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Foeniculum vulgare Mill. Aerial Parts (Italian 'Finocchio di Isola Capo Rizzuto' PGI): Valorization of Agri-Food Waste as a Potential Source of Lipase Inhibitors and Antioxidants

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Abstract: A large amount of waste material derives from the horticultural industry. These plant matrices constitute a valuable source of active secondary metabolites with a wide spectrum of potential applications, including both human health and veterinary science. Italy is one of the leading European producers of fennel, and the 'Finocchio di Isola Capo Rizzuto' is a protected geographical indication (PGI) product, typical of the Calabria region. In this study, the waste material from this PGI Italian fennel was investigated for the first time as a potential source of bioactive compounds. Both bulbs and aerial parts were extracted with ethanol through maceration, and the phenolic content was assessed, together with the antioxidant properties. Moreover, the nutraceutical value was investigated by evaluating the potential anti-obesity effects. To this end, fennel extracts were studied for their inhibitory effects on pancreatic lipase enzyme, which plays a pivotal role in dietary fat absorption. The aerial part extract demonstrated DPPH radical scavenging (IC_{50} = 293.13 ± 22.98 µg/mL) and lipid peroxidation inhibitory activities (IC_{50} = 43.26 ± 1.90 µg/mL), and it was also effective in inhibiting pancreatic lipase (IC_{50} = 3.51 ± 0.09 mg/mL). Moreover, a significant positive correlation was highlighted between observed biological properties and fennel phenolic constituents. Obtained results show that 'Finocchio di Isola Capo Rizzuto' PGI by-products are a good candidate for further investigations as a potential source of antioxidant and anti-obesity agents useful as functional ingredients.

Keywords: fennel; agri-food wastes; anti-obesity; phenolics; nutraceutical properties; food supplements; animal feed; animal welfare and health

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1. Introduction

Waste material from cultivated plants constitutes a rich source of high added-value molecules, such as secondary metabolites, that are characterized by a wide spectrum of potential applications [1,2]. The increasing number of studies dealing with the valorization of plant waste material reflects the interest in a circular economy, which promotes the shift from a linear economic scheme—in which resources are extracted for production and consumption without any plan for reusing waste—to a circular economy. This last model, also

known as a "bioeconomy", is based on reuse and recycling to create a closed-loop system, with the goal of minimizing the use of resource inputs and the generation of waste and pollution [3]. The production of minimally processed vegetables, in particular, generates a large quantity of waste material. Interestingly, the bioactive compound concentrations in these matrices are comparable to those of the edible part of the plant, as underlined by Baiano and colleagues. These authors assessed different extraction techniques to optimize the sustainable extraction of phenolic compounds from fennel by-products using water as solvent [4].

Foeniculum vulgare Mill. (Apiaceae), commonly known as "fennel", is an aromatic plant native to the southern Mediterranean countries and currently cultivated worldwide. It also grows wild in Asia, North America and Europe. This plant has been used since ancient times for culinary purposes, but *F. vulgare* has been extensively used also as a medicinal plant in traditional medicine [5]. The bulbs, together with foliage and seeds, are widely used in the culinary traditions of many countries: fennel seeds are an anise-flavored spice, while the bulb is a crisp vegetable that can be grilled, braised or eaten raw. The bulbs are typically used in Mediterranean cuisine, both raw and cooked [6].

A number of pharmacological properties have been explored and reported for this species, such as antimicrobial [7], antioxidant [8], anti-inflammatory [9], antidiabetic [10], and hypolipidemic effects [11]. Moreover, different studies also highlighted the potential anti-obesity activity of *F. vulgare* extracts and essential oil, which were demonstrated to regulate obesity and lipid disorders in vivo [12–15]. The effects of aromatherapy based on the use of *F. vulgare* essential oil for the reduction of appetite in obese patients were explored as well [16]. In addition to the pharmacological properties, some potential environmental applications have been reported for other properties of *F. vulgare*, such as repellent, insecticidal, acaricidal, larvicidal and nematicidal properties [5,17–19]. Moreover, potential applications of this species have been considered in the veterinary field. Safaei-Cherehh and colleagues evaluated the effects of *F. vulgare* Mill. diet supplementation on the growth and health status of male broilers [20]. Moreover, the effects of a diet supplemented with different levels of *F. vulgare* seed powder were assessed on male lambs of Kermani sheep, and it was demonstrated to increase animal growth and muscle mass [21].

Worldwide, the total harvested area of fennel has been recently reported to be equal to 1.96 Mha [22]. As recently reported by Fortis and coworkers, in the EU-27 countries, Italy is largely the leading producer of fennel and other vegetable bulbs, with 524 thousand tons in 2019, and, as the leading producing province, Crotone (Calabria, in southern Italy) alone accounts for almost one-fifth of the whole Italian production of fennel [23]. The particular fennel harvested in this province, 'Finocchio di Isola Capo Rizzuto', has been awarded by the European Union with the Protected Geographical Indication (PGI) label. It refers to fresh hybrids/varieties of the species *Foeniculum vulgare* Mill., subspecies *capillaceum*, var. dulce or azoricum [24]. The production area includes the municipalities of Mesoraca, Cutro, Isola di Capo Rizzuto, Crotone, Rocca di Neto and Strongoli in the province of Crotone and the municipalities of Botricello and Belcastro in the province of Catanzaro. These areas of Calabria are characterized by mild climatic conditions both in winter and spring, and by a sandy loam soil with a water table very close to the surface, which allows low dry matter content, thus making the fennel succulent and crunchy. Moreover, the cultivation technique and the lightness of the soil allow obtaining a very white product. The 'Finocchio di Isola Capo Rizzuto' PGI differs from other fennel varieties due to some main characteristics, such as the absent or extremely attenuated fibrousness, which increases its palatability, a stronger scent compared to other cultivars, and a persistent aroma in the mouth after ingestion. This fennel is an ingredient in many traditional recipes in its production area, and it is also currently well known among chefs and gastronomy experts nationwide for its organoleptic properties and wide range of possible culinary uses. It can be consumed fresh or baked and used for the production of sweets products and as a food preservative [25]. In the production area, the traces of the marketing of this product date back to the beginning of the Twentieth century. Successively, this product gained importance across the entire

Italian fruit and vegetable market, mainly in the period between November and May. The harvest is carried out by hand. Approximately, 5000 hectares of land are cultivated, with a maximum field production of 60 tons per hectare [25,26].

Considering that fennel is one of the crops that lead to high amounts of phenol-rich agri-food wastes [27], and taking into account previous studies underlining a potential anti-obesity activity of *Foeniculum vulgare* Mill. Refs. [12,14], the aim of this study was to assess the potential of 'Finocchio di Isola Capo Rizzuto' PGI by-products, also considering that no reports about the antioxidant and anti-obesity properties of this PGI Italian fennel are present in the literature to date.

Both bulbs and aerial parts of the plant were extracted through maceration, and the phenolic content was assessed. The radical scavenging, lipid peroxidation inhibitory properties and inhibitory activity on nitric oxide (NO) production were evaluated as well. Moreover, the potential anti-obesity effects were assessed through the study of the inhibitory properties on pancreatic lipase enzyme, which plays a pivotal role in dietary fat absorption. Finally, a correlation analysis was performed in order to verify a correlation between observed biological properties and fennel phenolic content. To the best of our knowledge, this is the first study dealing with the antioxidant and anti-obesity properties of the horticultural waste from the Italian 'Finocchio di Isola Capo Rizzuto' PGI fennel.

