

EFFECT OF HARVESTING DATE, DRYING METHOD, AND STORAGE PERIOD ON THE ESSENTIAL OIL PERCENTAGE AND COMPOSITION OF *Aloysia triphylla* (L'Her.) Britton LEAVES.

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ABSTRACT

Aloysia triphylla (L'Her.) Britton (Verbenaceae) is an aromatic shrub native to Argentina and Chile; known as herb louisa or lemon verbena. It is an important medicinal and aromatic plant that contains high quality essential oil of lemon scent that has an antifungal activity, and is used as a sedative, antispasmodic, anti diarrhea, and to add flavor to food and beverages. A study was carried out during two successive seasons of 2005/2006 and 2006/2007 at the Experimental Station of Medicinal and Aromatic Plants, Fac. Agric., Mansoura Univ. The research aimed to study the effect of drying method (shade and oven) and the length of storage period (2, 4 and 6 months) on essential oil percentage and composition of the leaves of two harvesting dates (June and September).

The physical and chemical properties of the oil were determined and are included in this study.

The results showed that the oil percentage obtained from September harvest was higher than that of June harvest in both seasons. Shade drying gave the highest oil % followed by oven drying method at 45°C compared to the fresh leaves. In both June and September cuts, increasing storage period of the dried leaves of lemon verbena decreased the essential oil percentage in both drying methods.

The G.L.C. of the essential oil of the fresh leaves revealed a total of 20 compounds. The total identified compounds constituted 83.5 % and 76.8 % in the oil of shade and oven dried leaves, respectively in June cut, while constituted 87.9 % and 85.9 % in the same order in September oil. The most important compounds in both cuts were 1,8-cineole, geranial, limonene, linalool, and spathulenol and accounted for 49 % of June oil, while summed 54.3 % in September. The method of drying affected the proportion of the oil components in the two cuts.

The relative concentrations of each constituent changed with the length of the storage period. The percentage of some compounds increased (linalool and spathulenol), others decreased (1, 8- cineol, geranial, and limonene), while others changed slightly in their percentages (geranyl acetate and menthone). Some compounds were not present in the oil of June (α -pinene, camphene, citronellal, ocimene, and sabinene), while were present after storage. In September oil, citronellal and curcumene were found in the control, but disappeared after storage.

INTRODUCTION

An increasing interest in the cultivation and production of aromatic plants has been recognized in Egypt to cover the increasing demands of the local industries as well as, for export and local purposes. Drying process of medicinal and aromatic plants is very important to meet the requirements for quality of plants and make them available for foreign and local markets. *Aloysia triphylla* (L'Her.) Britton (Verbenaceae), commonly known as lemon

verbena is native to Argentina and Chile. Lemon verbena leaves contain high quality essential oil of lemon scent and its main constituents are limonene, spathulenol, geranial, citral, and linalool. The essential oil of lemon verbena contains several physiologically active substances, and is used as antispasmodic, antipyretic, sedative, stomachic, and diuretic, (Keville, 1999 and Figueiredo *et al.*, 2004).

Volatile aroma compounds are the most sensitive components in the process of herb drying. The effect of drying method on the essential oil content and composition were studied by many investigators. Guenther (1961) stated that the direct exposure of plants to the sun tended to break the stalks and made the leaves brittle. Balbaa *et al.* (1974) reported that shade drying is a satisfactory method for quality, a practical and economic method, and increased glycosidal content in *Digitalis lanata* leaves. Omidbaigi *et al.* (2004) reported that the oil content of the shade dried flowers of Roman chamomile was the highest compared to sun-dried and oven-dried flowers at 40°C. On the contrary, Sefidkon *et al.* (2006) mentioned that the drying method had no significant effect on oil composition of *Saturia hortensis*.

The length of storage affected the essential oil content and composition of many medicinal and aromatic plants. Singh *et al.* (1994) reported that storage period length affected the percentage of *Cymbopogon* essential oil and the relative percentages of its constituents. Baritoux *et al.* (1992) found that the losses of total essential oil of basil after drying were 19, 62 and 66 % at 3, 6 and 7 months storage, respectively. Shala (2007) worked on sage plants and found that the percentages of thujone, methyl chavicol and eugenol were not affected after six months storage, while linalool, terpinene, α -pinene, β -pinene and camphene percentages were declined, but the percentages of camphor, borneol, cineole, and linalyl acetate increased at the end of storage period. These changes depend on condition of plant material, method and conditions of drying and storage (i.e. temperature and humidity), and the chemical composition of the essential oil (Paakkonen *et al.*, 1990 and Kotb & Eid, 1996).

