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## ***In vitro* antimalarial activity of six *Aspidosperma* species from the state of Minas Gerais (Brazil)**

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### **ABSTRACT**

Ethnomedicinal informations point to some *Aspidosperma* species (Apocynaceae) as antimalarial plants in Brazil and have motivated the evaluation of six species which were collected in the state of Minas Gerais: *A. cylindrocarpon* Müll. Arg., *A. parvifolium* A. DC., *A. olivaceum* Müll. Arg., *A. ramiflorum* Müll. Arg., *A. spruceanum* Benth. ex Müll. Arg. and *A. tomentosum* Mart.. A total of 23 extracts of different plant parts in different solvents were assayed in vitro against chloroquine-resistant (W2) and chloroquine-sensitive (3D7) strains of *Plasmodium falciparum*. All the extracts were shown to be active with IC<sub>50</sub> values in the range of 5.0 ± 0.28 µg/mL to 65.0 ± 4.2 µg/mL. TLC profile of the extracts revealed the presence of alkaloids in the six species assayed. These results seem to confirm the popular use of *Aspidosperma* species to treat human malaria in Brazil and seem point to alkaloids as the putative active compounds of the assayed species.

**Key words:** antimalarial activity, *Plasmodium falciparum*, *In vitro* assays, *Aspidosperma* spp., Apocynaceae.

### **INTRODUCTION**

Malaria is caused by protozoa of the genus *Plasmodium* and remains as one of the most serious parasitic diseases in the tropical and subtropical regions of the world because of its morbidity, mortality and drug resistance. It is estimated that 3.3 billion people were at risk of malaria in 2010, what represents approximately half of the world's population. There were 216 million cases of malaria

and an estimated 655,000 deaths in 2010, and the disease was present in 106 countries and territories. However, the World Malaria Report 2011 has shown positive aspects in the fight against this disease: a decrease of more than 25% is reported in mortality rates, more than 25% globally since 2000, and by 33% in the WHO African Region. (WHO 2011).

In Brazil, more than 350,000 cases were reported annually for the period of 2001-2007, with a maximum of 607,730 cases, in 2005. A decrease of approximately 25% in the number of reported cases

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was recorded since 2006, what is considered as a consequence of public actions related to vigilance, prevention and control. The majority (97%) of the reported cases occur in the Legal Amazon Region and is mostly caused by *P. vivax*. However, it causes concern the information that, in 2009 and 2010, approximately 16% of the confirmed cases were by *P. falciparum*, the most deadly *Plasmodium* species, (MS 2008, WHO 2011).

Quinine, the first drug used in malaria chemotherapy, was isolated in 1820, by the French scientists Pelletier and Caventou, from the bark of *Cinchona* species (Rubiaceae) which were used by Peruvian Indians, in South America and whose plant material were taken to Europe by Jesuits, in the XVII century. The structure of quinine was established by Rabe in 1908, and its synthesis was accomplished in 1944 by Woodward and Doering (Figure 1). However, its synthetic production by industrial means is both complex and costly. Quinine is still used today, and it is currently obtained by extraction from *Cinchona* spp, which grow wild in South America and are cultivated in Java (Boulos et al. 1997).

