

Investigation of the Antienzyme and Antimicrobial Properties of the Fruit Extracts of the Oleaster (*Elaeagnus angustifolia* L.) Under In Vitro Conditions

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Investigation of the Antienzyme and Antimicrobial Properties of the Fruit Extracts of the Oleaster (*Elaeagnus angustifolia* L.) Under *In Vitro* Conditions

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Abstract

Oleaster fruit, which is a rich source of nutrients, is also an important source of antioxidants. Not only the fruit but also all parts of the plant such as roots, bark, flowers and leaves have medicinal properties. For this reason, this plant is utilized in many sectors such as food, medicine and perfumery. PPO, an enzyme commonly found in fruits and vegetables, causes enzymatic browning, resulting in loss of color, odor, taste, nutritional and economic value of foods. At the same time, it is of great importance to prevent microbiological spoilage that causes food spoilage. In this study, the inhibition effect of the oleaster fruit against the PPO enzyme and its antimicrobial effect against 4 pathogenic bacteria and one yeast cell were investigated by well diffusion technique. At the end of the study, it was observed that there was no inhibition effect and antimicrobial effect of oleaster fruit. As a result, it was determined that the extraction and inhibition method changes the inhibition depending on the enzyme and the effect on the enzyme in anti-enzyme studies. In addition, in antimicrobial studies, it was observed that the extraction method, especially the strain used, affected the antimicrobial study result.

Keywords: *Elaeagnus angustifolia* L., PPO inhibition, Antibacterial, Antifungal

1. Introduction

The Latin name *Elaeagnus angustifolia* L. is also known as Russian Olive (1). This thorny plant in tree or shrub form is a perennial plant that can reach a length of seven meters with leaves covered with silver scales, single-seeded, brown fruit (2). The inside of the oleaster fruit, which has a similar appearance to dates, is dry, white and has a sweet taste (1). Oleaster, which is distributed in Asia (central and western regions), the Gobi Desert, the Alps, around the Mediterranean and in our country (Black Sea, Marmara, Southern Anatolia and Southeastern Anatolia), can be found naturally and can also be cultivated (3). The production of oleaster, which is a tree growing in temperate regions, is around 6000 tons in our country (1). The fruit is a rich source of nutrients, chemical compounds, minerals and antioxidants (4).

This plant, which has a high health value, can grow even in arid environments, salty and calcareous soils, can improve soil conditions by binding the free nitrogen of the air with nodules in its roots (3), and has important environmental effects such as erosion control and wind stopping (1). Decoctions and infusions of fruits, flowers, leaves and bark are traditionally used in the treatment of various diseases. Raw or boiled fruit is consumed to treat some diseases (5). Since all parts of the plant such as root, bark, flower, leaf and fruit have medicinal properties, this plant is also utilized in the food, medicine and perfumery industry (6). In Iranian folk medicine, oleaster fruit is used as a painkiller, and recent pharmacological studies have determined that it has anti-inflammatory and antioxidant properties (5). In addition, many antimicrobial and antienzyme studies have been conducted to determine the pharmacological properties of *Elaeagnus angustifolia* (7; 8). It has been reported that fruit and leaf extracts of oleaster showed inhibition effect on AChE, BChE, Tyrosinase, α -Amylase and α -Glucosidase enzyme activities depending on the extraction method (7). In different studies, it was stated that the oleaster leaf extract had an inhibition effect on α -Amylase and α -Glucosidase enzyme activities (9). In another study, it was reported that the components isolated from *Elaeagnus angustifolia* L. generally did not show inhibition effect on α -Amylase and α -Glucosidase enzyme activity (10). As can be understood from the literature, polyphenol oxidase enzyme, which has pharmacological potential, is also an important enzyme in the food enzyme industry. This enzyme, which is widely found in fruits and vegetables, causes enzymatic browning by catalyzing the oxidation of phenolic compounds to quinones that produce brown pigments (11). Some substances show the ability to reduce o-quinones that cause color change to phenolic forms. Thus, the browning reaction stops and the color does not deteriorate (12). In recent studies, the PPO enzyme inhibitory effects of various plant extracts such as damson plum bark extract (12), rosehip fruit extract (13) were investigated and it was determined that they had inhibitory effects on PPO.