2. Materials and Methods

2.1. Chemicals

The utilized standards (ascorbic acid, quercetin, chlorogenic acid, propyl gallate, indomethacin, L-NAME and orlistat) were obtained from Sigma-Aldrich S.p.a. (Milano, Italy), as well as 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), β-carotene, linoleic acid, Tween 20, Dulbecco's modified Eagle's medium (DMEM), L-glutamine, fetal bovine serum, penicillin/streptomycin solution, trypan blue, PBS, lipopolysaccharide from *Escherichia coli*, Griess reagent, Tetrazolium salt MTT, type II crude porcine pancreatic lipase and 4-nitrophenyl caprylate (NPC). All other reagents were supplied by VWR International s.r.l. (Milan, Italy).

2.2. Plant Material and Extraction

The bulbs and the aerial parts of *F. vulgare* Mill. subsp. *capillaceum* 'Finocchio di Isola Capo Rizzuto' PGI, regulated by Protected Geographic Indication (PGI) under European Union law, were kindly provided by the local farm Azienda Agricola Mercurio Fausto Luigi, located in Le Castella, Isola di Capo Rizzuto, Crotone, Italy, in January 2023. A voucher specimen was identified by Dr. Carmine Lupia, Department of Health Sciences of the "Magna Græcia" University of Catanzaro, and deposited at the Mediterranean Ethnobotanical Conservatory, Sersale (CZ), Italy (number 54 Apiaceae family).

The aerial parts were immediately separated from the bulbs to carry out the analyses. The two fresh samples were extracted with ethanol through maceration (72 h × 3 times, plant to solvent ratio 1:10 g/mL). Then, the solutions were filtered using qualitative filter paper (particle retention 10–20 µm, VWR International, Leuven, Belgium) and dried using a rotary evaporator IKA® RV 10 (VWR International, Milan, Italy) at 40 °C to preserve the chemical composition of the extracts. Three extractions were performed, and results were expressed as mean ± SD.

2.3. HPLC-DAD Analyses

Phenolic acids and flavonoids of bulb and aerial part extracts were analyzed quantitatively using an Agilent Technologies 1260 Infinity PDA system equipped with a Gemini C18 (Phenomenex, Torrance, CA, USA) column (250 × 4.6 mm, 5 µm particle size). The mobile phases used were water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B). The elution gradient was 10% B in A, increasing to reach 40% B at 40 min and 60% B at 60 min. The flow rate was 1 mL/min, and the injection volume was 25 µL. UV spectra of

each extract were conventionally recorded at 210, 280, 310 and 350 nm. All analyses were run in triplicate.

The quantification of the phenolic acid components in the extract was made by HPLC-PDA against a calibration curve obtained with chlorogenic acid (Sigma-Aldrich) at eight different concentrations in the linear range of 250–1.9 μg/mL. The correlation coefficient (R^2) of standard curve in the linear plot was R^2 = 0.9992 (y = 67,032x + 99.537), indicating good linearity between peak areas and concentrations within the used concentration range. The quantification of flavonoids was made against a calibration curve obtained with quercetin-3-O-glucoside (Extrasynthese) at five different concentrations in the linear range 1000–62.5 μg/mL. The correlation coefficient (R^2) of the standard curve in the linear plot was R^2 = 0.9991 (y = 52,775 + 746.91).

2.4. ESI-MS/MS Analyses

Compound identification was carried out with a 1100 Series Agilent LC/MSD Trap System VL. An Agilent Chemstation (LC/MSD TrapSoftware 4.1, Agilent Technologies: Santa Clara, CA, USA, 2002) was used for the acquisition and processing of the data. All analyses were carried out using an ESI ion source in the negative and positive mode with the following settings: capillary voltage, 4000 V; nebulizer gas (N2), 15 psi; drying gas (N2), 350 °C, 5 L/min. Full scan spectra were acquired over the range of 100–2200 m/z with a scan time of 13,000 m/z/s. Automated ESI-MS/MS was performed by isolating the base peaks (molecular ions) using an isolation width of 4.0 m/z, threshold set at 100 and ion charge control on, with max acquisition time set at 300 ms. Different collision energies were conventionally used (1.0, 10.0 and 30.0 V) for MS/MS fragmentation. Extracts were dissolved in MeOH at the concentration of 20–30 ppm and injected at a flow rate of 10 μL/min (KD Scientific Syringe Pump, KD Scientific Inc., Holliston, MA, USA).

2.5. DPPH Free Radical Scavenging Activity

The radical scavenging activity of fennel was assessed with the in vitro 1,1-diphenyl-2-picrylhydrazil (DPPH) test [28]. It is the most common method used to verify the antioxidant ability of pure compounds and herbal extracts. DPPH is a stable radical due to the localization of the spare electron in the molecule. The single electron on its nitrogen atom can be reduced to the corresponding hydrazine by taking a hydrogen atom from the antioxidants. Following this transformation, the solution color changes from intense purple to pale yellow, and this reaction can be detected by UV-vis spectroscopy [29]. The observed discoloration at 517 nm is considered an indicator of the antioxidant potential [30]. For this assay, an ethanolic 0.1 mM solution of the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical was prepared. Different concentrations of each extract (ranging from 5 to 1000 μg/mL, 200 μL) were added to the DPPH solution (800 μL). The mixtures were incubated at room temperature in the dark for 30 min, and then the absorbance was measured at 517 nm using a UV-vis spectrophotometer (Jenway 6300, Cole Parmer, Cambridgeshire, UK). The scavenging of the DPPH radical by hydrogen donation from the antioxidant compounds is visually noticeable as a discoloration from purple to yellow. The assays were run in triplicate, and ascorbic acid was utilized as positive control.

2.6. β-Carotene Bleaching Activity

The antioxidant properties of *F. vulgare* samples were also explored using the β-carotene bleaching test, as previously described [31]. Briefly, a 0.2 mg/mL β-carotene solution was prepared in a round-bottom flask by dissolving β-carotene in chloroform. Chloroform was then removed under vacuum, and an emulsion was prepared by adding linoleic acid (0.02 mL), 100% Tween 20 as an emulsifier (0.2 mL) and distilled water (100 mL) and by vigorous shaking. The extracts were tested at different concentrations (1–100 μg/mL), and to assess their ability to inhibit lipid peroxidation, 4.8 mL of prepared emulsion was added to 0.2 mL of each sample. Test solutions were placed in a water bath at 45 °C, and absorbance was measured at 470 nm at the initial time and after 30 and 60 min of incubation

with a UV-vis spectrophotometer (Jenway 6300, Cole Parmer, UK). Each experiment was performed in triplicate. Propyl gallate was used as positive control.

