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Aspidosperma species : a review of their chemistry and biological activities

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Ethnopharmacological relevance

Species of *Aspidosperma* are known popularly as “peroba, guatambu, carapanaúba, pau-pereiro” and “quina”. The genus can be found in the Americas, mainly between Mexico and Argentina. Many species of *Aspidosperma* are used by the population in treating cardiovascular diseases, malaria, fever, diabetes and rheumatism. The phytochemical aspects of the species of the genus *Aspidosperma* have been studied extensively. The monoterpene indole alkaloids are the main secondary metabolites in *Aspidosperma* species, and about 250 of them have been isolated showing a considerable structural diversity. Several of them have showed some important pharmacological activities. *Aspidosperma subincanum* Mart. and *Aspidosperma tomentosum* Mart. (Apocynaceae) are Brazilian species widely used by the population to treat diabetes mellitus, hypercholesterolemia. The pharmacological activities of both species have been investigated and the biological properties described can be related to their isolated indole alkaloids. However, more pharmacological studies are needed in order to justify the use of these species in folk medicine. In this review, we present reports mainly focused on chemical and biological studies and their relationship with the ethnopharmacological use of both *Aspidosperma* species.

Aim of the study

The aim of this review is to present their ethnopharmacological use as correlated to their biological activities as described for the extracts and isolated compounds from *Aspidosperma subincanum* Mart. and *Aspidosperma tomentosum* Mart. In addition, some aspects related to the biosynthetic pathways are discussed, also NMR assignments and some synthesis information about indole alkaloids from both *Aspidosperma* species are included.

Material and methods

The bibliographic search was made in theses and dissertations using some databases such as ND LTD (Networked Digital Library of Theses and Dissertations), OATD (Open Access Theses and Dissertations) and

Google Scholar. More data were gathered from books, Brazilian journals and articles available on electronic databases such as, Google Scholar, PubChem, Scifinder, Web of Science, SciELO, PubMed and Science Direct. Additionally, the Google Patents and Espacenet Patent Search (EPO) were also consulted. The keywords *Aspidosperma*, *Aspidosperma subincanum*, *Aspidosperma tomentosum*, indole alkaloids were used in the research. The languages were restricted to Portuguese, English and Spanish and references were selected according to their relevance.

Results

Aspidosperma subincanum Mart. and *Aspidosperma tomentosum* Mart. (Apocynaceae) are Brazilian species widely used by the population to treat a few diseases. Extracts and isolated compounds of both species have shown antitumor and antimalarial activities.

The antitumor activity of isolated compounds has been extensively studied. However, the antiplasmodial activity needs to be investigated further as well as the anti-inflammatory, anti-hyperlipidemic and anorexigenic activities. From *A. subincanum* twenty-one indole alkaloids were isolated and some of them have been extensively studied. From the leaves and bark of *A. tomentosum* four alkaloids and one flavonoid were isolated. Furthermore, CG-MS analysis of seeds, branches, leaves and arils identified nine indole alkaloids. Stemmadenine has been proposed as a precursor of indole alkaloids obtained from some species of *Aspidosperma*. Many of the biosynthetic steps have been characterized at the enzymatic level and appropriate genes have been identified, however, other steps have yet to be investigated and they are still controversial. Some isolated alkaloids from *Aspidosperma subincanum* and *A. tomentosum* were identified only by mass spectrometry. In many cases, their NMR data was either not available or was incomplete. The described meta-analysis of the available NMR data revealed that the chemical shifts belonging to the indole ring might be used to characterize this class of alkaloids within complex matrices such as plant extracts. The biological activities and the structural complexity of these compounds have stimulated the interest of many

groups into their synthesis. In this review, some information about the synthesis of indole alkaloids and their derivatives was presented.

Conclusions

Aspidosperma subincanum and *Aspidosperma tomentosum* are used by the population of Brazil to treat many diseases. A few biological activities described for the extracts and isolated compounds of both species are in agreement with the ethnopharmacological use for others species of *Aspidosperma*, such as, antimalarial, the treatment of diabetes and other illnesses. These species are sources of leading compounds which can be used for developing new drugs. In addition, other biological activities reported and suggested by ethnopharmacological data have yet to be investigated and could be an interesting area in the search for new bioactive compounds.

Keywords: *Aspidosperma*, Apocynaceae, indole alkaloids, biological activity, antitumoural activity, antiplasmodial activity

1- Introduction

The traditional knowledge of plants continues to play an important role in the development of new medicines, e.g., the discovery of artemisinin and quinine obtained from *Artemisia annua* L. (Asteraceae) and *Cinchona* spp. (Rubiaceae), respectively (Schenkel et al., 2004, Cui and Su, 2009; Achan et al., 2011). The Apocynaceae family is an important source of drugs used in modern medicine. Several substances from species of this family have been isolated and used in therapeutics (Raskin et al., 2002; Kato et al., 2002; Heijden et al., 2004; Bhadane et al., 2018). The genus *Aspidosperma* is one of the most important among the Brazilian genera of Apocynaceae. The genus can be found in the Americas, mainly between Mexico and Argentina. The species of *Aspidosperma* are known popularly as “peroba, guatambu, carapanaúba, pau-pereira” and “quina” (Pereira et al., 2016). Many of them have been used by the population for treatment of cardiovascular diseases (Ribeiro et al., 2015), malaria (Dolabela et al., 2012), fevers, diabetes and rheumatism. The phytochemical aspects of species of the *Aspidosperma* have been extensively studied and the indole alkaloids are the main secondary metabolites found in *Aspidosperma* and they are considered to be good taxonomical chemical markers for this genus (Pereira et al., 2007, Dolabela et al., 2012) and the biological activities observed for the species of *Aspidosperma* have been attributed to them (Chierrito et al., 2014). The indole alkaloids (Fig. 1) aspidoscarpine (**1**), apparicine (**2**), ramiflorine A (**3**) and ramiflorine B (**4**) isolated from the *Aspidosperma* genus have showed antiprotozoal activity. The compounds (**1**), and (**2**) which were isolated from stem bark of *A. olivaceum* showed IC_{50} 5.4 ± 2.5 $\mu\text{g/mL}$ and 3.0 ± 1.4 $\mu\text{g/mL}$, respectively, against *Plasmodium falciparum* chloroquine resistant blood parasites (W2 clone) (Chierrito et al., 2014). H-17- α -Ramiflorine A (**4**) and H-17- β -ramiflorine B (**5**) obtained from stem bark of *Aspidosperma ramiflorum* showed *in vitro* activity against promastigote forms of *Leishmania (L.) amazonensis*, with LD_{50} values of 18.5 ± 6.5 $\mu\text{g/mL}$ and 12.6 ± 5.5 $\mu\text{g/mL}$, respectively (Tanaka et al., 2007; Cunha et al., 2012).

Others classes of natural products have also been isolated from *Aspidosperma* species, such as flavonoids, saponins and organic acids (Fig. 1). The triterpenes and steroids (**5 - 8**), organic acid (**11**) and flavonoids (**12 - 14**) were isolated from a methanolic extract of *Aspidosperma cylindrocarpon* Müll.Arg. stems

(Guimarães et al., 2013). During the bioguided fractionation of *Aspidosperma fendleri* Woodson leaves a mixture of two saponins, i.e. quinovic acid 3-O- β -rhamnopyranoside (**9**) and quinovic acid 3-O- β -fucopyranoside (**10**) were isolated. These saponins showed hypotensive and bradycardic activity (Estrada et al., 2015).

Fig. 1 – Chemical structures of some compounds isolated from the *Aspidosperma* genus.

The species *Aspidosperma subincanum* and *Aspidosperma tomentosum* are used by the population in Brazil to treat diseases. Some of the indole alkaloids isolated from both species have been used as lead compounds for the development of antitumour drugs (Fig. 2) such as elliptinium (**15**), datelliptium (**16**), retelliptine (**17**) and pazelliptine (**18**) (Le Pecq et. al, 1976; Le Pecq and Paoletti, 1982; Haider and Sotelo,

2002; Tylińska et al., 2010). Elliptinium (**15**) has already been commercialized as Celliptium® by Sanofi-Aventis (Cragg and Newman, 2005).

Fig. 2 - Antitumour drugs developed from *Aspidosperma*'s indole alkaloids.

The aim of this review is to present the ethnopharmacological use of some *Aspidosperma* species and biological activities of the isolated compounds from *Aspidosperma subincanum* and *Aspidosperma tomentosum*. Also some aspects relating to the biosynthetic pathways are discussed as well as NMR assignments. A few synthetic aspects about indole alkaloids from *Aspidosperma subincanum* and *Aspidosperma tomentosum* are also included. This article intended to provide an overview about both *Aspidosperma* species in different aspects aiming to be an important input in setting directions for future research with *Aspidosperma* species.

