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Chemical Composition, Characteristics Profiles and Bioactivities of Tunisian *Phalaris canariensis* Seed: A Potential Source of ω-6 and ω-9 Fatty Acids

Hichem BEN SALAH[1,*], Mouna KCHAOU[1], Rihab BEN ABDALLAH KOLSI[2], Raed ABDENNABI[2], Mohamed AYEDI[3], Néji GHARSALLAH[2], Noureddine ALLOUCHE[1]

[1]Laboratory of Organic Chemistry LR17ES08 (Natural Substances unit), University of Sfax, Faculty of Sciences of Sfax, Sfax, Tunisia
[2]Laboratory of Plant Biotechnology Applied to Crop Improvement, Faculty of Science, Sfax University, Sfax, Tunisia
[3]Olive Tree Institute, Sfax, Tunisia, UR: Cultivated Plant Protection and Environment
*Corresponding author: hichembensalah9@gmail.com

Abstract Seed oils of *Phalaris canariensis* extracted by ultrasonication and cold maceration were evaluated for their physical characteristics, fatty acid, sterol composition and total phenol contents as well as for their antioxidant, antibacterial and acetylcholinesterase activities. The physicochemical properties of ultrasonication and cold maceration oils respectively were: acid values (4 and 3.25) mg KOH/g, peroxide values (5.53 and 4.41) meq O_2 Kg^{-1}, iodine values (88.83 and 95.17) g/100 g of oil, saponification values (119.21 and 98.17) mg KOH/g, phenolic content (36.40 and 53.00) mg gallic acid equivalents/g extract, chlorophylls (0.52 and 0.60) mg/kg oil and carotenoids contents (1.92 and 1.88) mg/kg oil. Gas chromatography analysis revealed that linoleic (52.03 and 52.2%), oleic (31.75 and 31.84%) and palmitic (11.09 and 11.34 %) acids were the major fatty acids in the two oils. The main sterol found in the seed oils was β-sitosterol (48.01 and 47.86%). On the other hand, the evaluation of their DPPH radical scavenging, total antioxidant capacity, antibacterial and acetylcholinesterase activities showed interesting results. Thus, *Phalaris canariensis* seed oils could deserve further consideration and investigation as a potentially new multi-purpose product for agro-food, medicinal and cosmetic uses.

Keywords: *Phalaris canariensis, seed oil, chemical composition, physicochemical properties, biological activities*

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1. Introduction

Oils and fats have a rich history and are used principally for food and for a variety of industrial items. They are used to make soaps, candles, perfumes and other personal care and cosmetic products [1]. A large quantity of oils and fats, indispensable for human consumption and industrial purposes, is presently derived from plant sources. Recently, natural plant seeds have received much attention as sources of biologically active substances that have a wide range for pharmacological activities including antibacterial, antioxidant, anti-inflammatory, anti-diarrheal, anti-diabetic and anticancer effects [2,3,4]. Despite the wide range of vegetable oil sources, the world consumption is dominated by palm, soybean, rapeseed and sunflower oils with 38.1, 35.7, 17.8 and 18.2 million tons consumed per year, respectively [5]. Furthermore, worldwide interest is oriented for the recovery and exploitation of oils from natural plant resources. Hemavathy and Prabhakar reported that seed of some species of *Poaceae* can be the edible oil sources to meet the increasing demands for vegetable oil [6]. *Phalaris canariensis* L. (Canary seed) is a shrubby plant belonging to the *Poaceae* family with production practices and life cycle similar to those of other winter cereal crops such as wheat and oats [7]. *Phalaris canariensis* is one of the top four specialty crops grown in Western Canada with over 400 000 acres planted in this region. Currently this crop is solely used as food for caged and wild birds. However the unique composition and characteristics of *Phalaris canariensis* make it a promising cereal for food and industrial uses [8,9,10]. It is spread throughout the entire northern hemisphere, particularly in Asia, Europe, North America and North Africa. *Phalaris canariensis* commonly grows on soils rich in nutrients with a slightly acidic to neutral pH, and also in the wetlands. Traditionally, *Phalaris canariensis* is also used as a folk medicine for diabetes and hypertension treatments [7,10]. The main aim of the present work is to determine the physicochemical properties, fatty acids, sterols contents, UV/visible spectra and thermal profiles, as well as to evaluate the biological activities of *Phalaris canariensis* seed oil. This study represents the first report dealing with chemical composition and biological properties evaluations of *Phalaris canariensis* seed grown in Tunisia.

2. Materials and Methods

2.1. Seed Material

Mature seed of *P. canariensis* were collected from Beja, region in Tunisia (a city of the northwest of Tunisia with a semi-arid climate) in December 2013. A voucher specimen (Number LCSN 132) has been deposited in the Herbarium Laboratory of Organic Chemistry, Faculty of Sciences, Sfax University, Tunisia.

2.2. Oil Extractions

2.2.1. Ultrasonication

The powdered seed (300 g) of *P. canariensis* were powdered and extracted with hexane (200 ml) in ultrasonication bath at 40°C for 90 min. The mixtures were filtrated and the solvent was removed via a rotary vacuum distillation at 40°C.

2.2.2. Cold maceration

The powdered seed (300 g) of *P. canariensis* were extracted by maceration with hexane (200 ml) at room temperature (24 h three times). The mixtures had passed through a filter and were concentrated in a vacuum at 40°C.

The recovered seed oils were stored in a freezer (-20°C) for subsequent physicochemical analyses. The weight (g) of oils extracted with the two methods was determined to calculate the lipid content. The result was expressed as the lipid percentage in the dry seed powder.

