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Evaluation of monthly changes in essential oil yield and components of cherry laurel (*Prunus laurocerasus* L.) leaf

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SUMMARY: This research was carried out to examine the monthly changes in both the volatile oil content and volatile components of cherry laurel leaves by taking samples every month for 12 months in Turkey. Harvest periods significantly affected volatile oil content ($P < 0.01$). Depending on harvest periods, volatile oil ratios ranged from 0.19 to 0.35%. The months of August, July, and September yielded the highest volatile oil ratios. Benzaldehyde, phenol, benzoic acid, benzeneacetonitrile, pentadecanone, 1,54-dibromotetrapentacontane and, tetrapentacontane were determined as components in the volatile oil. The ratio of benzaldehyde, the main active ingredient, varied between 83.89 and 94.41%, depending on the harvest time. The cherry laurel leaf should be harvested in July, August, and September for high essential oil ratios and in May, June, and July for high benzaldehyde ratios. Due to the high concentration of benzaldehyde in its volatile oil, cherry laurel evergreen leaf can be considered a valuable source of raw materials for the fragrance and pharmaceutical sectors.

KEYWORDS*:* *Cherry laurel; Monthly variation; Volatile components; Volatile oil content.*

RESUMEN: ***Evaluación de cambios mensuales en el rendimiento de aceite esencial y componentes de la hoja de laurel cereza (*****Prunus laurocerasus** ***L.).*** Esta investigación se llevó a cabo para examinar los cambios mensuales tanto del contenido de aceite volátil como de los componentes volátiles de las hojas de laurel cereza tomando muestras cada mes durante 12 meses en Turquía. Los períodos de cosecha afectaron significativamente al aceite volátil ($P<0.01$). Dependiendo de los períodos de cosecha, las proporciones de aceite volátil oscilaron entre 0,19% y 0,35%. Agosto, julio y septiembre arrojaron los mayores índices de volatilidad del aceite. Como componentes del aceite volátil se determinaron benzaldehído, fenol, ácido benzoico, bencenoacetonitrilo, pentadecanona, 1,54-dibromotetrapentacontano y tetrapentacontano. La proporción de benzaldehído, principal ingrediente activo, osciló entre el 83,89% y el 94,41% según la época de cosecha. La hoja de laurel cereza debe cosecharse en julio, agosto y septiembre para obtener proporciones altas de aceites esenciales y en mayo, junio y julio para proporciones altas de benzaldehído. Debido a la alta concentración de benzaldehído en su aceite volátil, la hoja perenne de laurel cereza puede considerarse una valiosa fuente de materias primas para los sectores farmacéutico y de fragancias.

PALABRAS CLAVE: *Componentes volátiles; Contenido de aceites volátiles; Laurel cereza; Variación mensual.*

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1. INTRODUCTION

Turkey is a crucial region for the diversity of plants. Many diverse local or native fruit species are recognized, and many different fruit species are farmed. Cherry laurel, one of the plants originating in Turkey, is a valuable ornamental plant with attractive dark and evergreen leaves and white flower clusters in spring (Islam, 2002). The fruit of the cherry laurel is its most utilized component. Cherry laurel fruit has reportedly been shown to be superior to other fruits in terms of its strong antioxidant capacity, bioactive components, phenolic compounds, flavonoids, fatty acids, and some mineral substances, especially its vitamin C content (Çalışır ve Aydın, 2004). In addition to its direct use as food, its fruit is also used in traditional Turkish medicine to treat a range of diseases. It has been reported that the naturally occurring chemical components of cherry laurel fruit have significant potential for the treatment of cancer, cardiovascular, chronic, and neurodegenerative diseases (Çalışır and Aydın, 2004).

The medicinal antioxidant content in the leaves of the cherry laurel is greater than that of the fruit. Cherry laurel leaves are dried and made into tea in Turkey, and this tea is used to treat neurological disorders in Anatolia (Karataş and Uçar, 2018). In a study examining the effects of cherry laurel leaf extracts on cholinesterase, it was observed that these extracts effectively inhibited cholinesterase enzymes and showed neuroprotective properties with their antioxidant capacity (Orhan and Akkol, 2011). Cherry laurel leaves are employed in Turkish traditional medicine for their analgesic, antispasmodic, narcotic, and sedative properties, as well as for the treatment of asthma, coughs, and dyspepsia (Karataş and Uçar, 2018). Moreover, its aqueous and ethanolic extracts exhibited antifungal, antinociceptive, and anti-inflammatory action without producing stomach ulcers (Erdemoglu *et al.,* 2003). Stanisavljević *et al.* (2010) revealed that cherry laurel leaves contain numerous beneficial compounds. However, these compounds are lost while drying, so the leaves should be consumed fresh.

