

## Research Article

# Changes in the Total Polyphenolic Content and Antioxidant Capacities of Perilla (*Perilla frutescens* L.) Plant Extracts during the Growth Cycle

Francesco Gai,<sup>1</sup> Pier Giorgio Peiretti,<sup>1</sup> Magdalena Karamać,<sup>2</sup> and Ryszard Amarowicz<sup>2</sup>

<sup>1</sup>National Research Council, Institute of Science of Food Production, 10095 Grugliasco, Italy

<sup>2</sup>Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, 10-747 Olsztyn, Poland

Correspondence should be addressed to Ryszard Amarowicz; [r.amarowicz@pan.olsztyn.pl](mailto:r.amarowicz@pan.olsztyn.pl)

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Changes in the total polyphenolics and antioxidative capacity of the perilla (*Perilla frutescens* L.) plant, during the growth cycle, have been analyzed in this study. These parameters were evaluated at five morphological stages. The extracts characterized by the highest total phenolic compound content were obtained at the full flowering stage. The phenolic compound profile was characterized by the presence of three major compounds, with rosmarinic acid being the most abundant. Moreover, their contents were significantly different according to the growth stage. High Trolox equivalent antioxidant capacity values were found for the last two growth stages. The lowest ferric-reducing antioxidant power value was observed for the medium vegetative stage. The highest antiradical activity against DPPH<sup>•</sup> was observed for extracts obtained from the early vegetative stage. The antioxidant activity changes during the growth cycle, and this change may be useful to determine the optimal harvest time.

## 1. Introduction

Perilla (*Perilla frutescens* L.), which belongs to the *Lamiaceae* family, is a native plant to Asian countries, such as Korea, Japan, and China, and it has traditionally been used as a source of human and animal food [1] that is rich in fat [2–4] and good quality protein [5]. Consumption of perilla seeds or oil in humans has been reported without any adverse effects [6]. The positive effect of perilla seeds fed in term on the fatty acids composition in the muscle and lipid tissue was reported for pig [7] and in rabbits [8]. Perilla seeds are a rich source of  $\alpha$ -linolenic acid (approx. 60%), as well as phenolic compounds (flavones, cyanidins, and phenolic acids), sterols, and terpenoids [9, 10]. Bioactive compounds of perilla exhibit antiseptic, antimicrobial, anticarcinogenic, anti-inflammatory, antipyretic, and immunomodulatory properties [11–20]. Moreover, perilla leaf extracts have shown antimutagenic, antiproliferative, and antioxidative activities, as well as neuroprotective effects [21–26]. However, few studies have focused on the isolation, characterization, and

identification of perilla phenolic antioxidants [27, 28] which are known to be able to protect human organism against free radicals and reactive oxygen species and can retard the progress of many chronic diseases as well as lipid autoxidation in foods [29–31]. Chou et al. [32] examined water extracts from different anatomical parts of *Perilla frutescens* in order to establish their antioxidant activities and phenolic compounds and found that stem extracts had the highest DPPH free radical activity and the highest superoxide anion radical scavenging activity. The content of total flavonoids and phenolics of the stem extracts were found to be higher than those of other plant extracts. Peiretti [33] determined the proximate chemical composition, fatty acid composition, and gross energy content of perilla plants during the growth cycle. However, no study has been carried out so far regarding the antioxidant activities of the aerial part of perilla harvested at different growth stages. The aim of this study was to determine the phenolic compounds and antioxidant potential of perilla extracts during the growth cycle.

TABLE 1: Total phenolic contents of the perilla extract and fresh matter (FM) at different growth stages.

Growth stage	Plant height (cm)	Yield of extraction (%)	Total phenolics	
			(mg catechin eq./g extract)	(mg catechin eq./g FM)
Early vegetative	15	3.09	100.0 ± 11.1 <sup>a</sup>	3.09 ± 0.34 <sup>ab</sup>
Medium vegetative	30	2.52	54.3 ± 5.9 <sup>b</sup>	1.37 ± 0.15 <sup>c</sup>
Late vegetative	40	2.20	61.4 ± 7.8 <sup>b</sup>	1.35 ± 0.17 <sup>c</sup>
Early flowering	60	2.31	118.2 ± 12.0 <sup>a</sup>	2.73 ± 0.27 <sup>b</sup>
Full flowering	70	3.26	123.2 ± 12.7 <sup>a</sup>	4.02 ± 0.42 <sup>a</sup>

Means with the same letter are not significantly different ( $P < 0.05$ ).