The aim of this search was to study the effect of harvesting date, drying methods, and the length of storage period on the essential oil percentage and composition of lemon verbena leaves.

MATERIALS AND METHODS

Harvesting:

Fresh materials of lemon verbena were collected from 2 years old plants grown in the Experimental Station of Veget. and Flori. Dept., Fac. of Agriculture, Mansoura University. Plants were harvested at two different dates; the first of June and the first of September.

Drying:

Leaves were separated from the branches, and subjected to two methods of drying; oven drying at 45°C and shade drying at room temperature. The essential oil of the three fresh samples of lemon verbena leaves (50g each) were extracted using hydro distillation. Before storage, three samples (50g each) of each cut for each drying method were used as control, and were subjected to hydro distillation.

Storage:

The dried samples of both drying methods at each cut were placed in paper bags at room temperature for 2, 4 and 6 months. Three samples, 50g each, after each storage period was subjected to hydro distillation.

Essential oil extraction and determination

1- Oil percentage

The essential oil percentage was determined in the dried samples (50g) in both seasons by subjecting leaves to hydro distillation in Clevenger apparatus according to the method described by Egyptian Pharmacopoeia (1984).

2- Physical and chemical properties of oil

Samples of the two seasons and harvesting dates were mixed together and the essential oil was extracted to measure its general physical and chemical properties. These measurements were analyzed at the Laboratory of the Chemistry Dept., Fac. Agric., Mansoura Univ. The specific gravity, the refractive index and the optical rotation of the essential oil were determined according the methods described by Guenther (1949). The acid number, saponification number and ester number of the oil were determined by applying the methods described in Guenther (1972).

3- Gas Liquid Chromatography (GLC)

The resulted oil was dried over anhydrous-sodium sulphate and kept at refrigerator until Gas Liquid Chromatography analyses. The G.L.C. analysis was carried out at the Central Laboratory of Cairo Univ. The relative retention time (RT) of each peak was compared with the authentic sample to identify the unknown samples. The quantitative estimation for each component was based on the peak area measurement by triangulation (Guenther and Joseph 1978).

Statistical analysis

Simple experiment in a randomized complete block design with 3 replicates was adopted. Collected data were subjected to the statistical analysis of variance (ANOVA) according to Gomez and Gomez (1984) and means were compared using L.S.D at 5 % level.

RESULTS AND DISCUSSION

I) Physical and chemical properties of lemon verbena essential oil:

Identification of the physical properties (specific gravity, refractive index and optical rotation); and the chemical properties (acid number and ester number) of the essential oil of lemon verbena leaves are shown in Table (1).

II) Effects of drying method and storage period on essential oil percentage:

1- Effect of drying method on the essential oil (%) in the two cuts:

Data in Table (2) showed that, the method of drying affected the essential oil percentage in the two different harvesting dates (June and September). Shade drying gave the highest oil (%) followed by oven drying

method at 45°C compared to the fresh leaves. Fresh leaves, accordingly, would have less oil (%) than the dry ones, simply because they possess more water content. Although leaves in shade drying takes longer time to dry than oven drying, the later one have higher drying temperature (45°C) than the shade drying under room temperature, which may be the cause for loss of some of the volatile oil from the leaves during oven drying process. In this concern, Omidbaigi *et al.* (2004) reported that the flowers of Roman chamomile were dried by three different methods of sun-drying, shade-drying and oven-drying at 45°C as the oil content of the shade dried flowers was the highest compared to sun drying and oven drying at 45°C. It is also clear from the same table that the essential oil (%) was affected by harvesting date, the highest values were obtained from September cutting while the lowest was obtained from June cutting in both seasons, which might be attributed to the fact that *Aloysia triphylla* plants usually blooms during September; and thus the plant would contain high volatile oil percentage, which is similar to the case of *Melissa officinalis* plants (Bottcher *et al.*, 2000) as well as other essential oil containing plants.

Table (1): Pphysical and chemical properties of the essential oil of lemon verbena leaves (means of two cuts for two seasons).

