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Evaluation of lavender essential oils and by-products using microwave hydrodistillation and conventional hydrodistillation

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This study investigated the impact of two extraction methods, traditional hydrodistillation (TDH) and microwave-assisted hydrodistillation (MAH), on the essential oil yield and chemical profile of *Lavandula angustifolia* L., as well as the bioactive potential of the resulting wastewater. Essential oil composition was analyzed via GC–MS, revealing similar qualitative and quantitative profiles for both methods, with α -terpinolene and (–)-borneol as major constituents. Wastewater analysis via LC–MS/MS and spectrophotometric assays demonstrated the presence of significant total phenolic content (3.29–1.78 mg GAE/g) and 32 individual phenolics (463.1 μ g/kg for TDH; 479.33 μ g/kg for MAH). These findings suggest that both essential oil and wastewater obtained by either method possess considerable bioactive potential, with the MAH method potentially offering advantages over TDH for essential oil extraction. Further exploration of wastewater applications in various industrial sectors is warranted.

Keywords *Lavandula angustifolia*, By-product, Wastewater, Essential oil, Phenolic profile

The Lamiaceae family, encompassing approximately 245 genera and 7886 species, enjoys a near-global distribution¹. Among its most notable members is *L. angustifolia* (lavender), a species of significant economic and medicinal importance renowned for its substantial essential oil content². Essential oils, highly complex mixtures derived from various plant parts (roots, stems, leaves, flowers, seeds, bark, etc.), have experienced a surge in demand due to the growing global interest in pure and natural products^{3,4}. Lavender essential oil, in particular, has been extensively studied for its therapeutic applications in aromatherapy, demonstrating the potential to alleviate headaches, depression, and colds, as well as exhibiting sedative and balancing effects⁵.

Meeting this escalating demand across industries like cosmetics, health, and food necessitates efficient extraction methods to obtain essential oils from medicinal plants, including lavender. Conventional extraction techniques, such as distillation, solvent extraction, and mechanical processes, are often labor-intensive and time-consuming and may involve using environmentally harmful organic solvents^{3,6}. Consequently, there has been a growing interest in developing novel extraction techniques, such as ultrasound-assisted extraction, pressurized solvent extraction, microwave-assisted solvent extraction, and supercritical fluid extraction, that minimize environmental impact by reducing solvent consumption, shortening extraction times, and enhancing yields⁶.

Microwave-assisted hydrodistillation (MAHD) is an emerging technique that utilizes microwave energy to heat the extractant (typically water) in contact with the plant material. The elevated temperature and solvent properties facilitate the transfer of target analytes from the sample matrix into the extraction solvent. In essential oil extraction, MAHD induces the selective heating of water molecules within the plant's glandular and vascular systems. This leads to a rapid rise in temperature, often exceeding the boiling point of water, causing the rupture of cell walls and the release of essential oils into the surrounding medium⁷. This mechanism contrasts with classical

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solvent extraction, where the solvent diffuses into the matrix and extracts components through solubilization. MAHD's reduced reliance on high solvent affinity broadens the range of suitable solvents for extraction⁷.

The choice of extraction method significantly influences the yield, quality, and composition of the essential oil obtained⁸. In these extraction processes, three different products are obtained: (i) essential oils, (ii) discharged water (hydrolate, hydrosol, or plant juice) expressed as effluent, and (iii) post-extraction solid residue of the plant material used⁸. However, regardless of the method employed, essential oil extraction generates a substantial amount of by-products, primarily consisting of the aqueous portion of the decoction, which is frequently discarded as waste⁹. Many of these by-products are released into the environment untreated or repurposed as fuel, animal feed, or fertilizer¹⁰. Recent efforts have explored potential applications for these by-products to conserve energy and resources¹⁰. Numerous studies have revealed the presence of diverse bioactive compounds within these by-products, highlighting their potential value^{11–13}. The presence of water-soluble polyphenols, proteins, free amino acids, alkaloids, and vitamins in the extraction vessel and the solid residue during extraction was detected and present in significant quantities^{14–16}. The essential oil industry has the potential to utilize both residual water and solid residue.

This study aimed to evaluate the impact of two extraction methods, traditional hydrodistillation (TDH) and MAHD, on the essential oil content and by-product composition of lavender. The volatile constituents of the essential oil were identified via GC–MS. Furthermore, the by-products collected from both TDH and MAHD were analyzed to determine total flavonoid, total phenolic, total monomeric anthocyanin (TACc), and total proanthocyanidin content, as well as individual phenolic compounds (using LC–MS/MS) and antioxidant activity. This comprehensive comparative analysis provides valuable insights into the potential utilization of by-products generated during essential oil extraction from lavender.

Material and methods

Collection of plant material

Lavandula angustifolia flowers were collected from the lavender field in Marmara Ereğlisi/Tekirdağ during the summer of 2023. The samples were stored at -20°C until further analyses and recorded in Istanbul University Faculty of Science Herbarium under collection number 41819.

Extraction of essential oil

Essential oil isolation was carried out using two different methods (Fig. 1), microwave and conventional, as follows:

Traditional hydrodistillation (TDE)

A modified version of the method of European Pharmacopoeia 9th Edition¹⁷ was used for the traditional hydrodistillation. *L. angustifolia* essential oil was obtained via hydrodistillation of 100 g of dried flowers, sieved to a particle size between 80 and 100 mesh, in 500 mL of distilled water using a Clevenger-type apparatus for 4 h. The essential oil was gathered, centrifuged, and separated from the upper layer. Anhydrous sodium sulfate (Na_2SO_4) was used to remove moisture from the waste sample. Essential oil, the dehydrated waste sample, and vacuum-filtered wastewater were stored in amber bottles at $+4^{\circ}\text{C}$ until further experiments.

