Isolated Compounds and Semi-Synthetic Derivatives from *Miconia ferruginata* as Inhibitors of Cathepsins K and B and their Cytotoxic Evaluation

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Compostos Isolados e Derivados Semissintéticos de *Miconia ferruginata* como Inibidores das Catépsinas K e B e sua Avaliação Citotóxica

Resumo: O gênero *Miconia*, pertencente à família Melastomataceae, é amplamente distribuído na América tropical. Compostos isolados e derivados semissintéticos das folhas de *Miconia ferruginata* foram avaliados quanto à citotoxicidade em linhagens de células tumorais e contra importantes enzimas proteolíticas, conhecidas como catépsinas B e K. Entre os compostos avaliados, a mistura de ursolato (2a) e oleanato de metila (3a) mostraram citotoxicidade considerável contra as linhas celulares de melanoma (MDA-MB435) e câncer de cólon (HCT-8). Além disso, também foi demonstrado que a mistura dos ácidos ursólico (2) e oleanólico (3) inibiu a catépsina B, com valor de IC<sub>50</sub> de 13,02 μM. Por outro lado, a mistura dos compostos 2a e 3a mostrou atividade considerável contra a catépsina K, com valor de IC<sub>50</sub> de 1,42 μM. O composto 5,6,7-trihidroxi 4'-metoxiflavona (1) não mostrou atividade.

Palavras-chave: Cisteíno proteases; Melastomataceae; fitoquímica; triterpenos.

Abstract

*Miconia* genus, belonging to the Melastomataeceae family, is widely distributed in tropical America. Isolated compounds and semi-synthetic derivatives from *Miconia ferruginata* leaves were evaluated both for their cytotoxicity on tumor cell lines and against important proteolytic enzymes such as cathepsins B and K. Among the compounds evaluated, the mixture of methyl ursolate (2a) and oleanate (3a) showed considerable cytotoxicity against melanoma (MDA-MB435) and colon cancer (HCT-8) cell lines. In addition, it was also demonstrated that the mixture of ursolic (2) and oleanolic (3) acids inhibited cathepsin B, with IC<sub>50</sub> value of 13.02 μM. On the other hand, the mixture of compounds 2a and 3a showed considerable activity against cathepsin K, with IC<sub>50</sub> value of 1.42 μM. The compound 5,6,7-trihydroxy 4’-methoxyflavone (1) showed no activity.

Keywords: Cysteine proteinases; Melastomataceae; phytochemistry; triterpenes.
Isolated Compounds and Semi-Synthetic Derivatives from *Miconia ferruginata* as Inhibitors of Cathepsins K and B and their Cytotoxic Evaluation

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1. Introduction

Cancer is one of the leading causes of death worldwide, accounting for about 9.6 million deaths in 2018.\textsuperscript{1} In the search for new drug candidates with a variety of pharmacological activities, including the antitumor, natural products and their semisynthetic derivatives have been extensively investigated. Considering the period between 1940 and 2014, among the 174 approved anticancer drugs, 37% were composed of natural products and their semisynthetic derivatives.\textsuperscript{2}

Cathepsins are a group of protease enzymes originally discovered in the cell lysosome, with several ubiquitous members in the human body.\textsuperscript{3} Two important members of this group are the cathepsins K and B. Cathepsin K is a cysteine protease highly expressed in osteoclasts and the most important protease in bone degradation.\textsuperscript{4} Cathepsin B has been shown to have multiple roles in cancer, including tumor invasion, proliferation and metastasis of tumor cells.\textsuperscript{5} Yin et al. (2012)\textsuperscript{6} found up-regulation of cathepsin B in melanoma/fibroblast cell co-culture and proved its relationship with the progression and invasiveness of the
melanoma cells to other tissues. When a specific inhibitor of cathepsin B was tested in this coculture, complete inhibition of cell invasion was observed. The work accomplished by Mehrotra et al. (2018)\textsuperscript{7} demonstrated the overexpression of cathepsins B and G in liver metastasis from colon adenocarcinoma (LMCA) and also that these enzymes are located in the cytoplasm of these cells, demonstrating the involvement of these cathepsins in colon adenocarcinoma. Van Noorden et al. (1998)\textsuperscript{8} demonstrated that a selective inhibitor of cathepsin B reduced the number of tumors by 60% and the volume of metastatic colon tumors by 80%. Shimizu et al. (2013)\textsuperscript{9} identified a molecule (netrin-1) that is directly involved with the glioblastoma invasiveness and angiogenesis, and that these processes are mediated by activated cathepsin B. The authors demonstrated that when these cells were treated with a specific cathepsin B inhibitor, netrin-1-induced cell invasion and angiogenesis were inhibited. These findings show strong evidence that selective cathepsin B inhibitors can act to fight brain, colon and melanoma cancers. This and other studies demonstrate the great importance of the search for cathepsin B inhibitors for the discovery of new drugs.