Properties	Constant
Specific gravity	0.892
Optical rotation	-12
Refractive index	1.482
Acid number	4.5
Ester number	24.1

Table (2): Effect of drying method on essential oil (%) of lemon verbena in the two seasons 2005/06 and 2006/07.

Drying methods	1 st season		2 nd season	
	June cut	Sep. cut	June cut	Sep. cut
Fresh (control)	0.5	0.42	0.6	0.7
Shade drying	1.2	1.49	1.7	1.6
Oven drying at 45 °C	0.80	1.19	1.2	1.5
L.S.D. at 5 %	0.24	0.23	0.24	0.21

2- Effect of storage period on the essential oil (%) in the two cuts:

In both June and September cuts, increasing storage period of the dried leaves of lemon verbena decreased the essential oil percentage in both drying methods, as shown in Figs. (1 & 2). In June cut, oil % was decreased after two months of storage by 0.6 and 0.2 % in shade dried leaves in the first and second seasons, respectively while was decreased by 0.2 and 0.1 % in oven dried leaves in the same respective order. In September cut, oil % was decreased after two months of storage by 0.37 and 0.3 % in shade dried leaves in the first and second seasons, respectively while was decreased by 0.49 and 0.28 % in oven dried leaves in the same respective order. One may conclude that September cut had more oil % than June cut, and that the longer the storage period, the less the oil that could be extracted from the

leaves. Similarly, Fehr (1980) mentioned that the oil of anise, caraway and fennel fruits was decreased by 1, 2.8 and 0.5% respectively when stored for a month. On contrary, Shalaby *et al.* (1988) reported that the essential oil content of the stored samples of *Mentha arvensis* did not change throughout the storage period. However, the loss of the essential oil from air dried plant material during storage depends on condition of material, method and conditions of storage, length of storage period, and the chemical composition of the oil (Fehr, 1980 and Kotb & Eid, 1996).

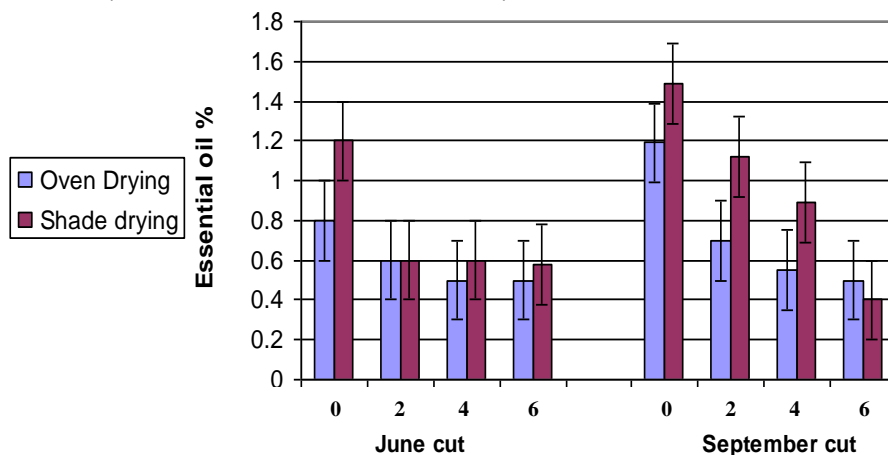


Figure 1: Effect of the drying method and storage period (months) on lemon verbena essential oil (%) in the two cuts of 2005/06 season.

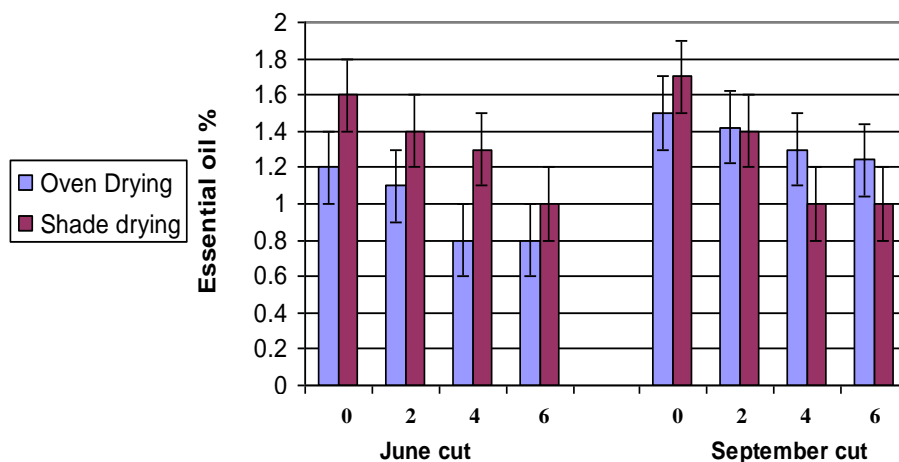


Figure 2: Effect of the drying method and storage period (months) on lemon verbena essential oil (%) in the two cuts of 2006/07 season.

