JOURNAL OF FOOD AND DRUG ANALYSIS 23 (2015) 225–233

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

By-product of *Lavandula latifolia* essential oil distillation as source of antioxidants

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ARTICLE INFO

Article history:
Received 19 May 2014
Received in revised form
9 July 2014
Accepted 10 July 2014
Available online 24 August 2014

Keywords:
antioxidant activity
by-product
interpopulation variability
spike lavender
total phenolic content

ABSTRACT

The objective of this work was to evaluate the antioxidant properties of *Lavandula latifolia* waste obtained after essential oil distillation. Samples of 12 wild populations of the *Lavandula* genus collected between 2009 and 2010 were hydrodistilled and their by-products were analyzed using the Folin–Ciocalteu, free radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl), and the ferric reducing antioxidant power (FRAP) methods. Rosmarinic acid, apigenin, and luteolin contents were analyzed by high-performance liquid chromatography–diode array detection. The mean of total phenolic content ranged from 1.89 ± 0.09 mg gallic acid equivalents/g dry weight to 3.54 ± 0.22 mg gallic acid equivalents/g dry weight. The average value of the half maximal effective concentration (EC_{50}) for scavenging activity ranged from 5.09 ± 0.17 mg/mL to 14.30 ± 1.90 mg/mL and the variability of the EC_{50} in FRAP ranged from 3.72 ± 0.12 mg/mL to 18.55 ± 0.77 mg/mL. Annual variation was found among this samples and the environmental conditions of 2009 were found to be more favorable. The plants collected from Sedano showed the highest antioxidant power. Our results show that rosmarinic acid and apigenin in *L. latifolia* contributed to the antioxidant properties of the waste. In conclusion, the by-product of the distillation industry could be valorizing as a source of natural antioxidants.

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http://dx.doi.org/10.1016/j.jfda.2014.07.003

1. Introduction

The genus *Lavandula* is a member of the Lamiaceae (Labiatae) family, which includes 39 species. This genus has a wide distribution from the Macaronesic region to all the Mediterranean regions and is scattered throughout the Northern parts of Africa, the Arabian Peninsula, and South Asia reaching India [1]. In particular, spike lavender (*Lavandula latifolia* Medik.) is a shrub that measures between 50 cm and 70 cm (height) and blossoms in mid-July [2]. *Lavandula latifolia* prefers limestone rocks or dry pastures on sunny hillsides and requires a basic alluvial substratum (between 20 m and 2050 m). The species is commonly found in the Iberian Peninsula, France, Italy, and former Yugoslavia [1].

Since ancient times, various species of the *Lavandula* genus have been used for medicinal and ornamental purposes. They are also used as a flavoring agent. In addition, they are used as a condiment and as a disinfectant, due to their antiseptic properties. The essential oil from the plants of this genus is extracted and used in perfumes. Additionally, in recent years, the potential of *Lavandula* oil as a bactericidal, bacteriostatic, and as an antifungal agent has been studied, with study results supporting its bactericidal and antifungal properties [3].

Lavandula × *intermedia* Emeric ex Loiseleur, *L. angustifolia* Mill., and *L. latifolia* Medik. are the most widely used species of this genus [1]. Nowadays, the plants of this genus are especially used for medicinal purposes; in addition, the flower spikes are distilled to obtain essential oils, which are widely used in the perfume industry. The genus possesses anti-inflammatory [4], antispasmodic, anticonvulsant [5], and sedative properties. In addition, it is also known to improve the quality of sleep and reduce anxiety and stress [6,7].

In the perfume industry, essential oil is extracted from the flowers of the plant spike lavender. This process of extraction generates large amounts of residue, with 50–100 tons of wastes generated every year [8]. This large volume of by-product generated during distillation is of growing concern. In some industries, the biomass is used for generating energy or for preparing compost [9]. However, this recycling system has the following disadvantages: recycling the by-product to energy requires a huge investment, and recycling to composting is not always satisfactory due to the antigerminative properties of some aromatic plants [10], which may also be transferred by the plant residue. The aerial parts of the plants of the genus *Lavandula* also have the ability to act as a natural antioxidant [11], acting as a free radical scavenger with a diverse content of polyphenols [12]. Torras-Claveria et al [13] identified the phenolic content of lavandin waste (*Lavandula* × *intermedia* Emeric ex Loiseleur) obtained after the distillation of essential oils. Rosmarinic acid was identified as the main compound in these wastes; in addition, important flavones (apigenin, luteolin, and chrysoeriol) were also identified. This indicates that other similar residues from the plants of this genus may also contain polyphenols, which can be used for various purposes.

