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Antimalarial activity of some Colombian medicinal plants

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Abstract

Antimalarial activity of 10 vegetal extracts (9 ethanolic extracts and 1 crude alkaloid extract), obtained from eight species traditionally used in Colombia to treat malaria symptoms, was evaluated in culture using *Plasmodium falciparum* chloroquine resistant (FcB2) strain and in vivo on rodent malaria *Plasmodium berghei*. The activity on ferriprotoporphyrin biomineralization inhibition test (FBIT) was also assessed. Against *Plasmodium falciparum*, eight extracts displayed good activity *Abuta grandifolia* (Mart.) Sandwith (Menispermaceae) leaves, *Acacia farnesiana* (L.) Willd. (Mimosaceae) leaves, *Acnistus arborescens* (L.) Schltdl. (Solanaceae) aerial part, *Croton leptostachyus* Kunth (Euphorbiaceae) aerial part, *Piper cumanense* Kunth (Piperaceae) fruits and leaves, *Piper holtonii* C. DC. (Piperaceae) aerial part and *Xylopia aromatica* (Lam.) Mart. (Annonaceae) bark with IC_{50} values ranging from <1 to 2.1 μg/ml, while in the in vivo model only *Abuta grandifolia* alkaloid crude extract exhibits activity, inhibiting 66% of the parasite growth at 250 mg/kg/day. In the FBIT model, five extracts were active (*Abuta grandifolia*, *Croton leptostachyus*, *Piper cumanense* fruit and leaves and *Xylopia aromatica*).

Keywords: Malaria; Antimalarials; Colombia; Medicinal plants; *Plasmodium*

1. Introduction

In Colombia, around 30 million people are exposed to malaria (Ministerio de Salud de Colombia, 2002). Traditional Colombian pharmacopoeia reports numerous plants to be used as antimalarials, but few have been scientifically evaluated. The aim of this study was to validate the antimalarial properties of eight frequently reported species: *Abuta grandifolia*, *Acacia farnesiana*, *Acnistus arborescens*, *Calea prunifolia* Kunth (Asteraceae), *Croton leptostachyus*, *Piper cumanense*, *Piper holtonii* and *Xylopia aromatica* (García-Barriga, 1992). Antimalarial activity was tested through classical antimalarial in vivo model (*Plasmodium berghei*) and through in vitro test on *Plasmodium falciparum* chloroquine resistant (FcB2) strain and on ferriprotoporphyrin (FP) IX biomineralization inhibition test (FBIT).

2. Methodology

Plants were collected in the neighborhood of Bogotá (Colombia). Voucher specimens were identified and deposited in the Herbario Nacional de Colombia, Universidad Nacional de Colombia (COL), and identities were confirmed by specialists. Dried plant parts were submitted to a percolation process with ethanol–water (70–30%) for 48 h at 25 °C (extraction yield: 10–20%). Crude alkaloid extract was obtained from *Abuta grandifolia* dried leaves as previously described (Muñoz et al., 2000). Antimalarial activity against FcB2 strain of *Plasmodium falciparum* was assessed as previously described (Deharo, 2000). Each test included positive control with chloroquine (Sigma, USA) and *Remijia peruviana* Standley (Rubiaceae) bark ethanolic extract, containing quinine and related compounds (Mongelli et al., 1995). To determine the activity against haem biomineralization process, the technique previously described by Deharo et al. (2002) was performed. *Remijia peruviana* and chloroquine were used as control.

In vivo antimalarial activity was determined against *Plasmodium berghei* ANKA according to Muñoz et al. (2000).

