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Antioxidant, Cytotoxic, and Enzyme Inhibitory Activities of *Agropyron repens* and *Crataegus monogyna* Species

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ABSTRACT

Objective: The aim of this study was to investigate antioxidant, enzyme inhibitory and cytotoxic activities of *Agropyron repens* and *Crataegus monogyna* methanol extracts with total phenolic and flavonoid contents.

Materials and Methods: Total phenolic and flavonoid contents of *A. repens* and *C. monogyna* methanol extracts were measured according to Folin Ciocalteu and aluminum nitrate methods, respectively. Antioxidant and enzyme inhibitory activities of the methanol extracts were tested spectrophotometrically. Also, cytotoxic activities of the methanol extracts against DLD-1 and CCD-18Co were investigated by using Alamar Blue assay.

Results: *C. monogyna* methanol extract with the highest total phenolic and flavonoid contents (68.13±0.34 µg GAEs/mg extract and 36.91±0.17 µg QEs/mg extract, respectively) had the best antioxidant activity in β-carotene-linoleic acid (IC_{50}: 32.72±0.15 µg/mL), CUPRAC ($A_{0.50}$: 282.69±0.25 µg/mL), $DPPH^{\cdot}$ (IC_{50}: 71.69±0.85 µg/mL), and $ABTS^{\cdot+}$ (IC_{50}: 40.43±0.55 µg/mL) assays. *A. repens* methanol extract showed the highest effect against AChE (18.73±0.47 %), BChE (37.59±1.07 %), urease (89.18±0.84%), α-glucosidase (6.71±0.23 %), whereas *C. monogyna* methanol extract showed the highest effect against tyrosinase (30.52±1.00%) and α-amylase (37.24±0.06 %). Also, *A. repens* (IC_{50}: 57.38 µg/mL) and *C. monogyna* (IC_{50}: 54.04 µg/mL) methanol extracts showed close cytotoxic activity on DLD-1.

Conclusion: Antioxidant, cytotoxic, and enzyme inhibitory activities of *A. repens* and *C. monogyna* methanol extracts were investigated with total phenolic and flavonoid contents in this study. The results obtained with this study strengthen the potential of the studied plants as a new source for the discovery of antioxidant, cytotoxic, and enzyme inhibitor agents.

Keywords: *Agropyron repens, Crataegus monogyna*, antioxidant activity, cytotoxic activity, enzyme inhibitory activity

INTRODUCTION

Pharmacologically, medicinal plants have always been at the forefront of almost all civilizations. Medicinal plants are used to treat diseases and prevent possible epidemics, and additionally to flavor and to preserve foods. Also, medicinal plants are considered as rich sources of traditional medicines, and most synthetic medicines are produced from these plants. Secondary metabolites produced by plants are generally responsible for the biological properties of plant species used worldwide (1,2). Compounds such as alkaloids, tannins, flavonoids, and phenolics found in plants are therapeutic for human health (3,4).

Agropyron species is a member of Poaceae. *Agropyron repens* (Quack grass) is known as 'Ayrık otu' in Turkey. It is often used in folk medicine as a diuretic in prostate disease, urinary infections, as well as calming of spasms and pain in the urinary tract (5). *A. repens* has been reported

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to be used in Bulgarian traditional medicine as antitussive, anti-inflammatory, and diuretic; in Kosovo traditional medicine as antirheumatic and antianemic; in Turkey traditional medicine to treat treatment of kidney stones and gastrointestinal diseases (6-8). *A. repens* was previously determined to contain phenol compounds, carbohydrates, pectins, saponins and to have anti-inflammatory, antiadhesive, and diuretic effects (9-11).

