forests

Article

Phytochemical Screening of Volatile Organic Compounds in Three Common Coniferous Tree Species in Terms of Forest Ecosystem Services

Martina Zorić [1,*], Saša Kostić [1], Nebojša Kladar [2], Biljana Božin [3], Verica Vasić [1], Marko Kebert [1] and Saša Orlović [1]

[1] Institute of Lowland Forestry and Environment, University of Novi Sad, Antona Čehova 13d, 21000 Novi Sad, Serbia; sasa.kostic@uns.ac.rs (S.K.); vericav@uns.ac.rs (V.V.); kebertm@uns.ac.rs (M.K.); sasao@uns.ac.rs (S.O.)
[2] Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia; nebojsa.kladar@mf.uns.ac.rs
[3] Center for Medical and Pharmaceutical Investigations and Quality Control, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia; biljana.bozin@mf.uns.ac.rs
* Correspondence: martinazoric@uns.ac.rs

Abstract: Multiple positive effects that forests have on human health and overall well-being have been reported widely in the literature. Still, multiple elements of this relationship remain unidentified and unexplained. In this study, the composition of leaf volatile organic compounds (BVOCs) content in three common coniferous species: the Austrian pine (*Pinus nigra*), Scots pine (*Pinus sylvestris*) and Spruce (*Picea abies*), was analyzed. The specificity of BVOCs content in the examined species and their genotypes is observed as a plant potential to evaporate these organic compounds and potentially improve human health and well-being. Principal component analysis applied on BVOCs content among species showed significant differences between compounds that have previously been characterized as having positive effects on human health by acting as anticancer, anti-inflammatory, antiviral and antibacterial. Variations among genotypes of the investigated species were observed in the content of BVOCs relevant for human health improvement, such as limonene, terpinolene, β-pinene, linalool, camphene, camphor, citronellol and α-cadinol. The observed intra- and inter-species variations in the BVOCs content provide an appropriate base for further research on the forest–human health relationship, breeding and selection of the most suitable genotypes for human health improvement, and could I mpact the sustainable management of forests.

Keywords: *Pinus nigra*; *Pinus sylvestris*; *Picea abies*; terpenes; BVOC; human health

Citation: Zorić, M.; Kostić, S.; Kladar, N.; Božin, B.; Vasić, V.; Kebert, M.; Orlović, S. Phytochemical Screening of Volatile Organic Compounds in Three Common Coniferous Tree Species in Terms of Forest Ecosystem Services. *Forests* **2021**, *12*, 928. https://doi.org/10.3390/f12070928

Academic Editors: Alan Ewert and Jillisa Overholt

Received: 9 June 2021
Accepted: 14 July 2021
Published: 15 July 2021

1. Introduction

In light of the on-going pandemic, a significant effort has been invested into finding the alternative strategies for human health improvement, among which nature visits and forest therapy or *Shinrin Yoku* showed the potential to enhance the immune response [1]. The basis of forest therapy lies in the relaxed time spent in forests as a means of restoring mental and physical balance or the act of engaging in various therapeutic activities while in forests, to improve health and well-being [2]. In certain countries, such as Korea, this term is defined by law as 'immune-strengthening and health-promoting activities utilizing various elements of the forest, such as fragrance and scenic view' [3]. Also, due to urbanization and the modern "sedentary" lifestyle, the overall societal need to maintain contact with nature is increasing, with forests playing an important role in this regard given their capacity to provide important recreational services [4].

A significant amount of medical research has shown the positive impact that trees and forests have on the human health, with studies proving its positive impact on the immune, cardiovascular and respiratory system, as well as proving its positive impact on reduction of symptoms of diabetes, mental disorders and stress (Table 1).

Table 1. Positive impacts of trees and forests on human health.

Impact	Description of Improvement	References
The immune system	Observed increment of natural killer (NK) cells	[5–7]
Cardio-vascular system	Observed lower blood pressure and heart rate	[8–11]
Respiratory system	Decreased symptoms of allergies and asthma	[7,12]
Diabetes	Observed decrement of blood glucose values	[13]
Mental disorders	Decreased symptoms of stress, depression, and anxiety	[3,6,8,10,14–17]
Chronic pain	Chronic pain decrement	[18]

A recent comprehensive investigation of global forest ecosystem services (ES) research has revealed that the published studies have assessed multiple forest functions, whereas the majority of research was focused on the regulating services, such as carbon storage and/or sequestration and climate regulation [19]. Since the general influence of the ecosystems on human well-being is well-recognized [20], further research on this subject would notably contribute to improving overall public awareness that ecosystems should be understood as an important determinant of human health [21].

Although the positive influence of trees and forests on human health is evident, the exact mechanism of human health improvement in these environments is still not fully explained [22]. A recently published review on the existing literature considering the forest–human health relationship highlights that it still remains largely unexplored [23] and more accurate descriptions of forest variables in future human health studies are needed in order to provide data for future changes in the forest management [24]. Some researchers suggest that the volatile organic compounds (VOCs) released from trees, also named phytoncides or biogenic volatile organic compounds (BVOCs), are the main factors for human health improvement [8,25]. In addition to the potential for direct human health improvement, these compounds also contribute to changes in the air quality [26] and therefore indirectly affect human health and well-being. The current literature on BVOCs analysis shows that the results describing content of these compounds in forest tree species were mostly used as chemotaxonomic indicators and biochemical markers of provenances in different species [27,28]. More recently, the role of the BVOCs composition in the air is being analyzed, especially in the urban areas trough impact on ozone formation [29–31] and the potential to reduce the content of harmful particulate matter (PM) in the urban air [32,33]. Further work on the ozone formation is highly valuable, especially in the field of forestry, since it is causing greater damage to the vegetation compared to other pollutant gases, which results in substantial economic loss for horticulture, agriculture and forestry [34].

BVOCs in the air include atmospheric trace gases of organic origin, such as terpenes (isoprenoids), alkanes, alkenes, aldehydes, ketones, alcohols, esters, ethers and acids [35]. Terpenes are the largest class of plant secondary metabolites, but still are largely unrecognized, although about 1000 new structures are being added every year [36]. The volatile terpenes mostly include classes of monoterpenes, sesquiterpenes and diterpenes, which differentiate based on the number of characteristic C5 units. Furthermore, monoterpenes (MT) with two C5 units and sesquiterpenes (SQT) with three C5 units are the most abundant in the air and are recognized to have an important role in forming secondary organic aerosols [37]. BVOCs, including terpenes, are described as highly reactive compounds when emitted in the air, and their lifetime is highly dependent on the environmental conditions [35,38–40] that are characteristic to each specific time and place.

Terpenes that originate from plants are produced as secondary metabolites, whose primary role is protection from stress, herbivores and pathogenic microorganisms [41]. Forests are recognized as a major source of these compounds [42], and although the content and type of terpenes is characteristic for each species, certain authors [40] emphasize that their content can also differ between individual trees.

The aim of our research was to define and investigate differences in the terpene emission potential of the selected tree species and their superior genotypes. The use of this "screening" methodology, implemented in the presented research, means the results would not be limited and restricted to a certain time and place, keeping in mind high dependability of BVOCs in the air on the different factors of the environment. Our research provides insight on the qualitative and quantitative composition of the blends of BVOCs that were found in the needles of three common coniferous tree species, Austrian pine (*Pinus nigra* J. F. Arnold), Scots pine (*Pinus sylvestris* L.) and Spruce (*Picea abies* (L.) Karst.) and its genotypes from Fruška gora mountain, Vojvodina Province, Serbia. The main aim of this study is to determine species- and genotype- dependent differences in the BVOCs emission potential among investigated species as a platform for further research that will estimate BVOCs impact on human health and well-being. Furthermore, the knowledge gained through this research on qualitative and quantitative BVOCs content in the examined material, observed as the genetically determined emission potential and the ability to form compound-specific BVOCs storage, will contribute to future breeding and selection of species and genotypes more suitable for human health and well-being improvement.

2. Materials and Methods

2.1. Plant Material and Location

The content of BVOCs in the needles of three common coniferous species, growing in the Fruška gora mountain, Austrian pine, Scots pine and Spruce were analyzed to investigate their potential to evaporate BVOCs that can have a positive impact on human health and well-being.

Fruška gora mountain is located in the Vojvodina Province, Republic of Serbia near the second largest populated city in this country, Novi Sad. Vojvodina, characterized with the high agriculture production, and as one of the most deforested regions in Europe, with less than 6.00% forests coverage of its total area [43]. The vicinity of natural area to the highly populated cities and the existence of multiple hiking trails on this mountain makes it a valuable protected nature resource, providing multiple ES for over 500,000 people. The individuals of investigated species are growing in the areas of the most popular visitor sites near the hiking trails (Figure 1).

Climate conditions in the sampling area are defined as temperate continental to modified continental, fully humid with warm summers [44]. The average altitude of the sampling area is 270 m. The ten-year average annual temperature is 11.2 °C, with an annual rank of 22.1 °C and average precipitation of 603.1 mm [45].

