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Phytochemical contents and *in vitro* activities of *Berberis thunbergii* hydroethanolic extract

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Abstract: *Berberis thunbergii*, also known as Japanese dogwood, has many medicinal properties due to its rich phytochemical components. Therefore, *B. thunbergii* hydroethanolic extract was investigated for its phytochemical content, antioxidant, anti-inflammatory, and enzyme inhibition activities. In LC-ESI-MS/MS and GC-MS/MS analysis, the main components of the extract were determined as chlorogenic and oleic acid, respectively. Total phenol and flavonoid amounts were found as 259.40±0.96 mg GAE/g extract and 2.60±0.57 mg QE/g extract, respectively. It was recorded that it could reduce iron as 6.38 mg TE/g extract in the FRAP test and had an activity effect of 85.26% in the DPPH˙ scavenging test. The anti-inflammatory property of the extract was found to be 60.80±2.22 µg/mL, while DFS was found to be 45.23±0.68 µg/mL. Additionally, in enzyme inhibition, it was observed that it inhibited urease at a low level of 44.19±0.22 µg/mL and xanthine oxidase (XO) at a high level of 0.91±0.21 µg/mL. It was recorded that it protected DNA with a 57.55% Form I value. Based on these results, it was determined that its high phenolic content also contributes to its high radical scavenging ability. Furthermore, the high XO inhibition of this extract suggests that further detailed studies will be required to demonstrate this property. Further *in vivo* and *in silico* studies may be conducted for this purpose. Furthermore, *B. thunbergii* is thought to have potential applications in the food, cosmetics, and medical fields.

Berberis thunbergii bitkisinin hidroetanolik ekstraktının fitokimyasal içerikleri ve *in vitro* aktiviteleri

Anahtar Kelimeler
Berberis thunbergii, Hidroetanolik ekstrakt, Fitokimyasal, *İn vitro* biyoaktivite

Öz: Japon kan otu olarak bilinen *Berberis thunbergii* çeşitli fitokimyasal bileşenleri nedeniyle birçok tıbbi özelliğe sahiptir. Bu nedenle, *B. thunbergii*'nin hidroetanolik ekstraktının fitokimyasal içeriği, antioksidan, anti-inflamatuar ve enzim inhibisyon aktiviteleri incelenmiştir. LC-ESI-MS/MS ve GC-MS/MS analizinde ekstraktın ana bileşenleri sırasıyla klorojenik ve oleik asit olarak belirlendi. Toplam fenol ve flavonoid miktarları sırasıyla 259.40±0.96 mg GAE/g ekstrakt ve 2.60±0.57 mg QE/g ekstrakt olarak bulunmuştur. FRAP testinde 6.38 mg TE/g ekstrakt düzeyinde demiri indirgediği ve DPPH˙ radikal süpürme testinde %85.26 oranında aktivite etkisine sahip olduğu kaydedilmiştir. Ekstraktın antiinflamatuar özelliği 60.80±2.22 µg/mL düzeyinde iken DFS nin 45.23±0.68 µg/mL bulundu. Enzim inhibisyonu açısından değerlendirildiğinde, üreazı düşük düzeyde (44.19±0.22 µg/mL) ve ksantin oksidazı (KO) yüksek düzeyde (0.91±0.21 µg/mL) inhibe ettiği gözlemlenmiştir. Ayrıca, DNA'yı %57.55 oranında (Form I değeri) koruduğu kaydedilmiştir. Bu sonuçlar doğrultusunda, yüksek fenolik bileşen miktarına sahip olması nedeniyle radikal giderme

yeteneğinin de yüksek olduğu belirlenmiştir. Ayrıca yüksek KO inhibisyonu göstermesi bu ekstraktın daha detaylı çalışmalarla bu özelliğinin ortaya koyulabileceği öngörülmektedir. Bunun için *in vivo* ve *in siliko* çalışmalar daha ileri çalışmalarda gerçekleştirilebilir. Ek olarak, *B. thunbergii*'nin gıda, kozmetik ve tıp alanlarında potansiyel uygulamalara sahip olduğu düşünülmektedir.

