Volatile organic compounds of *Tilia cordata* Mill. from Serbia, in terms of ecosystem services

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**Abstract:** It is considered that different plant organic compounds, known as phytoncides are creditable for positive effects of Forest therapy on human health. Ongoing pandemic has also put these compounds in research focus considering potential use for novel corona virus treatment. This research was conducted in order to examine the potential of *Tilia cordata* Mill. considering volatile organic compounds (VOCs) content for the use in Forest therapy. In order to determine qualitative and quantitative content of phytoncides, as well the variability among genotypes growing in the same environmental conditions, leaf samples from genotypes of *T. cordata* were collected from the Fruška gora (Serbia) during 2019. VOCs determined in fresh herbal material were analyzed by Headspace-GC/MS and VOCs determined in essential oil were obtained by GC/MS. The results showed the presence of 17 different phytoncides (monoterpane hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, aliphatic compounds and diterpenes) in all tested genotypes. According to the mean values of the obtained results, the most abundant classes of phytoncides among the examined genotypes were monoterpene hydrocarbons (87.05%), followed by aliphatic compounds (36.59%) and oxygenated sesquiterpenes (34.60%). The highest coefficient of variation (CV) among phytoncide content in tested *T. cordata* genotypes has been observed within oxygenated monoterpenes (43.12%), sesquiterpene hydrocarbons (28.18%), and diterpenes (27.04%). Some individual monoterpene hydrocarbons, such as o-cymene (19.92%) and α-pinene (17.40%) had the highest CV in terms of its presence in analyzed genotypes. In addition, the principal component analysis (PCA) showed more notable clustering within the VOCs determined in essential oil in comparison to VOCs determined in fresh herbal material. Considering the phytoncide content detected in *T. cordata* leaves, this species has high potential for the use in human health improvement within Forest therapy.

**Keywords:** Ecosystem services, *Tilia*, linden, phytoncides, VOCs, human health, forest therapy.

1. Introduction

In terms of ecosystem services, besides air purification, carbon fixation and oxygen supply, trees and forests have huge impact on human quality of life, health, and well-being. The results of different
medicinal research on forest therapy showed positive impact on (i) immune system function- increase in number and activity of Natural killer (NK) cells for cancer prevention (Li, 2010; Sunjaya et al. 2018); (ii) cardiovascular system- lowering blood pressure and heart rate (Li et al. 2011; Lee et al. 2014; Ochiai et al. 2015); (iii) respiratory system- allergies and asthma (Hansen et al. 2017; Sunjaya et al. 2018); (iv) diabetes- decreased levels of blood glucose (Ohtsuka et al. 1998); and (v) depression and anxiety (Li, 2010; Li et al. 2011; Lee et al. 2011; Ochiai et al. 2015).

Although the positive impact of forests on human health is obvious, the exact processes of human health improvement and the role of the plants in this process are still not fully explained. Some suggest that organic compounds derived from trees, called phytoncides, mostly monoterpenes and sesquiterpenes are the most merit forest part for human health improvement (Cheng et al. 2009; Li, 2010).

Four linden species are distributed naturally in Europe (i) Caucasian linden – *T. dasystyla* (Stev.); (ii) Silver linden – *T. tomentosa* (Moench.); (iii) Small-leaved linden – *T. cordata* (Mill.); and (iv) Large-leaved linden – *T. platyphyllos* (Scop.) (Kesić et al. 2020). *Tilia cordata* is a minor, broadleaved species with wide, but scattered distribution in Europe, characterized as a species with wide ecological tolerance and numerous ecosystem services (De Jaegere et al. 2016). Besides its natural distribution, *T. cordata* is a common species in parks, or other urban green areas in the Eastern Balkan region (Vaštag et al. 2018; Kesić et al. 2020). As flowers of *Tilia* species are widely used in the traditional medicine as herbal tea for cough treatment or restlessness, most of the previous research have put its focus on the organic composition of the dried inflorescence (Fitsiou et al. 2007).

Since the expected rise of the importance of *T. cordata* in the terms of forest management and ecosystem services (De Jaegere et al. 2016) and numerous positive health impact of forest therapy on human health (Ohtsuka et al. 1998; Li et al. 2009; Li, 2010; Lee et al. 2011; Li et al. 2011; Lee et al. 2014; Ochiai et al. 2015), this paper presents preliminary research on the potential use of *T. cordata* in forest therapy. The chemical profiling of volatile organic compounds (VOCs) and essential oils in leaves of three *T. cordata* genotypes are presented. These results will suggest whether this species has potential for human health improvement and will serve as a basis for further research in selection of suitable tree species and genotypes for forest therapy.

