Biological activity and quassinoid content of fruits from the amazonian medicinal plant *Picrolemma sprucei* (Simaroubaceae)

Atividade biológica e teor de quassinoides dos frutos da planta medicinal amazônica *Picrolemma sprucei* (Simaroubaceae)

[1,2]**Rodrigo César N. Amorim**; [3,6]**Márcia Rúbia S. Melo**; [1,2,5]**Luiz Francisco R. Silva**; [4]**Valter F. Andrade-Neto**; [3]**Wanderli Pedro Tadei**; [1,]*****Adrian M. Pohlit**

[1]Amazonian Active Principles Laboratory (LAPAAM), Department of Technology and Innovation (CoTI), National Institute for Amazonian Research (INPA), Manaus, AM, Brazil.

[2] Graduate Program in Biotechnology, Federal University of Amazonas (UFAM), Manaus, AM, Brazil.

[3]Malaria and Dengue Laboratory, Department of Society, Environment and Health (CSAS), INPA.

[4]Malaria and Toxoplasmosis Biology Laboratory, Department of Microbiology and Parasitology, Center for Biosciences, Department of Microbiology and Parasitology, Federal University of Rio Grande do Norte (UFRN), Natal, RN, Brazil.

[5]Tropical Medicine Foundation – Heitor Vieira Dourado (FMT-HVD), Manaus, AM, Brazil.

[6]School of Health (ESA), State University of Amazonas (UEA), Manaus, AM, Brazil.

***Corresponding author**: e-mail address: ampohlit@inpa.gov.br. Phone: +55 92 3643-3177; FAX: +55 92 3643-3079.

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Resumo

Picrolemma sprucei Hook. f. (Simaroubaceae) é uma planta medicinal utilizada por toda a região Amazônica no tratamento da malária. Nenhum trabalho anterior foi publicado sobre a atividade biológica ou composição química dos frutos desta espécie. No presente estudo, a atividade antimalárica dos frutos de *P. sprucei* foi avaliada. Além disso, um método analítico simples e rápido baseado em cromatografia em camada delgada-fotodensitometria (CCD-FD) é utilizado para a determinação semi-quantitativa do teor de quassinóides isobruceina B (**1**) e neosergeolida (**2**) nos frutos de *P. sprucei*. Extratos etanólicos do fruto inteiro, pericarpo e sementes (caroços) de *P. sprucei* exibiram elevada atividade *in vitro* (CI_{50} = 0,6, 1,5 e 0,2 mg/mL, respectivamente) frente à cepa K1 do parasita da malária humana *Plasmodium falciparum*. Frações obtidas em hex, $CHCl_3$, AcOEt, BuOH e $MeOH-H_2O$, por partição liquid-líquido dos extratos etanólicos das sementes e pericarpos, também proveram elevada atividade antimalárica (CI_{50} = 0,01-3,7 mg/mL para frações obtidas das sementes e CI_{50} = 0,01-9,1 mg/mL para frações obtidas dos pericarpos). As frações clorofórmicas apresentaram as maiores atividades antimaláricas (CI_{50} = 10 ng/mL), provavelmente devido ao teor elevado de quassinóides. A fração clorofórmica do extrato da semente exibiu 39,8 % (m/m) de **1** e 3,5% (m/m) de **2** enquanto a fração clorofórmica do extrato do pericarpo continha 5,0 % (m/m) de cada quassinóide. A atividade antiplasmodial das frações foi presumivelmente devida à presença de quassinóides e não-quassinóides ativos. A polpa da fruta e o caroço tem potencial como agentes fitoterápicos.

Abstract

Picrolemma sprucei Hook. f. (Simaroubaceae) is a medicinal plant that is used throughout the Amazon region in the treatment of malaria. No previous report has been published on the biological activity or chemical composition of the fruit of this species. In the present study, antimalarial effects of the fruit from *P. sprucei* was evaluated. Furthermore, a rapid and simple thin-layer chromatography-photodensitometry (TLC-PD) analytical method is utilized for the semi-quantitative determination of the quassinoids isobrucein B (**1**) and neosergeolide (**2**) in *P. sprucei* fruit. EtOH extracts of the whole fruit, pericarp and seeds (pits) of *P. sprucei* exhibited high *in vitro* inhibitory activity (IC$_{50}$ = 0.6, 1.5 and 0.2 mg/mL, respectively) against the K1 strain of the human malaria parasite *Plasmodium falciparum*. Hex, CHCl$_3$, EtOAc, BuOH and MeOH-H$_2$O fractions obtained by liquid-liquid partitioning of seed and pericarp EtOH extracts provided seed (IC$_{50}$ = 0.01-3.7 mg/mL) and pericarp (IC$_{50}$ = 0.01-9.1 mg/mL) fractions exhibiting high inhibitory activity against *P. falciparum*. CHCl$_3$ fractions had the highest *in vitro* antimalarial activities (IC$_{50}$ = 10 ng/mL), probably due the high quassinoid content. The CHCl$_3$ fraction of the seed extract exhibited 39.8 % (m/m) of **1** and 3.5% (m/m) of **2** while the CHCl$_3$ fraction of the pericarp extract contained 5.0 % (m/m) of each quassinoids. The antiplasmodial activity of the fractions was due presumably to quassinoid and non-quassinoid active components. *P. sprucei* fruit pulp and pit have potential as phytotherapeutic agents.