## 2. Materials and Methods

**2.1. Plant Material and Environmental Conditions.** The biological experiment was conducted in the Western Po Valley near Cuneo, North West Italy (longitude 7°E, latitude 44°N). Perilla seeds, purchased from the Manitoba Seed Expert of Manitoba Inc. (Winnipeg, Canada), were sown in May. No irrigations or fertilisers were applied after sowing. Herbage samples were collected from June to July, using edging shears (0.1 m cutting width), from the early vegetative (plant height 15 cm) to the full flowering stage (plant height 70 cm), from randomly located 2 m<sup>2</sup> subplots in 3 × 8 m<sup>2</sup> plots, with three replicates cut to a 1 to 2 cm stubble height. Fresh samples of the whole plants were immediately frozen, using a laboratory lyophilizer (5 Pascal, Trezzano sul Naviglio, Italy), and were then freeze-dried and ground to pass a 1 mm screen.

**2.2. Chemicals.** Sodium persulfate, ferrous chloride, the Folin-Ciocalteu phenol reagent, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), (+)-catechin, rosmarinic acid, and apigenin were purchased from Sigma (Poznań, Poland). Acetonitrile, trifluoroacetic acid, and methanol were obtained from the P.O.Ch. Company (Gliwice, Poland).

**2.3. Extraction.** The phenolic compounds were then extracted from ground plants using 80% (v/v) methanol, as described by Amarowicz and Raab [34]; methanol was evaporated in a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland) and the remaining aqueous solution was lyophilized.

**2.4. Determination of the Total Phenolic Content.** The TPC of the extracts was determined using Folin-Ciocalteu's phenol reagent [35]. The results were expressed as mg catechin equivalent per g extract or plant fresh matter (FM).

**2.5. Trolox Equivalent Antioxidant Capacity.** The Trolox equivalent antioxidant capacity (TEAC) was determined using the Re et al. [36] method. The results were expressed as  $\mu\text{mol}$  Trolox equivalent per g extract or plant FM.

**2.6. Ferric-Reducing Antioxidant Power.** The ferric-reducing antioxidant power (FRAP) assay was performed, as described

by Benzie and Strain [37]. The FRAP value was expressed as  $\mu\text{mol Fe}^{2+}$  equivalent per g extract, or plant FM, using the  $\text{Fe}^{2+}$  calibration curve.

**2.7. Scavenging of the DPPH Radical.** The antiradical activity of the extracts against DPPH radical was determined as described in Amarowicz et al. [38].

**2.8. HPLC Analysis.** The phenolic compounds present in the extract were analyzed using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan), consisting of two LC-10AD pumps, an SCTL 10A system controller and an SPD-M 10A photodiode array detector, and a prepacked Luna C18 column (4 × 250 mm, 5  $\mu\text{m}$ ; Phenomenex, Torrance, CA, USA). Elution was conducted at condition described by Peiretti et al. [39].

**2.9. Statistical Analysis.** All the biological experiments and chemical determinations were performed in triplicate. The results are reported as mean value ± SD values. ANOVA and Duncan's test were performed at a  $P < 0.05$  level to evaluate the significance of differences among mean values.

## 3. Results and Discussion

**3.1. Total Phenolic Contents.** The TPC of the perilla plant extracts and of the fresh matter (FM) is reported in Table 1. The extracts were characterized by a significant difference between the stages ( $P < 0.05$ ), with the highest TPC contents being observed for the early vegetative and for the early and full flowering stages (100, 118 and 123 mg catechin eq./g extract, resp.). Similar statistical differences were found among the stages when TPC was expressed according to the plant FM, with the lowest content being found for the late vegetative stage (1.37 mg catechin eq./g plant FM). The TPC results obtained in the present research are consistent with the values reported in the literature, even though some differences have emerged which might be due to the use of different phenolic compound standards or different extraction methods.

Deng et al. [40] investigated *Perilla frutescens* leaves and another 55 vegetables, and they determined the TPC in both lipophilic and hydrophilic extracts. The perilla leaves showed a TPC of 10.9 and 3.5 mg gallic acid equivalent (GAE)/g in the lipophilic and hydrophilic fractions, respectively, for a total of 14.4 mg GAE/g. Li et al. (2013) determined the TPC of

TABLE 2: Individual phenolic compounds in the perilla extracts at different growth stages.

