Journal homepage
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Anbar Journal of Agricultural Sciences
(University of Anbar – College of Agriculture)

EFFECT OF DISTILLATION DURATION ON ESSENTIAL OIL YIELD AND COMPOSITION OF LAVENDER LAVANDULA ANGUSTIFOLIA GROWN IN IRAQ

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Article info
Received: 2024-11-16
Accepted: 2025-01-17
Published: 2025-06-30

DOI-Crossref:
10.32649/ajas.2025.186647

Cite as:
Alhasan, A. S., Alrawi, M. Sh., Al-Ameri, D. T., and Almehemdi, A. F. (2025). Effect of distillation duration on essential oil yield and composition of lavender lavandula angustifolia grown in Iraq. Anbar Journal of Agricultural Sciences, 23(1): 339-353.

Abstract
Lavender is a medicinal and aromatic crop grown worldwide for essential oil (EO) production that is used in the pharmaceutical, ecofriendly pesticides, foods, perfumery, cosmetics, and other industries. However, the quantity and quality of lavender EO is affected by several factors including the extraction process. This study examined the effect of distillation time (DT) on the quantity and quality of the EO obtained from dried lavender flowers through the hydro-distillation process. Different distillation times ranging from 10 to 180 min were applied in isolating the EO from dried lavender flowers using a Clevenger-type apparatus while gas chromatography-mass spectrometry (GC-MS) identified the chemical compositions derived from the oils. The maximum EO of 2.15% was obtained after 150 min distillation and the minimum (0.16%) at 10 min. In all the analyzed samples, the chemical composition of the isolated EO revealed the dominance of cyclohexanol (4.64-16.33%), cyclohexanone (3.42-10.77%), eucalyptol (1.04-6.06%), caryophyllene oxide (2.94-5.23%), caryophyllene (2.89- .74%), nerol oxide (1.12-

196%), trans-carveol (0.32-1.66%), and β-ocimene (0.46-0.89%). The results suggest that distillation time affects the yield of EO and the quantity of some constituents in dried lavender flowers.

Keywords: Hydrodistillation, Essential oil, Medicinal and aromatic plants, Cyclohexanone, Eucalyptol, Lavender.

تأثير مدة الاستخلاص على حاصل الزيت العطري ومكوناته لنبات الخزامى المنزرع بالعراق

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الخلاصة

نبات الخزامى من النباتات الطبية والعطرية والتي تزرع في العالم لغرض انتاج الزيوت العطرية والتي بدورها تستخدم في مختلف الصناعات الغذائية والطبية والدوائية وكذلك في صناعة المبيدات الصديقة للبيئة، اضافة الى استخدام زيت الخزامى في صناعة مواد التجميل وفي صناعة أرقى انواع العطور. ان زيت العطري لنبات الخزامى يتاثر كما ونوعا بالعديد من العوامل ومن اهم تلك العوامل هو عامل طرائق ومدة الاستخلاص للزيت العطري. حيث يعد الوقت الذي يستغرقه الاستخلاص من اهم العوامل المؤثرة على نوعية وكمية الزيت العطري المنتج من الازهار المجففة لنبات الخزامى. لذلك نفذت هذه الدراسة بهدف معرفة تاثير استخدام الاوقات المختلفة في عملية الاستخلاص (10، 15، 30، 60، 90، 120، 150، و180 دقيقة) للأزهار المجففة وباستخدام جهاز الاستخلاص الكلافنجر لغرض استخلاص الزيت العطري للنبات. ولقد استخدم جهاز كروماتوغرافيا الغاز (GC-MS) لغرض عزل وتشخيص المكونات الكيميائية الداخلة في تكوين الزيت العطري لنبات الخزامى. لقد اظهرت نتائج التجربة تسجيل اعلى حاصل للزيت العطري بمعدل 2.15% عند وقت الاستخلاص (150 دقيقة)، في حين سجل أدنى معدل لحاصل الزيت 0.16% عند وقت استخلاص (10 دقائق). كما اظهرت نتائج التحليل الكيميائي باستخدام تقنية GC-MS الى وجود فروقات معنوية في تركيز مختلف المواد الكيميائية الداخلة في تكوين الزيت العطري لنبات الخزامى. غير ان أكثر المواد الكيميائية ظهورا في جميع اوقات الاستخلاص كانت هي Cyclohexanol، Cyclohexanone، Eucalyptol، Caryophyllene oxide، Caryophyllene، trans-Carveol ،Nerol oxide، ومادة β-Ocimene. لقد اظهرت نتائج تجربتنا تفسير واضح في اهمية عامل وقت الاستخلاص في تحديد كمية ونوعية الزيت العطري المستخلص من الازهار الجافة لنبات الخزامى.

