Araştırma Makalesi / Research Article

Association of the phenolic content, DNA protective activity and some antioxidant properties in the Achillea arabica Kotschy Flower

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Abstract
Pharmacological herbs are our first medicines in history and we take them into our bodies in different ways by consuming tea, spices, or raw. Nowadays, due to the side effects of more effective synthetic medicines, the tendency to use pharmacological plants has increased and the benefits are provided from plants in the treatment of routine diseases and to protect from them. In our daily life, stress and adverse environmental conditions may cause oxidative stress by increasing the concentration of reactive oxygen species (ROS) in a cell and oxidative stress is known to be associated with cancer and various neurodegenerative diseases such as Parkinson's and Alzheimer's. Antioxidants protect the cell against diseases by regulating the ROS concentration and herbal phenolics are known as antioxidants and strong candidates for this. In addition, studies on plant components and active ingredient have gained more importance during the Covid-19 epidemic period as they provide important data for vaccine development studies. In this study, it was aimed to obtain important data for advanced studies. For this purpose, *Achillea arabica*, one of the pharmacological members of the Asteraceae family, was collected from Ağrı/Patnos and stored by converting into herbarium material. Some biological activities such as the determination of some in vitro antioxidant properties, DNA protective activity, and the phenolic content by HPLC were evaluated in the DMSO extract prepared from the flowers of the herbarium plant. The results of the study were evaluated together and correlated.

Anahtar kelimeler: *Achillea arabica*, Antioxidant, DNA protection, HPLC, Phenolic

Achillea arabica Kotschy Çiçeğindeki Fenolik İçerik, DNA Koruyucu Aktivite ve Bazı Antioksidan Özelliklerin İlişkisi

Öz
Farmakolojik şifalı bitkiler tarihteki ilk ilaçlarımızdır ve vücudumuza çay, baharat veya çiğ tüketerek farklı şekillerde alırız. Günümüzde daha etkili sentetik ilaçların yan etkileri nedeniyle farmakolojik bitkilerin kullanımına olan eğilim giderek artarak özellikle rutin hastalıkların tedavisinde ve bunlardan korunmada bitkilerden faydalanılmaktadır. Günlük yaşamımızda stres ve olumsuz çevresel koşullar, hücrede reaktif oksijen türlerinin konsantrasyonunu artırarak kanser veya Parkinson ve Alzheimer gibi çeşitli nörodejeneratif hastalıklarla ilişkili olduğu bilinen oksidatif strese neden olabilir. Antioksidanlar, reaktif oksijen türlerinin konsantrasyonunu düzenleyerek hücreyi hastalıklara karşı korur ve antioksidan olarak bilinen bitkisel fenolikler reaktif oksijen türlerinin süpürülmesi için güçlü adaylardır. Ayrıca bitki ve etken madde ile ilgili çalışmalar, Covid-19 salgını döneminde aşı geliştirme çalışmaları için önemli veriler sağladıkları için daha da önem kazanmıştır. Bu çalışma ile ileri araştırmalarda kullanılabilecek faydalı verilerin elde edilmesi amaçlanmıştır. Bu amaçla çeşitli farmakolojik özellikleri bilinen Asteraceae familyasının önemli üyelerinden biri olan *Achillea arabica*, Ağrı / Patnos'tan toplanmış ve herbaryum materyaline dönüştürülerek depolandı. Herbaryum bitkisinin çiçeklerinden DMSO ekstresi hazırlandı ve hazırlanan ekstrelerde, bazı *in vitro* antioksidan özellikler, ekstrenin DNA koruyucu aktivitesi ve HPLC ile farklı fenolik içerikleri kantitatif olarak belirlendi ve elde edilen sonuçlar ilişkilendirilmeye çalışıldı.

Keywords: *Achillea arabica*, Antioksidan, DNA koruma, HPLC, Fenolik

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1. Introduction

Plants were used by humankind throughout history due to the biological effects on many different diseases and people tried to cure their diseases or protect from them by consuming the plants with different methods such as extracting with infusion, making tea or spice, or eating them directly. The healing effects of the plants are associated with the antioxidant content, secondary metabolite, and active molecules in their structure [1, 2] and are known to have an important role in the treatments of many diseases from diabetes mellitus to many cancer types [3]. Active molecules that can be produced in various ways are essential components of pharmacological production and plants are one of the main sources for active molecule discovery. Thanks to these discovered molecules, today's effective medicines have been produced. However, side effects known in synthetic medicines have directed society to benefit from plants in the treatment of routine diseases such as sputum, cough, sinusitis, edema, and wound healing [4, 5]. In addition to this, biological properties and active molecules determined previously can provide important data for the study about pharmacological production and vaccine development [3]. In parallel with the onset of the Covid-19 pandemic, the treatment methods with new approaches, the discovery of active molecules, and various biological properties in the plants have become very popular.

