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DETERMINATION OF TOTAL PHENOLIC ACID CONTENT AND ANTI-ATHEROGENIC ACTIVITY OF OIL OBTAINED FROM FRESH ARUGULA (*Eruca sativa L.*) BY COLD-PRESSING

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Abstract: Cold-pressed oils have attracted great interest due to their high content of bioactive compounds and potential health benefits. This study aims to determine the total phenolic acid content and anti-atherogenic activity of cold-pressed wild arugula (*Eruca sativa L.*) oil. Total phenolic acid content was analyzed using spectrophotometric and chromatographic methods, while anti-atherogenic activity was evaluated by in vitro tests measuring antioxidant capacity and lipid peroxidation inhibition. The results show that *Eruca sativa L.* oil contains significant amounts of phenolic acids, which contribute to its antioxidant potential. Furthermore, the oil exhibited promising anti-atherogenic activity, suggesting its potential role in cardiovascular health. These findings highlight the importance of cold-pressed wild arugula oil as a functional food ingredient with potential therapeutic applications.

Keywords: *Eruca sativa L.*, Cold pressed oil, Phenolic acids, Anti-atherogenic activity, Antioxidant potential

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1. Introduction

It is an annual herbaceous plant belonging to Brassicaceae family, genus Eruca MILLER, taxon *Eruca sativa* (*E. sativa*) MILLER (Ahmed et al., 2013; Sadig et al., 2013; Rizwana et al., 2016). *E. sativa* is commonly known as arugula and its leaves are consumed (Gozukara et al., 2019). It has been used as a medicine to treat different diseases since ancient times (Sadig et al., 2013).

E. sativa L. (Arugula) oil can be used as an oil plant with 35% oil content in its seeds (Acar et al., 2016) and as a medicinal plant because its leaves contain flavonoids such as quercetin, kaempferol, isorhamnetin (Nicoletti et al., 2007; Caruso et al., 2018).

E. sativa plant 100 grams approximately; 3.65 g carbohydrate; 2.58 g protein; 1.6 g dietary fiber; 0.305 mg niacin; 0.437 mg pantothenic acid; 0.073 mg pyridoxine; 0.086 mg riboflavin; 0.44 mg thiamine; 15 mg vitamin C; 2373 IU vitamin A; 0.43 mg vitamin E; 108.6 ug vitamin K; 27 mg sodium; 369 mg potassium; 160 mg calcium; 0.076 mg copper; 1.46 mg iron; 47 mg magnesium; 0.321 mg manganese; 52 mg phosphorus; 0.3 ug selenium; 0.47 mg zinc and 1424 ug carotene-ß; 3555 ug lutein-zeaxanthin. Palmitic acid, linoleic, linolenic, oleic, stearic and erucic acid are the fatty acids it contains (Garg and Sharma, 2014). The fatty acid profile is approximately as follows: palmitic acid 2.80%; stearic acid 30.8%; oleic acid 17.8%; linoleic acid 1.44%; linolenic acid 6.78%; erucic acid 47.0%.

It has anticarcinogenic (Koubaa et al., 2015; Bell and Wagstaff, 2019), anti-inflammatory (Koubaa et al., 2015, Katsarou et al., 2016), antiulcer (Koubaa et al., 2015) activity. It helps to remove toxic substances from the body and strengthens the immune system and stomach (Sadig et al., 2013; Shabnam 2015).

Serum paraoxonase (PON1, EC 3.1.8.1) is an enzyme found in the blood. It belongs to a group of enzymes called esterases. It is physically bound to a type of cholesterol called high-density lipoprotein (HDL). It is named for its ability to break down paraoxon, which is an organophosphate that is produced in the body from the insecticide parathionate (Mackness et al., 2002). The assessment of PON1 activity has traditionally relied on the use of paraoxon and phenylacetate as substrates. Another type of paraoxonase enzyme, paraoxonase 3 (PON 3), is found in the blood. However, PON3 has almost no paraoxonase activity with paraoxon as substrate (Ng et al., 2005). It also has almost no arylesterase activity when phenylacetate is used as a substrate. Some studies have suggested that the role of PON3 may be different from that of PON1. Furthermore, there is less serum human PON3 than PON1 (Draganov and La Du, 2004). PON1 is one of the main enzymes that may be responsible for a significant portion of the antioxidant properties of HDL. Numerous studies have shown that HDL protects LDL and cell membranes against damage from oxidation (Draganov and La Du, 2004). Oxidised LDL has been

