Characterization of Chemical Compounds in Volatile Oil and Ethyl Acetate Extract of *Lavandula angustifolia* by GC-MS

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Abstract: *Lavandula angustifolia* is an important aromatic plant and rich source of linalool. *L. angustifolia* and its bioactive compounds have been reported to possess several bioactivities such as antioxidant, antifungal, antibacterial and antidiabetic etc. The aim of the present study is to characterize chemical compounds present in volatile oil and ethyl acetate extract of *L. angustifolia* aerial parts by gas chromatography mass spectroscopy (GC-MS). The hydrodistillation of aerial parts of *L. angustifolia* yielded 1.56±0.27% *v/w* volatile oil. The GC-MS analysis of volatile oil of *L. angustifolia* yielded 74 chemical compounds, and lavandulyl isobutyrate (52.61%), and linalool (5.41%) were identified as major compounds. Ultrasonication was utilized for extraction of *L. angustifolia* using ethyl acetate as solvent and extraction yield was found to be 3.59±0.73% *w/w*. The GC-MS analysis of ethyl acetate extract of *L. angustifolia* yielded 40 chemical compounds and the major compounds were dotriacontane (8.33%), linalool (7.09%), eucalyptol (6.42%), linalyl acetate (4.69%), 1,4-cineole (4.62%), and *trans*-linalool oxide (4.51%). The present study explores the chemical composition of volatile oil and ethyl acetate extract of *L. angustifolia*.

Keywords: *Lavandula angustifolia*, Lamiaceae, GC-MS, Linalool, Aromatic Plant, Hydrodistillation, Ultrasonication

1. Introduction

Aromatic medicinal plants are major source of volatile oils, and volatile oils and extracts of these plants have been utilized for several years as source of medicines, fragrance, food additives and in aromatherapy (Ali, 2001). Volatile oils are also source of bioactive components such as linalool, carvone, menthol, fenchone, eucalyptol, eugenol, camphor, etc. Volatile oils are emerged as source of medicinal agents and known for several beneficial bioactivities such as antibacterial, antifungal, antioxidant, sedatives and anticancer (Ahamad et al., 2019). Kurdistan is mountainous region and habitat of several aromatic plants.

Lavandula angustifolia Linn. belongs to Lamiaceae family and is abundantly growing in Iraqi Kurdistan region (Hamad et al., 2013). *L. angustifolia* is commonly known as Lavender and source of high quality fragrance.

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L. angustifolia is a source of linalool, and linalool is used industrially for chemical synthesis of vitamin A and E (Kamatou et al., 2008). *L. angustifolia* is found throughout the world specifically in Mediterranean countries such as Italy, France, Spain Iran, and Turkey. Essential oils of *L. angustifolia is* extensively used in fragrance and aromatherapy (Babatabar et al., 2020). *L. angustifolia* has several bioactivities *viz.* antidiabetic (Nasiri Lari et al., 2020; Kulabas et al., 2018), antianxiety (Sayed et al., 2020), antibacterial and antioxidant (Hamad et al., 2013; de Rapper et al., 2016; Giovannini et al., 2016; Insawang et al., 2019), antifungal (Behnam et al., 2006), and anti-inflammatory (Cardia et al., 2018). The aim of present study to characterize chemical composition of volatile oil and ethyl acetate extract of *L. angustifolia* by GC-MS method.

2. Materials and Methods

2.1 Plant Materials and Chemicals

The fresh aerial parts of *Lavandula angustifolia* Linn. (1 kg) was collected in March 2021 from Erbil, Kurdistan Region, Iraq. The authenticity of all the accession was ascertained by Dr. Raad A Kaskoos, Faculty of Pharmacy, Hawler Medical University, Erbil, Iraq. For future reference, the plant sample was archived in the Faculty of Pharmacy, Tishk International University, Erbil, Iraq (voucher number: PRL/2021/06). The dimethyl sulfoxide (DMSO), ethyl acetate, and hexane were of analytical grade.

2.2 Isolation of Volatile Oil

The volatile oil from fresh aerial part of *L. angustifolia* (500 g) was isolated by using Clevenger apparatus. The plant sample in small pieces was placed in round bottom flask with water (1000 mL) and glycerin (2 mL) mixture, and hydrodistilled for 6 hours. After complete isolation of volatile oils, the assembly is switched off and volatile oil was collected from graduated tube in eppendorf. The volatile oil was kept in refrigerator at 2-4 ºC until further use.