2- Material and methods

The bibliographic search regarding both species was made in theses, dissertations and monograph from the using the databases ND LTD (Networked Digital Library of Theses and Dissertations), OATD (Open Access Theses and Dissertations) and Google Scholar. Additional data were gathered from books, Brazilian Journals and articles available on electronic databases such as, Google Scholar, PubChem, Scifinder, Web of science, SciELO, PubMed and Science Direct. Google Patents and Espacenet patent search (EPO) were also consulted. The keywords *Aspidosperma*, *Aspidosperma subincanum*, *Aspidosperma tomentosum*, "levantamento etnobotânico", indole alkaloids were used in the research. The languages were restricted to Portuguese, English and Spanish and references were selected according to their relevance. The range date from 1950 to 2018.

3- Botany and traditional use of *Aspidosperma* species

The *Aspidosperma* genus is one of the most important among the Brazilian genera of Apocynaceae (Pereira et al., 2016). Species of this genus can be found from Mexico to Argentina with the exception of Chile and on the Antillean island of Hispaniola (Marcondes-Ferreira, 1988; Marcondes-Ferreira and Kinoshita, 1996). In Brazil, there are six different biomes: Amazon rainforest, Atlantic rainforest, Cerrado, Pantanal, Pampa and Caatinga. The *Aspidosperma* species occur in four of these biomes with exception of Pantanal and Pampa (Koch et al., 2015, Flora do Brasil 2020). Marcondes-Ferreira (1999) reported that there are two centers of diversity of *Aspidosperma* species. The first one is “Amazônica” that comprises the legal Amazon and Central America while the second is the “Atlântico” which includes the Eastern part of Brazil, Argentina, Paraguay and Bolivia. The number of species in this genus is controversial (Guimarães et al., 2012). The Plant List (2013) for the genus *Aspidosperma* included 66 species, while The Flora do Brasil 2020 (2018), reported 56 species and some of them are endemic (21 species) with the greatest concentration found in the Amazon Region.

Furthermore, Pereira and co-workers (2016) reported that the species of *Aspidosperma* are difficult to identify because some of them are very similar to each other, and have overlapping morphological characteristics. In addition, the latter features such as the shape and size of the leaves, pilosity, flowers and fruits led some researchers to describe some as new species. However, these existing features represent diverse stages of a continuous variation within the species.

The *Aspidosperma* species are known in the Brazilian Amazon region as “paracanaúba”, “Carapanaúba” (mosquito’s tree) and “casca de caepana” (Oliveira et al., 2015). In other regions of Brazil they are known as “peroba, guatambu, pau-pereira” and “quina” (Silva, 2014; Pereira et al., 2016) (Table 1). Many species of *Aspidosperma* have the same vernacular name and are used by the Amazon native people (indigenous people, “caboclos” and riverside people) to treat malaria and other related diseases (Table 1). It suggests the difficulty in characterizing the etnospecies using morphological features (Oliveira et al., 2015; Pereira et al. 2016).

A. subincanum is a tree 15 to 20 meters high. In Brazil, this species is found in the Amazon Rainforest, Cerrado and the Atlantic Rainforest (Flora do Brasil, 2020). *A. tomentosum* is an endemic species found in the Cerrado areas (Flora do Brasil 2020). It is a tree that can reach a height of 5 to 8 meters, with a cork bark and a thick shell (Lorenzi, 2009). The wood is used for making furniture, decorative objects and household items (Lorenzi, 2009; Aquino et al., 2013). The tree is also considered to be ornamental, and can be used in landscaping (Oliveira et al., 2011). The traditional uses of some species, including *A. tomentosum* and *A. subincanum*, are described in Table 1.

Table 1 Ethnopharmacological use of *Aspidosperma* species obtained from the literature survey

Region associated to the use	Described Specie	Vernacular name	Use	Part plant	Preparation/ administration	Reference
Assuncion market places, Paraguai	<i>A. quebracho-blanco</i> Schltdl.	Quebracho-blanco	to regulate fertility	bark	decoction	Arenas & Ozorero, 1977
Southern Pará and northeast of Rondônia, Brazil	<i>A. nitidum</i> Benth. ex Müll.Arg	Carapanaúba	malaria	bark	decoction	Brandão et al., 1992
Yanomani indigenous communities, Brazil	<i>A. nitidum</i> Benth. ex Müll.Arg	Hura-sihi	malaria	bark	decoction/drunk	Milliken & Albert, 1996
Roraima, Brazil	<i>A. nitidum</i> Benth. ex Müll.Arg	Carapanaúba	malaria	bark	decoction	Milliken, 1997
	<i>A. excelsum</i> Benth.	Carapanaúba	malaria	bark	decoction	
Izoceño-Guarani, Bolivia (Chaco)	<i>A. quebracho-blanco</i> Schltdl.	Ivacaro-guaru	appendicitis	bark	decoction	Bourdy et al., 2004
Quilombo Sangrador, Maranhão, Brazil	<i>A. subincanum</i> Mart.	Carrasco	diseases associated with the digestive tract	bark	bottle	Monteles et al., 2007
Cariri Paraibano, Paraíba, Brazil	<i>A. pyriforme</i> Mart. & Zucc.	Pau-pereiro, pereiro	inflammation of the urinary tract, dermatite	bark	decoction/ intern use	Agra et al, 2010
Nova Xavantina, Mato Grosso, Brazil	<i>A. tomentosum</i> Mart.	Guatambu	hypercholesterolemia, anorexic, diuretic	bark	water bottle/ decoction	Silva et al., 2010
Cuiabá, Mato Grosso, Brazil	<i>A. subincanum</i> Mart.	Guatambu	diabetes	bark	water bottle ,tea	Pinto et al., 2013
Curaçá, Bahia, Brazil	<i>A. polyneuron</i> Müll. Arg.	Pau-pereiro	belly ache	bark, leaves	maceration	Jesus, 2013
Communities of Boca do Agre, Amazonas, Brazil	<i>Aspidosperma</i> sp.	Paracanaúba, Carapanaúba	liver, anemia, malaria	bark	maceration	Silva, 2014
Manacapuru, Amazonas, Brazil	<i>A. excelsum</i> Benth.	Carapanaúba	inflammation, diabetes, liver, high blood pressure, contraceptive	leaves, bark	tea, maceration	Vasquez et al., 2014
Xapuri, Acre and Pauini, Amazônia, Brazil	<i>A. nitidum</i> Benth. ex Müll.Arg	Carapanaúba-preta, Carapanaúba-amarela	malaria/liver/fever	bark	decoction	Ferreira, 2015
	<i>A. excelsum</i> Benth.	Pariquima	malaria/fever		decoction	

	<i>A. megaphyllum</i> Woodson (Accepted name <i>A. myristicifolium</i> (Markgr.) Woodson)	Paracanaúba, Carapanaúba	malaria/ liver		decoction	
Uapes River, São Gabriel da Cachoeira, Amazonas, Brazil	<i>Aspidosperma</i> sp.	Carapanaúba	malaria	bark	decoction/bottle	Trivellato, 2015
Julião Community, Manaus, Amazonas, Brazil	<i>Aspidosperma</i> sp.	Carapanaúba	liver, stomach, anemia, malaria	bark	not decribed	Veiga et al., 2015
Girau do Ponciano, Alagoas, Brazil	<i>A. pyrifolium</i> Mart. & Zucc.	Pereiro	gastritis	bark	not described	Santos et al., 2015
Valley of Juruena Region, Legal Amazon, Mato Grosso, Brazil	<i>A. polyneuron</i> Müll. Arg.	Peroba	Local pain	not described	not described	Bieski et al., 2015
Quilombola communities of Oriximina	<i>Aspidosperma excelsum</i> Benth.b (Syn. <i>A.marc-gravianum</i>) and <i>Aspidosperma rigidum</i> Rusbyb <i>Apocynaceae</i>	carapanaúba	fever (1);migraine(1), body pain(1)	bark	not described	Oliveira et al., 2015
São Gabriel da Cachoeira indigenous communitites from Amazonas, Brazil	<i>A. schultesii</i> Woodson	Carapanaúba Kome-yahpuri	malaria	bark	decoction 1 cup 3X a day for adults; ¼ cup to children with sugar	Kffuri et al., 2016
Rio verde, Goiás, Brazil	<i>A. polyneuron</i> Müll.Arg.	Peroba	diabetes	bark	tea	Gonçalves, 2016
Loreto, Northeast of Peru	<i>A. excelsum</i> Benth.	Remo caspi (de bajo)	malaria/leishmamia	cortex	not described	Kvist et al., 2016
	<i>A. rigidum</i> Rusby	Remo caspi (de alto)	malaria	not described	not described	
Middle region of Negro River, Amazonas, Brazil	<i>A. nitidum</i> Benth. ex Müll.Arg	Carapanaúba	malaria	bark	decoction	Tomchinsky et al., 2017
	<i>A. schultesii</i> Woodson	Carapanaúba	malaria	bark	not described	
	<i>Aspidosperma</i> sp.	Carapanaúba	malaria	bark	not described	

4- Biological activities of *Aspidosperma subincanum* Mart. extracts and isolated compounds

The extracts obtained from *Aspidosperma subincanum* as well as the isolated and/or identified compounds from this specie have showed biological activities that can be associated with traditional uses for this species or others species belonging to the same genera.

