CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL OF LEAVES FRESH Syzygium cumini COLLECTED IN THE AMAZON REGION IN SEASONS DIFFERENT.


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ABSTRACT
Essential oils are distributed in a limited number of families, as Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Apioaceae, Cupressaceae, Poaceae, Zingiberaceae and Piperaceae. The leaves of Syzygium cumini are used in folk medicine as a remedy against diabetes, dysentery, indigestion, gastrointestinal disorders, anti-inflammatory agent, antipyretic and anti-emic. The leaves of S. cumini were collected at AM-010 Road, Km 26, Itacoiatiara-Manaus sense. The essential oils were obtained by hydro distillation of fresh material Clever apparatus (6 h). The samples analyzed in GC-MS to identify the chemical constituents. The α-Pinene is the substance with the highest percentage in area among those identified in the essential oil from the leaves of S. cumini. The area values α - Pinene ranged from 17.99% 10/2013 in maximum and 56.14% 02/2014. The results of DPPH inhibition of essential oil samples were kept constant even with varying composition of the major compounds. The chemical composition of essential oil of fresh leaves of S. cumini suffers variations in percentage values in the area of major compounds depending on the time in which they are collected. The α-Pinene was the major substance in almost all the analyzed periods.

KEYWORDS: Seasonality, Myrtaceae, GC-MS and α - Pinene

INTRODUCTION
Among the natural products used in therapeutic approaches, essential oils often used in aromatherapy are described as products with great therapeutic and pharmacological potential. Essential oils are natural, volatile and complex compounds that are characterized by a strong odor being synthesized by aromatic plants during the secondary metabolism and usually extracted from plants found in hot countries such as the Mediterranean and the tropics, which represent an important part of the pharmacopoeia traditional. The botanical genera that produce compounds that are the essential oils are distributed in a limited number of families, as Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Apiaceae, Cupressaceae, Poaceae, Zingiberaceae and Piperaceae. Some essential oils have high toxicity, repellent action, feeding inhibitors, and exert influence on the development of living organisms, such as insects. The chemical composition of essential oils can be divided into two classes. The first, based on biosynthesis, which are derivatives of terpenoids, formed via the acetate-mevalonic acid; and second, in whom they are located, the phenylpropanoid derivatives, aromatic compounds formed via the acid shikimic. The essential oils are extracted from different parts of plants, e.g., leaves, bark, flowers, buds, seeds, fruits, roots. May be used various extraction methods among them, steam distillation, expression, and so forth. The steam distillation method has been widely used, particularly for production-scale commercial. There are 17,500 species of herbs between higher plants and nearly 3,000 essential oils are known, of which 300 are commercially important for pharmaceuticals, cosmetics and perfume. An industry has been recognized for a long time that some essential oils have antimicrobial properties, antiviral, antiparasitic and insecticide. These properties are possibly related to the function of these compounds in plants. Syzygium cumini (L) Skeels belongs to the Myrtaceae family and sub Myrtoideae family is originally from India and Java, was introduced in many tropical countries in Africa and America Latina. in Brazil is found in various states of the Southeast, Northeast and North. It is also found in some subtropical regions like Florida and California in the United States.

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The leaves of *S. cumini* are used in folk medicine to treat diabetes, dysentery, indigestion, gastrointestinal problems, and weight loss, and as an anti-inflammatory agent, antipyretic and antiemetic. The objective of this study was to evaluate the effect of seasonality of chemical constituents present in the essential oil from the leaves of *S. cumini* and determine its antioxidant activity.

**Experimental Section**

The leaves of *S. cumini* (Myrtaceae) were collected at AM-010 road, Km 26 (3° 03' 48.50" S, 58° 37' 14.01" O, 20m), Itacoiara - Manaus sense, to obtain the essential oils. Samples were collected from a single tree at 7:30 a.m. every two months totaling 9 collections in the period from August / 2013 to December / 2014 to analyze the seasonal variation of yield and chemical constituents of essential oils. The leaves were collected in the period in which the plant had inflorescence, fruits and without fruit in order to evaluate the effect of seasonality in the essential oil yield.

**Essential oil extraction**

Essential oils from the leaves were obtained by hydro distillation of fresh material (1000 g) in Clevenger apparatus (6 h). The leaves were placed whole into the distillation flask along with 7.0 liters of distilled water. Then the oil was centrifuged for 10 minutes at 3500 revolutions per minute (rpm) for separation and removal of water. The essential oils obtained were kept in amber vials capped under refrigeration 2 °C until they are analyzed. The yields of essential oils were calculated based on the volume of essential oil obtained from the leaves divided by the sample mass used (v/m).

