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ICP-OES AND LC-ESI-MS/MS ANALYSES, ENZYME INHIBITION AND DNA PROTECTION POTENTIAL OF *PELARGONIUM QUERCETORUM* AGNEW

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ABSTRACT. *Pelargonium quercetorum* Agnew extract affects activities of acetylcholinesterase (AChE) and carbonic anhydrase I-II enzymes (hCA I-II) was investigated under *in vitro* conditions. IC_{50} values of *P. quercetorum* Agnew on hCA I-II and AChE activity were determined as 0.144±0.0720, 0.209±0.0593, and 0.062±0.0097 mg/mL, respectively. Rutin and shikimic acid was found to be the main phenolic component in *P. quercetorum* Agnew flower extract from the results of LC-ESI-MS/MS analysis. Rutin was found to be the main phenolic component in *P. quercetorum* Agnew leaf extract from the results of LC-ESI-MS/MS analysis. ICP-OES analysis showed that the leaves of *P. quercetorum* Agnew were rich in potassium. The DNA protective effect on plasmid DNA was demonstrated by using extracts obtained from leaf and flower tissues. Consequently, based on the findings of the current study, it could be anticipated that clinical trials related to *P. quercetorum* Agnew could be completed and the plant could be used pharmacologically.

Keywords: *Acetylcholinesterase; Carbonic anhydrase; DNA protection; ICP-OES; P. quercetorum Agnew*

INTRODUCTION

P. quercetorum Agnew is a species that belongs to *Pelargonium genus* of the Geraniaceae family in the Geraniales order. This species is distributed in Southeastern Anatolia Region of Türkiye [1]. *P. quercetorum*

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Agnew (Hakkari geranium), popularly known by the names Tolk or Tolik, is used for cooking and medical purposes [2]. There have been very few studies on *P. quercetorum* Agnew in the literature. Such as, chemical content of *P. quercetorum* Agnew was analyzed using GCxGC-TOF/MS, and 23 different compounds with different proportions were reported [3]. The main chemicals were found to be tetracosane, heneicosane, and 2-methyleicosane. Similarly, analysis of volatile components in oil extract of *P. quercetorum* Agnew using GC-MS revealed 26 compounds, and alpha pinene, alpha-fenchyl acetate, and limonene were found to be the dominant compounds [4]. Besides, different extracts of the plant showed antioxidant [5], anti-growth/cytotoxic activity on non-small cell lung cancer cells [3], human MCF-7, MDA-MB-231 breast cancer cells [6] and anthelmintic activity [5]. Despite all these studies, the research on *P. quercetorum* Agnew is limited. The fact that the plant grows only in a specific region limits its potential for use. Results obtain from current study will have contributed to many researchers recognize plant material and evaluate its potential uses in pharmacy, alternative medicine, and cosmetics. Therefore, the phenolic content, elemental analysis, DNA protective effect, and enzyme inhibitory effect of *P. quercetorum* Agnew were addressed.

Almost all mechanisms in the living system are coordinated through enzymes [7]. So, interfering with their activity is used in the diagnosis and treatment of many diseases [8]. Acetylcholinesterase and carbonic anhydrase enzymes used in the present study are some of these enzymes. Acetylcholinesterase (AChE, E.C.3.1.1.7) is a carboxylic ester hydrolase that catalyzes hydrolysis of choline esters called acetylcholine acetyl hydrolase [9]. Acetylcholinesterase inhibitors such as tacrine, donepezil, rivastigmine, and galantamine have been reported to be utilized in the treatment of Alzheimer's disease (AD) [10; 11]. The inhibitors of this enzyme were mentioned to stop the hydrolysis of acetylcholine, its usual substrate, thereby increasing cholinergic transmission [12]. In addition to AD inhibitors of the enzyme were reported to be used in myasthenia gravis and glaucoma diseases [13; 14]. That's why inhibition effects of many different plant extracts such as *Crinum moorei Hook*.f, *Galanthus ikariae* L., Narcissus 'Sir Winston Churchill', *Sarcococca coriacea* (Hook.f.) *Sweet*, *Corydalis bulbosa* DC., *Fritillaria imperialis* L., marigold [15] on AChE enzyme activity have been investigated. Besides, inhibitory effects of secondary metabolites from plant extracts on AChE enzyme have also been studied [16]. However, new and different inhibitors continue to be investigated because the existing inhibitors of the enzyme have a large number of side effects in the treatment of specified diseases [16] and cannot provide fully the desired effect. The other enzyme used in the present study was carbonic anhydrase, a clinically important enzyme similar to acetylcholinesterase. Carbonic anhydrase (CA,

