http://dx.doi.org/10.5935/0103-5053.20140112

J. Braz. Chem. Soc., Vol. 25, No. 8, 1326-1330, 2014.
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0103 - 5053 $6.00+0.00

Article

Chemical Composition and Multivariate Analysis of the Volatile Oil of *Dalbergia frutescens* (Vell.) Britton (Fabaceae)

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A composição química do óleo volátil das folhas de três matrizes de *D. frutescens*, coletadas no período de um ano foi determinada por cromatografia gasosa-espectrometria de massas (GC-MS) e cromatografia gasosa com detector de ionização de chama (GC-FID). Os óleos voláteis da espécie foram caracterizados predominantemente por norisoprenóides (β-damascenona, β-ionona e α-ionona), seguida da ocorrência de sesquiterpenos. Através da análise multivariada foi observado que os compostos β-damascenona e β-ionona influenciaram de forma intensa a composição dos óleos voláteis e que espécies coletadas na mesma cidade apresentaram composição química similar.

The chemical composition of the volatile oil from the leaves of three specimens of *D. frutescens*, collected over a period of one year, was determined by gas chromatography-mass spectrometry (GC-MS) and gas chromatography with flame ionization detection (GC-FID). These essential oils were characterized by the presence of norisoprenoids (β-damascenone, β-ionone, and α-ionone), as well as sesquiterpenes. Multivariate analysis showed that the compounds β-damascenone and β-ionone exerted the greatest influence on spatial and temporal differences in the composition of the oils. Samples obtained from specimens located in the same city showed similar chemical compositions.

Keywords: damascenone, norisoprenoids, seasonality

Introduction

The intrinsic value of the services provided by biodiversity to society is well known. Biological diversity is responsible for supplies of raw materials, nutrient cycling, pollination, control of agricultural pests, and climate regulation, amongst other benefits. About 1.4 million species have been described to date, of which 250,000 are plants. Estimates indicate that there are at least 5.5 million species worldwide, but the extinction rate increases every year,[1] as a result of which there is an urgent need for an improved understanding of interspecies relationships and the interactions between different species and the environment.

Brazil has become a major supplier of bioactive species due to its vast biodiversity and the existence of environmental conditions favorable for sustainable development. The country possesses renewable sources of chemical compounds with different pharmacological properties, including antioxidant, anticancer, antidiabetic, anti-HIV, antibacterial, antifungal, and antimalarial activities, amongst others.[2-5]

Studies of plants native to southern Brazil have mainly considered those species that have economic value due to the quality of their wood, including *Dalbergia frutescens* (Fabaceae). Previous studies of this species have shown

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the presence of isoflavones with antiprotozoal activity,[4] and recent work has identified the compounds linalool, β-damascenone, α-ionone, geranyl acetone, and β-ionone as characteristic components of the volatile oil of *D. frutescens*. Environmental variables such as temperature, cloudiness, directly influence the content of volatile oil while precipitation inversely influences.[5]

The objective of this work was to perform a chemical characterization of the volatile oil of *Dalbergia frutescens* obtained from three specimens growing at different locations in the State of Santa Catarina, in order to evaluate the temporal and spatial variability of the chemical compounds found in this species and contribute to understanding local biodiversity.

Experimental

Plant material

Samples of *Dalbergia frutescens* leaves were collected between March 2010 and February 2011, during the second half of each month, between 2 pm and 3 pm. Three separate specimens of *D. frutescens* (labeled BI, BII, and BIII) were employed. Specimens BI and BII were located in Chapecó (at 27°03'29.8"S, 52°39'36.4"W and 27°03'15.07"S, 52°39'32.97"W, respectively), and specimen BIII was located in the city of Concórdia (at 27°17'37.88"S, 52°04'02.57"W). During the collection period, the temperature varied according to season, with averages of 24.6 °C (summer) and 12.3 °C (winter). After collection, the samples were stored in a freezer at −10 °C until the volatile oil was extracted.

Voucher specimens were labeled and deposited at the herbarium of the Universidade Federal do Rio Grande do Sul: ICN 173123 (BI), ICN 173124 (BII), and ICN 173125 (BIII).

Analysis of the volatile oils

Fresh leaves of *D. frutescens* were crushed into small fragments and submitted to water distillation for 3 h, using a modified Clevenger-type apparatus. The volatile oil was dried over anhydrous sodium sulfate, giving an average yield of 0.0021% (percentage of oil, expressed as w/w with respect to the fresh leaves).

