

## Examination of the Inhibition Effect of Extracts of *Berberis crataegina* Fruit on Elastase, Xanthine Oxidase, Tyrosinase, and Calcium Oxalate Crystal

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### *Berberis crataegina* Meyve Ekstraktlarının Elastaz, Ksantin Oksidaz, Tirozinaz ve Kalsiyum Oksalat Kristali Üzerindeki İnhibisyon Etkisinin İncelenmesi

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#### Abstract

Research is being conducted on the effect of various plants and plant parts on enzymes and kidney stones. Calcium oxalate crystal is the main component of kidney stones that damage the urinary system and can result in both surgical operations and financial strain. Furthermore, certain enzymes that function excessively might lead to many health issues. Many illnesses, including hyperuricemia caused by the accumulation of uric acid crystals, for example, can be due to excessive activity of the xanthine oxidase enzyme. Furthermore, one field of research that protects against skin aging is the reduction of elastase activity. Studies are also being carried out in the fields of food and cosmetics to prevent pigmentation by suppressing the tyrosinase enzyme. Therefore, herbal medicines that can be used as inhibitors of these enzymes, whose excessive activity causes various disorders, attract attention. In this study, the inhibition effect of two different fruit extracts of *Berberis crataegina* (methanol and ethanol) on calcium oxalate crystals and xanthine oxidase, elastase, and tyrosinase enzymes *in vitro* was investigated. Additionally, the amount of monomeric anthocyanins and total phenolic and flavonoid contents were evaluated. It was observed that both extracts of *B. crataegina* fruit inhibited the formation of calcium oxalate crystals. However, ethanol extract was found to outperform methanol extract in inhibiting the enzymes xanthine oxidase, elastase, and tyrosinase. Due to all these features, *B. crataegina* fruit is one of the natural resources that can be used effectively in food, cosmetics, and health applications.

**Anahtar Kelimeler:** *B. crataegina* fruit; Elastase inhibition; Xanthine oxidase inhibition; Tyrosinase inhibition; Total phenolic content; Total flavonoid content

#### Öz

Çeşitli bitki ve bitki kısımlarının enzimler ve böbrek taşları üzerindeki etkisi üzerine araştırmalar yapılmaktadır. Kalsiyum oksalat kristali, idrar sistemine zarar veren böbrek taşlarının ana bileşenidir ve hem cerrahi operasyonlara hem de maddi sıkıntılara neden olabilir. Ayrıca bazı enzimlerin aşırı çalışması birçok sağlık sorununa yol açabilir. Örneğin ürik asit kristallerinin birikmesinden kaynaklanan hiperürisemi de dahil olmak üzere birçok hastalık, ksantin oksidaz enziminin aşırı aktivitesinden kaynaklanabilir. Ayrıca cilt yaşlanmasına karşı koruma sağlayan araştırma alanlarından biri de Elastaz aktivitesinin azaltılmasıdır. Gıda ve kozmetik alanında da tirozinaz enzimini baskılayarak pigmentasyonun önlenmesine yönelik çalışmalar yürütülmektedir. Bu nedenle aşırı aktivitesi çeşitli rahatsızlıklara neden olan bu enzimlerin inhibitörü olarak kullanılabilir bitkisel ilaçlar dikkat çekmektedir. Bu çalışmada *Berberis crataegina*'nın iki farklı meyve ekstresinin (metanol ve etanol) kalsiyum oksalat kristalleri ve ksantin oksidaz, elastaz ve tirozinaz enzimleri üzerindeki *in vitro* inhibisyon etkisi araştırıldı. Ayrıca monomerik antosiyaninlerin miktarı ve toplam fenolik ve flavonoid içerikleri de değerlendirildi. *B. crataegina* meyvesinin her iki ekstresinin de kalsiyum oksalat kristallerinin oluşumunu engellediği gözlemlendi. Bununla birlikte, etanol ekstresinin, ksantin oksidaz, elastaz ve tirozinaz enzimlerini inhibe etmede metanol ekstresinden daha iyi performans gösterdiği bulunmuştur. Tüm bu özellikleri nedeniyle *B. crataegina* meyvesi gıda, kozmetik ve sağlık uygulamalarında etkin bir şekilde kullanılabilir doğal kaynaklardan biridir.

**Keywords:** *B. crataegina* meyvesi; Elastaz inhibisyonu; Ksantin oksidaz inhibisyonu; Tirozinaz inhibisyonu; Toplam fenolik içerik; Toplam flavonoid içeriği

#### 1. Introduction

Alkaloids, phenolic compounds, flavonoids, minerals, and terpenoids are notable chemical components found in medicinal plants. These compounds play a role in preventing the emergence of many diseases by protecting against oxidative damage due to their antioxidant properties (Abdulhafiz et al. 2020, Kabir et al. 2016,

Mathur and Velpandian 2009). Plants have several health-promoting qualities, which might make them helpful in the treatment of various diseases in addition to the antioxidant qualities of the components they contain (El-Saadony et al. 2023). Among these characteristics are the suppression of certain enzymes whose overactivity results in harm and the prevention of calcium oxalate

crystallization, which is the cause of kidney stones. Globally, the frequency of urolithiasis illness is increasing (Romero et al. 2010, Wong et al. 2015). In addition to the increasing incidence of this disease, the frequent need for surgical intervention as a solution to this disease also brings a significant cost to the economy (Wong et al. 2015). By 2030, it is predicted that the rising incidence of urolithiasis as a urological issue will have a substantial negative impact on the US healthcare system and contribute \$1.24 billion to the national debt (Antonelli et al. 2014, Raheem et al. 2017, Stamatelou et al. 2003).