2.3 Preparation of Extraction

The fresh aerial part of *L. angustifolia* (500 g) were dried under shade provided with proper ventilation. After drying, the plant sample was pulverized in a mixed grinder. The coarse powder of plant sample was extracted using ultrasonicator (Elma, Germany). The coarse powder of plant sample was placed in a stoppered conical flask and extracted with ethyl acetate (250 mL) in an ultrasonicator at 200 W power for 30 minutes at 30 ºC temperature. The filtrate is concentrated further in a rotary evaporator (Buchi, Switzerland) at 35 ºC. The filtered extract was then air dried and kept in the refrigerator at 2-4 ºC until needed.

2.4 GC-MS Instrumentation

The GC-MS method was used to analyze the chemical composition of volatile oil and ethyl acetate extract of *L. angustifolia.* The test samples (volatile oil and ethyl acetate extract) were run on Agilent Bench Top GC-MS (Agilent Technologies, Wilmington, DE, USA) equipment using a DB-5 glass capillary column with specification of 30 m × 0.25 mm i.d.; film thickness of 0.25 µm. The carrier gas was helium, and the flow rate was fixed at 1 mL/min. The oven temperature was set to 50 °C for 1 minute and then isothermally maintained at 320 °C for 2 minutes, while the injector port temperature was kept at 280 °C. The split ratio was retained at 1:5 for injecting the *L. angustifolia* (volatile oil in hexane 1:1 mixture, and ethyl acetate extract in DMSO, 0.5 µL each). Data was collected at 70 eV with 1.5 second scanning durations in the mass range of 50-1000 amu and run times of 32 minutes for

volatile oil and 40 minutes for ethyl acetate extract. Chem station software was used to handle the chromatography and mass spectra.

2.5 Identification of Chemical Compounds

The individual chemical constituents were identified by comparing their Kovats Index (K.I.) to those found in the literature and mass fragmentation pattern of spectra obtained by GC-MS was compared to those stored in the spectrometer database of NIST, NBS 54 K.L, WILEY8 libraries. Further identification of chemical compounds was done on the basis of published literatures (Adams, 2007; Ali, 2001; Najibullah et al., 2021; Hamad et al., 2013; Guo and Wang, 2020; Bialon et al., 2019; 4. Wesołowska ET AL., 2019). The percent composition of individual compounds or peaks was calculated on the basis of respective area.

3. Results and Discussion

3.1 GC-MS Analysis of Volatile Oil of *L. angustifolia*

Hydrodistillation is most preferred method for isolation of volatile oil at laboratory scale. Hydrodistillation of fresh aerial parts of *L. angustifolia* yielded 1.56±0.27% v/w volatile oil. The GC-MS analysis of volatile oil of *L. angustifolia* yielded seventy-four chemical compounds which constitute about 98.25% of total volatile oil (Table 1 and Figure 1). Lavandulyl isobutyrate (52.61%), linalool (5.41%), isoverbanol acetate (4.33%), limonene-1,2-diol (3.44%), *p*-cymene (2.77%), 1,1,5,6-tetramethylindane (2.54%), globulol (1.94%), 6-hepten-3-one, 4-methyl (1.90%), 4-hydroxyhexan-3-one (1.86%), and pentadecanoic acid, methyl ester (1.41%) were found as major chemical compounds in volatile oil from aerial parts of *L. angustifolia.* The other chemical constituents those are less than 1% are also presented in Table 1.

Table 1: Chemical composition of volatile oils from aerial parts of *L. angustifolia*