Microwave-assisted hydrodistillation (MAH)

The microwave-assisted hydrodistillation procedure was carried out using the method described by Fan et al.¹⁸. A Milestone Ethos 1600 (Sorisole, Italy) was used for MAH trials. 100 g dried *L. angustifolia* sample was moistened

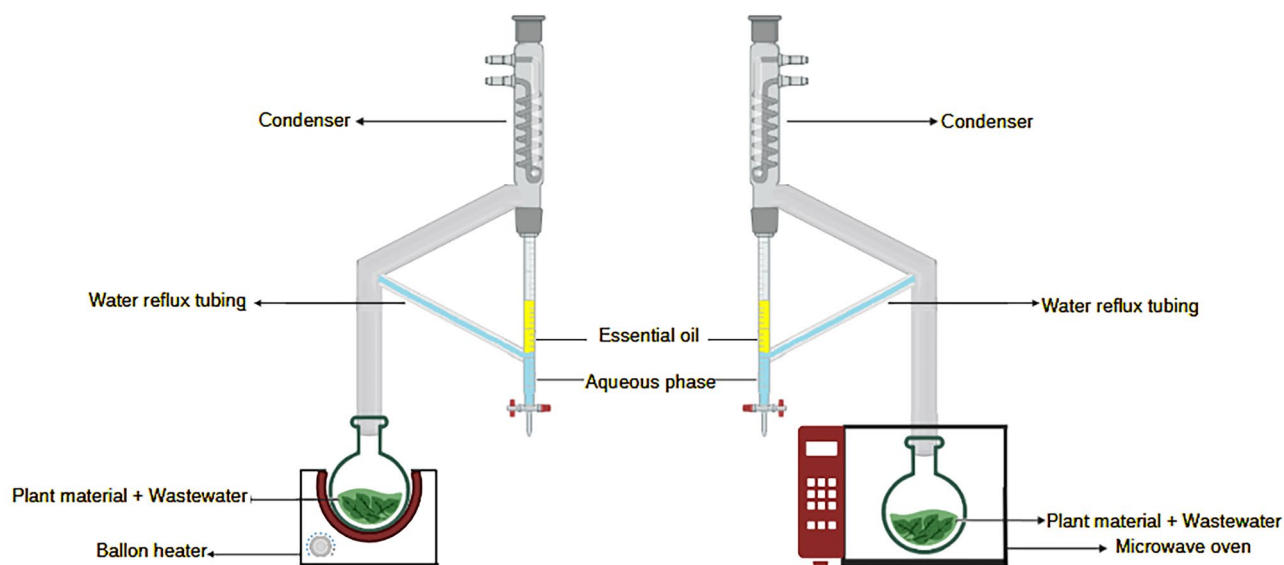


Fig. 1. Diagram of microwave-assisted and traditional hydrodistillation process.

with 500 mL distilled water and transferred to the extraction vessel. The microwave digestion power was set to 1000 W, and the program was set as follows: The temperature was increased to 100 °C in 10 min and then maintained at this level for 30 min. After gathering essential oil, storing essential oil and wastes were the same as TDE methods.

GC–MS analysis of essential oil

The method described by Fan et al.¹⁸ was followed for essential oil analysis. For the analysis of the plant sample, both gas chromatography-mass spectrometry (GC–MS) and gas chromatography with flame ionization detection (GC–FID) were used. The analysis was conducted on a GC–FID (model 7890A) and MS (model 5975C) from Agilent. An HP-5 capillary column (30 m × 0.25 mm inner diameter × 0.25 µm layer thickness) was used for GC–FID analysis. The injection volume was 0.2 µL with a syringe capacity of 0.5 µL at a split ratio of 1:40, using nitrogen as the carrier gas at a 1 mL/min flow rate. The oven temperature was programmed in the following manner: started at 50 °C for 5 min, increased at a rate of 5 °C/min to 190 °C and further increased at a rate of 15 °C/min to 220 °C for 5 min. Temperatures were maintained at 220 °C for the injector and 230 °C for the detector. MS analysis was performed using an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm) to separate the compounds. A temperature of 210 °C was set for the injector and detector. The column temperature was initially set at 40 °C for 3 min, then increased to 90 °C at 3 °C per minute and held for 4 min, then increased to 115 °C at 3 °C per minute and held for 10 min, then increased to 140 °C at 2 °C per minute and held for 8 min, and finally increased to 210 °C at 3 °C per minute and held for 5 min. The carrier gas was helium. Within the 45–500 AMU mass range, the ionization energy was set at 70 eV with a scan time of 0.3 s. Agilent GC–MS solution software was used for GC–MS system management, GC and mass spectrometry parameter settings, and data acquisition and processing. FID peak area normalization was used to express relative percentages of oil components. Identification by GC–MS was performed by comparing mass spectra with WILEY-NIST data libraries and by comparing calculated retention indices with those reported in the literature (NIST Chemistry WebBook, <https://webbook.nist.gov/chemistry>) relative to C8–C28 n-alkanes analyzed under similar GC–MS conditions.

Waste-water analysis

The analysis focused on the wastewater's polyphenol profile, including total phenols, total flavonoids, total monomeric anthocyanins (TAcC), total proanthocyanins, and antioxidant activity assessed through three different assays.

Liquid chromatography-tandem mass spectrometry (LC–MS/MS) analysis of phenolic profile

LC was conducted on an Agilent 1260. MS/MS analyses were performed on an Agilent 6460 triple quadrupole LC–MS equipped with an electrospray ionization (ESI) interface. In all, 32 different polyphenols were investigated by LC–MS/MS. For information on the method, please review our previously published articles^{19,20}.

Total phenolic content

The Folin-Ciocalteu method proposed by Magalhães et al.²¹ evaluated the phenolic content in the wastewater. For this method, 50 µL of extract and 50 µL of Folin-Ciocalteu reagent (1:5, v/v) were added to each well. This was followed by adding 100 µL of sodium hydroxide solution (0.35 M). The absorbance was measured at 760 nm (Epoch, Microplate Reader, Biotek Instruments Inc., USA) after 3 min. The results were expressed regarding gallic acid equivalents (mg GAE/g).

Total flavonoid content

A modified version of the method of Zhishen et al.²² was used for the total flavonoid analysis of the wastewater. 1 mL of wastewater was mixed with 0.3 mL AlCl₃·6 H₂O (10%) solution after adding 0.3 mL NaNO₂ (5%). This was followed by adding an amount of NaOH solution (1 M, 2 mL) and water (2.4 mL), and the final mixture obtained was stirred in the mixer. The absorbance was measured at 510 nm (Epoch, Microplate Reader, Biotek Instruments Inc., USA). The results were presented as quercetin equivalents (mg QE/g).

