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New Facts on the Antimicrobial Essential Oil of *Satureja kitaibelii*

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The objective of the present study was to assess the difference in antimicrobial activity of *Satureja kitaibelii* Wierzb. ex Heuff. essential oil at three growth stages. In addition, using statistical and chemometric tools, we tried to explain why the essential oil from a certain vegetation stage shows the highest antimicrobial activity. *S. kitaibelii* essential oils demonstrated minimum inhibitory concentration values from 160 to 10000 μg mL^{-1}, and minimum microbicidal concentration values from 630 to 20000 μg mL^{-1}. Geraniol, borneol, limonene and *p*-cymene are the dominant compounds of *S. kitaibelii* essential oil. The most abundant compound, geraniol, possesses antimicrobial activity in a range of MIC values from 40 to 5000 μg mL^{-1} and MMC values from 80 to 10000 μg mL^{-1}. The highest activity of essential oil for all tested strains of microorganisms was recorded in November. Results of statistical analysis indicate that the percentage of dominant compounds of essential oils does not affect the antibacterial activity of essential oils. Chemometric analyses leads to the conclusion that borneol, spathulenol, caryophyllene oxide and limonene can be the main contributors to the antibacterial activity of essential oil from November and that their mutual ratio is important. These results may represent a new methodological approach for future research on essential oils.

Introduction

The massive use of antibiotics has led to resistance, a serious problem affecting public health. The increase in bacterial resistance to antibiotics and the lack of new antibiotics coming to market have led to the need to find alternative strategies to deal with infections caused by drug-resistant bacteria.[1] The secondary metabolites of plants represent a huge storehouse of the most structurally diverse compounds, many of which are proven to be active against a number of microbial species.[2] Among compounds of natural origin, essential oils from aromatic and medicinal plants can be a powerful tool to reduce bacterial resistance. An important property of essential oils and their components is hydrophobicity, which allows them to partition with the lipids present in the cell membrane of bacteria and mitochondria, making them more permeable by disrupting cell structures.[3] The genus *Satureja* L. includes more than 30 species with a large number of polymorphic species and a number of intraspecific species. *Satureja* species, due to the presence of secondary metabolites such as terpenoids, flavonoids, tannins, and steroids, have long been known for their medicinal properties. They are used as traditional folk remedies to treat various ailments such as spasms, muscle pain, nausea, indigestion, diarrhoea and infectious diseases.[4–6] *Satureja kitaibelii* Wierzb. ex Heuff. is an aromatic species that inhabits dry limestone areas in eastern Serbia. The aerial parts of this species are commercially available under the name "Rtanj tea" and have been used in traditional medicine for many years to treat diarrhoea, nausea, spasms, digestive problems, respiratory and infectious diseases, and as a culinary herb in Mediterranean cuisine.[7] According to a literature review, the essential oil, and extracts of *S. kitaibelii* have several pharmacological activities such as antimicrobial, antioxidant, antihyperglycemic and anti-inflammatory activities.[8,9]

In previous work, we investigated essential oil (EO) from natural populations *Satureja kitaibelii* at the Kravlje village, southeastern Serbia, during different stages of development, with emphasis on chemotaxonomy.[10] A review of studies on the pharmacological activity of *S. kitaibelii* essential oil reveals no data on the antimicrobial activity of *S. kitaibelii* essential oils from the different phenological stages. The available literature also lacks data on the components of essential oil, which could be carriers of antimicrobial activity in a certain development phase. The objective of the present study was to assess the difference in antimicrobial activity of *S. kitaibelii* essential oil at three growth stages (six months), in order to determine the optimum harvesting period to obtain the most potent antibacterial content of essential oil. In addition, using statistical

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and chemometric tools (Multivariate analysis of variance and Principal component analysis), we tried to explain why the essential oil from a certain vegetation stage shows the highest antimicrobial activity. More precisely, which components of essential oil are carriers of antimicrobial activity in that stage of development. According to the authors' knowledge, this is the first report on the antimicrobial activity of *S. kitaibelii* essential oils depending on the different phenological stages.

