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THE ANTIMICROBIAL ACTIVITIES OF OLIVE LEAF EXTRACT AGAINST SOME PATHOGENIC BACTERIA

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ABSTRACT

This study was designed to investigate the antibacterial activities of olive leaf extract against five pathogenic bacteria *(Bacillus cereus, Salmonella typhimurium ATCC 14028, Pseudomonas aeruginosa ATCC 9027, Staphylococcus aureus ATCC 25923* and *Bacillus subtillus ATCC 6633*). The olive leaf extract, at a concentration of 300 ppm, produced the highest inhibitory potential on *Salmonella typhimurium ATCC 14028*, *Bacillus subtillus ATCC 6633* and *Bacillus cereu*s with a zone of inhibition of 18.0 mm, 15.0 mm and 15.0 mm, respectively. While increasing concentration extracts at 600ppm had the greatest activities in *Salmonella typhimurium ATCC 14028*, *Bacillus subtillus ATCC 6633* and *Bacillus cereus* with a zone of inhibition of 20.0 mm. These results, therefore, inferred the antibacterial efficacy of the olive leaf extracts.

Key words: Olive leaf extracts, antibacterial, pathogenic bacteria.

INTRODUCTION

Olive (*Olea europaea*) leaf has been widely used in folk medicine for several thousand of years within European Mediterranean islands and countries (Gucci *et al*., 1997). Historically, olive leaf was used for the treatment of malaria and associated fever (Benavente-Garcia *et al*., 2000). Olive leaves extracts (OLE) are rich in phenolic components (De Nino *et al*., 1997), oleuropein being the most prominent phenolic compound that may reach concentrations of 60–90 mg g^{-1} of dry matter (Ryan *et al*., 2002).The major physiological substances of olive leaf are hydroxytyrosol, tyrosol, caffeic acid, p-coumaric acid, vanillic acid, vanillin, oleuropein, luteolin, diosmetin, rutin,verbascoside, luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin-7-glucoside (Bianco and Uccella, 2000; Tasioula-Margari and Ologeri, 2001).

Several reports have been published on olive leaf and presented the following: olive leaf offered a capacity to lower blood pressure and increase blood flow in the coronary arteries (Khayyal *et al*., 2002). The phenolic compounds extracted from olive leaf possessed antimicrobial activity against *Helicobacter pylori, Campylobacter jejuni, Staphylococcus*

aureus (Sudjana *et al*., 2009). Pereira *et al*., (2007) reported that the antimicrobial properties of phenolic compounds in olive products refer to compounds obtained from olive fruit, particularly hydroxytyrosol and oleuropein.

Despite the many reports on olive leaf and its phenolic compounds, the combined effects of olive leaf phenolics in terms of antimicrobial activities have not been studied (Lee and Lee, 2010).

Pathogenic bacteria constitute a major cause of morbidity and mortality in humans. Sharma *et al*., (2005) reported that the emergence and spread of bacterial resistance made the treatment of infectious diseases more problematic. The antimicrobial activity of a plant is highly related to secondary substances that are synthesized and produced by these plants (Cowan, 1999).

Secondary metabolites are substances of low molecular weight, which were not the products of the primary metabolic pathway of the producing organism and at first thought to be with no advantage to the plant. Nowadays it is believed that they have vital functions (Kant *et al*., 2010).The utilization of plant extracts and phytochemicals, with known antibacterial characteristic, may be of immense significance in therapeutic treatments.Several studies have been conducted in different countries to substantiate such efficiency (Almagboul *et al*.(1985) and Rakholiya and Chanda, 2012).

This study has been aimed to determine the antimicrobial activity of olive leaf extracts against five pathogenic bacteria (*Bacillus cereus, Salmonella typhimurium ATCC 14028, Pseudomonas aeruginosa ATCC 9027, Staphylococcus aureus ATCC 25923 and Bacillus subtillus ATCC 6633).*

MATERIAL AND METHODS

Olive Leaf Extract (OLE) Preparation

Olive leaves were collected and put in plastic bags. The plant material was then dried at room temperature and powdered (20 mesh). Ground powdered leaves were extracted as reported by Hassan *et al*. (2013) using ethanol (70% v/v) at 20% (w/v) concentration. The mixture was mixed usng magnetic stirar for three hours and filtered through whatman no.4, and then membrane filter (0.45 um). To obtain the solid residues of the olive leaf extract, the extracts were dried in rotary evaporator under lower temperature.

Pathogenic indicators

The used bacterial indicators were, *Bacillus subtillus ATCC 6633, Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 9027, Salmonella typhimurium ATCC 14028, Bacillus cereus*. These pathogens were kindly provided by the staff members of The National Institute of Oceanography and Fisheries (NIOF), Alexandria branch.