Total monomeric anthocyanin content (TAcC)

The wastewater's total anthocyanin content (TAcC) was measured using the pH differential method described in the previous procedure with some modifications using three replications²³. One milliliter of aliquots of wastewater were diluted 1:4 in separate solutions: 25 mM KCl buffer at pH 1.0 and 0.4 M C₂H₂NaO₂ buffer at pH 4.5. The absorbance of these solutions was then recorded at 520 nm and 700 nm using a microplate reader (Epoch, Microplate Reader, Biotek Instruments Inc., USA). The results were expressed as mg cyanidin-3-O-glucoside equivalents (mg C3-G/g) based on the following equation:

$$\text{Anthocyanin content} = A \times MW \times DF \times 10^3 / \epsilon \times 1$$

where, A = (A_{520 nm}–A_{700 nm}) pH 1.0—(A_{520 nm}–A_{700 nm}) pH 4.5, MW = 449.2 g/mol (molecular weight of cyanidin-3-glucoside), DF = dilution factor, ϵ = molar absorptivity (assumed to be 1 in this case).

Total proanthocyanidin

The Vanillin-HCl method determined Total proanthocyanidin content based on a previous study by Zurita et al.²⁴. 200 µL of the extract was incubated for 20 min at room temperature by adding 800 µL of vanillin reagent,

and absorbance measurements were performed at 500 nm (Epoch, Microplate Reader, Biotek Instruments Inc., USA). The results were recorded as milligrams of catechin equivalent (mg CAE/g) per gram dry weight (DW).

Antioxidant capacity

2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), the cupric ion reducing antioxidant capacity (CUPRAC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were used to detect the antioxidant activity of wastewater produced during essential oil extraction.

According to Re et al.²⁵, a modified method was used for ABTS wastewater analysis. The results were expressed in terms of Trolox equivalents (mg TE/g).

The method proposed by Apak et al.²⁶ was used to determine the CUPRAC of wastewater. The results were presented in terms of Trolox equivalents (mg TE/g).

The DPPH assay was based on the 96-well plate assay described by Herald et al.²⁷ with some modifications. The results were presented as mg Trolox equivalent antioxidant capacity (TAC) per g of samples (mg TEAC/g).

Greenness assessment tools

Greenness assessment tools provide highly visual semi-quantitative information on the green character of an entire analytical methodology, considering every relevant step from collection and storage to sample preparation and instrumental analysis (LC-MS/MS, HPLC, GC-MS, GC-FID). The ComplexGAPI method²⁸, the BAGI method²⁹, and the AGREEp method³⁰ were applied in our study.

Statistical analysis

All experiments were carried out in triplicate. Results are presented as mean $\pm$ standard deviation (SD). Data were analyzed using a t-test followed by the Holm-Sidak method using GraphPad Prism version 8 (San Diego, CA, USA). Differences were considered significant at $P < 0.05$. Principal component analysis (PCA) was performed using SPSS 26.0 software. PCA was used to determine the main components of the LC-MS/MS results of *L. angustifolia*.

Results and discussion

As complex matrices comprising hundreds of diverse compounds, essential oils necessitate meticulous analysis to determine their volatile profiles. These profiles are crucial for understanding the therapeutic and aromatic properties of medicinal plants like *L. angustifolia*, as their chemical composition can be influenced by various factors, including season, the plant's developmental stage, climate conditions, harvest time, extraction parameters, and extraction techniques⁶.

In this study, we employed two distinct extraction methods, TDH and AH, to investigate the volatile profile of *L. angustifolia* essential oil. The results in Table 1 reveal the presence of 50 different volatile compounds. While thirty-five of these compounds were detected in both extractions, 15 compounds exhibited method-specific detection. Specifically, eight compounds, including 1-borneol (2.11%), 2-cyclohexen-1-one (0.35%), 2-methyl-3-phenylpropanal (0.45%), 4-carvomenthenol (4.97%), α -bisabolol (0.69%), β -myrcene (0.21%), β -ocimene (0.11%), and hexyl isovalerate (0.24%), were exclusively identified in the MAH extract. Conversely, seven compounds, namely 1-hexanol (0.87%), α -pinene (0.88%), β -pinene (0.16%), cyclooctane (0.07%), levomenol (0.80%), geraniol (0.43%), and hexyl valerate (0.24%), were unique to the TDH extraction.

Despite these differences, both TDH and MAH yielded essential oil with α -terpinolene (25.60% and 24.25%, respectively) and (-)-borneol (19.55% and 19.37%, respectively) as the predominant volatile constituents (Fig. 2). This consistency in major compound extraction suggest that both methods are effective in capturing the characteristic aroma and therapeutic potential of *L. angustifolia*, even though they may differ in their efficiency for extracting minor or trace components. These findings align with previous studies, such as that by Hajhashemi et al.³¹, which identified camphor (9.5%), borneol (11.5%) and 1,8-cineole (65.4%) as major volatiles in lavender leaves. However, some variations were observed, such as the detection of α -terpineol (0.5%) in their study but not in ours, emphasizing the potential influence of methodological or sample-specific factors on essential oil composition.

Essential oil extraction from medicinal plants, such as lavender, inevitably generates substantial by-products, including wastewater. However, previous research has revealed that the organic components of this wastewater possess diverse bioactivities, including anti-inflammatory, antibacterial, antioxidant, antifungal, and enzymatic activities³². This study compared the chemical profile of essential oil and wastewater obtained from *L. angustifolia* using different extraction methods (TDH and MAH). Table 2 details the phytochemical profile of the wastewater extract, with the MAH method yielding higher total content than the TDH method. According to the results, the total phenolic and total flavonoid content of MAH extraction were significantly higher than that of TDH extraction. The higher phenolic and flavonoid content in MAH extracts compared to TDH can be attributed to enhanced cell disruption, reduced thermal degradation, increased mass transfer, and selective heating mechanisms facilitated by microwave energy, leading to more efficient extraction of these bioactive compounds^{33–36}. DPPH, CUPRAC, and ABTS assays evaluated the wastewater's antioxidant activity. Results revealed significant differences in antioxidant activity between the MAH (7.619 ± 0.83 mg TEAC/g, 7.847 ± 0.59 and 7.790 ± 0.80 mg TE/g) and TDH (5.603 ± 0.58 mg TEAC/g, 3.869 ± 0.17 and 4.291 ± 0.43 mg TE/g) methods. Furthermore, the MAH method also resulted in the highest total proanthocyanidins (0.548 ± 0.03 mg CAE/g) and TacC (0.023 ± 0.002 mg C3-G/g). Our results highlight that the choice of extraction method significantly impacts the essential oil yield but also the bioactive composition of the resulting wastewater. Microwave-assisted hydrodistillation (MAH) consistently outperformed traditional hydrodistillation (TDH) in extracting a more comprehensive array of bioactive compounds and producing wastewater with superior antioxidant activity. This

