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Determination of cytotoxic, anti-acetylcholinesterase and antioxidant activity of some medicinal *Artemisia* spp.

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Abstract

The study aims to investigate the antioxidant activities, phenolic compounds, acetylcholinesterase enzyme inhibition and cytotoxic effects of two different *Artemisia* spp. (*Artemisia dracunculus* L. and *Artemisia dracunculoides* Pursh) cultivated in Türkiye organically, for the first time. The total phenolic and total flavonoid contents of the plants were determined spectrophotometrically while antioxidant activity DPPH•, CUPRAC, and FRAP was determined using the colorimetric method. And also, acetylcholinesterase *in vitro* enzyme inhibition activity and anti-cancer activity against human melanoma (VMM917, CRL-3232), lung carcinoma (A549, CCL-185), and normal human fibroblast (hGF, PCS-201-018) cells were studied. Total phenolic (225-324 µg GAE/mL extract) and total flavonoid contents (0.066-0.085 µg QE/mL extract), antioxidant activity Color Scavenging Concentration 50% (DPPH• (CSC_{50}: 1.371-1.655 mg/mL), CUPRAC (0.246-0.344 µM CTEAC) and FRAP (462.133-726.661 µM CTEAC)). *A. dracunculus* and *A. dracunculoides* extracts inhibited 40.09 ± 0.65%, and 39.48 ± 3.68% of acetylcholinesterase activity. It was determined that demonstrated the selective effect of *A. dracunculus* and *A. dracunculoides* on the cytotoxicity of A549 and hGF cells. According to our results, both tarragon plant species, it may be said that it is not only consumed as food but also used for therapeutic purposes will be beneficial for health.

Keywords: *Artemisia dracunculus*, *Artemisia dracunculoides*, acetylcholinesterase, lung carcinoma, human melanoma

1. Introduction

In recent years, there has been a remarkable increase in the amount of pharmacological and chemical studies on members of *Artemisia* L. (Asteraceae) because of their medicinal properties [1–3]. Among the species, *Artemisia dracunculus* L. which is an important medicinal and aromatic plant has been used in the treatment of various diseases such as malaria, liver disease, neoplasms, inflammatory diseases, and infections.

Tarragon or dragon's-wort (*Artemisia dracunculus* and *Artemisia dracunculoides* Pursh), known as "tarhun"in Anatolia are plants of the Asteraceae family. *Artemisia dracunculus* (tarragon) is an edible spice plant as both fresh (leaves) and dried (herb). Most of these plant species are edible and used as a flavoring in food in many traditional recipes. It is also used for medicinal purposes [4]. The essential fatty acids [5–7] phenolic compounds, flavonoids [8] carotenoids [9], tannins [10], and mineral compounds [11] in the tarragon plant were suitably used for therapeutic purposes.

Cancer is one of the diseases with the highest mortality rate following cardiovascular diseases worldwide. Among cancer types, lung cancer having the second highest mortality rate in both sexes is the most common type behind prostate cancer in men and breast cancer in women [12]. In addition, skin cancer is one of the most common types of cancer among all cancer types. There are many methods in the treatment of cancer diseases such as chemotherapy, radiation therapy, surgery and immunotherapy including utilization [13]. However, these methods are known to damage healthy cells during cancer treatment [14]. In order to reduce these side effects, researchers are increasing their interest in the application of alternative medicines and natural and herbal compounds. It was

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determined that the activity of AChE increased in the cortex and hippocampus in brains with AD; therefore, the amount and activation of ACh decreased. Nowadays, acetylcholinesterase inhibitors (AChE) are the most widely used AD treatment with drugs. Most of the inhibitors are plant origins. In recent years, studies on the treatment of AD have been planned according to strategies aimed at the discovery and development of new AChE's that are more effective and have fewer side effects and may also have protective properties as well as therapeutic effects.

In this study, it was investigated whether or not tarragon's could be a natural inhibitor of the acetylcholinesterase enzyme. While antioxidant activity was determined by the colorimetric method with dropping on TLC plate in the study and phenolic compounds of tarragon plants were analyzed in PDA-HPLC. To the best of our knowledge, in this study is the first investigation of tarragon on lung carcinoma (A549, CCL-185), human melanoma (VMM917, CRL-3232) and human normal fibroblast (hGF, PCS-201-018) cells.

2. Material and Methods

2.1. Chemicals and reagents

HPLC- grade methanol, absolute ethanol, acetonitrile solutions, all phenolic compounds standards and all chemicals used for antioxidant testing were purchased from Sigma-Aldrich (Munich, Germany). Electric eel acetylcholinesterase (EC 3.1.1.7, type-VI-S), acetylthiocholine iodide, and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and donepezil were purchased from the Sigma (St. Louis, MO). Dimethyl sulfoxide (DMSO, D2650), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, M2128) and cisplatin (P4394) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals and solutions used in the cell culture studies were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), Gibco (NY, USA) and Sigma-Aldrich (St. Louis, MO, USA).