E.C.4.2.1.1) is found in different cell types and encoded by different gene families. In mammals, this enzyme has 16 isoenzymes containing Zn^{2+} ion at its active site [17; 18]. Although the enzyme is known to catalyze many reactions, its major role in metabolism is to catalyze the conversion of CO_2 to HCO_3^-, thereby creating an important buffering in the living system as well as transporting CO_2 [7; 18]. Inhibitors and activators of carbonic anhydrase enzyme have potentials to be used as drugs. It was reported that the inhibitors of the enzyme can be used for the treatment of epilepsy, glaucoma, edema, obesity and cancer, while its activators can be used for the treatment of Alzheimer's disease [19; 20; 21]. However, it was also stated that the current inhibitors of the enzyme (sulfonamides, acetazolamides, dorzolamides, methazolamides and brinzolamide) used in the treatment of above-mentioned diseases have numerous side effects [22; 23]. For this reason, investigations have been going on to find natural or synthetic new inhibitors of the enzyme.

Exposure of DNA to hydrogen peroxide (H_2O_2) causes breakage of open-ended DNA and DNA breaks (chromatid and chromosome breaks) [24]. In the meantime, genetic disorders may occur as a result of breaks in the DNA chain. Irreversible DNA damage can lead to carcinogenesis, aging and other degenerative diseases [25]. Therefore, it is extremely important to investigate the effects of the active ingredients of plants on DNA protection. Since there is no study in the literature on how *P. quercetorum* Agnew influences genetic material, its DNA protective activity has been determined. The aim of this study was to determine the phenolic compound content, elemental analysis, antienzyme activity, and DNA protective activity of *P. quercetorum* Agnew to determine if it has potential for use in the medical, food, and cosmetic industries. Considering the aforementioned causes, in the current study, *in vitro* effect of *P. quercetorum* Agnew extract on the activities of acetylcholinesterase and carbonic anhydrase I-II, which play a critical role in metabolism, was evaluated for the first time. Furthermore, *P. quercetorum* Agnew was examined conducting phenolic compound analysis by LC-ESI-MS/MS and elemental analysis by ICP-OES. Additionally, the effect on DNA protection of *P. quercetorum* Agnew extract was evaluated by working in different concentrations in both flower and leaf tissue.

RESULTS AND DISCUSSION

In this study, *in vitro* effect, *P. quercetorum* Agnew on human erythrocyte carbonic anhydrase I-II and acetylcholinesterase enzymes were investigated to determine the being enzyme inhibitory properties. IC_{50} values calculated to determine the inhibition effect of *P. quercetorum* Agnew on hCA I-II and

AChE activities were 0.144±0.0720, 0.209±0.0593 and 0.062±0.0097 mg/mL, respectively. Thus, it was revealed that *P. quercetorum* Agnew has a better inhibition effect on AChE enzyme activity. On the other hand, carbonic anhydrase appeared to have a relatively similar inhibition effect on both isoenzymes, but it was more effective on hCA I enzyme activity (Figure 1, Table 1).

