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Supercritical CO_2 Extracts and Volatile Oil of Basil (*Ocimum basilicum* L.) Comparison with Conventional Methods

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Abstract: Interest in new products from aromatic plants as medical and nutritional compounds is increasing. The aim of this work was to apply different extraction methods, including the use of supercritical carbon dioxide extraction, and to test the antioxidant activity of basil (*Ocimum basilicum* L.) extracts. In vitro efficacy assessments were performed using enzymatic assays. Essential oil obtained by hydrodistillation and volatile oil obtained from supercritical fluid extraction were analyzed by gas chromatography to quantify components. The total phenolic content in the extracts ranged from 35.5 ± 2.9 to 85.3 ± 8.6 mg of gallic acid equivalents and the total flavonoid content ranged from 35.5 ± 2.9 to 93.3 ± 3.9 micromole catechin equivalents per gram of dry weight of extract. All the extracts showed an antioxidant activity with 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), and the reducing power test. Extracts obtained from methanol had a higher antioxidant capacity per the DPPH test results (IC_{50} = 3.05 ± 0.36 mg/mL) and the reducing power test assay 306.8 ± 21.8 µmol of trolox equivalents per gram of extract (TE/g) compared with ethanolic or supercritical fluid extracts. However, using the ABTS assay, the extract obtained by supercritical fluid extraction had a higher antioxidant capacity with an IC_{50} of 1.74 ± 0.05 mg/mL. Finally, the examined extracts showed practically no acetylcholinesterase (AChE) inhibitory capacity and a slight inhibitory activity against tyrosinase.

Keywords: *Ocimum basilicum*; supercritical fluid extraction; phenolic and flavonoids content; antioxidant activity; in vitro efficacy tests

1. Introduction

Basil is an aromatic plant belonging to the Lamiacecae family, used as a culinary herb and for ornamental purposes. The genus *Ocimum* contains between 50 to 150 species of plants and is found in tropical regions of Asia, Africa, and South and Central America [1–9]. Basil has been used in traditional

medicine for treating problems like headaches, coughing, diarrhea, poor kidney function, and warts. Many authors have referenced basil as a medicinal plant. Basil extract and essential oil from various parts including the leaves, flowers, and roots, have been used to determine antimicrobial, antioxidant, anticancer, antidiabetic, anti-inflammatory, analgesic, sedative, and hypoglycemic activities [1,5,6,9–15], or to estimate the cost of manufacturing the extracts [16].

Consumer preference for natural products has increased. Consequently, the interest in new antioxidant sources, and aromatic herbs in particular, has also increased [17–19]. Other vegetable extracts have exhibited important activities [20–22]. Herbs can be applied as natural food preservatives to prevent the deterioration of foodstuff quality that occurs during processing and storage, mainly due to oxidative processes. Extraction techniques aim to not only extract active biocompounds from herb samples [23], but also to increase the concentration of the compound of interest. Classical extraction techniques are generally based on the extractive potential of various solvents, using heating or mixing. The disadvantages of conventional extraction techniques include the need for expensive purity solvents, long extraction times, and evaporation of significant amounts of solvents, low selectivity, and potential decomposition of thermolabile compounds [24,25]. These problems can be solved by using other extraction techniques, such as green techniques. Usually, green extraction methods use safer solvents and less toxic chemicals. Particularly, the use of supercritical fluids in the extraction of volatile oils and extracts has increased since 2000 due to the expected advantages of the supercritical extraction process. These processes have received increased attention for the production of high-value plant extracts for the pharmaceutical, cosmetics, and food industries [26–28]. Specifically, supercritical fluid extraction is considered a simple, rapid, selective, and convenient method. Supercritical fluid extraction is also a solvent-free and environmentally-friendly sample pretreatment technique. In this study, a number of factors that influence extraction yields of basil extracts were studied and a comparison with conventional extraction techniques was completed.