2.2. Providing of plant material and extract preparation

Tarragon is one of the important medicinal and aromatic plants which had significant expansions in its usage areas recently. The best-known species of tarragon are *Artemisia dracunculus* and *Artemisia dracunculoides* [15]. The species are grown as culture plants in Türkiye. In this study, *Artemisia dracunculus* and *Artemisia dracunculoides* were grown in ecological conditions, and organic production conditions in the Bayburt Province of Türkiye. Random Blocks Trial Design was used in the field experiment, which was prepared in three replications with a distance of 40 x 40 cm between rows. Since the plants are not flowering plants, they were harvested in June 2020 when they reached maturity.

After plants samples were at dried room temperature in a cool place, it was ground to a powder a using blender. 3.5 g of the powdered was weighed for each plant samples and 35 ml of ethanol was added, and then extracted by shaking vigorously Heidolph MR HEI-Standard, Schwabach, Germany for 24 hours. At the end of the time, the extracts were filtered with filter paper and then a syringe filter (Whatman, NY 0.45 μm) respectively. The extracts were divided into 4 parts (cytotoxic activity, antioxidant activity, phenolic compounds, and enzyme inhibition). Then, extracts were stored in the dark and at room temperature till analysis time.

2.3. Antioxidant Activity

2.3.1. Spectrophotometric Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The total phenolic content of tarragon plant extracts was evaluated using the Folin-Ciocalteu reagent [16]. In this method, 50 μL of sample solution was diluted with 2.5 mL of pure water. 250 μL, 0.2 N Folin-Ciocalteu reagent was added. Then 750 μL Na_2CO_3 (7.5%) was added to the vortexed. After 2 hours of incubation at room temperature, absorbance values were measured at 760 nm.

Gallic acid antioxidant standard, which was prepared at five different concentrations (500–250–125–62.5–31.25 μg/mL), was used for the calibration graph. The amount of phenolic substance was expressed in microgram gallic acid equivalent per milliliter extract (μg GAE /mL extract).

Flavonoids in the tarragon plants were determined with the method which was developed by Fukumoto and Mazza [17]. The study was carried out in three parallels. Initially, an equal volume (250 μL) of the sample was pipetted into the tubes and 2.1 mL of methanol was added. Finally, 50 μL of 1M ammonium acetate (CH_3COONH_4) and 10% aluminum nitrate nonahydrate ($Al(NO_3)_3.9H_2O$) were added respectively, except for the sample blank. The mixtures were mixed with a vortex. After 40 minutes, absorbance values were read at 415 nm. Quercetin was preferred as standard. It was prepared at six different concentrations (0.25 mg/mL). A graph was drawn with the absorbance values corresponding to the concentration. According to the drawn graph, the total flavonoid substance amount of

the extracts was determined as microgram quercetin equivalent per milliliter extract (µg QE /mL).

2.3.2. Colorimetric DPPH• Radical Scavenging Activity, FRAP and CUPRAC Antioxidant Activity Test

In the study, the DPPH• antioxidant activity method [18] which is commonly used in the literature was carried as colorimetric [19] instead of spectrophotometric. Preliminary tests were applied for tarragon plant extracts with 2000 µM freshly prepared DPPH• solution. Then initial concentrations of each sample were determined. Plant extracts and antioxidant standard (Trolox®) were studied at 5 different concentrations. Measurements of samples, reagent blanks and sample blanks were performed in triplicate. DPPH• solution was transferred at equal volumes onto the solutions. The mixtures in the tubes were mixed with vortex and incubated for 60 minutes at room temperature. At the end of the incubation, the mixtures were dropped onto a TLC plate (Merck Silica gel 60) as triplicates (15 µL). After 5 min incubation, the formed colors on TLC plates were scanned via scanner and transferred to the computer. Images were shown as Jpeg files. The color result of each spot-on TLC plates was determined with Image J software. The color values were plotted against sample concentrations for the calculation of Color Scavenging Concentration 50% (CSC_{50}) values. CSC_{50} is the concentration of the sample which increases the color value (intensity of color) to half of its maximum at complete DPPH• reduction. When calculating the CSC_{50} values of the samples, their 1st degree derivative was taken.

