Industrial Crops and Products 87 (2016) 255–260

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Total phenolic and flavonoid contents and antioxidant activity of extracts from different populations of lavandin

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ARTICLE INFO

Article history:
Received 31 October 2015
Received in revised form 20 April 2016
Accepted 21 April 2016
Available online 29 April 2016

Keywords:
Lavandula × *intermedia*
Variation
Antioxidant activity
Medicinal plant

ABSTRACT

Plants naturally are a rich source of secondary metabolites and novel therapeutic compounds. These compounds are well known for their various beneficial effects on human health. In this study, variation in total phenolic and flavonoid contents and antioxidant activities (DPPH radical-scavenging and β-carotene bleaching assays) of methanol extracts from the inflorescence parts of 30 lavandin populations collected from western Iran was studied. Results indicated that there were significant differences ($p \leq 0.05$) among populations for total phenolic and flavonoid contents. Total phenolic content varied from 31.45 to 105.39 (mg GAE/100 g dry wt), and total flavonoid content ranged from 71.62 to 28.19 (mg QE/100 g dry matters). Furthermore, results of the antioxidant activities by both methods indicated that the extracts have good antioxidant activities. Results obtained in this study revealed that there is an antioxidant potential of the extracts variability among the populations of lavandin. In conclusion, variability among the populations of lavandin for total phenolic and flavonoid contents and antioxidant potential in both assays can be used in selection programs for production of lavandin with suppressing antioxidant activity.

1. Introduction

Antioxidants being non-enzymatic defenses of the organism against reactive O,N-species, are essential for human health. Most antioxidant compounds are introduced to the organism through diet. Therefore, it is desirable to establish methods that can directly measure the total antioxidant capacity of plant extracts (Berker et al., 2007). The defensive effects of natural antioxidants in herbs are related to three major groups: vitamins, phenolics, and carotenoids (Thaipong et al., 2006).

Herbs have been widely used from ancient times in medicine, cosmetics and for preserving and improving the flavor of foods (Sánchez-Vioquea et al., 2013). The genus *Lavandula*, belonging to the family Lamiaceae, contains many different species. Various *Lavandula* species are geographically grown in many countries which three are economically important: *Lavandula angustifolia*, *Lavandula latifolia*, and *Lavandula* × *intermedia* (Bajalan and Ghasemi Pirbalouti, 2015; Landmann et al., 2007). Lavandin (*Lavandula* × *intermedia*), which result from natural crosses between *L. latifolia* and *L. angustifolia* species, is grown more abundantly and is more prolific than *L. angustifolia* (Yohalem and Passey, 2011). Lavender extracts are used in food and pharmaceutical industries because of their health-promoting and nutraceutical effects. Hsu et al. (2007) found that the aqueous extracts of *L. angustifolia* Vera and *Lavandula stoechas* contained a potent tyrosinase inhibitor and the extracts can be suitable as food bleaching agents. Kovatcheva-Apostolova et al. (2008) found that the addition of *Lavandula vera* extract to minced chicken reduced lipid oxidation and the loss of α-tocopherol during the storage of cooked meat, confirming the antioxidant activity of the extract in a real food system. Lavender extracts and essential oils with phytotoxic and insecticidal properties may also be valuable in the agrochemical industry (Haig et al., 2009; Pavela, 2005). *Lavandula* × *intermedia* is used in soaps, washing agents and perfumes, but is also added as a flavor to food and beverages. Additionally lavender is regarded as a pharmaceutical plant with predominantly sedative effects employed in aromatherapy (Landmann et al., 2007; Gonçalves and Romano, 2013).

To our knowledge, no documented reports on variation of total phenolic and flavonoid contents and antioxidant activities by two methods of the extracts from different populations of lavandin in western provinces of Iran are available. The aim of this study was to compare the efficiency of DPPH and β-carotene assays to estimate antioxidant activities and their correlations with total phenolics, and total flavonoid contents in different populations of lavandin and to assess the relationships between variations of total phenolic

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http://dx.doi.org/10.1016/j.indcrop.2016.04.059
0926-6690/

Table 1
Collection site, geographical and soil characteristics of different populations of *Lavandula × intermedia*.