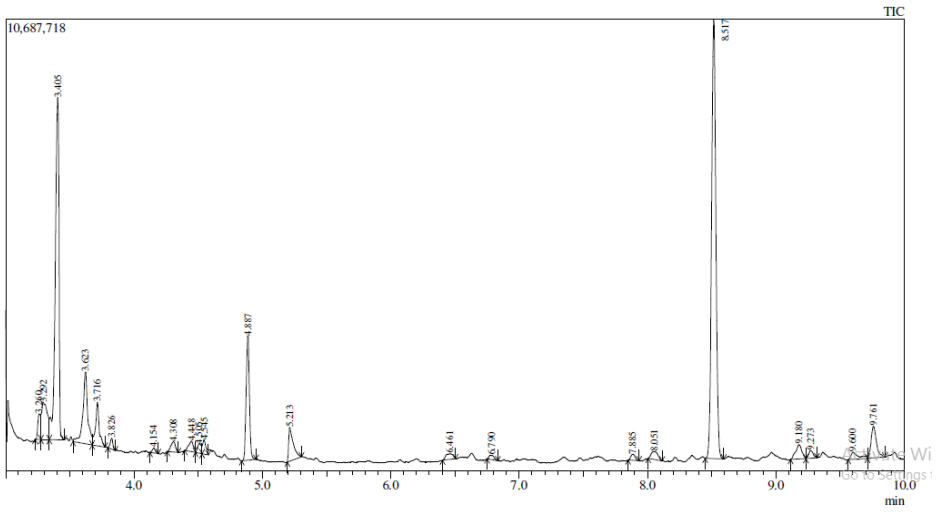
S.No.	Chemical compounds	RT	RI	% Composition
1.	Propanoic acid	3.260	683	0.12
2.	4-Hydroxypentan-2-one	3.292	818	0.32
3.	4-Hydroxyhexan-3-one	3.405	846	1.86
4.	Acetic acid, pentyl ester	3.623	859	0.51
5.	*n*-Dibutyl ether	3.716	865	0.24
6.	Dimethyl sulfone	4.448	914	0.08
7.	*α*-Pinene	4.887	934	0.60
8.	*β*-Pinene	5.213	980	0.23
9.	*p*-Cymene	8.517	1025	2.77
10.	Eucalyptol	9.180	1030	0.13

11.	Cyclooctanone	9.273	1081	0.05
12.	*β*-Ocimene	9.761	1074	0.25
13.	Fenchone	10.682	1096	0.26
14.	Linalool	11.023	1107	5.41
15.	6-Hepten-3-one, 4-methyl	11.475	1113	1.90
16.	α-Fenchol	11.654	1120	0.21
17.	2-Methyl-1-phenyl-propan-2-ol	11.751	1124	0.15
18.	3,4-Dimethyl-cyclohexanol	11.823	1126	0.16
19.	Linalol oxide, trifluoroacetate	11.935	1128	0.60
20.	Pinocarveol	12.255	1139	0.10
21.	Camphor	13.615	1149	0.21
22.	Isoneral	13.821	1151	0.23
23.	Isoborneol	13.909	1154	0.26
24.	4-Menthen-8-ol	14.302	1156	0.68
25.	3-Thujanol	14.531	1157	0.12
26.	*p*-Menthan-8-ol	14.592	1162	0.25
27.	2-(4-Methylphenyl)-propan-2-ol	15.006	1172	0.45
28.	Menthol	15.217	1173	0.10
29.	2-Methylisoborneol	15.602	1180	0.49
30.	Terpinen-4-ol	15.756	1182	0.63
31.	3,5,5-Trimethyl-cyclohexene	15.900	1183	0.06
32.	*α*-Terpineol	16.008	1185	0.06
33.	Verbenone	16.182	1204	0.77
34.	Citronellol	16.268	1212	0.42

35.	Isobornyl formate	16.468	1228	0.36
36.	Linalyl acetate	16.629	1257	0.76
37.	*trans*-carvone oxide	17.090	1277	0.21
38.	Bornyl acetate	17.231	1285	0.21
39.	Thymol	17.375	1290	0.12
40.	Lavandulyl acetate	17.447	1290	0.39
41.	Limonene dioxide	17.557	1294	0.80
42.	Isoverbanol acetate	17.707	1306	4.33
43.	1,1,5,6-Tetramethyl-indane	17.894	1311	2.54
44.	*cis*-2,3-Pinanediol	17.998	1313	0.98
45.	Limonene-1,2-diol	18.103	1321	3.44
46.	*n*-Undecanol	18.274	1355	0.13
47.	Decanoic acid	18.451	1380	0.53
48.	*cis*-Jasmone	18.779	1394	0.13
49.	D-8-Hydroxy-carvotanacetone	18.925	1395	0.23
50.	*E*-Caryophyllene	19.092	1418	0.37
51.	Lavandulyl isobutyrate	19.606	1423	52.61
52.	γ-Muurolene	19.701	1477	0.30
53.	Lavandulyl isovalerate	19.919	1505	0.29
54.	*trans*-2-α-Bisabolene epoxide	20.104	1522	0.40
55.	*trans*-Nerolidol	20.256	1564	0.51
56.	Caryophyllene epoxide	20.311	1572	0.88
57.	Spathulenol	20.419	1576	0.16
58.	Caryophyllene oxide	20.508	1580	0.27
59.	Globulol	20.884	1583	1.94