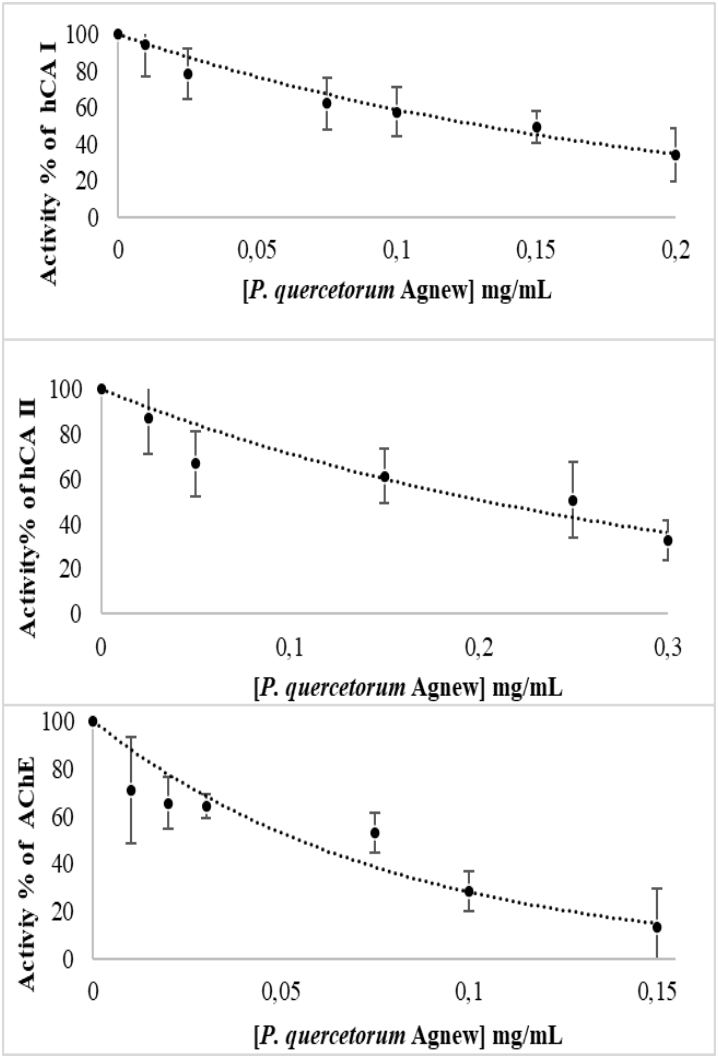

Figure 1. Effect of *P. quercetorum* Agnew on hCA I, II and AChE enzyme activity

Table 1. Anti-enzyme activity of *P. quercetorum* Agnew

	hCA I	**hCA II**	**AChE**
IC_{50} (mg/mL)	0.144±0.0720	0.209±0.0593	0.062±0.0097
K_i (mg/mL)	0.0363±0.0053	0.277±0.12880	0.0035±0.002

It was stated that phenolic compounds cause inhibition by forming hydrogen bonds with the water molecules around Zn^{2+} located at the active site of carbonic anhydrase enzyme [21; 26; 27; 28]. *P. quercetorum* Agnew appeared to exhibit a similarly powerful inhibition effect on both isoenzymes of carbonic anhydrase enzyme. The isoenzyme I and II of the carbonic anhydrase enzymes share about 60% similarity [29]. Therefore, although methanol extracts of *P. quercetorum* Agnew exhibit similar effects on activities of hCA I and hCA II enzymes, there was about two-fold difference in their IC_{50} values. Consequently, due to similar inhibition effects, it is thought that the phenolic compounds in the extract interact with water molecules around Zn^{2+} in the active region of both isoenzymes by hydrogen bonding. A detailed analysis of the narrow groove-shaped active site of acetylcholinesterase (AChE), the other enzyme investigated in the present study, would reveal that its active region can be divided into several subregions: omega lobe (Cys69-Cys96), peripheral anionic subunit (Asp74, Tyr124, Ser125, Trp286, Tyr337, Tyr341), anionic subunit (Trp86, Tyr133, Glu202, Gly448, Ile451), oxyanion hole (Gly121, Gly122, Ala204), catalytic triad (Ser203, His447, Glu334) and acyl bonding pocket (Trp236, Phe295, Phe297, Phe338) [29]. Acetylcholinesterase enzyme has 14 aromatic amino acids at the active center [30; 31]. In this study, plant extract was used and it contained multiple phenolic compounds. Therefore, clamp formation by aromatic amino acid at the active center of the enzyme and multiple phenolic acids in plant extract of *P. quercetorum* Agnew through non-covalent interactions at different locations in the enzyme molecule at the same time, and thus increasing the effect of the inhibitor, is an expected situation. As a result, a low concentration of *P. quercetorum* Agnew had an inhibition effect on AChE.

In the LC-ESI-MS/MS analysis performed to the phenolic compound profile of the *P. quercetorum* Agnew extract was analyzed by using 45 different standards in-our-house library (48). Results showed that *P. quercetorum* Agnew leaf extract had 627.4441 ng/mL shikimic acid, 116.9617 ng/mL gallic acid, 4.7379 ng/mL hyperoside, 123.1750 ng/mL quercetin-3-glucoside, 1633.5768 ng/mL rutin, 473.4672 ng/mL hesperidine, 108.6753 ng/mL kaemerol-3-glucoside, and 66.7999 ng/mL fisetin while other standards could not be detected (Figure 2, Table 2). It was determined first time using LC-ESI-MS/MS analysis in the present study that dominant compound of *P. quercetorum* Agnew leaf extract is rutin (Table 2, Figure 2).

