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Research Article

The Essential Oil Components in Lemon Verbena Significantly Vary According to Diurnal Variations and Drying Process

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ABSTRACT

This study investigated the effects of harvesting time, dried, and or fresh leaf on essential oil components in lemon verbena (*Lippia citriodora* H.B.K.). Essential oil was obtained from fresh and dried leaves harvested at different times using the hydro-distillation method. The essential oil components were determined using a GC-MS device. A two-way analysis of variance (ANOVA) was applied to the essential oil components determined at all harvesting times (HT), on dry or fresh (DF) leaves. Except for Myrcene and Guaiol, the essential oil content of Limonene, ß-Citral, α-Citral, Caryophyllene oxide, ß-Guaiene, Geranyl acetate, Prenylacetone, Linalool, Verbenol, Humulene, Curcumene, Carvone, Nerolidol, Spathulenol, and α-Cadinol were significantly affected by the HT×DF interactions ($P<0.05$). It was found that especially for the cosmetic industry, Limonene should be obtained from fresh leaves harvested at 12(pm), β-Citral from fresh leaves harvested at 12(am), and α-Citral from dry leaves harvested at 6(pm), which are active compounds derived from the volatile oil of lemon verbena. According to the PCA results obtained from the study, we can clearly state that harvesting time has differently influenced each active compound in the essential oil of lemon verbena. This study has provided important insights into the application areas of the active compounds derived from the essential oil of lemon verbena.

Keywords: Lemon verbena, essential oil components, harvesting times, PCA.

1. INTRODUCTION

The genus *Lippia* (Verbenaceae), which includes about 200 species, is native to South and Central America as well as Africa[1]. Lemon verbena, *Lippia citriodora* H.B.K. (Syn: Aloysia triphylla), is a medicinal and ornamental shrub primarily cultivated for the lemon-like aroma emitted from its leaves. The principal medicinal component of lemon verbena comprises its leaves, which contain between 0.9% and 1.5% of the total essential oil, composed primarily of citral, neral, and limonene[2]. The essential oil of lemon verbena is utilized in the fields of perfumery and cosmetics, as well as in antimicrobial and insecticidal activities[3,4,5,6]. The antioxidant activity of lemon verbena leaf extracts has garnered significant interest in the food industry[7,8].

In general, medical and aromatic plants are genetically controlled in terms of secondary metabolism and biosynthetic activity. However, these plants are significantly influenced by factors such as environment, climatic conditions, geographic location, agronomic conditions, growth stages, and harvesting[9]. In addition to this information, various studies have reported that the essential oil contents of some medicinal and aromatic plants may vary according to daily changes (temperature, light, etc.)[10,11,12]. Determining the harvest times during the day is crucial for the volatile oil content, depending on the intended use (medicinal, culinary, perfumery, plant protection, etc.) of medicinal and aromatic plants[13]. Indeed, some studies have reported that the essential oil contents of certain medicinal plants vary according to the harvest times

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during the day[14,15,16,17]. Therefore, in the present study, we investigated the variations in the essential oil content of Lippia citriodora H.B.K. leaves, both dried and fresh, according to the harvest time of the day.

2. MATERIALS and METHODS

2.1. Plant Material

The leaves of lemon verbena, aged 6-7 years, cultivated for landscaping purposes on the campus of Hatay Mustafa Kemal University (36°19'N 36°11'E), were manually harvested during the flowering period. Harvests were conducted at 6 am, 12 pm, 6 pm, and 12 am to determine the variation in the content of the plant essential oil based on the time of harvest during the day.

2.2. Obtaining Essential Oils

The fresh leaves were divided into 6 separate samples, each consisting of randomly selected 100 g of leaves. Three fresh leaf samples were dried at 35°C in a drying cabinet to obtain dried leaves. The other three fresh leaf samples were immediately used to obtain essential oil. In the hydro-distillation method, Neo-Clevenger apparatus was used, and the hydro-distillation was carried out for 4 hours.

