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Determination of *In Vitro* Antioxidant, Antimicrobial Properties and COX-1 Enzyme Inhibitory Activity of Mentha Pulegium

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ABSTRACT

People have used medicinal and aromatic plants and spices for centuries in order to treat diseases. *Mentha pulegium* is utilized as food in the world and in Turkiye and also a medicinal plant in traditional treatments. In this context, this study was carried out to examine the antioxidant and antimicrobial properties of the *Mentha pulegium* plant and its effects on cyclooxygenase-1 enzyme. While antioxidant properties were investigated spectrophotometrically by using 2,2-diphenyl-1-picrylhydrazyl, free radical test and Cupric Reducing Antioxidant Capacity assay methods, antibiotic effect was determined by using disc diffusion method. The effects on cyclooxygenase-1 enzyme were determined colorimetrically using commercial kits. The results showed that *Mentha pulegium* plant had a significant antioxidant effect, but it did not have any antibiotic effect on *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans* strains. On the other hand, it exhibited a very weak inhibitory effect on the cyclooxygenase-1 enzyme. Consequently, the *Mentha pulegium* plant has an important antioxidant property and showed a very weak inhibitory effect on the cyclooxygenase-1 enzyme.

***Keywords**: Lamiaceae, antibiotic, analgesic, anti-inflammatory, herbal extract*

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INTRODUCTION

Cyclooxygenases (COXs) enzymes are pro-inflammatory proteins that drive inflammation in tissue and are also known to be involved in tumor development and cancer cell growth. Two isoforms of COX can be found in metabolism. Of these, COX-1 is constitutively active in many tissues, especially in the gastrointestinal tract. COX-2 is usually stimulated against proinflammatory factors [1]. Both COX isoforms are involved in the inflammatory response and have been shown to be overexpressed in various cancers. COXs are target molecules of non-steroidal anti-inflammatory agents (NSAIDs). Thus, inhibition of COXs has become a common biological target in the development of NSAIDs and cancer drugs [2]. Enzyme inhibition is necessary for the treatment of different diseases and is one of the important method used for the develop of new drugs [3]. Safety concerns with non-selective NSAIDs, the failure of COX-2-specific agents, and the absence of any COX-1-specific drugs provide a tremendous opportunity to develop alternative COX-1 and COX-2 blockers without any adverse affects and safety problems. Therefore, natural compounds and their derivatives have been widely accepted as COX inhibitors [4]. Free radicals have an important role in the development of chronic and neurodegenerative diseases [5]. Increased levels of free radicals can promote oxidative stress and lead to dangerous cellular and molecular damage. By this way, different illness such as inflammation, lung and cardiovascular diseases, and cancer may develop in organisms [6] Plants contain many biological active molecules such as phenols and polyphenols with antioxidant properties [7] On the other hand, its use has been limited due to the toxic effects of synthetic antioxidants [8]. For this reason, there has been an increasing interest in medicinal plants with antioxidant properties. In addition, the antimicrobial properties of herbal extracts have constituted the basis of many applications, including preservation of foods, pharmaceuticals, alternative and natural treatments [9]. The development of antibiotics is one of the most important discoveries in the history of medicine and has gained vital importance in the treatment of surgery, organ transplantation and various microbial diseases. However, the widespread and indiscriminate use of antibiotics causes the development of antibiotic resistance in some microorganisms and has emerged as a potential threat today. The World Health Organization (WHO) indicates that antibiotic resistance is the most common problem found in many people today [10]. Investigating the antimicrobial activities of plant-derived antibiotics (PDA) is gaining popularity due to their environmental friendliness and various modes of action on pathogens. PDAs have multiple target sites and different effects on pathogens and microorganisms. This reduces the chance of microorganisms and pathogens developing resistance to these compounds [11]. However, there are studies showing that plants rich in flavonoids and also having antioxidant, antimicrobial and analgesic effects[12-14]. The family Lamiaceae is generally in the form of herbs and shrubs and consists of annual or perennial plants. This family grows almost everywhere in the world and includes about 220 genera and 3500 species, mostly spread over the Mediterranean region. 38 genera and 400 species grow in Turkey and 240 of these species are endemic [15]. The Lamiaceae family has aromatic plant species used in folk and modern medicine, pharmaceutical and food industries [16] *Mentha pulegium* L. (*M. pulegium*) is one of the species found in Turkey and one of the *Mentha* species of the family Lamiaceae and is commonly known as Pennyroyal which grows in the places with an altitude higher than 2000 m [17]. Extracts obtained from this genus have been used traditionally for centuries in the treatment of many diseases such as inflammatory

diseases, cough, genital tumors, ulcers, spleen sclerosis. [18]. The studies have revealed that *M. pulegium* extracts have antibacterial [19], and analgesic effects [20]. Today, *M. pulegium* is used as a commercial product in food and beverage aroma, cough, kidney problems and headache [21]. In the Bitlis region, *M. pulegium* is mixed with yogurt to treat stomach cramps, acid reflux and nausea, and it is also consumed in meals and as tea. Studies on the biological activities of this plant are limited.