2. Materials and Methods

2.1. Plant Material

Ocimum basilicum L. was purchased from a commercial supplier in Portugal in March 2016. Only the aerial parts of the plant leaves and flowers were used for these experiments. Extractions with fresh plants were performed in the first three days after acquiring the plant. The remaining plants were dried in an oven (LSIS-B2V/VC111 1900 W, Cejl, Czech Republic) at 40 °C for 72 h and stored in vacuum packages at –18 °C. The flowers and leaves were ground (IKA WERKE-M20, Staufen, Germany) at low temperatures to avoid loss and thermal degradation of secondary metabolites. A set of standard sieves was used to determine the particle size distribution.

2.2. Supercritical Fluid Extraction and Conventional Extractions

To obtain the volatile oil, supercritical fluid extraction (SFE) with carbon dioxide (CO_2) and 80 g basil with a 0.6 mm mean particle size was performed for 2.5 h, at a flow rate of 1.0 kg/h CO_2, at 313 K and 9.0 MPa, in a flow apparatus using a two-stage fractional separation technique [29,30]. Separation was performed at 7.0 MPa, 263 K in the first separator, whereby waxes were mainly collected. A second separation was performed at 2.0 MPa and 273 K, from which the volatile oil (SFEO) was obtained. The amount of volatile oil obtained was assessed gravimetrically with an uncertainty of ±0.1 mg. Purity CO_2 (99.995%) was supplied by Air Liquide (Lisboa, Portugal). Due to this SFE equipment limitations (maximum pressure of 30.0 MPa) and to obtain basil extracts at higher pressures, SFE experiments were carried out in a flow apparatus from Applied Separations, SpeedTM SFE, which allows extraction to be performed at temperatures up to 393.2 K and pressures up to 60.0 MPa [31–33].

SFE was completed using 15 g samples of basil with a 0.6 mm mean particle size. The conditions of extraction were as follows: a CO_2 flow rate of 0.10 kg/h, pressure of 40.0 MPa, and a temperature of 313 K. The supercritical carbon dioxide extracts (SFEE) were collected in a separator at atmospheric pressure.

For comparison, conventional hydrodistillation was conducted in a Clevenger-type apparatus for 2.5 h, using 40 g of dry plant material, with the same particle size as that used in the supercritical extraction conditions. This essential oil (hydrodistillation with dried plants, HDD) was compared with that obtained by hydrodistillation with fresh plants (HDF). The basil extracts, by conventional extraction, were isolated by Soxhlet. A total of 20 g of basil with a particle size of 0.6 mm were extracted either with 250 mL of methanol (MeOH) (Sigma Aldrich 99.8%, Steinheim, Germany) or with ethanol (EtOH) (Panreac 99.5%, Barcelona, Spain) for 3 h at the solvent boiling point, siphoning at least five times per hour. The extract was filtered, the solvent was removed by reduced pressure evaporation in a rotary evaporator (Büchi, model R-205, Flawil, Switzerland), and the residue was dried to constant weight.

2.3. Gas Chromatography and Gas Chromatography-Mass Spectrometry Analysis

Essential oil, SFE volatile oil, and waxes were analyzed by gas chromatography (GC), for component quantification, using a Perkin Elmer Autosystem XL gas chromatograph (Perkin Elmer, Shelton, CT, USA) equipped with two columns of different polarities: a DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm; J and W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column (30 m × 0.25 mm i.d., film thickness 0.15 µm; J and W Scientific Inc.). For component identification, a gas chromatograph coupled to mass spectrometry (GC-MS) was used, consisting of a Perkin Elmer Autosystem XL gas chromatograph interfaced with a Perkin-Elmer Turbomass mass spectrometer (software version 4.1; Perkin Elmer, Shelton, CT, USA). The components were identified based on their comparative retention times relative to C_9–C_{27} *n*-alkane indices and GC-MS spectra from a lab-made library constructed based on the analyses of reference oils, laboratory-synthesized components, and commercially-available standards [34].

