

## Artigo

**Chemical Analysis of Essential Oil and Hydrolates of Leaves, Inflorescences and Stems of *Piper chimonanthifolium* Kunth**

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<http://rvq.sbq.org.br>**Análise Química de Óleos Essenciais e Hidrolatos de Folhas, Inflorescências e Caules de *Piper chimonanthifolium* Kunth**

**Resumo:** Plantas do gênero *Piper* são frequentemente utilizadas comercialmente. A espécie *Piper chimonanthifolium* Kunth. apresenta poucos estudos em literatura quanto a sua composição química e atividades biológicas. Óleos essenciais de outras espécies deste gênero apresentam boas atividades biológicas, principalmente contra microrganismos. Este trabalho apresenta uma comparação da composição química de óleos essenciais e hidrolatos de folhas, inflorescências e caules desta espécie. As amostras foram obtidas por co-destilação e analisadas por cromatografia com fase gasosa acoplada à espectrometria de massas (CG-EM). As amostras mostraram presença de monoterpenos e sesquiterpenos, com predominância de monoterpenos. A piperitona, um monoterpeno com atividades biológicas anteriormente relatadas na literatura, foi a substância com maior destaque. Além desta substância, destacaram-se: *L*-linalol, *cis*- $\beta$ -ocimeno, *trans*- $\beta$ -cimeno, 1-felandreno,  $\alpha$ -pineno, limoneno e espatulenol. Algumas dessas substâncias podem exibir variação na composição de acordo com fatores ambientais e podem ser agentes de controle biológico, demonstrando importância ecológica. As análises por CG-EM evidenciaram diferenças na composição química entre folhas, inflorescências e caules, além de demonstrarem potencial biológico para esta espécie.

**Palavras-chave:** Atividade biológica; Óleo essencial; Monoterpeno; Piperaceae; Piperitona.

**Abstract**

Plants of the genus *Piper* are widely used commercially. *Piper chimonanthifolium* Kunth. is a species with few studies on chemistry and biological activities. Essential oils of other species of this genus show good biological activities, especially against microorganisms. This work presents a comparative analysis of chemical composition of essential oils and hydrolates of leaves, inflorescences and stems of this specie. Samples were obtained by co-distillation and analyzed by gas chromatography-mass spectrometry (GC-MS). The samples were composed of monoterpenes and sesquiterpenes, with predominance of monoterpenes. Piperitone, a monoterpene with important biological activities, was the most important substance and in addition to this compound, *L*-linalool, *cis*- $\beta$ -ocimene, *trans*- $\beta$ -ocimene, 1-phellandrene,  $\alpha$ -pinene, limonene, and spathulenol stood out. Some of these substances can exhibit a variation in the composition according to environmental factors or may be biological control agents, demonstrating ecological importance. The chemical characterization showed chemical differences between leaves, stems and inflorescences and demonstrated biological potential for this species.

**Keywords:** Biological activities; Essential oil; Monoterpenes; Piperaceae; Piperitone.

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## Chemical Analysis of Essential Oil and Hydrolates of Leaves, Inflorescences and Stems of *Piper chimonanthifolium* Kunth