Growth stage	Plant height (cm)	Compounds (mg/g extract)		
		1*	2*	3 (rosmarinic acid)
Early vegetative	15	15.31 ± 1.65 <sup>a</sup>	4.44 ± 0.42 <sup>b</sup>	57.93 ± 3.05 <sup>a</sup>
Medium vegetative	30	9.71 ± 1.27 <sup>b</sup>	2.72 ± 0.27 <sup>c</sup>	26.44 ± 2.06 <sup>b</sup>
Late vegetative	40	12.87 ± 1.20 <sup>ab</sup>	5.92 ± 0.71 <sup>a</sup>	56.89 ± 2.79 <sup>a</sup>
Early flowering	60	10.42 ± 0.53 <sup>b</sup>	6.46 ± 0.58 <sup>a</sup>	66.17 ± 4.90 <sup>a</sup>
Full flowering	70	10.54 ± 0.94 <sup>b</sup>	6.14 ± 0.72 <sup>a</sup>	55.12 ± 5.09 <sup>ab</sup>

\* Expressed as apigenin; means with different letters in the same column are significantly different ( $P < 0.05$ ). Numbers of compounds are connected with peak numbers in Figure 1.

TABLE 3: Individual phenolic compounds in the perilla fresh matter (FM) at different growth stages.

Growth stage	Plant height (cm)	Compounds (mg/g FM)		
		1*	2*	3 (rosmarinic acid)
Early vegetative	15	0.472 ± 0.051 <sup>a</sup>	0.137 ± 0.013 <sup>b</sup>	1.784 ± 0.094 <sup>ab</sup>
Medium vegetative	30	0.246 ± 0.031 <sup>c</sup>	0.069 ± 0.007 <sup>c</sup>	0.671 ± 0.052 <sup>d</sup>
Late vegetative	40	0.287 ± 0.017 <sup>c</sup>	0.124 ± 0.015 <sup>b</sup>	1.195 ± 0.059 <sup>c</sup>
Early flowering	60	0.240 ± 0.013 <sup>c</sup>	0.149 ± 0.013 <sup>b</sup>	1.521 ± 0.113 <sup>b</sup>
Full flowering	70	0.348 ± 0.030 <sup>b</sup>	0.203 ± 0.024 <sup>a</sup>	1.815 ± 0.170 <sup>a</sup>

\* Expressed as apigenin; means with different letters in the same column are significantly different ( $P < 0.05$ ). Numbers of compounds are connected with peak numbers in Figure 1.

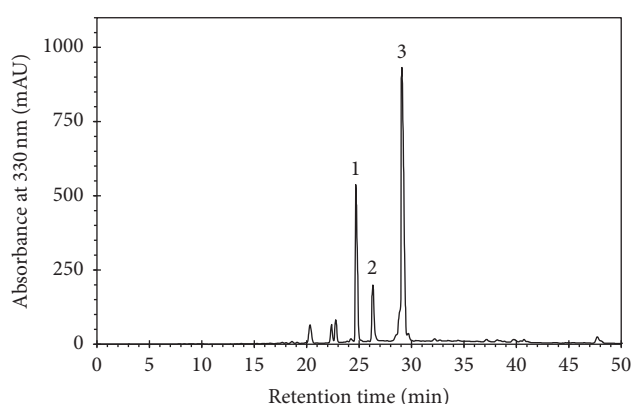


FIGURE 1: HPLC chromatogram of the phenolic compounds present in the perilla extract.

infusions from 223 medicinal plants, and among these they found 12.9, 4.2, and 5.2 mg GAE/g for the seed, stem, and leaves of *Perilla frutescens*, respectively.

Saita et al. [41] determined the polyphenol concentration of two varieties of *Perilla frutescens*, var. *crispa*, and found that the TPC values were 4.6 mg/mL and 1.7 mg/mL in red and green perilla, respectively.

Hong et al. [27] evaluated the TPC levels of *Perilla frutescens* leaves extracted by means of various solvents (70% ethanol, water, or methanol) and under different fractionation conditions (*n*-hexane, chloroform, ethyl acetate, and *n*-butanol). A good TPC level, expressed as GAEs, was found in the ethyl acetate fraction (227.38–373.92 mg/g).