Gas chromatography-mass spectrometry (GC-MS) and gas chromatography with flame ionization detection (GC-FID) analyses

Qualitative analysis of the volatile oil was performed using a gas chromatograph coupled to a mass spectrometer. The gas chromatograph (Model GC-2010, Shimadzu) was equipped with a fused silica RTX-5MS capillary column (Restek, 30 m × 0.25 mm × 0.25 μm). The carrier gas was helium (1.0 mL min^{-1}), the injector temperature was 240 °C, and the oven temperature was programmed from 50 to 290 °C at 4 °C min^{-1}. Sample solutions (1 mg mL^{-1}) were injected in split mode (1:10). The mass spectrometer was operated in electron ionization mode, at 70 eV, scanning the mass range 40-600 Da at a rate of 2.94 scan s^{-1}. The ion source temperature was 260 °C and the interface was kept at 280 °C.

Quantification of the volatile constituents was performed using a GC-FID operated under conditions similar to those employed for the GC-MS. The peak area data were processed using the GCMS Post-Run Analysis software package.

Linear retention indices (LRI) were calculated using the method of Van den Dool for a homologous series of C_7-C_{30} n-alkanes injected under the same conditions. Identification of the components was achieved by matching the mass spectra with McLafferty and Stauffer (Wiley) and National Institute of Standards and Technology (NIST) library data. Identifications were also made by comparison of the retention indices with private reference libraries and values reported in the literature.[6] All the analyses were performed in triplicate.

Statistical analysis

The concentrations of the chemical compounds identified were subjected to analysis of variance (ANOVA), and the significance of the differences between the means was determined using Tukey's test, where values with probability less than 5% were considered significant. In this statistical procedure, differences between the compositions of the volatile oils obtained from the different specimens were evaluated on a monthly basis.

Seasonal trends in the chemical composition were investigated by multivariate statistical analysis of the measurement data. These analyses employed the mean contents of the five characteristic compounds of *D. frutescens* in four seasons, for each sample, giving a 5 × 12 matrix. These data were first submitted to hierarchical clustering analysis, using the nearest neighbor method and the minimum Euclidean distance as dissimilarity measure.[7] The results obtained from the cluster analysis were then complemented by application of factor analysis and principal component analysis. All statistical procedures were performed using Statistica 7.0 software.

Results and Discussion

Comparative analysis of the chemical composition of the *D. frutescens* volatile oil from different specimens

Considering all the samples collected over the course of the year, 92 compounds were identified in the volatile oil of *D. frutescens*, with the identified compounds contributing 93.43% (annual average) of the total. The seasonal variations of these compounds are given in Supplementary Information Tables S1-S3 for the three different specimens, and the chromatograms are shown in Supplementary Information Figures S1-S36.

The compounds linalool (LRI 1097), β-damascenone (LRI 1385), α-ionone (LRI 1430), geranyl acetone (LRI 1455), and β-ionone (LRI 1489) were present in the volatile oil of this species for most months and for all three sources of *D. frutescens* leaves.

There was greater prevalence of β-damascenone in the autumn months (24.22%), with lower concentrations in spring, when the concentration of β-ionone was highest (26.77%) for all specimens. These two compounds were the most common in most months, for all three specimens, with β-ionone being the largest component in spring, summer, and autumn. β-damascenone is produced biosynthetically from xanthophylls, giving rise to β-carotene,[8] while β-ionone is obtained by cleavage of α- and β-carotene.[9] These differences in the levels of these two compounds can therefore be explained by different phenological growth stages.

The presence of α-ionone as the largest component, especially in the spring months, was only observed for specimen BIII. This could have been due to different soil characteristics, because in Concórdia (specimen BIII) the main soil type is Udorthent Eutrophic, while in Chapecó (specimens BI and BII, located approximately 80 km distant from BIII) the Hapludox soil type predominates.[10]

The compounds α-ionone and β-ionone showed similar seasonal patterns, with maxima in the spring, when there was greater occurrence of new leaves, and there were gradual decreases in concentrations during subsequent seasons. According to Rao *et al.*,[11] there is an increase in the percentage of linalool during months characterized by the presence of young and growing leaves, as also observed in this study.

The compounds α-cedrene (LRI 1412), pentadecanal (LRI 1711), pentadecan-2-one (LRI 1841), and farnesyl acetone (LRI 1921) were characteristic of specimen BII, and pentadecan-2-one (LRI 1841) was characteristic of specimen BIII.