Crataegus species is a member of Rosaceae and widely grown in Europe, America, and Asia. The genus *Crataegus* consists of 200 species around the world and represented by 21 species in Turkey. *Crataegus* (Hawthorn) is known as 'Alıç' in Turkey (12). *Crataegus* (hawthorn) species are widely used in folk medicine in the therapy of diseases such as congestive heart failure, angina, hypertension, arrhythmia. *Crataegus* species have been reported to be used in traditional Chinese medicine to remove blood stasis, improve circulation, treat diarrhea, indigestion, hyperlipidemia, hypertension and abdominal pain; in European traditional medicine in the therapy of heart problems in associated with their antiatherosclerotic, cardiotonic, antispasmodic, and hypotensive properties; in Turkey traditional medicine as a diuretic agent for the treatment of intestinal disorders (13). It has been reported that *Crataegus* species indicated immunostimulant, radical scavenging, antiviral, anti-lipoperoxidant, antimicrobial, anti-inflammatory, antihyperlipidemic, hepatoprotective, gastroprotective, and hypoglycemic activities in relation to containing phenolic compounds, proanthocyanins, triterpenoids, and flavonoid glycosides (12,14).

Investigating the effects of medicinal plants on health is important for the discovery or design of new drugs, and studies in this area have been increasing in recent years. Therefore, the aim of this study is to investigate the antioxidant, cytotoxic, and enzyme inhibitory activities of *A. repens* and *Crataegus monogyna* methanol extracts with total phenolic and flavonoid contents.

MATERIALS AND METHODS

Plant Materials

A. repens and *C. monogyna* were collected from Konya, Turkey in 2017. The plant species were identified by Dr. Ergün Kaya at Muğla Sıtkı Koçman University, Muğla, Turkey. The voucher specimen has been deposited at Plant Molecular Genetics and Biotechnology Laboratory, Department of Molecular Biology and Genetics, Muğla Sıtkı Koçman University with voucher no EK.1688 (for *A. repens*) and EK.1687 (for *C. monogyna*).

Extraction

The aerial parts of *A. repens* and *C. monogyna* were extracted with methanol at room temperature for 24 h and four times. Solvent was evaporated under vacuum by an evaporator to obtain the methanol extracts. All extracts were stored at +4°C until analysis.

Instruments

Antioxidant and enzyme inhibitory tests were measured by using a 96-well microplate reader, SpectraMax 340PC384 (Molecular Devices, Silicon Valley, California, USA). Softmax PRO v5.2 software (Molecular Devices, Silicon Valley) was used to calculate and measure the bioactivity data. A 96-well microplate reader (MultiskanGo, Thermo Scientific Co., MA, USA) was used to analyze cytotoxic activity studies. Cytotoxic activity results were measured and calculated by using GraphPad Prism (GraphPad Software v5.0, USA).

Total Phenolic and Flavonoid Contents

The phenolic contents of extracts were tested based on the method reported by Slinkard and Singleton (15). Results were given as a microgram of gallic equivalents (GAEs) using the following equation that was obtained from standard gallic acid graph:

Absorbance=0.0104[gallic acid (µg)] - 0.0263 (r^2, 0.9974)

Total flavonoid contents of extracts were measured by using the aluminum nitrate method (16). Results were given as microgram quercetin equivalents (QEs) using the following equation that was obtained from standard quercetin acid graph:

Absorbance=0.0158[quercetin (µg)] - 0.0306 (r^2, 0.9993)

Antioxidant Activity

β-carotene-linoleic acid, metal chelating, cupric reducing antioxidant capacity (CUPRAC), 1,1-diphenyl-2-picrylhydrazyl free radical ($DPPH^{\cdot}$), and (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt cation radical ($ABTS^{\cdot+}$) scavencing assays were performed for measurement of antioxidant activities of the extracts (17). The graph of the inhibition percentage (%) versus the concentration (µg/mL) was used to calculate the IC_{50} values of the extracts. The graph of the absorbance versus the concentration (µg/mL) was used to calculate 0.50 absorbance ($A_{0.5}$) values of the extracts. The antioxidant activity results were stated as 50 % inhibition concentration (IC_{50}) for β-carotene-linoleic acid, ABTS and DPPH scavenging, inhibition percentage (%) at 400 µg/mL concentration for metal chelating assay and $A_{0.50}$ which corresponds to the concentration producing 0.500 absorbance for CUPRAC assay.

Enzyme Inhibitory Activity

Acetylcholinesterase (AChE), butyrylcholinesterase (BChE), urease, and tyrosinase inhibitory activities of the extracts were carried out as reported in our previous study (18). α-Amylase and α-glucosidase inhibitory activities were screened according to the method previously reported by Deveci et al. (19). Galantamine, Kojic acid, Thiourea and Acarbose were used as standards. The enzyme inhibitory activity results were stated as IC_{50} and inhibition percentages (%).

