ANADOLU UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

European Journal of Life Sciences 2022; 1: 107-111

Research Article

Volatile compositions of *Tilia platyphyllos* Scop. infusions by headspace-solid-phase microextraction (HS-SPME), antioxidant activity

Damla Kırcı[1], Gözde Öztürk[2], Betül Demirci[2]

[1]Selçuk University, Faculty of Pharmacy, Department of Pharmacognosy, Konya, Türkiye.
[2]Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir, Türkiye.

✉ Damla Kırcı
damla.kirci@selcuk.edu.tr

https://doi.org/10.55971/EJLS.1207835

Received: 21.11.2022
Accepted: 12.01.2023
Available online: 24.02.2023

ABSTRACT

The genus *Tilia* (Tiliaceae) represents 45 species, of which six species are European. *Tilia* sp. are simple, cordate or deciduous trees with long, silicate shaped, 5-valve fruits and fragrant flowers. It is used as a medicinal tea in traditional medicine for colds, coughs, and hypertension and as an antioxidant.

Within the scope of this research, *Tilia platyphyllos* Scop. in the culture form from Eskişehir was obtained, and infusions were prepared at different times (including 5 min., 10 min., 15 min., and 30 min., respectively). Headspace-solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) was used to examine the volatile components in the infusion extracts. Major volatile compounds of infusion extracts determined as *E)-β*-ocimene (15.7-45.9%) and limonene (11.7-33.4%), respectively. Also, terpinolene (26.0%) were identified as the main compounds for 30 min infusion. After then, the infusion extracts were lyophilized, and the antioxidant activity of the infusion extracts were performed by DPPH· radical scavenging effect. It was determined that inhibition percentages were relatively high in the concentration range of 10-0.02 mg/mL (5.60-72.45%).

Our first research was the chemical composition and biological activity of the time-dependent *T. platyphyllos* infusions.

Keywords: *Tilia platyphyllos* Scop., volatile compounds, infusion, antioxidant activity

1. INTRODUCTION

Tilia sp. (Tiliaceae) are trees with simple, cordate leaves or very tall, silicate-shaped, deciduous trees with 5-valve-blooming fruits and fragrant flowers. The genus *Tilia*is represented by 45 species, 6 of which are European. It is distributed in Central-Southern Europe, especially in Ukraine, France, Sweden and Northern Iran [1]. In Turkey, it grows in provinces such as Bolu, Zonguldak, Kastamonu, Çanakkale, Eskişehir, Isparta, Trabzon, İzmir, Kırklareli, Sakarya, Tekirdağ [2]. Generally, these species are widely grown in parks and gardens [3].

Due to its pleasant taste, linden or dried flower states of the linden tree (*T. cordata* Miller or *T. platyphyllos* Scop.) are traditionally often used in the form of herbal tea. It has been determined that *Tilia* species contain essential oil, mucilage, condensed tannins, procyanidin dimers, flavonoids, phenolic acids, amino acids, carbohydrates and saponins [4]. Thanks to these compounds it carries, Commission

E has approved the use of linden flower in coughs associated with colds and colds [5]. The British Herbal Compendium reported that it is used in upper respiratory tract diseases, colds, cough, hypertension and anxiety [6].

In this study, *Tilia platyphyllos* Scop. in the culture form from Eskişehir was obtained, and infusions were prepared at different times (including 5 min., 10 min., 15 min. and 30 min., respectively). Headspace-solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) was used to examine the volatile compounds in the infusion extracts.

2. MATERIALS AND METHODS

2.1. Plant material

The *Tilia platyphyllos* material was collected in 2019 from Eskisehir province, Turkey. The plant was identified by Prof. Dr. Yavuz Bülent KÖSE (Anadolu University, Department of Pharmaceutical Botany). The sample is kept in Anadolu University, the Herbarium of Pharmacy Faculty with archive number 52. The Inflorescences of the plant was dried in the shade.

2.2. Extraction

Distilled water boiled at 80°C was added to the dried flowers. It was kept in different brewing times (5 min., 10 min., 15 min. and 30 min., respectively), filtered and infusions were prepared. After lyophilization, the infusions were used in biological activity studies.

