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ORIGINAL PAPER

Effect of bioactive compounds on antiradical and antimicrobial activity of extracts and cold-pressed edible oils from nutty fruits from Macedonia

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Abstract
The chemical composition, antiradical and antimicrobial activity of cold-pressed edible oils from nutty fruits (almond and walnut) and oils from poppy seed and wheat germ from Macedonia were studied. Regarding the fatty acid composition, the highest level of oleic acid was determined in almond oil ($67.6 \pm 0.02\%$) whereas poppy seed oil was the richest sources of linoleic acid with abundance of $72.3 \pm 0.06\%$. The highest level of α-tocopherol (23.8 ± 0.01 mg/100 g of oil) was quantified in almond oil while γ-tocopherol was the most abundant in walnut and wheat germ oil. Wheat germ oil was the richest source of phytosterols (3894 ± 155.0 mg/kg) with domination of β-sitosterol and campesterol. Although DPPH radical is less sensitive against phenolic compounds in comparison to ABTS radical, its relationship with tocopherols and tocotrienols was indicative. Poppy seed oil had the lowest level of tocopherols, but it indicated the highest antibacterial activity against *Listeria monocytogenes* and antifungal activity against *Candida albicans*.

Keywords Bioactive compounds · Extracts · Cold-pressed oils · Nutty fruits · Antiradical activity · Antimicrobial activity

Introduction

Edible oils are important foodstuff due to their high level of energy, essential polyunsaturated fatty acids and vitamin-E-active compounds [1]. The daily intake of edible oils decreases the risk of coronary heart diseases, degenerative diseases and cancer [2]. In addition to essential fatty acids and vitamin E, the chemical composition of edible oils from different plants can also include minor amounts of polar components such phenolic as compounds powerful antioxidants responsible for human health benefits [3].

Phenolic compounds have a determinant role in taste formation and they contribute to astringency and bitterness. Anthocyanins are phenolic compounds that are responsible for colour formation of fruits and vegetables. Phenolic compounds have an important role in fruit juice processing industry since they increase the turbidity and sedimentation of drinks such as fruit juices and wines [4].

Although they have a remarkable antioxidant activity, phenolic compounds usually are removed from edible oils during the refining process as thermally unstable compounds.

The first article which included the chemical composition and antioxidant activity of ten cold-pressed edible oils from Macedonia was published by Kostadinović Veličkovska et al. [5]. They applied DPPH and TEAC assay for determination of the antioxidant activity of the oils under study. According to their findings, both assays gave completely different results of antioxidant activity of the oils. DPPH assay indicated the highest antioxidant activity of oils which

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were the richest sources of vitamin-E-active compounds. However, TEAC assay showed the highest antioxidant activity for oils which had the highest level of polar compounds such total phenolic compounds and flavonoids.

The chemical structure of phenolic compounds presented in cold-pressed edible oils allows protonation from one of the hydroxyl groups which results stabilization of alkyl radicals from fatty acids [6].

The cold pressed oils from poppy seed, almond, walnut and wheat germ are valuable oils regarding their nutritional and pharmacological activities. It is known since a long time that the oils are edible with potential antiradical activity but no research has focused on their use as antimicrobial and antifungal drugs. This study aims to investigate bioactive components of the oils in terms of fatty acid composition, vitamin-E-active compounds, phytosterols and total phenolic content. Furthermore, the main object of this study was to find the impact of bioactive compounds on antiradical, antibacterial and antifungal activities of the oils produced from the region of Macedonia.

Materials and methods

Harvesting and selection of plant material

Seeds from poppy (*Papaver somniferum* L.) were collected in August 2014 from the experimental sown fields of the Štip valleys. Almonds (*Prunus dulcis* L.) were collected in September 2014 from the sown fields of the Srumica valleys. Walnuts (*Juglans regia* L.) were collected in October 2014 from the sown fields of the Povardarje valleys. Wheat (*Triticum* spp.) was collected at the end of June 2014 from the sown fields of the Ovče Pole valleys. All plant materials were organically produced from the territory of Macedonia.

Purification and cold pressing

In order to produce cold-pressed edible oils, the cold pressing experiment was performed in triplicate. The purification process of poppy seeds started with removal of broken or damaged seeds. Almonds and walnuts were roasted in microwave oven at 190 °C. Wheat germs were obtained from milling of collected wheat.