The FRAP reagent was prepared freshly in the colorimetric method [20] as in the spectrophotometric method [21]. In the study, the Trolox antioxidant standard was studied at 5 different concentrations (1000, 500, 250, 125, 62.5 µM) to draw the standard calibration graph. Samples, reagent blanks and sample blanks were measured in triplicate. Pipetting of all samples was carried out as in the spectrophotometric method [21]. First, 50 µL of the extract was transferred to the test tubes and 1.5 mL FRAP reagent was added. The solutions were vortexed and kept for 20 minutes. Then the mixtures were dripped onto a TLC plate (Merck silica gel 60) as triplicates (15 µL). Also, the same amount of reagent blank and sample blank was dropped onto the TLC plate. It was left to dry at room temperature for 7 minutes. Then, it was transferred with the scanner device to the computer and saved as a JPEG file. The color values of the images were analyzed with the Image J program. graphs of the color values and concentrations were drawn using Microsoft Excel. µM Color TEAC (CTEAC) values were calculated for each sample with equality of the standard antioxidant Trolox plot [20].

As in the other tests, the CUPRAC antioxidant activity test was performed in triplicate. Trolox as a standard antioxidant was studied at 6 different concentrations (1000-500-250-125-62.5-31.25 µM). In this method, the tubes were added equal volume of Cu (II) chloride solution, neocuproin solution, ammonium acetate buffer (pH=7) and analysis solutions, respectively. The final solution volume was completed to 1.03 mL with distilled water. The tubes were stored at room temperature for 30 minutes [22]. At the end of the waiting period, 15 µL of the mixtures in the tubes were dropped onto the TLC plate in triplicate for all the samples. Because of pure water in the CUPRAC reagent, the TLC plates were left for 10 minutes and thus it was dried. Then the plates were scanned via a scanner, transferred to the computer and saved as JPEG files. Image J program was used in the determination of color values. Graphs were drawn based on average color values. The µM Color TEAC (CTEAC) values of each extract were calculated [23].

2.4. Phenolic and Flavonoid Compounds Analysis by RP-HPLC-PDA

The phenolic and flavonoid contents of tarragon plants were determined by RP-HPLC-PDA. The HPLC analyses were conducted on a Shimadzu Liquid Corporation LC 20AT HPLC system equipped with a PDA detector, C_{18} column (250 mm × 4.6 mm, 5 µm; GL Sciences). The mobile phases include acetic acid (2%) in water (A), acetonitrile (30%) in water (B), and the flow rate was selected as 1mL/min, injection volume of 20 µL, a column at 30ºC, were used and the detection range from 250, 280, 320 and 360 nm. The gradient was as follows: 0 min, 95% solvent A; 8 min, 15% solvent A; 10 min, 21% solvent A; 20 min 52% solvent A; 35 min 67% solvent A; 50 min 90% solvent A; 60 min 5% solvent A [24]. In order to confirm the phenolic compounds of the samples and to determine the concentrations, the retention times were compared with the real standards and ultraviolet absorption spectrum data. Samples were passed through a 0.45 µm filter before were given to the device.

2.5. Acetylcholinesterase Enzyme Inhibition

The extracts prepared for cell culture and antioxidant activity were also used for enzyme inhibition. Ellman method was modified with minor modification and applied [25,26]. Briefly, 50 µL of plant extract in various concentrations, 50 µL acetylcholinesterase (0.22 U/mL) enzyme, and 3 mL 0.1M phosphate buffer (pH = 8) 30 by mixing after minutes incubated. After 100 µL of 0.01 M 5.5'-dithiobis (2-nitrobenzoic acid) (DTNB) to the tube, adding 50 µL of 0.75 mM acetylthiocholine iodide the reaction was initiated.

The absorbance of the yellow compound [5-thio-2-nitrobenzoate dianion (TNB)] formed as a result of the reaction of thiocholine with chromogenic DTNB, which is released due to hydrolysis of acetylthiocholine, was measured in a spectrophotometer at 412 nm. Donepezil and ethanol were used as positive control and negative control respectively.

2.6. Cell Culture

Lung carcinoma (A549, CCL-185), human melanoma (VMM917, CRL-3232) and human normal fibroblast (hGF, PCS-201-018) cells were purchased from ATCC (Manassas, VA, USA). Both cells were cultured in Rosewell Park Memorial Institute-1640 Medium (RPMI-1640) with 10% heat inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were incubated at 37°C supplied with 5% CO_2 [27].

2.6.1. Drug Preparation and Treatment

Extracts of *A. dracunculus* and *A. dracunculoides* and cisplatin were prepared in ethanol. All of the extracts and cisplatin concentrations were diluted with their solvents for cytotoxicity studies. Cisplatin was preferred as a positive control in this work [28]**.** Final solvent concentrations were adjusted to a maximum of 0.5 % for the medium.