2.3. GC/MS Analysis

The analysis of the main components of the essential oils of each sample was performed using Thermo Scientific ISQ Single Quadrupole Gas Chromatography-Mass Spectrometry device (Milan, Italy) fitted with TR-FAME capillary column (5% Phenyl Polysilphenylene-siloxane, 60 m × 0.25 mm i.d. × 0.25 µm film thickness). Helium (99.9%) was used as the carrier gas at a flow rate of 1 ml/min. Mass spectra were recorded at 70 eV, the with a mass range was from 1.2 to 1,200 m/z. Scan Mode was used for data collection. MS transfer line, ionization source and the injection port temperatures were 250, 230, and 220 ℃, respectively. The samples were injected with a 150-split ratio. The injection volume was 1 µl. The oven temperature was initially 50 ℃, increased to 220 ℃ at a rate of 3 ℃ min^{-1}.

2.4. Data Analysis

In the present study, Grubbs test was conducted to check for outlier variability in some major and minor essential oil content data obtained from the study, followed by the application of two-way ANOVA test via XLSTAT. As a result of this test, Tukey pairwise grouping was conducted on the essential oil contents found to be significant ($P < 0.05$). In addition, principal component analysis was performed to major and minor essential oil components according to harvesting times.

3. RESULTS and DISCUSSION

3.1. Effects of Main Factors on Essential Oil Components in Lemon Verbena

The results showing the effects of harvest times (HT) and dry or fresh (DF) leaf treatments on certain major and minor essential oil contents of lemon verbena are presented in Table 1. The effect of HT and DF on the characteristics of Limonene, ß-Citral, α-Citral, Caryophyllene oxide, Geranyl acetate, Linalool, Humulene, Curcumene, Carvone, and Spathulenol was found to be significant ($P < 0.01$). The effect of DF was significant for ß-Guaiene ($P < 0.01$), Prenylacetone ($P < 0.01$), Nerolidol ($P < 0.01$) and α-Cadinol ($P=0.02$), while the effect of HT was not significant. For the Verbenol content, DF was not significant, while HT was significant ($P = 0.02$). Both the HT and DF factors did not have a significant effect on the essential oil contents of Myrcene and Guaiol. The highest essential oil content of Limonene was determined at 12(pm), while the lowest was observed at 12(am). It was determined that fresh leaves in the DF treatments offered a higher content of Limonene and ß-Citral. In contrast to the Limonene content, it was found that in the HT treatments, the ß-Citral and α-Citral content was highest at 12(am) and lowest at 12(pm). In contrast to the content of Limonene and ß-Citral, the highest content of α-Citral and Caryophyllene oxide in the DF treatments was found in dry leaves. In the HT treatments, the highest content of Caryophyllene oxide and ß-Guaiene emerged at 12(pm), while the lowest content of Caryophyllene oxide and ß-Guaiene was observed at 6(am) and 6(pm), respectively. In the HT treatments, the highest Geranyl acetate content was determined at 6(pm), while the lowest Geranyl acetate content was found at 12(pm). Between the DF applications, similar to the Limonene and ß-Citral contents, the highest essential oil contents of Geranyl acetate, Linalool, Carvone, and Spathulenol were obtained from fresh leaves. The effect of HT treatments resulted in the highest essential oil contents of Prenylacetone and Linalool at 6(pm), while the lowest was found at 6(am). With the DF treatments, the highest contents of Verbenol, Humulene, and Curcumene were obtained from dry leaves. The effect of HT applications resulted in the highest Humulene content at 6(am), while the lowest was observed at 12(pm). In the HT, the highest Curcumene and Carvone contents were determined at 12(pm), while the lowest was observed at 6(pm). In the HT applications, the highest Nerolidol and α-Cadinol contents were found at 6(pm), while the lowest were observed at 12(pm). The highest Spathulenol content occurred at 6(am), while the lowest was observed at 6(pm). Similar to the results obtained from present study, it has been reported in several studies that the essential oil contents vary according to daily changes in many medicinal and aromatic plants[10,14,15,18,19,20]. In addition, Dos Santos et al. (2019)[18] have indicated that

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the essential oil contents vary between dry and fresh leaves, similarly. Soltanbeigi and Maral (2025)[21] reported that distillation time and whether the plant material is fresh or dried are critical factors affecting both the yield and chemical composition of essential oils. In their study, they found that drying significantly increased the essential oil content (2.06 to 2.46 times) but did not significantly affect the total yield