In studies performed by Ribeiro and co-workers (2015), the oral administration of the ethanolic extract from the bark of *A. subincanum* (EEAS) in rats at 60 and 120 mg/Kg showed it significantly increased diuresis in a dose dependent manner. The cumulative urinary excretions at 24h after treatment with EEAS 60 mg/kg and 120 mg/kg were 7.57 ± 0.7 and 10.37 ± 0.9 mL, respectively. These values were significantly higher ($p < 0.001$) when compared to the control group (4.37 ± 0.4 mL). After 24h, the cumulative urinary volume of animals treated with EEAS at 120 mg/kg was not different from the diuresis observed with animals treated with furosemide (diuretic index of 2.4 and 2.5, respectively). EEAS showed a greater natriuretic than kaliuretic effect. The Na^+/K^+ ratio for furosemide is approximately 1,0, meaning that it eliminates the two electrolytes equally, while, EEAS at 60 and 120 mg/kg showed a lower excretion of K^+ than Na^+ (The Na^+/K^+ ratio was 1.33 and 1.43, respectively). According to the research, the EEAS induced a dose-dependent urinary excretion and the observed action appeared to involve prostaglandins with a consequent increase in the glomerular filtration rate and natriuresis.

The ethanolic extracts of branches of *A. subincanum* showed acetylcholinesterase inhibitory activity (IC_{50} 233.11 ± 12.50), antioxidant activity in the lipid peroxidation assay (39.0 ± 3.4), and the ethanolic extracts of leaves showed antimicrobial activity against *S. aureus* ATCC 25923 with IC_{50} 211 ± 5.32 (Rocha et al., 2018).

The antimalarial activity of the ethanolic extract of the bark of *A. subincanum* against *P. falciparum* (W2) was demonstrated by Oliveira and co-workers (2013). The extract showed CI_{50} 25.28 ± 1.52 $\mu\text{g/mL}$ against *P. falciparum* (W2), the toxicity in HepG2 was CC_{60} $383,82 \pm 42,31$ $\mu\text{g/mL}$ resulting in SI 15.19), while chloroquine (positive control) showed an IC_{50} of 0.10 ± 0.03 $\mu\text{g/mL}$, and cytotoxicity in HepG2 with CC_{60} (pg/mL) $271.55 \pm 13,41$ corresponding to an SI of 2629.11.

In studies of acute and subchronic toxicity of a stem bark ethanolic extract, oral administration of doses up to 300 mg/kg in rats did not show signs of toxicity, but doses from 500 to 2500 mg/kg lead to conditions related to central nervous system stimulant effects such as piloerection, tremors, convulsions, cyanoses and death at an estimated LD₅₀ 1129 ± 154 mg/kg. Intraperitoneal administration showed an LD₅₀ 397 ± 15 mg/Kg. Furthermore, serious changes in the hematological, biochemical and behavioral parameters, as well as a deleterious effect on the vital organs of rats after 30 days exposure (daily) to 5 and 100 mg/Kg of extracts, were not observed (Santos et al., 2009).

In 1959, Woodward and co-workers isolated the alkaloids ellipticine (**25**) and N-methyl-tetrahydroellipticine (**26**) from the bark of *A. subincanum*. Both alkaloids have been isolated from other species of Apocynaceae (Burnell and Della Casa, 1967; Pereira et al., 2007; Miller and McCarthy, 2012; Rocha e Silva et al., 2012). Antitumour activity of ellipticine (**25**) has been studied against different types of cancer cells (Le Pecq et al., 1974; Haugwitz et al., 1993; Jurayj et al., 1994; Rocha e Silva et al., 2012). In work carried out by Stiborová and co-workers (2001) it was demonstrated that ellipticine (**25**) showed cytotoxicity against several human cancer cell lines including breast adenocarcinoma MCF (IC₅₀ 1.25 ± 0.13 µm), leukemia HL-60 (IC₅₀ 0.67 ± 0.06 µm), leukemia CCRF-CEM (IC₅₀ 4.70 ± 0.48 µm), neuroblastoma IMR-32 (IC₅₀ 0.27 ± 0.02 µm), neuroblastoma UKF-NB (IC₅₀ 0.44 ± 0.03 µm), and glioblastoma U87MG (IC₅₀ 1.48 ± 0.62 µm). The proposed mechanisms of antitumour activity involved (i) intercalation into DNA, (ii) inhibition of DNA topoisomerase II activity, and (iii) covalent binding to DNA *in vitro* and *in vivo* after enzymatic activation by cytochrome P450 (Miller and McCarthy, 2012; Stiborová and Frej, 2014). Other modes of action have been described such as kinase inhibition, interaction with p53 transcription factor, bio-oxidation and adduct formation (Miller and McCarthy, 2012). According to Stiborová and co-workers (2011), the pharmacological efficiency and/or genotoxic side effects of ellipticine are dependent on its activation by CYPs and peroxidases in target tissues.

De Andrade-Neto and co-workers (2007) described the activity *in vitro* of ellipticine (**25**) against *P. falciparum* K1 strain (IC₅₀ 73 nM), where chloroquine-diphosphate salt and quinine-salt were used as positive control (IC₅₀ 890 nM and 12 nM, respectively). This activity was confirmed by Rocha e Silva and co-workers

(2012), where ellipticine (**25**) was active *in vitro* against *P. falciparum* K1 and 3D7 strains, with IC₅₀ values of 0.81 μ M and 0.35 μ M, respectively. The toxicity of ellipticine (**25**) against murine macrophages was CC₆₀ > 4.1 X 10⁻² μ g/mL resulting in SI >5.0 X 10² and 1.2 X 10³, respectively. The chloroquine-diphosphate (positive control) showed IC₅₀ 0.13 μ M and IC₅₀ 0.058 μ M against *P. falciparum* K1 and 3D7 strains, respectively, and quinine sulphate (positive control) showed IC₅₀ 0.16 μ M and IC₅₀ 0.11 μ M against *P. falciparum* K1 and 3D7 strains, respectively. In the same study, this research group evaluated ellipticine (**25**) against *P. falciparum* chloroquine resistant FcM29-Cameroon strain (IC₅₀ 1.13 μ M) and *in vivo* on *P. berghei* (NK65 strain). For this study, the infected female Webster Swiss mice treated orally and subcutaneously with ellipticine (dose 50 mg/kg/day). On the 5th and 7th day after inoculation with parasites, blood was microscopically examined. Ellipticine (**25**) was active at an oral dose of 50 mg/kg/day with 100% inhibition and the mean survival time of the animals was > 40 days (similar to the control chloroquine). Also, it had good oral activity on day 5 and 7 at 10 mg/kg/day via subcutaneous injection with 77% and 70% inhibition, respectively. The mechanism of action proposed involved inhibitory effects on the formation of hemozoin in *P. falciparum* and interaction of DNA with the formation of covalent DNA adducts mediated by ellipticine oxidation with cytochrome P450 and peroxidases (Rocha e Silva 2012; Chong and Sullivan 2003).

However, ellipticine (**25**) showed mutagenic activity (DeMarini et al., 1983, Gupta, 1990; DeMarini et al., 1992; Stiborová et al., 2001; Stiborová et al., 2011). Ellipticine (**25**) leading to a reduced body weight in mice when it was administered at three different doses (10 mg, 20 mg and 30 mg/Kg weight body). At the lowest dose, ellipticine decreased the body weight of mice by 18% over 4 weeks. The middle dose led to a 33% reduction in body weight after 3 weeks and the higher dosage showed a 28% reduction in body weight (Ellies and Rosenberg, 2010).

N-methyl-tetrahydroellipticine (**26**) led to the inhibition of the *E. coli* cyclopropane fatty acid synthase (CFAS) *in vitro* (IC₅₀ 5.07 μ M), where the dioctylamine (IC₅₀ 4 $\pm$ 0.6 μ M) was used as a positive control (Guianvarc'h et al., 2008). While the physiological role of the CFAS enzyme has not been fully defined in any species, it has been suggested that different organisms use this modification to facilitate adaptation to

environmental conditions or processes requiring changes in membrane structure and function. In this way, for intracellular pathogens, cyclopropanation may play a role in survival in physiologically hostile and nutrient-poor compartments within the host cell. This would be of particular relevance to *Leishmania* species (Oyola et al., 2012). Also, the antimalarial activity of (**26**) was shown by Montoia and co-workers (2014). Compound (**26**) presented activity against *Plasmodium falciparum* K1 and 3D7 strains, IC₅₀ 4.2 µM and 13 µM, respectively. Chloroquine diphosphate (positive control) showed an IC₅₀ of 0.33 µM against *P. falciparum* K1 and IC₅₀ 0.11 µM against *P. falciparum* 3D7. Quinine sulfate (positive control) showed an IC₅₀ of 0.12 µM against *P. falciparum* K1 and IC₅₀ 0.15 µM against *P. falciparum* 3D7.