\textit{Miconia} is one of the most representative genus from the Melastomataceae family, with more than 1,050 species occurring from western Mexico and the Caribbean to Uruguay and northern Argentina.\textsuperscript{10} In Brazil, this genus ranks fifth position in diversity and is represented by 276 species, out of which 121 are endemic.\textsuperscript{11} Previous phytochemical investigations on this genus have revealed that the main constituents include flavonoids and triterpenes.\textsuperscript{12} Extracts obtained from \textit{Miconia} and their isolated compounds have demonstrated the therapeutic potential of this genus. Some pharmacological properties were evaluated with \textit{in vitro} and \textit{in vivo} preclinical studies, as antimicrobial,\textsuperscript{13-17} analgesic,\textsuperscript{18-20} anti-inflammatory,\textsuperscript{20,21} insecticide\textsuperscript{22} and antitumor\textsuperscript{23-27} activities.

The aim of the current study was to evaluate the \textit{in vitro} cytotoxicity of isolated compounds and semi-synthetic derivatives from \textit{Miconia ferruginata} leaves against human cancer cell lines, namely, MDA-MB435, melanoma; HCT-8, colon and SF-295, glioblastoma, using the MTT assay. In addition, the compounds were also evaluated against cathepsins K and B, which are related with osteoporosis and tumor processes, respectively.

## 2. Material and Methods

### 2.1. General experimental procedures

1D and 2D NMR experiments were performed on a Bruker DRX-400 (400 MHz, 9.4 T) and a Bruker ARX-200 (200 MHz, 4.7 T) spectrometers using deuterated solvents ($\text{CDCl}_3$ and acetone-$d_6$ with TMS as internal standard). Mass spectrometry (MS) was performed on Bruker microTOF-Q III apparatus. The electrospray source was operated in the positive mode. High-purity nitrogen (>98%) was used as desolvation (200°C; 4 L min$^{-1}$) nebulizer and collision gas. Nebulizer pressure was kept at 0.4 bar and the capillary voltage set at 4500 V. The Q-TOF conditions were as follows: End plate offset: -500 V; Funnel 1: 200 Vpp; Funnel 2: 200 Vpp; Hexapole RF: 200 Vpp; Collision RF: 400 Vpp; Transfer Time: 70 μs; Pre Pulse Storage: 5 μs; Ion Energy Quadrupole: 5eV; Rolling Average: 2 x 1Hz. The mass spectra were acquired and processed using a Bruker Compass DataAnalysis Software (Bruker Daltonik, GmbH).

### 2.2. Plant material

\textit{Miconia ferruginata} (SisGen code A59B2F2) leaves were collected in September 2008 at the Campus de Ciências Exatas e Tecnológicas of the Universidade Estadual de Goiás (UEG), Anápolis, GO, Brazil (latitude 16°38’15”S, longitude 48°94’51”W). The species was identified by Dr. Mirley Luciene dos Santos and a voucher specimen (HUEG 5794) was deposited in the Herbarium of the Universidade Estadual de Goiás.

