Received 3 Dec 2010; Accepted 26 Mar 2010; Available online 29 Oct 2010.

Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
20(6): 1003-1006, Dez. 2010

Short Communication

Antileishmanial activity of 3-(3,4,5-trimethoxyphenyl) propanoic acid purified from Amazonian *Piper tuberculatum* Jacq., Piperaceae, fruits

Maria Goretty P. R. Ferreira,[#,1] Anderson M. Kayano,[2] Izaltina Silva-Jardim,[1] Tatiany O. da Silva,[1] Juliana P. Zuliani,[1,2] Valdir A. Facundo,[3] Leonardo de A. Calderon,[1,2] Alexandre de Almeida-e-Silva,[1,4] Pietro Ciancaglini,[5] Rodrigo G. Stábeli[*,1,2]

[1]Instituto de Pesquisas em Patologias Tropicais de Rondônia, Fundação Oswaldo Cruz, Fiocruz Noroeste, Rua da Beira 7671, BR364, km 3.5, 76812-245 Porto Velho-RO, Brazil,
[2]Centro de Estudos de Biomoléculas Aplicadas a Medicina Prof. Dr. José Roberto Giglio, Núcleo de Saúde, Universidade Federal de Rondônia, BR 364, km 9.5, 76800-000 Porto Velho-RO, Brazil,
[3]Laboratório de Química Orgânica, Núcleo de Ciência e Tecnologia, Universidade Federal de Rondônia, BR 364, km 9.5, 76800-000 Porto Velho-RO, Brazil,
[4]Laboratório de Bioecologia de Insetos, Núcleo de Ciência e Tecnologia, Universidade Federal de Rondônia, BR 364, km 9.5, 76800-000 Porto Velho-RO, Brazil,
[5]Departamento de Química, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, 14040-901 São Paulo-SP, Brazil.

RESUMO: "Atividade antileishmania do 3,4,5-trimetoxi-dihidrocinâmico purificado das frutas de *Piper tuberculatum* Jacq., Piperaceae, amazônica". A atividade leishmanicida do ácido 3,4,5-trimetoxi-dihidrocinâmico (TMPP) isolado do extrato hidroalcoólico de frutos de *Piper turbeculatum* Jacq. amazônica foi testado em ensaios *in vitro* utilizando formas promastigotas de *Leishmania amazonensis*. O TMPP foi utilizado em culturas de *L. amazonensis* nas concentrações de 1600 a 6,25 µg/mL. A viabilidade celular das formas promastigotas foi observada em 24, 48, 72 e 96 h para cálculo da CI50. O TMPP apresentou efeito leishmanicida dose dependente para as formas promastigotas de *L. amazonensis* apresentando CI50 de 145 µg/mL.

Unitermos: *Piper tuberculatum* Jacq., Piperaceae, ácido 3,4,5-trimetoxi-dihidrocinâmico, *Leishmania amazonensis*, atividade leishmanicida.

ABSTRACT: Leishmanicidal activity of the 3-(3,4,5-trimethoxyphenyl) propanoic acid (TMPP) isolated from EtOH extracts of the Amazonian *Piper turbeculatum* Jacq. fruits was evaluated *in vitro* using *Leishmania amazonensis* promastigotes. The TMPP was assayed at concentrations of 1600 to 6.25 µg/mL for 24, 48, 72 and 96 h. Promastigotes viability was analyzed and the IC50 of TMPP was 145 µg/mL.

Keywords: *Piper tuberculatum*, Piperaceae, 3-(3,4,5-trimethoxyphenyl) propanoic acid, *Leishmania amazonensis*, leishmanicidal activity.

INTRODUCTION

Leishmaniasis is a broad group of parasitic diseases with a wide spectrum of morbidity. It is currently estimated that 350 million people are at risk, and that more than two million new cases occur annually (Reithinger et al., 2007). Leishmaniasis treatments present many problems such as potentially toxic drugs with parenteral administration, several collateral effects, resistance to medication and the need for long term treatment (Santos et al., 2008).

The above mentioned, in addition to the emergence of drug resistance and the absence of oral preparations, encourages innovation in the development of new drugs against leishmaniases parasites. Many researches have been done in order to find new compounds from the biodiversity with potential leishmanicidal activity (Calderon et al., 2009; Rocha et al., 2009; Flores et al., 2008; Paula-Júnior et al., 2006).