كلمات مفتاحية: نبات الخزامى، النباتات الطبية والعطرية، الزيوت العطرية، الأوكاليبتول، سيكلوهكسانون.

Introduction

Lavandula angustifolia is a perennial shrub in the family Lamiaceae. It is native to the Mediterranean region, and grown worldwide as an essential oil crop (11). Nowadays, lavender is one of the most important medicinal and aromatic crops traditionally utilized to treat different human diseases. *Lavandula angustifolia* Mill is also used in various industries such as food, pharmaceutical, ecofriendly pesticides, perfumery, and cosmetics. Global, lavender plantations and production have increased in the last few years (12), with most lavender essential oil production concentrated in European countries, the United States, Australia, and North Africa (38).

Lavender is widely cultivated under field and greenhouse conditions for its essential oil, which has been shown to have antifungal, antioxidants, sedative, antidepressant, and antimicrobial properties (14). It is also grown and sold as an ornamental plant for the garden (23). Also, *L.angustifolia* Mill is a well-known good source of nectar for honeybees, and lavender honey is specially produced in European countries (21). Moreover, lavender essential oils have been reported as a possible solution for controlling honey bee diseases (24). Lavender essential oil yield and composition have been widely studied due to its medicinal and economic importance. The essential oil consists mainly of monoterpenes including linalool, 1,8-cineole, and camphor (18). The high amounts of linalool and low level of 1,8-cineole make the oil of high quality and much sought after by the medicinal, pharmaceutical, and cosmetic industries. Thus, the essential oil yield and composition determine the commercial value of the medicinal and aromatic plant (10 and 14).

The chemical constituents of lavender essential oil are influenced by soil types, water quality, environment, genotype, and extraction process (35). The oil can be easily obtained from lavender flower utilizing steam or through hydrodistillation. The quantity and quality of the oil in lavender and other medicinal and aromatic plants are influenced by distillation time (9, 19 and 29). Different compounds in the essential oil may be lost if distillation times are too short and at high boiling points, while lengthy distillation times may imbue the oil with an unpleasant odor. In general, while maximum essential oils from lavender are obtained in the first 25 minutes of distillation, the other compositions of the oil need between 50 - 80 minutes of distillation (29). Thus, this investigation focused on the impact of the duration of hydrodistillation on the yield of essential oils and the chemical composition of dried lavender flowers.

Materials and Methods

Plant materials: The dried lavender flowers (Syrian cultivar) used for essential oil production were obtained from a nursery at the Department of Horticulture, College of Agriculture, Al-Qadisiyah province, Iraq. The experiment was conducted from 15

April to 15 June 2023 at the medicinal and aromatic lab, Horticultural Department, Agricultural College, University of Al-Qadisiyah, Iraq.

Essential oil extraction: The essential oil was extracted via hydrodistillation using a Clevenger-type apparatus. The extraction process was applied using 100g of dried lavender flowers. Distillation times (DT) of 10, 15, 30, 60, 90, 120, 150, and 180 minutes were used for this study, each measured from the moment of the first drop of oil into the separator. The essential oil was immediately collected at the end of each DT treatment, and each DT was carried out in the three replicates. The isolated essential oils were separated from water, and stored in a freezer at -5 ºC until GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) analysis: Analyses of the essential oils of the dried lavender flowers were performed on a GC-MS (Model QP 2010, Shimadzu, Japan) at the Research Lab, College of Science, University of Al-Qadisiyah. The GC-MS was equipped with a DP5-MS capillary column (30m × 0.25 mm and a film thickness of 0.25 mm) operated utilizing the following conditions: injector temperature of 240 ºC; column temperature of 60-120 ºC at 3 ºC/min, then maintained at 240 ºC at 20 ºC/min for 5 min. Helium was utilized as a carrier gas at a flow rate of 1 mL/min. The essential oils components were identified by comparing their mass spectra with those stored in the MS databases (Willey, NIST 2002), and the constituent percentages measured based on the peak area (1).

Statistical analysis: The effects of the three-replicated distillation times were determined using non-linear regression analysis performed with Excel Program (Microsoft).

Results and Discussion

The EO of medicinal and aromatic plants can be derived or destroyed by DT. Furthermore, the oils must be extracted within the appropriate time to release all their active constituents. The results in Table 1 indicate that DT of lavender essential oils significantly impacted their yield and constituents. In this study, the EO yield concentration of 0.16-2.15% was low at 10 min DT, increased at 15- and 30-min, increased again at 60- and 90-min, and attained a maximum rate at 150 min DT.

