Leishmanicidal activity of onopordopicrin isolated from the leaves of Brachylaena discolor

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ABSTRACT

Brachylaena discolor (Asteraceae) is a medicinal plant used in Mozambique to treat stomachache, tuberculosis, and diabetes. To discover new lead compounds with leishmanicidal activity, a methanolic extract of the leaves of this plant was investigated. Through fractionation, employing several chromatographic methods, two sesquiterpene lactones onopordopicrin (1) and its germacroneolide epoxide derivative (2), together with other ten known phenolic compounds derivatives (3-12) were isolated. The structures of the isolated compounds were determined by spectroscopic methods, mainly 1D and 2D NMR experiments, as well as by comparing their spectroscopic data as reported in the literature. Compounds 1 and 2 were evaluated for their leishmanicidal activity using the Colorimetric method-XTT. Compound 1 showed a significant leishmanicidal activity against Leishmania amazonensis and L. braziliensis (IC50 values 39.6 and 27.9 μM, respectively) compared with miltefosine (12.5 and 12.0 μM, respectively), a currently used agent to treat leishmaniasis. While compound 2 was inactive against both stains tested with IC50 values >50μM compared with the same agent. Compounds 3-12 were not assayed for leishmanicidal activity. This is the first study reporting the above-mentioned activity of onopordopicrin (1). The results could suggest that compound 1 is a promising lead structure to treat leishmaniasis.

Keywords: Asteraceae, Brachylaena discolor, Onopordopicrin, Germanacronolides, Antileishmanial activity.

Introduction

Out of more than 5,500 plant species recorded in Mozambique, about 10% are used for medicinal purposes, including the treatment of infections and other diseases.1,2 Traditional medicine has remained as the most affordable and available source to solve health problems in rural communities all over the world. According to WHO, 80% of the world’s population still rely on such remedies for their basic healthcare needs. In addition, the knowledge provided by the traditional uses of plants to treat different illnesses has been helpful in the search for new biologically active metabolites.3,4 Based on such knowledge, phytochemical studies on Brachylaena (Asteraceae) species has led to the identification of a large number of sesquiterpene lactones. The sesquiterpene lactones are considered the characteristic secondary metabolites of the genus, exhibiting mainly antibacterial activity.5,6 The genus Brachylaena is composed of 13 to 20 plant species, mostly distributed in the Southern part of Africa.6,10 Brachylaena discolor D.C. (var. discolor) is an evergreen shrub or small tree usually 4 to 10 m in height. In Mozambique, the plant is found in the Southern part of the country and grows in coastal woodland and bushland, and on the margins of evergreen forests. The roots and leaves have been used for medicinal purposes for the treatment of stomachache, tuberculosis, and diabetes.4,11-13

Previous phytochemical investigation on the aerial parts of B. discolor (var. discolor) led to the isolation of a sesquiterpene lactone, onopordopicrin (1).5 In a continuing study of phytochemical constituents derived from Mozambican traditional medicinal plants, an extract of the leaves of B. discolor was investigated. In this paper, we wish to report the isolation and leishmanicidal activity of compound 1 and 2 assayed against Leishmania amazonensis and L. braziliensis, which is the first time compounds 1 and 2 are assayed for antileishmanial activity. The structure of compound (1) was established by high resolution NMR and MS experiments, and confirmed by the comparison of its experimental spectroscopic and physical data with those reported in the literature.14 Metabolite (1) has been found in larger amounts in various plants of the Asteraceae family15-18 and has been reported to possess cytotoxic and antibacterial activities.14,19

Materials and Methods

General Experimental Procedures

1D and 2D NMR spectra were recorded at room temperature with a Bruker Advance II 400 spectrometer. The chemical shifts (δ) are reported in ppm relative to solvent residual signals (δH 7.26 and δC 77.0 for CDCl3; δH 3.31 and δC 47.0 for CD3OD; δH 2.50 and δC 39.5 for DMSO-d6), while the coupling constants (J) are expressed in Hz. HRES-MS were performed with a Waters Acquity UPLC + Waters XEVO-G2 system spectrometer. The IR spectra data were recorded on a Bruker Alpha-P ART-IR spectrophotometer. The melting point measurements were carried out on a Gallenkamp instrument. The column chromatography (CC) was performed using silica gel 60 (230–400 mesh, Merck) and gel permeation on Sephadex LH-20 (GE-Healthcare)
Analytical TLC plates were visualized under UV lamp at 254 nm and spraying with vanillin followed by heating. All solvents used were of analytical grade.