One of the most prevalent crystalline stones in the human urinary system is calcium oxalate. Of the three hydrated states of calcium oxalate crystals, which are calcium oxalate monohydrate, calcium oxalate dihydrate, and calcium oxalate trihydrate, the most thermodynamically stable is calcium oxalate monohydrate, which also happens to be the hardest to excrete in urine (Xie et al. 2007). A buildup of uric acid crystals in the blood and excessive uric acid crystal synthesis can also result in conditions like gout and hyperuricemia (Dong et al. 2016, Wang et al. 2014). Additionally, hypertension, heart disease, and renal failure are known to be linked to hyperuricemia (Borghi et al. 2015, Borghi and Cicero 2017, Bove et al. 2017, Gustafsson and Unwin 2013, Nakanishi et al. 2003).

The metal flavoprotein enzyme xanthine oxidase catalyzes the oxidative hydroxylation of hypoxanthine and xanthine to uric acid during purine catabolism (Šmelcerović et al. 2017). It's the enzyme that leads the body to generate uric acid. Reactive oxygen species are produced together with uric acid during this xanthine oxidase process. Thus, oxidative damage and several illnesses resulting from it can be caused by increased xanthine oxidase activity (Šmelcerović et al. 2017). Numerous illnesses, including gout, hyperuricemia, arthritis, renal problems, hypertension, diabetes, obesity, cardiovascular problems, inflammations, and chronic heart failure, may be linked to excessive xanthine oxidase activity (Cengiz et al. 2012, Kelley et al. 2010, Lin et al. 2000, Liu et al. 2008).

There might be major adverse effects from the xanthine oxidase inhibitors employed (Cengiz et al. 2012, Liu et al. 2008, Umamaheswari et al. 2009). Thus, xanthine oxidase inhibitors are being explored for their potential health benefits, particularly natural inhibitors (Abdulhafiz et al. 2020). Flavonoids such as chrysin, quercetin, and medicinal herbs have gained popularity recently for their ability to naturally inhibit xanthine oxidase (Lin et al. 2015, Nile and Park 2015, Takahama et al. 2011, Zhang et

al. 2018). Since they have little adverse effects, researchers are looking into using natural materials like fruits and plants as xanthine oxidase and calcium oxalate inhibitors.

The elastic protein elastin is present in connective tissues such as the skin, arteries, and lungs. It plays a crucial role in preserving tissue structure during retraction or stretching (Daamen et al. 2007, DeBelle and Alix 1999, Liyanaarachchi et al. 2018). Skin aging results from the degradation and dysregulation of proteins like collagen, fibronectin, and elastin caused by an increase in the activation of proteolytic enzymes like elastase and collagenase (Wittenauer et al. 2015). The structure of the enzyme elastase is serine protease (Tousif et al. 2023). By suppressing enzymatic activity, delaying the aging process of the skin, and promoting the integrity of healthy connective tissue, plants, and their diverse components may offer encouraging outcomes.

The fundamental factor that determines the color of skin, hair, and eyes is melanin, which has functions that include protecting against damaging UV radiation and eliminating dangerous medications and chemicals (Lee et al. 2013, 2016). The copper-containing enzyme tyrosinase is extensively distributed in several animals and is crucial to the formation of melanin and, consequently, to enzymatic browning. Overproduction of melanin may result in DNA damage, gene mutation, age spots, and freckles, as well as dermatological issues including cancer development (Ahn et al. 2006, Iozumi et al. 1993, Li et al. 2003, Pillaiyar et al. 2017, Ünver et al. 2006, Yang et al. 2006). Moreover, undesirable enzymatic browning of plant-derived foods by Tyrosinase causes a decrease in nutritional quality and economic losses in food products (Parvez et al. 2007). Tyrosinase inhibitors are consequently useful in the food and agricultural sectors, as well as in cosmetic and medicinal applications, as depigmentation agents and anti-browning substances (Zolghadri et al. 2019). Researchers are searching for novel, powerful tyrosinase inhibitors for utilization in food and cosmetics since conventional techniques of inhibiting tyrosinase's function are inadequate (Parvez et al. 2007).

Fruits of *B. crataegina* are edibles that have antioxidant and protective qualities that can effectively prevent oxidative DNA damage and lipid peroxidation (Charehsaz et al. 2015). In this study, the total phenolic, total flavonoid, and monomeric anthocyanin amounts of two different extracts of *B. crataegina* will be determined, as well as the possible benefits will be investigated by determining the inhibition effect on calcium oxalate crystal and xanthine oxidase, elastase and tyrosinase enzymes.

## 2. Materials and Methods

### 2.1. Preparation of Fruit Extracts

*B. crataegina* fruit was collected from Kayseri/Tomarza in the last week of August. Fatma MUNGAN KILIÇ, an associate professor at Mardin Artuklu University's Department of Plant and Animal Production, made the identification. The plant is deposited in the Mardin Artuklu University herbarium with herbarium number MARIUM 41. *B. crataegina* fruit was dried in the shade. Following the addition of 400 milliliters of methanol and 400 milliliters of ethanol to 40 grams of fruit samples, two distinct solutions were produced by mixing for four hours in a magnetic stirrer. After separating both extracts through filter paper, their solvents were removed from the evaporator.