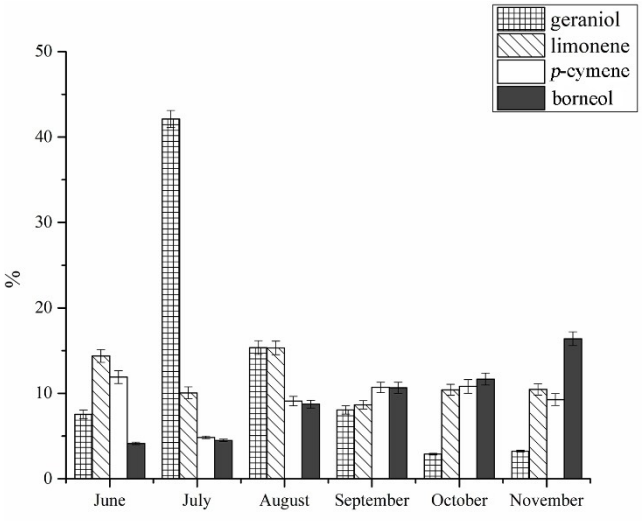

Figure 1. Changes in the content of the most abundant components of *S. kitaibelii* essential oil during different stages of development (%)

Results and Discussion

Antimicrobial activity

It is evident that the carriers of the antibacterial activity of the essential oils are the dominant class of compounds, in most of the studied plant species.[11] In previous work, we found that oxygenated monoterpenes were the most abundant class of compounds in the oil (21.3–61.2 %) and were dominated by geraniol (2.9–42.1 %) and borneol (4.1–16.4 %). The second abundant class of compounds, monoterpene hydrocarbons (24.1–51.1 %), was mainly dominated by limonene (8.7–15.3 %) and *p*-cymene (4.9–11.9 %). These four compounds are also the most abundant in *S. kitaibelii* EO (Figure 1,[10]). However, the importance of minor components of essential oil should not be ignored. In our study of the antimicrobial activity of six essential oils from *Achillea* and *Artemisia* species, we concluded that the minor compounds or a combination thereof were possibly responsible for the antibacterial activity of EOs.[12]

The results of the antimicrobial test show that the investigated EOs, selected components and some of their combinations exhibited activity against all tested microorganisms (Table 1). *S. kitaibelii* EOs demonstrated minimum inhibitory concentration (MIC) values from 160 to 10000 $\mu g\,mL^{-1}$ and and minimum microbicidal concentration (MMC) values from 630 to 20000 $\mu g\,mL^{-1}$. The most abundant compound, geraniol, possesses antimicrobial activity in a range of MIC values from 40 to 5000 $\mu g\,mL^{-1}$ and MMC values from 80 to 10000 $\mu g\,mL^{-1}$.

Table 1. Antimicrobial activity of essential oils and some components (MIC/MMC in $\mu g\,mL^{-1}$).

Strains Samples	*S. aureus*	*B. cereus*	*E. faecalis*	*S. enteritidis*	*E. coli*	*E. aerogenes*	*P. aeruginosa*	*C. albicans*
June	320/20000	1250/ 10000	2500/ 10000	2500/2500	2500/5000	5000/10000	2500/2500	1250/1250
July	320/20000	320/10000	2500/ 10000	2500/2500	1250/2500	10000/ 10000	2500/2500	630/1250
August	630/20000	320/10000	2500/ 10000	2500/2500	2500/2500	5000/10000	5000/5000	630/1250
September	630/20000	630/5000	2500/ 10000	1250/1250	2500/2500	10000/ 10000	2500/5000	630/1250
October	320/20000	320/10000	2500/ 10000	1250/1250	2500/2500	10000/ 10000	2500/2500	630/1250
November	160/10000	320/5000	1250/ 10000	630/1250	320/630	5000/10000	630/630	630/630
Geraniol	160/10000	80/5000	2500/5000	80/80	40/80	5000/10000	80/160	160/320
Thymol	160/20000	80/2500	40/5000	40/40	40/40	1250/5000	80/160	80/160
Limonene	630/10000	630/5000	1250/ 10000	320/320	630/320	10000/ 10000	1250/1250	320/320
Borneol	630/10000	630/5000	2500/ 10000	1250/1250	1250/630	10000/ 10000	1250/1250	630/320
Geraniol-limonene 4:1	630/10000	630/5000	5000/5000	1250/1250	630/630	5000/10000	2500/2500	320/320
Geraniol:limonene 1:1	5000/ 10000	1250/1250	2500/ 10000	630/1250	2500/ 10000	5000/20000	2500/2500	320/320
Streptomycin	0.5/0.5	0.5/0.5	4.0/4.0	4.0/4.0	16.0/16.0	0.5/0.5	8.0/8.0	Nystatin 16.0/ 16.0
Chloramphenicol	1.0/8.0	1.0/4.0	2.0/4.0	4.0/8.0	16.0/16.0	1.0/1.0	4.0/16.0	