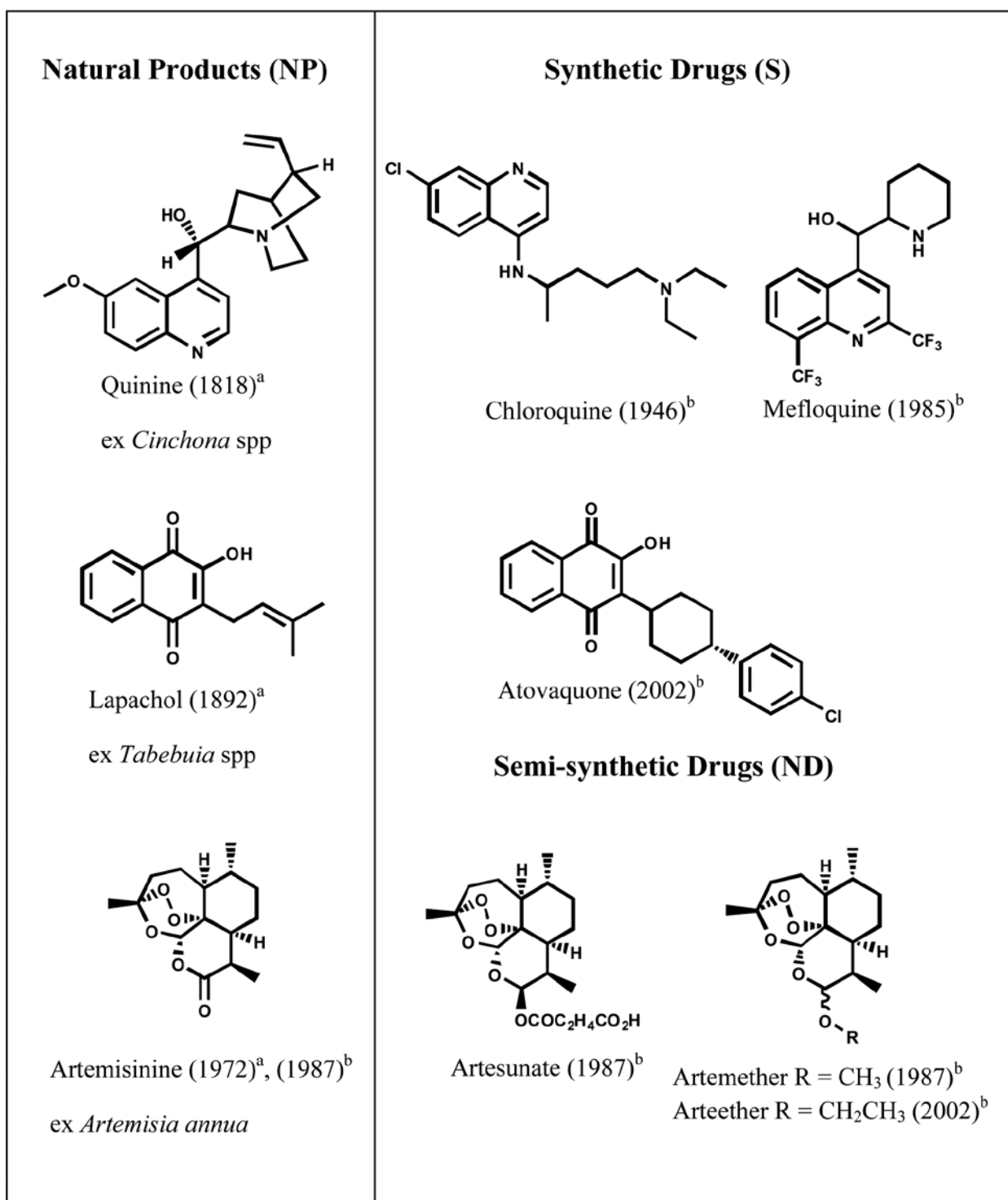
Chloroquine (CQ), a synthetic 4-aminoquinoline developed in the 1940's, represents the most widely used antimalarial drug. However, since the 60's, its effectiveness declined at an accelerating rate, as consequence of the resistance of *P. falciparum* strains which, associated with the resistance of the parasites vectors (*Anopheles*, Culicidae) to insecticides, resulted into significative increase in malaria-related morbidity and mortality. Mefloquine, as quinine, is a 4-quinolinomethanol, but totally synthetic, and was introduced in 1985 (Figure 1). (Guerin et al. 2002, Fidock et al. 2004).

Presently, artemisinin, the active compound from the traditional Chinese drug *Artemisia annua* (Asteraceae), and its semi-synthetic derivatives artemether, artether and artesunate are the most used drugs in malaria therapeutics. However artemisinin based monotherapy represents a risk for the

development of resistance to this drug what led to its withdrawal from the market. Indeed, resistance of *P. falciparum* to artemisinin was confirmed at Cambodia-Thailand border in 2009. (WHO 2010).

Artemisinin-based Combination Therapy – ACTs are recommended and five pharmaceutical formulations are presently marketed. One is based on artemether (artemether-lumefantrine), and all the others are based on artesunate in combination with amodiaquine (2 formulations), mefloquine (one formulation) and one with sulfadoxine + pyrimethamine (WHO 2009) (Figure 1). Atovaquone, the last antimalarial drug introduced in clinics, is a totally synthetic naphthoquinone (Baggish and Hill 2002). In this case, the contribution of natural products was also important once atovaquone was developed after an active natural product as template: the prenylnaphthoquinone lapachol, present mainly in species of the genus *Tabebuia* (Bignoniaceae) which occur in many Southamerican countries (Castellanos et al. 2009, Wagner and Seitz 1998). Atovaquone-proguanil (Malarone®) is one of the non-artemisinin combination therapies in clinical use (WHO 2008). Structures of naturally occurring antimalarial drugs and the synthetic derived ones cited here are shown on Figure 1.

The quest of new antimalarial drugs is needed and the investigation of plants traditionally and/or popularly used to treat malaria is considered a valuable strategy (Ginsburg and Deharo 2011, Bourdy et al. 2007, Wright 2005). Two approaches can be explored: one is focused on the search of naturally occurring bioactive compounds, potential new drugs or templates, and the second one aims at the validation of traditionally used plant remedies which could lead to the development of effective and safe phytomedicines. In the second approach, identification of active compounds is necessary to be used as biomarkers in the standardization of extracts that must be submitted to pre-clinical and clinical studies to guarantee the standard quality required for phytomedicines (Oliveira et al. 2009a, Wagner 2004).



