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Research Article

Determination of volatile compounds and antioxidant properties of summer linden (*Tilia platyphyllos* Scop.) at different harvesting times

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Abstract: This study aimed to determine the volatile compound profile and antioxidant activity levels of *Tilia platyphyllos* Scop., which naturally grows in the Sindel Plateau of Şarkikaraağaç district, Isparta province, during the 2022 vegetation period. Plant samples were collected during three distinct phenological stages (pre-flowering–June, flowering–July, and post-flowering–September). Volatile compound analyses were conducted using Solid Phase Microextraction (SPME) and Gas Chromatography-Mass Spectrometry (GC-MS), and compound identification was performed using the Wiley, NIST Tutor, and FFNSC libraries. A total of 54 compounds were identified in the pre-flowering stage, 42 during the flowering stage, and 51 in the post-flowering stage. Hexane was the dominant compound across all three stages, while Allyl bromide and Carvacrol appeared at varying concentrations. Notably, Carvacrol reached a concentration of 10.27% in the post-flowering stage, indicating increased biosynthesis during this period. Antioxidant activity was assessed using DPPH and CUPRAC assays, revealing significant variations in phenolic content and antioxidant capacity across the phenological stages. The post-flowering stage exhibited the highest values in both total phenolic content and CUPRAC activity. These findings indicate that *T. platyphyllos* demonstrates phenologically dependent biochemical compositions, and the post-flowering stage represents the most suitable harvest time in terms of medicinal and pharmacological potential.

INTRODUCTION

Due to its rich geographical characteristics, Türkiye is among the leading countries in the world in terms of plant diversity. The flora of Türkiye harbors numerous plant species used in various sectors, particularly in food additives, herbal products, cosmetics, and perfumery (Bayram *et al.*, 2010). One of the plant genera with such versatile uses is *Tilia*, a member of the Malvaceae family.

The genus *Tilia* is represented in the flora of Türkiye by four taxa: large-leaved lime (*Tilia platyphyllos*), Caucasian lime (*Tilia rubra*), silver lime (*Tilia tomentosa*), and small-leaved lime (*Tilia cordata*) (Güner *et al.*, 2012; Davis, 1967). All these species hold significant value in numerous fields such as medicine, pharmacy, food, cosmetics, and perfumery due to their rich

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content of essential oils, aromatic compounds, and other secondary metabolites (Başer *et al.*, 1993). Furthermore, the ethnobotanical use of these species is widespread throughout Türkiye (Başer, 2008; Baytop, 1999; Kargıoğlu *et al.*, 2008; Sezik *et al.*, 2001; Tuzlacı & Erol, 1999; Yeşilada *et al.*, 1999)

The part of the *Tilia* species used as a drug is the inflorescence, composed of flowers and bracts. Although *T. cordata* and *T. platyphyllos* are listed in pharmacopeias for their medicinal use, it is known that other species are also used similarly among the public (Yeşilada *et al.*, 2020). The chemical composition of *Tilia* species includes essential oils such as linalool, germacrene, and geraniol (0.01–0.02%), flavonoids like tiliroside, astragalin, and isoquercitrin, approximately 10% mucilage, derivatives of caffeic acid, and tannins (Baytop, 1999; Tanker *et al.*, 2004; Yeşilada, 2011).

The drug prepared using a 2% infusion method is traditionally used for ailments such as the common cold, cough relief, chest soothing, asthma, and abdominal pain (Kültür, 2007). Moreover, linden tea is rich in flavonoid-structured polyphenols derived from catechin and its derivatives, which have been reported to show stronger antioxidant effects than vitamins C and E against free radicals and support the immune system (Georgiev *et al.*, 2017; Vinson *et al.*, 1995). Flavonol glycosides (quercetin, kaempferol) and compounds such as hydroxycinnamic acid exhibit antioxidant activity approximately 20 times stronger than that of vitamin C (Craig, 1999; Leri *et al.*, 2015).

When applied as a compress, the infusion prepared from linden flowers is reported to have soothing and anti-discharge effects on the eyes, and when used as a hair rinse, it is believed to strengthen the hair. However, caution should be exercised as the immediate consumption of linden tea after meals may hinder digestion, and excessive intake should be avoided (Baytop, 1984).

Tilia bracts are widely used among the public for the treatment of the common cold, stomach disorders, urinary tract infections, antispasmodic purposes, expectorant effects, cough, sore throat, and liver and gallbladder ailments (Ateş, 2021). In Türkiye, the annual production of *Tilia* flowers ranges between 40 and 80 tons, with a total of 135 tons produced in 2018, generating an economic value of approximately 15 million TL (BAKKA, 2020). In this context, *Tilia* species stand out not only for their medicinal and aromatic properties but also for their high economic value.