2.4. Determination of Total Phenolic and Flavonoid Content

Spectrophotometric (BIO-RAD Model 680 Microplate Reader, Tokyo, Japan) quantitative determination of the total phenolic content (TPC) in plant extracts was performed using the Folin-Ciocalteau (Sigma Aldrich 2M, Switzerland) micro method (Microtiter plate reader, BIO-RAD Model 680). Gallic acid (GA) (Sigma Aldrich, 98%, China) was used as the standard and the calibration curve, which was adapted from previous studies [35–37] within a concentration range of 12.5 to 600 mg/L. The absorbance calibration curve vs. the concentration of the standard was used to quantify TPC content. For the assay, 30 µL aliquots of extracts were transferred to 96-well microtiter plates (Nunc-Imuno Plate, Roskilde, Denmark), then 150 µL of Folin-Ciocalteau phenol reagent (2M) (1:10 *v*/*v* with water) were added after 4 min. Next, 120 µL of sodium carbonate solution (0.25 mg/L) was added to each well to a final volume of 300 µL. The reaction mixture was placed in the dark for 30 min at 40 °C and the absorbance was recorded at A_{665} against a blank sample. The analysis was performed at least in triplicate and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract (mg GAE/g).

The total flavonoid content was measured by using an aluminum chloride colorimetric assay ($AlCl_3$-Merck, 98%), with minor modifications to the previously reported method [38,39]. The calibration curve was performed with catechin (Sigma Aldrich, 98%, China) as a standard. The flavonoid contents were measured as micromole catechin equivalents (µmol CE) per g of extract (µmol CE/g). Then, 25 µL of the dissolved extract were transferred to a microtiter (Nunc) and 7.5 µL of 5% $NaNO_2$ (*w*/*v*) (Panreac, 98%) were added to the wells. After 5 min, 7.5 µL of 10% $AlCl_3$ in ethanol were added to the mixture. Finally, after 5 min, 100 µL of 1M NaOH (Merck, P.A., Darmstadt, Germany) were added and, after 10 min, the absorbance was measured against the reagent's blank in triplicate at 750 nm using a microtiter plate reader (FLUOstar OPTIMA, Offenburg, Germany).

2.5. Antioxidant Activity Determinations

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich, Steinheim, Germany) assay was used as described previously [37] to determine the radical scavenging activity of the extracts, with appropriate modifications. A fresh stock solution of 100 µM DPPH in methanol was prepared daily. Aliquots of 30 µL of the test extract were dissolved in methanol at concentrations ranging from 0.05 to 1.3 mg/mL and mixed in a microtiter plate (Nunc) with 270 µL of DPPH solution that was previous transferred in triplicate to a 96-well microtiter plate. The absorbance was measured at 510 nm by using a microtiter plate reader (FLUOstar OPTIMA) after 40 min of incubation at room temperature in the dark. Blank and control solutions were also prepared and measured. By using the same procedure, positive controls of trolox solution (Sigma Aldrich, 98%, China) and ascorbic acid (Panreac, 99%, Barcelona, Spain) in methanol were also completed for the same concentration range. The half maximal inhibitory concentration value (IC_{50}) was obtained from the linear range of the data. According to Equation (1), A is the absorbance, s is the sample, b is the blank, and c the control:

$$\text{IC50 } (\%) = \left[1 - \left(\frac{A_s - A_b}{A_c - A_b}\right)\right] \times 100 \quad (1)$$

For the 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) (Sigma Aldrich, ≥98%, China) assay, the experiments were performed by using the different decolorization ability of ABTS that indicates diverse scavenging activities according to a previously-described method [6,7], with some modifications. A total of 2.5 mM potassium persulfate (Acros Organics, 99%, Geel, Belgium) solution was prepared with distilled water and 10 mL of this solution were used to prepare a 7.4 mM ABTS stock solution, which was reacted for 16 h at room temperature in the dark. The stock solution was diluted with methanol until a 1.0 absorbance was attained at 730 nm in the microplate reader (BIO-RAD Model 680). To determine IC_{50} values, 20 µL of the extract dissolved in methanol, at concentrations ranging from 0.05 to 1.3 mg/mL, were mixed in a microtiter plate (Nunc) with 280 µL of ABTS solution following the procedure described for DPPH. The same procedure was followed for the positive controls of the solutions using the trolox and ascorbic acid.