2. Material and methods

2.1. Plant material

All analyzed samples were collected from Fruška gora mountain in Serbia, during June 2019 (Table 1). Samples were taken as a tip of the branches with fully formed leaves from three healthy and representative genotypes of *T. cordata* Mill. All samples were south, and light exposed. Sampling has been done during noon on a sunny day with no precipitation, with temperature range of min 18°C and max 31°C. In order to ensure full plant examination, the samples were taken from three different parts of the tree canopy: lowest point, middle point, and top point. After cutting, samples were stored in paper bags and delivered to the lab and analyzed within 12 hours.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Diameter (m)</th>
<th>Height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype 1</td>
<td>45° 08’ 49.7''</td>
<td>19° 46’ 54.0''</td>
<td>0.60</td>
<td>20</td>
</tr>
<tr>
<td>Genotype 2</td>
<td>45° 08’ 49.1''</td>
<td>19° 46’ 41.5''</td>
<td>0.50</td>
<td>18</td>
</tr>
<tr>
<td>Genotype 3</td>
<td>45° 08’ 43.3''</td>
<td>19° 46’ 44.2''</td>
<td>0.65</td>
<td>25</td>
</tr>
</tbody>
</table>
2.2. Phytoncide analysis

The essential oils were isolated by hydrodistillation with Clevenger apparatus for 2.5 hours. After the solvent removal, the yield of extraction was determined gravimetrically. The qualitative and quantitative analyses of the essential oil was carried out on HP-5ms capillary column (30 m x 0.25 mm; film thickness 0.25 µm) by Agilent 6890B GC-FID instrument coupled to Agilent 5977 MSD. The samples were injected in splitless mode and at inlet temperature of 220°C. The oven temperature was set at 60°C and increased at a rate of 3°C/min up to 246°C. Helium was the carrier gas (1 ml/min) while the temperature of the MSD transfer line was set to 230°C.

Dried samples of collected plant material were crushed and sealed in headspace vials (20 mL) prior to analysis. Vials were incubated at 100°C for 20 min in a headspace sampler (5977HSS, Agilent Technologies) and injected to gas chromatograph in splitless mode. The inlet temperature and oven heating program were the same as in the case of EO analysis. Data were collected in scan mode (m/z = 50 – 550), while the identification of compounds was performed using NIST (v14) mass spectral database and comparison of relative retention indices (RT).

2.3. Data analysis

The analysis of data related to the chemical profiles of the VOCs and essential oils was performed using the R environment (R Development Core Team, 2011). Phytoncide data were expressed as the mean percentage of the relative amounts, with Standard Deviation (SD) and Coefficient of Variation (CV). Data with normal distribution (χ² test; p ≥0.05), and with no statistically important differences between the standard deviation of the genotypes (Levene’s test; p ≥0.05), were analyzed by Principal Component Analysis (PCA) as previously described in Nikolić et al. (2007). Data were analyzed in R environment (R) with the use of software packages "prcomp" (Version 4.0.2) and "ggplot2" (Version 3.3.2).

3. Results

Data considering the phytoncide content in the genotypes of *T. cordata* with mean, SD and CV for each compound are presented in Table 2. Seventeen different compounds occurred in all three tested genotypes of *Tilia cordata* from Serbia, among which monoterpene hydrocarbons were the major constituents, followed by aliphatic compounds and oxygenated sesquiterpenes in all three tested genotypes. The main VOCs in all genotypes were β-ocimene, terpinolene and limonene, while the most abundant compounds present in the essential oils were α-cadinol, heneicosane and epi-α-muurolol.

![Figure 1. Results of the principal component analysis (PCA) performed on phytoncide content in all analyzed genotypes.](image)
According to the mean values of the obtained results, the most abundant classes of phytoncides among the examined genotypes were monoterpenic hydrocarbons (87.05%), followed by aliphatic compounds (36.59%) and oxygenated sesquiterpenes (34.60%). The highest CV has been observed within oxygenated monoterpenes (43.12%), sesquiterpene hydrocarbons (28.18%), and diterpenes (27.04%). The highest CV among detected compounds in all tested genotypes was observed for α-bisabolol (51.46%), linalool (43.12%), α-cadinol (28.61%), cis-β-farnesene (28.18%), and epi-13-manool (27.04%). Some of the analyzed monoterpene hydrocarbons, such as o-cymene (19.92%) and α-pinene (17.40%) also showed high CV in terms of their presence in different genotypes.