1. INTRODUCTION

Antioxidants are organic molecules in low quantities that stop various substances from oxidizing by free radicals. Due to their exceptional effectiveness over extended periods, synthetic antioxidants were employed in the food sector for preservation during the past century. Nonetheless, recent studies have revealed the carcinogenic risks of synthetic antioxidants, and many nations have started to impose regulatory limitations on their usage [1]. The food sector and pharmaceutical treatment have recently increased their need for natural antioxidants found in herbs. Consequently, there is an increasing focus on natural antioxidants, particularly those derived from plants [2]. Some plant phenolics are commercially synthesized and have lately been identified as antioxidants. In this regard, understanding the biological availability and necessary concentrations of these antioxidants that offer dietary protection is essential. Natural antioxidants can be used as reducing agents, free radical scavengers, medicinal herbs, and singlet oxygen suppressors. Bioactive substances, including lignans, flavones, isocatechins, coumarins, isoflavones, catechins, anthocyanins, and flavonoids, are responsible for these plant antioxidant properties. Natural antioxidants with few or no adverse effects are being studied pharmacologically more often these days for application in preventative medicine. Many nations have successfully employed these spices in their local remedies due to their well-known health benefits (diuretic, expectorant, laxative, antibacterial, and antipyretic) [3].

Berberis (Berberidaceae) comprises about 500 species and is widely distributed in many regions, including central and southern Europe, the northeastern United States, southern Asia, and northern Pakistan [4, 5]. *B. thunbergii*, known as Japanese dogwood, is an ornamental plant with bright red leaves that can grow up to 2 m tall and is a deciduous structure known as a woody shrub [6]. *Berberis* species are known for their numerous medicinal properties, including antiviral, antimicrobial, antifungal, antioxidant, anti-inflammatory, antiemetic, antipyretic, sedative, and antihypertensive properties, gallbladder stones, ischemic heart disease (IHDS), cardiac arrhythmias, and cardiomyopathies [4, 5]. *B. thunbergii* is rich in phytochemical constituents such as tannins, steroids, alkaloids, terpenoids, proteins, carbohydrates, flavonoids, phenolic compounds, and essential oils [6, 7]. In previous studies, the MTT antioxidant, lipid peroxidation, and COX-1 and COX-2 enzyme inhibition of the methanol extract of *B. thunbergii* var. *atropurpurea* were investigated [8]. In the study by Villinski et al. (2003) [6], the antibacterial activity of the ethanolic extract of *B. thunbergii* was determined. In the other study, silver nanoparticles of *B. thunbergii* were formed, and DPPH˙ scavenging and pancreatic cancer activity were investigated [9]. In the study by Fernández-Poyatos et al. (2019) [7], the antioxidant, enzyme inhibition, and phytochemical contents of the methanol and water extracts of *B. thunbergii* were determined.

In our study, we aimed to evaluate antioxidant (FRAP and DPPH˙ scavenging), anti-inflammatory, DNA protection, and enzyme inhibition (urease and XO) activities of *B. thunbergii* hydroethanolic extract, which has been investigated in a limited number of studies. Also, the contents of *B. thunbergii* extracts were determined by total phenol, total flavonoid, LC-ESI-MS/MS, and GC-MS/MS analysis. The results obtained in this study will contribute new data to the literature. As a result of the tests performed on the *B. thunbergii* hydroethanolic extract, it is recommended that the molecule be used as a drug precursor in the pharmaceutical industry.

2. MATERIAL AND METHOD

2.1. Plant Material and Extraction

The *B. thunbergii* fruits were collected on February 16, 2024, on the Şehit Bülent Yurtseven Campus of Iğdır University/Turkey at 420851.1286 latitude and 4409496.2388 longitude. It was taken to the Iğdır University Research Laboratory Application Center (ALUM) and dried in a suitable ventilated environment. After drying, it was pulverized using a grinder, and 25 grams of the sample was weighed on a precision balance and transferred to a 250 mL Erlenmeyer. 50 mL of ethanol and 50 mL of pure water were added, and the opening was closed with aluminum foil. The extract-solvent mixture was filtered after seven days, and a rotary evaporator was used to remove the solvent. 2 grams of crude *B. thunbergii* hydroethanolic extract (BTHE) were obtained. It was stored at +4 degrees for more accurate analysis.

2.2. LC-ESI-MS/MS and GC-MS/MS Analysis

We used the Agilent 1260 Infinity II LC-ESI-MS/MS equipment to do the content analysis of the *B. thunbergii* hydroethanolic extract. A 100 mm × 3.0 mm, 2.7 µm Agilent Poroshell120 EC-C18 reversed-phase analytical column was employed for the chromatographic separation [10, 11]. 34 phenolic standards were used in the LC-ESI-MS/MS analysis.