2.3. Chemical composition

2.3.1. Headspace-Solid Phase Microextraction (HS-SPME)

Headspace-solid phase microextraction (HS-SPME) is a simple to use and solvent-free extraction technique. It is a sensitive, fast and inexpensive method that is widely used in the analysis of volatile components. It is a method based on the adsorption of volatile components onto the fiber coated with a polymeric stationary phase and the heat desorption of the components attached to the fiber at the gas chromatography injection port. Volatile components in the sample are adsorbed onto the fiber from the headspace of the samples, either directly or in a closed environment. When equilibrium is reached, usually after a period of 2-30 minutes, the fiber containing the adsorbed components is removed from the sample. These components are recovered by thermal desorption at a GC injector port and analyzed with the appropriate column and detector [7].

2.3.2. Gas Chromatography (GC)/Mass Spectrometry (MS)

GC/MS conditions were described previously. Identification of the volatile components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC-MS Library, MassFinder 4.0 Library), and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data was used for the identification as also previously reported [7,8].

2.4. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

Serial dilutions were carried out with stock solutions (10 mg/mL) of the infusions to obtain the concentrations of 0.5-0.001 mg/mL. Diluted solutions were mixed with DPPH· and allowed to stand for 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm at room temperature using a microplate spectrophotometer. The experiment was performed three times and average absorption was noted for each concentration. Ascorbic acid was used as a positive control. The percentage of inhibition was calculated using equation [9].

$A_{control}$: Absorbance of control

A_{sample}: Absorbance of sample

$$\text{Percentage Inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

3. RESULTS AND DISCUSSION

3.1. Chemical composition

The chemical compositions of the infusions were analyzed by gas chromatography/mass spectrometry (GC/MS) methods. The results of the analyzes were listed in Table 1.

(*E*)-*β*-Ocimene was determined as the major compound found in common in each sample (43.0%, 45.9%, 15.7%, 18.7%, 41.2%). Benzene acetonitrile (10.7%) was found as major component for 5 min. infusion of *T. platyphyllos*, Limonene (11.7%) and benzene acetonitrile (7.7%) were found as major components for 10 min infusion. Limonene (22.5%) was found as major constituent for 15 min. infusion. Limonene (34.4%) and terpinolene (26.0%) were defined as the main compounds for 30 min infusion. The percentage of limonene and terpinolene increased in the 30 min infusion, and their structure is cyclic monoterpenes. Also, ocimene, which is an acyclic monoterpene, was decreased depending on the time in the infusions.

In previous research, the essential oils of the inflorescences and leaves of *T. platyphyllos* were obtained from five different provinces in Kosovo. *n*-Heneicosane (3.59-13.50%), *n*-pentacosane (13.90-19.61%), *n*-nanocosane (1.49-13.98%), and *n*-nonanal (3.30-8.97%) were identified as the main components [10]. In the other research, tricosan (18,12%), heneicosan (10,06%), and pentacosan (6,08%) were found to be the main components of essential oil of *T. platyphyllos* linden blossoms [11]. A high amount of hydrocarbons (47.5–66.5%) was found in the essential oil of *T. platyphyllos* flowers [12].

The essential oil and the infusion of *T. platyphyllos* flowers were extracted with dichloromethane and were analyzed by GC/MS. The main component of essential oil was 2-phenyl ethanol with 26.07%. In the infusion of *T. platyphyllos*, 2-phenyl ethanol and 2-phenylethyl butanoate represented 29.48% and 12.11%, respectively [13].

A high amount of hydrocarbons was identified in the essential oils of *T. platyphyllos* flowers, while major components of monoterpenes were found in the infusions of *T. platyphyllos* flowers.

3.2. 1,1-Diphenyl-2-picrylhydrazyl (DPPH·) radical scavenging activity

The antioxidant capacity of infusions was tested. The % inhibition values of the samples and positive control ascorbic acid were given in Figure 1. It was

Table 1. Volatile components of *Tilia platyphyllos* Scop.

