Aspidosperma Species as Sources of Antimalarials. Part III. A Review of Traditional Use and Antimalarial Activity*

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- *Aspidosperma* spp.
- Apocynaceae
- malaria
- *Plasmodium falciparum*
- antimalarial activity

Abstract

Several plant species belonging to the genus *Aspidosperma* are traditionally used in Brazil and other Meso- and South American countries for the treatment of malaria and fevers. These traditional uses were motivation for this review. A literature survey completed for this review has identified scientific bibliographical references to the use of 24 *Aspidosperma* species to treat malaria/fevers and to 19 species that have had their extracts and/or alkaloids evaluated, with good results, for *in vitro* and/or *in vivo* antimalarial activity. Indole alkaloids are typical constituents of *Aspidosperma* species. However, only 20 out of more than 200 known indole alkaloids isolated from this genus have been assayed for antimalarial activity. These data support the potential of *Aspidosperma* species as sources of antimalarials and the importance of research aimed at validating their use in the treatment of human malaria.

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Introduction

Natural products and malaria

Malaria remains one of the most prevalent infectious diseases worldwide and is, therefore, a global health problem despite substantial efforts to control the disease over the past few decades. Approximately 3.3 billion people are at risk, and 250 million cases each year were reported in the period 2006–2008, primarily in Africa [1]. In the Americas, malaria transmission occurs in 21 countries. *P. vivax* caused 77% of all cases reported in 2008, but *P. falciparum* was responsible for almost 100% of all cases in Haiti and the Dominican Republic [1]. Brazil reported the highest number of malaria cases (603 532) in the region in 2005, primarily in the Brazilian Legal Amazon Region, where 10–15% of the population is at risk. Brazil was among the 30 highest-burden countries for malaria [2]. However, a decrease of approximately 25% in the number of reported cases has been recorded since 2006 [3,4].

Historically, plants have had a remarkable role in therapeutics and were the principal source of drugs until the 19th century. Quinine, isolated in 1820, from *Cinchona* species (Rubiaceae), was the first antimalarial drug introduced in chemotherapy and remained the only clinical weapon until the 1940s, when chloroquine, a synthetic 4-aminoquinoline, became available. Efficient and inexpensive, chloroquine was widely used until the 1960s, when resistance to the drug by *P. falciparum* became widespread in the malaria-endemic countries, causing a strong increase in mortality rates. The antimalarial drugs in current use are artemisinin, the active compound from *Artemisia annua* L. (Asteraceae), a traditional plant used for millennia in China, and its semisynthetic derivatives artemether, artesunate, and arteether [5]. Artemisinins are currently the most effective drugs for antimalarial chemotherapy and have been globally adopted for the treatment of *P. falciparum* malaria. The most recently introduced antimalarial drug is atovaquone, a synthetic naphthoquinone based on lapachol. Lapachol, a prenylnaphtoquinone, was first isolated from *Tabebuia impetiginosa* (Mart. ex DC.) Standl. (synon. *T. avellanedeae* Lor. ex Griseb.), a South American representative of the Bignoniaceae [6].

The emergence of *P. falciparum* strains resistant to artemisinin and its derivatives would cause a resurgence of human malaria to high levels in many

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countries. For this reason, R&D on new antimalarial drugs is urgent. Plants continue to represent a valuable source of drugs. A review of all small molecules that have been approved as pharmaceutical agents within the 25-year period from 1/1981 to 6/2006 has demonstrated that approximately 50% of these molecules originated from natural products [7]. Investigations that begin by screening plants used in traditional medicines are particularly valuable. In the past decade, a substantial number of publications have focused on the screening of either extracts or natural products for antimalarial activity [8]. However, as we have noted previously, very few highly active antimalarial natural products have been evaluated for cytotoxicity and in *in vivo* assays. This lack of previous study limits the potential of these products as bases for the development of new antimalarial drugs [5,9]. The relevance of these results should not, however, be underestimated. Active naturally occurring antimalarial compounds might be useful as bases for semisynthetic derivatives, as agents for direct use if their structures are too complex for an economically and/or technologically viable total synthesis, or as templates for the total synthesis of structurally related compounds [5]. Moreover, bioactive natural products from medicinal plants might be useful as biological markers in the development of efficient and safe phytomedicines [5,9], a new approach of growing interest that would provide malaria-endemic countries with good-quality herbal medicines of low cost, that would be locally and sustainably produced [8]. High-tech methods are available to standardize phytopreparations [10,11], and new molecular biological assays can serve to screen extracts and plant constituents as well as to evaluate their pharmacological profiles, elucidate the synergistic effects of the constituents of an extract and, thus, gain a better understanding of the various mechanisms underlying their pharmacological effects [11–14].

Several *Aspidosperma* species have a history of medicinal uses, including the treatment of human malaria and/or fevers in Brazil as well as in other Meso- and South American countries [15–19]. The present review reports the results of a literature survey on *Aspidosperma* species traditionally used to treat malaria and/or fevers as well as data on those previously evaluated for antimalarial activity.