After pressing at 40 °C, the fresh cloudy oils were purified from solid impurities in the tanks by sedimentation within 20 days at 18 °C. The quantities of plant material were collected only for needs of this experiment and yield of each cold-pressed oil was lower than 500 g. After sedimentation, the collected oils were filtrated by using a protection filter and bottled in dark 250 mL bottles.

Extraction of phenolic compounds from cold-pressed oils

A liquid–liquid extraction (LLE) system was used to extract the phenolic compounds present in the oils. According to Carrasco-Pancorbo et al. [7], 9 g of each oil was dissolved in 6 mL of hexane, and the solution was extracted successively with four portions from 3 mL of methanol/water (60:40, v/v) solution. The combined extracts of the hydrophilic layer were brought to dryness in a rotary evaporator under reduced pressure and temperature of 40 °C. At the end, the residue was redissolved in 0.5 mL of methanol/water (60:40, v/v). All the measurements were done using five times diluted extract with methanol.

Analytical methods

Determination of the fatty acid composition

Capillary gas chromatography was used for determination of fatty acid composition. The procedure of preparation of fatty acid methyl esters included dissolving of two drops of each oil dissolved in 1 mL of heptane. Furthermore, 50 µL of sodium methylate with concentration of 2 mol/L was added and solution was homogenized. In addition, all samples were diluted with 100 µL of distilled water and after centrifugation, the lower phase was removed. The upper phase was mixed with 50 µL of 1 M HCl and samples were centrifuged once again. For elimination of water traced, sodium sulphate anhydride was added. The upper phase of solution was inserted in GC vials and fatty acid methyl esters were analyzed by CP7420 Select FAME column, 100 m × 0.25 mm internal diameter with 0.25 µm film thickness. Analyzes were performed on Agilent 6890 equipped with KAS4Plus and FID. The temperature programmed started from 150 to 240 °C with a rate of 1.5 °C/min and maintained isotherm at 240 °C for 20 min. The injector and detector temperature were 260 °C. Hydrogen was used as the carrier gas at an average velocity of 25 mL/min [5].

Determination of tocopherols

For determination of tocopherols, 100 mg of cold pressed oils was dissolved in 1 mL of heptane. The analyses of tocopherols were performed with an HPLC instrument equipped with a L6000 pump, a Merck-Hitachi F-1000 fluorescence detector with excitation wavelength on 295 nm and emission wavelength on 330 nm and a Diol phase HPLC column 25 cm × 4.6 mm ID (Merck, Darmstadt, Germany). The flow rate was 1.3 mL/min and the injection volume 20 µL. For

separation was used mobile phase of heptane and TBME in ratio 95:5 [5].

Determination of phytosterols

The procedure for identification and quantification of phytosterols started with saponification of 250 mg of oil with a solution of ethanolic potassium hydroxide by boiling under reflux. Furthermore, unsaponifiable matter was isolated by solid-phase extraction on an aluminium oxide column (Merck, Darmstadt, Germany) by retention of fatty acid anions while sterols passed through. Thin-layer chromatography was used for separation of sterol fraction from other unsaponifiable matter (Merck, Darmstadt, Germany). After re-extraction from the TLC material, the composition of the sterol fraction was analysed by GLC using betulin as internal standard. For this purposes, a SE 54 CB column was used (50 m long,0.32 mm ID, 0.25 μm film thickness) (Macherey–Nagel, Düren, Germany). For separation of the compounds, hydrogen was used as carrier gas in split ratio 1:20. Injection and detection temperatures were adjusted to 320 °C. The oven temperature program was 245–260 °C at 5°C/min. The identification of separated peaks was performed by their mass spectra and standard compounds such β-sitosterol, campesterol, stigmasterol and mixture of sterols isolated from rape seed oil such brassicasterol or by a mixture of sterols isolated from sunflower oil such Δ7-avenasterol, Δ7-stigmasterol, and Δ7-campesterol.