Microwave-assisted hydrodistillation			Traditional hydrodistillation		
RT	Essential oils	Area (%)	RT	Essential oils	Area (%)
8.049	Camphane	0.46 ± 0.001	5.889	1-Hexanol	0.87 ± 0.001
9.063	1-Octen-3-ol	0.74 ± 0.001	7.467	α-Pinene	0.88 ± 0.001
9.283	3-Octanone	0.21 ± 0.001	8.051	Camphane	0.61 ± 0.001
10.172	Hexyl acetate	0.73 ± 0.001	8.926	β-Pinene	0.16 ± 0.001
10.519	O-Cymene	0.63 ± 0.001	9.071	1-Octen-3-ol	0.48 ± 0.001
10.804	Eucalyptol	6.55 ± 0.002	9.285	3-Octanone	0.13 ± 0.001
11.34	β-Ocimene	0.11 ± 0.001	10.184	Hexyl acetate	0.53 ± 0.001
11.696	Gamma-Terpinene	0.29 ± 0.001	10.523	O-Cymene	0.83 ± 0.001
12.209	Linalyl oxide	2.19 ± 0.002****	10.828	Eucalyptol	9.37 ± 0.002****
12.745	Trans-Linalool oxide	2.01 ± 0.003	11.696	Gamma-Terpinene	0.27 ± 0.001
13.489	α-Terpinolene	25.60 ± 0.006****	12.231	Linalyl oxide	2.81 ± 0.003
13.531	Ho-trienol	0.45 ± 0.001	12.766	Trans-Linalool oxide	2.26 ± 0.001
14.001	1-Terpineol	0.16 ± 0.001	13.474	α-Terpinolene	24.25 ± 0.005
14.848	Camphor	8.28 ± 0.009	13.524	Ho-trienol	0.65 ± 0.001
14.901	Propanoic acid	0.81 ± 0.001	13.995	1-Terpineol	0.07 ± 0.001
15.365	Pinocarvone	0.12 ± 0.001	14.839	Camphor	8.57 ± 0.002
15.775	(-)-Borneol	19.65 ± 0.02	14.897	Propanoic acid	0.74 ± 0.001
15.803	1-Borneol	2.11 ± 0.003	15.363	Pinocarvone	0.05 ± 0.001
16.057	4-Carvomenthenol	4.97 ± 0.005	15.747	(-)-Borneol	19.37 ± 0.007
16.262	Crypton	1.05 ± 0.001	16.044	3-Cyclohexen-1-ol	5.46 ± 0.001
16.461	Butanoic acid	2.57 ± 0.002	16.274	Crypton	1.00 ± 0.001
16.591	Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde	0.26 ± 0.001	16.439	Butanoic acid	2.46 ± 0.001
17.02	Bicyclo[3.1.1]hept-3-en-2-one	0.22 ± 0.001	16.584	Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde	0.21 ± 0.001
17.343	Trans-(+)-Carveol	0.21 ± 0.001	17.014	Bicyclo[3.1.1]hept-3-en-2-one	0.13 ± 0.001
17.966	Hexyl 2-methylbutanoate	1.10 ± 0.001	17.329	Trans-(+)-Carveol	0.21 ± 0.001
18.039	2-Methyl-3-phenylpropanal	0.45 ± 0.001	17.633	(-)-Borneol acetate	0.90 ± 0.001
18.112	Hexyl isovalerate	0.24 ± 0.001	17.957	hexyl 2-methylbutanoate	0.94 ± 0.001
18.193	2-Cyclohexen-1-one	0.35 ± 0.001	18.031	2-Methyl-3-phenylpropanal	0.46 ± 0.001
18.632	Linalyl acetate	2.76 ± 0.005	18.106	Hexyl valerate	0.24 ± 0.001
19.207	Phellandral	0.07 ± 0.001	18.181	Carvone	0.32 ± 0.001
19.598	(-)-Borneol acetate	0.34 ± 0.001	18.534	Geraniol	0.43 ± 0.001
19.778	Lavandulyl acetate	1.38 ± 0.001	18.586	Linalyl acetate	0.35 ± 0.001
20.129	Carvacrol	1.58 ± 0.001	19.098	Cyclooctane	0.07 ± 0.001
21.065	Hexyl tiglate	0.21 ± 0.001	19.201	Phellandral	0.11 ± 0.001
22.153	Neryl acetate	0.13 ± 0.001	19.768	Lavandulyl acetate	0.95 ± 0.001
22.774	Geranyl acetate	0.19 ± 0.001	20.108	Carvacrol	0.94 ± 0.001
22.859	Hexanoic acid	0.67 ± 0.001	21.056	Hexyl tiglate	0.21 ± 0.001
25.051	Trans-β-Farnesene	0.16 ± 0.001	22.149	Neryl acetate	0.13 ± 0.001
26.67	β-Myrcene	0.21 ± 0.001	22.77	Geranyl acetate	0.23 ± 0.001
28.859	Caryophyllene oxide	0.59 ± 0.001	22.853	Hexanoic acid	0.46 ± 0.001
30.986	Bisabolol oxide	0.34 ± 0.001	25.046	Trans-β-Farnesene	0.06 ± 0.001
31.519	Caryophyllenol-II	0.08 ± 0.001	28.852	Caryophyllene oxide	0.47 ± 0.001
31.938	α-Bisabolol	0.69 ± 0.001	30.981	Bisabolol oxide B	0.25 ± 0.001
34.834	2-Pentadecanone	0.13 ± 0.001	31.929	Levomenol	0.80 ± 0.001
Continued					