2.7. Inhibition of Nitric Oxide (NO) Production

The in vitro inhibitory effects of fennel extracts on nitric oxide production were tested on the murine macrophage cell line RAW 264.7 stimulated with LPS. The use of Griess reagent allowed indirectly verifying the release of nitric oxide by detecting the presence of nitrite, a stable oxidized product, in the cell culture medium [32]. This reagent is a mixture of sulfanilic acid and 1-naphthylamine and is widely used for detecting nitrite ions [33]. A reaction occurs between the dinitrogen trioxide (N_2O_3) deriving from acidified nitrite or from the autoxidation of NO and sulfanilamide. The product of this reaction is a diazonium derivative that in turn interacts with N-1-naphthylethelene diamine to give a colored diazo product that can be detected at 540 nm [34].

Cells (RAW 264.7 murine macrophages, ATCC no. TIB-71, LGC Standards Srl, Milan, Italy) were cultured in Dulbecco's modified Eagle Medium—high glucose (DMEM) supplemented with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin/streptomycin solution in a humidified incubator at 37 °C with 5% CO_2. The assay was performed using 96-well microtiter plates as previously reported [32]. Cells were removed from the culture flask by scraping and seeded into microtiter plates with a density of 1×10^5 cells per well. The day after, sample wells were treated with fennel extracts diluted in the cell culture media at final concentrations ranging from 25 to 1000 µg/mL in 0.5% v/v DMSO, and stimulated with 1 µg/mL LPS. The presence of nitrite in the cell culture supernatant was estimated after a further 24 h, adding the same volume (100 µL) of the Griess reagent and verifying the absorbance at 550 nm using a microplate reader Stat fax 3200 (Awareness Technology Inc., Palm City, FL, USA). Both the NO synthase inhibitor L-NAME (N^G-nitro-L-arginine methyl ester) and indomethacin were used as positive controls. Moreover, the cytotoxic activity was verified with the well-established MTT test [35]. Briefly, following cell supernatant removal, a 0.5% MTT solution in phosphate-buffered saline was added to each well and allowed to incubate for 4 h. Then, the tetrazolium salt solution was removed, and DMSO (100 µL/well) was added. The absorbance was measured at 550 nm 30 min later. Three replicates were performed for each sample.

2.8. Pancreatic Lipase Inhibition

The potential anti-obesity effects of fennel extracts were studied in vitro with the pancreatic lipase inhibitory assay, as previously reported [36]. The enzyme activity was assessed by monitoring the hydrolysis of 4-nitrophenyl caprate (4-NPC) to 4-nitrophenol. To perform the test, a 1 mg/mL type II crude porcine pancreatic lipase water solution and a 5 mM NPC solution were prepared. The extracts were tested at different concentrations, ranging from 0.125 to 5 mg/mL. Briefly, 25 µL of each sample was added to 25 µL of enzyme solution, 25 µL of NPC and 1 mL of tris-HCl buffer (pH 8.5). The absorbance was measured at 412 nm after 25 min of incubation at 37 °C. The well-known lipase inhibitor orlistat was used as a positive control, and three tests were performed for each analysis.

2.9. Statistical Analyses

The HPLC-DAD analyses were performed in triplicate, and results were expressed as mean ± SD. All the experiments aimed at assessing the biological properties of investigated plant extracts were carried out in triplicate, and results were expressed as mean ± SEM. Raw data were fitted through nonlinear regression in order to calculate the IC_{50} values (namely, the concentration providing 50% inhibition). Then, differences between the IC_{50} values were evaluated through univariate analysis. Firstly, data were checked for normality of data using the Shapiro–Wilk test and homogeneity of variance using Levene's test. Statistical differences were then assessed through one-way analysis of variance (ANOVA). Two post hoc tests were used, depending on the comparison we wanted to perform. A post hoc test is useful to find out which groups are different from each other [37]. The

Bonferroni post hoc test was used to perform pairwise post hoc comparisons, and the Dunnett's multiple comparison test was used to compare treated groups and the control, respectively. Differences were considered statistically significant at $p \leq 0.05$. Finally, to properly observe whether there was an association between the observed variables and to estimate how strong this relationship was, we performed a correlation analysis [38]. As we analyzed normally distributed data, the correlation between the investigated phytochemicals and the biological parameters was investigated using the Pearson correlation coefficient, considering as variables the amount of each identified and quantified phenolic, and the percentages of inhibition detected at the highest tested concentration for each performed biological assay. The PAST4 software (version 4.15) was used.

3. Results

3.1. Extraction Yields and Phenolics

The fresh aerial parts and the bulbs of *F. vulgare* Mill. subsp. *capillaceum* 'Finocchio di Isola Capo Rizzuto' PGI were extracted with ethanol at room temperature using the maceration technique. A better yield was obtained for the bulb extract (3.3 ± 0.3% *w*/*w*) compared to the aerial parts sample (2.6 ± 0.2%). A great difference could be observed between our results and those previously reported for wild fennel, *Foeniculum vulgare* subsp. *piperitum* (28.3% for the leaf hydroalcoholic extract and 6.6% for the seed extract) [39]. Fennel seeds were also extracted with ethanol by Dina and colleagues, who reported a yield percentage equal to 4% [14]. Bayazen and coworkers performed the extraction of the leaf for 25 h using an orbital shaker and utilizing four different solvents, namely methanol, chloroform, water and a hydroalcoholic solution. The authors obtained the highest yield for the methanolic extract (246.6 mg/g) and the lowest one for the chloroform sample (28.1 mg/g) [40].

The extract obtained from the aerial parts of 'Finocchio di Isola Capo Rizzuto' PGI fennel showed a higher amount of phenolics compared to the bulb extract (Table 1). Quercetin glucuronide was the most abundant component (3.421 ± 0.061 μg/g), followed by chlorogenic acid (1.957 ± 0.045 μg/g). Other phenolic acids, together with the flavonoid quercetin-3-glucuronide, were also identified (Figure 1, Table 2). As regards the bulb extracts, traces of some phenolic compounds were detected (Table 1). The observed trend is consistent with the results reported for *Foeniculum vulgare* Mill. by Crescenzi and coworkers [41], who analyzed the metabolite profiles of different parts (bulb, leaves, stems and little stems) of fennel waste and detected the highest flavonoid content in the leaf extracts.

Table 1. Phenolic content of *F. vulgare* Mill. subsp. *capillaceum* 'Finocchio di Isola Capo Rizzuto' PGI.

Peak	Compound	Aerial Parts	Bulb
		μg/g DW	
1	Neochlorogenic acid	0.064 ± 0.012 ^{g}	-
2	Chlorogenic acid	1.957 ± 0.045 ^{b}	0.149 ± 0.001 ^{f}
3	Feruloyl quinic acid	0.699 ± 0.010 ^{d}	0.062 ± 0.002 ^{g}
4	Quercetin-3-glucuronide	3.421 ± 0.061 ^{a}	-
5	3,5-Dicaffeoylquinic acid	1.129 ± 0.022 ^{c}	0.053 ± 0.003 ^{g}
6	Quercetin-4′-*O*-glucoside	0.295 ± 0.011 ^{e}	-
7	4,5-Dicaffeoylquinic acid	0.260 ± 0.032 ^{e}	-

Data are expressed as mean of three repetitions ± SD (n = 3). Different letters indicate significant differences ($p < 0.05$, Tukey's test).

