Research Square

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Application of Different Drying Techniques on the Chemical Composition and Antimicrobial Activities of the lemon verbena (Lippia Citrioodora Kunth palau) Essential Oil

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Abstract

This study examined various drying techniques to assess their impact on the chemical composition and biological activities of lemon verbena, particularly in its application within the pharmaceutical industry. The drying techniques employed included sun drying, shade drying, oven drying at temperatures of 30, 40, and 50°C, microwave drying at power levels of 100 and 300W, and freeze drying. Components of hydro-distilled essential oils were analyzed, and their antibacterial efficacy was assessed against six bacterial strains. Findings indicated that the essential oil extracted from samples dried using a microwave at 300W exhibited the highest concentration of secondary metabolites, reaching 88.6%, and contained 34 active compounds. The essential oil extracted from sun-dried samples exhibited a low concentration of secondary metabolites, quantified at 66.49%, comprising 25 distinct bioactive compounds. D-limonene was identified in the essential oil derived from all drying techniques, with its concentration ranging from 10.67% in samples dried in an oven at 40 and 50°C to 6.51% in those dried by sunlight. Largest diameter of the non-growth halo (73mm) was related to *Streptococcus pyogenes* by treatment with essential oil of samples dried in an oven (30°C). The essential oil from sun-dried samples exhibited no inhibitory effect against *Staphylococcus aureus*. In contrast, the antimicrobial activity of all essential oils demonstrated a dose-dependent inhibitory effect. Overall, lemon verbena essential oils are composed of chemical compounds with significant potential for combating bacterial infections.

1 Introduction

Medicinal plants are defined as those flora that contain compounds within one or more of their parts, which can be utilized for therapeutic applications or serve as precursors in developing beneficial pharmaceuticals. For millennia, these plants have served as a vital resource for healing practices in communities across the globe [1]. Various phytochemicals, including alkaloids, carbohydrates, fatty acids, glycoside derivatives, phenolics, flavonoids, polystyrenes, steroids, and essential oils, have been recognized in medicinal plants [2, 3]. Essential oil, recognized as a key active component, is a volatile substance with a low molecular weight, sourced from medicinal plants. It exhibits various properties, including antibacterial, antimicrobial, antifungal, antiviral, antioxidant, and cytotoxic effects, suggesting its potential utility across various fields such as medicine, food production, cosmetics, and agriculture [4, 5]. The quantity and quality of secondary metabolites, such as essential oils, in medicinal plants are significantly influenced by the plant's intrinsic biochemical properties and various external factors. These include cultivation conditions, geographical and environmental influences, the timing of the harvest, and subsequent post-harvest processes like drying. Consequently, these factors are crucial for determining these plants' biological, medicinal, and culinary applications [6, 7].

The drying process serves to inhibit microbial proliferation and mitigate certain biochemical alterations within the plant by lowering moisture content to approximately 10–14%, contingent upon the specific type of plant organ. This reduction in humidity not only influences the visual and aromatic properties of the plant but also results in modifications to the quantity and composition of essential oils. Numerous techniques are available for drying plants, including microwave, freeze, hot-air, sun, shade, and oven drying [4]. The most commonly employed methods for drying are shade, sun, and hot-air treatments, primarily because of their cost-effectiveness [8]. The drying process must aim to minimize the degradation of plant quality concerning active compounds, coloration, fragrance, and taste [9]. The drying methods can significantly influence plants' nutritional and qualitative attributes, including their vitamin content, coloration, chlorophyll levels, total flavonoid concentration, total phenolic content, and antioxidant activity [8]. For example, Pinela et al. [10], reported that the oven drying methods had the highest content of phenolic compounds in *Tuberaria lignosa*, among other methods.

Researchers showed that pre-drying operations, such as solarization and hot air flow, can increasing the quality of the product and reducing the drying time compared with the traditional methods (sun and shade). Their results showed that with increasing air temperature and air velocity, the drying time of lemon verbena in the dryer significantly decreased [11].

In another studty examined different drying methods on limone verbena leaves. Results showed the highest and lowest drying times belonged to shade drying (53 h) and vacuum drying at 60 °C (3 h) respectively. Glandular trichomes were

vulnerable to oven whereas vacuum drying and freeze drying preserved them. The maximum essential oil content (1.0 ml/100 g D.M.) obtained from vacuum-dried (60 ˚C) and oven-dried (40 ˚C) samples. Freeze drying preserved the highest amount of oxygenated monoterpenes, especially citral (64.7%) but the highest amounts of limonene and 1,8-cineole (8.2 and 7.2% respectively) were determined in vacuum-dried (60 ˚C) leaves. Apart from the standard oven drying at 40 ˚C, vacuum drying at 60 ˚C can be considered as an alternative approach to dry lemon verbena in a significantly shorter drying time [12].

Essential oils possess antibacterial characteristics that are effective against a wide variety of microorganisms, attributed to their diverse antimicrobial compounds. Consequently, these compounds may serve as a viable approach for preventing and managing numerous microbial infections [13, 14]. The essential oils of *Zataria multiflora* and *Origanum vulgare* killed 85.00% and 80.00% of *Escherichia coli* O157:H7, respectively [2]. The savory essential oil demonstrated considerable antimicrobial efficacy against Gram-positive and Gram-negative bacterial strains [15]. The volatile compounds derived from rosemary, cassia, and tea trees exhibited the most effective broad-spectrum antibacterial activity, while the volatiles from clove demonstrated negligible antimicrobial properties [16]. Oregano, clove, thyme, and arborvitae indicated very strong antibacterial activity against *Escherichia coli*, *Salmonella typhimurium*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Enterococcus faecalis* at both full strength and reduced concentrations [17].

The Iranian plateau is recognized as a significant source of numerous medicinal plants, and their cultivation has been on the rise within the country. This expansion is driven by the increasing demand from the pharmaceutical, food, health, and cosmetic sectors for these plants as essential raw materials in their production processes [18]. One of the medicinal plants cultivated in Iran is lemon verbena (*Lippia Citrioodora* Kunth palau) [19]. It is a perennial shrub in the Verbenaceae and native to South America, and that is main active ingredient is essential oil. The essential oil derived from the desiccated leaves of lemon verbena is recognized for its therapeutic applications in addressing anxiety, insomnia, stress, nervous exhaustion, psoriasis, multiple sclerosis, rheumatism, enterocolitis, anorexia, dyspepsia, intestinal parasites, as well as exhibiting antibacterial, antifungal, antiviral, and antioxidant properties, in addition to its role in the prevention of asthma episodes [13]. Hematian et al. [20] indicated that the most important constituents of lemon verbena were found to be geranial (27.21%), neral (20.01%), spathulenol (7.28%), limonene (6.86%), caryophyllene oxide (6.77%), α-curcumene (4.64%), and 1,8-cineole (4.61%). Also, researchers reported that the most important constituents of lemon verbena essential oil were geranial, neral, limonene, and 1,8-cineole when the plants grew in Iran [21]. Its essential oil content and composition depend on the plant's developmental stage, ontogenesis, harvest season, and pedoclimatic conditions [22]. The essential oil derived from lemon verbena leaves, which contains compounds such as spathulenol, Ar-curcumene, trans-caryophyllene oxide, and neral, exhibits antimicrobial properties effective against both Gram-negative and Gram-positive bacterial strains [13].

Most studies concerning the drying processes of lemon verbena leaves have predominantly utilized conventional techniques [23, 24], while innovative methods such as infrared or gamma radiation have been employed to a lesser extent [25, 26]. This situation highlights the need to focus on expanding the cultivated area of medicinal plants to enhance regional cultivation practices, particularly given the insufficient data regarding the optimal quality of lemon verbena products. This study seeks to explore three primary objectives: first, to assess how various drying techniques influence the phytochemical properties of lemon verbena; second, to evaluate the impact of these drying methods on the composition of essential oils derived from lemon verbena; and third, to examine the effects of drying processes on the antimicrobial efficacy of the essential oil extracted from lemon verbena cultivated in the Sistan region of Iran.

2 Materials and Methods

2.1 Experimental design, plant material, and treatments

The experiment was conducted as a completely randomized design with three replications. Lemon verbena samples were collected from the medicinal plants collection in the Agriculture Institute, Research Institute of Zabol, Zabol, Iran. First, aerial

parts of lemon verbena were harvested with sterilized scissors. Then the leaves were placed in the open air for 10 min to reduce the plant respiration. The leaves were kept in cotton cloth and plastic at the normal temperature (4°C, 12 h) of the refrigerator. After that, leaves dried in different methodes. The sample drying method had eight levels in 3 replicate for each treatment. Different methods of drying samples based on reaching 10% moisture are as follows (Fig. 1): Drying in the shade, drying in the sun, drying with an oven at temperatures of 30°C, 40°C, and 50°C, drying by microwave with power of 100 W and 300 W, and drying with a freeze dryer (temperature − 10°C and pressure 2 mm Hg). To determine how moisture is removed from the samples in each of the mentioned methods, the weight of samples was measured at specific time intervals by a precision scale (accuracy 0.001 g), and their mass changes were recorded.

2.2 Evaluation of photosynthetic pigments

The content of photosynthetic pigments (chlorophyll *a*, chlorophyll b, total chlorophyll, and carotenoids) was measured according to Arnon's method with a slight change. The dried leaves (0.5 g) were finely crushed by liquid nitrogen. Then, it was grinded with 10 ml of 80% acetone. The light absorption in the extract was read at 663, 645, and 470 nm with a UV-visible spectrophotometer (T-60, PG Instrument, UK). The content of pigments was reported in mg g^{-1} dry weight [27].

Chorophyll a= (12.25 ×A_{663}-2.798) ($\frac{V}{W\times\ 1000}$)

Chorophyll b= (21.50×A_{645}-5.10) ($\frac{V}{W\times\ 1000}$)

Total Chl =)7.15 A_{663} + 18.71 A_{645})× ($\frac{V}{W\times\ 1000}$)

C= (1000 A_{470} – 1.82 Chl a -85.02 Chl b)/ 198 × ($\frac{V}{W\times\ 1000}$)

In these formula A:the absorbance with a UV-visible spectrophotometer, V: last volum of extract, W: the weight of plant sample, Chorophyll a: the amount of Chorophyll a, Chorophyll b: the amount of Chorophyll b, Total Chl: the amount of total Chorophyll, C: the amount of cartenoeid.

2.3 Measurement of total phenol, total flavonoid, and antioxidant activity

First, the dried leaves were powdered using an electric grinder. Then methanolic extract from dry samples was prepared by cold maceration method with a ratio of 1:10 w/v sample and 70% methanol solvent. The samples were kept for 48 h in the solvent and on the shaker (120 rpm) at room temperature and in the dark. After that, the extract was filtered by a Whatman paper NO.1, and the resulting extract was used to measure total flavonoid, phenol, and antioxidant activity [28].