III) Effect of drying method and storage period on the components of lemon verbena essential oil:

1. Effect of drying method on essential oil components in two cuts:

The results of G.L.C. analyses are shown in Table (3) and Figure (3) which show the effect of drying method on the essential oil constituents in June and September cuts. The G.L.C. of the essential oil of the fresh leaves revealed a total of 20 components. They are divided into 13 oxygenated compounds (1,8- cineole, α -thujone, linalool, spathulenol, terpinene 4-ol, geranyl acetate, α -terpinol, neral, citronellal, geranial, menthone, bornyl acetate, and pulegone) and 7 hydrocarbon compounds (α -pinene, β -pinene, curcumene, camphene, sabinene, myrcene, and limonene). Eighteen of these compounds were identified in fresh June oil and accounted 76 % of the oil, while twenty compounds were identified in fresh September oil and accounted 88 % of the oil. The identified compounds constituted 83.5 and 76.8 % in the oil of shade and oven dried leaves in June cut, while constituted 87.9 and 85.9 % in the same order in September oil. Some constituents were found in September oil but not in June oil as sabinene and curcumene were found in September oil, while were absent in June oil. Ten compounds in both cuts were found to constitute around 70 % of the total oil composition; 1,8-cineole, linalool, spathulenol, terpinene 4-ol, α - terpineol, neral, citronellal, geranial, limonene, and pulegone. Among these ten compounds, percentages of spathulenol, terpinene 4-ol, α -terpinol, neral, and pulegone were higher in June oil than in September oil, while the other 5 compounds were higher in September oil than June oil. The five top compounds in fresh June oil accounted 49.9 % of the oil as were spathulenol (14.6 %), linalool (13.4 %), geranial (8.9 %), neral (5.5 %), and limonene (7.6 %). The five top compounds in fresh September oil accounted 54.9 % of the oil as were linalool (15.3 %), limonene (11.2 %), geranial (9.5 %), spathulenol (9.3 %), and 1,8- cineole (8.9 %).

The method of drying affected the proportion of the oil components in the two cuts. As a result of drying the leaves, the results were inconsistent for the proportion of each constituent, since in some cases the percentage of one constituent increased, other component decreased, while were not markedly changed in others. In June cut, percentages of linalool, spathulenol, limonene, 1,8-cineol, and geranial were higher in the oil of shade dried leaves than in oven one, but pulegone, increased in proportion in dried leaves than in fresh ones, and in oven dried than in shade dried ones. In September oil, percentages of linalool, limonene, and geranial were higher in oil of the oven dried leaves than shade ones. However, in most cases, oil of shade dried leaves had higher percentages for most components. In June oil, the sum of percentages of 1,8-cineole, geranial, limonene, linalool, and spathulenol were 49, 61.5, and 49.2 % from fresh, shade, and oven dried leaves, respectively while were 54.3, 57.7, and 56.3 % in the same respective order in September cut.

These results are in agreement with those of Omidbaigi *et al.* (2004) who reported that the oil content of the shade dried flowers of Roman chamomile was the highest compared to sun-dried and oven-dried at 40°C. Asekun *et al.* (2007) reported that method of drying affected the oil composition of *Mentha longifolia*. In addition, the results are very similar to those of Figueiredo *et al.* (2004) who reported that the total main components in lemon verbena essential oil were 1,8- cineole, geranial, limonene, linalool, and spathulenol, and represented more than 50 % from the total components in lemon verbena essential oil.

2. Effect of storage period on lemon verbena essential oil components in two cutting dates:

Data in Table (4) and Figure (4) show the effect of storage period (2, 4 and 6 months) on the percentage of lemon verbena essential oil components of shade dried leaves of June cut.

Table (4): Effect of drying method and storage period on lemon verbena essential oil components (%) of June cut.