**3.2. Individual Phenolic Compounds.** The individual phenolic compounds contained in the perilla extracts were separated

by means of HPLC, and the resulting chromatograms showed the presence of three major peaks (1–3), with retention times of 24.8, 26.3, and 29.5 min (Figure 1). The UV-DAD spectra of compounds 1, 2, and 3 were characterized by maxima at 266 and 334 nm (1), 280 and 334 nm (2), and 330 nm (3) and were very similar to the spectrum of apigenin and rosmarinic acid (Figure 2).

The individual phenolic compounds, quantified by means of HPLC analysis on the perilla plant extracts or FM, are reported in Tables 2 and 3, respectively. The contents of the individual phenolic compounds, in both the extract and the plant, differed significantly according to the growth stage.

Compound 3 was identified, on the basis of the UV spectrum and retention time of the standard, as rosmarinic acid. The results revealed that this compound was the most abundant phenolic constituent. Compound 3 contents in the extract ranged from 26.4 to 66.2 mg/g of extract, at the medium vegetative and early flowering stages, respectively. As far as the perilla FM data are concerned, the rosmarinic acid content ranged from 0.67 to 1.82 mg/g of plant FM at the medium vegetative and full flowering stage, respectively.

In the perilla extracts (Table 2), individual phenolic compounds 1 and 2, expressed as apigenin equivalents, ranged from 9.7 to 15.3 mg/g of extract and from 2.7 to 6.5 mg/g of extract, respectively. In the perilla FM (Table 3), compound 1 ranged from 0.24 to 0.47 mg/g of plant FM, while compound 2 ranged from 0.07 to 0.20 mg/g of plant FM.

Jun et al. [42] investigated the antioxidant activities of various extracts from *Perilla frutescens* var. *acuta* leaves and, on the basis of HPLC analysis, found that rosmarinic acid was the most abundant phenolic acid in subfraction 3 of perilla leaves.

Zhu et al. [43], by means of the supramolecular technique and solvent extraction, identified, in *Perilla frutescens* leaf