Thus, *Piper* is a genus that is both economically

**E-mail: stabeli@fiocruz.br, Tel. +55 69 3219 6010, Fax.+55 69 3219 6000.*

ISSN 0102-695X
DOI: 10.1590/S0102-695X2010005000033

and ecologically significant and includes a fascinating array of vegetal species for the study of natural products in organic chemistry, pharmaceutics products, and ethnobotany. The Piper genus includes more than a hundred species and is one of the largest genera of basal angiosperms distributed pantropically as shrubs, herbs, and lianas. The greatest diversity of *Piper* species occurs in the tropics of the Americas (about 700 spp.), followed by Southern Asia (300 spp. approximately) (Jaramillo & Manos, 2001).

Extracts from *Piper* are employed by people throughout the tropics for many purposes, such as antimicrobial, antifungal , antipyretic, aromatic, diuretic, emetic, fish bait, food, hallucinogen, ornamental, perfume, spices, styptics, sudorific, as well as many other medicinal recipes (Wadt et al., 2004). These uses demonstrate some of the array of biological properties of the chemicals present in these species, which have potential pharmacological applications (Kato & Furlan, 2007). Most of the compounds isolated from the *Piper* species are essential oils, lignans, phenylpropanoids, and sesquiterpenes (Facundo et al., 2008). Some of these molecules have economic value, such as sassafras oil or Safrole, an important raw material for the chemical industry (Rocha & Ming, 1999).

The *Piper tuberculatum* Jacq., Piperaceae, is popularly known in the Northeast of Brazil as "pimenta-longa" or "pimenta-d'arda" (Facundo et al., 2008). Local communities have traditionally used this species as an anesthetic sedative for toothache, antidote for snake bite, stimulants, and stomach problems (Chaves et al., 2003). Recent studies showed that different extracts and isolated molecules from *P. tuberculatum* present antifungal (Lago et al., 2004), antitumor (Bezerra et al., 2006), antiplatelet aggregation (Fontenele et al., 2009), insecticide (Pohlit et al., 2004) and hypotensive properties (Duarte et al., 2004).

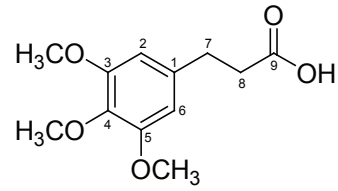
In this short communication we report the preliminary *in vitro* antileishmanial activity of 3-(3,4,5-trimethoxyphenyl) propanoic acid (TMPP) purified from *Piper tuberculatum* Jacq fruits.

MATERIAL AND METHODS

Fresh fruits of *Piper tuberculatum* Jacq., Piperaceae, were collected around the city of Porto Velho-RO, Brazil (Southwestern Amazon). A voucher specimen (211724) was deposited in the herbarium of the Instituto Nacional de Pesquisas da Amazônia, Manaus-AM, Brazil. The fruits (1.5 kg) were dried, powdered and extracted with 95% EtOH (3x 1.0 L). The extract was concentrated, delipidated by *n*-hexane, and partitioned with EtOAc. The EtOAc layer was concentrated and the fractions were chromatographed on silica gel column (200-300 mesh, 80 g) and eluted with *n*-hexane and subsequently by *n*-hexane/EtOAc mixtures (19:1, 9:1 and 2:1, v/v, increasing polarity) to give more eighteen fractions. Fraction 12 (124.2 mg) was re-chromatographed in silica gel (20 g) eluted with *n*-hexane/EtOAc (4:6) and yielded the purified compound TMPP (56.5 mg). The chemical identity of the 3-(3,4,5-trimethoxyphenyl) propanoic acid (**1**) was performed on the basis of their ESI-MS,1D and 2D NMR data according to Facundo et al. (2008). TMPP (10 mg) was diluted in 2000 µL of 20% dimethyl sulfoxide (DMSO) in phosphate buffer solution (PBS) and used in leishmanicidal assay.

The promastigotes of *Leishmania amazonensis* PH8 strain (IFLA/BR/67/PH8) were harvested on the 5th day of growth (stationary phase) for the *in vitro* assays. The parasites (5x 10^5 per well) were incubated at 24 ºC for 96 h in duplicate on RPMI culture medium with or without 1600, 800, 400, 200, 100, 50, 25.5, 6.25 µg/mL of TMPP. Viability of parasites was determined daily by erythrosin B (0.04% in PBS) dye exclusion.

All experiments were performed in duplicate, and the results expressed as logarithmic number of cells per milliliter and as a percentage of growth inhibition. Two positive controls were prepared with 1600 µg/mL Pentamidine® (pentamidine isethionate) and 1600 µg/mL Glucantime® (meglumine antimoniate). Inhibitory Concentrations (IC) were calculated by Probit Analysis (Minitab 14).