3.2. Effects of Interactions (HT×DF) on Essential Oil Components in Lemon Verbena

Interaction results of Limonene, β-Citral, α-Citral and Caryophyllene oxide are given in Figure 1. The highest Limonene, β-Citral, α-Citral and Caryophyllene oxide were obtained from fresh leaves harvested at 12(pm), fresh leaves harvested at 12(am), dry leaves harvested at 6(pm) and fresh leaves harvested at 12(pm), respectively. Among these results, the highest Limonene and Caryophyllene oxide was determined similar interactions. Interaction results of β-Guaiene, Geranyl acetate, Prenylacetone and Linalool are given in Figure 2. The highest β-Guaiene content among the interactions was in fresh leaves harvested at 12(pm) whereas the lowest was in fresh leaves harvested at 6(pm). In terms of Geranyl acetate, highest value was determined in fresh leaves harvested at 12(am). Yet, similar result was obtained from fresh leaves harvested at 6(pm). The highest Prenylacetone was determined in dry leaves harvested at 12(pm) while the highest Linalool was in fresh leaves harvested at 6(pm). Interaction results of Verbenol, Humulene, Curcumene and Carvone are given in Figure 3. The highest values for these four essential oil contents were determined in different interaction treatments. The highest Verbenol, Humulene and Curcumene was found in dry leaves harvested at 6(pm), 6(am) and 12(pm), respectively. The highest Carvone was obtained from fresh leaves harvested at 12(pm). Interaction results of Nerolidol, Spathulenol and α-Cadinol are given in Figure 4. The highest Nerolidol and α-Cadinol contents was found in fresh and dry leaves harvested at 6(pm). The highest Spathulenol was obtained from fresh leaves harvested at 6(am). In general, all interaction results were different from each other in terms of all investigated essential oil components. Some studies have reported that essential oil components are affected by daily variations and whether the leaves are dry or fresh, showing differences[14,15,18]. Indeed, similar results were obtained from the present study as well.

Table 1. Changes of major and minor essential oil components in *Lippia citriodora* H.B.K. according to main effects.

ITEMS	Harvesting times (HT)				Dry or Fresh (DF)		SEM			P values		
	12$^{(am)}$	6$^{(am)}$	12$^{(pm)}$	6$^{(pm)}$	Dry	Fresh	HT	DF	HT×DF	HT	DF	HT×DF
Limonene	24.50^{d}	29.46^{b}	31.82^{a}	26.53^{c}	27.01^{b}	29.14^{a}	0.13	0.09	0.19	<.01	<.01	<.01
ß-Citral	15.07^{a}	11.41^{c}	10.48^{d}	14.76^{b}	12.76^{b}	13.10^{a}	0.07	0.05	0.09	<.01	<.01	<.01
α-Citral	18.25^{a}	14.95^{c}	13.59^{d}	17.87^{b}	16.33^{a}	16.00^{b}	0.04	0.03	0.06	<.01	<.01	<.01
Caryophyllene oxide	8.46^{c}	7.50^{d}	12.49^{a}	8.78^{b}	9.94^{a}	8.67^{b}	0.07	0.05	0.10	<.01	<.01	<.01
ß-Guaiene	6.40^{ab}	6.38^{b}	6.64^{a}	5.95^{c}	6.36	6.32	0.06	0.04	0.08	<.01	0.56	<.01
Geranyl acetate	2.09^{b}	1.91^{c}	1.82^{d}	2.17^{a}	1.82^{b}	2.17^{a}	0.02	0.01	0.03	<.01	<.01	<.01
Myrcene	0.23	0.38	0.31	0.30	0.33	0.28	0.06	0.04	0.08	0.71	0.36	0.93
Prenylacetone	0.66^{bc}	0.61^{c}	0.79^{ab}	0.89^{a}	0.73	0.75	0.04	0.03	0.06	<.01	0.63	<.01
Linalool	0.56^{bc}	0.52^{c}	0.75^{ab}	0.87^{a}	0.59^{b}	0.76^{a}	0.05	0.04	0.07	<.01	<.01	<.01
Verbenol	0.36	0.28	0.27	0.37	0.35^{a}	0.29^{b}	0.02	0.02	0.03	0.40	0.02	0.03
Humulene	0.52^{b}	0.62^{a}	0.41^{c}	0.56^{ab}	0.60^{a}	0.46^{b}	0.02	0.01	0.03	<.01	<.01	<.01
Curcumene	5.11^{ab}	4.98^{b}	5.24^{a}	4.00^{c}	4.92^{a}	4.74^{b}	0.04	0.03	0.06	<.01	<.01	<.01
Carvone	0.53^{b}	0.36^{c}	0.63^{a}	0.33^{c}	0.36^{b}	0.57^{a}	0.02	0.01	0.03	<.01	<.01	<.01
Nerolidol	0.25^{ab}	0.23^{b}	0.20^{b}	0.31^{a}	0.23	0.26	0.02	0.01	0.03	<.01	0.12	0.03
Guaiol	0.14	0.13	0.15	0.13	0.13	0.15	0.01	0.01	0.02	0.93	0.23	0.97
Spathulenol	0.53^{b}	0.68^{a}	0.41^{c}	0.35^{c}	0.46^{b}	0.52^{a}	0.02	0.01	0.02	<.01	<.01	<.01
α-Cadinol	0.31^{b}	0.34^{ab}	0.31^{b}	0.39^{a}	0.35	0.33	0.02	0.01	0.02	0.02	0.24	0.02