Two combinations of geraniol-limonene have shown antimicrobial activity in a range of MIC values from 320 to 5000 μg mL^{-1} and MMC values from 320 to 20000 μg mL^{-1}. Reference antibiotic streptomycin was active in concentrations between 0.5 and 16 μg mL^{-1}, chloramphenicol was active in concentrations between 1 and 16 μg mL^{-1}, while nystatin was active in concentration 16 μg mL^{-1}. Essential oil terpenes show antimicrobial activity by themselves; however, this activity does not always agree with the activity of the essential oil itself. This fact could indicate that complex mixtures of these terpenes determine synergistic or antagonistic relationships between them. Synergism could be defined as a combination of EOs that have multiple modes of action, respecting the principle that the effect of combined substances is greater than the sum of known and unknown chemical components. Antagonism can be thought of as a reduction in the biological activity of a mixture of components compared to the individual activity of each component alone.[13]

Based on MIC and MMC values for all tested microorganisms during the studied phenological stages (essential oils only), the following sequence can be established, MIC values: *E. aerogenes* > *P. aeruginosa* > *E. faecalis* > *E. coli* > *S. enteritidis* > *C. albicans* > *B. cereus* > *S. aureus* and MMC values: *S. aureus* > *E. aerogenes* > *E. faecalis* > *E. coli* > *P. aeruginosa* > *E. coli* > *S. enteritidis* > *C. albicans*. The MIC values of Gram-positive strains were lower compared to Gram-negative strains, which is expected.

However, the MMC values of Gram-positive strains were found to be higher compared to Gram-negative strains, which was not expected. The capability of *Satureja* EOs to inhibit pathogenic microbes has been well-reported in several studies. For instance, *S. calamintha* EO was reported as having good antibacterial activity against four bacterial strains and important antifungal activities compared against four bacterial strains [H]. Another study proved the high effectiveness of the *S. sahendica* and *S. spicigera* EOs to inhibit *C. albicans*, while *P. aeruginosa* was the most resistant bacteria.[2]

Our results of antimicrobial activity are higher, compared to the literature data.[14,15] As we know, the chemical composition of EOs determines antimicrobial activity, but a variety of laboratory methods can be used for antimicrobial susceptibility testing. The basic methods are the disk-diffusion and broth or agar dilution methods.[16] On the other hand, if we are talking about the broth dilution method the inoculum size, the type of growth medium, the inoculum preparation method and the incubation time can influence MIC and MMC values. Of course, broth dilution has been standardized by CLSI, but we should always consider the specifics of each laboratory separately.[17]

One of the main goals of this research was to assess the difference in antimicrobial activity of *S. kitaibelii* at three growth stages, in order to determine the optimum harvesting period to obtain the most potent antibacterial content of essential oil. Results are presented in Figure 2. The highest activity of essential oil for all tested strains of microorganisms was recorded in November (after the flowering stage of development). During this month, only the borneol content is the highest, if we consider the four dominant components of the essential oil (Figure 1,[10]). In a paper on the chemical composition and antimicrobial activity of *S. cuneifolia* essential oils, depending on the stage of plant development, the authors suggest that oil isolated during the flowering period has a high concentration of biologically active components required for strong antimicrobial effects.[18] *S. thymbra* and *S. parnassica* EOs obtained during the flowering period were the most effective, exhibiting the lowest MIC values.[19] In the investigation of the evaluation of changes in the chemical composition and antimicrobial and antioxidant activities of *S. cuneifolia* essential oils, an increase in antimicrobial activity during the maturation period was observed.[20]

