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In vitro Biological Activities of Different Extracts from *Alcea dissecta*

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ABSTRACT

Objective: *Alcea* genus belongs to Malvaceae family and this genus is represented by 85 taxa in the world and 21 taxa in the Flora of Turkey. The flowers of *Alcea* genus contain plenty of mucilage and are used for the treatment of respiratory diseases such as asthma and cough. *Alcea dissecta* is known as 'Govik, Hiro, Hero' in Turkey, and the flowers and leaves of this species have been used in the treatment of asthma, injury, colds, and gastrointestinal diseases in Turkey. To the best of our knowledge, there is no report on the effect of extraction methods on the biological activity of this plant. In addition, although this species is being used as a medical plant, there is no study of the antioxidant, anti-urease, esterase, and anticholinesterase activity of the plant. Therefore, the aim of this study was to evaluate *in vitro* antioxidant, anti-urease, esterase, anticholinesterase activities of *Alcea dissecta* using a variety of extracts.

Methods: The antioxidant activities of different extracts were examined by DPPH, ABTS, FRAP, and CUPRAC methods. The total phenolic compounds contained in the extracts were determined using the Folin-Ciocalteu reagent (FCR) method. Anti-urease and anticholinesterase activities of different extracts were evaluated by indophenol and Ellman methods respectively. In addition, esterase activities of plant extracts were determined.

Results: In the present study, ethanol:water (1:1, v/v) and chloroform extracts obtained maceration method showed stronger DPPH and ABTS radical scavenging activity than other extracts. The chloroform extract obtained Soxhlet method was found to have higher FRAP and CUPRAC values than other extracts. It was also found that the ethanol extract obtained maceration method showed the most potent anti-urease and anticholinesterase activity. According to the results, the strongest inhibitory effect on both hCA I and II isoenzymes was shown by the petroleum ether extract obtained Soxhlet method.

Conclusion: As a result, it was determined that different plant extracts have antioxidant, anti-urease, esterase, anticholinesterase activities. In addition, the data obtained from this study will shed light on future research on the biological activities of this species.

Keywords: *Alcea dissecta*, biological activity, medicinal plant

1. INTRODUCTION

Medical plants have gained considerable importance in the food and pharmaceutical industries due to their therapeutic properties (1). Phenolic and flavonoid compounds found in medicinal plants are known to be effective in the treatment of diseases caused by oxidative stress. Therefore, a great number of scientific studies on medicinal plants and extracts/compounds from these plants have increased during recent years (2). It is now known that gastric and duodenal ulcers are commonly caused by *Helicobacter pylori*. This organism releases urease that converts urea into ammonia. This enzyme produces an alkaline environment that makes it suitable for bacterial growth and the manifestation of the disease (3). For the past 20 years, the recommended first-line therapy for *H. pylori* eradication consisted of a combination of a proton pump cell inhibitor, omeprazole, and antibiotics including amoxicillin and clarithromycin. However, the increased *H. pylori* resistance of these antibiotics (especially clarithromycin) has made this treatment an unattractive option (4). In recent years, numerous studies have been carried out on the anti-urease activity of plant extracts, partially purified fractions, and natural compounds (5).

Alzheimer's disease is a chronic neurodegenerative disease that prevents memory, speech, problem-solving, and daily activities. One way to treat Alzheimer's disease is to inhibit

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the acetylcholinesterase (AChE) enzyme, which is responsible for ACh hydrolysis. In addition to Alzheimer's disease, AChE inhibition is considered promising for dementia, myasthenia gravis, glaucoma, and Parkinson's disease. The number of studies on the use of medicinal plants as an inhibitor of acetylcholinesterase, which is important in Alzheimer's disease, has increased in recent years (6, 7).