The reducing power test was performed by using a microtiter reader following a previously reported method [38,39] with some modifications. A total of 25 µL of different concentrations of the extracts ranging from 0.1 to 1.3 mg/mL were mixed in a microplate (Nunc) with 25 µL of sodium phosphate buffer (200 mM, pH 6.6) and 25 µL of 1% (w/v) aqueous potassium ferricyanide III ($K_3Fe(CN)_6$, Sigma-Aldrich, ≥97%). The mixture was incubated at 50 °C for 20 min, and 25 µL of trichloroacetic acid (10% w/v) were added. A volume of 50 µL of this solution was transferred to the microtiter plate, subsequently adding 50 µL of deionized water and 50 µL of ferric chloride (0.1% w/v). The absorbance was measured at 655 nm. The same procedure was followed by using trolox concentrations as positive controls and the final results were expressed as µmol of trolox equivalents per g of extract (µmol TE/g).

2.6. Acetylcholinesterase and Tyrosinase Activity

Acetylcholinesterase activity was assayed as described, with some modifications [40,41]. In a microtiter plate (Costar, Cambridge, MA, USA)), a mixture of 98 µL of HEPES solution (50 mM, pH 8.0) with 30 µL of extract and 7.5 µL of acetylcholinesterase (AChE) was prepared. After 15 min of incubation at 25 °C, the reaction was started with the addition of 22.5 µL of acetylthiocholine iodide (AChI, 1.20 mM) and 142 µL of 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB).The microplate was then read at 405 nm every 30 s for 3 min by a microtiter plate reader (TECAN Infinito M200, Mannedorf, Switzerland). All tests were performed in triplicate at a sample and a positive control concentration of 50 µg of extract/mL, and tacrine was used as the reference standard for this procedure.

Enzyme activity was calculated as a percentage of the initial velocities compared to the assay by using buffer without any inhibitor [42]. Tyrosinase (Tyr) activity was assayed as described [43]

with appropriated modifications. In a microtiter plate (Costar), a mixture of 180 µL of L-tyrosine (0.45 mM,) with 10 µL of the extract was incubated at 30 °C for 5 min (min). Tyrosinase (10 µL, 0.5 U/mL) was added and the mixture was incubated again at 30 °C for 5 min. The reaction was followed by a microtiter plate reader (TECAN Infinito M200) at 450 nm, at 2 min intervals for 12 min. Kojic acid was used as the positive control. The reaction was performed as previously described in a 50 mM phosphate buffer at pH 6.8, with 0.5 mM L-tyrosine, 10 mg/mL of kojic acid, and 5000 U/mL of mushroom tyrosinase at 30 °C [42]. Experimental conditions were also based on previous studies [44,45]. After incubation, absorbance were measured at 450 nm every 2 min, for a total of 10 min using 96-well reader [44]. The enzyme inhibition activity was calculated using Equation (2) and the rate of inhibition using Equation (3) [42]:

$$\text{Enzyme inhibition activity } (\%) = \left(\frac{\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}}{\text{OD}_{\text{Control}}} \right) \times 100 \quad (2)$$

$$\text{Rate of inhibition} = \frac{\text{Corrected absorbance}}{\text{time (min)}} \quad (3)$$

All tests were performed in triplicate at a sample and positive control concentration of 50 µg of extract/mL and kojic acid was used as the reference standard for this procedure.

2.7. Statistical Analysis

All results are presented as mean value ± standard deviation (SD). Correlation and regression analyses were performed with the Excel software 2013 package (Microsoft Corporation, under Academic License, Microsoft of Portugal). Correlations were considered statistically significant at $p < 0.05$ according to Tukey HSD and Scheffé test.

3. Results and Discussion

3.1. Extraction Yield

The methodologies to obtain volatile oils with SFE from herbaceous matrices are well documented in the literature, specifically the selection of the working extraction conditions, such as pressure, temperature, flow rate, and particle size [27,46–48]. Additionally, to obtain an extract comparable to conventional organic solvent extraction, other operational conditions must be selected [24,49–51]. The basil extraction yields obtained by different methodologies are presented in Table 1.

