

ANTI-GIARDIAL ACTIVITY OF THE HEXANE EXTRACT OF *MAYTENUS GONOCLADA* MART.FERNANDO C. SILVA^{1,2*}, HAENDEL G. N. O. BUSATTI², MARIA A. GOMES³, LUCIENIR P. DUARTE¹, GRÁCIA D. F. SILVA¹ AND SIDNEY A. VIEIRA FILHO⁴

¹Departamento de Química, Universidade Federal de Minas Gerais, Belo Horizonte MG, Brazil, ²Faculdade de Farmácia, Universidade de Itaúna, Itaúna MG, Brazil, ³Departamento de Parasitologia, Universidade Federal de Minas Gerais, Belo Horizonte MG, Brazil, ⁴Departamento de Farmácia, Universidade Federal de Ouro Preto, Ouro Preto MG, Brazil.

Email: fcsquimico@ufmg.br, fcsquimico@yahoo.com.br

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ABSTRACT

Plants of the *Maytenus* genus have been used in folk medicine in Brazil for treatment of gastrointestinal motility disturbances such as diarrhea. The present study was performed to evaluate anti-giardial activity of the hexane extract and triterpenes isolated from the branches of *Maytenus gonoclada* Mart. (Celastraceae). The hexane extract and triterpenes were examined for anti-giardial activity using the colorimetric method against *Giardia lamblia* (ATCC 30888). The hexane extract exhibited anti-giardial activity. This result may partly explain and support the use of plants of the *Maytenus* genus for the treatment of diarrhea in folk medicine in Brazil.

Keywords: Celastraceae, *Maytenus gonoclada*, *Giardia lamblia*, Diarrhea.

INTRODUCTION

Giardia lamblia is a cosmopolitan protozoan that causes intestinal infections in humans, known as giardiasis^{1,2}. Symptomatic disease is characterized by diarrhea, and weight loss³. *Giardia* infection occurs by eating food or drinking water that has been contaminated by the organism -usually from stool. Giardiasis has been included in the WHO Neglected Diseases Initiative⁴. In underdeveloped countries, the prevalence of giardiasis ranges from 20 to 60%, and the estimate is that there are about 200 million symptomatic cases in Africa, Asia, and South America⁵.

The main drugs used to treat this illness are metronidazole, furazolidone, benzimidazoles, and nitazoxanide. Although these drugs are effective, most of them have side effects such as headache and nausea beyond this many microorganisms already are resistant to these drugs³. Recently, many others drugs have been developed, and various synthetic and natural substances have been tested in the search for new compounds giardicidal^{6,7}.

Plants of the *Maytenus* genus have been used in folk medicine in Brazil and other countries to treat a variety of illnesses. Crude ethanol extract of *Maytenus rigida* barks showed anti-diarrheal activity⁸. The components of *Maytenus ilicifolia* have potential use in the treatment of diarrhea⁹. These results support the popular applications of *Maytenus* genus for the treatment of diarrhea in Brazilian folk medicine.

As part of our search for bioactive compounds from Brazilian medicinal plants, we have investigated *Maytenus gonoclada* Martius (Celastraceae), that is commonly known as "tuzinho", can be found in regions of "cerrado" and rupestrian fields of Southeastern and Northeastern Brazil. In our previous studies, the occurrence of six pentacyclic triterpenes of the friedelane series and fungal activity of a triterpene was reported¹⁰. The objective of the study was to determine the anti-giardial potential of the hexane extract and triterpenes isolated from the branches of *M. gonoclada*. The triterpenes tested were 3-oxofriedelane (1), 3 β -hydroxyfriedelane (2), 3,11-dioxofriedelane (3), 3,16-dioxofriedelane (4) e 3-oxo-12 α -hydroxyfriedelane (5) (Figure 1). The compounds (1) and (2), (3) and (4) were tested as mixture.

MATERIALS AND METHODS

Plant material

Branches of *Maytenus gonoclada* Mart. (Celastraceae) was collected in October 2004, in Serra da Piedade, Caeté, Minas Gerais, Brazil. The specie was identified by Profa. Dra. Rita Maria Carvalho-Okano from the Departamento de Biologia Vegetal da Universidade Federal de

Viçosa (Viçosa, Minas Gerais, Brazil). A specimen is deposited in the Herbarium of the Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, under the voucher number 60280.

Preparation of extract

The air-dried powdered branches were packed in Soxhlet apparatus and extracted with hexane for 72 h. The extract was dried at 60 °C in vacuum distillation till it was condensed. A hot air oven was used at 50 °C till solid mass was obtained.

