*Cold-pressed oregano (*Origanum vulgare*) oil: a rich source of bioactive lipids with novel antioxidant and antimicrobial properties*

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ORIGINAL PAPER

Cold-pressed oregano (*Origanum vulgare*) oil: a rich source of bioactive lipids with novel antioxidant and antimicrobial properties

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Abstract In this investigation, cold-pressed oregano (*Origanum vulgare*) oil (OO) was studied for its lipid classes, fatty acid profile, tocols and phenolics contents. Radical scavenging potential against DPPH· and galvinoxyl radicals was determined. Antimicrobial properties of OO against foodborne bacteria, food spoilage fungi and dermatophyte fungi were also evaluated. The level of neutral lipids in OO was the highest, followed by glycolipids and phospholipids. The main fatty acids in OO were linoleic, oleic, stearic and palmitic acids. γ-Tocopherol constituted 32.1 % of total measured tocols followed by α-tocotrienol (25.8 %) and γ-tocotrienol (21.3 %). OO contained high amounts of phenolic compounds (5.6 mg/g as GAE). OO had strong antiradical action wherein 72 % of DPPH· radicals and 60 % of galvinoxyl radical were quenched after 60 min of incubation. Rancimat assay showed that induction time (IT) for OO/sunflower oil blend (1:9, v/v) was 6 h, while OO/sunflower oil blend (2:8, v/v) recorded higher IT (8 h). OO inhibited the growth of all tested microorganisms. The highest antimicrobial activity of OO was recorded against the dermatophyte fungi including *T. mentagrophytes* (42 mm) and *T. rubrum* (38 mm), followed by food spoilage fungi including *A. flavus* (36 mm) and *C. albi* (32 mm) with minimal lethal concentration (MLC) ranging between 40 and 320 μg/mL. OO exhibited also broad-spectra activity against foodborne pathogen bacteria (*S. aureus*, *E. coli, S. enteritidis* and *L. monocytogenes*) with MLC ranging between 160 and 320 μg/mL. The results suggest that OO could be used economically as a valuable natural product with novel functional properties in food, cosmetics and pharmaceutical industries.

Keywords Lipid classes · Tocols · Antiradical · Food spoilage fungi · Dermatophyte fungi

Introduction

Herbs with high levels of plant-based bioactive compounds are increasingly used in novel foods, pharmaceuticals and cosmetics. Many herbs rich in bioactive chemicals were recognized to have biological activities and possess many health-promoting effects [2]. Consumers are also looking for natural products and preservatives for healthier lifestyles. Many medicinal plants have become attractive to scientists as natural bioactive agents that could be safer than synthetic sources [1]. Therefore, herbs are being sought for their biological value as antioxidant and antimicrobial agents [8].

Origanum species (family Lamiaceae) are a group of plants rich in bioactive compounds [17, 21]. Oregano (*Origanum vulgare* L.) is a perennial herb with a worldwide distribution [14]. Oregano is commonly utilized as a spice in diets and in conventional medicine for the treatment of cough, cold and digestive disorders [39]. The herb has many therapeutic properties and antimicrobial activities [15]. Oregano's aroma

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is due to the presence of essential oil that is accumulated in leaf trichomes. The essential oil of *O. vulgare* is well recognized for its antioxidant and antimicrobial potential [5, 11]. The activities of *O. vulgare* essential oil are mainly attributed to the carvacrol and thymol [17, 32, 38].

Microbial spoilage is an important factor affecting both the product shelf life and cost [35]. Foodborne pathogens are responsible for 76 million cases of foodborne illness, 325.000 hospitalizations and 5000 deaths in the USA annually [18]. Yeasts are able to spoil many foods causing changes in odor, color, taste and texture. *Candida, Pichia, Rhodotorula, Torulopsis, Saccharomyces, Zygossacharomyces, Hansenula* and *Trichosporon* are important food-spoiling yeasts [35]. On the other hand, candidiasis caused by *Candida* species has increased substantially in the past years. *Candida* species are opportunistic pathogens that cause local and systemic infections in predisposed persons, commonly affecting immunologically compromised patients and those undergoing prolonged antibiotic treatment [41]. The difficulties associated with the management of *Candida* infections necessitate the discovery of new antifungal agents. Plant-derived natural products may offer potential lead to new compounds, which could act on these yeasts [13].

Increased interest on cold-pressed oils has been recognized as these oils have high levels of lipid-soluble bioactives with nutritive properties. The cold pressing technology is becoming an interesting substitute for traditional practices because of consumers' desire for natural products. Cold pressing is a technique that involves no chemical or heat or refining treatments. Thus, cold-pressed oils usually contain a high level of lipophilic phytochemicals including natural antioxidants [31].

Recently, cold-pressed oils have been internationally marketed, wherein bio-information on their chemical composition, antioxidant potential and antimicrobial properties has not been reported. Such data are of importance for the evaluation of the nutritional and health impact of these oils. It is hard to find any data in the literature on cold-pressed *Origanum vulgare* oil (OO). As a continuation of the efforts in developing edible oils with health-beneficial characteristics, this study was carried out to (1) describe the composition of lipid classes, fatty acids and tocols of OO, (2) determine the total phenolics content, in vitro antiradical activity and antioxidant potential in food model (Rancimat assay) of OO; and (3) estimate the antimicrobial properties of OO. The results might be used to develop functional products rich in bioactive molecules with a desirable shelf life.