Total phenolic compounds (TPC)

The total phenolic content of oil extracts was determined with Folin–Ciocalteau reagent. For each sample, 50 μL of diluted (1:5) oil extract were added to 750 μL water and 50 μL of Folin–Ciocalteu reagent. The solution a with total volume of 850 μL was incubated in the dark for 5 min. Then, 150 μL of 20% sodium carbonate solution was added and samples were incubated in the dark for 1 h. Reference solution was prepared with distilled water instead oil extracts and treated with the Folin–Ciocalteu reagent in the same way as the assayed samples. The samples turned to a blue colour with different degrees, depending on the content of phenolic compounds in the samples. The absorbance at 750 nm was recorded against the absorbance of the reference solution. The measurements were performed in duplicate [3, 5].

The content of total phenolic compounds was calculated using a calibration curve of gallic acid (the linearity range: 1–10 mg/L, $R^2 = 0.9943$) (Table 1). The total level of electron-rich components (mainly known as total phenolic content) for each sample was determined in terms of gallic acid equivalents.

Determination of antiradical activity

The antiradical activity of the four oils was measured spectrophotometrically by using DPPH and ABTS radicals. The antiradical activity using DPPH assay was estimated for the whole oils and methanol extracts. The TEAC assay (Trolox equivalent antiradical assay) was estimated for methanolic oil extracts [3].

DPPH radical scavenging assay (DPPH assay) For estimation of the antiradical activity of whole oils, the stock solution of DPPH radical (0.1 g/L) was dissolved in hexane. All measurements were done by direct incorporation of 5 μL pure oil in 995 μL DPPH reagent. Incubation time of 30 min at 37 °C was necessary for decreasing of DPPH absorbance at 517 nm. For the calculation of the radical scavenging ability a calibration curve was prepared using a stock solution of α-tocopherol (100 mg/L) in hexane and different concentrations (1–10 mg/L) were used (the linearity range: 1–10 mg/L, $R^2 = 0.991$) (Table 1).

For estimation of the antiradical activity of methanol extracts of oils, the same DPPH protocol was used, but DPPH reagent was dissolved in methanol (0.1 g/L). All samples were performed by addition of 50 μL extract and 950 μL DPPH reagent. For this prurpse, DPPH reagent was five time diluted. The incubation time of 30 min at 37 °C was necessary for decreasing the absorbance at 517 nm. The radical scavenging ability was calculated using a calibration curve with Trolox (the linearity range: 0.5-6 mg/L, $R^2 = 0.998$) (Table 1). Radical scavenging ability was expressed as percentage of decolarization of DPPH radical [3, 5].

Trolox equivalent antiradical capacity assay (TEAC assay) The chromophore ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) was dissolved in distilled

Table 1 Calibration curves

water to 7 mM concentration and leaving the mixture to stand in the dark at room temperature for 12–14 h before use. For initiation of ABTS radical cation ($ABTS^{\bullet+}$), 4.9 mM potassium persulphate solution was used. Furthermore, the $ABTS^{\bullet+}$ solution was diluted with water in a 1:9 v/v ratio (10 mL is quantitatively transferred into 100 mL calibrated flask and diluted) to 0.7 mM. The concentration of the resulting blue-green ABTS radical solution was adjusted to an absorbance of 0.80 ± 0.020 at 735 nm.

A 245 µL volume of reagent was transfered into a quartz cuvette with subsequent addition of 100 µL of oil extract. The decrease in absorbance at 735 nm was measured after 30 min and incubation at 37 °C. The estimation of the antiradical activity with TEAC assay was calculated using a calibration curve of Trolox (the linearity range: 0.5–6 mg/L, $R^2 = 0.997$) (Table 1).

Antimicrobial assays

The samples of four cold-pressed oils were investigated for their "in vitro" antibacterial and antifungal properties using a disk-diffusion method in Petri dishes. The four oils were tested for antibacterial activity against two Gram-positive bacterial strains: *Listeria monocytogenes* (ATCC 13076), and *Staphylococcus aureus* (ATCC 49444), and against two Gram-negative bacterial strains: *Salmonella enteritidis* (ATCC 13076) and *Escherichia coli* (ATCC 25922), and for antifungal activity using *Candida albicans* (ATCC 10231).

In brief, each microorganism was suspended in Mueller Hinton (MH) broth and diluted approximately to 10E6 colony forming unit (cfu)/mL. They were "flood-inoculated" onto the surface of MH agar and MH Dextroxe Agar (MDA) and then dried. Six-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 60 µL of each oil were delivered into the wells. The plates were incubated at 37 °C and the diameters of the growth inhibition zones were measured after 24 h. Gentamicin (10 µg/well) was used as positive control. The controls were performed with only sterile broth and with only overnight culture and 10 µL of 70% ethanol.