Cell Culture

DLD-1 (colorectal cancer), and CCD-18Co (human colon fibroblast cell line) were cultivated in RPMI-1640 and EMEM growth mediums (ATCC, Virginia, USA), respectively and incubated with 1% penicillin/streptomycin, 10% fetal bovine serum (FBS), 2 mM L-glutamine (Sigma, St. Louis, Missouri, USA) in 5% CO_2 at 37°C and 90-95 % humidity.

Cell Viability Assay

$1x10^4$ cells were put into 96-well plate with growth medium and incubated in 5% CO_2 at 37°C for 24h until attached to the

bottom. Then, different concentrations (between 1 µg/mL and 1000 µg/mL) of the extracts were added to each well. Viability and proliferation of the cells were tested according to the previously described Alamar Blue assay (20). The results were measured by using 96-well microplate reader at 570 nm and 610 nm. The sigmoidal plot of the inhibition rate (%) versus the log concentration (µg/mL) was used to calculate the IC_{50} values of the extracts.

Statistical Analysis

Antioxidant, cytotoxic, and enzyme inhibitory activity results were the average of three parallel sample measurements. The data were registered as the mean ± S.E.M.

RESULTS

Total Phenolic and Flavonoid Contents

Total phenolic and flavonoid contents of *A. repens* and *C. monogyna* methanol extracts were measured according to Folin Ciocalteu and aluminum nitrate methods, respectively. Total phenolic contents of *A. repens* and *C. monogyna* methanol extracts were calculated as 24.57±0.22 and 68.13±0.34 µg GAEs/mg extract. Total flavonoid contents of *A. repens* and *C. monogyna* methanol extracts were recorded as 9.31±0.41 and 36.91±0.17 µg QEs/mg extract (Table 1).

Antioxidant Activity

Antioxidants have different mechanisms of action, so more than one method is need to be used to test antioxidant properties. Therefore, antioxidant activities of *A. repens* and *C. monogyna* methanol extracts were screened by using five different assays, namely, β-carotene-linoleic acid, metal chelating, CUPRAC, scavenging of ABTS cation radical and DPPH free radical assays and results are summarized in Table 2.

β-carotene-linoleic acid method is an important test system that demonstrates the ability of antioxidant compounds to inhibit linoleic acid oxidation. The degree of the bleaching caused

Table 1. Total phenolic and flavonoid contents of the extracts[a].

	Total phenolic content (µg GAEs/mg extract)[b]	Total flavonoid contents (µg QEs/mg extract)[c]
***A. repens* methanol**	24.57±0.22	9.31±0.41
***C. monogyna* methanol**	68.13±0.34	36.91±0.17

[a] Values represent the means ± SEM of three parallel sample measurements (n=3) analyzed 3 times. T test was used to determine significant differences between means, *p* values *<0.05* were regarded as significant.
[a] Values expressed are means ± S.E.M. of three parallel measurements *(p<0.05)*.
[b] GAEs, gallic acid equivalents.
[c] QEs, quercetin equivalents.

Table 2. Antioxidant activities of the extracts.

	Antioxidant Activity				
	β-carotene-linoleic acid assay	$DPPH^{\cdot}$ assay	$ABTS^{\cdot+}$ assay	CUPRAC assay	Metal chelating assay
Extracts	IC_{50} (µg/mL)[a]	IC_{50} (µg/mL)[a]	IC_{50} (µg/mL)[a]	$A_{0.50}$ (µg/mL)[b]	Inhibition (%)[c]
***A. repens* methanol**	77.62±0.09	>400	127.78±0.99	>400	13.09±0.99
***C. monogyna* methanol**	32.72±0.15	71.69±0.85	40.43±0.55	282.69±0.25	NA[e]
α-Tocopherol[d]	2.10±0.08	37.18±0.41	38.51±0.54	66.72±0.81	NT[f]
BHA[d]	1.34±0.04	19.80±0.36	11.82±0.09	24.40±0.69	NT[f]
EDTA[d]	NT[f]	NT[f]	NT[f]	NT[f]	95.20±0.13

[a]: IC_{50} values represent the means ± SEM of three parallel measurements (n=3) analyzed 3 times. T test was used to determine significant differences between means, *p* values *<0.05* were regarded as significant.
[b]: $A_{0.50}$ values represent the means ± SEM of three parallel measurements *(p<0.05)*.
[c]: Inhibition % of 400 µg/mL concentration of the extracts.
[d]: Standards
[e]: NA: Not active.
[f]: NT: Not tested.