Carbonic anhydrases (CAs, EC 4.2.1.1) as ubiquitous widespread metalloenzymes catalyze the crucial reaction for all living organisms: the hydration process of CO_2 to HCO_3^- and H^+ (8, 9). Fifteen isoenzymes of CA encoded from the α-CA gene family have been identified and characterized for human beings as cytosolic forms (hCA I, II, III, VII, and XIII), membrane-bound forms (hCA IV, IX, XII, and XIV), mitochondrial forms (hCA VA and VB). hCA VI isoform was found in saliva. The three other ones (CA VIII, X, and XI) are determined as noncatalytic ones (10). Activation and inhibition investigations about CA catalytic activity are vital for treating many diseases and defects (11). The inhibitors of CA isoforms are carried out to discover and design drugs for some diseases such as glaucoma and epilepsy. Therefore, there is a great requirement in the pharmaceutical disciplines to develop new therapeutic agents (12, 13).

The selection and extraction method of solvent for the extraction of plant material to determine the potential activity of the extract is one of the most important factors since the solvent polarity and extraction method determines which compounds will be extracted and which will not. Thus, in many newly studied plants, various extracts are prepared using different extraction methods and solvents (2, 14).

Alcea genus belongs to Malvaceae family, and this genus is represented by 85 taxa in the world and 21 taxa in the Flora of Turkey (15, 16). *Alcea dissecta* is a perennial plant and mainly grows in calcareous fields and roadside areas in the eastern parts of Anatolia, North West Iraq, and Palestine (17). The Malvaceae family contains high amounts of polyphenol compounds, known as chemotaxonomic markers. Also, cyclopropane acids not found in other families are found in this family. The flowers of *Alcea* genus contain plenty of mucilage and are used to treat respiratory diseases such as asthma and cough (18). *Alcea dissecta* is known as 'Govik, Hiro, and Hero' in Turkey. The flowers and leaves of this species are used in the treatment of asthma, injury, cold and gastrointestinal diseases in Turkey (19-21).

To the best of our knowledge, there is no report on the effect of extraction methods on the biological activity of this plant. In addition, although this species has been used as a medical plant, there is no study of the antioxidant, anti-urease, esterase, and anticholinesterase activity of the plant. Therefore, the aim of this study was to evaluate in vitro antioxidant, anti-urease, esterase, anticholinesterase activities of *Alcea dissecta* using a variety of extracts.

2. METHODS

2.1. Identification of plant material

Alcea dissecta was collected from Tunceli province of Turkey and taxonomically identified by Dr. Ahmet DOĞAN. The voucher specimens, representative samples of the plant material, were archived in the herbarium of the Faculty of Pharmacy, Marmara University, and documented with the herbarium number of MARE:19141.

2.2. Preparation of Alcea dissecta extracts

Aerial parts of *Alcea dissecta* were dried at 25°C in the shade. Dried parts of the plant were treated with a mechanical grinder (Renas, RBT1250) for fine powder and proper weight. The two extraction methods were performed to gain crude extracts from the aerial parts of the plant. (i) Maceration: Plant powder (20 g) was extracted for 72 h with the use of petroleum ether (MP) (200 mL), chloroform (MC) (300 mL), ethanol (ME) (400 mL) and ethanol-water (1:1, *v/v*) (MEW) (400 mL). (ii) soxhlet extraction: 20 gram of plant powder was extracted in Soxhlet apparatus (300 mL) with petroleum ether (SP), chloroform (SC), ethanol (SE) and ethanol-water (1:1, *v/v*) (SEW). Eight different extracts from the plant were concentrated by rotary vacuum evaporator and lyophilized device. All obtained extracts were stored at 4°C for future analysis.