Materials and methods

Oils and chemicals

Three different samples of cold-pressed OO and extra virgin olive oil were purchased from a local market in Mekkah (KSA). The total phenolics content (TPC) in olive oil as determined by the Folin-Ciocalteu was 3.6 mg/g as gallic acid equivalents (GAE). Neutral lipid (NL) standards were obtained from Sigma (St. Louis, MO, USA). Standards used for glycolipids (GL) including monogalactosyldiacylglycerol (MGD), digalactosyldiacylglycerol (DGD), cerebrosides (CER), steryl glucoside (SG) and esterified steryl glucoside (ESG) were of plant origins and purchased from Biotrend Chemikalien GmbH (Köln, Germany). Standards used for phospholipids (PL) including phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI) from bovine liver and phosphatidylcholine (PC) from soybean were purchased from Sigma (St. Louis, MO, USA). Standards used for tocols were purchased from Merck (Darmstadt, Germany).

Methods

Column chromatography (CC) and thin-layer chromatography (TLC) of lipid classes

Fractionation of lipid classes and subclasses

Cold-pressed OO was separated into neutral and polar lipid classes by elution with solvents over a glass column (20 mm × 30 cm) packed with a slurry of activated silicic acid (70–230 mesh; Merck, Darmstadt, Germany) in chloroform (1:5, w/v). NL were eluted with three times the column volume of chloroform [26]. The major portion of GL was eluted with five times the column volume of acetone and that of PL with four times the column volume of methanol. The amount of the lipid classes obtained was determined by gravimetry. By means of TLC on silica gel F_{254} plates (thickness = 0.25 mm; Merck, Darmstadt, Germany), a further characterization of the GL and PL subclasses was carried out with the following solvent system chloroform/methanol/25 % ammonia solution (65:25:4, v/v/v). For the characterization of NL subclasses, TLC plates were developed in the solvent system *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v). For detection of the lipids, TLC plates were sprayed with the following agents: for the marking of all lipids with sulfuric acid (40 %), for the marking of GL with α-naphthol/sulfuric acid and for the marking of PL with the molybdate-blue reagent [29]. Each spot was identified with lipid standards as well as their reported retention factor (R_f) values. Individual bands were visualized under ultraviolet light, scraped from the plate and recovered by extraction with chloroform/methanol (2:1, v/v). Fatty acid composition of OO as well as NL, GL and PL was determined by GLC/FID as described below.

Quantitative determination of lipid subclasses

For the quantitative determination of NL classes, individual bands were scraped from the plate and recovered by extraction with 10 % methanol in diethyl ether, followed by diethyl ether. For the quantitative estimation of GL subclasses, the acetone fraction obtained by CC was separated by TLC in the above-given solvent system. The silica gel regions with the corresponding GL classes were scraped out followed by hexose measurement photometrically at 485 nm using the phenol/sulfuric acid in acid-hydrolyzed lipids [29]. The percent distribution of each component was obtained from the hexose values. From the extinction values, the quantitative amount was determined and related to their portion of the GL fraction. The determined portion was set into relation with the amount of oil, which had been separated by CC into the main lipid fractions. For the determination of the PL, the methanol fraction from CC was also separated by TLC in the above-given solvent system and after scraping out of the individual PL subclasses brought to reaction with the hydrazine sulfate/sodium molybdate reagent at 100 °C for 10 min and photometrically analyzed at 650 nm. From the obtained extinction values via a calibration chart for phosphorus, the amount of PL was calculated. The individual values were put into relation to the PL fraction (methanol fraction from CC) and to the amount of oil.

Gas chromatography (GC) analysis of fatty acid methyl esters (FAME)

Fatty acids of OO and lipid classes were transesterified into FAME using *N*-trimethylsulfoniumhydroxide (Macherey–Nagel, Düren, Germany) according to Arens et al. [3]. FAME were identified on a Shimadzu GC-14A equipped with flame ionization detector (FID) and C-R4AX chromatopac integrator (Kyoto, Japan). The flow rate of the carrier gas helium was 0.6 mL/min and the split value with a ratio of 1:40. A sample of 1 µL was injected on a 30 m × 0.25 mm × 0.2 µm film thickness Supelco SPTM-2380 (Bellefonte, PA, USA) capillary column. The injector and FID temperature were set at 250 °C. The initial column temperature was 100 °C programmed by 5 °C/min until 175 °C and kept 10 min at 175 °C, then 8 °C/min until 220 °C and kept 10 min at 220 °C. A comparison between the retention times of the samples with those of an authentic standard mixture (Sigma, St. Louis, MO, USA; 99 % purity specific for GLC), run on the same column under the same conditions, was made to facilitate identification.

HPLC analysis of tocols

For tocols analysis, a solution of 250 mg of OO in 25 mL *n*-heptane was directly used for the HPLC [31]. The HPLC analysis was conducted using a Merck Hitachi low-pressure gradient system, fitted with an L-6000 pump, a Merck-Hitachi F-1000 Fluorescence Spectrophotometer (The detector wavelength was set at 295 nm for excitation, and at 330 nm for emission) and a D-2500 integration system; 20 µL of the samples was injected by a Merck 655-A40 Autosampler onto a Diol phase HPLC column 25 cm 9 4.6 mm ID (Merck, Darmstadt, Germany) using a flow rate of 1.3 mL/min. The mobile phase used was *n*-heptane/*tert*-butyl methyl ether (99:1, v/v).