2.2. Plant Material Collection

Analyzed plant material was collected from National Park Fruška gora (Serbia) during June 2019. For each of three tree species (*P. nigra*, *P. sylvestris* and *P. abies*), three individual genotypes were investigated to define their potential for emission of different BVOCs. The genotypes of the investigated species were fully formed and medium aged, with characteristics representing their natural population. No visible signs of pathogens or mechanical injuries were present. A detailed description of the selected genotypes is shown in the Table 2.

Samples were taken as the tip of branches with fully formed needles. In order to ensure full plant examination, samples were taken from three different parts of the canopy of each genotype: lowest point, middle point and the top point. Collected plant parts were south and light exposed. Sampling was carried out during noon in specific environmental conditions as previously described [46].

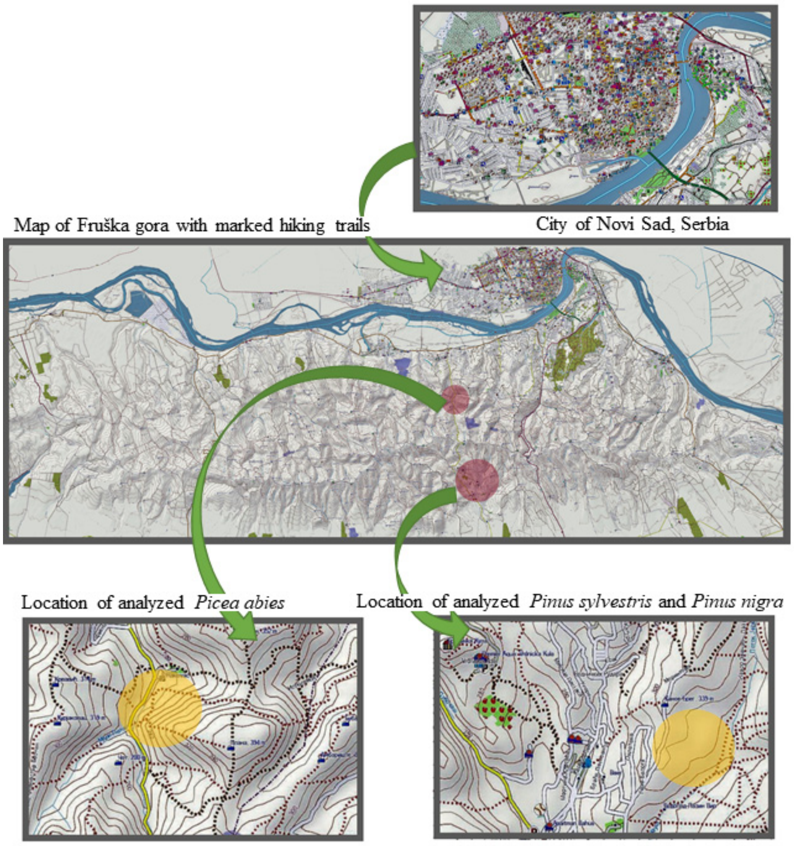

Figure 1. Location of the analyzed species and the map of hiking trails on Fruška gora mountain.

Table 2. Details of the investigated *Pinus nigra*, *Pinus sylvestris* and *Picea abies* genotypes.

Species	*Picea abies*			*Pinus nigra*			*Pinus sylvestris*		
Genotype	**Gen. 1**	**Gen. 2**	**Gen. 3**	**Gen. 1**	**Gen. 2**	**Gen. 3**	**Gen. 1**	**Gen. 2**	**Gen. 3**
Latitude	45°10′50″	45°10′51″	45°10′50″	45°08′06″	45°08′06″	45°08′06″	45°08′07″	45°08′07″	45°08′06″
Longitude	19°46′15″	19°46′15″	19°46′16″	19°48′11″	19°48′12″	19°48′10″	19°48′15″	19°48′16″	19°48′16″
Diameter (cm)	21	22	19	16	14	18	18	19	19
Height (m)	20	18	15	17	19	20	16	20	19

After cutting, samples were stored in paper bags, delivered to the lab and analyzed within 12 h. The voucher specimens were determined and deposited at the Herbarium of Department of Pharmacy, Faculty of Medicine, University of Novi Sad, under the following voucher numbers: PN-07/2019, PS-08/2019 and PA-09/2019.

2.3. BVOCs Analysis

The chemical profiling of Austrian pine, Scots pine and Spruce was performed by analyzing the content of primary BVOCs and essential oils. Primary BVOCs were investigated using the headspace sampling technique gas chromatography–mass spectrometry (Headspace-GC/MS), while the essential oils were characterized qualitatively and quantitatively by gas chromatography coupled to mass spectrometry and, flame ionization detection (GC-MS/FID).

The essential oils were isolated by hydrodistillation with a Clevenger apparatus for 2.5 h. After the solvent removal, the yield of extraction was determined gravimetrically. The qualitative and quantitative analyses of the essential oils were carried out on HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 µm) on Agilent 6890B GC-FID instrument coupled to Agilent 5977 MSD. The samples were injected in splitless mode, 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.

Prior to the analysis of primary BVOCs, dried samples of collected plant material were crushed and sealed in headspace vials (20 mL). 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. Mass spectral data were collected in scan mode (m/z = 50–550), while the identification of compounds was performed using the NIST (v14) mass spectral database and by comparison to relative retention indices (RT), as well as literature data [47].

2.4. Data Analysis

The two-way analysis of variance (ANOVA) has been carried out to conclude if there are significant differences among the mean values of BVOCs content in the examined species and among its genotypes. In order to observe clustering and variation of detected BVOCs among species, as well as between the examined genotypes of the species, the principal component analysis (PCA) was performed. PCA was carried out in the R environment [48]) with the use of R packages "prcomp" (Version 4.0.2) and "ggplot2" (Version 3.3.2).

3. Results

3.1. BVOCs Composition in the Examined Species

The results of the data analysis confirmed the presence of 60 different compounds, mainly terpenes, in three examined species: Austrian pine, Scots pine and Spruce. Values were obtained with different types of the analysis: primary BVOCs (Headspace-GC/MS), while the essential oils were obtained by gas-chromatography coupled to mass spectrometry and flame ionization detection (GC-MS/FID).

Complete data of phytochemical screening of the examined species, together with the mean values (Mean%) with standard deviation (SD%) and coefficient of variance (CV%) for each detected compound is given in the Supplementary Materials.

Compounds classified as terpenes dominated in all of the examined material. Among them, monoterpenes (MT) were the most abundant. According to the mean values for BVOCs content in each of the examined species (Figure 2), the most abundant classes of analyzed species were monoterpene hydrocarbons, followed by sesquiterpene hydrocarbons in the Austrian and Scots pine. The second most represented terpene class in Spruce were oxygenated monoterpenes, while the least present were aliphatic compounds in all of the examined species. The most abundant individual compound in all of the examined species was α-pinene.

The results of the two-way ANOVA among individual BVOCs content showed statistically significant differences in the analyzed species, with the value of $p = 5.2 \times 10^{11}$. The content of limonene was the highest in Spruce (6.00%), followed by Austrian pine (1.83%), while Scots pine had the smallest amount (1.72%) of this particular compound. The results have shown the highest presence of α-terpineol in Spruce (1.87%), compared to Austrian pine (0.13%) and Scots pine (0.22%). The highest amount of camphor was detected in Spruce (0.56%), lower value was detected in Austrian pine (0.33%), while Scots pine was characterized with the lowest value of this terpene (0.05%). Isoborneol, citronellol and eugenol were only detected in Spruce in small amounts 0.04%, 0.25% and 0.07%, respectively. Terpinen-4-ol and linalool were the most present in Spruce in the concentrations of 0.38% and 0.17%, respectively. α-cadinol and camphene were the most present in Spruce (8.44% and 8.35%), followed by Scots pine (2.28% and 5.34%) and Austrian pine in small amount (0.92% and 1.38%).

The principal component analysis (PCA) in Figure 3 showed that the first two principal components (PC1 and PC2) explain more than 50% of the data variability considering BVOCs content in needles of the examined species.