Microwave-assisted hydrodistillation			Traditional hydrodistillation		
RT	Essential oils	Area (%)	RT	Essential oils	Area (%)
			34.832	2-Pentadecanone	0.05 ± 0.001
	Monoterpene hydrocarbon	27.3		Monoterpene hydrocarbon	27
	Oxygenated monoterpene alcohol	28.64		Oxygenated monoterpene alcohol	26.67
	Oxidized monoterpene aldehyde	0.33		Oxidized monoterpene aldehyde	0.32
	Oxygenated monoterpene ester	4.8		Oxygenated monoterpene ester	1.66
	Oxygenated monoterpene ether	8.74		Oxygenated monoterpene ether	12.18
	Oxidized monoterpene furanoid ether	2.01		Oxidized monoterpene furanoid ether	2.26
	Oxidized monoterpene ketone	8.62		Oxidized monoterpene ketone	9.07
	Oxygenated monoterpene phenol	1.58		Oxygenated monoterpene phenol	0.94
	Sesquiterpene hydrocarbon	1.21		Sesquiterpene hydrocarbon	1.06
	Oxygenated sesquiterpene alcohol			Oxygenated sesquiterpene alcohol	0.8
	Oxidized sesquiterpene ether	0.77		Oxidized sesquiterpene ether	0.72
	Alcohol	0.93		Alcohol	0.87
	Ester			Ester	54.39
	Hydrocarbon	2.28		Hydrocarbon	0.07
	Ketone	0.34		Ketone	0.18
	Organic acid	4.05		Organic acid	3.66
	Terpenoid aldehyde	5		Terpenoid aldehyde	1.1

Table 1. Composition of *L. angustifolia* essential oil. *RT: retention time; ****P < 0.0001.

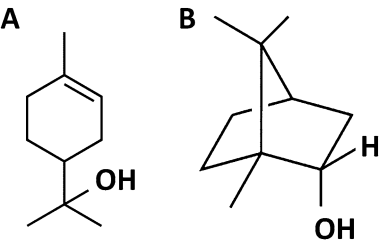


Fig. 2. Molecular structure of (A) α-terpinolene and borneol (B).

Phytochemical analysis	Microwave-assisted hydrodistillation	Traditional hydrodistillation
Total phenolic content (mg GAE/g)	3.280 ± 0.18****	1.778 ± 0.18
Total flavonoid content (mg QE/g)	3.226 ± 0.30****	1.458 ± 0.08
Total proanthocyanidin (mg CAE/g)	0.548 ± 0.03	0.267 ± 0.01
TAcC (mg C3-G/g)	0.023 ± 0.002	0.007 ± 0.01
DPPH (mg TEAC/g)	7.619 ± 0.83****	5.603 ± 0.58
CUPRAC (mg TE/g)	7.847 ± 0.59****	3.869 ± 0.17
ABTS (mg TE/g)	7.790 ± 0.80****	4.291 ± 0.43

Table 2.. Phytochemical profile of *L. angustifolia* wastewater. ****P < 0.0001.

suggests that MAH, as an innovative extraction technique, offers a more efficient and comprehensive approach to essential oil isolation, yielding a richer source of valuable compounds in both the oil and its by-product. Beyond its efficacy in extracting bioactive substances, MAH also presents several operational advantages over TDH, including reduced extraction time, minimized solvent usage, and lower energy consumption. These findings underscore the potential of MAH as a sustainable and economically viable alternative to conventional methods for essential oil production.

Several studies have investigated the composition and bioactivity of lavender by-products, specifically wastewater generated during essential oil extraction. Our research builds upon these findings, providing a more comprehensive analysis of the chemical profile and antioxidant activity of *L. angustifolia* wastewater. Previous studies have reported lower total phenolic and flavonoid content in lavender wastewater compared to our findings. For example, Turrini et al.³⁷ found levels of 0.75 mg GAE/g and 0.002 mg QE/g, respectively, using traditional distillation. This discrepancy may be attributed to differences in extraction methods, plant varieties, or

environmental conditions. Ciocarlan et al.³⁸ focused on the antimicrobial activity of *L. angustifolia* by-products, demonstrating their effectiveness against various pathogens, including *Bacillus* sp., *B. subtilis*, *Xanthomonas campestris*, *Pseudomonas fluorescens*, *Candida utilis*, *Erwinia carotovora*, *E. amylovora*, *Penicillium chrysogenum*, *Aspergillus niger*, *Alternaria alternata*, and *Pseudomonas aeruginosa*³⁸. Our study builds upon previous research by Turrini et al.³⁷ and Ciocarlan et al.³⁸, providing a more in-depth analysis of lavender wastewater. Specifically, we assessed the total phenolic and flavonoid content and identified and quantified individual phenolic and flavonoid compounds. Furthermore, our study investigated the antioxidant activity of the wastewater, offering a more comprehensive understanding of its bioactive potential.