Extraction, quantification and characterization of phenolic compounds

Aliquots of OO and extra virgin olive oil (1 g) were dissolved in *n*-hexane (5 mL) and mixed with 10 mL methanol–water (80:20, v/v) in a glass tube for two min in a vortex [27]. After centrifugation at 3000 rpm for 10 min, the hydroalcoholic extracts were separated from the lipid phase by using a Pasteur pipette then combined and concentrated *in vacuo* at 30 °C until a syrup consistency was reached. The oily residue was redissolved in 10 mL methanol–water (80:20, v/v), and the extraction was repeated twice. Hydroalcoholic extracts were redissolved in acetonitrile (15 mL), and the mixture was washed three times with *n*-hexane (15 mL each). Purified phenols in acetonitrile were concentrated *in vacuo* at 30 °C then dissolved in methanol for further analysis. Aliquots of phenolic extracts were evaporated to dryness under nitrogen. The residue was redissolved in 0.2 mL water, and diluted (1:30) Folin-Ciocalteu's phenol reagent (1 mL) was added. After 3 min, 7.5 % sodium carbonate (0.8 mL) was added. After 30 min, the absorbance was measured at 765 nm using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). Gallic acid was used for the calibration, and the results of triplicate analyses are expressed as parts per million of gallic acid.

UV spectrum (200–400 nm) of diluted extracts (1 mg/mL) was recorded by spectrophotometry (Jenway 4605-UV–Vis spectrophotometer).

Radical scavenging activity (RSA) of OO and olive oil toward DPPH·

RSA of OO and extra virgin olive oil was assayed with DPPH· radical dissolved in toluene [30]. Toluene solution of DPPH· radicals was freshly prepared at a concentration of 10^{-4} M. The radical, in the absence of antioxidant compounds, was stable for more than 2 h of normal kinetic assay. For evaluation, 10 mg of OO or olive oil (in 100 µL toluene) was mixed with 390 µL toluene solution of DPPH· radicals, and the mixture was vortexed for 20 s at ambient temperature. Against a blank of pure toluene without

DPPH·, the decrease in absorption at 515 nm was measured in 1-cm quartz cells after 1, 30 and 60 min of mixing using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). RSA toward DPPH· radicals was estimated from the differences in absorbance of toluene DPPH· solution with or without sample (control), and the inhibition percent was calculated from the following equation:

$$\%\text{Inhibition} = \left[(\text{absorbance of control} - \text{absorbance of test sample})/\text{absorbance of control}\right] \times 100.$$

RSA of OO and olive oil toward galvinoxyl radical

A miniscope MS 100 ESR spectrometer (Magnettech GmbH, Berlin, Germany) was used in this analysis [27]. Experimental conditions were as follows: measurement at room temperature; microwave power 6 db; centerfield 3397 G; sweep width 83 G; receiver gain 10; and modulation amplitude 2000 mG. Ten mg of oil (in 100 µL toluene) was allowed to react with 100 µL of toluenic solution of galvioxyl (0.125 mM). The mixture was stirred on a vortex stirrer for 20 s then transferred into a 50 µL micro pipette (Hirschmann Laborgeräte GmbH, Ederstadt, Germany), and the amount of galvinoxyl radical inhibited was measured exactly after total incubation time of 60 min after the addition of the galvinoxyl radical solution. The galvinoxyl signal intensities were evaluated by the peak height of signals against a control. A quantitative estimation of the radical concentration was obtained by evaluating the decrease of the ESR signals in arbitrary units after 60-min incubation using the KinetikShow 1.06 Software program (Magnettech GmbH; Berlin, Germany). The reproducibility of the measurements was 5 % as usual for kinetic parameters.

Rancimat assay

The antioxidant potential of OO blends with sunflower oil (1:10 and 2:8 w/w, OO: sunflower oil) was tested with Rancimat method according to Ranalli et al. [33]. The Rancimat apparatus was operated at 120 °C. A dry air flow of 20 L/h was passed through the oil sample (5 g). The volatile oxides coming from the oxidation of the oil or blend dissolved in cold milli-Q water (60 mL), causing an increase in the electrical conductivity. The time (h) taken to reach a specific conductivity value, corresponding to the flex point of the peroxidation curve, was considered as the induction time (IT). The higher the induction time, the higher the antioxidant potency of the compounds. Tests were performed in triplicate.

Antimicrobial activity

Bacteria and fungi strains The antimicrobial activities of OO were assessed against pathogenic bacteria and fungi including *Staphylococcus aureus* (ATCC8095), *Salmonella enteritidis* (ATCC 13076), *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 15313), *Candida albicans* (ATCC 10231), *Aspergillus flavus, Trichophyton mantigrophytes* and *Trichophyton rubrum*. Stock cultures of bacteria were maintained on nutrient agar slants at 4 °C, while fungi and *Candida* yeast were maintained on potato dextrose agar slants at 4 °C.

Determination of antimicrobial activity The antimicrobial activity of OO was determined by agar well diffusion method as described by Torres et al. [37]. For each bacterial or fungal strain, sterilized Mueller–Hinton and potato dextrose agar mediums containing 0.1 % tween 80 were poured into sterilized *Petri* dishes, left to solidify at room temperature (22 °C), then swabbed from fresh bacterial or fungal strain culture. Wells in the center of agar plate were created using a sterile cork borer (9 mm) and different concentrations of OO samples were transferred to the wells. Plates with pathogenic bacteria or *Candida albicans* were incubated at 37 and 30 °C for 24 h, respectively. Other pathogenic fungi were incubated at 30 °C for 48–72 h. The antimicrobial activity was determined by measuring the clear zones diameter (CZD) around each well in mm. Distilled water without test compounds was used as a control. The antibacterial activity of bacterial antibiotics was assessed by the agar disk diffusion method [25] by measuring CZD around each disk in mm.

