INTRODUCTION

Giardia lamblia is a cosmopolitan protozoan that causes intestinal infections in humans, known as giardiasis. Symptomatic disease is characterized by diarrhea, and weight loss. 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 Initiative. 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 America.

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. However, many other drugs have been developed, and various synthetic and natural substances have been tested in the search for new compounds. Antigiardial Activity of the Hexane Extract of Maytenus gonoclada Mart. (Celastraceae). The hexane extract and triterpenes were examined for antigiardial activity using the colorimetric method against Giardia lamblia (ATCC 30888). The hexane extract exhibited antigiardial 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.

Key words: Celastraceae, Maytenus gonoclada, Giardia lamblia, Diarrhea.

MATERIALS AND METHODS

Plant material

Branches of Maytenus gonoclada Mart. (Celastraceae) were collected in October 2004, in Serra da Piedade, Caeté, Minas Gerais, Brazil. The species 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 skeleton.

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 %. To quantify the sample's action, 4 x 10⁶ trophozoites of Giardia were grown in culture plates of 24-well (Nunc) in CO₂ atmosphere at 37 °C for 24 h.

Antigiardial 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 μL 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 0.886 to 50 μmol/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 blue. 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 μg/mL). The giardicidal activity of the hexane extract appeared to be dose-
inhibited about 90% of the culture growth (Table 1). However the triterpenes, 3-oxofriedelane (1), 3β-hydroxyfriedelane (2), 3,11-dioxofriedelane (3), 3,16-dioxofriedelane (4) e 3-oxo-12α-hydroxyfriedelane (5) isolated from the hexane extract did not show effect on the giardia cultures (data not showed).

![Chemical structures of the friedelanes isolated from branches of M. gonoclad.](image)

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

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

<table>
<thead>
<tr>
<th>Hexane extract (µg/mL)</th>
<th>Inhibition (%)</th>
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</thead>
<tbody>
<tr>
<td>0.14 ± 0.06</td>
<td>12.72 ± 0.06</td>
</tr>
<tr>
<td>0.29 ± 0.02</td>
<td>14.59 ± 0.02</td>
</tr>
<tr>
<td>0.58 ± 0.03</td>
<td>34.75 ± 0.03</td>
</tr>
<tr>
<td>1.16 ± 0.05</td>
<td>57.12 ± 0.05</td>
</tr>
<tr>
<td>2.32 ± 0.07</td>
<td>79.04 ± 0.07</td>
</tr>
<tr>
<td>4.64 ± 0.08</td>
<td>87.17 ± 0.08</td>
</tr>
<tr>
<td>9.28 ± 0.03</td>
<td>89.89 ± 0.03</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Antigiardial activities have been described for pentacyclic triterpenes, such as quinoxamethides. It is speculated that the mechanism of action of triterpenes is due to a disruption on the microorganism’s cellular membrane. The hexane extract showed interesting giardical 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 antigiardial test, probably because of low polarity of these compounds that not disperse in the culture medium. The development of other solvents is necessary, since these compounds may have potential giardical property. Another hypothesis is the possible synergistic associations of friedelane triterpenes for the giardical properties of the hexane extract of branches from *Maytenus gonocladia.*

**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 antigiardial activity. These results may partly explain and support the use of the *Maytenus* genus for the treatment of diarrhea in Brazilian folk medicine.

**REFERENCE**