Other compounds frequently observed in the volatile oil of *D. frutescens* were methyl hexadecanoate (LRI 1925), which occurred from September to December, and pentadecanal (LRI 1711), which only occurred during the winter months (June-August). Pentadecanal and pentadecan-2-one were only found as characteristic compounds for specimens BII and BIII. Although these specimens were growing in different locations, they were similar in size and both were close to roads and crops, which could have influenced the composition of the oil. This behavior was similar to that of α-ionone.

The compounds 3-hexenol (LRI 859) and 1-hexanol (LRI 871) also showed similar behavior for BII and BIII, and different behavior for BI, and only BI showed higher amounts of these constituents in the winter months. This could have been related to the time of occurrence of these compounds, which was limited to the developmental stage when leaf senescence began. The detection of high concentrations of these constituents could have been linked to enzymatic degradation of unsaturated fatty acids, producing compounds such as aldehydes, ketones, and low molecular weight alcohols, as reported by Hatanaka.[12] The different amounts of these compounds detected in BI, compared to BII and BIII, could be explained by the different sizes of the specimens, as well as the type of environment. The BII and BIII specimens were both small, with few branches, and were close to other trees, which probably limited light capture and growth. An additional factor is that they were both very close to farming environments.

Secondary metabolites detected in the volatile oil of *D. frutescens* included norisoprenoids, which were present in most months, with mean seasonal concentrations ranging from 31.18% to 47.78%. Oxygenated sesquiterpenes showed high concentrations in spring (22.17%) and winter (18.48%), while sesquiterpene hydrocarbons showed higher concentrations in autumn (16.14%), followed by summer (12.57%).

The oxygenated compounds showed highest concentrations in spring (89.95%), followed by winter and summer (80.70% and 79.39%, respectively). Similar results were reported by Mohammad *et al.*,[13] who found that high ambient temperature promoted higher levels of oxygenated compounds such as bisabolol oxide A and B and bisabolene oxide in the essential oil of chamomile (*Matricaria chamomilla* L.).

Multivariate analyses

Chemical similarities between the *D. frutescens* volatile oils obtained from the different specimens in the various collection periods were investigated using hierarchical cluster analysis (CA), factor analysis (FA), and principal component analysis (PCA).

The original data matrix used in these analyses was composed of the means of the five characteristic compounds of *D. frutescens* (β-damascenone, β-ionone, α-ionone, linalool, and geranyl acetone) for each season and specimen. Factor analysis showed that the original data matrix could be reduced to two principal components that explained 90.79% of the data. The application of CA and FA resulted in the identification of five distinct groups (Figure 1).

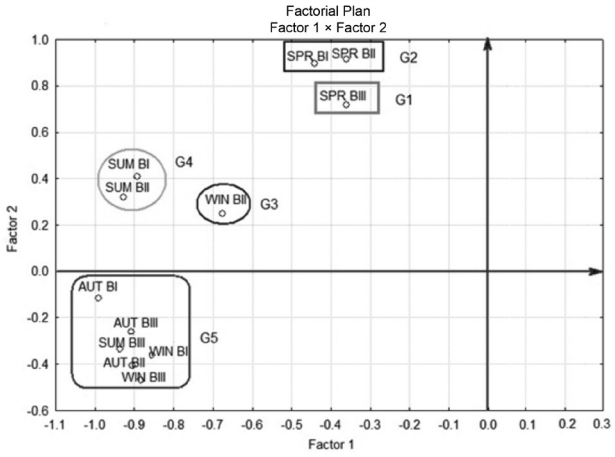

Figure 1. Graph representing the relationship between factors 1 and 2, and grouping of variables obtained from factor analysis.

The first group (G1) only contained BIII in spring, and was characterized by low levels of linalool and β-damascenone (means for the spring months of 2.77% and 8.67%, respectively), as well as the highest levels of α-ionone and β-ionone (15.63% and 32.35%, respectively). The second group (G2), composed of BI and BII in spring, differed from G1 due to high levels of linalool (10%).

The third group (G3), composed only of BII in winter, had a mean linalool contribution of 5.58% and low levels of β-ionone and β-damascenone (8.17% and 8.75%, respectively), but the highest average geranyl acetone contribution obtained considering the four seasons and the three specimens (7.05%).

The fourth group (G4) was composed of BI and BII in summer. The main features of this group were average levels of β-damascenone between 15 and 16%, β-ionone between 15 and 18%, and linalool between 9 and 10%.

Finally, considerable similarity between the specimens was observed for autumn and winter. Group 5 (G5) was composed of these seasons, with the exception of BII in winter (which was in G3) and BIII in summer (G5). The chemical compositions obtained for BI and BIII were more similar in autumn and winter, as reflected in G5. The key features of G5 were the presence of linalool at percentages lower than 5%, low percentages of β-ionone (below 14%), and high levels of β-damascenone (exceeding 17%).

