Chemical and Biological Investigations of *Pilocarpus spicatus* essential oils

[Estudios químicos y biológicos del aceite esencial de *Pilocarpus spicatus*]

Adriana P. OLIVEIRA¹*, Rodrigo A. S. CRUZ¹, Gisele S. BOTAS¹, Marcelo S. GONZALEZ², Marcelo G. SANTOS², Lenise A. TEIXEIRA², Leandro M. ROCHA¹.

1. Laboratório de Tecnologia de Produtos Naturais, Faculdade de Farmácia, Universidade Federal Fluminense, Rua Mário Viana 523, Santa Rosa, CEP 24241-000, Niterói, RJ, Brazil. 2. Laboratório de Controle Microbiológico, Faculdade de Farmácia, Universidade Federal Fluminense, Rua Mário Viana 523, CEP 24241-000, Niterói, RJ, Brazil. 3. Departamento de Biologia Geral, Universidade Federal Fluminense, Morro do Valonguinho SN, Centro, CEP 24020-140, Niterói, RJ, Brazil. 4. Departamento de Ciências, Faculdade de Formação de Professores, Universidade Estadual do Rio de Janeiro, Dr. Francisco Portela 794, CEP 24435-000, São Gonçalo, RJ, Brazil.

Abstract

Essential oil was obtained by steam distillation of aerial parts of *Pilocarpus spicatus* Saint-Hilaire (Rutaceae) from the northern coast of Rio de Janeiro State and examined by GC–MS. A total of 17 components were identified accounting for 96.06% of the oil composition. The major components were limonene (41.87%), 2-undecanone (11.0%) and sabine (10.78%). *P. spicatus* essential oil had inhibitory effects on the growth of bacteria (*Escherichia coli* and *Staphylococcus aureus*) and showed anticholinesterase activity in TLC assay. In addition, the volatile oil was toxic to larvae of the brine shrimp.

Keywords: *Pilocarpus spicatus*; antibacterial activity; anticholinesterase activity; cytotoxic activity; essential oil

Resumen

El aceite esencial fue extraído por arraste de vapor de las partes aéreas de *Pilocarpus spicatus* Saint-Hilaire (Rutaceae) de la costa Norte del estado de Rio de Janeiro y examinado por GC-MS. Fueron identificados 17 componentes que corresponden al 96,06% de la composición química del aceite. Los componentes mayoritarios fueron el limoneno (41,87%), 2-undecanona (11,0%) y sabineno (10,78%). El aceite esencial de *P. spicatus* tiene efecto inhibitorio sobre el crecimiento bacteriano (*Escherichia coli* y *Staphylococcus aureus*) y presentando también actividad anticolinesterasa en ensayo de TLC. Adicionalmente el aceite volátil ha demostrado ser tóxico para las larvas de *Artemia salina*.

Palabras Clave: *Pilocarpus spicatus*; actividad antibacteriana; actividad anticolinesterasa; actividad citotóxica; aceite esencial

List of abbreviations: CG–MS - Gas chromatography-mass spectrometry, TLC – thin layer chromatography

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*Contactos | Contacts: passosoliv@hotmail.com

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INTRODUCTION

Pilocarpus spicatus Saint-Hilaire (Rutaceae) is one of the various Pilocarpus spp. called ‘jaborandi’. Nowadays, the great importance of Pilocarpus spp. is due to the extraction of the alkaloid pilocarpine from the leaves (Pinheiro, 2002). The Rutaceae has secretory cavities lysigenous and schizo-lysigenous containing essential oil (Simões and Spitzer, 1999). The leaves of Pilocarpus microphyllus Stapf ex Wardleworth and Pilocarpus pennatifolius Lem produce 0.25-0.50% of essential oil (Lorenzi and Mattos, 2002).

In this way, a lot of essential oils from different species of the Pilocarpus genus have been analyzed and chemical substances as terpenoids, alcohols, aldehydes, hydrocarbons aliphatic and aliphatic ketones have been described (Craveiro et al., 1979; Santos et al., 1997; Andrade-Neto et al., 2000, 2002; Santos et al., 2004). In addition, chalepin is a coumarin extracted from P. spicatus essential oil. Experiment in vitro showed that binding of chalepin to glycosomal glyceraldehyde-3-phosphate dehydrogenase of T. cruzi, a protozoan exclusively transmitted by hematophagous triatomines (Rhodnius prolixus), disrupted flagellate development (Mafezoli et al., 2000; Pavão et al., 2002). Moreover, we observed a variety of effects of P. spicatus essential oil on Rhodnius prolixus -a vector of Chagas disease-which indicates their secondary metabolites, nowadays under investigation in our laboratory, as good candidates for the study of insect physiology, vector control population and perhaps, blockage of protozoan development in triatomine hosts (Melo et al., 2007).