shown to play a key role in the development of atherosclerotic lesions, and HDL has been shown to protect LDL from oxidation, thereby preventing the production of oxidised LDL. The protective effect of HDL may be attributed to its associated enzymes, including PON1 (Draganov and La Du, 2004; Mackness et al., 2002). In addition to its role in protecting LDL, PON1 has been reported to play a significant role in the breakdown and inactivation of homocysteine thiolactone, a toxic substance produced when homocysteine is broken down. This enzyme has also been shown to activate and inactivate certain drugs. PON1 also plays a role in other diseases, such as diabetes, familial hypercholesterolemia, chronic renal failure, obesity, metabolic syndrome and sepsis (Draganov and La Du; Goswami et al., 2009; Aviram and Rosenblat,2005; Camps et al., 2009).

PON1 is a glycoprotein enzyme synthesized by the liver, hydrolyzes aromatic carboxylic acid esters, has the ability to hydrolyze paraoxon, the active metabolite of paration, an organic phosphorus insecticide, and is tightly bound to HDL (Azarsız, 2000). PON1 is an important liver enzyme which is bound to HDL and plays a protective role against hydrolysis of organophosphate agents (OP) and nerve gases, formation of lipid peroxides by oxidation of LDL and bacterial endotoxins. Paraoxanase was initially studied in the field of toxicology due to its ability to hydrolyze organophosphate compounds, but in recent years, it has gained popularity in recent years due to its antioxidant effects, as it is thought that it can be protected from the risk of chronary heart disease. Paraoxanase also reveals the importance of the antioxidant properties of the enzyme as HDL reduces lipid peroxides by these enzymatic mechanisms during the oxidation of LDL, which constitutes the initial stage of the arterosclerosis process, and thus the accumulation of lipid peroxides (Durrington and Mackness, 2001, Carey et al., 2005). Paraoxonase enzyme is one of the defense mechanisms in the body against arterosclerotic diseases (Gencer, 2008).

It has long been known that HDL in serum is protective against the development of atherosclerosis. The mechanisms of this protective effect include reverse cholesterol transport, increasing the synthesis of some vasodilator molecules such as nitric oxide, inhibiting inflammation and thrombosis formation, decreasing the synthesis of adhesion molecules, stimulating endothelial repair and preventing oxidative changes in LDL. However, which components of HDL are involved in all these mechanisms is controversial. The recent discovery that PON1 is a component of HDL has gained importance in clarifying these controversial mechanisms. In order to better examine the function of PON1 in this mechanism, it must first be obtained in pure form from serum. It is known that there are many other proteins in the complex formed by PON1 with HDL. Previous studies reported a wide range of substrates for PON1, suggesting that the enzyme could not be obtained in a sufficiently pure form. Therefore, in this study, it was aimed to completely separate PON1 from other HDL components using appropriate purification steps from serum and to better define the structural and functional properties of the pure enzyme (Bayrak, 2009).

In this study, it was aimed to investigate the total phenolic acid content and paraoxanase enzyme inhibition activity of *E. sativa* L. oil.

2. Materials and Methods

2.1. Plant Samples

Fresh arugula (*Eruca sativa* L.) was taken and the oil was extracted by cold pressing. The extracted oils were stored at 2-8 ^{o}C until biological property studies were carried out.

2.2. Total Phenolic Acid Content

2.4 mL of distilled water was added to the tubes. Then 40 µL Eruca sativa L. oil and 200 µL Folin ciocalteu solution were added. Subsequently, 600 µL of the sample was equilibrated to room temperature, with an incubation interval ranging from 30 seconds to 7.5 minutes following the preceding step. Then saturated sodium carbonate was added. 760 µL of distilled water was added. Vortexed. It was kept for 2 hours at room temperature in the dark and read at 765 nm in a spectrophotometer (Singleton and Rossi, 1965; Turkes et al., 2014).

2.3. hPON1 activity

In this study, PON1 (Paraoxonase-1) enzyme activity was evaluated by spectrophotometric method using phenylacetate substrate. The measurement was carried out based on the absorbance increase of the product formed by hydrolysis of phenylacetate to p-hydroxybenzene at 405 nm wavelength in the reaction mixture containing Tris-HCl buffer (pH 8.0) and 1 mM $CaCl_2$. Potential inhibitors such as Eruca sativa L and Kojic acid were added separately and in combination and enzyme inhibition levels were calculated and % inhibition rates were compared with the control group (Güzel et al., 2025).

3. Results

3.1. Phenolic Acid Content

Total phenolic matter content is given in Table 1. Phenolic acid content of arugula oil was 330.23±9.12 GAE/L.

