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Antimalarial Activity of Alkaloids from *Pogonopus tubulosus*

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The antimalarial activity of the Bolivian medicinal plant *Pogonopus tubulosus* (D.C.) Schumann was evaluated by *in vitro* testing on trophozoite stages of resistant and sensitive strains of *Plasmodium falciparum* and by *in vivo* tests on *P. berghei* and *P. vinckei petteri* in mice. The bark of this medicinal plant yielded three alkaloids: tubulosine, psychotrine, cephaeline. Tubulosine showed an interesting activity *in vitro* with an IC_{50} of 0.006 μg/mL against the sensitive strain of *P. falciparum* and an IC_{50} of 0.011 μg/mL against the resistant strain of *P. falciparum*. This compound had good *in vivo* antimalarial activity with an ED_{50} of 0.05 mg/kg/day on *P. vinckei petteri* strain and an ED_{50} of 0.45 mg/kg/day on *P. berghei*.

Keywords: antimalarial; *Plasmodium falciparum*; *P. berghei*; *P. vinckei*; tubulosine; psychotrine; cephaeline; *Pogonopus tubulosus*.

INTRODUCTION

Pogonopus tubulosus (D.C.) Schumann (Rubiaceae), a tree that grows in the southern South American subtropical rain

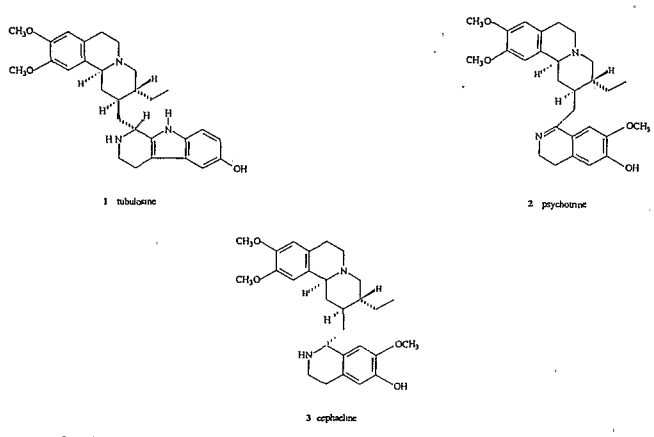
alkaloid compounds **1** (0.157 g), **2** (0.024 g) and **3** (0.018 g) in a pure state. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz respectively. 2D experiments were performed using standard Bruker microprograms. More details of the isolation procedure and copies of the original spectra

in mice described by Peters (1980) was used. This work was carried out as previously described (Deharo *et al.*, 1993). Mice were inoculated with *P. berghei* NK 65 or *P. vinckei petteri* 279BY on day 1 of the experiment and inoculated daily for 4 consecutive days with the extract or drug under test. On day 5 of the test, a blood smear was taken. ED_{50} values were computed by comparing the parasitaemias present in infected controls with those of test animals.

RESULTS AND DISCUSSION

A preliminary *in vivo* antimalarial bioassay showed that the activities were concentrated in the crude alkaloid mixture extracted from stem bark, and to a lesser extent in the petroleum extracts. Chemical study of the latter extract is in progress.

Identifications of **1**, **2** and **3** were carried out by comparing their physical and spectral data ($[\alpha]_D$, U.V., S.M., ^{1}H-NMR); with literature data for **1** and **2** (Ma *et al.*, 1990; Budzikiewicz *et al.*, 1954), and for **3**, with an authentic sample available in our laboratory. ^{13}C-NMR spectrum and 2 D ^{1}H-NMR experiments provided further confirmation for their structure.



Tubulosine **1** was previously isolated from the same species (Brauchli *et al.*, 1964); psychotrine **2** has already been detected in the ornamental species *Pogonopus speciosus* (Ma *et al.*, 1990); however this is the first reported occurrence of cephaeline **3** in this genus, which, having only 2 to 3 species seems chemically homogeneous.

