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Antibacterial and Antioxidant Activities of *Ocimum basilicum* L. Against Mastitis Pathogens

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Abstract. The most common bacterial agents cause inflammation in the mammary gland (mastitis), which disease cause to less yields in cows. These bacteria are *Staphylococcus aureus* and Coagulase-negative *Staphylococci* (CNS). Therefore, these bacteria were selected in this study. The purpose of this study is to research the antibacterial and antioxidant potential of the various extracts of *Ocimum basilicum* L. The mastitis agents used in the study are 7 bacteria in total; 2 of them are *Staphylococcus aureus* and the other 5 bacteria are CNS. The antibacterial activities studies were carried out using Kirby-Bauer disc diffusion technique. The other antibacterial activity test performed within the scope of this study was minimum inhibitory concentration (MIC) test. Antioxidant activities were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. This study showed that the largest inhibition zone was obtained from the methanol extract of *O. basilicum.* Result of this study is 9 mm. The lowest MIC value was recorded as 3250 µg/mL. It was found that *O. basilicum* used in the study had antibacterial activity against mastitis pathogens, and high antioxidant activity could be obtained from the water extracts (72%). Consequently, *O. basilicum* is a good candidate in developing new antibacterial and antioxidant agents.

***Keywords*:** Antibacterial activity, Antioxidant activity, Mastitis, *Ocimum basilicum.*

Mastitis Patojenlerine Karşı *Ocimum basilicum* L.' un Antibakteriyel ve Antioksidan Aktiviteleri

Özet. En yaygın bakteriyel ajanlar meme bezlerinde iltihaplanmaya (mastitis) neden olmakta, ki bu hastalık ineklerde düşük verime neden olur. Bu bakteriler *Staphylococcus aureus* ve koagülaz-negatif *Staphylococci* (KNS)'dir. Bu çalışmanın amacı, *Ocimum basilicum* L.'nin çeşitli özütlerinin antibakteriyel ve antioksidan potansiyellerinin araştırılmasıdır. Çalışmada kullanılan mastitis etkenleri toplam 7 bakteri olup, bunlardan 2 tanesi *Staphylococcus aureus*, diğer 5 bakteri ise KNS'dir. Antibakteriyel aktivite çalışmaları Kirby-Bauer disk difüzyon metodu kullanılarak gerçekleştirilmiştir. Bu çalışma kapsamında yapılan diğer bir antibakteriyel aktivite testi, minimum inhibitör konsantrasyon (MİK) testidir. Antioksidan aktivite çalışmaları 2,2-difenil-1-pikrilhidrazil (DPPH) radikal süpürme yöntemi ile belirlenmiştir. Çalışma en geniş inhibisyon zonunun, *O. basilicum*'un metanol özütünden sağlandığını göstermiştir. Bu çalışmanın sonucu 9 mm'dir. En düşük MİK değeri 3250 µg/mL olarak kayıt edilmiştir. Çalışmada kullanılan *O. basilicum*, mastitis patojenlerine karşı antibakteriyel aktiviteye sahiptir ve yüksek antioksidan aktivite sulu özütlerden elde edilmiştir (%72). Sonuç olarak, yeni antibakteriyel ve antioksidan ajanların geliştirilmesinde, *O. basilicum*' un iyi bir aday olduğunu ortaya koymaktadır.

***Anahtar Kelimeler*:** Antibakteriyel aktivite, Antioksidan aktivite, Mastitis, *Ocimum basilicum.*

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1. INTRODUCTION

The milk from a healthy cow under hygienic conditions contains only a limited number of microorganisms in the teat canal. The main sources of contamination can be environment, milking equipment and even more milker his/herself [1]. The most common bacterial agents causing inflammation in the mammary gland (mastitis) are coliform bacteria, *Staphylococcus* and *Streptococcus* [2]. Among these agents, *Staphylococcus aureus* has been commonly reported for many years [3]. Coagulase-negative *Staphylococci* (CNS) have been more isolated in recent years in comparison with the far past. This indicates that mastitis has attracted much research interest recently [4].