3.3. Evaluation of Harvesting Times (HT) with PCA

As seen in Figure 5, it was observed that the variance was significantly reduced with PC-F1 (55.22%) and PC-F2 (25.25%), while with PC-F3 (19.53%), the variance was completely eliminated. PC-F1 and PC-F2 together account for 80.47% of the total variance (Figure 6). The levels of certain major and minor essential oil components and harvesting times are presented in Figure 6. Upon examining the graph, it was observed that the harvesting times are distributed across all four quadrants. From this observation, it can be inferred that all harvesting times affect the examined essential oil components differently. The PCA analysis clearly demonstrated that each essential oil may vary according to the harvesting time.

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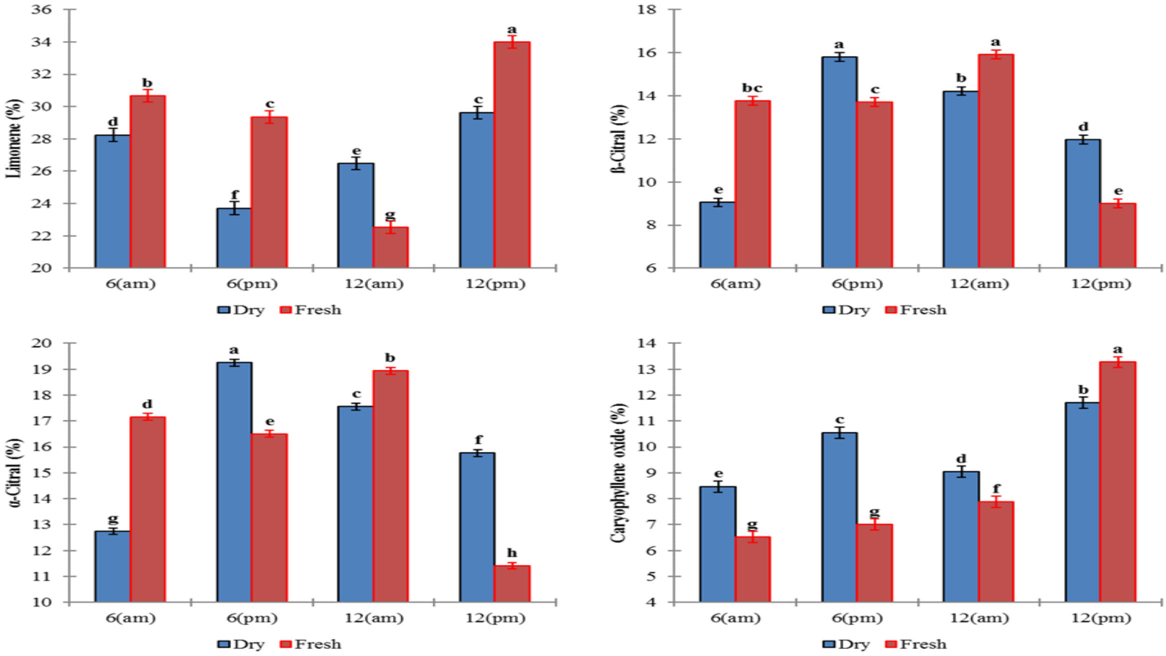

Figure 1. Changes of Limonene, β-Citral, α-Citral and Caryophyllene oxide in *Lippia citriodora* H.B.K. according to interaction effects.