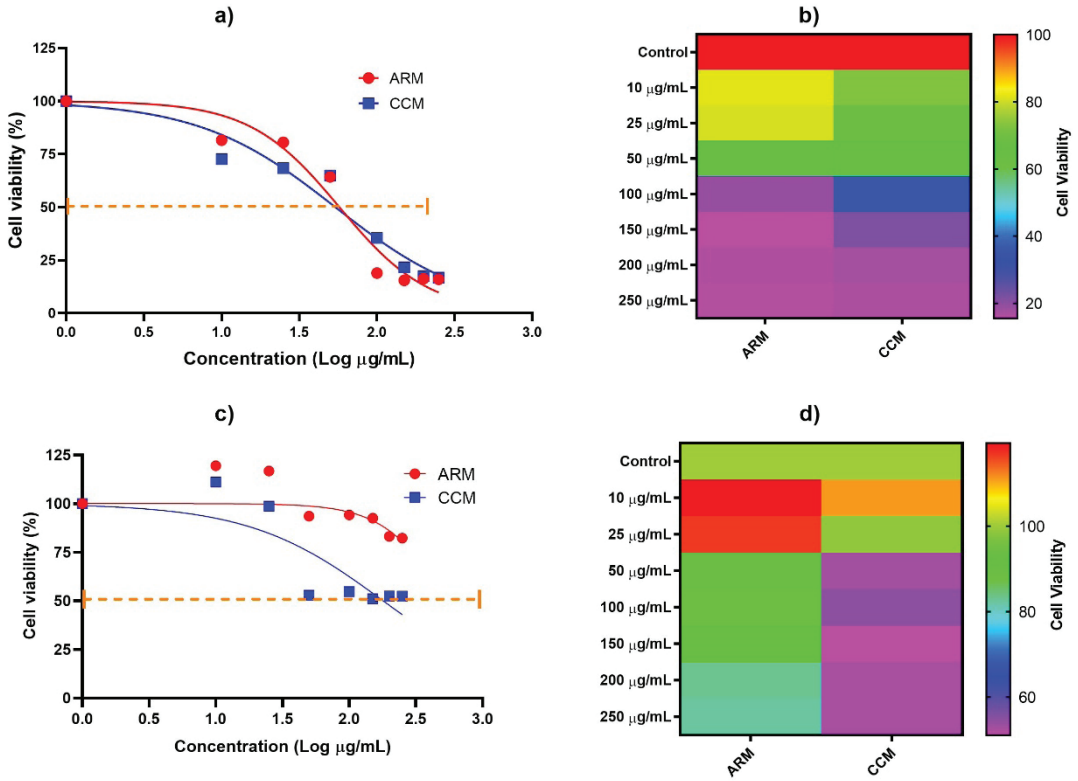

Figure 1. Cytotoxic effects of *A. repens* and *C. monogyna* methanol extracts on DLD-1 and CCD-18Co a) IC_{50} values on DLD-1 b) Heat Map analyses of dose-dependent inhibition against DLD-1 cells. Cell viability decreased from red to pink color c) IC_{50} values on CCD-18Co d) Heat Map analyses of dose-dependent inhibition against CCD-18Co. Cell viability decreased from green to pink color. ARM: *A. repens* methanol extract, CMM: *C. monogyna* methanol extract.

by lipid peroxyl radicals formed in the method in the color of β-carotene is inhibited by antioxidant compounds are tested. IC_{50} values of *A. repens* and *C. monogyna* methanol extracts were found as 77.62±0.09 and 32.72±0.15 µg/mL in the β-carotene-linoleic acid assay.