**Leishmanial assay**

The activity was measured on in vitro cultures of Leishmania parasite in promastigote forms of complex L. amazonensis (clon 1: Lama, MHOM/BR/76/LTB-012) and complex L. braziliensis (strain M2904 C192 RIA), cultivated at 26°C in Schneider medium (pH 6.8) supplemented with inactivated (56°C x 30min) calf bovine serum (10%). Parasites in logarithmic phase of growth, at a concentration of 1×10^6 parasites/mL, were distributed on 96 micro well plates and different concentration of compounds 1 and 2 (100, 50, 25, 12.5, 6.2, 3.1, 1.5 μM) were added. The micro well plates were incubated for 72hrs at 26°C after which a solution of XTT (1mg/mL) in PBS (pH 7.0 at 37°C) with PMS (Sigma Aldrich, 0.06mg/mL), was added (50μL/well) and the incubation continued for 4hrs at 26°C. All assays were carried out as triplicates. DMSO (1%) and miltefosine were used as negative and positive control. The optical density of each well was determined with a Synergy HT microplate reader, at 490–540nm. The IC₅₀ values were calculated using the Gen5 program (BioTek).²⁰

**Plant material**

The leaves of B. discolor were collected in Magduse District, Maputo Province, Mozambique, in August 2014. The plant was identified locally and a voucher specimen under accession number 225 is kept at the Herbarium of the Botanical Garden of Eduardo Mondlane University.

**Extraction and isolation of compounds**

The air-dried and powdered leaves of B. discolor (350g) were extracted 3 times for 24h with 200ml MeOH. The combined extracts were evaporated to dryness under reduced pressure to give a crude MeOH extract (22.5g). This was redissolved in a mixture of CHCl₃/MeO (10:90) and then extracted with n-heptane, CHCl₃, and EtOAc to yield 10.5g, 1.1g, and 2.5g, respectively, of organic fractions. The EtOAc fraction (2.3g) was subjected to column chromatography (CC) on silica gel eluted with DCMe:EtOAc mixtures in different ratios (0-100%) and then EtOAc:MeOH (95:5) to yield 5 fractions (A-E). Fraction D (697mg) was subjected to Sephadex LH-20 chromatography eluted with a mixture of MeOH:CHCl₃ (1:1) to afford 5 subfractions (D1-D5). Subfraction D2 (304mg) was passed through Sephadex LH-20 using the same solvent system as previously employed to afford two main subfractions (D21 and D22). The subfraction D22 (95.0mg) was chromatographed on Sephadex LH-20 (100% MeOH) giving hydroxytyrosol (22.7mg), dihydroxyisinic acid (31.7mg) and 6'-O-acetyl homoplagmitin (2.6mg). After passing the fraction D5 (180.1mg) through Sephadex LH-20 (100% MeOH), quercitin-7-O-galactopyranoside (24.2mg), quercetin-3-O-glucoside-7,3'-diacetyl ether (13.9mg), quercetin-3-O-β-D-galactopyranoside (9.0mg), and eupafolin (7.0mg) were isolated. Fraction E (300 mg) was submitted to Sephadex LH-20 MeOH:CHCl₃ (1:1) to afford subfractions E1-E4. The Sephadex LH-20 (100%) fractionation carried out with subfraction E3 (145.2mg) resulted in the isolation of onoporidin (10.2mg). Fraction E4 (48.0mg) was subjected to Sephadex LH-20 fractionation with a mixture of MeOH:CHCl₃ (1:1) to yield 3'-hydroxykwanin (11.0mg), and luteolin (17.0mg). Fraction B (198.7 mg) was submitted to silica gel in a mixture of DCMe:EtOAc (0-100%) to give 1 (88.9 mg) and 2 (68.6mg) as the main components.

**Results and Discussion**

The leaves of B. discolor were air-dried at room temperature, and the dried leaves were macerated with MeOH. The crude methanolic extract was then fractionated between n-heptane, CHCl₃, and EtOAc. Repeated chromatographic purifications of the EtOAc extract yielded twelve known metabolites. Besides the isolation of compound (1), the sesquiterpene lactone germacroneol epoxide derivative (2) was isolated, along with the following phenolic compounds: hydroxytyrosol (3),[(3,12)-dihydroxyisinic acid (4),][[2,6']-O-acetyl homoplagmitin (5),][[23]-onoporidin (6),][24]-3'-hydroxykwanin (7),][23]-heteolin (8),][26]-quercetin 3-O-glucoside-7,3',4'-trimethyl ether (9),][27]-quercetin-3-O-β-D-galactopyranoside (10),][28]-eupafolin (11),][29]-quercetin-7-galactopyranoside (12).][30] The structures of the isolated compound are presented in Figure 1.