No.	A. n.[a]	Collection site	Location (UTM)	Altitude (m a.s.l)	CaO (%)	N (%)	P (mgr/kg)	K (ppm)	pH	E. C. (dS m^{-1})	Soil texture
1	Do1	Dorud, Lorestan Province, Iran	39 S 322031 E 3707483 N	1467	16.1	0.14	10.3	395	7.7	0.75	Clay Loam
2	Do2	Dorud, Lorestan Province, Iran	39 S 322031 E 3707483 N	1467	16.1	10.4	10.3	395	7.7	0.75	Clay Loam
3	Do3	Dorud, Lorestan Province, Iran	39 S 322031 E 3707483 N	1467	16.1	0.14	10.3	395	7.7	0.75	Clay Loam
4	Kh1	Khoramabad, Lorestan Province, Iran	39 S 254029 E 3711858 N	1218	15.2	0.20	11.5	448	7.8	0.82	Clay Loam
5	Kh2	Khoramabad, Lorestan Province, Iran	39 S 254029 E 3711858 N	1218	15.2	0.20	11.5	448	7.8	0.82	Clay Loam
6	Kh3	Khoramabad, Lorestan Province, Iran	39 S 254029 E 3711858 N	1218	15.2	0.20	11.5	448	7.8	0.82	Clay Loam
7	Bo1	Borujerd, Lorestan Province, Iran	39 S 290598 E 3753058 N	1561	14.8	0.17	13.1	460	7.3	1.21	Clay Loam
8	Bo2	Borujerd, Lorestan Province, Iran	39 S 290598 E 3753058 N	1561	14.8	0.17	13.1	460	7.3	1.21	Clay Loam
9	Bo3	Borujerd, Lorestan Province, Iran	39 S 290598 E 3753058 N	1561	14.8	0.17	13.1	460	7.3	1.21	Clay Loam
10	Al1	Alashtar, Lorestan Province, Iran	39 S 246199 E 3750496 N	1605	12.8	0.23	12.5	418	7.9	0.58	Clay Loam
11	Al2	Alashtar, Lorestan Province, Iran	39 S 246199 E 3750496 N	1605	12.8	0.23	12.5	418	7.9	0.58	Clay Loam
12	Al3	Alashtar, Lorestan Province, Iran	39 S 246199 E 3750496 N	1605	12.8	0.23	12.5	418	7.9	0.58	Clay Loam
13	Az1	Azna, Lorestan Province, Iran	39 S 356163 E 3702673 N	1875	15.9	0.14	10.1	394	7.4	0.85	Clay Loam
14	Az2	Azna, Lorestan Province, Iran	39 S 356163 E 3702673 N	1875	15.9	0.14	10.1	394	7.4	0.85	Clay Loam
15	Az3	Azna, Lorestan Province, Iran	39 S 356163 E 3702673 N	1875	15.9	0.14	10.1	394	7.4	0.85	Clay Loam
16	Ali1	Aligoodarz, Lorestan Province, Iran	39 S 378785 E 3697711 N	2040	15.8	0.18	8.50	509	7.8	0.71	Clay Loam
17	Ali2	Aligoodarz, Lorestan Province, Iran	39 S 378785 E 3697711 N	2040	15.8	0.18	8.50	509	7.8	0.71	Clay Loam
18	Ali3	Aligoodarz, Lorestan Province, Iran	39 S 378785 E 3697711 N	2040	15.8	0.18	8.50	509	7.8	0.71	Clay Loam
19	Ma1	Malayer, Hamedan Province, Iran	39 S 296591 E 3799319 N	1754	14.2	0.15	11.5	388	7.9	0.69	Silty Clay
20	Ma2	Malayer, Hamedan Province, Iran	39 S 296591 E 3799319 N	1754	14.2	0.15	11.5	388	7.9	0.69	Silty Clay
21	Ma3	Malayer, Hamedan Province, Iran	39 S 296591 E 3799319 N	1754	14.2	0.15	11.5	388	7.9	0.69	Silty Clay
22	Jo1	Jowkar, Hamedan Province, Iran	39 S 287136 E 3811449 N	1702	15.3	0.18	10.8	385	7.6	0.76	Sandy loam
23	Jo2	Jowkar, Hamedan Province, Iran	39 S 287136 E 3811449 N	1702	15.3	0.18	10.8	385	7.6	0.76	Sandy loam
24	Jo3	Jowkar, Hamedan Province, Iran	39 S 287136 E 3811449 N	1702	15.3	0.18	10.8	385	7.6	0.76	Sandy loam
25	Na1	Nahavand, Hamedan Province, Iran	39 S 258059 E 3785878 N	1671	13.7	0.22	12.6	410	7.5	0.42	Sandy clay
26	Na2	Nahavand, Hamedan Province, Iran	39 S 258059 E 3785878 N	1671	13.7	0.22	12.6	410	7.5	0.42	Sandy clay
27	Na3	Nahavand, Hamedan Province, Iran	39 S 258059 E 3785878 N	1671	13.7	0.22	12.6	410	7.5	0.42	Sandy clay
28	Ha1	Hamedan, Hamedan Province, Iran	39 S 273217 E 3855800 N	1810	12.1	0.13	10.4	402	7.8	0.68	Silty Clay
29	Ha2	Hamedan, Hamedan Province, Iran	39 S 273217 E 3855800 N	1810	12.1	0.13	10.4	402	7.8	0.68	Silty Clay
30	Ha3	Hamedan, Hamedan Province, Iran	39 S 273217 E 3855800 N	1810	12.1	0.13	10.4	402	7.8	0.68	Silty Clay