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Table 1
Antimalarial activity of selected plants

Scientific determination voucher number	Part of plant tested and extract type	*Plasmodium falciparum* IC_{50} (μg/ml)	*Plasmodium berghei* (%inhibition)	FBIT IC_{50} (mg/ml)
Abuta grandifolia Sandwith (Menispermaceae) CMSJG94	Leaves alkaloid crude extract	<1	66 ± 11	0.09 ± 0.02
Acacia farnesiana (L.) Willd. (Mimosaceae) Col. 488661	Bark ethanolic extract	1.3 ± 0.2	32 ± 5	>10
	Leaves ethanolic extract	24 ± 4	I	>10
Acnistus arborescens (L. Schltd. (Solanaceae) Col. 359328	Aerial part ethanolic extract	1.3 ± 0.2	I	>10
Calea prunifolia Kunth. (Asteraceae) Col. 468655	Aerial part ethanolic extract	32 ± 5	I	>10
Croton leptostachyus Kunth. (Euphorbiaceae) Col. 344160	Aerial part ethanolic extract	2.1 ± 0.2	Toxic	1.1 ± 0.1
Piper cumanense H.B. & K. (Piperaceae) Col. 468660	Fruit ethanolic extract	<1	Toxic	2.8 ± 0.5
	Leaves ethanolic extract	<1	I	1 ± 0.1
Piper holtonii C. DC. (Piperaceae) Col. 468663	Aerial part ethanolic extract	<1	I	>10
Xylopia aromatica (Lam.) Mart. (Annonaceae) Col. 468667	Aerial part ethanolic extract	<1	I	1.1 ± 0.1
Chloroquine diphosphate		0.09 ± 0.01	98 ± 2	0.014 ± 0.005
Remijia peruviana Standl. (Rubiaceae)	Bark ethanolic extract	0.85 ± 0.1	92 ± 7	0.7 ± 0.1

I, inactive; against *Plasmodium berghei*, chloroquine was administrated at 3 mg/kg, plant extracts at 250 mg/kg.

Each test included a positive control with chloroquine (3 mg/kg) and *Remijia peruviana* (250 mg/kg). This work was conducted according to the Resolution 008430/93 of the Colombian Ministry of Health for laboratory animal use and care.

3. Results

Results are listed in Table 1.

Abuta grandifolia leaf crude alkaloids together with *Croton leptostachyus*, *Acnistus arborescens*, *Piper holtonii* aerial parts, *Piper cumanense* fruit and leaves, *Acacia farnesiana* and *Xylopia aromatica* bark ethanolic extract displayed good activity against FcB2 strain with IC_{50} values ranging from <1 to 2.1 μg/ml, displaying a comparable activity with *Remijia peruviana* bark. *Acacia farnesiana* leaves extract and *Calea prunifolia* were inactive against *Plasmodium falciparum*.

In the FBIT, *Croton leptostachyus*, *Piper cumanense* (fruits and leaves) and *Xylopia aromatica* were active. The strongest activity was with *Abuta grandifolia* alkaloids, eight times more active than *Remijia peruviana*.

In vivo, only the alkaloidic fraction of *Abuta grandifolia* was weakly active, inhibiting 66% of the parasite growth at 250 mg/kg.

4. Discussion

Abuta grandifolia is present in Amazonia and Putumayo departments of Colombia, being used by the *Sionas* natives in form of a leaf infusion to treat malarial fevers. It is widely used through South America as antimalarial, and claimed to possess many other medicinal properties (García-Barriga, 1992). Some alkaloids (krukovin and limacrin) isolated from this plant have been reported to be active against *Plasmodium falciparum* (Steele et al., 1999). We showed here that the alkaloidic fraction of *Abuta grandifolia* is also active against *Plasmodium falciparum*. In the FBIT it was 10 times more active than *Remijia peruviana*. Thus, the antiplasmodial activity of this extract is directly related with the inhibition haemozoin formation, making it of interest for further studies. It also inhibits 66% of the parasite growth in vivo at 250 mg/kg. Despite this elevated dose, no sign of toxicity was detected in treated mice. The in vitro activity allows a bioguided fractionation process, which may lead to a more active compound for in vivo evaluation.