The cherry laurel leaf is brilliant and bright. Several chemicals, such as benzaldehyde, benzyl alcohol glucose, sambunigrin, quercetin, and tannin, have been identified in the leaves (Duke,1992). The chemical composition of the volatile oils extracted from the leaves of Mediterranean cherry laurel has been inadequately studied. The results of only two studies conducted on this subject have been documented. Mchedlidze and Kharebava (1988) reported that cherry laurel fruit and leaves contain benzaldehyde as the main component at a rate of 94.6 and 95%, respectively. Stanisavljevic *et al.* (2010) revealed that the volatile oil extracted from the leaf of the *P. laurocerasus* L. var *serbica* species contains 99.7% benzaldehyde and a negligible amount of E)-2-hexenal and (Z)-ocimenone.

Seasonality, growth stage, temperature, water availability, and ultraviolet radiation are the most influential factors on the synthesis and chemical composition of volatile oils in medicinal plants, according to some researchers (da Cruz *et al.,* 2022; Hadj Larbi *et al.,* 2023). Prior studies dealing with volatile oil quantity and quality show that seasonal variation may significantly affect different plants (Melito *et al.,* 2019; Schoss *et al.,* 2023). However, a study examining the seasonal changes in the volatile oil content in cherry laurel leaves, which has evergreen leaves, has not been found in the literature.

Gabriel (1971) first developed the biplot method, which is used to graphically examine two-way data. Yan (2014) developed divergent perspectives on the application of this method to various data sets. The most prevalent application of the biplot method in agricultural research is the interpretation of G x E interactions in multi-environment trials (Kaplan *et al.,* 2017). In the subsequent periods, genotype x trait interaction, salt stress x genotype interaction, genotype x disease scores, genotype x seed chemical characteristics etc. were analyzed in a variety of two-way agricultural datasets (Akcura *et al*., 2019).

This study was conducted to examine the monthly variations in the volatile oil ratio and volatile compositions of the leaves of the cherry laurel under Mediterranean climate conditions for 12 months, from March 2021 to February 2022. In this study, biplot analysis was performed on a dataset of harvest time x volatile oil qualities from multiple perspectives. Biplots were created to determine I) the relationships between the examined volatile oil properties, II) the changes in the properties depending on harvest dates, III) the most suitable harvest date based on the examined properties, and IV) the most suitable volatile property based on the harvest dates, using the two-way data.

2. MATERIALS AND METHODS

The sampling plant is located between 25° 40′-27°30′ east longitudes, 39° 27′-40°45′ north latitudes, and 20 meters above mean sea level. Due to its position, the climate of Çanakkale Province exhibits features of a transitional climate. It embodies the characteristics of the Mediterranean climate in terms of its general disposition. The minimum temperature recorded for many years is -4.2 °C in February, and the maximum temperature is +35.8 °C in August. The annual average temperature is 14.7 °C, and the average humidity is 72.6%. The average temperature ranged between 6.32 °C and 28.7 °C between March-2021 and February 2022, when the experiment was conducted. The highest precipitation was recorded in February at 123.9 mm (Figure 1; Anonymous, 2022)

The materials used were leaves from ten-year-old *P. laurocerasus* trees located in the landscape garden of Çanakkale Onsekiz Mart University Terziolu Campus, Faculty of Agriculture. During each sampling month, approximately 300 g of mature leaf samples were collected from the trees. The first sample was collected between 8:00 and 8:30 a.m. on 03.02.2021. After this date, leaf samples were taken from the same tree between 8:00 and 8:30 in the morning on the 2nd day of each month for a year (until 02.02.2022). Within thirty minutes of each month's harvest, three replicates of 100 grams of leaf samples were taken. After this, 1000 ml balloons with a volume of 1000 ml each were filled with three replicates of fresh leaf samples that had been diced with a blender. Each balloon was distilled for three hours using an S-H Clevenger equipment and 600 cc distilled water. At the end of the distillation, the ratios of volatile oil per 100 g of wet weight were determined.

All samples were then refrigerated to -18 °C for analysis by gas chromatography and mass spectrometry (GC MS). Using a GC MS instrument, the volatile components of three replicate samples of volatile oil per month were identified. For volatile oil component analysis in this work, a Restek Rxi-5MS GC Capillary Column (30 m x 0.25 mm x 0.25 m) and a Shimadzu GCMS-QP2020 NX GC-MS instrument were used. In the study, the temperature of the column was raised to