To measure the total phenolic content based on the Folin-Ciocalteu method [29]. About 200 µl of methanolic extract was mixed with 400 µl of Folin-Ciocalteu reagent (diluted with distilled water in a 1:10 ratio) and 400 µl of 7% sodium carbonate solution. After incubating the resulting mixture at room temperature for 30 min, the absorbance was read at 765 nm using a UV-visible spectrophotometer. Finally, the total phenolic content was calculated as mg gallic acid per g of dry weight using an equation obtained from the calibration curve of gallic acid at different concentration: 10, 50, 150, and 200 mg mL^{-1}. The calibration curve formul was Y = 0.0192 X + 0.04311, R^2 = 0.7924

A colorimetric method of aluminum chloride measured the total flavonoid content of methanolic extract. Methanolic extract (500 µL) was mixed with 10% aluminum chloride solution (100 µL), 1 M potassium acetate (100 µL), and distilled water (2.8 mL). The resulting mixture was incubated at room temperature for 40 min. Then, the absorbance of the reaction mixture was read at 415 nm using a UV-visible spectrophotometer. The standard curve was drawn based on quercetin solution with different concentrations (50, 150, 450, and 550 mg mL^{-1}). Flavonoid content was reported in mg quercetin per g of dry weight [30]. The calibration curve formul was Y = 2.1647x − 2.8775, R^2 =0.9825.

Antioxidant activity was determined using a free radical scavenging assay described by Barros et al. [31]. This method used 2,2-Diphenyl-1-picrylhydrazyl (DPPH) as a free radical. Methanolic extract (250 µL) was added to 750 µL of DPPH solution (2 mg of DPPH dissolved in 50 mL of pure methanol). The resulting mixture was incubated in the dark and at room temperature for 30 min. After that, the absorbance of the reaction mixture was read at 517 nm using a UV-visible spectrophotometer. Finally, the inhibition percentage of free radicals was obtained with the following formula:

The percentage of DPPH free radical scavenging = $(A_c-A_s) / Ac \times 100$

Where in A_c and A_s are the absorbance of the control and the absorbance of the sample, respectively.

2.4 Identification of essential oil constituents

The dried samples were powdered using an electric grinder. Then, the essential oil was extracted by water distillation method by the Clevenger type device for 3 h. The obtained essential oil was stored in opaque glass in a refrigerator at 4°C temperature, until the essential oil components were identified.

The essential oil's compounds were identified using gas chromatography-mass spectrometry (GC-MS) analysis. The compounds were analyzed on a 6890 N Agilent gas chromatograph coupled with a 5975 C Agilent mass-selective detector (Agilent Technologies, Avondale, PA, USA). A 7683 Agilent autosampler and 2 µL of the essential oil were injected in split less mode at 250^{o}C into a 30 m × 0.25 mm × 0.5 µm DB-5 MS capillary column and were operated by MSD Chemstation Software (Agilent Technologies). The temperature program used for the chromatographic separation was as follows: 50^{o}C for 2 min, temperature increase at 25^{o}C/min to 100^{o}C and hold for 2 min, then temperature increased at 5^{o}C min^{-1} to 290^{o}C where it was finally held for 5 min. The carrier gas was helium (99.99%) and was kept at a constant flux of 1.0 ml /min. The mass spectrometer operated in electronic impact ionization mode and the energy of the electrons was kept at 70 eV. The interface was kept at 290^{o}C. The mass spectrums were obtained at a mass ratio scan range from 100 to 400 m/z to determine the appropriate mass.

2.5 Investigation of antimicrobial effects

The biological effect of lemon verbena extracts dried by different methods on the growth of foodborne pathogenic bacteria including two Gram-negative bacteria, *Escherichia coli* (PTCC 1533) and *Pseudomonas aeruginosa* (ISIRI 275) and four Gram-positive bacteria, *Streptococcus pyogenes* (ATCC® 19615™), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (PTCC 1112), and *Bacillus cereus* (ATCC 1177) was investigated by the disc diffusion method [32]. The antimicrobial susceptibility test was performed using the standard disc diffusion method on the Mueller-Hinton agar medium. A digital caliper measured the diameter of the non-growth halo and the results in mm were reported according to the CLSI standard [33]. All studied bacteria did a kill time study following exposure to the tested essential oils. It was conducted with the MIC value obtained formerly. The enzyme-linked immunosorbent assay or ELISA reader method (ELISA approach, model: Bio-Tek Instruments) was used [33]. Initially, bacterial cultures were prepared at a concentration of 10^6 CFU/mL in Mueller-Hinton broth. Subsequently, essential oils at their respective MIC concentrations were added to the bacterial cultures. At predetermined time intervals (24 hours), samples were collected from the bacterial suspensions. To determine the survival of viable bacterial cells, the dilution and plating method on agar medium was used to enumerate the Colony Forming Units per milliliter (CFU/mL) at each time point. For this assay, 10 microliters of the kit's reagent were added to 90 microliters of each bacterial suspension sample and incubated for 48 hours at 37°C. Optical density (OD) was read at 450 nm wavelength by using an ELISA reader aproach. A decrease in absorbance over time indicated a reduction in metabolic activity and, consequently, the killing effect of the essential oils. Survival curves were plotted based on both the CFU counts and the ELISA absorbance changes to determine the time required for a significant reduction in bacterial population, which was defined as the kill time. As a result, the MIC (Minimum Inhibitory Concentration) was determined (the lowest concentration of essential oil, that inhibits the visible growth of bacteria) [33].

2.6 Statistical Analysis

The data obtained from this experiment were analyzed as One-way ANOVA analysis with SPSS software (version 16). The factor was eight levels of the method of drying samples. The mean values were compared with the LSD test ($P \leq 0.05$).

3 Results

3.1 Photosynthetic pigments

The analysis of variance showed that the simple effects of sample drying methods were significant in the content of photosynthetic pigments (Table 1). Mean comparison indicated that the highest content of chlorophyll *a* was obtained in samples dried in the oven at 30°C (16.71 mg g^{-1} D.W.). The lowest chlorophyll *a* content was related to the leaves dried in the freeze dryer with an average of 6.46 mg g^{-1} D.W. (Fig. 2A).

Table 1
Analysis of variance of the drying method on photosynthetic pigments, total phenol, total flavonoids, and Antioxidant activity in Lemon verbena.

Source of variation	Mean of squares							
	df	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Carotenoid	Total phenol	Total flavonoid	Antioxidant activity
Sample drying method	7	**11.25	*19.96	**21.77	*8.27	259771.56*	58955.22*	959.09**
Error	48	5.47	2.7	15.41	4.454	33.013	87.11	240.19
Coefficient of variation (%)		24.48	12.6	19.25	18.41	4.26	5.88	22.57

*, $P \leq 0.05$; **, $P \leq 0.01$.

The most chlorophyll b content was observed in the samples dried in the shade (8.14 mg g^{-1} D.W.), which did not significantly differ from the freeze dryer drying method (samples with values of 8.06 and 8.05 mg g^{-1} D.W., respectively). The lowest amount of chlorophyll b (0.773 mg g^{-1} D.W.) was related to the samples dried in the freeze dryer, which was not significantly different from the samples dried in the oven at 50°C with an average of 1.195 mg g^{-1} (Fig. 2B).

The greatest content of total chlorophyll was obtained in leave samples dried in the oven at 30°C (average 21.23 mg g^{-1} D.W.). The lowest amount of total chlorophyll (average 7.23 mg g^{-1} D.W.) was related to the samples dried in the freeze dryer (Fig. 3A).

The sample drying method affected carotenoid content. The highest carotenoid content was observed in samples dried in the freeze dryer (average 4.8 mg g^{-1} D.W.), which did not have a significant difference with the samples dried in the oven at 40°C and 50°C (with values of 4.78 and 4.6 mg g^{-1} D.W., respectively). The lowest carotenoid content (0.79 mg g^{-1} D.W.) was related to the samples dried in the Shade, which was not significantly different from those dried in the microwave 100 W (Fig. 3B).

3.2 Total phenol, total flavonoid, and antioxidant activity

The analysis of variance showed that the simple effects of the sample drying method were significant in the total phenol, total flavonoid, and antioxidant activity (Table 1). Mean comparison indicated that the highest total phenol content was obtained in the samples dried in the microwave at 300 W (average 974.53 mg Gallic acid g^{-1}D.W.). The samples dried in the

sun (72.03 mg Gallic acid g^{-1} D.W.) had the lowest phenol content, which was not significantly different from the samples dried in exposed to sunlight (Fig. 4A).

The drying methods affected the total flavonoid content. The samples dried in the freeze dryer had higher total flavonoid content (366.24 mg Quercetin g^{-1}D.W.). The lowest flavonoid content (17.04 mg Quercetin g^{-1}D.W.) was related to the samples dried in the sun (Fig. 4B).

The mean comparison for the drying method showed that the lowest antioxidant activity was obtained in samples that were exposed to sun light (48.23%). The samples dried in the microwave at 100 W (97.22%) had the greatest antioxidant activity (Fig. 4C).

3.3 Essential oil phytochemical analysis

Phytochemical analysis of the essential oil obtained from the samples dried in the shade condition was performed by Gas chromatography-mass spectrometry (GC–MS) (Fig. 5A). The compounds identified in the essential oil of samples dried in the shade are reported in Table 2. In this essential oil, 29 bioactive compounds were identified that accounted for 71.45% of the total essential oil content. As shown in Table 2, the most important compounds were: citral (11.05%), linalool (10.41%), trans limonene oxide (7.36%), germacrene D (7.33%), D-limonene (6.88%), trans-chrysanthemal (5.48%), eucalyptol (5.34%), alpha-terpineol (2.19%), caryophyllene (1.96%), o-cymene (1.71%), levomenthol (1.62%), and tau-cadinol (1.42%).

Table 2
Components of essential oil obtained from Lemon verbena dried in shade.

No.	Compound	Retention time	Compound amount (%)
1	beta.-Phellandrene	9.83	0.52
2	o-Cymene	10.62	1.71
3	D-Limonene	10.69	6.88
4	Eucalyptol	10.76	5.34
5	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	11.28	0.8
6	3-Methyl-2-(2-methyl-2-butenyl)-furan	11.61	0.13
7	Linalool	11.65	10.41
8	Furan, 3-(4-methyl-3-pentenyl)	11.70	0.12
9	cis-p-Mentha-2,8-dien-1-ol	12.25	0.43
10	trans Limonene oxide	12.3	7.36
11	Isoneral	12.33	0.12
12	trans-Chrysanthemal	12.47	5.48
13	2-((3,3-Dimethyloxiran-2-yl)methyl)-3-methylfuran	12.70	1.26
14	Levomenthol	12.77	1.62
15	alpha.-Terpineol	13.032	2.19
16	Santolina triene	13.121	0.2
17	cis-Isopiperitenol	13.166	0.28
18	Carvone	13.73	0.47
19	Citral	13.94	11.05
20	1-Pentene, 4,4-dimethyl-1,3-diphenyl-1-(trimethylsilyloxy)-	14.10	1.9
21	alfa.-Copaene	15.39	0.7
22	beta.-Bourbonene	15.53	0.9
23	Caryophyllene	15.96	1.96
24	Germacrene D	16.60	7.33
25	alpha.-Muurolene	17.13	0.12
26	Caryophyllene oxide	17.38	0.33
27	(1S,3aS,4S,5S,7aR,8R)-5-Isopropyl-1,7adimethyloctahydro-1H-1,4-methanoinden-8-ol	17.92	0.18
28	tau.-Cadinol	18.18	1.42
29	tau.-Muurolol	18.33	0.24
Total			71.45

The chromatogram of essential oil obtained from *L. citriodora* dried in exposed to sunlight is shown in Fig. 5B. The compounds identified from this essential oil by the GC–MS are shown in Table 3. As is evident in the results, 25 bioactive compounds have been identified in this essential oil, which accounted for 66.49% of the total essential oil. As shown in Table 3, the most important bioactive compounds of this essential oil were: isogeranial (17.42%), trans-limonene oxide (14.99%), D-limonene (6.51%), geranyl acetate (5.39%), and eucalyptol (2.19%).

