Crude Ethanolic Extract, Lignoid Fraction and Yangambin from *Ocotea duckei* (Lauraceae) Show Antileishmanial Activity

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Crude ethanolic extract, lignoid fraction and the purified compound yangambin were obtained from *Ocotea duckei* (Lauraceae) and their antileishmanial activity was tested against promastigote forms of *Leishmania chagasi* and *Leishmania amazonensis* cultivated in Schneider medium, supplemented with 20% of fetal bovine serum. All substances presented antileishmanial activity with IC_{50} values of 135.7 μg/mL for the crude ethanolic extract, 26.5 μg/mL for the lignoid fraction and 49.0 μg/mL for yangambin on *L. chagasi*. For *L. amazonensis* the IC_{50} values were 143.7 μg/mL, 48.2 μg/mL and 64.9 μg/mL for the crude ethanolic extract, the lignoid fraction, and the purified compound yangambin, respectively. The crude ethanolic extract, lignoid fraction, and yangambin caused an inhibition higher than Glucantime®, a reference drug used for the treatment of leishmaniasis.

Key words: Leishmania, *Ocotea duckei*, Antileishmanial Activity

Introduction

Leishmaniasis constitutes a complex group of infective parasitic diseases caused by protozoans of the genus *Leishmania*, which are distributed in tropical and subtropical regions of the world. Data from WHO reveal that leishmaniasis threatens 350 million people of 88 countries, of which 72 are developing countries, totalling 2 million new cases and 57 thousand deaths per year (World Health Organization, 2004). There are regions in Brazil where these illnesses are endemic, spreading over urban and suburban areas, becoming a serious public health problem (Brandão-Filho and Shaw, 1994).

The clinical forms of leishmaniasis depend on the association among the virulent characteristics of the infective *Leishmania* and host susceptibility to the illnesses (Pearson and Souza, 1996). Leishmaniasis is categorized as cutaneous, mucocutaneous, diffuse cutaneous, and visceral. This latter occurs when the parasite migrates to liver, spleen, lymphonodes, and bone marrow. In Brazil, *Leishmania* (*Leishmania*) *chagasi* has been deemed as the main causative agent of the visceral form of leishmaniasis, while *Leishmania* (*L.*) *amazonensis* is more associated with the cutaneous forms of the disease (Marzochi and Marzochi, 1994).

The chemotherapy of leishmaniasis is based on the administration of pentavalent antimonials (Glucantime® and Pentostan®), pentamidine, or amphotericin B (Berman, 1997). These chemical agents have shown to generate a high rate of toxicity in humans and augmentation of parasites resistance to the drugs (Boelaert *et al.*, 2002; Lira *et al.*, 1999).

The drugs are parentally injected into the patient and a clinical supervision throughout the high cost treatment is always needed. These drawbacks show the urgent need of new therapeutic agents for the treatment of leishmaniasis.

The popular use of plants has been widely practiced against many parasitoses including leishmaniasis (Franca *et al.*, 1996). Several compounds isolated from plants have already been identified as a antileishmanial agents, a fact showing that plants yet to be investigated represent an important source of new drugs against parasitoses (Rocha *et al.*, 2005; Rosa *et al.*, 2003; Carvalho and Ferreira, 2001; Araújo *et al.*, 1998). The aim of the present work is to identify compounds having therapeutic potential against leishmaniasis by investigating the activity of derivatives of compounds extracted from *Ocotea duckei* (Lauraceae) on promastigote forms of *Leishmania* (*L.*) *chagasi* and *Leishmania*

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(*L.*) *amazonensis*. Derivatives of the compounds isolated from this plant, known popularly in Brazil as 'louro-de-cheiro', have been found to exhibit different biological activities (Araújo *et al.*, 2001; Herbert *et al.*, 1997; Serra *et al.*, 1997; Dias *et al.*, 2003).

Materials and Methods

Plant material

The plant used in the present study, *Ocotea duckei* Vattimo (Lauraceae), was collected in March 2004 near the city of Santa Rita, State of Paraíba, Brazil, and the material was identified by Dr. Maria de Fátima Agra, Botany Sector of the Laboratory of Pharmaceutical Technology (LTF), João Pessoa, PB, Brazil. A voucher specimen is deposited in the Herbarium Prof. Lauro Pires Xavier (registry no. JPB-4309) of the Systematic and Ecology Department, Federal University of Paraíba. The crude ethanolic extract (CEE) used in the experiments was obtained from the stem bark and leaves of the plant. The lignoid fraction (LF) and the purified yangambin (Yg) [7*S*,7′*S*-di-(3,4,5-trimethoxyphenyl) furofuran] (Fig. 1) were