2.3. Physicochemical Properties of Seed Oils

2.3.1. Acid Value

The acid value of seed oil was estimated according to the standard (ISO 660, 1996).

2.3.2. Iodine Value

The iodine value was identified according to the standard (ISO 3961, 1996).

2.3.3. Saponification Value

The saponifiction value was defined according to the standard (ISO 3657, 1996).

2.3.4. Spectroscopic Indices (K_{232}, K_{270})

The spectroscopic indices, K_{232} and K_{270}, were determined according to the official methods described in European Regulation EC 2568/91 and amendments [11].

2.3.5. Chlorophylls and Carotenoids Contents

Following the procedures mentioned by [12], 1g of *P. canariensis* oil was placed in a falcon tube and filled until 10 mL with hexane. The chlorophyll fraction was measured using a UV/Vis spectrophotometer (Shimadzu Co., Kyoto, Japan) at 630, 670 and 710 nm and the carotenoids fraction at 470 nm. The concentration of pigments was expressed using the following equations:

$$Chlorophyllscontent(mg/kg) = \frac{Abs670-(Abs630+Abs710)}{0.1086\times L} \quad (1)$$

$$Carotenoids\ content(mg/kg) = \frac{Abs470\times 10^3\times 25}{2000\times 7.5} \quad (2)$$

where Abs is the absorbance and (L) is the thickness of the UV spectrophotometer curve (1 cm).

2.4. Fatty Acid Composition

Fatty acid methyl esters (FAMEs) analysis was carried out after performing alkaline treatment obtained by dissolving the oil (0.05 g) in hexane (1 mL) and adding a solution of potassium hydroxide (1 mL; 2N) in methanol [13].

FAMEs were analyzed by gas chromatography using a Shimadzu 17A gas chromatograph equipped with a flame ionization detector (FID) and a capillary column. The operation conditions were as following: The column temperature was programmed from 180 to 240°C at 5°C/min and the injector and detector temperatures were set at 250°C, the carrier gas was nitrogen with a flow of 1 mL/min. The chromatographic separation was achieved by injecting 1 µL of solution into a capillary column of 30 m length, 0.32 mm of diameter, with a film thickness of 0.25 µm. The polar stationary phase was cyanopropylmethyl/ phenylmethyl-polysiloxane (1:1, v/v). Peaks were identified by comparing their retention times with those of authentic reference compounds. The fatty acid composition was expressed in percentage of each fatty acid in the lipid fraction.

2.5. Sterol Composition

Unsaponifiable matter of *P. canariensis* seed oil was extracted and determined in the oil sample according to IOC [14]. Briefly, 1 g of oil was refluxed with 10 mL of 10 % ethanolic potassium hydroxide for 1 hour. After saponification, 10 mL of distilled water was added and the unsaponifiable matter was extracted three times with 20 mL portion of ether. The ether extract was dried with Na_2SO_4 and evaporated. The sterol fraction obtained by TLC separation from the unsaponifiable of the oil was derivised with silanizing mixture (pyridine-hexamethyldisilazane-trimethylchlorosilane 9:3:1, v/v/v) in the ratio of 50 µL for every mg of sterols. Further sterol analysis determination was carried out by CPG (Agilent Technologies 7890 A) equipped with a HP-5 column (30m × 0.32 mm × 0.25 mm i.d) under the following operating condition: column temperature 267°C, detector temperature 290°C, injector temperature 280°C, carrier gas: helium (1 mL/min).

2.6. ^{1}H-NMR profile

In order to strengthen the chemical composition study of the two extracted *P. canariensis* oils, ^{1}H-NMR spectroscopy was used. ^{1}H-NMR spectra were obtained using a Bruker Ascend 400 NMR Spectrometer operating at 400 MHz in $CDCl_3$. In a typical experiment, 15 mg of

seed oil was dissolved in 1 ml $CDCl_3$ in an NMR tube and readings were taken between 0–14 ppm. The residual chloroform resonance was used as the internal reference. Coupling constants are given in Hertz. The chemical shifts are expressed in δ (ppm).

2.7. UV/Vis spectrophotometric analyses

Absorbances of oil solutions were measured with UV/Vis spectrophotometer (Shimmadzu Co., Kyoto, Japan) using hexane as a blank. The oil was diluted with hexane to bring the absorbance reading within the range of the calibration curve.

2.8. Thermal Characteristics (DSC Profile)

The thermal characteristics of seed oil were measured by using a differential scanning calorimeter (DSC 4000, Perkin Elmer). A flow of nitrogen gas (1.5 ml/min) was used in the cell cooled by helium (1.5 ml/min) in a refrigerated cooling system. The instrument was calibrated for temperature and heat flow with mercury (melting point, m.p. = -38.834°C, ΔH= 11.469 J/g), gallium (m.p. = 29.760°C, ΔH= 28.7 J/g) and indium (m.p. = 156.598°C, ΔH= 28.5 J/g). The oil samples (15 mg) were introduced in DSC- pan (aluminium open, TA Instruments T70529) with an empty reference pan. The sample and reference pans were then placed inside the calorimeter and kept at -70°C for 2 min. The temperature was augmented from -70 to 70°C at a rate of 5 °C/min. The samples were then kept at 70°C for 1 min, and then decreased again, at the same rate, down to -70°C. The scans were carried out at 5°C/min.