Table 3
Components of essential oil obtained from Lemon verbena dried in sun.

No.	Compound	Retention time	Compound amount (%)
1	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	9.40	0.34
2	5-Hepten-2-one, 6-methyl	10.21	0.33
3	D-Limonene	11.38	6.51
4	Eucalyptol	11.47	2.19
5	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1- methylethyl)	12.18	0.49
6	Linalool	12.72	0.5
7	Citral	13.034	0.83
8	cis-p-Mentha-2,8-dien-1-ol	13.56	1.82
9	Limonene oxide - trans	13.63	14.99
10	trans-Chrysanthemal	13.88	0.79
11	Isoneral	14.008	0.33
12	Isogeranial	14.35	17.42
13	Terpinen-4-ol	14.46	1.59
14	alpha.-Terpineol	14.69	1
15	D-Carvone	15.72	1.59
16	Geranyl acetate	17.97	5.39
17	alfa.-Copaene	18.26	1.37
18	beta.-Bourbonene	18.49	1.15
19	Caryophyllene	19.26	1.8
20	Geranyl propionate	19.84	1.67
21	Alloaromadendrene	20.19	0.54
22	Caryophyllene oxide	22.49	1.63
23	Cedrol	23.70	0.36
24	tau.-Cadinol	24.22	0.95
25	tau.-Muurolol	24.49	0.91
Total			66.49

The chromatogram of essential oil extracted from *L. citriodora* dried in the oven at 30°C, 40°C, and 50°C is shown in Fig. 6A, B, and C, respectively. In the essential oil extracted from samples dried in the oven at 30°C, 25 biologically active

compounds were identified, constituting 79.93% of the total essential oil (Table 4). As shown in this table, the most important constituents of this essential oil were caryophyllene oxide (23.02%), caryophyllene (18.36%), D-limonene (10.37%), tau-cadinol (4.74%), eucalyptol (3.96%), and isogeranial (1.5%).

Table 4
Components of essential oil obtained from Lemon verbena dried in the oven at 30°C.

No.	Compound	Retention time	Compound amount (%)
1	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	9.40	0.94
2	5-Hepten-2-one, 6-methyl	10.21	0.55
3	D-Limonene	11.38	10.37
4	Eucalyptol	11.47	3.96
5	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1- methylethyl)	12.18	0.45
6	Linalool	12.72	0.34
7	Citral	13.034	0.33
8	cis-p-Mentha-2,8-dien-1-ol	13.56	0.55
9	Limonene oxide - trans	13.63	0.43
10	trans-Chrysanthemal	13.88	0.61
11	Isoneral	14.008	0.91
12	Isogeranial	14.35	1.5
13	Terpinen-4-ol	14.46	0.24
14	alpha.-Terpineol	14.69	0.53
15	D-Carvone	15.72	0.4
16	Geranyl acetate	17.97	0.56
17	alfa.-Copaene	18.26	0.28
18	beta.-Bourbonene	18.49	3.59
19	Caryophyllene	19.26	18.36
20	Geranyl propionate	19.84	3.63
21	Alloaromadendrene	20.19	1.15
22	Caryophyllene oxide	22.49	23.02
23	Cedrol	23.70	1.03
24	tau.-Cadinol	24.22	4.74
25	tau.-Muurolol	24.49	1.46
Total			79.93

The results of the phytochemical analysis of the essential oil obtained from *L. citriodora* dried in the oven at 40°C are reported in Table 5. In this essential oil, 31 bioactive compounds have been identified that constituted 71.65% of the total essential oil content. As shown in Table 5, the most important components in this essential oil were: caryophyllene (17.1%),

D-carvone (14.93%), D-limonene (10.67%), cedrol (4.64), eucalyptol (4.51%), spathulenol (2.04%), alpha-terpineol (1.74%), alfa-copaene (1.61%), tau-cadinol (1.46%), bergamotol (1.42%), and beta-bourbonene (1.27%).

Table 5
Components of essential oil obtained from Lemon verbena dried in the oven at 40°C.

No.	Compound	Retention time	Compound amount (%)
1	(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	9.40	0.24
2	5-Hepten-2-one, 6-methyl	10.33	0.37
3	o-Cymene	11.29	0.26
4	D-Limonene	11.41	10.67
5	Eucalyptol	11.48	4.51
6	Linalool	12.72	0.38
7	trans-Verbenol	13.04	0.33
8	cis-p-Mentha-2,8-dien-1-ol	13.56	0.35
9	Limonene oxide - trans	13.63	0.42
10	trans-Chrysanthemal	13.88	0.54
11	Isoneral	14.01	0.53
12	2-((3,3-Dimethyloxiran-2-yl) methyl)-3-methylfuran	14.24	0.53
13	Isogeranial	14.35	0.81
14	Carveol	14.63	0.25
15	alpha-Terpineol	14.70	1.74
16	cis-Isopiperitenol	14.88	0.26
17	Geraniol	15.28	0.23
18	D-Carvone	15.75	14.93
19	alfa-Copaene	18.26	1.61
20	(-)-beta-Bourbonene	18.50	1.27
21	Caryophyllene	19.26	17.1
22	Alloaromadendrene	20.19	0.58
23	tau-Cadinol	21.49	1.09
24	Caryophyllene oxide	22.49	0.92
25	Bergamotol	23.36	1.42
26	Cedrol	24.007	4.64
27	tau-Cadinol	24.23	1.46
28	(-)-Spathulenol	24.30	2.04
29	alpha-Cadinol	24.49	1.04
30	Cedrol	24.60	0.23
31	alpha-Atlantone	25.91	0.2
Total			71.65

As seen in Table 6, 34 bioactive compounds were identified in the essential oil obtained from the samples dried in the oven at 50°C, accounting for 77.33% of the total essential oil. As shown in this table, the most important constituents of this essential oil were 2,6-octadienal, 3,7-dimethyl (19.02%), D-carvone (15.39%), D-limonene (10.67%), caryophyllene (4.98%), cedrol (4.77%), eucalyptol (4.51%), and alpha-terpineol (1.74%).

Table 6
Components of essential oil obtained from Lemon verbena dried in the oven at 50°C.

No.	Compound	Retention time	Compound amount (%)
1	(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	9.40	0.24
2	5-Hepten-2-one, 6-methyl	10.33	0.37
3	beta-Myrcene	10.43	0.19
4	Benzene, 1-methyl-3-(1-methylethyl)	11.28	0.26
5	D-Limonene	11.39	10.67
6	Eucalyptol	11.47	4.51
7	Linalool	12.72	0.38
8	trans-Verbenol	13.04	0.33
9	cis-p-Mentha-2,8-dien-1-ol	13.56	0.35
10	Limonene oxide - trans	13.63	0.42
11	trans-Chrysanthemal	13.88	0.54
12	Isogeranial	14.008	0.56
13	Isoneral	14.01	0.53
14	2- (3,3-Dimethyloxiran-2-yl) methyl)-3-methylfuran	14.24	0.53
15	Isogeranial	14.35	0.81
16	Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6- trimethyl-, acetate	14.61	0.4
17	alpha-Terpineol	14.70	1.74
18	cis-Isopiperitenol	14.88	0.26
19	trans-p-Mentha-2,8-dienol	15.19	0.63
20	cis-Isopiperitenol	15.26	0.24
21	Geraniol	15.28	0.23
22	Oxiranecarboxaldehyde, 3-methyl-3-(4-methyl-3-pentenyl(	15.57	0.65
23	D-Carvone	15.75	15.39
24	2,6-Octadienal, 3,7-dimethyl	16.09	19.02
25	alfa-Copaene	18.26	1.61
26	(-)-beta-Bourbonene	18.50	1.32
27	Caryophyllene	19.26	4.98
28	Geranyl propionate	19.84	1.1
29	Alloaromadendrene	20.19	0.58
30	tau-Cadinol	21.49	1.09
31	Caryophyllene oxide	22.49	0.92
32	Bergamotol	23.36	1.42

No.	Compound	Retention time	Compound amount (%)
33	Cedrol	23.42	4.77
34	tau-Cadinol	24.22	1.64
Total			77.33

The essential oil extracted from samples dried with a 100 W microwave (Fig. 7A), a 300 W microwave (Fig. 7B), and a freeze dryer (Fig. 7C) was analyzed by GC–MS. In the essential oil extracted from samples dried in the microwave at 100 W, 34 biologically active compounds were identified, constituting 74.15% of the total essential oil (Table 7). As shown in this table, the most important constituents of this essential oil were 2,6-octadienal, 3,7-dimethyl (20.02%), D-carvone (13.30%), D-limonene (8.65%), caryophyllene (5.08%), cedrol (3.7%), eucalyptol (3.51%), beta-bourbonene (3.32%), and alfa-copaene (2.61%).

Table 7
Components of essential oil obtained from Lemon verbena dried in 100 W microwave.

No.	Compound	Retention time	Compound amount (%)
1	(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	9.43	0.22
2	5-Hepten-2-one, 6-methyl	10.53	0.47
3	beta-Myrcene	10.63	0.12
4	Benzene, 1-methyl-3-(1-methylethyl)	11.38	0.26
5	D-Limonene	11.49	8.65
6	Eucalyptol	11.52	3.51
7	Linalool	12.82	1.38
8	trans-Verbenol	13.14	0.53
9	cis-p-Mentha-2,8-dien-1-ol	13.66	0.55
10	Limonene oxide - trans	13.93	0.52
11	trans-Chrysanthemal	13.89	0.34
12	Isogeranial	14.018	0.36
13	Isoneral	14.21	0.33
14	2- (3,3-Dimethyloxiran-2-yl) methyl)-3-methylfuran	14.34	0.33
15	Isogeranial	14.35	1.11
16	Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6- trimethyl-, acetate	14.71	0.94
17	alpha-Terpineol	14.80	0.74
18	cis-Isopiperitenol	14.98	0.36
19	trans-p-Mentha-2,8-dienol	15.20	0.63
20	cis-Isopiperitenol	15.36	0.44
21	Geraniol	15.38	0.23
22	Oxiranecarboxaldehyde, 3-methyl-3-(4-methyl-3-pentenyl)	15.67	0.55
23	D-Carvone	15.85	13.30
24	2,6-Octadienal, 3,7-dimethyl	16.19	20.02
25	alfa-Copaene	18.36	2.61
26	(-)-beta-Bourbonene	18.60	3.32
27	Caryophyllene	19.36	5.08
28	Geranyl propionate	19.94	1.13
29	Alloaromadendrene	20.29	0.68
30	tau-Cadinol	21.59	1.15
31	Caryophyllene oxide	22.59	0.92
32	Bergamotol	23.65	1.40

No.	Compound	Retention time	Compound amount (%)
33	Cedrol	23.72	3.7
34	tau-Cadinol	24.20	1.60
Total			74.15

The results of the phytochemical analysis of the essential oil obtained from *L. citriodora* dried in the microwave at 300 W are reported in Table 8. In this essential oil, 34 bioactive compounds have been identified that constituted 88.6% of the total essential oil. As shown in Table 8, the most important components in this essential oil were: isoneral (7.72%), linalool (7.33%), citral (6.88%), D-limonene (6.59%), cis-p-mentha-2,8-dien-1-ol (5.76%), o-cymene (5.48%), D-carvone (5.34%), caryophyllene oxide (5.26%), bergamotol (4.37%), eucalyptol (4.06%), aromadendrene oxide (3.48%), limonene oxide, trans (3.45%), epiglobulol (3.14%), trans-chrysanthemal (2.39%), isoneral (1.96%), and santolina triene (2.19%).