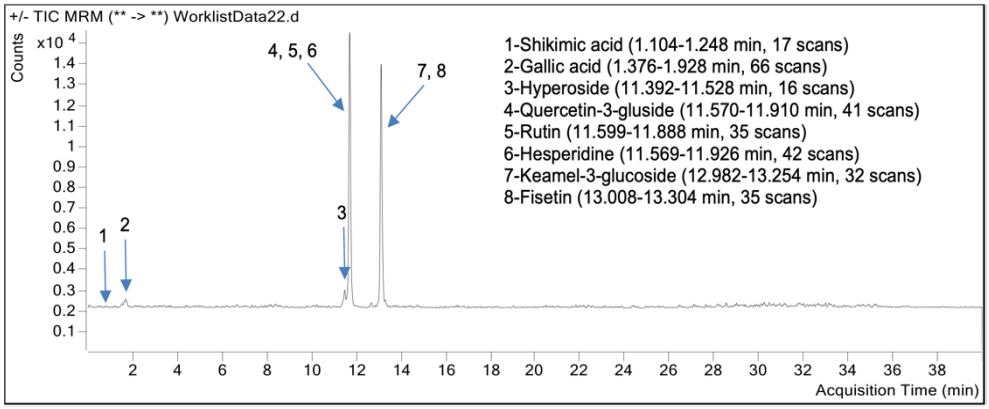

Figure 2. LC-ESI-MS/MS analysis of *P. quercetorum* Agnew leaf extract

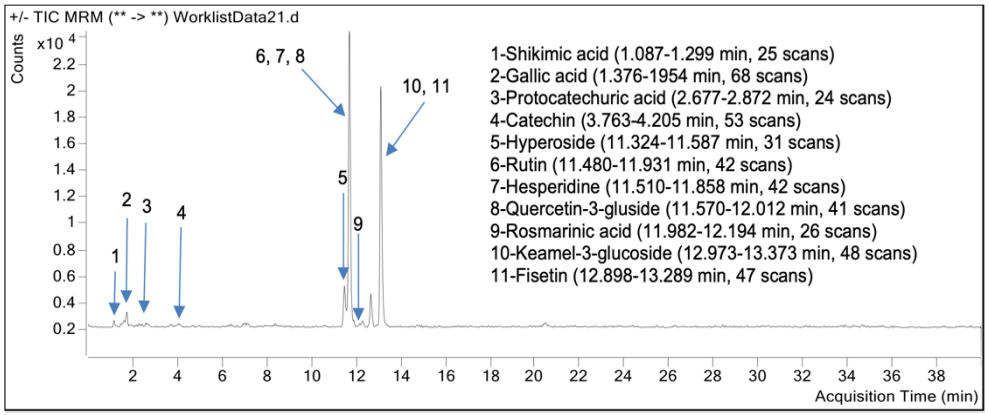

Figure 3. LC-ESI-MS/MS analysis of *P. quercetorum* Agnew flower extract

P. quercetorum Agnew flower extract had 3364.8076 ng/mL shikimic acid, 307.3271 ng/mL gallic acid, 6.2348 ng/mL protocatechuic acid, 379.4268 ng/mL catechin, 141.4570 ng/mL hyperoside, 252.6943 ng/mL quercetin-3-glucoside, 1787.7070 ng/mL rutin, 134.5509 ng/mL rosmarinic acid, 656.0438 ng/mL hesperidine, 181.2362 ng/mL kaemerol-3-glucoside, and 183.4773 ng/mL fisetin while other standards could not be detected (Figure 3, Table 2). It was determined first time using LC-ESI-MS/MS analysis in the present study that dominant compounds of *P. quercetorum* Agnew flower extract is rutin and shikimic acid (Figure 3, Table 2). Similarly, different extracts of *Pelargonium endlicherianum*, another Pelargonium species, prepared by different solvents contained apocynin and gallic acid, but apocynin was found

to be dominant. Moreover, it was stated in the same study that all extracts had vanillic acid in trace amounts, while only the ethyl acetate extract contained a low concentration of caffeic acid [5]. In *Pelargonium graveolens*, another *Pelargonium* species, flavonoid analysis was performed by library scanning using the LC MS device. It was reported that *P. graveolens* has nine flavonoids [32].

Table 2. Analysis of phenolic compounds in *P. quercetorum* Agnew by LC-ESI-MS/MS

	Compound name	**RT**	**Flower ng/mL**	**Leaf ng/mL**
1	Shikimic acid	1.155	3364.8	627.444
2	Gallic acid	1.733	307.33	116.962
3	Protocatechuic acid	2.736	6.2348	ND
4	Catechin	4.077	379.43	ND
5	Hyperoside	11.477	141.46	4.738
6	Quercetin-3-glucoside	11.706	252.69	123.175
7	Rutin	11.676	1787.7	1633.58
8	Rosmarinic acid	12.092	134.55	ND
9	Hesperidine	11.696	656.04	473.47
10	Kaempferol-3-glucoside	13.109	181.24	108.67
11	Fisetin	13.110	183.48	66.80

Mineral content of *P. quercetorum* Agnew was analyzed with ICP-OES. The mineral analysis of the leaf sample of *P. quercetorum* Agnew revealed that it is rich in potassium (3.21%), calcium (0.77%), phosphorus (0.23%) and magnesium (0.22%). Moreover, *P. quercetorum* Agnew was also found to contain 18 different minerals at low rates (Table 3). It was found that *P. quercetorum* Agnew contained alkaline metal, alkaline earth metals, 3A, 4A and 5A group and transition metals. In a related study examining mineral contents in different parts of *P. quercetorum* Agnew, a relative of *P. graveolens*, different parts of the plant were found to contain calcium (2.47 mg/100g), magnesium (0.35 mg/100g), sodium (1.34 mg/100 g), potassium (1.46 mg/100 g), phosphorus (0.18 mg/100g), nitrogen (1.06 mg/100g), chlorides (1.34 mg/100g) and sulphur (0.081 mg/100 g) based on analyses using flame photometer. Although both varieties seem to have similar minerals, it could be stated that *P. quercetorum* Agnew has a richer mineral content in terms of both quantity and variety of the minerals.