2.9. Total Phenolic Content

The total phenolic content in oils was determined according to the Folin-Ciocalteau procedure [15]. Shortly, 100 µL of diluted sample was added to 2 mL of 2 % Na_2CO_3 aqueous solution. After 2 min, 100 µL of 50 % Folin- Ciocalteau reagent was added into the mixture. After incubation for 30 min at room temperature in the dark, the absorbance of all samples was read at 750 nm using a UV/Vis spectrophotometer. The analysis was performed in a triplicate and the total phenolic content was expressed in mg gallic acid equivalents per gram extract (mg GAE/g extract).

2.10. Total Antioxidant Capacity

The antioxidant activity of the oils was assessed by the phosphomolybdenum method [16]. The assay is based on the reduction of Mo(VI)–Mo(V) by the oil sample and subsequent formation of a green phosphate/Mo(V) complex at acid pH. 0.1 mL of sample at different concentrations was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. After that, the samples were cooled at room temperature and the absorbance of each solution was measured at 695 nm using a UV/Vis spectrophotometer against blank (methanol, 0.1 mL). The antioxidant capacity was expressed in mg vitamin E equivalents per gram extract (mg antioxidant/g extract).

2.11. DPPH Radical Scavenging Assay

The DPPH radical-scavenging effect was determined using the procedure described by [17]. Oil sample (50µL) at various concentrations in methanol was placed in test tubes with 5 mL of a 0.004 % methanol solution of DPPH. After incubation for 30 min at room temperature, the absorbance was read against a blank at 517 nm. The inhibition of free radical DPPH was calculated using the following formula:

$$\mathrm{PI}(\%) = [\frac{Abscontrol - Abssample}{Abscontrol}] \times 100 \qquad (3)$$

where $Abs_{control}$ is the absorbance of the control reaction (containing all reagents except the sample) and Abs_{sample} is the absorbance of the test sample.

The radical scavenging activity was expressed as IC_{50} (µg/mL): Inhibitory concentration corresponding to 50 % DPPH inhibition. The BHT and vitamin E were used as positive control and all tests were carried out in a triplicate.

2.12. Determination of Antibacterial Activity

The agar diffusion method was used for the determination of antibacterial activities of *P. canariensis* seed oils according to the method described by [18]. The bacterial cultures were first grown on Muller Hinton agar (MH) plates at 37°C for 18 to 24 h prior to seeding onto the nutrient agar. One or several colonies of the respective bacteria were transferred into API suspension medium (bioMerieux) and adjusted to 0.5 McFarland turbidity standards with a Densimat (bioMerieux). The inocula of the respective bacteria were streaked into MH agar plates using a sterile swab and were then dried at 37°C during 15 min. A sterile filter disc having 6 mm of diameter was fixed at the surface of MH agar and 5 µl of the oil was dropped onto each Whatman paper disc. The treated Petri dishes were incubated at 37°C for 18 to 24 h. The antibacterial activity was evaluated by measuring the clear zone surrounding the Whatman paper. Standard discs of the antibiotic ampicillin (BIO-RAD) were applied as a positive antibacterial control.

2.13. Acetylcholinesterase Enzyme Inhibitory Activity

Inhibition of AChE by plant extracts was evaluated as described by [19]. The assay is based on the spectrophotometric measurement of the increase in yellow color produced by thiocholine when it reacts with the dithiobisnitrobenzoate ion (DTNB). The rise in absorbance value due to the spontaneous hydrolysis of the substrate was corrected by subtracting the ratio of the reaction before adding the enzyme from the rate after the enzyme addition. Tacrine was used as positive control. Percentage inhibition by oil extracts and tacrine were calculated using the equation below:

$$\mathrm{PI}(\%) = [\frac{Abscontrol - Abssample}{Abscontrol}] \times 100 \qquad (4)$$

where $Abs_{control}$ is the absorbance of the control and Abs_{sample} is the absorbance of the test sample.

3. Results and Discussion

3.1. Physicochemical Properties of Oils

Table 1 reports the comparison of physicochemical properties of *P. canariensis* seed oils with those of soybean oil. The ultrasonication extraction method yielded 6.43 % of oil against 4.92 % for the cold maceration extraction. These yields were lower than those of other plant oils such as *Lauris nobilis* and soybean [20].