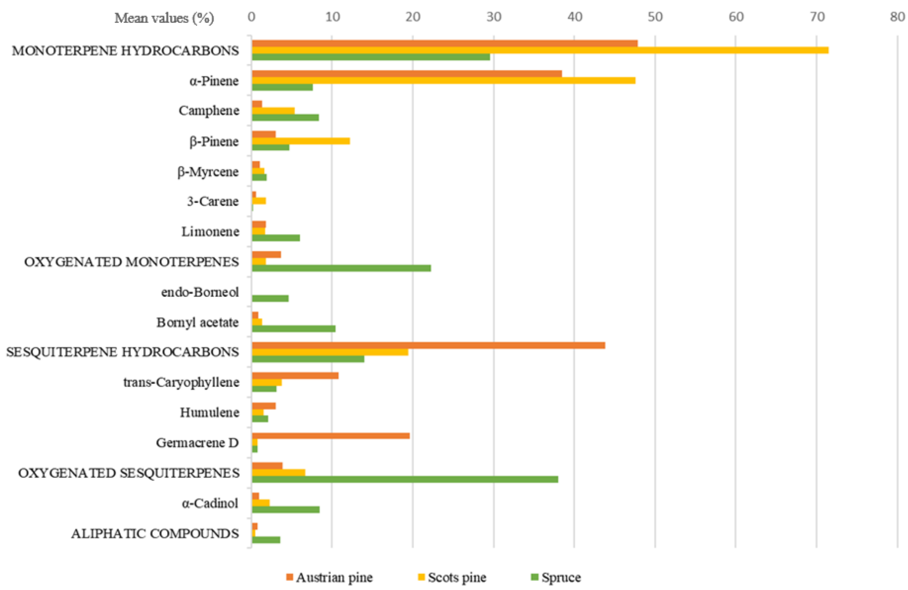

Figure 2. Major BVOCs in the examined species based on the mean values (%) of the obtained results.

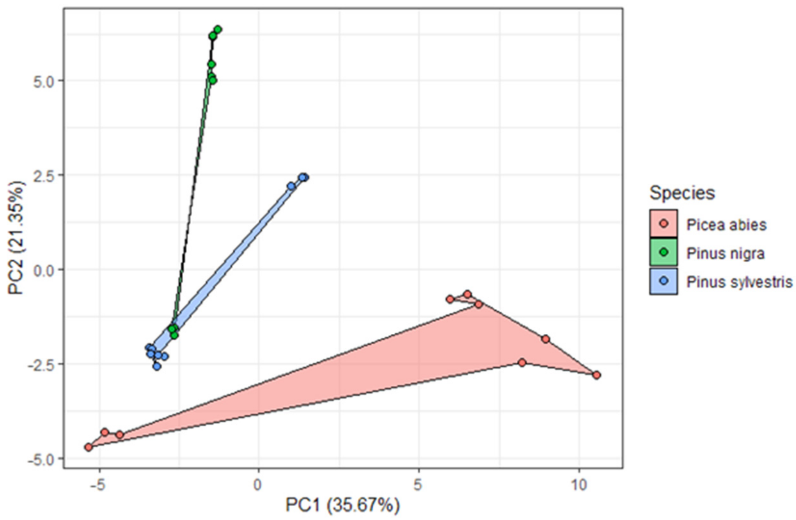

Figure 3. Score plot showing results of the principal component analysis (PCA) performed on the BVOCs content in all of the analyzed genotypes of the three examined species. Dots are representing the position of the obtained results provided in the Supplementary Materials, for each tested genotype within the investigated species, for three types of the conducted analysis (VOCs determined form fresh herbal material (Headspace-GC/MS 1) and essential oils determined from fresh and dry herbal material (GC/MS Fresh and GC/MS Dry)).

PCA analysis of the detected BVOCs in the analyzed species showed grouping based on the species, with the highest prevalence evident in Spruce, considering the detected number of different BVOCs. A small amount of compound content overlapping is evident in the examined *Pinus* species. PCA analysis of BVOCs content in the examined species showed clear separation of Spruce (*P. abies*) from *Pinus* species.

Figure 4 shows the correlation of the detected BVOCs in the examined species. The first component (PC1), with 35.67% of the variance represents the negative correlation between monoterpenes (MT) (e.g., camphene, camphor, α-pinene) and sesquiterepenes (SQT) (e.g., α-copaene, epicubenol, α-farnesene) of the detected compounds in the examined species. The second component (PC2), with less variance (21.35%), shows negative correlation among certain aliphatic compounds (e.g., eicosane, heneicosane) with certain MT (β-ocimene, camphor, 3-carene).

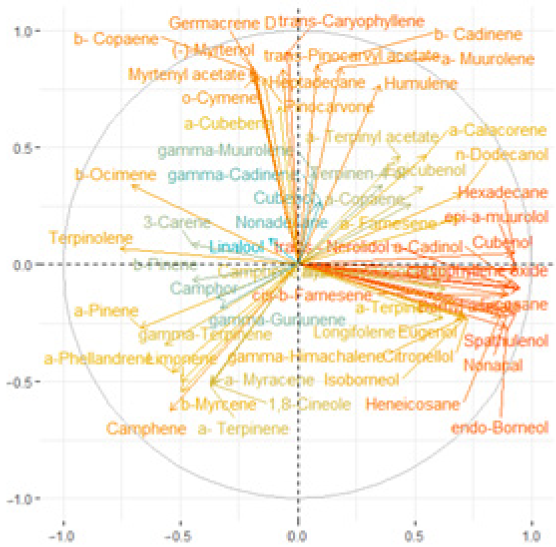

Figure 4. Loading plot connected to the score plot showing VOCs content variation among the investigated species.

3.2. *Variation of BVOCs Content among Genotypes of the Investigated Species*

The two-way analysis of variance (ANOVA) showed relevant statistical difference in the intra-species variations of BVOCs content in the investigated species, with $p = 4.5 \times 10^{164}$ for Austrian pine, $p = 6 \times 10^{163}$ for Scots pine, and $p = 6.4 \times 10^{87}$ for Spruce. The results of BVOCs content in the genotypes of the investigated species showed presence of 46 different compounds in the Austrian pine, 42 in Scots pine and 47 in Spruce. Complete results of the analysis, together with the mean values of the BVOCs content in the investigated species, coefficient of variation (CV) and standard deviation (SD) can be found in the Supplementary Materials.

The PCA performed on the BVOCs content in the tested genotypes of the investigated species showed that first two principal components (PC1 and PC2) explain 92.85% variability for Austrian pine (Figure 5a), 92.85% for Scots pine (Figure 5b) and 85.72% for Spruce (Figure 5c).

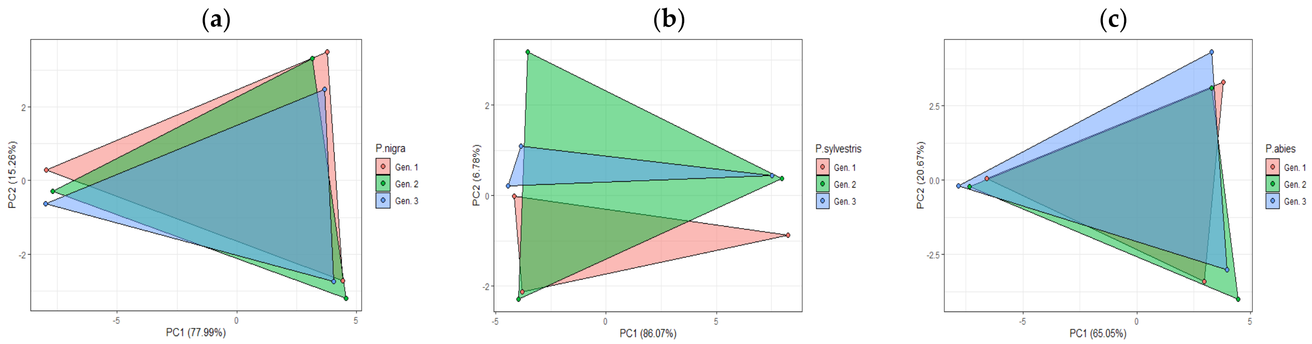

Figure 5. PCA score plot performed on the BVOCs content in the examined genotypes of the examined species: (**a**) Austrian pine; (**b**) Scots pine, (**c**) Spruce.

The loading plot of the PCA analysis performed on the genotypes of the Austran pine (Figure 6a) defines the major variability between the content of MT: limonene, terpinolene, β-pinene, 3-carene, linalool, β-myrcene and camphene. The loading plot of PCA analysis for Scots pine (Figure 6b) also showed high variability between the content of certain MT: terpinolene, camphene and limonene. The highest variations among Spruce genotypes were also observed within the detected BVOCs belonging to the MT: camphor, linalool, citronellol and SQT: α-cadinol, α-copaene and trans-caryophyllene (Figure 6c).