Components	Shade				Oven			
	Control	2 month	4 month	6 month	Control	2 month	4 month	6 month
α-pinene	----	1.42	1.62	2.50	2.20	2.54	2.92	0.82
β-pinene	0.02	4.73	5.45	5.67	2.24	3.42	3.79	8.38
1,8-cineole	9.54	8.39	7.97	7.58	6.08	7.43	7.03	4.00
Bornyl acetate	1.27	1.22	1.09	0.09	1.08	1.06	0.97	0.05
Camphene	----	2.29	2.35	4.75	----	-----	4.21	---
Citronellal	----	2.40	2.92	5.47	2.06	2.67	5.52	1.07
Geranial (citrals b)	8.88	6.50	4.77	2.60	7.43	4.28	3.63	2.16
Geranyl acetate	2.75	0.88	0.66	0.60	0.25	0.55	0.66	0.79
Limonene	12.61	8.55	7.87	6.57	10.2	6.85	6.27	5.19
Linalool	12.70	13.0	15.98	18.62	11.31	12.97	14.03	17.19
Menthone	0.71	0.99	1.03	0.40	0.66	1.62	0.94	0.59
Myrcene	4.18	0.15	0.13	0.03	0.17	0.04	0.02	0.02
Neral (citrals a)	3.73	3.09	2.50	1.92	4.27	3.24	2.65	2.63
Ocimene	----	1.78	1.84	1.32	-----	2.67	2.02	0.40
Pulegone	6.39	6.36	5.21	1.59	9.59	8.09	7.13	6.68
Sabinene	----	0.57	0.69	0.87	----	0.22	0.23	1.50
Spathulenol	10.9	11.45	14.5	16.33	10.9	12.29	12.35	13.44
Terpinene 4-ol	2.39	4.19	4.07	4.21	3.02	5.57	4.06	3.75
α-terpineol	0.83	2.17	1.61	1.19	2.26	1.69	1.31	2.52
α-thujone	1.53	1.29	1.19	0.75	0.88	1.04	1.06	6.84

Data revealed that the relative concentrations of each constituent changed with the length of the storage period. The compounds could be divided into four groups in their response to extending the storage period. The first group was the compounds that decreased in their percentages with increasing the storage period; myrcene, bornyl acetate, 1,8- cineole, neral, geranial and limonene. The second group included the compounds that increased in percentage with extending storage period; β-pinene, linalool, pulegone, spathulenol, and terpinene 4-ol. The third group included compounds that were not found in the control and were presented after

storage and continued to increase with extending the storage period. This group included in addition to camphene, ocimene and sabinene (were not present in both controls of room and oven), α -pinene and citronellal (were not present in the control of room oil only). The last group included compounds that either have irregular trend, or their percentages were slightly affected by the storage period as geranyl acetate, menthone, α -terpinol, and α -thujone.

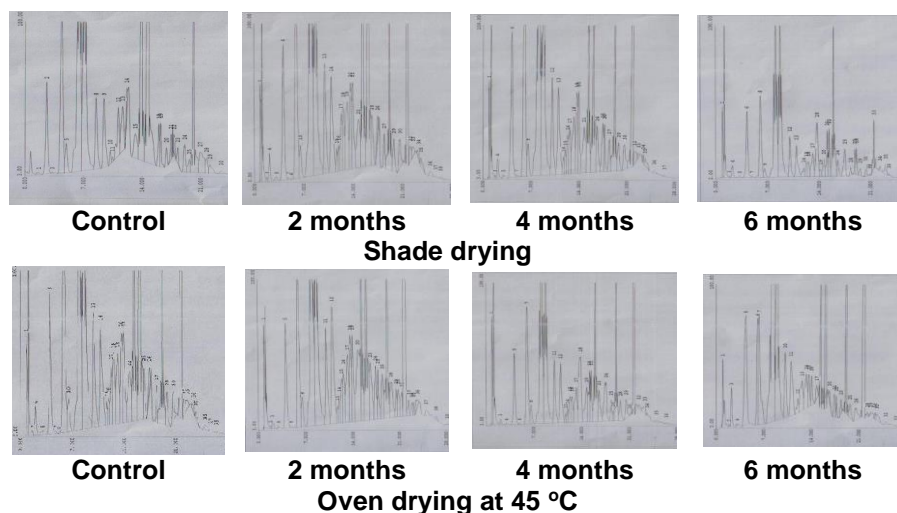


Fig (4): G.L.C. of dried lemon verbena essential oil at shade and oven at 45 °C in June cut.

