

Chemical Composition of Cactus Pear Seed Oil: phenolics identification and antioxidant activity

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Received April 26, 2021
Reviewed March 13, 2022
Accepted April 18, 2022

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Objectives: The chemical composition of cactus pear seed oil (*Opuntia ficus-indica* [L.] Mill.) was analyzed in terms of its fatty acid composition, tocopherol content, phenolic identification, and the oil's phenolic-rich fraction antioxidant power was determined.

Methods: Fatty acid profiling was performed by gas chromatography coupled to an FI detector. Tocopherols and phenolic compounds were analyzed by LC-FLD/UV, and the oil's phenolic-rich fraction antioxidant power was determined by phosphomolybdenum, DPPH assay and β -carotene bleaching test.

Results: Fatty acid composition was marked by a high unsaturation level ($83.22 \pm 0.34\%$). The predominant fatty acid was linoleic acid ($66.79 \pm 0.78\%$), followed by oleic acid ($15.16 \pm 0.42\%$) and palmitic acid ($12.70 \pm 0.03\%$). The main tocopherol was γ -tocopherol (172.59 ± 7.59 mg/kg). In addition, Tyrosol, vanillic acid, vanillin, ferulic acid, pinoresinol, and cinnamic acid were identified as phenolic compounds in the analyzed seed oil. Moreover, the oil's phenolics-rich fraction showed a significant total antioxidant activity, scavenged DPPH up to 97.85%, and effectively protected β -carotene against bleaching (97.56%).

Conclusion: The results support the potential use of cactus pear seed oil as a functional food.

Keywords: cactus pear, seed oil, tocopherols, phenolics-rich fraction, *opuntia ficus-indica*, antioxidant activity

INTRODUCTION

Cactus pear (*Opuntia ficus-indica* [L.] Mill.), belonging to Cactaceae originating from Mexico, is now a widespread species in Mediterranean countries. In Morocco, nopals are consumed for recreative and therapeutic purposes giving them a high scientific, social, and economic value.

Cactus pear seeds (CPS) are part of the fruit, and they are considered a potent source of bioactive compounds. For instance, many investigators contributed to CPS characterization. The oil content varies between 10% and 13% of the seed

weight [1, 2]. The first attempt at cactus pear seed oil (CPSO) characterization was performed by Sawaya and Khan [1], reporting a high unsaturated fatty acid content (82%). In another study, it has been reported that linoleic acid is the major fatty acid in CPSO, and palmitic acid is the main saturated fatty acid [3]. Furthermore, El Mannoubi et al. reported that CPSO contains, in addition to α -tocopherol and δ -tocopherol, high amounts of γ -tocopherol [4]. A comparative study of the seeds' phenolic content at two different stages of fruit ripening has been reported by Saïdani's team [5]. Furthermore, Chougui et al. reported that cactus pear defatted seed extract is charac-

terized by a complex profile of phenolic compounds using a combined approach of liquid chromatography coupled to mass spectrometry and nuclear magnetic resonance [6]. A recent study outlined the presence of aroma compounds in CPSO using olfactometric and dynamic Headspace-GC/MS [7].

The present work aims to characterize the Moroccan CPSO composition in terms of fatty acids, tocopherols, and phenolic compounds using GC/FID and LC/FLD/UV and in addition assessing the antioxidant power of its polyphenolics-rich fraction.

MATERIALS AND METHODS

1. Chemicals

All solvents used were HPLC-analytical grade and purchased from Sigma-Aldrich, Germany. Standard phenolic compounds used are cinnamic acid, vanillic acid, sinapic acid, coumaric acid, pinoresinol, ferulic acid, tyrosol, apigenin, syringic acid, 3,4-dihydroxyhydrocinnamic acid, vanillin, luteolin, 3-(4-hydrophenyl) lactic acid, quinic acid and 1,3-dihydroxynaphthalene (1,3-DHN) and were analytical grade purchased from Sigma-Aldrich. The fatty acid methyl-ester standard was a 37-fatty acid mixture (Supelco® 37 Component FAME Mix, Sigma Aldrich), and the tocopherol standard was a mixture of α - β - γ - δ -tocopherol (Tocopherols-mixed, FCC, FG, Sigma-Aldrich).