Determination of minimum lethal concentrations (MLC) The MLC of OO was determined according to the dilution method described by Jobran and Finegold [12]. For pathogenic bacteria or *Candida albicans*, serial of twofold concentrations of OO (20, 40, 80, 160, 320 and 640 µL) were pipetted into tubes containing 4 mL of LB or potato dextrose broth medium (containing 0.1 % tween 80), respectively. Each tube was inoculated with 0.4 mL (0.5 McFarland medium) of a standardized suspension of bacterial test species containing 1×10^6 cell/mL. For fungi, liquid media was inoculated with fungus and incubated for approximately 48 h at 30 °C. Subsequently, the culture was filtered through a thin layer of sterile sintered Glass G2 to remove mycelia fragments. The titer of spores of each fungus was determined microscopically using a hemocytometer. A suspension containing the spores was used for inoculation of PDA medium. Serial of twofold concentrations of OO were pipetted into tubes containing 4 mL of PD (containing 0.1 % tween 80). Each tube was inoculated with 1×10^6 of prepared spores.

Table 1 Lipid classes (g/kg TL) composition of OO

Neutral lipid subclass	R_f values × 100[a]	G/kg TL	Glycolipid subclass	R_f values × 100[b]	g/kg TL	Phospholipid subclass	R_f values × 100[b]	g/kg TL
MG	14	5.55 ± 0.08	SQD	6	0.20 ± 0.04	PS	4.7	0.66 ± 0.03
DG	39	8.69 ± 0.12	DGD	17	0.44 ± 0.06	PI	11	0.77 ± 0.04
FFA	56	15.6 ± 0.11	CER	29–35	2.36 ± 0.09	PC	20	2.58 ± 0.09
TG	79	877 ± 2.44	SG	41	2.45 ± 0.07	PE	30	1.14 ± 0.08
STE	95	6.62 ± 0.08	MGD	64	0.11 ± 0.03			
			ESG	76	1.44 ± 0.09			

Results are given as the average of triplicate determinations ± SD

MAG monoacylglycerols, *DAG* diacylglycerols, *TAG* triacylglycerols, *FFA* free fatty acids, *STE* sterol esters, *SQD* sulphoquinovosyldiacylglycerol, *DGD* digalactosyldiacylglycerol, *CER* cerebrosides, *SG* steryl glucoside, *MGD* monogalactosyldiacylglycerol, *ESG* esterified steryl glucoside, *PS* phosphatidylserine, *PI* phosphatidylinositol, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine

[a] Solvent system used in TLC development: *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v)

[b] Solvent system used in TLC development: chloroform/methanol/ammonia solution 25 % (65:25:4, v/v/v)

All inoculated tubes were incubated at appropriate temperature and time for each microorganism. After the incubation period, 0.1 mL from each tube was subcultured on LB agar or PDA plates and incubated at appropriate temperature and time for each microorganism. The lowest concentration of tested OO which gave a viable count less than 0.1 % of the original inocula (1×10^6 cell/mL) was assumed as the MLC.

Antibiotics such as augmentin (30 µg), chloramphenicol (30 µg), flucoral (fluconazole, 100 µg/mL) and mycosat (nystatin BP, 100 µg/mL) were used as standards for comparison in antibacterial and antifungal tests, respectively. All work was carried out under subdued light conditions. All experimental procedures were performed in triplets if the variation was routinely less than 5 % and the mean values (±SD) were determined.

Results and discussion

The current necessity of discovering new antioxidant and antimicrobial compounds in all fields of health promotion and microbial control has stimulated research regarding the characteristics and functionality of plant-based bioactive compounds [1, 35]. To the best of our knowledge, we report for the first time on the composition and biological properties of cold-pressed OO.

Lipid classes of OO

The proportion of lipid classes and subclasses presented in OO as well as R_f values of these subclasses are shown in Table 1. The level of NL was the highest (ca. 91 %), followed by GL (0.76 %) and PL (0.5 %). Subclasses of NL contained triacylglycerol (TAG), free fatty acids (FFA), diacylglycerol (DG), esterified sterols (STE) and monoacylglycerol (MG) in decreasing order. Significant amount of TAG was found (ca. 96 % of total NL) in OO followed by a lower level of FFA (ca. 1.7 % of total NL), while DG and STE were recovered in lower levels. Subclasses of GL found in OO (Table 1) were sulphoquinovosyldiacylglycerol (SQD), digalactosyldiglycerides (DGD), cerebrosides (CER), sterylglycosides (SG), monogalactosyldiglycerides (MGD) and esterified sterylglycosides (ESG). CER, SG and ESG were the main components and made up about 89 % of the total GL. TLC fractions of PL revealed that the major PL subclasses were PC followed by PE, PI and PS, respectively (Table 1). About a half of total amount of PL in OO was PC and a quarter was PE, while PI and PS were measured in lower quantities.