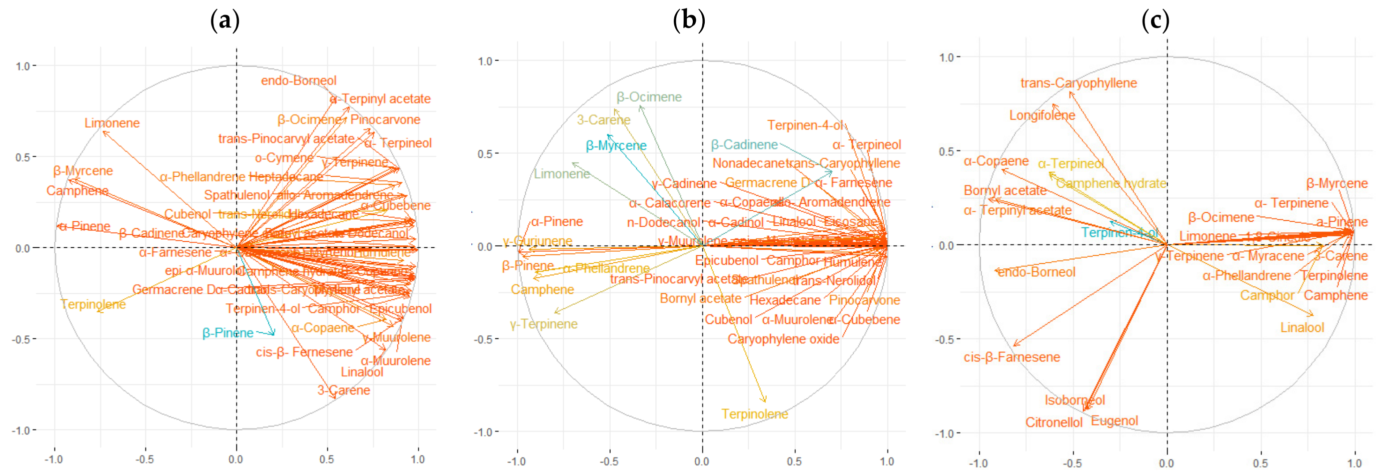

Figure 6. PCA loading plot performed on the BVOCs content in the genotypes of the examined species: (**a**) Austrian pine, (**b**) Scots pine, (**c**) Spruce.

4. Discussion

A significant amount of research regarding ecosystem services (ES) has contributed to the acknowledgment of the importance of natural environments in terms of the human health and well-being in global society [49]. Previous research on the health-related ES has focused attempts on defining the benefits that humans gain from natural environments [21,50,51]. In recent years, a number of papers were published investigating the relation of BVOCs and positive impact of forests on human health [42,52–54]. Furthermore, it was reported that certain VOCs can have long- and short-term negative effects on human health and the majority of them have anthropogenic origin (AVOCs) [55]. On the other hand, it is known that during the photochemical reactions with AVOCs, VOCs of organic origin (BVOCs), including terpenes, can contribute to the creation of new air compounds, of which some can harm human health [56]. Taking this into consideration, certain authors [57] are questioning, predominately, the undoubtable role of trees in urban air quality improvement and are calling for further research on this subject. Furthermore, many studies are reporting pharmacological activities of the terpenes providing new data and an insight about new chemotherapeutic characteristics of these compounds [52]. Human health and ecosystems are complex domains of research; therefore, some authors [23] suggest that an overly simplistic approach in the research of this forest–human health relationship should be avoided, whilst a multidisciplinary approach that considers all the variables affecting the final results should be used.

4.1. The BVOCs Content in the Investigated Species and Their Genotypes

Our research aim presented in this paper is to define and to investigate differences in the potential of the selected tree species and their genotypes to evaporate BVOCs. With the use of the "screening" methodology, the results would not be limited to a certain time and place, keeping in mind high dependability of BVOCs in the air on the environmental conditions.

The results of the presented research have shown the presence of 60 different BVOCs in three examined tree species, of which the majority belongs to the monoterpenes (MT) and sesquiterpenes (SQT) groups. The highest number of different BVOCs was observed in Spruce (47), followed by Austrian pine (46) and Scots pine (42). Although the number of different BVOCs among investigated species does not differ significantly, the two-way ANOVA revealed relevant differences between the mean values of the BVOCs content among the investigated species.

The most dominant terpenes in the Austrian pine were α-pinene (MT), germacrene D and trans-caryophyllene (SQT). Previous phytochemical analysis with similar methodology on the content of terpenes in Austrian pine matches with our results, reporting dominance of α-pinene and limonene as MT and trans-caryophyllene among SQT, as major detected compounds [58]. During research on the impact of herbivore on the BVOCs content and emission of Austrian pine, previously published research [59] reported 3-carene, α-pinene and myrcene as major emitted terpenes from the control group of plants. Our results have shown that the most abundant terpenes in the needles of Scots pine were monoterpenes: α-pinene, β-pinene and γ-muurolene, whilst others [40] reported α-pinene, β-pinene, camphene and limonene as major terpenes of this species. Considering Scots pine terpene emission measurements, it was reported that MT were the highest of the total terpene emission during long-term measurement, and that 3-carene, α-pinene and myrcene were dominant MT, while trans-caryophyllene was dominant among detected SQT [38]. The results of the analysis of Spruce terpenes showed dominance of SQT: bornyl acetate and MT: camphene and α-pinene. Our results match with previously reported α-pinene, β-pinene, camphene, myrcene, limonene and bornyl acetate as dominant terpenes in Spruce [40]. The measurements of the Spruce terpenes emission and their content in the air [38] showed prevalence of MT: α-pinene, limonene and camphene, while dominant SQT were trans-caryophyllene and α-humulene.

Considering the obtained results, higher dominance of MT in the majority of the presented cases can be justified by higher reactivity of SQT in the air. Previously published results [35] reported that MT lifetime in the air during the day can last up to 3 h, while the lifetime of SQT is less than 4 min. During long-term measurements of air concentrations of MT and SQT, certain authors [56] reported higher concentrations of MT, as well as the significant amount of SQT. Their further research [60] showed higher content of SQT in the air compared to MT. The difference in the results of the terpene content in the air is a consequence of the multiple different factors that influence the concentration of BVOCs in the air. Their biosynthesis in plants is highly dependent on the genetic traits, as well on the impact of abiotic and biotic stressors, such as ozone or the presence of the insects, mites and pathogens [35,61,62]. Finally, the higher content of the other chemical compounds in the air that BVOCs could react with, mostly those with anthropogenic origin, such as nitrogen oxides and sulfur dioxide, would result with the formation of the secondary organic aerosols in the air [63] and decrement of the lifetime of certain BVOC.

Complex correlation among functional and genetical traits and BVOCs emission in tree species, as well as their pollution mitigation capacity and high reactivity with other air compounds has been previously described [61]. It was previously concluded that there are results claiming a significant difference in the emission of terpenes from trees, not only among different tree species, but also that different individuals within the same species-genotypes have their own terpene profile [40]. The results of our research also support this thesis. The results we have gained not only show significant differences in terpene composition within different coniferous species, they also show significant differences among the genotypes from the same species. Major intra-variations in Austrian pine genotypes were detected in the content of MT: limonene, terpinolene, β-pinene, linalool and camphene. Similar variability has been observed among the Scots pine genotypes and their BVOCs content. High inter-variability within this species has also been observed on the content of MT: terpinolene, camphene and limonene. High variations within BVOCs content in Spruce were recorded in the content among MT: camphor, linalool, citronellol and SQT: α-cadinol, α-copaene and trans-caryophyllene. The majority of these compounds were previously characterized to have a positive effect on human health (Table 3).

Table 3. Literature evidence relative to biological activities of BVOCs detected in the investigated species.

Phytoncide	Effects	References
α-Pinene	Anti-inflammatory Anticancer Sedative	[64–69] [64,65,70,71] [68,72,73]
Limonene	Anticancer Anti-inflammatory	[74–78] [41,78]
Terpinolene	Anticancer Anti-inflammatory	[79] [41,80]
β-Pinene	Anti-depressant Anticancer Antimicrobial	[81,82] [64,70,71] [64]
Linalool	Anti-depressant Anti-inflammatory Antimicrobial Anticancer	[81] [15,83] [75] [84]
Camphene	Anticancer	[85]
Camphor	Antiviral Anti-inflammatory	[86] [87]
Citronellol	Anticancer Anti-inflammatory	[88] [89]
α-Cadinol	Antiviral Anticancer	[90] [91]

4.2. Health Effects of the Detected Terpenes

The results of our research show that monoterpenes (MT) and sesquiterpenes (SQT) were characterized as major BVOCs components in all of the examined material. The PCA on the BVOCs content in the examined species showed grouping within species, and clear prevalence of the Spruce in comparison to *Pinus* species in considering the overall BVOCs content. The results also show the presence of terpenes previously characterized as health improving agents in Spruce that were not detected in the investigated *Pinus* species. Since Spruce has more versatile terpene composition compared to the *Pinus* species, it can be concluded that this species has more potential for human health improvement through the impact of individual terpenes, as well as through a greater possibility of creations of new blends that impact human health. Detected BVOCs characterized with the potential for human health and well-being improvement based on previously published results are listed in Table 3.

Based on the obtained results, the α-pinene is found to be the major BVOCs constituent of *Pinus* species, with measured mean values for Scots pine (47.58%) and Austrian pine (38.52%). Previously published results indicate that α-pinene has anticancer and neuroprotective impact on humans [64]. Other research also suggest that α-pinene and γ-terpinene have sedative and anesthetic properties [72]. Although the results have shown higher concentrations of α-pinene in the *Pinus* species, higher values of γ-terpinene were detected in Spruce.

