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Antimicrobial activity of olive leaf aqueous extract

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ABSTRACT

The leaves of the olive tree (*Olea europaea* L.) have been used for medicinal purposes since ancient times and research has suggested that olive leaf extracts have antimicrobial properties. The olive leaf is the first botanical mentioned in the Bible. In this study, Olive leaf aqueous extracts were screened for their antimicrobial activity against pathogenic bacteria (*Staphylococcus aureus* PTCC 1431, *Salmonella typhimurium* PTCC 1639, and *Escherichia coli* PTCC 1399, *Klebsiella pneumonia* PTCC 1053, *Bacillus cereus* PTCC1274). Olive leaf extract was found to be most active against *Salmonella typhimurium* PTCC 1639 with inhibitory, 11.5 mm. These findings suggest an antimicrobial potential for olive leaves.

Keywords: olive leaves, antimicrobial activity, *Olea europaea* L.

INTRODUCTION

The olive leaf is the first botanical mentioned in the Bible. Throughout the history of civilization, the olive plant has been an important source of nutrition and medicine. The first formal report of medicinal use was made in 1854, when olive leaf extract (OLE) was reported to be effective in treating fever and malaria. OLE contains compounds with potent antimicrobial activities against bacteria, fungi, and mycoplasma [1]. The reports describing antimicrobial properties of phenolic compounds in olive products refer to compounds obtained from olive fruit, particularly hydroxytyrosol and oleuropein [2]. Antibiotics are important biochemicals produced by microorganisms and widely employed in current medical use for a long time in semi-synthetic forms. Unfortunately, uncontrolled use of antibiotics, caused from either patients or prescriptions made without cell cultures analyses, increased resistance of bacteria. Increment in resistance and some other problems caused an increasing interest in antimicrobial plant extracts [3]. Sudjana et al, showed the antimicrobial activity of commercial *Olea europaea* (olive) leaf extracts against *Campylobacter jejuni*, *Helicobacter pylori* and methicillin-resistant *Staphylococcus aureus* (MRSA). The authors also demonstrated these extracts play a role in regulating the composition of the gastric flora by selectively reducing levels of *H. pylori* and *C. jejuni* [4]. In this study, Olive leaf aqueous extracts were screened for their antimicrobial activity against some pathogenic bacteria (*Staphylococcus aureus* PTCC 1431, *Salmonella typhimurium* PTCC 1639, and *Escherichia coli* PTCC 1399, *Klebsiella pneumonia* PTCC 1053, *Bacillus cereus* PTCC1274) by Agar well diffusion method [5].

MATERIALS AND METHODS

Preparation Aqueous Extract of Olive Leaf (OLE):

Olive leaves used in this study were collected in winter 2011 from Gilan province (Northern Iran). Leaves were washed to remove impurities such as dust and then dried in an air oven for 3 days. Then, they were ground by grinder and sterilized with Tendalization method. One liter water was added to 50 grams powder obtained from leaves and put on the shaker to be solved thoroughly. Finally, obtained solution was passed through filter [6].

Standard Strains

The standard strains used in this study were *Staphylococcus aureus* PTCC 1431, *Salmonella typhimurium* PTCC 1639, and *Escherichia coli* PTCC 1399, *Klebsiella pneumonia* PTCC 1053, *Bacillus cereus* PTCC1274. The strains were obtained from collection center of fungi and bacteria, Tehran, Iran.

Antimicrobial assay

Agar well diffusion method [5,7] used to detect antimicrobial activities of olive leaf aqueous extract. The plates were poured with 20 ml Mueller Hinton Agar (Merck; Germany). The pathogenic strains (*Staphylococcus aureus* PTCC 1431, *Salmonella typhimurium* PTCC 1639, and *Escherichia coli* PTCC 1399, *Klebsiella pneumonia* PTCC 1053, *Bacillus cereus* PTCC1274) were adjusted to a density of 10^9 CFU/ml by adding sterile water and spread on the surface of MHA. Wells of 7 mm in diameter were cut into these agar plates and Dried extract were dissolved in DMSO at the concentration 10, 15, 25, 30, 50 mg/ml, placed into each well. The culture plates were incubated at 37°C for 24 h and Antibacterial activity was evaluated by measuring the diameter of the inhibition zone and presented in millimeter. Statistical analyses were performed using SPSS software.

RESULTS AND DISCUSSION

Table 1 showed the antibacterial activity of olive leaf aqueous extracts measured by the agar diffusion method against selected pathogenic bacteria. The olive leaf extracts showed good inhibitory effects on pathogenic bacteria. Many studies confirm positive role of olive leaf in inhibitory pathogenic bacteria. Markin et al, also reported that water extract of olive leaf with a concentration of 0.6% (w/v) killed *E.coli*, *P. aeruginosa*, *S. aureus* and *K. pneumonia* in 3h exposure. *B. subtilis* on the other hand was inhibited only when the concentration was increased to 20% (w/v) possibly due to spore forming ability of this species [8]. In another study, Korukluoglu et al, investigated the effect of the extraction solvent on the antimicrobial efficiency of *S. aureus*, *E. coli*, *S. enteritidis*, *S. thypimurium* and some others. They reported that solvent type affected the phenolic distribution and concentration in extracts, and antimicrobial activity against tested bacteria. As ethanol extracted OLE showed the highest antimicrobial efficiency against *E. coli* and *S. enteritidis*, acetone extracted OLE showed the highest antimicrobial efficiency against *S. thypimurium* [9].

Table 1- Antibacterial activity of olive leaf aqueous extracts at different concentration (Inhibition zones in millimeter)

pathogenic bacteria Extract mg/ml	<i>S. aureus</i> PTCC 1431	<i>S. typhimurium</i> PTCC 1639	<i>E. coli</i> PTCC 1399	<i>K. pneumonia</i> PTCC 1053	<i>B. cereus</i> PTCC1274
10	-	1	-	-	-
15	1	2	-	2	1
25	3.5	4	2	4.5	2.1
35	7.2	8.5	6	7.2	7
50	9	11.5	8.2	10	9.5

Sudjana et al, studied antibacterial activity of olive leaf extract with large variety of bacteria. Results indicated that OLE did not present broad-spectrum antibacterial activity, but had appreciable activity on *H. pylori* and *C. jejuni* [4]. In study Faiza et al, The olive extracts showed an unusual combined antibacterial and antifungal action and ethyl acetate and acetone extracts revealed a wide range of antimicrobial activity [10]. In study Pereira et al, tested the antimicrobial activity of aqueous olive leaf extract against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *Bacillus cereus*, bacteria, *Candida albicans* and *Candida neoformans*, fungi. They revealed that the growth rates of *S. aureus* and *E. coli* were decreased while OLE concentration increased and the OLE showed a IC₂₅ (25% inhibitory concentration) value of 2.68 and 1.81 mg/mL for *S. aureus* and *E. coli*, respectively [2]. In study Owen et al, Phenolic compounds within olive leaf extract have shown antimicrobial activities against several microorganisms including: *E. coli*, *S. aureus*, *K. pneumoniae*, *B. cereus*, *S. typhi* and *V. parahaemolyticu* [11]. In our study, olive leaf aqueous extract showed good Antimicrobial abilities and highest inhibition of 11.5 mm against *Salmonella typhimurium* PTCC 1639.

CONCLUSION

OLE is a potent source of polyphenols having antioxidant, antimicrobial, antiinflammatory and antiviral properties. The results clearly indicated that using olive leaves had the beneficial effect in controlling the microbial infections.

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