Subclinical mastitis infection continues to spread among the cows, while clinical mastitis is seen as sporadic cases. And hence the disease can be transmitted to other cows in the herd [5]. Mastitis leads to high economic losses in livestock breeding industry. It is reported that because of mastitis, worldwide annual losses reach 35 billion dollars [6].

The prolonged use of antibiotics in the treatment of mastitis causes the development of antibiotic resistant in bacterial strains, thus triggers increasing in dose of antibiotics, which finally leads to the occurrence of antibiotic in milk [7]. The development of antibiotic resistance in *S. aureus* via the contaminated milk affects the environment to a great extent, and such bacteria can infect humans via direct contact or food chains [8]. For this reason, today's scientists focus on discovery and application of new antibiotics against bacterial agents that have developed resistance to many antibiotics.

Ocimum basilicum L. belongs to *Lamiaceae* family. This is an annual, herbaceous plant that is 15-60 cm height in pink or white blooms. The plant is originally native to Africa, Asia and America, and it is also cultivated in the Southwest Asia and Europe (especially the Mediterranean countries) including Turkey [9]. More than 65 species of this genus are commercially cultivated in many countries [10]. In the food industry, its spices or volatile oils are widely used in soft drinks, candies, vines, meat and flavouring products [11]. It is also used in perfume making [12]. The plant has sedative, flatulence and diuretic effects [13]. The volatile oils obtained from this plant have been studied since 1930 and over 200 chemical compounds have been found [14]. It is formed by terpenoids and phenylpropanoids such as citral, eugenol, methyl eugenol and chavicol [15]. The extracts of the leaves of *Ocimum basilicum* exhibit antiviral, anticancer, antiaging, antioxidant and antibacterial properties [16-20]. Such pharmacological effetcs come from its polyphenolic contents [21].

The most common bacterial agents cause inflammation in the mammary gland (mastitis), which disease cause to less yields in cows. These bacteria are *Staphylococcus aureus* and Coagulase-negative *Staphylococci* (CNS). Therefore, these bacteria were selected in this study. This study aims to analyse the biological activities of *O. basilicum* against mastitis pathogens and to contribute some information on these activities in the extant literature. Although several studies on the biological activities of these species are already exist in the literature [22-24], the antioxidant and antibacterial activities of *O. basilicum*, which is widely grown and used in Mugla/Turkey, against mastitis pathogens have not been extensively investigated.

2. MATERIALS and METHODS

2.1. Plant material

The leaves of *Ocimum basilicum* was collected from local markets (C2 region/Mugla/Turkey). The material was identified by Dr. Neslihan Balpinar and stored at the Department of Biology at Mugla Sitki Kocman University. The identification of plant material was made according to “Flora of Turkey and the East Aegean Islands” [25-27].

2.2 Microorganisms

The mastitis agents in this study are 7 bacteria in total; 2 of them are *S. aureus* and the other 5 bacteria are Coagulase-negative *Staphylococci* (CNS). All mastitis pathogens were supplied from Dr. Zafer Cantekin's Project that has been completed previously [50]. The diagnosis of the pathogens was performed using biochemical tests with classical culture methods [28].

2.3 Preparation of the plant material

The leaves of *Ocimum basilicum* used in the analysis of the antibacterial activity were washed several times under running and distilled water. The distilled water was sterile. The leaves of plant were dried at room temperature in a laboratory and then powdered in a blender. During the preparation of the samples, all the materials were kept at room temperature.

2.4 Extraction process

The powdered plant leaves (50g) were processed using a soxhlet extractor (Isotex). In this process, it was used the solvents of ethanol, methanol and water. The extracts were evaporated under a fume hood and transferred into sterilized opaque falcon tubes containing their own solvents. The concentrations of all extracts were set at 150 mg/mL, and they were preserved in a refrigerator until analysis. The temperature was +4˚C.

2.5 Cultivation of microorganisms

Mastitis agents were used as a source of microorganisms. The extracts were analysed one by one against mastitis agents. The bacteria used in the study were cultivated in Mueller-Hinton Broth (MHB, Merck) medium. Incubation was carried out at 37˚C for 24 h [29].