Fatty acid profile of OO and lipid classes

Fatty acid profiles of OO and lipid classes (NL, GL and PL) are presented in Table 2. Nine fatty acids were identified in OO, wherein linoleic and oleic acids were the main fatty acids. Both fatty acids accounted for 83 % of the total FAME. OO contained significant levels of monounsaturated fatty acids (MUFA, 44/100 g total FAME) which is comparable to the hemp, cranberry, blueberry, onion and milk thistle cold-pressed seed oils but was much lower than that of 81 and 82 % in the carrot and parsley cold-pressed oils [22, 23]. OO had a polyunsaturated fatty acids (PUFA) content of 41.1/100 g of total fatty acids (Table 2). This PUFA content was lower than that in the cranberry (67.6/100 g), onion (64–65/100 g), milk thistle (61/100 g) and blueberry (69/100 g) cold-pressed oils [24]. Palmitic and stearic were the major saturated fatty acids (SFA), comprising together about 15 % of total FAME. OO contained about 14.8 g of SFA per 100 g of total FAME, which is lower than that of

Table 2 Fatty acid composition (relative content, %) of OO and its lipid classes

	OO	Neutral lipids	Glycolipids	Phospholipids
C 10:0	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.01	0.06 ± 0.02
C 12:0	0.03 ± 0.01	0.03 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
C 14:0	0.03 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	0.04 ± 0.01
C 16:0	8.95 ± 0.24	8.65 ± 0.21	8.97 ± 0.19	9.10 ± 0.22
C 16:1	0.52 ± 0.05	0.53 ± 0.06	0.51 ± 0.04	0.50 ± 0.06
C 18:0	5.76 ± 0.19	5.45 ± 0.15	6.22 ± 0.15	6.25 ± 0.11
C 18:1*n*-9	43.5 ± 1.99	43.8 ± 1.85	43.1 ± 1.88	43.1 ± 1.24
C 18:2*n*-6	39.9 ± 1.15	40.1 ± 1.22	39.8 ± 1.23	39.7 ± 1.26
C 18:3	1.27 ± 0.07	1.37 ± 0.04	1.27 ± 0.05	1.21 ± 0.06
ΣSFA	14.81	14.20	15.32	15.49
ΣMUFA	44.02	44.33	43.61	43.60
ΣPUFA	41.17	41.47	41.07	40.91

Results are given as the average of triplicate determinations ± SD

30.8/100 g of total FAME in the cardamom cold-pressed oil and comparable to that of 13.8 and 15.9/100 g of total FAME found in the cold-pressed milk thistle and roasted pumpkin cold-pressed seed oils, respectively [24]. The SFA levels were higher than those of 7.4–9.7/100 g of total FAME in the parsley, onion, hemp, mullein and cranberry cold-pressed seed oils [22].

Fatty acids of NL and polar lipids (GL and PL) were not different significantly from each other, wherein linoleic and oleic acids were the main fatty acids. The ratio of unsaturated fatty acids to SFA, however, was not significantly higher in NL than in the corresponding polar fractions (GL and PL). For the obtained results, it could be said that OO contains high level of MUFA and PUFA. MUFA and MUFA have been shown to reduce LDL (low density lipoproteins) cholesterol and retain HDL (high density lipoproteins) cholesterol [27]. Literature illustrates the health-promoting benefits of PUFA, in alleviating many diseases including inflammatory, heart diseases, cardiovascular, atherosclerosis and diabetes. Fatty acid profile of OO evinces the lipids as a good source of the nutritionally essential fatty acids. The fatty acid profile and high levels of MUFA and PUFA make the OO a special material for nutritional applications.

Tocols composition

From our results, OO was characterized by high amounts of unsaponifiables (23.7 g/kg). Levels of α-, β-, γ- and δ-tocopherols in OO were 180.4, 60.4, 650 and 117.6 mg/100 g oil, respectively. In addition, amounts of α-, γ- and δ-tocotrienols were 521, 58.9, and 430 mg/100 g oil, respectively. γ-Tocopherol constituted ca. 32.1 % of total tocols followed by α-tocotrienol (25.8 % of total tocols) and γ-tocotrienol (21.3 % of total tocols). Other tocols were measured in lower levels. α- and γ-Tocopherols proved to be the major tocopherols in edible oils and fats. γ-Tocopherol were found in high levels in camelina, linseed, cold-pressed rapeseed and corn oil [34]. α-Tocopherol is the most efficient antioxidant of tocol isomers, while β-tocopherol has 25–50 % of the antioxidative potential of α-tocopherol, and γ-isomer 10–35 %. Levels of tocols detected in OO may contribute to the stability of the oil toward oxidation.

RSA of OO in comparison with extra virgin olive oil

The model of scavenging stable free radicals is widely used to evaluate the antioxidant properties in a relatively short time, as compared to other methods. Two or more radical systems are needed to better study the antiradical potential of antioxidants or bioactive extracts. Ramadan and Moersel [28] developed a simple experiment using toluene to dissolve fat or oil samples as well as the free radicals.

Antiradical properties of the OO and extra virgin olive oil (as a standard edible oil that contain high levels of antioxidants and bioactives) were compared using stable $DPPH^{\cdot}$ and galvinoxyl radicals. Figure 1 shows that OO had stronger antiradical action than olive oil. After 60 min of incubation with $DPPH^{\cdot}$ radicals, 72 % of $DPPH^{\cdot}$ radicals were quenched by OO, while olive oil was able to quench only 45 % (Fig. 1a). ESR results showed also the same pattern (Fig. 1b), wherein OO quenched 60 % of galvinoxyl radical and olive oil deactivated about 38 % after 60 min of reaction. Regarding the composition of OO and olive oil, they have different patterns of fatty acid and lipid-soluble bioactives.

