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Research article

ESSENTIAL OIL COMPOSITION AND TOTAL FLAVONOID CONTENT OF ALOYSIA CITRIODORA PALAU UNDER DIFFERENT CULTIVATION SYSTEMS

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ABSTRACT: As a spice and medicinal plant, *Aloysia citriodora* Palau (Family, Verbenaceae) is applied in nutritional and pharmacological purposes. According to the findings, many parameters relating to growth location and cultivation conditions, may affect on the secondary metabolites of the herb and result in different percentage of concerned constituents. Accordingly, present study was performed to compare the chemical composition profiles of essential oil and total flavonoid content of *Aloysia citriodora* Palau leaves under three different cultivation situations. To this, three cutting batch of herb were cultivated in garden, green house and hydroponic systems. Essential oil was then extracted by hydrodistillation and analyzed using Agilent technologies model 7890A gas chromatograph connected to a mass detector. Total flavonoid content was determined using Dowd method. Major essential oil constituents for the garden sample were Geranial (16.70%), Neral (13.46%) and Limonene (12.41%) followed by Caryophyllene oxide (9.30%), alpha-Curcumene (7.90%) and 1, 8-Cineole (5.49%). Main constituents were as Geranial (26.94%), Neral (21.46%) and alpha-Curcumene (9.15%) followed by trans-Caryophyllene (5.39%) for the green house sample and as Geranial (21.57%), Neral (18.25%), alpha-Curcumene (8.23%) followed by trans-Caryophyllene (6.20%) and Limonene (6.07%) for the hydroponic system. The total flavonoid content for garden, green house and hydroponic systems were determined as 7.01, 7.70 and 4.85 mg (QE)/g dry plant leaves. Despite some differences in volatile oil components of mentioned samples, findings of current work emphasizes that these cultivation conditions do not much create a significant difference in major essential oil ingredients. However results revealed the inappropriateness of hydroponic system versus other mentioned methods.

Key words: *Aloysia citriodora* Palau, Essential oil, Flavonoid, Lemon verbena

INTRODUCTION

Aloysia citriodora Palau, commonly known as Lemon verbena, is a flowering plant related to the family Verbenaceae [1]. The herb is indigenous to South America and now is cultivated in many parts of the world under different conditions [2]. From the past, it was reputed to be effective in cold, asthma, spasms, flatulence, fever, colic, diarrhea, insomnia as well as anxiety [3-4]. As a spice and medicinal plant, Lemon verbena involves several flavonoid compounds and phenolic acids [5-6]. The leaves also exhibit antispasmodic, antipyretic digestive, stomachic and sedative properties [7]. In addition to the mentioned therapeutic activities, *Aloysia citriodora* Palau may possess antimicrobial properties due to its essential oil composition [8-9]. The oil is also applicable in pharmaceutical industry as well as perfumery and cosmetics [10]. Several investigations have been performed on the analysis and identification of *Aloysia citriodora* Palau phenolic compounds and total essential oil constituents [1-2, 5, 11-12]. According to the results, it has been remarked that many parameters relating to growth location and cultivation conditions may affect on the essential oil composition and result in different percentage of the constituents [13]. Today, one of the surest ways to obtain certain herbal bioactive ingredients is the cultivation of exact species of a genus. Hence, cultivation planning and improving the quality and performance of farm processes reach the producers to the best results.

This fact may be due to producing different amount of secondary metabolites. Accordingly, present study was carried out to compare the chemical composition profile of essential oil and total flavonoid content of *Aloysia citriodora* Palau leaves under three different cultivation situations.

MATERIALS AND METHODS

Identification and Cultivation of the herb

A cutting batch of *Aloysia citriodora* Palau was authenticated by Mrs. S. Khademian, the herbalist of School of Pharmacy, Shiraz University of Medical Sciences. For future reference, a sample of herb was pressed and deposited in the School of Pharmacy Herbarium with a respective voucher name. Cutting batch was divided into three samples. Each sample was then propagated in an outdoor garden, green house and a hydroponic system. Hydroponic medium which is an appropriate cultivation system for lands with low natural resources needs less water and even no soil [14]. Ordinary soil was considered for garden and was enriched only with compost (2.5 Kg/10 m^2). In the selected soil for green house, urea (15-20 g/20 m^2) was added every 15 days by a cyclone water pump. For the hydroponic system, cuttings were cultivated in pots containing washed sand: perlite (2:1). NPK water soluble fertilizer (20-20-20) along with aluminium sulfate (20 g) was added to 2.5 m^3 water in a reservoir. Plant watering was performed two times a day. Moreover, iron fertilizer and carbendazim (20 g), a systemic benzimidazole fungicide were added to the water every 15 days.