<sup>a</sup>: Year of isolation; <sup>b</sup>: Year of introduction in therapeutics; NP: Natural Products; S\*: Synthetic drug derived after a natural product as template, ND: Natural Product derivatives.

**Figure 1** - Plant-derived antimalarial drugs: natural products (NP), synthetic drugs (S) and semi-synthetic drugs (ND, Natural Derivatives).

The first step in the investigation of a traditionally and/or popularly used plant for treatment of human malaria aiming at the isolation of antimalarial compounds is the *in vitro* evaluation of its extracts against cultures of *P. falciparum* strains in human erythrocytes. This is the most recommended methodology although other approaches are available. (Oliveira et al. 2009a, Fidock et al. 2004). Once the *in vitro* activity is confirmed, extracts with  $IC_{50} \leq 50$   $\mu\text{g/mL}$  deserve further investigation for isolation of antiplasmodial compounds (Oliveira et al. 2009a).

*Aspidosperma* Mart. & Zucc. (tribo Plumeriae) is found from Mexico to Argentina (Marcondes-Ferreira Neto 1988). This genus belongs to the Apocynaceae family and includes 24 species that have been traditionally used for the treatment of human malaria in Brazil and other South American countries. (Brandão et al. 1992, Milliken and Albert 1996, 1997, Bertani et al. 2005, Mariath et al. 2009).

Chemically, *Aspidosperma* species are characterized by the presence of alkaloids. Plants of this genus have afforded more than two hundred indolomonoterpenoid alkaloids, mainly in the period between 1965 and 1980. (Pereira et al. 2007). However, there are relatively few reports on the biological activities of these alkaloids (Oliveira et al. 2009a, b). A relevant one is elliptinium acetate, an antitumoral drug (Celliptium®) that was developed from ellipticine, a pyridocarbazol alkaloid originally isolated from *Ochrosia elliptica* (Australian evergreen shrub), and that occurs in other Apocynaceae genera such as *Aspidosperma* (Cragg and Newman 2005).

Extracts from various *Aspidosperma* species were evaluated for other biological/pharmacological activities. *A. ramiflorum* and *A. olivaceum* were assayed against bacteria, fungi and yeasts when stems and leaves extracts from *A. ramiflorum* showed weak antifungal activity against *Cladosporium cladosporioides* and *C. sphaerospermum*. Inhibition of bacteria growth was observed in cultures of *Escherichia coli* (Agripino et al. 2004). A methanol extract from *A. ramiflorum* stem bark disclosed moderate antibacterial

activity against *Bacillus subtilis* and *Staphylococcus aureus*. Subfractions of this extract showed moderate to high activity against these bacteria as well as to *E. coli*, and *Pseudomonas aeruginosa*. Two alkaloids, ramiflorines A and B, were isolated from *A. ramiflorum* and were identified as responsible for its bactericidal effect (Tanaka et al. 2006).

An alkaloidal extract from *A. ramiflorum*, rich in ramiflorines A and B, showed activity against promastigotes of *Leishmania (V.) braziliensis* and *L. (L.) amazonensis* (Ferreira et al. 2004). Subsequently, ramiflorines A and B were isolated from an *A. ramiflorum* alkaloidal extract and were submitted to further assays against promastigotes of *L. (L.) amazonensis*, confirming the antileishmanial effect (Tanaka et al. 2007).

An ethanol extract from *A. polyneuron* roots exhibited antifungal activity against *Cladosporium herbarium*. However the substance responsible for this activity was not identified (Ferreira et al. 2003). Activity against *Proteus mirabilis* was observed for an ethanol extract from *A. polyneuron* that is rich in alkaloids (Granato et al. 2005).

A pro-erectile effect was observed in mice treated with fractions derived from an ethanol extract from *A. ulei* bark (Campos et al. 2006) and a bark extract from *A. quebracho* blanco was able to stimulate human receptors related to penile erection (Sperling et al. 2002). These two extracts were rich in alkaloids.

*A. nitidum* is used in the Amazon region as antiinflammatory as well as for treatment of fevers and malaria. An ethanol extract from the trunkwood disclosed anti-inflammatory and antinociceptive activities and afforded the following substances: a mixture of  $\beta$ -sitosterol and stigmaterol, pentacyclic triterpenes ( $\beta$ -amyrin and lupeol), an inositol (L-2-O-methyl-4 chiroinositol) and a  $\beta$ -carboline alkaloid (harman-3-carboxylic acid) (Pereira et al. 2006).