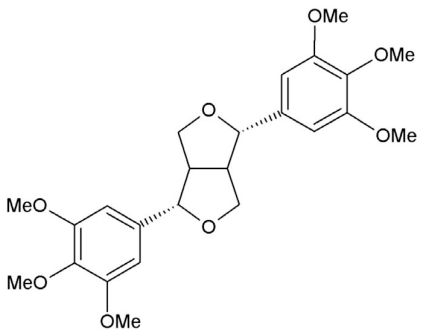

Fig. 1. Chemical structure of yangambin [7*S*,7′*S*-di-(3,4,5-trimethoxyphenyl) furofuran], a furofuran lignan found in the lignoid fraction obtained from *Ocotea duckei* Vattimo (Lauraceae).

obtained as described by Barbosa-Filho *et al.* (1999). The ethanolic extract, the lignoid fraction, and the purified compound yangambin were diluted in dimethyl sulfoxide (DMSO; Vetec, Brazil). A final content of DMSO of 1.0% in the test solution was shown to have no effect on the parasite growth by including solvent controls alongside the tests.

Reference drug

Meglumine antimoniate (Glucantime®; Aventis Pharma, Brazil) was utilized as the reference drug for comparison with the results obtained from the plant derivatives used here. The drug was supplied by the Academic Hospital Lauro Wanderley of the Federal University of Paraíba, João Pessoa, Paraíba, Brazil.

Parasite culture

Promastigote forms of *Leishmania chagasi* (MCAN/BR/99/JP15) were isolated from a dog spleen, an animal that was captured in João Pessoa and clinically diagnosed as having visceral leishmaniasis (Rocha *et al.*, 2004). *Leishmania amazonensis* (IFLA/BR/67/PH8) promastigotes were kindly given by Dr. Maria Norma Melo (Federal University of Minas Gerais, Brazil). The parasites are deposited in the cryobank of Federal University of Minas Gerais, Brasil.

The promastigote forms of *Leishmania* were maintained in agar-blood culture medium, 'Novy & MacNeal-Nicolle' (NNN) associated to the Schneider medium, supplemented with 20% (v/v) of heat-inactivated fetal bovine serum (FBS), streptomycin (50 μg/mL), and penicillin (1000 U/mL) at 26 °C.

Evaluation of antileishmanial activity

L. chagasi and *L. amazonensis* promastigotes ($1 \cdot 10^6$ parasites/mL) were incubated at 26 °C for 72 h in Schneider medium supplemented with 20% FBS in the presence of several concentrations of the CEE, LF and the purified compound Yg. The growth of promastigote forms was evaluated simultaneously in the presence of Glucantime®. Controls were performed using cultures in the presence or absence of DMSO and the parasites alone.

In order to estimate cell growth, aliquots were taken and diluted in isoton solution (10.5 g of citric acid plus 7.0 g of NaCl, 5.0 mL of formalin, and 1000 mL of distilled water), being further quantified in a Neubauer counting chamber. Growth inhibition was calculated by comparing the number of cells obtained from control to the number of cells in the presence of the tested compounds. The antileishmanial activity is expressed as IC_{50}/72 h, which is the concentration that causes 50% of reduction in culture growth. All experiments were

done at least twice and each experiment was performed in triplicate.

Statistical analysis

Possible differences among the assays were evaluated by using Student's *t*-test. The values of $P \leq 0.05$ were considered as significant. The IC_{50} values were obtained by the probit model of regression using the SPSS package program.

Results and Discussion

The obtained products from *Ocotea duckei* presented antileishmanial activity against promastigote forms of *L. chagasi* and *L. amazonensis* (Fig. 2). The inhibition of promastigotes growth caused by the CEE, LF, and Yg was statistically larger than that caused by Glucantime®. The IC_{50} values for *L. chagasi* were 135.7 μg/mL for the crude ethanolic extract, 26.5 μg/mL for the lignoid