The value of *L. latifolia* by-product can be increased by using it as a source of natural antioxidants. These natural antioxidants could be extracted for animal feed or as a natural food preservation agent in the food industry. Previous studies have reported the toxicity of food preservatives such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) in animals [14,15]. Although recent studies by the European Food Safety Authority [16,17] reported that the acceptable daily intake of BHA and BHT is not generally exceeded in food products, the safety of these authorized and widely used additives is still controversial. At present, intake of antioxidants and identifying new sources of natural antioxidants are a priority. As a result, many studies evaluating the antioxidant content of plants have been carried out in recent years [12,13,18].

In order to exploit spike lavender as a source of natural antioxidants, it is essential to understand the variability in the antioxidant content of different wild populations of *L. latifolia*, so as to select those with a high content of antioxidants. The objective of this work was to study the variability of the antioxidant capacity and content of polyphenols among different populations of the genus and seasons in the aerial parts of the plant and in the hydrodistilled residue. In this way, it would be possible to revalue the waste of the distilling industry, thereby reducing production costs and preserving the environment. Antioxidants and phenols are influenced by climatic conditions [19], and therefore the populations were studied for a period of 2 years.

Table 1 – Geographical coordinates of collected populations of *Lavandula latifolia*.

Populations	Province	Locality	Latitude (N)	Longitude (W)	Altitude (m)
LL-01	Soria	Dévanos	41°54′06″	1°55′01″	968
LL-02	Soria	Velamazán	41°29′05″	2°47′22″	936
LL-03	Segovia	Moral de Hornuez	41°27′15″	3°37′56″	1133
LL-04	Segovia	Fuentidueña	41°26′41″	3°57′38″	844
LL-05	Burgos	Sedano	42°41′18″	3°44′13″	750
LL-06	Burgos	Santibáñez del Val	41°58′38″	3°29′08″	953
LL-07	Burgos	Gumiel de Izán	41°46′23″	3°40′40″	899
LL-08	Valladolid	Quintanilla de Onésimo	41°37′14″	4°20′09″	879
LL-09	Palencia	Aguilar de Campoo	42°45′33″	4°13′50″	913
LL-10	Soria	Tejado	41°33′40″	2°13′26″	1066
LL-11	Palencia	Cevico Navero	41°52′21″	4°11′36″	916
LL-12	Palencia	Reinoso de Cerrato	41°56′57″	4°22′28″	876

2. Materials and methods

2.1. Plant material

The aerial parts of 12 wild populations of *L. latifolia* were collected during the blossom phase in the summer of 2009 and 2010 in 12 locations from Castilla and León (Spain). Voucher specimens of these populations were deposited in the Botany Laboratory, Herbarium of Campus of Palencia (PALAB), University of Valladolid.

Table 1 presents the data on the province, locality, and geographical coordinates of the sampling sites. The plant material was dried for 4 weeks at room temperature, in the dark, after collection. When the drying process was completed, the leaves and flowers were separated from the stems and only the mix of leaves and flowers was used for further analysis. Part of the raw plant was grounded using a grinder (grinder type ZM 1; Retsch, Haan, Germany) and preserved in a glass flask for further analyses.

2.2. Chemical and reagents

Methanol was obtained from Sigma-Aldrich (St. Louis, MO, USA). The Folin–Ciocalteu reagent was supplied by Panreac (Barcelona, Spain). Sodium carbonate and trichloroacetic acid (TCA) were obtained from Fluka (Steinheim, Switzerland). Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, and ferric chloride ($FeCl_3 \cdot 6H_2O$) were supplied by Sigma-Aldrich. Phosphate buffer (pH 6.6) was prepared from sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 2H_2O$; Merck, Darmstadt, Germany) and disodium hydrogen phosphate ($Na_2HPO_4 \cdot 2H_2O$; Panreac).

2.3. Distillation

The essential oils were isolated from 180 g of dried material of each population by hydrodistillation in 2 L of water for 150 minutes using a Clevenger-type apparatus. The by-product obtained (i.e., waste plant material obtained after removing the essential oils through distillation) was dried in an oven at 32°C for 48 hours and used for further analysis. The dry by-product was grounded using the ZM 1 grinder (Retsch) and preserved in a glass flask until further use.

2.4. Preparation of extracts

For each population, three homogeneous samples of 0.5 g of the grounded plant material or grounded by-product were mixed with 15 mL of methanol. Following the addition of methanol, the mixture was vigorously shaken for 5 minutes and allowed to decant for 1 hour. Subsequently, the methanolic extracts were separated and stored at −18°C until the analysis of total phenolic content and antioxidant activity by DPPH and ferric reducing antioxidant power (FRAP) methods.