2.6.2. Measurement of Cell Viability

Cell viability was evaluated using a colorimetric MTT [3-(4,5-d i m e t h y l t h i a z o l -2- y l) - 2,5-d i p h e n y l t e t r a z o l i u m b r o m i d e] assay [29]. Briefly, $5x10^3$ cells A549, VMM917 and hGF cells were seeded into the well of sterile 96-well cell culture plates. After overnight incubation, the media on the plates were replaced with fresh media for A549, VMM917 and hGF. Then the plates were treated with various concentrations of the plant extracts (0.9–125 µg/mL) in A549, VMM917 and hGF for 72 h. Cisplatin was treated at different concentrations (0.25–32 µM) on both A549, VMM917 and hGF cell lines for 72h. Subsequently, 10 µL of MTT dye (0.25 mg/mL) was added to each well for 2 h. At the end of the time, the dye was removed and 200 µL of DMSO was added to each well to dissolve the formazan crystals. Absorbance values were read at 570 nm in a microplate reader (Molecular Devices Versamax, CA, USA) [28]. Optical densities were used to evaluate the percentage of viabilities in treated cells compared to untreated control cells. A logarithmic plot of log concentrations against cell viability was drawn to determine the IC_{50} value. The IC_{50} values of *A. dracunculus* and *A. dracunculoides* extracts in both cell lines were used to calculate the selectivity index with the following formula [27].

$$Selectivity\ Index = hGF\ cells\ IC_{50} / Cancer\ cells\ IC_{50}$$

2.7. Statistical Analysis

All experiments were conducted as three repeats and the results were given as arithmetic mean and ± standard deviation. Statistical analyzes were carried out using SPSS (Statistics Program for Social and Science) software (Version13.0.1). One-Way ANOVA test and post-hoc Tukey test were used in the study. *p* value less than 0.05 was considered as statistically significant.

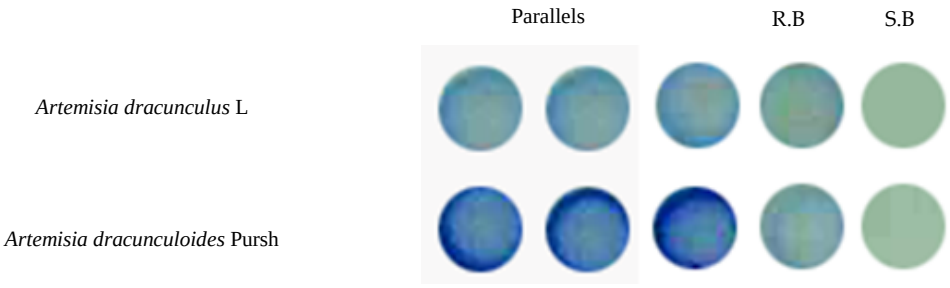
3. Results and Discussion

3.1. Antioxidant Activity

In our study, total phenolic and total flavonoid were determined spectrophotometrically, while antioxidant activity was determined colorimetrically. The results of the antioxidant activity test were given in Table 1. The results showed that the *A. dracunculus* sample has the lower total phenolic and flavonoid contents than *A.*

Table 1. Colorimetric and Spectrophotometric Antioxidant Activity, Test Results of the Plant Extracts

	Colorimetric Antioxidant Activity Tests			Spectrophotometric Antioxidant Activity Tests	
Samples	**DPPH (mg/mL) CSC50**	**FRAP (µM CTEAC)**	**CUPRAC (µM CTEAC)**	**Total Phenolic Substance µg GAE /mL extract**	**Total Flavonoid Substance µg QE /mL extract**
Artemisia dracunculus L.	1.371 ± 0.120	462.133 ± 0.201	0.246 ± 0.060	225 ± 0.090	0.066 ± 0.010
Artemisia dracunculoides Pursh	1.165 ± 0.100	726.661 ± 0.270	0.304 ± 0.080	324 ± 0.110	0.085 ± 0.020



Three-parallel, reagent blank (R.B) and sample blank (S.B) images of the samples in the colorimetric FRAP antioxidant activity test

dracunculoides. The total phenol and flavonoid content values were determined at 324 μg GAE/mL extract and 0.085 μg QE/mL extract in *A. dracunculoides* while the values were measured at 225 μg GAE/mL extract and 0.066 μg QE/mL extract in *A. dracunculus*, respectively. In addition, DPPH, FRAP, and CUPRAC antioxidant activity assays were performed as colorimetric for the plant samples. The highest activity values of DPPH, FRAP and CUPRAC tests were founded in *A. dracunculoides*. The extracts of *A. dracunculoides* exhibited inhibition of DPPH activity than the *A. dracunculus* and CSC_{50} inhibition was measured as 1.371 and 1.162 mg/mL extract, respectively. Values of FRAP and CUPRAC tests in extracts of *A. dracunculoides* were determined as 726.661 and 0.304 respectively. However, the values were measured as 462.133 and 0.246 μM CTEAC for *A. dracunculus*.