Table 1. Phenolic acid content of *Eruca sativa L*. oil

Sample	Total Phenolic Content (GAE/L)
Eruca sativa L. oil	330.23±9.12

3.2. Paraoxanase Results

In this study, the inhibition effect of Eruca sativa L. oil on paraoxonase enzyme activity was studied. IC50 value for arugula oil was determined as 350.39±9.77 with the help of % activity graph (Table 2, Figure 1). In our study, Lineweaver-Burk graph was drawn (Figure 2) and it was determined that the inhibition type was competitive. As a result of inhibition studies, it was observed that arugula oil strongly inhibited the hPON1 enzyme.

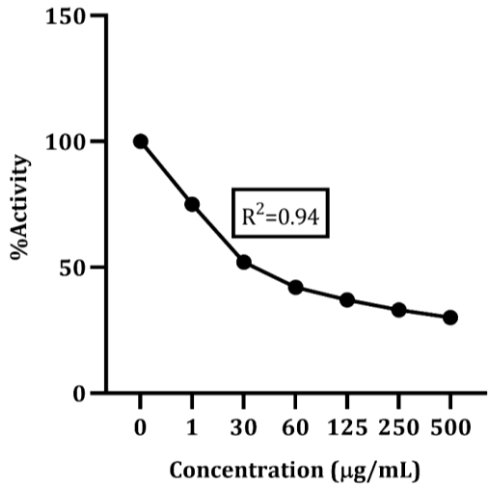

Figure 1. hPON1 Percent Activity in Response to Eruca sativa L. Oil Treatment.

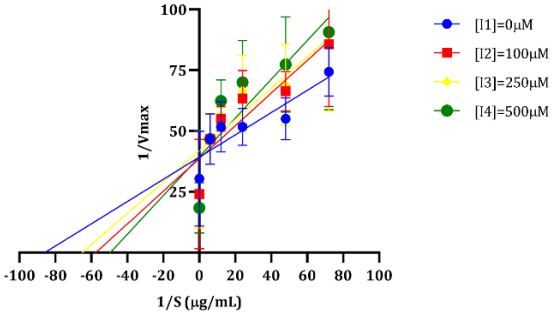

Figure 2. Lineweaver–Burk plot depicting the effect of Eruca sativa L. oil on the catalytic kinetics of human paraoxonase 1 (hPON1), suggesting potential modulation of enzyme activity.

Table 2. Inhibitory effect of *Eruca sativa L.* on Paraoxanase (hPON1).

	Inhibition against paraoxanase IC50 (µg/mL)	Inhibition against paraoxanase R^2
Eruca sativa L. Oil	350.39±9.77	0.94

4. Discussion

The specific enzyme known as PON1 is present in various tissues throughout the human body, including those located in the brain, heart, liver, kidney, lung, and small intestine. The predominant site of synthesis of this enzyme is the liver during metabolic processes, and it subsequently binds to HDL, which subsequently circulates in the bloodstream (Turkes et al., 2019). A body of literature exists which demonstrates that PON1 is directly responsible for a number of antiatherogenic functions of HDL. These functions include the protection of lipoproteins and cells against oxidative stress, as well as the prevention of atherogenesis and lipoprotein peroxidation (Jaouad et al., 2003). The protective effects of PON1 are related to the enzyme level in serum. Recent studies have reported low PON1 levels in patients with atherosclerosis, diabetes and hypercholesterolemia. Conversely, the over-expression of PON1 has been observed to impede the progression of cardiovascular diseases. In addition, PON1 has been found to stimulate HDL efflux in macrophages and inhibit LDL biosynthesis (Richter et al., 2008). In another study, PON1 was shown to be transported from HDL to the outer surface of the cell membrane to protect metabolism against oxidative stress-related conditions such as coronary artery disease, diabetes, and obesity (Aviram and Rosenblat, 2008).

In this study, the phenolic acid content of *E. sativa L.* oil was examined and found to be 330.23±9.12 GAE/L. In addition, as a result of hPON1 inhibition study, it was observed that *E. sativa L.* oil strongly inhibited the hPON1 enzyme.

5. Conclusion

In summary, this study focused on investigating the in vitro effects of *E. sativa L.* oil on PON1 activity, which has a role in serious cases such as cardiovascular diseases. Decreased activity of PON1 is an important risk factor for atherosclerosis. This study should be supported by in vivo studies. Further studies are needed as there is no universal conclusion on whether PON1 can be used as a biomarker for cardiovascular disease.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	E.Ö.	İ.D.
C	60	40
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	60	40
SR	60	40
PM	60	40
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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