2.5. Determination of total phenolic content

The total phenolic content of the extracts was measured using the Folin–Ciocalteu method based on a colorimetric assay described by Singleton and Rossi [20]. Approximately 1 mL of each extract (with a concentration of 5 mg/mL for the plant and 10 mg/mL for the by-product) was mixed with 1 mL of the Folin–Ciocalteu reagent. After 2 minutes, 1 mL of saturated solution of sodium carbonate was added and finally 7 mL of Milli-Q water was added. After allowing the mixture to react for 90 minutes in the dark at room temperature, the absorbance was measured at 725 nm in a JASCO V-530 UV–VIS spectrophotometer (JASCO, Tokyo, Japan). The total phenolic content in each extract was determined through a calibration curve of gallic acid (0.01–0.08 mM; correlation coefficient > 0.99). The total phenolic content was expressed as milligram of gallic acid equivalent/gram of dry weight plant or dry weight by-product (mg GAE/g).

2.6. Free radical scavenging activity (DPPH method)

The free radical scavenging activity was determined using the method reported by Pereira et al [21].

The analysis was carried out with 300 μL of six different concentrations of methanolic aqueous dissolutions (0.66–16.66 mg/mL) mixed with 2.7 mL of DPPH radical (6×10^{-5} mol/L in methanol). A blank solution was also prepared with 300 μL of Milli-Q water and 2.7 mL of DPPH solution. The mixture was vigorously shaken and allowed to rest for 60 minutes in the dark at room temperature. A colorimetric evaluation was then carried out using a spectrophotometer at 517 nm. The free radical scavenging ability was measured as a percentage of DPPH decoloration using the following equation:

$$\%\text{Scavenging effect} = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100 \quad (1)$$

where A_{DPPH} is the absorbance of the blank solutions and A_S is the absorbance of each sample concentration tested. The extract concentration providing 50% inhibition (i.e., half maximal effective concentration or EC_{50}) was calculated. Lower EC_{50} value means a higher antioxidant activity.

2.7. Ferric reducing antioxidant power

Six different concentrations of the methanolic aqueous dissolutions (0.66–16.66 mg/mL) were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) $K_3[Fe(CN)_6]$ solution. The mixtures were incubated for 20 minutes in a water bath at 50°C. The incubated mixtures were allowed to cool at room temperature. Once cooled 2.5 mL of 10% (w/v) TCA solution was added. The solutions were mixed thoroughly, aliquots of 2.5 mL were withdrawn, and 0.5 mL of 0.1% (w/v) $FeCl_3 \cdot 6H_2O$ solution was added. The absorbance was measured at 700 nm. The same procedure was followed for the blank but with Milli-Q water instead of the sample. The extract concentrations needed to provide an absorbance of 0.5 (EC_{50}) were calculated. A lower EC_{50} value means a higher antioxidant activity.

2.8. High-performance liquid chromatography analysis

For high-performance liquid chromatography analysis, 0.5 g of sample obtained from each by-product was dissolved in petroleum ether for 24 hours, and then filtered and extracted in a soxhlet apparatus with methanol as solvent for 150 minutes. The methanolic extracts were concentrated under

vacuum at 50°C using a rotary evaporator and were then resuspended in 5 mL of acetonitrile/water (1/1).

High-performance liquid chromatography analysis was performed in an Agilent Technologies 1200 series high-performance liquid chromatograph with a diode array detector, which was equipped with a ZORBAX Eclipse XDB-C18 column (150 mm × 4.6 mm i.d., 5 μm; Agilent Technologies, Palo Alto, CA, USA). The column was thermostated at 25°C and the injection volume was 20 μL. The solvents were (A) water:acetic acid (98:2) and (B) acetonitrile. The flow rate was 1.2 mL/minute. The linear gradient used is as follows: from 10% to 22% B (10 minutes), from 22% to 38% B (2 minutes), isocratic for 5 minutes, finally from 38% to 100% B (2 minutes) and then isocratic for 5 minutes. Compound identification was done by comparing their retention times and UV–VIS spectra with their respective pure standards at a wavelength of 254 nm, 280 nm, or 350 nm depending on the maximum absorption of each compound. The phenolic compounds were quantified using the external standard method and the respective calibration curve of each quantified phenolic compound.