2. Plant material

CPSO was freely provided by the Argan Oil Company, Casablanca, Morocco. *Opuntia ficus-indica* (L.) Mill. seeds were separated from fresh fruit collected in the summer period (June-August). Separated seeds were dried at room temperature and then cold-pressed using an oil extraction machine, avoiding any use of a solvent system. Extracted seed oil was bottled in a 40 mL glass bottle and stored in the dark at 6°C under a nitrogen cushion until use.

3. Fatty acid methyl ester identification

Fatty acid methyl ester analysis was implemented based on the method described by Moumen et al. [8]. Fatty acid methyl esters (FAMES) were obtained by transesterification of 10 mg of CPSO in boron trifluoride (10% in methanol) by heating at 70°C for 90 min.

FAMES obtained were identified using a HP6890 Series-GC (Germany) coupled to a flame ionization detector (FID).

Prepared samples (0.5 μ L) were injected in a 30 m \times 250 μ m \times 0.25 μ m Factor Four VF-Wax MS capillary column (Agilent P/N: CP9205) using an automatic injection system. The obtained signals were integrated using an Agilent ChemStation GC system. A comparison of obtained peaks was made with an authentic FAMES standard solution (10 mg/mL) that was run on the same column with the same conditions. The quantification of identified FAMES was computed automatically by a software signal integrator.

4. Tocopherol analysis

Fat-soluble tocopherols were identified and quantified by using a normal phase high-performance liquid chromatography (NP-HPLC) to bypass the saponification step.

Two-fold CPSO dilution in hexane was injected (10 μ L) in a 150 \times 3.05 mm i.d. UPTISPHERE NH₂ 3 μ m column (INTERCHROM, USA) using an Agilent 1200 (Agilent Technologies, USA) equipped with an automatic sample injector.

The chromatographic separation was made by isocratic elution mode, where the solvent (hexane/isopropanol, 99:1; v/v) flow rate was sustained at 0.5 mLmin⁻¹ during an elution time of 25 min. Peak detection was made by use of a fluorescence detector (FLD) at excitation/emission wavelengths of 298 nm and 325 nm, respectively. Data were collected using the Agilent Chem Station LC system software.

Chromatogram peaks were identified by comparison with a freshly prepared standard tocopherol mixture, and the quantification of identified tocopherols was made using a standard curve of tocopherols.

5. Phenolic compound analysis

1) Phenolic compound extraction

Phenol extraction was performed using a liquid/liquid mode according to the method described by Brenes et al. [9]. One gram of CPSO was mixed with 1 mL methanol 80%, vortexed for 10 min, and centrifuged at 3,800 rpm for 15 min. The supernatant was pipetted into a volumetric flask, and the extraction was performed three times per sample. The final volume obtained was made up to 5 mL and stored at 4°C. The extract was analyzed on the same day of the extraction.

2) Total phenol determination

Total phenolic compound determination was performed as per the method described by Ainsworth and Gillespie [10] with some modifications. In summary, 5 mL of sodium carbonate solution (10%) was added to a mixture of CPSO phenolic extract/Folin-Ciocalteu reagent (1/1 mL). Tubes were agitated and incubated in the dark at room temperature for 60 min. Absorbance was measured against the blank at 750 nm using an UV-Visible spectrophotometer (Ultra Spec 400, Pharmacia Biotech, England). Caffeic acid was used as a standard, and results were expressed as mg equivalent of caffeic acid per kg of oil.

3) Reverse phase high-performance liquid chromatography (RP-HPLC) phenol analysis

RP-HPLC for phenolic compound characterization was performed using the Agilent 1200 automatic-injection apparatus (Agilent Technologies, USA) coupled to a UV detector.