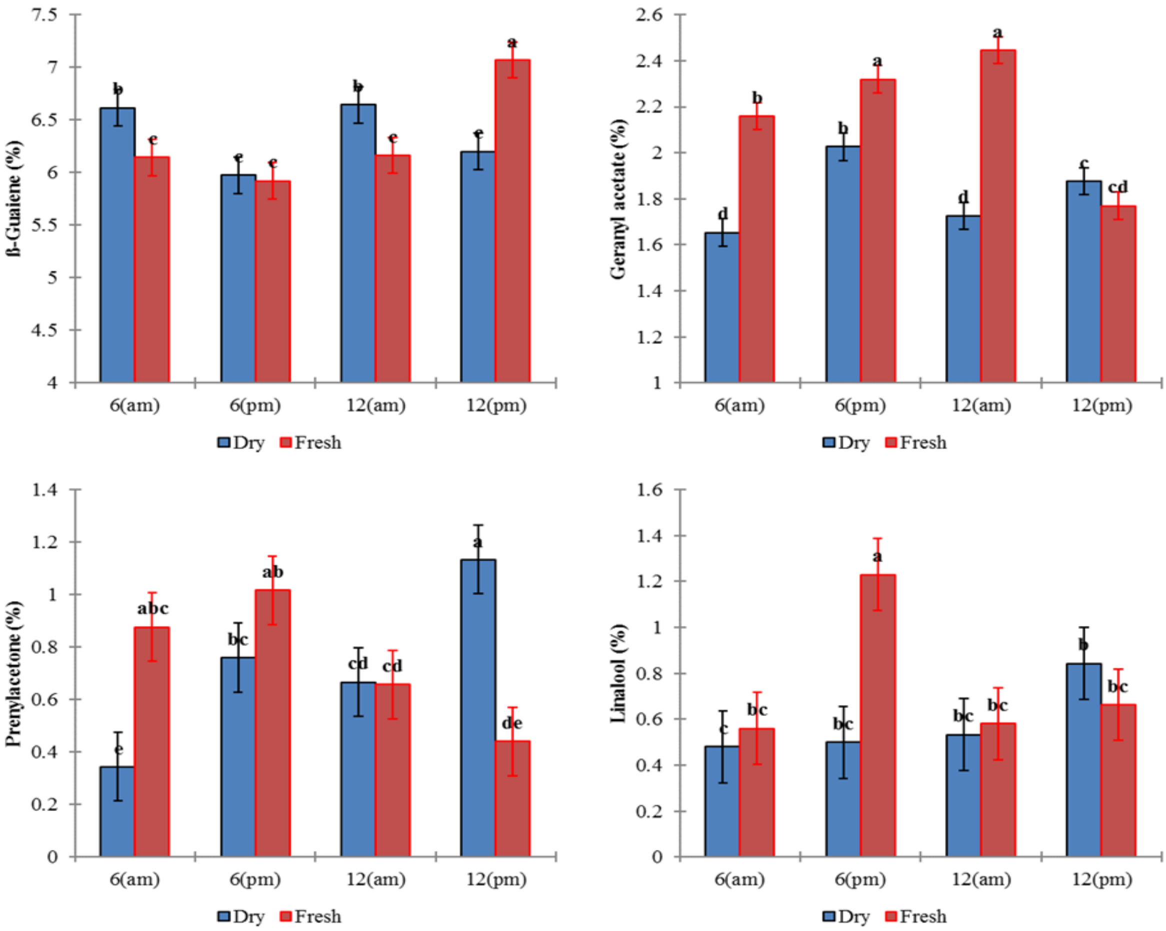

Figure 2. Changes of β-Guaiene, Geranyl acetate, Prenylacetone and Linalool in *Lippia citriodora* H.B.K. according to interaction effects.