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## 1. Introduction

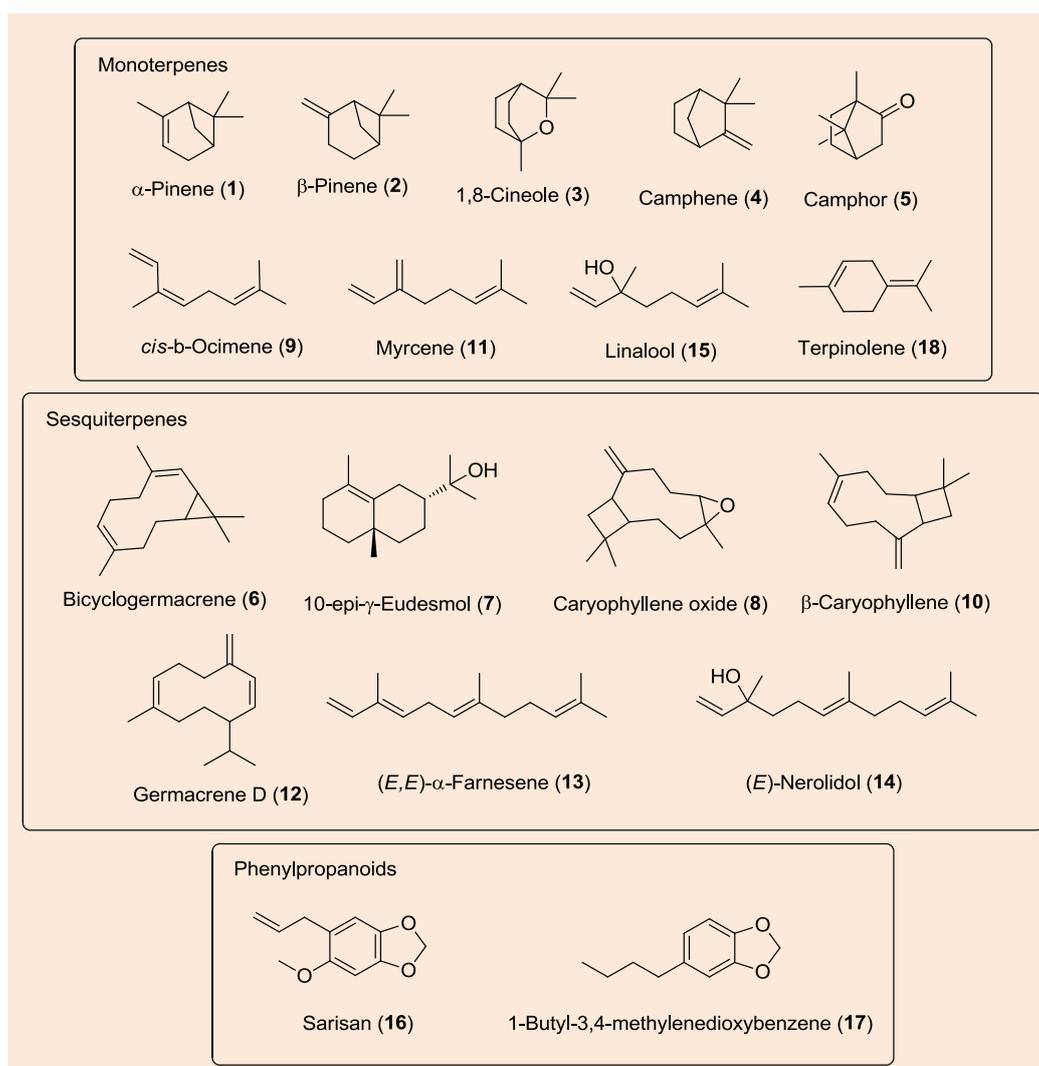
The Piperaceae family is distributed in tropical and subtropical regions of both hemispheres, and includes about 4,000 species and the genus *Piper* L. has the highest number of species.<sup>1</sup> This genus is representative in commercial use, with the Indian specie *Piper nigrum* L. being the most widespread in the world for producing black

pepper.<sup>2</sup> Species of this genus are often used not only in human diets, but also for other purposes, such as a perfumery fixer. They have several uses in folk medicine because their substances have many biological and pharmacological activities.<sup>3</sup> *Piper aduncum* L., known as “fake jaborandi”, is reported in folk medicine as diuretic, wound healing and anti-hemorrhagic,<sup>4</sup> *Piper regnellii* (Micah) C. DC has antifungal activity<sup>5</sup> and *Piper hispidinervum* C. DC. is used in the manufacture of fixative for fragrances and as

natural insecticides.<sup>6</sup>

Studies of essential oils of *Piper* are very important, since this genus is characterized by aromatic species and present a great variety of substances, among them terpenoids and arylpropanoids. The essential oil from a sample of *P. aduncum*, a species used in folk medicine for the treatment of respiratory and inflammatory diseases, presented predominance of monoterpenes (1-3).<sup>7</sup> *Piper malacophyllum* (C. Presl) C. DC. (4, 5), *Piper arboreum* Aubl. (6-8), *Piper dilatatum* Rich. (9, 10) and *Piper hispidum* Kunth (2). presented monoterpenes and sesquiterpenes in their essential oils.<sup>8</sup> These were also the main substances found in study about essential oil of 10 Piperaceae species of the Brazilian Atlantic Forest (1, 7, 10-14).<sup>9</sup>

Several studies in toxicity against bacterial, fungal and protozoal organisms have been done with species of this genus.<sup>10</sup> *P. malacophyllum*, *P. aduncum* (15) and *Piper tuberculatum* Jacq. exhibited high antifungal action, whereas the former species have antiparasitic and good antibacterial activities.<sup>8b, 11</sup> A study of the essential oil from *Piper solmsianum* C.DC. and its major compound, sarisan (16), in mice behavior showed exciting and depressant effects in tested animals.<sup>12</sup> The essential oil of *Piper corcovadensis* (Miq.) C.DC. (17, 18) exhibited a strong oviposition deterrent activity in *Aedes aegypti*.<sup>13</sup> The structural formula of the major substances in the essential oils of this species are in Figure 1.