Table 8
Components of essential oil obtained from Lemon verbena dried in 300 W microwave.

No.	Compound	Retention time	Compound amount (%)
1	(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	9.40	0.38
2	1-Octen-3-ol	9.78	0.71
3	beta.-Phellandrene	9.83	1.43
4	5-Hepten-2-one, 6-methyl	9.92	0.36
5	o-Cymene	10.62	5.48
6	D-Limonene	10.69	6.59
7	Eucalyptol	10.76	4.06
8	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	11.28	0.96
9	Linalool	11.65	7.33
10	cis-p-Mentha-2,8-dien-1-ol	12.25	5.76
11	Limonene oxide, trans	12.3	3.45
12	Isoneral	12.33	1.96
13	trans-Chrysanthemal	12.49	2.39
14	Levomenthol	12.78	1.05
15	Isoneral	13.01	7.72
16	alpha-Terpineol	13.32	0.16
17	Santolina triene	13.8	2.19
18	trans-p-Mentha-2,8-dienol	14.19	0.26
19	cis-Isopiperitenol	14.26	0.79
20	Geraniol	15.28	1.26
21	Oxiranecarboxaldehyde, 3-methyl-3-(4-methyl-3-pentenyl)	15.57	0.25
22	D-Carvone	15.73	5.34
23	Citral	15.94	6.88
24	1-Pentene, 4,4-dimethyl-1,3-diphenyl-1))trimethylsilyloxy	16.09	0.22
25	alfa-Copaene	18.26	1.48
26	(-)-beta-Bourbonene	18.50	0.42
27	Caryophyllene	19.26	1.71
28	Geranyl propionate	19.84	0.23
29	Alloaromadendrene	20.19	0.56
30	tau-Cadinol	21.49	0.61
31	Caryophyllene oxide	22.49	5.26
32	Bergamotol	23.36	4.37

No.	Compound	Retention time	Compound amount (%)
33	Aromadendrene oxide	23.42	3.48
34	Epiglobulol	24.22	3.14
Total			88.6

As seen in Table 9, 34 bioactive compounds were identified in the essential oil obtained from the samples dried in the freeze dryer, accounting for 82.31% of the total essential oil. As shown in this table, the most important constituents of this essential oil were 2,6-octadienal, 3,7-dimethyl (16.32%), D-carvone (14.16%), caryophyllene oxide (10.26%), D-limonene (8.37%), bergamotol (7.32%), caryophyllene (5.4%), eucalyptol (3.01%), aromadendrene oxide (2.48%), alpha-terpineol (1.82%), tau-cadinol (1.61%), and alfa-copaene (1.48%).

Table 9
Components of essential oil obtained from Lemon verbena dried in freeze dryer.

No.	Compound	Retention time	Compound amount (%)
1	(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	9.40	0.17
2	5-Hepten-2-one, 6-methyl	10.33	0.07
3	beta-Myrcene	10.43	0.44
4	Benzene, 1-methyl-3-(1-methylethyl)	11.28	0.2
5	D-Limonene	11.39	8.37
6	Eucalyptol	11.47	3.01
7	Linalool	12.72	0.17
8	trans-Verbenol	13.04	0.66
9	cis-p-Mentha-2,8-dien-1-ol	13.56	0.41
10	Limonene oxide - trans	13.63	0.22
11	trans-Chrysanthemal	13.88	0.19
12	Isogeranial	14.008	0.46
13	Isoneral	14.01	0.46
14	2- (3,3-Dimethyloxiran-2-yl) methyl)-3-methylfuran	14.24	0.54
15	Isogeranial	14.35	0.72
16	Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6- trimethyl-, acetate	14.61	0.81
17	alpha-Terpineol	14.70	1.82
18	cis-Isopiperitenol	14.88	0.20
19	trans-p-Mentha-2,8-dienol	15.19	0.26
20	cis-Isopiperitenol	15.26	0.79
21	Geraniol	15.28	0.44
22	Oxiranecarboxaldehyde, 3-methyl-3-(4-methyl-3-pentenyl)	15.57	0.65
23	D-Carvone	15.75	14.16
24	2,6-Octadienal, 3,7-dimethyl	16.09	16.32
25	alfa-Copaene	18.26	1.48
26	(-)-beta-Bourbonene	18.50	0.42
27	Caryophyllene	19.26	5.4
28	Geranyl propionate	19.84	0.21
29	Alloaromadendrene	20.19	0.46
30	tau-Cadinol	21.49	1.61
31	Caryophyllene oxide	22.49	10.26
32	Bergamotol	23.36	7.32

No.	Compound	Retention time	Compound amount (%)
33	Aromadendrene oxide	23.42	2.48
34	Epiglobulol	24.22	1.14
Total			82.31

The results of the present experiment showed that, drying conditions (temperature, air flow intensity, device power, etc.) affect the presence or absence of some compounds, as well as their amount. For example, the D-limonene and Eucalyptol was found in all drying methods, with the highest amount in the oven drying method and the lowest amount in the sun drying method. On the other hand, the Isogeraniol compound, which had a considerable amount in the sun-dried sample (17.42%), was found at about 1% in other methods, and was not found at all in the two shadow and microwave methods (300 W). Other comparisons can be understood by considering Table 10. The use of innovative, new or existing drying technologies in preserving the active compounds was included in this paper. It was found that (i) no single drying method can be effectively used for the dehydration of all herbs; (ii) heat treatment can lead to biochemical changes (Maillard reaction), which increases the antibacterial activity; and (iii) innovative combined drying methods are promising in the production of herbs with high antioxidant activity and higher yields of total volatile concentrations.

Table 10
Components of essential oil obtained from Lemon verbena in different drying methods.

No.	Compound	sun	shade	300 W microwave	100 W microwave	Freeze dryer	Oven 30	Oven 40	Oven50
1	(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	0.34	-	0.38	0.22	0.17	0.94	0.24	0.24
2	1-Octen-3-ol	0.33	-	0.71	-	-	-	-	
3	5-Hepten-2-one, 6-methyl	-	0.8	-	0.47		0.55	0.37	0.37
4	Bicyclo [3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)	0.49	-	-	-	-	0.45		-
5	beta.-Phellandrene	-	0.52	1.43	-	-	-	-	
6	beta-Myrcene	-	-	-	0.12	0.44	-	-	0.19
7	5-Hepten-2-one, 6-methyl	-	-	0.36	-	-	-	-	-
8	Benzene, 1-methyl-3-(1-methylethyl)	-	0.12	-	0.26	-	-	-	-
9	O-Cymene	-	1.71	5.48	-	-	-	0.26	-
10	D-Limonene	6.51	6.88	6.59	8.65	8.37	10.37	10.67	10.67
11	Eucalyptol	2.19	5.34	4.06	3.51	3.01	3.96	4.51	4.51
12	Limonene oxide - trans	14.99	-	-			-	0.42	
13	trans-Verbenol	-	-	-	-	0.66	-	0.33	0.33
14	Isopropyl-2-5-methylbicyclo[3.1.0]hexan-2-ol	-	-	0.96	-	-	-	-	-
15	trans-Verbenol	-	-	-	0.53	-	0.34	-	-
16	Linalool	0.5	10.41	7.33	1.38	0.17	-	0.38	0.38
17	cis-p-Mentha-2,8-dien-1-ol	1.82	0.43	5.76	0.55	0.41	0.55	0.35	0.35
18	Limonene oxide, trans	-	7.36	3.45	0.52	0.22	0.43	-	0.42
19	Terpinen-4-ol	1.59	-	-	-	-	0.24	-	-
20	alpha.-Terpineol	1	-	-	-	-	0.53	-	-
21	Isogeranial	17.42	-	-	1.11	0.72	1.5	0.81	0.81
22	Isoneral Cis-	0.33	-	1.96	0.36	-	0.91	0.53	-
23	trans-Chrysanthemal	0.79	5.48	2.39	0.34	0.19	0.61	0.54	0.54
24	2- (3,3-Dimethyloxiran-2-yl-methyl)-3-methylfuran	-	1.26	-	0.33	0.54	-	0.53	0.53
25	Levomenthol	-	1.62	1.05	-	-	-	-	-
26	Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6- trimethyl- acetate	-	1.9	-	0.94	0.81	-	-	0.4
27	Isoneral	-	0.12	7.72	0.33	0.46	-	-	0.53
28	trans-p-Mentha-2,8-dienol	-	-	-	0.63	0.26	-	-	-

No.	Compound	sun	shade	300 W microwave	100 W microwave	Freeze dryer	Oven 30	Oven 40	Oven50
29	alpha-Terpineol		2.19	0.16	0.74	1.82	-	1.74	1.74
30	Santolina triene	-	0.2	2.19	-	-	-	-	-
31	trans-p-Mentha-2,8-dienol	-	-	0.26	-	-	-	-	0.63
32	cis-Isopiperitenol	-	0.28	0.79	0.44	0.79	-	0.26	0.24
33	Geraniol	-	-	1.26	0.23	0.44	-	0.23	0.23
34	Oxiranecarboxaldehyde, 3-methyl-3-(4-methyl-3-pentenyl	-	0.13	0.25	0.55	0.65	-	-	0.65
35	D-Carvone	1.59	0.47	5.34	13.30	14.16	0.4	14.93	15.39
36	Carveol	-	-	-	-	-	-	0.25	-
37	Citral	0.83	11.05	6.88	-	-	0.33	-	-
38	1-Pentene, 4,4-dimethyl-1,3-diphenyl-1))trimethylsilyloxy	-	-	0.22	-	-	-	-	-
39	2,6-Octadienal, 3,7-dimethyl	-	0.18	-	20.02	16.32	-	-	19.02
40	alfa-Copaene	1.37	0.7	1.48	2.61	1.48	0.28	1.61	1.61
41	(-)-beta-Bourbonene	1.15	0.9	0.42	3.32	0.42	3.59	1.27	1.32
42	Caryophyllene	1.8	1.96	1.71	5.08	5.4	18.36	17.1	4.98
43	Germacrene D	-	7.33	-	-	-	-	-	-
44	Geranyl acetate	5.39	-	-	-	-	0.56	-	-
45	Geranyl propionate	1.67	-	0.23	1.13	0.21	3.63	-	1.1
46	Alloaromadendrene	-	-	0.56	0.68	0.46	1.15	0.58	0.58
47	tau-Cadinol	-	1.42	0.61	1.15	1.61	-	-	1.09
48	Caryophyllene oxide	1.63	0.33	5.26	0.92	10.26	23.02	0.92	0.92
49	Bergamotol	-	-	4.37	1.40	7.32	-	1.42	1.42
50	Aromadendrene oxide	0.54	-	3.48	-	2.48	-	-	-
51	Epiglobulol	-	-	3.14	-	1.14	-	-	-
52	Cedrol	0.36	-	-	3.7	-	1.03	4.64	4.77
53	alpha-Cadinol	-	-	-	-	-	-	1.04	
54	tau-Cadinol	0.95	-	-	1.60	-	4.74	1.46 + 1.09	1.64
55	(-)-Spathulenol	-	-		-	-	-	2.04	-
56	tau.-Muurolol	0.91	0.24	-	-	-	1.46	-	-
57	alpha-Atlantone	-	-	-	-	-	-	0.2	-

3.4 Antimicrobial effects of lemon verbena essential oil

The analysis of variance indicated that different essential oils of lemon verbena have a significant effect on the inhibition of bacterial growth. The results of the antimicrobial activity of essential oil extracted from lemon verbena dried by different methods via measuring the growth inhibition halo diameter are reported in Table 11. The largest diameter of the non-growth halo was obtained from *S. pyogenes* bacteria as a result of treatment with essential oil extracted from lemon verbena dried in the oven at 30°C. Also, the essential oil extracted from lemon verbena dried in the sun had no inhibitory effect on *S. aureus* (Table 11).