Stress or environmental pollution components such as smoke and heavy metals are known to cause oxidative stress associated with cancer or neurodegenerative diseases by increasing the concentration of the reactive oxygen species (ROS) in a cell [6-8]. To protect from the aforementioned diseases, the reduction of heavy metals or the scavenging of free radicals are important for the continuation of life and plants are one of the most reliable sources for this. As a matter of fact, previous studies have stated that secondary metabolites, terpenoids, and antioxidant phenolics can regulate the cellular ROS concentration and play a protective role against many diseases caused by oxidative stress [1, 2, 9].

It has been reported that the Asteraceae family, one of the flowering plant families, includes 1600 genera and 25000 species [10, 11] and in addition to being consumed as food, the members of this family were reported to have antimicrobial activity thanks to the flavonoids and terpenoids in their structure [12]. They can also be used as garden and landscape plants [10] and the members of the Asteraceae family are used for herbal medicine production [3] and the elimination of pollutants in urban areas [10].

Achillea arabica (A. arabica) is one of the important members of the Asteraceae family and has been reported to have more than 130 genera in the world in previous studies [13] and 24 of 48 species in Turkey flora have been stated to be endemic [14]. *Achillea* species are known to have antioxidant, antiproliferative capacity, antimicrobial and anticancer activity and can be used for therapeutic purposes [15]. Importance of this genus used in a versatile way is emphasized [13]. Many studies, which also include *Achillea* species, reported that plant content may be changed depending on geographical variables such as altitude, climate, fertilization, soil type, and mineral content of the soil, environmental factors [13] or cultivation techniques such as various agricultural practices and culture area [13, 16]. This suggests that the biological effects of the plants may change depending on various conditions.

In the light of the given data, it was aimed to provide valuable data in this study for advanced pharmacology and alternative medicine studies by investigating some biological properties in the extract prepared from the flowers of *A. arabica* herbarium material. In this context, first of all, some *in vitro* antioxidant activities were investigated. In this context, the scavenging activity of DPPH and ABTS radicals and the activities of metal reduction for cupric and ferric iron using CUPRAC and FRAP methods, respectively were evaluated. To validate the results observed in the antioxidant studies, the protective effect of the extract on pUC18 DNA against hydrogen peroxide (H_2O_2) were investigated and finally, in order to evaluate the obtained data, the phenolic concentrations of the extract were determined by HPLC.

2. Material and Method

2.1 Plant collection and identification

A. arabica was collected from Ağrı/Patnos land in September 2020 (39° 08′ 12″ N, 42° 52′ 7″ E; 1450 m). The plant was dried in the shade and converted into herbarium material (Herbarium code: A.SAVCİ-2) and stored then in Muş Alparslan University, Center Research and Application Center. The identification of the plant used in this study was performed by Dr. Murat KURŞAT who is a member of Bitlis Eren University

2.2. *In Vitro* Antioxidant Tests

2.2.1. Sample Preparation for Antioxidant Assays

Herbarium material was pulverized using liquid nitrogen. The resulting powder material was incubated in pure water (PW) and methanol (MetOH) for 48 hours at room temperature by mixing and was filtered. Solvents were then evaporated using a rotary evaporator under a suitable vacuum. Using the obtained material, PW extract and MetOH extract were prepared at the 1 mg/mL concentrations and used in the antioxidant tests.

2.2.2. Cupric Ion (Cu^{2+}) Reduction Test

Cupric ion (Cu^{2+}) reduction test was performed using the CUPRAC method modified by Gulcin (2006) [17]. According to this, 0.25 mL of 0.01 M $CuCl_2$ was added in test tubes and then neocuprin solution and 1 M ammonium acetate buffer (pH: 6.5) were transferred into test tubes at the same volumes. 10µL, 20 µL, and 30 µL volumes from 1mg/mL samples were added on. The mixture was vortexed and incubated for 30 min, and absorbance values were then recorded at 450 nm. Increasing absorbance values show the reduction capacity of cupric ions.