Data in Table (5) and Figure (5) show the effect of storage period (2, 4 and 6 months) on the percentage of lemon verbena essential oil components of shade dried leaves of September cut.

In September cut, constituents were divided into three groups. The first group was those compounds that their percentages decreased with extending the storage period; β -pinene, 1,8-cineole, geranial, limonene, α -terpineol and α -thujone. The second group were constituents that increased in percentages with extending the storage period; pulegone, spathulenol, and terpinene 4-ol. This group included also α -pinene, camphene, linalool, and neral, but linalool and α -pinene increased in their percentages for four months of storage and decreased thereafter, camphene increased for four months and disappeared from the oil after six months of storage, while neral increased after two months only of storage and then decreased thereafter. The third group was compounds that vanished after storage; citronellal and curcumene. The last group included compounds that either have irregular trend, or their percentages were slightly affected by the storage period; bornyl acetate, geranyl acetate, menthone, myrcene, and sabinene. The previous results by Singh *et al.* (1994) are in agreement with the results of this work, since they showed that the essential oil composition during storage changed with length

of the storage period; some increased, some decreased, while others remained constant.

Table (5): Effect of drying method and storage period on lemon verbena essential oil components (%) of September cut.

Contents	Shade				Oven			
	Control	2 month	4 month	6 month	Control	2 month	4 month	6 month
α -pinene	1.63	2.39	4.10	0.97	1.38	1.82	1.84	1.56
β - pinene	5.01	5.59	4.88	2.40	4.64	6.80	4.88	4.77
1,8-cineole	9.49	9.48	8.06	6.20	9.17	11.26	8.19	6.61
Bornyl acetate	0.56	1.12	1.61	0.50	0.83	1.24	0.90	0.66
Camphene	2.12	2.62	3.84	-----	2.70	2.98	2.40	-----
Citronellal	5.12	----	---	----	5.52	---	----	---
Curcumene	2.69	----	---	----	2.14	---	----	---
Geranial (citral b)	8.83	7.53	6.33	6.27	9.57	8.04	7.53	6.86
Geranyl acetate	0.34	0.34	0.17	0.06	0.39	0.48	0.50	0.13
Limonene	12.27	9.53	7.19	6.61	12.45	9.36	8.61	6.92
Linalool	13.05	15.49	15.51	12.47	15.58	16.64	14.03	12.78
Menthone	0.93	0.95	1.36	0.77	0.96	1.32	0.87	0.82
Myrcene	0.02	0.09	0.17	0.09	0.02	0.12	0.13	0.09
Neral (citral a)	2.78	3.15	1.72	1.29	2.57	3.19	2.90	1.93
Pulegone	1.51	6.91	6.68	5.94	1.37	5.36	3.99	1.55
Sabinene	0.82	0.62	0.68	0.53	0.50	0.82	0.46	0.54
Spathulenol	9.54	12.39	12.96	14.01	9.53	10.84	12.36	13.48
Terpinene 4-ol	2.75	2.84	3.22	3.51	2.85	3.88	5.26	7.36
α -terpineol	1.61	1.22	1.00	0.80	1.50	1.42	1.23	0.98
α -thujone	2.39	1.50	1.09	0.97	2.19	1.90	1.29	0.92

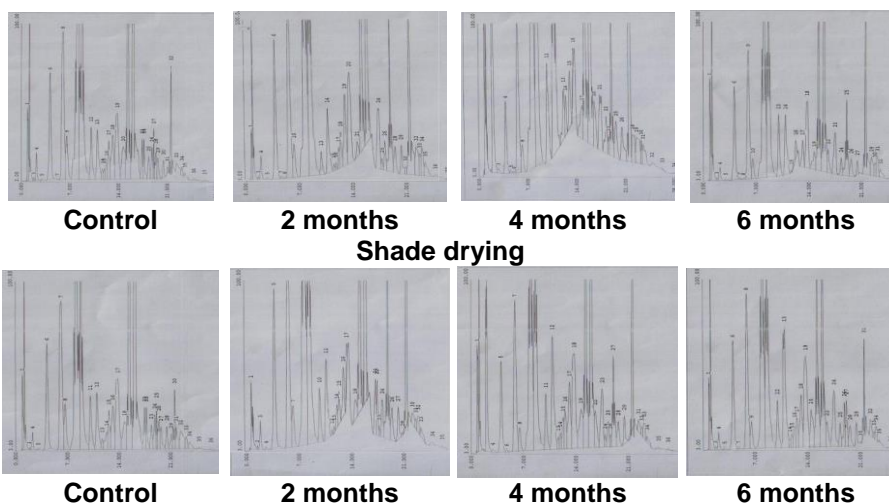


Fig (5):G.L.C. of dried lemon verbena essential oil at shade and oven at 45 °C in September cut.