1

RESULTS AND DISCUSSION

The 3-(3,4,5-trimethoxyphenyl) propanoic acid (**1**) (TMPP) interfered with the promastigote replication. On the third day of incubation, a significant decrease in the parasite growth induced by TMPP (1600 and 800 µg/mL) and the presence of some dead parasites was observed. After 96 h of incubation, the calculated inhibitory concentrations were 145 µg/mL and 703 µg/mL for IC50 and IC90, respectively. The Pentamidine® eliminated 100% of promastigotes after 24 h of incubation at 1600 µg/mL. However, Glucantime® at 1600 µg/mL inhibited only approximately 22.5% , similar to the growth inhibition by 200 µg/mL of TMPP (Figure 1). It is believed that antimony, like glucantime, may be a prodrug and is converted to trivalent antimony after administration, interfering in the process of β-oxidation of fatty acids and of glycolysis in the parasite (Croft & Coombs, 2003). For over ninety years the drug based on antimony is the first

choice in the treatment of leishmaniasis. The demand for new drugs for leishmaniasis cure, which affect the life cycle of the parasite, is necessary, to replace the treatment of first choice. The present results suggest that the TMPP was more efficient than Glucantime® and may be a new bioactive leishmanicidal compound.

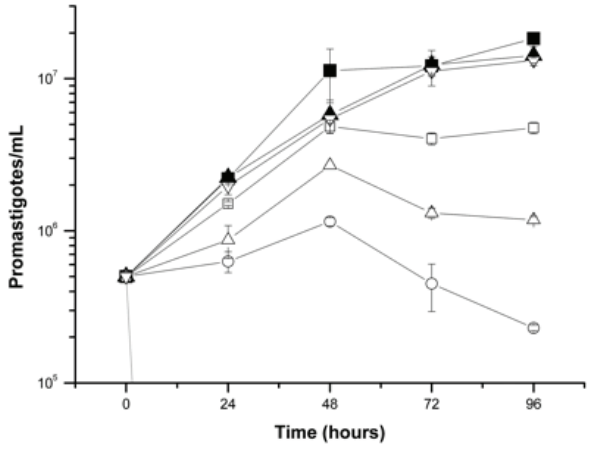


Figure 2. Growth curve of *L. amazonensis* promastigotes forms in the following conditions: control (■); 1600 µg/mL of Glucantime® (▲); 1600 µg/mL of Pentamidime® (---); and TMPP at 200 µg/mL (▼), 400 µg/mL (□), 800 µg/mL (▲) and 1600 µg/mL (○). The experiment started with 5x 10^5 parasites in all samples.

The Piperaceae family is under investigation by many laboratories and presents important perspectives for drugs development (Calderon et al., 2009). This is the first report of leishmanicidal activity of TMPP. Other natural products with leishmanicidal activity were isolated from other *Piper* species, such as methyl 3,4-dihydroxy-5-(3'-methyl-2'-butenyl)benzoate (Flores et al., 2008), several prenylated benzoic acids (Flores et al., 2009), kavapyrone and some chalcones (Flores et al., 2007).

Although additional tests involving this natural product, such as pharmacological and toxicological studies, are required for the elucidation of the TMPP mechanism of action in the promastigote replication, these results might be useful in the identification of natural products' candidates for the development of new antileishmanial drugs.

CONCLUSION

The 3-(3,4,5-trimethoxyphenyl) propanoic acid (**1**) *purified* from *Piper tuberculatum* Jacq., Piperaceae, fruits inhibited the growth of *L. amazonensis* promastigotes with an IC50 value of 145 µg/mL.

ACKNOWLEDGEMENTS

The authors are grateful Dr. J. Gomes from Instituto Nacional de Pesquisa da Amazônia (INPA) for identification of plant material, and CT-Amazonia/CNPq, FINEP and Pronex/CNPq/Seplad/RO for the financial support.

REFERENCES

Bezerra DP, Castro FO, Alves APNN, Pessoa C, Moraes MO, Silveira ER, Lima MAS, Elmiro FJM, Costa-Lotufo LV 2006. *In vivo* growth-inhibition of Sarcoma 180 by piplartine and piperine, two alkaloid amides from *Piper*. *Braz J Med Biol Res 39*: 801-807.

Calderon LA, Silva-Jardim I, Zuliani JP, Silva AA, Ciancaglini P, Silva LHP, Stábeli RG 2009. Amazonian biodiversity: a view of drug development for leishmaniasis and malaria. *J Braz Chem 20*: 1011-1023.

Chaves MCO, Junior AGF, Santos BVO 2003. Amides from *Piper tuberculatum* fruits. *Fitoterapia 74*: 181-183.

Croft SL, Coombs GH 2003. Leishmaniasis-current chemotherapy and recent advances in the search for novel drugs. *Trends Parasitol 19*: 502-508.

