Antifungal piperamides from *Piper mollicomum* Kunth (Piperaceae)

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**ABSTRACT:** The phytochemical study on dichloromethane extracts of leaves, stem and roots of *Piper mollicomum* Kunth (Piperaceae) led to isolation of the known piperamides tembamide (1), (R)-(−)-tembamide acetate (2) and riparin I (3). Compounds 1 and 2 displayed moderate in vitro antifungal activity against *Cladosporium cladosporioides* (5.0 μg) and *Cladosporium sphaerospermum* (1.0 μg) by direct bioautographic analyses and compound 3 was inactive up to 100.0 μg.

**1. Introduction**

Piperaceae family belongs to the Piperales order and it is one of the most primitive families among Angiosperms. It is predominantly a tropical family, comprising the genera *Piper*, *Peperomia*, *Sarchorrhachis* and *Ottonia*, from which *Piper* and *Peperomia* are the most representative ones with approximately 2000 and 1700 species, respectively¹,². Piperaceae species have long been used as food additive and folk medicine agents mainly due to their antimicrobial properties, including potent fungicidal action³.

*Piper* species usually possess strong and pleasant aroma and spicy flavor, which explain their use as condiments, flavorings and medicinal materials. *Piper* compounds are often grouped into seven general classes, including amides, lignans, neolignans, flavonoids, kavalactones, butenolides, and volatile oils⁴,⁵. Amides are one of the most characteristic constituents of *Piper* species⁶. The biological activities presented by *Piper’s* amides (also known as “piperamides”) have inspired...
synthetic studies towards the preparation of analogs of these substances, in order to evaluate their potential for commercial and medical use\(^7\). As result, many of these analogs revealed promising antifungal and insecticidal properties\(^8-10\).

Known as “jaborandi-manso” or simply “jaborandi”, the species *Piper mollicomum* Kunth is a small shrub of 1.0 to 1.5 m in height, found in Brazil in the states of São Paulo, Minas Gerais, Rio de Janeiro, Espírito Santo, Bahia, Santa Catarina, Ceará, Paraíba, Pernambuco, Mato Grosso and Goiás. The fruits of *P. mollicomum* Kunth are popularly used to treat stomach problems, and its roots, when chewed, are useful for anesthetizing toothaches\(^11\). Nevertheless, few studies with *P. mollicomum* Kunth have been performed so far. From *P. mollicomum* methanolic extracts, the chromenes methyl 2,2-dimethyl-2H-chromene-6-carboxylate (4) and methyl 8-hydroxy-2,2-dimethyl-2H-chromene-6-carboxylate (5), along with the dihydrochalcone 2',6'-dihydroxy-4'-methoxychalcone (6), were isolated and displayed antifungal properties against the fungi *Cladosporium cladosporioides* and *Cladosporium sphaerospermum*\(^12\).

The present study aimed to further investigate the chemical composition of *P. mollicomum* Kunth dichlorometane extracts. The compounds tembamide (1), (R)-(−)-tembamide acetate (2) and riparin I (3) were isolated and characterized and compounds 1 and 2 were found to display in vitro antifungal activity against *Cladosporium cladosporioides* and *C. sphaerospermum*.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Tembamide (1), (R)-(−)-tembamide acetate (2), riparin I (3), methyl 2,2-dimethyl-2H-chromene-6-carboxylate (4), methyl 8-hydroxy-2,2-dimethyl-2H-chromene-6-carboxylate (5) and 2',6'-dihydroxy-4'-methoxychalcone (6) isolated from *Piper mollicomum* Kunth.

2. Experimental

2.1 General procedures

Optical rotation was measured on a Perkin-Elmer 241 polarimeter. \(^1\)H and \(^1\)C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker DRX. CDCl\(_3\) (Aldrich) was used as solvent and TMS as internal standard. Chemical shifts were reported in \(\delta\) units (ppm) and coupling constants (\(J\)) in Hz. MS spectra were obtained on a GGEM SHIMADZU mass spectrometer (70 eV) apparatus GCMS-QP5050A, equipped with BPX5 capillary column (30m x 0.25mm ID). Silica gel
The chromatograms were then fractionated by similar procedure. When crude extracts from stems (1.31 g) were subjected to silica gel column chromatography (Si-CC) in Ilhéus, Bahia, under the code “PIPER-007”, and identified as *Piper mollicomum* Kunth by Dr. André Márcio Araújo Amorim (Bahia State University of Santa Cruz – UESC). The collected material was dried in an oven (50 °C) for 48 hours, and their separated parts (stem, leaf and root) were pulverized in a knife mill.