Introduction

According to the World Health Organization (WHO), about 3.3 billion people live in regions where there is a risk of malaria infection. At least 219 million people are infected by malaria parasite every year causing an estimated 660.000 deaths. The economic costs of malaria are also high and it is estimated that annual economic growth is diminished by as much as 1.3% in poor and developing countries where malaria transmission is intense as is the case in Brazil (WHO, 2012). Today, with the exception of a few countries in Central America, countries having high incidences of malaria in general must also deal with increasing resistance of malaria parasites to antimalarial drugs. A decrease in sensitivity of several strains of *Plasmodium falciparum* to artemisinin *in vitro* was observed in China and Vietnam (Dondorp et al., 2009). These facts are of concern especially in poor regions with high incidence of malaria as is the case of the Brazilian Amazon where human malaria is endemic. In this region of dense forest and high biodiversity, plants with medicinal potential are intensively used by the local population to treat malaria (Milliken, 1997).

The genus *Picrolemma* (Simaroubaceae) is composed of only two species—*P. huberi* (syn. *Cedronia granatensis* Cuatr. and syn. *P. granatensis* Cuatr.) and *P. sprucei* Hook. f. (syn. *P. pseudocoffea* Ducke). Both species are native to and found exclusively in the Amazon region (Cronquist, 1944). Know in the Brazilian Amazon by the common name "caferana", *P. sprucei* is used in traditional medicine in Peru, Brazil and French Guyana to treat malaria (Milliken, 1997; Silva et al., 2009). Our research group in collaboration with others has demonstrated the antimalarial (Andrade-Neto et al., 2007), anthelmintic (Nunomura et al., 2006), larvicidal (Pohlit et al., 2004), cytotoxic (Quignard et al., 2004; Silva et al., 2009, Cavalcanti et al., 2012) and antitumor (Silva et al., 2009, Cavalcanti et al., 2012) activities of caferana stem and root extracts, the isolated quassinoid components isobrucein B (**1**) and neosergeolide (**2**) (figure 1) found in this plant and semi-synthetic derivatives of **1** and **2** (Amorim and Pohlit, 2006). Agronomic studies towards the domestication of caferana and improving the process of obtaining this plant in a sustainable fashion are under development to further allow the use of this plant as a therapeutic agent (Silva, Amorim and Pohlit, 2009). There are no data in the literature on the chemical composition or biological activity of *P. sprucei* fruit. The aim of the present work was to evaluate the antimalarial potential and chemical composition of extracts and fractions of the fruit of *P. sprucei*.

Materials and methods

Plant materials, preparation of extracts and fractions: Collection was performed in Manaus, Amazonas State, Brazil in the period February-April, 2006. Voucher specimens were deposited at the INPA Herbarium (INPA 223883). Dried whole fruit (191.26 g), pericarp (16.29 g) and dried seeds (18.69 g) were separately ground in a mincer and macerated in EtOH (100 mL, 3 × 1 week). The filtrates from these extractions were evaporated to dryness under vacuum. Each extract was dissolved in a mixture of
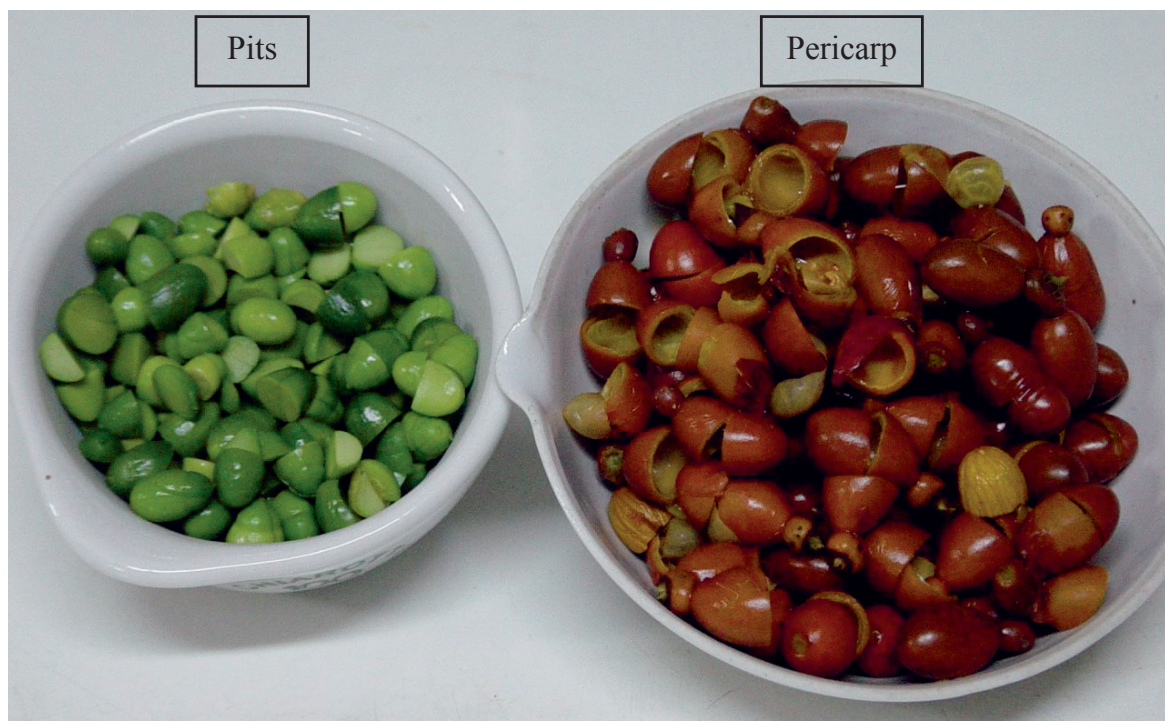
Figure 1. Fruits from *P. sprucei*.