### 2.2. Calcium oxalate inhibition experiments

CaC<sub>2</sub>O<sub>4</sub> crystal was created by mixing equal volumes of CaCl<sub>2</sub>·2H<sub>2</sub>O and Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub> solutions prepared as 20x10<sup>-4</sup> M in a magnetic stirrer at 600 rpm and 37 °C (Akin et al. 2019). Then, the effect of methanol and ethanol extracts prepared at concentrations of 0.5 mg/mL, 1 mg/mL, 2 mg/mL, and 4 mg/mL on the growth of CaC<sub>2</sub>O<sub>4</sub> crystals was examined. This was accomplished by adding 1 mL of *B. crataegina* fruit methanol and ethanol extracts, each produced at concentrations of 0.5, 1.0, 2.0, and 4.0 mg/mL, to equal volumes of CaC<sub>2</sub>O<sub>4</sub> solutions made independently in three parallel methods. To ascertain the impact of fruit extracts applied at varying doses on calcium oxalate, pH measurements were made. Making use of a UV-VIS spectrophotometer set at 625 nm, the impact of extracts applied at various quantities on turbidity was measured. After that, the influence of the extracts on the size and shape of the crystals was investigated using SEM and FTIR analyses of pure calcium oxalate crystals and CaC<sub>2</sub>O<sub>4</sub> crystals that were produced by adding methanol and ethanol extracts to CaC<sub>2</sub>O<sub>4</sub> solutions.

### 2.3. Determination of total phenolic content and total flavonoid content

The Folin-Ciocalteu technique was employed to measure the amount of total phenolic in the methanol and ethanol extracts of *B. crataegina* fruit as gallic acid equivalent (Capanoglu et al. 2013). The aluminum chloride technique was used to quantify total flavonoid levels as routine equivalents (Zhishen et al. 1999).

### 2.4. The quantity of monomeric anthocyanin

The Çağındı (2016) technique was modified to ascertain the quantity of anthocyanin (Çağındı 2016). Five grams of the fruit were mixed with 50 milliliters of alcohol

(methanol, ethanol) to determine the monomeric anthocyanins in the methanol and ethanol extracts. Whereas the methanol extract yields a dark purple tint, the ethanol extract produces a dark pink color. Following the filtering process, one milliliter of each extract was taken individually and diluted ten times with water to create distinct solutions. For the ethanol and methanol extracts, six distinct solutions were made. Three of them had their pH values adjusted to 1, while the remaining solutions had their pH values adjusted to 4.5. The volume of these solutions was completed to 25 mL and the pH was controlled "pH 1 and pH 4.5". Absorbances were then recorded at 515 nm and 700 nm. Using the formula below, the quantity of monomeric anthocyanin was determined (Çağındı 2016).

$$\text{Amount of Monomeric Anthocyanin (mg / L)} = A(MW)(DF) * 1000 / (\epsilon)(L)$$

A: Absorbance difference measured at pH values of 1.0 and 4.5

$$A = (A_{515} - A_{700}) pH_{1.0} - (A_{515} - A_{700}) pH_{4.5}$$

MW: Anthocyanin molecular weight ( $MA_{\text{malvidin-3-glucoside}} = 493.5 \text{ g / mol}$ )

DF: Dilution factor

$\epsilon$ : Molar absorption coefficient (28000)

L: The measuring cuvette's thickness (cm)

### 2.5. Xanthine oxidase enzyme inhibition assay

Xanthine Oxidase derived from bovine milk was purchased from Sigma Aldrich. By altering Wang et al.'s methodology, the inhibitory of this fruit's methanol and ethanol extracts on the xanthine oxidase enzyme was ascertained (Wang et al. 2021). Different enzyme concentrations were adjusted with 50 mM potassium phosphate buffer pH 7.5. Using DMSO at a concentration of 1 mg/mL, a stock solution of the fruit extracts was created, and it was diluted ten times with distilled water. Enzyme solution (10  $\mu$ L), 50 mM potassium phosphate buffer (50  $\mu$ L), and extracts (100  $\mu$ L at different concentrations) were added to the 96-well plate. Subsequently, 60  $\mu$ L 3 mM hypoxanthine was added to each well as substrate. In the inhibition tests, allopurinol served as a positive control. Incubated for 20 minutes at 37 °C. Ultimately, a multi-reader spectrophotometer was used to quantify the absorbance change at 7 minutes using spectrophotometric monitoring of the uric acid content at 295 nm (Wang et al. 2021).

### 2.6. Elastase enzyme inhibition assay

A stock solution containing 75  $\mu$ g/mL of porcine pancreatic elastase was produced in Tris-HCl (pH 8). For ten minutes at room temperature, extract solutions (20  $\mu$ L of 10% DMSO), 80  $\mu$ L of Tris-HCl, and 10  $\mu$ L of elastase stock were incubated in a microplate. Each well was then filled with 15  $\mu$ L of the substrate solution (pH 8, N-

succinyl-Ala-Ala-p nitroanilide in Tris-HCl). At 405 nm, absorbance was measured to track the process (Eun Lee et al. 2019, Lee et al. 1999, Medina-Pérez et al. 2021). The protease inhibitor phenylmethylsulfonyl fluoride (PMSF) was employed as a positive control in this study.

### 2.7. Tyrosinase enzyme inhibition assay

Tyrosinase from mushrooms was purchased from Sigma Aldrich. Utilizing L-DOPA as the substrate, the Dopacram method was used to conduct an enzyme inhibition experiment on tyrosinase. 50  $\mu$ L of sodium phosphate buffer (pH 6.8), 20  $\mu$ L of tyrosinase solution, and 15  $\mu$ L of extract were combined. The mixture was incubated for 15 minutes at 25 °C. Following the addition of 20  $\mu$ L L-DOPA, it was incubated for 10 minutes at 25 °C. At 492 nm absorbance measurement was made. Tyrosinase activity was tested for how extracts affected it (Yırtıcı et al. 2022). Tropolone was used as a positive control in tyrosinase

enzyme inhibition investigations. The enzyme activity in the absence of extract was used as a control, and the absorbance that resulted from the addition of extract was calculated as a percentage of the control. Calculated IC<sub>50</sub> values indicate the 50% change in enzyme activity.