In this study, the essential oil components and antioxidant properties of large-leaved linden (*Tilia platyphyllos*) individuals naturally distributed in the flora of the Şarkikaraağaç district of Isparta were determined at different harvest periods.

MATERIAL and METHODS

Material

This research was conducted in the Sindel Plateau region, located within the boundaries of Gedikli Village in the Şarkikaraağaç district of Isparta province (Figure 1). The plant material of the study consists of the species *Tilia platyphyllos* Scop., belonging to the Malvaceae family, which was collected from the specified area in 2022 (Figure 1). General information regarding the taxon collected from the study area is presented in Table 1.

Plant specimens were transported to the Herbarium of the Faculty of Forestry at Isparta University of Applied Sciences in airtight bags to protect them from environmental factors. Before analysis, the samples were dried in shaded, well-ventilated conditions under controlled temperatures ranging from 25 to 40°C. The dried specimens were then stored in a dry, cool environment, typically at 4°C temperature to prevent moisture exposure and preserve their integrity.



Figure 1. A view of the Linden Habitat in the Sindel Plateau.

Table 1. Site information of the sampling areas.

Collection Location	Elevation	Exposure	Harvesting Period	Collection Date
Şarkikaraağaç, Gedikli Village, Sindel Plateau, Isparta	1580 m	Northeast	Pre-flowering (Bud Stage)	04.06.2022
Şarkikaraağaç, Gedikli Village, Sindel Plateau, Isparta	1580 m	Northeast	Flowering	01.07.2022
Şarkikaraağaç, Gedikli Village, Sindel Plateau, Isparta	1580 m	Northeast	Post-flowering (Seed Dormancy)	11.08.2022

Methods

Field studies

A field study was planned and carried out to determine the distribution area and collect specimens of the *Tilia platyphyllos* species used in this research. The bracts forming the plant material were collected from the linden trees during three different phenological stages: pre-flowering, flowering, and post-flowering periods.

A sufficient amount of plant material for identification, volatile component, and antioxidant analyses was packed under appropriate conditions to avoid direct sunlight during transportation. A portion of the collected material was pressed and prepared as herbarium specimens in the Forest Botany Laboratory of the Faculty of Forestry at Isparta University of Applied Sciences, to confirm species identification. The remaining part will be stored under suitable conditions in the same laboratory for use in volatile component and antioxidant analyses. The samples will be preserved in plastic bags at room temperature in a laboratory environment.

GC-MS analysis

The floral aroma components of the bract were identified using Headspace Solid-Phase Microextraction (HS-SPME) combined with Gas Chromatography-Mass Spectrometry (GC-MS). Based on the solid-phase microextraction (SPME, Supelco, Germany) method, 2 g of bract samples were placed into a 10 mL vial and incubated at 60°C for 30 minutes. Volatile compounds in the headspace were then absorbed using a fused silica fiber coated with 75 µm Carboxen/Polydimethylsiloxane (CAR/PDMS), and immediately injected into the capillary column (Restek Rx-5 Sil MS 30 m × 0.25 mm, 0.25 µm) of a GC-MS system compatible with HS-SPME (Shimadzu 2010 PLUS). The oven temperature was initially held at 40°C for 2 minutes, then increased to 250°C at a rate of 4°C per minute. The injector and detector

temperatures were set at 250°C. Electron Impact Ionization (EI) at 70 eV was used, with helium as the carrier gas at a flow rate of 1.61 mL/min. Identification of volatile compounds was performed using the Wiley, NIST, Tutor, and FFNSC spectral libraries.

Antioxidant analyses (%)

A defined amount of dried and ground plant material, 2 g, was placed into an appropriate container. Subsequently, 20 mL of ethanol was added. The resulting mixture was incubated at room temperature for 24 hours in a shaking incubator operating at 120 rpm. Following the extraction period, the mixture was filtered using Whatman filter paper or centrifuged to obtain a clear extract. The obtained ethanolic extract was then reserved for antioxidant activity analyses.

The antioxidant activities of the species were analyzed using two different methods: DPPH and CUPRAC.