It could be concluded that in both cuts (June and September) drying leaves of lemon verbena in the shade is recommended for obtaining higher essential oil quantity and quality. Also increasing the storage period from 2 to 6 months caused a decrease in the essential oil % and that its components changed with the length of storage period.

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تأثير موعد القطف وطريقة التجفيف وفترة التخزين على نسبة وتركيب الزيت العطري في أوراق نبات اللوزا (الفربيانا الليمونية)

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نبات اللوزا شجيرة متساقطة الأوراق موطنها الأصلي الأرجنتين وشيلي وتعرف باسم عشب اللوزا أو الفربيانا الليمونية ، وهي نبات طبي وعطري هام يحتوي على زيت طيار عالي الجودة له رائحة الليمون ، وهو مضاد للفطريات ويستخدم كمهدئ و كمضاد للتقلصات كما يستخدم لإعطاء النكهة للأطعمة والمشروبات. ولقد تم عمل دراسة خلال الموسمين الزراعيين المتتاليين ٠٦/٢٠٠٥ و ٠٧/٢٠٠٦ في المزرعة التجريبية للنباتات الطبية والعطرية بكلية الزراعة جامعة المنصورة ، ويهدف البحث إلى دراسة تأثير طريقتا التجفيف (الظل في الغرفة والفرن على ٤٥ م) وطول فترة التخزين (٢ و ٤ و ٦ شهور) على نسبة وتركيب الزيت العطري بالأوراق في مواعيد للحصاد (يونيو وسبتمبر) ، كما تضمنت الدراسة تقدير الخواص الطبيعية والكيميائية للزيت.

ويمكن تلخيص النتائج المتحصل عليها في التالي:-

- أظهرت النتائج أن نسبة الزيت في قطفة سبتمبر أعلى من قطفة يونيو في كلا الموسمين. ووجد أن أعلى نسبة زيت تم الحصول عليها من الأوراق التي تم تجفيفها في الظل ثم تلاها الأوراق التي جففت في الفرن بالمقارنة مع الأوراق الطازجة، كما أدت زيادة فترة تخزين الأوراق إلي نقص نسبة الزيت في طريقتي التجفيف في يونيو و سبتمبر.
- أوضح التحليل الكروماتوجرافي للزيت الناتج من الأوراق الطازجة التعرف على ٢٠ مركبا ، وقد شكلت المركبات التي تم التعرف عليها ٨٣,٥ و ٧٦,٨ % من زيت الأوراق المجففة في الظل والفرن على الترتيب في زيت يونيو ، بينما كانت ٨٧,٩ و ٨٥,٩ % بنفس الترتيب في زيت سبتمبر. وقد كانت أهم المركبات التي تم التعرف عليها في كلتا القطفتين هي سينول، جيرانيال، ليمونين، لينالول، سباتولينول وبلغ مجموع نسبتها ٤٩ % من زيت يونيو و ٥٤,٣ % في زيت سبتمبر.
- أثرت طريقة التجفيف في نسبة مكونات الزيت في كلتا القطفتين. ولقد تغيرت نسبة كل مركب مع زيادة فترة التخزين حيث زادت نسبة بعض المركبات (لينالول وسباتولينول)، في حين إنخفضت في البعض (سينيول وجيرانيال وليمونين)، ولم تتغير إلا بنسبة طفيفة في البعض الأخر(جيرانيال أسيتات ومنتون). بعض المركبات لم تكن موجودة في عينة الكونترول في زيت يونيو مثل كامفين وأوسيمين وسابينين وألفا-باينين ولكنها ظهرت بعد التخزين، إلا أن سترونيلال والكامفين كانا موجودين في عينة الكونترول زيت قطفة سبتمبر ولكنهما إختفيا بعد التخزين.

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