Phytochemical investigation

Preliminary phyto-chemical tests were carried out with branches of hexane extract of *M. gonoclada* for the isolation of constituents like pentacyclic triterpenes of the friedelane skeleton¹⁰.

Cultures and growth conditions

G. lamblia strain Portland (ATCC 30888) was used in all experiments. It was maintained axenically at 37 °C in Diamond's modified TYI-S-33 medium supplemented with heat inactivated bovine serum at 10 %¹¹. To quantify the sample's action, 4 x 10⁵ trophozoites of *Giardia* were grown in culture plates of 24-well (Nunc) in CO₂ atmosphere at 37 °C for 24 h¹².

Anti-giardial Assay

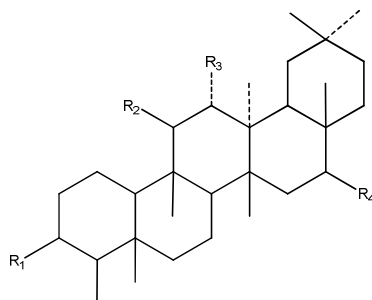
The hexane extract and triterpenes were dissolved in 1 mL of dimethylsulfoxide (DMSO) to reach 5 to 40 mg/ml. Of these solutions, 100 μ L were added to 10 mL of culture medium to the final concentration ranging from 0.05 to 0.4 mg/mL. Aliquots of these mentioned solutions were added to the plates containing trophozoites to reach the final concentration of 0.086 to 50 μ mol/L. After incubation, plates were washed with buffered saline pH 7.2, and then, the attached trophozoites were fixed with methanol. This way, cells that were kept in the plates were quantified by colorimetric assay with methylene blue¹⁰. Absorbance was determined in 655 nm in a microplate reader (Bio-RAD, Model 3550). Metronidazole was used as the reference drug, culture medium with trophozoites and DMSO was the negative control, and culture medium was the blank. The 50 % inhibitory concentration (IC₅₀) was determined for each sample and meant that the minimal concentration inhibits 50% of the culture growth. Experiments were performed in triplicate and repeated twice.

RESULTS

Hexane extract showed high giardicidal activity (IC₅₀ of 1.02 μ g/mL). The giardicidal activity of the hexane extract appeared to be dose-

dependent, because the concentration of $9.28 \pm 0.03 \mu\text{g/mL}$ inhibited about 90% of the culture growth (Table 1). However the triterpenes, 3-oxofriedelane (1), 3 β -hydroxyfriedelane (2), 3,11-

dioxofriedelane (3), 3,16-dioxofriedelane (4) e 3-oxo-12 α -hydroxyfriedelane (5) isolated from the hexane extract did not showed effect on the giardia cultures (data not showed).



Subst.	R ₁	R ₂	R ₃	R ₄
1	=O	H	H	H
2	—OH	H	H	H
3	=O	=O	H	H
4	=O	H	H	=O
5	=O	H	—OH	H

Fig. 1: Chemical structures of the friedelanes isolated from branches of *M. gonoclada*.

Table 1: Inhibition growth of *Giardia lamblia* by hexane extract

Hexane extract ($\mu\text{g/mL}$)	Inhibition (%)
0.14 \pm 0.06	12.72 \pm 0.06
0.29 \pm 0.02	14.59 \pm 0.02
0.58 \pm 0.03	34.75 \pm 0.03
1.16 \pm 0.05	57.12 \pm 0.05
2.32 \pm 0.07	79.04 \pm 0.07
4.64 \pm 0.08	87.17 \pm 0.08
9.28 \pm 0.03	89.89 \pm 0.03

DISCUSSION

Antigiardial activities have been described for pentacyclic triterpenes, such as quinonamethides³. It is speculated that the mechanism of action of triterpenes is due to a disruption on the microorganism's cellular membrane¹³. The hexane extract showed interesting giardicidal effect, signaling to the stratification of compounds of extract as a mean to obtain more active substances. Five friedelane triterpenes were purified. Unfortunately these compounds showed no activity in the anti giardial test, probably because of low polarity of these compounds that not disperse in the culture medium¹⁰. The development of other solvents it is necessary, sincethese compounds may have potential giardicidal property. Another hypothesis is the possible synergistic associations of friedelane triterpenes for the giardicidal properties of the hexane extract of branches from *Maytenus gonoclada*.

CONCLUSION

Plants belonging to the *Maytenus* genus are routinely used in folk medicine to treat a variety of illnesses. This genus is a source of important bioactive secondary metabolites. Among the compounds isolated from their species, triterpenes are of great medicinal interest. The hexane extract exhibited significant anti giardial activity. These results may partly explain and support the use of plants of the *Maytenus* genus for the treatment of diarrhea in Brazilian folk medicine.

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