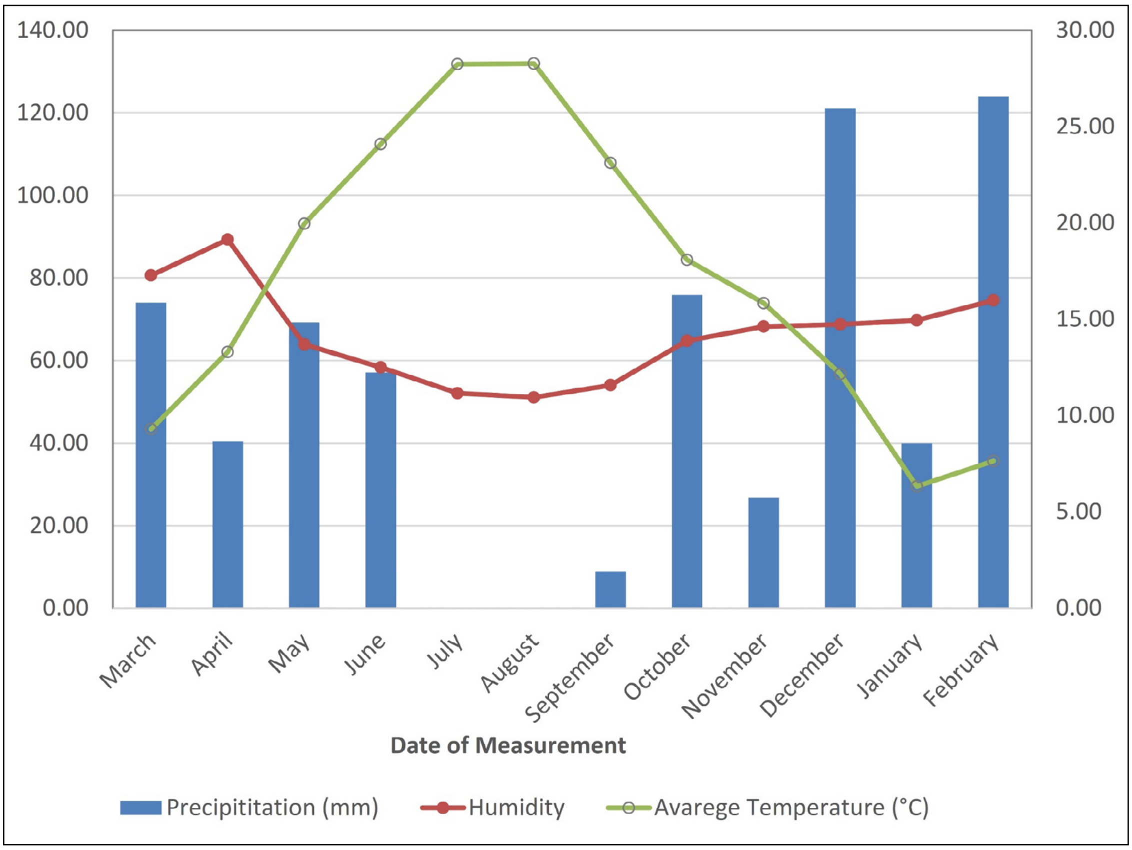

Figure 1. Average temperature, humidity and total precipitation values for the sample months

40 °C for the first 3 minutes, and then to 240 °C at a rate of 5 °C/min, where it remained for 10 minutes. 1.0 µl diluted volatile oil samples (1/100 hexane, v/v) was injected automatically in the split method (1:25). During detection in the GC-MS system, an electron ionization system with an ionization energy of 70 eV was used. Helium was used as carrier gas. The temperature of the injector and MS contact was fixed to 250 °C. The relative retention index values of the components detected by GC-MS were evaluated using the standard n-alkane series (C8-C24), and characterized based on electronic libraries (FFNSC 3, W9N11, NIST11) and literature values (Adams, 2007).

The collected data were subjected to variance analysis by the experimental design of randomized blocks. The Tukey multiple comparison test was employed to compare the means of statistically significant characteristics (SAS Institute, 2000). In recent years, the most widely used method to visually evaluate two-way data in many different branches of science is Biplot analysis (Yan, 2014). In this method, two-way matrix data consisting of the factors in the rows and the factors in the columns are positioned on the PCA-based Biplot according to the relationship between them (Akçura, 2011). Using two-way data generated by averaging the volatile oil content, volatile components, and sample dates, biplot analyses were conducted from a different view. Figure 4a was developed to display the relationships between the studied volatile oil traits and to decide which traits are favorable at which harvesting time. Figure 4b was constructed to illustrate the stability of varying the volatile oil content and volatile components according to the mean coordinate axis of the harvest dates. Figure 4c was constructed to compare the examined traits at various harvest dates to the optimal volatile oil trait. Figure 4d was created to evaluate the various harvest dates based on the optimal harvest date based on the analyzed volatile oil variables. The GGEbiplot Software Version 8 was used for biplot analyses (Yan, 2014).

3. RESULTS AND DISCUSSION

The influence of harvest dates on the volatile oil concentration and volatile components of leaf samples was statistically significant ($P < 0.01$). Figures 2 and 3 represent heat map bar graphs of volatile oil ratio and volatile components according to harvest dates, and Table 1 provides their respective means.