Our results confirmed that OO was characterized by higher amounts of phenolic compounds (5.6 mg/g, respectively as GAE) than extra virgin olive oil (3.6 mg/g as GAE). To detect bioactive phenolic compounds in OO, absorptive spectra between 200 and 400 nm were screened. Chemical structure of phenolic compounds, specifically the aromatic ring, produces strong absorbance in the UV region associated with electronic transitions of the molecule which provides a unique spectrum [10]. Phenolic compounds in OO exhibit two major absorption bands in the UV/visible region: a first band in the range between 320 and 380 nm and a second band in 255–280 nm range. Absorption at 220 nm may be due to the presence of flavones or flavonol derivatives. The UV spectra of phenolic acids showed differences over the scan range of 200–400 nm. Ferulic acid displays a maximum absorbance at 215, 287 and 312 nm. Moreover, *p*-coumaric acid exhibits a maximum absorbance at 286, 209 and 220 nm [10].

Phenolic compounds have been reported to be present in edible oils, which is very important for the oxidative

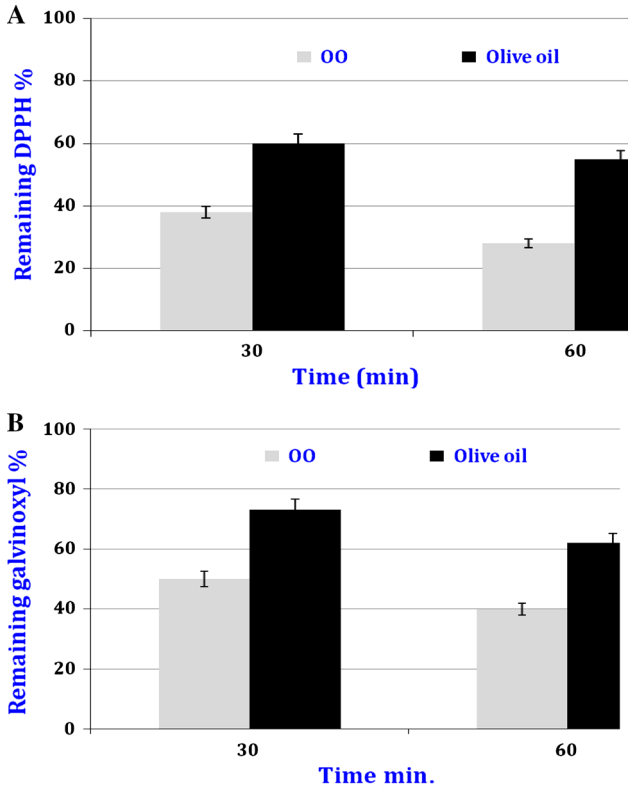

Fig. 1 Scavenging effect at different incubation times of cold-pressed OO and olive oil on **a** DPPH radical as measured by changes in absorbance values at 515 nm and on **b** galvinoxyl radical as recorded by ESR

stability of the PUFA of these oils. Additionally, oils rich in natural antioxidants may play a role in their health-promoting activities. The antioxidant effect of phenolic compounds is mainly due to their redox properties and is the result of various mechanisms: free radical scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity [6]. The total phenolic amount of OO was higher than that of 1.73–2.0 mg GAE/g oil for the red raspberry, blueberry and boysenberry cold-pressed seed oils, and that of 1.8–3.4 mg GAE/g oil for the parsley, onion, cardamom, mullein and milk thistle cold-pressed seed oils [24]. On the other hand, tocols levels in oils may have a great effect on their RSA. Increasing ring methyl substitution led to an increase in scavenging activity against the $DPPH^{\cdot}$ radical, and also to a decrease in oxygen radical absorbance capacity [19].

The stronger RSA of OO compared to olive oil may be due to (1) the differences in content and profile of unsaponifiable materials (2) the diversity in structural characteristics of potential antioxidants present, (3) a synergism of antioxidants with other bioactive components and (4) different kinetic behaviors of antioxidants. From the results we can suggest that OO may be used in different food or pharmaceutical applications to provide nutrition and health benefits.

Rancimat assay

It was important to assess the antioxidant potential of OO in a food system such as edible oils. Rancimat is an accelerated aging test, wherein air is passing through the sample in the reaction vessel at constant high temperature. In this reaction, fatty acids are oxidized and volatile as well as secondary oxidations products are formed, which are transported into the measuring vessel by the air stream and absorbed in the measuring solution (milli-Q water). Electrical conductivity of the measuring solution is increasing due to the absorption of the reaction products. In our study, induction time (IT) of sunflower oil was 3 h. The IT for OO/sunflower oil blend (1:9, v/v) was 6 h, while OO/sunflower oil blend (2:8, v/v) recorded the highest value (8 h). Considering the results of Rancimat assay, the antioxidant activity of OO in sunflower oil blend was superior wherein high levels of tocols and phenolic compounds in OO can explain high antioxidant capacity.

Antimicrobial activity of OO

Antimicrobial properties of herbs have been recognized since ancient times for food preservation. Foodborne illness caused by consumption of contaminated foods with pathogenic bacteria and/or their toxins has been of great concern to public health. Controlling pathogenic microorganisms would reduce foodborne outbreaks and assure consumers a continuing safe, wholesome and nutritious food supply. Herbs are generally applied to food, which is a nutrient-rich environment for most bacteria. Plant extracts have shown antimicrobial potential against many pathogenic microorganisms. *O. vulgare* have presented prominent results as antimicrobial agents to be applied in food bioconservation systems. Studies reported antimicrobial activity in *Origanum* species, while *O. vulgare* extracts and essential oil had shown positive results in inhibiting the growth of pathogen microorganisms and in the synthesis of microbial metabolites [4, 36].