Essential oil and ethanolic extract preparation

Leaves of the herb grown in aforementioned three cultivation systems were picked and dried. They were subsequently grounded and subjected to hydrodistillation for 3 hrs using a Clevenger-type apparatus for the extraction of the essential oil [8]. On the other hand, leaves (10 g) were also macerated in 100 ml of 96% ethanol at room temperature for 48 hrs. Respective extracts were subsequently filtered, dried and kept in 4°C for further steps.

Analysis of the essential oil

Initially GC/FID oil analysis was performed to get a desirable analytical condition. The work was done on a gas chromatograph Agilent technologies model 7890A apparatus attached to HP-5 column (25 m × 0.32 mm, 0.52µm film thickness) and connected to a flame ionization detector (FID). Nitrogen gas was used as carrier gas with a flow rate of 1 ml/min and split ratio was 1:30. The injector temperature was 250°C, and detector temperature was 280°C, while column temperature was linearly programmed from 60 to 250°C (at rate of 5 °C/min) and held for 10 min at 250°C. Solutions of anhydrous and diluted essential oil samples were consecutively injected. The above method was then applied for GC/MS analysis. This process was carried out by using Agilent technologies model 7890A gas chromatograph connected to a mass detector (Agilent technologies model 5975C). The gas chromatograph was equipped with a DB-5MS capillary column (phenyl methyl siloxan, 30 m × 0.25 mm i.d., Agilent technologies). Helium was used as carrier gas with the same flow rate as for GC/FID. The mass spectrometer was acquired in EI mode (70 eV) in a mass range of 30–600 m/z. The interface temperature was 280°C. Identification and quantification of components via GC/MS was based on comparison of their mass spectra with Willey (nl7) and Adams libraries spectra as well as with those reported in literatures [15].

Determination of the total flavonoids

The total flavonoid content was determined using Dowd method [16]. Solution of 5 ml of 2% aluminium trichloride ($AlCl_3$) (Sigma-Aldrich) in methanol (Merck) was mixed with the same volume of different concentrations of quercetin. Absorption readings at 415 nm (PG instrument T90 spectrophotometer) were taken after 10 min against a blank sample consisting of a 5 ml extract solutions with 5 ml methanol in the absence of $AlCl_3$. By preparing a standard curve of quercetin (Sigma-Aldrich) in concentrations of 0–80 mg/L, the total flavonoid content of these samples was determined. The mean of three readings for each sample was considered and expressed as mg of quercetin equivalents (QE)/g of dry plant leaves.

RESULTS AND DISCUSSION

We have carried out an investigation on the essential oil composition of Lemon verbena herb cultured on different conditions. The extraction of essential oil yielded 1.33 % (V/W) for all three samples cultivated in garden, green house and hydroponic systems. Table 1 represents the chemical composition of essential oil for *Aloysia citriodora* Palau which was cultured in mentioned systems (in the order of elution from the HB5-MS column).

Table 1. Chemical composition of *Aloysia citriodora* Palau essential oil cultivated in mentioned media