Table 1. Tukey test results for monthly averages of volatile oil content and active constituents[a] in cherry laurel leaf

Date of measurement	Volatile Oil Content (%)	Benzaldehyde (%)	Phenol (%)	Benzene-acetonitrile (%)	Benzoic acid (%)	Pentadecanone (%)	1,54-Dibromotetra-pentacontane (%)	Tetrapenta-contane (%)
RRI[b]		953	983	1138	1151	2024	2996	3094
March	0.19±0.013 g	93.37±0.61 ab	0.32±0.01	0.27±0.06 g	2.03±0.03 a	0.16±0.01 b	0.29±0.01 def	2.92±0.11 de
April	0.21±0.010 fg	92.99±0.56 ab	0.35±0.02	0.29±0.08 g	1.82±0.05 ab	0.11±0.01 b	0.30±0.01 de	3.05±0.14 cde
May	0.22±0.02 efg	93.89±1.30 a	0.34±0.02	0.34±0.04 g	1.78±0.09 abc	0.13±0.02 b	0.34±0.04 cd	3.64±0.13 a
June	0.25±0.012 c-f	92.96±1.39 ab	0.35±0.02	0.32±0.04 g	1.78±0.03 abc	0.11±0.01 b	0.39±0.03 ab	3.48±0.07 ab
July	0.31±0.012 ab	94.31±0.95 a	0.40±0.03	0.33±0.05 g	1.56±0.15 bcd	0.12±0.02 b	0.42±0.01 a	3.45±0.21 ab
August	0.35±0.031 a	90.61±0.70 abc	0.38±0.01	3.40±0.4 f	1.30±0.05 de	0.09±0.01 b	0.41±0.01 a	3.33±0.12 abc
September	0.30±0.011 bc	86.95±0.59 cde	0.37±0.03	6.94±0.4 de	1.05±0.21 ef	0.09±0.01 b	0.41±0.01 a	3.27±0.14 bcd
October	0.28±0.012 bcd	86.36±0.90 cde	0.35±0.01	8.07±0.4 cd	0.81±0.03 f	0.87±0.08 a	0.38±0.03 abc	3.17±0.14 b-e
November	0.27±0.014 b-e	84.67±1.99 e	0.33±0.01	9.07±0.6 bc	0.90±0.04 ef	0.84±0.03 a	0.36±0.02 bc	3.12±0.12 b-e
December	0.24±0.013 def	83.89±1.14 e	0.32±0.08	11.2±0.34 a	0.92±0.21 ef	0.85±0.04 a	0.26±0.01 ef	2.99±0.15 cde
January	0.21±0.011 fg	85.82±0.21 cde	0.30±0.08	9.50±0.50 b	1.16±0.21 def	0.85±0.05 a	0.25±0.02 f	2.88±0.12 e
February	0.19±0.010 g	89.33±2.23 bcd	0.29±0.05	6.25±0.22 e	1.34±0.11 cde	0.08±0.02 b	0.27±0.01 ef	2.82±0.19 e
Mean	0.25	89.6	0.34	4.66	1.37	0.35	0.34	3.17
Significantly	**	**	ns	**	**	**	**	

[a]: Values are mean ± standard deviation (n=3). [b]: RRI, relative retention indices were calculated against n-alkanes **: In the ANOVA analysis, $p < 0.01$ is significant. The differences among mean values shown on the same line with the same letter are not significant ($p < 0.01$). ns: not significant. Differences were determined using the Tukey test.