The major constituents of the EO were cyclohexanol, cyclohexanone, eucalyptol, caryophyllene oxide, caryophyllene, nerol oxide, trans-carveol, and β-ocimene (Fig 1). Cyclohexanol concentrations, ranging from 4.64-16.33%, were highest at 10 min distillation time (16.13%), decreasing gradually at higher DTs to stabilize at a minimum concentration at 180 min (4.64%) (Table 1). The concentration of cyclohexanone (range 3.42-10.77%) was also the highest at 10 min DT (10.77%), and decreased in stages with longer distillation duration to stabilize again at the minimum value at 180 min (3.42%). Also, the concentration of eucalyptol was highest at 10 min distillation time (6.06%) and lowest at 180 min (1.04%). The density of caryophyllene oxide (ranging from 2.94-5.23%) was the highest when distilled for 90 min (5.42%), and further increases in the duration did not significantly alter its concentrations. However, its lowest concentration was reached at 10 min distillation time (2.94%).

The concentration of caryophyllene (range 2.89-3.74%) was lowest at 10 min distillation time, reaching the maximum at 120 min, then to a lower value at 180 min. Nerol oxide density was lowest at 10-30 min distillation time, increasing at 60-90 min distillation time; further increases in DT did not affect its density. For trans-carveol, its concentration (range 0.32-1.66%) was lowest at 180 min DT (0.32%) and the highest at 30 min (1.66%). Its concentration at 60 min distillation was not significantly different from that at 30 min. The concentration of β-ocimene (range 0.46-0.89%) was the lowest at 150-180 min distillation time, increasing at 30-60 min, then declining to reach a minimum at 120 min.

The fitted non-linear regression models shown in the plots of Figures 2, 3, and 4 show the constituents and essential oil yield of lavender at any given DT. They describe the relationship between DT and essential oil concentrations (%) of caryophyllene and caryophyllene oxide (Fig. 2A, B, C), cyclohexanone, cyclohexanol, and β-ocimene (Fig. 3A, B, C), and of eucalyptol, nerol oxide, and trans-carveol from dry material (Fig. 4A, B, C), with potential maximum contents of 2.15%, 3.74%, 5.26%, 10.77%, 16.13%, 0.89%, 6.06%, 1.96%, and 1.66%, respectively. The polynomial non-linear regression model was the most suitable for the concentrations of β-ocimene, caryophyllene oxide, caryophyllene, eucalyptol, nerol oxide, and trans-carveol. Further, it represented the best model for explaining the relationship between DT and the concentrations of cyclohexanone and cyclohexanol (Fig. 3B, C), while the logarithmic nonlinear model was the most apt for that between DT and lavender essential oil yield (Fig. 2A) .

The essential oil content of lavender generally ranges between 0.5 to 9.62%, and is influenced by factors such as soil type, water quality, geographical location, cultivar and genotype, propagation method, and climate conditions (4 and 16). In this study, the maximum lavender EO yield reached 2.15%, lower than found in most prior studies (15, 25, 28, 35 and 38). Moreover, the concentrations of β-ocimene, caryophyllene oxide, caryophyllene, eucalyptol, nerol oxide, and trans-carveol noted in this study were also lower than that reported by Wesolowska et al. (29 and 30) in Poland, and Zheljazkov et al. (38) in the US. The variations in the chemical constituents of essential oils in this study can most likely be attributed to unfavorable environmental conditions during the lavender growing season in Iraq.

The composition of lavender oil is determined by a variety of environmental and genetic factors, as well as postharvest processing factors. There is no unanimity in the literature on the optimal period for steam distillation of lavender biomass, with most papers recommending 2-4 hours. Comparing literature data on lavender oil production and composition is problematic because to the varying lengths of the distillation process used. It is unclear how the DT influences essential oil yield and composition, which are important criteria for producers, processors, and end users. Recent investigations on plants from the same family as lavender demonstrated that distillation durations may significantly modify the essential oil content and, in certain cases, the bioactivity of peppermint (8 and 34), Japanese cornmint (33), oregano (36), lavender (38), garden sage (37), and rosemary (32). Where, they discovered that DT alters the essential oil content of fresh or dried biomass, producing oils from these species with increased or decreased concentrations of certain targeted oil constituents to fulfill specific market demands. Wesołowska et al. (29 and 30) found that DT significantly affects the components of lavender oil. The essential oil obtained after two hours of steam distillation is suitable for medical usage. Zhang et al. (31) obtained the same distillation time from basil leaves, extracting the best essential oil after two hours.