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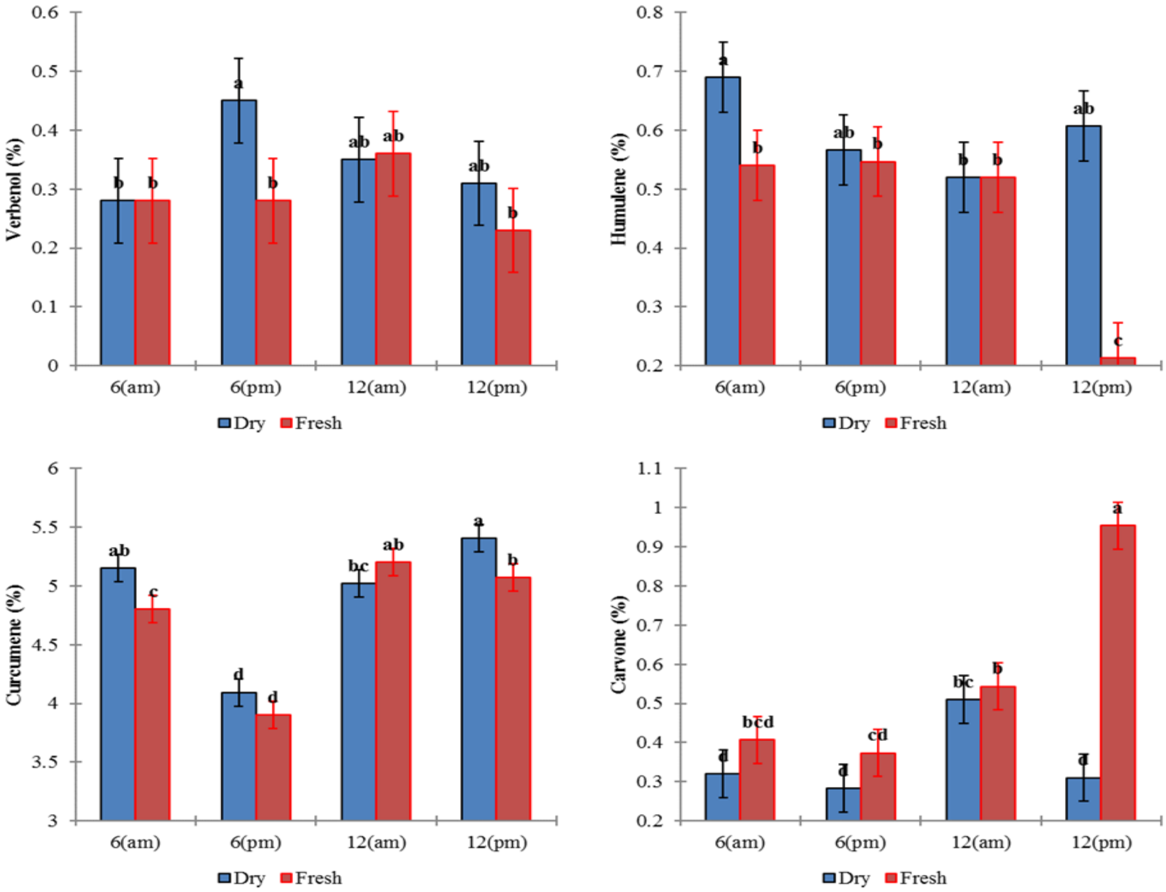

Figure 3. Changes of Verbenol, Humulene, Curcumene and Carvone in *Lippia citriodora* H.B.K. according to interaction effects.