MeOH (45 mL) and H_2O (5 mL) (9:1) and partitioned sequentially with Hex, $CHCl_3$, EtOAc and n-BuOH (3 × 10 mL of each solvent). These fractions were evaporated to dryness under vacuum. The procedure used for the isolation of **1** and **2** (figure 1) from the roots and stems of *P. sprucei* has been described previously (Silva, Amorim and Pohlit, 2009).

TLC-photodensitometric determination of 1 and 2 in extracts and fractions: Extracts and fractions of *P. sprucei* fruit were first analyzed qualitatively for the presence of **1** and **2** by normal-phase thin-layer chromatography (TLC) (20 × 20 cm Merck F_{254} plates (Merck, Darmstadt, Germany) which were cut to obtain 10 × 20 cm plates). Et_2O and *i*-PrOH (9:1) were used as mobile phase. Quassinoids **1** and **2** were semi-quantified in extracts and fractions by external calibration TLC-photodensitometry on a Hewlett-Packard Image Scanner III high resolution optical scanner using GE Healthcare Lab Scan software version 6.0 (Uppsala, Sweden). Spots were visualized qualitatively for the presence or absence of detectable quassinoids using an ultraviolet (UV) lamp (254 nm) and compared to spots corresponding to **1** and **2**. For quantification, TLC plates were conveniently developed by spraying with phosphomolybdic acid (Merck, Darmstadt, Germany) solution (20.0 g of phosphomolybdic acid in 100 mL of ethyl alcohol followed by filtering) then spraying with *p*-anisaldehyde (Merck, Darmstadt, Germany) solution (0.5 mL *p*-anisaldehyde, 50 mL of glacial CH_3CO_2H, and 1.0 mL conc. H_2SO_4). The R_F values for **1** and **2** were 0.6 and 0.4, respectively, under these conditions. The limits of quantification (LOQ) for **1** and **2** by TLC-densitometry in the extracts and fractions of *P. sprucei* fruit were 0.08 and 0.12 μg, respectively. For qualitative analyses performed with illumination of TLC F_{254} plates with UV lamp (254 and 366 nm) the limit of detection (LOD) was 0.03 μg for **1** and 0.06 μg for **2**.

Parasite culture and *in vitro* antimalarial tests: Chloroquine-resistant *P. falciparum* strain K1 was acquired from MR4 (Malaria Research and Reference Reagent Resource Center, Manassas, Virginia, USA) and was used in the *in vitro* tests. Parasites were maintained in continuous culture in A+ human erythrocytes using RPMI medium supplemented with 10 % human serum (Trager and Jensen, 1976). The antiparasitic effect of the extracts and fractions was measured by growth inhibition percentage (Carvalho and Krettli, 1991). Trophozoite-stages in sorbitol-synchronized blood (Lambros and Vanderberg, 1979) were cultured at 1 % parasitaemia and 2.0 % hematocrit and then incubated with samples, diluted with DMSO (≤ 0.02 % final concentration) in culture medium (RPMI 1640) for 24 h at 37 ºC. The antiparasitic effect was measured using an optical microscope.