2.3. Quantification of total phenolic contents

Each extracts were prepared at 5 mg/mL concentrations. 5 µL sample from the extracts was taken in a tube and 225 µL distilled water was added on it. After this process, 5 µL Folin-Ciocalteu reagent was diluted with distilled water (1:3, *v/v*), and 15 µL of 2% sodium carbonate solution was added to the prepared mixture. Then the mixture was placed in a shaking water bath at room temperatures for 2 h, and the absorbance was determined against the reference molecule at 760 nm. 25 mg gallic acid was dissolved with water in a 25 mL flask, and a stock solution of 1 mg/mL was prepared. Then the working solutions at different concentrations (0.05-0.40 mg/mL) were prepared by diluting this stock solution with water. Gallic acid solutions prepared with 0.05-0.40 mg/mL concentrations were evaluated with Folin-Ciocalteu reagent to determine phenolic content. For the further procedures: (i) concentrations against the absorbance were plotted, (ii) a calibration curve was prepared, and (iii) the correct equation was obtained. The calibration equation for gallic acid is $A = 35.06x + 0.1214$ ($R^2 = 0.9966$). The total phenolic contents of the extracts were stated as mg gallic acid equivalents/g extract (22).

2.4. In vitro bioactivity assays

2.4.1. 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay

240 µL 0.1 mM DPPH solution (9.9 mg radical was dissolved in methanol in 250 mL flask) was added to 10 µL sample of the extracts at 5 mg/mL, 4 mg/mL, 3 mg/mL, 2 mg/mL, 1 mg/mL and 0.5 mg/mL concentrations. The prepared mixture was stirred for 1 minute and placed at 25°C for 30 min. The mixture absorbance was determined against the reference at 517 nm. The control sample was carried out under the same conditions using 10 µL of methanol instead of experimental and standard materials and the control sample was measured daily. The % DPPH radical scavenging activity was calculated by the formula:

$$\% \text{ DPPH radical inhibition} = [(A_0 - A_1)/A_0] \times 100]$$

A_0: The absorbance of the control solution

A_1: Absorbance of plant extracts and standard solutions.

IC_{50} is the extract/standard concentration that causes a fifty percent reduction in the DPPH radical concentration. The IC_{50} value was calculated using the correct equation obtained by placing the % radical scavenging activity against the concentrations studied. The data gained from the investigation was given as IC_{50}=mg/mL. The investigation was performed three times, and the averages of the data and standard deviation were calculated (23).

2.4.2. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\cdot+}$) scavenging assay

The 7 mM ABTS ammonium salt (0.38 g of ammonium salt was dissolved in a 100 mL flask with water) and reacted with 2.45 mM potassium peroxidisulfate (0.066 g potassium peroxidisulfate was dissolved in a 100 mL flask with water) for 12 hours at room conditions for preparing $ABTS^{\cdot+}$ stock solution. Then the $ABTS^{\cdot+}$ stock solution was diluted with water at 734 nm to prepare 0.70 (±0.02) absorbance working solution. 50 µL of extracts were prepared at 5 mg/mL concentrations, then 50 µL of $ABTS^{\cdot+}$ working solution and 150 µL distilled water were added to the prepared extracts. The mixture absorbance was determined against the reference at 734 nm for 6 min. The control sample was prepared under the same conditions with the use of 50 µL distilled water instead of experimental and standard materials. 10 mM stock trolox solution was prepared to obtain trolox standard curve equation. Then, working solutions with 1 mM, 0.8 mM, 0.6 mM, 0.5 mM, 0.4 mM and 0.2 mM were prepared by diluting the stock solution with 75 mM phosphate buffer (pH 7.4). The control sample was measured daily. ABTS radical scavenging determination was applied to trolox solutions prepared at different concentrations (0.2-1 mM). The calibration equation for trolox is A = 37.22x + 1.66 (R^2 = 0.9899). The results from this study were given as mM trolox/g extract (24).