[a] Association name.

and flavonoid contents and the environmental factors involved in different geo-ecological regions.

2. Materials and methods

2.1. Plant material and site description

Inflorescence parts of *Lavandula × intermedia* were collected according to completely randomized design with three replications from the plants growing sites in the Zagros regions, Western Iran. All the plants grown in the regions had the same phenotype. To be more exact about sampling, three samples were collected from each plant and they were mixed then samples were labeled (Bajalan and Ghasemi Pirbalouti, 2015). The location was recorded using a Global Positioning System (GPS, Vista Garmin) receiver (Table 1).

2.2. Extraction method

Preparation of extracts was adapted from the methods described by Kowalski (2009). A total of 10 g inflorescence powder of lavandin was stirred with 100 ml of methanol into conical flasks. The samples were then shaken for 24 h, and achieved extracts were filtered by filter paper, properly protected and stored in a fridge (4 °C).

2.3. Total phenolic content

Total phenolic was determined by the Foline–Ciocalteu method. 0.1 ml of sample was mixed with 2 ml of sodium carbonate (2%) freshly prepared, the whole was vigorously mixed on a vortex. After 5 min, 100 ml of Foline–Ciocalteu reagent (1 N) were added to the mixture, all was left for 30 min at room temperature and the reading of absorbance (SPECORD 200 Plus) is performed against a blank at 750 nm. A calibration curve was performed in parallel under the same operating conditions using gallic acid as a positive control. The results are expressed as mg gallic acid equivalent per gram of dry extract (mg GAE/100 g dry wt) (Vermerris and Nicholson, 2006).

2.4. Total flavonoid content

Total flavonoid content was determined according to El-Haci et al. (2013) method. Each sample (500 ml) was mixed with 2 ml of distilled water and subsequently with 150 ml of a $NaNO_2$ solution (15%). After 6 min, 150 ml of aluminum chloride ($AlCl_3$) solution (10%) was added and allowed to stand for 6 min. Then, 2 ml of NaOH solution (4%) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then determined at 510 nm versus prepared water blank. Results were expressed as catechin equivalent per gram of dry extract (mg QE/100 g dry wt).

2.5. Antioxidant activities

2.5.1. DPPH radical-scavenging assay

The antioxidant capacity of the extracts was studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the method proposed by Alothman et al. (2009). An aliquot (10 μl) of extract was mixed with 90 μl of distilled water and 3.9 ml of 25 mM DPPH• methanolic solution. The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. The absorbance was measured later, at 515 nm, against a blank of methanol without DPPH•. Results were expressed as percentage of inhibition of the DPPH radical. The percentage of inhibition of the DPPH radical was calculated according to the following equation:

$$\%\ \text{inhibition of DPPH} = (\text{Abs control} - \text{Abs sample}/\text{Abs control}) \times 100$$

where Abs control is the absorbance of DPPH• solution without extracts.