**Gas chromatography**

Quantitative analysis by GC was performed using a Shimadzu model GC-2010 Plus for chromatographic analysis of the components used was DB-5MS column, 30 m x 0.25 mm, film thickness 0.25 internal micrometers. Equipped with a flame ionization detector (FID), the oven temperature was programmed 100-140 °C at 10 °C / min, 140-180 °C at 2.5 °C / min and 180-250 °C at 20 °C / min, the chromatographic run was terminated after 50 min. The temperatures of the injector port and detector were maintained at 220 to 280 °C, respectively. Was used as a helium gas at a flow rate of 2 ml min⁻¹ and inlet pressure in split mode 30 p.s.i. (1:30). The injection volume was 0.5 μL of a solution diluted (1/100) oil in hexane. Amount of each compound was calculated from the areas of the peaks in the GC column elution order and expressed as a percentage of the total area of the chromatogram. Analyses were performed in triplicate.

**Gas chromatography analysis coupled with mass spectrometry - GC-MS**

The extracted oil was analyzed by GC-MS in Shimadzu equipment coupled to a mass spectrometer Shimadzu QP2010. System ion source operating at 220 °C and impact energy of 70 eV equipped with the same column and temperature program as described for the experiments GC with the following parameters: carrier gas = helium; flow rate = 2 ml min⁻¹; split mode (1:30); Volume injected = 0.5 μL of a solution diluted (1/100) oil in hexane.

**Identification of the chemical components of the essential oil**

The identification of the components was made based on GC retention index - FID in reference to a homologous series of C₁₁⁻C₃₃ n-alkanes and calculated using the equation Van den Dool & Kratz [20], interpretation of their respective spectra masses as aid library database (Nist08)[21] and by comparison with data from literature.[22]

**Determination of Antioxidant Activity**

Sample solutions (1.0 mg / ml) were diluted to final concentrations of 250, 125, 50, 25, 10 and 5 / ml in ethanol. One ml of an ethanol solution of 0.3 mM DPPH was added to 2.5 ml of different concentrations of the sample solutions, letting react at room temperature in a dark chamber. After 30 min the absorbance values were measured at 518 nm in a multimode DTX detector 800, and then were converted to percentage of the antioxidant activity (AA). Ethanol (1.0 mM) and the sample solution (2.5 ml) were used as blank. DPPH solution (1.0 ml, 0.3 mM) plus ethanol (2.5 ml) was used as negative control. The positive controls used were those standard solutions. IC₅₀ values were calculated by linear regression plots where the abscissa represents the concentration of the samples tested and sorted average percentage of antioxidant activity of three separate tests according to literature.[23]

**Statistical analysis**

The relative percentages of compounds identified in oils of *S. cumini* from the nine collection periods and data antioxidant activity were subjected to analysis of variance (ANOVA) using the Tukey test (P <0.05) for comparison values, using the statistical program Assistat 7.7.

**Results and Discussion**

**Essential oil yield**

The results of yields (%) obtained during the study period can be seen in Table 1.
Table 1. Essential oil yield of *S. cumini* (%) and precipitation volume (mm).

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Date</th>
<th>Used leaves Mass (g)</th>
<th>Essential Oil Volume (ml)</th>
<th>Yield (%) (V/M)</th>
<th>Precipitation volume INMET (mm)</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27/08/2013</td>
<td>1007.07</td>
<td>0.73</td>
<td>0.072</td>
<td>128</td>
<td>Fruitless</td>
</tr>
<tr>
<td>2</td>
<td>17/10/2013</td>
<td>1056.68</td>
<td>0.85</td>
<td>0.08</td>
<td>99</td>
<td>Green fruit and inflorescence</td>
</tr>
<tr>
<td>3</td>
<td>11/12/2013</td>
<td>1004.71</td>
<td>1.02</td>
<td>0.102</td>
<td>222</td>
<td>Green fruit and ripe</td>
</tr>
<tr>
<td>4</td>
<td>18/02/2014</td>
<td>1003.02</td>
<td>0.87</td>
<td>0.086</td>
<td>474</td>
<td>Ripe fruits</td>
</tr>
<tr>
<td>5</td>
<td>25/04/2014</td>
<td>1000.47</td>
<td>0.72</td>
<td>0.072</td>
<td>338</td>
<td>Fruitless</td>
</tr>
<tr>
<td>6</td>
<td>18/06/2014</td>
<td>1016.27</td>
<td>0.91</td>
<td>0.089</td>
<td>221</td>
<td>Fruitless</td>
</tr>
<tr>
<td>7</td>
<td>23/08/2014</td>
<td>1027.91</td>
<td>0.88</td>
<td>0.085</td>
<td>79</td>
<td>Fruitless</td>
</tr>
<tr>
<td>8</td>
<td>24/10/2014</td>
<td>1000.13</td>
<td>0.91</td>
<td>0.091</td>
<td>265</td>
<td>Green fruit and inflorescence</td>
</tr>
<tr>
<td>9</td>
<td>05/12/2014</td>
<td>1017.52</td>
<td>1.33</td>
<td>0.13</td>
<td>208</td>
<td>Green fruit and ripe</td>
</tr>
</tbody>
</table>