### 2.3. Extraction, isolation and reaction

In a previous work\textsuperscript{22}, we described the isolation of 5,6,7-trihydroxy 4’-methoxyflavone (1) and a mixture of ursolic (2) and oleanolic (3) acids from ethanolic extract of \textit{M. ferruginata} leaves. Briefly, air-dried (45°C) and powered leaves (1485 g) were extracted at room temperature with ethanol (5 L) in a macerating process that yielded the crude ethanolic extract (MFLE, 405.11 g). Part of this extract (112.62 g) was dissolved in a mixture of EtOH/H$_2$O (1:3 v/v) and then subjected to liquid-liquid partition with hexane, CH$_2$Cl$_2$, and EtOAc. The solvents were removed under reduced pressure, yielding the hexane (MFLE-H, 19.63 g),
dichloromethane (MFLE-D, 0.86 g), ethyl acetate (MFLE-A, 24.3 g) and hydroalcoholic (MFLE-W, 65.19 g) fractions. The MFLE-D fraction (0.86 g) was subjected to column chromatography (CC) (3 x 16 cm, silica gel 230-400 mesh, eluted with 9.5:0.5 CH₂Cl₂/acetone, isocratic) to yield 5 subfractions (D1-D5). Subfraction D5 (233 mg) was subjected to column chromatography (3 x 15 cm, silica gel 230-400 mesh, eluted with 9:1 CH₂Cl₂/acetone, isocratic) to yield 10 subfractions (D5.1-D5.10). Subfraction D5.3 (20.1 mg) was chromatographed (Sephadex-LH20, 2.4 x 66 cm, eluted with 7:3 MeOH/CH₂Cl₂, isocratic), resulting in the isolation of 1 (13.5 mg). The MFLE-H fraction (2.0 g) was subjected to vacuum column chromatography (7 x 26 cm, silica gel 70-230 mesh, eluted with hexane, CH₂Cl₂, AcOEt and MeOH). The solvents were removed under reduced pressure, yielding the hexane (MFLE-HH, 0.05 g), dichloromethane (MFLE-HD, 0.53 g), ethyl acetate (MFLE-HA, 0.68 g) and methanol (MFLE-HM, 0.47 g) subfractions. Subfraction MFFE-HA (0.68 g) was subjected to column chromatography (2 x 19 cm, silica gel 230-400 mesh, eluted with 8:2 CH₂Cl₂/AcOEt → MeOH, gradient) to yield 5 subfractions (HA1-HA5). Subfraction HA4 (124 mg) was subjected to column chromatography (2.5 x 11.5 cm, silica gel 230-400 mesh, eluted with 9.5:0.5 CH₂Cl₂/Acetone → Acetone, gradient), resulting in the mixture of 2 and 3 (63 mg).

The mixture of 2 and 3 (63 mg) was methylated with diazomethane affording 2a and 3a (62.4 mg, 96% yield). The diazomethane solution was prepared by dissolving 1.0 g of p-tolylsulfonylmethyl nitrosamine (diazald) in 15 mL of ethyl ether. The solution was cooled in an ice bath and transferred to a distillation flask, where 1.0 g of potassium hydroxide was added to 10 mL 96% ethanol. The system was allowed to stand for 5 minutes in an ice bath and then distilled in a 65°C thermal blanket. The product was collected over ethyl ether in an ice bath. Methylation was performed by adding the diazomethane solution to the material to be methylated until gas (N₂) was no longer released.

2.4. Cytotoxicity assay

Isolated compounds and semi-synthetic derivatives were tested for cytotoxicity against three tumor cell lines: MDA-MB435, melanoma; HCT-8, colon and SF-295, glioblastoma. All cell lines were obtained from the National Cancer Institute (NCI) and were cultivated in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% antibiotics (penicillin and streptomycin), at 37°C, in an atmosphere containing with 5% CO₂. The MTT assay was used for the cytotoxicity evaluation. Cells were plated at a concentration of 0.1 x 10⁵ cells/100 μL for adhered cells. Substances were added at 5 μg mL⁻¹ (single concentration). The plates were incubated for 72 hours. Before the end of the incubation period, they were centrifuged, and the supernatant was removed. Then, 200 μL of MTT solution (tetrazolium salt) was added and the plates were incubated for 3 h. The absorbance was read after dissolution of the precipitate with DMSO on a 550-nm plate spectrophotometer. Results were analyzed according to their means and standard errors on the GraphPad Prism software. Each sample was analyzed from two experiments performed in duplicate.