The Genus *Aspidosperma*

▼

Taxonomy and geographic distribution

The genus *Aspidosperma* Mart. & Zucc. belongs to the family Apocynaceae, one of the ten largest angiosperm families [20]. The Apocynaceae s.l. belong to the order Gentianales and have a primarily pantropical/subtropical distribution with few temperate representatives [21].

Aspidosperma species are of outstanding economic interest as sources of valuable timber. They are found from Mexico to Argentina. The number of species in this genus is controversial. Woodson [22] recognizes 52 species, whereas Marcondes-Ferreira and Kinoshita [23] have proposed an infra-generic division for this genus with 43 species, 32 of them occurring in Brazil. However, in the "Lista de Espécies da Flora do Brasil (LEFB)", 51 species are listed with accepted names; 21 of these species are endemic to Brazil, and 90 synonyms are reported [24].

Traditional use in treating malaria

Data obtained by searching for *Aspidosperma* species with reported traditional uses as antimalarials and/or febrifuges as well as those species that have been experimentally evaluated for antimalarial activity are shown in **Table 1**. The table includes the accepted taxonomic nomenclature, synonyms, and occurrence, according to Koch and collaborators [20], LEFB [24], Lorenzi (1992) [15], TROPICOS® Specimen Data Base [25], and The Plant List [26]. The reported uses for treating malaria/fevers are based on the scientific literature. Medicinal uses described on commercial Internet sites, as well as uses other than for malaria and/or fevers, have not been included. Reported antimalarial activity may refer to *in vitro* assays with different *Plasmodium* species, *in vivo* assays in different animals with different *Plasmodium* species, or clinical assays in humans. Details of these findings are shown in **Table 2**.

Our literature survey has identified 24 *Aspidosperma* species (**Table 1**) used to treat malaria/fevers, representing approximately 50% of the representatives of this taxon [24]. In addition to these 24 species, **Table 1** includes two other species, *A. cylindrocapon* and *A. macrocarpon*, for which no reports have been found on their use to treat malaria/fever but that have been evaluated for antimalarial activity [27,28].

The "Dicionário das plantas úteis do Brasil e das exóticas cultivadas" (Dictionary of useful plants from Brazil and of the exotics cultivated), by Pio-Corrêa (1874–1934) [17], was our first source of information on the antimalarial use of plants belonging to the genus *Aspidosperma*. More than 50 *Aspidosperma* species are listed within this six-volume collection, with taxonomic data and vernacular names. Most of the applications described there are as sources of timber, whereas only three species, *A. discolor, A. polyneuron*, and *A. gomezianum*, are identified as plants used to treat malaria and/or fevers.

The botanical names shown for the 26 *Aspidosperma* species in **Table 1** are those originally reported in the literature. However, it should be emphasized that these are not the presently accepted names in certain cases. This is the situation for *A. marcgravianum* Woodson (*A. marckgravianum*), which is not presently an accepted name [24] but a synonym of *A. excelsum* Benth. together with *A. nitidum* Benth. ex Müll. Arg., according to Koch and collaborators [20] and LEFB [24], but is described as a synonym for *A. excelsum* only by the The Plant List [26]. In the same source [26], however, *A. nitidum* Benth. ex Müll. Arg. is an accepted name, with *A. acquaticum* Ducke as a synonym. Another contradiction is found for *A. album* (Vahl) Benoist ex Pichon and *A. desmanthum* Benth. ex Müll. Arg., cited as accepted names for distinct species [20,24,26], whereas the second species represents a synonym for the first one [25]. Furthermore, *A. parvifolium* A. DC. is the accepted name of a species that has *A. tambopatense* A.H. Gentry and *A. vargasii* A. DC. as synonyms [20,24], whereas the latter synonym is also cited as a distinct species [25,26]. A similar situation is observed for *A. tomentosum* Mart., an accepted name having *A. gomezianum* A. DC. as a synonym [25, 26], although both of them are also reported as distinct species [20,26]. These observations show that several controversies remain in the taxonomy of *Aspidosperma* species.

Phytochemistry and antimalarial activity

Chemically, *Aspidosperma* species are characterized by the presence of alkaloids and have yielded more than 200 indole alkaloids. The phytochemical investigation of several Brazilian *Aspidosperma* species, primarily in the period between 1960 and

Table 1 Literature survey on *Aspidosperma* species, their traditional use to treat malaria and/or fevers, and reported antimalarial activity.