Determination of Antiradical Activity (DPPH)

Antiradical activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, as described by Shimata *et al.* (1992). For the assay, 0.25 mL of the sample (at a concentration of 250 ppm) was mixed with 1 mL of 0.2 mM DPPH solution and thoroughly vortexed. The mixture was kept in the dark at room temperature for 30 minutes, after which the absorbance was measured at 517 nm. The calculation was performed using the following Equation (1):

$$SRS\ (\text{mmol TR g}^{-1}\ \text{extract}) = \frac{\Delta A}{\varepsilon_{TR}} \times \frac{V_m}{V_s} \times \text{Sf} \times \frac{V_E}{m} \quad (1)$$

ε_{TR}: Molar absorptivity of the Trolox compound in the DPPH method (2.168×10^4 L mol^{-1} cm^{-1}),

V_s: Volume of the sample (mL),

V_m: Total volume of the method (4 mL),

S_f: Dilution factor (if applicable),

V_E: Volume of the extract (mL),

m: Mass of the extract (g).

Cupric Ion Reducing Antioxidant Capacity (CUPRAC)

The CUPRAC (Cupric Ion Reducing Antioxidant Capacity) assay was conducted following the method proposed by Apak *et al.* (2004). For this assay, a solution containing 0.01 M $CuCl_2$, 7.5 mM neocuproine, 1 M ammonium acetate buffer (pH 7.0), and 1 mL of distilled water was added to the 100 ppm extract solution. Ethanol was used as the control, and all samples were incubated in the dark for 1 hour. The absorbance was measured at 450 nm using a spectrophotometer. The antioxidant capacity was expressed as Trolox Equivalent (TE) per gram of dry extract using the following Equation (2):

$$CUPRAC\ (\mu\text{mol TE g}^{-1}) = \frac{A}{\varepsilon_{TR}} \times \frac{V_m}{V_s} \times D_f \times \frac{V_E}{m} \times 1000 \quad (2)$$

A: Absorbance at 450 nm,

ε_{TR}: Molar absorptivity of the Trolox compound in the CUPRAC method (1.67×10^4 L mol^{-1} cm^{-1}),

V_m: Total volume of the measured solution (mL),

V_s: Volume of the sample (mL),

D_f: Dilution factor,

V_E: Volume of the prepared extract (mL),

m: Mass of the dry herb material (g).

FINDINGS

Findings on Volatile Components of Flowers

In this study, the volatile compound profile of *Tilia platyphyllos* naturally growing in Sindel Plateau, located within the boundaries of Gedikli Village in the Şarkikaraağaç district of Isparta province, was analyzed during three different phenological stages: pre-flowering, flowering, and post-flowering. The analysis was conducted using the Solid Phase Microextraction (SPME) method.

As a result of the SPME analysis, 54 different volatile compounds were identified in the pre-flowering stage, 42 in the flowering stage, and 51 in the post-flowering stage. Among the identified compounds, the major components detected in the pre-flowering stage were hexane (52.21%), allyl bromide (18.84%), and p-cymene (8.88%), respectively. During the flowering stage, the dominant compounds were hexane (60.46%), allyl bromide (23.89%), and carvacrol (2.30%). In the post-flowering stage, hexane (43.78%), allyl bromide (17.53%), and carvacrol (10.27%) were found to be the most abundant constituents (Table 2).

Based on the chemical classification of the volatile compounds, it was determined that all phenological stages predominantly contained aromatic hydrocarbons (pre-flowering: 80.08%; flowering: 83.89%; post-flowering: 58.55%), monoterpene hydrocarbons (pre-flowering: 12.08%; flowering: 6.96%; post-flowering: 11.47%), and oxygenated monoterpene (pre-flowering: 9.31%; flowering: 4.25%; post-flowering: 11.88%) (Figure 2).

Table 2. Volatile compound percentages of *Tilia platyphyllos*.