We can certainly assume that the dominant components of the essential oil, which are most intensively produced in the flowering phase, determine the antimicrobial activity. Another explanation lies in the fact that minor components (most often after the flowering stage), as well as possible interactions

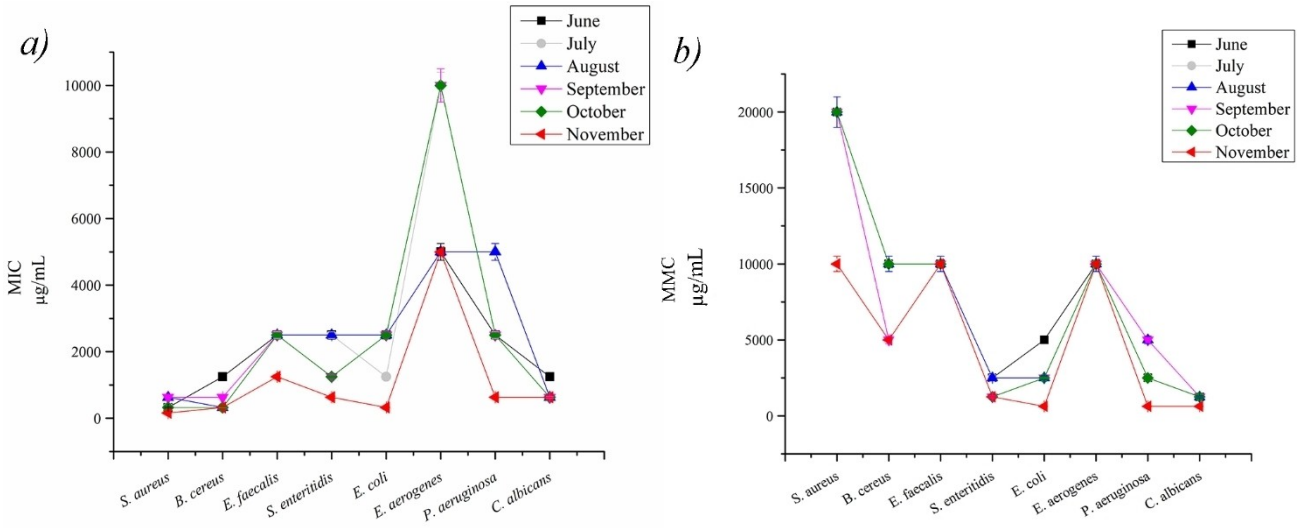

Figure 2. a) MIC values, b) MMC values, of *S. kitaibelii* EOs during different growth stages

among other active compounds, can also affect the microbiological properties of essential oils. These statements are at the level of assumptions and speculation, so the evidence is lacking. We can accept the fact that it is difficult to analyze a complex mixture, such as an essential oil, with many different classes of compounds. However, it is necessary to find adequate methodological procedures and try to give a realistic explanation.

Statistical analysis by Multivariate analysis of variance (MANOVA)

In order to try to explain the results of our study, we used statistical and chemometric tools. First, it should be established whether the content of the four dominant components of the essential oil has an effect on antimicrobial activity. MANOVA was used to determine the effect of the content of essential oil compounds on antimicrobial activity. It was done using Wilks' Lambda test. The results are shown in Table 2. The *p* value ranges from 0.222 to 0.984, and for all samples is greater than the significance level alpha = 0.05. Based on the results, the *p* values obtained for Gram-negative bacteria are higher than for Gram-positive bacteria, which indicates a greater deviation. Accordingly, we cannot reject the null hypothesis, *i.e.*, the fact that the percentage of dominant compounds of essential oils does not affect the antibacterial activity. This is confirmed by the fact that the antibacterial activity of the essential oil is not a simple addition of the antibacterial activities of its components.[21]

Table 2. MANOVA test.