Table 1. The extraction yields from basil (*Ocimum basilicum* L.) using different extraction methods.

Extraction Method	Sample Identification	Yield (%)
Hydrodistillation (Fresh plant)	HDF	0.35 ± 0.02
Hydrodistillation (Dry plant)	HDD	0.32 ± 0.02
SFE (9.0 MPa, 40 °C)	SFEO	0.39 ± 0.02
Soxhlet (Methanol)	MeOH	17.8 ± 0.9
Soxhlet (Ethanol)	EtOH	9.6 ± 0.4
SFE (40.0 MPa, 40 °C)	SFEE	2.2 ± 0.1

The extraction yield from *Ocimum basilicum* L. isolated by hydrodistillation of fresh (HDF) and dried plants (HDD) were 0.35 ± 0.02% and 0.32 ± 0.02% (*w*/*w*), respectively. For SFE from dried basil at 9.0 MPa and 40 °C, the volatile oil yield was 0.39 ± 0.02% (*w*/*w*) in the second separator. The total wax content (not presented in Table 1), recovered in the first separator of SFE, was 0.10 ± 0.01%

The operational conditions used in SFE were selected considering several experimental works [24,30,33]. The volatile oil and essential oil yields were different from the data reported in other studies [47,49,50]. Basil genotypes differed significantly with respect to their chemical composition

and oil content, ranging from 0.07% to 1.92% in dry matrices [4]. The volatile oil exhibited a light yellow color with a pleasant fragrance as opposed to the essential oil that was pale yellow.

The Soxhlet extraction with the two solvents and the comparison with supercritical fluid extraction (40.0 MPa and 40 °C) showed that the highest extract yield was obtained with methanol (17.8 ± 0.9%) and the lowest was achieved with SFE, probably due to the lower polarity of CO_2.

3.2. Quantitative Analysis of the Essential Oil and the Volatile Oil

The chemical composition of the volatile compounds was analyzed by GC. The results are presented in Table 2. Methyl eugenol (29–34%), linalool (12.6–18.8%), methyl chavicol (12.6–19.9%), and cineole (5.8–11%) were found in different concentrations as the dominant volatile compounds in *Ocimum basilicum* L. Previous studies reported that the chemical composition of the oil was significantly different with diverse basil genotypes [4,52–56]. The main differences in the essential oils from fresh and dry plants can be observed in the increase of 1,8-cineole (7.6% to 11%) and methyl chavicol (13.5% to 19.9%). In contrast, the levels of linalool remained constant (18.1% to 18.8%) in HDF when compared to HDD (Table 2).

When comparing the two obtained oils, that from the dry plant showed a decrease in the levels of 1,8-cineole, linalool, and methyl chavicol, as well as a significant increase in phytol acetate 2 (0.3% to 5.4%) in HDDF with respect to SFEO. Moreover, an increase in phytol acetate 2 was observed in SFEO. No difference in wax content was observed in the oil, suggesting that extraction with supercritical CO_2 followed by two-stage separation procedure allowed the isolation of the pure basil volatile oil; whereas in the first separator, a white mass consisting of waxes was collected [56]. Paraffinic compounds ranging between C_{27} and C_{32} were the principal waxes, obtained in the first separator, and presented in Table 2.

Table 2. Percentages of compounds from the essential oil samples of fresh (HDF) and dried (HDD) basil, SFE volatile oil (SFEO), and waxes.