In 1961, Büchi and co-workers isolated ellipticine (**25**), *N*-methyl-tetrahydroellipticine (**27**), 1,2-dihydroellipticine (**27**), olivacine (**29**), and 3,4-dihydroolivacine (**30**) from methanolic extracts of *A. subincanum* bark. The antitumour activity of olivacine (**29**) has been described (Jasztold-Howorko et al., 2013). It possessed similar antitumour activity like ellipticine (Tylinska et al., 2010). The main mechanism of the antineoplastic action proposed is related to the stabilization of the DNA-enzyme complex and/or inhibition of topoisomerase II (Tylinska et al., 2010). Olivacine (**29**) has shown antiprotozoal activities, it showed 98% inhibition of *Trypanosoma cruzi* epimastigotes forms (Y strain) *in vitro* at 10 µg/mL.

However, it was not effective in mice suggesting, according to the authors, an inactivation of the drug by the host (Leon et al., 1978). In assays carried out by Rocha e Silva and co-workers (2012), olivacine (**29**) inhibited growth of *P. falciparum* 3D7 and K1 strains (IC₅₀ 1.2 µM and 1.4 µM, respectively). Chloroquine-diphosphate (positive control) showed an IC₅₀ of 0.13 µM and 0.058 µM against *P. falciparum* K1 and 3D7 strains, respectively. Quinine sulphate (positive control) showed an IC₅₀ of 0.16 µM and 0.11 µM against *P. falciparum* K1 and 3D7 strains, respectively, while the artemisinin showed IC₅₀ values of 2.1 nM and 1.1 nM against *P. falciparum* K1 and 3D7 strains, respectively. Female Webster Swiss mice infected with *P. berghei* (NK65 strain), were treated orally and subcutaneously with doses of 100 mg/kg/day. The parasitaemia was evaluated on days 5 and 7 after inoculation. The percentage of parasite inhibition after oral treatment was 97% and 90% on day 5 and 7, respectively. Using the subcutaneous treatment, the suppression of

parasitaemia observed was 14% and 55% after 5 and 7 days, respectively. The mechanism of action proposed was the same as discussed above for ellipticine (Rocha e Silva et al, 2012).

In 1965, Gilbert et al. reported the isolation of uleine (**21**) and olivacine (**29**) from the ethanolic extract of bark and cork of *A. subincanum*. The structure of uleine was proposed in 1959 by Büchi and Warnhoff.

Dolabela and co-workers (2015) reported the activity of uleine (**21**) against *P. falciparum* W2 and 3D7 strains in different assays using cloroquine and mefloquine as positive controls. In the microscopic method, uleine (**21**) showed an IC_{50} of $0.75 \mu\text{g/mL} \pm 0.10$ and $11.90 \pm 0.10 \mu\text{g/mL}$ against *P. falciparum* 3D7 and W2 strains, respectively. In this assay, cloroquine showed IC_{50} $0.02 \pm 0.002 \mu\text{g/mL}$ and $0.0013 \pm 0.0001 \mu\text{g/mL}$ against the W2 and 3D7 strains, respectively, and mefloquine IC_{50} $0.016 \pm 0.002 \mu\text{g/mL}$ and $0.048 \pm 0.0007 \mu\text{g/mL}$, respectively). The mechanism proposed involves the inhibition of haemozoin formation (Oliveira et al., 2010).

Uleine (**21**) showed inhibitory activities *in vitro* on human AChE, (hrAChE, IC_{50} $279.0 \pm 4.5 \mu\text{M}$) and butyrylcholinesterase from human serum (hBChE, IC_{50} $24.0 \pm 1.5 \mu\text{M}$). Tacrine was used as positive control (IC_{50} $0.34 \pm 0.03 \mu\text{M}$ and $0.02 \pm 0.06 \mu\text{M}$ with rAChE and hBChE, respectively). In addition uleine (**21**) at $250 \mu\text{M}$ showed an aggregation inhibition of beta-amyloid of $40.1 \pm 7.4\%$ while curcumine ($10 \mu\text{M}$, positive control) showed $34.4 \pm 1.1\%$ inhibition (Seidl et al., 2017). In 2010, Maes and Maes patented the use of uleine (**21**) for the prevention and/or the treatment of infectious diseases, especially AIDS.

In 1967 Gaskell and Joule isolated dasycarpidone (**19**), 20-*epi*-dasycarpidone (**20**) uleine (**21**), and 20-*epi*-uleine (**22**) from *A. subincanum*. Dasycarpidone (**19**) exhibited antiplasmodial activity *in vitro* against *P. falciparum* KI strain (IC_{50} $1.67 \mu\text{M}$) and this alkaloid did not inhibit the growth of NIH3T3 murine fibroblasts ($IC_{50} > 50 \mu\text{g/mL}$) (Rocha e Silva et al., 2012). Chloroquine-diphosphate, quinine sulphate and artemisinin were used as positive controls.

Biological activity of dasycarpidone (**19**) and 20-*epi*-uleine (**22**) was not described in the literature consulted. In 1970, the same researchers described the isolation of subincanine (**32**) and its structural elucidation. Borris and co-workers (1983) described the isolation of uleine (**21**), 20-*epi*-uleine (**22**) and

limatinine (12-hydroxy-N-acetyl-aspidospermatidine, **24**) from *A. subincanum* bark. In 2009, Santos and co-workers isolated oleic acid and guatambuine (u-alkaloid C, *N*-methyln-tetrahydroolivacine, **31**) from the stem bark of *A. subincanum* (Santos et al., 2009, Ribeiro et al., 2015). Guatambuine (**31**) showed antitumour activity (Simone et al., 2006). Subincanadine E (pericine, **38**) was first isolated from the *Picralima nitida* cell suspension culture line in 1982 by Arens and co-workers. In the same studies, the authors described the binding of this compound to an opiate receptor and its analgesic properties. Later, Kobayashi and co-workers (2002) isolated six new indole alkaloids, subincanadine A-F (**34-39**), from the bark of *A. subincanum* Mart. Subincanadine E (**38**) showed *in vitro* cytotoxicity against both murine lymphoma L1210 and human epidermoid carcinoma KB cells (LD₅₀ 0.3 µg/mL and 4.4 µg/mL, respectively) and an opiate agonist activity in a 3-H-naloxone binding study (IC₅₀ 0.6 µmol/L) (Kobayashi et al., 2002; Chen et al., 2009). Subincanadine F (**39**) exhibited potent cytotoxicity *in vitro* against murine lymphoma L1210 cells (IC₅₀ 2.4 µg/mL) and human epidermoid carcinoma KB cells (IC₅₀ 4.8 µg/mL) (Kobayashi et al., 2002). Subincanadine G (**40**) was isolated by Ishiyama and co-workers (2005), but no biological activity was reported for this compound. Although the alkaloids compactinervine, 1, 2-dihydropirazol and des-N-methyluleine were described in a review article by Pereira and co-workers (2007) in *Aspidosperma subincanum*, they were not described in other references consulted.

5- Biological activities of *Aspidosperma tomentosum* Mart. extracts and isolated compounds

The extracts obtained from *Aspidosperma tomentosum* as well as the isolated and/or identified compounds from this specie have showed antiplasmodial, antimicrobial, inflammatory, antinociceptive and antitumor activities by *in vitro* assays.

In research carried out by Albernaz and co-workers (2010), the dichloromethane extract of the *Aspidosperma tomentosum* root (DAtr) was active against *Plasmodium falciparum* FcB1 strain (IC_{50} 6.7 $\mu\text{g/mL}$) and showed no toxicity against the NIH-3T3 cells (IC_{50} 452.25 $\mu\text{g/mL}$) resulting in an SI 67.5. In this study chloroquine was used a positive control. In addition, according the same authors, DAtr was active against clinical isolates of *Candida krusei* LMGO 174 (MIC 31.25 $\mu\text{g/mL}$) and *Cryptococcus neoformans* LMGO 02 (data not shown).

Ethanollic extracts of trunk wood, leaves, fruits and seeds (EtOH-P-Tr, EtOH-P-L, EtOH-P-F, and EtOH-P-Se, respectively) were evaluated *in vitro* against chloroquine-resistant (W2) and sensitive (3D7) clones of *P. falciparum* by Dolabela and co-workers (2012). The extracts EtOH-P-Tr, EtOH-P-L, EtOH-P-F and EtOH-P-Se were active with IC_{50} values ($\mu\text{g/mL}$) of 26.50 ± 3.50 , 23.75 ± 1.06 , 20.52 ± 1.41 and 24.51 ± 3.56 , respectively, against W2, and of 25.00 ± 4.24 , 27.00 ± 5.66 , 38.55 ± 1.06 and 3.03 ± 0.20 , respectively, against 3D7 strains. Chloroquine (IC_{50} 0.02 ± 0.002 $\mu\text{g/mL}$ against *P. falciparum* W2 and IC_{50} 0.0013 ± 0.0001 $\mu\text{g/mL}$ against *P. falciparum* strains W2 and 3D7, respectively) and mefloquine (IC_{50} 0.0165 ± 0.002 $\mu\text{g/mL}$ and IC_{50} 0.048 ± 0.0007 $\mu\text{g/mL}$ against *P. falciparum* strains W2 and 3D7, respectively) were used as positive controls and the cytotoxicity was evaluated in Vero cell cultures.