RRI[a]	Component	A %[b]	B %	C %	D %
1203	Limonene	tr[c]	**11.7**	**22.5**	**34.4**
1266	(*E*)-*β*-Ocimene	**43.0**	**45.9**	**15.7**	**18.7**
1290	Terpinolene	-	-	-	**26.0**
1360	1-Hexanol	3.8	3.5	-	-
1452	*α*-*p*-Dimethyl styrene	5.3	2.7	-	2.8
1553	Linalool	-	-	1.5	1.4
1719	Borneol	-	-	0.5	-
1957	Benzene acetonitrile	**10.7**	**7.7**	-	-
2091	*cis*-Methyl isoeugenol	2.3	-	-	-
2400	Tetracosane	-	1.8	-	-
2500	Pentacosane	-	2.5	-	-
2600	Hexacosane	-	2.9	-	-
2700	Heptacosane	-	3.6	-	-
2900	Nonacosane	-	3.7	-	-
	Total	**85.6**	**92.6**	**75.9**	**96.5**

[a]RRI: Relative retention indices calculated against n-alkanes; [b]%, calculated from the TIC; chromatograms; [c]tr: Trace amount (<0.1%); A: 5 min. infusion; B: 10 min. infusion; C: 15 min. infusion; D: 30 min. infusion.

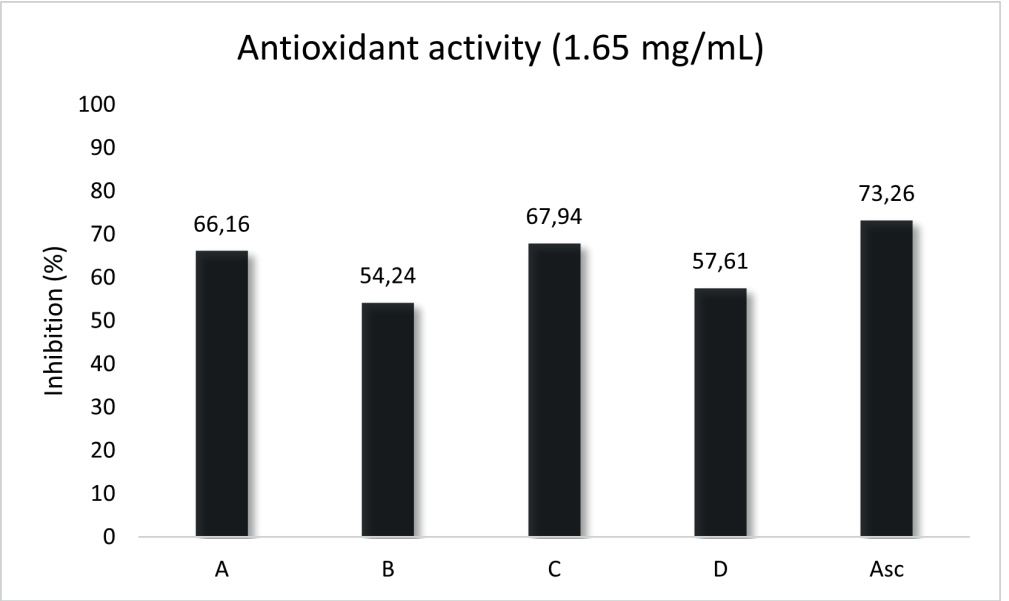

Figure 1. % Inhibition values of *Tilia platyphyllos* Scop. infusions
A: 5 min. infusion; B: 10 min. infusion; C: 15 min. infusion; D: 30 min. infusion; Asc.: Ascorbic acid.

studied in the concentration range of 10-0.02 mg/mL. According to the antioxidant activity results, all infusions showed as high effects as ascorbic acid.

In previous study, the antioxidant effects of *T. platyphyllos* infusions and methanol extract were determined by the DPPH· radical scavenging method. It has been reported that there is lower antioxidant activity in infusions (0.03 mmol/g) compared to the extract and essential oil [14]. In another study, it was determined that the antioxidant activity of ethanol extracts (IC_{50} =105±1 µg/mL) was high [15].

4. CONCLUSION

According to our results, the volatile components of *T. platyphyllos* infusions were rich in monoterpenes. The infusion of *T. platyphyllos,* 15 min., was found to have high antioxidant activity. To the best of our knowledge, the volatile components of *T. platyphyllos* infusions were evaluated for the first time by using the HS-SPME method. Also, the changes in the contents depending on the infusion times were revealed.Our research was the first on the biological activity of time-dependent infusions.