Up to now, crude extracts and/or alkaloidal fractions from 11 *Aspidosperma* species have been evaluated for antimalarial activity: *A. excelsum*

(Kvist et al. 2006, Brandão et al. 1992, Carvalho et al. 1991), *A. macrocarpon* (Mesquita et al. 2007), *A. megalocarpon* (Weniger et al. 2001), *A. nitidum* (Becker 1949, Brandão et al. 1992, Carvalho et al. 1991), *A. oblongum* (Cabral et al. 1993), *A. olivaceum* (Brandão et al. 1985), *A. polyneuron* (Wasicky et al. 1942), *A. pyriform* (Bourdy et al. 2004, Weniger et al. 2001), *A. quebracho-blanco* (Bourdy et al. 2004, Mariath et al. 2009), *A. ramiflorum* (Krettli et al. 2008), and *A. rigidum* (Kvist et al. 2006).

Only 14, among hundreds of known alkaloids from *Aspidosperma* were tested: two were isolated from *A. desmanthum* and *A. vargasii* (Andrade-Neto et al. 2007), 11 other ones were previously obtained from *A. megalocarpon* and *A. pyriform* (Mitaine-Offer et al. 2002) and one from *A. parvifolium* (Oliveira et al. 2010).

We report here the evaluation of the antiplasmodial activity of extracts from six *Aspidosperma* species with reputation of use to treat malaria or fever in different regions of Brazil.

## MATERIALS AND METHODS

### PLANT MATERIAL

Plant material from *A. parvifolium* was collected in the municipality of Paracatú, state of Minas Gerais, and all the others were collected in the Pampulha Campus of the Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, Minas Gerais, Brazil. Dr. J. A Lombardi has done the taxonomical identification of the species (present address: Departamento de Botânica, Instituto de Biociências de Rio Claro, UNESP, Av. 24-A 1515, CEP 13506-900, Rio Claro, SP, Brazil). Voucher specimens are deposited in the BHC, UFMG and the code numbers are shown in Table I.

### EXTRACTION

The different plant parts were separated, dried in an oven with circulating air at 40°C and milled. Powdered plant materials were extracted by

percolation with ETOH 96 °GL and/or by Soxhlet extraction successively with dichloromethane and ETOH after addition of 5% ammonium hydroxide to the powdered plant material (v/w). Extracts were concentrated in a rotavapor to give the crude dry extracts whose phytochemical profiles were analysed by TLC (Table I).

### ANTIPLASMODIAL ASSAY

*P. falciparum* clones (W2 and 3D7) were kept in continuous cultures in human erythrocytes suspended in RPMI 1640 supplemented with 10% human serum, using the candle jar/Petri dish method as described by Trager and Jensen (1976). The antiplasmodial effects of the extracts and controls were determined according to the microscopic methodology (Rieckmann et al. 1978) with minor modifications. (Carvalho et al. 1991). Briefly, a suspension of ring-stage parasites in sorbitol-synchronized blood cultures (100 µL) containing predominantly trophozoites was added to 96-well culture plates at 1% parasitemia and 2.5% hematocrit and then incubated with the test samples in various concentrations for a total of 72h, at 37 °C. Negative control of parasite culture and positive control in which chloroquine and mefloquine were added to the parasite culture were run simultaneously. Stock solution of the test samples in DMSO (10 mg/mL) were diluted in complete medium to a final concentration of 0.002% (v/v) and stored at -20°C. After 24h and 48h incubation periods. The culture medium was replaced with fresh medium with or without test samples/control drugs. After further 24h incubation, Giemsa stained smears were microscopically examined to determine the parasitemia by counting 5,000 erythrocytes. All experiments were performed three times, and each sample was tested in triplicate. The results were expressed as the means IC<sub>50</sub> and IC<sub>90</sub> and the Graph Pad Prism 4.0 program was used to statistically compare the inhibition of the two different *P. falciparum* clones.

## CYTOTOXICITY ASSAY

Vero cells were exposed to different concentrations of extracts for 48 and 72 h. After incubation, cell viability was assessed by the MTT assay (Merck solution 2 mg/mL<sup>-1</sup> in PBS) (Twentyman and Luscombe 1987). Each sample was assayed in four replicates for concentrations ranging from 500 to 0.125 µg/mL. The cytotoxicity of each sample was expressed as CC<sub>50</sub>, i.e. the concentration of sample that inhibited cell growth by 50%.

## RESULTS AND DISCUSSION

The six species we had assayed, *A. cylindrocarpon* Müll. Arg., *A. parvifolium* A. DC., *A. olivaceum* Müll. Arg., *A. ramiflorum* Müll. Arg., *A. spruceanum* Benth. ex Müll. Arg. and *A. tomentosum* Mart.,

were phytochemically investigated previously and the isolation of indolomonoterpenoid alkaloids was described for all of them (for a review see Pereira et al. 2007).