Recent research presented the potential that BVOCs in general have for the treatment of viral infections [92], while a previous study showed the positive impact of these compounds, among which α-cadinol was found in the investigated species for antiviral therapy [90]. The highest content of this compound has been detected in Spruce. Additionally, high content of germacrene D, that has anticancer properties [93] in Austrian pine and β-pinene, characterized as anticancer and neuroprotective [64] in Scots pine, corresponds to the observation that natural environments with Scots pine and Austrian pine as dominant species could contribute to the upgrade of the therapeutic methods dealing with certain diseases and afflictions that significantly affect global society, such as cancer, depression and anxiety.

One of the major BVOCs constituent of Spruce is camphene, previously characterized with anticancer activity [85], together with α-pinene, as one of the three major BVOCs constitutes of this species. This indicates that Spruce volatiles could be beneficial for further research on prevention and therapeutic methods of treatment. The content of α-terpineol, that was characterized as anticancer [94], antioxidant [95], anti-inflammatory [96] and antibacterial constituents [9] differed among species. The highest mean value of this compound was observed in Spruce. Interestingly, citronellol, described as an anticancer [88] and anti-inflammatory agent [89], was only detected in Spruce, although in small amount.

4.3. Health Forest Ecosystem Services and Terpenes

A recent review paper on health-related ES highlights that within the overall research on this subject, fourteen direct health-related ES were defined, while placing emphasis on the fact that the majority of these did not specify which human health outcomes are affected by these ES [21]. None of this research observed forest BVOCs as a potential for human health and well-being improvement. The influence of certain positive effects of forest BVOCs may be limited, since the content of these compounds in the air is highly dependent on the environmental conditions [61,97], but medical reports claim that even the mild interaction may be beneficial for individual human well-being [53], which created the need for further research.

Considering the research on natural environments and humans, certain authors [49] claim that the main challenge is to apply the knowledge on the nature and health relationship in such manner that a fuller potential of these relationships is realized. Although the link between the ES and social benefits is evident, especially in terms of the human health improvement and the current pandemic, there is still a major difference among the approaches and the methodologies between ecosystem and health researchers [21]. Considering current medical research on this subject, there is a need for more in-depth investigation of the relationship between environment, human health and detailed forest characteristics [98]. Still, the major constrain in the investigations that are considering the impact of forests on human health is the lack of the support of forestry research, which could contribute to the more accurately defined potential of specific habitats and species for preventive medicine and clinical practice guidelines [23,24].

An additional constraint in considering the final definition of the role of BVOCs in terms of health-related ES is the lack of the large studies that assess the influence of forest BVOCs on human health in real-life situations [53]. The results presented in this paper may prove to be a good basis upon which further research can be implemented.

By observing detected BVOCs content as plant potential to evaporate these compounds that act as potential health and well-being improvement agents, the presented research aims to investigate if there are BVOCs that could improve human health which are present in the investigated species and their inter- and intra- variations. While the investigated species show evident potential for human health improvement, there is a need for further research in order to provide enough information for future investigation and valuation of health-related forest ES.

5. Conclusions

Multiple different BVOCs, previously characterized as potential human health and well-being improving agents, were detected in the needles of the investigated species and their genotypes. Furthermore, the results of the conducted statistical analyses confirmed that there is a relevant statistical difference among the content of BVOCs in three investigated species: Austrian pine, Scots pine and Spruce. Moreover, high variations among genotypes of the investigated species were observed in the content of BVOCs relevant for human health improvement, such as limonene, terpinolene, β-pinene, linalool, camphene, camphor, citronellol and α-cadinol.

The high intra- and inter- variations of BVOCs content that have previously been characterized as having positive effects on human health by acting as anticancer, anti-inflammatory, antiviral, antibacterial and neuroprotective agents, indicates that there is a basis for further research aimed at providing a better understanding of tree BVOCs in health-related ES.

Primarily, the presented research is intended to sharpen the role of ecosystem services, specifically forest health ES and forest BVOCs for human health improvement, by linking different disciplines and state of the art knowledge in order to provide a basis for further research and to remove current barriers in this research field.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12070928/s1, Table S1: BVOCs content in *Pinus nigra* presented as the mean values (%) with SD (%) and CV (%), Table S2: BVOCs content in *Pinus sylvestris* presented as the mean values with SD (%) and CV (%), Table S3: BVOCs content in *Picea abies* presented as the mean values with SD (%) and CV (%).

Author Contributions: Conceptualization, M.Z. and S.O.; Formal analysis, M.Z. and S.K. Methodology, M.Z., N.K., B.B. and S.O.; Supervision, B.B. and S.O.; Writing—original draft, M.Z.; Writing—review & editing, M.Z., S.K., N.K., V.V. and M.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-9/2021-14/200197.

Data Availability Statement: The data presented in this study are available in the Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Larenas-Linnemann, D.; Rodríguez-Pérez, N.; Arias-Cruz, A.; Blandón-Vijil, M.V.; Del Río-Navarro, B.E.; Estrada-Cardona, A.; Gereda, J.E.; Luna-Pech, J.A.; Navarrete-Rodríguez, E.M.; Onuma-Takane, E.; et al. Enhancing innate immunity against virus in times of COVID-19: Trying to untangle facts from fictions. *World Allergy Organ. J.* **2020**, *13*, 100476. [CrossRef]
2. Lee, I.; Choi, H.; Bang, K.S.; Kim, S.; Song, M.K.; Lee, B. Effects of forest therapy on depressive symptoms among adults: A systematic review. *Int. J. Environ. Res. Public Health* **2017**, *14*, 321. [CrossRef] [PubMed]
3. Jung, W.H.; Woo, J.M.; Ryu, J.S. Effect of a forest therapy program and the forest environment on female workers' stress. *Urban. For. Urban. Green.* **2015**, *14*, 274–281. [CrossRef]
4. Dudek, T.; Kasprzyk, I.; Dulska-Jeż, A. Forest as a place for recreation but also the source of allergenic plant pollen: To come or avoid? *Eur. J. Res.* **2018**, *137*, 849–862. [CrossRef]
5. Li, Q.; Morimoto, K.I.; Kobayashi, M.; Inagaki, H.; Katsumata, M.; Hirata, Y.; Hirata, K.; Suzuki, H.; Li, Y.; Wakayama, Y.; et al. Visiting a forest, but not a city, increases human natural killer activity and expression of anti-cancer proteins cells, and intra-cellular anti-cancer proteins in lymphocytes. In the present study, we investigated how with a trip to places in a city. *Int. J. Immunopathol. Pharm.* **2008**, *21*, 117–127. [CrossRef] [PubMed]
6. Li, Q. Effect of forest bathing trips on human immune function. *Environ. Health Prev. Med.* **2010**, *15*, 9–17. [CrossRef]
7. Sunjaya, A.F.; Sunjaya, A.P. Protective Effects of Phytoncides against Cancer. *Adv. Sci. Lett.* **2018**, *24*, 6837–6840. [CrossRef]
8. Li, Q.; Otsuka, T.; Kobayashi, M.; Wakayama, Y.; Inagaki, H.; Katsumata, M.; Hirata, Y.; Li, Y.; Hirata, K.; Shimizu, T.; et al. Acute eVects of walking in forest environments on cardiovascular and metabolic parameters. *Eur. J. Appl. Physiol.* **2011**, *111*, 2845–2853. [CrossRef]
9. Lee, J.; Tsunetsugu, Y.; Takayama, N.; Park, B.J.; Li, Q.; Song, C.; Komatsu, M.; Ikei, H.; Tyrväinen, L.; Kagawa, T.; et al. Influence of forest therapy on cardiovascular relaxation in young adults. *Evid. Based Complement. Altern. Med.* **2014**, *2014*. [CrossRef]
10. Ochiai, H.; Ikei, H.; Song, C.; Kobayashi, M.; Takamatsu, A.; Miura, T.; Kagawa, T.; Li, Q.; Kumeda, S.; Imai, M.; et al. Physiological and psychological effects of forest therapy on middle-aged males with high-normal blood pressure. *Int. J. Environ. Res. Public Health* **2015**, *12*, 2532. [CrossRef]
11. Song, C.; Ikei, H.; Miyazaki, Y. Sustained effects of a forest therapy program on the blood pressure of office workers. *Urban. For. Urban. Green.* **2017**, *27*, 246–252. [CrossRef]
12. Hansen, M.M.; Jones, R.; Tocchini, K. Shinrin-yoku (Forest bathing) and nature therapy: A state-of-the-art review. *Int. J. Environ. Res. Public Health* **2017**, *14*, 851. [CrossRef]
13. Ohtsuka, Y.; Yabunaka, N.; Takayama, S. Shinrin-yoku (forest-air bathing and walking) effectively decreases blood glucose levels in diabetic patients. *Int. J. Biometeorol.* **1998**, *41*, 125–127. [CrossRef]
14. Lee, J.; Park, B.J.; Tsunetsugu, Y.; Ohira, T.; Kagawa, T.; Miyazaki, Y. Effect of forest bathing on physiological and psychological responses in young Japanese male subjects. *Public Health* **2011**, *125*, 93–100. [CrossRef] [PubMed]