2.9. Statistical analysis

The statistical analysis of the data was performed using SPSS version 15.0 (IBM SPSS Inc., Chicago, IL, USA). A general analysis of variance (ANOVA) was performed to check the differences between distilled and nondistilled plant material. The ANOVAs for the plant material were performed to evaluate populations and seasons. The Pearson correlation coefficients among the total phenolic content, free radical scavenging activity, and FRAP were also determined. To study the relationship between antioxidant capacity and phenolic compounds in the by-product, a stepwise regression was performed.

3. Results

3.1. Total phenolic content

The total phenolic content in the nondistilled plant material was higher than the phenolic content in the by-product for all studied populations (Table 2). The phenolic content in the nondistilled material ranged from 2.67 ± 0.14 (LL-2) mg GAE/g to 8.27 ± 0.50 (LL-8) mg GAE/g of dry plant for the samples collected in 2009 and from 2.40 ± 0.12 (LL-1) mg GAE/g to 6.83 ± 1.74 (LL-6) mg GAE/g of dry plant for the samples collected in 2010. For the by-product, the phenolic content of the populations ranged from 1.89 ± 0.09 (LL-3) mg GAE/g to 3.54 ± 0.22 (LL-5) mg GAE/g of dry by-product for the samples collected in 2009 and from 1.97 ± 0.16 (LL-3) mg GAE/g to 2.60 ± 0.23 (LL-9) mg GAE/g of dry

Table 2 – Total phenolic content, EC_{50} of scavenging activity, and EC_{50} of ferric reduction antioxidant power of the plant and the by-product of the 12 populations of *Lavandula latifolia* studied.

Populations	Plant material 2009			By-product 2009		
	TP (mg GAE/g)	DPPH EC_{50} (mg/mL)	FRAP EC_{50} (mg/mL)	TP (mg GAE/g)	DPPH EC_{50} (mg/mL)	FRAP EC_{50} (mg/mL)
LL-1	3.70 ± 0.21	4.56 ± 0.15	6.19 ± 0.24	2.55 ± 0.39	8.25 ± 1.14	9.37 ± 0.51
LL-2	2.67 ± 0.14	4.28 ± 0.38	3.92 ± 0.13	1.91 ± 0.34	10.55 ± 1.05	7.45 ± 0.31
LL-3	3.77 ± 0.18	2.94 ± 0.26	2.93 ± 0.05	1.89 ± 0.09	10.41 ± 0.48	7.61 ± 0.24
LL-4	3.13 ± 0.28	4.39 ± 0.16	3.36 ± 0.23	2.09 ± 0.13	8.97 ± 0.30	6.17 ± 0.07
LL-5	5.15 ± 0.46	2.83 ± 0.04	2.17 ± 0.06	3.54 ± 0.22	6.33 ± 0.14	4.35 ± 0.12
LL-6	3.70 ± 0.44	3.82 ± 0.07	3.39 ± 0.03	2.05 ± 0.12	8.03 ± 0.45	5.21 ± 0.15
LL-7	4.36 ± 0.11	3.62 ± 0.08	3.24 ± 0.02	2.62 ± 0.09	5.09 ± 0.17	3.72 ± 0.12
LL-8	8.27 ± 0.50	1.85 ± 0.05	1.79 ± 0.01	2.00 ± 0.11	11.90 ± 0.05	8.43 ± 0.17
LL-9	4.93 ± 0.20	3.18 ± 0.08	6.21 ± 0.03	2.45 ± 0.16	8.23 ± 0.59	5.61 ± 0.08
LL-10	5.07 ± 0.33	3.61 ± 0.96	2.79 ± 0.28	2.13 ± 0.12	9.67 ± 0.41	7.25 ± 0.24
LL-11	4.09 ± 0.22	3.86 ± 0.10	3.28 ± 0.07	2.36 ± 0.07	8.42 ± 0.53	5.76 ± 0.27
LL-12	4.00 ± 0.26	3.86 ± 0.11	3.42 ± 0.16	2.15 ± 0.13	10.14 ± 0.37	7.44 ± 0.14
Populations	Plant material 2010			By-product 2010		
	TP (mg GAE/g)	DPPH EC_{50} (mg/mL)	FRAP EC_{50} (mg/mL)	TP (mg GAE/g)	DPPH EC_{50} (mg/mL)	FRAP EC_{50} (mg/mL)
LL-1	2.40 ± 0.12	5.69 ± 0.35	5.83 ± 0.44	2.14 ± 0.16	12.63 ± 0.86	18.55 ± 0.77
LL-2	3.06 ± 0.22	3.19 ± 0.05	3.67 ± 0.15	2.11 ± 0.13	11.26 ± 1.21	10.85 ± 0.99
LL-3	3.79 ± 0.33	3.24 ± 0.02	3.64 ± 0.13	1.97 ± 0.16	14.30 ± 1.90	11.03 ± 1.72
LL-4	4.50 ± 0.49	3.59 ± 0.16	3.91 ± 0.32	2.00 ± 0.12	13.87 ± 0.67	11.03 ± 0.63
LL-5	6.30 ± 0.36	3.07 ± 0.08	3.55 ± 0.39	2.36 ± 0.17	9.21 ± 0.61	7.88 ± 0.61
LL-6	6.83 ± 1.74	4.06 ± 0.44	3.82 ± 0.43	2.29 ± 0.14	11.20 ± 0.39	9.65 ± 0.30
LL-7	4.57 ± 0.42	3.22 ± 0.21	3.98 ± 0.19	2.16 ± 0.05	10.29 ± 0.52	9.38 ± 0.74
LL-8	4.79 ± 0.36	2.94 ± 0.09	3.36 ± 0.10	2.49 ± 0.23	9.22 ± 0.65	7.97 ± 0.60
LL-9	4.05 ± 0.35	3.11 ± 0.08	3.33 ± 0.16	2.60 ± 0.23	9.99 ± 0.66	8.70 ± 0.78
LL-10	3.29 ± 0.30	4.01 ± 0.26	4.47 ± 0.26	2.52 ± 0.30	9.64 ± 1.09	9.49 ± 1.08
LL-11	2.88 ± 0.18	4.83 ± 0.28	5.39 ± 0.22	2.57 ± 0.28	9.81 ± 1.22	8.35 ± 1.38
LL-12	3.16 ± 0.17	5.33 ± 0.16	4.42 ± 0.23	2.50 ± 0.20	8.48 ± 0.51	8.71 ± 0.43