Table 3. Elemental analysis of *P. quercetorum* Agnew

Element	AVG (mg/kg)±SD	%
Al	193.7750±1.2468	0.0194
As	0.0000±0.0000	0
B	48.8165 ± 0.4232	0.0049
Ba	1.0374 ±0.0117	0.0001
Bi	0.0000±0.0000	0
Ca	7707.1867 ± 45.3395	0.77
Cd	0.0995 ± 0.0130	0.000001
Co	0.0640 ± 0.0377	0.000006
Cr	0.5635 ± 0.0215	0.000056
Cu	5.8670 ± 0.1360	0.00059
Fe	151.1622 ±1.8136	0.015
K	32098.3169 ± 154.1542	3.21
Zn	21.0820 ± 0.4345	0.0021
Mg	2192.8934±453.3947	0.22
Mn	35.9738±0.4798	0.0036
Mo	0.8862±0.0064	0.000089
Na	481.0847±0.7179	0.048
Ni	1.2680±0.0189	0.00013
P	2253.8071±42.3168	0.23
Pb	1.5450±0.1039	0.00015
Sb	0.3227 ±0.1095	0.000032
Se	0.0000±0.0000	0
Sn	0.7547 ±0.0117	0.000075
Sr	3.6554 ±0.0091	0.00036
Tl	0.0000±0.0000	0
Li	1.2642±0.0015	0.00013

There are diverse studies on the antioxidant capacity (5) and cancer activity (3; 6) of *P. quercetorum* but up to present, no study on plasmid DNA protection was found in the literature, we especially achieved the protective potential of total extract of *P. quercetorum* on plasmid DNA against the Fenton solvent DNA harmful effect. The DNA protective activity of ethanol-water extracts from *P. quercetorum* Agnew was achieved by using pBR322 plasmid DNA (Figure 4). In current study, the ability of extracts belongs to flower and leaf tissue at different concentrations to prevent DNA damage was handled. According to the obtained result, it was determined that H_2O_2 damaged form I of plasmid and transformed it into form II-form III (Figure 4-Line 9) and in Line 10 plasmid DNA without fenton solvent was observed

compact and only supercoil DNA was found. There was no found significantly DNA protecting effect of rosmarinic acid (Figure 4-Line 7) and tocopherol (Figure 4-Line 8) extract concentration (1 mg/mL) used at current study. However, when the two substances are compared, it can be said that rosmarinic acid has a slightly more protective effect.

On the other hand, it was observed that 2 and 3 mg/mL concentrations of both leaf and flower tissues have no DNA protective effect. Smear form was seemed and it was estimated that plant extracts almost destroyed the plasmid DNA (Figure 4). Hydrogen peroxide, a type of free radical, converts guanine to 8 hydroxy guanine and causes DNA damage (33; 34). However, it was observed that the concentrations of both tissues at 1 mg/mL preserved the form I and form III form of DNA. In this case, it can be said that the damage of OH radicals in the Fenton solution is significantly prevented (Figure 4). Under these circumstances, linear DNA bands were not formed in any 1 mg/mL of the extract's concentration of both tissues in both *P. quercetorum* Agnew. In other words, it has been determined that extracts protect DNA against the damaging effects of H_2O_2 on DNA. As a result of this study, it was concluded that extracts at different concentrations and solved in different solvents protect DNA at different levels. And it can be seen that extracts such as rosmarinic acid and tocopherol, which are some well-known DNA protective agents, do not protect DNA. Because the phytochemicals in plant extracts can also have an oxidative effect on DNA damage (35). It was observed that the extract obtained from *P. quercetorum* Agnew has a low effect on plasmid DNA protective activity. It was found that *P. quercetorum* Agnew extract in low concentration (1 mg/mL) in ethanol-water solvent mixture had a better protection effect.