15. Lee, S.C.; Wang, S.Y.; Li, C.C.; Liu, C.T. Anti-inflammatory effect of cinnamaldehyde and linalool from the leaf essential oil of Cinnamomum osmophloeum Kanehira in endotoxin-induced mice. *J. Food Drug Anal.* **2018**, *26*, 211–220. [CrossRef] [PubMed]
16. Chun, M.H.; Chang, M.C.; Lee, S.J. The effects of forest therapy on depression and anxiety in patients with chronic stroke. *Int. J. Neurosci.* **2017**, *127*, 199–203. [CrossRef]
17. Bielinis, E.; Takayama, N.; Boiko, S.; Omelan, A.; Bielinis, L. The effect of winter forest bathing on psychological relaxation of young Polish adults. *Urban. For. Urban. Green.* **2018**, *29*, 276–283. [CrossRef]
18. Han, J.W.; Choi, H.; Jeon, Y.H.; Yoon, C.H.; Woo, J.M.; Kim, W. The effects of forest therapy on coping with chronic widespread pain: Physiological and psychological differences between participants in a forest therapy program and a control group. *Int. J. Environ. Res. Public Health* **2016**, *13*, 255. [CrossRef] [PubMed]
19. Acharya, R.P.; Maraseni, T.; Cockfield, G. Global trend of forest ecosystem services valuation—An analysis of publications. *Ecosyst. Serv.* **2019**, *39*. [CrossRef]
20. Maund, P.R.; Irvine, K.N.; Dallimer, M.; Fish, R.; Austen, G.E.; Davies, Z.G. Do ecosystem service frameworks represent people's values? *Ecosyst. Serv.* **2020**, *46*, 101221. [CrossRef]
21. Oosterbroek, B.; de Kraker, J.; Huynen, M.M.T.E.; Martens, P. Assessing ecosystem impacts on health: A tool review. *Ecosyst. Serv.* **2016**, *17*, 237–254. [CrossRef]
22. Zorić, M.; Đukić, I.; Kljajić, L.; Karaklić, D.; Orlović, S. The Possibilities for Improvement of Ecosystem Services in Tara National Park. *Topola* **2019**, *203*, 53–63.
23. Doimo, I.; Masiero, M.; Gatto, P. Forest and wellbeing: Bridging medical and forest research for effective forest-based initiatives. *Forests* **2020**, *11*, 791. [CrossRef]
24. Pagès, A.B.; Peñuelas, J.; Clarà, J.; Llusià, J.; López, F.C.I.; Maneja, R. How should forests be characterized in regard to human health? Evidence from existing literature. *Int. J. Environ. Res. Public Health* **2020**, *17*, 1027. [CrossRef]
25. Cheng, W.W.; Lin, C.T.; Chu, F.H.; Chang, S.T.; Wang, S.Y. Neuropharmacological activities of phytoncide released from *Cryptomeria japonica*. *J. Wood Sci.* **2009**, *55*, 27–31. [CrossRef]
26. Grote, R.; Samson, R.; Alonso, R.; Amorim, J.H.; Cariñanos, P.; Churkina, G.; Fares, S.; Le Thiec, D.; Niinemets, Ü.; Mikkelsen, T.N.; et al. Functional traits of urban trees: Air pollution mitigation potential. *Front. Ecol. Environ.* **2016**, *14*, 543–550. [CrossRef]
27. Nikolić, B.; Ristić, M.; Tešević, V.; Marin, P.D.; Bojović, S. Terpene chemodiversity of relict conifers *Picea omorika*, *Pinus heldreichii*, and *Pinus peuce*, endemic to Balkan. *Chem. Biodivers.* **2011**, *8*, 2247–2260. [CrossRef]
28. Sadeghi, H.; Tahery, Y.; Moradi, S. Intra- and inter-specific variation of turpentine composition in Eldar pine (*Pinus eldarica* Medw.) and black pine (*Pinus nigra* Arnold). *Biochem. Syst. Ecol.* **2013**, *48*, 189–193. [CrossRef]
29. Calfapietra, C.; Fares, S.; Manes, F.; Morani, A.; Sgrigna, G.; Loreto, F. Role of Biogenic Volatile Organic Compounds (BVOC) emitted by urban trees on ozone concentration in cities: A review. *Environ. Pollut.* **2013**, *183*, 71–80. [CrossRef]
30. Bonn, B.; Kreuzwieser, J.; Sander, F.; Yousefpour, R.; Baggio, T.; Adewale, O. The uncertain role of biogenic VOC for boundary-layer ozone concentration: Example investigation of emissions from two forest types with a box model. *Climate* **2017**, *5*, 78. [CrossRef]
31. Fitzky, A.C.; Sandén, H.; Karl, T.; Fares, S.; Calfapietra, C.; Grote, R.; Saunier, A.; Rewald, B. The Interplay Between Ozone and Urban Vegetation—BVOC Emissions, Ozone Deposition, and Tree Ecophysiology. *Front. Glob. Chang.* **2019**, *2*, 1–17. [CrossRef]
32. Saxena, P.; Ghosh, C. A Sustainable Way to Mitigate Ozone Pollution by Reducing Biogenic Vocs Through Landscape Management Programme. *Int. J. Eng. Trends Technol.* **2018**, *56*, 87–91. [CrossRef]
33. Mei, P.; Malik, V.; Harper, R.W.; Jiménez, J.M. Air pollution, human health and the benefits of trees: A biomolecular and physiologic perspective. *Arboric. J.* **2021**, *43*, 19–40. [CrossRef]
34. Juráň, S.; Grace, J.; Urban, O. Temporal changes in ozone concentrations and their impact on vegetation. *Atmosphere* **2021**, *12*, 82. [CrossRef]
35. Kesselmeier, J.; Staudt, M. D077_An Overview on Emission, Physiology and Ecology. *J. Atmos. Chem.* **1999**, *33*, 23–88. [CrossRef]
36. Ashour, M.; Wink, M.; Gershenzon, J. *Biochemistry of Terpenoids: Monoterpenes, Sesquiterpenes and Diterpenes*; Blackwell Publishing Ltd.: Oxford, England, 2018; Volume 40, ISBN 9781119312994.
37. Hodzic, A.; Kasibhatla, P.S.; Jo, D.S.; Cappa, C.D.; Jimenez, J.L.; Madronich, S.; Park, R.J. Rethinking the global secondary organic aerosol (SOA) budget: Stronger production, faster removal, shorter lifetime. *Atmos. Chem. Phys.* **2016**, *16*, 7917–7941. [CrossRef]
38. Wang, M. *Characteristics of BVOC Emissions from a Swedish Boreal Forest Using Chambers to Capture Biogenic Volatile Organic Compounds (BVOCs) from Trees and Forest Floor*; Lund University, Faculty of Science, Department of Physical Geography and Ecosystem Science: Lund, Sweden, 2018; ISBN 9789185793891.
39. Hellén, H.; Praplan, A.P.; Tykkä, T.; Ylivinkka, I.; Vakkari, V.; Bäck, J.; Petäjä, T.; Kulmala, M.; Hakola, H. Long-term measurements of volatile organic compounds highlight the importance of sesquiterpenes for the atmospheric chemistry of a boreal forest. *Atmos. Chem. Phys.* **2018**, *18*, 13839–13863. [CrossRef]
40. Kopaczyk, J.M.; Warguła, J.; Jelonek, T. The variability of terpenes in conifers under developmental and environmental stimuli. *Environ. Exp. Bot.* **2020**, *180*, 104197. [CrossRef]
41. Kim, T.; Song, B.; Cho, K.S.; Lee, I.S. Therapeutic potential of volatile terpenes and terpenoids from forests for inflammatory diseases. *Int. J. Mol. Sci.* **2020**, *21*, 2187. [CrossRef]