Artemisia L. genus having small shrubs and herbs, belongs to the family of Asteraceae, is a traditional medicinal plant and is used in the treatment of many diseases [30]. There are many studies in the literature about members of *Artemisia*. One of the aims of the current study is to reveal the phytochemical properties of two species (*A. dracunculus* and *A. dracunculoides*) of the *Artemisia* genus. The relevance of polyphenols rests in their potential properties as an antioxidant, anti-inflammatory, anti-microbial, and anti-tumoral [31–33].

Contents of phenolic and flavonoid of the *A. dracunculus* and *A. dracunculoides* were compared based on spectrophotometric measurements (Table 1). The total phenolic content was evaluated based on the Folin-Ciocalteu colorimetric method using gallic acid as standard [16]. Mumivand et al. [34] reported the total phenolic content of tarragons (*A. dracunculus*) obtained from 12 different parts of Iran as 40.9–96.52 mg GAE/g. In another study, Ismail et al. [35] determined that total phenol and flavonoids in *A. dracunculus* as 28.5 and 1.54mg/g respectively. These results are higher than those our present. Because, there are many factors (e.g., different developmental stages of plants, genetic diversity, differences between different plant parts) that affect the chemical composition of plants. One of them is environmental factors due to location [36].

There are different methods to determine the antioxidant activity of the plants. In this study, the colorimetric dropping method was preferred. Because, it was cheaper cost due to using reagents and solvents less than the spectrophotometric method. In addition, there is no need for a costly spectrophotometer device. DPPH, FRAP and CUPRAC antioxidant tests were applied to plant extracts. The antioxidant activity values in all the methods were measured higher for *A. Dracunculoides* than *A. dracunculus*. Behbahani et al. [37] found that the IC_{50} value of *A. dracunculus* was 0.065 mg/ml in essential oil extracts. In another study was reported that DPPH values of *A. dracunculus* were determined as 94.2 μg/mL in essential oil extracts [38].

Carvalho et al. [39] reported that total phenolic contents of six different *Artemisia* species varied from 0.22 to 0.39 mg of GAE g^{-1}.

Table 2. Phenolic Compounds

Standards	*Artemisia dracunculus* L. μg phenolic / g sample	*Artemisia dracunculoides* Pursh μg phenolic / g sample
Gallic Acid	n.d.	n.d.
Protocatechuic Acid	n.d.	n.d.
Chlorogenic Acid	**281.938**	**365.523**
p-OH Benzoic Acid	15.294	n.d.
Epicatechin	n.d.	n.d.
Caffeic Acid	n.d.	331.381
Syringic Acid	n.d.	40.586
m-OH Benzoic Acid	n.d.	n.d.
Rutin	n.d.	n.d.
Ellagic Acid	n.d.	n.d.
p-Coumaric Acid	n.d.	44.519
Ferulic Acid	**407.751**	**399.833**
Myricetin	n.d.	n.d.
Resveratrol	n.d.	n.d.
Daidzein	n.d.	n.d.
Luteolin	n.d.	n.d.
Quercetin	n.d.	n.d.
t-Cinnamc Acid	n.d.	n.d.
Apigenin	n.d.	n.d.
Hesperidin	n.d.	n.d.
Rhamnetin	n.d.	n.d.
Chrysin	n.d.	n.d.
Pinocembrin	n.d.	n.d.
CAPE	n.d.	n.d.
Curcumin	n.d.	n.d.

*n.d.: not detected

3.2. Phenolic Compounds by RP-HPLC-PDA

HPLC-PDA analysis was performed to determine the phenolic profiles in the leaves of *A. dracunculus* and *A. dracunculoides*. Twenty-five standards (gallic acid, protocatechuic acid, chlorogenic acid, *p*-OH benzoic acid, epicatechin, caffeic acid, syringic acid, *m*-OH benzoic acid, rutin, ellagic acid, *p*-coumaric acid, ferulic acid, myricetin, resveratrol, daidzein, luteolin, quercetin, *t*-cinnamc acid, apigenin, hesperidin, rhamnetin, chrysin, pinocembrin, caffeic acid phenyl ester (CAPE), curcumin) were identified and quantified (Table 2). It was identified ferulic acid (407.751μg/g sample), chlorogenic acid (281.938 μg/g sample) and *p*-OH benzoic acid (15.294 μg/g sample) in the *A. dracunculus* while chlorogenic acid (365.523μg/g), ferulic acid (399.833 μg/g sample) and caffeic acid (331.381μg/g sample) in the *A. dracunculoides* as a major compound.

Table 3. Acetylcholinesterase Enzyme Inhibition

Sample	Inhibition of AChE activity %	
***Artemisia dracunculus* L.**	1.35 (mg/mL)	40.09 ± 0.65
***Artemisia dracunculoides* Pursh**	1.16 (mg/mL)	39.48 ± 3.68
Donepezil	62.5 (µg/mL)	79.50 ± 0.20

In addition, syringic acid (40.586 µg/g) and *p*-coumaric acid (44.519 µg/g sample) were also detected in the A. *dracunculoides*.