The acid value of oil is dependent on the amount of free fatty acids present or on the degree of hydrolysis of the oil. Acid value of oil suitable for edible purposes should not exceed 4 mg KOH/g [21]. The acid values of *P. canariensis* oils (4.00, 3.25) were higher than that of soybean oil (1.72) which might be attributed to the presence of large amounts of free acids in the crude oils of *P. canariensis*. The acid values suggest that these oils could be recommended for industrial use. The assessment of hydroperoxides (primary oxidation products) is usually carried out with determination of peroxide value and specific extinctions K_{232} and K_{270} [22]. Peroxide value is a measure of the reaction rate of lipid oxidation, which leads to rancidity. Normally, oils become rancid when the peroxide value ranges from 20.0 mg/g oil to 40.0 mg/g oil [21]. Peroxide values of oils obtained using sonication and cold maceration were 5.53 and 4.41 meq O_2/Kg oil, respectively. These peroxide values were higher than that of soybean oil (1.52 meq O_2/Kg oil) and this can be attributed to the presence of higher amounts of polyunsaturated fatty acids such as linoleic acid (Table 2). The K_{232} is usually considered as an indicator of the oil autoxidation, but the K_{270} is a more useful quantity that measures the presence of conjugated dienes and trienes [22]. The *P. canariensis* seed oils showed low absorptivity values at 232 nm (0.58, 0.44) and at 270 nm (0.42, 0.32), thus containing little quantities of primary (hydroperoxides) and secondary oxidation products. The K_{232} and K_{270} for *P. canariensis* seed oils were lower than those of soybean seed oil (2.78 and 0.73) and of *Pinus halepensis* seed oil (0.51 and 1.50) [22,23]. The *P. canariensis* seed oils are thus stables and would not easily go rancid. Saponification value is an indicator of the average molecular weight and hence the chain length. It is inversely proportional to the mean molecular weight of fatty acids present in the lipid [24]. The saponification values of the examined oils (119.21, 95.17) were lower than that of soybean (179.45) but higher than that of jojoba (92.00) [25]. The lower saponification values suggest that the mean molecular weight of fatty acids is lower or that the number of ester bonds is less. This might imply that the fat molecules did not interact with each other. Iodine value represents the degree of unsaturation in oil and could be used to quantify the amount of double bonds present in the oil which reflects the susceptibility of oil to oxidation. The iodine values (88.83, 98.17) of *P. canariensis* seed oils placed them in the semi-drying oil group and this reflected the presence of high percentage of unsaturated fatty acids in these seed oils. However, these values were close to 94.35, 95.12, 97.35, and 102.02 of some edible oils reported by [26]. Hence, *P. canariensis* oils can be considered as a good assets for human nutrition, especially since they exhibited a low amount of saturated fatty acids that are involved in cardiovascular diseases.

Table 1. Physicochemical properties of *P. canariensis* seed oils

Parameter	Ultrasonication oil	Maceration oil	Soybean oil[a]
Yield (%)	6.43 ± 0.21	4.92 ± 0.13	-
Acid value (mg KOH/g)	4.00 ± 0.15	3.25 ± 0.10	1.72 ± 0.08
Peroxide value (meq O_2/Kg)	5.53 ± 0.18	4.41 ± 0.11	1.52 ± 0.05
Saponification value (mgKOH/g)	119.21 ± 0.54	95.17 ± 0.62	179.45 ± 0.68
Iodine value (g/100g of oil)	88.83 ± 0.32	98.17 ± 0.68	122.56 ± 0.98
K 232	0.58 ± 0.05	0.44 ± 0.02	2.78 ± 0.03
K 270	0.42 ± 0.01	0.32 ± 0.02	0.73 ± 0.02
Chlorophylls (mg/ kg oil)	0.52 ± 0.02	0.60 ± 0.03	-
Carotenoids (mg/ kg oil)	1.92 ± 0.1	1.88 ± 0.09	-
State at ambient temperature	Liquid	Liquid	Liquid
Color	Yellow	Yellow	Dark yellow

Values expressed are means ± SD of three parallel measurements ($p<0.05$).
[a] Nehdi, 2011 [22].

Table 2. Fatty acid compositions (%) of *P. canariensis* seed oils

Fatty acid	Retention time (min)	Ulrasonication oil (%)	Maceration oil (%)	Soybean oil (%)
Saturated				
Myristic (C14:0)	2.59	0.12	0.13	0.12
Palmitic (C16:0)	3.60	11.09	11.34	15.65
Stearic (C18:0)	5.55	1.39	1.39	4.98
Arachidic (C20:0)	9.82	1.06	0.91	0.55
Monounsaturated				
Palmetoleic (C16:1)	5.65	0.09	0.10	0.12
oleic (C18:1) ω9	5.92	31.75	31.84	20.98
Eicosenoic (C20:1)	6.23	0.05	0.06	0.32
Polyunsaturated				
Linoleic (C18:2) ω6	6.47	52.03	52.20	50.17
Linolenic (C18:3) ω3	7.60	2.01	2.02	8.18
SAFA		13.66	13.77	21.30
MUFA		31.89	32.00	21.42
PUFA		54.04	54.22	58.35
P/S		3.95	3.93	2.73

SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

3.2. Fatty Acid Composition

The *P. canariensis* oils were subjected to GC analysis in order to determine the fatty acid composition (Table 2). The results showed the principal fatty acid components to be linoleic, oleic, palmitic and linolenic acids in decreasing order, while stearic, arachidic, palmetoleic, myristic and eicosenoic acids were present in low concentrations. The most abundant fatty acids in *P. canariensis* seed oils were linoleic acid (52.03%, 52.20%), oleic acid (31.75%, 31.84%) and palmitic acid (11.09%, 11.34%). The presence of this large amount of ω6-linoleic and ω9-oleic acids classified *P. canariensis* seed oils into the group "oils low in palmitic acid and high in oleic and linoleic acids". The most important members of this group are sunflower, safflower, olive, palm and sesame oils [27]. The percentages of saturated (13.7%) and unsaturated (86%) fatty acids of the *P. canariensis* seed oils were similar to those of *Pinus pinea* (13.7% and 87.9%) reported by [23]. The Poly-unsaturated / Saturated (P/ S) ratio of *P. canariensis* seed oils (3.9) was higher than that of soybean (2.7). A high ratio of P/S, attributed to the high level of PUFA, is regarded positively in the reduction of serum cholesterol and atherosclerosis and the prevention of heart diseases [28]. Interest in PUFA as health promoting nutrients has expanded dramatically in recent years. A rapidly growing literature illustrates the benefits of PUFA, in relieving cardiovascular, inflammatory conditions, heart disease, atherosclerosis, autoimmune disorders, diabetes and other diseases [29]. The fatty acid composition and high amounts of PUFA make the *P. canariensis* seed oils a special component for nutritional applications.