DPPH EC_{50} = extract concentration providing a 50% inhibition of scavenging activity; EC_{50} = half maximal effective concentration; FRAP EC_{50} = extract concentration needed to provide an absorbance of 0.5 of ferric reduction antioxidant power; mg GAE/g = milligram of gallic acid equivalents per gram of dry matter; TP = total phenol content.

Table 3 – Percentages of the sum of squares obtained in the analysis of variance by type of plant material using population, year, and year by population interaction as sources of variation.

	df	Total phenols	*df*	DPPH	*df*	FRAP
Plant material						
Population	11	55.57*	11	70.19*	11	58.8*
Year	1	0.85*	1	2.76*	1	6.62*
Year × population	11	33.71*	11	18.88*	11	32.1*
Residual	192	9.87	48	8.18	120	2.5
By-product						
Population	11	50.04*	11	37.04*	11	48*
Year	1	0.8**	1	20.85*	1	35.3*
Year × population	11	29.98*	11	31.28*	11	12.7*
Residual	192	19.21	48	10.83	120	4.02

* Values significant at $p < 0.001$.
** Values significant at $p < 0.01$.
df = degrees of freedom; DPPH = 2,2-diphenyl-1-picrylhydrazyl; FRAP = ferric reducing antioxidant power.

by-product for the samples collected in 2010. The loss of phenolic compounds by hydrodistillation ranged between 24% and 89% depending on the population and the season.

Table 3 shows the sum of squares from the ANOVA expressed as percentages, indicating the contribution to the variability for the studied factors (i.e., population, season, and the interaction between season and population) and "residual" expresses the percentage of the variability not explained by these factors. The effect of the season on total phenolic content and the effect of genotypic variability were statistically significant, but the effect of the season was much lower than that of the population effect. The interaction between year and population also had a strong effect on phenolic content (Table 3), meaning that the annual season variation has not been the same for all of the populations studied. The percentages of influence of each variable for both plant and by-product showed that the population was the most important variable followed by the interaction between season and population, with season being the less influential variable.

A comparison of these results with that obtained by other authors is neither simple nor accurate because the results vary depending on the extraction method and the solvent used for the different concentrations of plant material [22]. However, comparison with other studies is necessary to have more representative results. For other *Lavandula* species such as *L. angustifolia*, Miliauskas et al [23] reported total phenolic content of 5.4 ± 0.2 mg GAE/g of plant extract (methanolic extracts), which is similar to that obtained in this study for *L. latifolia*.