In studies realized by Aquino and co-workers (2013), the crude ethanolic extract (CEE) of the stem bark of *A. tomentosum* and its fractions (Hexane 100%, Hex : CHCl_3 50%, CHCl_3 : EtAcO 50%, EtAcO 100%, CHCl_3 : MeOH 5%, and CHCl_3 : MeOH 10%) showed antinociceptive and anti-inflammatory activities in mice. The acetic acid-induced abdominal writhing, the hot plate test and the catalepsy test were carried out to evaluate the antinociceptive activities. In the first test, the animals were treated with CEE and its fractions

(100 mg/kg, p.o.). The abdominal contortions were induced by an intraperitoneal injection of a 0.6% acetic acid solution 40 min after the treatment. Dipyrone (100 μ mol/kg, p.o.) was used as a positive control. The extracts CEE, Hexane 100%, Hex : CHCl₃ 50%, CHCl₃ : EtAcO 50%, CHCl₃ : MeOH 5%, and CHCl₃ : MeOH 10% produced a significant decrease in abdominal writhing response, with 53.3%, 42.2%, 54.7%, 36.1%, 59.7%, 50.8%, and 29.2% inhibition, respectively. Dipyrone showed 64.1% inhibition. In the hot plate test, only the animals treated with the Hex : CHCl₃ 50%, CHCl₃ 100%, and CHCl₃ : MeOH 5% fractions showed a significant increase of latency time at 30 min. According to the authors, this result indicated the ability of the extracts to influence the central mechanism of pain. Therefore, they performed the hot plate test in the presence of an opioid receptor antagonist. Animals treated with morphine showed a significant increase in latency at 30 to 120 min. In the presence of naloxone, an opioid receptor antagonist of morphine, the effects of the Hex : CHCl₃ 50%, CHCl₃ 100%, and CHCl₃ : MeOH 5% fractions were completely blocked. The fractions that showed significant results in the hot plate test were evaluated in the catalepsy assay. The animals treated did not show catalepsy condition, when a strong cataleptic effect was induced during the four-hour period of the study. Therefore, the authors suggested that these fractions may be acting by a mechanism of action dependent on opioid receptors and the extracts are not acting through the blockade of dopamine receptors in the striatum and nucleus accumbens. To evaluate the anti-inflammatory activities Aquino and co-authors (2013) conducted the ear capsaicin oedema and hioglycolate-induced peritonitis assays. In the first assay, the CEE and all fractions, except for the CHCl₃ : MeOH 10% fraction, were able to produce an antioedematogenic activity. In the second assay, only the EtOAc 100% fraction was not able to significantly inhibit leukocyte migration into the peritoneal cavity. According to the authors, these results suggested that *A. tomentosum* has antinociceptive and anti-inflammatory activities.

In studies realized by Kohn and co-workers (2015), the ethanolic extract of leaves inhibited the replication of avian metapneumovirus (aMPV) *in vitro* showing an IC₅₀ of 45.86 $\pm$ 0.62 μ g/mL; the cytotoxicity was evaluated against CER (chicken embryo related) cells (CC₅₀ 64.9 $\pm$ 0.05 μ g/mL, SI 1.5) (Kohn et al., 2015).

In this assay, the controls consisted of untreated infected (virus titer), treated non-infected (extract control), and untreated non-infected (cell control) cells.

The dichloromethane extracted from the root was active against clinical isolates of *Candida krusei* LMGO 174 (MIC 31.25 µg/mL) (Albernaz et al., 2010). Kohn and workers (2006) reported the antiproliferative activity of the ethanolic extracts of leaves against MCF7 (breast), NCIADR (breast), NCI460 (lung) and UACC62 (skin) cancer cell lines in the sulphorodamine B assay in a concentration dependent way. In this study, doxorubicin was used as positive control.

Concerning the phytochemical aspects *A. tomentosum*, Ardnt and co-workers (1967) isolated the alkaloids uleine (**21**), 3-*epi*-uleine (**22**) and limatinine (12-hydroxy-N-acetylaspidospermatidine, **24**) from the chloroformic extract of leaves. In research carried out by Aquino (2006), the presence of nine indole alkaloids was characterized in different parts of the plant using GC/MS analysis. Uleine (**21**) was found in leaves branches and seeds, aspidospermine (1-acetyl-12-methoxyaspidospermidine, **41**), 1,2-dehydroaspidospermidine (**43**), quebrachamine (**44**) and rhazinilam (**45**) were characterized in seeds and arils. The dasycarpidone (demetylen-oxo-uleine, **19**), nor-uleine (N-demethyluleine, **23**) and 3-oxo-eburnamonine (**33**) were detected only in the branches as well as 5,21-dehydrorhazinilam (**46**) was found at seeds and 1-acetyl-aspidospermidine (N-acetylaspidospermidine, demethoxy-aspidospermine, **42**) was detected only in arils.

In an assay completed by Deutsch and co-workers (1994), aspidospermine (**41**) and quebrachamine (**44**) induced contractions of human prostatic tissue, rabbit corpus spongiosum and cavernosum and guinea pig vas deferens. Aspidospermine (**41**) was both cytotoxic (starting at 75 µM) and genotoxic (starting at 50 µM) in studies performed by Coatti and co-workers (2016). In this assay, methyl methanesulfonate was used a positive control. In addition, the aspidospermine (**41**) showed antiprotozoan activity against an chloroquine-resistant and sensitive strain of *Plasmodium falciparum*, in vitro on chloroquine-resistant and sensitive strain of *Plasmodium falciparum* (Mitaine-Offer et al., 2002) and exhibits activity inhibitors of *Trypanosoma cruzi* trypanothione reductase (Galarreta et al., 2008).

Quebrachamine (**44**) and rhazinilam (**45**) showed cytotoxicity against A549 human lung adenocarcinoma cell line ($> 30.0 \mu\text{M}$ and $\text{IC}_{50} 0.35 \mu\text{M}$, respectively) and to HT29 human colon adenocarcinoma grade II cell line ($30.0 \mu\text{M}$ and $\text{IC}_{50} 0.35 \mu\text{M}$, respectively) in biological assay (Wu et al., 2009). In this assay, docetaxel was used a positive control with $\text{IC}_{50} 4.95 \times 10^{-4} \mu\text{M}$ against A549 human lung adenocarcinoma cell line and $\text{IC}_{50} 3.34 \times 10^{-4} \mu\text{M}$ to HT29 human colon adenocarcinoma grade II cell line. However, Décor and co-workers (2006) showed that although rhazinilam (**45**) showed in vitro cytotoxicity toward various cancer cell lines in the low micromolar range, it was not active in vivo. David and co-workers (1994) showed that rhazinilam (**45**) mimics the effects of taxol in vitro assays with mammalian cells. However, rhazinilam (**45**) alters microtubule stability differently than taxol, with distinct mechanisms of action at the molecular level. The compound 1, 2-dehydroaspidospermidine (**43**) was isolated from the others species. It was isolated from leaves of *Rhazya stricta* Decne (Apocynaceae) (Smith and Wahid, 1963), the above-ground path of *Vinca minor minor* (Apocynaceae) (Mokrý et al., 1967) and from other *Aspidosperma's* species (Pereira et al., 2007). In addition, the flavonoid isorhamnetin (**14**) was obtained from stem bark (Aquino et al., 2013).

In a study carried out by Dong and co-workers (2014), the results showed that isorhamnetin (**14**) inhibited the generation of reactive oxygen species, and the decrease of glutathione levels induced by arachidonic acid and iron. In addition, isorhamnetin (**14**) revealed protective effects against amyloid β -induced cytotoxicity and amyloid β aggregation (Iida et al., 2015).

Fig. 3 - Indole alkaloids isolated from *A. subincanum* and *A. tomentosum*.

6- Indole alkaloids isolated from *A. subincanum* and *A. tomentosum*

6.1 Biosynthetic aspects

The indole alkaloids are found mainly in eight plant families, among which the Apocynaceae, Loganiaceae, Rubiaceae and Nyssaceae (O'Connor and Maresh, 2006) with a great diversity of structures.

In some species, the MIA (Monoterpene Indol Alkaloid) biosynthetic pathway is known. In *Catharanthus roseus* (vinblastine and vincristine) and *Rauvolfia serpentina* (reserpine) this pathway has been best characterized at the molecular level and the spatial organization of MIA biosynthesis is almost completely known. In *Camptotheca acuminata* and *Ophiorrhiza pumila* some studies have also been carried out for characterizing early steps in camptothecin biosynthesis (De Luca et al., 2012).

Monoterpene indole alkaloids are derived from tryptamine (**60**) and secologanin (**55**) which combine in a Pictet-Spengler reaction to form strictosidine (**61**). Tryptamine (**60**) is obtained from the shikimate-chorismate-indole pathway. Previous studies dealing with aromatic amino acids have provided a comprehensive view on intermediate metabolites (Tzina and Galili, 2010). The biosynthesis of tryptamine initiates by the shikimate pathway, leading to the synthesis of chorismic acid (Fig. 5). The next step includes a transfer of an amino group of glutamine to chorismic acid (57) to generate anthranilic acid (**58**). A sequence of complex reactions leads to L-tryptophan (**59**). Tryptamine (**60**) is obtained after decarboxylation of L-tryptophan (Dewick, 2009; Tzina and Galili, 2010; St-Pierre, 2013; Pan et al., 2016).