Table 11
Inhibitory halo diameter (mean ± SD mm) of six bacterial strains for different lemon verbena essential oils.

Tested bacteria	Shade	Sun	Oven 30°C	Oven 40°C	Oven 50°C	Microwave 100 W	Microwave 300 W	Freeze dryer
Bacillus cereus	11 ± 1	30 ± 1.56	63 ± 2.08	30 ± 1.26	51 ± 0.61	42 ± 0.85	43 ± 1.70	10 ± 1.04
Escherichia coli	11 ± 0.45	25 ± 1.35	19 ± 1.45	35 ± 0.94	28 ± 1.71	29 ± 0.86	31 ± 0.64	22 ± 1.25
Pseudomonas aeruginosa	13 ± 0.66	11 ± 0.6	51 ± 0.7	10 ± 1.09	18 ± 0.55	29 ± 1.04	33 ± 1.80	12 ± 0.94
Streptococcus pyogenes	20 ± 0.97	50 ± 1.86	73 ± 1.52	40 ± 1.3	28 ± 0.81	19 ± 0.86	21 ± 0.78	12 ± 0.86
Enterococcus faecalis	11 ± 0.65	13 ± 1.27	5 ± 0.65	10 ± 1.15	39 ± 0.86	18 ± 0.98	38 ± 1.34	21 ± 0.90
Staphylococcus aureus	10 ± 0.65	0 ± 0.5	15 ± 0.88	3 ± 0.72	21 ± 0.77	21 ± 1.26	10 ± 0.97	10 ± 1.16

The results of the antibacterial test in different concentrations showed that the growth rate of the studied bacteria decreases with increasing the concentration of essential oil obtained from lemon verbena dried by different methods (Table 12). The antibacterial test results of essential oil extracted from lemon verbena dried in the shade showed that, at the 100 ppm concentration, the lowest optical density (OD) rate (0.235) was related to *P. aeruginosa* and the highest optical density (0.261) was for the *B. cereus*. At the 3.1 ppm concentration, the maximum optical density rate of bacteria was equal to 0.997, which was related to *S. pyogenes* bacteria (Table 12).

Table 12
Minimal Inhibition Concentration of essential oil extracted from Lemon verbena dried by different methods against several bacteria tested.

Essential oil obtained from different drying methods		**Essential oil concentration (ppm)**					
	Tested bacteria	**3.1**	**6.25**	**12.5**	**25**	**50**	**100**
Shade	*Bacillus cereus*	0.383	0.370	0.331	0.296	0.265	0.261
	Escherichia coli	0.548	0.449	0.334	0.294	0.291	0.237
	Pseudomonas aeruginosa	0.500	0.493	0.444	0.332	0.292	0.235
	Streptococcus pyogenes	0.997	0.509	0.405	0.289	0.263	0.246
	Enterococcus faecalis	0.518	0.456	0.423	0.281	0.276	0.236
	Staphylococcus aureus	0.492	0.456	0.403	0.272	0.270	0.243
Sun	*Bacillus cereus*	0.345	0.326	0.321	0.289	0.261	0.213
	Escherichia coli	0.361	0.354	0.321	0.259	0.241	0.189
	Pseudomonas aeruginosa	0.398	0.367	0.351	0.289	0.245	0.213
	Streptococcus pyogenes	0.456	0.421	0.356	0.321	0.269	0.231
	Enterococcus faecalis	0.498	0.465	0.435	0.415	0.365	0.321
	Staphylococcus aureus	0.512	0.489	0.453	0.421	0.389	0.356
Oven 30°C	*Bacillus cereus*	0.363	0.311	0.306	0.254	0.241	0.212
	Escherichia coli	0.365	0.321	0.272	0.254	0.226	0.221
	Pseudomonas aeruginosa	0.423	0.411	0.406	0.326	0.241	0.233
	Streptococcus pyogenes	0.422	0.394	0.321	0.245	0.212	0.191
	Enterococcus faecalis	0.436	0.335	0.299	0.238	0.206	0.175
	Staphylococcus aureus	0.465	0.363	0.325	0.316	0.223	0.189
Oven 40°C	*Bacillus cereus*	0.363	0.337	0.320	0.263	0.242	0.209
	Escherichia coli	0.442	0.334	0.286	0.277	0.208	0.202
	Pseudomonas aeruginosa	0.400	0.379	0.348	0.243	0.206	0.195
	Streptococcus pyogenes	0.620	0.569	0.528	0.309	0.221	0.165
	Enterococcus faecalis	0.347	0.328	0.322	0.240	0.209	0.190
	Staphylococcus aureus	0.394	0.363	0.298	0.222	0.212	0.192

Essential oil obtained from different drying methods		Essential oil concentration (ppm)					
	Tested bacteria	**3.1**	**6.25**	**12.5**	**25**	**50**	**100**
Oven 50°C	*Bacillus cereus*	0.328	0.302	0.268	0.243	0.203	0.198
	Escherichia coli	0.389	0.346	0.302	0.298	0.229	0.221
	Pseudomonas aeruginosa	0.402	0.389	0.321	0.265	0.225	0.197
	Streptococcus pyogenes	0.398	0.365	0.302	0.268	0.245	0.202
	Enterococcus faecalis	0.369	0.345	0.305	0.289	0.265	0.235
	Staphylococcus aureus	0.406	0.389	0.365	0.321	0.298	0.264
Microwave 100 W	*Bacillus cereus*	0.638	0.617	0.521	0.339	0.296	0.286
	Escherichia coli	0.557	0.552	0.520	0.429	0.346	0.284
	Pseudomonas aeruginosa	0.510	0.456	0.421	0.316	0.305	0.287
	Streptococcus pyogenes	0.502	0.462	0.456	0.390	0.351	0.295
	Enterococcus faecalis	0.635	0.624	0.578	0.348	0.268	0.230
	Staphylococcus aureus	0.645	0.621	0.569	0.340	0.310	0.286
Microwave 300 W	*Bacillus cereus*	0.356	0.321	0.296	0.280	0.246	0.236
	Escherichia coli	0.425	0.369	0.325	0.265	0.235	0.198
	Pseudomonas aeruginosa	0.425	0.369	0.325	0.265	0.236	0.213
	Streptococcus pyogenes	0.428	0.421	0.398	0.368	0.345	0.321
	Enterococcus faecalis	0.369	0.345	0.325	0.256	0.236	0.221
	Staphylococcus aureus	0.458	0.421	0.361	0.321	0.289	0.236
Freeze dryer	*Bacillus cereus*	0.458	0.457	0.281	0.280	0.274	0.247
	Escherichia coli	0.498	0.485	0.460	0.309	0.257	0.231
	Pseudomonas aeruginosa	0.562	0.542	0.423	0.264	0.236	0.226
	Streptococcus pyogenes	1.052	0.976	0.432	0.392	0.278	0.243
	Enterococcus faecalis	0.566	0.538	0.322	0.245	0.225	0.213
	Staphylococcus aureus	0.554	0.473	0.437	0.365	0.293	0.256

The antibacterial test results of essential oil extracted from lemon verbena dried in the sun on the studied bacteria indicated that at the 100 ppm concentration, the highest and lowest optical density rates were equal to 0.356 and 0.189, which were

related to *S. aureus* and *E. coli*, respectively. At the 3.1 ppm concentration, the highest and lowest optical density rates were equal to 0.512 and 0.345, which *S. aureus* and *B. cereus* have grown in this concentration, respectively (Table 12).

The antibacterial activity results of essential oil extracted from samples dried in the oven at 30°C showed that at the concentration of 100 ppm, the highest amount of optical density (0.233) was related to the *P. aeruginosa* strain and the lowest amount of optical density (0.175) was obtained in the *E. faecalis* strain. At the concentration of 3.1 ppm, the highest and lowest amount of optical density rates were equal to 0.465 and 0.363, which were related to *S. aureus* and *B. cereus* bacteria, respectively (Table 12).

The antimicrobial activity results of essential oil extracted from samples dried in the oven at 40°C indicated that, at the 100 ppm concentration, the greatest and lowest optical density rates were equal to 0.209 and 0.165, which were related to *B. cereus* and *S. pyogenes*, respectively. At the 3.1 ppm concentration, the maximum optical density rate was equal to 0.620, which was related to *S. pyogenes* strain (Table 12).

The antibacterial test results of essential oil extracted from lemon verbena dried in the oven at 50°C showed that at the concentration of 100 ppm, the highest and lowest optical density rates were equal to 0.264 and 0.197, which were related to *S. aureus* and *P. aeruginosa*, respectively. At the concentration of 3.1 ppm, the greatest and lowest optical density rates were equal to 0.406 and 0.328, which were related to *S. aureus* and *B. cereus*, respectively (Table 12).

The antibacterial activity results of essential oil extracted from lemon verbena dried in the microwave at 100 W indicated that at the concentration of 100 ppm, the maximum amount of optical density (bacterial growth) was equal to 0.295, which was related to *S. pyogenes* bacteria. At the concentration of 3.1 ppm, the highest and lowest optical density rates were equal to 0.638 and 0.502, which were related to *B. cereus* and *S. pyogenes*, respectively (Table 12).

The antibacterial test results of essential oil extracted from samples dried in the microwave at 300 W showed that at the concentration of 100 ppm, the highest amount of optical density absorption (0.321) was related to the *S. pyogenes* strain and the lowest amount (0.198) was obtained in the *E. coli* strain. At the concentration of 3.1 ppm, the highest and lowest optical density rates were equal to 0.458 and 0.356, which were related to *S. aureus* and *B. cereus*, respectively (Table 12).

The antimicrobial activity results of essential oil extracted from lemon verbena dried in the freeze dryer indicated that at the concentration of 100 ppm, the greatest and lowest optical density rates were equal to 0.256 and 0.213, which were related to *S. aureus* and *E. faecalis*, respectively. At the concentration of 3.1 ppm, the maximum and minimum optical density rates were equal to 1.052 and 0.458, which were related to *S. pyogenes* and *B. cereus*, respectively (Table 12).

