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Short communication

Antimalarial activity of *Cinchona*-like plants used to treat fever and malaria in Brazil

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Abstract

For centuries, malaria was treated with the bark of *Cinchona calisaya* and *Cinchona succirubra* plants named "quinas" in Brazil, from which the quinine molecule was isolated. Other plant species known also as "quinas" are used to treat fever and malaria, like *Deianira erubescens* (roots and leaves), *Strychnos pseudoquina* (bark), and *Remijia ferruginea* (bark). Based on this popular knowledge, we evaluated the in vivo antimalarial activity of the ethanol crude extracts of these plant species in mice infected with *Plasmodium berghei*. Only *Remijia ferruginea* showed antimalarial activity, reducing parasitaemia and mortality at the highest dose tested. Its phytochemical analysis showed the presence of alkaloids but not quinine. The other two plant species were inactive.

Keywords: Antimalarial tests; Ethnopharmacology; *Cinchona*; *Remijia ferruginea*; *Plasmodium berghei*

1. Introduction

Malaria is a human protozoan disease widespread in the Amazon region. In Brazil, most malaria cases at present are caused primarily by *Plasmodium vivax*, followed by *Plasmodium falciparum* (Ministry of Health/Funasa, 2002). Several derivatives of the quinine molecule are used to treat acute symptomatic malaria including chloroquine, amodiaquine and mefloquine or, to prevent *Plasmodium vivax* late relapses, as primaquine and other 8-aminoquinolines (Greenwood, 1995; Ridley, 2002). At present, malaria chemotherapy is complicated by drug-resistant strains of *Plasmodium falciparum*, including in the Amazon region (Segurado et al., 1997), thus, new antimalarials are needed.

Quinine is an alkaloid first identified and isolated from the barks of the Peruvian plants *Cinchona calisaya* Wedd. and *Cinchona succirubra* Pav. ex Klotzsch (Rubiaceae) (Bruce-Chwatt, 1988), popularly known as "quinas" in Brazil. Quinine has been used to treat human malaria for over 350 years (Meshnick, 1997; Meshnick and Dobson, 2001), especially in cases of chloroquine-resistant *Plasmodium falciparum* (Ridley, 2002). The antimalarial activity of about 40 medicinal plant species used in Brazil have been tested in vivo and in vitro (Carvalho et al., 1991; reviewed in Krettli et al., 2001). In this paper, we studied species known as "quinas" used to treat fever and malaria, namely, *Deianira erubescens* Cham. and Schltdl. (Gentianaceae; roots and leaves), *Remijia ferruginea* (A.St.-Hil.) DC. (Rubiaceae; bark) and *Strychnos pseudoquina* A.St.-Hil. (Loganiaceae; bark) (Wasick, 1944; Correa, 1975; Balbach, 1980) for their antimalarial activity.

2. Material and methods

2.1. Plant samples

The roots and leaves of *Deianira erubescens* and the bark of *Remijia ferruginea* and *Strychnos pseudoquina*, popularly used as remedies, were collected and dried at room temperature. Plant samples were collected in the "cerrado" (savanna-like) vegetation of the Serra do Cabral, northern Minas Gerais, in July 2000. Voucher herbarium specimens

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were prepared and deposited in the herbarium BHCB, Federal University of Minas Gerais. Plants were identified by one of us (J.R.S.) as *Deianira erubescens* Cham. and Schltdl. (BHCB 47133), *Remijia ferruginea* (A.St.-Hil.) DC. (BHCB 47136), and *Strychnos pseudoquina* A.St.-Hil. (BHCB 4720).

2.2. Ethanol extract preparation

Plant samples (100 g each) were pulverized, then extracted by percolation with 80% ethanol, as described earlier by Brandão et al. (1997). The dried extracts (4.82 g from root and 23.1 g from leaves of *Deianira erubescens*, 6.75 g from *Remijia ferruginea* bark, and 11.2 g from *Strychnos pseudoquina* bark) were stored at 4 °C and tested as a fresh water suspension in Tween 20 at 1% final concentration as antimalarial drug.

2.3. Phytochemical screening

For identification of alkaloids, the dried extract was solubilized in ethanol and water (1:2), alkalinized to pH 9.0 with NH_4OH and extracted with CH_2Cl_2. The extracts were chromatographed (TLC) using concentrated dichloromethane phase and applied on silica gel. The solvent system used was toluene–ethyl acetate–diethylamine (7:2:1) or chloroform–diethylamine and Draggendorff/$NaNO_2$ or 10% ethanol/H_2SO_4 (and UV-365 nm) as a spray reagent. Quinine (Sigma Ref. 1125, St. Louis, MO, USA) was used as the reference compound. For the identification of flavonoids, saponins and tannins, solvent systems and detection methods as described by Wagner and Bladt (1996) were used.

2.4. Malaria parasites

The *Plasmodium berghei*, strain NK-65 was originally received from the New York University Medical School. It was maintained through weekly blood passages in mice, by intraperitoneal route (i.p.), using 3.8% sodium citrate as anticoagulant and also in liquid nitrogen with glycerolyte.