Table 3. Sterol compositions of *Phalaris canariensis* seed oils

Sterol	Ulrasonication oil (%)	Maceration oil (%)
Cholesterol	0.18	0.16
α-Cholestanol	13.41	13.44
Brassicasterol	0.32	0.21
24-Methylenecholesterol	0.20	0.17
Campesterol	24.32	24.43
Campestanol	0.35	0.22
Stigmasterol	2.09	2.32
Δ -7-Campesterol	0.57	0.50
Δ-5-23-Stigmastadienol	2.03	2.09
Clerosterol	1.11	1.03
β- Sitosterol	48.01	47.86
Sitostanol	0.69	0.84
Δ -5-Avenasterol	4.66	4.58
Δ-5-24 Stigmastadienol	0.78	0.77
Δ-7-Stigmastenol	0.71	0.74
Δ-7-Avenasterol	0.50	0.56

3.3. Sterol Composition

Phytosterols (sterols) are the main compounds of unsaponifiable matters of the oils. Levels of phytosterols in vegetable oils are used for the identification of oils, oil derivatives and for the determination of the oil quality [29]. The sterol compositions of *P. canariensis* seed oils are exhibited in Table 3. Sixteen sterols were detected and identified. β-sitosterol was the major sterol found in the two oil samples (48.01 %, 47.86 %), followed by compesterol (24.32 %, 24.43 %) and α-cholestanol (13.41 %,13.44 %). The concentration of sterols has been reported to be less influenced by environmental factors and/or by cultivation of new breeding lines. The main phytosterol in plants is β-sitosterol which has been scientifically proven to lower blood LDL cholesterol by 10-15 % as part of a healthy diet [30].

3.4. ^{1}H-NMR Profile

For scientists, NMR spectroscopy is nowadays considered as one of the most important analytical tools. As shown in Figure 1, the ^{1}H-NMR spectra of *P. canariensis* oils recorded in the solvent $CDCl_3$ have similar spectral profiles. The chemical shifts of signals appeared in ^{1}H-NMR spectra are displayed in Figure 1. Most of these signals are assigned to non equivalent groups of protons that are common to fatty acyl chains. A peak was observed as a triplet (2.29- 2.39 ppm) related to α-carbonyl methylene protons. Other characteristic peaks were observed at (0.83 - 0.93 ppm) for terminal methyl protons, a strong signal at (1.22 - 1.40 ppm) related to methylene protons of the carbon chain, a signal at (1.56 - 1.67 ppm) representing β-carbonyl methylene protons and a signal at (5.30 - 5.42 ppm) due to olefinic hydrogen. The proton NMR data are in close agreement with the iodine values and the fatty acid compositions of *P. canariensis* seed oils.

3.5. UV/Vis Spectrophotometric Analyses

P. canariensis seed oils showed some absorbance in the UV-C (100-290 nm), UV-B (290-320 nm) and UV-A (320-400 nm) ranges (Figure 2). In the UV-B and the UV-A ranges, the wavelengths of ultraviolet light are responsible for the most cellular damage [22,23].

P. canariensis seed oils shield against UV-A and UV-B radiations as indicated by the absorbance at 290–400 nm (Figure 2). Thus, they may be used in the formulation of UV protectors which protect against both UV-A and UV-B. The optical transmission of *P. canariensis* seed oils, especially in the UV range (290-400 nm) was comparable to that of *Nigella sativa* [31] and titanium dioxide preparations which can be used as sun protection factors for UV-B (SPF) and UV-A (PFA).

Green pigments, particularly chlorophyll content, usually measured at 630, 670 and 710 nm were negligible (0.52 and 0.60 mg/Kg oil; Table 1). The low amount of green pigments does not impart undesirable color to the oil and may be unable to promote oil oxidation, especially in the presence of light. *P. canariensis* seed oils contained yellow colors, due partly to the presence of carotenoids (1.92 and 1.88 mg/Kg oil). The latters are beneficial, since they simulate the appearance of butter without the use of primary colorants such as carotenes and annattos, commonly used in the oil and fat industry [24].