It has well established that P. spicatus essential oil collected in the state of Ceará (Brasil) displays in vitro antibacterial activity against Pseudomonas aeruginosa and Staphylococcus aureus (Santos et al., 1997). However, the chemical composition of P. spicatus essential oil showed qualitative and quantitative variation by the influence of local environmental conditions of soil and seasonal period of collections (Taveira et al., 2003).

A variety of plants has been reported to show acetylcholinesterase (AChE) inhibitory activity and so may be relevant to the treatment of neurodegenerative disorders such as Alzheimer’s disease (AD). (Mukherjee et al., 2007). Numerous essential oils and their monoterpene constituents have been investigated for their effects on AChE, and have shown inhibitory activity. Although, several extracts of plants and essential oils already have been investigated, this is the first account in the Pilocarpus genus.

The brine shrimp lethality test (BST) is a simple and efficient method used to predict compounds or extracts as cytotoxic agents and that may have anticancer activity (Meyer et al., 1982). Although several essential oils have been tested against brine shrimp, P. spicatus essential oil cytotoxicity has not been investigated yet. In this work, studies were carried out to elucidate the chemical composition and analyses antibacterial, anticholinesterase and cytotoxicity activities in vitro of P.spicatus essential oil from Rio de Janeiro State (Brazil).

MATERIALS AND METHODS

Plant material

Aerial parts of P. spicatus Saint-Hilaire (Rutaceae) was collected in Sandy Coastal Plains (Restinga de Jurubatiba National Park) located on the northern coast of Rio de Janeiro State, Brazil (October 2004) and was identified by Dr. Marcelo Guerra Santos. The dried specimens were deposited in the herbarium of the Faculdade de Formação de Professores, UERJ (M. Guerra Santos 1.824) and of the Museu Nacional, UFRJ (M. Guerra Santos 1.406).

Extraction of the essential oil

The essential oil was obtained by steam distillation (1.37 kg of fresh plant) during 4 h in a Cleavenger-type apparatus (yield 0.42% v/w), and stored at 4°C until tested and analyzed.

Gas chromatography/mass spectrometry analysis

Essential oil was analyzed by a GCMS-QP5000 (SHIMADZU) gas chromatograph equipped with a mass spectrometer using electron ionization. The gas-chromatographic (GC) conditions were as follows: injector temperature, 260°C; FID temperature, 280°C; carrier gas (Helium), flow rate 1 mL/min and split injection with split ratio 1:40. Oven temperature was initially 60°C and then raised to 240°C at a rate of 3°C/min. One microliter of each sample, dissolved in CH2Cl2 (1:100 mg/μL), was injected at ZB5MS column (i.d. = 0.25 mm, length 30 m, film thickness = 0.25 mm) column was used. The mass spectrometry (MS) conditions were ionization
The chemical composition of *P. spicatus* essential oil was analysed by GC/MS and 17 components were identified, as shown in Table 1. The major components were limonene (41.87%), 2-undecanone (11.0%) and sabinene (10.78%).
Table 1. Chemical composition of *Pilocarpus spicatus* essential oil

<table>
<thead>
<tr>
<th>Compounds a</th>
<th>RI(min.) b</th>
<th>KI c</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 α-Pinene</td>
<td>6.613</td>
<td>935</td>
<td>5.08</td>
</tr>
<tr>
<td>2 Sabinene</td>
<td>7.983</td>
<td>974</td>
<td>10.78</td>
</tr>
<tr>
<td>3 β-Pinene</td>
<td>8.162</td>
<td>979</td>
<td>1.43</td>
</tr>
<tr>
<td>4 β-Myrcene</td>
<td>8.570</td>
<td>989</td>
<td>4.95</td>
</tr>
<tr>
<td>5 α-Terpinene</td>
<td>9.679</td>
<td>1017</td>
<td>2.04</td>
</tr>
<tr>
<td>6 Limonene</td>
<td>10.275</td>
<td>1031</td>
<td>41.87</td>
</tr>
<tr>
<td>7 (Z)-β-Ocimene</td>
<td>10.472</td>
<td>1037</td>
<td>1.05</td>
</tr>
<tr>
<td>8 (E)-β-Ocimene</td>
<td>10.915</td>
<td>1047</td>
<td>0.97</td>
</tr>
<tr>
<td>9 γ-Terpinene</td>
<td>11.432</td>
<td>1059</td>
<td>2.70</td>
</tr>
<tr>
<td>10 Terpinolene</td>
<td>12.626</td>
<td>1083</td>
<td>1.84</td>
</tr>
<tr>
<td>11 Terpen-4-ol</td>
<td>17.086</td>
<td>1180</td>
<td>7.90</td>
</tr>
<tr>
<td>12 Undecanone-2</td>
<td>22.384</td>
<td>1292</td>
<td>11.00</td>
</tr>
<tr>
<td>13 Germacrene D</td>
<td>30.650</td>
<td>1477</td>
<td>1.34</td>
</tr>
<tr>
<td>14 Viridiflorene</td>
<td>31.261</td>
<td>1491</td>
<td>1.13</td>
</tr>
<tr>
<td>15 γ-Cadinene</td>
<td>32.230</td>
<td>1514</td>
<td>0.67</td>
</tr>
<tr>
<td>16 δ-Cadinene</td>
<td>32.412</td>
<td>1519</td>
<td>0.60</td>
</tr>
<tr>
<td>17 Elemol</td>
<td>33.493</td>
<td>1545</td>
<td>0.71</td>
</tr>
<tr>
<td>Total</td>
<td>96.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Compounds listed in order of elution from a ZB-5 MS column.
b Retention time (minutes).
c Kovats Index on ZB-5 MS column in reference to n-alkanes (Adams, 1995).

