Cytotoxic and antiprotozoal compounds from *Piper cernuum*

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Abstract

In this work was conducted a bioactivity-guided fractionation of hexane extract from leaves of *P. cernuum,* aiming isolation of cytotoxic and antiprotozoal compound. Using several chromatographic and spectroscopic methods were purified/identified two sesquiterpenes (**1** and **2**) as lignans (**3** and **4**), being the first occurrence of **1 – 3** in *P. cernuum*.

Introduction

The *Piper* genus, typically found in tropical zones worldwide, is represented by approximately 2000 species[1]. Chemically, this genus was composed by different classes of natural products such amides, chromenes, benzoic acids etc, with pharmacological potential[2]. As part of our continuous studies aiming identification of antiprotozoal and cytotoxic derivatives from Brazilian plant species, the bioactive extracts from leaves from *P. cernuum* were chemically investigated using a bioactivity-guided approach.

Results and Discussion

Dried leaves (62.8 g) and branches (24.8 g) of *P. cernuum* were individually extracted using an automatized system (ASE350) to afford the respective hexane (2.35g and 0.42g) and MeOH (3.33g and 1.97g) crude extracts.

After evaluation, was detected cytotoxic potential (against B16F10Nex2 cells – murine melanoma) in hexane extract from leaves (HEL) and branches (HEB) as well as antiprotozoal activity (against promastigote forms of *Leishmania infantum*) in HEL. Initially, part of HEL (1.17 g) was subjected to fractionation over SiO_2 (hexane:EtOAc:MeOH) to afford 11 fractions (F1 – F11), in which cytotoxic and antiprotozoal activities were concentrated at F2 (43 mg), F7 (232 mg), F8 (144 mg) and F9 (59 mg), as showed in Table 1.

After NMR analysis, fraction F2 was composed by a mixture of **1** and **2** (dihydroagarofuran derivatives). Part of F7 (200 mg) was purified by CC over SiO_2 (hexane:EtOAc) to afford hinokinin (**3** – 30 mg) and cubebin (**4** – 60 mg), which NMR data was compared to the literature. Additionally, NMR and HPLC analysis of F8 and F9 indicated the presence of **3** and **4** in different proportions.

Table 1. Cytotoxic (tested at 200 μg/mL against B16F10Nex cells) and antiprotozoal (tested at 100 μg/mL against promastigotes of *L. infantum*) evaluation of fractions F1 – F11 from HEL of *P. cernuum*.

	Citotoxic viability (%)	Antiprotozoal evaluation (%)
F1	>100	0
F2	**10**	0
F3	>100	0
F4	80	0
F5	60	0
F6	40	**100**
F7	**10**	**100**
F8	**10**	**100**
F9	**10**	**90**
F10	25	0
F11	20	0

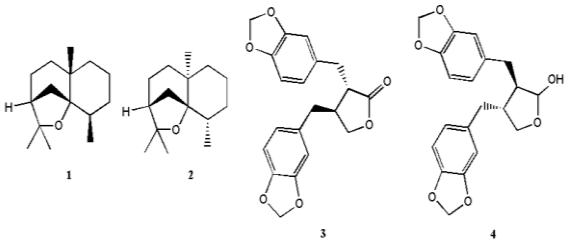

Figure 1. Compounds **1 – 4** isolated from bioactive HEL from *P. cernuum*.

As previously reported, compounds **3** and **4** displayed antiprotozoal activity[1], justifying the obtained results. Otherwise, this is the first occurrence of **1** and **2** in *P. cernuum* as well as the evaluation of their cytotoxic potentials against tumoral cells.

Conclusions

The present study aimed the identification of bioactive (cytotoxic and antiprotozoal) compounds from hexane extract from leaves of *P. cernuum*. Using a bioactivity-guided approach was possible the identification of two unusual sesquiterpenes (**1** and **2**) and two lignoids (**3** and **4**), being the first occurrence of **1 – 3** in *P. cernuum*.

Aknowledgements

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[2] Fernando, A.; et al. *Proc. Chem*. **2014**, *13*, 79-84.