In our study, the antimicrobial activity of OO was examined against four bacterial and four yeast strains selected on the basis of their relevance as food or human pathogens. The examination of antimicrobial activity of the OO by the agar diffusion method revealed that OO inhibited the growth of all microorganisms tested. Representative pictures of the inhibitory effect of OO against tested microorganisms in comparison with the control are shown in Fig. 2. As presented in Table 3, OO exhibited wide spectrum antimicrobial activity against foodborne pathogen bacteria (*S. enteritidis*, *L. monocytogenes*, *E. coli* and *S. aureus*) and

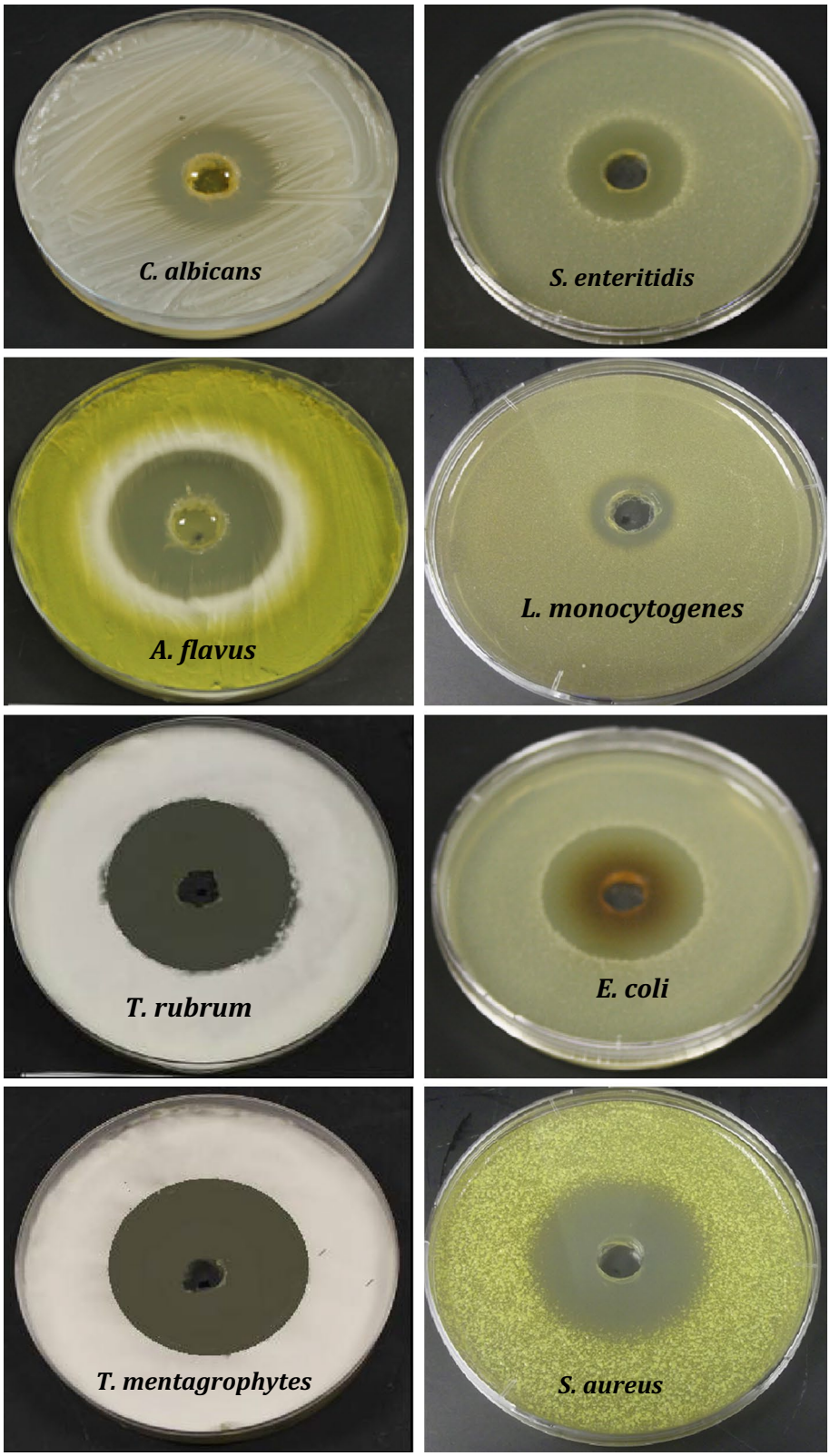

Fig. 2 Antimicrobial activity of OO against studied microorganisms indicated by clear zone diameter

food spoilage fungi (*C. albicans* and *A. flavus*) as well as the dermatophyte fungi (*T. mentagrophytes* and *T. rubrum*). The antibacterial activity against foodborne pathogen bacteria measured as clear zone diameter (CZD) were 35, 33, 30 and 15 mm for *S. aureus*, *E. coli, S. enteritidis* and *L. monocytogenes*, respectively. The highest antibacterial

Table 3 In vitro antibacterial and antifungal activities of OO recorded by clear zone diameter (CZD, mm) and minimal lethal concentration (MLC, μg/mL)