2.5. Inhibition assay of Cathepsin B and Cathepsin K

The methodology applied consists of the determination of the enzymatic inhibition by detecting the increase in fluorescence that occurs with the hydrolysis of the fluorogenic substrate Z-FRMCA, which is detected on a spectrofluorometer with excitation slit adjusted to λex = 380 nm and emission λem = 460 nm. The protease inhibition activity was performed in triplicates, in which samples were diluted in DMSO, yielding a final compound concentration of 125 µM. A volume of 5 µL was transferred to each well of the ELISA plates (96 wells) and the MIX 1 and enzyme in 3 mM concentration in buffer (100 mM sodium acetate with 5 mM EDTA and pH 5.5) pre-activated with DTE (5.0 mM) were added to the analytes. The enzyme was in contact with the compounds for 5 minutes and then 10 µM of MIX 2 (Z-FRMCA fluorogenic substrate in buffer) was added and then read. For compounds with significant inhibition (greater than or equal to 80%) against the enzymes tested, IC₅₀ values were determined using nine different inhibitor concentrations (0.25 - 125 µM) and constructing a concentration-response curve by the Microcal Origin 6.0 non-linear regression method.
3. Results and Discussion

Compounds 1, 2 and 3 were isolated from the ethanolic extract of *M. ferruginata* leaves and the mixture of 2 and 3 was submitted to a methylation reaction to yield the semi-synthetic derivatives 2a and 3a. The chemical structures of the identified compounds are shown in Figure 1.

The discussion about the structural characterization of this compounds is described in our previous work. Briefly, the $^1$H NMR spectrum of 1 exhibited the presence of a singlet at δ$_H$ 3.98 (s, 3H), indicating the presence of an OCH$_3$. Two doublets appeared at δ$_H$ 7.03 (d, 2H, $J = 8.4$ Hz, H-3'/5') and δ$_H$ 7.96 (d, 2H, $J = 8.4$ Hz, H-2'/6') and two singlets appeared at δ$_H$ 6.79 (s, 1H, H-8) and δ$_H$ 6.68 (s, 1H, H-3). Another singlet was also observed at δ$_H$ 13.14, characteristic of flavonoids containing a hydroxyl group at the C-5 position. These observations suggested the presence of a flavone containing a substituent at the C-4' position and a tri-substituted A ring. The HSQC experiment showed position of five protons, which were found to be linked with 131.9 (C-2'/6'), 118.9 (C-3'/5'), 106.6 (C-3), 92.6 (C-8) and 58.6 (OCH$_3$). The three-bond correlation of H-2'/6' with C-2 (δ C 165.0) and H-3 with C-1' (δ C 123.0) in the HMBC experiment confirmed the connection of fragment B with C-2. Based on HMBC experiment, all the carbons, except for C-5 and C-6, were assigned. These data were consistent with the structure of 5,6,7-trihydroxy-4'-methoxy flavone, commonly known as scutellarein 4'-methyl ether.

The $^1$H NMR spectrum of 2 and 3 showed the presence of several signals in the region between δ$_H$ 0.77 and 1.25 (s), characteristic of methyl hydrogens; two signals at δ$_H$ 5.25 and 5.24 (m) characteristic of olefinic hydrogen, and also one signal at δ$_H$ 3.21 (m), which agree with carbinolic hydrogens. All these data suggest the mixture of two triterpenes. The $^1$H NMR spectrum of the methylated sample, 2a and 3a, showed signals centered on δ$_H$ 5.22 and δ$_H$ 5.21 (t, 1H, $J = 3.6$ Hz), referring to the olefinic H-12 characteristic of ursan and oleanonic skeletons, suggesting that the sample was a mixture of these triterpenes. The signal referring to carbinolic H-3 appeared centered on δ$_C$ 3.18 (dd, 1H, $J = 11.3$ and 4.3 Hz), and the signals referring to methyl hydrogens between δ$_C$ 1.10 and 0.69. The presence of two singlets in δ$_C$ 3.59 and δ$_C$ 3.57 was also observed, and is related to methoxy hydrogens. The HSQC experiment showed the correlation of hydrogen at δ$_H$ 5.22 with the carbon signal at δ$_C$ 122.59, referring to the C-12 vinyl carbon of the oleanonic acid. The signal at δ$_C$ 5.21 correlated with carbon at δ$_C$ 125.80, characteristic of the C-12 of the ursolic acid. The presence of oxygenated carbon was observed with a chemical shift in δ$_C$ 79.28, and the presence of carbonyl typical of C-28 methyl ester was observed at δ$_C$ 178.03. The ESI-MS in the positive mode of the mixture of 2a and 3a indicated the presence of an ion fragment at m/z 471.3703 [M+H]$^+$, which corresponds to the methyl ursolate (2a) and oleanate (3a) ($C_{31}H_{50}O_{3}$).