	Compounds	Wilks' value	*p* value
Gram-negative bacteria	*p*-cymene	0.724	0.968
	limonene	0.485	0.876
	γ-terpinene	0.375	0.804
	borneol	0.625	0.939
	terpinene-4-ol	0.624	0.938
	geraniol	0.665	0.952
	caryophyllene	0.625	0.939
	germacrene D	0.625	0.939
	spathulenol	0.625	0.939
	caryophyllene oxide	0.803	0.984
Gram-positive bacteria	*p*-cymene	0.547	0.695
	limonene	0.615	0.761
	γ-terpinene	0.154	0.222
	borneol	0.752	0.877
	terpinene-4-ol	0.342	0.466
	geraniol	0.615	0.761
	caryophyllene	0.752	0.877
	germacrene D	0.752	0.877
	spathulenol	0.752	0.877
	caryophyllene oxide	0.385	0.517

Chemometric analysis by Principal component analysis (PCA)

Finally, the key question follows: which essential oil components are the carriers of antibacterial activity in November? PCA gave two Eigenvalues of more than 1 (9.462 and 5.628), explaining 94.3% of the variability (Table 3).

Due to unsatisfactory value, the correlation matrix was subjected to the Varimax rotation with Kaiser normalization. The principal components are rotated so that the total sum of squares of the loadings along each new axis is maximised.[22] Two factors gave more than 95% of the cumulative variability (Table 3), so they were used to explain the results (Table 4).

The higher the loading of a variable implies a larger contribution to the variation, accounting for the Varimax rotated principal components.[23] The main contributors to the first factor with the highest variability 60.3% (Table 5) are borneol, caryophyllene oxide, spathulenol, with negative values, and *S. aureus*, *S. enteritidis*, *E. coli*, and *P. aeruginosa* with positive values (Table 4). If the concentrations of borneol, spathulenol and caryophyllene oxide are increased, then MICs of *S. aureus*, *S. enteritidis*, *E. coli*, and *P. aeruginosa* are decreased, which indicates better antibacterial activity.

Ghavam[24] has shown in his research that sesquiterpene such as (−) spathulenol, seems to be one of the main reasons for antibacterial activity. Also, caryophyllene oxide was effective against Gram-positive bacteria and Gram-negative bacteria.[25] In the case of the second factor with a variability of 38.8%, the main contributor is limonene with a negative value, and *E. aerogenes* with a positive value (Table 4). From this point, it can be said when the concentration of limonene is high, the MIC of *E. aerogenes* is low. The results showed that limonene effectively inhibited bacterial growth.[26] From the scores on the first principal component, it can be written that the MICs of *S. aureus*, *S. enteritidis*, *E. coli*, and *P. aeruginosa* on the first principal component loadings are higher for essential oil from August, and lower for essential oils from September, October, and particularly November. When the second component is

Table 3. Eigenvalues and cumulative variability before and after Varimax rotation.

	Before Varimax rotation		
	PC1	PC2	PC3
Eigenvalue	9.462	5.628	0.910
Variability (%)	59.137	35.176	5.687
Cumulative %	59.137	94.313	100.000
	After Varimax rotation		
	PC1	PC2	PC3
Eigenvalue	11.470	4.393	0.136
Variability (%)	71.689	27.458	0.852
Cumulative %	71.689	99.148	100.000

Table 4. The loadings and the scores of the first two principal components after the Varimax rotation.

	The loadings			The scores	
Compound	**D1**	**D2**	**EOs**	**F1**	**F2**
p-cymene	−0.329	0.942	August	5.165	−0.953
limonene	0.720	−0.685	September	−0.647	2.782
γ-terpinene	0.671	0.737	October	−1.733	1.555
borneol	−0.997	−0.028	November	−2.784	−3.383
terpinen-4-ol	0.022	0.999			
geraniol	0.908	−0.415			
caryophyllene	0.874	−0.484			
germacrene D	0.869	−0.494			
spathulenol	−0.843	0.532			
caryophyllene oxide	−0.867	0.495			
S. aureus	0.993	0.062			
S. enteritidis	0.929	−0.363			
E. coli	0.898	0.416			
E. aerogenes	−0.243	0.968			
P. aeruginosa	0.957	−0.276			

Table 5. Percentage of variance after Varimax rotation.

	D1	**D2**	**D3**
Variability (%)	60.3	38.8	0.9
Cumulative %	60.3	99.1	100.0

considered, the MIC of *E. aerogenes* is higher for essential oils from September and October, and lower for MICs from August and particularly November. The principal component analysis shows that the concentrations of borneol, spathulenol, caryophyllene oxide and limonene are the main factors affecting the activities of essential oils from August to November against Gram-positive and Gram-negative bacteria. All the presented evidence supports the fact that these four essential oil components can be the main contributors to the antibacterial activity of essential oil from November.