2.2.3. Ferric ion (Fe^{3+}) reduction test

The Ferric ion reduction capacities of the extracts were tested using the FRAP method developed by Oyaizu (1986) [18]. According to this, the same volumes of the phosphate buffer (pH: 6.6) and [$K_3Fe(CN)_6$] were transferred into test tubes, and then distilled water at 40% ratio were added on. After the incubation for 20 min at 50 ^oC. by adding the trichloracetic acid (TCA) solution, total volume was completed to 8.5 mL. After vortexing and centrifugation briefly, an ideal volume from the upper phase of the samples was taken and transferred into the other tubes. After adding distilled water at the same volume and $FeCl_3$ solution at 20% ratio, absorbance values were recorded spectrophotometrically at 700 nm [18].

2.2.4. DPPH Radical Scavenging Test

DPPH (1,1-Diphenyl 2-picrylhydrazyl) radical scavenging method proposed by Blois (1958) [19] was performed to evaluate the radical scavenging activities of the extracts. This method is a common method to measure the radical scavenging activities of various factors, and the principle of the method is based on the reduction of DPPH radicals in alcohol. In this study, 10µL, 20µL, and 30µL volumes of 1 mg/mL extracts were transferred into the test tubes and total volumes were completed to 4 mL by adding the ethanol/DPPH solution (v/v: 3/1). Samples were incubated for 30 min at room temperature and absorbance values were recorded at 517 nm. The percentages of DPPH radical scavenging were calculated using the following equation;

$$\text{DPPH Radical Scavenging (\%)} = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) \text{x } 10 \qquad (1)$$

2.2.5. ABTS Radical Scavenging Test

The ABTS (2.2′-azinobis (3-ethylbenzthiazoline-6-sulfonate) radical scavenging was performed according to the method proposed by Wu, Chang, Chen, Fan. (2009) [20], and the ABTS radical scavenging activities of the extracts were evaluated. In this test, 2.45 mM potassium persulphate ($K_2S_2O_8$) and 7 mM ABTS solution were reacted at the same volumes and by incubating the samples for 16 hours at room temperature, ABTS radical solution was created. ABTS radical solution was diluted using methanol to achieve the desired control absorbance value at 734 nm. Distilled water and radical solution were transferred into the tubes at the same volumes and the different concentrations of the samples were added on (0.25mg/mL, 0.5 mg/mL, and 1 mg/mL). After the incubation at room temperature for 2 hours, absorbance values were recorded at 734 nm.

2.3. DNA Protective Activity Test

The protective effect of the extract on pUC18 DNA was evaluated by agarose gel electrophoresis [21]. Since DMSO is known to be a powerful solvent, stock extract solution was prepared by dissolving 200 mg of crude extract in 1 mL DMSO (Sigma Aldrich, 99.5% D4540, CAS Number: 67-68-5). New extract concentrations were formed at 1 mg/mL, 0.5 mg/mL, and 0.25 mg/mL concentrations by re-diluting the stock solutions in DMSO and the new extracts were used in the DNA preservation study. The different concentrations of the new extract were mixed with the other components as shown in Table 1 and incubated for 24 hours at 37 ºC. Thus, electrophoresis samples were ready for use. 5µl from the electrophoresis samples were transferred into the wells of the agarose gel by coloring with loading buffer, and run at 40 volts for 2 hours. The electrophoresis product was visualized using BIORAD ChemiDoc XRS Imaging system.

Table 1. The component and quantities of electrophoresis samples

Wells	DNA(µL)	H_2O_2(µL)	DMSO(µL)	PW extract(µL)	Extract(µL) / Concentration
Sample 1	15	-	-	15	-
Sample 2	15	5	-	10	-
Sample 3	15	5	10	-	-
Sample 4	15	-	10	5	-
Sample 5	15	5	-	-	10 / 0.25 mg/mL
Sample 6	15	5	-	-	10 / 0.5 mg/mL
Sample 7	15	5	-	-	10 / 1 mg/mL
Sample 8	15	-	-	5	10 / 0.25 mg/mL
Sample 9	15	-	-	5	10 / 0.5 mg/mL
Sample 10	15	-	-	5	10 / 1 mg/mL

2.4. Phenolic Tests by HPLC

2.4.1 Sample preparation for HPLC

10 g from the flowers of the herbarium material were taken and pulverized with the aid of liquid nitrogen and then incubated by mixing in the HPLC buffer for 2 hours. After centrifugation at 2000 rpm, prepared mixture was used in the determination of the quantitative phenolic concentrations.