The antibacterial and antifungal activity tests of four oils is shown in Table 3. The antibacterial activity is ranked from no activity (−: inhibition diameter < 10 mm), low (+: inhibition diameter between 10 and 15 mm), moderate (++: inhibition diameter between 15 and 20 mm) and high activity (+++: diameter inhibition ≥ 20 mm).

All tests were performed in triplicate and clear halos greater than 10 mm were considered as positive results.

Statistical analyses

used by significance level of all statistical analyses set at 0.05. The significant differences between the amount of fatty acids, tocopherols, phytosterols, total phenolic compounds and antiradical activity measured by DPPH and ABTS radicals mean values was 5% by a one-way ANOVA using Tukey's test. For this purposes, SPSS v.16.0 software, IBM Corporation, USA was used.

The ANOVA results were classified using letters (different letters mean significant differences among results). The letters are a, b, c and d according to the decrease of the result values.

Results and discussion

Bioactive compounds in poppy seed, almond, walnut and wheat germ cold-pressed edible oils

Fatty acid composition

The fatty acid composition of examined oil is presented at Table 2. The most dominant saturated fatty acid in four samples of oils was palmitic acid with levels between 5.9 and 9.3%. Regarding unsaturated fatty acid, the most dominant was monounsaturated oleic acid in roasted almond oil with level of 67.6% and polyunsaturated linoleic acid with level of 21.0. On the other hand, the level of unsaturated fatty acid in the cold pressed oil was significantly different. The polyunsaturated linoleic acid was most dominant in this oil with abundance of 60.7% and oleic acid was present with level of 17.9%. The results obtained for this oil were in excellent agreement with those published in the work of Tapia et al. [2]. According to their findings, the fatty acid composition of four varieties of walnuts was almost identical to the fatty acid composition of walnut oil from Macedonia with linoleic acid as the most abundant fatty acid with percentages between 59 and 60.6%. The second most abundant was oleic acid with level between 12.3 and 16.9%. Similar results for the fatty acid composition were published by Sielicka et al. [3]. The polyunsaturated linoleic acid and monounsaturated oleic acid were the most abundant fatty acid with 59.3 and 19.8%, respectively [3]. Poppy seed oil had very similar fatty acid composition with 72.3% of linoleic and 14.4% of oleic acid, but did not contain α-linolenic acid. However, wheat germ oil consisted almost equal abundance of oleic and linoleic acid with 38.1 and 37.7%, respectively. According to the results of Hassanien et al. [8] the most abundant fatty acid in wheat germ oil was linoleic acid with 57.9% and oleic acid with 12.8% [8]. The significant amount of

Table 2 Fatty acid composition of cold pressed edible oils (%). Fatty acid analyses were performed in duplicate and the variation between duplicates was less than 1%

