Screening Effects of Methanol Extracts of The *Diplotaxis tenuifolia* and *Reseda lutea* on The Enzymatic Antioxidant Defense Systems and Aldose Reductase Activity

***Diplotaxis tenuifolia* ve *Reseda lutea* Metanol Özütünün Antioksidan Savunma Sistemi Enzimleri ve Aldoz Redüktaz Aktivitesi Üzerinde Olan Etkisinin İncelenmesi**

ABSTRACT

The aim of this study is to investigate the effects of methanol extracts from flowers and leaves of *Diplotaxis tenuifolia* and *Reseda lutea* on the activity of the aldose reductase (AR), catalase (CAT), glutathione-S-transferase (GST) and glutathione peroxidase (GPx). In this study, total phenolic and flavonoid contents of the plant samples were evaluated by Folin–Ciocalteu and aluminum chloride colorimetric methods. Also, the effects of extracts on CAT, GST, GPx and AR enzyme activities were investigated by kinetic assays. The highest phenolic and flavonoid contents were detected in methanol extract of *D. tenuifolia* leaves with 144.49±0.29 mg gallic acid equivalent/L and 250.485±0.002 quercetin equivalent/L respectively. The best activity profile for GST and GPx are observed in the extract of leaves belonging to *D. tenuifolia* with IC_{50} values of 121±0.05 and 140±0.001 ng/mL, respectively. According to the results, methanol extracts from leaves of *R. lutea* and *D. tenuifolia* have not shown significant activity potential on the AR enzyme. By the way, none of the studied extracts have shown any reasonable CAT activation potential. Results indicated that leaves of *D. tenuifolia* have good effect on the antioxidant enzymatic defense system which it make to be considered as a good source of food for daily diet.

Key words: *Diplotaxis tenuifolia*, *Reseda lutea*, Antioxidant enzymes, Aldose reductase

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ÖZ

Bu çalışmada *D. tenuifolia* ve *R. lutea*'nın çiçek ve yapraklarından elde edilmiş olan metanol özütlerinin aldoz redüktaz (AR), katalaz (CAT), glutatyon-S-transferaz (GST) ve glutatyon peroksidaz (GPx) enzimlerinin aktiviteleri üzerinde olan etkilerinin araştırılması amaçlanmıştır. Bu çalışmada, bitki örneklerinin toplam fenolik ve flavonoid içeriği; Folin-Ciocalteu ve alüminyum klorür reaktiflerinin yardımıyla kolorimetrik yöntemlerle değerlendirilmiştir. Ayrıca özütlerin CAT, GST, GPx ve AR enzimlerinin aktiviteleri üzerindeki etkileri kinetik analizler ile araştırılmıştır. En yüksek miktarda fenolik ve flavanoid içeriği sırasıyla 144.49±0.29 mg gallic asit eşdeğeri/L ve 250.485±0.002 quercetin eşdeğeri/L tespit edilmiştir. GST ve GPx için en iyi aktivite profilleri sırasıyla 121±0.05 ve 140±0.001 ng/mL IC_{50} değerleri ile *D. tenuifolia* yaprak özütünde gözlemlenmiştir. Elde edilen sonuçlara göre, *R. lutea* ve *D. tenuifolia*'nın yapraklarından elde edilen metanol özütleri, AR enzimi üzerinde önemli ölçüde bir aktivite potansiyeli göstermemiştir. Bununla beraber, çalışılmış olan çiçek ve yaprak özütlerinin hiçbiri yeterli düzeyde CAT aktivasyonu gösterememiştir. Çalışma sonucunda, *D. tenuifolia*'nın yapraklarının antioksidan enzimatik savunma sistemi üzerinde iyi bir etkiye sahip olduğu gösterilmiştir. Bu sebeple günlük diyet için iyi bir besin kaynağı olarak kabul edilebilir.