Preparation of pathogenic bacterial indicators:

The pathogenic bacteria indicators were grown in nutrient broth and incubated at 38°C for 24 h. The cells were centrifuged at 7000 rpm, and standardized to OD 600 nm 0.1 and stored at 4 °C until ready for use (Cwala *et al*., 2011).

Screening for antimicrobial activity:

The well-cut diffusion technique was used to test the ability of the different concentration from the crude extract to inhibit the growth of indicator bacteria. Fifty millimeters of nutrient agar medium inoculated with indicator microorganism were pored into plates. After solidifies, wells were punched out using 0.5 cm cork

borer, and each of their bottoms was then sealed with two drops of sterile water agar. One hundred micro-liters of tested compounds were transferred into each well. All plates were incubated at 38°C for 24 h, the detection of clear inhibition zone around the wells is an indication of antimicrobial activities of the different isolates. (El-Masry *et al*., 2002).

RESULTS AND DISCUSSION

Table (1) and Figer (1) showed the antibacterial activity of olive leaf extracts (300 and 600 ppm) measured by inhibition zone (mm) of the isolates against some references of bacterial pathogens. In general results indicated that olive leaf extracts showed good inhibitory effects on pathogenic bacteria.

Olive leaf extract (300 ppm) showed good antimicrobial abilities and the highest inhibition of 15 mm against *Bacillus subtillus ATCC 6633* and against 12 mm *Staphylococcus aureus ATCC 25923*, 12 mm against *Pseudomonas aeruginosa ATCC 9027*, 18 mm against *Salmonella typhimurium ATCC 14028*, 15 mm against *Bacillus cereus*. However, increasing of concentration olive leaf extract (600 ppm) showed a marked increase in inhibition zone presented Figer (1).

Many studies confirm the positive role of olive leaf extracts in inhibitory pathogenic bacteria. Markin *et al*, (2003) reported that water extract of olive leaf with a concentration of 0.6% (w/v) killed *Staphylococcus. aureus* in 3 h exposure and *Bacillus. subtilis*. On the other hand it was inhibited only when the concentration was increased to 20% (w/v) possibly due to spore forming ability of this species. Pereira et *al*., (2007) revealed that the growth rates of *S. aureus* were decreased while OLE concentration increased .Sudjana *et al*. (2009) studied the antibacterial activity of OLE with a large variety of bacteria. In another study, Korukluoglu *et al*. (2010) investigated the effect of the extraction solvent on the antimicrobial efficiency of *S. aureus, S. thypimurium* and they reported that solvent type affected the phenolic distribution and concentration in extracts, and antimicrobial activity against tested bacteria.

Owen *et al*. (2003) added that phenolic compounds within the OLE have shown antimicrobial activities against several microorganisms including *Staphylococcus aureus, Bacillus Cereus* and *Salmonella typhimurium*.

In our study, the ethanolic olive leaf extract of two concentrations showed good antimicrobial abilities and highest inhibition against pathogenic bacteria (Bacillus *cereu, Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus aureus* ATCC 25923).

Table (1): Antibacterial efficiency of OLE at different concentrations (Inhibition zones in millimeter) against some references bacterial pathogens:

Bacterial Pathogens	Concentration (ppm)	
	300	600
Bacillus cereu	15	20
Salmonella typhimurium ATCC 14028	18	20
Pseudomonas aeruginosa ATCC 9027	12	15
Staphylococcus aureus ATCC 25923	12	15
Bacillus subtillus ATCC 6633	15	20

Figer (1): Antibacterial activity of OLE at different concentrations against various bacterial pathogens.

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الملخص العربي

النشاط المضاد للميكروبات لمستخلص اوراق الزيتون ضد بعض انواع البكتريا الممرضة

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اجريت هذه الدراسة على المستخلص الايثانولى لأوراق الزيتون لتوضيح نشاطه المضاد للبكتريا على خمسة انواع من البكتريا الممرضه وهي *Salmonella typhimurium* ATCC 14028 *(Bacillus cereus*, *Pseudomonas aeruginosa* ATCC 9027 , *Staphylococcus aureus* ATCC 25923 and *Bacillus subtillus* ATCC 6633).

أوضحت النتائج أن مستخلص اوراق الزيتون تركيز 300 جزء فى المليون اظهر تثبيط عالى ضد كلا من *Salmonella typhimurium* ATCC 14028 , *Bacillus subtillus* ATCC 6633 and *Bacillus cereus* بلغ 18مم ، 15 مم و 15مم على التوالى فى منطقه التثبيط وبزياده تركيز المستخلص (600 جزء فى المليون) ادى لزياده منطقه التثبيط 20مم لكل من الانواع السابقه . هذه النتائج يستدل منها على فعاليه مستخلص اوراق الزيتون كمضاد للميكروبات .