R. Time	Compound	Pre-flower %	Bloom Time %	Post-flowering %	Formula	Category
1.369	Ethyl alcohol	0.77	1.13	1.99	CH_3CH_2OH	AAI
1.440	2-Propanone	0.21	0.31	0.53	C_3H_6O	AAI
1.736	Propyl bromide	0.92	-	1.08	$CH_3CH_2CH_2$	AH
1.806	Hexane	**52.21**	**55.12**	**43.78**	C_6H_{14}	AH
1.911	Allyl bromide	**18.84**	**25.70**	**17.53**	C_3H_5Br	AH
2.538	1-Pentene-3-ol	0.99	2.05	1.38	$C_5H_{10}O$	AAI
2.697	Pentanal	1.11	2.12	2.06	$C_5H_{10}O$	AA
2.870	2-Butanone	0.39	0.33	0.80	C_4H_8O	AAI
4.619	Hexanal	0.56	1.04	1.53	$C_6H_{12}O$	AA
6.122	(E)-2-Hexenal	0.32	0.18	0.49	$C_6H_{10}O$	AA
7.702	Heptanal	0.15	0.23	0.42	$C_7H_{14}O$	AA
8.526	α-Thujene	0.23	0.36	0.61	$C_{10}H_{16}$	MH
8.765	α-Pinene	0.37	0.58	0.94	$C_{10}H_{16}$	MH
9.352	Camphene	0.06	0.08	0.19	$C_{10}H_{16}$	MH
9.721	(E)-2-Heptenal	0.08	0.07	0.10	$C_7H_{12}O$	AA
9.850	Benzaldehyde	0.08	0.26	0.31	C_7H_6O	AA
10.245	Sabinene	0.04	0.10	0.16	$C_{10}H_{16}$	MH
10.399	β-Pinene	0.09	0.15	0.22	$C_{10}H_{16}$	MH
10.799	6-Methyl-5-hepten-2-one	0.06	0.14	0.17	$C_8H_{14}O$	AAI
10.941	β-Myrcene	0.35	0.51	1.02	$C_{10}H_{16}$	MH
11.511	α-Phellandrene	0.12	0.17	0.16	$C_{10}H_{16}$	MH
11.625	δ-3-Carene	0.09	0.14	0.22	$C_{10}H_{16}$	MH
11.948	α-Terpinene	0.02	0.13	0.09	$C_{10}H_{16}$	MH
12.245	p-Cymene	**8.88**	2.89	5.56	$C_{10}H_{14}$	MH
12.430	Limonene	1.19	0.79	1.77	$C_{10}H_{16}$	MH
12.542	Eucalyptol	0.52	0.28	0.76	$C_{10}H_{18}O$	OM
13.151	1,2-Nonadiene (CAS)	0.44	-	-	C_9H_{16}	MH
13.578	γ-Terpinene	0.19	0.28	0.66	$C_{10}H_{16}$	MH
14.102	3,5-Octadien-2-one	0.05	-	0.23	$C_8H_{12}O$	AAI
15.268	Linalool	0.84	0.50	0.75	$C_{10}H_{18}O$	OM
15.435	Nonanal	0.32	0.30	0.54	$C9H_{18}O$	AA
16.988	Camphor	0.02	-	0.11	$C_{10}H_{16}O$	OM

19.318	Decanal	0.06	0.08	0.14	$C_{10}H_{20}O$	AA
20.863	p-Cymene-2,5-dione	0.26	0.11	0.40	$C_{10}H_{12}O_2$	OM
21.836	Tetradecane	0.11	0.15	0.32	$C_{16}H_{34}$	AH
22.346	Pentadecane	0.03	-	0.12	$C_{18}H_{38}$	AH
15.004	Thymol	0.05	-	0.08	$C_{10}H_{14}O$	OM
22.613	5-Isobutylnonane	0.07	-	0.11	$C_{13}H_{28}$	AH
22.779	Carvacrol	7.77	**3.47**	**10.27**	$C_{14}H_{30}$	OM
23.496	Hexadecane	0.15	0.17	0.47	$C_{16}H_{34}$	AH
23.867	Heptadecane	0.09	0.60	0.27	$C_{17}H_{36}$	AH
24.150	Octadecane	0.05	0.08	0.24	$C_{18}H_{38}$	AH
24.361	α-Cubebene	0.06	-	0.12	$C_{15}H_{24}$	SH
17.479	Eicosane	0.07	0.05	0.16	$C_{20}H_{42}$	AH
26.822	trans-Caryophyllene	0.25	0.51	0.12	$C_{15}H_{24}$	SH
28.770	β-Ionone	0.01	-	-	$C_{13}H_{20}O$	OM
28.841	Curcumene	0.04	-	-	$C_{15}H_{22}$	SH
29.083	Docosne	0.08	0.10	0.26	$C_{22}H_{46}$	AH
29.712	β-Bisabolene	0.11	0.08	0.12	$C_{15}H_{24}$	SH
29.964	2-Isopropyl-5-methyl-1-heptanol	0.02	-	0.08	$C_{11}H_{24}O$	AAI
30.035	δ-Cadinene	0.02	-	0.04	$C_{15}H_{24}$	SH
30.136	Calamenene	0.05	-	0.11	$C_{15}H_{22}$	SH
30.525	2,6,11,15-Tetramethylhexadecane	0.06	0.07	0.19	$C_{20}H_{42}$	AH
38.385	Ethylhexyl chloroformate	0.09	0.73	0.22	$C_9H_{17}ClO_2$	AAI
43.000	Benzeneacetaldehyde	-	0.10	-	C_8H_8O	AA
TOTAL	100	100	100	100		
Number of compound		54	42	51		
AA: Aromatic alcohol		4.70	4.38	7.11		
AAI: Aromatic aldehyde		2.56	4.69	5.06		
AH: Aromatic hydrocarbon		80.08	83.89	58.55		
MH: Monoterpene hydrocarbon		12.08	6.96	11.47		
OM: Oxygenated monoterpene		9.31	4.25	11.88		
SH: Sesquiterpene hydrocarbon		0.98	0.59	1.24		