1 mL of methanol was added to 50 mg of BTHE. Then, 1 mL of hexane was added to remove lipophilic components, and liquid-liquid extraction was performed. Centrifugation was performed at 9000 rpm for 10 min to clarify the phase separation. 100 µL of the methanol phase obtained after centrifugation was carefully taken, and 450 µL of ultrapure water and 450 µL of methanol were added to make a total volume of 1 mL. The extract was filtered

through a membrane filter with a 0.25 µm pore diameter to prevent particle interference. The prepared samples were injected into the LC-ESI-MS/MS system in the final stage. The device conditions used during the analysis were: Injection volume: 5.12 µL, Flow rate: 0.400 mL/min, Method time: 30.00 min, Column temperature: 40.0 °C. The mobile phase transition rate during the method time was used as follows: 75% A (water) + 25% B (methanol) at 3 min, 50% A + 50% B at 12 min, 10% A + 90% B at 16 min, 10% A + 90% B at 21 min, 97.5% A + 2.5% B at 24 min.

The analysis of the BTHE extract was performed on a GC-MS/MS device, as our previously published article described. This analysis used the Agilent 7000 A GC/MS Triple Quad with 7693 Autosampler, 7697A Headspace Sampler, and 7890 GC [12, 13]. As a procedure, the initial temperature was determined at 50 °C and maintained for two minutes, then increased at 3 °C/min to 140 °C, 4 °C/min to 220 °C for 10 min, and constant at 4 °C/min to 270 °C, and finally continued at 270 °C for 30 min. The ion temperature of the MS detector is 280 °C. The filtered sample with a 0.22 µm disposable syringe filter was run with a 1 mL He gas flow by injecting a volume of 1 µL at a ratio of 1:10. Agilent HP-5 (5%-phenyl)-methyl polysiloxane) (30m x 0.25 mm x 0.25 µm) was carried out on the GC column. 30 mg of the sample was taken, 2 mL MeOH was dissolved, and 2 mL *n*-hexane was added. Then, 1 ml KOH solution (1M) was added and mixed with a vortex device at 2500 rpm for 30 seconds. It was taken from the upper phase (*n*-hexane phase), which contained fatty acid methyl esters and was filtered with 0.22-micron PVDF and injected.

2.3. Total Phenolic and Flavonoid Content Assays

The total phenolic (TP) and flavonoid (TF) contents of plant extract were obtained by Golmakani, Mohammadi et al. (2014) [14] using the methods applied.

To determine the TP content, 500 µL of Folin-Ciocalteu reagent was mixed with 100 µL of extract (or standard gallic acid) solution (1024 µg/mL). One minute after, 1.5 mL of 20% Na_2CO_3 was added, and the mixture was allowed to sit at room temperature for two hours in the dark. At 760 nm, the absorbance values of the mixed solutions were determined. Based on the gallic acid calibration graph, the TP content values of the samples were converted to mg gallic acid equivalent (GAE) using the formula y = 0.0022x + 0.0408 (R^2 = 0.99).

500 µL extract and standard quercetin (1024 µg/mL) solutions were mixed with 1.5 mL methanol, 100 µL 10% $AlCl_3$, 100 µL 1 M CH_3COOK, and 2.8 mL deionized water to determine the TF concentration. Absorbance measurements were taken at 415 nm after the combination was allowed to sit at room temperature in the dark for half an hour. Using the formula y = 0.0061x + 0.0046 (R2 = 0.99), which was derived from the quercetin calibration graph, the TF content values of the samples were converted to mg quercetin equivalent (QE).

2.4. DPPH• Scavenging Activity

In this study, DPPH˙ scavenging activity was evaluated by measuring the DPPH˙ scavenging activity not quenched in the environment by the interaction of antioxidant species. 10 mL of a 100 µg/mL DPPH solution was mixed with 10 mL of the plant extract (250 ppm) or standard (ascorbic acid, 10 ppm). It waited 30 minutes until the interaction reaction of the antioxidant species in the plant content with DPPH˙ was completed, and the potential values were measured by directly immersing these samples in a DPPH-selective PVC membrane biosensor (DPPH-SPMB). The following formula (1) was used. The results were expressed as percentages [15, 16].

$$\%\text{DPPH}^{\cdot}\ \text{scavenging} = \frac{[(E1-E0)-(E2-E0)]}{E1-E0} x100 \qquad (1)$$

E_0 is the potential value of the plant sample. E1 is the potential value of the DPPH• standard solution, and E_2 is the potential value of the DPPH˙ scavenging activity remaining in the medium after 30 minutes of reaction.