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Within the scope of our study, the inhibitory effect of methanol extract of oleaster fruit on PPO enzyme activity and antimicrobial effects against various pathogenic bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29242, *Escherichia coli* ATCC 25922) and yeast (*Candida albicans* ATCC 10231) cells were also investigated. Thus, it was tried to find a solution to the problem caused by PPO enzyme, which causes economic losses as well as color, odor, taste and nutritional values of foods.

2. Materials and Methods

Samples of the oleaster to be used in the study were obtained commercially from local markets. After separating the fruit from the core, methanol extract was prepared and this extract constituted the sample of the inhibition and antimicrobial study.

Preparation of methanol extract: 5 grams of oleaster fruits were weighed and homogenized with 50 mL of methanol. For this purpose, it was kept in a shaker incubator (200 rpm, room temperature) overnight in the dark. Then it was filtered with filter paper and placed in tared flasks and the solvent was removed by evaporation. The extract concentration was prepared as 1 mg/mL by dissolving in 10% DMSO. Dilutions during the study were carried out with distilled water. The whole study was carried out at 4°C.

2.1. Enzyme extraction

Jang and Moon's method was modified and used in enzyme extraction (14). The banana discs were homogenised (7) (Velp, Scientifica, OV5/ Europe) in a ten fold amount of chilled 50 mM K-phosphate buffer (pH 7.5) for 2 min using a homogenizer. The homogenate was filtered through whatman filter paper and the filtrate was centrifuged (Thermo Scientific Heraeus Megafuge 16 R, Germany) at 6700 rpm for 60 min at 4°C. The supernatant solution was used in experiments.

2.2. Determination of Polyphenoloxidase (PPO) Enzyme Activity

For this purpose, the method used by Sakiroglu (1994) (15) and Kim and Kim (2011) (16) was utilized. 50 mM pH 7.5 phosphate buffer, 0.1 M catechol solution (1,2 dihydroxy benzene), 10 µL PPO were used and the enzyme activity at 420 nm wavelength for 1 min was determined in a spectrophotometer (VWR-UV-6300 PC, Double Beam Spectrophotometer). Within the scope of the inhibition study, enzyme activity was examined at at least five different inhibitor concentrations. Reaction medium without inhibitor was considered as control. Inhibition effect was determined by plotting %Activity versus [I] (17).

2.2. Determination of Antimicrobial Effect

Well agar diffusion method was used to determine the antimicrobial effect (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29242, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231) (18). Bacterial strains used for this purpose were activated in Tryptic Soy Broth and fungi in Sabouraud Dextrose broth medium at 37°C for 18-24 hours and their concentrations were adjusted according to 0.5 McFarland standard. Then, each bacterial strain was inoculated separately into Mueller-Hinton medium, and after the absorption of the bacterial solution into the medium, 0.6 cm diameter wells were made on the medium with at least 2 cm between each well. 30 µL of each sample was transferred to the wells, the amikacin antibiotic disk was placed on the petri surface and allowed to be absorbed into the medium for approximately 20 minutes. On the other hand, similar procedures were applied to determine the antifungal effect of the dilutions using Sabouraud Dextrose agar medium and the antifungal agent nystatin. The petri dishes were then inverted and incubated at 37°C for 18-24 hours under aerobic conditions. At the end of incubation, the diameters of the transparent zones were measured with a digital caliper and the severity of antimicrobial effect was determined.

3. Results and Discussion

As a result of the study, it was determined that there was no inhibition effect of oleaster fruit against PPO enzyme (Figure 1). In a study, the correlation between the chemical composition and high antioxidant potential of leaf extracts compared to fruit extracts in terms of phenolic and pigment content of oleaster was confirmed and all

extracts showed a promising effect against tyrosinase (7). Tyrosinase enzyme used in the study in question is another synonym of PPO enzyme, but the enzyme activity method and inhibition technique of the enzyme used differ from our study. Therefore, the results obtained in both studies are not similar. Similarly, in a different study, the fact that the components obtained from the leaf extract of oleaster leaf did not show inhibition effect on α -Amylase and α -Glucosidase enzyme activities confirms our study (10). Therefore, it can be said that the inhibition effect and dosage depend on the extract and inhibition method as well as the extracted parts of the plant.