		***Ocimum basilicum* L.**			
Components	**RI**	**HDF**	**HDD**	**SFEO**	**Waxes**
				2nd S	**1st S**
α-Pinene	930	0.3	0.6	t	t
Camphene	938	0.1	0.2	t	t
Sabinene	958	0.2	0.4	0.1	t
1-Octen-3-ol	961	0.2	0.2	0.1	t
β-Pinene	963	0.6	1.3	0.2	t
β-Myrcene	975	0.8	0.7	0.2	t
1,8-Cineole	1005	7.6	11.0	5.8	t
trans-β-Ocimene	1027	1.5	0.8	0.3	t
γ-Terpinene	1035	0.2	0.2	0.1	t
trans-Sabinene hydrate	1037	0.1	0.1	0.1	t
Terpinolene	1064	0.4	0.4	0.1	t
Linalool	1074	18.1	18.8	12.6	t
Camphor	1102	0.7	0.9	0.5	t
Borneol	1134	0.6	0.6	0.4	t
Terpinen-4-ol	1148	0.3	0.4	0.1	t
α-Terpineol	1159	0.9	1.0	0.7	t
Methyl chavicol (Estragole)	1163	13.5	19.9	12.6	t
Bornyl acetate	1265	0.4	0.5	0.3	t
Eugenol	1327	6.9	3.9	6.5	t
trans-Methyl cinnamate	1346	0.1	0.2	0.2	t
α-Copaene	1375	0.1	t	0.1	t
Methyl eugenol	1377	34.0	30.1	29.4	1.3
trans-α-Bergamotene	1434	2.8	2.4	5.6	0.1

Table 2. *Cont.*

		Ocimum basilicum L.			
Components	**RI**	**HDF**	**HDD**	**SFEO**	**Waxes**
				2nd S	**1st S**
α-Humulene	1447	0.5	0.3	0.6	0.4
trans-β-Farnesene	1455	1.3	1.1	3.4	1.3
Germacrene D	1474	1.4	0.3	1.7	1.6
Bicyclogermacrene	1487	0.5	0.1	0.5	0.2
γ-Cadinene	1500	0.4	0.3	0.6	0.3
β-Sesquiphellandrene	1508	0.6	0.4	0.9	0.7
Spathulenol	1551	0.2	0.1	0.2	t
T-Cadinol	1616	1.7	0.9	1.1	0.3
Phytol acetate 2	2101	0.4	0.3	5.4	3.9
n-Heptacosane	2700	t	0.1	0.2	17.8
n-Nonacosane	2900	t	t	0.2	15.4
n-Triacontane	2000	t	t	0.1	8.3
n-Hentriacontane	3100	t	t	0.1	11.6
n-Dotriacontane	3200	t	t	0.2	23.8
Identified Compounds		97.4	98.5	91.2	87.2
Grouped components					
Monoterpene hydrocarbons		4.2	4.7	1.1	t
Oxygen-containing monoterpenes		28.4	32.9	20.2	t
Sesquiterpene hydrocarbons		7.7	5.0	13.5	4.6
Oxygen-containing sesquiterpenes		2.3	1.3	6.7	4.4
Phenylpropanoids		54.4	53.9	48.5	1.3
Others		0.4	0.7	1.2	76.9

RI: In-lab calculated retention index relative to C_9–C_{27} *n*-alkanes on the DB-1 column; t: trace (<0.05%); 1° S and 2° S are the first and second separator, respectively.

3.3. Caracterization of Plant Extract

3.3.1. Total Phenolic and Flavonoid Content and Antioxidant Activity

The total phenolic and flavonoid content of the basil extracts determined by using MeOH, EtOH, and SFEE (40.0 MPa, 40 °C) are presented in Figure 1.