P. quercetorum Agnew is widely used among the local population in the context of alternative medicine as a stomach stimulant or as a treatment for indigestion (2). However, studies on this plant in the literature are very limited. With this study, we aimed to reveal the important chemical metabolites of the plant. The secondary metabolites, such as shikimic acid, gallic acid, protocatechuic acid, catechin, hyperoside, quercetin-3-glucoside, rutin, hesperidine, kaemerol-3-glucoside, and fisetin are quite important chemical compounds in chosen plant in current study. Therefore, it is thought that the determined metabolites will help to clarify the relationship with the therapeutic disease. One of these chemicals or its mixture in the form of a mixture may have created the current effect. In addition, the secondary metabolites are important in the prevention of a variety of disease. The existence of the above-mentioned secondary metabolites is responsible for the antidiuretic, anti-inflammatory, antianalgesic, anticancer, antiviral, antimalarial, antibacterial, and anti-fungal properties (36). Usually, plants are utilized to find and test ionic

elements that are extremely useful in the development of novel medications. The elements that come out of the plant are very important for the functioning of the protein and especially the enzymes that serve in the metabolism. In this context, it can be said that the plant, which is the subject of the study, has an important nutritional element content. Moreover, in order to expand the potential for use of the plant in traditional medicine and ethnobotany, it was desired to investigate its effects on AChE and CA I-II enzyme activities and its potential for circular DNA protection. As a result of the study, it is thought that the plant may have an important potential effect in the treatments of Alzheimer's, glaucoma, epilepsy, gastric, spinal disorders, obesity and cancer. For the manufacture of important plants, phytochemical analysis is also significant and has commercial interest in both research institutes and pharmaceutical businesses.

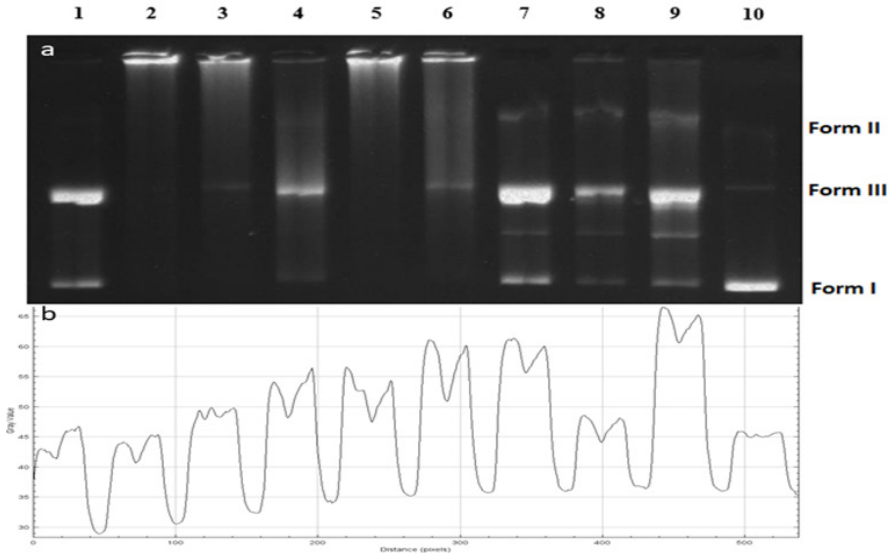

Figure 4. DNA protection gel image (a) and plot analysis (b) of flower and leaf extracts 1. DNA+Fenton Reagent+Flower extract (1 mg/mL), 2. DNA+Fenton Reagent+Flower extract (2 mg/mL), 3. DNA+Fenton Reagent+Flower extract (3 mg/mL), 4. DNA+Fenton Reagent+Leaf extract (1 mg/mL), 5. DNA+Fenton Reagent+Leaf extract (2 mg/mL), 6. DNA+Fenton Reagent+Leaf extract (3 mg/mL), 7. DNA+Fenton Reagent+Rosmarinic acid (1 mg/mL), 8. DNA+Fenton Reagent+alpha Tocopherol (1 mg/mL), 9. DNA+Fenton Reagent, 10. pBR322 DNA

CONCLUSIONS

P. quercetorum Agnew is rich in rutin, potassium (3.21%), calcium (0.77%), phosphorus (0.23%) and magnesium (0.22%). Thus, extracts can also be used as alternative natural sources for the treatment of different diseases. In the present study, methanol extract of *P. quercetorum* Agnew showed different effects on hCA I-II, AChE activity and also lower concentration of leaf and flower extract was found as a DNA protective effect. Synthetic drugs have fairly expensive synthesis and characterization even many side effects. Therefore, *P. quercetorum* Agnew may be preferred instead of synthetic drugs in diseases treatment such as glaucoma, epilepsy, gastric, neurological disorders, obesity, cancer, Alzheimer's treatment.

