

EVALUATION OF ANTIMICROBIAL ACTIVITY IN OLIVE (*OLEA EUROPAEA*) LEAF EXTRACT

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ABSTRACT

In this study the antimicrobial activity of Olive Leaf Extracts (OLE) was screened against five gram-positive and five gram-negative bacteria. The antimicrobial activity was evaluated using disc diffusion and microdilution methods. The diameters of inhibition zones of the olive leaf extract were observed 13.33 ± 2.08 mm against *S. typhimurium* and 21.67 ± 1.53 mm against *B. cereus*. There was a similarity between the inhibition zones of olive leaf extract and Gentamicin discs against *S. typhimurium*, *P. vulgaris* and *P. aeruginosa*. While Minimum Inhibitory Concentrations (MIC) of olive leaves extract against, *L. monocytogenes*, *E. coli* O157, *E. sakazakii* and *P. aeruginosa* was ≥ 32 mg mL⁻¹, the MIC against the other bacteria (*B. cereus*, *S. aureus*, *E. faecalis*, *P. vulgaris*, *E. coli*, *S. typhimurium*) that used in this study was ≥ 16 mg mL⁻¹. It's considered that some other works should be conducted about using olive leaf extract in food industry as a natural antimicrobial food additive as well as medicine and pharmaceutical industry.

Keywords: Olive Leaf Extract, Antimicrobial Activity, Minimum Inhibitory Concentration

1. INTRODUCTION

It is known that the olive fruit, its oil and the leaves of the olive tree have a rich history of nutritional and medicinal uses (Soni *et al.*, 2006). Oleuropeosits (oleuropein), flavones, flavonols and substituted phenols (tyrosol, hydroxytyrosol) are some phenolic compounds in the olive leaf extract (Benavente-García *et al.*, 2000). It has been reported by many researchers that the olive leaf extract has an antimicrobial activity because of its high phenolic content (Markin *et al.*, 2003; Owen *et al.*, 2003; Pereira *et al.*, 2007; Sudjana *et al.*, 2009; Aytul, 2010).

In recent years, its observed that the consumer demand for natural, organic and no preservative contained foods has increased. For this reason, the

demands for olive leaf extract have been increasing for utilization as food materials, food additives because of its high phenolic content (Lee and Lee, 2010). The researches about using volatile oils and plant extracts as a natural food additives to prevent or retard the spoilage of foods become widespread (Bubonja-Sonje *et al.*, 2011). The aim of this study is to investigate the antimicrobial activity of olive leaf extract by using agar disc diffusion and microdilution broth methods.

2. MATERIAL AND METHODS

2.1. Olive Leaf Extract

Olive Leaf Extract (OLE), produced by Deva Trade, was purchased from local health food company, mutaFTarım® (İzmir, TURKEY). According to the

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label, the OLE was produced in accordance with the Ministry of Food, Agriculture and Livestock Registration (No: TR-45-K-000 088-Manisa, TURKEY) and minimum oleuropein and olive leaf polyphenols content was 50 mg mL⁻¹ in OLE.

2.2. Microorganisms Used

Five Gram-positive and five Gram-negative bacteria were used in this study. These bacteria are *Listeria monocytogenes* (ATCC 7644), *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212), *Proteus vulgaris* (ATCC 8427), *Escherichia coli* (ATCC 25292), *Pseudomonas aeruginosa* (ATCC 27853) *Enterobacter sakazakii* (ATCC BAA-894), *Escherichia coli* O157 (ATCC 43894), *Salmonella typhimurium* (ATCC 14028).

2.3. Determination of Antimicrobial Activity with Disc Diffusion Method

Antimicrobial activity of olive leaf extract (*Olea europea*) was researched by disc diffusion method using the Kirby-Bauer technique⁽¹⁰⁾ on Mueller-Hilton agar (Oxoid CM337). Combined phenolics in the olive leaf extract were dissolved in %1 Dimethyl Sulfoxide (DMSO) and sterilized by filtration through 0.45 µm millipore filters. All assays were carried out under aseptic conditions and performed twice to check the results. Suspension of the tested microorganisms (10⁶ CFU/µL) was spread on the solid media plates. Then the 6-mm diameter paper discs (Oxoid CT0998B) were impregnated (800 µg/disc) with 20 µL of the olive leaf extract and placed on the inoculated agar and they were incubated at 37°C for 24 h. As control Gentamicin 10 µg (Oxoid CT0024B) and Ciprofloxacin 5 µg (Oxoid CT0425B) were used. The antimicrobial activities were evaluated by measuring the zones of inhibition against the test organisms (Bauer *et al.*, 1966).

2.4. Minimum Inhibitory Concentration

The antimicrobial activity of the extract was determined for the majority of organisms using the broth microdilution assay following the methods described by the Clinical and Laboratory Standards Institute for bacteria (Anonim, 2012). Six different concentration were tested in the microdilution method. OLE was tested in doubling dilutions ranging from 256 to 8 mg mL⁻¹ (512 mg mL⁻¹ for olive leaf extract) for broth assays and prepared, inoculated at 1% (v/v) with an inoculum of 10⁷ CFU/mL and incubated for 24 h at 37°C. The MIC was determined by observing the lowest concentration of extract that inhibited visual bacterial growth.

2.5. Statistical Analysis

Statistical analysis of the data was performed using SPSS Package Program. Statistical significance level was taken as 95%. When Analysis of Variance (ANOVA) revealed a significant effect ($p < 0.05$), the data means were compared by the least significant difference (Duncan's Multiple Range test) test.

3. RESULTS

In this study, olive leaf extract was tested for antibacterial activity against five Gram-positive and five Gram-negative bacteria and it was presented in **Table 1**.