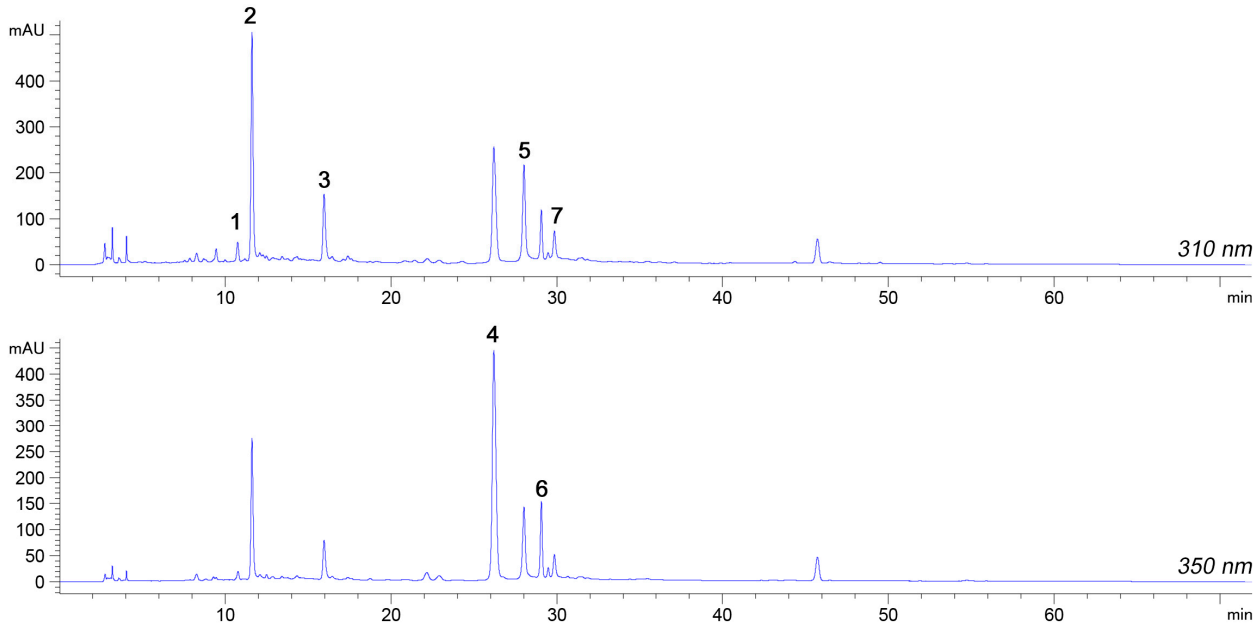

Figure 1. HPLC-DAD chromatograms of aerial parts of 'Finocchio di Isola Capo Rizzuto' PGI. For peak naming, see Table 2.

Table 2. MS fragmentation and UV-vis absorption data of compounds detected in the aerial parts and bulbs of 'Finocchio di Isola Capo Rizzuto' PGI by HPLC-DAD (310 and 350 nm).

Peak	Name	UV (λ max, nm)	ms1	ms2
			m/*z* (%)	
1	Neochlorogenic acid	328, 296 sh, 244	353 (100) $[M-H]^-$	191.05(100) $[M-162-H]^-$, [quinic acid-H]$^-$ 179.03(1) $[M-174-H]^-$ [caffeic acid-H]$^-$
2	Chlorogenic acid	326, 296 sh, 242	353 (100) $[M-H]^-$	191.05(100) $[M-162-H]^-$, [quinic acid-H]$^-$ 179.03(1) $[M-174-H]^-$ [caffeic acid-H]$^-$ 173.04(3) [quinic acid-H_2O-H]$^-$
3	Feruloyl quinic acid	324, 296 sh, 238	367 (100) $[M-H]^-$	191.05(100) $[M-176-H]^-$ 173.04(6) [quinic acid-H_2O-H]$^-$
4	Quercetin-3-glucuronide	354, 300 sh, 264, 234	477 (100) $[M-H]^-$	301.03(100) $[M-176-H]^-$, [Aglycone-H]$^-$
5	3,5-Dicaffeoylquinic acid	328, 298 sh, 244	515 (100) $[M-H]^-$	353.08 (2) [M-162 (caffeoyl)-H]$^-$ 191.05 (100) $[M-354–H]^-$ [quinic acid-H]$^-$ 179.03 (7) [caffeic acid-H]$^-$
6	Quercetin-4′-*O*-glucoside	348, 298, 266	463 (100) $[M-H]^-$	301.03(100) $[M-162-H]^-$, [Aglycone-H]$^-$
7	4,5-Dicaffeoylquinic acid	326, 300 sh, 244	515 (100) $[M-H]^-$	353.08 (2) [M-162 (caffeoyl)-H]$^-$ 191.05 (100) $[M-354-H]^-$ [quinic acid-H]$^-$ 179.03 (7) [caffeic acid-H]$^-$

3.2. Antioxidant Activity

The antioxidant potential of fennel extracts was assessed using two different in vitro assays: the DPPH test and the β-carotene bleaching test. The radical scavenging activity of 'Finocchio di Isola Capo Rizzuto' PGI extracts is reported in Figure 2.

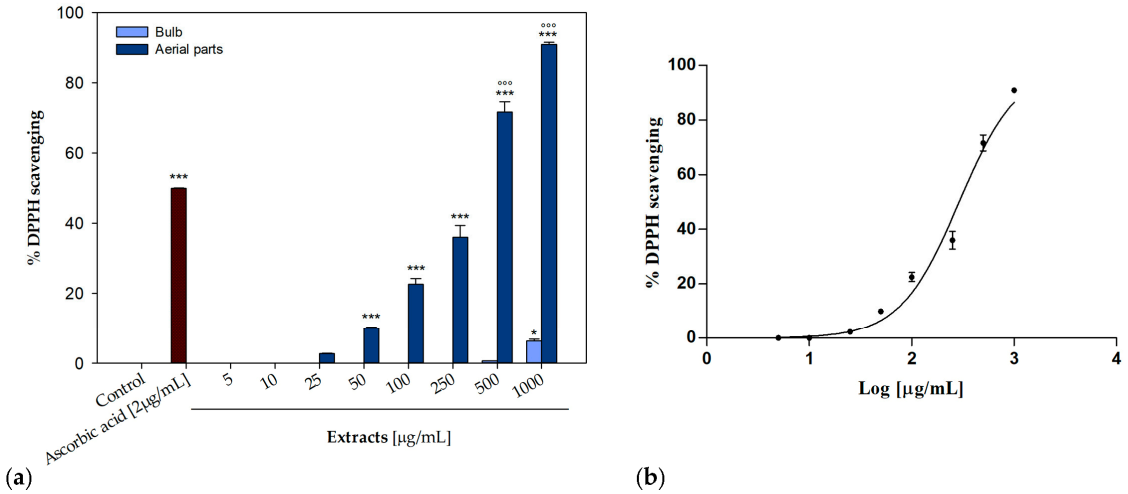

Figure 2. (**a**) Concentration-dependent radical scavenging activity of *F. vulgare* Mill. subsp. *capillaceum* 'Finocchio di Isola Capo Rizzuto' PGI extracts. (**b**) Non-linear regression analysis of DPPH scavenging activity of aerial parts sample. Data are expressed as mean ± S.E.M. (n = 3). Ascorbic acid (2 µg/mL) was used as positive control. Significant difference vs. control: *** $p < 0.001$; * $p < 0.05$; Significant difference vs. positive control: °°° $p < 0.001$ (Dunnett's multiple comparison test).