Secologanin (**55**) is obtained via the secoiridoid route (Dewick, 2009; Pan et al., 2016) where isopentenyl diphosphate (IPP) is a precursor. In plants, IPP can be produced by the mevalonate pathway (MVA) and by methylerythritol phosphate pathway (MEP). In *Aspidosperma* species the preferential route is not known. According to Pan et al. (2016), the MEP pathway is the major route for the biosynthesis of secologanin in *Catharanthus roseus*.

In most MIAs, the tryptamine skeleton is conserved and secologanin may be reorganized. Considering the different structures derived from the secologanin unit, MIAs can be classified in 3 types (Fig. 4). In the

Apocynaceae family, the 3 types can be found (Szabó, 2008; St-Pierre et al., 2013). However, in this review, the alkaloids isolated from *A. subincanum* and *A. tomentosum* will be classified as an uleine-like scaffold (*Corynan type*, type I), an ellipticine-like scaffold (*Corynan type*, type I) and related pyridocarbazoles, a subincanadine-like scaffold (*Corynan type*, type I), and an aspidospermine-like scaffold (*ibogan type*, type III), according to their structure to facilitate the discussion.

Fig. 4 – Types of skeleton of monoterpene indole alkaloids.

In *Cataranthus roseus*, tryptamine (**60**) and secologanin (**55**) are synthesized in cytoplasm and need to be transported to the vacuole where strictosidine (**61**) is obtained (Payne et al., 2017). In *Aspidosperma* species is not known where the synthesis takes place. According to Szabó (2008), strictosidine (**61**) was isolated from *Anthocephalus cadamba* (Rubiaceae), *Strychnos mellodora* (Loganiaceae), *Catharanthus roseus* (Apocynaceae) and *Rhazya stricta* (Apocynaceae).

4, 21-Dehydrogeissoschizine (**62**) is formed through strictosidine aglycone cyclization (Dewick, 2008). 4, 21-Dehydrogeissoschizine (**62**) serves as the intermediate for the formation of preakuammicine (**63**). The mechanism to explain the formation of preakuammicine (**63**) remains unknown (O'Connor and Maresh; 2006; Benayad et al., 2016). Furthermore, preakuammicine (**63**) has not been isolated from plant material and the enzymes that can explain the proposed biosynthetic mechanism are not known. Preakuammicine (**63**) is reduced to form stemmadenine (**64**). During this conversion the C3-C7 bonding is maintained (Fig. 6) and the indole ring aromaticity is re-established.

Stemmadenine (**64**) has been proposed as a precursor of indole alkaloids obtained from some *Aspidosperma* species (Fuller, 1974; O'Connor and Maresh, 2006; Dewick, 2009, Szabó, 2008). The biogenetic relationship between a precursor like stemmadenine (**64**) and the alkaloids apparicine (**2**), uleine (**21**), guatambuine (**31**), ellipticine (**25**) and olivacine (**29**) was suggested by Kutney and co-workers (1969a) and later put forward by Potier and Janot (1973). Nevertheless, it is still missing experimental data regarding the biosynthetic pathways of alkaloids identified in *A. subincanum* and *A. tomentosum*.

Fig. 5- Schematic steps of strictosidine (58) biosynthesis. The isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) precursors may be synthesized by the mevalonate pathway (MEV) and the non-mevalonate pathway (MEP). The

condensation of secologanin with tryptamine in a Mannich-like reaction produces strictosidine (Pan et al., 2016; St-Pierre et al., 2013; Szabó, 2008; Dewick, 2009). Solid arrows represent a one step reaction. The broken arrows represent multiple reactions.

Fig. 6- Schematic steps of stemmadenine (58) biosynthesis (Dewick, 2009; Mujib et al., 2012). Solid arrows represent a one step reaction. The broken arrows represent multiple reactions.

In the proposed biosynthetic pathway of uleine (21) from stemmadenine (64) the loss of an ester function and the formation of double bonding at C16-C17 occurs (Fig. 7). The C5-C6 bond is broken with the loss of C6 (original C2 of tryptophane) and a C7-C21 bond is formed (Kutney et al., 1969b; Kansal and Potier, 1986; Dewick, 2009).

Fig. 7- Proposed uleine-like scaffold and ellipticine-like scaffold biosynthetic pathways from stemmadenine (Potier and Janot, 1973; Fuller, 1974; Kansal and Potier, 1986).

In the proposal of the biosynthetic formation of ellipticine (**25**) a stemmadenine-like precursor may undergo decarboxylation of the ester function at C-16 with formation of the methylene group in C16, similar to that proposed for uleine (Fig. 7). The biosynthetic pathway of the subincanadines A-G (**34-40**) is proposed in Fig. 8. A stemmadenine-type alkaloid may be a biogenetic precursor of the subincanadines. The biogenetic pathway proposed involves decarboxylation of the ester function at C-16 similar to the biosynthesis of apparicine (**2**) from stemmadenine (**60**) as proposed by Kutney and co-workers (1969b). The formation of subincanadine D (**37**) and subincanadine E (**38**) involves decarboxylation of the ester function at C-16 and the formation of the exocyclic methylene group. In the formation of subincanadine A (**34**), B (**35**), C (**36**) and G (**40**) the formation of an N4-C16 bond was proposed. In the biosynthesis of subincanadine F the loss of two-carbons (C16 and C17) was proposed, as well as the formation of a C2-C14 bond and oxidation at C-15 (Kobayashi et al., 2002; Kutney et al., 1969b).

Fig. 8- Proposed subincanadine biosynthetic pathway (Kobayashi et al., 2002; Kutney et al., 1969b).

The biosynthetic pathway of aspidospermine (**41**) from stemmadenine (**64**) involves a rearrangement to form dehydrosecodine (Fig. 9), which serves as a common intermediate for the Aspidosperma and the Iboga skeletons (Dewick, 2009).

dasycarpidone (**19**) making the carbons unshielded. To understand this effect it is necessary to consider the resonance structure (Fig. 10):

Fig. 10- Resonance structure of dasycarpidone.

However, the H-15 of dasycarpidone (**19**) is shifted downfield compared to uleine (**21**). The anisotropic effect of the carbonyl group at C-16 in dasycarpidone (**19**) makes H-15 more shielded.

The ellipticine-guatambuine-like scaffold has the pyrido[4.3.b]carbazole moiety in common. In ellipticine (**25**) and olivacine (**29**) the atoms of four rings (A, B, C and D) are coplanar. In the ^1H NMR spectrum, the ellipticine piperidine moiety (D ring) is associated with signals at δ 8.3 (doublet, H3), δ 7.9 (d, H14) and δ 9.95 (H21). In olivacine (**29**) the same ring shows signals at δ 8.5 (doublet, H3) and δ 7.9 (d, H14). In the ^{13}C NMR spectrum, the chemical shift of the methyl group of C18 of olivacine (**29**) is shifted downfield (δ 21.9) compared to the same carbon (δ 14.1) in ellipticine (**25**). This difference can be explained by the electron withdrawing effect of the nitrogen (N4).

In guatambuine (**31**) and *N*-methyltetrahydroellipticine (**26**) the atoms of three rings (A, B and C) are nearly coplanar and the heterocyclic ring D is in a half-chair conformation. The C19 and C14 atoms of the heterocyclic ring D are located in the same plane as the rings A, B and C, while atoms C3 and N4 are located above and below, respectively (Simone et al., 2006). The chemical shifts of similar carbons of both compounds are similar.

The seven subincanadines (**34-40**) provide interesting architectures, with three different subgroups. The first subgroup has a 1-azaniatricyclo[4.3.3.0]undecane moiety (subincanadine A-C and G), the second subgroup has a 1-azabicyclo[5.2.2]undecane (subincanadine D-E) and the third has a 1-azabicyclo[4.3.1]decane (subincanadine F) moiety. In all subgroups we can observe that the chemical shifts of the indole system (rings A and B) in ^{13}C NMR occur in similar regions with small variations. In subincanadine A (**34**) and C (**36**), the heterocyclic ring C shows a twisted conformation and the heterocyclic rings D and E

have envelope conformations. In subincanadine A (**34**) and B (**35**) the hydroxyl group at C15 displaces the C14 signal downfield when compared with subincanadine C (**36**). The chemical shift of C17 at subincanadine A (**34**) and B (**35**) appears upfield when compared with subincanadine C (**36**) due to the electron withdrawing effect of the hydroxyl group. The change in configuration of C16 in subincanadine B (**35**) makes the heterocyclic ring C adopt a boat conformation that contributes to the higher energy of the system. Due to the increase in ring tension, the signals of C2, C3, C5, C6 and C16 appear upfield, when compared with the isomeric alkaloid subincanadine A (**34**).