Positive control wells containing the reference anti-malarial drug chloroquine in standard concentrations (Rieckmann et al., 1978; WHO, 2001) were used for each experiment. The stock solutions were further diluted with complete medium (RPMI 1640 plus 10 % human serum) to each of the concentrations used (100 to 0.0001 mg/mL in seven dilutions). The half-maximal inhibitory (IC_{50}) responses as compared to the drug-free controls were estimated by interpolation using Microcal Origin® software. Each duplicate experiment was repeated three times and blood smears were read blind (Rieckmann et al. 1978).

Results and Discussion

The yields (as a percentage of extracted plant material) of whole fruit, pericarp and seed EtOH extracts were 12.0, 8.2 and 5.8 % m/m, respectively, based on extracted plant materials. **1** and **2** could be detected in whole fruit, pericarp and seed extracts by UV illumination of TLC plates after elution. All EtOH extracts contained comparable amounts of the quassinoids **1** and **2** according to TLC-PD analysis (table 1). The order of decreasing antiplasmodial activity among the EtOH extracts was pit > whole fruit > pericarp.

Liquid-liquid was successful at concentrating quassinoids and antiplasmodial activity into the fractions. $CHCl_3$ fractions obtained from pericarp and seed extracts presented yields of 14.3 and 15.9 % m/m, respectively, based on the mass of fraction obtained from the mass of extract that was partitioned. The $CHCl_3$ fraction of the seed extract exhibited a significant quantity of **1** (39.8 %, m/m) and also **2** (3.5 %, m/m) while the $CHCl_3$ fraction of the pericarp extract contained 5.0 % of each of these quassinoids (table 1). Other fractions (hex, EtOAc, n-BuOH and MeOH-H_2O) obtained during partitioning did not exhibit appreciable amounts of quassinoids **1** and **2** according to TLC analysis. In general, all seed and pericarp extracts and fractions exhibited significant *in vitro* inhibition of the K1 strain of *P. falciparum* used in tests. Quassinoid-rich $CHCl_3$ fractions derived from fruit extracts were the most active inhibitors of

Table 1 – Isobrucein B (1) and neosergeolide (2) content of Picrolemma sprucei EtOH extracts and fractions based on TLC-photodensitometry and activity against *Plasmodium falciparum.*

Part	Extract/fraction	Quassinoid Content (%)		*P. falciparum*
		1	**2**	(IC_{50} µg/mL)
whole fruit	EtOH extract	0.2-0.5	0.4-0.8	0.6
pericarp	EtOH extract	0.2-0.5	0.4-0.8	1.5
	Hex fraction	< 0.2	< 0.4	9.1
	$CHCl_3$ fraction	5.0	5.0	0.01
	EtOAc fraction	< 0.2	< 0.4	0.2
	n-BuOH fraction	< 0.2	< 0.4	1.1
	MeOH-H_2O fraction	< 0.2	< 0.4	0.6
pit	EtOH extract	0.2-0.5	0.4-0.8	0.2
	Hex fraction	< 0.2	< 0.4	3.7
	$CHCl_3$ fraction	39.8	3.5	0.01
	EtOAc fraction	< 0.2	< 0.4	0.1
	n-BuOH fraction	< 0.2	< 0.4	0.3
	MeOH-H_2O fraction	< 0.2	< 0.4	0.1
chloroquine				0.03
1				0.003
2				0.002

P. falciparum. The antimalarial activity of both CHCl$_3$ fractions was comparable to that of chloroquine and about an order of magnitude less than that of pure **1** or **2**. High antiplasmodial activity (low IC$_{50}$ values) was exhibited by several fractions which did not contain detectable amounts of quassinoids. This observation is consistent with the notion that there are other antimalarial components in *P. sprucei* fruit besides **1** and **2**. The presence of non-quassinoid-related antiplasmodial activity has not been reported previously for *P. sprucei* and should be investigated in future work.
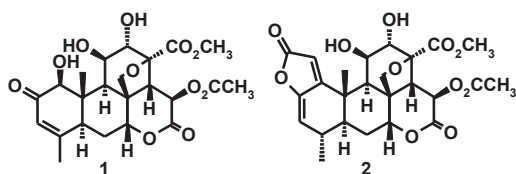

Figure 2. Quassinoids obtained from P. sprucei.

P. sprucei fruit extracts and fractions may be a good alternative for standardization of phytotherapeutic drugs, especially extracts containing quassinoids. It is possible that non-quassinoid compounds contribute to antimalarial activity in traditional remedies prepared from *P. sprucei* and/or modulate toxic effects associated with isolated quassinoids. In addition, standardized herbal remedies may be better tolerated and exhibit low or moderate toxicity. As shown before, quassinoids present in *P. sprucei* have strong antimalarial activity *in vivo* against *P. berghei* infection in mice at a very low dose (DE$_{50}$ 0.26 mg/kg/day) (Fandeur, Moretti and Polonsky, 1985).

Acknowledgements

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