***Aspidosperma* species**	**Local names in Brazil References: [15, 17, 20, 24]**	**Occurrence References: [15, 17, 20, 24–26]**	**Reported use to treat malaria/ fever**	**Reported anti-malarial activity**
A. album (Vahl) Benoist *ex* Pichon Syn. *A. desmanthum* Benth. ex Müll. Arg., *A. centrale* Markg. [20, 24, 26]	Cabeça-de-arara, Piquiá-marfim, Araracanga, Aracanga-preta, Mapa-rana	Bolívia, Colômbia, French Guiana, Guyana, Suriname, Venezuela, Brazil: Amazonia	Yes [62, 63]	No
A. auriculatum Markgr. [20, 26]	Carapanaúba Carapanaúba-amarela	Brazil: Endemic (PA)	Yes [64]	No
A. cuspa (Kunth) S. F. Blake ex Pittier Syn. *A. decipiens* Müll. Arg. (+ 11 synon.) [20, 26]	Guatambuzinho, Guatambú-branco, Amargoso, Peroba-de-Goiás	Bolivia, Colombia, Ecuador, Haiti, Venezuela, Paraguay, Peru, Republica Dominicana, Trinidad Tobago, Venezuela, Brazil: Amazonia, Caatinga, Cerrado, Atlantic Rainforest	Yes [65]	No
A. cylindrocarpon Müll. Arg. Syn. *A. brevifolia* Rusby [20, 26]	Peroba-iquira, Peroba de Lagoa Santa, Peroba de Minas, Peroba rosa	Bolivia, Paraguay Peru, Brazil: Amazônia, Cerrado, Atlantic Rainforest	No	Yes See ◘ **Table 2**
A. desmanthum Benth. ex Müll. Arg Accepted name Syn. *A. cruentum* Woodson, *A. chiapense* Matuda, *A. matudae* Lundell [20, 24, 26] Syn. *A.* album (Vahl) Benoist *ex* Pichon [25]	Araraíba, Peroba, Arraracanga, Amargoso, Pequiá-marfim	Belize, Bolivia, Colombia, Ecuador, French Guiana, Honduras, Mexico, Panama, Nicaragua, Brazil: Amazonia, Atlantic Forest	Yes [17, 63, 66]	Yes See ◘ **Table 2**
A. discolor A. DC. Accepted name Syn. *A. francisii* A. DC. [20, 26]	Cabo-de-machado, Quina, Carapanaúba-amarela, Pau-pereira, Peroba-de-rego, Peroba	Bolívia, French Guiana, Guyana, Peru, Suriname, Venezuela, Brazil: Amazonia, Caatinga, Cerrado, Atlantic Forest	Yes [17]	No
A. excelsum Benth. Accepted name Syn. *A. marcgravianum* Woodson, *A. nitidum* Benth. ex Müll. Arg. [20, 25, 26]	Carapanaúba, Carapanaúba-preta, Sapopema, Sapopemba	Costa Rica, Panama, Colombia, French Guiana, Guyana, Ecuador, Peru, Bolivia, Venezuela, Brazil: N, NE, SE, CW	Yes [67–70]	Yes See ◘ **Table 2**
A. gomezianum A. DC. Accepted name [20, 24] Non-cons., syn. of *A. tomentosum* Mart. [25]	Peroba-amarela, Pequiá-de-pedra, Pau-cetim, Guatambu, Ipê-peroba	Brazil: NE (Bahia), SE/Minas Gerais, Espirito Santo, Rio de Janeiro	Yes [17, 28]	No
A. macrocarpon Mart. Accepted name Syn. *A. macrocarpum* Mart., *A. duckei* Huber ex Ducke, *A. gardneri* Müll. Arg. (+ 6 other syn.) [20, 26]	Guatambú, Guatambú-do-cerrado, Peroba-do-campo, Pau-pereira, Pereiro	Bolívia, Ecuador, Peru, Brazil: Amazonia, Cerrado	No	Yes See ◘ **Table 2**
A. marcgravianum Woodson This name is a synonym of *A. excelsum* Benth and *A. nitidum* Benth. ex Müll. Arg. [24, 26]	Carapanaúba, Carana-paúba-preta	Bolivia, Colômbia, French Guiana, Guyana, Ecuador, Panamá, Peru, Suriname, Venezuela	Yes [71]	Yes See ◘ **Table 2**
A. megalocarpon Müll. Arg. Accepted name Syn. *A. lundellianum* Woodson, *A. saguinale* Bartl., *A. stegomeris* (Woodson) Woodson [22, 26]	Pequiá-marfim, Araracanga, Carapanaúba, Carapanaúba-preta	Central America from México to Colombia [25], Brazil: Endemic (Acre) [24]	Yes [36]	Yes See ◘ **Table 2**
A. nitidum Benth ex Müll. Arg. Accepted name Syn. *A. aquaticum* Ducke, Syn. of *A. excelsum* Benth. [24–26]	Carapanaúba, Maparaná	Peru [25], Brazil: Amazonas [25], Roraima [24]	Yes [72]	Yes See ◘ **Table 2**
A. oblongum A. DC. Accepted name Syn. *A. khulmannii* Markgr. [26]	Carapanaúba, Carapa-naúba- amarela	French Guiana, Guyana, Suriname, Venezuela, Brazil: Pará, Amazonas, Maranhão, Goiás	Yes [73] (*A. oblongum*)	Yes See ◘ **Table 2**
A. olivaceum Müll. Arg. Accepted name Syn. *A. bello-horizontinum Silveira* [20, 26]	Guatambú, Guatambu-mirim	Brazil: Endemic to Atlantic Rainforest (NE, SE, S) [24], Argentina [23, 25]	Yes [67]	Yes See ◘ **Table 2**
A. parvifolium A. DC. Accepted name Syn. *A. ingratum* K. Schum., *A. vargasii* A. DC., *A. tambopatense* A. H. Gentry [20, 25, 26]	Pau-Pereira, Peroba, Guatambú, Guatambú-rosa, Guatambú-marfim Amarelão	Argentina, Bolivia,Guyana, Paraguay, Peru, Venezuela, Brazil: Amazonia, Caatinga, Cerrado, Atlantic Rainforest	Yes [74]	Yes See ◘ **Table 2**