Additionally, Méndez-Tovar et al.³⁹ assessed the total phenolic content, antioxidant activity, and phenolic profiles of solid residues, a distinct by-product generated during essential oil extraction, from 12 different populations of *L. latifolia* collected across two seasons. Their findings revealed a total phenolic content of 3.54 mg GAE/g in these residues, which aligns with the range observed in our study for lavender wastewater. However, the individual phenolic levels, specifically apigenin, luteolin, and rosmarinic acid, were lower in their study (55.9, 102.6, and 3040 mg/kg, respectively) compared to our results. This discrepancy can be attributed to variations in both plant species (*L. latifolia* vs. *L. angustifolia*) and the type of by-product analyzed (solid residues vs. wastewater). Dobros et al.⁴⁰ demonstrated that the total phenolic content of extracts from various *L. angustifolia* cultivars obtained through different extraction methods (ultrasonically assisted extraction, maceration, and decoction), ranged from 14.89 to 32.82 mg GAE/g. Similarly, they found a range of 15.55 to 30.79 mg GAE/g for extracts from two *L. x intermedia* cultivars using different methods. These findings highlight the substantial impact of extraction methodology on the recovery of bioactive substances from lavender, suggesting that the choice of extraction technique can significantly influence the phenolic content of the final product. A separate study analyzing inflorescence powder samples of *L. x intermedia* collected from 30 different populations found a range of 0.31–1.03 mg GAE/g for total phenolic content and 0.28–0.71 mg QE/g for total flavonoid content⁴¹. In comparison to our findings, the study by⁴¹ reported lower total phenolic and flavonoid content in *L. x intermedia* inflorescence powder extract than what we observed in the *L. angustifolia* wastewater by-product. In contrast, a study by⁴² focusing on the total phenolic content and antioxidant activity (using the DPPH method) of *L. angustifolia* essential oil itself reported values of 1.22 ± 0.04 mg GAE/g and 6.522 ± 0.069 mg/mL, respectively. Our study, however, found higher total phenolic content (1.7776 mg GAE/g for TDH and 3.2803 mg GAE/g for MAH) and antioxidant activity (7.619 mg TE/g) in the wastewater by-product. This highlights a key finding: the by-product generated during essential oil extraction from *L. angustifolia* may contain a higher concentration of phenolic compounds and exhibit stronger antioxidant activity than the essential oil. This result underscores the potential value of this by-product as a source of bioactive compounds. This observation aligns with numerous other studies demonstrating the diverse biological activities of wastewater generated from essential oil isolation of various medicinal plants. These activities include not only antioxidant properties^{43–45} but also antiacetylcholinesterase⁴⁴, antifungal effects⁴⁵, and antibacterial⁴⁶. For instance, a study examining wastewater generated during essential oil isolation from various medicinal plants (including *Dittrichia viscosa*, *Salvia officinalis*, *Origanum vulgare*, *Thymus camphoratus*, *Foeniculum vulgare*, *Thymbra capitata*, *Thymus carnosus*, and *Thymus mastichina*) reported total phenolic content values ranging from 5.3 to 45.2 mg/mL⁴⁶. This wide range demonstrates plant species' significant impact on wastewater's phenolic composition. In our study, this species-specific variation can be attributed to the lower total phenolic content observed in *L. angustifolia* wastewater (1.7776 mg GAE/g for TDH and 3.2803 mg GAE/g for MAH).

This study aimed to evaluate the impact of different essential oil extraction methods (TDH and MAH) on the recovery of phenolic compounds from wastewater. Previous research suggests that the chosen distillation method can significantly affect phenolic content. Therefore, analyzing the phenolic profiles of wastewater samples obtained using these methods is crucial. In addition to standard spectrophotometric analyses, we employed LC–MS/MS to identify 32 individual phenolics in the wastewater samples. The results were further evaluated using PCA. LC–MS/MS analysis revealed that several individual phenolics, including gallic acid + epigallocatechin, (–)-epicatechin, peonidin chloride, epicatechin gallate + gallic acid gallate, and myricetin, were not detected in either sample. However, minor amounts of specific phenolics were found to be method-dependent. For instance, caffeic acid phenethyl ester, quercitrin, hesperidin, catechin + epicatechin, chlorogenic acid, and pinobanksin were only detected in the TDH wastewater, while quercetin, galangin, and trans-caffeic acid were exclusive to the MAH method. Notably, despite these differences in individual phenolic composition, the total phenolic content values for both methods were quite similar (489.322 µg/kg for TDH; 537.803 µg/kg for MAH).

This study compared the profile of polyphenols in wastewater extracts obtained using the TDH and MAH methods. The data are presented in Table 3. Both methods identified protocatechuic acid as the major phenolic compound, with concentrations of 141.852 ± 4.868 µg/kg and 181.397 ± 28.652 µg/kg in TDH and MAH extracts, respectively. While the overall distribution of phenolic compounds was similar between the two methods, p-coumaric acid was found in significantly higher concentrations in the TDH extract. Conversely, apigenin and luteolin were present at significantly higher concentrations in the MAH extract.

Phenolic compounds, as secondary metabolites in plants, possess various biological activities, making their qualitative and quantitative determination in different plant products crucial for understanding their potential health benefits. Our study revealed that *L. angustifolia* wastewater contains a notable diversity of individual phenolic compounds. Previous studies have reported that the *L. angustifolia* by-product is particularly rich in syringic acid (26.082 µg/g) and rutin (56.081 µg/g) compared to other targeted phenolics³⁷. In our study, rutin was identified as a major phenolic compound in lavender wastewater, consistent with previous reports highlighting its abundance in *L. angustifolia* by-products³⁷. While limited research specifically focuses on the phenolic content of lavender wastewater, studies on other types of lavender extracts have identified a diverse range of phenolic compounds. These include gallic acid, vitexin, luteolin 7-O-glucoside, umbelliferone, chlorogenic acid, and isocoumaroside, which have been found in extracts obtained using various extraction methods⁴⁷.

RT	Polyphenols	Microwave-assisted hydrodistillation	Traditional hydrodistillation
2.110	Gallic acid	6.53 ± 0.302	4.908 ± 0.333
2.240	Protocatechuic acid	181.397 ± 28.652****	141.852 ± 4.868
4.600	Neochlorogenic acid	2.676 ± 0.344	5.231 ± 0.072
4.610	Chlorogenic acid	nd	0.757 ± 0.003
5.170	trans-caffeic acid	10.457 ± 1.109	nd
7.560	Catechol	24.941 ± 2.121	26.688 ± 1.988
7.970	<i>p</i> -coumaric acid	3.908 ± 0.271	115.07 ± 2.782****
8.150	Gallocatechin + Epigallocatechin	nd	nd
8.740	Caffeine	2.117 ± 0.111	2.111 ± 0.066
9.160	trans-ferulic acid	1.071 ± 0.037	7.499 ± 0.115
9.170	(-)-Epicatechin	nd	nd
9.210	Catechin + epicatechin	nd	1.681 ± 0.166
10.12	Epicatechin gallate + gallocatechin gallate	nd	nd
10.30	Peonidin chloride	nd	nd
10.33	Rutin	36.527 ± 2.152****	19.297 ± 1.569
10.42	Quercetin	12.63 ± 1.144	nd
10.45	Hyperosid	2.037 ± 0.187	21.833 ± 1.193****
11.08	Hesperidin	nd	4.016 ± 0.167
11.08	Quercitrin	nd	0.173 ± 0.032
11.36	Myricetin	nd	nd
12.27	<i>t</i> -cinnamic acid	2.805 ± 0.085	2.246 ± 0.425
12.52	Cyanidin chloride	31.096 ± 2.894****	13.875 ± 1.123
12.52	Luteolin	127.007 ± 7.126****	66.731 ± 0.74
12.53	Kaempferol	34.497 ± 2.353**	19.654 ± 1.451
13.07	Naringenin	0.674 ± 0.003	0.691 ± 0.08
13.27	Pinobanksin	nd	0.907 ± 0.06
13.30	Apigenin	47.733 ± 0.995****	24.38 ± 1.238
15.03	Pinocembrin	2.791 ± 0.323	4.446 ± 0.266
15.11	Chrysin	5.503 ± 0.779	3.599 ± 0.278
15.27	Caffeic acid phenethyl ester	nd	0.007 ± 0.001
15.34	Galangin	0.28 ± 0.001	nd
16.80	Artepillin C	1.126 ± 0.052	1.67 ± 0.138