A total of 23 extracts were obtained from different parts of the six *Aspidosperma* species. Powdered dry plant materials were extracted by two different methodologies: 1) percolation with ethanol 96 °GL, and 2) Soxhlet extractions with dichloromethane followed by ethanol 96 °GL of the powdered plant material to which ammonium hydroxide had been added. Data on collection sites, popular names, code number of voucher specimens and classes of natural products detected by TLC phytochemical screening are shown in Table I. As expected, alkaloids were detected in all the six species by TLC analysis.

TABLE I  
Data on collection sites, local names, voucher specimens, plant parts extracted, extraction, phytochemical screening by TLC of the *Aspidosperma* species extracts (Apocynaceae).

<i>Aspidosperma</i> species	Collection sites	Local names (Lorenzi 1992, Pio Correa 1978)	Voucher numbers	Extraction Solvent/method/plant part	NP Classes (TLC) Wagner 2004
<i>A. cylindrocarpon</i> Müll. Arg.	BH/UFGM	peroba-de Minas, peroba-rosa, peroba-iquira	BHCB47812	ETOH-P-Tr	A, F, T, St, Sa
<i>A. olivaceum</i> Müll. Arg.	BH/UFGM	guatambú-marfim, pequiá-marfim, pequiá-branco		DCM-S-L	T, St, Sa
				ETOH-S-L	T, St
				ETOH-P-Tr	F, T, St
				DCM-S-Tr	A, Sa
				ETOH-S-Tr	T, St, Sa
				DCM-S-B	A, S e F
				ETOH-S-B	A, Sa, F
<i>A. ramiflorum</i> Müll. Arg.	BH-UFGM	matambú, guatambú, guatambú-amarelo, pequiá-doce, pequiá	BHCB848	ETOH-P-L	A, Sa, T, St, F
				DCM-S-L	A, Sa, T, St, F
				ETOH-S-L	A, Sa, T, St, F
				ETOH-P-Tr	A, F
				DCM-S-Tr	A, Sa, T, St, F
				ETOH-S-Tr	A
				DCM-S-B	A, Sa, T, St, F
<i>A. parvifolium</i> A. DC				ETOH-S-B	A, F
	Paracatu	guatambú, guatambú-branco, pequiá-marfim, peroba, paupereira, tambú	BHCB60345	ETOH-P-B	A, F, T, St, Sa

TABLE I (continuation)

<i>A. spruceanum</i> Benth. ex Müll. Arg.	BH-UFGM	amargoso, peroba, guatambú-rugoso, quina-da-mata, pequiá-marfim	BHCB46274	ETOH-P-L	F, Sa, T, St
				DCM- S-L	Sa, T, St
				ETOH- S-L	F, T, St, Sa
				DCM- S-Tr	A, Sa, T, St
				ETOH-S-Tr	A, Sa
				ETOH-P-Tr	A, Sa, T, St
				DCM- S-Tr	A, As, T, St
				ETOH-S-Tr	A, As, T, St
<i>A. tomentosum</i> Mart.		pau-pereira-do-campo, pereiro-do-campo, peroba-do-campo, peroba-do-cerrado	BHCB49751	ETOH-P-Tr	A, T, St, F
				ETOH-P-L	F, Sa, T, St, F
				ETOH-P-F	A, Sa, T, St, F
				ETOH-P-Se	A, Sa, T, St

BH: Belo Horizonte, B: trunk bark; F: fruits, L: leaves; Tr: trunk wood; Se: seeds; P: percolation; S: Soxhlet; NP: natural products, A: alkaloids; T: triterpenes; St: steroids; F: flavonoids; Sa: saponins.

*In vitro* assays were carried out by the microspic method (Rieckmann et al. 1978) with minor modifications (Carvalho et al. 1991). Extracts were assayed against chloroquine-resistant (CQR, W2) and sensitive (CQS, 3D7) clones of *P. falciparum* and all of them had shown to be active disclosing  $IC_{50}$  values in the range of  $5.0 \pm 0.28 \mu\text{g/mL}$  to  $65.0 \pm 4.2 \mu\text{g/mL}$  (Table II).

It is usually considered that extracts showing  $IC_{50} \leq 50 \mu\text{g/mL}$  deserve further investigations for the isolation and identification of active compound(s). All the extracts, except that one from *A. spruceanum* leaves (EtOH-P-L, W2  $IC_{50} = 65.0 \pm 4.2$ , 3D7  $IC_{50} > 100$ , 3D7), fall in this group (Table II).