2.5. Ferric Reducing Antioxidant Power Activity

The Ferric Reducing Antioxidant Power (FRAP) activity of Fe (III) ions in a dissolved environment was successfully measured using FRAP-SPMB. In our study, iron (III) reduction activity was evaluated by measuring the activity of Fe (III) ion that remained unreduced in the environment as a result of the reduction of Fe (III) ion to Fe (II) ion by antioxidant species. Fe (III) reduction activity results in spectrometer measurements that are expressed as trolox equivalent (mg/g extract). Therefore, in our study, we reported our results as trolox equivalent. For this purpose, a calibration curve for Fe (III)–equivalent trolox was created. Using this calibration curve, the Fe (III) reduction activity of the plant extract (250 ppm) was calculated potentiometrically [15, 16].

2.6. Anti-inflammatory Activity

The anti-inflammatory activity was assessed *in vitro* using the Kandikattu, Kumar et al. (2013) [17] technique to measure its impact on the denaturation of bovine serum albumin (BSA). The procedure involved adding 500 µL of the BSA solution (0.2% produced in ddH_2O) to 500 µL of extract or Diclofenac sodium (DFS) at several concentrations (1000, 500, 250, and 125 µg/mL) in tubes. Additionally, a control tube containing 500 µL of BSA and 500 µL of hydroethanolic was made. After 15 minutes of incubation at 37°C, the mixture was heated for five minutes at 72°C. In a UV-visible spectrophotometer, the absorbance was measured at 660 nm after cooling. IC_{50} (µg/mL) values were calculated using the absorbance values.

2.7. Xanthine Oxidase Inhibition

The 96-well microplate was filled with 50 µL of sample or allopurinol solution, 100 µL of substrate solution

(0.041 mM xanthine), and 50 µL of newly made enzyme solution (0.1 U/mL xanthine oxidase (XO) in PBS (pH 7.5)). For five minutes, the samples were incubated at 37°C. After that, 100 µL of 1 M HCl was added to halt the reaction. A UV-Vis spectrophotometer set at 292 nm was used to measure the absorbance [18]. The absorbance data were used to generate the IC_{50} (µg/mL) values.

2.8. Urease Inhibition

The indophenol technique was used to measure the materials' urease inhibition activity [19]. In a 96-well plate, 50 µL of 17 mM urea, 25 µL of 1 U urease (in 100 mM pH 8.2 Na-K buffer), and 10 µL of samples or thiourea at various concentrations were combined evenly. For fifteen minutes, the samples were incubated at 30ºC. Forty-five liters of phenol reagent (0.1% (w/v) sodium nitroprusside and 8% (w/v) phenol) and seventy liters of alkaline reagent (4.7% (v/v) NaOCl and 2.5% (w/v) NaOH) solutions are combined into the mixture. The mixture was stored for 50 minutes at 30ºC. Using a BIOTEK (Epoch2) microplate reader, the absorbance values of each combination were determined and displayed at 630 nm. The findings' IC_{50} values (µg/mL) were computed.

2.9. DNA Protection Activity

An induced DNA agarose gel electrophoresis assay was performed to evaluate the DNA protection activity of the extracts. In this test, plasmid DNA and H_2O_2 were made to react with UV radiation. The fragmentation ratio of the plasmid DNA molecules was measured by adding pure compounds to a test mixture using an electrophoresis gel tank and a gel imaging system. Results were calculated using the imaging system as the percentage of fragmentation as a positive control. Quercetin was used and compared to the negative control using only the DNA and H_2O_2 mixture [20, 21].

2.10. Statistical Analysis

Activity or enzyme levels ± standard deviations were used to evaluate *in vitro* enzyme inhibitory and antioxidant activities. All data were subjected to one-way ANOVA analysis as the analytical means recorded using the IBM SPSS 20.0 program had a normal distribution. This procedure subjects the data to the Tukey $HSD^{a,b}$ multiple comparison test. The value $p<0.05$ was used to assess the statistical significance level of the data.