3.2. *Free radical scavenging activity (DPPH)*

The scavenging activity of the aqueous methanolic dissolutions in the nondistilled plant material was higher than that in the by-product for all studied populations (Table 2). The value of the extract concentrations providing a 50% inhibition of DPPH free radicals varied from 1.85 ± 0.05 (LL-8) mg/mL to 4.56 ± 0.15 (LL-1) mg/mL for the nondistilled plant material collected in 2009 and from 2.94 ± 0.09 (LL-8) mg/mL to 5.69 ± 0.35 (LL-1) mg/mL for the samples collected in 2010. For the by-product, the average value of EC_{50} ranged from 6.33 ± 0.10 (LL-5) mg/mL to 11.90 ± 0.05 (LL-8) mg/mL for the samples collected in 2009 and from 8.48 ± 0.51 (LL-12) mg/mL to 14.30 ± 1.90 (LL-3) mg/mL for the samples collected in 2010. For the different populations studied, the increase in EC_{50} for the by-product in comparison with the plant material ranged from 29% to 84% depending on the population and season.

The effect of the season on scavenging activity was much lower than that of genotypic variability; however, both factors were statistically significant, and so is the interaction between year and population (Table 3). Average values of scavenging activity for samples collected in 2009 showed higher antioxidant capacity than populations collected in 2010; however, upon checking every population, we found that the results were more favorable for some cases in 2009, whereas 2010 was favorable is other cases because the interaction between year and population was very important and the samples were collected in different places under different weather and ecological conditions.

The lowest EC_{50} values were shown by LL-8 (1.85 ± 0.05 mg/mL for 2009 and 2.94 ± 0.09 mg/mL for 2010) and LL-5 (2.83 ± 0.04 mg/mL for 2009 and 3.07 ± 0.08 mg/mL for 2010) for both seasons in the nondistilled plant material. For the by-product, LL-7 and LL-5 showed the highest antioxidant capacity in 2009 with 5.09 ± 0.17 and 6.33 ± 0.14 mg/mL, respectively, whereas LL-12 and LL-5 showed the highest antioxidant capacity in 2010 with 8.48 ± 0.51 mg/mL and 9.21 ± 0.61 mg/mL, respectively. Miliauskas et al [23] found an inhibition of 35.4% ± 1.7% using a methanolic extract concentration of 2.5 mg/mL for *L. angustifolia*, which is very similar to those results obtained with the nondistilled plant of *L. latifolia*, because concentrations between 1.85 ± 0.05 mg/mL and 5.69 ± 0.35 mg/mL provided an inhibition of 50%.

3.3. *FRAP*

The samples analyzed with the FRAP method showed more antioxidant activity for the nondistilled plant extracts than for the by-product obtained from the wastes of all the analyzed populations (Table 2), except for LL-9 in 2009 where the antioxidant power was higher in the by-product. The variability of the EC_{50} value for the reducing power in the studied populations ranged from 1.79 ± 0.01 (LL-8) mg/mL to 6.21 ± 0.03 (LL-1) mg/mL for the nondistilled plant material collected in 2009 and from 3.33 ± 0.16 (LL-9) mg/mL to 5.83 ± 0.44 (LL-1) mg/mL for the material collected in 2010. For the by-product, the data ranged from 3.72 ± 0.12 (LL-7) mg/mL to 9.37 ± 0.51 (LL-1) mg/mL for the samples collected in 2009 and from 7.88 ± 0.61 (LL-5) mg/mL to 18.55 ± 0.77 (LL-1) mg/mL for the samples collected in 2010. The increase in the EC_{50} value for the by-product in comparison with the plant material ranged from 13% to 79% depending on the population and year of harvest.

With regard to the total phenolic content and scavenging activity, the effect of the population on reducing power was much higher than that of the season, although both were statistically significant, as was the interaction between year and population (Table 3). Samples collected in 2009 showed higher antioxidant power than samples collected in 2010.

Table 4 – Coefficients of Pearson correlation among total phenols, free radical scavenging activity, and ferric reducing antioxidant power.

		Plant		
		TP	DPPH	FRAP
By-product	TP		−0.6592 $p < 0.0005$	−0.4652 $p < 0.0220$
	DPPH	−0.4492 $p < 0.0277$		0.642 $p < 0.0007$
	FRAP	—	0.727 $p < 0.0001$	

DPPH = 2,2-diphenyl-1-picrylhydrazyl; FRAP = ferric reducing antioxidant power; p = statistical significance; TP = total phenols.

The populations with higher FRAP were LL-8 and LL-5 for 2009 and LL-9 and LL-8 for 2010 with values of 1.79 ± 0.01 mg/mL, 2.17 ± 0.06 mg/mL, 3.33 ± 0.16 mg/mL, and 3.36 ± 0.10 mg/mL, respectively, for the nondistilled plant. LL-7 and LL-5 in 2009 and LL-5 and LL-8 in 2010 with 3.72 ± 0.12 mg/mL, 4.35 ± 0.12 mg/mL, 7.88 ± 0.61 mg/mL, and 7.97 ± 0.60 mg/mL were the populations with higher FRAP, respectively, for the by-product.