42. Šimpraga, M.; Ghimire, R.P.; Van Der Straeten, D.; Blande, J.D.; Kasurinen, A.; Sorvari, J.; Holopainen, T.; Adriaenssens, S.; Holopainen, J.K.; Kivimäenpää, M. Unravelling the functions of biogenic volatiles in boreal and temperate forest ecosystems. *Eur. J. Res.* **2019**, *138*, 763–787. [CrossRef]
43. Pekeč, S.; Ivanišević, P.; Rončević, S.; Kovačević, B.M. Plan and program of shelterbelts establishment in Vojvodina. *Topola* **2008**, *181–182*, 61–70. (In Serbian)
44. Kottek, M.; Grieser, J.; Beck, C.; Rudolf, B.; Rubel, F. World map of the Köppen-Geiger climate classification updated. *Meteorol. Z.* **2006**, *15*, 259–263. [CrossRef]
45. Republički Hidrometeorološki Zavod. *Srbije Sezonski Bilten za Srbiju: Leto 2020. Godine*; Official Report; Republic Hydrometeorological Service of Serbia (RHMZ): Belgrade, Serbia, 2020. (In Serbian)
46. Zorić, M.; Kostić, S.; Kebert, M.; Kladar, N.; Božin, B.; Orlović, S. Volatile organic compounds of *Tilia cordata* Mill. from Serbia, in terms of ecosystem services. *Topola* **2020**, *4*, 21–28. [CrossRef]
47. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatograpy/Mass Spectrometry*, 4th ed.; Allured Pub Corp: Carol Stream, IL, USA, 2007; Volume 4, pp. 804–806.
48. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2011.
49. Van Herzele, A.; Bell, S.; Hartig, T.; Podesta, M.T.C.; van Zon, R. Health benefits of nature experience: The challenge of linking practice and research. *Forests* **2011**. [CrossRef]
50. Battisti, L.; Pille, L.; Wachtel, T.; Larcher, F.; Säumel, I. Residential greenery: State of the art and health-related ecosystem services and disservices in the city of Berlin. *Sustainability* **2019**, *11*, 1815. [CrossRef]
51. McFarlane, R.A.; Horwitz, P.; Arabena, K.; Capon, A.; Jenkins, A.; Jupiter, S.; Negin, J.; Parkes, M.W.; Saketa, S. Ecosystem services for human health in Oceania. *Ecosyst. Serv.* **2019**, *39*, 100976. [CrossRef]
52. Cho, K.S.; Lim, Y.R.; Lee, K.; Lee, J.; Lee, J.H.; Lee, I.S. Terpenes from forests and human health. *Toxicol. Res.* **2017**, *33*, 97–106. [CrossRef]
53. Antonelli, M.; Donelli, D.; Barbieri, G.; Valussi, M.; Maggini, V.; Firenzuoli, F. Forest volatile organic compounds and their effects on human health: A state-of-the-art review. *Int. J. Environ. Res. Public Health* **2020**, *17*, 6506. [CrossRef]
54. Wolf, K.L.; Lam, S.T.; McKeen, J.K.; Richardson, G.R.A.; Bosch, M.; van den Bardekjian, A.C. Urban trees and human health: A scoping review. *Int. J. Environ. Res. Public Health* **2020**, *17*, 4371. [CrossRef]
55. Soni, V.; Singh, P.; Shree, V.; Goel, V. *Effects of VOCs on Human Health*; Springer: Singapore, 2012; p. 12180.
56. Jaars, K.; Vestenius, M.; van Zyl, P.G.; Beukes, J.P.; Hellén, H.; Vakkari, V.; Venter, M.; Josipovic, M.; Hakola, H. Receptor modelling and risk assessment of volatile organic compounds measured at a regional background site in South Africa. *Atmos. Environ.* **2018**, *172*, 133–148. [CrossRef]
57. Eisenman, T.S.; Churkina, G.; Jariwala, S.P.; Kumar, P.; Lovasi, G.S.; Pataki, D.E.; Weinberger, K.R.; Whitlow, T.H. Urban trees, air quality, and asthma: An interdisciplinary review. *Landsc. Urban. Plan.* **2019**, *187*, 47–59. [CrossRef]
58. Dogan, G.; Bagci, E. Chemical Composition of Essential Oil of *Pinus nigra* subsp. *pallasiana* (*Pinaceae*) Twigs, From Different Regions of Turkey. *J. Essent. Oil-Bear. Plants* **2018**, *21*, 511–519. [CrossRef]
59. Heijari, J.; Blande, J.D.; Holopainen, J.K. Feeding of large pine weevil on Scots pine stem triggers localised bark and systemic shoot emission of volatile organic compounds. *Environ. Exp. Bot.* **2011**, *71*, 390–398. [CrossRef]
60. Hellén, H.; Schallhart, S.; Praplan, A.P.; Tykkä, T.; Aurela, M.; Lohila, A.; Lohila, A.; Hakola, H. Sesquiterpenes dominate monoterpenes in northern wetland emissions. *Atmos. Chem. Phys.* **2020**, *20*, 7021–7034. [CrossRef]
61. Grote, R.; Sharma, M.; Ghirardo, A.; Schnitzler, J.-P. A New Modeling Approach for Estimating Abiotic and Biotic Stress-Induced de novo Emissions of Biogenic Volatile Organic Compounds from Plants. *Front. Glob. Chang.* **2019**, *2*, 1–13. [CrossRef]
62. Simon, J.; Adamczyk, B. Plant Secondary Compounds in Forest Ecosystems under Global Change: From Defense to Carbon Sequestration. *Front. Plant. Sci.* **2019**, *10*, 831. [CrossRef]
63. Xu, L.; Pye, H.O.; He, J.; Chen, Y.; Murphy, B.N.; Ng, N.L. Large contributions from biogenic monoterpenes and sesquiterpenes to organic aerosol in the Southeastern United States. *Atmos. Chem. Phys. Discuss.* **2018**, *2018*, 1–47.
64. Salehi, B.; Upadhyay, S.; Orhan, I.E.; Jugran, A.K.; Baghalpour, N.; Cho, W.C.; Sharifi-rad, J. Therapeutic potential of α-and β-pinene: A miracle gift of nature. *Biomolecules* **2019**, *9*, 738. [CrossRef] [PubMed]
65. Matsuo, A.L.; Figueiredo, C.R.; Arruda, D.C.; Pereira, F.V.; Borin Scutti, J.A.; Massaoka, M.H.; Travassos, L.R.; Sartorelli, P.; Lago, J.H.G. α-Pinene isolated from *Schinus terebinthifolius* Raddi (*Anacardiaceae*) induces apoptosis and confers antimetastatic protection in a melanoma model. *Biochem. Biophys. Res. Commun.* **2011**, *411*, 449–454. [CrossRef]
66. Kim, D.S.; Lee, H.J.; Jeon, Y.D.; Han, Y.H.; Kee, J.Y.; Kim, H.J.; Shin, H.J.; Kang, J.; Lee, B.S.; Kim, S.H.; et al. Alpha-Pinene Exhibits Anti-Inflammatory Activity Through the Suppression of MAPKs and the NF-κB Pathway in Mouse Peritoneal Macrophages. *Am. J. Chin. Med.* **2015**, *43*, 731–742. [CrossRef]
67. Rufino, A.T.; Ribeiro, M.; Judas, F.; Salgueiro, L.; Lopes, M.C.; Cavaleiro, C.; Mendes, A.F. Anti-inflammatory and chondroprotective activity of (+)-α-pinene: Structural and enantiomeric selectivity. *J. Nat. Prod.* **2014**, *77*, 264–269. [CrossRef]
68. Khoshnazar, M.; Bigdeli, M.R.; Parvardeh, S.; Pouriran, R. Attenuating effect of α-pinene on neurobehavioural deficit, oxidative damage and inflammatory response following focal ischaemic stroke in rat. *J. Pharm. Pharm.* **2019**, *71*, 1725–1733. [CrossRef]
69. Rahbar, I.; Abbasnejad, M.; Haghani, J.; Raoof, M.; Kooshki, R.; Esmaeili-Mahani, S. The effect of central administration of alpha-pinene on capsaicin-induced dental pulp nociception. *Int. Endod. J.* **2019**, *52*, 307–317. [CrossRef] [PubMed]