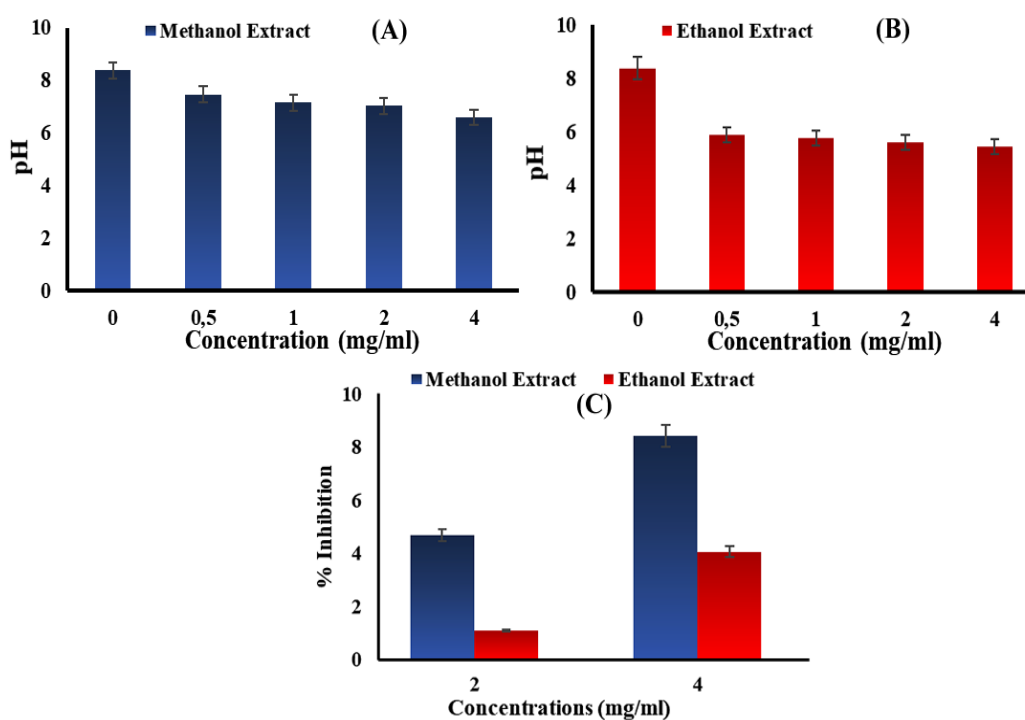
### 2.8. Statistical analysis

Every experiment was run three times. The results are displayed as mean  $\pm$  standard deviation. One-way ANOVA was used to evaluate the data for multiple mean comparisons. The significance was  $p < 0.05$ .

## 3. Results and Discussions

### 3.1. Calcium oxalate inhibition results

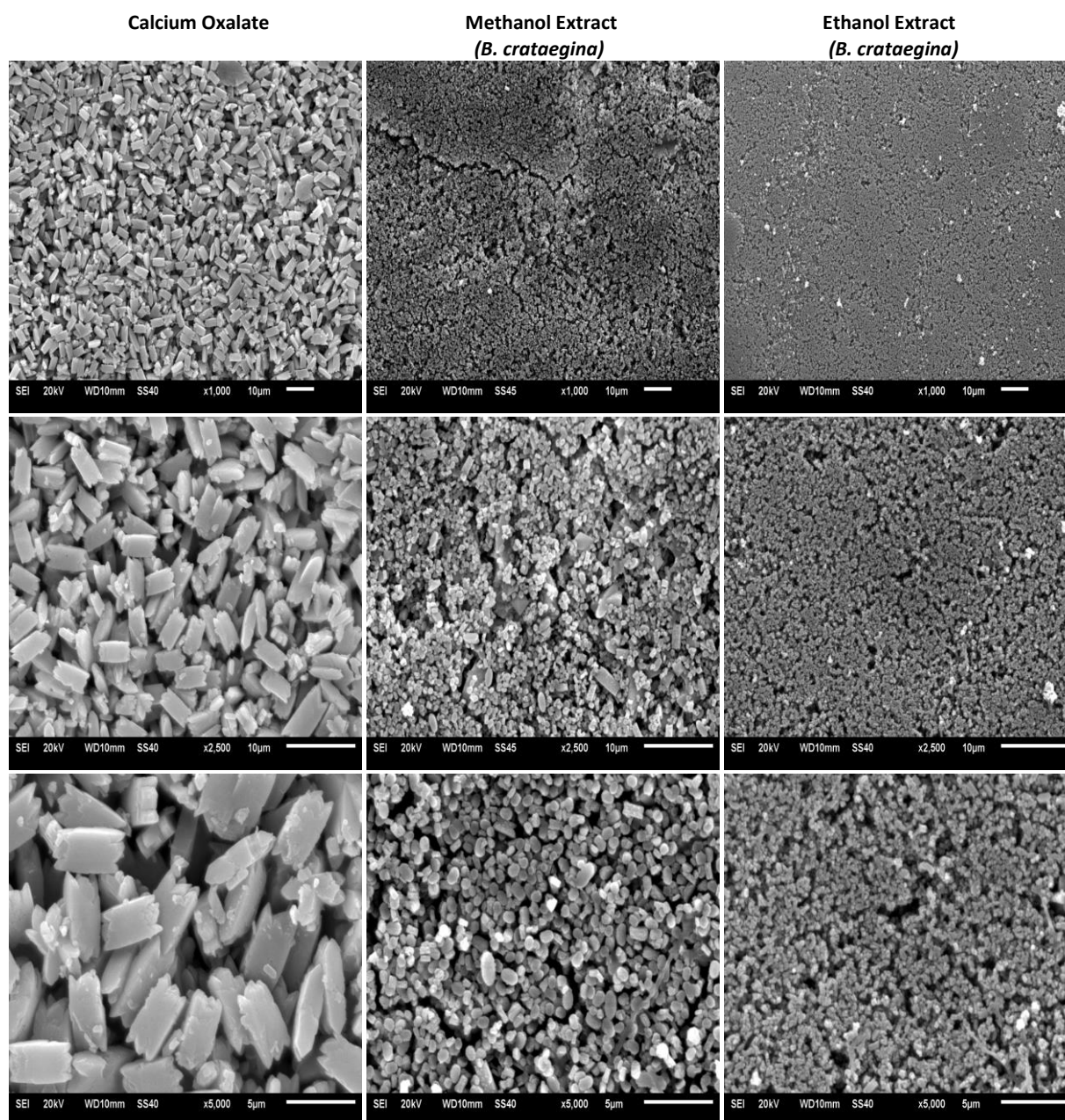
The pH changes of the solutions created by adding methanol and ethanol extracts of *B. crataegina* fruit to CaC<sub>2</sub>O<sub>4</sub> solutions are displayed in Figure 1.