Table 3. Phenolic profile of *L. angustifolia* wastewater (µg/Kg). *nd: not detected; ****P < 0.0001; **P < 0.001.

Additionally, flower extracts obtained through maceration have been shown to contain high amounts of gallic acid, ellagic acid, and chlorogenic acid⁴⁸. Moreover, the bioactive potential of wastewater from other plant species has also been investigated. For instance, studies on wastewater from *Rosa* species revealed a phenolic content of 0.37 mg/mL and flavonoid content of 7.2 to 7.8 mg/mL⁴⁹. This wastewater also contained various phenolic compounds, including brevifolincarboxylic acid, gallic acid, hyperoside, ellagic acid, catechin, glucogallin, epicatechin, chlorogenic acid, avicularin, miquelianin, and kaempferol, along with their derivatives. Notably, the total flavonoid content in *Rosa* wastewater was lower than that observed in our lavender wastewater, whereas the total phenolic content was higher. These discrepancies can be attributed to variations in plant species and the specific extraction methods employed, highlighting the complex interplay between these factors in determining the bioactive composition of wastewater.

In this study, principal component analysis (PCA) was employed to analyze the phenolic profile results of *L. angustifolia*. PCA is a statistical technique that simplifies complex datasets by reducing the number of variables

	Eigenvalue	Variability (%)	Cumulative (%)
PC1	15.352	46.522	46.522
PC2	7.329	22.209	68.731
PC3	5.979	18.119	86.850
PC4	2.925	8.863	95.713
PC5	1.415	4.287	100.000

Table 4. Eigenvalues and percentage of variance for phenolics of PCA analysis.

while retaining most of the original information. It achieves this by transforming the original variables into a new set of uncorrelated variables called principal components (PCs), which are linear combinations of the original variables. The eigenvalues and variance percentages of the PCA analysis are presented in Table 4 and Fig. 3. Eigenvalues indicate the amount of variance explained by each principal component, with higher values representing greater importance. Based on Kaiser's rule, eigenvalues greater than 1.0 are considered significant and retained as principal components⁵⁰. In our analysis, five principal components (PC1-PC5) met this criterion, with eigenvalues of 15.352, 7.329, 5.979, 2.925, and 1.415, respectively. Previous studies using PCA on lavender samples have reported varying numbers of principal components explaining the majority of the variance. For example, Gök and Erdoğan⁵¹ identified two principal components accounting for 98.29% of the total variance, while Komes et al.⁵² found that PC1 and PC2 explained 84.29% of the variance in their study of medicinal aromatic plants. These differences highlight the influence of sample composition and experimental conditions on the PCA results.

The Kaiser–Meyer–Olkin (KMO) test was employed to assess the suitability of the data for principal component analysis (PCA). The KMO statistic measures sampling adequacy, indicating the proportion of variance among variables that might be common variance. According to Kaiser⁵⁰, KMO values range from 0 (unacceptable) to 1 (excellent), with values above 0.7 considered suitable for factor analysis. Based on the KMO test results and the evaluation of the phenolic profile of *L. angustifolia*, five principal components were identified in this study. According to the PCA results obtained in our study, PC1 consists of gallic acid, neochlorogenic acid, p-coumaric acid, trans-ferulic acid, catechin + epicatechin, hesperidin, rutin, kaempferol, cyanidin chloride, luteolin, pinobanksin, apigenin, pinocembrin, chrysin, trans-cafeic acid and artepillin C, while PC2 consists of protocatechuic acid, chlorogenic acid, peonidin chloride, quercitrin, naringenin and caffeic acid phenethyl ester are the main components. In addition, PC3 was composed of epicatechin gallate + gallo catechin gallate, gallo catechin + epigallocatechin, (–)-epicatechin, hyperoside, myricetin, t-cinnamic acid, PC4 was composed of caffeine, quercetin, galangin, and for PC5 catechol alone constituted a major component.

In recent years, various tools have been developed to assess the greenness of analytical procedures^{28–30}. These approaches are often general and need to pay more attention to the analytical steps responsible for a non-green analytical procedure. The evaluation criteria are based on the ten principles of green sample preparation: selection of solvents, materials, and reagents; waste generation; energy consumption; sample size; and yield. The AGREeprep pictograms in Table 5 represent different levels of environmental sustainability. The central score of the pictograms is 0.57 for the microwave-assisted method and 0.55 for traditional hydrodistillation. The BAGI tool, on the other hand, presents a definitive pictogram and score using 10 BAGI standards, indicating the utility and effectiveness of an analytical procedure. The color of an approach, as shown in Table 5, indicates its proximity to meeting the requirements. A technique is considered applicable if it scores 60 or higher. Table 5 shows the proposed pictograms for BAGI indices. The microwave method scored 70, while traditional hydrodistillation scored 65. Green analytical chemistry properties are used to assess analytical methods in ComplexGAPI. The ComplexGAPI graph has been expanded to include pre-analytical procedures by adding hexagon to the GAPI. A color scale is used in this evaluation, with red indicating a high harmful impact on the ecosystem, while yellow