3. RESULTS

2.1. LC-ESI-MS/MS Analysis

The phenolic content of the BTHE was obtained by LC-ESI-MS/MS analysis (Figure 1). A total of 10 compounds were found (Table 1). According to the analysis results, chlorogenic acid (2797.41 µg/g extract), *trans*-ferulic acid (192.50 µg/g extract), isoquercitrin (129.07 µg/g extract), and hesperidin (85.48 µg/g extract) were detected in the highest amounts.

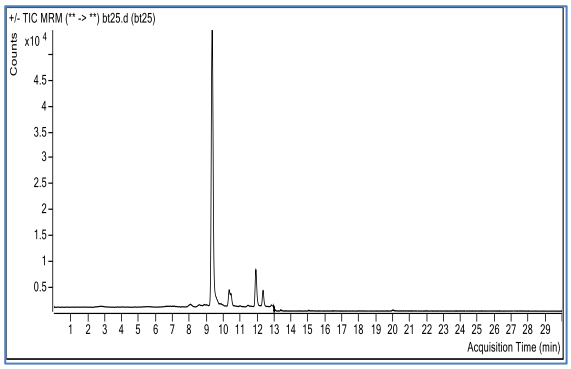


Figure 1. LC-ESI-MS/MS chromatogram of BTHE

Table 1. The LC-ESI-MS/MS analysis results of BTHE

No	RT, min.	Compound name	BTHE, µg/g extract
1	9.35	Chlorogenic acid	2797.41
2	11.44	Vanillic acid	23.11
3	10.35	Caffeic acid	38.37
4	10.48	Hydroxybenzaldehyde	2.10
5	11.90	Rutin	35.41
6	11.45	Polydatin	6.63
7	12.35	*trans*-ferulic acid	192.50
8	11.94	Isoquercitrin	129.07
9	11.90	Hesperidin	85.48
10	13.42	Kaempferol	7.00

RT: Retention time, n:1

2.2. GC-MS/MS Analysis

Fatty acid methyl esters of the hydroethanolic extract of *B. thunbergii* were determined by GC-MS/MS analysis (Figure 2), and it was discovered that it contained six fatty acids (Table 2). According to the analytical result, oleic acid methyl ester (40.91%), palmitic acid methyl ester (22.74%), linoleic acid methyl ester (16.58%), stearic acid, methyl ester (11.01%), oleic acid methyl ester isomer (5.44%) and benzene propanoic acid, 3,5-bis(1,1-dimethyl ethyl)-4-hydroxy- methyl ester (2.07%) were detected.

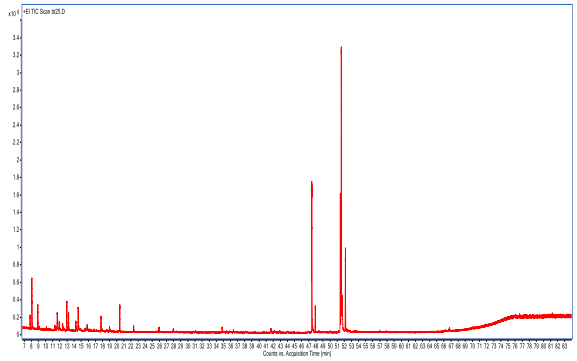


Figure 2. GC-MS/MS chromatogram of BTHE

Table 2. The GC-MS/MS analysis results of BTHE

No	RT, min.	Compound Name	BTHE, %
1	47.43	Palmitic acid, methyl ester	22.74
2	47.90	Benzenepropanoic acid, 3,5-bis(1,1-dimethyl ethyl)-4-hydroxy-, methyl ester	3.32
3	51.43	Linoleic acid, methyl ester	16.58
4	51.56	Oleic acid, methyl ester	40.91
5	51.70	Oleic acid, methyl ester-isomer	5.44
6	52.14	Stearic acid, methyl ester	11.01
Total			100

RT: Retention time, n:1

2.3. Total Phenol (TP) and Flavonoid (TF) Contents

This study shows the TP and TF contents of BTHE. According to this analysis's results, the hydroethanolic extract's total phenol content was found to be 259.40±0.96 mg GAE/g extract. In contrast, the total flavonoid content was 2.61±0.57 mg QE/g extract.

2.4. Antioxidant Activities

Antioxidant activities of BTHE are presented in Table 3. It reduced iron at the rate of 6.38 mg TE/g extract in the FRAP test and scavenged radicals at the rate of 85.26 % (ascorbic acid, 82.35 %) in the DPPH˙ scavenging test.

