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

Corresponding Author: Ebru Deveci  E-mail: edeveci@ktun.edu.tr
Submitted: 27.10.2020 • Revision Requested: 19.11.2020 • Last Revision Received: 23.11.2020 • Accepted: 25.11.2020 • Published Online: 13.12.2020
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 ‘Aliç’ 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, cardiotoxic, antiapoptotic, 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-liperoxidant, 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:

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

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:

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

Antioxidant Activity
β-carotene-linoleic acid, metal chelating, cupric reducing antioxidant capacity (CUPRAC), 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH), and (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt cation radical (ABTS•−) scavenging 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_{0.50}) 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 Acrabose 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 needed 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 by the linoleic acid is a measure of its antioxidative activity. The results showed that A. repens methanol extract had a higher antioxidant activity compared to C. monogyna methanol extract. The IC_{50} values of A. repens methanol extract were 77.62±0.09 µg/mL, while for C. monogyna methanol extract, it was 32.72±0.15 µg/mL.

**Table 1.** Total phenolic and flavonoid contents of the extracts.

| Extract                  | Total phenolic content (µg GAES/mg extract) | Total flavonoid contents (µg QEs/mg extract) |
|--------------------------|--------------------------------------------|---------------------------------------------|
| A. repens methanol       | 24.57±0.22                                 | 9.31±0.41                                   |
| C. monogyna methanol     | 68.13±0.34                                 | 36.91±0.17                                  |

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.

**Table 2.** Antioxidant activities of the extracts.

| Extract                  | β-carotene-linoleic acid IC_{50} (µg/mL) | DPPH IC_{50} (µg/mL) | ABTS IC_{50} (µg/mL) | CUPRAC A_{50} (µg/mL) | Metal chelating Inhibition (%) |
|--------------------------|----------------------------------------|----------------------|----------------------|-----------------------|-------------------------------|
| 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                            |
| α-Tocopherol              | 2.10±0.08                              | 37.18±0.41           | 38.51±0.54           | 66.72±0.81            | NA                            |
| BHA                       | 1.34±0.04                              | 19.80±0.36           | 11.82±0.09           | 24.40±0.69            | NT                            |
| EDTA                      | NT                                      | NT                   | NT                   | NT                    | 95.20±0.13                    |

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.

α-Tocopherol, α-Tocopherol

NT: Not tested.
by lipid peroxyl radicals formed in the method in the color of β-carotene is inhibited by antioxidant compounds are tested. IC\textsubscript{50} values of \textit{A. repens} and \textit{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\textsuperscript{•+} and DPPH\textsuperscript{•} 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\textsuperscript{•+} (IC\textsubscript{50} : 40.43±0.55 µg/mL) and DPPH (IC\textsubscript{50} : 71.69±0.85 µg/mL) radicals were observed in \textit{C. monogyna} methanol extract. Also, \textit{C. monogyna} methanol extract indicated near-standard activity in ABTS\textsuperscript{•+} 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 \textit{A. repens} methanol extract exhibited low metal chelating activity with an inhibition value of 13.09±0.99 % at 400 µg/mL concentration, \textit{C. monogyna} methanol extract showed no activity.

### Cytotoxic Activity

Cytotoxic activities of \textit{A. repens} and \textit{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\textsubscript{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. \textit{A. repens} (IC\textsubscript{50} : 57.38 µg/mL) and \textit{C. monogyna} (IC\textsubscript{50} : 54.04 µg/mL) methanol extracts showed similar cytotoxic activity on DLD-1.

**Table 3. Cytotoxic activities of the extracts.**

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

Figure 1. Cytotoxic effects of \textit{A. repens} and \textit{C. monogyna} methanol extracts on DLD-1 and CCD-18Co a) IC\textsubscript{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\textsubscript{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: \textit{A. repens} methanol extract, CMM: \textit{C. monogyna} methanol extract.
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 EDTA/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 and 1.23±0.17, 4.85±0.22, 3.6±0.15 % of ABTS, 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 A. repens methanol extract showed enzymes whereas C. monogyna methanol extract displayed the highest effect against AChE, BChE, urease, α-glucosidase and α-amylase in the digestive system, or to obtain results, A. repens methanol extract showed moderate enzyme inhibitory activity against urease enzyme. Also, 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 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.

Financial Disclosure: There are no funders to report for this submission.

Acknowledgements: Authors would like to thank Dr. Ergün Kaya (Faculty of Science, Department of Molecular Biology and Genetics, Muğla Sıtkı Koçman University) for the identification of the plant samples.

