Antiprotozoal Activity of Flavonoids Isolated from Mimosa tenuiflora (Fabaceae-Mimosoideae)

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Abstract. As result of the chemical study of the leaves and flowers of Mimosa tenuiflora (Willd.) Poir. (Fabaceae-Mimosoideae) eight flavonoids were isolated: 6-methoxy-4’-O-methylmaringenin (1), santin (2), 6-methoxynaringenin (3), tenuiflorin A (4), 5, 7, 4’-triacylxy-3, 6-dimethoxyflavone (5), 6-dimethoxy-4’-O-methylcapilarisine (6), 6-methoxycamperol (7) and tenuiflorin C (8). Antiprotozoal activity of these compounds as well as the tenuiflorina B (9) and 6-desmethoxy-capilarisine (10), isolated in a previous study was assessed against Entamoeba histolytica and Giardia lamblia trophozoites.

Keywords: Mimosa tenuiflora, Fabaceae, flavonoids, 2-phenoxycromones, antiprotozoal activity, tepescohuite.

Introduction

Parasitic infections constitute one of the most widespread human health problems, and more of them occur through contaminated food or water, therefore, these infections are more prevalent in developing countries. The human intestine is a major target of these ingested pathogenic microorganisms, resulting in severe infections, such as dysentery and diarrhea [1]. Two of the most common causes of such symptoms are intestinal protozoa Entamoeba histolytica and Giardia lamblia, the first cause amoebic dysentery, the second cause giardiasis [2].

Metronidazole is a classical and effective treatment for both diseases; however, resistance to drug as well as the risk of potential mutagenicity and carcinogenicity has been described. In prolonged treatment or high doses often cause side effects such as headache, dry mouth, metallic taste, glossitis and urticaria [1, 2]. Owing to these undesired side effects and taking into account the possibility of the development of resistant strains of the E. histolytica and G. lamblia against metronidazole, there is a clear need for new, effective, and safer antiprotozoal agents [1]. Recently different natural products that possessed antiprotozoal activity, has been reported, they are alkaloids [3], terpenoids [4], and flavonoids [5] among other. We are particularly interested in flavonoids since they are present in several of the plants used in traditional medicine to treat amoebic dysentery and severe diarrhea [6].

Mimosa tenuiflora (Willd.) Poir (Fabaceae), is known by the common name of “tepessohuete” [7]. The bark of this tree is used in Mexican traditional medicine to treat wounds caused by burns, prevention of inflammation and as an antimicrobial agent [8]. In Brazil the bark from Mimosa tenuiflora is used to prepare a sacramental drink called “jurema wine” [9]. The species has been chemically studied several times; triterpene saponins [10], indole alkaloids [9] and polysaccharides [11], have been isolated from its bark; while chalcones [12], flavonoids [13], phenoxicromones [14] and diterpene rhamnosides [13] have been obtained from its leaves and flowers.

In this study, we present the isolation and antiprotozoal activity of ten flavonoids isolated from the leaves of Mimosa tenuiflora, including five 2-phenoxycromones, that are rare natural products whose biosynthesis has been associated with flavonoids [15].

Results and Discussion

The chemical study of leaves and flowers from Mimosa tenuiflora lead us to obtain two flavonones: 6-methoxy-4’-O-methylmaringenin (1) [15] and 6-methoxynaringenin (3) [17]; three flavonols: santin (2) [16], 5, 7, 4’-Trihydroxy-3, 6-dimethoxyflavone (5) [18] and 6-methoxycamperol (7) [19], and three 2-phenoxycromones: tenuiflorin A (4) [14], 6-desmethoxy-4’-O-methylcapilarisine (6) [14] and tenuiflorin C (8) [14]. It is the first time that flavonols are isolated from this species.

The flavonones and flavonoids showed oxygenated functions at positions 5, 6, 7 and 4’; meanwhile, the 2-phenoxycromones showed a 5, 7 and 4’ oxygenated pattern.

Palabras clave: Mimosa tenuiflora, Fabaceae, flavonoids, 2-fenoxycromones, actividad antiprotozoaria, tepescohuite.

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In a previous study the antiamoebic and antigiardial activity of some flavanones, flavones, flavonols and catechins were evaluated [14]. Those compounds with hydroxy groups at C3, C5 and C7 resulted more active, the flavonoids with a substituent in C6 were inactive and when the degree of oxygenation increased in the B-ring the antiprotozoal activity decreased [23].