Table 1: Effect of distillation time on essential oil (%) and composition (%) of *L. angustifolia* (RT: retention time).

RT	Components	Distillation times (min)							
		10	15	30	60	90	120	150	180
7.959	**1-Heptanol**				1.76				
7.963	**1-Hexanol**								0.77
7.981	**Formic acid**		0.52						
10.407	**3-Octen-5-yne**				1.51				
10.955	**Camphene**		0.42	0.47				0.66	
10.965	**Cyclohexene oxide**				2.15			2.11	
10.968	**Norbornane**				1.23				1.1
12.113	**β-Pinene**								0.66
12.428	**1-Dodecen-3-ol**							0.78	
12.446	**1-Octen-3-ol**	0.93				0.67			
12.466	**2-Octen-1-ol**		1.12	0.98	2.39				1.26
12.853	**2,3-Diazabicyclo [2.2.1] hept-2-ene**				1.47				
12.857	**β-Myrcene**	1.16	0.86	1.4			1.06		0.88
13.424	**3-Dodecyne**							0.46	
13.740	**Acetic acid**	0.97	0.89	1.14				1.03	
13.770	**(+)-Sabinene**								1.37
14.063	**5-Allyl-4-[1-(p-aminophenyl) ethylidenehydrazono]-6-methyl-2-phenylpyrimidine**								2.9
14.065	**(2,4,6-Trimethyl-phenoxy)-acetic acid benzylidene-hydrazide**				2.99				
14.235	**D-Limonene**							0.88	
14.313	**Eucalyptol**	6.06	6.35	6.64	4.64	3.74	3.64	2.92	1.04
14.700	**trans-β-Ocimene**	0.60	0.70	0.77	0.88	0.55			0.43
15.066	**β-Ocimene**	0.61	0.69	0.89	0.74	0.74	0.64	0.54	0.46
15.422	**3-Octen-5-yne**				3.44				2.15
15.423	**γ-Terpinene**		0.63						
15.958	**Oxirane**	7.44							
16.554	**Cyclohexanol**	16.13	5.71	5.57	6.95	6.54	4.64	5.37	4.64
17.182	**Dehydrolinalool**	0.52							
17.250	**(1-Allylcyclopropyl) methanol**					1.57	1.46	1.62	
17.253	**Butanoic acid**			0.69					0.90
17.481	**7-Hydroxy-6-methyl-oct-3-enoic acid**	5.14		4.58			3.50		
17.503	**Trans-2,3-Epoxyoctane**							4.3	
17.519	**2-Decen-1-ol**		2.69						
17.522	**1,2-Epoxynonane**					4.48			
18.431	**(+)-2-Bornanone**	1.80						1.32	
18.453	**Camphor**					1.37			
18.456	**Spiro[bicyclo[2.2.1]heptane-2,2'-[1,3]dioxolan]-3-one**			2.16			1.27		
18.558	**1-Isopropenyl-4-methyl-1,2-cyclohexanediol**		8.38						
18.793	**2-(1-Hydroxy-1-methyl-ethyl)-1-methyl-cyclohexanol**								0.58
18.797	**Propanoic acid**		0.62						

