Characterization of Leaf Extracts of *Schinus terebinthifolius* Raddi by GC-MS and Chemometric Analysis


INTRODUCTION

*Schinus terebinthifolius* Raddi belongs to Anacardiaceae family and originates from South America. It is distributed throughout Brazil and is more commonly known as "aroeira." This plant is widely used in folk medicine due to its therapeutic properties, which include antimicrobial, anti-inflammatory, and antipyretic effects. The complexity and variability of the chemical constitution of the herbal raw material establishes the quality of the respective herbal medicine products. **Objective:** Thus, the purpose of this study was to investigate the variability of the volatile compounds from leaves of *S. terebinthifolius*. **Materials and Methods:** The samples were collected from different states of the Northeast region of Brazil and analyzed with a gas chromatograph coupled to a mass spectrometer (GC-MS). The collected data were analyzed using multivariate data analysis. **Results:** The samples' chromatograms, obtained by GC-MS, showed similar chemical profiles in a number of peaks, but some differences were observed in the intensity of these analytical markers. The chromatographic fingerprints obtained by GC-MS were suitable for discrimination of the samples; these results along with a statistical treatment (principal component analysis (PCA)) were used as a tool for comparative analysis between the different samples of *S. terebinthifolius*. **Conclusion:** The experimental data show that the PCA used in this study clustered the samples into groups with similar chemical profiles, which builds an appropriate approach to evaluate the similarity in the phytochemical pattern found in the different leaf samples.

**Key words:** *Schinus terebinthifolius*, GC-MS, fingerprint, chemometrics, principal component analysis

**SUMMARY**

- The leaf extracts of *Schinus terebinthifolius* were obtained by turbo-extraction.
- The extracts were partitioned with hexane and analyzed by GC-MS.
- The chromatographic data were analyzed using the principal component analysis (PCA).
- The PCA plots showed the main compounds (phellandrene, limonene, and carene), which were used to group the samples from a different geographical location in accordance to their chemical similarity.

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The major components identified by gas chromatography were interpreted in terms of the average variation.\[11\]

To execute the PCA analysis, each variable (the m/z intensity of each of variables and data dimensionality. The score plot of PCA is an observational approach to conduct a comprehensive evaluation of \textit{S. terebinthifolius}. The PCA is used to qualitatively analyze the samples by reducing the number for Weather Forecasting and Climate Studies,\[8\] from September to November, most of the Northeast region was in its dry season, except the states of Piauí, Maranhão, and the West of Bahia. The GC-chromatograms for the samples are presented in Figure 1. The qualitative analysis of the chromatograms showed small differences among the chemical profiles, especially regarding its main peaks.

The gas chromatographic analysis allowed the separation and identification of the volatile compounds where the ones with the highest concentrations were α-pinene, limonene, carene, and phellandrene [Table 1]. In addition to these main compounds, the extracts also presented myrcene, δ-elemene, α-cubebene, γ-cadinene, and α-copaene For samples collected in Bahia (BA), Piauí (PI), and Maranhão (MA), limonene was the compound with the highest percentage. Regarding the samples from Alagoas (AL), Paraiba (PB), Pernambuco (PE), Rio Grande do Norte (RN) and Sergipe (SE); carene was the compound with the highest percentage, while phellandrene was the major compound for the samples collected in Ceará (CE). Even though α-pinene does not represent the highest percentage in any of the samples, it is present in all of them and may, therefore, be a standard reference peak.

The majority of the compounds identified in the samples of this study are qualitatively similar to previously reported data for the leaves of \textit{S. terebinthifolius}. According to studies by Jamal and Agusta\[12\] in India, detected 3-carene, α-pinene, β-pinene, α-phenllandrene, limonene, sabine, p-cymene, β-cymene, δ-elemene, isocaryophyllene, and α-cubebene in their study with the leaves of this plant.

The studies described by Gundidza et al.\[13\] detected α-pinene (30.27%), camphene (0.58%), β-myrcene (6.60%), β-pinene (7.96%), myrcene (1.63%), α-phenllandrene (9.86%), α-terpinene (0.77%), sabine (40.66%), trans-β-ocimene (0.30%), γ-terpinene (0.77%), and 3-cyclohexen-1-ol (0.61%) when studying the composition of \textit{S. terebinthifolius} leaves.

The variability of the main compounds on different samples collected in the states of the Northeast region of Brazil may be related to the microclimates of each region since the amount of secondary metabolites on medicinal plants may vary depending on various factors such as seasonality, circadian period, age, development of the plant, and availability of nutrients and water in the soil.\[14\]

### Table 1: The major components identified by gas chromatography-mass spectrometry from samples of \textit{Schinus terebinthifolius}

<table>
<thead>
<tr>
<th>Collection local</th>
<th>α-pinene</th>
<th>Limonene</th>
<th>Carene</th>
<th>Phellandrene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salvador/BA</td>
<td>16.02±0.06</td>
<td>77.27±0.03</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Maceio/AL</td>
<td>20.66±0.02</td>
<td>12.36±0.01</td>
<td>53.80±0.02</td>
<td>4.76±0.03</td>
</tr>
<tr>
<td>Teresina/PI</td>
<td>5.36±0.05</td>
<td>30.06±0.01</td>
<td>1.71±0.01</td>
<td>14.07±0.04</td>
</tr>
<tr>
<td>João Pessoa/PA</td>
<td>3.40±0.06</td>
<td>4.98±0.01</td>
<td>47.52±0.04</td>
<td>3.42±0.01</td>
</tr>
<tr>
<td>São Luís/MA</td>
<td>4.52±0.05</td>
<td>44.17±0.01</td>
<td>2.11±0.01</td>
<td>16.14±0.04</td>
</tr>
<tr>
<td>Recife/PE</td>
<td>16.97±0.02</td>
<td>6.23±0.02</td>
<td>61.11±0.03</td>
<td>3.46±0.01</td>
</tr>
<tr>
<td>Fortaleza/CE</td>
<td>6.01±0.02</td>
<td>22.83±0.04</td>
<td>22.38±0.04</td>
<td>27.80±0.02</td>
</tr>
<tr>
<td>Natal/RN</td>
<td>17.55±0.03</td>
<td>ND</td>
<td>46.33±0.04</td>
<td>11.30±0.04</td>
</tr>
<tr>
<td>Aracaju/SE</td>
<td>16.49±0.05</td>
<td>ND</td>
<td>66.58±0.03</td>
<td>4.82±0.03</td>
</tr>
</tbody>
</table>