2.2 Plant material

The plant was collected in Firmino Alves, southern Bahia, Brazil, in April 2009, and a voucher specimen was housed in the Herbarium of the Executive Committee of the Cacao Plan (CEPLAC) in Bahia State University of Santa Cruz – UESC. The collected material was dried in an oven (50 °C) for 48 hours, and their separated parts (stem, leaf and root) were pulverized in a knife mill.

2.3 Obtaining extracts

The dried and pulverized leaves (28 g), stem (128 g) and roots (200 g) were thoroughly extracted by maceration with dichloromethane, and then concentrated under reduced pressure (38 °C) until complete removal of the solvent, affording the dichloromethane extracts of the roots (1.68 g), stem (1.31 g) and leaves (1.19 g).

2.4 Fractionating extracts

The crude extract from roots (1.68 g) was subjected to silica gel column chromatography (Si-CC) eluting with mixtures of hexane, ethyl acetate (EtOAc) and methanol (MeOH) with increasing polarities, yielding a total of 55 fractions. Two fractions eluted with EtOAc were further processed. The fraction 24 (45 mg) was treated with ethyl ether to provide *(R)-(−)-tembamide* acetate (2, 28 mg), and the fraction 26 (245 mg) was submitted again to Si-CC (hexane-EtOAc, 1:1) to produce tembamide (1, 31 mg). Additional amounts of 1 (6 mg) and 2 (5 mg) were isolated when crude extracts from stems (1.31 g) were fractionated by similar procedure.

The crude dichloromethane extract from leaves (1.19 g) was also submitted to Si-CC using mixtures of hexane, EtOAc and MeOH with increasing polarities to afford 87 fractions. Fractions 76 to 80, eluted with hexane-EtOAc (7:3), were grouped (13 mg) and submitted to prep-TLC (hexane-EtOAc, 6:4) to yield riparin I (3, 5 mg).

*Tembamide* (1). *1H NMR (500 MHz, CDCl3)*: δ 7.75 (d, 2H, *J* = 8.0 Hz), 7.51 (dd, 1H, *J* = 7.5, 7.0 Hz), 7.43 (dd, 2H *J* = 8.0, 7.0 Hz), 7.33 (dd, 2H *J* = 8.5 Hz), 6.90 (d, 2H, *J* = 8.5 Hz), 6.59 (br. s, 1H), 4.91 (dd, 1H, *J* = 8.0, 3.5 Hz), 3.90 – 3.86 (m, 1H), 3.81 (s, 3H), 3.54 – 3.49 (m, 1H); *13C NMR (125 MHz, CDCl3)*: δ 168.53, 159.18, 134.12, 133.85, 131.67, 128.60, 127.09, 126.96, 113.99, 73.33, 55.30 and 47.73; EM-IES (m/z): 294.1124 (M + Na)*; [C16H17NO3 requires (M + Na)* = 294.1095]. *(R)-(−)-Tembamide acetate* (2). [α]D = −43.9° (c 0.55), CHCl3. *1H NMR (500 MHz, CDCl3)*: δ 7.72 (d, *J* = 8.0 Hz, 2H), 7.50 (t, *J* = 7.0 Hz, 1H), 7.43 (dd, *J* = 8.0, 7.0 Hz, 2H), 7.33 (d, *J* = 8.5 Hz, 2H), 6.91 (d, *J* = 8.5 Hz, 2H), 6.41 (br. s, 1H), 5.94 (dd, *J* = 7.5, 5.5 Hz, 1H), 3.82 – 3.85 (m, 2H), 3.81 (s, 3H), 2.10 (s, 3H). *13C NMR (125 MHz, CDCl3)*: δ 170.76, 167.43, 159.76, 134.27, 131.58, 129.65, 128.62, 127.94, 126.86, 114.14, 74.34, 55.30, 45.01, 21.23; EM-IES (m/z): 336.1204 (M + Na)*; [C16H16O3Na requires (M + Na)* = 336.1201]. *Riparin I* (3). *1H NMR (500 MHz, CDCl3)*: δ 7.67-7.70 (m, 2H), 7.16 (d, *J* = 8.5 Hz, 2H), 7.46-7.49 (m, 1H), 7.38-7.42 (m, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 6.10 (br. s, 1H), 3.80 (s, 3H), 3.68 (q, *J* = 7.0 Hz, 2H), 2.88 (t, *J* = 7.0 Hz, 2H); *13C NMR (125 MHz, CDCl3)*: δ 167.42, 158.69, 159.31, 134.19, 133.75, 129.65, 128.55, 126.77, 114.14, 52.27, 41.27, 34.77; EM-IES (m/z): 255.1209 (M)*, 256.1322 (M + 1)*, 254.1201 referring to [(M – 2H) + 1]*; [C13H16NO3 requires (M)* = 255.1254, (M + 1)* = 256.1332, and (M-1)* = 254.1176].