Anahtar kelimeler: *Diplotaxis tenuifolia*, *Reseda lutea*, Antioksidan enzimler, Aldoz redüktaz

INTRODUCTION

Reactive Oxygen Species (ROS) is a term used to describe a number of reactive molecules and free radicals derived from molecular oxygen which generated by all aerobic species. These molecules are generated as by-products during the mitochondrial electron transport of aerobic respiration or by oxidoreductase enzymes and metal catalyzed oxidation. In normal physiological conditions, a number of defense mechanisms have evolved to provide a balance between production and removal of ROS, but alterations of the balance between ROS production and the capacity to detoxify reactive intermediates lead to oxidative stress. It has been caused to a wide variety of states, processess and metabolic diseases such as heart diseaes, severe neural disorders such

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as Alzheimer's and Parkinson's and some cancers (1,2). Under oxidative stress, an organism has a variety of defense mechanisms to prevent or neutralize negative ROS effects. These are mainly based on enzymatic such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) or non-enzymatic components such as vitamin E, vitamin C, glutathione and flavonoids (3). Glutathione-S-transferase enzyme is one of the phase II enzymes and plays a critical role in the detoxification and metabolism of many xenobiotic compounds (4). Glutathione peroxidase enzyme has important role as catalysis in reduction of hydro peroxides, including hydrogen peroxides, by using reduced glutathione. GPx also functions to protect the cell from oxidative damage. Several studies related the dysfunctional GPx with cancer (5). Catalase is a very important enzyme of living organisms which catalyzes the decomposition of hydrogen peroxide to water and oxygen. Aldose reductase is an NADPH dependent enzyme and it has been implicated in the formation of cancer and diabetic complications such as retinopathy, neuropathy, nephropathy and cardiovascular disorders (6).

Plants synthesize a vast range of organic compounds that are traditionally classified as primary and secondary metabolites. Primary metabolites are compounds that have essential roles associated with photosynthesis, respiration, growth and development. Other phytochemicals which accumulate in high concentrations in some species are known as secondary metabolites which possess antioxidant activity. Antioxidant compounds found in different parts of plants involved to phenolics, flavonoids, alkaloids, glycosides, tocopherols, carotenoids and ascorbic acid. These are structurally diverse and many are distributed among a very limited number of species within the plant kingdom (7). Secondary metabolite compounds have played an important role in treating and preventing human diseases. They are important sources for new drugs and are also suitable lead compounds for further modification during drug development (4).

Diplotaxis tenuifolia (L.) DC., commonly known as ''wild rocket'' belongs to the Brassicaceae family. It has originally found as a crop in Mediterranean and Middle Eastern countries and become popular largely due to the pungent aromas and tastes associated with it (8). In Turkish folk medicine *D. tenuifolia* is known as "Yabani Roka" and wildly distributed in North and West parts of Turkey. Phytochemical studies show that

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the aerial parts of *D. tenuifolia* contain significantly high concentration of flavonoids, tannins, glucosinolates, sterols and vitamin C (9).

The genus *Reseda* is one of the herbs in the Resedaceae family. In Turkey, this genus is represented by 15 species including *Reseda lutea* L. and *Reseda luteola* L. It is known as yellow mignonette or wild mignonette and has economic importance. It is widely used in carpet and rug industry as a source of natural dye due to its high luteolin content. In addition to its staining properties, luteolin has attracted great scientific interest because of its pharmacological activities. Luteolin displays numerous anti-inflammatory effects at micromolar concentrations which cannot be completely explained by its antioxidant capacities. Also, phytochemical analysis of aerial parts of *R. lutea* has shown the presence of flavonoid, anthocyanin and glucosides (10).

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 *D. tenuifolia* and *R. lutea* and determine their effects on the activity of the aldose reductase (AR), catalase (CAT), glutathione-S-transferase (GST) and glutathione peroxidase (GPx). These enzymes play the critical role on the antioxidant defense system.

EXPERIMENTAL

Chemicals materials

In this study, 4-aminoantipyrine (4-AP), hydrogen peroxide (H_2O_2) and sodium azide (NaN_3) were provided from Acros, USA. Ethylenediaminetetraacetic acid (EDTA), Folin-Ciocalteu reagent, reduced glutathione (GSH), glutathione reductase (GR), horse raddish peroxidase (HRP), catalase (CAT), gallic acid and quercetin hydrate were supplied from Sigma Aldrich, Germany. Lithium sulphate (Li_2SO_4) and Nicotineamide adenine dinucleotide phosphate (NADPH) was purchased from Gerbu, Germany. All other chemicals used were analytical grade and provided from Sigma Aldrich, Germany.