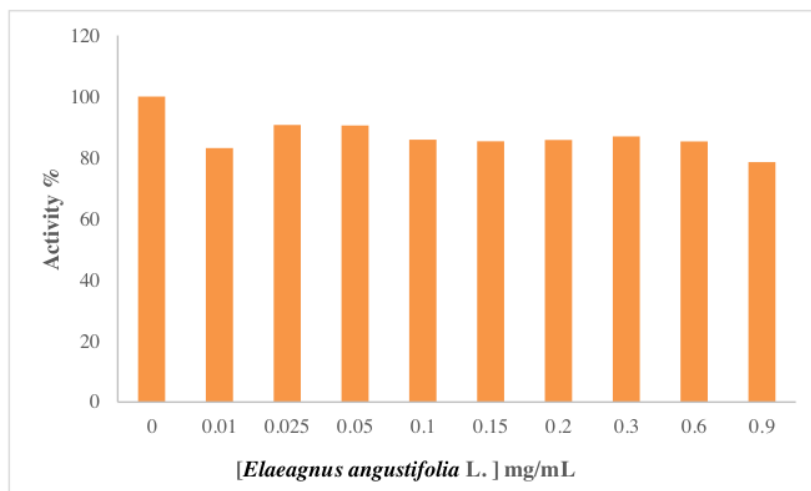


Figure 1. *Elaeagnus angustifolia* L. meyve ekstresinin PPO enzim aktivitesi üzerindeki etkisi

Similarly, it was observed that oleaster fruit had no antimicrobial effect against the pathogenic bacteria and yeast (11) is examined (Figure 2). In another study, it was observed that methanol extract of oleaster leaves did not form inhibition zone against *Escherichia coli* TCC 1122 and *Candida albicans* RSKK 02029. In the same study, 9 mm inhibition zone was formed against *Bacillus subtilis* RSKK 245, *Staphylococcus aureus* RSKK 2392, while 10 mm inhibition zone was formed against *Enterococcus faecalis* ATCC 8093 (19). In (17) other study, it was determined that oleaster ethyl extract (20) formed an inhibition zone of 15 mm for *E. coli*, 14 mm for *S. aureus* and 10 mm for *B. cereus*. Fruit hexane extract (6) showed no effect against *E. coli*, while fruit methanol extract showed no antibacterial effect against *B. cereus*. It was observed that the type of extract was also effective on the antimicrobial effect (20). When the literatures are examined, it is seen that the antimicrobial effect varies depending on the strain used, extraction method and raw material composition.

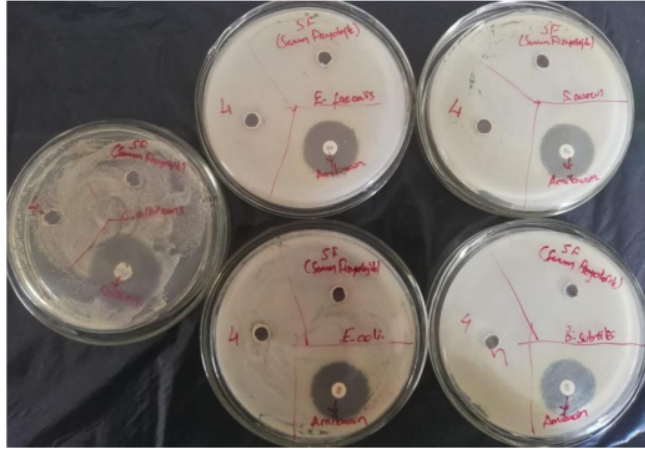


Figure 2. *Elaeagnus angustifolia* L. meyve ekstresinin Antimikrobiyal analiz sonucu

4. Conclusion

Although it is seen that the methanol extract of the fruit of the needle has no enzyme inhibitor and antimicrobial effect, it is thought that the extracts to be prepared with different solvents may give positive results. However, it is thought that it would not be appropriate to use the methanol extract for the studied bacteria and enzyme.

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