2.4.3. Ferric reducing/antioxidant power (FRAP) assay

The method of Benzie and Strain (1996) was applied to the extracts in order to estimate the ferric reducing ability. The FRAP reagent [25 mL 300 mM acetate buffer (pH 3.6), 2.5 mL of TPTZ solution and 2.5 mL 20 mM $FeCl_3 \cdot 6H_2O$] was kept at 37°C for 30 min. 190 µL FRAP reagent was mixed with 10 µL extract, and the mixture absorbance was determined at 593 nm after 4 min. 1 mM stock $FeSO_4$. $7H_2O$ solution was prepared to obtain the $FeSO_4$ standard curve equation. Subsequently, working solutions of 0.4 mM, 0.2 mM, 0.1 mM, and 0.05 mM were prepared by diluting the stock solution with water. $FeSO_4$. $7H_2O$ solutions prepared with different concentrations were evaluated with FRAP method. For the further procedures: (i) concentrations against the absorbance were plotted, (ii) a calibration curve was prepared, and (iii) the correct equation was obtained. The calibration equation for Fe^{2+} is A = 12.86x – 0.006610 (R^2 = 0.9986). FRAP values of the extracts were given as mM Fe^{2+}/mg extract (25).

2.4.4. Cupric ion reducing/antioxidant power (CUPRAC) assay

60 µL Cu(II)x$2H_2O$, 60 µL neocuproine, and 60 µL NH4Ac (1 M) were mixed. Then 60 µL of the extract and 10 µL of ethanol were added to the mixture. After the duration time of 60 min, the mixture absorbance was spectrophotometrically measured at 450 nm. 1 mM stock trolox solution was prepared to obtain trolox standard curve equation. Then, working solutions with 1 mM, 0.8 mM, 0.6 mM, 0.4 mM, 0.2 mM and 0.1 mM were prepared by diluting the stock solution with ethanol. Trolox solutions prepared with different concentrations were evaluated with the CUPRAC method. For the further procedures: (i) concentrations against the absorbance were plotted, (ii) a calibration curve was prepared and (iii) the correct equation was obtained. The calibration equation for trolox is A = 3.055x + 0.2344 (R^2 = 0.9933). CUPRAC values of the extracts were given as mM trolox/mg extract (26).

2.4.5. Anti-urease activity assay

Stock solutions (5 mg/mL) were prepared from different extracts obtained from the plant and these solutions were diluted to prepare working solutions at concentrations of 2 mg/mL. Working solution (100 µL) was taken and then urease (500 µL) was added on it. The mixture was incubated at 37°C for 30 min. Then, 1100 µL of urea was added on this mixture and kept in the incubator at 37°C for 30 min. R1 (1% phenol, 0.005% sodium nitroprusside) and R2 (0.5% NaOH, 0.1% sodium hypochlorite) reagents were added to the mixture, respectively. After the incubation period at 37°C for 2 h, the absorbance of samples was measured at 635 nm (27).

The % inhibition of urease was calculated by the formula:

$$\% \text{ enzyme inhibition} = [(A_0 - A_1)/A_0] \times 100]$$

A_0: The absorbance of the control solution

A_1: Absorbance of plant extracts and standard solutions.

2.4.6. Anticholinesterase activity assay

Inhibition activities of acetylcholinesterase (AchE) were measured using a microplate reader (AMR-100 Allsheng). Acetylcholinesterase derived from electric fish and acetylthiokolin iodide was used as enzyme and substrate, respectively. Yellow-colored 5,5-dithiobis – (2-nitrobenzoic acid) (DTNB) was used for the measurement of the activity. As a control, ethanol and galantamine, the alkaloid type drug isolated from the galanthus plant, were used as controls.

AChE % Inhibition Test: AChE (20 µL) and different concentrations of extracts (20 µL) were added to phosphate buffer solution (pH 8, 0.1 M, 40 µL). This mixture was incubated at 25°C for 10 min. After incubation, DTNB (100 µL) and AcI (20 µL) as substrate were added to the mixture. The same procedure was applied to the galantamine used as standard. 5-thio-2-nitrobenzoic acid was spectrophotometrically measured at 412 nm. Anticholinesterase activity of the extracts was calculated using the following equation as percentage inhibition relative to control (28).