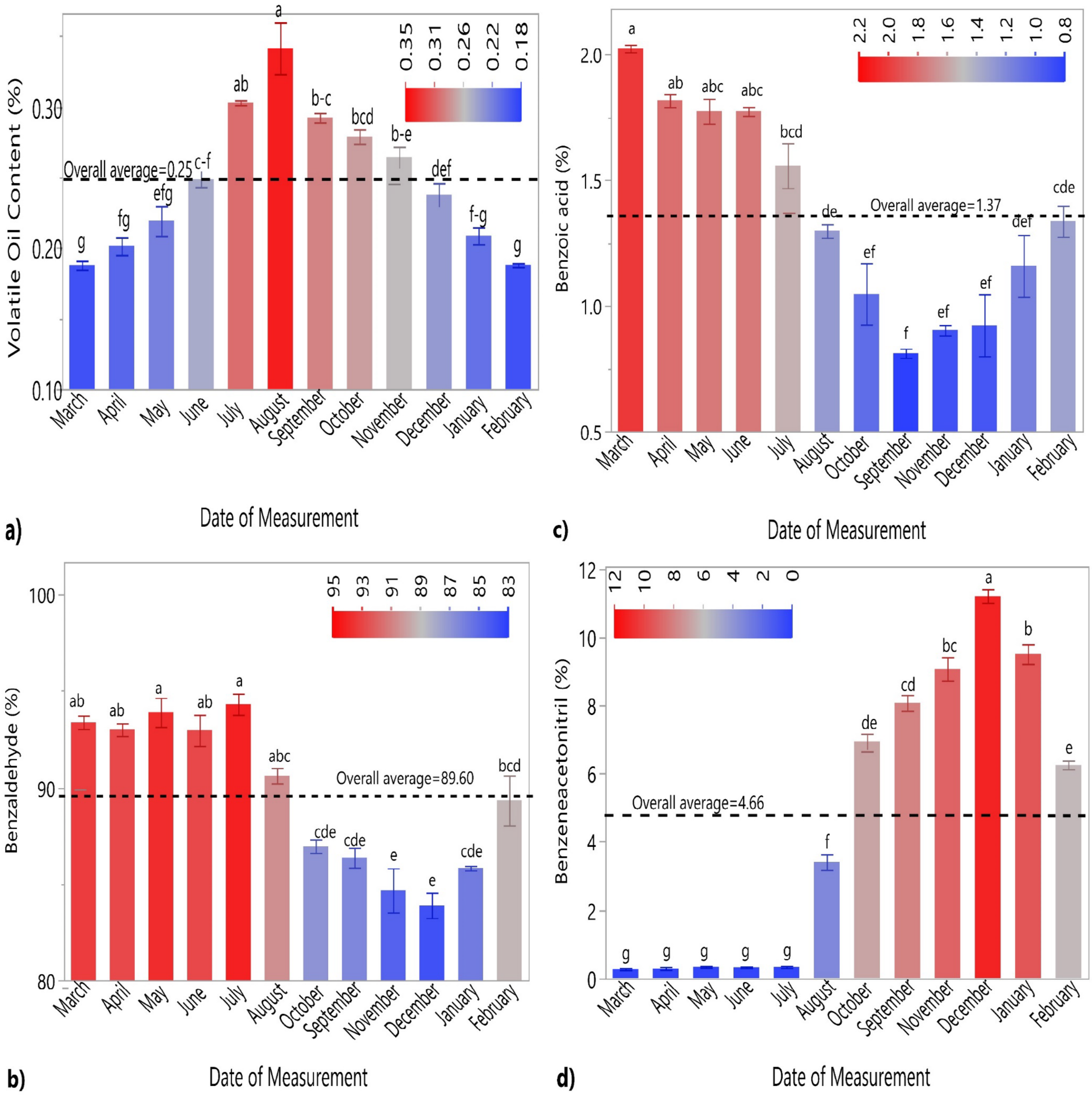

Figure 2. Charts with heat maps and Tukey test means for volatile oil content, benzaldehyde, and benzaldehyde derivatives based on cherry laurel leaf harvest time.
The differences among on the same chart with the same letter are not significant ($p < 0.01$). Differences were determined using the Tukey test. Each error bar is constructed using 1 standard error from the mean.

The percentage of volatile oil obtained from the leaves varied between 0.19 and 0.35%, while the average was 0.25%. The odor of the obtained clear and colorless volatile oil was similar to the smell of fresh green almonds. The highest percentage of volatile oil was obtained from the harvest in August, followed by the sample taken in July with 0.31%, and the sample taken in September with 0.30%. The first sample obtained in March and the last sample taken in February had the lowest volatile oil ratio of 0.19%. Although results of research using *P. laurocerasus* as a material have not been found, it has been reported that the volatile oil ratio in *P. persica* is 0.46, 0.45, and 0.42% in the rainy season, autumn, and summer seasons, respectively (Verma *et al.,* 2017). Similar to our findings, it was reported that some plant species should be harvested in the summer and autumn in Mediterranean climates in order to obtain high vol-