2.5. Antimalarial tests

We used the traditional suppressive test of Peters (1965) as modified by Carvalho et al. (1991). Briefly, adult Swiss albino mice weighing 18–20 g were inoculated by i.p. route with 1×10^5 *Plasmodium berghei*-infected red blood cells. The mice were randomly divided in groups of five per cage, and treated during 4 consecutive days with daily doses of the extracts, by oral route. Two control groups were used in each experiment, one treated with chloroquine at low non-curative doses (≤25 mg/kg, orally), the other group was kept untreated. On the 5th day after parasite inoculation, blood smears were prepared from all mice, fixed with methanol, stained with Giemsa, then microscopically examined (1000× magnification). Parasitaemia was determined in coded blood smears by randomly counting 2000–6000 erythrocytes in the case of low parasitaemia (≤10%); or up to 1000 erythrocytes in the case of higher parasitaemia. Overall mortality was monitored daily in all groups during a period of four weeks following inoculation. The inhibition of parasite growth in the drug-treated groups was calculated as follows: parasitaemia in the control (non-treated) group minus parasitaemia in the drug-treated group, divided by parasitaemia in the control (non-treated) group, expressed as percentages. The extracts were considered partially active if parasitaemia was reduced by 30% or more (Carvalho et al., 1991). All extracts were tested in three independent experiments at daily doses of 500 and 1000 mg/kg body weight.

2.6. Statistical analysis

The ANOVA and Student's *t*-test were used for comparison of average parasitaemia and the Kruskal–Wallis test by the Biostat 1.0 Software for Windows (MCT-CNPq) for survival analysis.

2.7. Ethical approval

The present work was approved by the Ethical Committee for Using Animals at Fundação Instituto Oswaldo Cruz, FIOCRUZ (CEUA P0094-01).

3. Results and discussion

The phytochemical screening of "quina" plants showed the presence of flavonoids and tannins in *Deianira erubescens* leaves, and tannins in its root; alkaloids were detected in the *Strychnos pseudoquina* and *Remijia ferruginea* barks. The antimalarial activity of these plants in *Plasmodium berghei*-infected mice is shown in Table 1 for one representative experiment in a total of three performed. Extracts from the bark of *Strychnos pseudoquina* and the root and leaves of *Deianira erubescens* were inactive and caused no significant reduction in parasitaemia or mortality. Extracts from *Remijia ferruginea* bark caused up to 48% inhibition of parasite growth at the dose of 1000 mg/kg, but only a borderline activity at the dose of 500 mg/kg in one experiment.

Malaria mortality was slightly but not significantly reduced by *Remijia ferruginea* in relation to the mortality in non-treated mice ($P > 0.05$). However, the mice survival time was reduced in groups treated with *Deianira erubescens* extracts from leaves and root (Table 1). Indeed, one dose of 4000 mg/kg of *Deianira erubescens* ethanol extract caused 20% mortality to non-malaria mice, whereas, similar doses of *Strychnos pseudoquina* and *Remijia ferruginea* bark extracts caused no mortality (not shown). Chloroquine, a standard antimalarial used in non-curative doses to mice, caused a significant reduction of parasitaemia (51–95%) and mortality (Table 1) in all experiments.

Table 1
Antimalarial activity of ethanolic extracts of *Cinchona*-like plants and chloroquine in mice infected with *Plasmodium berghei*

Plant species/part used or reference drug	Dose (mg/kg) orally 4×	Reduction of parasitaemia (%)[a]	Half-survival time in days ± S.D.	Antimalarial activity[b]
Strychnos pseudoquina				
Bark	1000	10	10 ± 2.0	None
	500	0	10 ± 1.5	
	0[c]		10 ± 2.0	
Remijia ferruginea				
Bark	1000	48*	12 ± 2.6	Partial
	500	34	11 ± 2.4	
	0[c]		10 ± 2.1	
Deianira erubescens				
Leaves	1000	0	9 ± 1.2	None
	500	0	9 ± 2.3	
	0[c]		10 ± 0.6	
Root	1000	0	8 ± 1.7^{+}	None
	500	18	9 ± 2.6	
	0[c]		11 ± 0.6	
Chloroquine (control)	25	95**	28 ± 3.0^{++}	Active
	12.5	51***	18 ± 1.5^{+++}	Partial
	0[c]		11 ± 1.0	

S.D.: standard deviation. The significance of parasitaemia inhibition evaluated by the Student's *t*-test was $^{*}P = 0.045$; $^{**}P = 0.0001$; $^{***}P = 0.001$; and, of mortality by the Kruskal–Wallis test was $^{+}P = 0.015$; $^{++}P = 0.007$; $^{+++}P = 0.04$.

[a] Reduction of parasitaemia in relation to control non-treated mice.

[b] Extracts which reduce ≥30% parasitaemia are considered partially active.

[c] Control non-treated group.

Cinchona species, a source of quinine, do not occur in Brazil. *Remijia ferruginea* known as "quina" has been used as a substitute of quinine to treat malaria (Wasick, 1944; Souza, 1951). Other plants named "quinas" are used in traditional medicine to treat fever and malaria. In the present study, the antimalarial effect of three species of "quinas" tested in rodent malaria proved to be disappointing. Only one, *Remijia ferruginea*, displayed some activity; *Strychnos pseudoquina* and *Deianira erubescens* were totally inactive. It has been previously shown that *Strychnos pseudoquina* extracts tested in *Plasmodium cathemerium*, an avian malaria, were inactive (Wasick, 1944). In an attempt to characterize the active compounds in *Remijia ferruginea*, we performed TLC tests of the alkaloid extract. We found no quinine. Other alkaloids are described in *Remijia* plants (McCalley, 2002), but further work has to be undertaken to elucidate whether they are responsible for the antimalarial activity of *Remijia ferruginea*.

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