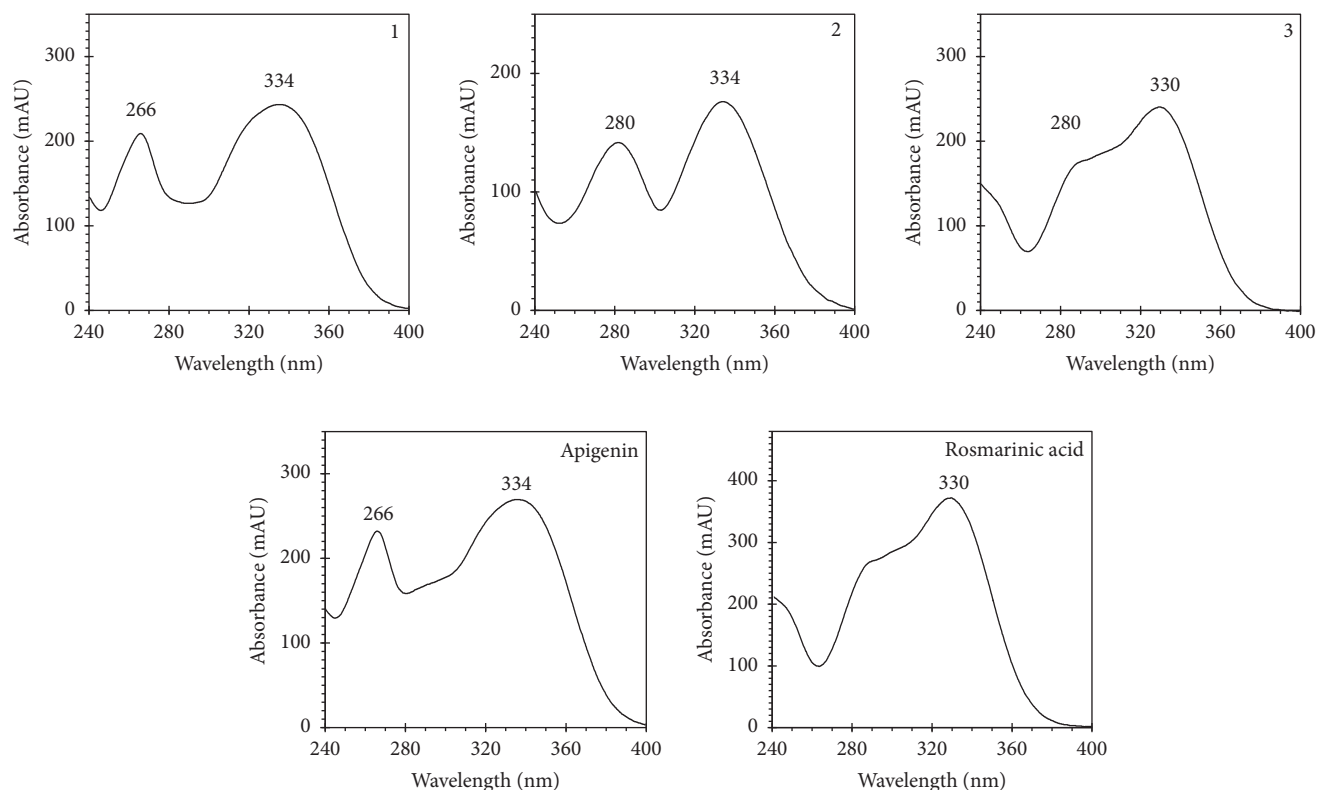


FIGURE 2: UV-DAD spectra of compounds 1–3 separated using HPLC.

extracts, phenolic acids (rosmarinic and caffeic acids) and flavonoids (glucuronides of luteolin and apigenin, shisonin, and malonylshisonin), and they found that rosmarinic acid was the most abundant phenolic compound.

Kang and Lee [44] investigated changes in the phenolic phytochemical contents in the leaves of *Perilla frutescens*, cv. Bora, for eight different harvest times over a period of two months. The profile was characterized by three phenolic acids (caffeic acid, rosmarinic acid, and rosmarinic acid methyl ester) and five anthocyanins methyl ester. These authors observed significant differences between the individual and total phytochemical contents, and the predominant constituent was rosmarinic acid. As far as the different harvest times are concerned, the lowest content was 39.0 mg/g on 17th of August (phenolic acid: 32.6 mg/g), while the highest content was found on 21st of September, with 82.5 mg/g (phenolic acid: 65.7 mg/g). They concluded that the optimal harvest time at which the phenolic compounds reach a maximum level was in mid-September.

Meng et al. [25] differentiated various *Perilla frutescens* cultivars harvested in China and Japan, compared their polyphenolic compounds, and quantified caffeic, coumaroyl tartaric, and rosmarinic acids, anthocyanin, and flavonoid contents.

Nakamura et al. [45] isolated large amounts of rosmarinic acid from the leaves of *Perilla frutescens*, var. *acuta f. viridis*. The antiradical activity of rosmarinic acid was found to be significantly higher than that of vitamin C. These authors

concluded that the presence of an orthodihydroxyphenyl group was most important for the scavenging effect.

Nakamura et al. [45] determined the apigenin, caffeic acid, catechin, ferulic acid, luteolin, and rosmarinic acid contents in the leaves and seeds of *Perilla frutescens*.

Tada et al. [46] isolated various antioxidants (flavones and derivatives of rosmarinic acid, caffeic acid, and coumarin) from the leaves and stems of *Perilla frutescens*, var. *Crispa*.

As far as the other parts of the plant are concerned, Lee et al. (2013) investigated the phenolic compound profiles of the methanolic extract of the seeds of fifteen *Perilla frutescens* cultivars. Nine phenolic compounds were elucidated: caffeic acid and its glucoside, luteolin and apigenin and their glucosides, rosmarinic acid and its glucoside, luteolin, apigenin and their glucosides, and chrysoeriol. The individual and total phenolic contents were remarkably different, especially those of rosmarinic acid and rosmarinic acid-3-*O*-glucoside, which were the predominant compounds in all the perilla cultivars. The rosmarinic acid-3-*O*-glucoside and rosmarinic acid contents ranged between 1.02 and 2.42 mg/g and 1.04 and 2.52 mg/g, respectively. Ha et al. (2012) isolated and identified five phenolic compounds (apigenin, luteolin, caffeic acid-3-*O*-glucoside, rosmarinic acid, and rosmarinic acid-3-*O*-glucoside) from the seeds of *Perilla frutescens*. Zhou et al. [47] isolated a total of 11 phenolic compounds (vanillic acid, caffeic acid and its derivatives, rosmarinic acid and its derivatives, apigenin, and luteolin and its glucoside), as well as tryptophan and sucrose, from cold-pressed *Perilla frutescens*, var. *argute*, seed flour.



TABLE 4: Trolox equivalent antioxidant capacity (TEAC) of the perilla extract and fresh matter (FM) at different growth stages.