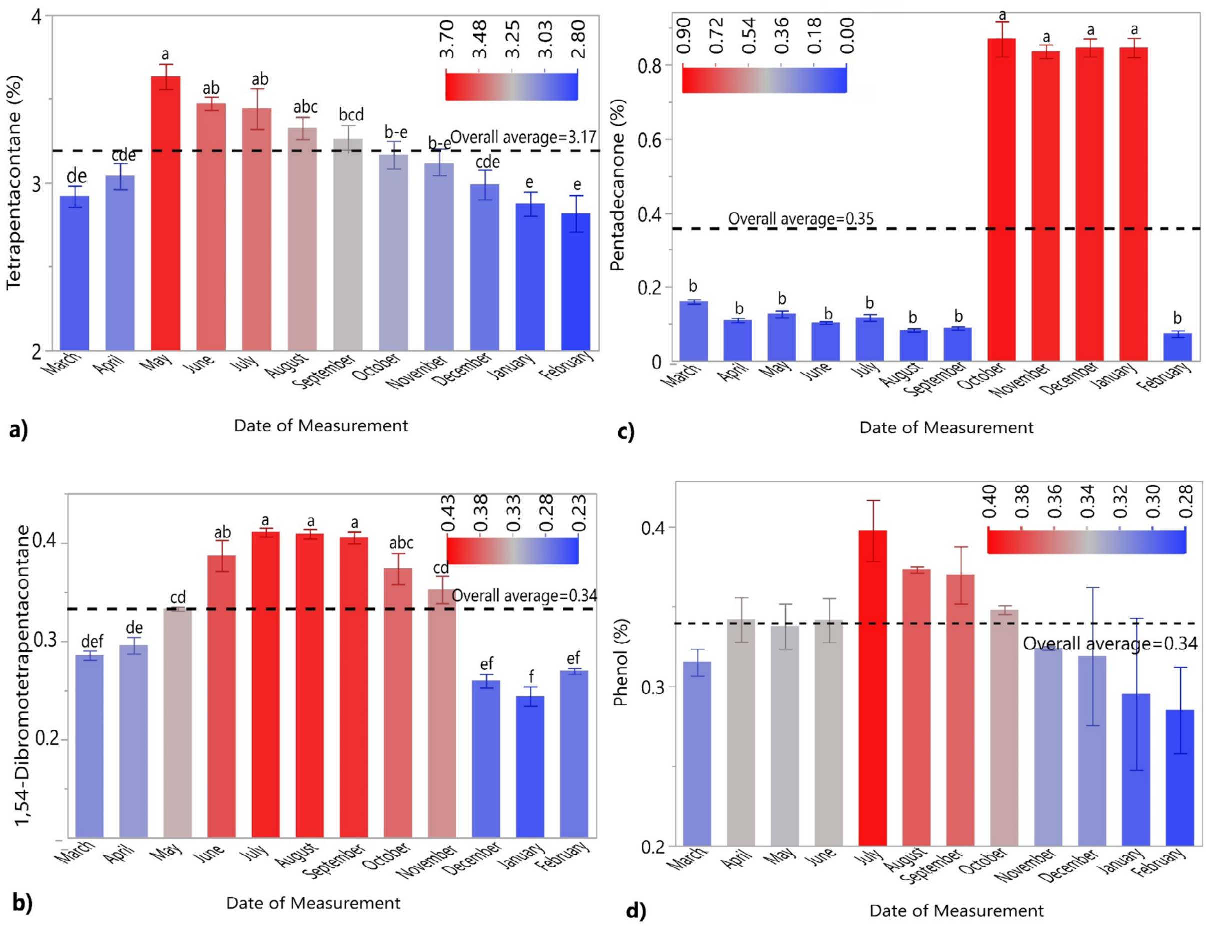

FIGURE 3. The Tukey test mean groups and heat map bar charts of the mean ratios of pentadecanone, tetrapentacontane, 1.54-dibromo tetrapentacontane, and phenol in cherry laurel leaf essential oils by harvest time
The differences among on the same chart with the same letter are not significant ($p < 0.01$). Differences were determined using the Tukey test. Each error bar is constructed using 1 standard error from the mean.

atile oil contents (Melito *et al.,* 2019; Serralutzu *et al.,* 2020).

In this study, the volatile oil content was discovered to contain benzaldehyde, phenol, benzoic acid, benzeneacetonitrile, pentadecanone, 1,54-dibromotetrapen, and tetrapentacontane. Changes in the proportions of the volatile oil's active compounds (excluding phenol) were statistically significant ($P < 0.01$) depending on the date of harvest. In this study, benzaldehyde, detected at a rate between 83.89 and 94.41%, was the most common active component in the volatile oil based on the harvest time. In the first six harvest periods, benzaldehyde ratios were found to be above average. The two highest benzaldehyde averages were 93.89 and 94.31 in the leaf samples collected in May and July, respectively. Similar to our findings, it has been reported that the primary active component of the volatile oil obtained from the leaves of cherry laurel (*P. laurocerasus*) and *P. phaeosticta* var. *phaeosticta* is benzaldehyde (Lazic *et al.,* 2009). As one of the most significant aromatic aldehydes, natural benzaldehyde has significant industrial use in the production of pigments for food flavorings, and perfumes. Additionally, natural benzaldehyde is a crucial intermediary for numerous medicinal compounds. It is also a volatile intermediate for many pharmaceutical products (Haffenden *et al.,* 2001). Today, fruit seeds, natural cinnamon oil, and bitter almond oil are used to create the naturally accessible benzaldehyde which is required in the food and perfume industries synthetically and through chemical reactions. However, it is difficult to create benzaldehyde from seed derivatives and it is necessary to wait for a specific time period before production may begin (Berger, 2007). Since the trees produce fruit at least once a year, raw materials must be supplied once a year to produce natural benzaldehyde. In addition, the natural benzaldehyde manufacturing process may result in many hazardous chemical compounds due to the various chemicals found in fruit seeds (Verma *et al.,* 2017). However, cherry laurel is an important raw material source that can always be used in the fragrance and medicine industry due to the high content of benzaldehyde in its large and evergreen leaf.