2.4.2. Determination of standard graph and phenolic content

To use in the preparation of standards, stock solutions were prepared by mixing in the 1% acetic acid and acetonitrile. Methanol was then dissolved in the homogeneous mixture at the rate of 1:1. Final concentrations of standards were adjusted to 10 mg/mL by dissolving in the stock solution and then new standard solutions were prepared by diluting at 10 mM, 25 mM, 50 mM, 75 mM, and 100 mM concentrations [22]. The previous prepared plant extract was diluted to 20 mg/mL and filtered with the aid of 0.45 µm membrane filter before loaded into HPLC. HPLC application was performed by

following the protocol shown in Table 2 and phenolic concentrations were determined using their chromatograms (Figure 3) and calibration curve was created by HPLC.

Table 2. HPLC operating conditions and gradient elution programme.

HPLC operating conditions	
Model	Agilent Technologies 1260 Infinity II
Colon	ACE 5 C18 (250x4.6 mm id)
Kolon Oven	G7130A
Dedector	1260 DAD WR
Pump	1260 Quat Pump VL
Mobile phase	A: %1 Acetic Acid B: Acetonitrile
Dedection	272, 280 ve 310 nm
Autosampler	1260 Vialsampler
Flow Rate	1 mL/dk
Colon Temperature	28 ºC
Injection	20 µl

Gradient elution		
Time (min)	A (%)	B (%)
0	90	10
25	60	40
39	40	60
50	10	90
55	90	10

2.5. Statistical Analysis

All experimental assays were repeated at least three times. In all antioxidant studies, the effects of the highest concentrations of the extracts were compared statistically due to observed important activities. Antioxidant effects of PW extract and MetOH extract were compared statistically with the effects of standard antioxidants. Statistical comparisons were performed by Nonparametric Kruskal-Wallis test, with a very high confidence interval, followed by Dunnett's multiple comparisons test. Heavy metal reduction and lipid peroxidation results were presented as Mean ± Standard Deviation and p values below 0.05 were considered significant. IC50 values showing DPPH and ABTS radical scavenging effects were compared with the same test and the differences between the means and p values were calculated (Table 3). In all comparison results, *; was used as a symbol of statistical significance, *P<0.05 (significant); **P<0.01 (very significant); ***P<0.001 and ****P<0.0001 (extremely significant); ns P>0.05 (not significant).

3. Results and Discussion

3.1. In vitro antioxidant results

Heavy metals and various adverse conditions that we are exposed to in daily life encourage the production of free radicals, known as ROS, and excessive radical accumulation causes oxidative stress [8, 23]. Herbal phenolics are one of the best reducers of the oxidative stress associated with diabetes and various neurodegenerative diseases [24]. In the study based on this idea, PW extract and MetOH extract were prepared from the flowers of herbarium material, and it was aimed to illuminate some antioxidant properties of the extracts by comparing to the standard antioxidants, which are Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), and Ascorbic acid (ACS). The heavy metal reduction results are presented as µg TE/mL extract (Figures 1A and 1B). Heavy metal reduction results and lipid peroxidation percentages in the highest concentrations of PW extract and MetOH extract were statistically compared with the standard antioxidants (Figures 1A, 1B, and 1C). No statistically significant difference was observed between the cupric ion reduction activities of the PW extract and MetOH extract against ASC standard, but significantly lower than other standards (Figure 1A). Contrary to this, it was observed that there was no significant difference between the iron reducing effects of both extracts against the BHA and BHT, but significantly lower activity compared to the ASC standard (Figure 1B). It was also observed that there was not significant difference between the percentages of the lipid peroxidation in MetOH extract against the ASC results (Figure 1C) and lipid peroxidation scavenging percentages of the MetOH extract and ASC standard were found to be similar and the other lipid peroxidation scavenging effects of the both extracts were found to be significantly lower than the standards (Figure 1C). In a study conducted on papaya root, it was emphasized that the DPPH and total

reduction percentages of papaya root extract were highly similar to the results of ascorbic acid. This similarity is consistent with the results of this study [25].