**Figure 1.** Structural formula of the major substances found in essential oils of *Piper* spp

Besides the characterization of essential oils, hydrolates obtained in the extraction may also be pharmacologically tested, so their characterization is also relevant.<sup>14</sup>

*Piper chimonanthifolium* Kunth. is a shrub, native from Brazil,<sup>1</sup> and there is a dearth of studies about its biological activities and chemistry. The aim of this study is the chemical characterization of essential oils and hydrolates of leaves, inflorescences and stems of *P. chimonanthifolium* due to the biological relevance of the essential oils of the genus *Piper*.

## 2. Materials and methods

### 2.1. Plant material

The plant material was collected on the campus of Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais state, Brazil. The plant material was dried in a forced air circulation oven with temperature of 45°C until constant weight. The specie was identified by MSc. Danielle Monteiro, from Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. The voucher is found at the Herbarium CESJ, under the register number 57540.

### 2.2. Essential oil extraction and obtaining of hydrolates

An aliquot of 370 g of leaves was added to a 12 L flask with 4 L of distilled water and was subjected to co-distillation using modified Clevenger for 1h and 30 min after boiling. Similarly, aliquots of 130 g of the inflorescences and stems were added to a 2 L flask in 1.5 L of distilled water and were extracted by 1h and 30 min. Therefore, six samples were obtained, leaves, inflorescences and stems essential oils and

their hydrolates, which were stored at -18°C for later analysis.

### 2.3. Gas chromatography-mass spectrometry

Samples were analyzed in the Plataforma Analítica of Farmanguinhos (FIOCRUZ – Rio de Janeiro state, Brazil), using a Hewlett-Packard 6890 gas chromatograph equipped with a fused silica capillary column (HP-5, 30 m × 0.25 mm, 0.25 µm film thickness), helium as carrier gas with a flow rate 1.0 mL/min; temperature programming from 70°C to 290°C (2°C/min), coupled to a Hewlett-Packard 5972 mass spectrometer. The MS operating parameters were: 70 eV, ion source 250 °C equipped with EI.

### 2.4. Identification of the oil and hydrolate substances

The compound identifications were carried out by comparison of their retention index (RI) with literature values; and the MS data with those from Wiley 275.1 mass spectral data base besides literature records.<sup>15</sup>

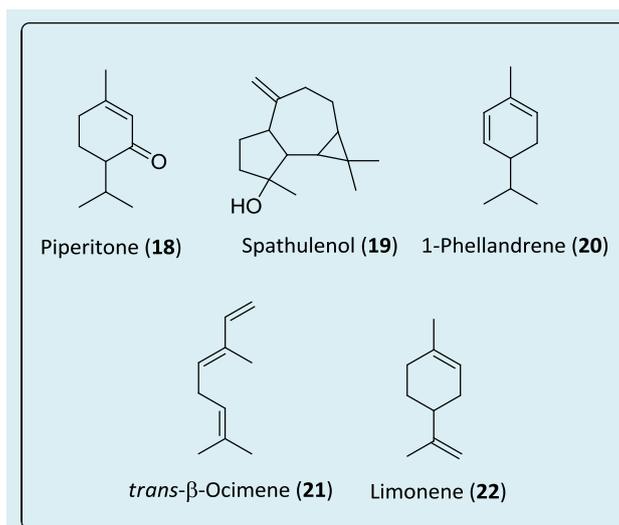
The RI was calculated using a GC data of a homologous series of saturated aliphatic hydrocarbons within C8 to C30, performed at the same column and conditions as used in the GC-MS analysis for the essential oils and hydrolates.