Oil type	Fatty acid						
	Saturated fatty acid (SFA)						
	C14:0	C16:0	C17:0	C18:0	C20:0	C22:0	C24:0
Poppy seed oil	0.03 ± 0.03^{a}	8.5 ± 0.03^{b}	0.05 ± 0.00^{a}	2.4 ± 0.01^{a}	0.1 ± 0.00^{a}	ND^{a}	ND^{a}
Walnut oil	ND^{a}	5.9 ± 0.02^{a}	0.05 ± 0.00^{a}	2.3 ± 0.01^{a}	0.1 ± 0.00^{a}	ND^{a}	ND^{a}
Almond oil	ND^{a}	6.4 ± 0.01^{a}	0.06 ± 0.00^{a}	2.8 ± 0.00^{ab}	0.2 ± 0.00^{a}	0.03 ± 0.00^{a}	ND^{a}
Wheat germ oil	0.04 ± 0.01^{a}	9.3 ± 0.03^{b}	0.09 ± 0.00^{b}	4.0 ± 0.01^{b}	1.0 ± 0.01^{b}	1.8 ± 0.01^{b}	1.0 ± 0.00^{b}
	Monounsaturated fatty acid (MUFA)						
	C16:1 D9	C17:1	C18:1 trans 2	C18:1 trans 1	C18:1 D9	C18:1 D11	C20:1 11	C20:1	C22:1 13
Poppy seed oil	0.1 ± 0.01^{a}	ND^{a}	14.35 ± 0.02^{b}	ND^{a}	14.4 ± 0.02^{a}	1.06 ± 0.01^{b}	ND^{a}	0.1 ± 0.01^{a}	ND^{a}
Walnut oil	0.1 ± 0.00^{a}	ND^{a}	ND^{a}	ND^{a}	17.9 ± 0.01^{a}	0.8 ± 0.00^{a}	ND^{a}	0.2 ± 0.00^{a}	ND^{a}
Almond oil	0.4 ± 0.01^{b}	0.09 ± 0.00^{a}	ND^{a}	ND^{a}	67.6 ± 0.02^{c}	1.0 ± 0.00^{b}	ND^{a}	0.1 ± 0.00^{a}	ND^{a}
Wheat germ oil	0.1 ± 0.00^{a}	0.06 ± 0.00^{a}	0.1 ± 0.00^{a}	0.1 ± 0.00^{a}	38.1 ± 0.04^{b}	1.0 ± 0.00^{ab}	0.04 ± 0.00^{a}	0.8 ± 0.00^{b}	0.1 ± 0.00^{a}
	Polyunsaturated fatty acid (PUFA)								
	C18:2	C18:3	C18:2 trans 1	C18:3 trans 2	C18:3 trans 1	C20:2 5,11	C20:2 11,14	C20:3 5,11,14	
Poppy seed oil	72.3 ± 0.06^{d}	0.9 ± 0.01^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	
Walnut oil	60.7 ± 0.01^{c}	11.7 ± 0.01^{c}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	
Almond oil	21.0 ± 0.01^{a}	0.4 ± 0.00^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}	
Wheat germ oil	37.7 ± 0.01^{b}	2.2 ± 0.00^{b}	0.6 ± 0.01^{a}	0.1 ± 0.00^{a}	0.5 ± 0.00^{a}	0.1 ± 0.00^{a}	0.1 ± 0.00^{a}	0.1 ± 0.01^{a}	

ND not detected

$^{a-c}$Significant difference at $p < 0.05$ among different oils in terms of the content of saturated, mono and poly unsaturated fatty acid

Table 3 Vitamin-E-active compounds in cold pressed oils (mg/100 g of oil)

Oil type	α-t	α-T3	β-t	γ-t	Plast 8	γ-T3	δ-t	Total
Poppy seed oil	1.9 ± 0.00^{a}	ND^{a}	0.03 ± 0.00^{a}	15.7 ± 0.01^{b}	0.17 ± 0.00^{a}	0.1 ± 0.00^{a}	0.2 ± 0.00^{a}	18.2 ± 0.00
Walnut oil	1.0 ± 0.01^{a}	ND^{a}	0.1 ± 0.00^{a}	21.9 ± 0.01^{c}	ND^{a}	0.1 ± 0.00^{a}	2.48 ± 0.01^{b}	25.5 ± 0.03
Almond oil	238 ± 0.01^{c}	0.3 ± 0.00^{a}	0.2 ± 0.00^{a}	1.6 ± 0.00^{a}	0.4 ± 0.05^{a}	0.2 ± 0.00^{a}	0.04 ± 0.01^{a}	26.5 ± 0.07
Wheat germ oil	5.8 ± 0.06^{b}	ND^{a}	0.5 ± 0.02^{a}	19.7 ± 0.04^{c}	0.6 ± 0.06^{a}	0.3 ± 0.02^{a}	5.6 ± 0.04^{b}	32.5 ± 0.24

HPLC analyses were performed in triplicate

Vitamin-E-active compounds

The identified and quantified vitamin-E-active compounds in four oils under study are presented in Table 3. The highest amount of α-tocopherol was presented in roasted almond oil with 23.8 mg/100 g oil. However, all other vitamin-E-active compounds were significantly higher in wheat germ oil with exception of γ-tocopherol which was quantified as the most dominant vitamin-E-active compound in roasted walnut oil with an amount of 21.9 mg mg/100 g oil. The results from vitamin-E-active compounds of walnut oil were in good different varieties of Tunisian walnuts in the range from 18.6 to 43.6 mg/L with dominance of γ-tocopherol. The cold-pressed edible oils from roasted almond and walnut had similar amounts of total vitamin-E-active compounds. However, the most dominant vitamin-E-active compound in almond oil was α-tocopherol (23.8 mg/100 g oil) and γ-tocopherol was the most abundant tocopherol (21.9 mg/kg oil) in walnut oil. Poppy seed oil had the lowest amount of vitamin-E-active compounds with dominance of γ-tocopherol.