Compounds 1 and 2 were evaluated for their antileishmanial activity. Compound 1 showed a promising antileishmanial activity against L. amazonensis and L. braziliensis strains with IC₅₀ values of 39.6 and 27.9μM, respectively. Miltefosine used to treat leishmaniosis, with IC₅₀ values 12.5 and 12.0μM, respectively, was used as positive control. Interestingly, compound 2 was inactive against both strains tested according to table 1. The difference in activity of these two metabolites could be attributed to the presence of two α, β-unasaturated carbonyl functionality in 1, while 1 has two of these functionalities 2 has only one, in addition to an epoxide moiety. The presence of a second α,β-unasaturated carbonyl functionality in 1 could influence the activity, as such activated methylene groups are commonly identified as responsible for many biological effects as a consequence of their electrophilic reactivity as Michael acceptors, which can react with, for example, the third groups present in protein targets. This is also supported by the fact that dehydrozaluzanin C, another sesquiterpene lactone isolated from Mannocia maronii (Asteraceae) with two α,β-unasaturated carbonyl groups, inhibits the growth of leishmanial promastigotes at concentrations ranging between 10.0 to 44.6μM.³² The (2-hydroxy)methyl acrylate side chain in 1, as well as the number of α,β-unasaturated carbonyl groups may be implicated for other biological activities. For example, in comparison of antiproliferative activity the lack of the side chain of 1 drastically lowers its activity.³² Sesquiterpene lactones are bioactive secondary metabolites, which are found in a variety of plants. The biological activities they possess could be useful for a range of conditions, from skin ulcer to atherosclerosis, neurodegeneration, and even cancer. They have also been proposed as lead compounds for the design of new anti-inflammatory drugs.³⁴ This class comprises a large group with more than 5000 known metabolites, mostly found in the family Asteraceae.³³ Sesquiterpene lactones are derived from the mevalonic acid pathway, and metabolites 1 and 2 have a germacrone terpenoid skeleton.³⁴ Compounds 1 and 2 could therefore share the same biosynthetic pathway, possessing an additional and unusual four carbon atom unit. However, the detailed biosynthetic mechanism is still not understood.

**Ongopelogin (1):** Colorless oil: [α]D²⁵ + 17.2 (c = 1.00, CHCl₃), m.p. 57–59°C, HR-ESMS m/z: 349.1651 [M+H]+ (calc. for C₁₉H₂₃O₉, 349.1650), IR film (cm⁻¹) 3450 (OH), 2925 (methylene), 1755 (C=O, γ-lactone), 1705 (C=O, unsaturated ester); [H-NMR (400 MHz, CDCl₃)]: 5.02 (1H, dd, J = 11.1, 4.0 Hz, H-1), 2.24 (1H, m, H-2), 2.24 (1H, m, H-3), 2.62 (1H, m, H-3), 4.85 (1H, d, J = 10.0 Hz, H-5), 5.14 (1H, dd, J = 10.0, 6.0 Hz, H-6), 3.11 (1H, m, H-7), 3.19 (1H, dd, J = 10.0, 4.0 Hz, H-1'), 2.58 (1H, m, H-9), 2.67 (1H, m, H-9a, 2.67 (1H, m, H-9b), 2.69 (1H, s, H-10b), 3.19 (1H, m, H-10a), 4.75 (1H, brd, d = 3.6 Hz, H-14a), 4.07 (1H, brd, d = 15.0 Hz), 3.51 (2H, brd, d = 14.0 Hz, brd, d = 3.6 Hz, H-14). The activity was measured on in vitro cultures of Leishmania parasite material...
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Table 1: IC₅₀ values in µM of tested compounds.

<table>
<thead>
<tr>
<th>Sample</th>
<th>L. amazonensis clon 1</th>
<th>L. braziliensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 1</td>
<td>39.6</td>
<td>27.9</td>
</tr>
<tr>
<td>Compound 2</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Miltefone</td>
<td>12.5</td>
<td>12.0</td>
</tr>
</tbody>
</table>

Conclusion

In this study, compound 1 showed remarkable leishmanicidal activity as compared to the miltefone, a currently used agent in leishmaniosis treatment. Thus, contributing for the suggestion of B. discolor as a medicinal plant to treat leishmaniosis ailment, besides the known traditional uses.

The replacement of the secondary Michael acceptor group at C-8 on compound 1 by an epoxide group at same carbon atom on compound 2 seems to be responsible for the leishmanicidal activity in both strains tested. However, further studies are required to clearly define the molecular mechanism of action underlying the leishmanicidal activity in compound 1 and not observed in compound 2 in both L. amazonensis and L. braziliensis strains assayed.

Conflict of interest

The authors declare no conflict of interest.

Authors’ Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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