4 Discussion

This research aimed to evaluate the effects of various drying techniques on the total phenolic content, total flavonoid content, antioxidant activity, and photosynthetic pigments in lemon verbena. The results indicated that the drying methods led to notable variations in the levels of chlorophyll, carotenoids, total phenols, flavonoids, and antioxidant activity. The post-harvest procedures for medicinal plants play a crucial role in their production, as these processes directly impact the quantity and quality of the active compounds present [3]. The drying processes, particularly those conducted in the shade, in an oven at 30°C, and via microwave at 100 W, resulted in a notable increase in chlorophyll levels in the leaf samples. Conversely, the samples dried in the oven at 50°C exhibited a lower chlorophyll content than the other temperature treatments. The observed phenomenon may be attributed to the heat sensitivity of chlorophyll. Research indicates that chlorophyll exhibits sensitivity to both elevated and reduced temperatures. The preservation of chlorophyll is contingent upon the temperature and the length of exposure during processing. Consequently, a reduction in chlorophyll content was noted in samples subjected to high-temperature oven drying. Therefore, the maximum and minimum levels of chlorophyll were recorded in samples processed through freeze-drying and those dried in an oven at 60°C, respectively [8]. The degradation process of chlorophylls a and b into pheophytin a and b, respectively, adhered to first-order kinetics, with chlorophyll exhibiting greater heat sensitivity than chlorophyll b [35]. The heat treatment process facilitates the substitution

of magnesium in chlorophyll with two hydrogen atoms when mild acids, such as oxalic or acetic acid, are present, resulting in the formation of pheophytin. This degradation is recognized as an indicator of quality loss in plant materials, prompting researchers to explore methods to prevent or reduce chlorophyll degradation [8].

Total phenols, classified as non-enzymatic antioxidants, are secondary metabolites that contribute to inhibiting lipid peroxidation and protein denaturation, as well as the scavenging of reactive oxygen species and the protection against DNA damage. The concentration of phenolic compounds in plants is affected by various factors, including genotype, analysis timing, processing methods, developmental stage, environmental conditions, and storage practices [35]. The primary factors that enhance the biosynthesis of phenolic compounds are elevated temperatures and increased intensity of solar radiation. It has been reported that total phenol content decreased in higher temperatures (80 and 100°C), which agreed with our results about samples dried in the oven at 50°C. Consistent with our findings regarding the elevated phenol levels in samples dried using microwave methods, the samples of *B. tuldoides* and *B. vulgaris* that underwent microwave drying exhibited the highest total phenol content [36]. Consistent with our findings, Singhal et al. [34] observed that the total phenol content in sun-dried *Bambusa vulgaris* shoots was the lowest when compared to dried samples using oven, microwave, and freeze-drying methods. The extended drying duration of the sun-dried samples, which subjected them to atmospheric conditions, led to the degradation of phenolic compounds through oxidation, thereby reducing the overall total phenol content [36]. Furthermore, the drying temperatures influenced the stability of phenolic compounds present in herbal extracts [37].

Flavonoids mitigate photo-oxidative damage resulting from ultraviolet absorption and inhibit the generation of reactive oxygen species by obstructing the enzymes responsible for free radical formation [38]. This alteration likely increased the absorption of ultraviolet light while reducing the production of reactive oxygen species in lemon verbena that was dried using a microwave at 100 W. Prior studies have indicated that elevated irradiance levels lead to an increase in flavonol concentrations [39]. It was noted that the presence of flavonoids was detected in samples dried using all methods, except those dried in a microwave at 100 W and in a freeze dryer. The findings indicate that the concentration of flavonoids in the extracts is influenced by the drying technique [40].

The essential oils derived from medicinal plants represent promising sources for discovering novel antimicrobial agents, particularly effective against bacterial pathogens [41]. The agar disk diffusion technique utilizing the essential oil derived from *Lippia grandis* Schauer demonstrated efficacy against 75% of the bacterial strains examined, with particular effectiveness noted against *S. aureus*, *E. faecalis*, and *E. coli* [33]. The findings of this study demonstrated that essential oils derived from lemon verbena can suppress bacterial proliferation, with their efficacy exhibiting variability, consistent with previous research [24]. This study suggests that a significant presence of lipophilic compounds, including linalool, citral, caryophyllene, β-caryophyllene oxide, and α-terpineol, may be primarily responsible for antibacterial properties. The initial interaction of essential oils with bacterial cells occurs at the membranes, which is closely linked to the hydrophobic nature of the essential oil components. This property enhances these molecules' ability to penetrate the membrane's phospholipid bilayer. Consequently, this destabilizes the plasma membrane's structure and alters its permeability to protons, ions, and other cellular elements [13, 42]. Furthermore, the phenolic constituents present in essential oils impede bacterial proliferation by altering the cell membrane's phospholipid bilayer, which increases its permeability [43]. The antibacterial properties of essential oils appear to stem from their molecular diversity, suggesting that multiple mechanisms work in concert to affect various cellular targets [44]. An essential oil's antimicrobial efficacy is attributed to its diverse constituents' characteristics and composition, which may function synergistically. The influence of minor compounds should not be underestimated, as their interaction with major components can result in a significantly enhanced effect beyond what would be anticipated from the primary constituents alone [33].

Our research findings indicate that the extent of the inhibitory effect of lemon essential oil on bacterial proliferation is influenced by the drying techniques employed. Specifically, when lemon verbena essential oil was dried in an oven at 30°C and applied at a concentration of 100 ppm, the *Pseudomonas aeruginosa* strain exhibited the greatest absorption level, indicative of bacterial growth. This strain is known for its significant intrinsic resistance to a wide range of antibacterial

agents, attributed to its highly selective outer membrane barrier [45]. The varying effects of essential oils on pathogen reduction may be attributed to the distinct compositions found in lemon verbena essential oils. The diverse biological activities essential oils exhibit are likely linked to their specific chemical makeup. Consequently, this biological variability can be partially explained by the differences in their chemical compositions. Also, inconsistencies between the present research and other studies might be due to the plant species utilized, the growth ecosystem (habitat, height, and temperature), and the conditions of experiments (temperature and pH), which were reported by Shariatifar et al. [41]. Several studies have shown that variation in essential oil compositions (due to geographical location, seasonal changes, drying methods, harvesting period, isolation and packaging methods, and essential oil extraction techniques) lead to changes in the biological activity, antimicrobial, and antioxidant properties of the essential oil [24, 46].

In other study fourteen compounds were identified, representing 85.2% of the total essential oil. The 59.90% of total essential oil components are consist of geranial, limonene, neral and 1,8-cineol [47]. In this essential oil, terpenoids are the main secondary metabolites that are commonly produced by different medicinal plants of Lamiaceae family [48]. Monoterpenes and sesquiterpenes, mostly identified from this essential oil, along with a number of triterpenes and their sulfated derivatives [49]. Conversely, the antibacterial properties of essential oils are largely ascribed to terpenoids, such as limonene, which are significant constituents of these oils. D-limonene has effectively inhibited Gram-negative and Gram-positive bacteria, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* [50, 51]. Limonene causes the destruction of the cell integrity and wall structure in bacteria, leads to damage to bacterial cell membranes, has an obvious effect on cell membrane permeability, affects respiration and energy metabolism by inhibiting the function of the respiratory chain complex, and hinders ATP synthesis by inhibiting the activity of the respiratory complex and ATPase. Consequently, this study suggests that the presence of D-limonene in the essential oils derived from various drying methods may contribute to the observed antibacterial properties of these oils, although we faced brief limitations statement to conduct practical experiments in real-world environments because of laboratory conditions.

5 Conclusion

This study presented optimal drying techniques, including total phenolic content, flavonoid levels, antioxidant capacity, chlorophyll concentration, and essential oil yield. It was observed that elevated temperatures resulted in greater degradation of chlorophyll. Overall, freeze drying either enhanced or preserved the levels of flavonoids, carotenoids, and chlorophyll b, whereas microwave drying at 300 W proved to be more effective in increasing the total phenolic content of *lemon verbena*. The phytochemical analysis revealed that the essential oil extracted from lemon verbena, which was dried in a microwave at 300 W, exhibited the highest yield at 88.6% and comprised 34 secondary active compounds. In contrast, the essential oil derived from lemon verbena dried under sunlight demonstrated a lower yield of 66.49% and contained 25 bioactive compounds. The notable antibacterial activity of lemon verbena essential oils obtained from different drying methods had a dose-dependent inhibitory effect on bacterial species. So the growth power of the studied bacteria was significantly decreased by increasing the concentration of essential oil from 1.3 to 100 ppm. The largest diameter of the non-growth halo was related to *S. pyogenes* bacteria as a result of treatment with essential oil extracted from lemon verbena dried in the oven at 30°C. Also, the essential oil extracted from lemon verbena dried in the sun had no inhibitory effect on *S. aureus*. The results obtained in this research indicated that the molecular profile of the essential oil of lemon verbena dried in different methods is variable depending on the type of drying method. D-limonene was one effective compound that was present in the essential oil obtained from all drying methods, which, along with other compounds (linalool, citral, caryophyllene, β-caryophyllene oxide, and α-terpineol), can be a reason for their antibacterial activity. Also, it is feasible that other constituents present at much-reduced concentrations can act synergistically with the main constituents. Overall, it is clear that the essential oil of lemon verbena contains chemical compounds that could be significant in dealing with micro-organisms. In general, our results suggest that the two drying methods, microwave (300 W) and freeze-drying, had the highest concentrations of essential oil and D-limonene (the most important commercial compound) in lemon verbena, respectively. The results of the present study demonstrated for the first time that *Aloysia citrodora* essential oil may prevented the growth of pathogenic microbes even in a variety of tolerance assays, highlighting their potential as preserver

in the food industry. However, further research is required to validate their activity in food systems and provide more insights on their mode of action.

Declarations

Author contributions

A and C: Conceptualization, Methodology, Software, Validation, Supervision, Visualization, Writing original draft. B and E: Formal analysis, Investigation, Resources, Data curation, Project administration, Writing original draft. B and D: Software, Writing, Review and preparation of final version. All authors contributed different sections to this research article. All authors have read and agreed to the published version of the manuscript.

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Data availability

The datasets generated and analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures



Figure 1

Appearance quality of dried *Aloysia citrodora* samples in different methods. (A) Shade, (B) Oven 30 ˚C, (C) Microwave 100, (D) Oven 40 ˚C, (E) Oven 50 ˚C, (F) Freeze dryer, (G) Sun, and (H) Microwave 300.