2.6 *In vitro* antimicrobial assay

The Bauer-Kirby method was used to this activity [30]. The various extracts of the leaves (150 mg/mL) were analysed by the disk diffusion method. The ethanol, methanol and water extracts were used at the analyses. Test bacteria inoculated on Mueller-Hinton Agar (MHA, Merck) medium were grown at an incubator. The temperature of this study was 37˚C (24 h). The turbidities of the cultures were set to 0.5 McFarland. Inhibition zones occurring after the incubation were recorded in mm scale. Standard antibiotic discs (ampicillin 10 µg; oxacillin 5 µg) were used as positive controls while solvents were tested as negative controls against the test pathogens. All measurements were performed in triplicate.

2.7 Minimum inhibitory concentration (MIC)

The other antibacterial activity test applied to bacteria was minimum inhibitory concentration (MIC). The experiments were performed by broth dilution assay as defined in CLSI standards [31, 32]. The MIC values of extracts were recorded after the incubation. The concentrations of extracts were set to 13000, 6500, 3250, 1625 and 812.5 µg/mL.

2.8 Detection of non-enzymatic antioxidant activities

In this study, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as free radical. Stable DPPH was used to determine the antioxidant capacities of the extracts. The 0.1 mL of extracts were added to 3.9 mL of the methanolic DPPH solution. The concentration of this solution was 0.1 mM. After waiting for 30 min, the absorbances were measured at a wavelength of 515 nm in a spectrophotometer. The methanolic DPPH solution was employed as a control while methanol was utilized as the blank. Trolox was used as reference in this assay. The results were given in percentage (%) [33].

3. RESULTS

The antibacterial activities of the various extracts obtained from *Ocimum basilicum* were tested against mastitis agents. The results were summarized and compared to the standard antibiotics in Table 1. Having taken a closer look at the data obtained from this study, it was seen that the highest inhibition zone was obtained from

the methanol extract, and this zone diameter was 9 mm. Most of extracts have antibacterial activities against the mastitis pathogens. These inhibition zones are lower than zones of antibiotics (Table 1). As shown in the results, the ethanol and methanol extracts exhibit a big inhibition zone (9 mm) against *Staphylococcus aureus*–18 and CNS–22. The solvents used as negative controls have no antibacterial activities against microorganisms (Table 1).

Table 1. The antibacterial activities of the various extracts of *Ocimum basilicum* (150 mg/mL) against mastitis pathogens.

Bacteria	Inhibition zone (mm)			Antibiotics (mm)		Solvents		
	EE	ME	WE	AM	O	E	M	W
S. aureus-17	7	-	-	18	10	-	-	-
S. aureus-18	8	9	-	12	8	-	-	-
CNS-22	8	9	8	-	-	-	-	-
CNS-32	-	-	-	10	7	-	-	-
CNS-33	8	8	-	8	7	-	-	-
CNS-36	-	-	7	-	-	-	-	-
CNS-37	-	-	-	-	-	-	-	-

CNS: Coagulase-Negative *Staphylococci*; (-): No inhibition; AM: Ampicillin (10µg); O: Oxacillin (5µg); EE: Ethanol extract; ME: Methanol extract; WE: Water extract

The minimum inhibitory concentration (MIC) results of the various extracts of *O. basilicum* are summarized in Table 2. In this study, different concentrations of extracts were worked for antibacterial activities. This test was applied to the extracts with antibacterial activity, and the lowest MIC value (3250 µg/mL) was found in the ethanol extracts (Table 2). The MIC values for CNS-22 and CNS-36 could not be determined until 13000 µg/mL concentration.

Table 2. The MIC results of the various extracts of *Ocimum basilicum* (µg/mL).

Bacteria	Ethanol extract	Methanol extract	Water extract
S. aureus-17	6500	(nt)	(nt)
S. aureus-18	6500	6500	(nt)
CNS-22	13000	6500	-
CNS-32	(nt)	(nt)	(nt)
CNS-33	3250	6500	(nt)
CNS-36	(nt)	(nt)	-
CNS-37	(nt)	(nt)	(nt)

(nt): not tested; (-): No MIC was observed at concentrations tested up to 13000 µg/mL.