70. Yoshida, N.; Takada, T.; Yamamura, Y.; Adachi, I.; Suzuki, H.; Kawakami, J. Inhibitory effects of terpenoids on multidrug resistance-associated protein 2-and breast cancer resistance protein-mediated transport. *Drug Metab. Dispos.* **2008**, *36*, 1206–1211. [CrossRef] [PubMed]
71. Chen, W.; Liu, Y.; Li, M.; Mao, J.; Zhang, L.; Huang, R.; Jin, X.; Ye, L. Anti-tumor effect of α-pinene on human hepatoma cell lines through inducing G2/M cell cycle arrest. *J. Pharm. Sci.* **2015**, *127*, 332–338. [CrossRef] [PubMed]
72. Pereira da Silva, H.N.; dos Santos Machado, S.D.; de Andrade Siqueira, A.M.; Cardoso Costa da Silva, E.; de Oliveira Canto, M.Â.; Jensen, L.; Vargas Flores da Silva, L.; Sena Fugimura, M.M.; de Sousa Barroso, A.; Veras Mourão, R.H.; et al. Sedative and anesthetic potential of the essential oil and hydrolate from the fruit of *Protium heptaphyllum* and their isolated compounds in *Colossoma macropomum juveniles*. *Aquaculture* **2020**, *529*, 735629. [CrossRef]
73. Woo, J.; Lee, C.J. Sleep-enhancing Effects of Phytoncide via Behavioral, Electrophysiological, and Molecular Modeling Approaches. *Exp. Neurobiol.* **2020**, *29*, 120. [CrossRef]
74. Hafidh, R.R.; Hussein, S.Z.; MalAllah, M.Q.; Abdulamir, A.S.; Abu Bakar, F. A high-throughput quantitative expression analysis of cancer-related genes in human HepG2 cells in response to limonene, a potential anticancer agent. *Curr. Cancer Drug Targets* **2018**, *18*, 807–815. [CrossRef]
75. Liu, X.; Cai, J.; Chen, H.; Zhong, Q.; Hou, Y.; Chen, W.; Chen, W. Antibacterial activity and mechanism of linalool against *Pseudomonas aeruginosa*. *Microb. Pathog.* **2020**, *141*, 103980. [CrossRef]
76. Vieira, A.J.; Beserra, F.P.; Souza, M.C.; Totti, B.M.; Rozza, A.L. Limonene: Aroma of innovation in health and disease. *Chem. Biol. Interact.* **2018**, *283*, 97–106. [CrossRef] [PubMed]
77. Blowman, K.; Magalhães, M.; Lemos, M.F.L.; Cabral, C.; Pires, I.M. Anticancer Properties of Essential Oils and Other Natural Products. *Evid. Based Complement. Altern. Med.* **2018**, *2018*. [CrossRef]
78. Yu, X.; Lin, H.; Wang, Y.; Lv, W.; Zhang, S.; Qian, Y.; Deng, X.; Feng, N.; Yu, H.; Qian, B. D-limonene exhibits antitumor activity by inducing autophagy and apoptosis in lung cancer. *Onco. Targets Ther.* **2018**, *11*, 1833. [CrossRef]
79. Rajivgandhi, G.; Saravanan, K.; Ramachandran, G.; Li, J.L.; Yin, L.; Quero, F.; Alharbi, N.S.; Kadaikunnan, S.; Khaled, J.M.; Manoharan, N.; et al. Enhanced anti-cancer activity of chitosan loaded *Morinda citrifolia* essential oil against A549 human lung cancer cells. *Int. J. Biol. Macromol.* **2020**, *164*, 4010–4021. [CrossRef]
80. de Christo Scherer, M.M.; Marques, F.M.; Figueira, M.M.; Peisino, M.C.O.; Schmitt, E.F.P.; Kondratyuk, T.P.; Endringer, D.C.; Scherer, R.; Fronza, M. Wound healing activity of terpinolene and α-phellandrene by attenuating inflammation and oxidative stress in vitro. *J. Tissue Viability* **2019**, *28*, 94–99. [CrossRef]
81. Guzmán-Gutiérrez, S.L.; Gómez-Cansino, R.; García-Zebadúa, J.C.; Jiménez-Pérez, N.C.; Reyes-Chilpa, R. Antidepressant activity of *Litsea glaucescens* essential oil: Identification of β-pinene and linalool as active principles. *J. Ethnopharmacol.* **2012**, *143*, 673–679. [CrossRef]
82. Linck, V.M.; da Silva, A.L.; Figueiró, M.; Caramão, E.B.; Moreno, P.R.H.; Elisabetsky, E. Effects of inhaled Linalool in anxiety, social interaction and aggressive behavior in mice. *Phytomedicine* **2010**, *17*, 679–683. [CrossRef]
83. Kim, M.G.; Kim, S.M.; Min, J.H.; Kwon, O.K.; Park, M.H.; Park, J.W.; Ahn, H.I.; Hwang, J.Y.; Oh, S.R.; Lee, J.W.; et al. Anti-inflammatory effects of linalool on ovalbumin-induced pulmonary inflammation. *Int. Immunopharmacol.* **2019**, *74*, 1–9. [CrossRef]
84. Iwasaki, K.; Zheng, Y.W.; Murata, S.; Ito, H.; Nakayama, K.; Kurokawa, T.; Sano, N.; Nowatari, T.; Villareal, M.O.; Nagano, Y.N.; et al. Anticancer effect of linalool via cancer-specific hydroxyl radical generation in human colon cancer. *World J. Gastroenterol.* **2016**, *22*, 9765–9774. [CrossRef] [PubMed]
85. Vallianou, I.; Hadzopoulou-Cladaras, M. Camphene, a Plant Derived Monoterpene, Exerts Its Hypolipidemic Action by Affecting SREBP-1 and MTP Expression. *PLoS ONE* **2016**, *11*, e147117. [CrossRef] [PubMed]
86. Sokolova, A.S.; Yarovaya, O.I.; Korchagina, D.V.; Zarubaev, V.V.; Tretiak, T.S.; Anfimov, P.M.; Kiselev, O.I.; Salakhutdinov, N.F. Camphor-based symmetric diimines as inhibitors of influenza virus reproduction. *Bioorg. Med. Chem.* **2014**, *22*, 2141–2148. [CrossRef]
87. Ehrnhöfer-Ressler, M.M.; Fricke, K.; Pignitter, M.; Walker, J.M.; Walker, J.; Rychlik, M.; Somoza, V. Identification of 1,8-cineole, borneol, camphor, and thujone as anti-inflammatory compounds in a *Salvia officinalis* L. Infusion using human gingival fibroblasts. *J. Agric. Food Chem.* **2013**, *61*, 3451–3459. [CrossRef] [PubMed]
88. Zhuang, S.R.; Chen, S.L.; Tsai, J.H.; Huang, C.C.; Wu, T.C.; Liu, W.S.; Tseng, H.C.; Lee, H.S.; Huang, M.C.; Shane, G.T.; et al. Effect of citronellol and the Chinese medical herb complex on cellular immunity of cancer patients receiving chemotherapy/radiotherapy. *Phyther. Res. Int. J. Devoted Pharm. Toxicol. Eval. Nat. Prod. Deriv.* **2009**, *23*, 785–790. [CrossRef] [PubMed]
89. Brito, R.G.; Guimarães, A.G.; Quintans, J.S.S.; Santos, M.R.V.; De Sousa, D.P.; Badaue-Passos, D.; De Lucca, W.; Brito, F.A.; Barreto, E.O.; Oliveira, A.P.; et al. Citronellol, a monoterpene alcohol, reduces nociceptive and inflammatory activities in rodents. *J. Nat. Med.* **2012**, *66*, 637–644. [CrossRef] [PubMed]
90. Wen, C.C.; Kuo, Y.H.; Jan, J.T.; Liang, P.H.; Wang, S.Y.; Liu, H.G.; Lee, C.K.; Chang, S.T.; Kuo, C.J.; Lee, S.S.; et al. Specific plant terpenoids and lignoids possess potent antiviral activities against severe acute respiratory syndrome coronavirus. *J. Med. Chem.* **2007**, *50*, 4087–4095. [CrossRef] [PubMed]
91. Abbas, M.M.; Abbas, M.A.; Kandil, Y.I. Cytotoxic activity of *Varthemia iphionoides* essential oil against various human cancer cell lines. *Acta Pol. Pharm. Drug Res.* **2019**, *76*, 701–706. [CrossRef]
92. Nadjib Boukhatem, M.; Mohamed Nadjib, B. Effective Antiviral Activity of Essential Oils and their Characteristic Terpenes against Coronaviruses: An Update. *J. Phar-Macol Clin. Toxicol* **2020**, *8*, 1138.

93. Quintans, J.S.S.; Soares, B.; Ferraz, R.C.; Oliveira, A.A.; Da Silva, T.; Menezes, L.A.; Sampaio, M.C.; Prata, A.D.N.; Moraes, M.; Pessoa, C.; et al. Chemical constituents and anticancer effects of the essential oil from leaves of *Xylopia laevigata*. *Planta Med.* **2013**, *79*, 123–130. [CrossRef]
94. Alves Batista, F.; Brena Cunha Fontele, S.; Beserra Santos, L.K.; Alves Filgueiras, L.; Quaresma Nascimento, S.; de Castro e Sousa, J.M.; Ramos Gonçalves, J.C.; Nogueira Mendes, A. Synthesis, characterization of α-terpineol-loaded PMMA nanoparticles as proposed of therapy for melanoma. *Mater. Today Commun.* **2020**, *22*. [CrossRef]
95. Khaleel, C.; Tabanca, N.; Buchbauer, G. α-Terpineol, a natural monoterpene: A review of its Max Musterman, Paul Placeholder What Is So Different About biological properties. *Open Chem.* **2018**, *1*, 91–102.
96. Held, S.; Schieberle, P.; Somoza, V. Characterization of α-terpineol as an anti-inflammatory component of orange juice by in vitro studies using oral buccal cells. *J. Agric. Food Chem.* **2007**, *55*, 8040–8046. [CrossRef] [PubMed]
97. Yáñez-Serrano, A.M.; Bourtsoukidis, E.; Alves, E.G.; Bauwens, M.; Stavrakou, T.; Llusià, J.; Filella, I.; Guenther, A.; Williams, J.; Artaxo, P.; et al. Amazonian biogenic volatile organic compounds under global change. *Glob. Chang. Biol.* **2020**, *26*, 4722–4751. [CrossRef] [PubMed]
98. Antonelli, M.; Donelli, D.; Carlone, L.; Maggini, V.; Firenzuoli, F.; Bedeschi, E. Effects of forest bathing (shinrin-yoku) on individual well-being: An umbrella review. *Int. J. Environ. Health Res.* **2021**. [CrossRef] [PubMed]