## 3. Results

In the analysis of essential oils, 29 substances were identified in leaves (13 monoterpenes and 16 sesquiterpenes) and in inflorescences (14 monoterpenes and 15 sesquiterpenes) and 22 in stem (12

monoterpenes and 10 sesquiterpenes), representing 88.96% (65.82% monoterpenes and 23.14% sesquiterpenes), 94.02% (86.78% monoterpenes and 7.24% sesquiterpenes) and 98.36% (73.57% monoterpenes and 24.79% sesquiterpenes) of the total chromatogram area, respectively. In the hydrolates, the three substances of leaves and the single substance of stems were

identified, totaling 100.00% of the total chromatogram area, as in inflorescences three substances were identified, representing 96.88% of the total area. All substances identified in hydrolates are monoterpenes. The structural formula of the major substances are described in Figure 2 and substances found in the samples are described in Table 1.



**Figure 2.** Structural formula of the major substances found in the samples of essential oils and hydrolates of *Piper chimonanthifolium* Kunth

**Table 1.** Substances found in essential oils and hydrolates of leaves, inflorescences and stems of *Piper chimonanthifolium* Kunth.

Substances	RT	LOA	LHA	IOA	IHA	SOA	SHA	RI	CRI
Monoterpenes	-	65.82	100.00	86.78	96.88	73.57	100.00	-	-
$\alpha$ -Pinene	7.77	4.10	-	0.90	-	6.80	-	939	934.1
$\beta$ -Pinene	8.99	0.80	-	0.40	-	4.60	-	980	980.3
$\beta$ -Myrcene	9.18	1.31	-	0.75	-	1.33	-	991	987.5
1-Phellandrene	9.74	4.57	-	1.30	-	3.76	-	1005	1008.0
$\alpha$ -Terpinene	10.00	-	-	1.76	-	-	-	1018	1017.1
<i>p</i> -Cimene	10.26	-	-	4.94	-	2.10	-	1026	1026.1
Limonene	10.39	-	-	-	-	7.17	-	1031	1030.7
<i>cis</i> - $\beta$ -Ocimene	10.48	15.70	-	7.70	-	7.20	-	1040	1033.8
<i>trans</i> - $\beta$ -Ocimene	10.80	0.89	-	7.71	-	4.20	-	1050	1044.9
$\gamma$ -Terpinene	11.22	0.70	-	3.40	-	0.90	-	1062	1059.6

Linalool oxide	11.50	0.40	10.30	0.60	11.37	-	-	1074	1069.3
$\alpha$ -Terpinollene	11.84	0.17	-	0.95	-	-	-	1088	1081.2
L-Linalool	12.37	0.20	-	31.14	25.44	2.90	-	1098	1099.7
<i>cis</i> -Menthenol	13.18	0.57	3.10	-	-	0.91	-	1121	1127.6
<i>trans</i> -Menthenol	13.50	1.21	-	1.73	-	-	-	1140	1138.6
Piperitone	16.97	35.20	86.60	23.50	60.07	31.70	100.00	1252	1260.4
Sesquiterpenes	-	23.14	-	7.24	-	24.79	-	-	-
$\alpha$ -Copaene	20.20	0.57	-	0.23	-	-	-	1376	1379.4
<i>trans</i> -Caryophyllene	21.43	2.09	-	0.73	-	1.62	-	1418	1426.9
$\alpha$ -Humullene	22.34	2.91	-	0.78	-	2.30	-	1454	1462.8
$\gamma$ -Muurolleene	22.72	0.68	-	0.21	-	-	-	1477	1477.9
<i>D</i> -Germacrene	22.94	0.98	-	0.45	-	-	-	1480	1486.6
$\beta$ -Selinene	23.15	1.78	-	0.17	-	-	-	1485	1494.9
$\delta$ -Cadinene	23.81	2.10	-	0.31	-	1.22	-	1524	1522.1
Cadina-1,4-diene	24.01	0.17	-	-	-	-	-	1532	1530.4
Calacorene	24.23	0.30	-	-	-	-	-	1542	1539.6
Nerolidol	24.75	0.80	-	2.00	-	1.70	-	1564	1561.2
Spathulenol	25.30	6.60	-	0.30	-	3.30	-	1576	1584.2
Caryophyllene oxide	25.47	-	-	0.47	-	4.85	-	1581	1591.2
Humulene epoxide II	26.11	1.60	-	0.68	-	5.02	-	1606	1618.9
Cubenol-1,10-di-epi	26.44	0.41	-	0.26	-	2.17	-	1614	1633.5
Cubenol-1-epi	26.62	0.48	-	0.16	-	1.15	-	1627	1641.4
t-Cadinol	26.76	1.07	-	0.23	-	-	-	1640	1647.6
$\alpha$ -Cadinol	27.09	0.60	-	0.26	-	1.46	-	1653	1662.1
Total	-	88.96	100.00	94.02	96.88	98.36	100.00	-	-

RT = retention time, LOA = leaf oil area (%), LHA = leaf hydrolate area (%), IOA = inflorescence oil area (%), IHA = inflorescence hydrolate area (%), SOA = stem oil area (%), SHA = stem hydrolate area (%), RI = retention index, CRI = calculated retention index.