18.830	**Citronellol epoxide (R or S)**				2.17				
18.974	**Nerol oxide**	1.12	1.23	1.34	1.96	1.82	1.60	1.54	1.41
19.184	**Pinocarvone**				0.80				
19.349	**Isobornyl formate**	3.79							1.2
19.353	**5-Caranol**		7.00	4.73		4.85	4.31	4.31	
19.462	**Isopulegol**	6.81	4.45	6.60			5.76	5.97	
19.741	**(-)-Terpinen-4-ol**	1.75				1.72	1.64	1.73	
19.768	**Sabinene hydrate**			2.33					
19.833	**Isopulegol**		7.40						
19.848	**3,7-dimethyl-3,7-diazabicyclo[3.3.1]nonane**								6.45
19.909	**4-Isopropyl-2-cyclohexenone**	1.83							
19.920	**2-Methyl-4,5-nonadiene**							3.47	
19.936	**Octahydro-2H-inden-2-one**			3.45		3.84	3.49		2.34
19.952	**5-Isopropylbicyclo[3.1.0]hexan-2-one**		2.15						
20.034	**Benzenemethanol**		0.68	0.53		0.76	0.82	0.72	1.08
20.072	**Cyclopentane**				2.01				
20.105	**3,5-Heptadienal**				1.24				
20.219	**α-Terpineol**	4.42		6.38					
20.283	**Cyclohexanol**		6.06			6.94	5.96	6.33	
20.284	**Butanoic acid**	0.69							
20.308	**Dehydrolinalool**								5.08
20.370	**β-cis-Ocimene**				4.85				
20.464	**2-Furanmethanol**				0.77	0.35			
20.525	**(-)-Myrtenol**				0.81				0.46
20.743	**2,4-Cycloheptadien-1-one**			0.66		0.65	0.73	0.62	
20.765	**Verbenone**				1.20				0.39
20.842	**2,6-Dimethyl-3,5,7-octatriene-2-ol,**	0.55	0.48	0.49		0.62	0.86		
21.241	**trans-Carveol**	0.62	1.41	1.66	1.45	0.96	0.46	0.43	0.32
21.478	**Norborneol**	0.86	0.76	0.40					0.32
21.508	**Neodihydrocarveol**				2.55				
21.546	**Geraniol**	0.63	1.18	2.59		2.91			0.80
21.658	**Phenol**	0.88	0.64	0.49	1.17		0.62		0.44
21.810	**Propanal**		2.04	1.62		1.61	1.83	1.59	
21.853	**Hexyl n-valerate**				3.57				2.77
21.921	**(-)-Carvone**						1.11		
21.939	**D-Carvone**		1.15	0.99		0.94		0.92	
21.983	**Butanoic acid**				3.59				1.96
22.497	**Cyclohexanone**	10.77	9.45	8.18	4.45	7.85	6.85	6.83	3.42
22.543	**Dichloroacetic acid**		7.24	7.43		7.86		7.25	
22.965	**β-Cyclocitral**					0.58	0.91	1.05	
23.208	**Acetic acid**			1.79		1.63	2.03	1.67	
23.383	**Benzene**		0.56					0.54	3.29
23.365	**Anethole**				0.97	1.01			
23.563	**1,4-Methanophthalazine**				4.20				
23.613	**1-Isopropenyl-3-propenylcyclopentane**	8.71							3.33
24.043	**3,5-Heptadienal**				2.68				3.20
24.078	**2-(2-Nitrophenoxy) benzaldehyde 4-isopropyl-3-methylphenoxyacetylhydrazone**								4.89

24.765	**3,7-Diacetyl-1,5-dimethyl-3,7-diazabicyclo-[3,3,1]nonan-9-one**				0.75				
24.807	**Cyclobutylcarboxylic acid**								1.32
24.813	**Hexyl tiglate**		0.31						
24.814	**3-Methyl-2-butenoic acid**				1.8				
25.632	**m-Eugenol**								0.67
25.853	**Ethanol**							3.05	
25.860	**4-Hexen-1-ol**	1.11	0.34						0.94
26.307	**Hydroxy-α-terpenyl acetate**					0.91			
26.308	**Acetic acid**						1.37		
26.311	**1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol**			0.61				0.59	
26.439	**Geranyl acetate**	1.80							
26.462	**Geraniol**			3.70	2.08	4.04			1.46
26.467	**Carvone**						3.9		
26.530	**Hexanoic acid**		0.32		1.26				0.83
26.761	**α-phellandrene**				1.00				0.83
27.654	**(-)-β-caryophyllene**	3.33	3.28				1.96		
27.658	**Caryophyllene**	2.89	3.06	3.05	3.33	3.11	3.74	3.26	3.12
27.672	**Naphthalene**								3.32
28.113	**α-pinene**								0.36
28.674	**cis-β-Farnesene**	0.87		1.24		1.15		1.20	
28.717	**Humulen-(v1)**				4.11				4.04
28.859	**4-Hexen-1-ol**								1.20
29.440	**(-)-β-Copaene**	0.86	0.60						
29.443	**1,6-Cyclodecadiene**				1.84				1.39
30.169	**Neryl (S)-2-methylbutanoate**		1.63						
30.192	**trans-β-Terpinyl butanoate**				3.57				3.38
30.503	**α-Santalol**						1.58		
30.514	**Teresantalol**			0.80		1.01		0.91	
32.266	**Caryophyllene oxide**	2.94	2.98	4.78	3.33	5.23	5.26	4.70	3.23
33.695	**Aromadendrene oxide**						0.70		
33.787	**τ-Cadinol**				0.86		1.87		0.74
33.796	**Octahydronaphthalene**		0.69	0.62		0.92		0.78	
34.057	**Geranyl tiglate**				0.96				
34.142	**2-Furanmethanol**								0.61
34.242	**Androstan-17-one**						0.89		
34.590	**1-Oxaspiro[2.5]octane**					1.12			
34.597	**Isolongifolol**			0.56			2.40		0.47
34.782	**1H-Indene**						0.78		
34.852	**α-Bisabolol**								0.42
35.593	**L-α-Terpineol**						1.01		
38.719	**Hexahydrofarnesyl acetone**				0.66		1.04		0.93
55.724	**Cholest-2-ene**				2.00				
	Essential Oil % (w/w)	0.16	0.46	1.03	1.50	1.80	1.99	2.15	2.11