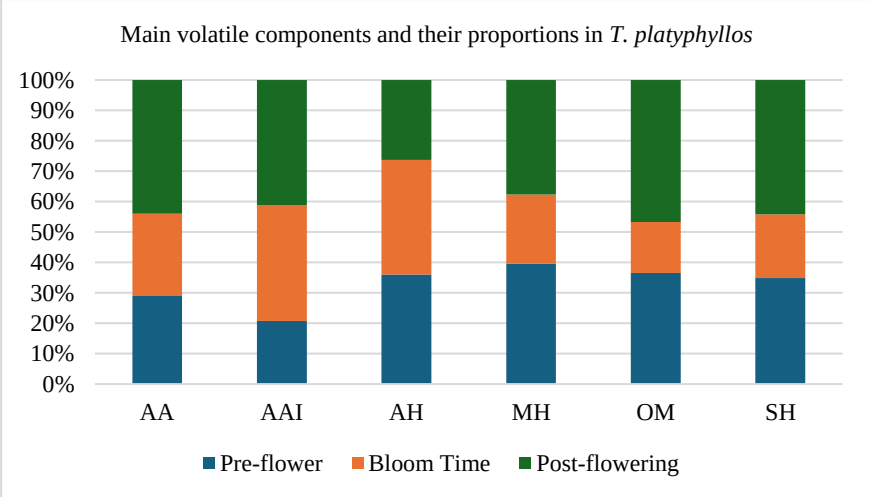


Figure 2. Main volatile components and their proportions in *T. platyphyllos*.

The quantitative relationships between the volatile compounds of *Tilia platyphyllos* flowers were evaluated using Pearson correlation analysis. The data obtained from the correlation matrix revealed the linear changes of compounds with each other during different phenological stages (pre-flowering, flowering, and post-flowering); these relationships were visualized through a heatmap (Figure 3). As a result of the analysis, very high positive correlations were observed between certain compounds. In particular, the correlation coefficient between Ethyl

alcohol, 2-Propanone, and Propyl bromide, which is close to 1.00, indicates that these compounds are synthesized through common biosynthetic pathways or respond similarly to environmental conditions. Negative correlations were observed between Hexane, Allyl bromide, and some monoterpenes, as well as aliphatic compounds. This suggests that these compound groups are produced through different enzymatic pathways or show opposite directional changes during the flowering stages. The heatmap also indicated that specific compound groups tend to cluster together. For example, the high correlation between oxygenated compounds (alcohols and ketones) and terpenoid compounds within their respective groups suggests that these groups are either produced together biochemically or triggered by similar environmental stimuli.