<table>
<thead>
<tr>
<th>Compound (%)</th>
<th>RI</th>
<th>genotype 1</th>
<th>genotype 2</th>
<th>genotype 3</th>
<th>Mean</th>
<th>SD</th>
<th>CV (%)</th>
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<tr>
<td>Monoterpenic Hydrocarbons</td>
<td></td>
<td></td>
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<tr>
<td>α-Pinene*</td>
<td>937</td>
<td>6.29</td>
<td>8.29</td>
<td>8.89</td>
<td>7.82</td>
<td>1.36</td>
<td>17.40</td>
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<tr>
<td>α-Cymene*</td>
<td>1022</td>
<td>3.23</td>
<td>4.39</td>
<td>4.83</td>
<td>4.15</td>
<td>0.83</td>
<td>19.92</td>
</tr>
<tr>
<td>Limonene*</td>
<td>1030</td>
<td>11.40</td>
<td>14.55</td>
<td>14.17</td>
<td>13.37</td>
<td>1.72</td>
<td>12.86</td>
</tr>
<tr>
<td>β-Ocimene*</td>
<td>1037</td>
<td>47.37</td>
<td>42.27</td>
<td>41.74</td>
<td>43.79</td>
<td>3.11</td>
<td>7.10</td>
</tr>
<tr>
<td>γ-Terpinene*</td>
<td>1060</td>
<td>3.33</td>
<td>3.87</td>
<td>3.93</td>
<td>3.71</td>
<td>0.33</td>
<td>8.91</td>
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<tr>
<td>Terpinolene*</td>
<td>1088</td>
<td>14.82</td>
<td>13.52</td>
<td>14.27</td>
<td>14.20</td>
<td>0.65</td>
<td>4.59</td>
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<td>Oxygenated Monoterpenes</td>
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<td>Linalool*</td>
<td>1099</td>
<td>6.49</td>
<td>4.24</td>
<td>2.66</td>
<td>4.46</td>
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<td>43.12</td>
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<td>Sesquiterpenic Hydrocarbons</td>
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<td>cis-β-Farnesene*</td>
<td>1444</td>
<td>2.11</td>
<td>3.34</td>
<td>2.11</td>
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<td>28.18</td>
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<td>Oxygenated Sesquiterpenes</td>
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<tr>
<td>Caryophyllene oxide**</td>
<td>1581</td>
<td>2.07</td>
<td>2.15</td>
<td>1.98</td>
<td>2.07</td>
<td>0.09</td>
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<td>epi-α-Muurolol**</td>
<td>1642</td>
<td>10.74</td>
<td>10.26</td>
<td>11.82</td>
<td>10.94</td>
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<tr>
<td>α-Cadinol**</td>
<td>1653</td>
<td>25.54</td>
<td>14.18</td>
<td>19.84</td>
<td>19.85</td>
<td>5.68</td>
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<tr>
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<td>1684</td>
<td>1.11</td>
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<td>1.35</td>
<td>1.74</td>
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<td>Aliphatic Compounds</td>
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<tr>
<td>n-Octane*</td>
<td>800</td>
<td>3.02</td>
<td>3.24</td>
<td>3.32</td>
<td>3.19</td>
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<td>1-Hexanol*</td>
<td>868</td>
<td>4.03</td>
<td>5.32</td>
<td>4.84</td>
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<td>13.78</td>
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<td>Heneicosane**</td>
<td>2100</td>
<td>25.85</td>
<td>26.01</td>
<td>27.19</td>
<td>26.35</td>
<td>0.73</td>
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<tr>
<td>Docosane**</td>
<td>2200</td>
<td>2.37</td>
<td>2.55</td>
<td>2.02</td>
<td>2.31</td>
<td>0.27</td>
<td>11.65</td>
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<tr>
<td>Diterpenes</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Epi-13-Manool**</td>
<td>2056</td>
<td>4.61</td>
<td>3.91</td>
<td>6.53</td>
<td>5.02</td>
<td>1.36</td>
<td>27.04</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>174.38</td>
<td>164.86</td>
<td>171.49</td>
</tr>
</tbody>
</table>

Note: The data shows content of VOCs determined in fresh herbal material (Headspace-GC/MS 1*) and VOCs determined in essential oil (GC/MS Fresh**).

Figure 1 shows that the first two principal components (PC1 and PC2) explain more than 98% of the data variability. The six analyzed samples were separated in three groups. The PCA results showed grouping of all three genotypes considering the VOCs content determined in fresh herbal material (Tilia cordata Headspace-GC/MS 1, Tilia cordata Headspace-GC/MS 2 and Tilia cordata Headspace-GC/MS 3). Considering the content of VOCs determined in essential oil, Genotype number 2 (Tilia cordata GC/MS Fresh 2) differed from the other two tested genotypes (Tilia cordata GC/MS Fresh 1 and Tilia cordata GC/MS Fresh 3).
The distribution of detected compounds in PCA biplot (Figure 2) shows that major variation was detected within the content of essential oils: epi-13-manool, epi-\(\alpha\)-muurolol, heneicosane and \(\alpha\)-cadinol.