In this context, the aim of this study is to determine the antioxidant and antimicrobial properties of methanol extract obtained from the leaves of *M. pulegium* and its inhibition affect on COX-1 enzyme.

Material and Method

Preparation of Plant Extracts

M.pulegium, which is the study material, was collected with its root without damaging the plant around the Koruk Village in Bitlis Province between May-July 2020. Methanol extract of the plant sample was prepared. The solvent was removed from the medium. After its removal, its solution of 7.98 mg/mL was prepared by taking some of the remaining part into ultra- distilled water. The prepared solution was used freshly. The collection and scientific diagnosis of the plants was made by Mehmet FIRAT, the instructor of the Biology Department of YYU Faculty of Education.

2,2-diphenyl-1-picrylhydrazyl, free radical (DPPH) Scavenging Activity

DPPH was performed according to the method specified by free radical scavenging activity [22]. Minor modification was made in the method. It was prepared and used in 25 mg/L DPPH methanol as free radical. The prepared stock solution of 7.98 mg/mL was diluted 10 times and 10, 20, 30, 40, and 50 µL of this solution were taken into the test tubes, respectively, and used in the DPPH test. The samples were incubated for 30 minutes in a dark environment at room temperature. At the end of the incubation, their absorbance was read on a spectrophotometer against blank (methanol) at 517 nm. The amount of DPPH scavenged from the reaction medium was calculated
$\% = [(A0 - A1)/A0] \times 100$ formula. Absorbance of A0 control was taken as absorbance of A1 samples.

Determination of Total Antioxidant Capacity by using Cupric Reducing Antioxidant Capacity assay (CUPRAC) Method

1 mL of copper (II) solution, neocuproine solution and ammonium acetate buffer were added into a glass tube, respectively. The antioxidant solution were taken into the test tubes from 7.98 mg/mL stock solution in 10, 20, 30, 40, 50 µL by being diluted 10 times, respectively, and distilled water was added. Total volume was completed to 4 mL. The resultant solution was kept under room conditions for 30 minutes with its cover closed.

Absorbance value was measured at 450 nm [23] Ascorbic acid (AsA) was used as standard. 4.44 $x10^{-4}$ M stock solution was taken from ascorbic acid and used. 50 µL of AsA stock solution was taken and normal test procedure was applied. Total antioxidant capacity was calculated as ascorbic acid.

Determination of Antimicrobial Properties

Antimicrobial activity was studied according to the disc diffusion method by National Committee for Clinical Laboratory Standard [24]. Bacterial isolates were inoculated in Mueller Hinton Broth (OXOID) and fungal strains in SD Broth (DIFCO) broth medium and activated by incubation at 35±2°C for 24 hours and their density was adjusted according to MCFarland 0.5 (108 CFU/mL) [25]. The bacteria were left in 100 µl of Mueller Hinton Agar (OXOID) and yeast fungus Sabouraud Dextrose Agar (OXOID) media and applied with a glass stirrer and kept for 15 minutes to dry. 25 µl plant extracts were impregnated into sterile standard discs with a diameter of 6 mm and left in the culture medium. Then, the samples were incubated at 37 ºC for 24 hours and their inhibition diameters were determined. In the antimicrobial study, an amount equivalent to the amount of plants in other tests was taken and dissolved in DMSO.

Detection of COX-1 Enzyme Inhibition

It was made using a commercial kit (Cayman Chemicals COX ovine/human Inhibitor Screening Assay Kit tem No. 560131). The study was performed in two repetitions. The absorbance values were read in the Micro Elisa plate reader at 70^{th} minutes. Calculations were made according to the kit procedure. In the study, plant extract was used by diluting 10 times.

RESULTS AND DISCUSSION

Historically, plants have been used as an important resource to meet people's needs such as shelter, fuel, and food. On the other hand, plants have always been the source of tradational medicine and modern medicine [26]. Due to the high cost of pharmaceutical medicine and safety concerns, there is a renewed interest in consuming plants as food and medicine [27]. Because medicinal products of plant origin are considered to be safer [28]. In parallel with the results obtained from the study [29], they showed *Mentha spicata* L. in their study with DPPH, ABTS free radical scavenging activity and FRAP method. In the same study, it was shown to have an antimicrobial effect on standard strains *Staphylococcus epidermidis, Escherichia coli, Candida glabrata*.

In this context, the present study investigated the antioxidant and antimicrobial properties of *M. pulegium* plant and its effect on COX-1 enzyme. Its antioxidant properties were examined spectrophotometrically with DPPH and CUPRAC methods. The results obtained from the study showed that the antioxidant activity of the methanol extract obtained from *M. pulegium* leaves reached 63% as a result of the DPPH test (Figure 1).