2.5. Anti-inflammatory Activity

The Inhibition of denaturation of proteins assay is a popular *in vitro* technique for assessing a compound's ability to reduce inflammation, especially phytochemicals. Proteins lose their tertiary structure if they are denatured by external stressors such as heat, pH shifts, or chemicals. As a result, their beneficial qualities are lost. Oxidative stress and inflammation frequently cause protein denaturation, which worsens tissue damage and leads to illnesses [22]. To ascertain if a molecule can prevent protein denaturation, heat or chemical stress is applied to a protein solution, often egg albumin or BSA. When the test substance is added to the protein solution, changes in absorbance, turbidity, or loss of enzyme activity are measured to determine the degree of denaturation [23]. By contrasting the absorbance or activity levels in the presence of the test compound with a control, the protective effects of the chemical are ascertained. By stabilizing proteins and limiting their denaturation, phytochemicals such as flavonoids, polyphenols, and alkaloids demonstrated their anti-inflammatory and protective properties in this test [24]. Bovine serum albumin (BSA) is the most often used protein model. It is heated to 70°C for ten minutes to denaturize the protein solution.

The anti-inflammatory activity of BTHE was seen, and it was observed that BTHE ($IC_{50,}$ 60.80±2.22 µg/mL) had a lower inhibition effect than DFS ($IC_{50,}$ 45.23±0.68 µg/mL) in Table 3. Since no such study on *B. thunbergii* was found in the literature search, this study is the first.

2.6. Urease Inhibition

Numerous eukaryotic and prokaryotic organisms, such as bacteria, plants, fungi, algae, and invertebrates, have nickel-containing amidohydrolase urease. Urea is hydrolyzed by urease to produce carbon dioxide and ammonia [25]. It is well known that *Helicobacter pylori* thrives in the stomach with an increased ammonia level. High ammonia concentrations caused by urease hyperactivity raise the pH of the stomach and can result in peptic and gastric ulcers, kidney stones, pyelonephritis, and hepatic coma [26-28].

Slicing synthetic small molecules to decrease urease activity is the most appealing and successful method of controlling its function. This avoids the gastrointestinal issues brought on by hyperactive urease [29]. Few synthetic urease inhibitors have progressed to the next stage of therapeutic research, such as urease inhibitors, despite a few papers on the topic up to this point [30].

As can be seen in Table 3, the urease inhibition results of BTHE were given, and it was observed that BTHE ($IC_{50,}$ 44.19±0.22 µg/mL) had a lower inhibition effect than thiourea ($IC_{50,}$ 18.79±0.12 µg/mL). Since no such study on *B. thunbergii* was found in the literature search, this study is the first.