These compounds were evaluated for their cytotoxic activities in MTT assay against human tumor cells line: MDA-MB435, melanoma; HCT-8, colon and SF-295, glioblastoma.

In general, no cytotoxicity against cell lines was observed to the flavone (1) and the mixture of triterpenes in the acid form (2 and 3) (Table 1). However, the methylated form of triterpenes (2a and 3a) showed cell growth inhibition higher than 95% in at least two cell lines: melanoma (MDA-MB435) and colon cancer (HCT-8).

Results obtained by Resende et al. support the indication of triterpenes ursolic and oleanolic acids as promising candidates in the prevention of cancer. In this study, ursolic and oleanolic acids

![Figure 1. Isolated compounds and semi-synthetic derivatives from *Miconia ferruginata*](image-url)
isolated from the aerial parts of *Miconia fallax* were evaluated for antimutagenic potential using the micronucleus test in peripheral blood and bone marrow of Balb/c mice. The results showed a significant reduction on micronucleus frequency in the groups concomitantly treated with the triterpenes and doxorubicin, an antineoplastic agent, compared to that treated with doxorubicin alone.26

Cunha *et al.*23 also evaluated the antitumor potential of the *Miconia fallax* species. In this study, *in vitro* tumor growth inhibition by the ethanol extract of the aerial parts of *M. fallax* was evaluated in culture media containing cells of the human uterine cervix adenocarcinoma cell line. Bioassay-guided fractionation of this extract furnished a mixture of ursolic and oleanolic acids. Both the ethanol extract and the mixture of the triterpenes produced dose-dependent tumor growth inhibition.23 Additionally, ursolic and oleanolic acids isolated from the methylene chloride extract of aerial parts of *M. fallax* were also reported to have a protective effect against colon carcinogenesis.24

The inhibition assay on cysteine proteases revealed that the mixture of compounds 2 and 3 inhibited cathepsin B. On the other hand, mixture of compounds 2a and 3a showed significant activity against cathepsin K. Compound 1 showed no significant activity against cathepsins (Table 2).

Table 1. Cytotoxic activity of compound 1, mixture of 2 and 3 and mixture of 2a and 3a

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cell-growth inhibition percentage GI % (5 μg mL⁻¹)¹</th>
<th>MDA-MB-435</th>
<th>HCT-8</th>
<th>SF-295</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.65 ± 4.99</td>
<td>35.73 ± 2.50</td>
<td>26.39 ± 2.06</td>
<td></td>
</tr>
<tr>
<td>2 and 3</td>
<td>15.08 ± 6.66</td>
<td>40.59 ± 0.49</td>
<td>32.98 ± 8.82</td>
<td></td>
</tr>
<tr>
<td>2a and 3a</td>
<td>98.10 ± 2.88</td>
<td>98.57 ± 0.24</td>
<td>69.89 ± 9.93</td>
<td></td>
</tr>
</tbody>
</table>

¹ GI % values shown were the average of two replicates. Human tumor cell lines: MDA-MB435, melanoma; HCT-8, colon and SF-295, glioblastoma

Ramalho *et al.* (2014)29 screened several natural triterpenes, including ursolic and oleanolic acids and some of their derivatives against cathepsin B and cathepsin L. According to these authors, ursolic and oleanolic acids do not inhibit cathepsin B in the concentration tested.29 In the present study, the mixture of these triterpenes inhibited cathepsin B with IC₅₀ value of 13.02 μM, suggesting that the compounds in the mixture behave differently against cathepsin B than when isolated.