In the second subincanadine subgroup, subincanadine D (**37**) has a hydroxyl group at C15 and the chemical shifts of C20, C14 and C16 are upfield whereas C2 is downfield when compared with subincanadine E (**38**). Subincanadine F (**39**) is included in the last subgroup. In subincanadine F (**39**), the carbonyl group at C15 is conjugated with the exocyclic double bond and this causes the signal of C20 to appear downfield when compared to the other subincanadines.

Table 2 – ¹H NMR assignments of indole alkaloids isolated from *Aspidosperma* species (Bonjoch et al., 1991; Kobayashi et al., 2002; Jácome et al., 2004; Bennasar et al., 2006; Henrique et al., 2010; Torres et al., 2013).

Hydrogen	19 CDCl ₃ , 500 MHz	20 CDCl ₃ , 500 MHz	21 CDCl ₃ , 360 MHz	22 CDCl ₃ , 360 MHz	25 CDCl ₃ :CD ₃ OD 1:1; 500 MHz	26 CDCl ₃ :CD ₃ OD 1:1; 500 MHz	29 MeOD, 500 MHz	31 MeOD, 300 MHz	34 DMSO-d ₆ , 500 MHz	35 DMSO-d ₆ , 500 MHz	36 Pyr-d ₅ , 500 MHz	37 Pyr-d ₅ , 500 MHz	38 CD ₃ OD, 500 MHz	39 CD ₃ OD, 500 MHz
1 (NH)	10.39 (<i>br</i> , 1H)	9.88 (1H, <i>s</i>)	8.25 (1H, <i>s</i>)	8.25 (1H, <i>s</i>)	Not related	Not related	Not related	7.85 (<i>br s</i> , 1H)	10.89 (1H, <i>s</i>)	10.9 (1H, <i>s</i>)	13.33 (1H, <i>s</i>)	12.15 (1H, <i>s</i>)	-	-
3a	2.36 (<i>tt</i> , 7.5)	2.88 (1H, <i>m</i>)	2.45 (1H, <i>m</i>)	2.4 (1H, <i>m</i>)	8.3 (1H, <i>d</i> , 7)	2.8 (2H, <i>t</i> , 6.5)	8.15 (1H, <i>d</i> , 6.3)	2.79 (1H, <i>ddd</i> , 5.7; 6.0; 11.7)	3.78 (1H, <i>m</i>)	3.90 (1H, <i>m</i>)	3.68 (1H, <i>m</i>)	3.51 (1H, <i>m</i>)	3.30 (1H, <i>m</i>)	4.22 (1H, <i>dd</i> , 4.8; 17.6)
3b	2.61-2.65 (<i>m</i>)	3.37 (1H, <i>d</i> , 8.7)	2.7 (1H, <i>d</i> , 3.0)	2.65 (1H, <i>m</i>)				3.19 (1H, <i>ddd</i> 5.7; 6.0; 11.7)	3.60 (1H, <i>m</i>)	3.73 (1H, <i>m</i>)	3.51 (1H, <i>m</i>)	2.71 (1H, <i>ddd</i> ; 15.8; 7.8; 5.5)	3.10 (1H, <i>m</i>)	4.13 (1H, <i>m</i>)
5a	2.34 (<i>s</i> , 3H)	2.8 (3H, <i>s</i>)	2.3 (3H, <i>s</i>)	2.25 (3H, <i>s</i>)	-	2.6 (3H, <i>s</i>)	-	2.54 (3H, <i>s</i>)	3.81 (1H, <i>m</i>)	3.82 (1H, <i>m</i>)	4.01 (1H, <i>dd</i> , 7.3; 11.9) 3.81 (1H, <i>dd</i> , 7.6; 11.9)	3.79 (1H, <i>d</i> , 13.1)	3.68 (1H, <i>d</i> , 13.4)	3.83 (1H, <i>m</i>)
5b	-	-	-	-	-		-		3.70 (1H, <i>m</i>)	3.71 (1H, <i>m</i>)		3.47 (1H, <i>m</i>)	3.39 (1H, <i>d</i> , 13.4)	3.65 (1H, <i>d</i> , 13.4)
6a	-	-	-	-	-		-		3.14 (1H, <i>m</i>)	3.11 (1H, <i>m</i>)	3.15 (1H, <i>m</i>)	4.03 (1H, <i>d</i> , 15.9)	3.88 (1H, <i>d</i> , 14.5)	3.24 (1H, <i>dd</i> , 6.4; 18.0)
6b	-	-	-	-	-		-		3.12 (1H, <i>dd</i> , 17.1; 6.7)	3.11 (1H, <i>m</i>)		3.12 (1H, <i>d</i> , 15.9)	3.12 (1H, <i>dd</i> , 1.4; 14.5)	3.21 (1H, <i>dd</i> , 6.4; 18.0)
9	7.72 (<i>d</i> , 8.1)	7.74 (1H, <i>d</i> , 8.0)	7.35 (1H, <i>d</i> , 8)	7.3 (1H, <i>d</i> , 7.8)	8.3 (1H, <i>d</i> , 7)	8.2 (1H, <i>d</i> , 7.8)	8.26 (1H, <i>d</i> , 7.8)	8.0 (1H, <i>d</i> , 7.8)	7.44 (1H, <i>d</i> , 8.2)	7.43 (1H, <i>d</i> , 7.5)	7.53 (1H, <i>d</i> , 7.5)	7.62 (1H, <i>d</i> , 7.8)	7.48 (1H, <i>d</i> , 8.0)	7.43 (1H, <i>d</i> , 7.9)
10	7.19 (<i>dt</i> , 7.5; 1.0)	7.39 (1H, <i>t</i> , 7.0)	7.1 (1H, <i>t</i> , 7.8)	7.1 (1H, <i>t</i>)	7.2 (1H, <i>ddd</i> , 7.2; 6.8, 1.5)	7.1 (1H, <i>t</i> , 7.8)	7.26 (1H, <i>m</i>)	7.19 (1H, <i>ddd</i> , 1.2; 6.6, 7.8)	7.05 (1H, <i>dd</i> , 8.02; 7.4)	7.02 (1H, <i>dd</i> , 7.8; 7.6)	7.21 (1H, <i>dd</i> , 7.5; 7.3)	7.29 (1H, <i>dd</i> , 7.8; 7.2)	7.05 (1H, <i>dd</i> , 8.0; 7.4)	7.01 (1H, <i>dd</i> , 7.9; 7.5)
11	7.38 (<i>dt</i> , 7.5; 1.0)	7.51 (1H, <i>t</i> , 7.0)	7.2 (1H, <i>t</i> , 7.8)	7.15 (1H, <i>t</i> , 8.0)	7.4-7.5 (<i>m</i>)	7.3 (1H, <i>t</i> , 7.8)	7.52 (1H, <i>m</i>)	7.37 (1H, <i>ddd</i> , 1.2, 6.6, 8.1)	7.15 (1H, <i>dd</i> , 7.6; 7.4)	7.11 <i>dd</i> (1H, 7.8; 7.2)	7.31 (1H, <i>dd</i> , 7.9; 7.3)	7.36 (1H, <i>dd</i> , 7.9; 7.2)	7.14 (1H, <i>dd</i> , 8.0; 7.4)	7.10 (1H, <i>dd</i> , 8.1; 7.5)
12	7.53 (<i>d</i> , 8.4)	7.6 (1H, <i>d</i> , 8.0)	7.55 (1H, <i>d</i> , 7.8)	7.55 (1H, <i>d</i> , 8.0)	7.4-7.5 (<i>m</i>)	7.4 (1H, <i>d</i> , 7.8)	7.52 (1H, <i>m</i>)	7.42 (1H, <i>dd</i> , 1.2, 8.1)	7.48 (1H, <i>d</i> , 7.6)	7.44 (1H, <i>d</i> , 7.2)	7.85 (1H, <i>d</i> , 7.9)	7.60 (1H, <i>d</i> , 7.9)	7.36 (1H, <i>d</i> , 8.0)	7.3 (1H, <i>d</i> , 8.1)
14a	1.26-1.34 (<i>m</i> , 3H)	2.06 (1H, <i>d</i> , 15.0)			7.9 (<i>d</i> , 7.0)	3.0 (2H, <i>t</i> , 6.5)	7.90 (1H, <i>d</i> , 6.3)	2.94 (2H, <i>m</i>)	2.05 (1H, <i>ddd</i> , 17.8; 10.9; 3.0)	2.54 (1H, <i>m</i>)	1.83 (1H, <i>m</i>)	2.59 (1H, <i>dd</i> , 14.5; 5.4)	2.41 (1H, <i>ddt</i> , 7.2; 7.2; 14.4)	4.11 (1H, <i>t</i> , 4.8)
14b	1.94-1.99 (<i>m</i> , 1H)	2.55 (1H, <i>m</i>)	2.05 (2H, <i>m</i>)	2.0 (2H, <i>m</i>)					1.82 (1H, <i>dd</i> , 17.8; 11.2)	2.24 (1H, <i>m</i>)	1.53 (1H, <i>m</i>)	2.00 (1H, <i>dd</i> , 14.3; 5.4)	1.82 (1H, <i>m</i>)	
15	2.08-2.11 (<i>m</i> , 1H)	2.86 (1H, <i>m</i>)	3.05 (1H, <i>s</i>)	3.98 (1H, <i>s</i>)	-	-	-	-	-	-	4.58	-	4.19 (1H, <i>br</i>)	-
17a	-	-	5.0 (1H, <i>m</i>)	4.95 (1H, <i>s</i>)	2.7 (3H, <i>s</i>)	2.4 (3H, <i>s</i>)	2.81 (1H, <i>s</i>)	2.41 (3H, <i>s</i>)	1.61 (3H, <i>s</i>)	1.66 (3H, <i>s</i>)	1.76 (3H, <i>s</i>)	6.59 (1H, <i>s</i>)	5.59 (1H, <i>s</i>)	-
17b	-		5.27 (1H, <i>s</i>)	5.2 (1H, <i>s</i>)								5.92 (1H, <i>s</i>)	5.56 (1H, <i>s</i>)	
18	0.88 (<i>t</i> , 3H)	0.91 (3H, <i>t</i> , 7.3)	0.85 (3H, <i>t</i> , 7.4)	1.08 (3H, <i>t</i> , 7.4)	3.2 (3H, <i>s</i>)	2.7 (3H, <i>s</i>)	3.07 (3H, <i>s</i>)	1.52 (3H, <i>d</i> , 6.3)	1.94 (3H, <i>d</i> , 6.9)	1.84 (3H, <i>d</i> , 6.9)	1.67 (3H, <i>d</i> , 6.5)	2.06 (3H, <i>d</i> , 6.3)	1.83 (3H, <i>d</i> , 6.8)	1.89 (3H, <i>d</i> , 7.1)
19	1.26-1.34 (<i>m</i> , 3H)	1.36 (2H, <i>m</i>)	1.15 (2H, <i>q</i> , 7.4)	1.25 (2H, <i>t</i>)	-	-	-	3.89 (1H, <i>q</i> , 6.3)	5.65 (1H, <i>qd</i> , 6.9)	5.10 (1H, <i>q</i> , 6.9)	5.41 (1H, <i>q</i> , 6.5)	5.97 (1H, <i>q</i> , 6.3)	6.08 (1H, <i>q</i> , 6.8)	7.04 (1H, <i>q</i> , 7.1)
20	2.75	2.72 (1H, <i>m</i>)	1.7 (1H, <i>m</i>)	Not related	-	-	-	-	-	-	-	-	-	-
21a	-	5.09 (1H, <i>s</i>)			9.5 (1H, <i>s</i>)	3.8 (2H, <i>s</i>)	8.84 (1H, <i>s</i>)	7.70 (1H, <i>s</i>)	4.31 (1H, <i>d</i> , 14.0)	4.19 (1H, <i>d</i> , 14.7)	4.32 (1H, <i>d</i> , 13.4)	4.62 (1H, <i>d</i> , 15.8)	4.24 (1H, <i>d</i> , 15.0)	4.60 (1H, <i>d</i> , 15.6)
21b			4.10 (1H, <i>s</i>)	4.0 (1H, <i>s</i>)					4.22 (1H, <i>d</i> , 14.0)	4.11 (1H, <i>d</i> , 14.7)	4.21 (1H, <i>d</i> , 13.4)	3.93 (1H, <i>d</i> , 15.8)	3.96 (1H, <i>d</i> , 15.0)	4.40 (1H, <i>d</i> , 15.6)
OH	-	-	-	-	-	-	-	-	7.03 (1H, <i>s</i>)	7.03 (1H, <i>s</i>)	-	-	-	-