EXPERIMENTAL SECTION

Chemicals and standards

Human erythrocyte AChE was purchased from Sigma Aldrich. Methanol was obtained from Merck and all other reagents were purchased from Sigma Aldrich or Fluka. Shikimic acid, gallic acid, protocatechuic acid, gentisic acid, catechin, 4-hydroxybenzoic acid, chlorogenic acid, 4-hydroxybenzaldehyde, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, salicylic acid, taxifolin, polydatine, trans-ferulic acid, sinapic acid, quercimeritrin, scutellarin, o-coumaric acid, cynarin, protocatechuic ethyl ester, hyperocide, quercetin 3-β-d-glucoside, rutin, resveratrol, naringin, rosmarinic acid, hesperidine, baicalin, kaempferol-3-glucoside, fisetin, oleuropein, trans-cinnamic acid, quercetin, naringenin, silibinin, hesperetin, kaempferol, tamarixetin, baicalein, biochanin a, chrysin, 5-hydroxyflavone, 6,2,4-trimetoxyflavone were obtained from sigma-aldrich (st. louis, mo, usa). HPLC grade methanol, ammonium formate and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure water (18 MΩ) was obtained from a Milli-Q water purification system (Millipore Co., Ltd.). pBR322 plasmid DNA (catalog number SD0041) was obtained from ThermoFisher Scientific.

Plant sample and extraction process

P. quercetorum samples Agnew species were obtained in the rocky and stony regions of the Pervari province of Siirt city in Türkiye's south-east. Dr. Mehmet Fidan, Associate Professor at Siirt University's Faculty of Arts and Sciences' Biology Department, identified the species. For extraction, tissue samples were dried and crushed. Methods outlined in prior research were adapted for our laboratory circumstances for the extraction process [22; 37]

350 mL methanol was added to about 7 g of sample to prepare the extract, and the mixture was kept at room temperature and in the dark on the magnetic stirring over ten days. Then the solution was filtered through a filter paper. Methanol was evaporated by an evaporator device (Heidolphl/Heizbad-hei-vap). Resulting samples were stored at -40°C until used.

Purification of human hCA I and II isoenzymes

As mentioned in our previous studies, hCA-I and hCA-II isozymes were purified with the help of sepharose-4B-tyrosine sulfanilamide affinity chromatography [20; 38; 39; 40; 41]. The erythrocyte solution used in the study was obtained from the blood bank in Siirt Ministry of Health Education and Research Hospital. A working temperature of 4°C was used throughout the study to maintain the stability of the enzymes. In purified enzymes and hemolysate, protein content was determined spectrophotometrically at 595 nm wavelength based on the method [42] Enzyme purity was determined by using SDS-polyacrylamide gel electrophoresis [43].

Determination of effect on carbonic anhydrase I-II and acetylcholinesterase enzymes of extracts of *P. quercetorum* Agnew

The activities of the carbonic anhydrase I-II isozymes were determined based on the method described by [44] Verpoorte et al. (1967). In this method, hCA I and II isozymes convert 4-nitrophenylacetate (NPA) to 4- nitrophenolate ion, and resulting product gives absorbance at 348 nm. The increase in absorbance was measured spectrophotometrically for three minutes (Shimadzu, UVmini-1280 UV-VI). The reaction medium without enzyme solution was used as a blank. For determination of the activity of AChE, a spectrophotometric method described by [45] Ellman et al. (1961) was used. In this method, acetylcholine iodine is used as substrate, the reaction was monitored spectrophotometrically at 412 nm. Enzymatic activity was assayed using at least five different inhibitor concentrations to determine IC_{50} values. The enzyme activity medium which did not have any inhibitor was used as control and its activity was considered 100%. Then, the inhibitor concentration that halved the enzymatic activity was calculated by drawing graph against % activity [19; 40; 41]. K_i values were determined based on the method described by [46] Zhang et al. 2020. K_M and V_{max} values were calculated by drawing Lineweaver-Burk graphs [47].

$$V = \frac{V_{max} \times S}{K_M \times \left(1 + \frac{I}{K_i}\right) + S} \quad (1)$$

According to the equation (1), I is the concentration of the compound, K_i is the inhibition constant, S is the concentration of the substrate, Inhibition constant (K_i) values were calculated using nonlinear regression according to the above equation [46].

Analysis with LC-ESI-MS/MS of extracts of *P. quercetorum* Agnew

Method validation and LC–ESI–MS/MS analysis in study was carried out in Iğdır University Research Laboratory Application and Research Center (ALUM). The values used in Validation parameters (LOD, LOQ, R^2 and Linearity Range etc.) of compounds by LC-ESI-MS/MS method, Yılmaz et al., are partners with our study [48]. To determine the method and to perform the content analysis, 1 mg/mL stock solutions in methanol of 45 standard phenolic compounds were prepared. Generally, the stock solutions diluted to a concentration of 8 µg/mL but scutellarin, hyperocide, quercetin-3-β-d-glucoside, kaempferol-3-glucoside, baicalein, baicalein, 5-hydroxyflavonen and flavone were diluted to 0.8 µg/mL concentration with 50% methanol/water (v/v; 1:1). Nine calibration levels were prepared by serial dilution of the standard mixture containing 45 phenolic compounds to plot the calibration curve with 50% methanol/water (v/v; 1:1). 10 mg of *P. quercetorum* Agnew dry extract was dissolved in 2 mL methanol and the solution was diluted to 2 mg/mL 50% methanol/water (v/v; 1:1). Subsequently, the solution was filtered through 0.45 µm filters and transferred into vials before LC-ESI-MS/MS analysis.