Duarte CDM, Verli H, Araujo-Junior JXD, Medeiros IAD, Barreiro EJ, Fraga CAM 2004. New optimized piperamide analogues with potent in vivo hypotensive properties. *Eur J Pharm Sci 23*: 363-369.

Facundo VA, Pollli AR, Rodrigues RV, Militão JSLT, Stabelli RG, Cardoso CT 2008. Fixed and volatile chemical constituents from stems and fruits of *Piper tuberculatum* Jacq. and from roots of *P. hispidum*. *Acta Amaz 3*8: 743-748.

Flores N, Cabrera G, Jiménez IA, Piñero J, Giménez A, Bourdy G, Cortés-Selva F, Bazzocchi IL 2007. Leishmanicidal constituents from the leaves of *Piper rusbyi*. *Planta Med 73*: 206-211.

Flores N, Jiménez IA, Giménez A, Ruiz G, Gutiérrez D, Bourdy G, Bazzocchi IL 2008. Benzoic acid derivatives from *Piper* species and their antiparasitic activity. *J Nat Prod 71*: 1538-1543.

Flores N, Jiménez IA, Giménez A, Ruiz G, Gutiérrez D, Bourdy G, Bazzocchi IL 2009. Antiparasitic activity of prenylated benzoic acid derivatives from *Piper* species. *Phytochemistry 70*: 621-627.

Fontenele JB, Leal LK, Silveira ER, Felix FH, Bezerra CF, Viana GS 2009. Antiplatelet effects of piplartine, an alkamide isolated from *Piper tuberculatum*: possible involvement of cyclooxygenase blockade and antioxidant activity. *J Pharm Pharmacol 61*: 511-515.

Jaramillo MA, Manos PS 2001. Phylogeny and patterns of floral diversity in the genus *Piper* (Piperaceae). *Am J Bot 88*: 706-716.

Kato MJ, Furlan M 2007. Chemistry and evolution of the Piperaceae. *Pure Appl Chem 79*: 529-538.

Lago JHG, Ramos CS, Casanova DCC, Morandim AA,

Bergamo DCB, Cavalheiro AJ, Bolzani VS, Furlan M, Guimarães EF, Young MCM, Kato MJ 2004. Benzoic acid derivatives from *Piper* species and their fungitoxic activity against Cladosporium cladosporioides and *C. sphaerospermum*. *J Nat Prod 67*: 1783-1788.

Paula-Junior W, Rocha FH, Donatti L, Fadel-Picheth CMT, Weffort-Santos AM 2006. Leishmanicidal, antibacterial, and antioxidant activities of *Caryocar brasiliense* Cambess leaves hydroethanolic extract. *Rev Bras Farmacogn 16*: 625-630.

Pohlit AM, Quignard ELJ, Nunomura SM, Tadei WP, Hidalgo AF, Pinto ACS, Santos EVM, Moraes SKR, Saraiva RCG, Ming LC, Alecrim AM, Ferraz AB, Pedroso ACS, Diniz EV, Finney EK, Gomes EO, Dias HB, Souza KS, Oliveira LCP, Don LC, Queiroz MMA, Henrique MC, Santos M, Lacerda Jr OS, Pinto PS, Silva SG, Graça YR 2004. Screening of plants found in the State of Amazonas, Brazil for larvicidal activity against *Aedes aegypti* larvae. *Acta Amaz 34*: 97-105.

Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B, Brooker S 2007. Cutaneous leishmaniasis. *Lancet Infect Dis 7*: 581-96.

Rocha LGR, Aragão CFS, Loiola MIB, Bezerril RA, Paiva NRF, Holanda CMCX, Brito MEF 2009. Evaluation of the leishmanicide action of ethanol extracts of *Crotalaria retusa* L. (Fabaceae). *Rev Bras Farmacogn 19*: 51-56.

Rocha SFR, Ming LC 1999. *Piper hispidinervum*: A sustainable source of safrole. In: Janick J (org.). *Perspectives on new crops and new uses*. Alexandria: ASHS Press, p.479-481.

Santos DO, Coutinho CE, Madeira MF, Bottino CG, Vieira RT, Nascimento SB, Bernardino A, Bourguignon SC, Corte-Real S, Pinho RT, Rodrigues CR, Castro HC 2008. Leishmaniasis treatment-a challenge that remains: a review. *Parasitol Res 103*: 1-10.

Wadt LHO, Ehringhaus C, Kageyama PY 2004. Genetic diversity of "Pimenta Longa" genotypes (*Piper* spp., Piperaceae) of the Embrapa Acre germplasm collection. *Genet Mol Biol 27*: 74-82.

[#] Taken in part from a memorial submitted by MGPRS to the Federal University of Rondonia in partial fulfillment of the requirement for the Master degree in Experimental Biology.