

CONSTITUENTS OF *Hololepis pedunculata* LEAVES AND THEIR TRYPANOCIDAL ACTIVITY

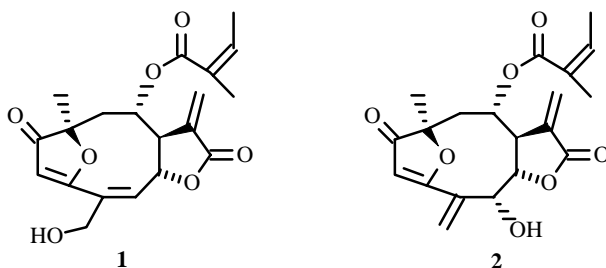
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Chagas' disease (caused by the protozoan haemoflagellate *Trypanosoma cruzi*) affects approximately 24 million people from Southern California to Argentina and Chile. Transmission by blood transfusion is an important source of infection in urban areas. Gentian violet is used to treat blood before transfusion, but is far from satisfactory for this purpose [1]. Searching for trypanocidal compounds to replace Gentian violet, an extensive *in vitro* screening of extracts of randomly collected species of Asteraceae family have been made [2]. Some results have already been published [3–5], and we reported here the phytochemical study of *Hololepis pedunculata*, one of these species that showed activity.

Aerial parts of *H. pedunculata* were collected at Serra do Cipo (Minas Gerais, Brazil) in April 1997 and identified by Prof. J. A. Lombardi. A specimen (voucher number 19089) is deposited in the Herbarium of the Instituto de Ciencias Biologicas of Universidade Federal de Minas Gerais.

The air dried plant leaves (200 g) were powdered and extracted successively with hexane:(C₂H₅)₂O (1:1) and C₂H₅OH:H₂O (7:3), at room temperature for five days. The residues, after solvents were removed, weighed 8.5 g and 65.0 g, respectively. The first one was submitted to silica gel column chromatography (170 g) and eluted with hexane, CH₂Cl₂, CH₃CO₂C₂H₅, and CH₃OH pures and with mixtures of increasing polarities. The fractions (57) were combined into 17 groups, and G5-G8 afforded friedelan-3 β -ol, α -friedelin, lupeol, α -amirin, and mixtures of sitosterol–stigmasterol and lupeyl–taraxasteryl acetates. G11 (2.5 g) was rechromatographed on a silica gel column (50 g) and eluted with CHCl₃–CH₃OH with increasing polarities. The fractions were assembled in 4 groups (G1'–G4'). G3' (700 mg) was submitted to flash chromatography (hexane–CH₃CO₂C₂H₅ 1:1) and two sesquiterpene lactones were isolated: centratherin or lychnophorolide A (1) [6], 25 mg, and isocentraterin (2) [7], 14 mg. According to Bohlmann [8], furanoheliangolides are considered one of the taxonomic markers of the Asteraceae family. However, these compounds were, to the best of our knowledge, not isolated before from this genus and species (studied here for the first time). G4' (100 mg) was rechromatographed under the same conditions as above and a mixture of goyazensolide [3] and 15-hydroxyeremantholide C [9], 6.6 mg, was obtained.



The 65 g of C₂H₅OH:H₂O extract was chromatographed on silica gel column eluted with CH₂Cl₂, CH₃CO₂C₂H₅, and CH₃OH pures and in mixtures of increasing polarities. Six groups of fractions were obtained. From G4'', after chromatography with silica gel, cellulose, and Sephadex LH-20, 3 mg of luteolin-7-*O*-rutoside [10] was isolated.

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TABLE 1. *In Vitro* Activity of Pure Sesquiterpene Lactones 1 and 2 Against the Trypomastigote Forms (Y Stain) of *Trypanosoma Cruzi*

Pure compound	Concentration (mol/L)×10 ⁻³	Result
1	4.0	A (DL)
	2.0	A (DL)
	1.0	A(DL)
2	2.8	A(DL)
	1.4	A(DL)
	0.7	I

A: absence of T. cruzi (active).

I: below 50% of trypomastigotes killed (inactive).

DL: discrete lysis of erythrocytes.

Controls used: DMSO (inactive) and Gentian Violet (active).

The bioassay using the Y strain of trypomastigote forms of *Trypanosoma cruzi* was carried out according to the method described by Chiari *et al.*[2]. G11 and G3' (from G11) showed activity in all tested concentrations but presented lysis of erythrocytes. Centratherin (**1**) killed all trypanosomas in all tested concentrations; isocentratherin (**2**) showed activity at two higher concentration, both showed only discrete lysis of erythrocytes (Table 1). The trypanocidal activity of isocentratherin is, to our best knowledge, reported here for the first time.

The mechanism of trypanocidal activity of sesquiterpene lactones is associated (as for antitumor activity) with the presence of the double bond conjugated with the carbonyl compound in the lactone ring [11].

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