For 9 of the 23 extracts, the  $IC_{50}$  values are lower than  $10 \mu\text{g/mL}$  and they can be considered as very active: five extracts from *A. olivaceum* (DCM-S-L, ETOH- S-L, DCM-S-Tr, DCM-S-B, ETOH-S-B), two from *A. ramiflorum* (DCM-S-L and DCM- S-B) and two from *A. spruceanum* (DCM-S-Tr and DCM- S-B). Interestingly, all these extracts were obtained by the traditional Soxhlet extraction method of the alkalinized powdered plant material,

leading to a more selective extraction once, in this condition, acidic compounds (carboxylic acids and phenols) are in their salt forms and, therefore, are not extracted resulting in a selective extraction of alkaloids along with neutral compounds.

Concerning the susceptibility towards the *P. falciparum* clones, 10 out of the 23 extracts showed no significant difference against CQR and CQS *P. falciparum* clones: one from *A. cylindrocarpon* (ETOH-P-Tr), four from *A. olivaceum* (ETOH-S-L, DCM-S-Tr, DCM-S-B, ETOH-S-B), two from *A. ramiflorum* (DCM-S-L, DCM-S-B), one from *A. spruceanum* (DCM- S-Tr) and two from *A. tomentosum* (ETOH-P-Tr and ETOH-P-L). Seven of the extracts were more active in the CQS (3D7) clone than in the CQR (W2) clone and the difference in the  $IC_{50}$  values is higher for those of *A. ramiflorum* ETOH-S-Tr (3D7  $0.98 \pm 0.03 \mu\text{g/mL}$  vs W2  $19.75 \pm 0.35 \mu\text{g/mL}$ ) and *A. tomentosum* ETOH-P-Se (3D7  $3.03 \pm 0.20 \mu\text{g/mL}$  vs W2  $24.51 \pm 3.56 \mu\text{g/mL}$ ). Nine of the extracts were more active in the CQR (W2) clone and a higher difference was observed for the DCM leaves *A. olivaceum* extract (DCM-S-L) which was prepared

**TABLE II**  
***In vitro* antimalarial activity of the six *Aspidosperma* species extracts against *Plasmodium falciparum* (W2/CQR, 3D7/CQS clones) and cytotoxicity in Vero cells cultures.**

Species Extracts	W2		3D7		Vero cells
	IC <sub>50</sub> (µg/ml) ± SD	IC <sub>90</sub> (µg/ml) ± SD	IC <sub>50</sub> (µg/ml) ± SD	IC <sub>90</sub> (µg/ml) ± SD	CC <sub>50</sub> (µg/ml)
<i>Aspidosperma cylindrocarpon</i>					
ETOH-P-Tr	44.0 ± 6.36	104.5 ± 6.36 2.3	39.0 ± 2.83	89.0 ± 7.07 2.3	>500
<i>A. olivaceum</i>					
DCM-S-L	7.0 ± 0.2	23.0 ± 0.2 3.2	25.5 ± 2.12	49.5 ± 6.3 1.9	>500
ETOH- S-L	7.0 ± 0.71	26.5 ± 0.71 3.8	5.0 ± 2.80	24.5 ± 2.10- 4.9	ND
DCM-S-Tr	<6	ND	<6	ND	>500
DCM-S-B	<6	ND	<6	ND	
ETOH-S-B	5.0 ± 2.8	24.5 ± 2.8 4.9	7.0 ± 0.42	26.5 ± 0.71 3.8	>500
<i>A. parvifolium</i>					
ETOH-P-B	32.75 ± 1.06	74.50 ± 1.34 2.3	20.51 ± 0.70	38.00 ± 4.26 1.8	>500
<i>A. ramiflorum</i>					
ETOH-P-L	32.8 ± 1.13	61.75 ± 1.13 1.8	20.5 ± 0.71	60.5 ± 0.71 3.0	ND
DCM-S-L	<6	ND	<6	ND	ND
ETOH-P-Tr	36.5 ± 0.20	100.0 ± 0.20 2.7	48.0 ± 1.1	103.0 ± 1.4 2.1	ND
DCM-S-Tr	ND	ND	9.5 ± 1.41	27.0 ± 1.41 2.8	>500
ETOH-S -Tr	19.75 ± 0.35	30.75 ± 0.35 1.5	0.98 ± 0.03	16.0 ± 2.8 16	ND
DCM- S-B	<6	ND	<6	ND	>500
<i>A. spruceanum</i>					
ETOH-P-L	65.0 ± 4.2	107.8 ± 0.71 1.6	>100	ND	ND
DCM- S-L	23.25 ± 0.35	47.0 ± 2.83 2.0	35.0 ± 4.2	63.5 ± 0.7 1.8	ND
ETOH- P-Tr	29.52 ± 0.71	ND	41.5 ± 2.12	101.5 ± 9.1 2.4	ND
DCM- S-Tr	<6.0	ND	< 6.0	ND	109,6
CLOR-P-Tr	37.0 ± 7.1	102.0 ± 2.8 2.8	>100	ND	ND
ETOH-P-B	26.25 ± 4.07	48.50 ± 3.18 1.8	14.0 ± 4.2	30.0 ± 2.83 2.1	ND
DCM- S-B	< 6.0	ND	15.75 ± 1.76	28.5 ± 0.71 1.8	ND
ETOH-S-B	28.01 ± 3.51	52.03 ± 2.83 1.8	19.0 ± 2.83	50.5 ± 2.12 2.6	ND
<i>A. tomentosum</i>					
ETOH-P-Tr	26.50 ± 3.50	54.75 ± 1.09 2.0	25.00 ± 4.24	61.00 ± 4.24 2.4	ND
ETOH-P-L	23.75 ± 1.06	54.75 ± 1.09 2.0	27.00 ± 5.66	47.00 ± 5.66 1.7	ND
ETOH-P-F	20.52 ± 1.41	37.53 ± 0.71 1.8	38.55 ± 1.06	99.54 ± 4.95 2.5	ND
ETOH-P-Se	24.51 ± 3.56	54.75 ± 1.09 2.2	3.03 ± 0.20	27.03 ± 0.20 9.0 1.5	>500
Positive controls					
Chloroquine	0.02 ± 0.002	ND	0.0013 ± 0.0001	0.0020 ± 0.0001 1.5	ND
Mefloquine	0.0165 ± 0.002	0.0895 ± 0.02	0.048 ± 0.0007	0.0975 ± 0.0007 2.0	ND

