In Vitro Antimalarial Activity of Crude Extracts of *Phothomorphe peltata* and *P. umbellata* (Piperaceae)

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Abstract: The *in vitro* antimalarial activity of hexane and methanol extracts of *Pothomorphe peltata* (L.) Miq. and *P. umbellata* (L.) Miq. (Piperaceae), two plants used in the Brazilian folk medicine in the treatment of malaria, was assessed against three *Plasmodium falciparum* strains with different patterns of sensitivity to the standard antimalarial drug chloroquine. Although both fractions had showed considerable activity in the system, methanol extracts of both plants were more effective in inhibiting plasmodial growth *in vitro* than the hexane ones.

Key words: antimalarial drugs, malaria, *P. peltata, P. umbellata, P. falciparum*

The worldwide spread of strains of *P. falciparum* resistant to antimalarial agents has had an enormous impact on malaria therapy. In Brazil, a country with a great flora diversity, many attempts to find out active compounds have been carried out with plants popularly used in regions where the disease is endemic (Brandão *et al.* 1992). Among them, two species of the *Pothomorphe, P. peltata* (L.) Miq. and *P. umbellata* (Piperaceae), are currently employed in Brazilian folk medicine to treat malaria, fever and hepatic dysfunction (Amorim *et al.* 1988; Hammer and Johns, 1993). In this report, we describe *in vitro* tests against three *P. falciparum* strains with different degrees of sensitivity to chloroquine, in an attempt to detect any potential component in the extracts of these plants, able to inhibit the development of parasites in the system.

The plants were collected and provided by INPA (Instituto Nacional de Pesquisas da Amazônia, Brazil) and FEEMA (Fundação Estadual do Meio Ambiente, RJ / Brazil), and identified by Dr. Elsie Franklin Guimarães, a specialist on Piperaceae from the Departamento de Botânica e Sistemática / Jardin Botânico - Rio de Janeiro. Voucher specimens of *P. peltata* are deposited at the herbarium of INPA under code 9389 (collector Wiliam Rodrigues, num-

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ber 2.845) while that of *P. umbellata* are deposited at the herbarium of FEEMA under code GUA- 41896 (collector Rogério Ribeiro de Oliveira, number 2.228). Both plants were dried at room temperature during thirty days, protected from sun light and humidity. For the extraction, fragmented dry leaves were used, since the leaves are the parts traditionally used in Brazilian folk medicine (Cruz, 1985; Vieira, 1992). A method of maceration which consisted of a sequential treatment overnight twice with hexane (grupo quimica) and thereafter with methanol (grupo quimica) was employed. The ratio plant /solvent was 1:4 (w/v). Each step consisted of a period of 24 hours in which the plant material was kept in contact with solvent, followed by evaporation under reduced pressure. The method allowed us to obtain hexane and methanol extracts of both plants. Before starting *in vitro* tests, aliquots of the hexane and methanol extracts were analyzed in a gas chromatography to rule out the possibility of contamination by residual solvents. In order to enable their use in the *in vitro* system, the extracts were dissolved in dimethyl sulphonyl oxide-DMSO (Sigma) at a concentration of 5㎎/ml. During assays, the stock solution of each extract was diluted in complete RPMI culture medium in order to obtain final concentrations ranging from 8 to 40μg/ml. Chloroquine diphosphate (as a standard antimalarial drug) was employed at concentrations ranging from 0.67 to 500ng/ml for determination of IC_{50} of *P. falciparum* strains. Three strains of *P. falciparum* were used throughout this work: two of them were African, one chloroquine-sensitive (D6) and the other one with a resistant phenotype to this drug (FCR3-provided by Dr. Daniel Camus-INSERM/France). The third strain used was isolated from a naturally infected patient from the Rondônia State, Brazil (kindly provided by Dr. Silvia di Santi, SUCEN-São Paulo/Brazil) and is chloroquine-resistant (S-20/87). The *in vitro* cultures of *P. falciparum* were carried out in human A^+ red blood cells, according to Trager and Jensen's methodology (1976). The procedure used for the *in vitro* tests employing the radioisotope microdilution method was basically those described by Desjardins *et al.* (1979). Statistical analysis was performed using The GraphPad Instat software for the Kruskal-Wallis Nonparametric ANOVA Test.