REFERENCES

1. Dar RA, Shahnawaz M, Qazi PH. General overview of medicinal plants: A review. J Phytopharmacol 2017; 6: 349-51.
2. Top R, Erden Y, Tekin S. The investigation of antioxidant and anti-cancer effects of some importance medical plants. BEU J Sci 2019; 8(2): 435-42.
3. Gintala R, Bhavsar R, Chigbu DJ, Jain P, Khan ZK. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. Antioxidants 2019; 8(2): e35.
4. Rawat D, Shrivastava S, Naik RA, Chhonker SK, Mehotra A, Koiri RK. An overview of natural plant products in the treatment of hepatocellular carcinoma. Anticancer Agents Med Chem 2018; 18(3): 1383-59.
5. Fersiuk K, Wójciak KM. The spectrophotometric analysis of anti-oxidant properties of selected herbs in vision-pro™ uv-vis. Appl Comput Sci 2019; 15: 49-62.
6. Leporatti ML, Ivancheva S. Preliminary comparative analysis of medicinal plants used in the traditional medicine of Bulgaria and Italy. J Ethnopharmacol 2003; 87:123-42.
7. Mustafa B, Haddari A, Krasniqi F, Hoxha E, Ademi H, Quave CL, et al. Medical ethnobotany of the Albanian Alps in Kosovo. J Ethnobiol Ethnomed 2012; 8: 6.
8. Sargin SA, Akçicek E, Selvi S. An ethnobotanical study of medicinal plants used by the local people of Alaşehir (Manisa) in Turkey. J Ethnopharmacol 2013; 150:860-74.
9. Grases F, Ramis M, Costa-Bauza A, March JG. Effect of Herniaria hirsuta and Agropyron repens on calcium oxalate urolithiasis risk in rats. J Ethnopharmacol 1995; 45: 211-4.
10. Mascolo N, Autore G, Capasso F, Menghini A, Fasulo MP. Biological screening of Italian medicinal plants for anti-inflammatory activity. Phytother Res 1987; 1: 28-31.
11. Rafaşanjany N, Lechtenberg M, Peteret F, Hensel A. Antiadhesion as a functional concept for protection against uropathogenic Escherichia coli. In vitro studies with traditionally used plants with antiadhesive activity against uropathogenic Escherichia coli. J Ethnopharmacol 2013; 145: 591-7.
12. Bardakci H, Celep E, Göz etl, Kany Y, Kırımızbekmez H. Phytochemical characterization and antioxidant activities of the fruit extracts of several Crataegus taxa. S Afr J Biol 2019; 124: 5-13.
13. Edwards JE, Brown PN, Talbot N, Dickinson TA, Shipley PR. A review of the chemistry of the genus Crataegus. Phytochemistry 2012; 79: 5-26.
14. Venskutonis PR. Phytochemical composition and bioactivities of hawthorn (Crataegus spp.): a review of recent research advances. J Food Bioact 2018; 4: 69-87.
15. Slinkard K, Singleton VL. Total phenol analyses: Automation and comparison with manual methods. Am J Enol Vitic 1977; 28: 49-55.
16. Park YK, Koo MH, Ikegaki M, Contado JL. Comparison of the flavonoid aglycone contents of Apis mellifera propolis from various regions of Brazil. Braz Arch Biol Technol 1997; 40: 97-106.
17. Çayan F, Tel-Çayan G, Deveci E, Öztürk M, Duru ME. Chemical profile, in vitro enzyme inhibitory, and antioxidant properties of Stereum species (Agaricomycetes) from Turkey. Int J Med Mushrooms 2019; 21(11): 1075-87.
Deveci et al. Bioactivities of *A. repens* and *C. monogyna*

18. Deveci E, Tel-Çayan G, Duru ME, Öztürk M. Phytochemical contents, antioxidant effects, and inhibitory activities of key enzymes associated with Alzheimer’s disease, ulcer, and skin disorders of *Sideritis albilora* and *Sideritis leptooclada*. J Food Biochem 2019; 43: e13078.

19. Deveci E, Tel Cayan G, Duru ME. *In vitro* antidiabetic activity of seven medicinal plants naturally growing in Turkey. Eur J Biol 2020; 79: 23-8.

20. Karakurt S, Adali O. Tannic acid inhibits proliferation, migration, invasion of prostate cancer and modulates drug metabolizing and antioxidant enzymes. Anticancer Agents Med Chem 2016; 16(6): 781-9.