![PCA biplot](image)

**Figure 2.** PCA biplot. 1- *Tilia cordata* Headspace-GC/MS1; 2- *Tilia cordata* Headspace-GC/MS2; 3-*Tilia cordata* Headspace-GC/MS3; 4- *Tilia cordata* GC/MS Fresh1; 5- *Tilia cordata* GC/MS Fresh2; 6- *Tilia cordata* GC/MS Fresh.

4. Discussion

Due to the numerous research on the forest ecosystem services in recent years, humanity tends to acknowledge the importance of natural environments considering their health and well-being (Van Herzele et al. 2011), and basic awareness of positive impact of nature on human health is becoming more present in the global society (Zorić et al. 2019), but still there is a need for further research on this topic.

VOCs and essential oils in *Tilia* species were subject of several previous research (Buchbauer et al. 1992; Toker et al. 1999; Ahmadi et al. 1999; Fitsiou et al. 2007; Kelmendi et al. 2020) mostly focused on the compounds found in the inflorescence. In presented research, the focus was put on the leaves, due to the fact that they last more than flowers which is notable for the use of this species in forest therapy. The VOCs are observed as the plant potential to evaporate organic compounds, known as phytoncides, that can improve human health (Cheng et al. 2009; Li, 2010). Previous studies defined the effects of certain organic compounds considering health improvement: (i) Anticancer- \(\alpha\)-pinene, limonene, terpinolene, \(\beta\)-ocimene, \(\alpha\)-cadinol, linalool, \(\alpha\)-bisabolol (Li et al. 2009; Kamatou et al. 2010; Aziz et al. 2016; Iwaski et al. 2016; Hafidh et al. 2018; Abbas et al. 2019; Rajivgandhi et al. 2020); (ii) Relieving symptoms of anxiety and depression- \(\alpha\)-pinene, \(\gamma\)-terpinene, linalool (Guzmán-Gutiérrez et al. 2012; Da Silva et al. 2020). (iii) Anti-inflamatory- \(\alpha\)-pinene, limonene, linalool, \(\alpha\)-bisabolol (Hirota et al. 2010; Kamatou et al. 2010; Lee et al. 2018; Kim et al. 2019). Also, recent research presented potential of phytoncides for novel corona virus treatment (Nadjib, 2020), while previous study showed positive impact of these compounds, among which \(\alpha\)-cadinol found in tested genotypes for antiviral therapy (Wen et al. 2007). All of the listed compounds were detected in all three tested genotypes. Some of them, such as \(\alpha\)-bisabolol, linalool, and \(\alpha\)-cadinol have shown high coefficient of variation among its content in tested genotypes which could provide a basis for further research on intra- and inter-variations, as it was previously done in similar research (Sadeghi et al. 2013; Čukanović et al. 2020) and possibility for selection of genotypes most suitable for forest therapy.
Since the concept of ecosystem services emphasizes equality of timber and non-timber product of forests in terms of forest management (Trudić et al. 2015), there is a need for further research on the forest ecosystem services provided by forests and tree species. Van Herzele et al. (2011) claim that the main challenge is to apply the knowledge on nature and health relationship in such manner that fuller potential of these relationships is realized. Considering the fact that forest trees are more affected by intensive climate changes (Kostić et al. 2019; McDowell et al. 2020), and that the other research suggest the importance of T. cordata Mill. in terms of its resilience to future climate changes (Latte et al. 2020) and forest management (De Jaegere et al. 2016), studies on Tilia species in terms of ecosystem services are highly valuable.

5. Conclusions

Due to the rising importance of Tilia species considering its resilience to the future climate changes and future forest management, we have analyzed the volatile organic compounds (VOCs) potential of the genotypes of Tilia cordata Mill. for the possible use in forest therapy. The composition of VOCs determined in Tilia cordata Mill. showed the presence of organic compounds that have previously been described as anticancer, anti-inflammatory, antidepressant, and antiviral agents. Also, the coefficient of variation among certain compounds had high values which provides a basis for further research on suitable genotypes for forest therapy. Considering climate changes and global health challenges that all of us are struggling with in these moments, it is clear that there is a need for finding an unobtrusive method for human health improvement with the sustainable use of nature.

Acknowledgments

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of the essential oil and hydrolate from the fruit of Protium heptaphyllum and their isolated compounds in Colossoma macropomum juveniles. Aquaculture 529: 735629.