continued

Table 1 *Continued*

Aspidosperma species	Local names in Brazil References: [15, 17, 20, 24]	Occurrence References: [15, 17, 20, 24–26]	Reported use to treat malaria/ fever	Reported anti-malarial activity
A. polyneuron Müll. Arg. Accepted name Syn. *A. dugandii* Standl., *A. peroba* Allemão ex Saldanha, A. *venosum* Müll. Arg. [20, 24, 26]	Peroba-rosa, Sobro, Peroba-amargosa	Argentina, Bolívia, Colombia, Paraguay, Peru, Venezuela, Brazil: Caatinga, Cerrado, Atlantic Rainforest	Yes [17, 75]	Yes See ▶ **Table 2**
A. pyrifolium Mart. Accepted name Syn. *A. bicolor Mart.* (+ several others) [26]	Pereiro-do-sertão, Pereiro-preto, Pau–de-coam, Pequiá-da-rest-inga	Argentina, Bolívia, Paraguay, Brazil: Caatinga, Cerrado	Yes [62]	Yes See ▶ **Table 2**
A. quebracho blanco Schltdl. Accepted name Syn. *A. quebracho* Griseb (+ several others) [26]	Quebracho-branco (Brazil), Quebracho-blanco (Argentina)	Argentina, Bolivia, Paraguay, Uruguay, Brazil: Mato Grosso	Yes [68, 72, 76, 77]	Yes See ▶ **Table 2**
A. ramiflorum Müll. Arg. Accepted name Syn. *Geissospermum ramiflorum* (Müll. Arg.) Miers [26]	Pau-pereira, Peroba, Guatambú-amarelo	Bolívia, Brazil: Amazonia Cerrado, Atlantic Rainforest	Yes [22]	Yes See ▶ **Table 2**
A. rigidum Rusby Accepted name Syn. *A. acreanum* Markg. (+ several others) [20, 26]	Carapanaúba, Aracaranga	Bolívia, Colombia, Costa Rica, Ecuador, Panamá, Peru, Venezuela [25], Brazil: Endemic (Amazonia, Cerrado) [24]	Yes [39]	Yes See ▶ **Table 2**
A. sandwithianum Markgr. Accepted name No synonyms [20, 26]	Carapanaúba	French Guiana, Guyana, Suriname, Brazil: Pará, Amazonas	Yes [71]	No
A. schultesii Woodson Accepted name No synonyms [20, 26]	Maku	French Guiana, Peru, Venezuela Brazil: Amazonas, Roraima, Mato Grosso	Yes [66, 71]	No
A. spruceanum Benth. ex Müll. Arg. Accepted name Syn. *A. igapoanum* Markgr., *A. melanocalix* Müll. Arg. (+ several others) [20, 26]	Amargoso, Araracanga, Guatambu, Peroba, Quina da mata, Pequiá marfim	Bolivia, Colombia, Ecuador, French Guiana, Mexico, Panama, Peru, Venezu-ela, Brazil: Amazonia, Cerrado, Atlantic Rainforest	Yes [78]	Yes See ▶ **Table 2**
A. tomentosum Mart Accepted name Syn. *A. camporum* Müll. Arg., *A. dasycarpon* A. DC. and others [20, 24, 26] Non-accepted name, syn. for *A. gomezianum* A. DC. fide Woodson, Jr. R. E., 1951 [25]	Guatambú-do-cerrado, Pereiro-do-campo	Bolívia, Paraguay, Brazil: Amazonia, Caatinga, Cerrado	Yes [79]	No
A. ulei Markg. Accepted name Syn. *A. occidentale* Markg. [20, 26]	Pitiá	Guyana, Suriname, Venezuela, Brasil: Amazonia, Atlantic Forest	Yes [22]	Yes See ▶ **Table 2**
A. vargasii A. DC. Accepted name [25, 26] Non-accepted name, syn. for *A. parvifolium* A. DC. [20, 24]	Amarelão	Bolívia, Colombia, Guyana, Peru, Suriname, Venezuela	Yes [62, 66, 71]	Yes See ▶ **Table 2**

1980, is highly valuable and can be appreciated in a 2007 review [29]. However, there are relatively few reports on the biological activities of these alkaloids [5, 30].