The phenolic extract of CPSO (10 μ L) was injected into a ZORBAX Eclipse XDB-C18 (3.5 μ m porosity, size 150 mm \times 4.6 mm, Agilent Technologies, USA) LC Column and eluted by solvent elution gradient of solution A (S_A = formic acid 1% in distilled water) and solution B (S_B = formic acid 1% in methanol) with a constant flow rate of 0.8 mL/min over 34 min. Elution's gradient was initially 100% S_A for 7 min, and S_B was integrated with a linear gradient 65:35 after 7 min, 55:45 after 12 min, 50:50 after 17 min, 40:60 after 20 min, and finally 5:95 after 25 min. Analyses were detected at 280 nm wavelength, and data were analyzed using Agilent Chem Station LC system software. CPSO phenolic compounds were identified by comparing the obtained peaks with those of freshly prepared mixed phenolic standards dissolved in 80% methanol. However, identified compounds were quantified using the mean of the internal standard (1–3 DHN), which was selected after a primary analysis.

6. Antioxidant activity of CPSO phenolic extract

1) Total antioxidant capacity

The total antioxidant effect of COPE phenolic extract was evaluated by the phosphomolybdenum test based on the protocol described by Kumaran and Joel Karunakaran [11]. The experiment quantifies the reduction of Mo (VI)-Mo (V) by COPE through a green phosphate/Mo (V) complexation at acidic pH.

COPE (0.3 mL) was mixed with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM Ammonium molybdate). Tubes were incubated at 95°C for 90 min.

After cooling to room temperature, absorbance was assessed at 695 nm against a blank tube (0.3 mL of methanol in the place of extract) using a JENWAY 6300 spectrophotometer, United Kingdom.

The antioxidant activity was expressed as mg.kg^{-1} equivalents of ascorbic acid using an ascorbic acid standard curve.

2) DPPH scavenging activity

The DPPH scavenging assay was performed according to the method described by Liu et al. [12] with some modifications. One milliliter of methanolic solution of DPPH 0.001% was added to 1.5 mL of COPE (16.6%, 10%, 3.3%, 1.6% and 1% v/v). The mixture was vortexed, and the absorbance was immediately determined using a spectrophotometer (JENWAY 6300, United Kingdom) at 517 nm. The absorbance was monitored at 30 min intervals during 90 min of incubation at room temperature in the dark. Ascorbic acid, a stable synthetic antioxidant, was used as a standard. All assays were performed in triplicate. The scavenging activity of the samples, expressed as DPPH scavenging percentage, was calculated according to the following formula:

$$\text{DPPH Scavenging percentage (\% S)} = 100[(A_B - A_S)/A_B]$$

Where A_B and A_S are the absorbance of blank and tested samples, respectively, at the measuring time.

3) Beta-carotene bleaching test

The antioxidant activity of COPE was evaluated using the β -carotene/linoleic acid test according to the protocol reported by Sahreen et al. [13] with minor modifications. In brief, 2 mg of β -carotene was solubilized in chloroform (10 mL) containing 20 mg of linoleic acid and 200 mg of tween 20. Chloroform was evaporated at 40°C after approximately 5 min of rotary evaporation. Therefore, 100 mL of distilled water was added, with careful shaking, to the dry β -carotene/linoleic acid emulsion. β -carotene aqueous solution (2.5 mL) was added to 0.5 mL of a serial dilution of COPE (16.6%, 10%, 3.3%, 1.6% and 1% v/v). The blank tube contained all reagents except β -carotene. Reaction tubes were incubated in a shaking water bath at 50°C and 150 rpm. β -carotene absorbance was monitored at 0, 30, 60, 90, and 120 min at 470 nm using a spectrophotometer (JENWAY 6300, United Kingdom). Ascorbic acid was used as a positive control. All measurements were performed in triplicate. The β -carotene protection percentage was calculated according to the formula:

$$\beta\text{-carotene protection (\% P)} = 100 (A_t/A_0)$$

Where A_t and A_0 are the absorbance of the tested sample at 120 min and 0 min, respectively.

7. Statistical analysis

All assays were carried out in triplicate, and descriptive statistics were expressed as mean \pm standard deviation (SD).