Plant materials

Plant samples of *D. tenuifolia* and *R. lutea* were harvested in July 2010 from Ankara-Turkey and were authenticated by Prof. Dr. Fatmagül Geven, in Department of Biology,

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Ankara University. The plant specimens with their localities and the necessary field records were written and numerated as voucher specimen number. The voucher number of *D. tenuifolia* and *R. lutea* are FG-2010-10 and FG-2010-13 respectively. They were deposited in herbarium department at Ankara University.

Extraction of plant

Different parts of fresh plant samples (flowers and leaves) were washed with tap water and dried at room temperature before analysis. For methanol extraction, 2gr of dried samples were weighed and ground into a fine powder with liquid nitrogen, then mixed with 20mL methyl alcohol at room temperature in 160 rpm for 24 h. Obtained extract was filtered over Whatman No. 1 paper and the filtrate was collected, then methanol was removed using a rotary evaporator at 40°C to obtain dry extract. The obtained product dissolved in DMSO and kept at dark (4°C) to be prevent oxidative damage until analysis (11).

Total Phenolics Determination

Total phenolic content of plant extracts were determined using the method of Slinkard and Singleton (12). Each plant extract solution (0.1 mL) was mixed with 2mL of a 2% (w/v) sodium carbonate solution and vortexed strongly. After 5min, 0.1mL of 50% Folin-Ciocalteu's reagent (w/v) was added and vortexed, then incubated for 1hr at room temperature. Afterwards, the absorbance of each mixture was measured at 750 nm with UV-vis spectrophotometer (HP 8453 A, USA). Results were evaluated using 50, 100, 200 ve 400 mg/L of Gallic acid (GA) as standard curve and recorded as milligrams (mg) GA equivalent /L of extract.

Total Flavonoid Determination

The total concentration of flavonoids in extracts was determined by employing the Aluminum Chloride Colorimetric method which was previously described (13). 0.1mL of each plant extract was separately mixed with 0.15mL of 95% ethanol, 0.01mL of 10% aluminum chloride, 0.01mL of 1M sodium acetate and 0.25mL of DMSO. The mixture was incubated at room temperature for 30 min and the absorbance of the reaction was measured at 415 nm with UV-vis spectrophotometer (HP 8453 A, USA). The standard curve was calculated by preparing quercetin solutions at different concentrations for 25,

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50, 100, 150 ve 200 mg/L. The total flavonoid content of the extract was expressed as milligrams (mg) quercetin equivalent /L of extract.

Isolation of cytosol from bovine liver

The bovine liver was obtained from slaughterhouse of Kazan, Ankara, Turkey. The liver samples were homogenized in 10mM potassium phosphate buffer (pH 7.0), containing 0.15 M KCl, 1.0 mM EDTA, and 1.0 mM of DTT, using a glass teflon homogenizer and then 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 (14). The prepared homogenates, containing 46.41mg protein/mL, were kept in ultralow freezer (-80°C) for the future consumptions. Here, the total protein content was determined by the Lowry method (15).

Isolation of Aldose Reductase Enzyme from Bovine Liver

The bovine liver were obtained from slaughterhouse of Kazan, Ankara, Turkey. The liver samples were cut into small pieces and washed with 1.0mM EDTA. Then it is weighed and homogenized with threefold 1.0mM EDTA 50µM PMSF and centrifuged at +4°C, 10,000 rpm for 30 min. To obtain a 40 % saturation, 22.6gr ammonium sulfate added to every 100mL supernatant solution and mixed for 5 min in magnetic stirrer and then centrifuged at +4°C, 10,000 rpm for 25 min. To obtain 50% and 75% saturations, the previous method was repeated adding 5.8gr and 15.9gr of ammonium sulfate to the 100mL supernatant solution respectively. The obtained pellets dissolved with 50mM sodium chloride and kept in deepfreeze at -80 °C (16).

Assay of Glutathione-S-transferase (GST)

GSTs activity was determined against the substrate, 1-chloro-2, 4-dinitrobenzene (CDNB), by monitoring the thioether formation at 340 nm (17). Briefly describing, the assay mixture contained plant extracts solution (final concentration in the range of 7- 476 ng/mL), 200mM potassium phosphate buffer (pH 6.5) with 50mM CDNB and 3.2mM GSH and bovine liver cytosolic fractions (0.782 mg protein/mL) was prepared and used as the enzyme source to measure GST activity. GSH-CDNB conjugate formation was followed

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in 250µL total volume assay by using multimode microplate reader (Specra Max M2e, USA) at 340 nm for 240 second. Initial rates of enzymatic reactions were determined as nanomoles of the conjugation product of GSH and reported as nmol/ minute/ mg protein.