Of the 26 *Aspidosperma* species included in ▶ **Table 1**, 19 have had their crude extracts and/or alkaloidal extracts assayed for antimalarial activity. Detailed data are shown in ▶ **Table 2**. Evaluations were performed as early as 1932, and several experiments were conducted before the methodology for the *P. falciparum* erythrocytic culture became available [31]. These experiments include *in vivo* assays in ducklings infected with *P. lophurae* [32], *in vitro* assays with *P. cathemerium* [33], and clinical assays in humans [34, 35] (▶ **Table 2**). Good activity (IC_{50} < 10 µg/mL) has been reported for extracts from *A. macrocarpon* [28], *A. megalocarpon* [36], *A. oblongum* [37], *A. olivaceum* [27], *A. quebracho blanco* [38], and *A. rigidum* [39]. A hydroethanolic extract from *A. pyrifolium* stem bark was inactive against the *P. falciparum* F32 strain [40]. However, a series of indole alkaloids isolated from the same species were shown to be active against *P. falciparum* FcM29 and Nigerian strains [41, 42]. Most likely, this controversy can be explained by a low content of alkaloids in the inactive crude extract. Extracts from *A. cylindrocarpon* [27, 43] and *A. macrocarpon* [28], the two species with no reported uses in treating malaria, have shown *in vitro* activity against three different *P. falciparum* clones (W2, 3D7, FcB1), with a selectivity index > 10 (SI = CC_{50}/IC_{50}) in every case (▶ **Table 2**). These results demonstrate the validity of the taxonomic approach for the selection

Table 2 Antimalarial activity of *Aspidosperma* extracts and alkaloids.

Aspidosperma species	Country of collection	Part of the plant	Extracts Compounds	Bioassay *Plasmodium* species/ strains	Results	References
A. cylindrocarpon	Brazil	Trunkwood	EtOH	*In vitro*/*P. falciparum* W2 and 3D7 strains	W2: IC_{50} = 44 µg/mL 3D7: IC_{50} = 39 µg/mL Cytotoxicity Vero cells CC_{50} > 500 µg/mL SI: W2 = 11.4 SI: 3D7 = 12.8	[27]
A. desmanthum	Brazil	Trunk bark	Aspidocarpine (**2**)	*In vitro*/*P. falciparum* K1	IC_{50} = 0.019 µM (0.07 µg/mL)	[45]
A. excelsum	Peru (Remo caspi)	Trunk bark	EtOH	*In vitro*/*P. falciparum* 3D7	IC_{50} = 42 µg/mL SI human lymphocyte inhibition IC_{50} > 100 µg/mL	[39]
A. macrocarpon	Brazil	Root bark	EtOH	*In vitro*/*P. falciparum* FcB1	FcB1: IC_{50} = 4.9 µg/mL Cytotoxicity MRC-5 CC_{50} = 79.2 µg/mL SI = 16.2	[28]
A. marcgravianum	Guyane	Leaves	Tetrahydrousambaresine (**17S**)	*In vitro*/*P. falciparum* FcM29	0.26 µg/mL (0.59 µM)	[32]
A. megalocarpon	Colombia	Trunk bark	MeOH	*In vitro*/*P. falciparum* D2 and F32 strains	MeOH extract D2 strain: IC_{50} = 8 µg/mL F32 strain: IC_{50} = 25 µg/mL Cytotoxicity U-937 human promonocitic cells: CC_{50} = 0.4 µg/mL SI: Pf/D2 = 0.05 SI: Pf/F32 = 0.02	[36]
A. megalocarpon	Colombia		Fendlerine (**13**), Aspidoalbine (**14**), Aspidolimidine (**15**)	*In vitro*/*P. falciparum* FcM29 and Nigerian strains	FcM29: IC_{50} = 25.6 to 59.2 µg/mL Nigerian strain: IC_{50} = 28.0 to 57.3 µg/mL	[41, 53]
A. nitidum	Brazil	–	–	*In vivo* Duckling/*P. lophurae*	Inactive	[32, 80]
A. nitidum	Brazil	Trunk bark	H_2O extract	*In vivo*/*P. berghei*	Inactive	[44]
A. oblongum	Brazil	–	EtOH	*In vitro*/*P. falciparum* W2 and D6	W2: IC_{50} = 4742.5 ng/mL D6: IC_{50} = 847.4 ng/mL	[37]
A. olivaceum	Brazil	–	EtOH Hexane-AcOEt	*In vivo*/*P. berghei*	Inactive	[67]
A. olivaceum	Brazil	Leaves, trunk wood, trunk bark	DCM and EtOH	*In vitro*/*P. falciparum* W2 and 3D7	W2: IC_{50} = 5.0 to 7 µg/mL 3D7: IC_{50} = 5.0 to 25.5 µg/mL	[27]
A. olivaceum	Brazil	–	Olivacine (**4**)	*In vitro*/*P. falciparum* 3D7 and K1	K1: IC_{50} = 1.4 µM 3D7: IC_{50} = 1.2 µM Citotoxicity murine macrophages: $CC_{50} > 4.1 \times 10^2$ µg/mL SI: K1 > 2.9×10^2 3D7 > 3.4×10^2	[48]
				In vivo/*P. berghei*	*In vivo*: active (50 mg/kg/day)	
A. parvifolium	Brazil	Trunk bark	EtOH extract, Uleine (**3**)	*In vitro*/*P. falciparum* W2 and 3D7	W2: IC_{50} = 32.8 µg/mL 3D7: IC_{50} = 20.5 µg/mL	[27]
				Uleine W2 and 3D7	W2: IC_{50} = 0.75 µg/mL (2.81 µM) 3D7: IC_{50} = 11.90 µg/mL (32.69 µM)	
A. polyneuron	Brazil	Trunk bark	Total alkaloids	*In vitro*/*P. cathemerium*	Active	[33]
A. pyrifolium	Bolívia	Stem bark	EtOH-H_2O (7 : 3)	*In vitro*/*P. falciparum* F32 strain FBIT	Inactive	[40, 81]
A. pyrifolium	Bolivia	Stem bark	*Eight alkaloids	*In vitro*/*P. falciparum* FcM29 and Nigerian strain	FcM29: IC_{50} = 3.2 to 28.5 µM Nigerian strain: IC_{50} = 5.1 to 22.6 µM	[42] [41]
A. quebracho-blanco	Argentina	Trunk bark	Total alkaloids	Human	Active	[35, 80]
A. quebracho-blanco	Argentina	Trunk bark	EtOH	Human	Inactive	[34, 80]
A. quebracho-blanco	Bolívia	Leaves, trunk bark	EtOH-H_2O (7 : 3)	*In vitro*/*P. falciparum* F32 strain FBIT	Trunk bark extract F32: IC_{50} = 3.9 µg/mL FBIT: IC_{50} = 1.22 mg/mL Leaf extract: inactive	[38]