Conclusions

In this study, combined chemical, antimicrobial, statistical and chemometric analysis of *S. kitaibelii* essential oil during different development phase, over the course of 6 months,were performed. The four most abundant essential oil components are geraniol, limonene, *p*-cymene and borneol. *S. kitaibelii* EOs demonstrated MIC values from 160 to 10000 $\mu g\,mL^{-1}$ and MMC values from 630 to 20000 $\mu g\,mL^{-1}$. The most abundant compound, geraniol, has antimicrobial activity with a range of MIC values from 40 to 5000 $\mu g\,mL^{-1}$ and MMC values from 80 to 10000 $\mu g\,mL^{-1}$. The highest activity of essential oil for all tested strains of microorganisms was recorded in November. Results of statistical analysis indicate that the percentage of dominant compounds of essential oils does not affect the antibacterial activity of essential oils. Chemometric analysis leads to the conclusion that borneol, spathulenol, caryophyllene oxide and limonene can be the main contributors to the antibacterial activity of essential oil from November.

Experimental Section

Plant material and chemicals

The aerial parts of *Satureja kitaibelii* Wierzb. ex Heuff. family Lamiaceae were collected in 2020 from a natural population at the Kravlje village (southeast Serbia). The plants were collected on the fifteenth of the month, at three different stages of development: the vegetative stage (June); the flowering stage (July and August); and after flowering stage (September, October, and November). Dr. Marija Marković did identification of plant material, and the voucher specimen (accession number 13220) is deposited at the Herbarium of the Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš (Herbarium Moesiacum Niš – HMN).

All chemicals, reagents, and standards were of analytical reagent grade and purchased from Sigma-Aldrich Chemical Company (Germany).

Oil isolation

Aerial parts of the plant (dried and pulverized) were subjected to hydro-distillation for 4 h, using the Clevenger-type apparatus to produce oil, according to the European Pharmacopoeia. The ratio of the plant material to water is 1:8. The resulting EOs essential oils were dried over anhydrous sodium sulfate and stored at 4 °C.

Gas chromatography, Gas chromatography/mass spectrometry,

The GC analysis of the oils was carried out on a GC HP5890 II apparatus, equipped with the split-splitless injector, HP-5MS

capillary column (30 m×0.25 mm, 0.25 μm flm thickness) with helium as the carrier gas (1 mL/min) and flame ionization detector. The operating conditions were set as follows: injector temperature of 250 °C and interface temperature of 280 °C; and temperature program from 50 °C (3 min) to 250 °C at a rate of 3 °C min^{-1}. GC-MS analyses were performed on an Agilent Technologies apparatus, Model GS 6890 N at 70 eV coupled with a mass-selective detector MSD 5975 C, under the same gas chromatograph conditions.

Identification of compounds

The identification of compounds was based on a comparison of Kovats retention indexes by the use of calibrated automated mass spectral deconvolution and identification system software AMDIS ver. 2.64 in combination with selective ion analysis (SIA) resolution method[27] compared with those from available literature,[28] and by comparing their mass spectra to those from Wiley 275 and NIST/NBS libraries, using different search engines. Retention indexes were obtained by co-injection with an aliphatic hydrocarbons C9–C28 standard mixture.

Antimicrobial testing

The activity of the essential oil samples, selected components, and some of their combinations (13 samples in total) were tested against seven different bacteria and one fungus. Laboratory control strain was obtained from the American Type Culture Collection (ATCC). Gram-positive strains were *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *and Enterococcus faecalis* ATCC 19433, while the studied Gram-negative bacteria were represented by *Salmonella enteritidis* ATCC 13076, *Escherichia coli* ATCC 25922, *Enterobacter aerogenes* ATCC 13048 and *Pseudomonas aeruginosa* ATCC 9027. *Candida albicans* ATCC 24433 was investigated fungal strain. The inocula of the bacterial and fungal strains were prepared from the overnight broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity (corresponding to 10^7–10^8 CFU/mL, depending on a genera-consensus standard by the Clinical and Laboratory Standards Institute.[29]