Both fennel extracts showed a concentration-dependent DPPH scavenging effect. The bulb extract demonstrated a significant inhibitory activity at 1000 µg/mL compared to the control ($p < 0.05$, Dunnett's multiple comparison test). The extracts from the aerial parts, in particular, induced a 90.88% inhibition at the highest tested concentration (1000 µg/mL), and the scavenging activity was significantly higher compared to the control also at 50 µg/mL ($p < 0.001$, Dunnett's multiple comparison test). Moreover, for this last sample, the inhibition percentages observed at the two highest tested concentrations were also significantly higher compared to the positive control (2 µg/mL ascorbic acid).

Nonlinear regression analysis was performed in order to deduce the IC_{50} parameter. Obtained results are summarized in Table 3.

Table 3. Antioxidant potential of *F. vulgare* Mill. subsp. *capillaceum* 'Finocchio di Isola Capo Rizzuto' PGI aerial part extract.

Sample	IC_{50} (µg/mL)		
	DPPH Test	β-Carotene Bleaching Test	
		30 min	60 min
Aerial parts	293.13 ± 22.98 ^{b}	43.26 ± 1.90 ^{b}	79.77 ± 1.75 ^{c}
Ascorbic acid *	2.00 ± 0.01 ^{a}	-	-
Propyl gallate *	-	1.00 ± 0.02 ^{a}	1.00 ± 0.02 ^{a}

Data are expressed as mean ± SEM (n = 3). Different letters along column (DPPH) or between columns (β-carotene bleaching test) indicate statistically significant differences ($p < 0.05$, Student's t test for DPPH and Bonferroni post hoc test for β-carotene). * Positive control.

The aerial part extract was able to scavenge the DPPH radical with an IC_{50} value equal to 293.13 ± 22.98 µg/mL. We previously reported the antioxidant potential of a hydroalcoholic extract of the leaves from wild fennel collected in Calabria (*Foeniculum vulgare* Miller ssp. *piperitum*). This sample exhibited DPPH scavenging activity with an IC_{50} value equal to 148 µg/mL [42]. Faudale and colleagues also found a higher radical scavenging activity in wild fennel samples compared to cultivated ones [43], and the hydroalcoholic extracts from the aerial parts of different samples from Italy showed DPPH scavenging activity with IC_{50} values ranging from 46.1 and 226.3 µg/mL.

The antioxidant activity was also evaluated through the β-carotene bleaching test, which is commonly used to determine the prevention of the autooxidation of emulsified

linoleic acid induced by tested samples. In this test, the aqueous emulsion of linoleic acid and β-carotene is discolored by the radicals generated by the oxidation of the fatty acid induced by thermal induction [44]. In line with the previous results, the extract of the aerial parts from 'Finocchio di Isola Capo Rizzuto' PGI was effective also in inhibiting lipid peroxidation, with an IC_{50} value of 43.26 ± 1.90 µg/mL after 30 min. The sample was effective also after 60 min of incubation, with an IC_{50} value equal to 79.77 ± 1.75 µg/mL (Table 2). The concentration-dependent inhibitory activity is illustrated in Figure 3.

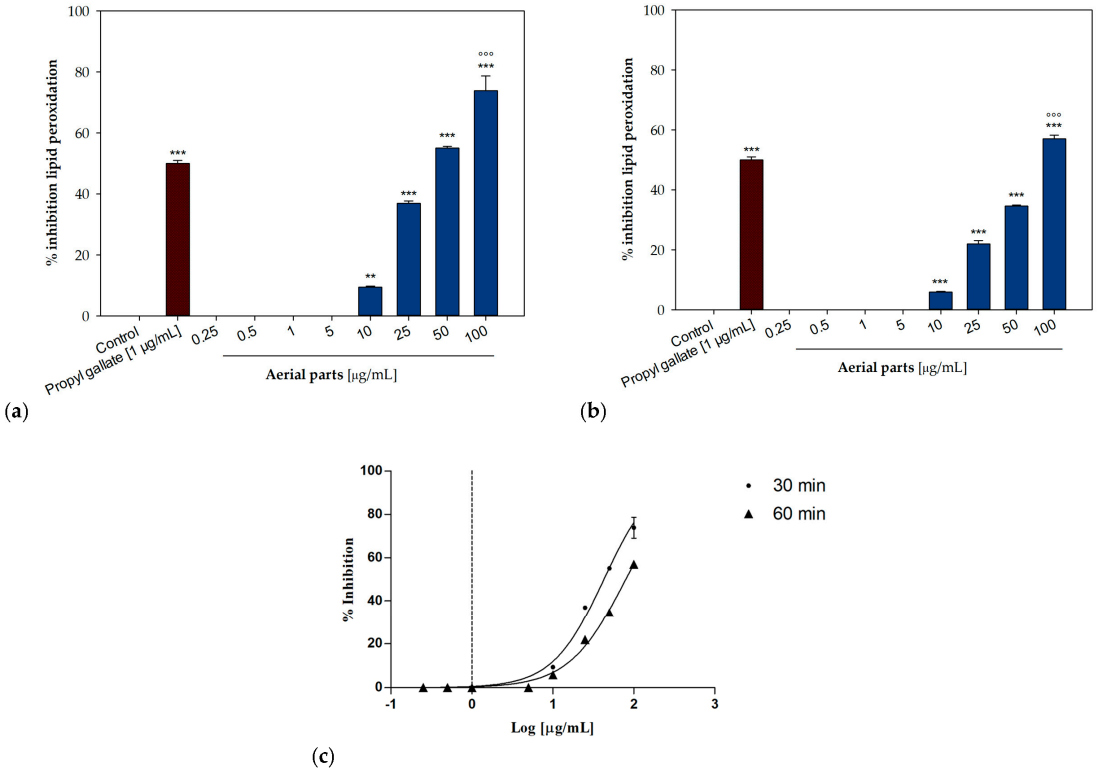

Figure 3. Concentration-dependent inhibition of lipid peroxidation induced by the extract of the aerial parts from 'Finocchio di Isola Capo Rizzuto' PGI after 30 (**a**) and 60 min of incubation (**b**). (**c**) Non-linear regression curves. Data are expressed as mean ± S.E.M. (n = 3). Propyl gallate (1 µg/mL) was used as positive control. Significant difference vs. control: *** $p < 0.001$; ** $p < 0.01$; significant difference vs. positive control: °°° $p < 0.001$ (Dunnett's multiple comparison test).

A very good inhibition percentage was observed at the highest tested concentration (100 µg/mL) with a value of 73.85 ± 4.83% after 30 min of incubation (Figure 3a), which was significantly higher compared to the positive control propyl gallate ($p < 0.001$, Dunnett's multiple comparison test). Even at the lower concentrations, 50, 25 and 10 µg/mL, this sample was effective, being able to significantly inhibit lipid peroxidation compared to the control (55.09%, 36.86% and 9.42%, respectively). This sample was effective also after 60 min of incubation (Figure 3b), even if to a lower extent, with an inhibition percentage equal to 57.01 ± 1.25% at the concentration 100 µg/mL, and percentages of inhibition ranging from 34.58% to 5.88% at the lower tested concentrations.

