ORIGINAL ARTICLE

J. Chem. Metrol. 18:2 (2024) 124-133

journal of chemical metrology

Determination of the effect of different drying methods on secondary metabolites of *Lavandula pedunculata* (Mill.) Cav. subsp. *cariensis* (Boiss.) Upson & S. Andrews by LC-HRMS

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(Received November 28, 2024; Revised December 21, 2024; Accepted December 25, 2024)

Abstract: This study shows the effects of different drying methods on the secondary metabolite composition of *Lavandula pedunculata* (Mill.) Cav. subsp. *cariensis* (Boiss.) Upson & S. Andrews were evaluated. MeOH extracts of dried plants which were dried with different techniques-sun, shade, oven and microwave- were analyzed by ~~by~~ LC-HRMS method. The findings revealed that the amounts of phenolic substances decreased after drying compared to the fresh sample; moreover, rosmarinic acid and syringic acid were found in quite high amounts at *L. pedunculata* subsp. *cariensis.*

Keywords: *Lavandula pedunculata* subsp. *cariensis*; LC-HRMS; seconder metabolite; rosmarinic acid. © 2024 ACG Publications. All rights reserved.

1. Introduction

Aromatic plant extracts contain a wide variety of functional bioactive components with potential applications in pharmaceutical, food, and cosmetic industries. Synthetic derivatives of those natural compounds, primarily polyphenols, exhibit antioxidant, antiprotozoal, antiparasitic, anti-inflammatory, antifungal, and antimicrobial properties [1-7].

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The article was published by ACG Publications
http://www.acgpubs.org/journal/journal-of-chemical-metrology July-December 2024 EISSN:1307-6183
DOI:http://doi.org/10.25135/jcm.119.2411.3382
Available online: December 26, 2024

The Lamiaceae (Labiatae) family, which encompasses most of the medicinal and aromatic plants worldwide, includes 7,886 species across approximately 245 genera. In Türkiye, this family is represented by 46 genera and 782 taxa [8]. Lamiaceae is an important family in ethnobotany due to its applications in medicine, chemistry, food, agricultural control, cosmetics, and various industrial fields [9-13].
The genus *Lavandula* L., belonging to the family Lamiaceae, comprises more than 41 taxa [14-16]. This genus is characteristic of the Western Mediterranean Region. *Lavandula* species hold significant economic value in the pharmaceutical, perfumery, food, and flavour industries [17,18]. These species are utilized as analgesic, antiseptic, antidepressant, carminative, wound healer, expectorant, and nerve and heart tonic, owing to their content of flavonoids, saponins, and essential oils [19,20].

In Türkiye, the genus *Lavandula* is represented by three species (*L. pedunculata*, *L. angustifolia*, and *L. stoechas*) [17]. The essential oil of *L. angustifolia* Mill. from Türkiye has been reported to contain 3,7-dimethyl-1,6-octadien-3-ol (42.07%), linalyl acetate (18.26%), camphor (5.89%), alpha-terpineol (4.83%), and eucalyptol (1,8-cineole) (3.73%) [21]. Studies have demonstrated that *L. angustifolia* essential oil is highly effective against various fungal and bacterial species [22]. The aqueous extract of the flowers of *L. pedunculata* (Mill.) Cav. exhibits antimicrobial [23] and antihyperglycemic [24] activities, primarily attributed to its high rosmarinic acid content. Moreover, coumarin, luteolin, caffeic acid, protocatechuic acid, herniarin, myricetin, apigenin, and chlorogenic acid have also been identified in the extracts of this plant [24].

L. pedunculata subsp. *cariensis,* locally known as "karan," grows naturally in the western and southern regions of Türkiye [16, 20]. Tea prepared from the dried flowers and leaves of *L. pedunculata* subsp. *cariensis* is traditionally used for treating cough, bronchitis, embolism, hypertension, as a mucolytic, sedative, and for alleviating stomachache and tremors [25]. The flowers and tips of the plant are also used as tea to treat ulcers [20].