**Figure 1.** The effect of *B. crataegina* fruit methanol (A) and ethanol (B) extracts on the pH change of calcium oxalate solution and the % inhibition (C) effect on calcium oxalate crystal.

Examining the impact of the fruit's methanol extract on the calcium oxalate solution's pH alteration, it is seen that the acidity rises with concentration and the pH value consistently drops. Examining the impact of the *B. crataegina* fruit ethanol extract on the pH change of the calcium oxalate solution, it is found that the pH value changes more at the 0.5 mg/mL concentration and lowers less at the other concentration. When Figure 1 (A) and (B) are examined together, it is clear that the addition of extracts causes the solution to change from basic to acidic.

The effect of concentration increase at 625 nm on turbidity measurement was evaluated using a spectrophotometer to assess the effect of methanol and ethanol extracts of *B. crataegina* fruit on the inhibition of calcium oxalate crystal. The % inhibition was computed and is shown in Figure 1 (C). SEM images of pure calcium oxalate crystal and SEM images (x1000-x5000) showing the inhibition effect of methanol extracts and ethanol extracts of *B. crataegina* fruit on calcium oxalate crystal are given in Figure 2. Calcium oxalate crystals with a regular crystal structure were found to be produced.



**Figure 2.** SEM images of calcium oxalate crystals (pure) and comparative SEM images of the inhibition effect of methanol and ethanol extracts of *B. crataegina* fruit on calcium oxalate crystals.

SEM pictures demonstrate the inhibitory impact of *B. crataegina* fruit methanol and ethanol extracts on calcium oxalate crystals. According to the pictures, adding fruit extracts causes the crystals of calcium oxalate to shrink. The fruit's ethanol extract's impact on calcium oxalate crystal examining SEM images reveals a very significant inhibitory impact.

FTIR analysis results of calcium oxalate crystals (pure) and calcium oxalate crystals formed by the effect of methanol and ethanol extracts are given in Figure 3. When the FTIR graph of the calcium oxalate crystal formed without adding fruit extract is studied, the bands at  $1130\text{ cm}^{-1}$  represent C-O stretching, and the peaks at  $1627$  and  $1319\text{ cm}^{-1}$  suggest carbonyl stretching bands ( $\text{C}=\text{O}$ ), according to the FTIR graphs in Figure 3. C-C stretching is responsible

for the band at  $885\text{ cm}^{-1}$ , which shows the existence of two carboxylate anions (Kesavan et al. 2012). The distinctive bands of calcium oxalate crystals are the bands of calcium oxalate that exist in pure media at  $1627$ ,  $1319$ ,  $947$ ,  $885$ , and  $666\text{ cm}^{-1}$  (Polat 2019). The creation of the monohydrate form is indicated by the tension at  $1627\text{ cm}^{-1}$ , which is ascribed to the asymmetric C-C (Polat, 2019). The presence of bands at  $666$  and  $516\text{ cm}^{-1}$ , absorption bands ( $\text{COO}^-$ ) at  $1627$ - $1634\text{ cm}^{-1}$  and  $1389\text{ cm}^{-1}$  ( $\text{COO}^-$ ) at  $1319\text{ cm}^{-1}$  indicate the formation of calcium oxalate trihydrate crystals (Conti et al. 2015, Kachkoul et al. 2020, Ouyang et al. 2005). In other words, it indicates the formation of a mixture of calcium oxalate monohydrate and calcium oxalate trihydrate crystals (Kachkoul et al. 2020).