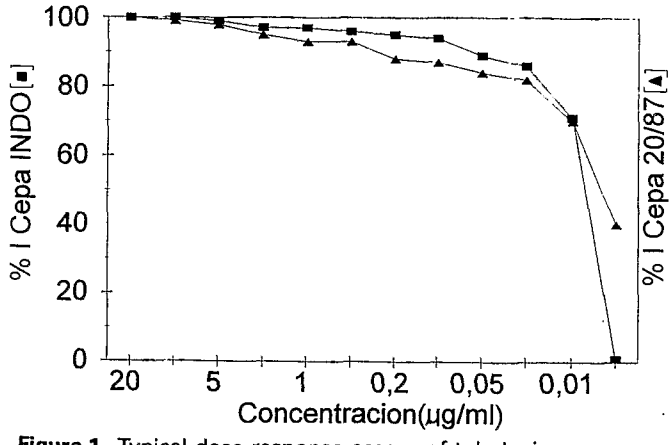


Figure 1. Typical dose-response assays of tubulosine on asynchronous cultures of *P. falciparum*. Each point is the mean of triplicate values. 20/87 chloroquine-sensitive strain (▲) and INDO resistant strain (■) after a 72 hr incubation.

Table 1. 50% inhibitory concentrations (µg/mL) of alkaloids from *Pogonopus tubulosus* against two isolates of *Plasmodium falciparum in vitro*

Drug	Isolate of *P. falciparum* 2087	Isolate of *P. falciparum* INDO
Chloroquine	0.02	0.08
Tubulosine **1**	0.006	0.011
Psychotrine **2**	0.14	0.39
Cephaeline **3**	0.027	0.011

Alkaloid **1** showed a good *in vitro* antiplasmodial activity with an IC_{50} of 0.006 µg/mL against the chloroquine-sensitive strain, and with an IC_{50} of 0.011 µg/mL against the resistant strains (Fig. 1 and Table 1). These activities are similar to those already published for this alkaloid against other strains, with an IC_{50} of 0.02 µg/mL with chloroquine resistant strain K-1 (Wright *et al.*, 1991). The *in vitro* activities of cephaeline (**3**) (Fig. 2 and Table 1) with an IC_{50} of 0.027 µg/mL against the chloroquine-sensitive strain, and with an IC_{50} of 0.011 µg/mL on the resistant strain were similar to tubulosine (**1**). Psychotrine (**2**) was less active (Fig. 3 and Table 1). Tubulosine (**1**) has been shown to have cytotoxicities against tumour cell lines. Its IC_{50} value on 9KB cells was 0.01 µg/mL (Ma *et al.*, 1990). These results suggest that compound **1** exhibits better selective toxicity against *Plasmodium* than that against mammalian cells; however its selectivity seems lower than that of chloroquine.

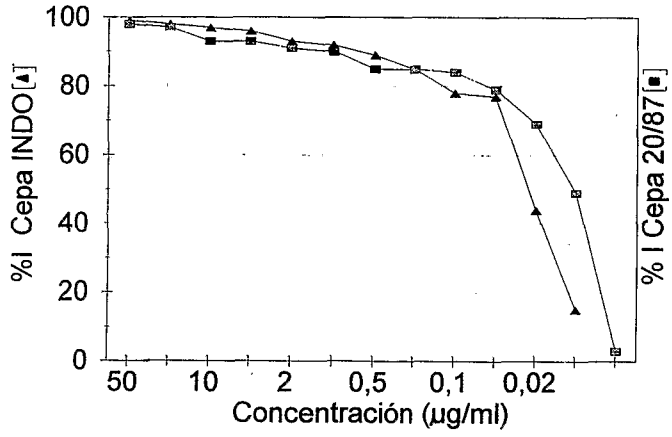


Figure 2. Typical dose-response assays of cephaeline on asynchronous cultures of *P. falciparum*. Each point is the mean of triplicate values. 20/87 chloroquine-sensitive strain (■) and INDO resistant strain (▲) after a 72 hr incubation.