Our results differ from those reported by Barros and coworkers, who investigated the antioxidant potential of the shoots, leaves, and inflorescences from fennel samples collected in Portugal [45]. In this study, samples were assayed for their DPPH radical scavenging activity, and IC_{50} values equal to 1.34, 7.88 and 7.74 mg/mL were detected. The same differences were observed for the lipid peroxidation inhibitory properties assessed with the β-carotene bleaching test, with IC_{50} values of 0.49, 1.14 and 1.29 mg/mL, high values compared to those obtained for 'Finocchio di Isola Capo Rizzuto' PGI, which were more effective. Majdoub and colleagues investigated the antioxidant activity of the water extracts

from two sweet fennel cultivars, 'Latina' and 'Doux de Florence', cultivated in Tunisia [46], and reported DPPH radical scavenging activity with IC_{50} values of 0.073 mg/mL for 'Latina' cultivar and of about 0.350 mg/mL for the second one.

3.3. In Vitro Inhibitory Properties of Nitric Oxide Production

The extracts were also evaluated for their ability to inhibit the production of nitric oxide. To this end, RAW 264.7 murine macrophages were stimulated with LPS and incubated in the presence of different concentrations of fennel samples.

Both samples showed a moderate inhibitory activity. At 1000 µg/mL, inhibition percentages of 14.26 ± 0.47 and 31.12 ± 1.51% were detected for bulb and aerial part extracts, respectively. This effect significantly decreased at 500 µg/mL (Table 4).

Table 4. Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 macrophages induced by *F. vulgare* Mill. subsp. *capillaceum* 'Finocchio di Isola Capo Rizzuto' PGI extracts.

Concentration (µg/mL)	Bulb	Aerial Parts
	NO Inhibition %	
1000	14.26 ± 0.47 ^{b}	31.12 ± 1.51 ^{a}
500	9.34 ± 0.66 ^{c}	9.19 ± 0.44 ^{c}

Data are expressed as mean ± S.E.M. (n = 3). Different letters indicate statistically significant differences with $p < 0.05$ (Bonferroni post hoc test).

At this concentration, both extracts induced about 9% inhibition of the nitric oxide production, with no statistically significant difference being detected between the two samples. Any biological effects were detected at lower concentrations. Overall, only a moderate inhibitory activity on NO was observed in the utilized cell line. The highest activity has been previously described for a leaf extract of wild fennel, *F. vulgare* subsp. *piperitum*, with an IC_{50} value equal to 389 ± 2.5 µg/mL calculated [39].

3.4. Pancreatic Lipase Inhibition

The inhibition of dietary fat absorption by pancreatic lipase inhibitors is considered a major target for the control of obesity [47]. The potential anti-obesity activity of 'Finocchio di Isola Capo Rizzuto' PGI extracts was here assessed in vitro through the study of the inhibitory potential on porcine pancreatic lipase. The inhibitory effects on the enzyme was detected by monitoring the release of the yellow chromogen *p*-nitrophenol that derives from the hydrolysis of 4-nitrophenyl caprylate (NPC), used as a substrate [36]. Orlistat, an effective gastrointestinal lipase inhibitor able to prevent dietary fat absorption by 30% through the inhibition of both pancreatic and gastric lipase [48], was used as positive control. Both bulb and aerial part extracts from 'Finocchio di Isola Capo Rizzuto' PGI demonstrated a concentration-dependent inhibitory activity. At the highest tested concentration, 5 mg/mL, the bulb sample induced a 35.37% inhibition of the enzyme (Table 5).

Table 5. Concentration-dependent pancreatic lipase inhibitory properties of *F. vulgare* Mill. subsp. *capillaceum* 'Finocchio di Isola Capo Rizzuto' PGI extracts.

Concentration (mg/mL)	Bulb	Aerial Parts
	Lipase Inhibition %	
5	35.37 ± 0.79 ^{c}	57.02 ± 1.91 ^{a}
2.5	17.35 ± 0.73 ^{d}	43.43 ± 1.20 ^{b}
1	8.88 ± 0.23 ^{e}	21.54 ± 1.37 ^{d}
0.5	0	13.40 ± 0.65 ^{d,e}
0.25	0	0
0.125	0	0

Data are expressed as mean ± SEM (n = 3). Different letters between columns indicate statistically significant differences with $p < 0.05$ (Bonferroni post hoc test).

Interestingly, the aerial parts sample demonstrated inhibitory effects significantly higher compared to the bulb sample, with an inhibition percentage equal to 57.02 ± 1.91% at the concentration 5 mg/mL ($p < 0.05$, Bonferroni post hoc test). The same trend was observed at all tested concentrations. Inhibition percentages equal to 43.43 ± 1.20%, 21.54 ± 1.37% and 13.40 ± 0.065% were detected at concentrations of 2.5, 1.0 and 0.5 mg/mL, respectively. The raw data were fitted through nonlinear regression in order to deduce the IC_{50} parameter, which was equal to 3.51 ± 0.09 mg/mL (Figure 4c).