Hydroxybenzoic acid, hydroxycinnamic acid and flavonols compounds found in plants can be considered therapeutic agents because they have protective effects against many diseases such as certain cancers, cardiovascular diseases and aging [40]. However, environmental factors and growing conditions can be affecting the chemical composition of plants; especially secondary metabolites [41]. In the present study, the phenolic composition of the *Artemisia* species was identified using RP-HPLC-PDA analysis.

The most important classes of bioactive substances of the tarragon plant are phenolic acid derivatives, flavonoids, essential oil, coumarins and alkamides [42–44]. The major phenolic compound in the extract of *A. dracunculus* was ferulic acid with 407.751 µg/g. It was followed by chlorogenic acid with 281.938 µg/g sample and *p*-OH benzoic acid with 15.294 µg/g sample. As in *A. dracunculus,* ferulic acid was determined as a major compound in the extract of *A. dracunculoides* with 399.833 µg/g. Other dominant compounds were chlorogenic acid (365. 523 µg/g sample), caffeic acid (331.381 µg/g sample) and syringic acid (40.586 µg/g sample). Ferulic acid was detected as the predominant component in both species. Lee et al. [45] reported that ferulic acid detected a lower value of 53.55 µg/g in *Artemisia absinthium* L. This may be associated with the species and environmental differences. In another study, it was reported that chlorogenic acid (0.44 mg/g) was detected in the methanol extract of leaves of *A. vulgaris* collected from Serbia in similar amounts as in the present study [46]. Chlorogenic acid, one of the hydroxycinnamic acid derivatives, is associated with a reduced risk of developing Alzheimer's disease, a common neurodegenerative disorder [47].

3.3. Acetylcholinesterase Enzyme Inhibition

One of the changes that happen in Alzheimer's disease is the rise in the activity of acetylcholinesterase (AChE), the enzyme responsible for the hydrolysis of acetylcholine from both cholinergic and non-cholinergic neurons of the brain [48] The AChE inhibition effects of samples are given in Table 3. Respectively, *A. dracunculus* and *A. dracunculoides* extracts inhibited 40.09 ± 0.65%, and 39.48 ± 3.68% of acetylcholinesterase activity at a concentration of about 1 mg/mL. The samples showed acetylcholinesterase inhibitory activities less than 50% although it has been shown to be effective against lung and melanoma cells.

Alzheimer's disease is the most common type of dementia. The treatment of Alzheimer's disease, which is multifactorial, requires multiple therapeutic approaches. By blocking the degradation of ACh using acetylcholinesterase inhibitors, it forms the most important therapeutic strategy by controlling the level of acetylcholine (ACh) as a neurotransmitter in cholinergic synapses [49]. To date, some *Artemisia* species have been determined by AChE inhibition effects. However, it has not been studied in *A. dracunculus* and *A. dracunculoides.* The inhibitory activity of these ethanolic samples was determined against the AChE enzyme at 1.35 and 1.16 mg/mL in vitro. As shown in Table 3, the highest