Gülçin et al [11] measured the FRAP in aqueous and ethanolic extracts of *L. stoechas* and obtained EC_{50} values of approximately 0.04 mg/mL for the ethanolic extracts and approximately 0.06 mg/mL for the aqueous extracts, showing reducing power values higher than those obtained for *L. latifolia* in this work.

As had already been shown by other authors [13,23], a negative correlation was found between the phenolic content and the EC_{50} values for DPPH antioxidant capacity (Table 4), both in the nondistilled plant extracts and in the by-product (−65.92%, $p = 0.0220$ and −44.92%, $p = 0.0277$, respectively. The lower the EC_{50} values, the higher antioxidant capacity). There was also a negative correlation between the phenolic content and the EC_{50} values for the reducing power method as reported previously [24]; however, this correlation was found only for the nondistilled plant (−46.51%, $p = 0.0220$) and not for the by-product. The correlation between EC_{50} values for the FRAP and DPPH method was statistically significant for both materials (plant and by-product) with a correlation of 64.20% ($p < 0.0007$) for the plant and a correlation of 72.70% ($p < 0.0001$) for the by-product.

3.4. *High-performance liquid chromatography analysis*

The three phenolic compounds identified in the hydrodistilled residue of spike lavender were apigenin, luteolin, and rosmarinic acid (Table 5). Rosmarinic acid was the predominant phenolic compound in all populations regardless of the year assessed, although its variation is important from year to year. The average content of all samples was higher in 2009 (189.18 mg/100 g of dry residue) than in 2010 (159.11 mg/100 g of dry residue); however, in some populations the opposite occurs (i.e., LL-3, LL-8, LL-10, and LL-12 had a higher average content). Higher rosmarinic acid content is a characteristic feature of the species in the family Lamiaceae [25,26].

Variations in the content of major phenolic compounds identified were highly significant ($p < 0.01$) for all three compounds among the collected samples. As with antioxidant

Table 5 – Phenolic compounds identified in by-products of 12 *Lavandula latifolia* populations.

Population code		LL-1	LL-2	LL-3	LL-4	LL-5	LL-6	LL-7	LL-8	LL-9	LL-10	LL-11	LL-12	Average	Standard deviation	Minimum	Maximum
Apigenin	2009	3.25	3.18	3.43	3.14	3.16	3.10	3.41	3.30	3.29	3.40	5.59	3.57	3.49	0.68	3.10	5.59
	2010	1.78	1.29	0.77	0.54	1.64	1.31	0.77	0.54	0.77	0.49	0.58	0.33	0.90	0.48	0.33	1.78
Luteolin	2009	4.94	4.91	4.59	5.55	4.39	4.56	4.70	5.29	6.04	6.70	10.26	6.26	5.68	1.62	4.39	10.26
	2010	4.00	5.91	3.25	3.92	3.88	2.87	2.98	3.61	2.73	3.64	3.42	2.08	3.52	0.94	2.08	5.91
Rosmarinic acid	2009	180	200	119	193	255	215	304	158	223	170	134	119	189	56	119	304
	2010	90	138	142	133	154	129	218	191	180	224	137	173	159	39	90	224

Results are expressed in mg/100 g of dry extract.

Table 6 – Stepwise regression analysis taking scavenging activity (DPPH) and FRAP as dependent variables and the polyphenolic content of apigenin, luteolin, and rosmarinic acid as independent variables.

	Step	Variable entered	Partial R^2	Model R^2	F	$p > F$
DPPH						
	1	Rosmarinic acid	0.5188	0.5188	23.72	<0.001
	2	Apigenin	0.1166	0.6354	6.72	0.017
FRAP						
	1	Rosmarinic acid	0.4016	0.4016	14.77	0.0009
	2	Apigenin	0.1772	0.5788	8.83	0.0073

DPPH = 2,2-diphenyl-1-picrylhydrazyl; F = F test to choose the predictive variables of the model; FRAP = ferric reducing antioxidant power; model R^2 = percentage of the model explained with the variables; partial R^2 = contribution of each variable to the model; $p > F$ = statistical significance.

capacity analysis, these data indicate that the presence of polyphenols in the hydrodistilled residue of *L. latifolia* depends on the population and the environmental characteristics.