These findings were supported by the results of the PCA procedure, which reduced the 12 initial variables to only 3 main components that explained 70.50% of the variability of the original data. Most of the variance (60.11%) was explained by the first two principal components, which were therefore used to prepare the PCA plot (Figure 2).

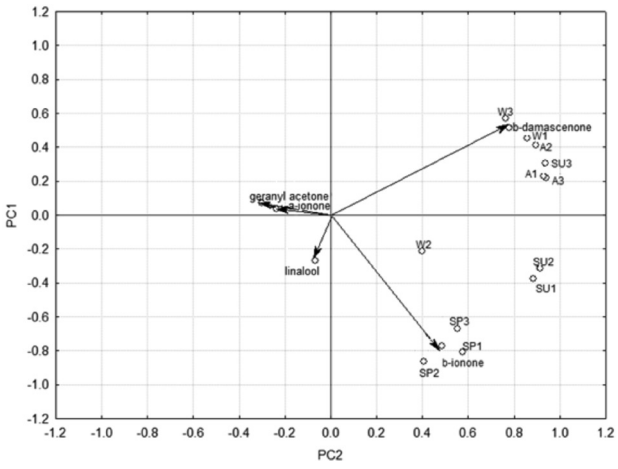

Figure 2. Principal component analysis of the chemical composition of volatile oil from *D. frutescens*.

Figure 2 shows that for all samples, the variables corresponding to the spring season were associated with high levels of β-ionone (due to their very close proximity to this variable). It can be seen that the variables corresponding to BI and BII in spring (SP1 and SP2) are shifted slightly to the left, which implies that these were also influenced by high concentrations of linalool, despite the greater influence of β-ionone.

The positions of the variables corresponding to BI and BII in summer (SU1 and SU2) reflected mean levels of β-ionone and β-damascenone, due to the intermediate positioning of SU1 and SU2 in the graph. The variable for BII in winter (W2) showed the same feature, but with a shift towards the origin of the graph, suggesting a relationship with geranyl acetone, as previously noted in the cluster analysis. Finally, grouping of the other variables near β-damascenone indicated high levels of this compound in the volatile oil of specimens BI and BIII in winter (W1 and W3), BIII in summer (SU3), and all three specimens in autumn (A1, A2, and A3).

Overall, the factor and principal component analyses confirmed the findings of the cluster analysis and demonstrated that the volatile oil of *D. frutescens* was strongly influenced by the levels of β-ionone and β-damascenone. These chemical constituents governed the separation of the variables, probably due to their high concentrations in the volatile oil.

Four of the five compounds detected in samples from the three specimens for almost all months are biosynthesized in degradation pathways involving oxidative cleavage of carotenoids and xanthophylls. These include the formation of α-ionone from α-carotene,[8,9] β-ionone from α-carotene and β-carotene,[8,9] geranyl acetone from the ζ-carotene,[14,15] and β-damascenone from neoxanthin.[16] The ubiquitous occurrence of these compounds in *Dalbergia frutescens* essential oil enables them to be considered as markers of this species.

Conclusions

Sesquiterpenes and norisoprenoids predominated in the volatile oil of *D. frutescens*, and characteristic compounds included linalool, β-damascenone, α-ionone, geranyl acetone, and β-ionone. All these compounds (except linalool) are biosynthesized by degradation of carotenoids,[8,9,14-16] resulting in high levels of β-damascenone and β-ionone, and low levels of other compounds. This study demonstrated that there can be significant differences in the chemical composition of volatile oils obtained from specimens of the same species at different locations, and that the differences can vary according to season.

The concentrations of norisoprenoids remained practically constant during the spring, summer, and autumn months, and diminished in the winter. The highest levels of oxygenated sesquiterpenes were obtained in spring and winter, while the opposite was observed for sesquiterpene hydrocarbons.

Statistical analysis using CA, FA, and PCA showed that, in general terms, the chemical composition of the volatile oil from specimens BI and BII, located in the city of Chapecó, was similar for most seasons (except winter). Application of PCA revealed that of the five characteristic compounds, β-damascenone and β-ionone were most influential in separating the groups, due to the presence of high concentrations of these compounds in the *D. frutescens* volatile oil.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgement

This work was supported by public call 013/2009, FAPESC (Fundação de Amparo à Pesquisa do estado de Santa Catarina)

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Submitted on: February 4, 2014
Published online: May 23, 2014