No.	Component	RI[1]	Identification	*Lippia citriodora* (Lam.) Kunth		
				Garden	Green house	Hydroponic
				%- Qual.	%- Qual.	%- Qual.
1	alpha-Pinene	935	MS-KI	0.75	-	0.22
2	Sabinene	975	MS-KI	1.88	0.48	1.06
3	6-Methyl-5-hepten-2-one	986	MS-KI	1.50	2.37	3.46
4	beta.-Myrcene	992	MS-KI	0.20	-	-
5	ortho-Cymene	1026	MS-KI	0.68	-	-
6	delta-Limonene	1031	MS-KI	**12.41**	3.89	**6.07**
7	1,8-Cineole	1034	MS-KI	**5.49**	1.49	1.91
8	trans-beta-Ocimene	1048	MS-KI	-	1.30	1.86
9	gamma-Terpinene	1063	MS-KI	1.16	0.27	0.31
10	trans-Chrysanthemal	1096	MS-KI	0.23	-	0.22
11	Linalool	1100	MS-KI	0.93	-	0.31
12	1,3,8-p-menthatriene	1117	MS-KI	0.21	-	-
13	cis-limonene oxide	1136	MS-KI	0.49	-	-
14	trans-limonene oxide	1140	MS-KI	0.22	-	-
15	cis-verbenol	1142	MS-KI	-	-	0.29
16	para-Menth-3-en-8-ol	1152	MS-KI	0.32	0.33	0.53
17	Citronella	1154	MS-KI	-	-	0.35
18	trans-beta-terpineol	1165	MS-KI	-	0.31	0.66
19	3-thujanol	1170	MS-KI	0.20	-	-
20	Borneol	1176	MS-KI	1.40	0.50	0.68
21	Terpinen-4-ol	1184	MS-KI	-	0.48	0.85
22	alpha-Terpineol	1193	MS-KI	-	-	0.99
23	beta-Fenchyl Alcohol	1193	MS-KI	1.69	0.74	-
24	gamma- Terpineol	1200	MS-KI	0.38	-	-
25	Octyl acetate	1212	MS-KI	-	-	1.03
26	trans-carveol	1221	MS-KI	0.54	-	-
27	Nerol	1230	MS-KI	0.35	0.48	0.81
28	Neral	1242	MS-KI	**13.46**	**21.46**	**18.25**
29	1-carvone	1247	MS-KI	0.34	-	-
30	Geraniol	1253	MS-KI	-	-	0.36
31	Piperitone	1254	MS-KI	0.25	-	-
32	Geranial	1273	MS-KI	**16.70**	**26.94**	**21.57**
33	Thymol	1293	MS-KI	0.86	-	-
34	Copaene	1380	MS-KI	-	0.67	0.63
35	alpha-Cubebene	1382	MS-KI	0.82	-	-
36	beta-Cubebene	1389	MS-KI	1.00	1.31	1.39
37	beta-Bourbonene	1390	MS-KI	0.54	-	0.23
38	Cedrene	1419	MS-KI	0.52	0.28	0.28
39	trans-Caryophyllene	1426	MS-KI	2.04	**5.39**	**6.20**
40	beta-Acoradiene	1460	MS-KI	0.22	0.42	0.54
41	Aromadendrene	1467	MS-KI	0.82	0.53	0.60
42	Alloaromadendrene	1473	MS-KI	0.49	0.35	-
44	alpha-Amorphene	1482	MS-KI	0.20	-	0.48
45	Geranyl propanoate	1483	MS-KI	0.19	-	0.24
46	alpha-Curcumene	1487	MS-KI	**7.90**	**9.15**	**8.23**
47	Zingiberene	1498	MS-KI	-	1.06	1.03
48	bicyclogermacrene	1501	MS-KI	-	3.30	1.85
49	beta-curcumene	1516	MS-KI	-	1.28	1.33
50	beta-sesquiphellandren	1521	MS-KI	1.11	0.81	0.92
51	delta.-Cadinene	1529	MS-KI	0.22	-	0.23

Table 1 cont…

52	Caryophyllen-5-ol	1565	MS-KI	0.30	1.70	1.86
53	Nerolidol	1568	MS-KI	1.16	-	-
54	trans-Sesquisabinene hydrate	1579	MS-KI	0.29	1.97	1.56
55	Spathulenol	1584	MS-KI	4.19	2.35	1.54
56	Caryophyllene oxide	1588	MS-KI	**9.30**	2.52	3.35
57	Unknown	1597	-	1.06	-	0.30
58	beta-himachalene oxide	1616	MS-KI	0.46	0.76	0.87
59	Unknown	1617	-	0.83	-	-
60	alpha.-copaene-8-ol	1630	MS-KI	1.64	1.27	1.53
61	alpha-eudesmol	1655	MS-KI	-	0.42	-
62	Unknown	1662	-	0.61	-	0.45
63	Unknown	1677	-	0.73	-	0.23
64	Calamenene	1693	MS-KI	-	-	0.37
65	1,4-cis-1,7-trans-Acorenone	1698	MS-KI	0.57	0.30	0.30
66	Unknown	1785	-	-	0.33	-
67	Unknown	1879	-	-	1.31	-
68	Cembrene C	2020	MS-KI	-	0.60	-
69	Unknown	2115	-	-	0.99	1.65
Total identification (%)				96.74	97.27	97.37
Monoterpene hydrocarbon (%)				22.78	7.43	11.43
Oxygen containing monoterpene (%)				40.04	53.68	50.60
Sesquiterpene (%)				36.64	35.36	36.02
Diterpene (%)				0.57	3.53	1.95

The significance of bold in components Limonene, Geranial, Neral, Caryophyllene oxide and alpha-Curcumene is to note those as the most abundant constituents.

[1] Compounds have been identified by combination of both mass spectra and retention indices. RI represents the retention indices which were calculated against C_8-C_{24} n-alkanes on mentioned column. Compounds have been sorted regarding retention indices on DB-5 MS capillary column.