$ABTS^{\cdot+}$ and $DPPH^{\cdot}$ radicals are the most widely used radicals in determining of radical scavenging activities. As it is seen in Table 2, the best scavenging activities on $ABTS^{\cdot+}$ (IC_{50}: 40.43±0.55 µg/mL) and $DPPH^{\cdot}$ (IC_{50}: 71.69±0.85 µg/mL) radicals were observed in *C. monogyna* methanol extract. Also, *C. monogyna* methanol extract indicated near-standard activity in $ABTS^{\cdot+}$ assay.

The reducing power is an important indicator to evaluate antioxidant activity and the electron donation capabilities of the methanol extracts were determined by using the CUPRAC method. When compared to the standards, both methanol extracts showed low cupric reducing power.

Transition metals accumulate in the body at high rates, contributing to oxidative damage and thus causing various abnormalities. Therefore, metal chelating activity is of great importance in explaining of antioxidant activity. When *A. repens* methanol extract exhibited low metal chelating activity with an inhibition value of 13.09±0.99 % at 400 µg/mL concentration, *C. monogyna* methanol extract showed no activity.

Table 3. Cytotoxic activities of the extracts.

	DLD-1	CCD-18Co
Extracts	**IC_{50} (µg/mL)**	**IC_{50} (µg/mL)**
***A. repens* methanol**	57.38	543.30
***C. monogyna* methanol**	54.04	179.60

Cytotoxic Activity

Cytotoxic activities of *A. repens* and *C. monogyna* methanol extracts were tested on DLD-1 (colorectal cancer) and CCD-18Co (human colon fibroblast cell line) according to Alamar Blue assay. Figure 1 represents the cytotoxic effects of the methanol extracts on DLD-1 and CCD-18Co. Table 3 shows the calculated IC_{50} values of the methanol extracts. As seen in Figure 1a and 1c, the methanol extracts inhibited the viability of DLD-1 and CCD-18Co dose-dependently. *A. repens* (IC_{50}: 57.38 µg/mL) and *C. monogyna* (IC_{50}: 54.04 µg/mL) methanol extracts showed similar cytotoxic activity on DLD-1.

Table 4. Enzyme inhibitory activities of the extracts[a].

Extracts	AChE		BChE		Tyrosinase		Urease		α-Amylase		α-Glucosidase	
	Inhibition (%)[b]	IC_{50} (µg/mL)	Inhibition (%)[b]	IC_{50} (µg/mL)	Inhibition (%)[b]	IC_{50} (µg/mL)	Inhibition (%)[b]	IC_{50} (µg/mL)	Inhibition (%)[c]	IC_{50} (µg/mL)	Inhibition (%)[d]	IC_{50} (µg/mL)
***A. repens* methanol**	18.73±0.47	>200	37.59±1.07	>200	NA[g]	>200	89.18±0.84	24.85±0.08	33.39±0.92	>500	6.71±0.23	>500
***C. monogyna* methanol**	10.92±0.66	>200	22.43±0.60	>200	30.52±1.00	>200	NA[g]	>200	37.24±0.06	>500	4.76±0.48	>500
Galantamine[e]	80.41±0.98	4.31±0.03	82.23±0.37	12.29±0.06	NT[f]	NT[f]	NT[f]	NT[f]	NT[f]	NT[f]	NT[f]	NT[f]
Kojic acid[e]	NT[f]	NT[f]	NT[f]	NT[f]	83.6±0.20	144.91±0.19	NT[f]	NT[f]	NT[f]	NT[f]	NT[f]	NT[f]
Thiourea[e]	NT[f]	NT[f]	NT[f]	NT[f]	NT[f]	NT[f]	78.93±0.18	7.87±0.18	NT[f]	NT[f]	NT[f]	NT[f]
Acarbose[e]	NT[f]	NT[f]	NT[f]	NT[f]	NT[f]	NT[f]	NT[f]	NT[f]	94.01±0.09	21.63±0.01	31.95±0.62	378.66±0.14

[a] Values represent the means ± SEM of three parallel sample measurements (n=3) analyzed 3 times. T test was used to determine significant differences between means, *p* values *<0.05* were regarded as significant.
[b]: Inhibition % of 200 µg/mL concentration of the plant extracts. [c]: Inhibition % of 500 µg/mL concentration of the plant extracts.
[d]: Inhibition % of 250 µg/mL concentration of the plant extracts. [e]: Standards. [f]: NT: not tested. [g]: NA: not active. AChE: Acetylcholinesterase, BChE: Butyrylcholinesterase.