continued

Table 2 *Continued*

Aspidosperma species	Country of collection	Part of the plant	Extracts Compounds	Bioassay *Plasmodium* species/ strains	Results	References
A. ramiflorum	Brazil	Leaves, trunk wood, trunk bark	DCM and EtOH	*In vitro*/*P. falciparum* W2 and 3D7	W2: IC_{50} = 19.7 to 36.5 µg/mL 3D7: IC_{50} = 1.0 to 48.0 µg/mL	[27]
A. ramiflorum	Brazil	–	–	*In vitro*/*P. falciparum* W1	W2: IC_{50} = 11 to 40 µg/mL	[82]
				In vivo/*P. berghei*	*In vivo:* partial activity	
A. rigidum	Peru (Remo caspi)	Trunk bark	EtOH	*In vitro*/*P. falciparum* 3D7	IC_{50} < 10 µg/mL SI humam lymphocyte inhibition 75%	[39]
A. spruceanum	Brazil	Leaves, trunk wood, trunk bark	DCM and EtOH	*In vitro*/*P. falciparum* W2 and 3D7	W2: IC_{50} = < 6.0 to 65.0 µg/mL 3D7: IC_{50} = < 6.0 to > 100 µg/mL	[27]
A. tomentosum	Brazil	Leaves, trunk wood, seeds, fruits	EtOH	*In vitro*/*P. falciparum* W2 and 3D7	W2: IC_{50} = 20.5 to 26.5 µg/mL 3D7: IC_{50} = 3.0 to 38.5 µg/mL	[27]
A. ulei	Brazil	Leaf, bark, trunk wood, root wood, root bark	Five alkaloids**	*In vitro*/*P. falciparum* K1	K1: IC_{50} = 16.7 to > 176.0 µM Citotoxicity NIH3T3 murine fibroblasts	[47]
A. vargasii	Brazil	Trunk bark	Ellipticine (**1**)	*In vitro*/*P. falciparum* K1	IC_{50} = 0.073 µM (0.018 µg/mL)	[45]
A. vargasii	Brazil	Bark	Ellipticine (**1**)	*In vitro*/*P. falciparum* 3D7 and K1	K1: IC_{50} = 0.81 µM 3D7: IC_{50} = 0.35 µM Citotoxicity murine macrophages: $CC_{50} > 4.1 \times 10^2$ µg/mL SI: K1 > 5.0×10^2 3D7 > 1.2×10^3	[48]
				In vivo/*P. berghei*	*In vivo:* active (50 mg/kg/day)	

*Aspidospermidine (**6**), 10-methoxy-aspidospermidine (**7**), N-formil-aspidospermidine (**8**), vallesine (**9**), (−)- aspidospermine (**10**), demethoxyaspidospermine (**11**), palosine (**12**), haplocine (**13**). ** 20-*epi*-dasycarpidone (**3**), 3,4,5,6-tetradehydro-*β*-yohimbine, 20(*E*)-*nor*-subincanadine E, 19*E*-hunteracine, 12-hydroxy-*N*-acetyl-21(*N*)-dehydroplumeran-18-oic acid

of plant species for antimalarial screening. Negative results were described for *in vivo* assays of *A. nitidum* crude extracts [32,44] (**Table 2**). These results are surprising as *A. nitidum* is one of the most frequently cited *Aspidosperma* species of those used to treat malaria/fevers in Brazil. Therefore, this species deserves further investigation with a focus on the alkaloids.

Interestingly, a strong interest in *Aspidosperma* species has resumed in the past two decades, and several species whose phytochemistry was intensively investigated during 1960–1980 have been reexamined, leading to the isolation of several known indole alkaloids. Several of these alkaloids have been evaluated for various biological/pharmacological effects [30]. A total of 20 *Aspidosperma* alkaloids have been assayed for antimalarial activity and have been isolated by Brazilian researchers, one from *A. desmanthum* [45], one from *A. vargasii* [45], one from *A. parvifolium* [27,46], five from *A. ulei* [47], and one from *A. olivaceum* [48]; additionally, a French-Bolivian group has evaluated 11 alkaloids from *A. megalocarpon* and *A. pyrifolium* [41].