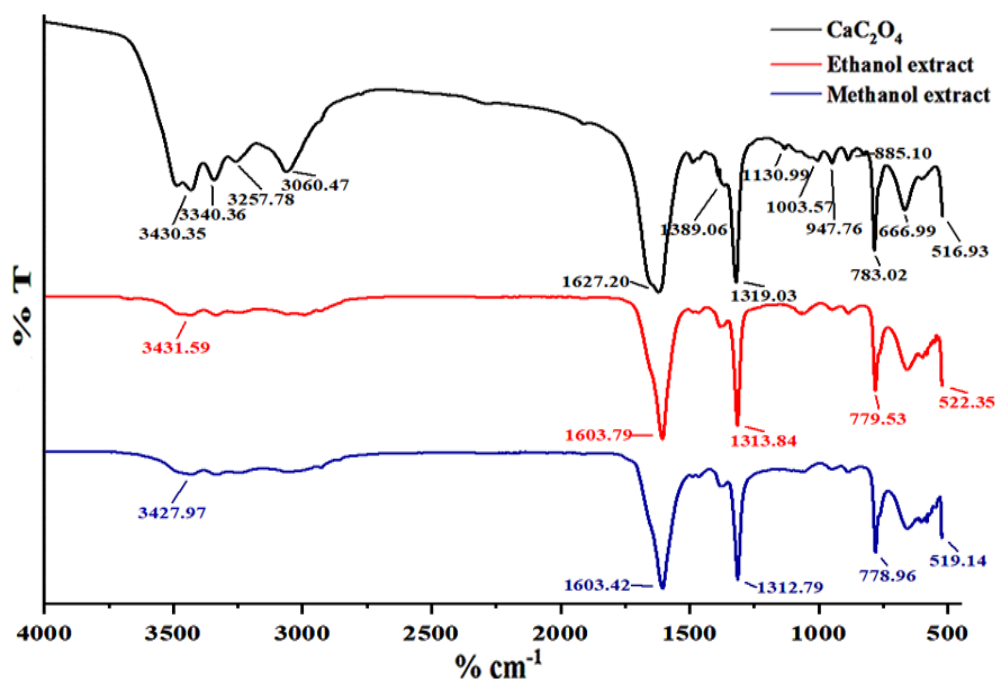


Figure 3. FTIR graphs of  $\text{CaC}_2\text{O}_4$  and  $\text{CaC}_2\text{O}_4$  crystals prepared with methanol and ethanol extracts of *B. crataegina* fruit

A single absorption peak arises between 3430 and 3000  $\text{cm}^{-1}$  of calcium oxalate crystals produced by the addition of methanol and ethanol extracts. This demonstrates that COD crystals are present. At 1603  $\text{cm}^{-1}$  and 1313  $\text{cm}^{-1}$ , two distinct absorption bands were detected. These confirm the formation of calcium oxalate dihydrate (Kachkoul et al. 2020, Yao et al. 2012). The formation of calcium oxalate dihydrate crystals is observed upon FTIR examination of calcium oxalate crystals resulting from the addition of methanol and ethanol extracts. The affinity of these crystals for renal cells is lower (Kachkoul et al. 2020).

### 3.2. Total phenolic content, total flavonoid content, and total monomeric anthocyanin results

The methanol and ethanol extracts of *B. crataegina* fruits were tested for total phenolic content, total flavonoid content, and total monomeric anthocyanin content. The findings are shown in Table 1.

Table 1. Total phenolic content, total flavonoid amount, monomeric anthocyanin amount of methanol extracts, and ethanol extracts of *B. crataegina* fruit

Samples	Total Phenolic Content (mg GAE/g extract)	Total Flavonoid Content (RE/g extract)	Total Monomeric Anthocyanin (mg MG/g extract)
Ethanol Extract	36.39±3.86	27.55±2.13	39.36±0.02
Methanol Extract	48.85±3.82	33.31±2.75	54.69±0.01

The results are given as mean values ± standard deviations in Table 1.

The data shown in Table 1 indicate that the methanol extract has higher levels of total flavonoid, total phenolic, and anthocyanin content.

### 3.3. Enzyme inhibition results

Table 2 displays the results of inhibition tests for xanthine oxidase, elastase, and tyrosinase of ethanol and methanol extracts of *B. crataegina* fruit.

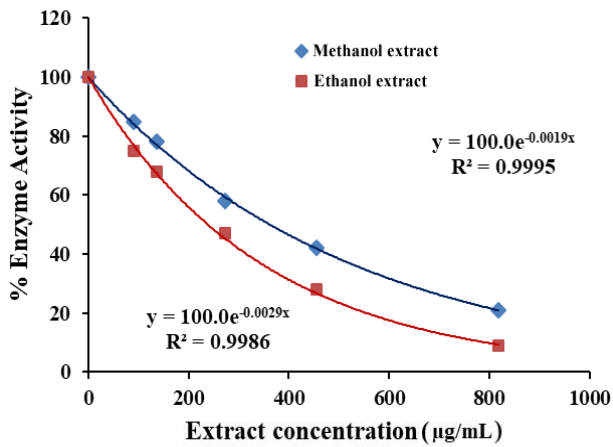
Table 2. Effect of methanol and ethanol extracts of *B. crataegina* fruit on enzymes ( $\text{IC}_{50}$  ( $\mu\text{g}/\text{mL}$ )).