It appears that high temperature exposure of plant material (70 °C) for 24 hours considerably reduces essential oil content, which could be attributed to the oil glands splitting during oven drying, particularly at higher temperatures. Peppermint (6), rosemary (7), bay leaf (26), and *Acorus calamus* L. (17) have all shown a similar pattern of reduced volatile oil content due to the impact of high temperature. Furthermore, distillation duration is a significant processing component in arranging active chemicals at the desired values, contributing to standardized manufacturing. Toker et al. (27) noted that 60 minutes distillation produced the highest essential oil output and carvacrol concentration from *Origanum minutiflorum*. As a result, hydrodistillation produced higher quality *Cananga* oil in terms of oxygenated compounds and ester concentration than steam-water distillation (20).

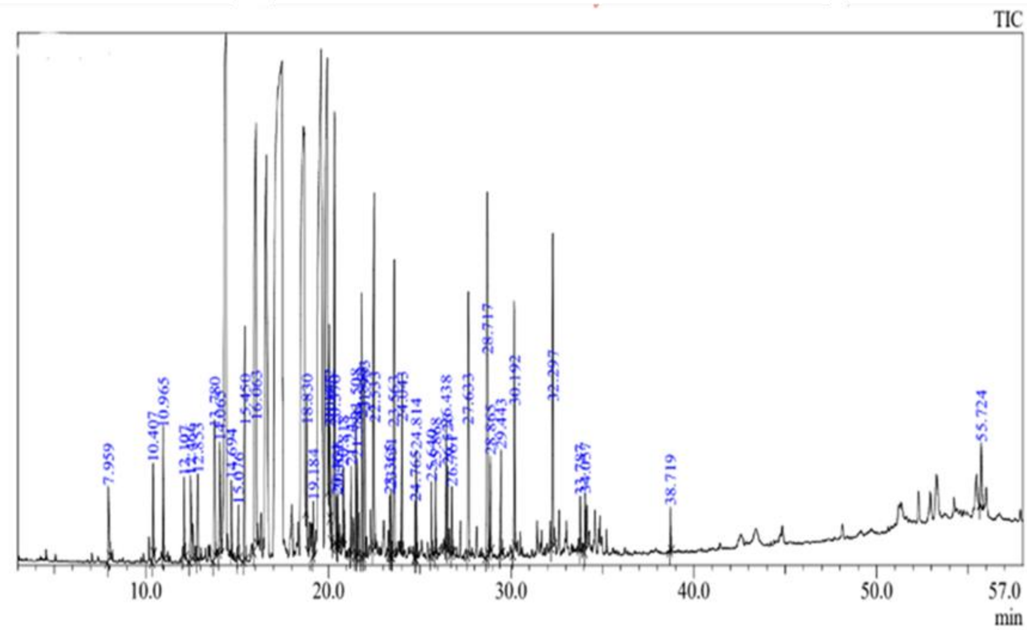

Figure 1: Constituents of essential oil components of L. angustifolia flowers identified by GC-MS.