Ellipticine (**1**) and aspidocarpine (**2**) (**Fig. 1**) have been isolated from the trunk bark of *A. vargasii* and *A. desmanthum*, respectively. Both of these species have been collected at the Ducke Reserve in Manaus, Amazonas state, Brazil, and they have shown remarkable *in vitro* activity against the multidrug-resistant K1 strain of *P. falciparum* (IC_{50} = 73 nM and 19 nM, respectively) [45]. Ellipticine (**1**), a pyridocarbazol alkaloid, was originally isolated from *Ochrosia elliptica* (an Australian evergreen shrub) and occurs in other genera of the family Apocynaceae, such as *Aspidosperma* and *Bleekeria* [49–51]. Ellipticine (**1**) and several related synthetic derivatives are highly cytotoxic to human cancer cell lines, and elliptinium acetate (9-OH-NME, Celliptium®) is an antineoplastic agent currently used in the treatment of metastatic breast cancer [49–51]. Recently, ellipticine (**1**) and olivacine (**5**) (**Fig. 1**) were assayed against *P. falciparum* strains K1 and 3D7. Olivacine disclosed lower *in vitro* antimalarial activity than ellipticine, and low cytoxicity for both agents was observed against murine macrophages. Ellipticine was also more active than olivacine in an evaluation of the effects in *P. berghei*-infected mice. Remarkably, 100% parasitemia suppression was observed at an oral dose of 50 mg/kg/day (four days), and no mortality or other signs of toxicity were reported [48]. Ellipticine (**1**) and its derivatives were described as the most active compounds, with IC_{50} values < 1 µg/mL (range 0.08 to 0.47 µg/mL), in a series of 184 randomly selected compounds belonging to several classes of natural products, either from plants or from marine organisms, or prepared as intermediates during the synthesis or semisynthesis of isolated products that were assayed against the *P. falciparum* FcM29 strain [52].

A total of 12 indole alkaloids were recently isolated from the EtOH extracts of various parts of *A. ulei* Markgr. Only five of these alkaloids were assayed for antimalarial activity against the multidrug-resistant K1 strain of *P. falciparum*. 20-*Epi*-dasycarpidone (**3**) (**Fig. 1**) showed moderate activity (IC_{50} 4.5 µg/mL, 16.7 µM), whereas 3,4,5,6-tetradehydro-*β*-yohimbine, 19*E*-hunteracine, 20(*E*)-nor-subincanadine E, and 12-hydroxy-*N*-acetyl-21(*N*)-dehydroplumeran-18-oic acid were inactive. Two of the known alkaloids isolated, uleine and olivacine, have previously

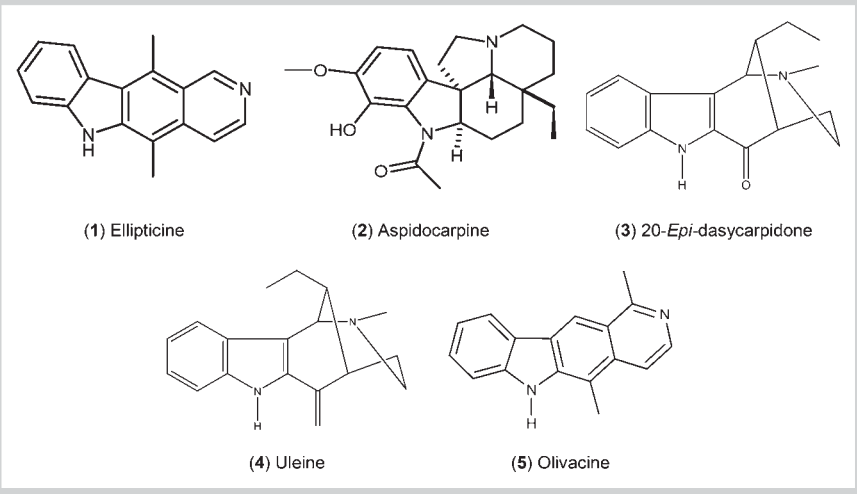

Fig. 1 Chemical structures of antimalarial alkaloids from *Aspidosperma* species: ellipticine from *A. vargasii* [45], aspidocarpine from *A. desmanthum* [45], uleine from *A. parvifolium* [46], olivacine from *A. olivaceum* [47] and 20-*Epi*-dasycarpidone from *A. ulei* [47].

been shown to be active [46,48]. The compounds were evaluated against NIH3T3 murine fibroblasts and showed no cytotoxicity up to a concentration of 50 µg/mL [47].