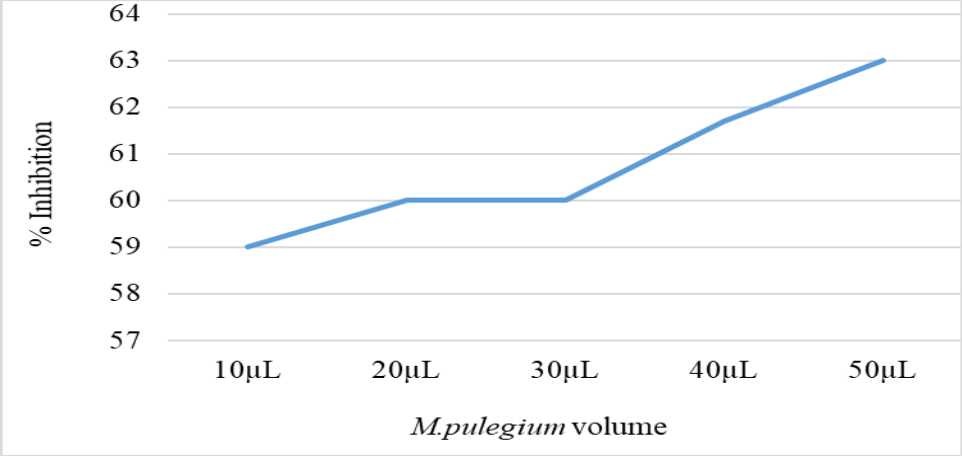

Figure 1. DPPH test findings of *M. Pulegium* plant

In the CUPRAC test, the total antioxidant capacity was determined by using plant extract in volumes ranging from 10 µL and 50 µL. The *M. pulegium* plant was found to have a higher antioxidant capacity than ascorbic acid (AsA), which is used as a positive control at all concentrations (Table 1).

Table 1. Findings of the *M.* Pulegium methanol extract and the CUPRAC test of AsA.

M. pulegium **(µL)**	**TAK (mmol AsA /g- *M. Pulegium*)**	**AsA (M)**	**TAK (mmol AsA /g)**
10 µL	1.532 ± 0.316	$4.44x10^{-4}$	0.329 ± 0.015
20 µL	1.495 ± 0.014		
30 µL	0.834 ± 0.041		
40 µL	0.663 ± 0.113		
50 µL	0.560 ± 0.034		

In the present study, no antimicrobial effect of *M. pulegium* plant could be detected on the standard strains used in the study (Table 2). Infectious diseases are accepted as one of the increasing concerns in the field of medicine in the world [30]. Unlike the results obtained, *Mentha* has also been reported to have antibacterial affect against gram+ and gram-strains containing *Pseudomonas aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, and Streptococcus aureus* standard strains [31].

The reason why the results are different may be due to the differences in concentration and solvent used, as well as the soil and climatic conditions in the region where the plant used in the study is grown. In addition, in underdeveloped and developing countries, synthetic drugs are both expensive and substandard to treat infectious diseases and exhibit adverse effects. This leads to the need to investigate new and safer natural antimicrobial agents in controlling and fighting microbial infections [32]. Plant-based drugs and herbal medicines also serve as prototypes for developing new, safer and more effective modern drugs [33].

Table 2. Antimicrobial effect findings *of M. Pulegium*

Menta pulegium **(mg/mL)**	**Microorganism**	**Antimicrobial effect**
	Staphylococcus aureus	-

7.98 mg/ml	*Pseudomonas aeruginosa*	-
	Escherichia coli	-
	Candida albicans	-

* - sign means no zone of inhibition

To the our best knowledge the information about the effect of Mentha genus on COX inhibition is very limited in the literature. In the present paper, the effect of *M. pulegium* plant on COX-1 enzyme was examined with a commercial kit for the first time. It was determined as 1.64% on COX-1 (Table 3).

Table 3. Inhibitory effect of *M. pulegium* on COX-1 enzyme.

Enzyme	**Inhibitor**	**% Inhibition Value**
COX-1	*M. pulegium*	% 1.64

Inflammatory diseases are one of the important health problems around the world. Inflammation, characterized by swelling, redness, and pain, is a complex biological response of vascular tissue under pathogenic and irritating conditions [34]. COXs 's have an important role in the synthesis of some hormones such as inflammation and platelet aggregation work-related prostacyclin and thromboxanes [35]. Long-term use of NSAIDs is known to cause various side affects like stomach lesions, kidney failure, and cardiovascular disorders [36,37]. This situation supports the need for the discovery of new drugs. Although not recognized as a drug target, new researches have revealed the role of COX-1 in angiogenesis and indicated that it is overexpressed in ovarian cancer [38]. However, there are very few known selective COX-1 blockers [4].

Researchers used NSAID indomethacin as positive control in their study evaluating the anti-inflammatory activity of *Mentha aquatica* extract and reported that *Mentha aquatic* produced 27% edema inhibition compared to this drug, which reduced edema response by 57% at a dose of 100 μg/cm^2 [39]. In a new study [40], researchers reported that essantial oil of *Mentha spicata* showed inhibition effect on COX-1 with 21.17 ± 0.85 μg/mL IC_{50} value.

CONCLUSION

In conclusion, the data of the presented study showed that *M. pulegium* had a good antioxidant potential at the concentration used, but did not have any antimicrobial effect on the strains used in the study. It had a very weak effect on the COX-1 enzyme. The obtain results showed that *M. pulegium* may be use a natural antioxidant in industry and medical aplications.

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