Growth stage	Plant height (cm)	TEAC	
		(mmol Trolox eq./g extract)	(mmol Trolox eq./g FM)
Early vegetative	15	0.379 ± 0.020 <sup>b</sup>	0.012 ± 0.001 <sup>a</sup>
Medium vegetative	30	0.308 ± 0.023 <sup>c</sup>	0.007 ± 0.001 <sup>b</sup>
Late vegetative	40	0.451 ± 0.051 <sup>ab</sup>	0.008 ± 0.001 <sup>b</sup>
Early flowering	60	0.507 ± 0.034 <sup>a</sup>	0.012 ± 0.001 <sup>a</sup>
Full flowering	70	0.475 ± 0.057 <sup>ab</sup>	0.015 ± 0.002 <sup>a</sup>

Means with the same letter in the same column are not significantly different ( $P < 0.05$ ).

TABLE 5: Ferric-reducing antioxidant power (FRAP) of the perilla extract and fresh matter (FM) at different growth stages.

Growth stage	Plant height (cm)	FRAP	
		(mmol Fe <sup>2+</sup> /g extract)	(mmol Fe <sup>2+</sup> /g FM)
Early vegetative	15	1.573 ± 0.151 <sup>a</sup>	0.048 ± 0.005 <sup>a</sup>
Medium vegetative	30	0.921 ± 0.072 <sup>b</sup>	0.023 ± 0.002 <sup>c</sup>
Late vegetative	40	1.699 ± 0.181 <sup>a</sup>	0.037 ± 0.004 <sup>b</sup>
Early flowering	60	1.660 ± 0.114 <sup>a</sup>	0.038 ± 0.003 <sup>b</sup>
Full flowering	70	1.759 ± 0.197 <sup>a</sup>	0.057 ± 0.006 <sup>a</sup>

Means with the same letter in the same column are not significantly different ( $P < 0.05$ ).

**3.3. Antioxidant Activity.** The TEAC radical scavenging abilities of different perilla growth stages are reported in Table 4. Significant differences can be observed between the stages ( $P < 0.05$ ) for both the extract and for the plant FM, with the highest TEAC value being in the early flowering stage (0.51 mmol Trolox eq./g of extract) and full flowering stage (0.015 mmol Trolox eq./g of FM), respectively.

The FRAP of the perilla extract and of the FM is shown in Table 5 for different growth stages, and the lowest content is found at the medium vegetative stage (0.92 mmol Fe<sup>2+</sup>/g of extract and 0.023 mmol Fe<sup>2+</sup>/g of FM, resp.). On the other hand, the highest FRAP content is found at the full flowering stage (1.76 mmol Fe<sup>2+</sup>/g of extract and 0.057 mmol Fe<sup>2+</sup>/g of FM, resp.). These results confirm the trend observed for the TEAC assay.

Finally, Figure 3 shows the antiradical activity of perilla extracts against DPPH radicals, expressed as EC<sub>50</sub> value, that is, the concentration required to scavenge 50% of DPPH radicals. The most active extracts are found in the early vegetative stage (EC<sub>50</sub> 34.5 µg/mL), and these are followed by the early and full flowering stages (EC<sub>50</sub> 41.7 and 42.9 µg/mL, resp.).

Jun et al. [42], investigated the antioxidant activities in the leaves of various *Perilla frutescens* var. *acuta* extracts, on the basis of the DPPH radical scavenging ability, ABTS radical cation scavenging ability, and reducing power. Samples were extracted with 80% ethanol and then sequentially fractionated according to the solvent polarity. The highest antioxidant activity was observed for the ethyl acetate fraction. EC<sub>50</sub> values of this fraction, for the DPPH<sup>•</sup> scavenging activity and reducing power, were 2.4 and 1.7 times lower than those of TBHQ, respectively.

Deng et al. [40] determined the antioxidant capacities of lipophilic and hydrophilic extracts of *Perilla frutescens* leaf and another 55 vegetables. The vegetables with the highest

total FRAP values, in decreasing order, were Chinese toon bud > perilla leaf > loosestrife > lotus root > sweet potato leaf, with values of 0.061, 0.045, 0.025, 0.024, and 0.021 mmol Fe<sup>2+</sup>/g, respectively. The vegetables with the highest total TEAC values were ranked as follows: Chinese toon bud > loosestrife > perilla leaf > cowpea > caraway, with values of 0.034, 0.025, 0.024, 0.021, and 0.018 mmol Trolox/g, respectively.