#### 4. Discussion

Piperitone (**18**) were found in all samples. This compound, a biologically active monoterpene, has insecticidal activity<sup>16</sup> and increases the nitrofurantoin activity against *Enterobacteria*.<sup>17</sup> This monoterpene is the

major compound in all hydrolates and in the essential oils of stem and leaf and the second most abundant substance in the essential oil of inflorescence. The piperitone was not observed in a study of 10 Piperaceae species from the Atlantic Forest.<sup>9</sup>

In addition to **18**, the other prominent substances are *cis*- $\beta$ -ocimene (**9**), spathulenol

(**19**), 1-phellandrene (**20**) and  $\alpha$ -pinene (**1**) in leaf essential oil, L-linalool (**15**), **9** and *trans*- $\beta$ -ocimene (**21**) in the essential oil of inflorescence, **9**, limonene (**22**) and **1** in the essential oil of stem (Table 1). These substances were also found in the study of Santos and coworkers.<sup>9</sup>

*trans*- $\beta$ -Ocimene (**21**) is present in great amounts in inflorescence and stem oils. In a study about the variation of the chemical substances of essential oil of *Houttuynia cordata* Thunb., another Piperales, this monoterpene had significant positive correlation with the altitude.<sup>18</sup>

*cis*- $\beta$ -Ocimene (**9**) was found in the three essential oils and was the second most abundant compound in the oil of leaf. This compound was found as major substance of *P. dilatatum* oil in a study of three *Piper* species collected in the region of Distrito Federal, Brazil.<sup>8a</sup> In the same study, spathulenol (**19**) was found in essential oil of three *Piper* species (*P. arboreum*, *P. dilatatum* and *P. hispidum*) and this sesquiterpene can be used as a food flavoring and in sophisticated perfumes.<sup>19</sup> This substance was also found in a study with six species of *Achillea* L., which reported high level of **19** with soil development, shown by the accumulation of CaCO<sub>3</sub>.<sup>20</sup> The substance **19** was described as the major compound of essential oil from *Scutia buxifolia* Reissek and showed interesting antioxidant activity and moderate antimicrobial activity.<sup>21</sup>

$\alpha$ -Pinene (**1**) is a monoterpene found also in *P. hispidum* essential oil<sup>8a</sup> and other Piperaceae species<sup>9</sup> and was described as insecticidal repellent<sup>22</sup> and cytotoxic selectively against tumor cells.<sup>23</sup> The linalool (**15**) was the major substance of inflorescences essential oil and was found in high concentration in inflorescence hydrolate but was absent or in low concentrations in the other samples, so may be thought of as a chemical marker for quality control of inflorescences. This compound shows the importance of chemical composition studies of different plant organs. This monoterpene has commercial importance in perfumery and cosmetics<sup>24</sup> and showed many biological

activities such as insecticide, antimicrobial,<sup>25</sup> anticonvulsive,<sup>26</sup> sedative,<sup>27</sup> neuroprotective,<sup>28</sup> Alzheimer's disease treatment<sup>29</sup> and protection against cigarette smoke-induced acute lung inflammation.<sup>30</sup> A study about the action of some essential oils against mosquitoes and ticks showed **1** and **15** as some of the major substances of essential oil of leaves of *Juniperus communis* L., and this oil presented activity against two species of tick and mosquito *Aedes aegypti* (L.).<sup>31</sup>

Limonene (**22**) is a monoterpene found only in the essential oil of stem that plays a modulatory role in the induction of the brown adipocyte-like phenotype as well as promotion of lipid metabolism.<sup>32</sup> This compound can be a kairomone, increasing the parasitism of *Dastarcus helophoroides* in Asian longhorned beetle, acting as an important biological control agent.<sup>33</sup>

## 5. Conclusion

In the analysis of essential oils of leaves, inflorescences and stems of *P. chimonanthifolium* monoterpenes and sesquiterpenes were found, with predominance of monoterpenes, and, in the hydrolates, only monoterpenes were found. Chemical differences were found between the analyzed parts of the plant and chemical characterization suggests pharmacological and cosmetic potential.

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