The literature on *L. pedunculata* subsp. *cariensis* is relatively scarce. A previous study demonstrated that the essential oil of *L. pedunculata* subsp. *cariensis* contains fenchone (26.7–31.7%) and camphor (33.7–41.0%) [20]. The plant is characterized by the prominence of camphor and bicyclo[2.2.1]heptan-2-one, which account for 33.7% and 22.6% of the essential oil from its flowers, respectively [26]. Additionally, total flavonoid and polyphenolic contents were detected in the hexane, water, and MeOH extracts of the flowers, roots, and leaves of *L. pedunculata* subsp. *cariensis* [27]. Although the biosynthesis of secondary metabolites in medicinal and aromatic plants is genetically controlled, it is also strongly influenced by environmental factors [28–30]. Moreover, drying methods and drying times significantly affect the nutritional composition and chemical components of many plant materials [31–33]. Therefore, optimizing these processes is essential to ensure the quality and cost-effectiveness of *L. pedunculata* subsp. *cariensis* before its commercial use. Various drying methods, such as sun drying, shade drying, microwave drying, vacuum drying, and oven heating, have been employed. The content of most plant phenolic compounds is highly sensitive to the drying methods and times. Hence, optimizing the drying conditions of *L. pedunculata* subsp. *cariensis* is crucial for its application in the food and pharmaceutical industries.

In this study, we report an analytical method for determining the differences in bioactive secondary metabolites in the methanol extracts of fresh and differently dried samples of *L. pedunculata* subsp. *cariensis.* The study also focuses on the standardization of these extracts for use in various cosmetic formulations and their incorporation into the final cosmetic product production process.

2. Experimental

2.1. Chemicals

Detailed information on the purity and origin of the reference materials and chemicals used in the study is given in the supporting information section.

2.2. Plant Material

The aerial parts of *L. pedunculata* subsp. *cariensis* (1.5 kg) were collected during the full-flowering period in September 2021 from Karacabey Longoz, Bursa, Türkiye (A2 Bursa: sandy places and roadsides, at 1 m elevation, 40°23'23.51" N, 28°26'49.20" E) and identified by Dr Selami Selvi. Voucher specimens were deposited at the Herbarium of Altınoluk Vocational School, Balıkesir University, Balıkesir, Türkiye (SV 2592).

2.3. Drying Conditions

Fresh aerial parts of *L. pedunculata* subsp. *cariensis* were collected during the full-flowering period from their natural habitat and then immediately exposed to different drying methods. The samples were divided into six batches, each including 500 g of fresh weight, with two replicates for each method. The drying methods utilized were fresh samples (L1), oven-drying at 40 °C (L2), oven-drying at 70 °C (L3), microwave drying at 600 W (L4), shade-drying at room temperature (L5), and sun-drying under direct sunlight (L6). In the shade-drying method the aerial parts of the plant were dried in a dry and dark room at room temperature under native airflow, in the absence of direct sunlight, for 4 weeks. In the sun-drying method, the aerial parts were dried on paper trays under direct sunlight in an open area for 1 week. For the oven-drying method samples were dried in a laboratory oven at 40 °C and 70 °C for 2 days. Microwave drying was performed at 600 W for 6 minutes.

2.4. Extractions of Samples

After the above-ground parts of the *L. pedunculata* subsp. *cariensis* were dried thoroughly in the different drying methods. From the different-dried grinded plant samples and fresh samples weighed approximately 50 grams, and then directly extracted with methanol for 15 days. Then, the plant parts were filtered from the MeOH using filter paper. The methanol was evaporated under a low vacuum by a rotary evaporator and obtained dried plant extracts. The yield of the extracts was determined as 13%, 17.2%, 15.6%, 18.8%, 18.3% and 15.2% from the fresh (L1), oven-drying at 40°C (L2), oven-drying at 70°C (L3), microwave-drying (L4), shade-drying (L5) and sun-drying (L6) plants, respectively. The extracts were stored at 4°C until they were utilized in experimental studies.