Enzyme Inhibitory Activity

Cholinesterase inhibitory activities of *A. repens* and *C. monogyna* methanol extracts were screened according to the Ellman method and the results are given in Table 4. *A. repens* displayed the best inhibitory activity against AChE (18.73±0.47 %) and BChE (37.59±1.07 %) at 200 µg/mL concentration.

Dopachrome method was used to test tyrosinase inhibitory activities of *A. repens* and *C. monogyna* methanol extracts. As it is given in Table 4, *C. monogyna* methanol extract showed low inhibitory activity against tyrosinase while *A. repens* methanol extract exhibited no activity.

Indophenol method was used for the measurement of urease inhibitory activity of *A. repens* and *C. monogyna* methanol extracts and results are summarized in Table 4. *A. repens* methanol extract (89.18±0.84 %) was found as better urease inhibitor by comparison with thiourea (78.93±0.18 %) at 200 µg/mL concentration.

Antidiabetic activities of *A. repens* and *C. monogyna* methanol extracts on α-amylase and α-glucosidase were determined. As it presented in Table 4, the highest α-amylase inhibitory activity was found in *C. monogyna* methanol extract (37.24±0.06 % at 500 µg/mL concentration) while the best α-glucosidase inhibitory activity was observed in *A. repens* methanol extract (6.71±0.23 % at 250 µg/mL concentration).

DISCUSSION

Medicinal plants, besides being used as taste, color, aroma and preservatives in foods for centuries, are excellent sources of natural antioxidants, and their bioactive compounds, especially phenolic substances, have the potential to reduce the risk of degenerative diseases such as diabetes, obesity, cardiovascular diseases and cancer (21). Antioxidant, cytotoxic, and enzyme inhibitory activities of *A. repens* and *C. monogyna* methanol extracts were investigated with total phenolic and flavonoid contents in this current study.

Phenolic compounds are one of the largest and most common secondary metabolite groups in the plant world with more than 8000 identified phenolic structures (22) Phenolic compounds can be found in all organs of plants and are involved in many functions, from skeletal components of different tissues to pigmentation (23). Phenolic compounds have diverse biological functions such as inhibition of lipid peroxidation, antioxidant and antimicrobial activities, inhibition of carcinogenesis, direct constrictive action on capillaries (24). Flavonoids are an essential group of naturally occurring phenolic compounds found in all vascular plants. It was well documented that flavonoids had antioxidant, cardioprotective, antidiabetic, anti-inflammatory, anti-allergic, antiviral, and anticancer effects (25). Plant phenolics and flavonoids have received greater attention since they have various biological properties. The highest amounts of total phenolic and flavonoid contents were found *in C. monogyna* methanol extract. Öztürk and Tunçel (26) reported the total phenolic contents of the methanol, ethyl acetate,

aqueous, and infusion extracts of *C. monogyna* in the range of 108.65 and 343.54 mg GAE/g extract. In a different study, total phenolic (361.39±3.78-398.48±0.98 mg GAE/g extract) and flavonoid (13.69±0.51-23.87±2.74 mg QE/g extract) contents of *C. orientalis, C. monogyna, C. pontica, C. turcicus, C. rhipidophylla* were investigated (12). Cosmulescu et al. found contents of 203.01±9.56 mg GAE/100 g FW phenolics and 147.98±7.29 mg QE/100 g FW flavonoids in the methanol extract of *C. monogyna* (27). Total phenolic content of *A. repens* methanol extract was calculated as 743 GAE mg/100 g extract by Dogan et al. (28).