Eleven known aspidospermane alkaloids (**Fig. 2**) were isolated from *A. pyrifolium* and *A. megalocarpon*, both collected in Bolivia [42,53] and both also occurring in Brazil [24–26]. Three of the active compounds, aspidospermine (**6**), 10-methoxy-aspidospermidine (**7**), and *N*-formyl-aspidospermine (**8**) (**Fig. 2**), were shown to be less cytotoxic to NIH 3 T3 cells (human fibroblasts), with calculated SI values of 22.7, 15.6, and 8.3, respectively [41]. The i*n vitro* antiplasmodial activity of *A. megalocarpon* bark extract against D2 and F32 strains of *P. falciparum* has been reported; subsequently, a very low SI (< 1) was reported in relation to cytotoxicity assays in U-937 human promonocytic cells [36].

Uleine (**4**) (**Fig. 1**) was first isolated from *A. ulei* in 1959 [54,55]. It is frequently found in species, e.g., *A. australe*, *A. dasycarpon*, *A. eburneum*, *A. excelsum*, *A. formosanum*, *A. gilbertii*, *A. gomezianum*, *A. multiflorum*, *A. nigricans*, *A. olivaceum*, and *A. parvifolium* [29], as well as in other apocynaceous representatives such as *Himathanthus lancifolius* [56].

A recent phytochemical reinvestigation of *A. parvifolium* trunk bark afforded four known indole alkaloids, uleine (**4**) (**Fig. 1**), *epi*-uleine, apparicine, and *N*-demethyluleine [57]. A bioguided fractionation of the trunk bark alkaloidal fraction demonstrated the *in vitro* activity of uleine against chloroquine-resistant *P. falciparum* (W2), with an IC_{50} at the ng level, at least against the W2 clone (**Table 2**) [27,46]. The antiplasmodial activity of uleine (**4**), an aspidospermane indole alkaloid, may be related to the inhibition of heme polymerization to give hemozoin, as demonstrated by its *in vivo* effect in the food vacuole of chloroquine-resistant *P. falciparum* (W2 clone) monitored by confocal microscopy [46].

Uleine (**4**) (**Fig. 1**) has been evaluated for cytotoxicity against the NCI 60 cancer cell line panel and was inactive in all of them [58], reinforcing its high potential as a leading antimalarial compound.

In summary, crude extracts, alkaloidal extracts, and isolated alkaloids from 19 *Aspidosperma* species have been evaluated for antimalarial activity, and positive results have been observed for most of them. These results disclose the high potential of *Aspidosperma* species as sources of antimalarial alkaloids. A substantial literature search, approximately 1031 articles, describes the antiplasmodial activity of plant extracts, but very few of these reports include the effect of pure constituents [14], a requirement for the development of new drugs. The development of new drugs is lengthy and extremely costly. However, if effective, safe, and locally produced phytomedicines are the goal, a more direct and less expensive route might certainly be pursued [5,59,60].

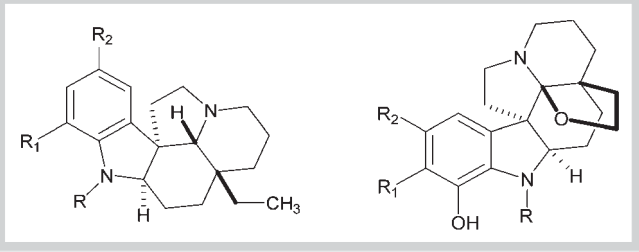

Fig. 2 Antimalarial alkaloids from *A. megalocarpon* and *A. pyrifolium* [41].

Conclusions

Our literature survey has identified 24 *Aspidosperma* species reported to be used to treat malaria/fevers. Of these, a total of 19 species have had extracts and/or isolated alkaloids evaluated for antimalarial activity by different assays, showing positive results. Only 20 of more than 200 known indole alkaloids from *Aspidosperma* species have been assayed for antimalarial activity, and variable levels of parasite inhibition have been observed. Among the assayed *Aspidosperma* alkaloids, uleine (**4**) (**Fig. 1**) appears the most promising as an antimalarial because it has shown good *in vitro* activity [27,46] and no cytotoxicity in several human cancer cell lines [58]. These findings have motivated a patent in Brazil [61]. However, recent results on the *in vivo* evaluation of ellipticine (**1**) (**Fig. 1**) appear to place this alkaloid in the leading position among natural antimalarials because it has, remarkably, shown 100% parasitemia suppression at an oral dose of 50 mg/kg/day and no signs of mortality or toxicity [48]. Our review also demonstrates a need for molecular genetic studies to facilitate the identification and the differentiation of *Aspidosperma* species and, therefore, to clarify the controversial question of synonyms. The high chemical diversity of alkaloids from *Aspidosperma* species and the small number of these alkaloids that have been as-

sayed for antimalarial activity coupled with the traditional use of several species of this taxon to treat malaria in Brazil as well as in other Meso- and South American countries make further investigations of the plants in this genus of great interest in the quest for natural antimalarial products. There is a need to isolate plant constituents to evaluate their pharmacological profile, to further pursue studies of synergistic effects, and to make these constituents available for use as markers in the standardization of extracts to be used in the development of effective, safe, and low-cost phytomedicines. These phytomedicines would then be available to Amazonian people, who inhabit the region where many of the *Aspidosperma* species occur and that have the highest incidence of malaria in Meso- and South America.

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Conflict of Interest

The authors declare that they do not have any conflict of interest in reference to the content of this article.

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