21. Patch CS, Sullivan DR, Fenech M. Health benefits of herbs and spices: Cardiovascular disease. Med J Aust 2006; 185(4): 7-9.

22. Tsao R. Chemistry and biochemistry of dietary polyphenols. Nutrients 2010; 2(12): 1231-46.

23. Ignat I, Volf I, Popa VI. A critical review of methods for characterization of polyphenolic compounds in fruits and vegetables. Food Chem 2011; 126: 1821-35.

24. Tanase C, Cosarca S, Muntean DL. A critical review of phenolic compounds extracted from the bark of woody vascular plants and their potential biological activity. Molecules 2019; 24(6): 1182.

25. Karak P. Biological activities of flavonoids: an overview. Int J Pharm Sci Res 2019; 10(4): 1567-74.

26. Öztürk N, Tunçel M. Assessment of phenolic acid content and antioxidative activity of vitamin E and Trolox: understanding of the factors that govern lipid peroxidation studies. J Enzyme Inhib Med Chem 2016; 31(S2): 46-50.

27. Lukyanova LD, Storozheva ZI, Proshin AT. Corrective effect of flavonoid containing preparation extralife on the development of Parkinson’s syndrome. Bull Exp Biol Med 2007; 144: 42-5.

28. Ghosezmazdeh A, Ghosezmazdeh N. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. J Med Plants Res 2010; 4(23): 2566-73.

29. Nunić M, Pavlović A, Bosco FL, Stanisavljević N, Zagorac DD, Akšić M. Antioxidant enzymes. Anticancer Agents Med Chem 2016; 16(6): 175-80.

30. Ma L, Xu GB, Tang X, Zhang C, Zhao W, Wang J, et al. Anti-cancer potential of polysaccharide extracted from hawthorn (*Crataegus*) on human colon cancer cell line HCT116 via cell cycle arrest and apoptosis. J Funct Foods 2020; 64: 103677.

31. Gholamhosseini A, ZohreRazmi Z. Screening the methanolic extracts of some plants for tyrosinase inhibitory activity. Toxicol Environ Chem 2012; 94: 310-8.

32. Baltas N, Karioqlu S, Tarakci C, Kolayli S. Effect of propolis in gastric disorders: inhibition studies on the growth of *Helicobacter pylori* and production of its urease. J Enzyme Inhib Med Chem 2016; 31(52): 46-50.

33. Baltas N, Yildiz O, Kolayli S. Inhibition properties of propolis extracts to some clinically important enzymes. J Enzyme Inhib Med Chem 2016; 31: 52-5.

34. Taslimi P, Gulcin I. Antidiabetic potential: *in vitro* inhibition effects of some natural phenolic compounds on α-glucosidase and α-amylase enzymes. J Biochem Mol Toxicol 2017; 31(10): e21956.

35. Rostami S. M.Sc Thesis. Investigation of anti proliferative and anti-inflammatory effects of common and endemic species of plants. Department of Biology, Institute of Science, Gazi University, Ankara, Turkey, 2013.

36. Doğan M, Akbulut H. Adjuvant Treatment of Colorectal Cancer. Türkiye Klinikleri J Med Oncol 2009; 2(3): 49-57.

37. Dellabona P, Moro M, Crosti MC, Casorati G, Corti A. Vascular attack and immunotherapy: a ‘two hits’ approach to improve biological treatment of cancer. Gene Ther 1999; 6: 153-4.

38. Terrero MN, Li S. Growth factor receptors: targets for gene therapy and immunotherapy for cancer treatment. Gene Ther Mol Biol 2004; 8: 175-80.

39. Alkan HO, Zengin G, Kaskan G. Antioxidant and *in vitro* some enzyme inhibitory activities of methylxan extract of cultivated *Lentimula edodes*. J Fungi 2017; 8(2): 90-8.

40. Chang TM. Tyrosinase and tyrosinase inhibitors. J Biocatal Bioconversion 2012; 1: 2.

41. Baltas N, Yildiz O, Kolayli S. Inhibition properties of propolis extracts to some clinically important enzymes. J Enzyme Inhib Med Chem 2016; 31: 52-5.

42. Taslimi P, Gulcin I. Antidiabetic potential: *in vitro* inhibition effects of some natural phenolic compounds on α-glucosidase and α-amylase enzymes. J Biochem Mol Toxicol 2017; 31(10): e21956.