The results of the stepwise regression between antioxidant capacity and phenolic compounds are shown in Table 6. This regression model was developed to predict the influence of the polyphenols on the antioxidant capacity of the by-product. For total phenolic content, none of the variables met the 0.05 significance ($p > F$) for entry into the model, which means that other phenolic compounds not measured and present in the methanolic extracts of spike lavender wastes are influencing the amount of total phenols. In the case of scavenging effect and ferric reduction antioxidant power, rosmarinic acid was ranked before the apigenin, for both models, which explains 51.88% and 40.16% (partial R^2) of variability, respectively. Despite this the models explained only 57.88% and 63.54% (model R^2) of variability, respectively, indicating that although these two compounds are responsible for part of the antioxidant activity of spike lavender waste, there are other compounds that affect this biological activity.

4. Discussion

Methanolic extracts of the by-product showed a considerably lower antioxidant activity than the plant material prior to distillation. The results found are in agreement with those found by Chizzola et al [27] who found a smaller amount of antioxidants in the by-products of leaves of *Thymus vulgaris* than in the nondistilled plant material. Water-soluble compounds and essential oil are extracted from the original plant material by the distillation process, and some of these compounds also contribute to the total antioxidant activity [28]. Moreover, the essential oil of spike lavender contains a proportion of antioxidants [29], and thus the by-product obtained after distillation had less antioxidant activity. By contrast, phenolic compounds are generally sensitive to prolonged exposure to heat and could be degraded with thermal treatments [30] such as hydrodistillation and the industrial process of essence extractions.

Although the waste of the distilling industry showed less antioxidant activity than the original plant, it would be possible to recover appreciable amounts of antioxidants from the hydrodistilled residue. The presence of rosmarinic acid in this residue is a characteristic of the Labiatae family [26], and our results confirm that this phenolic acid, as well as apigenin, contributes to the antioxidant properties of the by-product. However, these compounds do not explain all the antioxidant activity of the samples; other phenolic compounds detected in these samples but not identified could also have antioxidant properties.

There was a considerable variability in the total phenolic content, in the antioxidant capacity data, and in the FRAP among the populations studied. For all cases, except for the total phenolic content of the by-product of 2010, which presented medium values, the population LL-5 from Sedano (Burgos) is among the three populations with greater antioxidant capacity and phenolic content for the two seasons both in the nondistilled material and in the by-product. By contrast, the population LL-1 from Dévanos (Soria) is among the three populations showing less antioxidant activity for both years and in the three colorimetric methods except for the phenolic content of the by-product of 2009. This preliminary study showed that the population LL-5 could be selected by its highest antioxidant properties.

Phenols are secondary metabolites that are formed by the plant under conditions of stress such as drought, competition with other plants, and infection [31,32] among other aspects. The interannual variation was statistically significant in all cases although it was less important than population variation. Conditions in 2009 proved to be more favorable than those of 2010 due to a set of variables such as rainfall and temperatures. Irrigation causes a reduction in the antioxidant content of plants and fruits [33], and given that the 2009 season was a dry summer in Castilla and León with low rainfall and high drought conditions with respect to the overall mean, this could be responsible for the higher antioxidant activity of the samples collected in 2009.

Data of the latitude, longitude, and altitude were collected for every population; however, there was no correlation between these parameters and the experimental data. Other species of the Lamiaceae family noted for antioxidant activity could be *Mentha longifolia* L. ssp. *longifolia* with an EC_{50} of 57.4 ± 0.5 mg/L for antioxidant activity; its extracts had a phenolic content of 45 mg GAE/g of dry extract [34]; methanolic extracts of *Origanum vulgare* with an EC_{50} of 9.5 ± 0.5 mg/L for the antioxidant activity and with a phenolic content of 220 mg GAE/g dry extract [35]. The antioxidant activity of *L. latifolia* in comparison with the aforementioned species does not stand out, but it could be considered that the by-product of *L. latifolia* is a source of natural antioxidants.

5. Conclusion

The distillation of *L. latifolia* produces a nonprofitable waste and this by-product generated could be valorized and used as a source of natural antioxidants. To offer a homogeneous product to the industry, it would be necessary to standardize the production conditions and look at the factors that influence the antioxidant content. This study concludes that populations, seasons, and the population by season interaction influence the antioxidant properties of the *L. latifolia* by-product.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This project was carried out thanks to financial support from the National Institute for Instituto Nacional de Tecnología Agraria y Alimentaria (INIA) through project RTA2009-00062-C03-02. I. M.-T. was supported by a PhD fellowship from the Instituto Nacional de Tecnología Agraria y Alimentaria (INIA).

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