The *in vitro* antibacterial activity of *P. spicatus* essential oil in comparison with the reference standard included in the study, are shown in Table 2. All the tested strains were sensitive to the essential oil. The differences in inhibition zones diameters of *S. aureus* (CV = 5.0%) and *E. coli* (CV = 3.9%) were not statistically significant.

Table 2. Antibacterial activity of *Pilocarpus spicatus* essential oil

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Essential oil</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DD a</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>ATCC25923</td>
<td>12</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>ATCC36298</td>
<td>14</td>
</tr>
</tbody>
</table>

a Zone of inhibition (mm).
b Vancomycin 30 (μg/disc), c Ampicillin 10 (μg/disc).

In the chromatographic assay for acetylcholinesterase inhibitors, two white spots were seen, one on the base (false positive) and other on the middle of the plate (a true inhibitory result).

*P. spicatus* essential oil showed toxicity against brine shrimp nauplii (*Artemia salina L.*) with a lethal concentration 50% (the concentration of test compound that kills 50% of *A. salina*) value of 3.98 μg/mL. Positive control test was done using sodium lauryl sulfate whose LC50 is approximately 22 μg/mL and the blank test (50 μL DMSO) no lethality of brine shrimp was observed.

DISCUSSION

*P. spicatus* essential oil from Rio de Janeiro State presents monoterpenoids, sesquiterpenoids and aliphatic ketones. Regarding the previously reported chemical composition of many essential oils of *Pilocarpus* genus has been described (Craveiro et al., 1979; Santos et al., 1997; Andrade-Neto et al., 2000, 2002). However, here we note that (Z)-β-ocimene and viridiflorene were detected for first time in *P. spicatus*. About this, it is necessary to point out that environmental factors strongly influence the chemical composition of essential oil (Kaastra, 1982).

*P. spicatus* essential oil collected in the state of Ceará (Brasil) displays *in vitro* antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* but no activity against *E. coli* was reported (Santos et al., 1997). It is not clear whether the antibacterial effect may be caused mainly by a single active constituent or by the combined action of the many active constituents found in essential oil. Terpenoids are active against bacteria but the mechanism of action of terpenes is not fully understood although is speculated to involve membrane disruption by lipophilic compounds (Cowan, 1999). It has been reported by Sacchetti et al. (2005) that essential oil with high terpenoids percentages was probably more effective, as a consequence of higher specificity of the assay for lipophilic compounds.

Several monoterpenes and other terpenoids are known to inhibit AChE (Houghton et al, 2006). The principal monoterpene identified in *P. spicatus* essential oil is limonene (41.87%), which is known to be an inhibitor of AChE (Miyazawa et al, 1997). In this oil, there are others monoterpenes that have anticolinesterase activity as α-Pinene, β-Pinene (Miyazawa and Yamafuji, 2005), α-Terpine...
(Miyazawa et al., 1997), γ-Terpinene (Perry et al., 2002) and sesquiterpene alcohol elemol (Miyazawa et al., 1997).

_P. spicatus_ essential oil showed LC$_{50}$ = 3.98 µg/mL that is a value less than 1000 µL/mL, which suggests good cytotoxic activity potential and indicating that they may possess a significant anti-tumor activity since McLaughlin et al. (1998). The cytotoxic activity showed the _P. spicatus_ essential oil should be more studied as regards a possible antitumoral activity.

**CONCLUSION**

The present study is the first report of (Z)-β-ocimene and viridiflorene for _P. spicatus_ essential oil. In addition, showed antibacterial activity against Gram-negative ( _E. coli_ ) and Gram-positive ( _S. aureus_ ), anticholinesterase activity and cytotoxic activity in the BST. So, _P. spicatus_ essential oil might represent a valuable source for pharmaceutical applications.

**REFERENCES**


Kaastra RC. 1982. _A monograph of the Pilocarpine (Rutaceae). Flora Neotropica_ 33:198