2.5 LC-HRMS Analysis

2.5.1. Sample Preparation for LC-HRMS

200 mg of plant extract was weighed and dissolved in MeOH in an 8 mL volumetric flask by holding it in an ultrasonic bath for 30 minutes. After obtaining a completely clear solution, 200 µL of internal standard was added, the volumetric flask was completed with methanol and vortexed and left to rest at room temperature for 5 minutes. The solution in the volumetric flask was filtered through a 0.45 µm nylon filter and transferred to a glass autosampler bottle for 1.5 mL and left on the HPLC tray.

2.5.2. *Standard Solutions*

The standard solution mixtures were prepared in methanol and at ten different concentrations (0.01 mg/L, 0.05 mg/L, 0.1 mg/L, 0.3 mg/L, 0.5 mg/L, 1 mg/L, 3 mg/L, 5 mg/L, 7 mg/L, 10 mg/L). An internal standard was employed in the form of a 1000 mg/L stock solution of dihydrocapsaicin (purity 97%) in methanol.

2.5.3. *LC-HRMS Conditions*

Liquid chromatography-high-resolution mass spectrometry (LC-HRMS) utilizing an Orbitrap Q-Exactiv mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled with a Troyasil

C18 column (150 x 3 mm, 5 µ particle size) was used to determine the secondary metabolites of *L. pedunculata* subsp. *cariensis*. The combination of 1% formic acid and water (mobile phase A) and 1% formic acid and methanol (mobile phase B) was used as the mobile phase in the electrospray ionisation (ESI) mode of the device. The internal standard was Dihydrocapsaicin (97%). The gradient programs were composed of 90% A and 10% B for the initial 60 seconds, 10% A and 90% B for the subsequent 7–14 minutes, and 90% A and 100% B for the 14–22-minute interval. A column oven set at 25 °C with a mobile phase flow rate of 0.25 mL/min was used. The laboratory temperature was recorded as 23.0 ± 3.0 °C and the relative humidity as 50 ± 15% rh. The used MS conditions were as follows: sheath gas (Arb) 45, aux gas (Arb) 10, positive ion voltage: 3.50 kV, ion transfer tube temperature 300°C, vaporizer temperature 320°C. The identifications were made by comparing the retention times and target ions of the compounds in LC-ESI-HRMS. [34-38].

2.5.4. Method Validation

Specificity, accuracy, precision, LOD and LOQ were utilized as the method validation parameters herein. The EURACHEM/CITAC guide and in our previous studies was used for evaluation of sources and quantification [38-42].

2.5.4.1. Specificity

Specificity refers to the condition of detecting solely the peaks of target molecules at the identical retention time amidst contaminants, secondary metabolites, matrix constituents, and degradation products. The LC-HRMS method was selected to attain high specificity, requisite selectivity, and sensitivity within the matrix while eliminating the positive impacts of treatments. The specificity of the improved LC-ESI-HRMS method was determined using direct (blind) examination of all solvents generated, the plant extract, and the added target analytes.

2.5.4.2. Accuracy

Accuracy is a metric describing the measurement results and actual quantity proximity. The percent recovery value was used as an accuracy metric. This value was obtained using LC-ESI-HRMS data for each compound and the formula shown below.

Recovery % = Recovered concentration /injected concentration x 100

The studied compounds recoveries ranged from 81.55 to 102.22 %.

2.5.4.3. Linearity

A calibration curve was derived using standard calibration solutions to quantitatively define secondary metabolites via LC-ESI-HRMS. The curve was generated based on analyte results, with six repeated measurements performed using solutions at various concentrations. The regression coefficient (R^2) and the linear regression equation are provided in Table S1 in the supporting information.

2.5.4.4. Detection and Quantitation Limits

LOD and LOQ of the method for every compound was calculated according to the follows;

LOD or LOQ = κSDa/b, where 3 for LOQ and κ= 3 for LOD. SDa was the standard deviation of the intercept and was the slope [43-46].