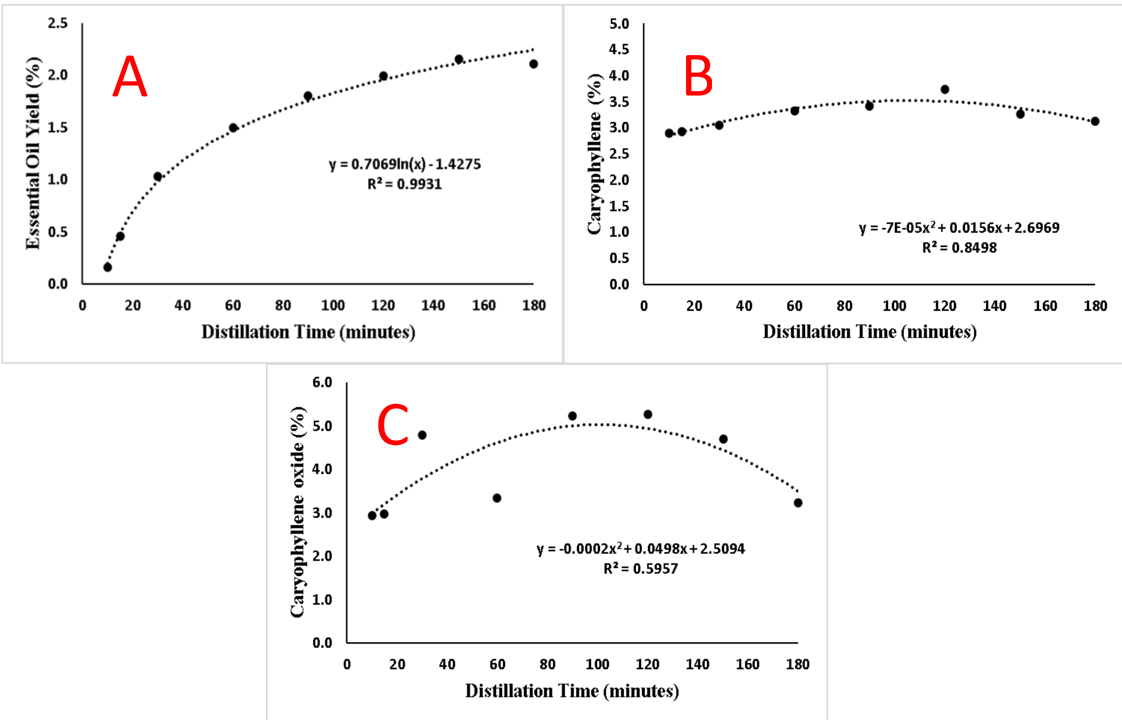

Figure 2: Distillation times and essential oil (yield (%) (A), and percentage concentrations of caryophyllene (B) and caryophyllene oxide (C).

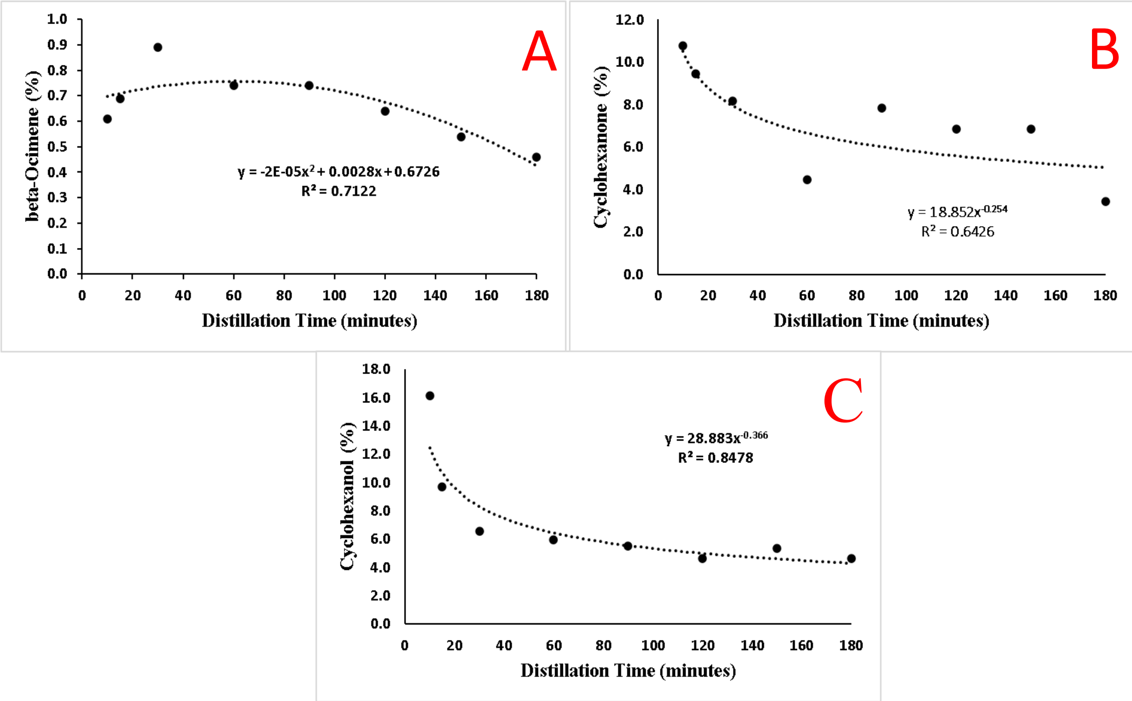

Figure 3: Distillation times and concentrations of β-ocimene (A), cyclohexanone (B), and cyclohexanol (C).