	Foodborne pathogen bacteria							
	S. enteritidis		*L. monocytogenes*		*E. coli*		*S. aureus*	
	CZD	MLC	CZD	MLC	CZD	MLC	CZD	MLC
Origanum vulgare (100 μL)	30	160	15	320	33	160	35	320
Augmentin (30 μg)	28	nd	28	nd	30	nd	40	nd
Chloramphenicol (30 μg)	20	nd	22	nd	27	nd	25	nd
Flucoral (100 μg/mL)	nd	nd	nd	nd	nd	nd	nd	nd
Mycosat (100 μg/mL)	nd	nd	nd	nd	nd	nd	nd	nd
	Food spoilage fungi				Dermatophytic fungi			
	C. albicans		*A. flavus*		*T. mentagrophytes*		*T. rubrum*	
	CZD	MLC	CZD	MLC	CZD	MLC	CZD	MLC
Origanum vulgare (100 μL)	32	320	36	320	42	40	38	40
Augmentin (30 μg)	nd	nd	nd	nd	nd	nd	nd	nd
Chloramphenicol (30 μg)	nd	nd	nd	nd	nd	nd	nd	nd
Flucoral (100 μg/mL)	35	nd	38	nd	35	nd	34	nd
Mycosat (100 μg/mL)	30	nd	40	nd	40	nd	38	nd

Each value represents mean of sample ± SD for $n = 3$

Diameter of inhibition zone was measured as the clear area centered on the agar well containing the sample

nd not determined

activity of the OO was recorded for *S. aureus* (35 mm), while the lowest was for *L. monocytogenes* (15 mm). The oil has broad-spectra activity against foodborne pathogen bacteria with MLC ranging between 160 and 320 μg/mL. Furthermore, compared to the antibiotic used in this study, the OO exhibited antimicrobial activity higher than or equal to that exhibited by tested antibiotics.

The antifungal activity of OO against food spoilage fungi (*C. albicans* and *A. flavus*) was initially tested by measuring the inhibition zone diameter, using a well diffusion method (Table 3). OO showed strong activity and high CZD values against *A. flavus* with the best CZD (36 mm). *A. flavus* causes different clinical manifestations of human aspergillosis such as *Cutaneous aspergillosis*, *Aspergillar otomycosis*, *Aspergillar onychomycosis*, invasive lung aspergillosis and aspergillar sinusitis [36].

Relatively lower CZD value (32 mm) was recorded for *C. albicans*, whereas the MLC was 320 μg/mL for both food spoilage fungi. Microbial spoilage is an important factor influencing food availability. Yeasts are widely able to spoil many foods products, wherein *Candida* and *Saccharomyces* are important food-spoiling yeasts [35]. *Candida* species are now at fourth ranks among microbes [41]. *Candida* genus is known as the yeasts most frequently involved in the etiology of mycotic infections [16]. Among *Candida* species, *C. albicans* is the organism most often associated with serious fungal infection and it is showing increased resistance to traditional antifungal agents [9]. The difficulties associated with the management of *Candida* infections necessitate the discovery of novel antifungal agents [13, 40]. Cleff et al. [7] demonstrated the antifungal effect of *O. vulgare* essential oil against *Candida* spp. suggest its administration may represent an alternative treatment for candidiasis.

Dermatophytic fungi including *Trichophyton rubrum* are anthropophilic fungi which frequently cause acute or chronic inflammatory tinea corporis which is a superficial fungal infection (dermatophytosis) of the arms and legs, especially on glabrous skin. The data in Table 3 and Fig. 2 showed that OO had a significant antifungal potential against the tested dermatophytic fungi (*T. mentagrophytes* and *T. rubrum*), whereas the CZD values were 38 and 42 mm, respectively. In addition, OO exhibited very low MLC values (ca. 40 μg/mL).

It is worth mentioning that the antimicrobial activity of OO against tested microorganisms recorded the same CZD without any changing when left for more than 10 days as an additional incubation period. Furthermore, no growth was observed when new agar plates or broth media were inoculated with loop from the clear zone area, indicating that the OO have a lethal effect.

In relation to OO composition, it appears that the antimicrobial activity of OO is mainly linked to the presence of significant proportion of tocols and phenolic constituents with high antioxidant activity. The variation in the effectiveness of OO against different bacterial strains may depend on the differences in the permeability of cell of those microbes.

In addition, the antimicarobial activity of OO might be due to the presence of outer membrane surrounding the cell wall in bacteria, which limits diffusion of hydrophobic substances through its lipopolysaccharide covering. Nazzaro et al. [20] reported that antimicarobial activity of the plant oils might targeted variety of microbial cell components, particularly the cell membrane, cytoplasm, and in some cases, they completely change the morphology.

Conclusions

Composition and properties of bioactive phytochemicals in herbs and medicinal plants are required to improve quality and nutritional value of the human diet. These are also important to improve utilization of food and pharmaceutical products. The present study was designed to investigate for the first time the composition and biological properties of cold-pressed OO. This report might serve as a milestone toward development of healthy oils with high nutritional value. It could be concluded that the OO is a good source of essential fatty acids and tocols. The high levels of linoleic and oleic acids make OO nutritionally valuable. The present study demonstrated that OO contained significant levels of natural antioxidants. Tocols and phenolics at the level estimated may be of nutritional importance as natural antioxidants and might directly react with and quench free radicals and prevent lipid peroxidation. The results confirmed that OO have potential antimicrobial activity and could be used in food, cosmetics and pharmaceutical products to inhibit microbial growth. Since tested dermatophytic fungi strains showed a very low MLC, OO could be used as a novel anti-yeast agent. In addition, OO could be used in the development of novel foods and drug formulations for the prevention of chronic diseases such as Alzheimer and Type II diabetes, which are linked to oxidative stress.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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