The data were expressed as mean±SD (n=3). ND: Not detected; SD: Standard deviation; BA: Bahia; AL: Alagoas; PI: Piauí; PB: Paraíba; MA: Maranhão; PE: Pernambuco; CE: Ceará; RN: Rio Grande do Norte; SE: Sergipe
In a study carried out by Barbosa et al.,[15] the oil content in the leaves of *S. terebinthifolius* showed some minor changes throughout the course of 1 year, but these changes were usually statistically insignificant. The oil content was higher (0.65%–0.69%) between March and September and lower (0.45%–0.55%) between October and February, which coincided with flowering and fruiting, respectively. The oil extracted from flowerless leaf petioles showed the presence of α-pinene, phellandrene, myrcene, δ-elemene, γ-cadinene, and α-copaene, corroborating with the results of this study.

The application of chemometric techniques provides a higher quality interpretation of the data, enabling the classification and discrimination of the samples.[16] To visualize the degree of similarity among the samples, the data were processed by PCA, to cluster samples with the same chromatographic characteristics. The chromatographic profiles obtained were organized in a data matrix containing 36 lines (samples) and 882 variables (ion fragment and retention time combined, m/z-time), and after the data pretreatment and alignment of the peaks, the chromatograms were evaluated by PCA.

The PCA was applied to evaluate the data of the compounds, determined by GC-MS, reducing the number of data set dimensions without losing relevant information. Obtaining a lower number of new variables (principal components) facilitates interpretation of the data. The analysis provided data clustering which allowed the identification of similar values among the samples. The main component 1 (PC1) explained 57% of the total variance of the data and the main component 2 (PC2) explained 19%. Thus, the PC1 × PC2 graph represents a variance of 76% of the plotted data, demonstrating that the sample points are distant [Figure 2]. In this way, samples with similar characteristics have nearby points on the graph.

The data were normalized between −0.2 and +0.2 for both PC1 and PC2. The results presented in Figure 2 shows that in PC1, PI and MA samples are grouped together in positive score values, along with BA sample, in negative score values. Analyzing the samples in PC2, it is possible to observe PB, RN, and CE samples in positive score values and SE, PE, and AL samples in negative score values. It can be observed in Figure 2, the clusters A, B, and C, which occurred because of the similarities between samples belonging to each group.

Analyzing the loading graph presented in Figure 3, which provides the variable that influences the clustering; the construction of the main components (PC1 and PC2) can be explained by the similarity of the samples in each group and therefore the projection of the samples in the score plot.[17] Figure 3 shows the loading plot for the model. It was observed that the grouping fragment for the first quadrant area is 93 m/z with a retention time of 4 min, which is characteristic for phellandrene; the second quadrant showed 68 m/z with a retention time of 6 min, which is characteristic for limonene, and the third quadrant showed 92 m/z with a retention time of 5 min, which is characteristic for carene. Therefore, samples in groups A (RN, PB, SE, PE, and AL), B (CE), and C (PI, MA, and BA) are grouped by the presence of carene, phellandrene, and limonene, respectively.

The scatter plot provided by the PCA was an important tool for this analysis since it allowed the comparison and exploration of complex data and multivariate design of the samples in a two-dimensional graph, where the chromatogram was treated not as a set of many peaks but as a single signal multivariable, enabling a comparison between them. Therefore, we are able to assume that the PCA was an appropriate approach to examine the similarity between the samples.
CONCLUSION

This paper determined the chromatographic profiles for S. terebinthifolius leaves using GC-MS. The data were treated by PCA. This analysis made it possible to investigate the similarity of the chemical compounds present in leaf samples of S. terebinthifolius from different states of the Northeast of Brazil.

Analyzing the chromatographic fingerprints from samples of S. terebinthifolius, it was possible to identify the main components in each sample and detect variations in their chemical composition.

The model obtained with PCA was effective in the interpretation of the data analyzed. Thus, the samples could be distributed into three groups by comparing the score and loading data obtained from the combination of main components.

Group A containing samples from the states of RN, PB, SE, and AL; Group B containing samples from the state of CE, and group C containing samples from the states of PI, MA, and BA; presented carene, phellandrene and limonene, as their main compounds, respectively. These markers guided the data clustering. Thus, plants belonging to the same group showed chemical similarities.

In conclusion, this study showed that the chemometric approach for the analysis of the GC-fingerprints from samples of S. terebinthifolius samples was able to their comparative analysis.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

11. Iorgulescu E, Voicu VA, Sârbu C, Tache F, Albu F, Medvedovici A. Experimental variability and data pre-processing as factors affecting the discrimination power of some chemometric approaches (PCA, CA and a new algorithm based on linear regression) applied to (+/-) ESI/MS and RPLC/UV data: Application on green tea extracts. Talanta 2016;155:133-44.