Results obtained with the radioisotope microdilution method, reproduced the reported chloroquine response pattern for the three *P. falciparum* strains (Table 1). It is interesting to note that, according to the IC_{50} values obtained, the highest degree of resistance to chloroquine was presented by the Brazilian S- 20/87 strain ($p<0.05$). Both hexane and methanol extracts of *P. peltata* inhibited *in vitro* parasitic growth. The extent of the inhibition varied, according to the extract and the parasite strain used (Table 2). All *P. falciparum* strains were more sensitive to the methanol extracts of this plant, but resistant strains of the parasite had their growth affected in a more pronounced degree when compared to the sensitive one ($p<0.05$). Results obtained with extracts of *P. umbellata* were more heterogeneous: each plasmodial strain was inhibited independently to its phenotypic resistance to chloroquine (Table 3). The growth inhibition has been observed with both extracts, but the hexane preparation affected in a similar degree D6 and FCR3 strains, while the resistant S-20/87 was inhibited with a higher concentration, as showed by the IC_{50} values obtained. Otherwise, the concentrations of the methanol extract able to inhibit the sensitive as well as the Brazilian

Table 1. *In vitro* response of *Plasmodium falciparum* strains to the standard antimalarial drug chloroquine (as expressed in concentration required to induce 50% of growth inhibition - IC_{50}) determined through the uptake of /H^3/ hypoxanthine.

P. falciparum strains	/H^3/ hypoxanthine IC_{50} (ng/ml)	Resistance Phenotype
	$\chi \pm SD$	Sensitive (S)/Resistant (R)
D6	6 ± 1.5	S
FCR3	36 ± 9.5	R
S-20/87	96 ± 14.0	R

Table 2. *In vitro* antimalarial activity (IC_{50}) of hexane and methanol extracts from *Pothomorphe peltata*, against three different strains of *Plasmodium falciparum.*

P. falciparum strains	Extracts		Resistance Phenotype
	hexane (μl)	methanol (μl)	Sensitive(S)/Resistant(R)
	$\chi \pm SD$	$\chi \pm SD$	
D6	37 ± 2.8	21 ± 1.4	S
FCR3	12 ± 1.4	3.85 ± 0.2	R
S-20/87	21.5 ± 2.1	5.6 ± 0.2	R

Table 3. *In vitro* antimalarial activity of hexane and methanol extracts from *Pothomorphe umbellata* against three different strains of *Plasmodium falciparum.*

P. falciparum strains	Extracts		Resistance Phenotype
	hexane (μl)	methanol (μl)	Sensitive(S)/Resistant(R)
	$\chi \pm SD$	$\chi \pm SD$	
D6	11.4 ± 1.2	5.6 ± 1.9	S
FCR3	11.4 ± 1.4	19.3 ± 0.3	R
S-20/87	15.0 ± 0.4	8.3 ± 1.3	R

resistant strains were close to each other and FCR3 strain seemed to be less sensitive to the action of the methanol fraction when compared to the hexane preparation. Data with hexane and methanol extracts from each plant were compared and independently of the plant and the phenotype of the strain, the concentrations of hexane extract able to inhibit parasitic development in this system were greater than those necessary to produce the same effect obtained with methanol extract ($p<0.05$), suggesting that active compounds may be more concentrated in this fraction.

In conclusion, data reported here indicates that both plants retain in the methanol fraction compounds with considerable inhibitory activity against the human malaria parasite. Further studies, including isolation and purification of the active compounds, could help to circumvent the growing pattern of resistance exhibited by *P. falciparum* strains.

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