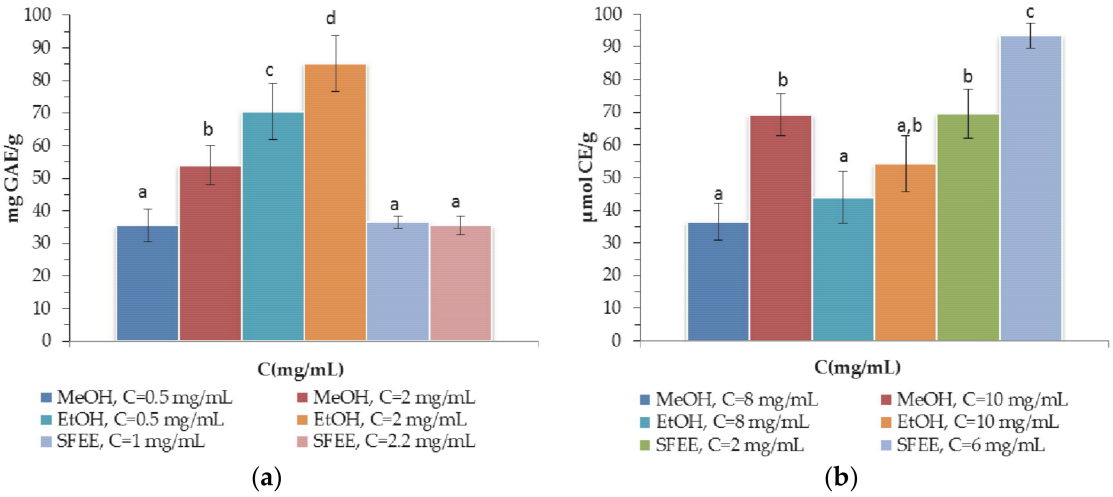

Figure 1. Content of total phenols and flavonoids in basil extract by using different extraction methods. Values with different letters are significantly different ($p \leq 0.05$) according to Tukey HSD and Scheffé tests. (**a**) Total phenolic compounds in basil extract expressed as mg GAE/g extract and (**b**) total flavonoid compounds in basil extract expressed as mol CE/g extract.

The total phenolic content was calculated based on the calibration curve of gallic acid and expressed as mg of gallic acid equivalents (GAE) per g of extract (mg GAE/g). Figure 1a shows that the highest amounts, 85.3 ± 8.6 and 70.4 ± 8.6 mg GAE, were observed in the ethanolic extracts (EtOH) at concentrations of 2.0 and 0.5 mg/mL, respectively. The lower content of phenols was determined in the SFEE, at 35.5 ± 2.9 mg of GAE, from the supercritical carbon dioxide extract (SFEE). The value of 175.6 ± 2.4 mg GAE/g was determined by in a previous study [57]; however, the origin and region of the plant can influence their composition and, consequently, the extract characteristics. The total flavonoid content in basil extract was calculated as micromole catechin equivalents (μmol CE) per g of extract (μmol CE/g) as shown in Figure 1b. The highest amount of flavonoids was found in the SFE extract, and the lowest values in the ethanol and methanol extracts.

The antioxidant activities of the extracts obtained with different extraction methods are presented in Table 3. The results for the DPPH and reduction power assays revealed that the most active radical scavenger was the MeOH extract. However, in the ABTS assay, the SFEE showed higher antioxidant activity when compared with the EtOH or MeOH extracts.

These results can be explained by the compounds responsible for the antioxidant activity not necessarily being the same for each extraction method, since the extracts are complex mixtures of compounds, thus, different interactions, either synergistic or antagonistic, may occur [58]. The antioxidant activity values were greater when compared with other extracts in the literature [3,50,57], which can be explained by the environmental conditions where basil is produced, as well as their genotype.

Table 3. Antioxidant activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) (IC_{50} in mg/mL), and reducing power (μmol TE/g) of basil extracts obtained by different extraction methods.

Sample	DPPH-IC_{50} (mg/mL)	ABTS-IC_{50} (mg/mL)	Reduction Power (μmol TE/g)
MeOH	3.05 ± 0.36 ^{a}	4.26 ± 0.22 ^{a}	306.8 ± 21.8 ^{a}
EtOH	3.99 ± 0.55 ^{a}	5.38 ± 0.23 ^{b}	285.1 ± 18.1 ^{a}
SFEE	5.63 ± 0.20 ^{b}	1.74 ± 0.05 ^{c}	111.7 ± 7.3 ^{b}
Trolox	0.471 ± 0.088 ^{c}	0.425 ± 0.084 ^{d}	—
Ascorbic acid	0.266 ± 0.022 ^{d}	0.331 ± 0.050 ^{e}	—

Values with different letters within the columns were significantly different ($p \leq 0.05$) according to Tukey HSD and Scheffé test.