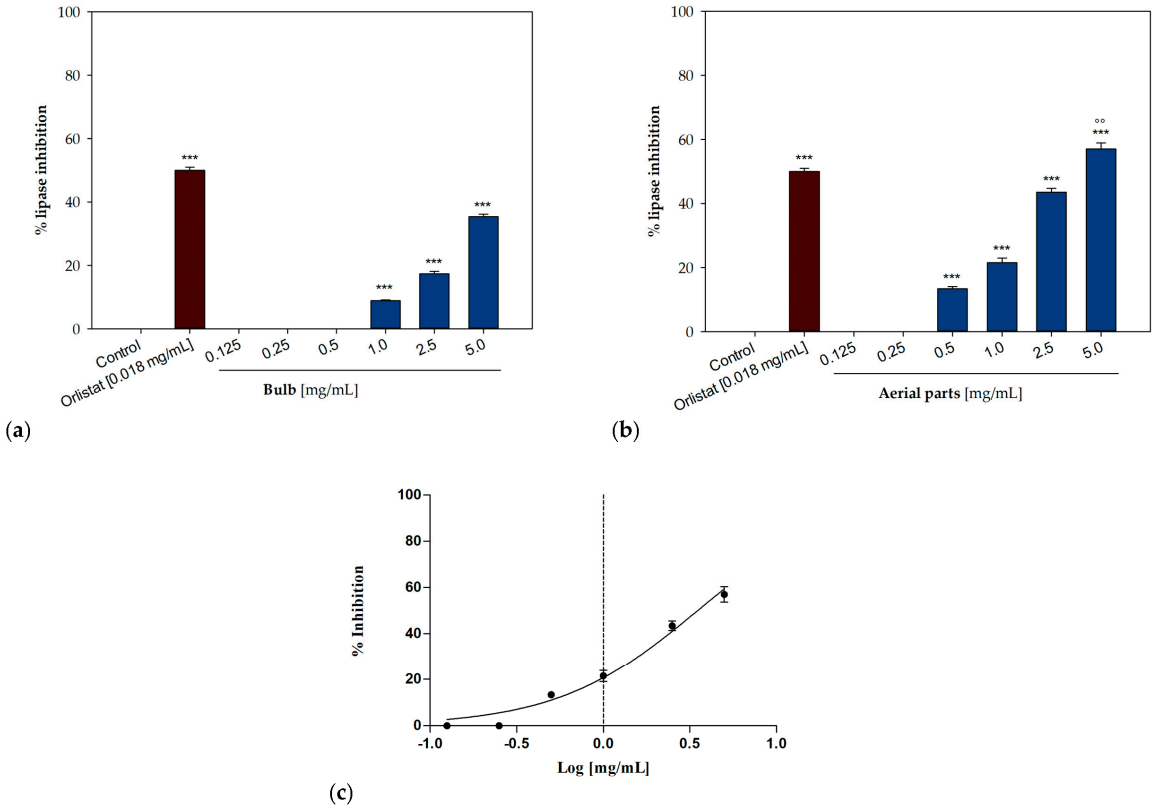

Figure 4. Concentration-dependent inhibition of pancreatic lipase induced by *F. vulgare* Mill. subsp. *capillaceum* 'Finocchio di Isola Capo Rizzuto' PGI bulb (**a**) and aerial part extract (**b**). (**c**) Non-linear regression analysis. Data are expressed as mean ± S.E.M. ($n = 3$). Orlistat (0.018 mg/mL) was used as positive control. Significant difference vs. control: *** $p < 0.001$; Significant difference vs. positive control: °° $p < 0.01$ (Dunnett's multiple comparison test).

These results are particularly interesting if compared to those we previously obtained for wild fennel samples, as the leaf hydroalcoholic extract from *Foeniculum vulgare* subsp. *piperitum* was not effective in inhibiting pancreatic lipase [49]. On the contrary, the beneficial effects of the essential oil from *Foeniculum vulgare* on obesity and related co-morbidities have been reported in some in vivo studies. The treatment with fennel extracts was demonstrated to reduce both the food intake and body mass index (BMI) in high-fat diet-induced obese rats. An improvement in dyslipidemia, hyperinsulinemia and hyperglycemia was also observed [13]. The anti-obesity activity of the ethanolic extracts from the seeds of the plant were tested in vivo on albino rats by Dina et al., demonstrating a significant reduction in rat body weight (12%) [14]. Elghazaly and colleagues described the anti-obesity effects of dried fennel seed powder in adult male albino rats. The administration of a high-fat diet caused a significant increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and the treatment with fennel induced a significant decrease in the activities of these enzymes [12]. Moreover, Al-Okbi and coworkers reported the beneficial properties of fennel volatile oil in a dyslipidemic rat model [11]. To the best

of our knowledge, the present work is the first study concerning the potential anti-obesity effects of waste material from the Italian 'Finocchio di Isola Capo Rizzuto' PGI fennel.

3.5. Correlation Analysis

For the assessment of the correlation between the identified phenolic constituents, the antioxidant activity, nitric oxide inhibitory properties and lipase inhibition, the Pearson coefficients were calculated. The correlation analysis measures the association between two variables. If data are correlated, a change in the magnitude of the first variable is associated with a change in magnitude of the second one, either in the same or in the opposite direction (positive and negative correlation, respectively). The Pearson coefficient is used for normally distributed data, while Spearman rank correlation is typically used when a non-normal distribution occurs [38]. As can be observed in Figure 5, a very strong positive correlation was highlighted between the detected flavonoids and phenolic acids and the radical scavenging percentages, with Pearson's correlation coefficients ranging from 0.97 to 0.99 ($p \leq 0.001$).

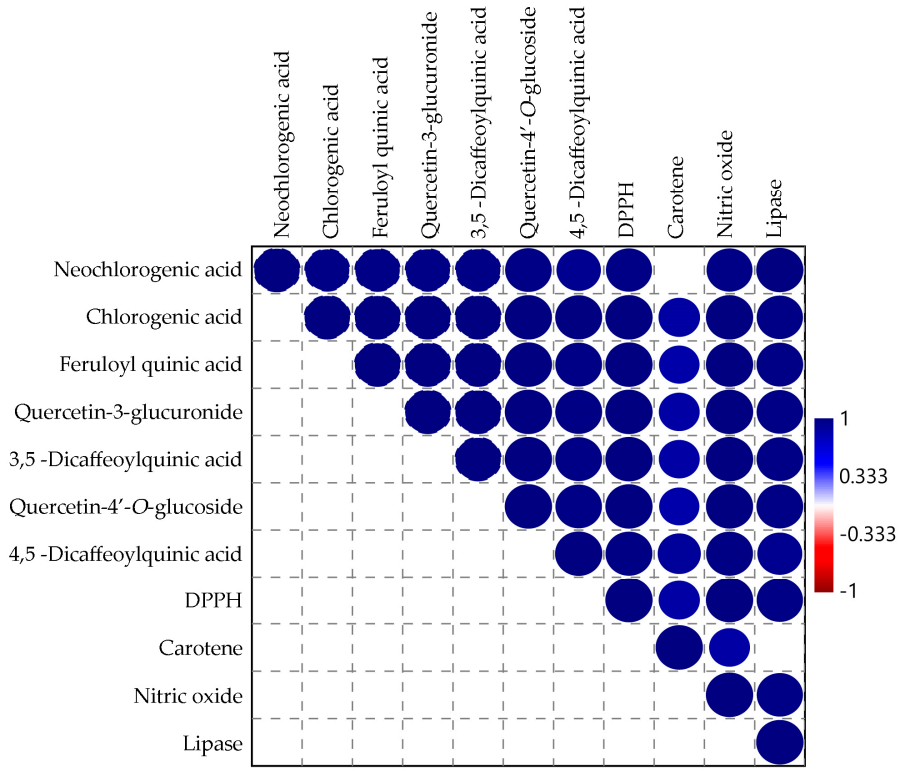

Figure 5. Pearson correlation among phenolic constituents and antioxidant, nitric oxide production inhibition and pancreatic lipase inhibitory activities.

Meanwhile, the Pearson's correlation coefficients between the lipid peroxidation inhibition percentages and the phenolic constituents (r ranging between 0.85 and 0.91, $p < 0.05$), indicated a strong positive correlation between the abundance of phenolic constituents and the antioxidant potential, with the exception only of neochlorogenic acid, for which no significant correlation was detected. The same trend was observed with the inhibition of nitric oxide production, which tended to increase with the phenolic abundance. Moreover, a significant positive correlation was also highlighted between the inhibition of the pancreatic lipase activity and the phenolic content ($p < 0.01$). While a significant positive correlation between lipase inhibition and DPPH scavenging percentages was highlighted (r = 0.979, $p < 0.001$), no significant correlation was detected with the lipid peroxidation inhibition.

4. Discussion

The agricultural sector produces around 140 billion tons of biomass per year, which not only has a negative impact on the environment, but also implies overall economic costs [50]. An innovative strategy aims to use the waste materials derived from agricultural production as matrices to obtain bioactive compounds useful as functional ingredients, thus integrating the concepts of agriculture, industrial production and the circular economy [51]. Such vegetable wastes also may be used as animal feed [52] and in the veterinary field [53,54].