Micro-well dilution assay

All tested oils were prepared in 100 % Dimethylsulfoxide (DMSO) in a microtiter plate over the range of 0.001–20.0 mg/mL in inoculated nutrient broth with a final volume of 100 μL. The final concentration of bacteria was 10^6 CFU/mL in each well. The microtiter plate was incubated at 37 °C for 24 h. All experiments were performed in triplicate. Streptomycin, chloramphenicol, and nystatin commercial antibiotics were used as a positive control and medium with DMSO as a negative control. Bacterial growth was determined by adding 20 μL of 0.5 % triphenyl tetrazolium chloride (TTC) aqueous solution. Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation, and minimum microbicidal concentrations (MMCs) as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture onto antibiotic-free media.[30] To determine MMC, the broth was taken from each well without visible growth and inoculated in Mueller Hinton agar for 24 h at 37 °C.

Statistical analysis of data

The statistical analyses were carried out using XLSTAT 2022.[31] In the first step of statistical evaluation, the Kolmogorov-Smirnov test (the significance level α was 0.05) was used to determine the distribution.[32] Before applying Principal component analysis (PCA), the data matrix was examined to detect outliers using Grubbs' test.[33] Outliers were discarded from PCA analysis. Ten compounds with average relative abundance ≥1 % were used for statistical analysis (p-cymene, limonene, γ-terpinene, borneol, terpinene-4-ol, geraniol, caryophyllene, germacrene D, spathulenol and caryophyllene oxide).

MANOVA

Some statistical methods are based on the analysis of variances that allow evaluation of the statistical significance of the experimental variables used in the research. MANOVA is one of those methods. It enables us to examine two or more 'parametric' dependent variables across one or more between-group independent variables.[34] Its application provides an opportunity to determine whether there are qualitative factors that significantly affect quantitative variables. In this study, MANOVA was used to compare essential oil compounds and their content's influence on antibacterial activity. MANOVA test was done through Wilks' Lambda test. The hypotheses used are as follows:

H_0: The percentage of the components has no significant effect on the antibacterial activity.

H_a The percentage of components has a significant effect on the antibacterial activity.

If the *p*-value is greater than the significance level alpha = 0.05, then we cannot reject the null hypothesis H_0.

Checking and preparing results for PCA

Principal component analysis was not applied to the whole set of data. Its first Kolmogorov-Smirnov test was used to check the normal distribution of the original dataset expressed through ACIs so that equal weight should be given.[32] The datasets were normally distributed.

Then, Grubbs' test was done, which is used to detect a single outlier in a univariate data set that follows an approximately normal distribution.[34] It showed outliers in the case of γ-terpinene, geraniol, *B. cereus* and *E. faecalis*, for essential oil samples obtained in June and July, and therefore these samples were thrown out and were not used for PCA analysis. Then the Grubbs' test was performed again to determine if there are any outliers among the used variables. Bacteria *B. cereus* and *E. faecalis* were found to be outliers and were not used for further analysis. A repeated Kolmogorov-Smirnov test established a normal distribution of the remaining results, which were further used for PCA analysis.

PCA is a very powerful technique in chemometrics that enables the grouping of samples. Original variables are reduced, and the new ones obtained after certain mathematical transformations give us better insight into groupings and correlations between analysed samples. Initial datasets must pass specific tests before PCA and be pre-processed depending on the results of the tests.[35] Groupings of samples can be observed in score plots, but better-visualizing solutions are biplots combining scores and loadings.[36] Components with Eigenvalues are selected to interpret results (Kaiser's rule or Kaiser-Guttman's rule).[37,38]

Author Contributions

All authors contributed to the study conception and design. The first draft of the manuscript was written by Dragoljub Miladinović, and all authors commented on previous version of the manuscript. Plant material collection and preparation were performed by Marija Marković and Dragoljub Miladinović. Statistical analysis was performed by Biljana Arsić and Marija Dimitrijević. Antimicrobial analysis was done by Tatjana Mihajlov Krstev. Review and editing were done by Ljiljana Miladinović. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: antimicrobial activity · essential oil · MANOVA · PCA · *Satureja kitaibelii*

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