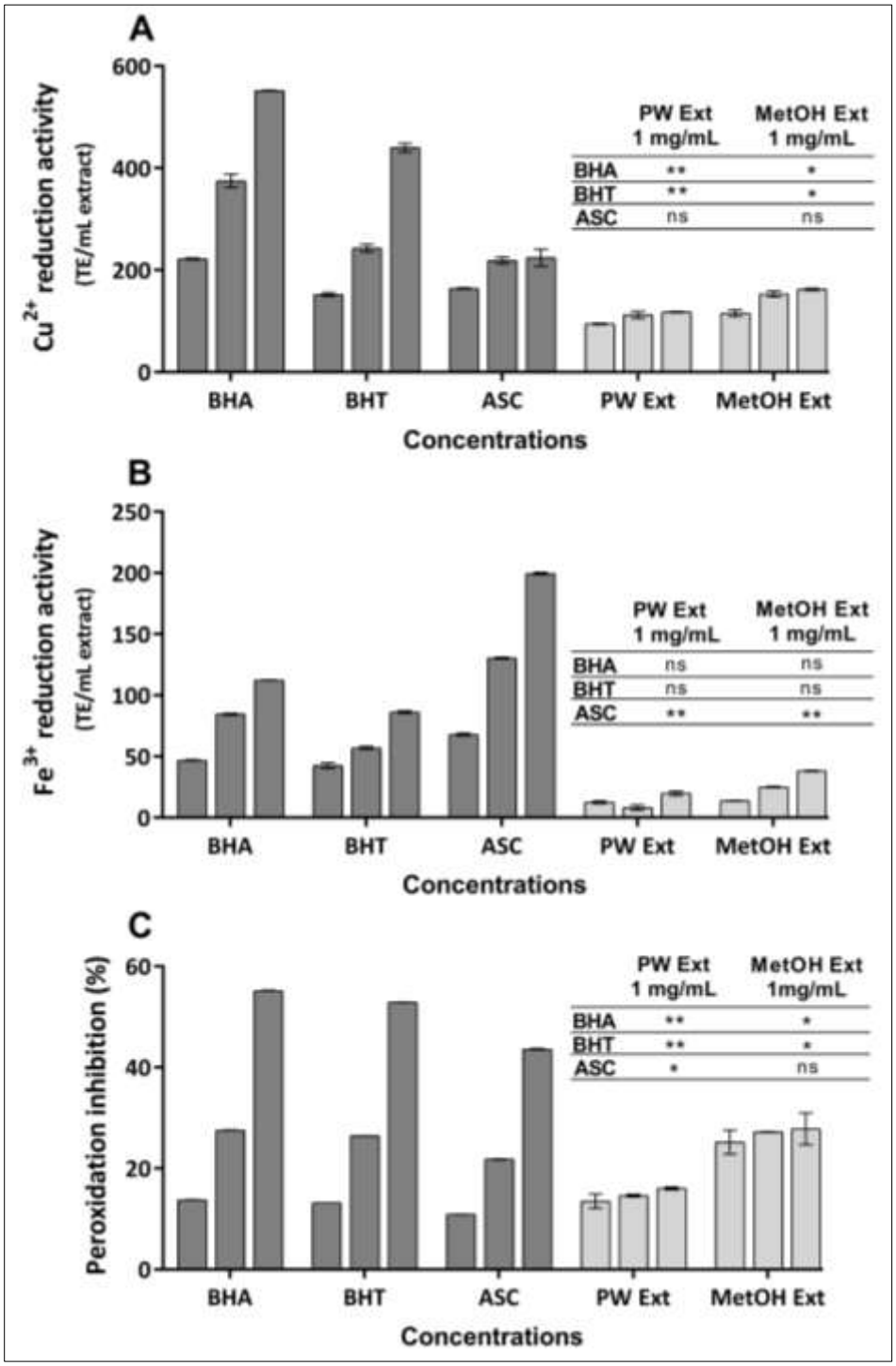

Figure 1. Heavy metal reduction activity and Lipid peroxidation inhibition percentages depending on extract concentrations in the extracts prepared from the *A.arabica* flowers. Heavy metal reduction results are expressed as TE/mL extract and lipid peroxidation results are expressed as percentages. A) Cupric ion (Cu^{2+}) reduction results, and B) Ferric iron (Fe^{3+}) reduction results and C) % Lipid peroxidation results.

