase IC_{50} (mg/mL extract)
***E. cyparissias* Stalk**	Free	14.505±0.5^{d}	12.02±0.08^{e}
	Esterified	2.938±0.02^{a}	11.32±0.22^{e}
	Bound	30.971±0.9^{g}	0.18±0.01^{a}
***E. cyparissias* Leaf**	Free	24.357±0.12^{f}	0.49±0.05^{a}
	Esterified	4.403±0.05^{b}	25.24±0.8^{f}
	Bound	4.595±0.128^{b}	31.80±0.94^{g}
***E. cyparissias* Flower**	Free	15.712±0.82^{e}	5.24±0.2^{c}
	Esterified	2.939±0.07^{a}	6.91±0.3^{d}
	Bound	12.60±0.21^{c}	3.35±0.41^{b}

et al., 2011) reported that 30 day of oral administration of extracts obtained from *E. hirta* leaves displayed antidiabetic activity in an animal-model experiment. The antidiabetic activity of the extracts obtained from *E. cyparissias* plant was assessed in the context of this study. Enzyme inhibitory activities of stalk, leaf and flower extracts are given in Table 3. The antidiabetic effect of the *Euphorbia* plant was calculated by measuring the α-amylase and α-glycosidase enzyme activities in the stalk, leaf and flower parts of the plant. According to the α-amylase enzyme activity measurement, the highest antidiabetic effect was determined in the esterified phenolic fractions in the flower and stalk. The α-glycosidase enzyme activity measurement depicted that the highest antidiabetic effect was present in the form of the bound phenolic fraction in the plant stalk. The lower IC_{50} means the stronger antidiabetic activity. Esterified phenolic extracts displayed the lowest IC_{50} values in stalk and flower organs as can be seen in Table 3.

CONCLUSIONS

There are more than 8000 individual phenolic compounds in plant species which establish one of the biggest classes of secondary metabolites. Phenolic compounds are involved in the defense mechanism of the plants against microorganisms and detrimental effects of abiotic stresses. In recent years phenolic compounds draw significant attention due to their antioxidant, antidiabetic and antiproliferative activities. Many studies were conducted to show their promoting effects on human health. However most of these studies focused on the free phenolic compounds rather than esterified and bound forms. *Euphorbia* is a genus comprising more than 2000 species and contains a significant amount of phenolic compounds. Until today, only free phenolic compounds extracted from *Euphorbia* species and their biological activities were evaluated. In the context of this study three different organs of *E. cyparissias* (stalk, leaf, flower) were used as phytochemical sources. Free, esterified, and bound forms of phenolics were isolated from these organs to evaluate their biological activities. The results showed that all three organs contain a significant amount of esterified and bound phytochemicals beyond their free forms. Esterified and bound natural compounds displayed strong antioxidant and antidiabetic activity that indicated their importance for the production of functional extracts. Especially esterified leaf extracts displayed strong biological activities and thus can be used for the production of functional extracts for food and pharmaceutical industries. Overall, the obtained results in this study showed the importance of *E. cyparissias* extracts to be used in pharmaceutical and food industries. The flowers, stalks and leaves can be used to produce phyto-tablets and food supplements after extensive *in vitro* and *in vivo* studies.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declare no conflict of interest.

Author contribution

The author collected the data, analyzed the results and wrote the paper.

Ethics committee approval

Ethics committee approval is not required.

Funding

No financial support received for this study.

Data availability

Data are available upon reasonable request.

Consent for publication

Not applicable.

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