Such added values were also explored for fennel by-products. Crescenzi and colleagues investigated the phytochemical content of the residual matrices and observed the major content of flavonoids and the highest antioxidant activity for the leaf extract, compared to the other plant parts [41]. Our results are in agreement with these data. The phenolic and flavonoid contents were here investigated with HPLC-DAD and ESI-MS/MS analyses, and the aerial parts demonstrated a higher abundance of both classes of metabolites compared to the edible part. Consistently, fennel has been reported to be one of the major vegetables that leads to high amounts of phenol-enriched agri-food wastes [27].

The goal of our study was to investigate the biological and nutraceutical potential of the residual plant matrix obtained from the cultivation of a fennel recently awarded by the European Union with the Protected Geographical Indication (PGI) label, and whose harvesting and production have gained particular importance in southern Italy. To the best of our knowledge, no reports about the 'Finocchio di Isola Capo Rizzuto' PGI antioxidant and anti-obesity properties have been reported to date. Both the bulbs and aerial parts of the plant were extracted with maceration using ethanol as solvent of extraction. Compared with the wild fennel, *F. vulgare* subsp. *piperitum* [39], lower yields were observed, namely 3.3% *w*/*w* and 2.6% for the bulb and aerial parts samples. Consistent with the literature, which includes fennel among the vegetables that lead to high amounts of phenol-rich agri-food wastes, the aerial parts exhibited a higher phenolic content compared to the bulb, with quercetin glucuronide and chlorogenic acid being the most abundant components. Our results are consistent with the study from Crescenzi and colleagues [41]. The authors investigated the phytochemical composition of the different parts of *Foeniculum vulgare* Mill. waste, with the leaves containing the highest flavonoid content compared to the bulbs and also the stems and little stems.

Antioxidant compounds act by donating electrons to free radicals, neutralizing them and minimizing the oxidative damage in biological processes [29]. The radical scavenging activity or fennel extracts was assessed through the evaluation of the ability to scavenge the DPPH radical. The antioxidant properties were studied also with a second assay, the β-carotene bleaching test, in order to detect the ability of samples to inhibit lipid peroxidation. In both assays, consistently with other results obtained for *F. vulgare* Mill., the aerial parts showed effectiveness. Faudale and colleagues investigated the DPPH radical scavenging activity of the hydroalcoholic extracts from the aerial parts of different fennel samples from Italy, and obtained IC_{50} values ranging from 46.1 to 226.3 μg/mL [43]. An IC_{50} value equal to 148 μg/mL was detected for the 'Finocchio di Isola Capo Rizzuto' PGI aerial parts sample. The extract from this residual plant matrix was also effective in inhibiting lipid peroxidation, with IC_{50} values equal to 43.26 ± 1.90 μg/mL and 79.77 ± 1.75 μg/mL after 30 and 60 min of incubation. These values are better than those reported for the aerial parts of some fennel samples from Portugal by Barros and coworkers [45].

With the aim of verifying the potential health beneficial properties of fennel waste materials, we also investigated the ability to inhibit the production of nitric oxide (NO), a well-known and important molecule produced in the human body that plays a role as an important regulator in a wide spectrum of physiological functions [55]. NO can be produced by two constitutive nitric oxide synthases, the endothelial (eNOS) and the neuronal isoforms (nNOS), and one inducible synthase (iNOS). Nitric oxide can react with several oxidative molecules like molecular oxygen, ROS, and thiols to yield various reactive nitrogen species [56]. The effects on NO production were assessed in RAW 264.7 murine macrophages previously stimulated with lipopolysaccharide and incubated in the presence

of different concentrations of fennel extracts. Nitric oxide undergoes a series of reactions with molecules such as superoxide and oxygen. The main end products of NO oxidation in vivo are nitrite and nitrate, whose sum is considered the best index of total NO production. The Griess reaction is a method that is widely used to measure the amount of nitrite as an index of the amount of NO in a medium [57–59]. Investigated fennel samples induced only moderate inhibitory effects on NO production in the utilized cell line. These effects were lower than those observed for the leaf extract of wild fennel, *F. vulgare* subsp. *piperitum* [39].

In addition to the antioxidant properties, one of the main goals of our study was to assess the potential applications of fennel waste residue as a source of anti-obesity active agents useful for the preparation of functional foods. This potential effectiveness was assessed in vitro through the study of the inhibitory potential on porcine pancreatic lipase, a key enzyme for dietary fat absorption. The modulation of human pancreatic lipase is considered a new tool in the discovery of active agents able to inhibit the absorption of fats and that could be useful in the treatment of obesity and other related metabolic disorders [47]. Both bulb and aerial part extracts of 'Finocchio di Isola Capo Rizzuto' PGI fennel demonstrated concentration-dependent inhibitory properties. The aerial parts, also in this case, demonstrated the best activity, with an IC_{50} value equal to 3.51 $\pm$ 0.09 mg/mL.

Previous studies dealing with *F. vulgare* demonstrated the biological potential of this plant species. Dina and colleagues investigated the anti-obesity and anti-hyperlipidemic activity of ethanolic extract of *Foeniculum vulgare* seeds in vivo, and the obtained results demonstrated a reduction in rat body weights [14]. On the contrary, we previously investigated the biological properties of a wild fennel sample, and the hydroalcoholic leaf extract from *Foeniculum vulgare* subsp. *piperitum* was not effective in inhibiting pancreatic lipase [49].

5. Conclusions

The production process in modern agriculture currently prefers paying attention to a specific part of the plant, which usually offers a single and faster product for the market. An impactful and standardized agriculture, based on intensive and mechanized criteria, derives from this choice. Plants are usually not utilized and valorized in their entirely, thus causing a great consumption of resources and a greater production of waste material. Often, only the fruits or seeds of the cultivated plants are used, while other plant parts, such as leaves, stem, and branches, are considered as waste [50]. On the other hand, the increasing attention towards a healthy and balanced diet and the use of food supplements with health beneficial properties has led to an increase in demand for these active natural compounds [51].

Fennel cultivation has been reported to lead to high amounts of phenol-rich agri-food wastes [27]. Taking into account this interesting information and previous studies reporting the potential anti-obesity activity of *Foeniculum vulgare* Mill. [12,14], this study was designed to evaluate the potentially beneficial properties of 'Finocchio di Isola Capo Rizzuto' PGI residues. This is the first study dealing with the antioxidant and anti-obesity properties of this PGI Italian fennel, to the best of our knowledge.

The extract from the aerial parts, the residual waste product derived from fennel cultivation, demonstrated in vitro antioxidant properties, being able to scavenge the DPPH radical and to inhibit lipid peroxidation. A modest ability to inhibit the production of nitric oxide was also observed in LPS-stimulated RAW 264.7 murine macrophages. Indeed, the aerial part extract was effective in inhibiting pancreatic lipase, the enzyme that plays a pivotal role in dietary fat absorption. Moreover, a significant positive correlation was highlighted between these biological properties and the higher phenolic content observed in the aerial parts of the plants compared to the bulbs.

In conclusion, the phenolic content detected in the aerial parts of the investigated plant suggests a possible re-use of this abundant waste material as a natural source of active phytochemicals. The antioxidant and lipase inhibitory potential demonstrated by

'Finocchio di Isola Capo Rizzuto' PGI fennel suggests a potential role as a functional ingredient with a wide range of applications, such as the production of functional foods and food supplements, thus giving new added value to this plant matrix.

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