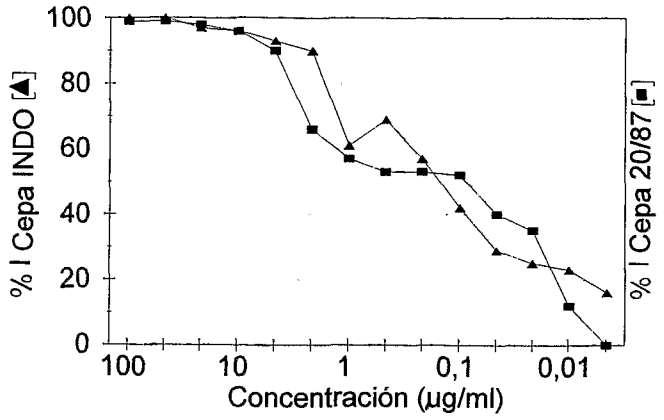


Figure 3. Typical dose-response assays of psychotrine on asynchronous cultures of *P. falciparum*. Each point is the mean of triplicate values. 20/87 chloroquine-sensitive strain (■) and INDO resistant strain (▲) after a 72 hr incubation.

Table 2. Antimalarial activity *in vivo* of tubulosine (1), psychotrine (2) and cephaeline (3)

Strain	Concentration	Parasitaemia	SEM	% Inhibition	Toxic deaths out of 10 mice at each dose
P. berghei NK 65	2.00	19	4	68	0
	1.00	22	5	63	0
	0.50	25	3	58	0
	0.10	34	1	42	0
	0.05	36	2	39	0
	control	59	4		
	IC_{50} of 1	=0.45 mg/kg/day			
P. vinckei petteri 279 BY	2.00	4	3	91	6
	1.00	5	2	89	0
	0.50	6	4	88	0
	0.10	12	3	74	0
	0.05	20	4	57	0
	control	45	4		
	IC_{50} of 1	≈0.05 mg/kg/day			
P. berghei NK 65	1.83	37	3	33	0
	0.1	39	3	28	0
	control	54	5		
	IC_{50} of 2	>2 mg/kg/day			
P. berghei NK 65	6.53	25	3	55	7
	3.13	43	4	21	0
	1.13	47	3	14	0
	control	54	5		
	IC_{50} of 3	=6 mg/kg/day			

Tubulosine (**1**) was also active *in vivo* with an ED_{50} value of 0.05 mg/kg/day on the *P. vinckei petteri* strain, at a lower concentration than its lethal dose (Table 2). All the mice were alive at the dose of 1 mg/kg/day. Mortality appeared at 2 mg/kg/day with 6/10 mice dead. This activity was confirmed on the *P. berghei* strain with an ED_{50} of 0.45 mg/kg/day but without toxicity at the doses tested (Table 2). This difference observed between the two *Plasmodium* species is reminiscent of the innate sensitivity of the same parasite species to chloroquine. Beauté-Lafitte *et al.* (1994), classified the different species of rodent malaria according to their CQ sensitivity. They found that *P. berghei* was less sensitive than *P. vinckei petteri*, and attributed these differences to a respective increase in the synchronicity of the infection, because the pharmacokinetics of chloroquine in the mouse are rapid and only the trophozoite stage is sensitive to the drug (Cambie *et al.*, 1991). It is therefore possible that the antimalarial effect of tubulosine is also stage-dependent. The two other alkaloids **2** and **3** are less active *in vivo* (Table 2).

The structure of tubulosine (**1**) is related to the strychnos

planar) conformation exists where the angles between the two planes containing an aromatic ring are nearly the same, conserving the electronic environment of the nitrogen atom with its free electron at a certain distance from the aromatic rings. Emetine like alkaloids (**2**, **3**) are more cytotoxic while the introduction of an indol moiety enhances the affinity for protozoan receptor. The relative *in vitro* inactivity of **2** in comparison with **3** can be explained by its double bond in ring C which enhances the coplanar conformation and electron environment. Structure/activity relationship studies indicate that minor changes in strychnos alkaloid structures may significantly affect their antiparasitic activities (Wright *et al.*, 1991, 1994). Our results show that tubulosine is more active than alkaloids of the usambarensine group which are relatively active *in vitro* and inactive *in vivo* (Wright *et al.*, 1991).

The results presented here lend support to the traditional common use of this plant as an antimalarial. However tubulosine, the principal antimalarial compound isolated from this species unfortunately presents little interest in the curative treatment of malaria because of its toxicity.

isolierung von emetin, cephaelin und psychotrin aus *Alangium lamarckii* und die identifizierung von almarsine mit

Mūnoz, V., Moretti, C., Sauvain, M. *et al.* (1994). Isolation of bis-indole alkaloids with antileishmanial and antibacterial