Zhu et al. [43] successfully separated a rosmarinic acid extract from *Perilla frutescens* leaves by means of the supramolecular technique and solvent extraction. This extract exhibited antiradical activity against DPPH<sup>•</sup> of 88.3%, at a concentration of 10 µg/mL, while the other subfractions exhibited a scavenging activity below 50%. The antioxidant activity of the total phenolics was 433.9 mg GAE/g for the rosmarinic acid extract, 116.9 mg GAE/g for the supernatant, and 170.7 mg GAE/g for the supramolecular complex.

In research of Gülçin et al. [29] anthocyanins from *Perilla pankinensis* were found to be effective on DPPH radical reduction, superoxide anion radical and H<sub>2</sub>O<sub>2</sub> scavenging, total reducing power, and metal chelating on the ferrous ion activities. *Perilla pankinensis*, at concentrations of 30 and 45 µg/mL, showed strong inhibition of the lipid peroxidation of linoleic acid peroxidation in acid emulsion system.

Li et al. [48] evaluated the antioxidant capacities of infusions of some parts of *Perilla frutescens*. They found 0.204, 0.039, and 0.028 mmol Fe<sup>2+</sup>/g for FRAP, 0.123, 0.017, and 0.057 mmol Trolox/g for TEAC assays, respectively, in the seeds, stems, and leaves.

Kim et al. [24] investigated the antioxidative activity of various enzymatic extracts from the leaves of *Perilla frutescens*, var. *japonica*, by measuring the DPPH, hydroxyl, and alkyl radical scavenging activities. The alcalase and promozyme extracts showed the highest DPPH radical scavenging activities with. IC<sub>50</sub> values were 109.7 and 77.3 µg/mL,

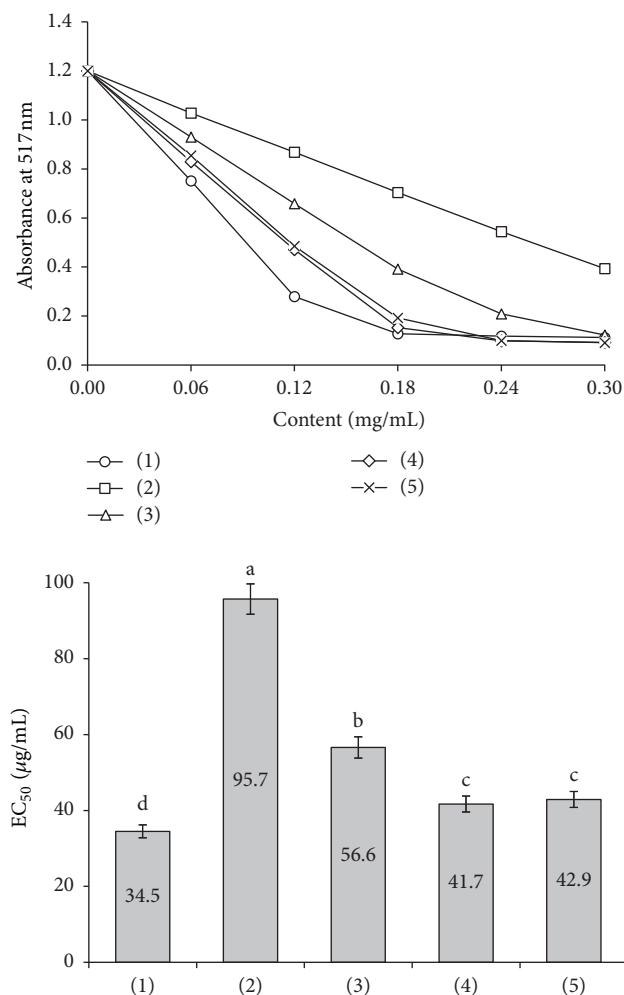


FIGURE 3: Antiradical activity of the extracts against the DPPH radical. (1) early vegetative; (2) medium vegetative; (3) late vegetative; (4) early flowering; (5) full flowering. Means of EC<sub>50</sub> with different letters are significantly different ( $P < 0.05$ ).

respectively. All the enzymatic extracts of the leaf scavenged hydroxyl radicals, and the IC<sub>50</sub> values of the pepsin and cellulase extracts showed the highest activity at 241.9 and 243.3 µg/mL, respectively. The α-chymotrypsin extract from the leaves showed the highest scavenging activity, and IC<sub>50</sub> value was 33.2 µg/mL, while the pepsin extracts showed a protective effect on H<sub>2</sub>O<sub>2</sub>-induced DNA damage and decreased cell death in PC-12 cells induced by hydrogen peroxide. These authors suggested that enzymatic extracts of the leaves can exert an antioxidative effect.