% inhibition = (Acontrol – Asample/Acontrol)x100

2.4.7. Esterase activity assay

The method was carried out to assay the esterase activity of hCA I and II (29). Changes of absorbance in 4-nitrophenyl acetate (NPA) to 4-nitrophenylate ion were spectrophotometrically recorded at 348 nm at 25°C for 3 min (30). Before kinetic studies, the reference measurement without enzyme was carried out, and then bioactivities of the extracts were investigated. Each concentration of the extracts was examined three times. % Activity (%) – [Inhibitor] graphs were determined for the extracts (31).

2.5. Statistical analysis

The antioxidant, anticholinesterase and anti-urease experiments were done in triplicates, and all data were shown as mean ± SD. The data were analyzed by Graphpad Prism 5 program. Statistical differences between the experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison test. Mean values were considered statistically significant when $p<0.05$.

3. RESULTS

3.1. In vitro evaluation of biological activities

3.1.1. Total phenolic contents

The total phenolic contents of different extracts were analyzed and presented in Table 1. The ethanol:water (1:1, *v/v*) and chloroform extracts prepared using the Soxhlet method were determined to contain higher phenolic contents than other extracts. In addition, chloroform and ethanol extracts obtained from the maceration method exhibited higher total phenolic contents than other extracts. When we compare extraction techniques and extracts, it was found that the maceration chloroform and ethanol extracts had the highest amount of phenolic content and maceration was the best method for total phenolic contents.

Table 1. *Total phenolic contents of different extracts from Alcea dissecta.*

Samples	Phenolics (mg GAE/g extract)	
	Soxhlet	Maceration
Petroleum ether	4.5±0.2	4.4±0.11
Chloroform	4.9±0.01	12.8±0.12
Ethanol	3.3±0.42	9.8±0.2
Ethanol:water (1:1, *v/v*)	5.8±0.7	4.7±0.3

Values are mean of triplicate determination (n = 3) ± standard deviation; GAE–Gallic acid equivalents.

3.1.2. In vitro antioxidant activity

The antioxidant activities of different extracts of the plant were shown in Table 2. The maceration ethanol:water (1:1, *v/v*) (IC_{50}: 0.09 mg/mL) and Soxhlet (IC_{50}: 0.13 mg/mL) extracts showed the strongest DPPH free radical scavenging activity. The petroleum ether extracts obtained from two extraction methods showed the lowest DPPH free radical scavenging activity. As shown in Table 2, the radical scavenging DPPH activities of all extracts showed lower than that of ascorbic acid (IC_{50}: 0.004 mg/mL) and butylated hydroxyanisole (BHA) (IC_{50}: 0.006 mg/mL).

The chloroform, ethanol, and ethanol:water extracts obtained from the Soxhlet method were found to have very close ABTS radical cation scavenging activity each other. When the results of all extracts were compared, it found that maceration chloroform extract (23.4 mM trolox/g extract) exhibited the highest ABTS radical cation scavenging activity. The petroleum ether extracts prepared using two extraction methods did not show ABTS radical cation scavenging activity in this study.

In the Soxhlet method, chloroform extract (0.47 mM Fe^{2+}/mg extract) showed stronger ferric reducing activity than other extracts. In maceration method, ethanol:water (1:1, *v/v*) extract (0.42 mM Fe^{2+}/mg extract) showed the highest ferric reducing activity. The petroleum ether extracts obtained from two extraction methods had the lowest ferric reducing activity. When the results of all extracts were compared, it was found that all extracts prepared by Soxhlet and maceration method exhibited close ferric reducing activity.

In Soxhlet method, chloroform (0.092 mM trolox/mg extract) and ethanol:water (1:1, *v/v*) (0.084 mM trolox/mg extract) extracts showed stronger cupric reducing antioxidant activity than other extracts. In maceration method, ethanol:water (0.075 mM trolox/mg extract) and chloroform (0.057 mM trolox/mg extract) extracts

exhibited the highest cupric reducing antioxidant activity. The petroleum ether extracts obtained from two extraction methods had the lowest cupric reducing antioxidant activity. When the results of the CUPRAC assay were examined, the all extracts showed lower cupric reducing antioxidant activity than BHA compounds.