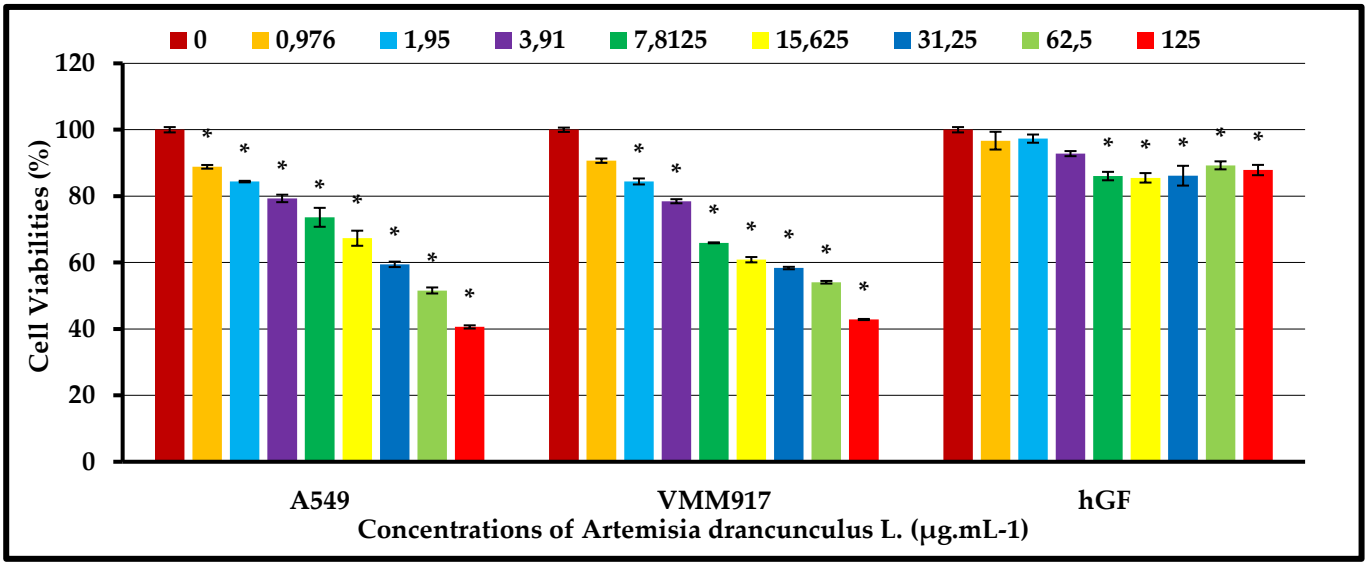


Figure 1. The cytotoxic effect of *A. dracunculus* on A549, VMM917, hGF cells for 72h. Cell viabilities (%) are shown in each cell after treatment with *A. dracunculus*, at various concentrations. Error bars are representative of the standard deviation of at least three independent experiments. * $p < 0.05$.

Table 4. IC_{50} values (μM) and selectivity index values calculated for, *A. dracunculus*, *A. dracunculoides*, cisplatin on A549, VMM917 and hGF cell lines (n=3)

Test compound/Cell Lines	A549	VMM917	hGF
***Artemisia drancunculus* L.**	53.17 ± 1.09	> 125	> 125
***Artemisia dracunculoides* Pursh**	46.66 ± 0.49	76.90 ± 1.63	> 125
Cisplatin	0.77 ± 0.06	6.86 ± 1.02	13.5 ± 0.71

Table 5. Selectivity index values calculated for *A. dracunculus*, *A. dracunculoides* and cisplatin on A549, VMM917 and hGF cell lines (n=3)

Cell Lines/Test compound	*Artemisia dracunculus*	*Artemisia dracunculoides* Pursh	Cisplatin
A549	> 2.35	> 2.68	17.50
VMM917	> 1	> 1.63	1.97

AChE inhibition was observed for *A. dracucunlus* with 40% inhibition at 1.35mg/mL concentration while it was measured for *A. dracunculoides* with 39.48% inhibition at 1.16 mg/mL concentration. Ferrante et al. [50] reported that among methanol, ethyl acetate, water and essential oil extracts of *Artemisia santonicum* L. was determined highest acetylcholinesterase inhibition effect in essential oil extract as IC_{50} 2.26 mg/mL.

3.4. Cytotoxity Effect

This study revealed the effects of *A. dracunculus* and *A. dracunculoides* extracts on cell viabilities in A549, VMM917 and hGF cells. *A. dracunculus* and *A. dracunculoides* induced cytotoxicity in all cells in a dose-dependent manner (Table 4, Fig. 1, Fig. 2) ($p < 0.05$). In all of the cytotoxicity studies, cisplatin, an anticancer drug known as a positive control, was used [28,48]. The cytotoxicity studies of cisplatin on A549, VMM917 and hGF cells and the IC_{50} values explained by all cells are given in Table 4. Demonstrated the selective effect of *A. drancunculus* and *A. dracunculoides* on the cytotoxicity of A549 and hGF cells. The selective index of the plant extracts was calculated using Formula 1, (Table 5).

Skin and lung cancer has increased in the last 20 years. The number of these cancers is increasing every year [51,52]. New methods and research are being tried in order to treat cancer and prevent the rapid increase in deaths due to cancer types every year [53–56]. There is a range of therapeutic approaches in the treatment of melanoma and lung cancer such as chemotherapy, photodynamic therapy, immunotherapy, targeted therapy and enzyme inhibition therapy. Conventional chemotherapy has been frequently used particularly in the of improved melanoma and nonsmall cell lung cancer (NSCLC), however, increased resistance to chemotherapeutic drugs and/or possible mutagenic effect of chemotherapeutics on healthy cells may reduce the success rate of treatment [53-56]. The side effects of chemotherapeutic drugs used in cancer treatment lead constantly researchers to the discovery of new drugs. Therefore, studies have increased on endemic, rare plant species and plants grown in extreme environments in recent years. Among them, species belonging to the genus *Artemisia* L. attracted the attention of researchers [3]. In a study on *A. dracunculus* L., it was associated with diabetes and was reported that increase insulin secretion