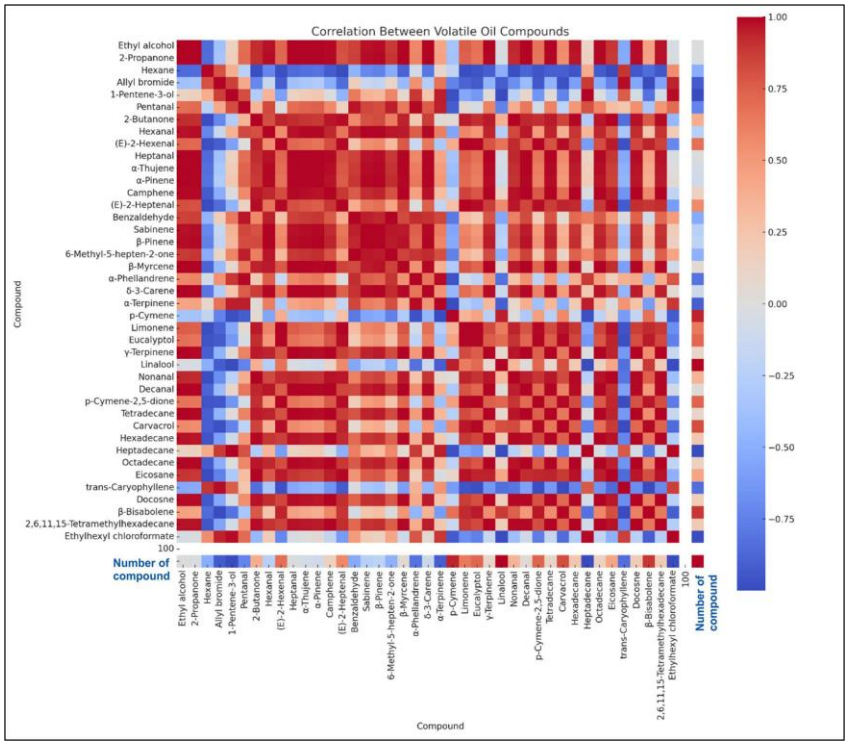


Figure 3. Correlation matrix heatmap.

Findings on Volatile Components of Flowers

As a result of the correlation analysis conducted, significant differences were observed between the total phenolic compound measurements of *Tilia platyphyllos* across different periods. Specifically, a very high positive correlation was found between the post-flowering and flowering period measurements (r = 0.987). This suggests that phenolic compounds in these two phenological stages remained at similar levels and were likely influenced by similar physiological or environmental factors.

On the other hand, negative and relatively weak correlations were found between the pre-flowering period and the other two periods (Post-Flowering – Pre-Flowering r = -0.027, Flowering – Pre-Flowering r = -0.185) (Table 3). This indicates that the phenolic content profile of the plant significantly differs before flowering (Table 4).

Table 3. Correlation analysis results of phenolic compound measurements.

	Post-Flowering	Flowering	Pre-Flowering
Post-Flowering	1	0.987 ($p \approx 0.05$)	-0.027 ($p > 0.05$)
Flowering	0.987 ($p \approx 0.05$)	1	-0.185 ($p > 0.05$)
Pre-Flowering	-0.027 ($p > 0.05$)	-0.185 ($p > 0.05$)	1

$p \approx 0.05$ indicates the borderline of statistical significance.
$p > 0.05$ denotes a lack of statistical significance.

The correlation matrix results reveal that the *Tilia platyphyllos* species exhibits strong and statistically significant positive relationships in antiradical activity among three variables (Post-Flowering, Flowering, and Pre-Flowering). The correlation coefficient between Post-Flowering and Flowering was found to be 0.87, between Post-Flowering and Pre-Flowering 0.98, and between Flowering and Pre-Flowering 0.99. The correlation coefficients being very close to 1 indicate that there are extremely strong linear relationships among the variables.

In particular, the correlation coefficient of 0.99 between Flowering and Pre-Flowering suggests that these two variables tend to change in nearly the same direction, demonstrating a very high structural relationship between them. Similarly, the Post-Flowering variable also shows a high positive correlation with both Flowering (r=0.87) and Pre-Flowering (r=0.98).

Table 4. Correlation analysis results of antiradical activity (DPPH).

	Post-Flowering	Flowering	Pre-Flowering
Post-Flowering	1	0.87 (p ≈ 0.25)	0.98 (p ≈ 0.08)
Flowering	0.87 (p ≈ 0.25)	1	0.99 (p ≈ 0.06)
Pre-Flowering	0.98 (p ≈ 0.08)	0.99 (p ≈ 0.06)	1

p ≈ 0.05 indicates the borderline of statistical significance.
p > 0.05 denotes a lack of statistical significance.

The correlation analysis conducted revealed the relationships between the copper reduction antioxidant capacity (CUPRAC) of *Tilia platyphyllos* and the variables of Post-Flowering, Flowering, and Pre-Flowering. According to the analysis results, there is a strong positive relationship between the Post-Flowering and Flowering variables (r = 0.974). This suggests that these two variables tend to increase together.

In contrast, strong negative correlations were found between Post-Flowering and Pre-Flowering (r = -0.911), and between Flowering and Pre-Flowering (r = -0.981). These findings indicate that as the Pre-Flowering variable increases, both Post-Flowering and Flowering variables tend to decrease. Specifically, the correlation coefficient of -0.981 between Flowering and Pre-Flowering indicates a very strong inverse linear relationship between these two variables (Table 5).

Table 5. Correlation analysis results of copper reduction antioxidant capacity (CUPRAC).