It is noted that samples 2 and 8 were those which showed an increase in oil yield in that study period was 0.80 and 0.91%, which coincided with the period where the plant was at the beginning of the inflorescence (Table 1). The results yield the samples 3 and 9 of 0.102 and 0.130%, respectively, were the highest values for the assessed period, corresponding to the time when the plant is in fruit ripening early. There is a decrease in the yield after the final period fruit production and the following samples a slight stabilization of the yield.

Literature data show that the yield of essential oil from the leaves of Melissa officinalis was affected by different stages of harvest, and leaves collected before and during flowering provided the best yields. The results are *S. cumini* confirming the literature data to give a yield increase in the period when the plant is in the inflorescence period.

Results found in the literature demonstrate the actual yield of essential oil from the leaves of *S. cumini* collected in June in the state of Kerala, South India, which was 0.04% lower than those obtained in this work compared to all values of the reporting period. The yield value of 0.08%, the essential oil from the leaves of *S. cumini* collected in the Botanical Garden of Medicinal Plants of Natural Products Research Laboratory (LPPN) Regional University of Cariri (URCA) in the state of Ceará to 09:30 coincides with the value of sample 2 of this work. But the samples of income values 3, 4, 6, 7, 8, and 9 were higher, showing that the collection time, geographical location of the plant, the collection period and seasonal and climatic variations affect the yield of oil essential.