The results obtained from this study showed that maceration and Soxhlet extraction techniques are the most suitable method to get the most powerful DPPH/ABTS and FRAP/CUPRAC activities. It was also found that chloroform was the most suitable solvent for obtaining high ABTS, FRAP, and CUPRAC values.

Table 2. *Effects of extracting solvents/methods on the antioxidant activity of Alcea dissecta extracts.*

Samples	DPPH (IC_{50}: mg/mL)		ABTS (mM trolox/g extract)		FRAP assay (mM Fe^{2+}/mg extract)		CUPRAC assay (mM trolox/mg extract)	
	Soxhlet	Maceration	Soxhlet	Maceration	Soxhlet	Maceration	Soxhlet	Maceration
Petroleum ether	0.61±0.02*	0.65±0.05*	NA	NA	0.09±0.01	0.08±0.03*	0.025±0.05*	0.020±0.02*
Chloroform	0.24±0.01*	0.41±0.09*	2.6±0.1*	23.4±0.6*	0.47±0.02*	0.26±0.02*	0.092±0.03*	0.057±0.07*
Ethanol	0.19±0.03*	0.47±0.01*	2.7±0.2*	1.3±0.3*	0.22±0.03*	0.15±0.04*	0.038±0.02*	0.033±0.05*
Ethanol:water (1:1, *v/v*)	0.13±0.01*	0.09±0.03*	2.8±0.3*	2.6±0.5*	0.32±0.04*	0.42±0.01*	0.084±0.07*	0.075±0.01*
Ascorbic acid	0.004±0.9	0.004±0.9	13±0.1*	13±0.1*				
BHT					1.1±0.12	1.1±0.12		
BHA	0.006±0.062	0.006±0.062					1.62±0.12	1.62±0.12

*BHA: butylated hydroxyanisole; DPPH: 2,2-diphenyl-1-picrylhydrazyl; CUPRAC: cupric ion reducing/antioxidant power; FRAP: ferric reducing antioxidant power; ABTS: 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid); BHT: butylated hydroxytoluene; NA: not activity; Values are mean of triplicate determination (n = 3) ± standard deviation; * P < 0.05 compared with the positive control*

3.1.3. Anti-urease activity

The percentage of inhibition of urease enzyme at 12.5 µg/mL concentration of different extracts was determined by using the indophenol method, and the results were shown in Table 3. In Soxhlet method, ethanol:water (1:1, *v/v*) (21.66%) and ethanol (7.91%) extracts showed stronger anti-urease activity than other extracts. In maceration method, ethanol (38.59%) and ethanol:water (1:1, *v/v*) (19.87%) extracts exhibited the highest anti-urease activity. The petroleum ether and chloroform extracts obtained from Soxhlet and maceration methods did not show anti-urease activity in this study. Comparing the activity results of all the extracts, it was found that maceration ethanol extract had the strongest anti-urease activity, and all extracts showed lower activity than thiourea compounds (76.05%). In the present study, the maceration ethanol method/solvent was the most suitable solvent and method to get the strongest anti-urease activity.