Acknowledgment

This study was financially supported as a Scientific Research Project (BAP 1905S077) by Anadolu University. Part of the manuscript was presented at the 5th International Eurasian Conference on Biological and Chemical Sciences. We thank Prof. Dr Yavuz Bülent Köse for helping identify the plant.

Author contribution

Concept: BD, GÖ, DK; Design: BD; Supervision: BD; Materials: GÖ; Data Collection and/or Processing: GÖ, DK; Analysis and/or Interpretation: BD, GÖ, DK; Literature Search: GÖ, DK; Writing: GÖ, DK; Critical Reviews: BD.

Source of funding

This work was financially supported by Research and Scientific Research Project Commission of Anadolu University (BAP1905S077).

Conflict of interest

The authors declared that there is no conflict of interest.

REFERENCES

1. Mabberley DJ. The Plant-Book. Cambridge: Cambridge University Press; 1997. ISBN: 0521414210.
2. Turkish Plants Data Service. Available at: https://www.tubives.com (Accessed on November 17, 2022)
3. Browicz K. Flora Europaea. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, editors. *Tilia* L. Cambridge: Cambridge University Press; 1976. p. 247-248. ISBN: 0521087171.
4. Yayalaci Y, Celik I, Bati B. Hepatoprotective and antioxidant activity of linden (*Tilia platyphyllos* L.) infusion against ethanol-induced oxidative stress in rats. J Membr Bio. 2014;247(2):181-188. https://doi.org/10.1007/s00232-013-9622-z
5. Blumenthal M, Goldberg A, Brinckmann J. Herbal Medicine. Expanded Commission E Monographs. Austin, TX: American Botanical Council; 2000. ISBN: 0967077214.
6. Bradley P. British Herbal Compendium. Bournemouth: British Herbal Medicine Association; 1992. ISBN: 9780903032124.
7. Demirci B, Demirci F, Başer KHC. Headspace-SPME and hydrodistillation of two fragrant *Artemisia* sp. Flavour Fragr J. 2005;19:395-398. https://doi.org/10.1002/ffj.1426
8. Demirci B, Kırcı D, Öztürk G, Demirci F. The Effect of extraction time on Origanum onites L. infusions and essential oils–biological evaluation, statistical principal component and hierarchial cluster analyses. Chem Biodiversity. 2022;19(12):e202200482. https://doi.org/10.1002/cbdv.202200482
9. Demirci F, Demirci B, Gürbüz İ, Yeşilada E, Başer KHC. Characterization and biological activity of *Achillea teretifolia* Willd. and *A. nobilis* L. subsp. *neilreichii* (Kerner) Formanek essential oils. Turk J Biol. 2009;33(2):129-136. https://doi.org/10.3906/biy-0808-1
10. Kelmendi N, Mustafa B, Zahiri F, Nebija D, Hajdari A. Essential oil composition of *Tilia platyphyllos* Scop. collected from different regions of Kosovo. Rec Nat Prod. 2020;14(5):366-371. http://doi.org/10.25135/rnp.171.19.11.1490
11. Prączko A, Góra J. The chemical composition of essential oil distillated from Linden blossoms. Żywność. 2000;4(25):90-94.
12. Toker G, Baser KHC, Kürkçüoğlu M, Özek T. The composition of essential oils from *Tilia* L. species growing in Turkey. J Essent Oil Res. 1999;11(3):369-374. https://doi.org/10.1080/10412905.1999.9701158
13. Rădulescu V, Oprea E. Analysis of volatile compounds of officinal Tiliae flos by gas–chromatography coupled with mass spectrometry. Farmacia. 2008;56(2):129-138.
14. Pogačnık L, Ulrih NP. Determination of antioxidants in Medicinal herbs. Bulletin of the Transilvania University of Braşov Series VI: Medical Sciences. 2011;4(53):95-102.
15. Jabeur I, Martins N, Barros L, et al. Contribution of the phenolic composition to the antioxidant, anti-inflammatory and antitumor potential of *Equisetum giganteum* L. and *Tilia platyphyllos* Scop. Food Funct. 2017;8:975-984. https://doi.org/10.1039/C6FO01778A