RESULTS

1. Fatty acid methyl ester determination

The fatty acid content in CPSO is reported in Table 1. The oil content was mainly characterized by the presence of unsaturated fatty acids (83%), including both monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs), resulting in an unsaturated/saturated ratio of 5. Linoleic acid (18:2) was the major fatty acid in CPSO (66%), followed by oleic acid (18:1)

Table 1. Fatty acid methyl esters content of cactus pear seed oil processed using gas chromatography coupled to flame ionization detector during 25 min of running time

Fatty acid	Relative content (%)
14: 0	0.094 \pm 0.006
16: 0	12.709 \pm 0.034
16: 1	0.607 \pm 0.020
18: 0	3.360 \pm 0.225
18: 1	15.160 \pm 0.425
18: 2	66.790 \pm 0.782
18: 3	0.268 \pm 0.012
20: 0	0.320 \pm 0.042
20: 1	0.208 \pm 0.017
22: 0	0.184 \pm 0.034
22: 1	0.183 \pm 0.025
24: 0	0.115 \pm 0.030
Σ UFA ^a	83.220 \pm 0.346
Σ SFA ^b	16.78 \pm 0.346
U/S ^c	4.960 \pm 0.124

Results are expressed as mean \pm SD (n = 3). ^aTotal content of unsaturated fatty acids in cactus pear seed oil. ^bTotal content of saturated fatty acids in cactus pear seed oil. ^cRatio of unsaturated to saturated fatty acids.

(15%). However, the α -linolenic acid (18:3) amount in CPSO was less than 0.3%.

2. Tocopherol analysis

The tocopherol content of CPSO was analyzed by normal phase high-performance chromatography (NP-HPLC) to maintain the stability of the tocopherol [14]. The tocopherol oil content is presented in Table 2. Total vitamin E in the analyzed CPSO was 180.42 \pm 7.68 mg/kg of oil. In the CPSO sample, γ -tocopherol was the major tocopherol in CPSO (172.59 \pm 7.59 mg/kg), succeeded by α -tocopherol (6.21 \pm 0.07 mg/kg), and a small amount of δ -tocopherol was detected (1.61 \pm 0.04 mg/kg). However, β -tocopherol was not detected in our sample.

3. Phenolic compound analysis

1) Total phenols

The total phenolic compound content of CPSO was assessed by the Folin–Ciocalteu method. Results showed that CPSO contains 316.0 \pm 19.5 mg equivalent of caffeic acid/kg of oil.

2) Identification of phenolic compounds

Phenolic compounds in CPSO were identified by RP-HPLC. The HPLC profile of the hydro-alcoholic extract of CPSO revealed a high diversity in the phenolic profile (Fig. 1).

The identification of the phenolic compounds was determined by referencing to retention times of standard phenols eluted under the same conditions (Fig. 1). The first peak corresponded to tyrosol, which was released at 9.57 min followed by vanillic acid at 10.95 min. Vanillin was the third identified compound at 11.95 min, and ferulic acid was released at 13.91 min. Pinoresinol and cinnamic acid were the last identified peaks at 19.20 and 22.11 min, respectively. However, this study did not

Table 2. Levels of fat-soluble tocopherols in cactus pear seed oil processed using normal phase high performance liquid chromatography during 25 min of elution time

Tocopherol	Concentration (mg/kg oil)
α -tocopherol	6.212 \pm 0.078
β -tocopherol	nd ^a
γ -tocopherol	172.597 \pm 7.594
δ -tocopherol	1.613 \pm 0.043
Total vitamin E	180.423 \pm 7.681

Results are expressed as mean \pm SD (n = 3). ^aNot detected.