	Post-Flowering	Flowering	Pre-Flowering
Post-Flowering	1	0.974 (*p*~0.14)	-0.911 (*p*~0.28)
Flowering	0.974 (*p*~0.14)	1	-0.981 (*p*~0.10)
Pre-Flowering	-0.911(*p*~0.28)	-0.981 (p~0.10)	1

p ≈ 0.05 indicates the borderline of statistical significance.
p > 0.05 denotes a lack of statistical significance.

DISCUSSION and CONCLUSION

The volatile component profile of *Tilia platyphyllos* was examined in detail across three different phenological stages, and the principal compounds exhibiting significant differences in each stage were identified. In the pre-flowering stage, the most dominant component of the essential oil was Hexane (52.21%), with Allyl bromide (18.84%) and p-cymene (8.88%) also being significant components. During the flowering stage, the proportion of Hexane increased to 55.12%, and Allyl bromide remained the second most abundant compound at 25.70%. The detection of Carvacrol at a concentration of 3.47% during flowering suggests that the synthesis of this phenolic compound begins during flowering and becomes dominant in the post-flowering stage, reaching 10.27%.

When compared with similar studies in the literature, these findings show both similarities and notable differences. For example, a study by Zrncic *et al.* (2010) on *Tilia cordata* reported high levels of monoterpenes and aromatic alcohols, such as linalool, benzyl alcohol, and *α*-terpineol, during the flowering stage. However, in that study, a compound like Hexane, which is aliphatic in nature, was not found in such high concentrations in the volatile fraction. This suggests that there could be significant variability in the volatile component profiles of different plant species (*T. cordata* and *T. platyphyllos*), influenced by sampling time, environmental factors, and extraction techniques (Kaurinovic *et al.*, 2011). Additionally, *Tilia tomentosa* flowers are rich in phenolic compounds, particularly bioactive compounds with antioxidant properties, highlighting their significant potential (Kıvrak *et al.*, 2017).

A particularly noteworthy finding of the present study is the consistently high concentration of Allyl bromide throughout all stages (17.53%–23.89%). While this compound is rarely observed in the volatile components of *Tilia* species in the literature, it has been reported that similarly structured halogenated aliphatic compounds can occasionally form due to environmental stress factors or exposure to anthropogenic pollutants (Santos *et al.*, 2015). This raises the possibility that specific chemical influences may be present in the habitat where the samples were collected.

Analyses conducted within the distribution area of *Tilia* revealed elevated levels of Allyl Bromide, which are attributed to the proximity of residential areas to waste disposal sites and the intensive coal-burning activities in the region. This finding indicates that anthropogenic pollution sources, particularly atmospheric and soil contamination, contribute to the accumulation of this compound in plant tissues. Organic compounds released into the atmosphere as a result of coal combustion adversely affect soil and air quality, leading to significant alterations in the chemical composition of *Tilia* populations. Therefore, the measured Allyl Bromide concentrations in plant materials collected near residential areas can be considered a biological indicator of environmental pollution.

The significant increase in Carvacrol, particularly in the post-flowering stage (10.27%), suggests that the biosynthesis of this phenolic compound intensifies during the maturation phase, and its potential antimicrobial and antioxidant effects may be amplified during this period. This finding aligns with data from Benincasa *et al.* (2017), who reported an increase in phenolic content after flowering in *Tilia tomentosa*.

The correlation analysis revealed significant changes in the total phenolic content of *Tilia platyphyllos* across the phenological stages. Specifically, the high positive correlation between the flowering and post-flowering stages ($r = 0.987$) suggests that total phenolic compounds in these stages are produced through similar biosynthetic pathways and maintain metabolic continuity. This finding is consistent with studies indicating that phenolic compounds increase at certain stages of plant development and continue to accumulate in response to environmental stress factors (Tattini *et al.*, 2004; Treutter, 2005).

In contrast, negative and weak correlations were observed between the pre-flowering stage and both the flowering ($r = -0.185$) and post-flowering ($r = -0.027$) stages. This indicates that total phenolic content is quite low during the pre-flowering stage. It has been suggested that phenolic metabolites typically increase during periods when the plant's defense mechanisms are actively engaged, and these defense systems may not yet be fully activated during the pre-flowering stage (Lattanzio *et al.*, 2006). Similarly, flavonoid and phenolic contents are known to increase in response to environmental stimuli (such as UV radiation, oxidative stress, etc.) during flowering and post-flowering stages (Agati *et al.*, 2012; Rice-Evans *et al.*, 1997).

These findings suggest that total phenolic accumulation is sensitive to phenological stages and that the post-flowering stage may be the most phenol-rich period in this species. This has significant implications for determining the optimal harvest time for plant material, indicating that the post-flowering stage is more suitable for pharmacological and food supplement

applications aimed at maximizing biological effects (Barros *et al.*, 2011; Kähkönen *et al.*, 1999).