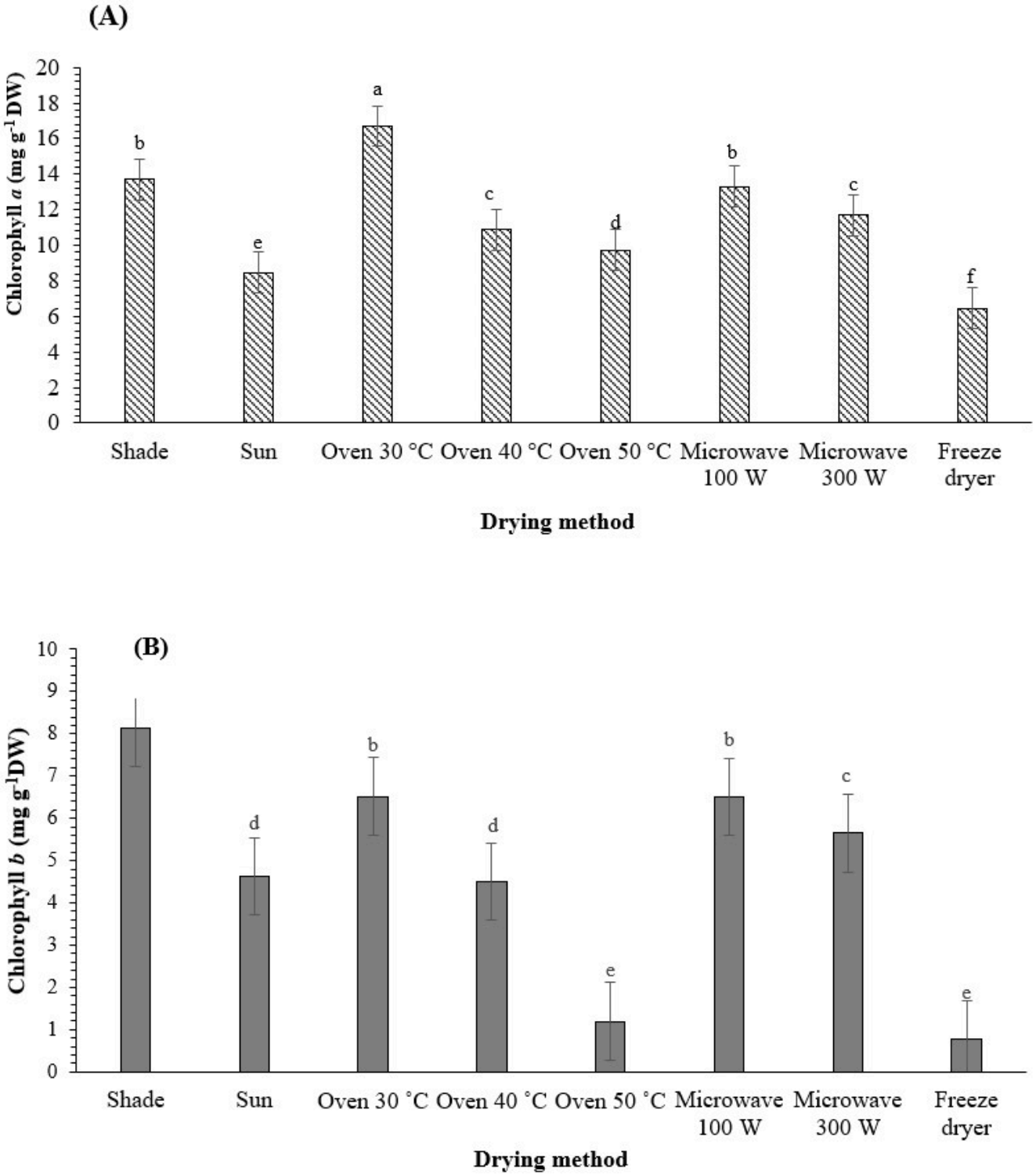


Figure 2

Mean comparison of the interaction of harvest time and drying method on the chlorophyll *a* (A) and chlorophyll *b* (B) in *Aloysia citrodora*. For each parameter, columns with the same letters do not differ significantly (Duncan multiple range test $P \leq 0.05$).

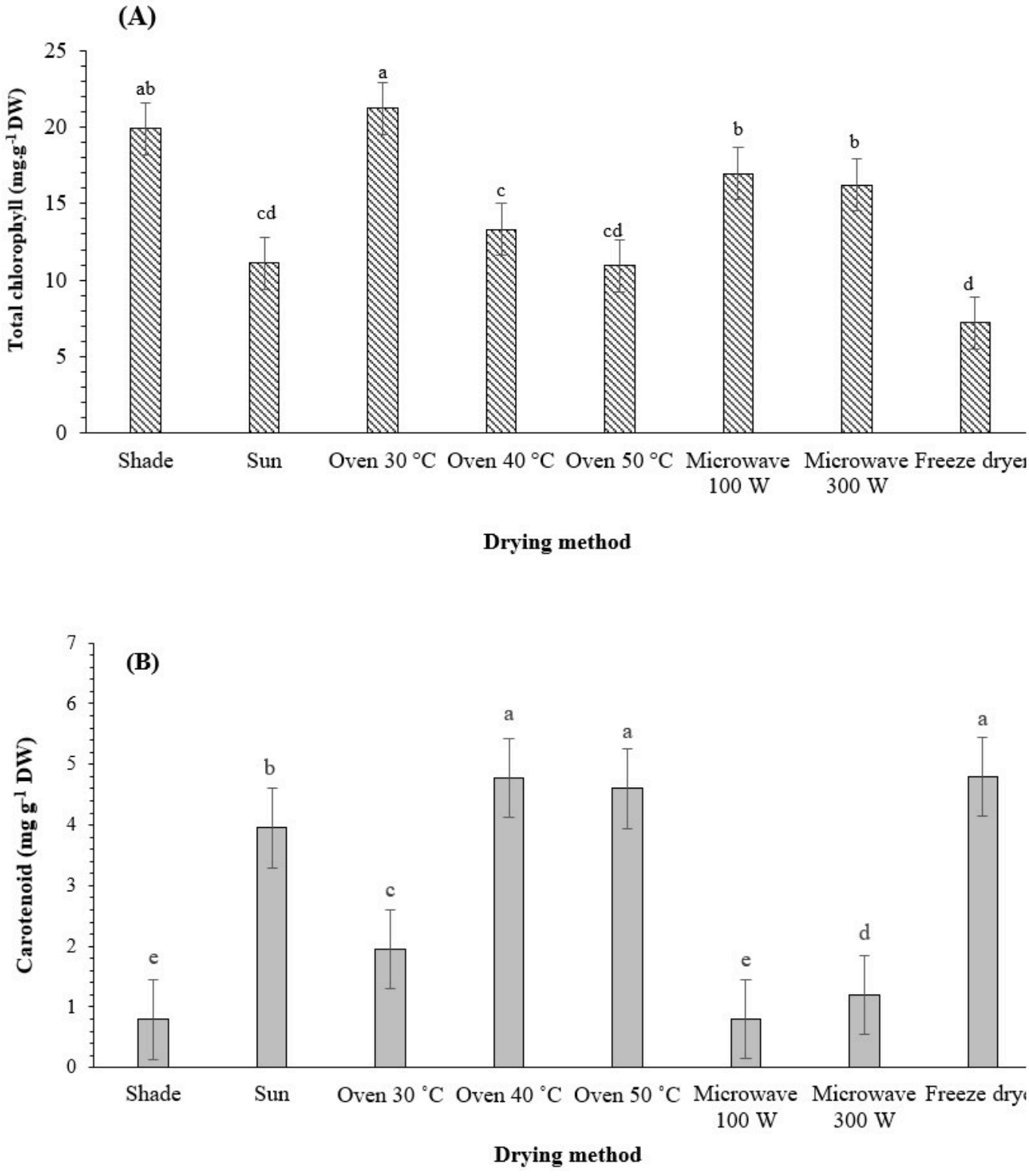


Figure 3

Mean comparison of the interaction of harvest time and drying method on the total chlorophyll (A) and carotenoid (B) in *Aloysia citrodora*. For each parameter, columns with the same letters do not differ significantly (Duncan multiple range test $P \leq 0.05$).

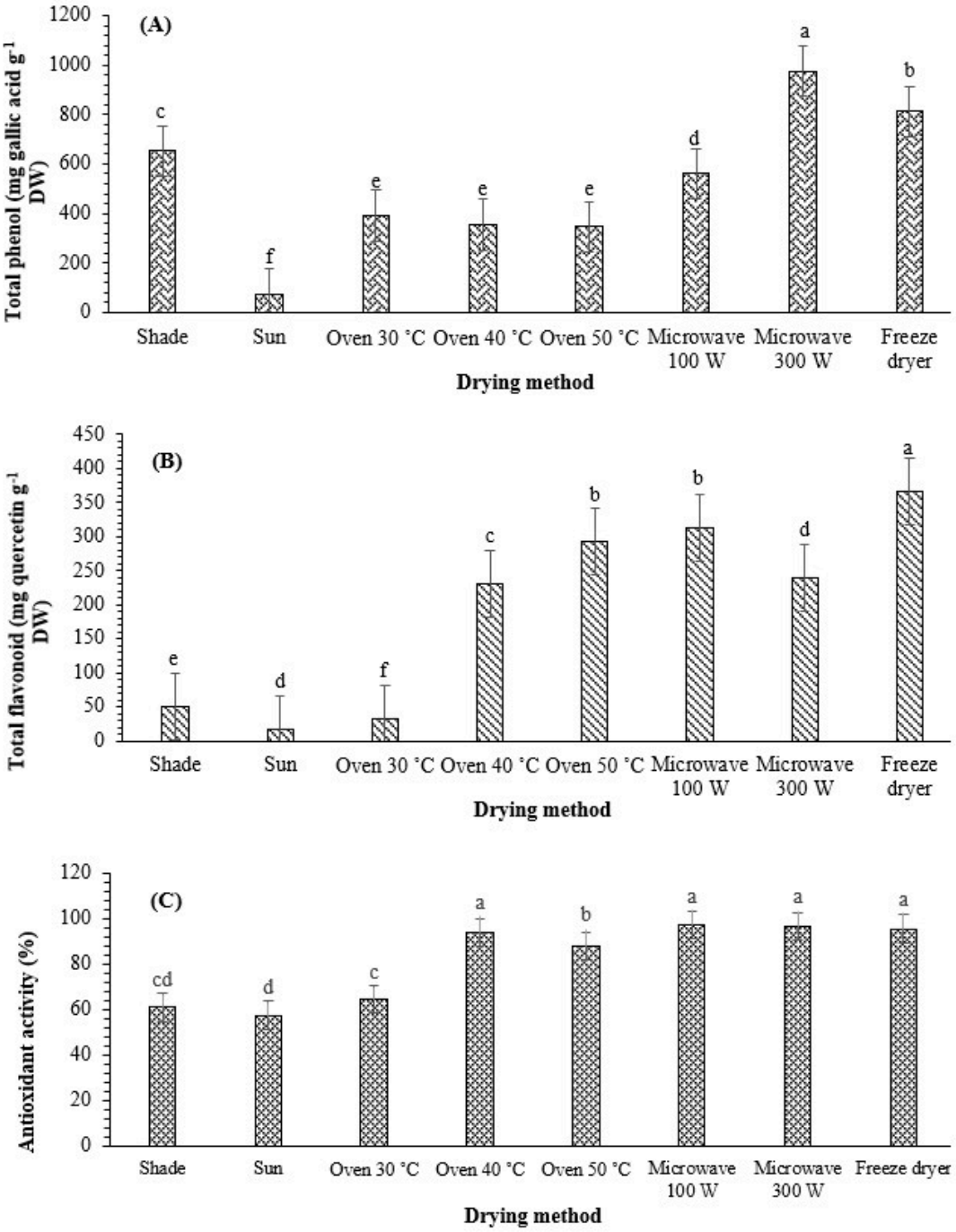


Figure 4

Mean comparison of the interaction of harvest time and drying method on the total phenol (A), total flavonoid (B), and antioxidant activity (C) in *Aloysia citrodora*. For each parameter, columns with the same letters do not differ significantly (Duncan multiple range test $P \leq 0.05$).