An Agilent Technologies 1260 Infinity II liquid chromatography System combined to a 6460 Triple Quad mass spectrometer was used for quantitative and qualitative analysis of 45 phytochemical compounds. Poroshell 120 EC-C18 (100 mm × 4.6 mm I.D., 2.7 µm) column was used for the chromatographic separation of the compounds [48]. Mobile phase flow rate, column temperature conditions and different mobile phases additives such as formic acid, ammonium acetate and acetic acid were applied together with acetonitrile, purified water, and methanol mobile phases to achieve the most ideal separation and ionization of the compounds. Thus, 0.1 % formic acid and 5 mM ammonium formate in water A mobile phase and 0.1 % formic acid and 5 mM ammonium formate in methanol B mobile phases were utilized in chromatographic separation. Using a flow rate of 0.4 mL/min, a gradient program of 15% for 1-12 min, 50% for 12-30 min, 90% for 30-32 min,

and 10% for 32-35 min was used in the B mobile phase, respectively. The injection volume was 4.0 µL, and the column temperature was maintained at 40°C [49]. An electrospray ionization (ESI) source operating in both negative and positive ionization modes was used to determine the mass-to-charge ratio (m/z) of the compounds. The ESI source parameters were set at capillary voltage to 4000 V, nebulizing gas (N_2) flow to 11 L/min, nebulizer pressure to 15 psi and gas temperature to 300°C to ensure ideal ionization of all compounds and achieve the ideal peak intensity. The precursor and product ions, collision energies and fragmentor voltage of each compound were determined for quantitative measurement as multiple reaction monitoring (MRM).

Mineral matter analysis with ICP-OES of *P. quercetorum* Agnew

This research was conducted out at Siirt University's Science and Technology Application and Research Center. The tests have been adjusted to our laboratory conditions. According to the Berghof microwave digestion speedwave MWS-2 device user manual V. 5.1, 0.3743 g of moisture-free ground material was weighed for the PTFE wet digestion system, and 6 mL nitric acid (65%) and 2 mL hydrogen peroxide (30%) were added to the sample. The sample was digested for 15+15 minutes at 180°C. After freezing, the samples were moved to volume containers with a final volume of 20 mL [50].

Two repetitive readings were performed with ICP-OES (device Perkin Elmer ICP-OES Optima 2100 DV) under the experimental conditions (Plasma power, 1300 W; nebulizer flow, 0.80 L/min; plasma flow, 15 L/min; auxiliary flow, 0.2 L/min; plasma view, axial; sample flow rate, 1.50 mL/min; delay time, 60 sec; source equilibration delay, 15 sec; plasma conditions, same for all elements; nebulizer start-up, Instant). Calibration solutions were prepared at five different concentrations ranging from 25.00 to 1000.00 ppb [51; 52].

DNA protective activity of extracts of different tissues of *P. quercetorum* Agnew

pBR322 plasmid DNA was used to measure the deleterious effects of Fenton's solution and protective effect of *P. quercetorum* Agnew extracts on DNA conformation. Extracts of leaf and flower tissues of the plant *P. quercetorum* Agnew were addressed to repair the DNA damage. Plant tissue extracts were prepared in a solution of a mixture of methanol (10%) and water (90%). Three different extract concentrations (1 mg/mL, 2 mg/mL, 3 mg/mL) were applied for each different tissue and for experimental design, negative (plasmid DNA only) and positive control (Fenton + plasmid DNA) groups were created. In addition, to compared results, a commercial extract with known

biological activities, antiviral, antibacterial, anti-inflammatory, antioxidant effects and DNA damage protective activity such as rosmarinic acid and alpha-tocopherol were used at a concentration of 1 mg/mL [53].

Accordingly, the reaction mixtures to be incubated was composed of 3 µL of pBR322 plasmid DNA, 5 µL of Fenton's solution (30 mM H_2O_2, 50 mM ascorbic acid and 80 mM $FeCl_3$) and 5 µL from different concentrations (1 mg/mL, 2 mg/mL, 3 mg/mL) of plant extracts were taken and the final volume was completed to 20 µL with distilled water. This mixture was then incubated at 37°C for 30 minutes. After incubation, 5 µL of 6x loading dye was added to the reaction mixture. 10 µL of the mixture was taken and loaded onto a 0.8% agarose gel stained with EtBr. The agarose gel was run at 100 volts for 60 minutes. Then the gel image was taken with the gel imaging device.

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