The biological activity of 2-phenoxychromones has been little studied; therefore we decided to evaluate their antiprotozoal activity against *Entamoeba histolytica* and *Giardia lamblia*. In this study we evaluated how the presence of an oxygen bridge between rings B and C affects the antiprotozoal activity. Results of this assay are shown in Table 1 [14].

Among the tested compounds, 4 (IC$_{50}$ = 41.1 µg/mL) was the most active against *E. histolytica* and 2 (IC$_{50}$ = 75.3 µg/mL) against *G. lamblia*. Unfortunately, these values are higher than those presented by kaempferol against both protozoa (*E. histolytica*: IC$_{50}$ = 7.9 µg/mL and *G. lamblia*: IC$_{50}$ = 8.1 µg/mL).

**Conclusions**

The antiprotozoal activity of the extracts and pure flavonoids were moderate. These results were consistent with the reported structural requirements of flavonoids for antiprotozoal activity [23]. Compounds with a C6 substituent showed IC$_{50}$ higher than kaempferol and the results with 2-phenoxychromones indicate that the presence of an oxygen atom between rings B and C of the flavonoid framework has no significant effect on the antiprotozoal activity.

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Sample</th>
<th>IC$_{50}$ µg/mL (E. histolytica)</th>
<th>IC$_{50}$ µg/mL (G. lamblia)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extracts</td>
<td>65.9</td>
<td>80.2</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>80.7</td>
<td>116.8</td>
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<tr>
<td></td>
<td>Methanol</td>
<td>73.5</td>
<td>95.5</td>
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<tr>
<td>Compounds</td>
<td>6-Methoxy-4’-O-methylnaringenin (1)</td>
<td>72.7</td>
<td>82.9</td>
</tr>
<tr>
<td></td>
<td>Santin (2)</td>
<td>69.7</td>
<td>75.3</td>
</tr>
<tr>
<td></td>
<td>6-Methoxynaringenin (3)</td>
<td>76.4</td>
<td>84.1</td>
</tr>
<tr>
<td></td>
<td>Tenuiflorin A (4)</td>
<td>41.1</td>
<td>108.6</td>
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<tr>
<td></td>
<td>4’,5,7-Trihydroxy-3,6-dimethoxyflavone (5)</td>
<td>69.8</td>
<td>77.1</td>
</tr>
<tr>
<td></td>
<td>6-Demethoxy-4’-O-methylcapillarisin (6)</td>
<td>80.7</td>
<td>91.8</td>
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<tr>
<td></td>
<td>6-Methoxykaempferol (7)</td>
<td>71.6</td>
<td>77.8</td>
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<td>Tenuiflorin C (8)</td>
<td>82.8</td>
<td>92.8</td>
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<td></td>
<td>Tenuiflorin B (9)</td>
<td>89.9</td>
<td>100.9</td>
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<tr>
<td></td>
<td>6-Demethoxycapillarisin (10)</td>
<td>78.7</td>
<td>86.6</td>
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<td></td>
<td>Kaempferol$^b$</td>
<td>7.9</td>
<td>8.1</td>
</tr>
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<td></td>
<td>Emetine$^c$</td>
<td>2.2</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Metronidazole$^c$</td>
<td>0.23</td>
<td>1.22</td>
</tr>
</tbody>
</table>

Results are expressed as mean ($n = 6$) IC$_{50}$ (Concentration that inhibited the growth of trophozoites in 50%).

$^a$Results are expressed as mean ($n = 6$), CI = 95% confidence intervals.

$^b$See ref. [23].

$^c$Positive controls.
The percentage of trophozoites surviving was calculated by Statistical analysis. and repeated at least three times. Experiments were performed in duplicate for each protozoan strain and in the log phase of growth. For assays were employed in the log phase of growth. The trophozoites were detached by chilling and 50 μL samples of each tube were subcultured in fresh medium for another 48 h. Without antiprotozoal samples. The final number of parasites was determined with a hemocytometer and the percentages of trophozoites were washed and incubated with the control culture. The results were confirmed by a hemocytometer and the percentages of trophozoites were calculated by comparison with the growth in the control group. The plot of probit against log concentration was made; the best straight line was determined by regression analysis and the 50% inhibitory concentration (IC50) values was calculated together with the 95% confidence limits [22].

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References