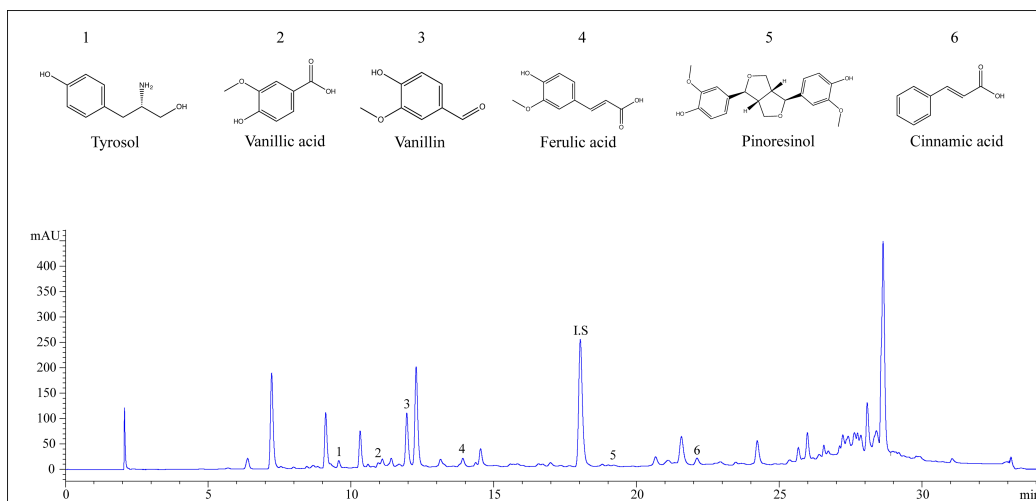


Figure 1. Typical chromatogram of cactus pear seed oil's phenolic compounds identified using a reverse phase high performance liquid chromatography during 30 min of elution time. 1: Tyrosol; 2: Vanillic acid; 3: Vanillin; 4: Ferulic acid; 5: Pinosresinol; 6: Cinnamic acid; I.S., Internal standard (1,3-Dihydroxynaphtalene).

Table 3. Levels of phenolic compounds in cactus pear seed oil identified using reverse phase high performance liquid chromatography during 34 min of elution time

Peak	Rt (min)	Phenolic compound	Concentration (mg/kg of oil)
1	9.57	Tyrosol	6.70 ± 0.08
2	10.95	Vanillic acid	1.70 ± 0.06
3	11.95	Vanillin	8.16 ± 0.05
4	13.91	Ferulic acid	2.99 ± 0.05
5	19.20	Pinosresinol	2.09 ± 0.07
6	22.11	Cinnamic acid	1.36 ± 0.03

Results are expressed as mean ± SD (n = 3).

identify major peaks because they did not correspond to the used phenolic standards. Quantification of identified phenols was determined using the internal standard calibration method, and the amounts of identified phenolic compounds were calculated by the relative response factor to the internal standard (1,3 dihydroxynaphtalene). Table 3 shows the amount of identified phenols in CPSO. Vanillin was the most identified phenol (8.16 ± 0.05 mg/kg) followed by tyrosol (6.70 ± 0.08 mg/kg). Cinnamic acid was the least identified compound (1.36 ± 0.03 mg/kg). The amounts of vanillic acid, pinosresinol, and ferulic acid were 1.69 ± 0.06, 2.09 ± 0.07, and 2.99 ± 0.05, respectively.

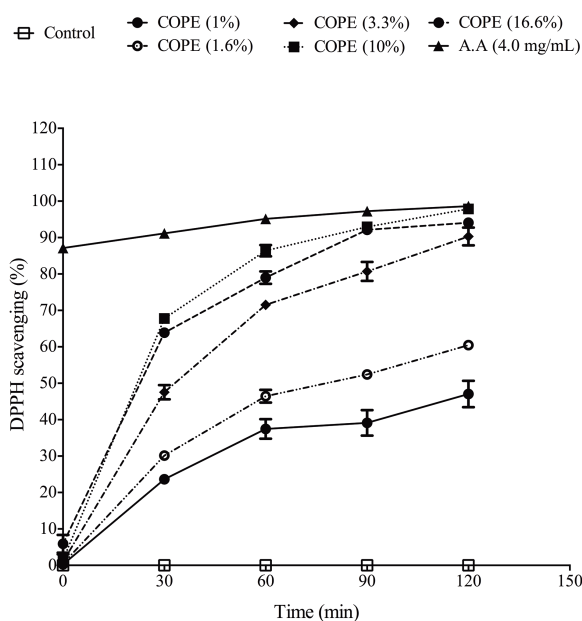


Figure 2. Antioxidant activity of cactus pear seed oil phenolic extract (COPE) at different concentration assessed by DPPH scavenging during 120 min incubation. Ascorbic acid (A.A) was used as standard antioxidant.