**CQR:** chloroquine-resistant, **CQS:** chloroquine-sensitive, **SD:** Standar Deviation, **ETOH:** ethanol, **DCM:** dichloromethane, **P:** percolation, **S:** Soxhlet extraction, **B:** trunk bark, **F:** fruits, **L:** leaves, **Tr:** trunk wood; Se: seeds; **IC<sub>50</sub>:** 50% inhibitory concentration, **IC<sub>90</sub>:** 90% inhibitory concentration, **CC<sub>50</sub>:** 50% cytotoxic concentration; **ND:** Not determined.

by Soxhlet extraction of alkalized plant material and was approximately 3 times more potent against the W2 strain ( $IC_{50} 7.0 \pm 0.71 \mu\text{g/mL}$ ) than against the 3D7 strain ( $IC_{50} 25.5 \pm 2.12 \mu\text{g/mL}$ ). Surprisingly, this extract did not show alkaloids on TLC, an indicative that the active compound(s) do(es) not belong to this natural product class. Besides, as mentioned, *A. olivaceum* was tested previously and negative results were reported for EtOH and Hexane-AcOEt extracts in assays in *P. berghei* infected mice. There is no information on the plant part used. (Brandão et al. 1985). Recently, the antimalarial activity of *A. ramiflorum* was reported *in vitro* against *P. falciparum* W2 clone ( $IC_{50}$  11 to 40  $\mu\text{g/mL}$ ). In vivo tests with *P. berghei* NK65 in mice showed that the crude extract and total alkaloids were partially active at 500 mg/kg and 250 mg/kg, respectively (Krettli et al. 2008).

The  $IC_{90}/IC_{50}$  ratios of the extracts towards both of the *P. falciparum* clones (W2 and 3D7) are in the range of 16.0 to 1.5 and for the antimalarial

drugs chloroquine and mefloquine the ratios are 1.5 and 2.0, respectively. For six of the 23 extracts assayed ratios  $> 2.0$  were calculated for each of the *P. falciparum* clones what allows one to infer that these extracts might be the most promising ones, particularly those related to the CQR clone: *A. ramiflorum* (ETOH-P-L and ETOH-S -Tr), *A. spruceanum* (ETOH-P-L, ETOH-P-B and ETOH-S-B) and *A. tomentosum* (ETOH-P-F).

The cytotoxicity of some of the 23 extracts was assessed in Vero cells culture by the MTT assay (Twentyman and Luscombe 1987) and no cytotoxic effect was observed up to the concentration of 500  $\mu\text{g/mL}$ , except for *A. spruceanum* dichloromethane trunkwood extract (DCM-S-Tr) which was moderately cytotoxic ( $CC_{50} = 109.6 \mu\text{g/mL}$ ) (Table III). The Selectivity Index ( $SI = CC_{50}/IC_{50}$ ) was calculated for some of the extracts and values higher than 10 were observed disclosing the potential of these species as sources of antimalarial compounds. (Table III).

TABLE III  
Selectivity index (SI) for some *Aspidosperma* species extracts which have been assayed for antiplasmodial activity (W2/CQR, 3D7/CQS) and cytotoxicity in Vero cells culture.