**Identification of major compounds and the effect of seasonality**

They identified 13 substances in essential oil samples of *S. cumini* leaves in the analyzed period, as shown in Table 2.
<table>
<thead>
<tr>
<th>Nº</th>
<th>Substance</th>
<th>27/08/2013</th>
<th>17/10/2013</th>
<th>11/12/2013</th>
<th>18/02/2014</th>
<th>25/04/2014</th>
<th>18/06/2014</th>
<th>23/08/2014</th>
<th>KI&lt;sub&gt;a&lt;/sub&gt;</th>
<th>KI&lt;sub&gt;b&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-Pinene</td>
<td>28,87</td>
<td>17,99</td>
<td>28,33</td>
<td>56,14</td>
<td>47,41</td>
<td>39,4</td>
<td>40,32</td>
<td>931</td>
<td>939</td>
</tr>
<tr>
<td>2</td>
<td>Camphene</td>
<td>1,07</td>
<td>0,45</td>
<td>0,63</td>
<td>1,02</td>
<td>1,23</td>
<td>1,28</td>
<td>1,15</td>
<td>947</td>
<td>954</td>
</tr>
<tr>
<td>3</td>
<td>β – Pinene</td>
<td>4,35</td>
<td>10,24</td>
<td>9,83</td>
<td>13,89</td>
<td>7,4</td>
<td>5,01</td>
<td>6,74</td>
<td>976</td>
<td>979</td>
</tr>
<tr>
<td>4</td>
<td>β - Myrcene</td>
<td>3,63</td>
<td>3,79</td>
<td>3,92</td>
<td>2,98</td>
<td>3,84</td>
<td>3,67</td>
<td>3,27</td>
<td>987</td>
<td>990</td>
</tr>
<tr>
<td>5</td>
<td>p-Cymene</td>
<td>0,81</td>
<td>0,37</td>
<td>0,45</td>
<td>1,04</td>
<td>0,89</td>
<td>0,85</td>
<td>0,74</td>
<td>1023</td>
<td>1024</td>
</tr>
<tr>
<td>6</td>
<td>Limonene</td>
<td>7,65</td>
<td>3,94</td>
<td>4,73</td>
<td>7,14</td>
<td>7,32</td>
<td>7,67</td>
<td>7,5</td>
<td>1027</td>
<td>1029</td>
</tr>
<tr>
<td>7</td>
<td>(Z) β - Ocimene</td>
<td>18,23</td>
<td>23,92</td>
<td>23,4</td>
<td>nd</td>
<td>14,63</td>
<td>17,98</td>
<td>17,1</td>
<td>1033</td>
<td>1037</td>
</tr>
<tr>
<td>8</td>
<td>(E) β - Ocimene</td>
<td>6,55</td>
<td>8,1</td>
<td>6,4</td>
<td>nd</td>
<td>2,66</td>
<td>4,59</td>
<td>4,14</td>
<td>1043</td>
<td>1050</td>
</tr>
<tr>
<td>9</td>
<td>α-Terpineol</td>
<td>7,05</td>
<td>5,15</td>
<td>6,78</td>
<td>5,63</td>
<td>5,38</td>
<td>6,47</td>
<td>6,09</td>
<td>1194</td>
<td>1188</td>
</tr>
<tr>
<td>10</td>
<td>Bornyl acetate</td>
<td>2,45</td>
<td>0,57</td>
<td>0,6</td>
<td>0,98</td>
<td>1,42</td>
<td>1,82</td>
<td>1,57</td>
<td>1281</td>
<td>1289</td>
</tr>
<tr>
<td>11</td>
<td>trans-Caryophyllene</td>
<td>6,54</td>
<td>12,38</td>
<td>6,44</td>
<td>1,95</td>
<td>3,15</td>
<td>4,63</td>
<td>5,24</td>
<td>1413</td>
<td>1418</td>
</tr>
<tr>
<td>12</td>
<td>α-Humulene</td>
<td>3,3</td>
<td>5,57</td>
<td>3,13</td>
<td>1,24</td>
<td>1,72</td>
<td>2,48</td>
<td>2,77</td>
<td>1449</td>
<td>1454</td>
</tr>
<tr>
<td>13</td>
<td>Caryophyllene oxide</td>
<td>1,34</td>
<td>1,14</td>
<td>0,49</td>
<td>5,94</td>
<td>1,27</td>
<td>1,19</td>
<td>1,35</td>
<td>1574</td>
<td>1583</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>91,84</td>
<td>93,61</td>
<td>95,13</td>
<td>97,95</td>
<td>98,32</td>
<td>97,04</td>
<td>97,98</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

KI<sub>a</sub> – Calculated Kovats index  
KI<sub>b</sub> – Kovats Literature Index [22]  
nd - Not detected
The α-Pinene was the substance with the highest percentage in area among those identified in the essential oil from the leaves of S. cumini during the evaluation period, except for the month of October 2013, where the majority was (Z)-β-Ocimene. This work can be seen that the highest concentration of α-Pinene occurred in the month February 2014.

The area values α - Pinene ranged from 17.99% in 10/2013, during which the plant was in the inflorescence period and 7 mm rainfall volume, 56.14% by 02/2014, during which plant had ripe fruits and the amount of rainfall was 348 mm. Area% results found in the literature for α - Pinene were 30.04% for essential oil sheets S. cumini collected in the state of Ceará and 31.85% of essential oil obtained from the leaves of S. cumini collected from a tree in flowering stage, located in the city of São Luis, Maranhão, in July 2010.[28] Another result found in the literature for the essential oil from the leaves of S. cumini demonstrated that area of value α - Pinene was 30.10%,[29] and the analysis of the essential oil composition of the fruit of S. cumini the percentage of α - Pinene was 4.56%.[30] This value 12.4-fold lower compared to that found in this study for essential oil of leaves S. cumini. The results may be different from those found in the literature due to several factors such as temperature, water regime, climatic and geographical conditions, vegetative stage, solar radiation, part of plants and seasonality that can influence the chemical composition of the oil of this species.[31]

The minimum camphene value was 0.45% in area between October 2013 and its maximum value 1.28% in area was in June / 2014. The percentage of camphene identified in the essential oil of S. cumini obtained from leaves collected in the Botanical Garden of Medicinal Plants of the Regional University of Cariri (URCA) in the state of Ceará, it was 1.17%,[26] being less than the maximum value found in this work. The result of literature 28 demonstrates percentage in camphene area of 1.56% of the essential oil obtained from leaves of S. cumini collected from a tree in flowering stage in the Federal University of Maranhão (UFMA), San Luis, Maranhão, in July 2010, an amount greater than the maximum value found in this work.