4. Antioxidant activity of the phenolic-rich fraction

The total antioxidant capacity of cactus pear seed oil's phenolic extract was assessed using the phosphomolybdenum method. This assay was quantitative, and results are expressed as mg equivalent of ascorbic acid. Results showed that phenolic

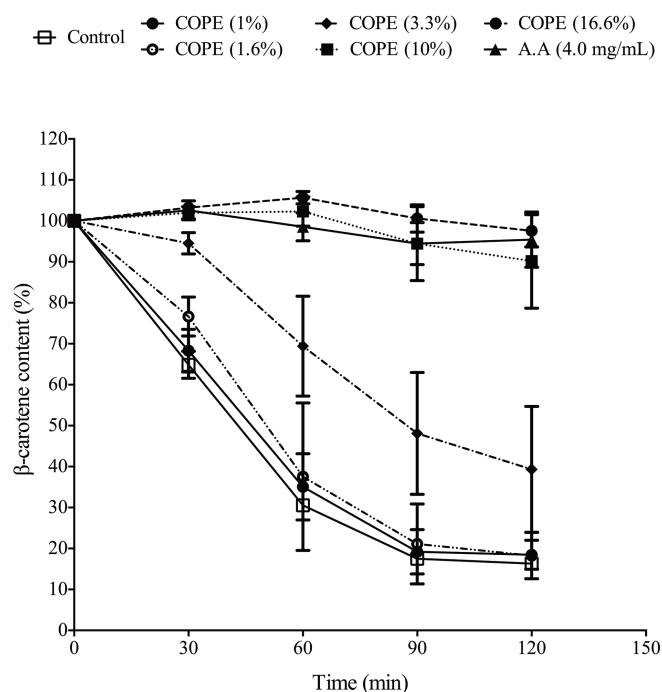


Figure 3. Antioxidant activity of cactus pear seed oil phenolic extract (COPE) at different concentration assessed by the β -carotene bleaching test scavenging 120 min incubation. Ascorbic acid (A.A) was used as standard antioxidant.

extract of cactus seed oil contains 334.80 ± 10.21 mg eq A.A/kg of oil.

The phenolic extract of cactus pear seed oil showed a scavenging capacity toward DPPH up to $97.86 \pm 0.26\%$ (Fig. 2). The IC_{50COPE} value, calculated from a regression curve, was 1.14% v/v.

The percentage of β -carotene protection by COPE is presented in Fig. 3. The β -carotene protection was dose-dependent, and the highest dose of COPE exhibited $97.57 \pm 2.92\%$ of protection after the incubation time (120 min). The $IC_{50COPE} = 4.45\%$ v/v. Ascorbic acid protected $95.43 \pm 3.89\%$ of the β -carotene tube contents.

DISCUSSION

1. Fatty acid methyl ester determination

Recorded CPSO fatty acid content agrees with the results found in German, Algerian and Tunisian cactus pear seed oils [2, 3, 6]. Tested oil linoleic acid and oleic acid content is less than those reported by Ennouri et al. [2] and higher than those found by Ramadan and Mörsel [3]. However, Chougui et al. [6] and Labuschagne and Hugo [15] reported the same amounts

of linoleic and oleic acids. The α -linolenic acid (18:3) in CPSO is less than 0.3%, inferior to the amount reported by Ramadan and Mörsel [3]. The difference in the fatty acid content in CPSO may be due to the habitat effect and/or the degree of fruit maturation [16]. Linoleic acid (ω -6) and α -linolenic (ω -3) are essential fatty acids that are fundamental for human structural and metabolic functions. Hartley demonstrated that ω -6 fatty acids may be significantly involved in the primary prevention of cardiovascular diseases [17], and Martins et al. reported that ω -6 could also protect gastric mucosa against ulceration [18]. In addition, oleic acid (ω -9) is the major monounsaturated fatty acid in CPSO. In a review paper, it has been reported that oleic acid could be beneficial against cancer, autoimmune diseases, and inflammation [19].

2. Tocopherols

The major tocopherol in CPSO is γ -tocopherol with no presence of β -tocopherol. Compared to our findings, previous studies reported higher values of tocopherol proportions in CPSO [3, 4]. However, another study showed that the tocopherol content in CPSO ranges from between 39 mg/kg and 500 mg/kg [20]. The same qualitative findings were reported previously [3].

Tocopherols are critical compounds of the unsaponifiable matter of vegetable oils significantly involved in preserving oil stability. However, even though tocopherols have antioxidant activity, it was reported that α -tocopherol could have a pro-oxidant effect in vegetable oil [21]. The antioxidant effect of tocopherols within vegetable oils was not correlated with high tocopherol content but instead related to the oil's optimal levels of tocopherols [22].