3.3.2. Acetylcholinesterase and Tyrosinase Activity

The acetylcholinesterase (AChE) and tyrosinase (Tyr) activities of basil extract using different extraction methods are shown in Figure 2. Figure 2a shows that, overall, the obtained extracts exhibited a very low inhibitory activity toward acetylcholinesterase when compared with the positive control. The SFEE extract did not present any activity, suggesting no possible inhibitory activity when compared with the values of 3% to 65% inhibitory activity in several MeOH extracts [40]. Moreover, a study reported that a 1 μg/mL hydro-methanol extract had an activity of 26.15% [59]. These results might have had some impact for cosmetic and dermatological purposes since a negative regulation of antimicrobial peptide exists in the skin through the cholinergic anti-inflammatory pathway via acetylcholine [60]. Some studies revealed that the β-adrenergic activation impairs cell motility and wound closure. The same study stated that the AChE released from keratinocytes also contributes to the regulation of local immune responses and potentially of infiltrating immune cells.

The examined extracts showed an inhibitory activity against tyrosinase (Figure 2b), although lower values were obtained when compared with the positive control (kojic acid). Tyrosinase inhibition may be related to antioxidant activity. Tyrosinase catalyzes the oxidation of phenols and its inhibition may lead to a decrease in melanin production by the rate-control step of its synthesis, for example, for potential cosmetic purposes.

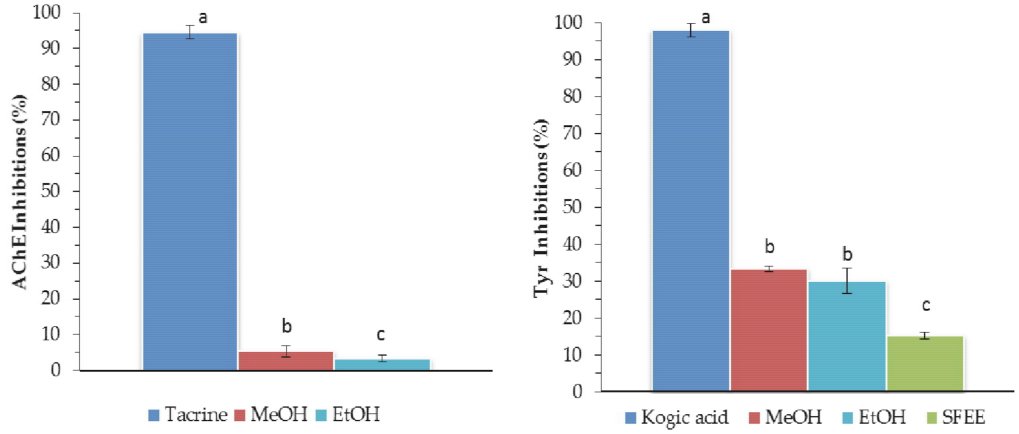

Figure 2. Acetylcholinesterase (AChE) and tyrosinase (Tyr) activity of basil extract from different extraction methods. Values with different letters were significantly different ($p \leq 0.05$) according to Tukey HSD and Scheffé tests. (**a**) AChE inhibition and (**b**) Tyr inhibition in basil extract.

4. Conclusions

The volatile oil obtained by supercritical carbon dioxide extraction of basil aerial parts was found to be similar to the essential oil obtained from hydrodistillation. The main differences in the composition were a significant increase in phytol acetate 2 in the SFEE compared with hydrodistillation oils. The composition of essential oil suggested that our *O. basilicum* chemotype is identifiable as Group 4, which is rich in estragole and linalool, by comparison with previous work [4]. The *O. basilicum* chemotype has important implications in terms of its biological properties.

From the comparison of the extracts obtained by methanol, ethanol, and supercritical CO_2, the highest yields were obtained with methanol and ethanol likely due to the higher polarity of these solvents. The methanol basil extract showed antioxidant activity, and the highest amounts of total phenolic compounds were found in the ethanol extract.

For enzyme inhibition, two enzymes (AChE and Tyr) were tested. Both enzymes are related to health problems, such as dementia and skin diseases. These extracts showed a low activity for the inhibition of the AChE enzyme. For the inhibition of TyrE, methanol and ethanol extracts of dried basil had the best activities and may be considered promising active ingredients for cosmetic purposes.

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