Oxidative stress plays an important role in the development and initiation of many diseases, comprising autoimmune diseases, inflammation, Parkinson's and neurodegenerative diseases, aging, cataracts, arteriosclerosis, and cancer (29). Studies have proven that oxidative damage is effective in the development of age-related and chronic diseases, and dietary antioxidant supplementation counteracts it and reduces the risk of disease (30). Antioxidants are substances that delay or prevent oxidation of an oxidizable substrate at low concentrations (31). In this study, antioxidant activities of *A. repens* and *C. monogyna* methanol extracts were screened by using five different assays and *C. monogyna* methanol extract was recorded to have the highest antioxidant activity in all activity assays excluding metal chelating assay. The highest antioxidant activity could be connected with the highest level of total phenolic and flavonoid contents. Many previous studies have proved that there is a positive relationship between the levels of total phenolic and flavonoid contents and antioxidant activity (12,18). There are studies on the antioxidant properties of *A. repens* and *C. monogyna* species in the literature. Scavenging activity of DPPH was found as 0.32±0.01 mmol Trolox/100 g FW in *C. monogyna* methanol extract (27). Antioxidant activity of the water, 80% ethanol: water and ethanol extracts of *C. monogyna* were studied by Nunes et al. (32). When 80 % ethanol: water extract exhibited the highest activity in total antioxidant activity (243.31±9.61 AAE/g dw), reducing power (177.86±7.54 mg TE/g dw), ferric reducing antioxidant power (225.52±10.91 mg TE/g dw) assays, the water extract (61.56±4.00 µg sample/mL) in DPPH radical scavenging assay. Rocchetti et al. reported the decoction, infusion, and methanolic extracts of leaves and twig of *C. tanacetifolia, C. szovitsii, C. orientalis* by using phosphomolybdenum (1.18±0.06-3.45±0.09 mmol TE/g), ABTS (81.35±5.28-515.54±6.29 mg TE/g), DPPH (74.20±1.26-393.69±0.48 mg TE/g), CUPRAC (200.51±2.71-708.09±13.35 mg TE/g), FRAP (97.84±1.10-399.02±2.03 mg TE/g) and metal chelating (11.90±1.68-48.95±1.01 mg EDTAE/g) assays (33). In a different report, antioxidant properties of 50% ethanol, 70% methanol, and water extracts of *C. monogyna* were tested according to DPPH and FRAP assays. 50% ethanol extract was found to have the highest activity in DPPH and FRAP assays with the value of 1955.9±2.8 and 1989.8±1.1 mM Trolox/g, respectively (34). In the research of Ferysiuk et al., water, aqueous ethanol (50:50) and ethanol extracts of *A. repens* scavenged 1.77±0.41, 2.92±0.18, 4.42±0.3 % of $DPPH^{\cdot}$ and 1.23±0.17, 4.85±0.22, 3.6±0.15 % of $ABTS^{\cdot+}$, respectively (5).

Colorectal cancer ranks 3rd after lung and breast cancer deaths in women, and lung and prostate cancer deaths in men. Considering the etiology of colorectal cancer, it is basically the genetic change process of the epithelial cells in the colon mucosa. The factors that trigger colon cancer include susceptibility to mutagenic effects, red meat consumption, bile acids, and insufficient intake of vitamins and minerals (35). Although the main treatment is surgery, recurrences occur in most of the patients within the first 3 years after surgery with only surgical treatment (36). Many different treatment modalities are used in cancer treatment to reduce mortality and increase survival. These can be listed as surgery, radiotherapy, chemotherapy, hormone therapy and new treatment methods, immunotherapy, signal transduction system inhibitors, gene therapy, and angiogenesis inhibitors. Chemotherapy is a form of treatment aimed mainly at killing cancer cells. However, the effectiveness of current chemotherapy agents in different cancer types is limited (37,38). For cancer treatment, many drugs, and new treatment methods have been developed in recent years, and studies to obtain new, natural and side effects free drugs from plants have gained importance. *A. repens* and *C. monogyna* methanol extracts showed close cytotoxic activity on DLD-1. There are only two reports on the literature related with cytotoxic activity of *Crataegus* species. The % inhibition values of HCT116 (colorectal cancer) by *Crataegus* L. polysaccharide extract were reported as in a range from 20% to 80% between 125 and 1000 µg/mL concentrations (39). Ganie et al. revealed that *C. songarica* methanol, ethanol and ethyl acetate extracts inhibited ~ 80%, 85%, 75% of SW480 (colorectal cancer) at 80 µg/mL concentration (40).