IC50 is expressed as the concentration of the competitive substance that inhibits the specific binding of any medicines at the rate of 50%, and the IC50 values of the extracts were found to be higher than the standard antioxidants. This means that the radical scavenging power of the extracts is weaker than the standards. IC50 values were calculated from the radical scavenging results. IC50 values of PW

extract and MetOH extract were compared statistically with the IC50 values of the standards, and the difference between the means of IC50 values and p values are shown in Table 3. ABTS radical scavenging activity of the MetOH extract is remarkable because of the similarity to the results of BHA and BHT standard antioxidants (p>.9999). This similarity indicates that the ABTS radical scavenging activity of MetOH extract is similar to the BHA and BHT. In general, the radical scavenging activities of the extracts were observed to be lower than standards. This result may indicate that the radical reducing force will increase depending on the concentration of MetOH extract, and the weakness in the antioxidant activities of the extracts can be attributed to the absence of many essential oil components and/or the cessation of the many enzymatic reactions in the herbarium material. As known, the extraction method used, fertilization, environmental condition, climate, and the shape of the raw material can alter the plant content and its biological activities [13]. A previous study has reported that the biological activity of the herbarium material is weaker than the fresh plant of the same species [26]. The DPPH radical scavenging activity of the standard and extract is consistent with previous studies. A study conducted on *Barringtonia acutangula* emphasized that the IC_{50} value of the bark extract was higher than that of the leaf and standard. In addition, this situation observed in this study may be another proof that enzymatic reactions are largely stopped in dry tissue [27].

Table 3. Statistical comparison results of DPPH and ABTS radical scavenging IC50 values. The table shows the difference between the IC50 means and p values.

Samples	DPPH radical scavenging		ABTS radical scavenging	
	Difference between IC_{50} means	p value	Difference between IC_{50} means	p value
PW Ext / BHA	9	.1371	7.333	.4461
PW Ext / BHT	6	>.9999	7.667	.3576
PW Ext / ASC	12	.0102	12	.0102
MetOH Ext / BHA	6	>.9999	4.333	>.9999
MetOH Ext / BHT	3	>.9999	4.667	>.9999
MetOH Ext / ASC	9	.1371	9	.1371

3.2. DNA Protective Activity Results

Some ROS such as H_2O_2 are well known to cause DNA damage [26] by destroying the primary form of DNA, which is known generally as form I. Form I can be converted into form II or form III with high concentrations of the ROS formed by severe conditions such as heavy metals [27]. This study was performed to verify the observed antioxidant activities and to illuminate the DNA protective effects of the antioxidants in the extract against H_2O_2. It was wondered whether the extracts could protect the DNA against H_2O_2 despite the weakness in heavy metal reduction activity and the radical scavenging activity. For this purpose, the protective effects and regulatory effects on pUC18 DNA were investigated in the environment with H_2O_2 and without H_2O_2, respectively. 5 mL of the samples whose components and amounts are given in Table 1 were taken and loaded into the wells of agarose gel, and the electrophoresis product was visualized with BIORAD ChemiDoc XRS imaging system (Figure 2). It is clear that pure pUC18 DNA has form I and form II structures (Figure 2, well: 1). In addition, form II was transformed to form III by the effect of H_2O_2 (Figure 2, well: 2) and form I and form II disappeared by the effects of DMSO only or H_2O_2 and DMSO together (Figure 2, wells; 3 and 4). In the current study, the effects of the different extract concentrations on pUC18 DNA were investigated. Regardless of the concentration, the extract could not prevent the reactivity of H_2O_2 and could not fix the damage caused by H_2O_2 (Figure 2, wells: 8, 9, 10). Although this result is quite thought-provoking, it is well known that the excessive amount of H_2O_2 in a cell is eliminated by the activities of glutathione peroxidase (GPx) and catalase (CAT) [6] however, these reactions may terminate in the herbarium material. This information may explain the reason why the extract does not show DNA protective activity and suggests data that, independent of enzymatic catalysis, H_2O_2 cannot be effectively eliminated in a cell. In addition, the high concentrations of the extract (0.5 ve 1 mg/mL) were observed to have a positive effect on form I in the absence of H_2O_2 (Figure 2, wells: 5,6,7). As a summary of the DNA protective activity result that supports the antioxidant activity result, there are some phenolics in the herbarium material, however it is understood that the DNA protective activity against H_2O_2 damage is more closely related to the

enzymatic activities that continue effectively in the cell. As a matter of fact, it is known that hydrogen peroxide is converted to water by the enzymatic activities of GPx and CAT in a cell [6].