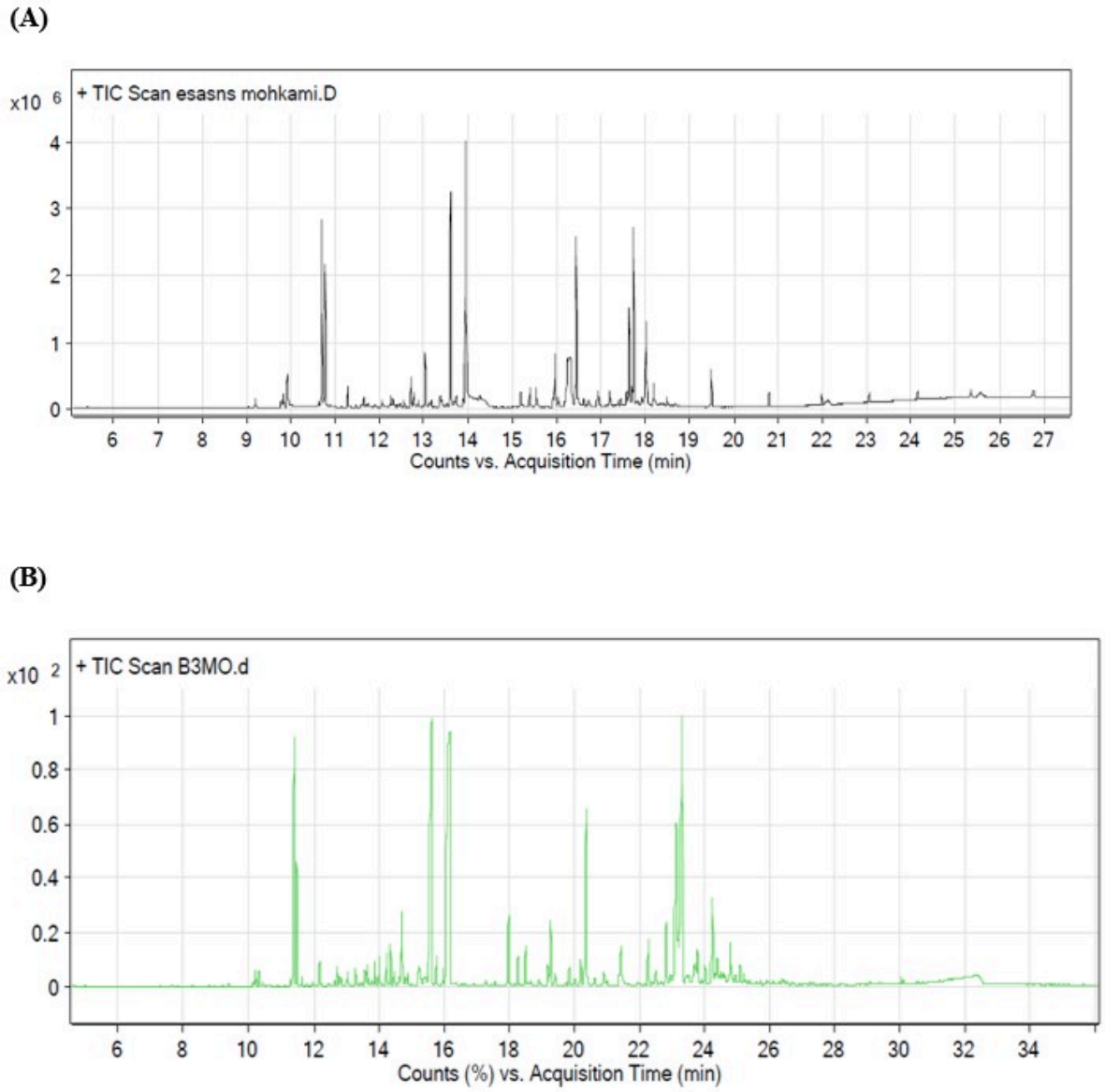


Figure 5

Gas chromatogram of essential oil obtained from *Aloysia citrodora* dried in shade (A) and sun (B).

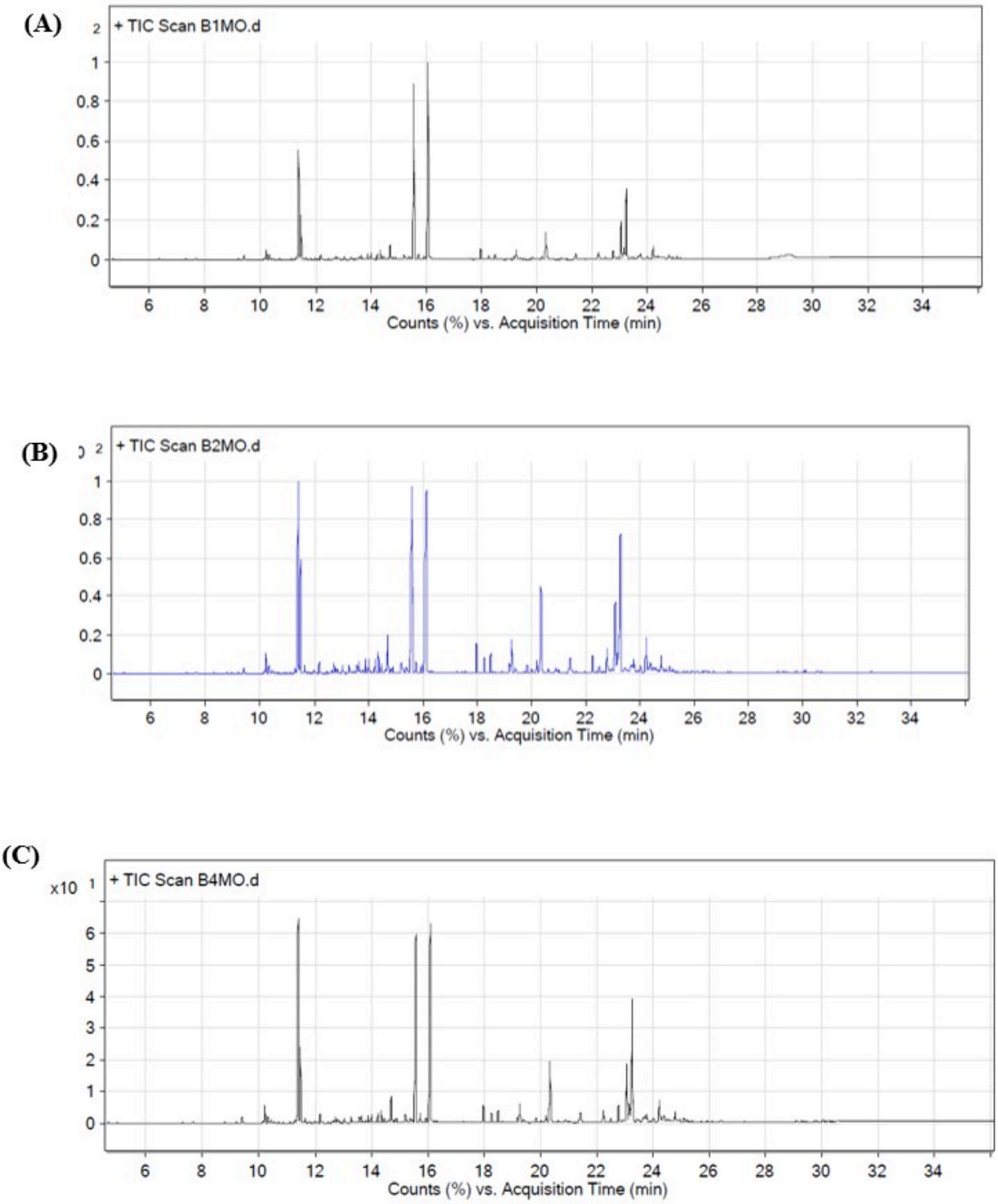


Figure 6

Gas chromatogram of essential oil obtained from *Aloysia citrodora* dried in the oven at 30 °C (A), 40 °C (B), and 50 °C (C).

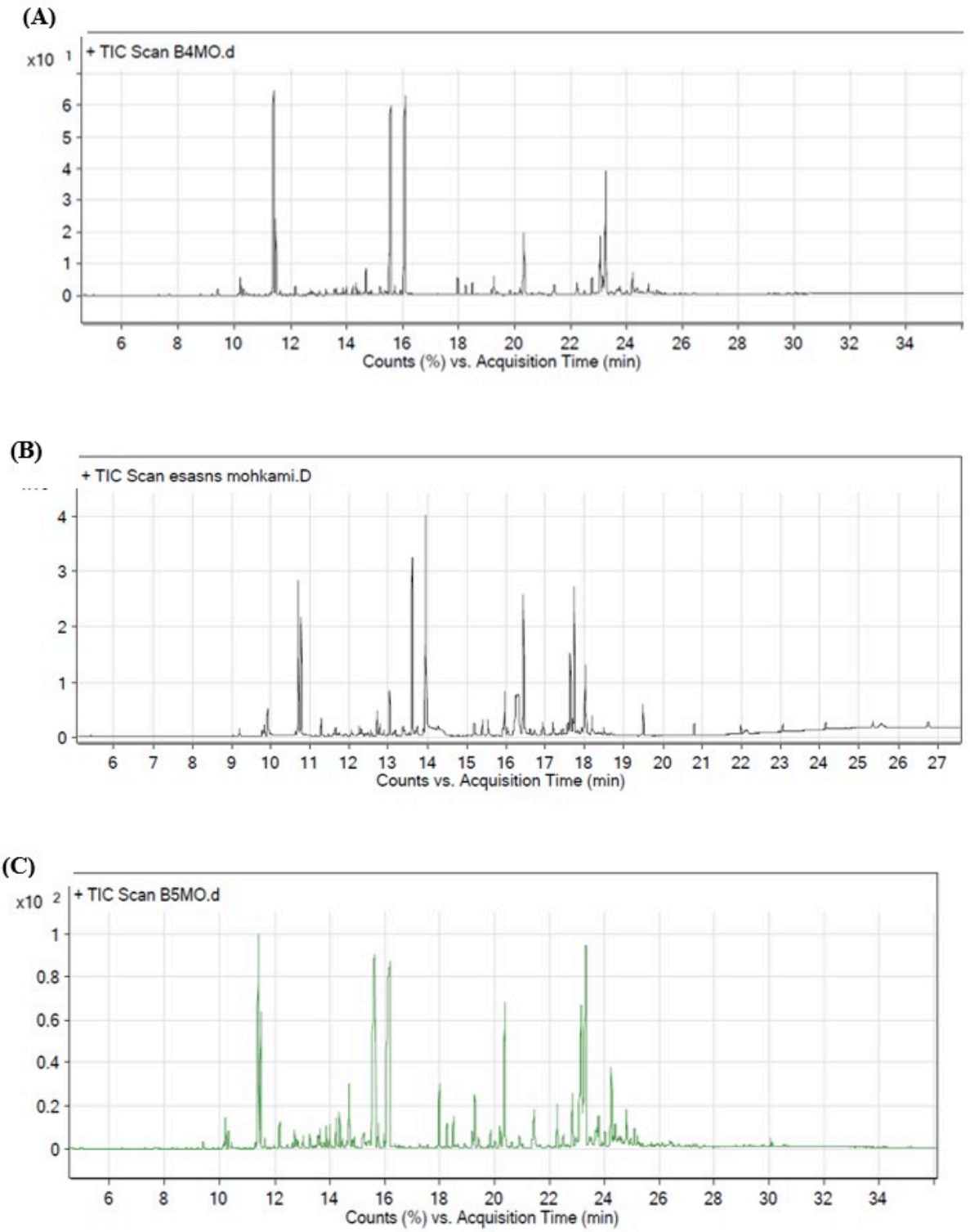


Figure 7

Gas chromatogram of essential oil obtained from *Aloysia citrodora* dried in 100 W microwave (A), 300 W microwave (B), and freeze dryer (C).