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Chemical composition and antibacterial activity of organic extra virgin olive oil from Iran

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Abstract

Purpose – The aim of this study was to investigate the antibacterial activity of original extra virgin olive oil in Iran on some food borne pathogens.

Design/methodology/approach – Microbial analysis tests including disk diffusion and detections of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were used. Also the chemical composition analysis of the oil was determined by GC-MS. Results of disk diffusion test confirmed antimicrobial activity for the oil in which *S.aureus* and *E. coli* were more resistant than *L. innocua*.

Findings – The MIC and MBC of organic extra virgin olive oil on the studied pathogens were in the range of 12.5-25 and 100 per cent v/v, respectively. The GC-MS analysis showed Z-9-Octadecenal (32.75 per cent), Oleic acid (15.78 per cent), Squalene (11.856 per cent), Phenol (8.392 per cent), Palmitic acid (1.884 per cent) as the main compounds (>0.7 per cent) in organic extra virgin olive oil, which have functional role in the biological activities.

Originality/value – This is the first study on organic extra virgin olive oil from Iran. According to the results, extra virgin olive oil has antimicrobial activity on foodborne pathogens.

Keywords Olive oil, Extra virgin, GC-MS, MIC, MBC

Paper type Research paper

Introduction

The use of antibiotics for the treatment and prevention of pathogenic bacterial diseases, not only cause drug resistance, but also leads to disturbing the normal microbial flora of useful gastrointestinal tract and make the body vulnerable to a variety of intestinal diseases such as diarrhea (Tenover and Arbiet, 1996). Because of their natural origin, herbal extracts are more compatible with body organism than antibiotics and their side effects are very scarce. One of the antimicrobial vegetable oils is olive oil (Visioli *et al.*, 2005). Having active phenol, alkaloid and unsaturated fatty acids with antibacterial properties has caused this oil to be used as natural preservatives in food and pharmaceutical industries. For example, olive oil contains a high content of oleic acid

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(Emery and Bucholtz, 1996). Phenolic materials in olive oil have antioxidative activities and remove free radicals and prevent cancer. Pure organic olive oils (extra virgin) contain the highest levels of polyphenols (Visioli and Galli, 2002). Researches have shown that extra virgin organic olive oil (EVOO) has inflammatory activity and also reduces the amount of thromboxane which delays platelet adhesion and prevents blood clotting. In spite of the fact that this oil contains about 75 per cent monounsaturated fatty acids, it is resistant to heat and oxidation (Amaral-Philips *et al.*, 1997). Most of the researches conducted on oils including olive oil have focused on antioxidant activities and there is few studies on antibacterial properties of these oils.

Geweely (2006) studied antifungal activity of olive oil on *C. albicans, A. fumigatus* and *M. canis* using disc diffusion method. Results showed that olive oil had a desirable inhibitory activity. Also Karaosmanoglu *et al.* (2010) studied antimicrobial activity of EVOO against *E.coli* 0157:H7, *L.monocytogenes* and *S.enteritidis*. While EVOO showed strong bactericidal activity, refined oil was found ineffective.

The growth of plants in different areas is influenced by weather conditions such as light, temperature, the growth in organic terms, type of soil and elements inside. These factors, also, affect antibacterial and antioxidant properties of the plant (Ogunniyi, 2006). According to this fact, in the current study, EVOO from Iran was analyzed for the chemical composition and antibacterial activity.

Materials and methods

Collection and preparation of olive oil

EVOO purchased from Fadak Integrated Agro-industrial Company (Qom-Iran) which had been produced by cold pressing. The oil complied with EU organic regulations (ECNO.834/2007).

GC/MS analysis

The chemical composition of EVOO was identified by gas chromatograph (GC) connected to mass spectrometer (GC-MS), respectively, with the models of Agilent Technologies 7890A and 5975C inert MSD Agilent Technologies equipped with HP-5 5 per cent phenyl methyl silox 325°C. The temperature of pendulum was 60 to 290°C with amplitude of 5°C/min. Injection temperature was 280°C and the ratio of mixture was 1 to 20. Injection was done with Split method using helium as a career gas with speed of 3 ml/min. Identification of the spectrums was performed with the help of retention indices alongside with normal hydro carbons C7-C25 under similar circumstances to oil injection (Zarai and Kadri, 2010).

Microorganisms

Pathogenic microorganisms including *L.innocua* (ATCC 33090), *S.aureus* (ATCC 25923) and *E. coli* (ATCC 25922) obtained from bacteriology department, Ferdowsi University of Mashhad. To determine the bacterial concentration, we used optical density as the indicator of bacterial growth using spectrophotometer. Standard solutions of microbial suspensions with optical density equal to 0.5 McFarland were provided (Akhondzadeh *et al.*, 2003).