Assay of Aldose Reductase (AR)

AR activity was determined against the substrate, DL-Glyceraldehyde, by monitoring the oxidation of NADPH to $NADP^+$ at 340 nm (18). Shortly explaining, the assay mixture consisted of plant extracts (5µl) solution (final concentration in the range of 7-476 ng/mL), AR enzyme (4.54 mg/mL) Li_2SO_4 (320 mM-400mM), NADPH (9×10^{-5} M) KP buffer (50mM, pH 6.2), DL-GA (6×10^{-4} M), were prepared and used as the enzyme source to measure AR activity. $NADP^+$ oxidation was followed in 0.25mL total volume assay by using multimode microplate reader at 340 nm for 4 min. Initial rates of enzymatic reactions were determined and reported as nmol/ minute/ mg protein.

Assay of glutathione peroxidase (GPx)

Glutathione peroxidase activity was measured by previously reported method (19,20). Also the GPx activity was measured against the substrate, tertiary butyl hydro-peroxides (t-BuOOH), and the decrease in nicotineamide 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 2.0mM GSH, 0.25mM NADPH, GSH-reductase (GR, 0.5 unit/ml) and 0.3mM t-BuOOH, in 50mM Tris-HCl (pH=8.0). The reaction was initiated by adding GPx and the change in absorbance was recorded at 340 nm for 5 min using multimode microplate reader.

Assay of catalase (CAT)

Catalase (CAT) inhibition was determined by monitoring a red quinoneimine dye remaining hydrogen peroxide (21,22). The assay was miniaturized for microplate application and contained plant extraction solutions with final concentration in the range of 7-476 ng/mL, 50mM phosphate buffer (pH 7.0), 20 U/mL purified bovine liver catalase and 0.0961mM H_2O_2. The reaction was stopped by NaN_3 and incubated at room temperature for 5 min, followed by the incubation with chromogen at room temperature for 40 min and then the absorbance was read at 520 nm. The enzyme activity was

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calculated with respect to hydrogen peroxide remnant that was determined by calibration curve constructed in the range of 9.61-307.6µM hydrogen peroxide.

Data analysis

The data analysis was performed using Graphpad Prism 6.0. The activity of extracts against enzyme targets were calculated as 50% inhibitory concentration, IC_{50} values, and obtained from dose response curves constructed. The enzyme calibration and the dose response curve construction were accomplished by 2-3 independent experiments, each in duplicates or triplicates using multimode microplate reader, in 96 well microplates.

RESULTS

Each extract was prepared by dissolving 2gr of dry samples in 20mL of methanol. The extraction yields for *D. tenuifolia* leave samples is %13.02 and for *R. lutea* flower and leave samples are %10.15 and %6.02 respectively (Table 1).

The total phenolic contents of extracts were determined by using the Folin-Ciocalteu's method. Additionally, the total amount of flavonoids in extracts were determined by employing the aluminum chloride colorimetric method. According to the results, the methanol extract of *D. tenuifolia* leaves have high amount of total phenolic and flavonoid contents. The results of total phenolic and flavonoid contents of methanol extracts of plant samples are listed in Table 1.

The activation percent profile of GST, GPx, CAT and AR enzymes and IC_{50} values of the methanol extracts of plant samples are presented in Table 2. 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 percentage of GST activity and IC_{50} values, the utilized final concentration of plant extracts in the assay was taken between 7-476 ng/mL. According to the results which presented in Table 2, the best activity effect was exhibited in the crude methanol extract of *D. tenuifolia* leaves with IC_{50} value of 121±0.05 ng/mL.

The activity of the GPx enzyme was determined as the amount of enzyme that converts 1µM of NADPH per minute in 1mL which is expressed as U/mg of total protein. The final

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concentration of plant extracts within concentration range of 7-476 ng/mL were used in the assay to calculate the percentage of GPx activity and IC_{50} values. The best activity profile for GPx is observed in the extract of leaves belonging to *D. tenuifolia* with IC_{50} value of 140±0.001 ng/mL.