Almost double quantity of β-tocopherol, plastochromanol 8, γ-tocotrienol and δ-tocopherol in wheat germ oil made

germ oil indicated the highest abundance of γ-tocopherol instead of α-tocopherol. This quantity of vitamin-E-active compounds was similar to wheat germ oil published by Hassanien et al. [8]. Furthermore, their results showed decreasing order of three main Vitamin-E-active compounds (α-tocopherol > β-tocopherol > γ-tocopherol).

Phytosterols

The richest source of phytosterols was wheat germ oil (3894.6 mg/kg oil) with abundance similar to poppy seed oil (3750.08 mg/kg oil). Except β-sitosterol with level of 2148 mg/kg oil, the most dominant phytosterol was campesterol with amount of 939.1 mg/kg oil (Table 4). Stigmasterol and sitostanol were presented in similar abundances around 120 mg/kg oil and all other phytosterols were present in levels below 100 mg/kg oil. According to the findings of Hassanien et al. [8] the total abundance of phytosterols in wheat germ oil was 23.8 μg/g oil [8]. Stigmasterol was present in significant amount in poppy seed oil (986.2 mg/kg oil) and delta-5-avenasterol was the second most abundant phytosterol in almond oil after β-sitosterol with level of 365.2 mg/kg oil. The lowest amount of phytosterols was quantified in walnut oil (2109 mg/kg oil). Apart from β-sitosterol, significant levels of delta-7-stigmastenol and delta-5-avenasterol were detected in walnut oil. All other phytosterols were presented in levels below 100 mg/kg oil. Czaplicki et al. [6] published almost double levels of total phytosterols (4840 mg/kg oil) in walnut oil from Poland in comparison to Macedonian walnut oil (2109 mg/kg oil) [6].

The ratios of particular phytosterols can be used as markers of origin and purity of the expensive oils [9]. As we can notice from Table 4, campesterol was presented ten times more in comparison to campestanol in cold pressed wheat germ oil [8]. However, the total level of phytosterols in cold-pressed wheat germ oil was very similar with hexane extracted wheat germ oil with level of 3.7 mg/g oil [10].

Total phenolic content (TPC) and antiradical potential of cold-pressed oils determinated by DPPH and ABTS radicals

There is significant impact of total phenolic content on antiradical activity of the examined oils. The methanolic extracts of roasted almond and walnut oil contained the highest amounts of phenolic compounds (558.8 and 524.8 mg/L, respectively). Significantly lower quantity was detected for poppy seed oil (368.2 mg/L) and the lowest total phenolic contents was determinate for wheat germ oil (61.6 mg/L) (Table 5).

According to DPPH antiradical assay, the highest antiradical potential was found for wheat germ oil (1418.9 mg of α-tocopherol/L oil) and the lowest antiradical potential for poppy seed oil (792.6 mg of α-tocopherol/L oil). The tendency of antiradical potential for the oils was in good agreement with vitamin-E-active compounds (Table 3). According to the finding of Shahidi and Shukla [11] α-tocopherol had the highest antioxidant activity following by β- and γ-tocopherol. The lowest antioxidant potential is characteristic for δ-tocopherol [11]. Although α-tocopherol was used as a standard for the calibration curve, DPPH assay of whole oil dissolved in hexane clearly indicated wheat germ oil as the oil with the highest antiradical potential. Almond oil had the highest level of α-tocopherol (23.8 mg/100 g oil) but its

Table 4 Determination of the amount of phytosterols (mg/kg)