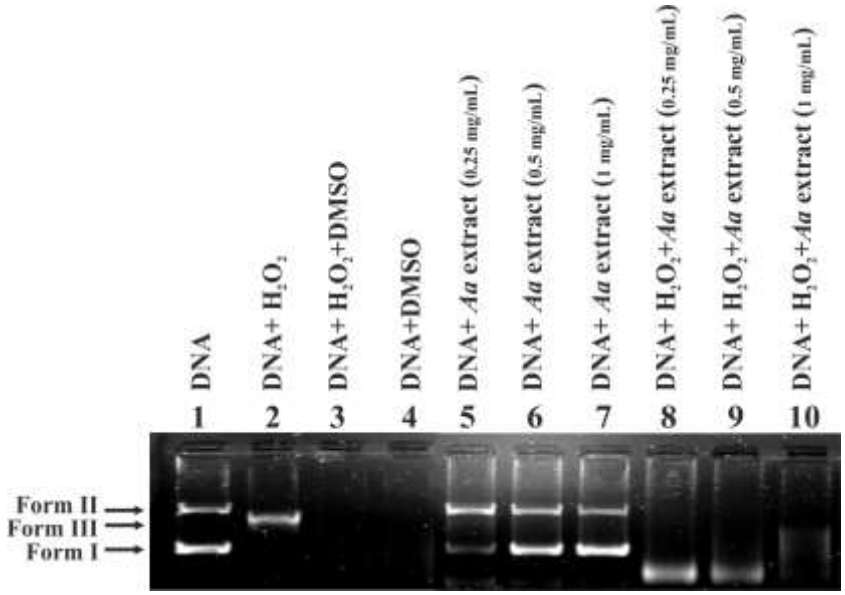

Figure 2. Agarose gel electrophoresis image showing the protective effect of the extract on pUC18 DNA. *Aa*: *Achillea arabica*

3.3. Phenolic concentration results

Plant phenolics are well-known antioxidants and play a protective role against many diseases by supporting the immune system [28]. The effects observed in the antioxidant studies and DNA protective activity were tried to confirm by determining the phenolic content of the extract, and the quantitative amounts of 16 different phenolics were investigated (Table 4). In the HPLC results, the total quantitative amount of the phenolics was detected as quite low (116.338 µg/mL). The reason for this may be the weakening of the antioxidant content of the herbarium material over time. As a result supporting this, we determined that the total phenolic content were quite high in the extracts prepared from the aerial parts of four different tanacetum species [28]. According to the determined phenolic content, the quantitative amount of quercetin was the highest (28.793 µg/mL) and the quantitative amount of 3,4-Dihydroxybenzoic acid was the lowest (0.584 µg/mL). Salicylic acid and Gallic acid could not be detected (Table 4).

Table 4. The µg/mL concentrations of 16 phenolics in the extracts obtained from *A. arabica* flowers.

Phenolic name	Concentration (µg/mL)
Ascorbic acid	13.079
Gallic acid	0.000
3,4-Dihydroxybenzoic acid	**0.584**
4 hydroxybenzoic acid	3.560
Trans-p-coumaric acid	23.720
Myricetin	3.671
Quercetin	**28.793**
Apigenin	5.834
Kaempferol	13.867
Curcumin	5.778
Catechol	11.332
Vanillin	1.775
Caffeic acid	0.787
Cinnamic acid	3.122
Rosmarinic acid	1.807
Salicylic acid	0.000
Total phenolic content	**116.338**

The parallelism between antioxidant activity and quantitative phenolic content has been highlighted in previous studies [26]. Phenolic molecules may be effective in the scavenging of some radicals by acting as an antioxidant, however, whether this mechanism is enzymatic or not remains largely unknown. Here, it was observed that there may be a relationship between antioxidant activity, phenolic content, and DNA protective activity.