3.6. Thermal Characteristics (DSC profile)

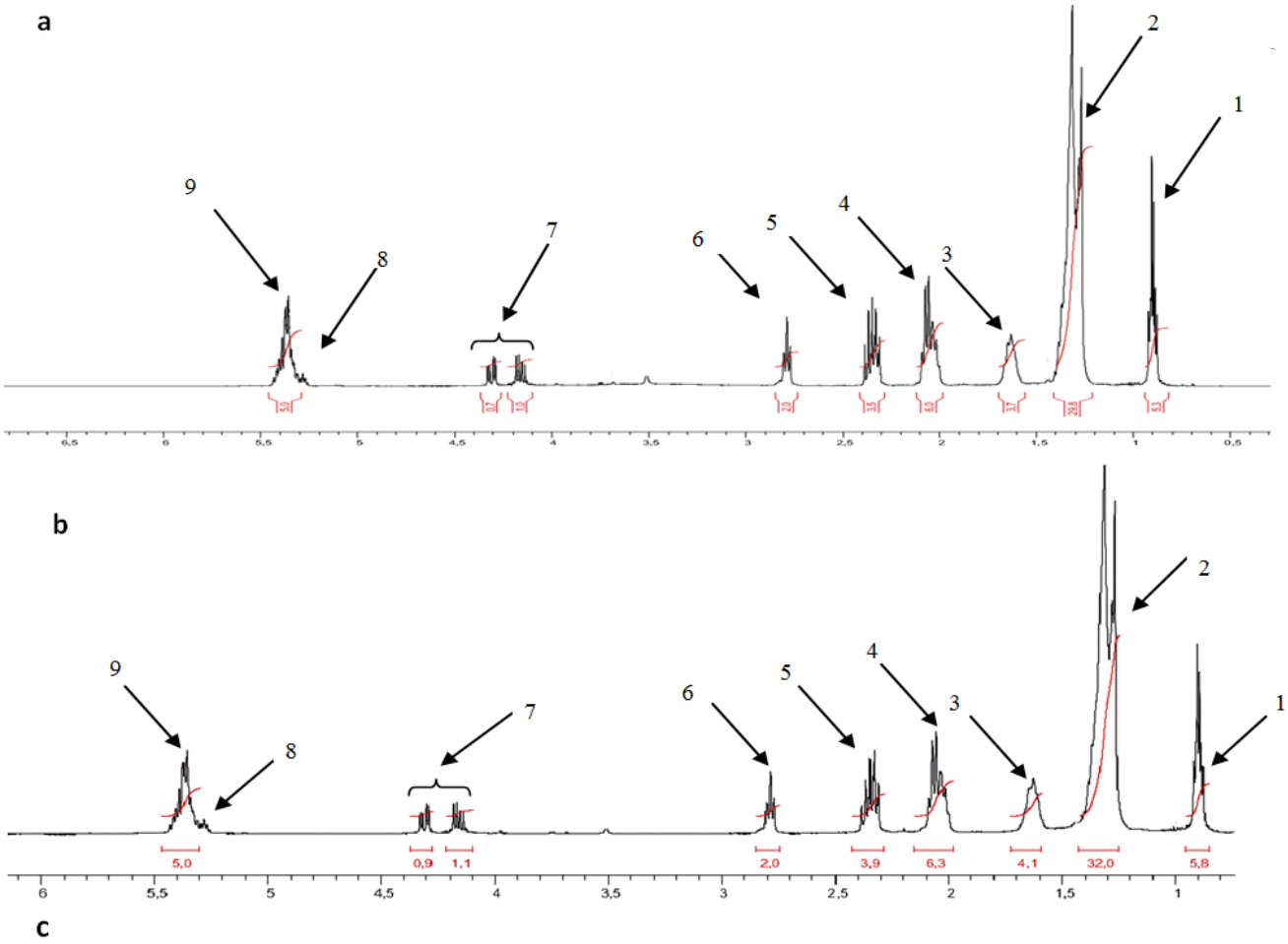
DSC provides information on the excess specific heat over a wide range of temperatures [22].

Any endothermic or exothermic events are registered as a peak in the chart, and its area is proportional to the enthalpy gained or lost, respectively. DSC curves (Figure 2)

for *P. canariensis* seed oils have similar profiles and showed two major peaks at -28.05°C with melting enthalpy 76.8 J/g (ultrasonication oil) and -27.47°C with enthalpy 62.3 J/g (maceration oil). These major peaks, due to PUFA presence, represented the melting triacyglycerols that pre-maturely melted. The DSC profile of *P. canariensis* oils was similar to that of Nigella seed oil [31]. The data obtained are useful to control fractionation of oil during production and may assist in identification of unknown seed oil samples.

3.7. Total Phenolic Content

Polyphenols are known for their antioxidant activity as radical scavengers and possible beneficial roles in human health, such as reducing the risk of cancer, cardiovascular diseases and other pathologies [32]. As illustrated in Table 4, the results showed a little difference in the total phenol content of the two *P. canariensis* oils. Their values (36.40 and 43.00) were higher than that of soybean oil cold-pressed (1.48) [33].



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Signal	Chemical shift (ppm)	Functional group
1	0.83-0.93	–C**H**$_3$ (satured oleic and linoleic acyl chains)
2	1.22-1.40	–(C**H**$_2$)n (acyl chains)
3	1.56-1.67	–O–CO–CH$_2$–C**H**$_2$– (acyl chains)
4	1.98-2.10	– C**H**$_2$–CH=CH– (acyl chains)
5	2.29-2.39	–O–CO–C**H**$_2$– (acyl chains)
6	2.76-2.81	C=CH–C**H**$_2$-CH=C (acyl chains)
7	4.13-4.32	–C**H**$_2$–OCOR (glycerol group)
8	5.25-5.30	C**H**–OCOR (glycerol group)
9	5.30-5.42	–C**H**=C**H**–(acyl chains)

Figure 1. a. ^{1}H-NMR spectrum of *P. canariensis* oil obtained with ultrasonication extraction; **b.** ^{1}H-NMR spectrum of *P. canariensis* oil obtained with maceration extraction; **c.** Chemical shifts (δ) and assignment of the main resonances in the ^{1}H NMR spectra of *P. canariensis* seed oils