Phytosterols	Walnut oil	Poppy seed oil	Almond oil	Wheat germ oil
Cholesterol	7.2 ± 0.4^{a}	1.9 ± 0.3^{a}	35.3 ± 1.4^{c}	12.1 ± 1.4^{b}
Brassicasterol	ND[a]	9.2 ± 1.9^{b}	ND[a]	ND[a]
24-Metylencholesterol	2.0 ± 2.0^{a}	92.7 ± 4.6^{c}	11.8 ± 1.45^{b}	83.7 ± 2.1^{d}
Campesterol	80.7 ± 2.3^{a}	587.9 ± 5.3^{c}	130.0 ± 7.4^{b}	939.1 ± 16.0^{d}
Campestanol	ND[a]	1.9 ± 0.2^{a}	ND[a]	92.7 ± 1.50^{b}
Stigmasterol	6.8 ± 0.4^{a}	986.2 ± 8.5^{d}	32.7 ± 3.1^{b}	122.5 ± 8.0^{c}
5,23-Stigmastadienol	16.1 ± 0.4^{a}	27.6 ± 1.6^{ab}	48.7 ± 2.1^{b}	62.2 ± 4.2^{c}
Chlerosterol	38.8 ± 0.4^{a}	46.7 ± 1.3^{b}	54.9 ± 0.7^{ab}	81.7 ± 11.5^{c}
β-Sitosterol	1476 ± 13.5^{a}	1739 ± 12.6^{b}	2396 ± 13.6^{c}	2148 ± 49.3^{d}
Sitostanol	14.0 ± 0.3^{b}	6.5 ± 0.1^{a}	54.9 ± 0.7^{c}	129.1 ± 28.1^{d}
Δ5-Avenasterol	118.8 ± 1.8^{b}	273.8 ± 4.3^{b}	$365.2 \pm 3.3^{b,c}$	70.7 ± 4.7^{a}
5,24-Stigmastadienol	28.4 ± 1.4^{a}	32.6 ± 2.1^{a}	60.4 ± 1.5^{b}	52.6 ± 9.5^{b}
Δ7-Stigmastenol	309.9 ± 3.5^{d}	10.7 ± 1.0	57.2 ± 2.8^{b}	78.7 ± 13.1^{c}
Δ7-Avenasterol	10.2 ± 0.6^{a}	13.4 ± 1.1^{ab}	19.3 ± 1.2^{a}	21.2 ± 5.5^{b}
Total	2109 ± 26.94	3750 ± 44.77	3266 ± 39.05	3894 ± 155.01

ND not detected

[a–d]Significant difference at $p < 0.05$ among different oils in terms of phytosterol content

Table 5 Total phenolic compounds, DPPH assay for the pure oils, DPPH for methanolic oil extracts and TEAC assay for methanolic oil extract

Samples	TPC assay (mg/L GAE)	DPPH assay for (mg of α-tocopherol/L oil)	DPPH assay for methanol extracts (mg Trolox/L oil)	TEAC assay for methanol extracts (mg of Trolox/L oil)
Almond oil	558.8 ± 10.3^{c}	952.9 ± 34.1^{b}	160.3 ± 7.1^{c}	174.0 ± 1.6^{c}
Walnut oil	524.8 ± 18.2^{c}	1191.4 ± 19.9^{c}	66.7 ± 1.0^{b}	138.8 ± 2.2^{b}
Poppy seed oil	368.2 ± 17.7^{b}	792.6 ± 4.1^{a}	56.5 ± 3.4^{b}	126.5 ± 4.9^{b}
Wheat germ oil	61.6 ± 3.8^{a}	1418.9 ± 16.0^{d}	27.9 ± 13.6^{a}	86.7 ± 21.1^{a}

Each value is the mean ± SD of two independent measurements

$^{a-d}$Significant difference at $p < 0.05$ among different oils in terms antiradical activity

antiradical activity determined by this antiradical assay was significantly lower (Table 5) due to the steric effect with DPPH.

The antiradical activity of methanol extracts estimated by ABTS radical was in a good correlation with the amount of total phenolic contents ($R^2 = 0.8789$). As can be seen from Table 5, the highest scavenger ability was found for the methanol extracts of roasted almond and walnut oil (174.0 and 138.8 mg Trolox/L of oil, respectively). Following the trend of the results estimated using TPC assay, lower scavenger ability than almond and walnut oils had poppy seed oil with 126.5 mg Trolox/L of oil. The lowest antiradical capacity was estimated for wheat germ oil of 86.7 mg Trolox/L of oil. However, if we compare the data from Table 5, we can notice that although poppy seed oil had approximately 30% less total phenolic content in comparison to walnut oil, the antiradical activity of their methanolic extracts determined by the two radicals was very similar. This can be explained by the fact that the position of hydroxyl groups in the structure of phenolic compounds is essential for the antioxidant activity of the oils [12–15]. Wheat germ oil had the lowest level of total phenolic compounds and the lowest antiradical activity of the methanolic extract measured by DPPH and ABTS radical, respectively. Consequently, we can conclude that the antiradical potential of these oils was influenced mainly by vitamin-E-active compounds [16].