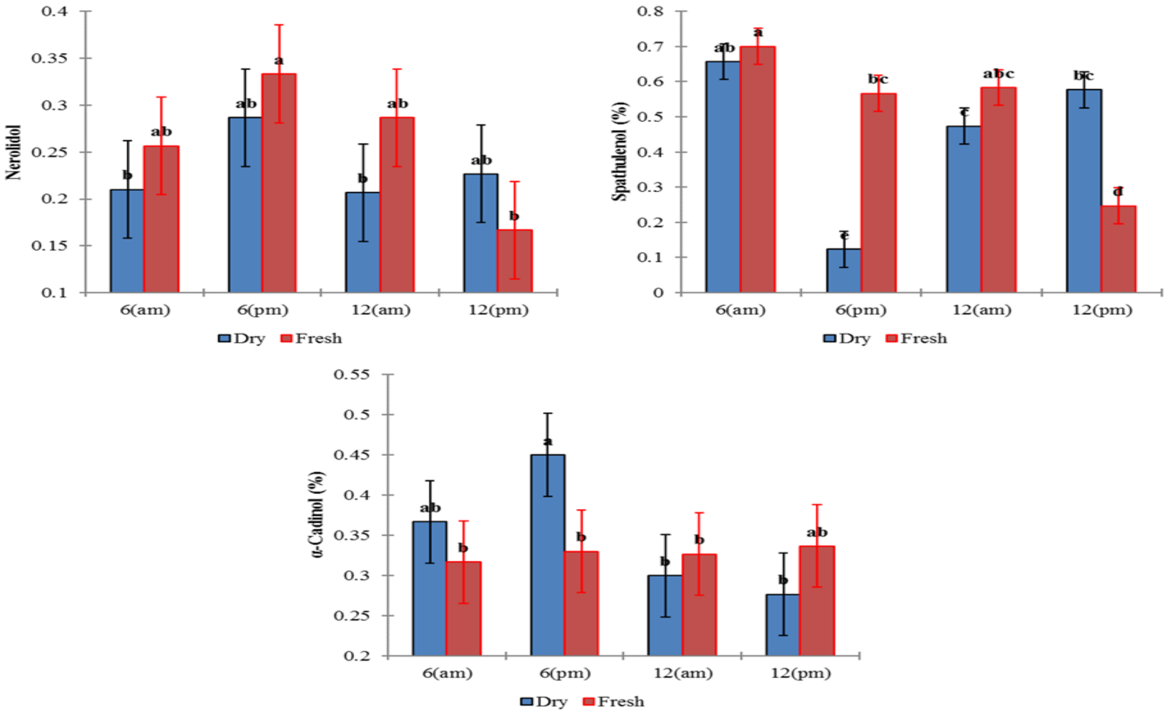

Figure 4. Changes of Nerolidol, Spathulenol and α-Cadinol in *Lippia citriodora* H.B.K. according to interaction effects.

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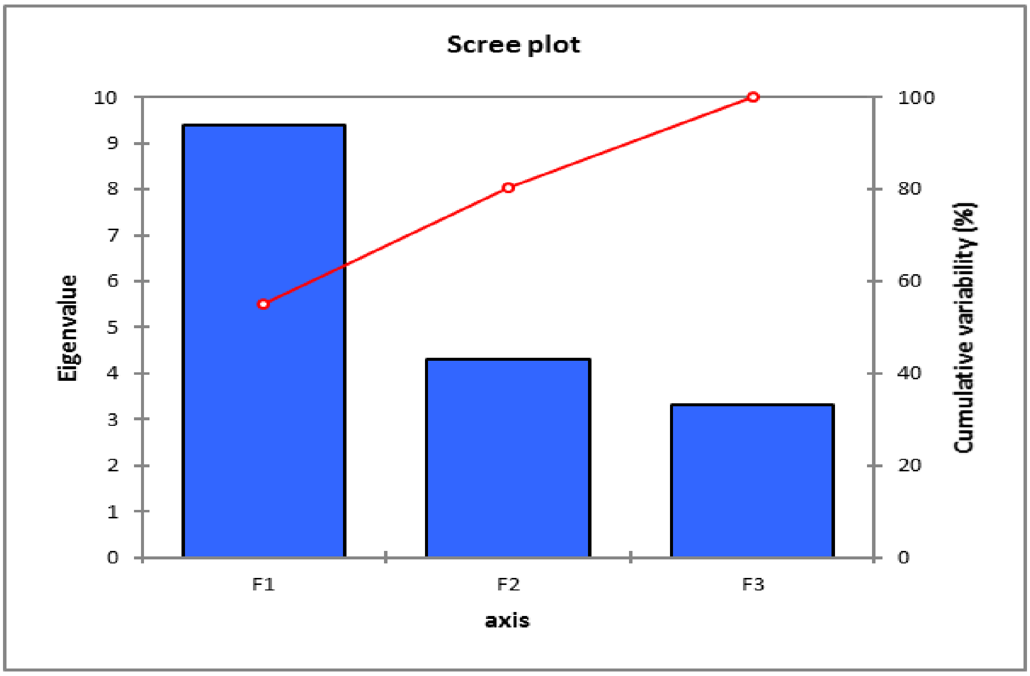

Figure 5. Principle component factors based on eigenvalues bar chart.