60.	Epicubenol	21.560	1627	0.27
61.	Cubenol	21.858	1641	0.22
62.	α-Muurolol	24.217	1645	0.49
63.	α-Cadinol	24.518	1653	0.20
64.	Bisabolol oxide B	25.185	1655	0.31
65.	α-Bisabolol	25.250	1683	0.47
66.	2-Methyloctadecane	26.393	1864	0.36
67.	Pentadecanoic acid, methyl ester	26.597	1877	1.41
68.	Patchoulane	26.700	1890	0.16
69.	Methyl linolenate	26.700	1893	0.16
70.	Methyl 9-octadecenoate	26.780	2073	0.05
71.	9-Octadecenoic acid (*Z*)	26.891	2074	0.26
72.	Methyl stearate	26.991	2099	0.13
73.	*Z*-Phytol	27.325	2114	0.07
74.	Dioctyl phthalate	31.944	2682	0.12

where, RT: retention time, and KI: Kovats index.



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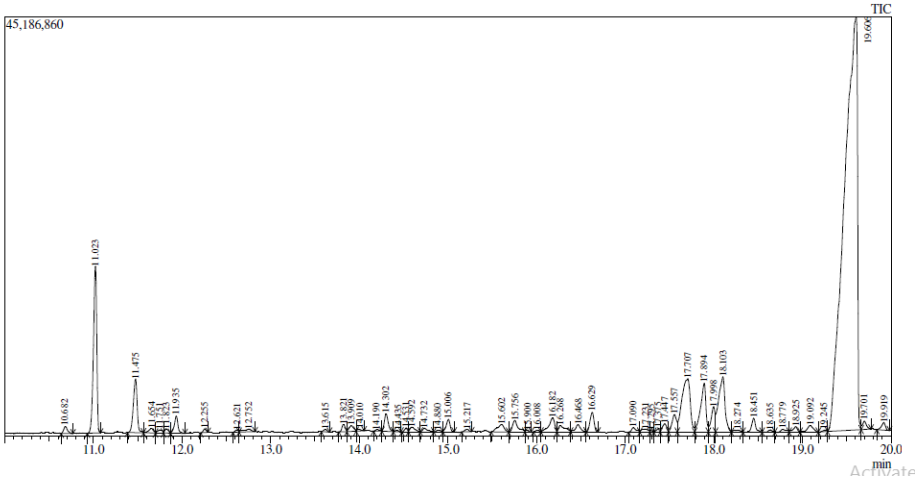

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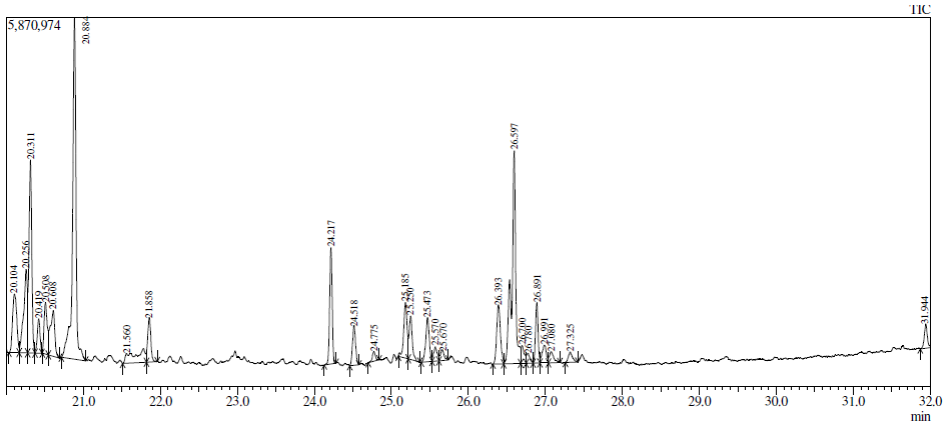