While phenol was identified at low concentrations, the influence of harvesting time was minor in terms of this active component. The variation range for phenol was between 0.29 and 0.40%. Similar to benzaldehyde, the ratios of benzoic acid in the first five harvest periods were above average. Benzoic acid is a key, medicinally-important acid which is generated from the interaction of benzaldehyde with oxygen, which was significantly affected by harvest time. The average level of benzoic acid in this study was 1.37%, while the first five harvest periods had benzoic acid levels which were above average. Benzoic acids are used as intermediates for pharmaceuticals, especially for antipyretic analgesic, ant-rheumatism, and antimicrobial activity (Bernhard *et al.,* 2014). Although the volatile oil samples obtained during the first harvest were stored at -18 °C, active ingredient analyses were carried out in the volatile oil at the end of 12 months in this study. The long storage period of the first samples may cause the partial formation of benzoic acid from benzaldehyde. Similar to our results, in a study using fruits, stems, leaves, barks, and trunk cores of *P. padus* Linn, a high rate of benzoic acid was detected (Zhu *et al.,* 2005).

Another volatile component that emerges from the transformation of benzaldehyde is benzeneacetonitrile (Abubacker and Devi, 2014). In this study, benzeneacetonitrile was identified on average at a rate of 4.66%. The final six leaf samples had benzeneacetonitrile ratios that were higher than the average. The ratio of benzeneacetonitrile was the highest in samples collected in December, whereas it was lowest in those collected in March.

Except for benzaldehyde and its derivatives, tetrapentacontane was discovered to be the fragrance component present in the volatile oil at the greatest rate in this research. Tetrapentacontane hydroxylation of liver enzymes during phase I metabolism, hair growth promoter, uric acid production, and arachidonic acid inhibitor in the human body are therapeutic and pharmaceutical benefits (Shunmugapriya *et al.,* 2017). The average amount of tetrapentacontane was determined as 3.17%, although it showed a significant change depending on the harvest times in the study. The precursor of this compound is 1,54-dibromotetrapentacontane, which was determined as 0.34% on average. In the studies on the essential oil content of the *Prunus* species used in this study, no research findings were found to show that the volatile component of tetrapentacontane was detected. However, it has been reported that both tetrapentacontane and 1,54-dibromotetrapentacontane are found at a rate of 6.71% in the volatile oil of *Citrus lemon* species with evergreen leaves, while 1,54-dibromotetrapentacontane is found in the volatile oil of *Alphitonia excelsa* (Tithi, 2022). In addition, it has been reported that tetrapentacontane is 11.5% in *Ephedra pachyclada* volatile oil (Khanal *et al.,* 2022), and 5.23% in *Aristolochia tagala* volatile oil (Mariyammal *et al.,* 2023).

Figures 4a, 4b, 4c, and 4d illustrate the biplots of harvest date x trait interactions which were produced to visualize the variations in volatile oil ratio and volatile substance ratios depending on harvest date. The biplot analysis explained 89.6% of the total variation. This explanation rate demonstrated that the variation in volatile oil concentration and active component content based on harvest date can be used to successfully analyze the biplot visually (Yan, 2014). According to the harvest period in this study, the amount of volatile oil and active components varied greatly (Figure 4a). According to their constructive relationships with one another, the active components were divided into 3 groups. The first group of active substances included pentadecanone and benzeneacetonitrile, which had the most positive correlation. The second group consisted of tetrapentacontane, phenol, 1,54-dibromotetrapentacontane, and volatile oil content. The third category contained benzaldehyde and benzoic acid. Regarding the first trait-group's traits, February stood out, whereas the second trait groups were highlighted by July and August. The months of April, May, and June stood out in the third trait group (Figure 4a).

Figure 4b shows the biplot designed to graphically demonstrate how the active components in volatile oils fluctuate over time depending on the time of collection. Evaluation of the stability of harvest dates is possible using the average coordinate axis resulting