It has been found that the more common structural modifications on triterpenes were at C-3 (OH group) and C-28 (COOH group), and the derivatives obtained displayed enhanced pharmacological activities, especially anticancer properties.29 Among the compounds evaluated, the mixture of 2 and 3 lost its activity towards cathepsin B when the C-28 (COOH) was transformed into the corresponding methyl esters 2a and 3a, confirming the importance of a free -COOH group for the activity against cathepsin B. Regarding cathepsin K, the behavior was the opposite. The change in the C-28 (COOH) by the treatment with diazomethane led to increased inhibition, confirming the importance of the -COOCH₃ group for the activity against cathepsin K. It is concluded that this triterpene moiety is important for both the enzymatic inhibition activity of the enzymes and cytotoxic effect, and

Table 2. Inhibitory activities (%) and IC₅₀ (μM) of compound 1, mixture of 2 and 3 and mixture of 2a and 3a against cathepsin B and cathepsin K

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibitory Activities (%)</th>
<th>IC₅₀ (μM)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cathepsin B</td>
<td>Cathepsin K</td>
</tr>
<tr>
<td>1</td>
<td>55.59 ± 5.20</td>
<td>17.94 ± 2.77</td>
</tr>
<tr>
<td>2 and 3</td>
<td>79.39 ± 0.19</td>
<td>10.79 ± 4.92</td>
</tr>
<tr>
<td>2a and 3a</td>
<td>39.37 ± 0.60</td>
<td>75.09 ± 1.47</td>
</tr>
</tbody>
</table>

ᵃ The values represent means of three replicates ± SD. nd: not determined
therefore stimulates the accomplishment of new structural modifications for the study of the activity structure relationship.

It was not possible to make a relationship between the cathepsin B inhibition effect and the cytotoxic effect observed in cancer cells, since ursolic and oleanolic acid esters were more effective than their respective acids against cancer cell lines, while with the effect of enzymatic inhibition against cathepsins B, the opposite was observed: the ursolic and oleanolic acids were more effective than the respective esters. That suggests that the mechanism through which inhibition of cell proliferation occurs does not involve inhibition of cathepsin B. Thus, the mechanism of ursolic and oleanolic acid action on these cancer cell lines should be further investigated.

4. Conclusions

Isolated compounds and semi-synthetic derivatives from *Miconia ferruginata* were evaluated for their inhibitory activities towards cathepsins K and B and their cytotoxic activities in MTT assay against human tumor cells line: MDA-MB435, melanoma; HCT-8, colon and SF-295, glioblastoma. Among the compounds evaluated, the mixture of methyl ursolate (2a) and oleanate (3a) showed considerable cytotoxicity against melanoma and colon cancer cell lines. Finally, our data reinforce the importance of the compounds as a source of new chemotherapeutics, but additional studies are necessary to understand the mechanisms of their antitumor effects. The mixture of ursolic (2) and oleanolic (3) acids inhibited cathepsin B, while the mixture of compounds (2a) and (3a) showed considerable activity against cathepsin K. These results are important to aid the design of new inhibitors with enhanced potency and affinity towards the target enzyme.

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References

Biology acids, and ursolic acid derivatives. extract of antibacterial activity of the methylene chloride
G.; da Silva, R.; da Silva Filho, A. Evaluation of the
Tozatti, M. G.; Andrade e Silva, M. L.; Martins, C. H. C. U.; from plant species of the
Rodrigues, J.; Michelin, D. C.; Rinaldo, D.; Zocolo, N. C.; Dias, A. L. T.; Marques, M. J.; Soares, M. G. Leishmanicidal and antimicrobial activity of primin and
N. C.; Dias, A. L. T.; Marques, M. J.; Soares, M. G. Antimutagenicity of ursolic acid and oleanolic acid from