Disc diffusion test

The disc diffusion assay was performed according to the Kirby-Bauer method (Manik *et al.*, 2013). Antibiotic discs (gentamycin, erythromycin and chloramphenicol)

containing 10, 15 and 30 μg antibiotic, as the positive controls, were obtained from Research and Production Laboratory of Roshd (Iran) (Hatami *et al.*, 2014).

Determining the minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was determined by micro-broth dilution. The EVOO was investigated in the domain of serial concentrations of 100 to 0.195 per cent v/v. To mix the oil with Muller Hinton Broth medium (Merck, Germany), 0.5 per cent tween 80 (Merck, Germany) was added to the oil and mixed by ultra-thoracic T25 basic LKA for 6 min. 0.5 μl of bacterial suspension with 1.5×10^8 cfu/ml was added to each sink of 96-well microplate other than sink number 12 which was considered as negative control and incubated in 37°C for 24 h. Then, its turbidity was measured by Elisa Reader ELX808 in the wavelength of 620 nm (Nasirpour *et al.*, 2014).

Determining the minimum bactericidal concentration

The wells without turbidity (in MIC experiment) were cultured on nutrient agar medium (Merck, Germany), and the first concentration in which bacterium growth was not observed would be considered as minimum bactericidal concentration (MBC) (Moreire *et al.*, 2005).

Data analysis

The experiments were conducted triplicate, and the average and standard deviation were calculated. Graphs for determining MIC range were drawn by Slide Write Software. Duncan test at $p < 0.05$ was done to determine significant differences.

Result and discussion

Disc diffusion analysis

According to Table I, the highest inhibition zone was gained for EVOO against *L. innocua*as (12 mm), while *E. coli* and *S. aureus* were more resistant. EVOO was used at two concentrations (50 and 100 per cent v/v), which showed similar antimicrobial activity. The effect of EVOO is compared with different antibiotics in Table I. Chloramphenicol (30 ppm) showed the highest inhibition zone on *E. coli* plate. Erythromycin (15 ppm) showed similar antimicrobial activity to EVOO on *L. innocua.*

Minimum inhibitory concentration and minimum bactericidal concentration

The MIC range for EVOO was between 12.5-25 per cent (v/v) on investigated pathogens (Figure 1). The bactericidal influence of EVOO on the investigated pathogens was observed only at 100 per cent concentration (MBC = 100 per cent v/v).

Chemical composition of EVOO

According to the results presented in Table II, GC-MS analysis represented seven compounds as the main constituents in which 9-octadecenal (32.75 per cent), Oleic acid (15.78 per cent), Squalene (11.856 per cent) and Palmitic acid (1.884 per cent) were dominant allocated compounds.

According to the results, EVOO exhibited antibacterial activity against pathogens including *L.innocua*, *S.aureus* and *E.coli*, and the MIC value was between 12.5 and 25 per cent. Also EVOO had bactericidal effect at 100 per cent concentration. This finding is in agreement with Geweely (2006) and Karaosmanoglu *et al.* (2010).

Based on the GC-MS analysis and other studies, major components of olive oil include unsaturated fatty acids, oleic acid, phenolic compounds, palmitic acid, aldehyde and

Microbs	*E. coli*		*S. aureus*		*L. innocua*	
Antimicrobial agent	Zone of inhibition (mm)	Concentration (%)	Zone of inhibition (mm)	Concentration (%)	Zone of inhibition (mm)	Concentration (%)
Extra virgin organic olive oil	7[a]	50	8[a]	50	12[a]	50
Extra virgin organic olive oil	9[a]	100	8[a]	100	12[a]	100
Erythromycin	–	–	–	–	12[a]	15 ppm
Gentamicin	–		22[b]	10 ppm	–	–
Chloramphenicol	30[c]	30 ppm	–	–	–	–

Note: [a, b, c]Data with the same letter for zone of inhibitions are not significantly different ($p < 0.05$)

Table I.
Inhibition zone of extra virgin organic olive oil and antibiotics on different pathogens by disk diffusion method

alkaloids. and all of them have antimicrobial and anticancer properties (Shunmugapriya and Uthayakumari, 2012).

Medina *et al.* (2006) studied the antimicrobial activity of different edible oils, and found that oils from olive fruit had strong bactericidal activity against both gram-positive and gram-negative bacteria. More than 4 log cycles reduction was observed within 1 h of exposure with Picual and Arbequina virgin olive oils for *L .monocytogenes*, *S. aureus*, *S.enterica*, *Yersinia* sp. and *C. perfringens*. Reduction of *E. coli* and *Shigella sonnei* was respectively 1-2 and 2-4 log cycles, respectively, during the same time. No reduction was observed with sunflower or corn oil. None of the oils, including olive oil, were inhibitory against *C. albicans*. The phenolic compounds in the olive oil were identified and their concentrations were estimated. The dialdehydic form of decarboxy methyl oleuropein and ligstrosidea glycons; hydroxytyrosol; and tyrosol were the phenolic compounds that statistically correlated with bacterial survival (Janakat *et al.*, 2015).