The diameters of inhibition zones of the olive leaf extract of was observed 13.33±2.08 mm against *S. typhimurium* and 21.67±1.53 mm against *B. cereus*. There was a similarity between the inhibition zones of olive leaf extract and Gentamicin discs against *E. coli*, *E. faecalis*, *P. vulgaris*, *P. aeruginosa* and *S. typhimurium*, (**Table 1**). The diameters of inhibition zones of Ciprofloxacin discs are larger than the diameters of inhibition zones of olive leaf extracts and Gentamicin discs ($p < 0.05$) (**Table 1**).

Minimum Inhibitory Concentrations (MIC) of olive leaf extract against some bacteriae were presented in **Table 2**. While MIC of olive leaf extract against, *L. monocytogenes*, *E. coli* O157, *E. sakazakii* and *P. aeruginosa* was ≥ 32 mg mL⁻¹, the MIC against the other bacteria that used in this study was ≥ 16 mg mL⁻¹ (**Table 2**).

4. DISCUSSION

The products of olive tree that can live for centuries are known for many years with their beneficial effects on health (Soler-Rivas *et al.*, 2000). Its reported by some researchers that the oleuropein which is included in these products has a lot of pharmacological properties including antioxidant, antimicrobial, anti-inflammatory, antiatherogenic anticarcinogenic and antiviral activities (Owen *et al.*, 2003; Visioli *et al.*, 2002; Micol *et al.*, 2005; Sanchez *et al.*, 2007).

Inhibition zones with diameter less than 12 mm were considered as having low antibacterial activity. Diameters between 12 and 16 mm were considered as moderately active and these with >16mm were considered as highly active (Indu *et al.*, 2006). According to this, the olive leaf extracts were moderately active against *S. typhimurium* and highly active against the other bacteria that used in this study (**Table 1**).

Table 1. Inhibition diameter zones (mm) on the tested bacteria of olive leaf extract (Olea Europea: OLE)

Microorganisms	Zone of inhibition (mm)		
	OLE 15µg	Ciprofloksasin 5µg	Gentamicin10µg
<i>L. monocytogenes</i>	19,33±0,58 ^b	29,50±0,71 ^a	14,50±0,71 ^c
<i>B. cereus</i>	21,67±1,53 ^b	29,00±1,41 ^a	15,00±1,41 ^c
<i>S. aureus</i>	18,67±1,53 ^b	26,00±1,41 ^a	15,50±0,71 ^b
<i>E. faecalis</i>	19,00±1,73 ^b	26,75±0,35 ^a	15,50±0,71 ^b
<i>P. vulgaris</i>	17,33±1,53 ^b	27,00±1,41 ^a	16,50±0,71 ^b
<i>E. coli</i>	18,00±1,00 ^b	21,50±0,71 ^a	16,75±0,35 ^b
<i>E. coli</i> O157	17,67±0,58 ^b	27,75±0,35 ^a	14,00±1,41 ^c
<i>S. typhimurium</i>	13,33±2,08 ^b	28,00±1,41 ^a	12,75±0,35 ^b
<i>E. sakazakii</i>	18,33±1,15 ^b	29,75±0,35 ^a	14,50±2,12 ^c
<i>P. aeruginosa</i>	18,00±1,73 ^b	28,50±2,12 ^a	16,50±0,71 ^b

a-c: Means in a same line with different letters are significantly different (p<0.05).

Table 2. Minimum Inhibitory Concentrations (MIC) of olive leaf extract against some bacteriae

Bacteria	Olive leaves extract concentrations (mg/mL)					
	256	128	64	32	16	8
<i>L. monocytogenes</i>	-	-	-	+	+	+
<i>B. cereus</i>	-	-	-	-	+	+
<i>S. aureus</i>	-	-	-	-	+	+
<i>E. faecalis</i>	-	-	-	-	+	+
<i>P. vulgaris</i>	-	-	-	-	+	+
<i>E. coli</i>	-	-	-	-	+	+
<i>E. coli</i> O157	-	-	-	+	+	+
<i>S. typhimurium</i>	-	-	-	-	+	+
<i>E. sakazakii</i>	-	-	-	+	+	+
<i>P. aeruginosa</i>	-	-	-	+	+	+

+, Growth observed; -, no growth observed.

Pereira *et al.* (2007), reported the olive leaves antimicrobial capacity in the following order *Bacillus cereus*>*Candida albicans*>*E. coli*>*S. aureus*>*P. aeruginosa* and there is no selectivity between gram-positive and gram-negative bacteria. They also reported that the antimicrobial mechanism of olive leaf extract is denaturing the proteins and effecting cell membrane permeability. Similarly, Lee and Lee (2010) reported that the combined phenolics mixture which was prepared from olive leaf extract showed inhibition effects against *B. cereus* and *S. enteritidis*. Owen *et al.* (2003) also reported that olive leaves presented antimicrobial activity against *E. coli*, *S. aureus*, *B. cereus*, *S. typhi* and *V. parahaemolyticus*. The results in our study, has similarity between the other studies (Markin *et al.*, 2003; Owen *et al.*, 2003; Pereira *et al.*, 2007; Sudjana *et al.*, 2009; Lee and Lee, 2010) about the antimicrobial activity of olive leaf extracts.

5. CONCLUSION

According to study, the olive leaf extract presented the highest antibacterial activity against *B. cereus* and the lowest antibacterial activity against *S. typhimurium* (Table 1). Also, the olive leaf extract was highly active against the all bacteria that used in this study except *S. typhimurium*. Because of the increase in consumer demand for natural antimicrobials in recent years, its considered that some other works should be conducted about using olive leaf extract in food industry as a natural antimicrobial food additive as well as medicine and pharmaceutical industry.

6. ADDITIONAL INFORMATION

6.1. Author's Contributions

All authors equally contributed in this study.

6.2. Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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