Acacia farnesiana is found in Valle del Cauca, Patía, Chicamocha and Tolima where warm and semi-warm climates predominate. Traditionally, leaves and bark are used for many medicinal indications including malaria, in form of an infusion or a decoction (García-Barriga, 1992). Glycosidal fraction of this plant has been shown to have bronchodilator and anti-inflammatory effects (Trivedi et al., 1986). In our model *Acacia farnesiana* bark ethanolic extract is active against *Plasmodium falciparum* while it is inactive in vivo and in the FBIT model. Nevertheless, because of antimalarial reputation, it would be interesting to submit the bark to antimalarial bioguided fractionation process.

Acnistus arborescens is largely distributed through Colombian Andes (Antioquia, Boyacá and Cundinamarca) from Ecuador to Venezuelans borders and the aerial parts are used, among others, as febrifuge (García-Barriga, 1992). Veras et al. (2004) showed that withaphysalins isolated from leaves of *Acnistus arborescens* displayed potent cytotoxic activities

against several cancer cell lines probably related to DNA synthesis inhibition. They could be responsible for the antiplasmodial activity detected herein against *Plasmodium falciparum* in vitro and could explain why our extract was inactive in the FBIT. *Plasmodium* is a fast developing cell, very sensitive to DNA replication inhibitors, but further studies are needed to determinate if withaphysalins are responsabile for the antimalarial activity.

Croton leptostachyus is mainly found in Antioquia, Boyacá, Cundinamarca and Tolima. Aerial parts are specifically used as febrifuge and against malaria (García-Barriga, 1992). 8,9-secokaurane diterpenes have been isolated from *Croton kongensis* exhibiting antimalarial activity against *Plasmodium falciparum* K1 multidrug resistant strain, with IC_{50} ranging from 1 to 2.8 μg/ml (Thongtan et al., 2003). In vitro and in vivo activity of *Croton mubango* was also evaluated on *Plasmodium falciparum* and *Plasmodium berghei*, respectively, with IC_{50} of 3.2 μg/ml for the aqueous extract in vitro and 60–80% of inhibition at a daily oral dose of 200 mg/kg (Mesia et al., 2005). In our hands, *Croton leptostachyus* ethanolic extract was active in vitro against *Plasmodium falciparum* and in the FBIT test but was toxic in the mice model. It should be interesting to determinate if products responsible for the activity are the same as that those responsible for the toxicity.

Piperaceae family is widely used in traditional medicine. Antimalarial activity has been already reported from many *Piper* spp., and related genus from this family (Jenett-Siems et al., 1999). We showed herein that *Piper cumanense* fruits and leaves (from Santander) and *Piper holtonii* aerial parts (from Cundinamarca) were active against *Plasmodium falciparum* in vitro but inactive in the in vivo model, and that *Piper cumanense* fruit was toxic at 250 mg/kg. Interestingly extract of leaves of this plant was as active as *Remijia peruviana* in the FBIT while extract of fruit was four times less active. Further studies should be carried out to isolate the products responsible for the in vitro antimalarial activity probably related to their aptitudes in impairing haemozoin formation.

Antimalarial use of *Xylopia aromatica* bark has been reported in the zone of Galeno in Boyacá (García-Barriga, 1992). Bioactive annonaceous acetogenins, xylopine and xylomatenin, have been isolated from the bark of *Xylopia aromatica*; these acetogenins showed cytotoxicities, comparable to adriamycin, against human solid tumor cell lines (Colman-Saizarbitoria et al., 1994). Oxoaporphine alkaloids, oxophoebine and liriodenine, have been isolated from *Xylopia aethiopica*; both showed selective toxicity against DNA repair and recombination deficient mutants of the yeast *Saccharomyces cerevisae* (Harrigan et al., 1994). We showed herein that *Xylopia aromatica* bark extract was active against *Plasmodium falciparum* in vitro but inactive in the in vivo model; this plant was 1.6 times less active than *R. periviana* in the FBIT. Further studies should be carried out to determine if the products responsible for the cytotoxicity are the same responsible for in vitro antimalarial activity.

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References

Colman-Saizarbitoria, T., Zambrano, J., Ferrigni, N.R., Gu, Z.M., Ng, J.H., Smith, D.L., McLaughlin, J.L., 1994. Bioactive annonaceous acetogenins from the bark of *Xylopia aromatica*. Journal of Natural Products 4, 486.