In general, gram-positive bacteria are more sensitive to olive oil than gram-negative ones (Medina *et al.*, 2007). Medina *et al.* (2009) compared the bactericidal effects of several olive phenolic compounds including dialdehydic form of decarboxy methylelenolic acid (EDA), EDA linked to tyrosol (TyEDA) or EDA linked to hydroxyl tyrosol (HyEDA) with synthetic disinfectants (glutaraldehyde and ortho-phthalaldehyde) against *Pseudomonas* fluorescens, *S. aureus*, *Enterococcus faecalis* and *E. coli*. Olive oil compounds with a dialdehydic structure exhibited strong bactericidal activity, and in the presence of organic material, stronger bactericidal activity than the synthetic disinfectants. The fact that these compounds are naturally present in olive opens the possibility to be also used in organic products and as bio-pesticides. The ability of phenolic fractions from EVOO to inhibit the growth of lactic acid bacteria and fungus was evaluated. Results showed that the inhibition of growth was dependent on concentration, pH (greater impact at lower pH) and type of microorganisms (Medina *et al.*, 2013).

The antibacterial activity of phenolic compounds is because of their ability to inflict bacterial membrane damage and disrupt the cell wall peptidoglycan, which cause loss of structural integrity and leakage of intracellular cytoplasmic constituents such as protein, glutamate, potassium and phosphate (Caturla *et al.*, 2005). Moreover, the hydroxyl group in phenolic compounds may bind the active sites of enzymes and change their substrate affinity. In addition, their lipid solubility and the degree of steric

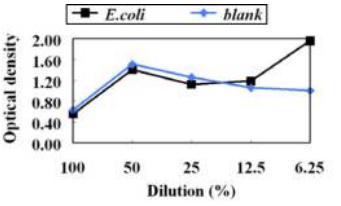

Figure 1
MIC of extra virgin organic olive oil on *E. coli*

Notes: Effect of extra virgin olive oil at different dilutions on bacterial growth (turbidity) in broth microdilution test is shown; the dilution in which bacterial growth is less than the blank (only extra virgin olive oil) gives the MIC

Table II.
GC/MS analysis of extra virgin organic olive oil

No.	Compound	Molecular formula	Compound nature	Activity	(%)	RI[a]
1	$C_{18}H_{34}O$	Z-9-octadecenal	Aldehyde compound	Antimicrobial	32.75	2009
2	$C_{18}H_{34}O_2$	Oleic acid	Mono unsaturated fatty acid	Anti-inflammatory, Antiandrogenic	15.78	2141
3	$C_{30}H_{50}$	Squalene	Carbohydrate	Antimicrobial	11.856	2790
4	$C_{24}H_{18}O$	1-(Phenylethynyl)-1-[2′-(phenylethynyl)phenyl] ethanol	Phenol compound	Antimicrobial	8.392	1122
5	$C_{18}H_{32}O$	(Z)-9,17-octadecadienal	Aldehyde compound	Antimicrobial	4.609	2080
6	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acid	Antimicrobial	1.884	1984
7	$C_{29}H_{50}O$	γ-sitosterol	Vitamin compound	Antimicrobial, Antioxidant	1.095	3408
8	$C_{21}H_{40}O_4$	(Z)-9-octadecenoic acid, 2,3-dihydroxypropyl ester	Alkaloid	Antimicrobial	0.783	2094

Notes: [a]Kovats retention index; main components of extra virgin olive oil (% w/v and RI) and their activity are shown

hindrance they cause may also contribute to their overall antimicrobial activity (Ceylan and Fung, 2004).

Several studies have shown that the composition of the phenolic fraction varies with cultivar, ripeness, climacteric conditions, and oil extraction process, and all of these have been reviewed by Gallina-Toschi *et al.* (2005). EVOO has a high content of unsaponifiables which include natural ingredients such as squalene, beta-sitostrerol and polyphenols. According to the results presented in Table II, 9octadecenal, oleic acid, squalene and palmitic acid are dominant allocated compounds. These components have inhibitory influences on polymerase activations in prokaryotes (Grossman *et al.*, 2001).

Conclusion

The study indicated that the amount of phenolic and formaldehyde material in EVOO from Iran is sufficient to show antimicrobial activity. Undoubtedly, more studies are needed to disclose the best method to obtain and apply the powerful tools that constitute the antimicrobial compounds provided by the olive fruits.

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