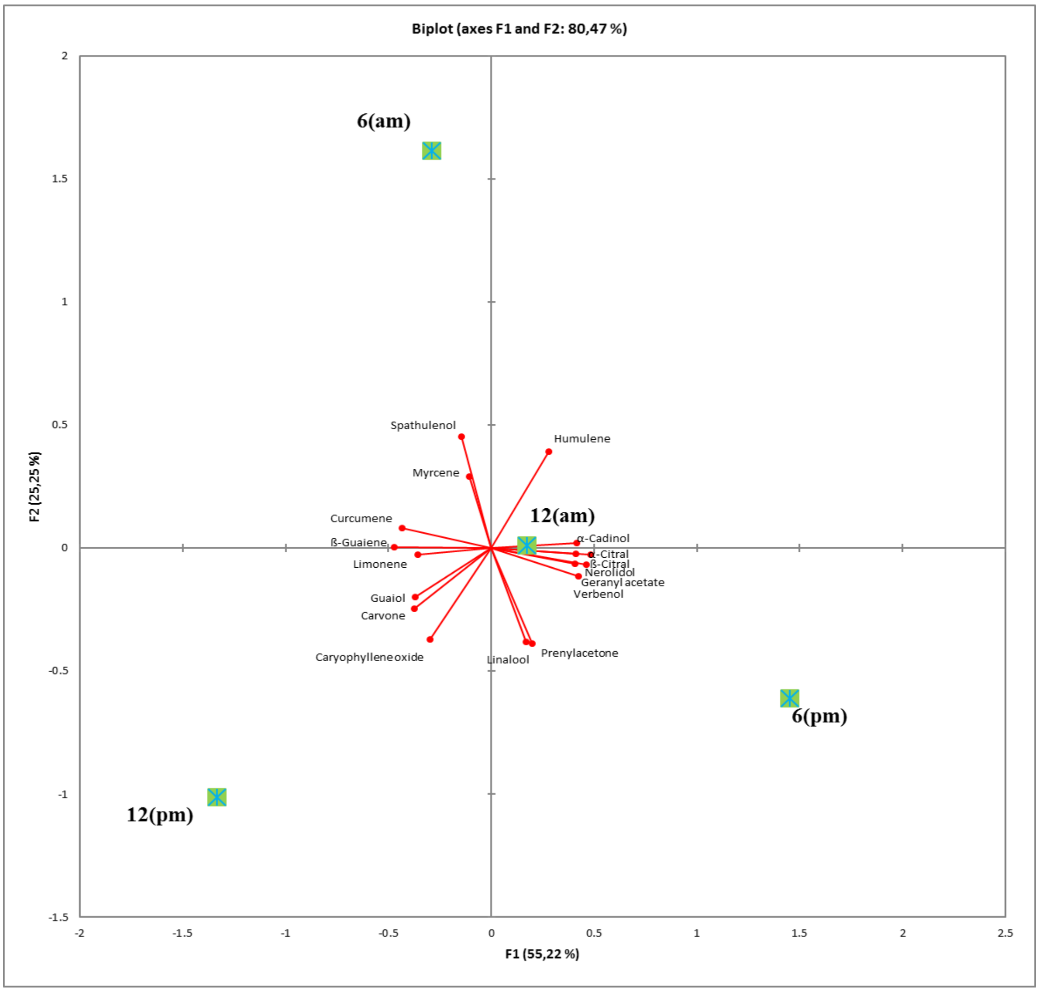

Figure 6. Biplot distribution graph of principal component analysis for major and minor essential oil components obtained from in *Lippia citriodora* H.B.K. harvested at different times in a day.

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4.CONCLUSION

According to the results obtained from the study, it was determined that the essential oil components in lemon verbena significantly vary based on the harvesting times and whether the leaf samples are fresh or dried. It was found that especially for the cosmetic industry, Limonene should be obtained from fresh leaves harvested at 12(pm), β-Citral from fresh leaves harvested at 12(am), and α-Citral from dry leaves harvested at 6(pm), which are active compounds derived from the volatile oil of lemon verbena. According to the PCA results obtained from the study, we can clearly state that harvesting times have different influence on each active compound in the essential oil of lemon verbena. This study has revealed key insights into the extraction and application of the active compounds from lemon verbena essential oil.

Acknowledgements

This study was conducted in Hatay, which was most affected by the Kahramanmaraş earthquake on February 6, 2023, and we would like to dedicate this study to people we lost in the earthquake.

Conflict of Interest

The authors declare that there is no conflict of interest with any person, institution, or company.

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