AR activity was determined using the substrate DL-Glyceraldehyde, by monitoring the oxidation of NADPH to $NADP^+$ at 340 nm. It is observed that methanol extracts from leaves of *R. lutea* and *D. tenuifolia* have not shown significant activity on the AR enzyme (Table 2). By the way, none of the studied extracts have shown reasonable CAT activity potential.

DISCUSSION

The aim of the present study was to evaluate the total amount of the phenolic and flavonoid contents of methanol extract obtained from flowers and leaves of *D. tenuifolia* and *R. lutea.* Furthermore, it was aimed to determine those extract effects on the activity of the aldose reductase (AR), catalase (CAT), glutathione-S-transferase (GST) and glutathione peroxidase (GPx). Phenolic compounds are having at least one or more aromatic rings with one or more hydroxyl groups attached (23). Many phenolic compounds and flavonoids have been reported to have potentials of antioxidant, anticancer, anti-atherosclerotic, antibacterial, antiviral, and anti-inflammatory activities (24). Flavonoids are phenolic compounds found throughout the plant kingdom. They have been shown to possess a variety of biological activities in organisms. Many flavonoids possess antitumor, anti-proliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidant and reversal of multidrug resistance activities (25,26,27). Different studies show that plant extracts with high polyphenol contents are known as good source of antioxidant activity (28,29,30).

In this study, for the first time, it was shown that methanol extract from leaves of *D. tenuifolia* contains high amount of total phenolic and flavonoid compounds. Results indicated that methanol extract from leave of *D. tenuifolia* have significantly effect on the GST and GPx activities. Therefore, it can be said that the leaves of *D. tenuifolia* have

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good effect on the antioxidant enzymatic defense system. However, it is found that the leave extracts of *D. tenuifolia* did not have any effect on the AR and CAT enzymes activities. It is also demonstrated that methanol extract from leaves of *R. lutea* contains more phenolic and flavonoid contents than its flower samples. But flower extract of *R. lutea* showed good effects on the GPx enzyme activity than the leave extract and the opposite of this situation is shown in the GST results.

In the previous study, *D. tenuifolia* was analyzed for active compounds and antitumor actions on colorectal cancer cells. Obtained results showed that *D. tenuifolia* is a good source of carotenoids, phenolics and glucosinolates compounds. Also, it has antitumor activities on the colorectal cancer (31). Marrelli (32) evaluated thirteen hydro alcoholic extracts of edible plants from Southern Italy for their *in vitro* antioxidant and antiproliferative activity on breast cancer MCF-7, hepatic cancer HepG2 and colorectal cancer LoVo. They showed that the lowest antioxidant activity was exhibited by *D. tenuifolia* (DT) extract. Also, they said that *D. tenuifolia* extract was able to induce an inhibitory activity of cell proliferation higher than 40%.

In the other study, the polyphenol content and the biological activities of the main organ of *D*. *simplex* extract was investigated. The analyzed extracts showed that flower extracts exhibit a potent *in vitro* antioxidant capacity using oxygen radical absorbance capacity and displayed a strong anti-inflammatory activity and inhibiting nitric oxide release. Findings suggested that the *Diplotaxis* flower is a valuable source of antioxidants and anti-inflammatory agents (33). Durazzo (34) studied the nutritional and antioxidant properties of wild rocket (*D*. *tenuifolia* (L.) DC.). They determined bioactive molecules content (vitamin C, quercetin, lutein) and showed bioactivity of polyphenolic extracts from the edible part of rocket in Caco-2 cells. Atta (35) isolated five main flavonoid glycosides from ethanol extract of *D. harra* and identified as quercetin, isorhamnetin 3-rhamnoside, isorhamnetin 3-*o*-rutinoside, isorhamnetin 3-glucosyl-4-rhamnoside and isorhamnetin 3-o-*β*-glucoside. Also, they evaluated alcoholic extract of plants against some bacterial strains that showed moderate antibacterial activity. Martínez-Sánchez (9) studied antioxidant compounds, flavonoids and vitamin C, and also antioxidant activity of four species from Brassicaceae vegetables used for salads such as Watercress (*Nasturtium officinale*), Mizuna (*Brassica rapa*), wild rocket (*D. tenuifolia*), and salad rocket (*Eruca*