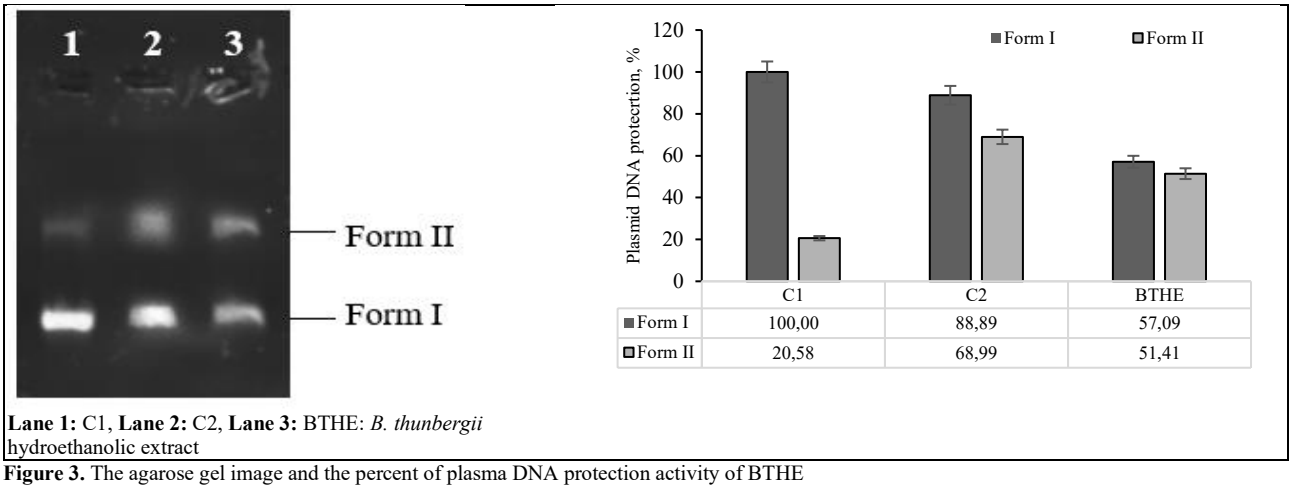
2.7. XO Inhibition

Animal hepatic organs are the primary expression site for the molybdenum-containing metalloenzyme XO [31]. It promoted the conversion of hypoxanthine to xanthine, producing uric acid [32, 33]. One significant contributing cause of gout is excessive uric acid. Anti-gout medications have been on the market for a few decades [34], and they fall into several categories, such as uric acid transporter inhibitors [35], urine alkalizing agents [36], XO inhibitors [37], anti-inflammatory analgesics [38], and others. The issue of excessive uric acid generation is not addressed by other pharmacological classes, except for XO inhibitors, which primarily target the inflammatory response triggered by uric acid [39]. XO inhibitors' primary effects are decreasing uric acid generation and inhibiting XO activity. XO activities are intimately linked to the therapeutic effects of XO inhibitors. It has long been challenging for researchers to create a dynamic, real-time process and method to track XO levels *in vivo* and evaluate drug effectiveness. The primary techniques to assess XO activities are radiation, colorimetry, spectrophotometry, and manometry [40].

The XO inhibition findings of BTHE are shown in Table 3, and it was found that BTHE (IC_{50}, 27.07±3.26 µg/mL) had a higher inhibition effect than allopurinol (IC_{50}, 0.91±0.21 µg/mL). This study is the first because the literature search yielded no similar research on *B. thunbergii.*

2.8. DNA Protection Activity

In the agarose gel image of DNA protection activity shown in Figure 3, the brightness of BTHE in Form I is lower than that of the controls. When the percentage form values were calculated, the percentage values of BTHE in Form I and Form II were determined as 57.09 and 51.41, respectively. According to this result, it was observed that in addition to its DNA protection feature, it also has a DNA degradation feature. When compared to C2, it was noted that BTHE's DNA protection activity was low.



Lane 1: C1, **Lane 2:** C2, **Lane 3:** BTHE: *B. thunbergii* hydroethanolic extract

Figure 3. The agarose gel image and the percent of plasma DNA protection activity of BTHE

Table 3. The antioxidant, anti-inflammatory, urease, and XO inhibition results of BTHE

Sample	FRAP (mg TE/g extract)	DPPH˙ scavenging, %	Anti-inflammatory, IC_{50} (µg/mL)	Urease inhibitory, IC_{50} (µg/mL)	XO inhibitory, IC_{50} (µg/mL)
BTHE	6.37±1.88^{d}	85.26±1.45^{b}	60.80±2.22	44.19±0.22	0.91±0.21
BHT	20.81±1.24^{c}	60.00±1.22^{e}	*nu*	*nu*	*nu*
BHA	23.22±1.20^{b}	47.06±1.50^{f}	*nu*	*nu*	*nu*
Trolox	18.73±1.22^{c}	66.67±1.02^{d}	*nu*	*nu*	*nu*
Gallic acid	27.38±1.21^{a}	75.63±1.22^{c}	*nu*	*nu*	*nu*
Ascorbic acid	22.45±1.20^{b}	89.35±1.23^{a}	*nu*	*nu*	*nu*
DFS	*nu*	*nu*	45.23±0.68	*nu*	*nu*
Thiourea	*nu*	*nu*	*nu*	18.79±0.12	*nu*
Allopurinol	*nu*	*nu*	*nu*	*nu*	27.07±3.26

BHA: Butylated hydroxyanisole, **BTHE:** *B. thunbergii* hydroethanolic extract, **FRAP:** Ferric reducing antioxidant power, **DFS:** Diclofenac sodium, **BHT:** Butylated hydroxytoluene, **XO:** Xanthine oxidase, **nu:** not used **n:**3, $p < 0.05$

4. DISCUSSION AND CONCLUSION

In a similar study, the main constituents in the HPLC-MS analysis of *B. thunbergii* methanol and aqueous extracts were determined to be berberine, rutin, and chlorogenic acid [7]. In a similar study, caffeic acid, rutin hydrate, procyanidin, quinic acid, protocatechuic acid, chlorogenic acid, and narcissin were determined in high amounts in the LC-HRMS analysis of the methanol extract of *B. thunbergii* [41]. A comparison of the literature shows that various extracts of *B. thunbergii* are rich in chlorogenic acid.