Meng et al. [25] investigated the antioxidant activity of water extracts of some *Perilla frutescens* cultivars harvested in China and Japan, on the basis of the inhibition of the DPPH free radical. The calculated TEAC value (23–167 µmol TE/100 mL) confirmed the high antioxidant activity of these leaf water extracts, due to the amount of total phenolics (4–29 µmol/100 mL). These results confirmed the high correlation between antioxidant activity and some *o*-dihydroxylated polyphenolic compounds.

Saita et al. [41] determined the antioxidant activities of green and red varieties of *Perilla frutescens*, var. *crispa* and found that the DPPH solutions required 7.9 and 29 µg/mL of red and green perilla, respectively, to scavenge 50% of DPPH radicals.

As far as the other parts of the plant are concerned, Sargi et al. [49] determined the antioxidant capacity of five seeds: white and brown perilla, chia, golden, and brown flax. The results showed that brown perilla exhibited a stronger antioxidant potential than white perilla and the other seeds. In the DPPH assay, the highest value was found for brown and white perilla (2.54 and 2.38 mmol TE/g, resp.) and the lowest value for golden flax (1.16 mmol TE/g). A similar pattern was found in the FRAP assay, in which brown and white perilla had the highest values (5.24 and 4.01 mmol TE/g, resp.) and golden flax the lowest (0.33 mmol TE/g). The highest value in the ABTS assay was also found in the brown perilla seed (4.06 mmol TEAC/g), while white perilla had a value of 3.32 mmol TE/g. Müller-Waldeck et al. [50] measured the TEAC values of five varieties of *Perilla frutescens*. The obtained values ranged from 0.032 to 0.056 mmol TE/g, which are lower than the results reported by Sargi et al. (2013) for white and brown perilla. This difference may be caused by the sample composition as an effect of factors such as climate conditions and geographic location. Lee et al. (2013) investigated the antioxidant properties of an 80% methanol extract (50 µg/mL) of the seeds of various *Perilla frutescens* cultivars. All the extracts showed powerful antioxidant activities against DPPH and ABTS radical scavenging activities, with the Yeupsil cultivar exhibiting the highest antioxidant activity (83% and 91%, resp.).

Finally, Tian et al. [51] evaluated the antioxidant potential of different *Perilla frutescens* oils from 11 areas in China using ABTS, DPPH, and the reducing power. The oils exhibited various degrees of scavenging ability.

#### 4. Conclusions

The present results indicate that the antioxidant activity of several components of perilla plant extracts changes during the growth cycle and may be useful in determining the optimal harvest time at which phenolic compounds reach their maximum content. The highest total phenolic compound content was found at the full flowering stage and was related to the rosmarinic acid content. This trend was also confirmed by the TEAC and FRAP values found for the full flowering stage. The highest antiradical activity against DPPH\* was observed for the early vegetative stage extract. However, further studies are needed to identify the specific antioxidant components of *Perilla frutescens*, which may constitute a new functional food and could be an important dietary source for the prevention of diseases caused by oxidative stress.

#### Conflicts of Interest

The authors declare that there are no conflicts of interest.

## Authors' Contributions

Francesco Gai and Pier Giorgio Peiretti designed and performed the biological experiments; Magdalena Karamać and Ryszard Amarowicz designed and performed the chemicals experiments; Francesco Gai, Pier Giorgio Peiretti, Magdalena Karamać, and Ryszard Amarowicz wrote the paper.

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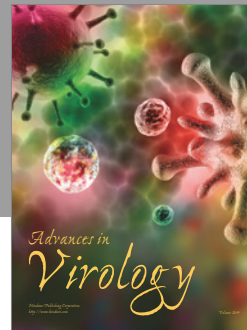
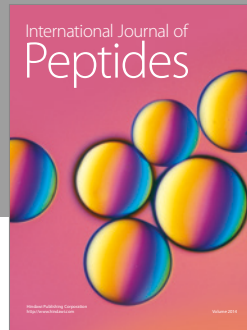
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