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sativa). They analyzed the characterization of phenolic compounds and they showed that the leaves of Watercress, Mizuna, wild rocket and salad rocket, presented high contents of antioxidant compounds such as flavonoids and vitamin C. Therefore they are good dietary sources of antioxidants with an important variability of bioactive compounds. However, no pharmacological studies have been performed with *R. lutea* extracts so far but *Reseda* species have been reported to possess various pharmacological effects such as anti-inflammatory, antioxidant, antibacterial and antimicrobial. For the first time, Benmerache (36) isolated six flavonoids from the aerial parts of *R. phyteuma*. They also found that the butanolic extract exhibited a good antioxidant and antimicrobial activities. *R. luteola* L. has been used as a dye due to its high luteolin content since ancient times. Woelfle (37) determined anti-proliferative and apoptosis-inducing effects of the *R. luteola* extract RF-40. They found that it contains 40% flavonoids, primarily luteolin, luteolin-7-O-glucoside and apigenin. Also it is observed that the isolated flavonoids dose-dependently, inhibited cell proliferation and induced apoptotic oligonucleosomes in PHA-stimulated peripheral blood mononuclear cells. Moreover, they showed that *Reseda* extract is an interesting raw material not only for dyeing purposes but also for further pharmacological investigation. In the other study, Berrehal (38) were investigated the methanolic and n-butanolic extracts of *R. duriaeana* and *R. villosa* for their antioxidant activity. They indicated that the methanolic and n-butanolic extracts of *R. duriaeana* exhibited better antioxidant activity than the respective extracts of *R. villosa*. This may be explained by the presence of more quercetin derivatives in *R. duriaeana*.

From a consideration of ethnobotanical information, seeds of 45 Scottish plant species were obtained from authentic seed suppliers. The n-hexane, dichloromethane (DCM) and methanol (MeOH) extracts were assessed for free radical scavenging activity in the DPPH assay. The obtain results displayed that the metanol extract of *R. Lutea* seeds' exhibited moderate levels of free radical scavenging activity. Also, the n-hexane extract was much less active than the MeOH and DCM extracts (39). Tawaha (40) have been determined the relative levels of antioxidant activity and the total phenolic content of aqueous and methanolic extracts of a total of 51 plant species of Jordanian. They indicated that aqueous and methanolic extracts of *R. lutea* have remarkably high total phenolic contents and showed good levels of antioxidant activity.

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As conclusion, in this research the biological potential of *D. tenuifolia* and *R. lutea* on the antioxidant defense system such as GST, GPx, CAT and AR enzymes were considered. It is shown that the methanol extract of *D. tenuifolia* leave has high amount of total phenolic and flavonoid compounds. Also, it is indicated that it has good activity potential on GPx and GST enzymes. It is suggested that these results might be related to the high content of fenolic and flavonoids found in the species. This work highlights the importance of *D. tenuifolia* as a good source of food for daily diet.

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Table 1 - The percent yield of dry products obtained from methanol extraction procedure with total polyphenol and flavonoid contents of each plant samples

Family	Species	Parts of plants	% Yield	TPC mg GAE/L	Flavonoid mgQE/L
Brassicaceae	*Diplotoxis tenuifolia*	Leaves	13.02	144.49±0.29	250.485±0.002
Resedaceae	*Reseda lutea*	Flowers	10.15	109.01±0.03	78.72±0.03
		Leaves	6.02	133.52±0.02	196.80±0.01

Table 2 - Gluthatione-S-transfrase (GST), Gluthatione peroxidase (GPx), Catalase (CAT), Aldose reductase (AR) percentage activities

Family	Species	Parts of plants	GST		GPx		CAT		AR	
			%	IC_{50} ng/mL	%	IC_{50} ng/mL	%	IC_{50} ng/mL	%	IC_{50} ng/mL
Brassicaceae	*Diplotoxis tenuifolia*	Leaves	72	121±0.05	84	140±0.001	ND	ND	5	231±0.0
Resedaceae	*Reseda lutea*	Flowers	36	149±0.004	84	490±0.05	ND	ND	ND	ND
		Leaves	70	403±0.015	ND*	ND	ND	ND	20	601±0.002

*ND: not determined

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