Table 3. *Urease inhibitory activity of different extracts from Alcea dissecta.*

Samples	Urease inhibition (%) (12.5 µg/mL)	
	Soxhlet	Maceration
Petroleum ether	NA	NA
Chloroform	NA	NA
Ethanol	7.91±2.04*	38.59±0.7*
Ethanol:water (1:1, *v/v*)	21.66±0.5*	19.87±0.34*
Thiourea	76.05±0.60	76.05±0.60

*Values are mean of triplicate determination (n = 3) ± standard deviation; * P < 0.05 compared with the positive control; NA: not activity*

3.1.4. Anti-cholinesterase activity

The percentage of cholinesterase enzyme inhibition at 500 µg/mL concentration of different extracts was determined by using the Ellman method, and the results are shown in Table 4. In the Soxhlet method, ethanol:water (1:1, *v/v*) (80.89%) and ethanol (73.81%) extracts exhibited a higher percentage of inhibition of cholinesterase enzyme than other extracts. In maceration method, ethanol (94.66%) and ethanol:water (1:1, *v/v*) (69.51%) extracts showed the strongest anticholinesterase activity. The Soxhlet petroleum ether and maceration petroleum ether and chloroform extracts did not show cholinesterase inhibitory activity. When the results were compared, it was determined that the maceration ethanol and Soxhlet ethanol:water (1:1, *v/v*) extracts had anticholinesterase activity very close to the galantamine compound (85.10%, 200 µg/mL). In the present study, maceration ethanol methods/solvents were the most suitable solvent and method to get the strongest anticholinesterase activity.

Table 4. *Anticholinesterase activity of different extracts from Alcea dissecta.*

Samples	Enzyme inhibition (%) (500 µg/mL)	
	Soxhlet	Maceration
Petroleum ether	NA	NA
Chloroform	33.78±2.08*	NA
Ethanol	73.81±0.77*	94.66±0.59*
Ethanol:water (1:1, *v/v*)	80.89±2.36*	69.51±0.92*
Galanthamine (200 µg/mL)	85.10±0.14	85.10±0.14

*Values are mean of triplicate determination (n = 3) ± standard deviation; * P < 0.05 compared with the positive control; NA: not activity*

3.1.5. Esterase Activity

It has been reported that carbonic anhydrase inhibitors have potential usages in eye disorders treatment, osteoporosis, diuretic, anti-obesity, and anticancer agents. It was reported that CA activity in rat erythrocytes was significantly decreased exposed to naringenin, a natural flavone. In addition, it is known that the extracts containing polyphenols and flavones obtained from various plants and fungal species inhibit the activities of CA I and II (9). Inhibitory effects of the different extracts from *Alcea dissecta* on hCA I and II were determined for the first time in this study. Bioactivities of the extracts against hCA I and II were performed using the esterase activity method. In the maceration method, petroleum ether extract showed a strong inhibitory effect on both hCA I (IC_{50}: 0.008 mg/mL) and II (IC_{50}: 0.025 mg/mL) isoenzymes. The chloroform extract did not show an inhibitory effect on hCA II, but this extract showed a strong inhibitory effect on hCA I (IC_{50}: 0.01 mg/mL) enzyme. In addition, ethanol and ethanol:water (1:1, *v/v*) extracts did not show an inhibitory effect on both hCA I and II isoenzymes. In Soxhlet methods, petroleum ether extract showed a strong inhibitory effect on both hCA I (IC_{50}: 0.003 mg/mL) and II (IC_{50}: 0.015 mg/mL) isoenzymes. The chloroform extract did not show an inhibitory effect on hCA II, but this extract showed a strong inhibitory effect on hCA I (IC_{50}: 0.028 mg/mL) enzyme. The ethanol:water (1:1, *v/v*) extract exhibited moderate inhibitory effect on both hCA I (IC_{50}: 0.105 mg/mL) and II (IC_{50}: 0.512 mg/mL) isoenzymes. In addition, ethanol extract did not show an inhibitory effect on both hCA I and II isoenzymes. According to the results, the strongest inhibitory effect on both hCA I and II isoenzymes was shown by Soxhlet petroleum ether extract. The effects of different extracts on the hCA isoenzymes are summarized in Table 5.