The antiradical activity values of *Tilia platyphyllos* were examined, and statistically significant positive relationships were identified across three different phenological phases: pre-flowering, flowering, and post-flowering. The correlation coefficient between the flowering and pre-flowering stages was found to be as high as 0.99, indicating that the antiradical activity in these two stages changes almost in parallel and is likely influenced by similar biochemical processes. These results are consistent with studies indicating that the production of phenolic compounds in plant materials varies according to the phenological phase, while biochemical continuity can be maintained between certain phases (Tattini *et al.*, 2004; Nicolle *et al.*, 2004).

Similarly, the post-flowering stage showed a high positive correlation with both the flowering ($r = 0.87$) and pre-flowering ($r = 0.98$) stages. This suggests that antiradical activity in *Tilia platyphyllos* is maintained through similar biological pathways from the start to the end of flowering, and the species exhibits a stable antioxidant capacity across its phenological phases. The biosynthesis of flavonoids and phenolic compounds is known to be sensitive to environmental stress factors and developmental stages (Treutter, 2005). Moreover, the high correlation values observed indicate that the production of these compounds is maintained within a specific physiological balance, and antiradical defense remains active throughout the development process.

These high correlations observed in our study provide important insights for determining the optimal harvest time of plant materials for medicinal and pharmacological applications. The biochemical similarities observed between phenological stages suggest that *Tilia platyphyllos* may exhibit similar antioxidant effects during different time periods, such as pre-flowering and flowering. These findings support the idea that plant material can be effectively harvested within a broader range of time, rather than at a specific period (Barros *et al.*, 2011; Wojdyło *et al.*, 2007).

The correlation analysis conducted on the CUPRAC (copper reduction antioxidant capacity) values of *Tilia platyphyllos* demonstrated that the phenological stages of the plant significantly and statistically affect antioxidant activity. According to the results, the positive and high correlation between the post-flowering and flowering stages ($r = 0.974$) indicates that these two stages show similar trends in antioxidant capacity, with CUPRAC levels increasing together during these periods. This suggests that the periods when antioxidant capacity reaches its peak coincide with increased synthesis of phenolic compounds and antioxidant enzymes during the flowering process (Treutter, 2005; Wojdyło *et al.*, 2007).

Conversely, the negative and strong correlations between the pre-flowering stage and both the post-flowering ($r = -0.911$) and flowering ($r = -0.981$) stages indicate that CUPRAC values were very low during the pre-flowering stage and change inversely as flowering begins. The increase in phenolic metabolites and antioxidant capacity is generally regarded as a plant defense mechanism against environmental stress factors (e.g., UV radiation, oxidative stress) (Agati *et al.*, 2012; Lattanzio *et al.*, 2006). The relatively low effect of these stress factors during the pre-flowering stage or the incomplete activation of defense systems could have contributed to the low CUPRAC values during this period.

The notably negative correlation of -0.981 between the flowering and pre-flowering stages suggests that antioxidant capacity is closely linked to developmental processes, and phenological transitions create sharp differences in the CUPRAC mechanism. These findings are consistent with the literature, which reports that plant antioxidant capacity can vary not only by species but also according to harvest time (Barros *et al.*, 2011; Kähkönen *et al.*, 1999). In this context, the flowering and post-flowering periods, when CUPRAC levels peak, can be considered the most suitable harvest times for potential pharmacological and functional food applications.

This study provides a detailed examination of the volatile compound profiles, total phenolic contents, antiradical activities, and antioxidant capacities determined by CUPRAC across the different phenological stages of *Tilia platyphyllos* flowers, revealing how the plant's biochemical dynamics change seasonally. The strong positive and negative correlations obtained offer important insights into the biosynthetic pathways involved in the synthesis of these compounds, as well as the influence of environmental factors. In this context, optimizing the harvest time based on scientific data is of great significance for the development of medicinal, aromatic, and functional products from *Tilia platyphyllos*. It is crucial for maximizing product quality, active ingredient yield, and biological activity. Future research, supported by molecular-level biosynthetic pathway analyses and validated under different ecological conditions, will contribute to a better understanding of the plant's industrial potential.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Ebru Hatice Tığlı Kaytanlıoğlu: Investigation, Resources, Software, Formal Analysis, and Writing - original draft. **Süleyman Mert Günal:** Investigation, Visualization. **Hüseyin Fakir:** Methodology, Supervision, and Validation.

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