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Figure 1 a,b,c: GC-MS spectrum of volatile oils from aerial parts of *L. angustifolia*

3.2 GC-MS Analysis of Ethyl Acetate Extract of *L. angustifolia*

The extraction of *L. angustifolia* aerial parts was performed on Ultrasonic bath using ethyl acetate as solvent at 35ºC temperature for 30 minutes. The extraction yield from aerial parts of *L. angustifolia* was 3.59±0.73% w/w based on dried weight of plant material. The GC-MS analysis of ethyl acetate extract of *L. angustifolia* yielded forty chemical compounds which constitute about 99.96% of total extract. The major chemical compounds in ethyl acetate extract of *L. angustifolia* are dotriacontane (8.33%), linalool (7.09%), eucalyptol (6.42%), linalyl acetate (4.69%), 1,4-cineole (4.62%), trans-linalool oxide (4.51%), isospathulenol (3.78%), and α-terpinene (3.21%). The components less than 3 percent includes hexyl butyrate (2.91%), tricyclene (2.90%), myrtenyl acetate (2.74%), phthalic acid (2.64%), oxirane, methyl (2.48%), *γ*-terpinene (2.38%), (Z)-β-ocimene (2.34%), 3-octanone (2.30%), *α*-thujene (2.10%), myrcene (2.28%), heneicosane (2.21%), and 4-isopropyl-2-cyclohexenone (2.01%). The minor components which are less than 2 percent are also presented in Table 2.

Ahamad et al. (2021) studied chemical composition of essential oil of *L. angustifolia* collected from Kashmir, India, by GC-MS method. The study results show presence of linalyl acetate (39.28 %), linalool (26.76 %), *trans-β*-caryophyllene (4.77 %), lavandulyl acetate (3.04 %) and 1,8-cineole (2.15 %) as major chemical compounds. In another study on chemical composition of *L. angustifolia* collected from Shaqlawa, Kurdistan Region, Iraqi. The study results show the presence of linalool

(24.63%), camphor (13.58%), linalyl acetate (8.89%), and borneol (6.41%) as major chemical components present in essential oil (Hamad et al., 2013). Both the above studies are conducted by same authors and the results shows variation in their chemical composition. This variation may be due to difference in the environmental conditions such as temperature, altitude, humidity and rainfall etc. (Ahamad et al., 2019). The present study explores and compare composition of essential oil and ethyl acetate extract of *L. angustifolia.* In the resent study, lavandulyl isobutyrate (52.61%), linalool (5.41%), isoverbanol acetate (4.33%), limonene-1,2-diol (3.44%), *p*-cymene (2.77%) were found as major chemical compounds in the volatile oil of *L. angustifolia*. The ethyl acetate extract of *L. angustifolia* contains dotriacontane (8.33%), linalool (7.09%), eucalyptol (6.42%), linalyl acetate (4.69%), 1,4-cineole (4.62%), trans-linalool oxide (4.51%), isospathulenol (3.78%), and α-terpinene (3.21%) as major chemical compounds. The results from volatile oil and ethyl acetate extract shows difference in chemical compounds. Linalool industrially used for preparation of high quality fragrance, and the present study shows presence of linalool in both volatile oil and extract.

Table 2: Chemical composition of ethyl acetate extract from aerial parts of *L. angustifolia*

S. No.	Chemical constituents	RT	KI	% Composition
1.	Oxirane, methyl	7.666	668	2.48
2.	Methyl 2,2-dimethylbutanoate	10.408	815	1.13
3.	3-Methoxy-4-methylheptane	15.963	935	1.11
4.	Tricyclene	18.355	923	2.90
5.	*α*-Thujene	20.036	927	2.10
6.	*α*-Pinene	20.110	934	1.91
7.	Sabinene	22.065	967	1.81
8.	*β*-Pinene	23.844	972	1.65
9.	3-Octanone	26.594	966	2.30
10.	Myrcene	27.228	983	2.28
11.	Hexyl acetate	29.056	1010	1.36
12.	1,4-Cineole	30.515	1016	4.62
13.	*α*-Terpinene	30.845	1017	3.21
14.	Eucalyptol	32.087	1031	6.42
15.	(*Z*)-*β*-Ocimene	33.337	1037	2.34
16.	(*E*)-*β*-Ocimene	34.556	1047	1.76