Deharo, E., 2000. Evaluación de la actividad antimalárica in vitro de productos naturales o de síntesis. In: Deharo, E., Gautret, Ph., Muñoz, V., Sauvain, M. (Eds.), Técnicas de laboratorio para la selección de sustancias antimaláricas, Primera edición. IRD-Cyted, La Paz, p. 187.

Deharo, E., García, R., Oporto, P., Sauvain, M., Gautret, Ph., Ginsburg, H., 2002. A non-radiolabeled ferriprotoporphyrin IX biomineralization inhibition test (FBIT) for the high throughput screening of antimalarial compounds. Experimental Parasitology 100, 252–256.

García-Barriga, H., 1992. Flora Medicinal Colombiana, vol. 3. Tercer Mundo Editores, Bogotá.

Harrigan, G.G., Gunatilaka, A.A., Kingston, D.G., Chan, G.W., Johnson, R.K., 1994. Isolation of bioactive and other oxoaporphine alkaloids from two annonaceous plants, *Xylopia aethiopica* and *Miliusa* cf. *banacea*. Journal of Natural Products 1, 68.

Jenett-Siems, K., Mockenhaupt, F.P., Bienzle, U., Gupta, M.P., Eich, E., 1999. In vitro antiplasmodial activity of Central American medicinal plants. Tropical Medicine and International Health 4, 611.

Mesia, G.K., Tona, G.L., Penge, O., Lusakibanza, M., Nanga, T.M., Cimanga, R.K., Apers, S., Van-Miert, S., Totte, J., Pieters, L., Vlietinck, A.J., 2005. Antimalarial activities and toxicities of three plants used as traditional remedies for malaria in the Democratic Republic of Congo: *Croton mubango*, *Nauclea pobeguinii* and *Pyrenacantha staudtii*. Annals of Tropical Medicine and Parasitology 99, 345.

Ministerio de Salud de Colombia, 2002. Boletín epidemiológico semanal comportamiento por regiones de la malaria en el 2001. Instituto Nacional de Salud, Sistema de Vigilancia en Salud Pública SIVIGILA, Bogota.

Mongelli, E., Desmarchelier, C., Coussio, J., Ciccia, G., 1995. Antimicrobial activity and interaction with DNA of medicinal plants from the Peruvian Amazon region. Revista Argentina de Microbiologia 4, 199–203.

Muñoz, V., Sauvain, M., Bourdy, G., Callapa, J., Bergeron, S., Rojas, I., Bravo, J.A., Balderrama, L., Ortiz, B., Gimenez, A., Deharo, E., 2000. A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part I. Evaluation of the antimalarial activity of plants used by the Chacobo Indians. Journal of Ethnopharmacology 69, 127–137.

Steele, J.C., Simmonds, M.S., Veich, N.C., Warhurst, D.C., 1999. Evaluation of the anti-plasmodial activity of bisbenzylisoquinoline alkaloids from *Abuta grandifolia*. Planta Medica 5, 413.

Thongtan, J., Kittakoop, P., Ruangrungsi, N., Saenboonrueng, J., Thebtaranonth, Y., 2003. New antimycobacterial and antimalarial 8,9-secokaurane diterpenes from *Croton kongensis*. Journal of Natural Products 66, 868–870.

Trivedi, C.P., Modi, N.T., Sarin, R.K., Rao, S.S., 1986. Bronchodilator and anti-inflammatory effect of glycosidal fraction of *Acacia farnesiana*. Indian Journal of Physiology and Pharmacology 30, 267–268.

Veras, M.L., Bezerra, M.Z., Braz-Filho, R., Pessoa, O.D., Montenegro, R.C., Pessoa, C.O., Moraes, M.O., Costa-Lutufo, L.V., 2004. Cytotoxic epimeric withaphysalins from leaves of *Acnistus arborescens*. Planta Medica 70, 551–555.