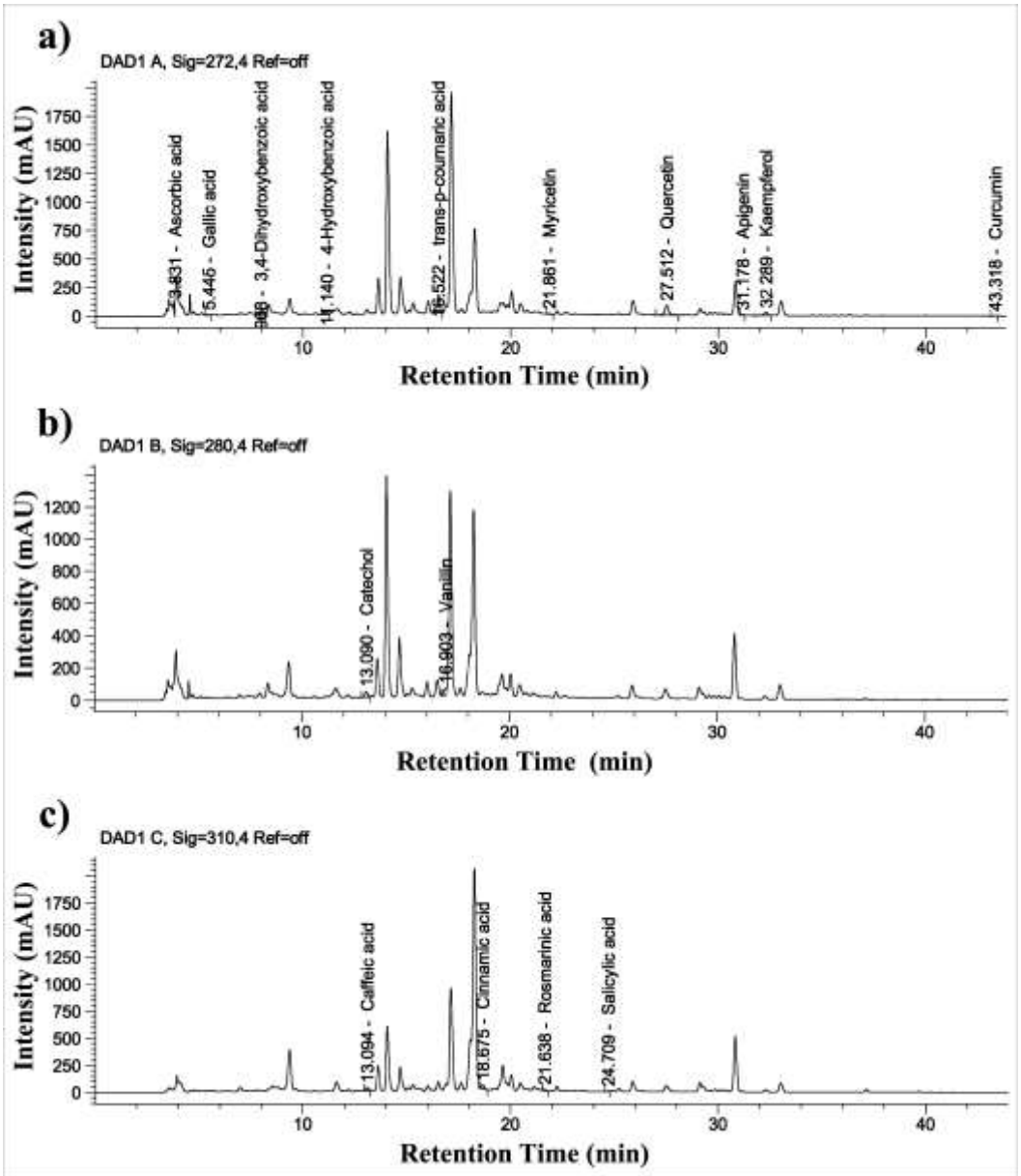

Figure 3. HPLC chromatogram showing the retention times of 16 different phenolic

4. Conclusion

Eventually, although the activities of the cupric ion reduction and the ABTS radical scavenging, and the lipid peroxidation percentages in the MetOH extract were found to be similar to the standards, the ferric iron reduction and radical scavenging activities were significantly lower than the standards. The reason for this may be the termination of enzymatic reactions depending on the herbarium material. Studies emphasizing that fresh plant extracts have stronger antioxidant properties. The extract prepared from herbarium material could not prevent DNA damage caused by H_2O_2 at all extracts concentrations, but had a relatively positive effect on DNA stabilization in the absence of H_2O_2. This effect may be also related to the termination of many enzymatic reactions in the herbarium material as well as the antioxidant activity results. The phenolic content of the extract prepared from the flowers of the *A.*

arabica herbarium plant was observed to be quite low in the HPLC study performed. DNA protective activity, which is not observed even at high concentrations, suggests that DNA protective effect and phenolic content may not be related. Despite some insufficient biological activities observed in the studies performed, obtained data may provide useful information for further studies. For this reason, the features of *A. arabica* not yet revealed should be investigated.

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Author's Contributions

All contributions belong to the author in this paper.

Statement of Conflicts of Interest

The author has no conflict of interest with any other person about this study.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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