Antibacterial and antifungal activity

The results from antibacterial and antifungal activity of the oils are presented in Table 6. Although cold-pressed poppy seed oil had the lowest level of vitamin-E-active compounds and the lowest antiradical activity measured by DPPH assay, it looks that this oil is the most interested as microbiologically active oil which indicated low antibacterial activity against gram positive bacteria *Listeria monocytogenes* and moderate antifungal activity against *Candida albicans*. Furthermore, cold-pressed almond and walnut oil showed low antifungal activity against *Candida albicans*. Although

Table 6 Antibacterial and antifungal activity (inhibition zone expressed in mm) a of four investigated oils

Samples	Inhibition zone in diameter (mm)				
	Staphylococcus aureus	*Listeria monocytogenes*	*Salmonella enteritidis*	*Escherichia coli*	*Candida albicans*
Almond oil	–	–	–	–	+
Walnut oil	–	–	–	–	+
Poppy seed oil	–	+	–	–	++
Wheat germ oil	–	–	–	–	–

antiradical activity measured by DPPH radical for whole oil, it was not active against any of examined bacteria and fungi. None of four cold pressed oils showed activity against gram-positive bacterial strain *Staphylococcus aureus* (ATCC 49444), and two gram-negative bacterial strains *Salmonella enteritidis* (ATCC 13076) and *Escherichia coli* (ATCC 25922).

Generally speaking, our results showed that antiradical activity measured by different radicals and assays is opposite to the antimicrobial activity for the four examined oils. Furthermore, no relationship of the examined bioactive compounds to the antibacterial activity of oils was found. Only few researchers published antimicrobial activity of edible oils. The antimicrobial activity of different edible oils obtained from cold-pressing of nuts such hazelnut, peanut, umbrella nut and walnut was related to long chain unsaturated fatty acids such linoleic and oleic acid which exhibit inhibitory activity against many bacteria such *S. aureus* [17, 18]. Poppy seed oil had the highest level of linoleic acid ($72.28 \pm 0.06\%$) in comparison with all other analyzed oil which can lead us to conclusion that may be linoleic acid is reason for the antimicrobial activity of poppy seed oil. Linoleic and oleic acid were reported as a potent antibacterial compounds which selectively inhibit enzymes in *S.*

particular β-sitosterol, campesterol, avenasterol and stigmasterol) and antimicrobial activity [19]. Beside published results for antimicrobial activity of wheat germ, our study did not show any antimicrobial potential of wheat germ oil as the richest oil with phytosterols [20]. Karaman et al. [21] demonstrated an antimicrobial activity of almond by-products and especially indicated antibacterial activity against *E. coli*, *L. monocytogenes* and *S. aureus*. According to their findings, the antimicrobial activity of the oils depends only of the type of the plant material and bioactive compounds presented in the oils [21–23]. The antioxidant and antimicrobial activity of oils from the seeds of *Amorpha fruticosa* L., *Caragana microphylla* Lam and *Elaeagnus angustifolia* L. against *B. subtilis*, *E. coli*, *S. aureus*, *P. solanacearum* and *B. thuringiensis* was connected with high level of unsaturated fatty acids which can support our statement that poppy seed oil as richest source of linoleic acid had the strongest antimicrobial potential [24].

Conclusions

In this work we have shown that particular bioactive compounds had different impact on antiradical and antimicrobial potential of the examined cold-pressed oils. The results from antiradical activity against DPPH radical indicated Vitamin-E-active compounds as the most powerful bioactive compounds. However, polyphenolic compounds were the most active against ABTS radical. Furthermore, poppy seed oil as the richest source of linoleic acid had the highest antimicrobial activity which can lead us to conclusion that may be linoleic acid in combination with other bioactive compounds as pytosterols and polyphenolics can be active against *Listeria monocytogenes* and *Candida albicans* by perforation and/or reduction in membrane fluidity with final cytoplasmatic membrane damage.

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Compliance with ethical standards

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

Research involving human and animal participants This article does not contain any studies with human or animal subjects.

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