<i>Aspidosperma</i> species	Extracts	$CC_{50}$ ( $\mu\text{g/mL}$ ) (Vero cells)	SI (W2)	SI (3D7)
<i>A. cylindrocarpon</i>	ETOH-P- Ca-L	>500	>11.36	>12.82
<i>A. olivaceum</i>	DCM-S-L	>500	>71.43	>19.61
	DCM-S-B	>500	>83.33	>83.33
	ETOH-S-B	>500	>100.00	>71.43
<i>A. parvifolium</i>	EtOH-P-B	>500	>15.27	>24.38
<i>A. ramiflorum</i>	DCM-S-L	>500	ND	>52.63
	DCM-S-Tr	>500	>83.33	>83.33
<i>A. spruceanum</i>	DCM-S-Tr	109.6	18.27	>18.27
<i>A. tomentosum</i>	ETOH-P-Se	>500	>20.4	>165.02

$CC_{50}$ : 50% Cytotoxic Concentration; **W2**: *P. falciparum* clone resistant to chloroquine and sensitive to mefloquine; **3D7**: *P. falciparum* clone sensitive to chloroquine; **SI**: Selectivity Index; **DCM-S L**: Leaves EtOH extract prepared in Soxhlet; **ETOH-S L**: Leaves EtOH extract prepared in Soxhlet; **ETOH-P-TR**: Trunk wood EtOH extract obtained by percolation; **DCM-S-Tr**: Trunk wood DCM extract obtained in Soxhlet; **DCM-S-B**: Trunk bark DCM extract obtained in Soxhlet; **ETOH-P-B**: Trunk bark EtOH extract obtained by percolation; **ND**: Not Determined.

The observed antiplasmodial activity may be attributed to indolomonoterpenoid alkaloids which were isolated previously from the six evaluated species. Indeed, antimalarial *in vitro*

activity was reported for alkaloids isolated from five *Aspidosperma* species: *A. Vargasii* A. DC., *A. desmanthum* Benth. (Andrade-Neto et al. 2007), *A. pyrifolium* Mart. and *A. megalocarpon* Müll.

Arg. (Mitaine-Offer et al. 2002) as well as from *Geissospermum sericeum* (Sagot.) Benth. & Hook.f. which belongs also to the Apocynaceae family and is used to treat malaria in Brazil, Guiana and Suriname (Steele et al. 2002). We have previously reported on the isolation and identification of alkaloids from *A. parvifolium* (Jácome et al. 2004) and more recently on the antiplasmodial activity of uleine, the major alkaloid from this species. (Oliveira et al. 2010)

Our results seem to confirm the popular use of *Aspidosperma* species to treat malaria in Brazil and other Central and Southamerican countries. The great chemical diversity of alkaloids from *Aspidosperma* species (Pereira et al. 2007), the low number of those that were assayed for antiplasmodial activity and the traditional use of several *Aspidosperma* species to treat malaria/fever turn this investigation of great interest. Bioguided isolation of antiplasmodial compounds from the *Aspidosperma* species assayed is on progress.

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UFMG, Belo Horizonte, Brazil. The other authors have participated in the antiplasmodial bioassays under the supervision of MMP, in the Instituto Evandro Chagas – IEC, PA, Brazil.

#### RESUMO

Informações etnomédicas mostram que algumas espécies de *Aspidosperma* (Apocynaceae) são utilizadas contra a malária no Brasil e motivaram a avaliação de 6 espécies que foram coletadas no Estado de Minas Gerais: *A. cylindrocarpon* Müll. Arg., *A. parvifolium* A. DC., *A. olivaceum* Müll. Arg., *A. ramiflorum* Müll. Arg., *A. spruceanum* Benth. ex Müll. Arg. and *A. tomentosum* Mart. Um total de 23 extratos de diferentes partes das plantas, em diferentes solventes, foram testados *in vitro* contra cepas de *Plasmodium falciparum* resistente a cloroquina (W2) e sensível a cloroquina (3D7). Todos os extratos mostraram-se ativos apresentando valores de  $CI_{50}$  na faixa de  $5,0 \pm 2,8 \mu\text{g/mL}$  a  $65,0 \pm 4,2 \mu\text{g/mL}$ . O perfil por CCD dos extratos revelou a presença de alcalóides nas 6 espécies avaliadas. Esses resultados parecem confirmar o uso popular de espécies de *Aspidosperma* no tratamento da malária humana no Brasil e parecem indicar os alcalóides como possíveis compostos ativos das espécies testadas.

**Palavras-chave:** atividade antimalárica, *Plasmodium falciparum*, ensaios *in vitro*, *Aspidosperma* spp., Apocynaceae.

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