2.5.4.5. Identification of Uncertainty Sources

The uncertainty parameter was detected as weighing the sample, repeatability and calibration graph. Detailed equations suitable for the assessment method are given in our previous studies [38-43]. The overall standard measurement uncertainty of chemicals in plant extracts was calculated using the following equation.

$$u_{Combined} = \sqrt{(u_{standard})^2 + (u_{weighing})^2 + (u_{recovery})^2 + (u_{curve})^2}$$

$u_{combined}$: combined uncertainty
$u_{standard}$: uncertainty from purity of standard
$u_{weighing}$: weighing
$u_{recovery}$: precision
u_{curve}: calibration curve

The uncertainty value of measurement results is described in Table S1 in supporting information.

3. Results and discussion

The main components in the of *L. pedunculata* subsp. *cariensis* MEOH extracts were determined as rosmarinic acid (29177.91-15211.13 mg/kg), syringic acid (20825.97-10542.35 mg/kg), luteolin 7-*O*-glucoside (1151.89-273.73 mg/kg) and apigenin 7-*O*-glucoside (1374.60- 850.65 mg/kg) by LC-HRMS measurements. Following these compounds, caffeic acid, acacetin, apigenin, hispidulin, and vanillic acid were identified as the most abundant components (Table 1). These quantified compounds' LC-HRMS chromatogram of the methanol extracts of *L. pedunculata* subsp. *cariensis* are given in supporting information (Figure S1-S6).

According to the results from the aerial parts treated with five drying methods, the group dried using microwave drying at 600 W had the highest rosmarinic acid content. The syringic acid content showed a significant increasing trend in microwave, oven-drying at 70°C and sun-drying, which might result from oxidative degradation or biochemical reactions. As a result, drying under the sun and shade had an effect on the increase of apigenin 7-*O*-glucoside content, moreover it can be mentioned that the drying method has a moderate effect on the amounts of some phenolic components of *L. pedunculata* subsp. *cariensis* extracts. The highest extract yield from *L. pedunculata* subsp. *cariensis* species was obtained from microwave-drying (18.8%), shade-drying (18.3%) and oven-drying at 70°C (17.2%). It clearly shows that each drying method has its own opportunities and challenges.

Other authors have analyzed the effects of drying on *Lavandula* species. For example, Vareltzis et al. [47] compared the effects of spray-dried against freeze-dried, when drying *L. stoechas*. It was found that freeze-dried peel had higher phenolic content, as compared to spray-dried. Also, the antioxidant activity values changed for this reason and were higher in freeze-dried samples. Another study by Caser et al. [48] analyzed fresh and dried *L. angustifolia* flowers to identify potential differences in their effect on the phytochemical profile. Hot-air drying preserved the phenolic content and antioxidant capacity of phenolic contents, indicating that phenolic compounds are generally more sensitive to heat-pump drying. In *L. angustifolia* flowers air-dried at 22 °C and 27 °C, Dobros et al. [49] found lower values of caffeic acid (170–247 mg/100 g DM) and rosmarinic acid (252–108.2 mg/100 g DM) than the values obtained in this study. Similarly, Duda et al. [50] reported reduced values of caffeic acid (3.04–6.17 mg/100 g dry plant) and rosmarinic acid (5.10–6.50 mg/100 g dry plant), likely due to differences in extraction solvents (1:1 ethanol:water solution).

In addition, the extract yield obtained from oven-drying methods (40 °C and 70 °C) was slightly lower than the values obtained from microwave extraction. In general, the observed values align with the available literature on medicinal plant extracts [51–58]. The effects of drying methods on the phenolic contents and extract yield of the methanolic extract of *L. pedunculata* subsp. *cariensis* showed that extract

yield and phenolic content increased with microwave drying. This increase might result from intense heat or microwave energy facilitating the release of cell wall phenolic compounds and the breakdown of cellular components [54]. A significant decrease in luteolin-7-*O*-glucoside and caffeic acid content was observed when the drying temperature was increased from 40 °C to 70 °C during oven-drying. This decrease may be attributed to high temperatures leading to the degradation of phenolic compounds, especially heat-sensitive ones.