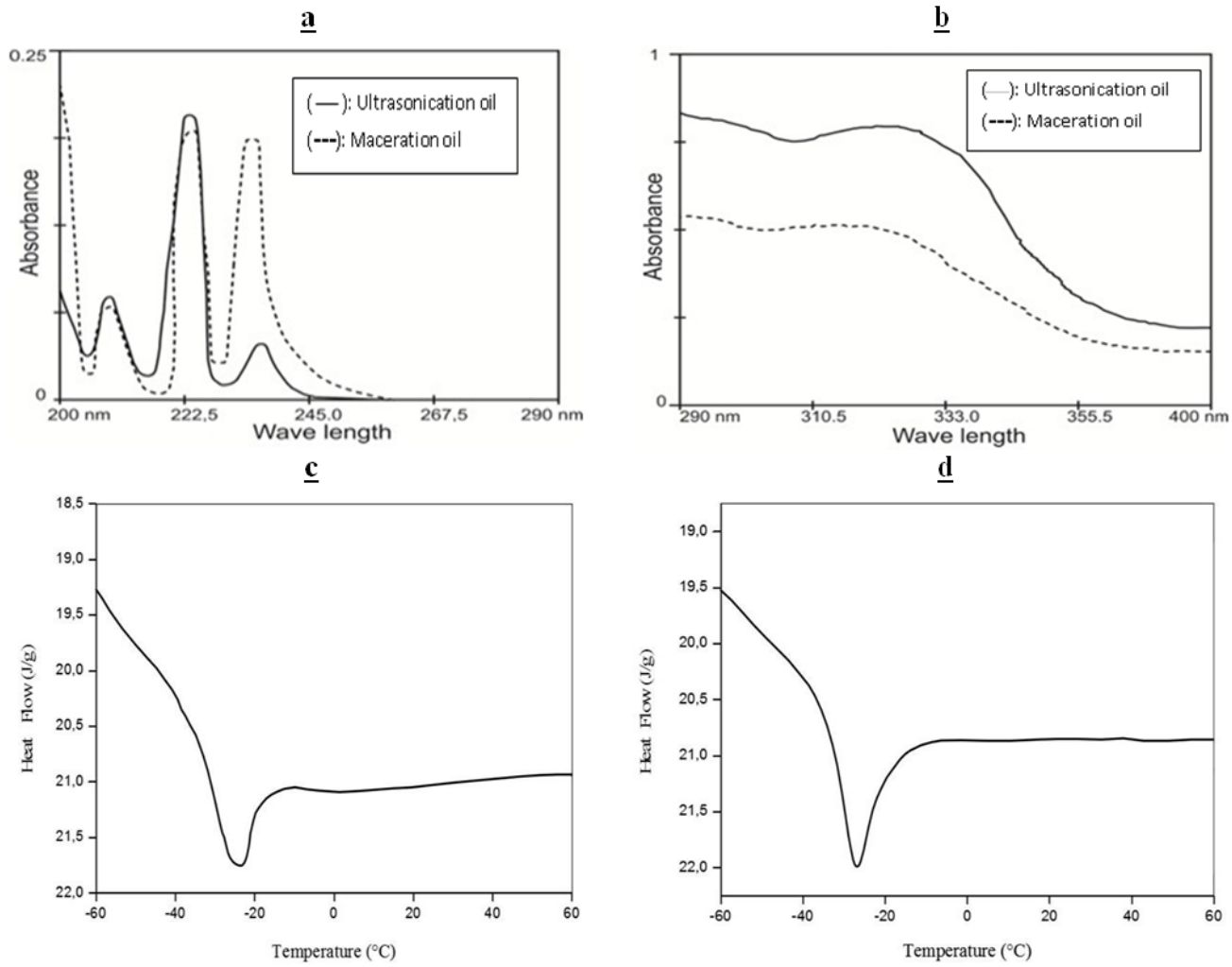

Figure 2. a. UV spectrum of *P. canariensis* seed oils: scans (λ= 200–290 nm) of oil diluted 1:1000 in hexane; **b.** UV spectrum of *P. canariensis* seed oils: scans (λ= 290–400 nm) of oil diluted 1:100 in hexane; **c.** DSC profile of ulrasonication oil of *P. canariensis;* ***d.*** DSC profile of maceration oil of *P. canariensis*

Table 4. Phenolic content, total antioxidant capacity (TAC), DPPH radical-scavenging assay and acetylcholinesterase inhibitory activity of *P. canariensis* seed oils

Extracts	Phenolic content (mg GAE/g extract)	TAC (mg antioxidant/g extract)	DPPH IC_{50} (µg/ml)	% AChE Inhibition
Ultrasonication oil	36.40 ± 0.72	59.80 ± 0.63	220.0 ± 1.10	55.4 ± 0.20
Maceration oil	43.00 ± 0.86	98.00 ± 0.98	610.00 ± 3.05	60.0 ± 0.71
BHT	-	-	26.00 ± 0.08	-
Vitamin E	-	-	17.00 ± 0.13	-
Tacrine	-	-	-	80.5 ± 0.10

Values expressed are means ± SD of three parallel measurements ($p<0.05$).

3.8. Total Antioxidant Capacity

CAT assays expressing different aspects of the antioxidant action and providing a broader view of the antioxidant potential of *P. canariensis* seed oils. The CAT of various oils of *P. canariensis* was evaluated by the phosphomolybdenum method and the results were given in Table 4. In the presence of antioxidant compound, Mo (VI) is reduced to Mo (V) by formation of a green phosphomolybdenum complex (V) which shows a maximal absorption at 695 nm. A high absorbance value indicated that the sample possesses a significant antioxidant activity. The antioxidant capacity value of maceration oil (98.0 mg antioxidant /g extract) was higher than that of ultrasonication oil (59.8 mg antioxidant/g extract). This result is in full agreement with the amount of polyphenols (Table 4). Thus, *P. canariensis* seed oils could be used as a natural antioxidant agent. This property may contribute to the reduction of the risk of chronic diseases such as cancer and cardiovascular disease and could be useful for the food drug industry [32].