2.5 Antifungal assay by direct bioautography

The microorganisms used in the antifungal assays *Cladosporium cladosporioides* (Fresen.) de Vries SPG 140 and *C. sphaceliferum* (Penzig) SPC 491 were grown in Sabouraud dextrose agar, and maintained at the Instituto de Botânica, São Paulo, SP, Brazil. Both fungi were cultured in Sabouraud medium before the assay. Direct bioautography assays were performed in triplicate in agreement with the literature procedure6,13. Ten microliters of dichloromethane solutions prepared for each pure compound, corresponding to 100, 50, 25, 10, 5, and 1 µg, were applied to TLC plates and eluted with hexanes-EtOAc (7:3) followed by complete removal of the solvent at room temperature. The chromatograms were then sprayed with a spore suspension (3 x 107 cells/mL) in glucose and salt solution and incubated for 48 h in the darkness in a moistened chamber at 25 °C.
Clear inhibition zones appeared against a dark background, indicating the minimal amount of compound required to inhibit the growth of each fungus (Table 1). Nystatin was used as the positive control (detection limit 1 µg).

3. Results and discussion

The phytochemical study on dichloromethane extracts of the leaves, stem and roots of *Piper mollicomum* Kunth led to the isolation of the known compounds tembamide (1), (R)-(−)-tembamide acetate (2) and riparin I (3) (Figure 1), from which only 1 and 2 were found to exhibit remarkable antifungal properties against *Cladosporium cladosporioides* (5 µg) and *C. sphaerospermum* (1 µg) through direct bioautography analyses. When tested against *C. sphaerospermum*, compounds 1 and 2 were shown to be as potent as nystatin, the positive control (Table 1). The acetate 2 could be easily hydrolysed by fungal enzymes and this could be one likely explanation for the same antifungal activity observed for both compounds 1 and 2. In spite of being already known chemical entities, the compounds 1-3 are reported here, for the first time, as chemical constituents of *P. mollicomum* Kunth. In addition, bearing in mind that only 1 and 2 were active against the assayed fungi, but not 3, one can hypothesize that such antifungal activity may be related to the oxygenation at the benzylic carbon, explaining why 3 was inactive against the both fungi assayed.

Tembamide is widely described as a chemical constituent of plants belonging to the Rutaceae family, such as *Clausena brevistyla* Oliver [14], *Zanthoxylum ekmanii* (URB.) ALAIN [15], *Aegle marmelos* [16], *Clausena lansium* [17], and *Feroniella lucida* [18]. This amide was also obtained by hydrolysis from tembamide acetate isolated from the dichloromethane fraction of *Piper guayranum* (Piperaceae) [19], a plant species traditionally used in Indian medicine due to its hypoglycemic activity [20]. In addition, tembamide displayed one of the most potent activities against HIV virus among 67 substances isolated from the bark and root of *Zanthoxylum ailanthoides* (Rutaceae) [21]. Isolated from *Zanthoxylum capense* (Rutaceae), tembamide was also evaluated along with (R)-(+) -tembamide acetate in tests of antibiotic modulators [22]. Its synthesis has been previously described by Aguirre et al. (2001) and Kamal et al. (2004) [23, 24].

According to literature data, riparine I, isolated from the fruits of *Aniba riparia* (Nees) Mez (Lauraceae), presented anxiolytic effects [25] and it was shown to be a potent muscle relaxant [26].

Our findings call attention to the importance of *P. mollicomum* Kunth as a source of bioactive natural compounds for further studies on promising antimicrobial and pharmacological agents.

<table>
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<th>compounds</th>
<th>Antifungal Activity (µg) a</th>
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<tr>
<td></td>
<td></td>
<td><em>C. cladosporioides</em></td>
<td><em>C. sphaerospermum</em></td>
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<tr>
<td>Tembamide (1)</td>
<td>5.0</td>
<td>1.0</td>
<td></td>
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<tr>
<td>(R)-(−)-Tembamide acetate (2)</td>
<td>5.0</td>
<td>1.0</td>
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<tr>
<td>Riparin I (3)</td>
<td>&gt; 100.0</td>
<td>&gt; 100.0</td>
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<tr>
<td>Nystatin</td>
<td>1.0</td>
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a Minimum amount required for inhibition of fungal growth on thin layer chromatography (TLC) plate.

4. Conclusions

The present study reports on the chemical constituents of *Piper mollicomum* Kunth, in which the piperamides tembamide, (R)-(−)-tembamide acetate and riparin I, bioactive compounds commonly isolated from Rutaceae species. Tembamide and (R)-(−)-tembamide acetate showed antifungal activity as potent as nystatin.
against *Cladosporium* strains by means of direct bioautographic analyses.

5. Acknowledgements

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6. References