Table 5. *Esterase activity of different extracts from Alcea dissecta on hCA I and II*

Samples	IC_{50} (mg/mL)	
	hCA I	hCA II
Maceration petroleum ether	0.008	0.025
Maceration chloroform	0.01	NA
Maceration ethanol	NA	NA
Maceration ethanol:water (1:1, *v/v*)	NA	NA
Soxhlet petroleum ether	0.003	0.015
Soxhlet chloroform	0.028	NA
Soxhlet ethanol	NA	NA
Soxhlet ethanol:water (1:1, *v/v*)	0.105	0.512

Values are mean of triplicate determination (n = 3) ± standard deviation; NA: not activity

4. DISCUSSION

Some reports on the biological activities of some *Alcea* species have been previously presented. Antioxidant and antimicrobial activities of dichloromethane, methanol, and water extracts from aerial parts (leaves, flowers, stems, roots) of *Alcea setosa* were investigated. According to the results, it was found that methanol extract from the leaves of the plant had higher DPPH radical scavenging (1 mg/mL, 72%) activity than other extracts. It was also found that these extracts did not show antimicrobial activity against *Pseudomonas aeruginosa* CMUL 241, *Escherichia coli* CMUL 577, *Staphylococcus aureus* CMUL 491, *Candida albicans* ATCC 10231 strains (32). In another study, it was found that methanol extract from *Alcea rosea* aerial parts showed strong and selective cytotoxic activity on HeLA (IC_{50}: 14.48 µg/mL) cells and C6 cell lines (37.63 µg/mL) (33). As a result of the *in vivo* experiment of water extract from flowers of *Alcea aucheri*, this extract has been disclosed to have strong anxiolytic and sedative properties (34). The antioxidant activities of flower, seed, and leaves methanol extracts from *Alcea hyrcana* were investigated by DPPH, nitric oxide, hydrogen peroxide, ferrous chelating, reducing power, and hemoglobin induced linoleic acid methods. In this study, leaves extract showed strong ferrous chelating (IC_{50}: 0.11 mg/mL) and nitric oxide (IC_{50}: 0.45 mg/mL) radical activity as well as seed extracts were found to have strong DPPH (IC_{50}: 421 µg/mL) and hydrogen peroxide (IC_{50}: 160.6 µg/mL) activity including, total phenolic contents (68.9 mg gallic acid/g extract) (35).

Total phenolic content and antioxidant activity of methanol extract from flowers of *Alcea pallida* were investigated, and the amount of phenolic content of the flower extract was determined as 2.82 mg GAE/g extract. In addition, this extract showed strong ABTS (83.68 µmol trolox/g extract) radical cation scavenging activity (36).

Unlike the above studies, to date, there have been no reports in the literature on the antioxidant, esterase, anti-urease, and anticholinesterase activity of *Alcea dissecta*. Therefore, the purpose of this study was to evaluate for the first time *in vitro* biological activity of this species' extracts obtained using different extraction methods. When the biological activities of *Alcea dissecta* plant were compared with other *Alcea* species, as in other species, it was found that polar (especially the ethanol: water and ethanol) solvent has been found to exhibit strong biological activity and total phenolic contents.

5. CONCLUSION

It is important to select an appropriate extraction technique to standardize plant products and phytochemical analysis. When different extraction methods and different solvents are used, different compounds are obtained, and in this case, the biological activity of the plants changes. In this study, *in vitro* antioxidant, esterase, anti-urease and anticholinesterase activities of different extracts from *A. dissecta* aerial parts were first time investigated. According to the results of this study, maceration ethanol:water (1:1, *v /v*) and chloroform extracts showed stronger DPPH and ABTS radical scavenging activity than other extracts. The soxhlet chloroform extract was found to have higher FRAP and CUPRAC values than other extracts. It was also found that the maceration ethanol extract showed the most potent anti-urease and

anticholinesterase activity. According to the results, the strongest inhibitory effect on both hCA I and II isoenzymes was shown by Soxhlet petroleum ether extract. Therefore, different extracts from this species may be a natural resource candidate for the pharmaceutical and food industry due to their antioxidant, anticholinesterase, anti-urease, esterase activities.

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