17.	γ-Terpinene	34.952	1059	2.38
18.	*cis*-Linalool oxide	35.838	1065	1.90
19.	*trans*-Linalool oxide	37.072	1072	4.51
20.	Linalool	38.261	1099	7.09
21.	Camphor	38.340	1125	1.64
22.	Hexyl butyrate	38.399	1171	2.91
23.	Isoneomenthol	38.440	1182	1.03
24.	4-Isopropyl-2-cyclohexenone	38.500	1165	2.01
25.	Hexyl butyrate	38.525	1171	0.99
26.	*α*-Terpineol	38.535	1175	0.37
27.	Myrtenyl acetate	38.560	1235	2.74
28.	1-Chlorodecane	38.626	1239	1.85
29.	Linalyl acetate	38.758	1256	4.69
30.	1-Tridecene	38.800	1287	1.46
31.	*α*-Selinene	38.830	1494	1.70
32.	*α*-Panasinsen	38.884	1518	1.05
33.	Isospathulenol	38.97	1619	3.78
34.	Diethyl isophthalate	39.000	1638	1.24
35.	1-Octadecene	39.020	1778	0.51
36.	Nonadecane	39.044	1900	1.57
37.	Dibutyl phthalate	39.095	1909	1.98
38.	Phthalic acid	39.160	1917	2.64
39.	Heneicosane	39.263	2100	2.21
40.	Dotriacontane	39.510	3200	8.33

where, RT: retention time, and KI: Kovats index.

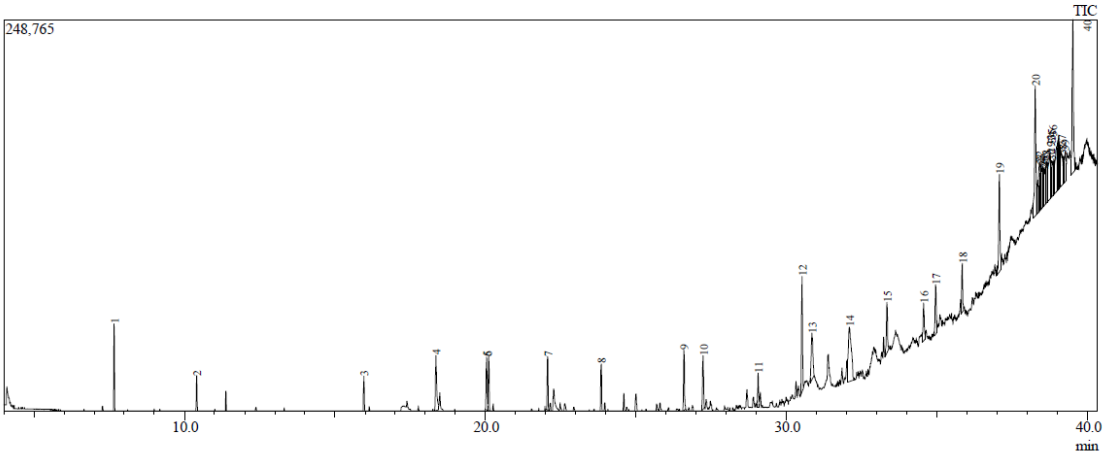

Figure 2: GC-MS spectrum of ethyl acetate extract from aerial parts of *L. angustifolia*

4. Conclusion

Hydrodistillation method was applied for isolation of volatile oils from fresh aerial parts of *L. angustifolia*. GC-MS analysis of volatile oils of *L. angustifolia* and results shows the presence of lavandulyl isobutyrate, linalool, isoverbanol acetate, limonene-1,2-diol and *p*-cymene as major compounds. Ultrasonication was used for extraction purpose and the GC-MS analysis of ethyl acetate extract shows the presence of dotriacontane, linalool, eucalyptol, linalyl acetate, 1,4-cineole (4.62%) and trans-linalool oxide as main compounds. The present study successfully characterizes chemical compounds of *L. angustifolia*, and the findings will help scientific community in their study on this medicinal plant.

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