In a similar study, analysis of the fatty acid content of the hexane extract of the fruits of *B. crataegina* by GC-MS revealed palmitic acid (9.48%), stearic acid (3.18%), oleic acid (14.07%), linolenic acid (35.25%), α-linolenic acid (37.57%), tricosanoic acid (0.44%) [41].

The total bioactive compounds of many *Berberis* species have been documented in earlier research. Nonetheless, Berberis fruits were the subject of the majority of research [42-44]. In their study, Fernández-Poyatos et al. (2019) [7] found the TPC and TFC results of the *B. thunbergii* methanol extract as 216.00±6.00 mg GAE/g and 46.00±1.00 mg RE/g, respectively. In contrast, the TPC and TFC content of the water extract was found as 194.00±1.00 mg GAE/g and 20.60±0.50 mg RE/g, respectively. When we compared the results of our study with the results in the literature, it was determined that the hydroethanolic extract had a higher phenolic content than the methanol and water extracts.

In a recent study, the TFC and TPC contents were found in methanol (80%, 13.94±0.5 mg CE/g extract for TFC, 53.86±0.40 mg GAE/g extract for TPC), ethanol (80%, 12.2±0.34 mg CE/g extract for TFC, 46.73±1.10 mg GAE/g extract for TPC), and water (10.03±0.47 mg CE/g extract for TFC, 41.76±0.95 mg GAE/g extract for TPC) extracts of *B. orthobotrys* [45]. The literature shows that lower phenolic content was obtained than in our results.
In a recent study, the EC_{50} values of DPPH˙ scavenging activities were found in methanol (80%, 203.15±2.67), ethanol (80%, 270.3±2.16), and water (412.41±6.06) extracts of *B. orthobotrys* and BHA (51.5±2.50), BHT (60.2±2.00) [45]. In the previous study, the FRAP and DPPH˙ scavenging activity results of the *B. thunbergii* methanol and water extracts were found to be

620.00±10.00 mg TE/g for FRAP, 549.00±6.00 mg TE/g for FRAP, 429.00±6.00 mg TE/g for DPPH˙, and 360.00±20.00 mg TE/g for DPPH˙ scavenging [7]. The results of the same plant in the literature are lower than those of the hydroethanolic extract obtained in our study.

In the study conducted by Vignesh, Pradeepa Veerakumari et al. (2021) [46], the anti-inflammatory effects of *B. tinctoria* leaf, stem, and root extracts in methanol, water, ethyl acetate, petroleum ether, and chloroform were investigated, respectively, and the standard used was aspirin. According to this standard, the stem part of the plant showed a lower effect. In contrast, they observed that leaf and fruit parts' ethyl acetate and methanol extracts had higher anti-inflammatory effects. Ethyl acetate and methanol extracts have been reported in the literature to have high anti-inflammatory activity, but in our study, the hydroethanolic extract was found to have

lower activity. This is thought to be due to the different phenolic compounds contained due to the use of different plant species or their different amounts.

This study observed that the *B. thunbergii* hydroethanolic extract examined had a high phenolic content and the highest amount of chlorogenic acid among these phenolic compounds. It was recorded to contain a high amount of oleic acid among fatty acids. In addition, it was determined that its iron-reducing property was lower than the standards, with 6.38 mg TE/g extract value, and its radical scavenging activity was higher than the other standards except for the ascorbic acid standard, with 85.26%. According to this result, it was seen that it had radical scavenging properties. Its anti-inflammatory property was determined by the BSA denaturing method, and it was found that it denatured BSA at a lower rate than the standard DFS used. In enzyme inhibitions, it was observed that the urease inhibition property was weak, but the XO inhibition property was very high. Accordingly, it is thought to lead to further studies as an XO inhibitor. In addition, it was calculated that its DNA protection activity was low. According to all these results, this extract's high xanthine oxidase inhibition suggests that further detailed studies will be needed to demonstrate this property. Future studies may require *in vivo* and *in silico* studies.

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Conflict of interest
The authors declare that any known financial conflicts or personal relationships did not impact the work presented in this journal.

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