Samples	XO Inhibition $\text{IC}_{50}$ ( $\mu\text{g}/\text{mL}$ )	Elastase Inhibition $\text{IC}_{50}$ ( $\mu\text{g}/\text{mL}$ )	Tyrosinase Inhibition $\text{IC}_{50}$ ( $\mu\text{g}/\text{mL}$ )
Ethanol Extract	239.02±16.71	138.35±28.10	635.45±20.30
Methanol Extract	364.81±20.15	297.49±25.02	781.09±25.12

$\text{IC}_{50}$ : extract concentration that inhibits enzyme activity by 50% (mean value ± standard deviation)

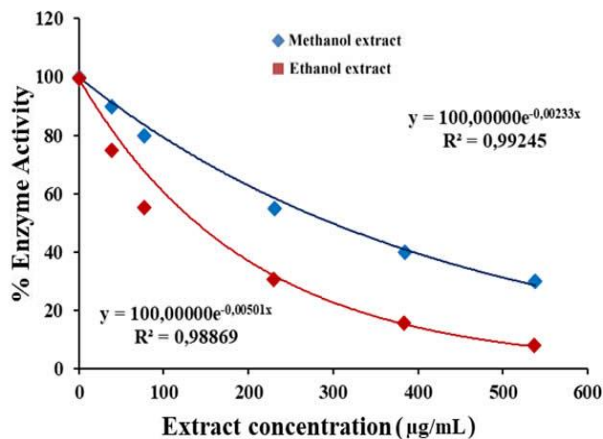
The study determined that PMSF had an inhibition value of 28.88  $\mu\text{g}/\text{mL}$  ( $\text{IC}_{50}$ ) against the elastase enzyme, Tropolon had an inhibition value of 40.00  $\mu\text{M}$  ( $\text{IC}_{50}$ ) against the tyrosinase enzyme, and Allopurinol had an inhibition value of 6.18  $\mu\text{M}$  ( $\text{IC}_{50}$ ) against the xanthine

oxidase enzyme. The xanthine oxidase enzyme was more inhibited by the ethanol extract. The fruit's methanol and ethanol extracts' impact on the xanthine oxidase enzyme's activity is displayed in Figure 4.



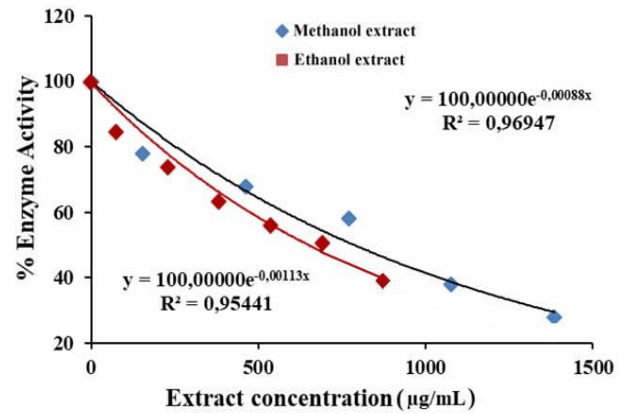
**Figure 4.** Xanthine oxidase enzyme inhibition graphs of methanol and ethanol extracts

With increasing concentration, the enzyme's activity was consistently reduced by both *B. crataegina* fruit extracts. Compared to methanol extract, ethanol extract inhibited the Elastase enzyme more. The effect of methanol and ethanol extracts of *B. crataegina* fruit on the elastase enzyme is given in Figure 5.



**Figure 5.** Elastase enzyme inhibition graphs of *B. crataegina* fruit extracts

When compared to methanol extract, ethanol extract had a stronger inhibitory impact on the Tyrosinase enzyme. Among these enzymes, fruit extracts showed the greatest effect on the elastase enzyme. The effect of methanol and ethanol extracts of *B. crataegina* fruit on tyrosinase is given in Figure 6. All enzymes were inhibited by *B. crataegina* extracts, and this effect grew with concentration. But of all these enzymes, fruit extracts had the biggest impact on the elastase enzyme. Both extracts of *B. crataegina* fruit showed an increasing inhibition effect on  $\text{CaC}_2\text{O}_4$  crystallization with increasing concentration. The inclusion of both extracts resulted in a degradation of the calcium oxalate crystal structure, as seen by the SEM pictures, and the calcium oxalate crystals assumed an oval form. Particularly the ethanol extract showed greater prevention of crystallization in SEM pictures.



**Figure 6.** Tyrosinase enzyme inhibition graphs of *B. crataegina* fruit extracts

The turbidity measurement-based efficiency test revealed that the methanol extract had a higher inhibitory impact. FTIR results of both extracts gave similar results. The same components in the fruit may be responsible for this situation. This can be explained by the higher concentration of this component, which is effective on  $\text{CaC}_2\text{O}_4$ , in the methanol extract. *B. crataegina* fruit's high anthocyanin content may account for its influence on calcium oxalate crystals, according to research on fruits with this compound published in the literature (Akin et al. 2019, Khawsuk et al. 2018, Sansores-España et al. 2022, Tayefi-Nasrabadi et al. 2012). Previous research has suggested that *Berberis vulgaris* root bark extract is a helpful agent in avoiding calcium oxalate crystal buildup and that *Berberis trifoliata* extract may be protective against the development of kidney stones (Bashir et al. 2010, Pérez-Hernández et al. 2018). It was found in this investigation that  $\text{CaC}_2\text{O}_4$  crystallization was suppressed by both extracts of the *B. crataegina* fruits.

It was discovered that the methanol extract had greater levels of total monomeric anthocyanins, total flavonoids, and total phenolic compounds than the ethanol extract. It was previously reported that *B. crataegina* fruit is rich in anthocyanins and, with this feature, may be a favorite fruit in food coloring production (Demirci et al. 2022). Furthermore, some earlier investigations have reported that acidic solvents prevent calcium oxalate crystals from growing and nucleating (Akyol 2016, Akyol et al. 2016, Oliveira et al. 2013). The *B. crataegina* fruit's ethanol extract was more acidic and effective in preventing calcium oxalate from occurring. However, due to the high anthocyanin content of the methanol extract, it had a blocking effect on  $\text{CaC}_2\text{O}_4$  crystallization.