The area results β - Pinene and β - Myrcene had variations during the analysis period, and 4.35 to 13.89% for β - Pinene and 2.98 to 3.92% for β - Myrcene as Table 2. the maximum value of β - Pinene, 13.89% obtained in this study was lower than that reported in the literature.[29] which was 20.50% for the essential oil from the leaves of S. cumini collected in September 1981 trees Campus Pici of the Federal University of Ceará. And the value was higher than that found for the essential oil from the leaves of S. cumini collected on the campus of the Cariri Regional University, which was 8.26%.[30] The value of area β - Myrcene 3.92% was greater than the results reported in the literature was 2.78%.[26] The results show that the same plant produces different amounts percentages in area of the same substance, even being collected from equal parts of this species, which in the case of the papers presented are the leaves of S. cumini, only collected in different regions and even in this work where the leaves were collected from the same plant but in different periods showed variation in the content (%) of its constituents.

The (Z) β - Ocimene and (E) β - Ocimene were not detected in February / 2014. The essential oils from the leaves Lippia alba (Mill.) NE Brown (Verbenaceae) from growing in the Garden of the City of São Luiz Gonzaga, RS, obtained from samples taken in (Jan (112.2 mm), April (338.1 mm), July (71.3 mm) and October (342.0 mm)) in 2005, has not identified the presence of (E) β - Ocimene in January and July, months of lower volume of rainfall, being found only in the months of April and October months of higher precipitation volumes.[32] This phenomenon is opposite to that found in essential oils from the leaves of S. cumini, wherein the months of higher precipitation volumes these substances were not detected.[33] Being isomers, (Z) β - Ocimene and (E) β - Ocimene presented the same variations during the study period. Values found in the literature for the essential oil from the leaves of S. cumini were 26.85% for (Z) β - Ocimene and 11.13% for (E) β - Ocimene, 26% to 9.00 (Z) β - Ocimene and 9.50% to (E) β - Ocimene.[29] The values found in this study in October / 2013 are lower than 23.95% (Z) β - Ocimene and 8.1% (E) β - Ocimene in relation to the essential oil of leaves of S. cumini collected in the Botanical Garden of Medicinal Plants of the Regional University of Cariri (URCA) in the state of Ceará, but are higher than values found in the essential oil from the leaves of S. cumini collected in September 1981 trees Pici Campus of the Federal University of Ceará.[29] This work the highest values of these substances were found in October / 2013.

![Figure 1](image-url) Check up the structures of the major compounds of the essential oil obtained from the leaves of S. cumini.

Antioxidant activity of essential oil

It was conducted to test the antioxidant activity (AA) of the essential oil of S. cumini with samples from different periods of the year. The results can be seen in Table 3.
Table 3. Essential oil AA *S. cumini*, n=3

<table>
<thead>
<tr>
<th>Date of Collection</th>
<th>% inhibition DPPH</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>27/08/2013</td>
<td>39.56</td>
<td>± 1.15</td>
</tr>
<tr>
<td>17/10/2013</td>
<td>39.67</td>
<td>± 0.56</td>
</tr>
<tr>
<td>11/12/2013</td>
<td>39.06</td>
<td>± 0.59</td>
</tr>
</tbody>
</table>

The results of DPPH inhibition in essential oil samples during the study period remained constant even with the composition of substances ranging majority in the study period (Table 2). The results obtained are less than 50%, which shows low activity inhibition facing the DPPH radical. Literature\(^{[34]}\) results show essential oil antioxidant activity *Tetraclinis articulata* give IC\(_{50}\) values of 88.44 ± 3.27 to 119.44 ± 25.05 mg / ml in different regions of samples. Due to the presence of Thymol and Carvacrol in the composition isomers essential oil which have a hydroxyl group that undergoes oxidation. These substances were not detected in the essential oil of *S. cumini*. The essential oil of *Pinus nigra* ssp Arnould *dalmatica* has a low antioxidant activity due to the dominant presence of absence of monoterpene and phenolic groups which are responsible for the great effect of DPPH sequestration\(^{[35]}\) as is the case with essential oil *S. cumini*.

CONCLUSION

It was found that the period of collection of fresh leaves of *S. cumini* influence on essential oil yield. which results in increased income primarily in inflorescence period and fruit production. The essential oil chemical composition of fresh leaves of *S. cumini* suffers variations in% values in the area of major compounds depending on the time in which they are collected, and the α-Pinene the major substance in almost all the analyzed periods. The essential oil of *S. cumini* showed low antioxidant activity inhibiting 39.43% of DPPH radical.

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