However, some of the concentrations declined with higher DTs, depending on the boiling points of each component. Genetic factors (22), origin, environmental conditions, harvesting time (3 and 5), distillation time and type (36), some growth inputs and production techniques (2 and 13), and flower growth stage (20) have all been linked to essential oil composition in medicinal and aromatic plants.

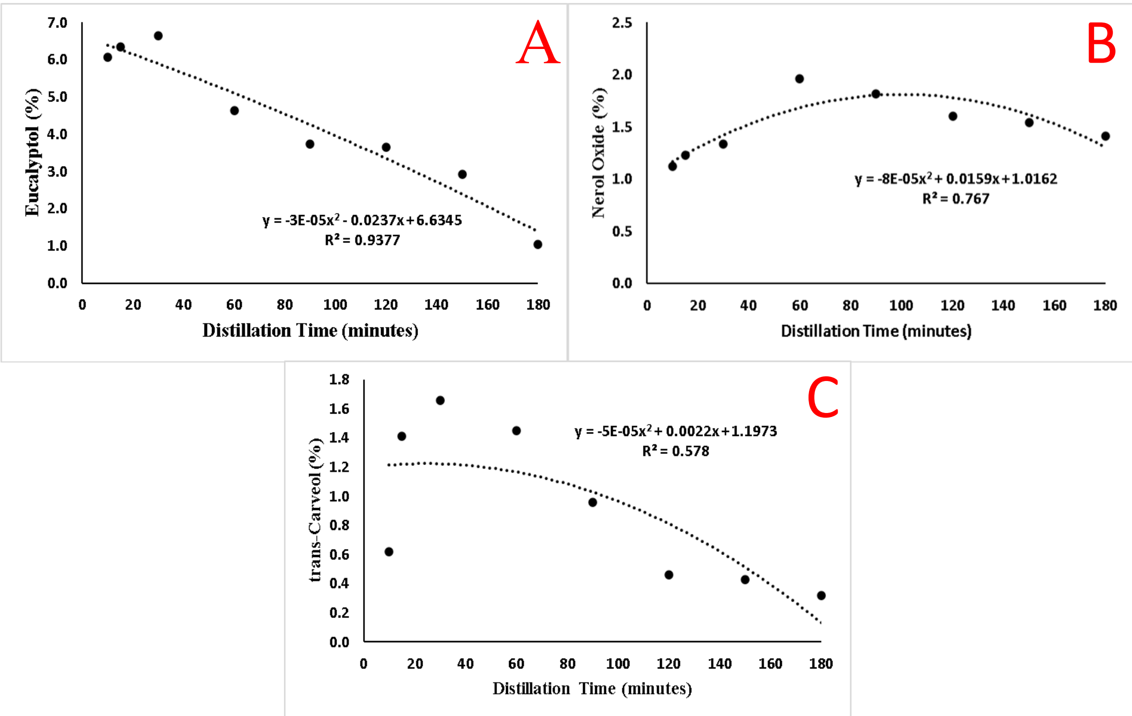

Figure 4: Distillation times and concentrations of eucalyptol (A), nerol oxide (B), and trans-carveol (C).

Conclusions

This study investigated the effects of hydrodistillation times on essential oil yield and constituents of dry lavender flowers. The maximum essential oil yield (2.15%) was obtained after 150 min distillation, while the minimum (0.16%) was reached at 10 min. The highest concentrations of eucalyptol, cyclohexanone, trans-carveol, and cyclohexanol can be obtained at very short DT (less than 30 min). However, high concentrations of β-ocimene can be attained when the flowers are distilled at most at 90 minutes. In general, hydrodistillation duration can be utilized as a simple method to obtain maximum essential oil yields with variable chemical profiles.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Alhasan, A. S.: methodology, writing—original draft preparation; Al-Ameri, D. T., Almehemdi, A. F., and Alrawi, M. Sh. writing and review. All authors have read and agreed to the published version of the manuscript.

Funding:

This research received no external funding.

Institutional Review Board Statement:

The study was conducted following the protocol authorized by the Head of the Ethics Committee, University of Anbar, Iraq Republic.

Informed Consent Statement:

No Informed Consent Statement.

Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

Acknowledgments:

None.

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