Numerous research has examined the impact of various solvents on total phenolic and total flavonoid amounts in the literature, underscoring the significance of the solvent during the extraction process (Addai et al. 2013, Ezez and

Tefera 2021). Compared to the ethanol extract, the methanolic extract of *B. crataegina* fruit had a higher concentration of flavonoid and phenolic components. This research can add to the body of knowledge in this area. Fruits and plants can dissolve in a variety of solvents, depending on what's in them. Because these studies can direct different applications and analyses, they contribute to optimization studies that are sample-specific. The methanol extract contained a greater concentration of dissolved anthocyanin. Anthocyanin compounds dissolve more readily in methanol than in ethanol, according to experiments published in the literature (Ju and Howard 2003, Sasikumar et al. 2021). These investigations validate our findings.

It was determined that methanol and ethanol extracts of *B. crataegina* fruit had an inhibitory effect on the xanthine oxidase enzyme. However, ethanol extract ( $IC_{50}=239.02 \mu\text{g/mL}$  for ethanol extract and  $IC_{50}=364.81 \mu\text{g/mL}$  for methanol extract) gave more effective results. The reason why ethanol extract is more effective can be attributed to different possibilities. The first possibility is that the substance exhibiting the inhibitory effect could not be phenolic in origin. Secondly, it has a phenolic character and is more soluble in ethanol. Yet, the extract performance cannot be determined only by looking at the total quantity of flavonoids or phenolic chemicals. It is crucial to consider the activity of the phenolic component it contains. These findings suggest that phenolic chemicals dissolved in ethanol rather than methanol may have more activity.

Both extracts of *B. crataegina* have a xanthine oxidase inhibition effect that increases with concentration. It can be utilized as a natural inhibitor in light of the negative effects of medications employed as xanthine oxidase inhibitors (Cengiz et al. 2012, Liu et al. 2008, Umamaheswari et al. 2009). Although there are studies examining the inhibition effect of various plants on xanthine oxidase Orbán-Gyapai et al. (2015), there is no study in the literature examining the effect of *B. crataegina* fruit on the xanthine oxidase enzyme. It appears that *B. crataegina* fruit may be useful in preventing a variety of illnesses that could arise from xanthine oxidase overactivity. This work adds to the body of knowledge by investigating the effects on xanthine oxidase of both *B. crataegina* fruit extracts.

Elastase enzyme breaks down Elastin due to increasing activation, which ages the skin (Wittenauer et al. 2015). Additionally, it is recognized that elastase contributes to the development of arthritis and some inflammatory diseases (Yeşilada and Küpeli 2002). Herbs and herbal

products are being researched for healthy connective tissue and prevention of skin aging. *B. crataegina* contains berberine and berberine inhibits the activity of the elastase enzyme (Tanaka et al. 1993, Yeşilada and Küpeli 2002). Both methanol and ethanol extracts showed an increasing inhibition effect on the elastase enzyme with increasing concentration. The ethanol extract showed more than twice the inhibitory effect of the methanol extract ( $IC_{50}=138.35 \mu\text{g/mL}$  for  $B_{\text{ethanol}}$ ,  $IC_{50}=297.49 \mu\text{g/mL}$  for  $B_{\text{methanol}}$ ). This fruit can thus be utilized as an anti-aging component in a range of pharmaceutical, cosmetic, and medical goods.

According to earlier research, the tyrosinase enzyme is inhibited by *Berberis aristata*, one of the *Berberis* species, which may help prevent hyperpigmentation in human skin (Biswas et al. 2016). Additionally, it has been noted that berberine can be utilized to treat and prevent problems related to skin pigmentation by inhibiting tyrosinase activity and melanin formation (Song et al. 2015). In this study, the tyrosinase enzyme inhibition of the ethanol extract of *B. crataegina* fruit was determined as  $IC_{50}=635.45 \mu\text{g/mL}$ , and the tyrosinase enzyme inhibition of the methanol extract was determined as  $IC_{50}=781.09 \mu\text{g/mL}$ . It was found that *B. crataegina* fruit extracts exhibited increasing inhibition effects with increasing concentration.

It is anticipated that *B. crataegina* fruit will be useful in treating or mitigating disorders brought on by the overactivity of the xanthine oxidase enzyme, as well as having the capacity to block calcium oxalate crystals. It was also determined that its anti-aging properties were better than its anti-hyperpigmentation properties. However the fruit's bioavailability may have a different impact on the human body, all these predictions ought to be backed by in vivo animal studies. Considering that the *B. crataegina* plant is a thorny shrub plant and that it is difficult to access this plant in urban life, it is thought that it may be beneficial to produce it for medical or pharmacological purposes.

#### **4. Conclusions**

Elastase, and tyrosinase enzyme activity can be inhibited by both *B. crataegina* fruit extracts. *B. crataegina* fruit can be useful in treating or mitigating disorders brought on by the overactivity of the xanthine oxidase enzyme, as well as can block calcium oxalate crystals. Furthermore, it has been revealed that this fruit, which is high in flavonoids and phenolic compounds, may be utilized as a natural source that is useful for health. Because of this characteristic, fruit has significant potential for use in the pharmaceutical, cosmetic, and medical fields.



#### Declaration of Ethical Standards

The author declares that comply with all ethical standards.

#### Credit Authorship Contribution Statement

Author: Conceptualization, methodology, data curation, writing- original draft preparation, visualization, investigation, supervision, validation, writing- reviewing and editing.

#### Declaration of Competing Interest

The author declares that I have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data Availability Statement

The author declares that the main data supporting the findings of this work are available within the article.

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