In Alzheimer's disease (AD), the acetylcholine level decreases with the loss of neurons and axons. For this reason, increasing the acetylcholine level is important in the therapy of AD. Acetylcholine level can be increased by suppressing cholinesterase enzymes that break down acetylcholine. AChE and BChE are enzymes that are encoded by different genes but differ from each other, especially due to their substrate selectivity and differences in some catalytic mechanisms. Studies have reported that increases in acetylcholine levels due to cholinesterase inhibition may improve unconsciousness in the early stages of AD (41,42). Tyrosinase is an important enzyme in hyperpigmentation problems such as skin spots caused by excessive melanin synthesis in the body and such as psoriasis and vitiligo caused by insufficient melanin synthesis. Agents that inhibit this enzyme can be used in the treatment of hyperpigmentation problems (43,44). Urease is an enzyme catalyse the hydrolysis of urea to ammonia and bicarbonate. Inhibition of urease is especially important in the treatment of urinary and gastrointestinal tract infections. Urease inhibitors are very important for *Helicobacter pylori*, an anaerobic bacterium that has recently caused stomach reflux, ulcers and gastritis. In fact, urease activity has an essential role in buffering the acidic pH in the stomach, in food intake, and in enhancing the ability of *H. pylori* to colonize the gastric epithelium. Urease inhibition is very important for the treatment of diseases associated with *H. pylori* (45,46). Diabetes mellitus, characterized by insulin

deficiency or ineffectiveness, is a lifelong metabolic disease. In type 2 diabetes, the level of sugar in the blood increases due to both insufficient insulin secretion and decreased insulin sensitivity (47). One of the treatment methods to reduce blood sugar is to delay the passage of glucose into the blood by inhibiting the activity of carbohydrate digestive enzymes such as α-glucosidase and α-amylase in the digestive system, or to allow them to pass into the blood regularly (48). According to obtained results, *A. repens* methanol extract displayed the highest effect against AChE, BChE, urease, α-glucosidase enzymes whereas *C. monogyna* methanol extract showed the highest effect against tyrosinase and α-amylase enzymes. Previously, tyrosinase inhibition values of *C. monogyna* and *C. oxyacantha* were reported as ~40% and ~50%, respectively (49). α-Amylase (IC_{50}: 10.71±0.11 mg/mL), α-glucosidase (IC_{50}: 10.72±0.43 mg/mL), AChE (IC_{50}: 69.59±1.12 mg/mL) and BChE (IC_{50}: 132.70±2.12 mg/mL) inhibitory activities of *Crataegus* L. methanol:water extract were investigated by Nowicka and Wojdylo (50). The decoction, infusion and methanolic extracts of twig and leaves of *C. tanacetifolia, C. szovitsii, C. orientalis* were tested for their inhibitory activities against AChE (3.62±0.25-4.33±0.05 mg GALAE/g), BChE (1.43±0.05-5.21±0.07 mg GALAE/g), tyrosinase (9.80±2.39-128.78±0.94 mg KAE/g), α-amylase (0.11±0.01-0.66±0.02 mmol ACAE/g) and α-glucosidase (3.01±0.11-33.57±0.02 mmol ACAE/g) (27). This study can be assumed as the first investigation on AChE, BChE, α-amylase, α-glucosidase, and urease inhibitory activities of *A. repens* and *C. monogyna* methanol extracts.

CONCLUSION

Antioxidant, cytotoxic, and enzyme inhibitory activities of *A. repens* and *C. monogyna* methanol extracts were investigated with total phenolic and flavonoid contents in this current study. It was determined that *C. monogyna* methanol extract with the highest total phenolic and flavonoid contents had the best antioxidant activity in all studied assays except metal chelating assay. When the extracts showed moderate enzyme inhibitory activities, *A. repens* methanol extract showed superior inhibitory activity against urease enzyme. Also, *A. repens* and *C. monogyna* methanol extracts showed close cytotoxic activity on DLD-1. This study can be considered as the first investigation on cytotoxic and enzyme inhibitory activities of *A. repens* and *C. monogyna* species. It is thought that this study will further contribute to the biological values of these plants, which are used for different purposes in folk medicines.

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