3.9. DPPH Radical Scavenging Assay

Antioxidant activity in food and biological systems can be expressed in terms of radical scavenging ability using DPPH. DPPH assay is extensively used to evaluate the antioxidant capacity of many plant extracts. As shown in Table 4, the *P. canariensis* seed oils were able to reduce the stable radical DPPH to the yellow colored DPPH-H

with IC_{50} values of sonication oil (220.00 ± 1.10 µg/ mL) and maceration oil (610.00 ± 3.05 µg/ mL). BHT and vitamin E exhibited high antioxidant activity with IC_{50} values of 26.00 ± 0.08 µg/ mL and 17.00 ± 0.13 µg/ mL, respectively. The radical scavenging activity of these oils could be related to tocopherols and phenolic compounds, which contribute to their electron transfer/hydrogen donating ability [10].

3.10. Determination of Antibacterial Activity

The antibacterial activity of *P. canariensis* seed oils was evaluated with disc-diffusion assay method on several strains of bacteria including Listeria monocytogenes, Micrococcus luteus, Bacillus subtilis, Salmonella typhimurium, Salmonella sp, Escherichia coli and Enterococcus faecalis. The obtained results (Table 5) indicated that both oils of *P. canariensis* showed a moderate antibacterial activity only against Bacillus subtilitis and Salmonella sp. Meanwhile, it was observed that the antibacterial activity of ultrasonication oil was stronger than that of maceration oil. In general, the higher resistance of Gram-negative bacteria to external agents is due to the presence of lipopolysaccharides in their outer membranes, which make them inherently resistant to antibiotics, detergents and hydrophilic dyes [2]. The main mechanism reported for antimicrobial activity of plant extracts is due to the membrane disruption by phenolic compounds [35].

Table 5. Antibacterial activity of *P. canariensis* seed oils using agar disc

Strains	DD[a]	DD[b]	DD[c]
Bacterial strains Gram (+)			
Listeria monocytogenes	-	-	19 ± 1.06
Micrococcus luteus	-	-	22 ± 1.06
Bacillus subtilitis JN 934392	20± 1.03	15 ± 0.65	26.0 ± 0.6
Bacterial strains Gram (-)			
Salmonella typhimurium	-	-	18 ± 0.35
Salmonella sp	15 ± 0.45	12 ± 0.15	16 ± 0.70
Escherichia coli ATCC 2592	-	-	22 ± 0.8
Enterococcus faecalis	-	-	14 ± 0.25

Average ± SD were obtained from three parallel measurements ($p<0.05$).
[a]DD: Disc Diameter of inhibition (halo size) in (mm), oil obtained with ultrasonication 100 µg/disc.
[b]DD: Disc Diameter of inhibition (halo size) in (mm), oil obtained with maceration 100 µg/disc.
[c]DD: Disc Diameter of inhibition zone of ampicillin (10 µg/disc), was used as positive control for bacteria.
" - " : not active.

3.11. Acetylcholinesterase Enzyme Inhibitory Activity

Plants have been used traditionally to enhance cognitive function and to alleviate other symptoms associated nowadays with Alzheimer's disease [36]. The results as acetycholinesterase inhibitory activity of tested *P. canariensis* seed oils were resumed in Table 4. The AChE inhibition of oils was found to increase the dose-dependently, the results were expressed as percentage inhibition values. The ultrasonication and maceration oils showed to be able to inhibit 55.4 % and 60.0 % of AChE activity, respectively. Tacrine was used as a standard and it shows strong inhibition of acetylcholinesterase with 80.5 %. According to [37] AChE inhibition values higher than 50 % indicate potent inhibition. This inhibitory action of *P. canariensis* seed oils against acetylcholinesterase enzyme may be related to the presence of sterols, phenolic, and polyunsaturated fatty acid (PUFA) compounds in these oils. Indeed, sterols and phenolic compounds have been shown to possess anti-acetylcholinesterase properties [38]. Occasional studies have looked for effects of dietary PUFA on brain membrane-associated activities that influence neurotransmitter function, such as acetylcholinesterase [39]. Conceivably, dietary PUFA induced changes in membrane functions could modify the neurotransmission across affected synapses in visual circuits in the retina and/or brain and contribute to the observed visual changes [40]. In this study, in vitro inhibition of acetylcholinesterase by *P. canariensis* seed oils was reported for the first time. The results indicate that these oils may offer great potential for the treatment of Alzheimer Disease.

4. Conclusions

In this paper, a comparative study was made on seed oils of *P. canariensis* extracted using cold maceration and ultrasonication methods. Extensive physicochemical properties, chemical composition, antioxidant, antibacterial and acetylcholinesterase activities of these two oils were carried out for the first time. The results showed that *P. canariensis* is an important source of essential fatty acids, notably linoleic and oleic acids. However, these two polyunsaturated fatty acids, beneficial for the human body, are very requested in vegetable oils. Regarding the results found herein, *P. canariensis* seed oils from Tunisia could be used in different fields such as nutrition, cosmetics, pharmaceutical and paint products.

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List of Abbreviations

AChE, acetylcholinesterase enzyme; ASA, American soybean association; BHT, butylated hydroxytoluene; CAT, total antioxidant capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DSC, differential scanning calorimeter; FAME, fatty acid methyl esters; GC, gas chromatography; ^{1}H-NMR, *proton* nuclear magnetic resonance; IOC, international olive council; IUPAC, international union of pure and applied chemistry; MUFA, monounsaturated fatty acids; ω, omega; PUFA, polyunsaturated fatty acids; SAFA, saturated fatty acids; UV/Vis, ultraviolet/visible.

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