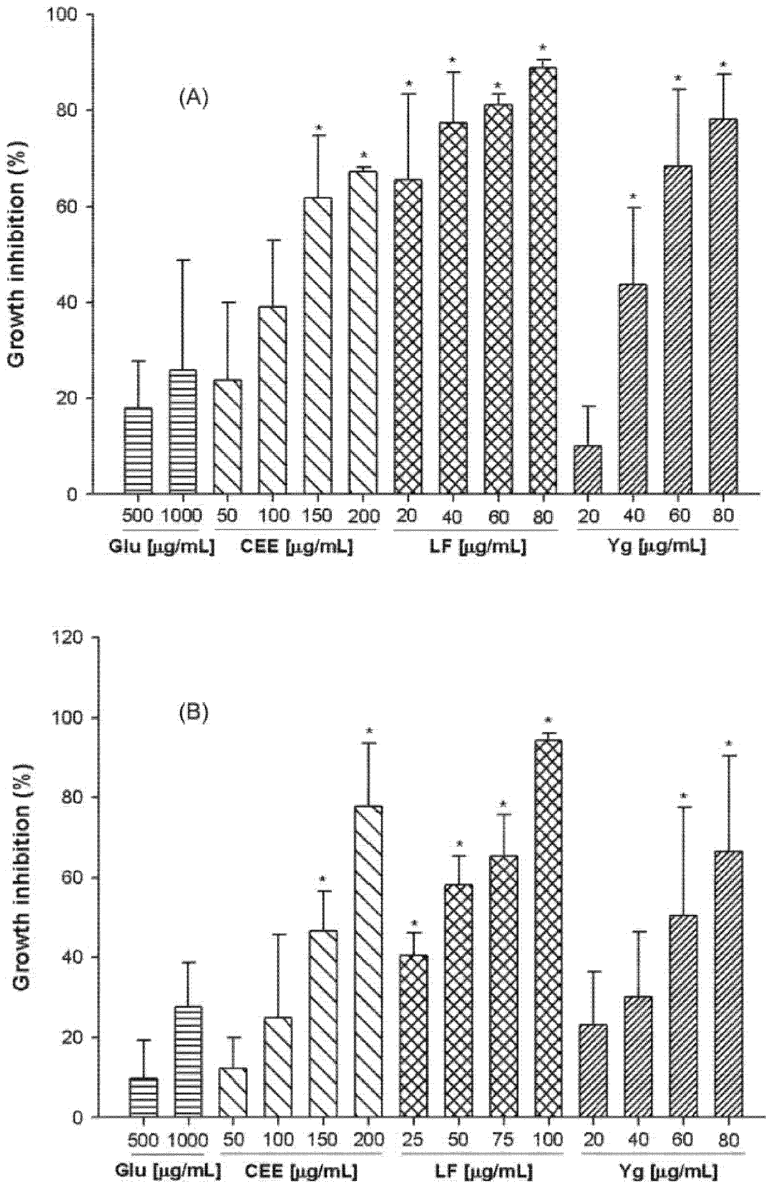

Fig. 2. Effect of different concentrations of crude ethanolic extract (CEE), lignoid fraction (LF) and the purified compound yangambin (Yg) obtained from *Ocotea duckei* against promastigote forms of *Leishmania chagasi* (A) and *Leishmania amazonensis* (B) in comparison with Glucantime® (Glu). The parasites were cultivated in Schneider medium at 26 °C for 72 h. The initial number of cells was $1 \cdot 10^6$ promastigotes/mL. The columns represent the mean of two independent experiments performed in triplicate. * $P \leq 0.05$ (in relation to Glucantime®).

Table I. Antileishmanial activity of crude ethanolic extract, lignoid fraction and the purified compound yangambin. IC_{50} values obtained from a minimum of two separate experiments performed in triplicate are shown.

Drug	IC_{50} [μg/mL]	
	L. chagasi	*L. amazonensis*
Crude ethanolic extract	135.7	143.7
Lignoid fraction	26.5	48.2
Yangambin	49.0	64.9

fraction, and 49.0 μg/mL for yangambin. For *L. amazonensis* the IC_{50} values were 143.7 μg/mL, 48.2 μg/mL, and 64.9 μg/mL for the crude ethanolic extract, the lignoid fraction, and the purified compound yangambin, respectively (Table I).

The lignoid fraction showed high activity against *L. chagasi* and *L. amazonensis* (Fig. 2). The inhibition of *Leishmania* promastigotes growth caused by the lignoid fraction might be partly due to yangambin, since this is the most concentrated lignan found in that fraction (Barbosa-Filho *et al.*, 1999). However, other lignans present in the LF like sesartemin, 4′-*O*-demethylepiyangambin, and syringaresinol might enhance the antileishmanial effect.

Antileishmanial activity of the lignan yangambin has not yet been reported, but other pharmacological activities have been reported with respect to its anticonvulsive (Pachú *et al.*, 1993), analgesic (Almeida *et al.*, 1995), and antiallergic (Serra *et al.*, 1997) effects.

Glucantime® has been a first choice drug employed for treating leishmaniasis in Brazil despite its serious side effects (Boelaert *et al.*, 2002). Therefore, it is quite important to search for new efficacious antileishmanial drugs that may have low toxicity for the mammalian hosts. Assays on toxicity of the lignan yangambin in mice did not show any lethal effect up to 48 hours after the treatment, when using a 1 g/kg dose (Pachú *et al.*, 1993). Recent studies on its mutagenic potential showed that this lignan at contents of up to 1.5 mg/plate did not induce mutations of the bacterium *Salmonella typhimurium* (Marques *et al.*, 2003). These data associated to the antileishmanial effect of the lignoid fraction and yangambin observed in the present work reinforce the therapeutic potential of this class of compounds.

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