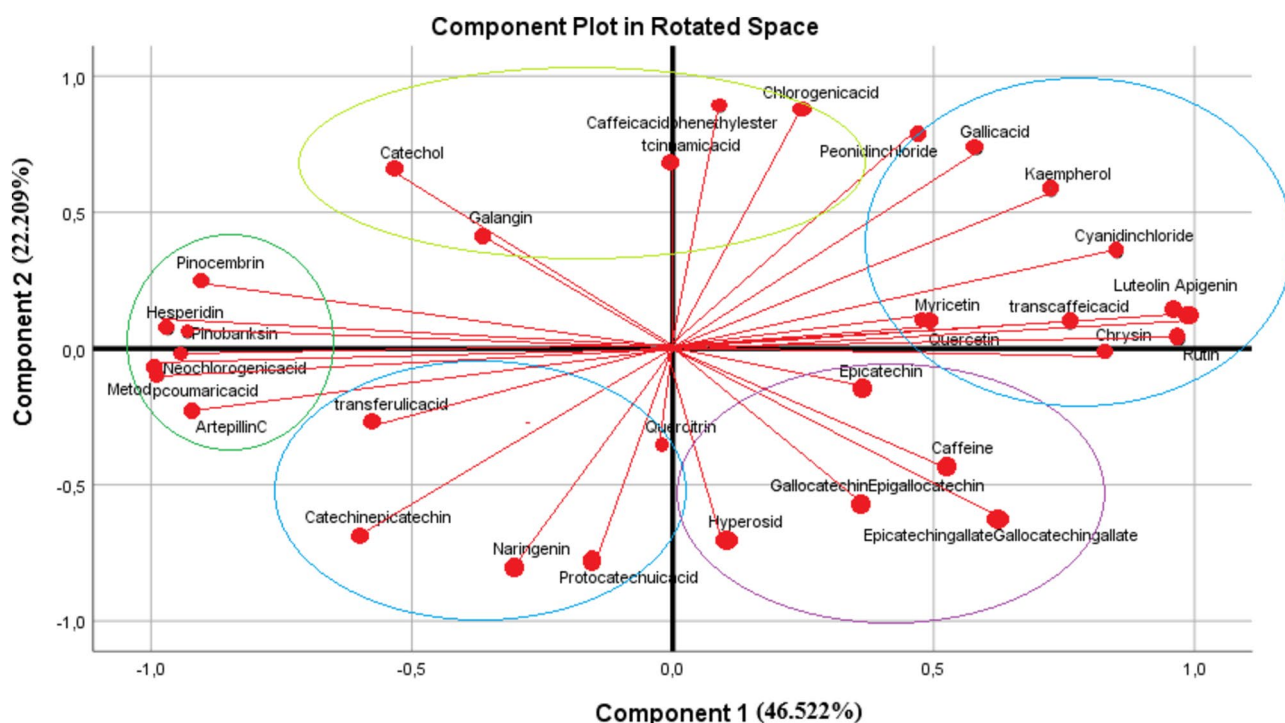


Fig. 3. PCA score plot based on the individual phenolic results of MAH and TDH methods.

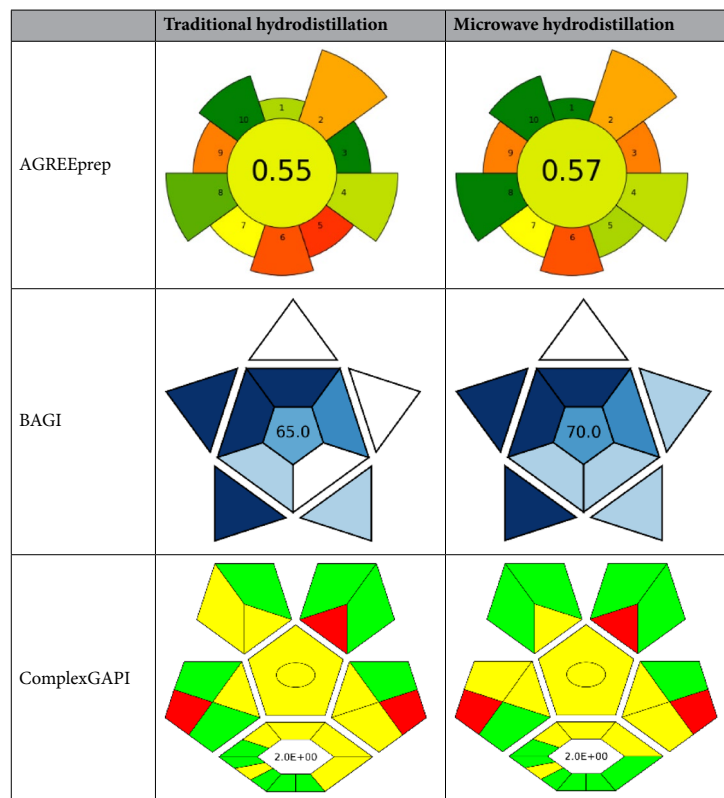


Table 5. Green assessment tool pictograms. AGREeprep: analytical greenness metric for sample preparation; BAGI: blue applicability grade index; ComplexGAPI: complementary green analytical procedure index.

and green reflect moderate and low effects, respectively. As shown in Table 5, the microwave method has three red zones, 11 yellow zones, and 11 green zones; the traditional method has three red zones, 10 yellow zones, and 12 green zones.

Conclusion

Essential oils are complex and economically valuable mixtures preferred in various industries due to their diverse effects. However, large-scale production generates significant amounts of by-product wastewater, which is often considered waste. Developing environmentally friendly production methods is crucial, especially for essential oils with high-volume production, like lavender. This study investigated the impact of different extraction methods (TDH and MAH) on the essential oil and wastewater obtained from *L. angustifolia*. Based on the GC–MS data, both methods yielded significant proportions of α -terpinolene and (–)-borneol. Notably, the chosen extraction method significantly affects not only the essential oil content but also the composition of the wastewater. This suggests the potential for utilizing wastewater in various industrial applications beyond just the essential oil itself, such as the food, cosmetic, or even health sectors.

Data availability

The data used in this study is available from the corresponding author on reasonable request.

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Author contributions

Conceptualization, sample preparation, formal analysis and visualization, writing—original draft preparation SK, Conceptualization, writing—original draft preparation, review and editing, Project administration YCG; formal analysis FB, NY, BG; writing, review and editing NEB. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

Ethical approval

Plant sampling were comply with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Additional information

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