The antioxidant activities of the various extracts of *O. basilicum* were also examined and the data obtained from this examination were summarized in Table 3. The water extract of *O. basilicum* had the highest antioxidant activity (72%). On the other hand, activities of the ethanol and methanol extracts were 13.6% and 23.4%, respectively (Table 3).

Table 3. The antioxidant activities of the various extracts of *Ocimum basilicum* (%) and their trolox equivalent.

Plant material	EE		ME		WE	
(150 mg/mL)	DPPH (%)	TE	DPPH (%)	TE	DPPH (%)	TE
Ocimum basilicum	13.6	1.3	23.4	1.67	72	2.1

EE: Ethanol extract; ME: Methanol extract; WE: Water extract; TE: Trolox equivalent (mM TE/g DW); DW: dry weight

4. DISCUSSION

Our study showed that most of the ethanolic extracts of the plant inhibited the development of the mastitis pathogens and the inhibition zones were 7-8 mm (Table 1). Suppakul et al. [34] noted that the essential oil of sweet *Ocimum basilicum* had a broad antimicrobial spectrum. Wannissorn

et al. [17] reported that the volatile oil obtained from *O. basilicum* had remarkable antibacterial effects. However, Bozin et al. [18] and López et al. [35] reported that Gram-positive bacterial strains showed a high susceptibility to *O. basilicum* essential oils. In other study, Shafique et al. [36] tested the leaf extracts of *O. basilicum* against 8 bacteria using disc diffusion assay, and found that the extracts of the plant were highly effective against Gram-positive bacteria. Yahya [37] applied the concentrations of 0.34-10.96 mg/mL of *O. basilicum* to *Staphylococcus aureus*, and observed that the development was inhibited. For the concentrations of 20 and 100 mg/mL, Yahya found the inhibition zones of 12 and 20 mm, respectively. Unnithan et al. [38] stated that the extracts of *O. basilicum* had significant antibacterial activity against Gram-positive bacteria (*S. aureus*) when compared to Gram-negative ones (*Escherichia coli*). These results support our findings. In the extant literature, antimicrobial mechanisms of volatile oils and extracts of herbs are explained by degrade cell membranes [39], cytoplasmic membrane damage [40], membrane protein damage [41], leakage of cell contents [39], coagulation of cytoplasm [42] and proton motive power reduction [43]. Antimicrobial activities of the essential oils of the plant are related to the presence of high linalool content [34].

In this study, the water extracts of plant did not inhibit the growths of many mastitis pathogens (Table 1). Some components such as camphor, eugenol, estragole, 1,8-cineole and linalool that have antimicrobial characteristics are known to be biologically active [44]. These differences indicates that essential oil composition is associated with environmental and genetic factors, chemotypes and nutritional status of the plant [45].

The lowest minimum inhibitory concentration (MIC) value (3250 µg/mL) in our study was obtained from ethanol extracts (Table 2). Alsabri et al. [46] determined the MIC value of *Arbutus pavarii* against *S. aureus* to be 4.86 mg/mL. Alsabri's result is higher than result of this study.

In the present study, the antioxidant activities of various extracts of *O. basilicum* were also analysed (see Table 3). The highest antioxidant activity of *O. basilicum* was determined as 72% for the water extract. The antioxidant activity is due to phenolic and flavonoid compounds in the composition [20]. In another study, it has been reported that *O. basilicum* have a high monoterpene level (93%), especially contains methyl chavicol (85%), eugenol (0.7%) and phenolic compounds, and hence it has antioxidant activity [47]. Zhang et al. [48] reported a large number of components belonging to *O. basilicum*. In the development period, the seasonal changes in temperature and humidity generally affect the chemical composition of its volatile oils, antimicrobial and antioxidantive activities of *O. basilicum* [49].

5. CONCLUSION

The study suggests that *Ocimum basilicum* which is grown in Mugla/Turkey can be employed as natural antibacterial agent against mastitis pathogens. However, further investigations concerning bioactive and phytochemical compounds of this plant are needed. In addition, the wider bacterial populations must be included in the study.

Conflicts of interest

The authors stated that did not have conflict of interest.

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