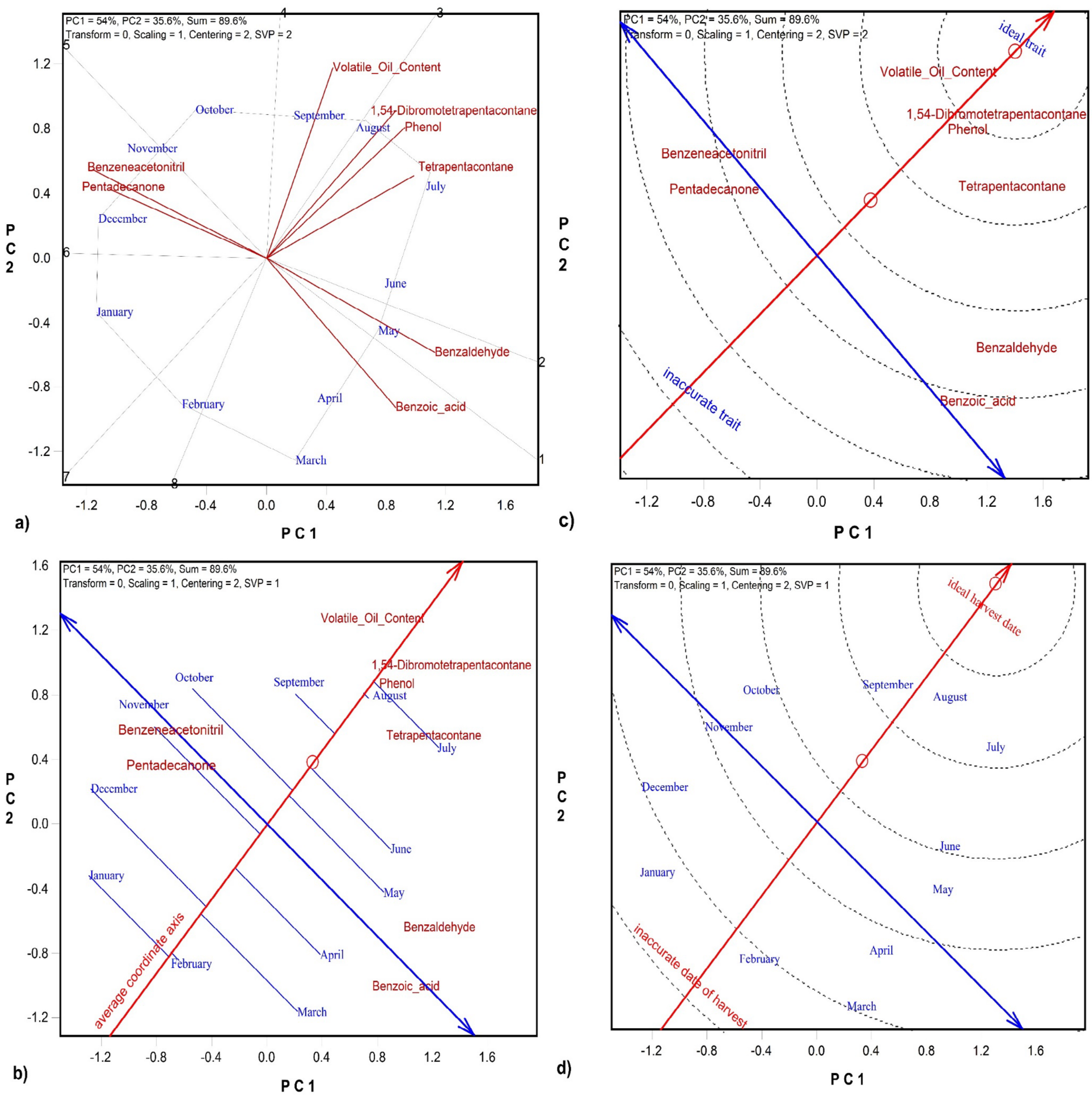

Figure 4. Biplots comparing harvest time x the qualities of cherry laurel leaf essential oil [a) Which-one-where, b) Evaluation of harvest times using the average harvest time axis, c) Ideal qualities of essential oil, and d) Ideal harvest time]

from characteristics and harvest dates (Akçura, 2023). In terms of volatile oil content and active components, the months of August, September, and July, which were closest to the mean axis in the positive direction, outperformed the other months. On the other hand, February and January were the months with the least negative effect on trait changes. One of the main advantages of biplot analysis is the opportunity to assess the ideal characteristics and optimum harvest period concerning the characteristics assessed in the research (Yan, 2014). Among the properties investigated in this study, volatile oil content, phenol, and 1,54-dibromotetrapentacontane were located closest to the ideal trait section. On the other hand, benzeneacetonitrile and pentadecanone, were the furthest from the ideal trait section in terms of explaining the variation (Figure 4c). With respect to harvest time, the months closest to the ideal harvest time were August, July, and September, respectively. In terms of both the volatile oil content and the majority of the active component ratios in the volatile oil, the harvests from August, July, and September were superior to those from other months (Figure 4d).

4. CONCLUSIONS

According to this study, the ratios of volatile oils in the green leaves of cherry laurel range from 0.19 to 0.35%, depending on the time of harvest. It has been determined that the main active ingredient in the volatile oil is benzaldehyde, varying between 83.89 and 94.41%, depending on harvest time. The volatile oil also contains the active components of phenol, benzoic acid, benzeneacetonitrile, pentadecanone, 1,54-dibromotetrapentacontane and tetrapentacontane. The harvests from the months of July, May, and June were shown to have the highest concentrations of benzaldehyde. The biplot analysis approach revealed that July, August, and September were the best for harvesting in terms of volatile oil content and the majority of volatile oil active components. The medicinally significant tetrapentacontane and 1,54-dibromotetrapentacontane volatile components, which were previously found in the fruits of *Prunus* species, are the components with the highest ratio after benzaldehyde and its derivatives, which is one of the study's most significant findings. Since cherry laurel has evergreen broad leaves and the volatile oil obtained from its leaf contains a high percentage of natural benzaldehyde, the industry will be able to use this plant more effectively in the future.

5. DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

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