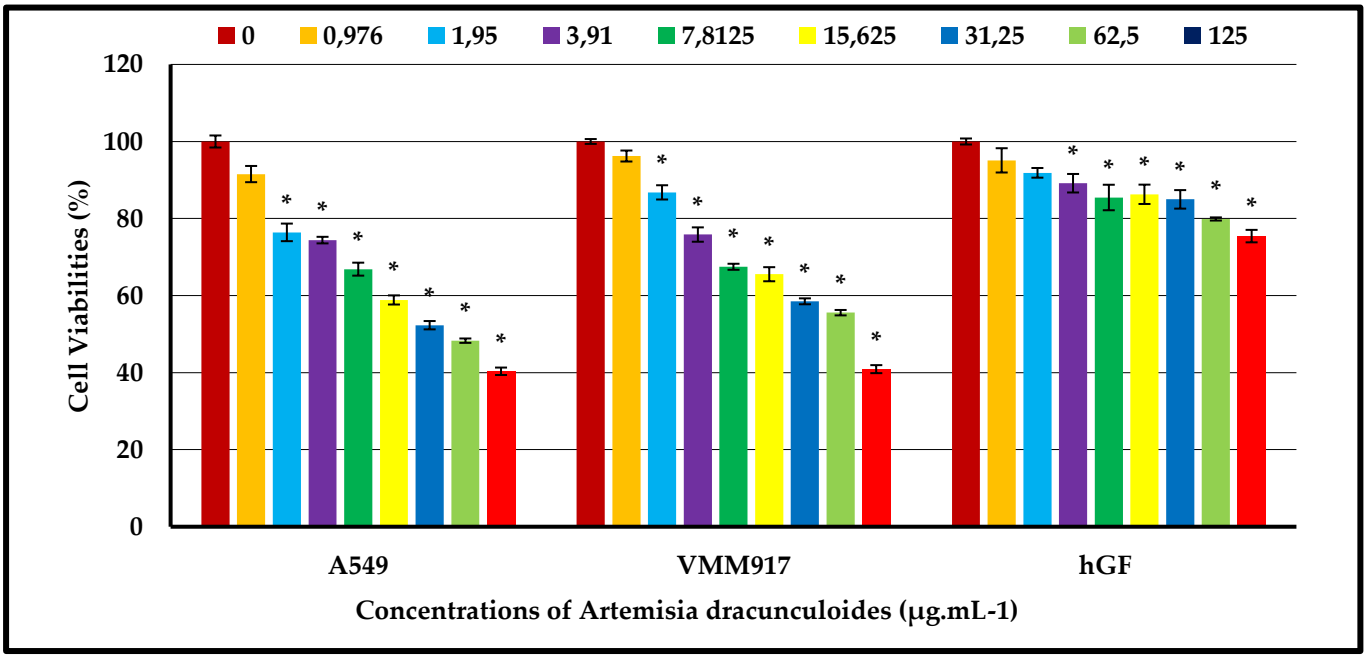


Figure 2. The cytotoxic effect of *A. dracunculoides* on A549, VMM917, hGF cells for 72h. Cell viabilities (%) are shown in each cell after treatment with *A. dracunculoides* at various concentrations. Error bars are representative of the standard deviation of at least three independent experiments. * $p < 0.05$.

and protect β cell number. In addition, it suppressed LPS/INFy-induced inflammation [57]. In another study on the same species of *Artemisia*, an MTT test was performed on cell viability at concentrations between 7–500 μg/mL on MCF-7, T-47D, MDA-MB-231, MCF-10 cancer and Hu-02 fibroblast cells. Although IC_{50} values are not calculated, the extract has been shown to cause a reduction in cancer cells at a concentration of 500 μg/mL compared to control cells [58]. In addition to in vitro studies, in vivo studies on *Artemisia dracunculus* were also carried out, it has been shown that *Artemisia dracunculus* causes a decrease in cytokines through IL-17 and IL-23, which are known as inflammatory parameters in an experimental rat model [59].

4. Conclusion

In this study, it was determined that, total phenolic and flavonoid contents, antioxidant activity, phenolic compounds, acetylcholinesterase enzyme inhibition, and cytotoxic effect of two species of *Artemisia*. Total polyphenols, flavonoids, FRAP, CUPRAC and the antioxidant activities and polyphenol contents of the plants were found to be very close. DPPH antioxidant capacities, were investigated in plant extracts. It is clearly seen that plant extracts have at a certain level efficient inhibition properties on AChE, so these plants can be considered for drug design in the treatment of Alzheimer's disease. There are a number of drugs developed chemically or biotechnologically. It is clearly seen that there is no study on VMM917 and A549 cancer cells associated with *A. dracunculus* and *A. dracunculoides* in the literature. In this respect, the results of the study suggest that the use of extracts at certain doses will be a pioneer in evaluating its potential novel chemotherapeutic drug.

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Conflicts of Interest:

The authors declare no conflict of interest.

Author Contributions

ZC: Planning of the study, formal analyses, writing-editing article.

EAD: cytotoxic activity analyses.

ZA: Colorimetric Antioxidant activity analysis.

YK: HPLC studies.

BG: Cultivation of the plant used in the study.

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