Tocopherols are cellular antioxidants involved in maintaining redox homeostasis [23]. Additionally, it was reported that γ -tocopherol could attenuate inflammatory events in a rat model via decreasing systemic levels of pro-inflammatory eicosanoids [24].

3. Phenolic compounds

This work embodies a contribution to CPSO phenolic compound profiling by dosing total phenols followed by qualitative and quantitative profiling using HPLC. All previous works reported a variable total phenolic content in seeds. Tunisian cactus pear seeds contain up to 328 mg rutin equivalent/100 g dry

weight [25]. However, total phenolic content in Algerian cactus pear seeds varied from 480 mg to 890 mg equivalents of gallic acid/kg of defatted seeds [6]. These variances could be due to the cultivar's type, the fruit's geographical origin, the degree of ripening, storage conditions, extraction protocol, and also to the analytical method.

Phenolic compounds are secondary metabolites in plants with no nutritive value but are critical for allelopathic interaction and plant protection [26, 27]. In edible oils, phenolics and more specifically cinnamic acids prevent fatty acid oxidation, protecting the oil from becoming rancid [28, 29].

In this work, we were able to identify six phenolic compounds without the identification of major HPLC profile pics. Here we report the presence of tyrosol, vanillic acid, vanillin, ferulic acid, pinoselinol, and cinnamic acid.

Phenolic compounds are also involved in preventive and curative effects against human diseases. For instance, vanillin was a potent antioxidant, antimetastatic, and anti UV β [30-32] and tyrosol has a neuroprotective effect [33, 34]. Moreover, ferulic acid was a hypocholesterolemic, antithrombotic and antiatherosclerotic agent [35]. Vanillic acid and cinnamic acid have been shown to be an antioxidant [36], antihyperglycemic, cardioprotective and antitumoral [37-40].

4. Antioxidant effect

In the present work, CPSO phenolic extract has been used to assess the total antioxidant capacity using phosphomolybdenum and free radical scavenging power using DPPH and β -carotene bleaching tests.

DPPH is routinely used to evaluate the free radical scavenging capacity of chemical compounds. This assay enlightens the effect of the antioxidant molecules toward a stable free radical through a known mechanism involving a hydrogen atom transfer coupled to a fast electron transfer. Phenolic compounds scavenge DPPH by their ability to form o-quinone intermediates upon free radical H-atom abstraction and its subsequent disproportionation [41]. The β -carotene bleaching assays are also useful for assessing the antioxidant capacity of natural extracts. However, this assay is polarity-dependent and less applicable for non-polar extract [42]. This assay is based on the capacity of antioxidant molecules to scavenge linoleic acid peroxide (LOO \bullet) produced during the incubation at 50°C [43].

Vegetable oils are exposed to rancidity through fatty acid oxidation. The presence of phenolic compounds in vegetable

oil prevents this autoxidation, consequently preventing oil rancidity. The mechanism behind the improved oil's stability by phenolic compounds involves the hydrogen atom donating to lipid radicals [28, 44]. On the other hand, phenolic compounds in vegetable oils are also interesting because of their health effects. Accordingly, phenolic compounds could prevent the development and progression of coronaropathies, diabetes mellitus, and cancer [45]. Benayad et al. reported that phenolic compounds extracted from *Opuntia ficus-indica* flowers had antioxidant and anti-inflammatory effects evaluated using the ORAC technique and the RAW 264.7 macrophage cell line (NO production), respectively [46].

CONCLUSION

The present work reports the chemical composition of CPSO, shedding light on the phenolic content by identifying six phenolic compounds. This promotes the potential use of CPSO as a nutraceutical and functional food. However, more advanced studies should be carried out to explore the composition of CPSO accurately.

ACKNOWLEDGEMENT

Authors are thankful to Richard Gaëton for its meticulous care of HPLC machine.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This work has been supported by a scholarship from Académie de Recherche et d'Enseignement Supérieur - Commission de la Coopération au Développement - Wallonie Bruxelles (ARES-CCD) for supporting this work by a scholarship.

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