Totally forty six, thirty five and forty four compound were identified garden, green house and hydroponic systems. Major essential oil constituents for the garden sample were Geranial (16.70%), Neral (13.46%) and Limonene (12.41%) followed by Caryophyllene oxide (9.30%) and α-Curcumene (7.90%) as well as 1, 8-Cineole (5.49%). Major constituents were as Geranial (26.94%), Neral (21.46%) and α-Curcumene (9.15%) followed by trans-Caryophyllene (5.39%) for the green house sample and as Geranial (21.57%), Neral (18.25%), α-Curcumene (8.23%) followed by trans-Caryophyllene (6.20%) and Limonene (6.07%) for the hydroponic system. Main ingredients for these samples are all presented at levels of more than 5%. According to these findings, the total Citral (Neral + Geranial) content was in the highest amount for green house sample (48.5% of total constituents). So this sample is clearly enriched in aldehydes as is not much different to previous studies [2]. On the contrary, garden sample exhibited the lowest amount of Citral fraction but highest amount of Limonene. Obviously, considering the Citral content in these samples may be of beneficial. As flavor and perfume materials from natural source, these components are widely applied in pharmaceutical and perfumery as well as cosmetics industries [17]. In addition to the constituents, Table 1 also involved contents of monoterpene hydrocarbon, oxygen containing monoterpene and sesquiterpene as well as diterpene for each sample. Accordingly, the percentage of monoterpene hydrocarbons involved lowest amount in green house sample. In contrast, garden sample contained highest amount of monoterpene hydrocarbon fraction among three cultivated samples, which is regarded to the amount of Limonene (12.7%) as well as 1, 8 cineol (5.49%). Overall, findings on the profile of dominant terpenes in leaf essential oil of Lemon verbena samples are partly in line with some previous reports [7, 18-19], but much different to those of others [20]. Comparing to the essential oil of Lemon verbena seeds, the major difference is in the amount of Geranial as the most abundant ingredient in seeds and absence or negligible in our samples [21]. Among those mentioned samples, the maximum content of cyclic monoterpene hydrocarbons fraction was 29.29% of total essential oil for the garden sample. While this fraction is represented to have lowest amount in green house sample (7.49%), the amount of aliphatic monoterpene hydrocarbons in that sample was in the highest level (52.55%).

The total content of aliphatic monoterpene hydrocarbons for garden and hydroponic system was 33.35% and 48.00%, respectively. Hydroponic system involved 13.79% of cyclic monoterpene hydrocarbons fraction in the total essential oil. The total amount of sesquiterpene hydrocarbons was similar in three mentioned samples (about 36% of total oil), while a prior investigation reported lower amounts [22]. The most prominent sesquiterpene hydrocarbon was found as Caryophyllene oxide (9.30%) for garden sample. Despite the fact that this compound has been reported in previous studies, but it was found in lower amounts [22-24]. This compound was also significantly decreased in green house and hydroponic samples. In contrast, α-Curcumene was found as the main sesquiterpene hydrocarbon in those samples (Table 1).

Other than essential oil extraction, samples were also subjected to alcoholic extraction via maceration in ethanol 96% for 48 hrs. The extraction yield for garden, green house and hydroponic systems were determined as 8.99% (W/W), 7.23% (W/W) and 10.05% (W/W) respectively. The total flavonoid content for Lemon verbena samples was determined by using the standard curve which was prepared by quercetin (0-80 mg/L). Herein, the total flavonoid for garden, green house and hydroponic systems are carried out and listed in Table 2. Results revealed that hydroponic medium may be of less beneficial for the extraction of total flavonoids.

Table 2. Total flavonoid content in samples (Garden, Green house, Hydroponic system)

	Garden sample			**Green house sample**			**Hydroponic sample**		
	7.04	6.93	7.06	7.69	7.67	7.76	4.85	4.87	4.83
Total flavonoid content (mg (QE)/g dry plant leaves) [a]	7.01±0.05			7.70±0.03			4.85±0.01		

CONCLUSION AND RECOMMENDATION

The study of the total volatile oil from fresh *Aloysia citriodora* Palau leaves cultivated and harvested in three various media confirmed the previous reports on majority of mentioned constituents. Despite some differences in volatile oil components of mentioned samples, findings in current work emphasizes that these cultivation situations does not much create a significant difference in major essential oil ingredients. However, a major component, Linalool was found in lower amounts in green house sample regarding to higher amount of α-Curcumene. In contrast, result for the evaluation of flavonoids was more considerable. Although the yield of ethanolic extraction for hydroponic system sample was the most, results revealed the inappropriateness of hydroponic system versus other mentioned methods.

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