Table 1. Phenolic compounds of MEOH extracts of *L. pedunculata* subsp. *cariensis* quantified by LCESI-HRMS (mg/kg)

No	Compounds	L1	L2	L3	L4	L5	L6	U%
2	(-)-Epigallocatechin	186.64	83.21	16.22	143.70	104.15	160.69	3.09
3	Fumaric acid	495.75	157.78	84.45	149.02	112.78	119.65	2.88
4	Chicoric acid	254.72	44.75	8.09	79.86	61.39	56.66	2.28
5	Caffeic acid	938.67	854.66	357.06	838.49	751.79	973.63	3.74
6	(+)-t*rans* taxifolin	1.64	1.35	0.59	1.04	1.15	1.58	3.35
7	Luteolin-7-*O*-rutinoside	16.63	10.42	12.96	10.54	13.09	20.87	3.06
8	Vanilic acid	317.77	249.91	125.41	186.37	154.47	198.37	3.49
9	Luteolin-7-*O*-glucoside	1151.89	773.05	273.73	971.38	724.38	1126.76	4.14
10	Syringic acid	20825.97	10542.35	1629.35	17943.02	12878.18	16464.28	3.71
11	Rosmarinic acid	29177.91	15211.13	1969.79	25180.04	18594.02	24988.13	3.77
12	Apigenin-7-*O*-glucoside	994.73	881.19	850.65	947.21	992.28	1374.6	3.59
13	Ellagic acid	0.92	<LOD	0.51	0.52	<LOD	<LOD	4.2
14	Quercetin	0.12	0.60	0.40	0.30	0.41	0.56	2.95
15	Herniarin	28.86	31.77	74.35	26.81	33.28	62.98	3.89
16	Naringenin	14.58	30.14	35.59	35.32	45.67	35.25	4.2
17	Luteolin	128.35	78.15	88.19	77.6	91.5	156.89	3.42
18	Apigenin	286.27	396.52	571.75	412.76	462.71	578.00	2.87
19	Hispidulin	234.28	104.82	359.86	103.47	119.4	242.02	3.41
20	Isosakuranetin	1.90	2.26	5.04	14.26	7.14	2.75	3.98
21	Caffeic asit phenethyl ester	0.07	0.81	1.41	1.09	0.30	0.62	3.13
22	Chrysin	0.64	0.53	0.57	0.56	0.68	1.38	3.24
23	Acacetin	505.75	421.95	505.85	587.11	615.60	606.81	3.98

L1: fresh, L2: oven-drying at 40°C, L3: oven-drying at 70°C, L4: microwave, 600 W, L5: shade-drying, L6: sun-drying

4. Conclusions

This study was conducted to develop and validate an LC-HRMS method for investigating the effects of drying on cosmetically significant lavender species. Fresh lavender species exhibit remarkable phytochemical properties and antioxidant activities, which are valuable for food and pharmacological applications. Optimizing drying methods may enhance the preservation of bioactive compounds. Microwave and shade-drying methods emerged as effective approaches for achieving high extraction yields. In this research, a notable reduction in luteolin-7-*O*-glucoside content was observed when the drying temperature increased from 40 °C to 70 °C during oven-drying. Overall, the microwave-drying method produced extracts with higher rosmarinic acid content. Based on these findings, it can be concluded that each drying method has its own advantages and disadvantages. For lavender, combining

microwave and sun-drying methods could provide promising and sustainable applications for obtaining extracts enriched in phenolic compounds.

Acknowledgements

This work was supported by Balıkesir University Scientific Research Projects Unit (BAP) [grant number 2020/044].

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/ journal-of-chemical-metrology

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