Table 3 ^{13}C NMR assignments of indole alkaloids isolated from *Aspidosperma* species (Bonjoch et al., 1991, Jácome et al., 2004; Henrique et al., 2010; Torres et al., 2013).

Carbons	19 MeOH, 125 MHz	20 CDCl ₃ , 125 MHz	21 CDCl ₃ , 90 MHz	22 CDCl ₃ , 90 MHz	25 CDCl ₃ :CD ₃ OD 125 MHz	26 CDCl ₃ :CD ₃ OD 1:1; 125 MHz	29 CD ₃ OD 125 MHz	31 CD ₃ OD 90 MHz	34 DMSO-d ₆ , 125 MHz	35 DMSO-d ₆ , 125 MHz	36 Pyr-d ₅ , 125 MHz	37 Pyr-d ₅ , 500 MHz	38 CD ₃ OD, 125 MHz	39 CD ₃ OD, 125 MHz
2	132.9	134	135.10	136.5	141.9	138.7	141.5	138.1	129.85	131.39	131.32	135.48	139.87	132.26
3	46	46.5	46.30	46.4	137.3	52.5	137.3	48.1	57.56	58.67	58.81	45.17	48.67	51.78
5	44	42.1	44.40	44.8	-	45.4	-	42	46.72	46.19	46.65	56.62	60.07	57.38
6	-	-	-	-	-	-	-	-	17,51	17.82	18.21	20.44	22.69	20.64
7	119.9	113.5	107.80	110.8	124.8	120.1	127.7	121.3	104.99	103.82	103.14	108.14	110.15	112.52
8	127	126.7	129.40	128.50	123.6	124.1	123.4	123.7	125.52	125.35	126.63	127.82	130.87	128.7
9	122	120.7	119.50	119.6	123.8	122.3	122.4	120.1	118.57	118.53	118.75	111.31	120.06	118.72
10	121.1	123.9	119.80	119.90	119.5	118.4	120.9	119.2	119.33	119.26	119,99	119.11	121.72	120.41
11	126.9	128.4	122.70	122.8	127.3	124.6	129.3	125.5	122.34	122.1	123.04	122.76	125.17	123.28
12	112.7	113.8	110.70	111.9	110.7	110.6	112.1	110.6	112.38	112.43	113.11	118.32	116.25	112.17
13	138.1	138.2	136.60	135.9	143.1	140.8	144.4	139.9	137.02	136.81	137.9	135.98	139.92	137.53
14	30.1	26.6	34.70	28.5	116.9	27.3	117.2	25	31.36	32.29	26.42	36.42	28.31	45.43
15	46.3	44.2	39.50	38.5	133.4	128.6	134.6	128.8	84.31	83.74	44.96	71.56	44.28	189.24
16	193.6	190.9	138.70	142.1	108.4	114.9	112.8	116.9	74.44	74.55	78.11	145.48	114.75	NT
17	-	-	106.70	104.7	11.2	12.1	12.6	12.9	18.9	17.89	20.79	117.81	123.15	NT
18	11.6	11,3	11.80	12.2	14.1	14.7	21.9	20.3	12.33	11.91	14.28	14.27	16.04	13.92
19	24.8	24,7	24.30	23.5	122.2	126.3	160.1	59.7	121.43	116.71	121.18	131.23	131.15	144.11
20	49.6	46.4	46.10	44.7	129.0	122.2	124.4	130.0	132.68	131.39	133.17	135.68	133.49	128.32
21	56.2	58.1	56.50	54.9	148.3	56.5	116.6	115.9	64.17	62.45	64.21	53.06	55.72	51.68

7- Synthetic approaches

Due to the wide range of important biological activities shown by indole alkaloids and their complex structure, there is much interest in the syntheses of this group of compounds.

Some synthetic approaches were used for the synthesis of compounds with an ellipticine-guatambuine skeleton (Kansal and Potier, 1986; Miller and McCarthy, 2012; Gataullin, 2009; Jasztold-Howorko et al., 2013). The first synthesis of ellipticine (**25**) was described by Woodward and co-workers (1959). The investigation into the anticancer activity of ellipticine derivatives uncovered several compounds that have been evaluated in clinical trials. Studies of the synthetic pathways to obtain the ulein-like scaffold have been carried out by different groups; many derivatives have been synthesized and their biological activities investigated (Gracia et al., 1994). The antitumour activity of olivacine (**29**) has been described and many derivatives have been synthesized (Rocha e Silva, et al., 2012; Jasztold-Howorko et al., 2013). Overall, the broad interest in compounds with a subincanadine-like scaffold can be related to the remarkable moiety and the biological activity of subincanadine E and F (Liu et al., 2006; Bennasar et al., 2009; Sadlowski, 2015).

8- Discussion

The *Aspidosperma* species provide high quality wood and are thus exploited in many regions of Brazil for this purpose (Lorenzi, 2009). The species can be found in different Brazilian biomes where various species are endemic. The taxonomic identification considering only the morphological aspects is very difficult. New techniques, such as a DNA barcoding, would be useful to help the identification of species. Some Brazilian conditions, such as the huge biodiversity, the geographical isolation, the endemic diseases, and the cultural diversity, contributed to their use in traditional medicine and the species' selection to treat specific diseases. However, the ethnopharmacological studies in Brazil are very arduous to realize and many researchers are abandoning their research lines considering the uncertainties and difficulties encountered. The species found in the Amazon region are widely used by the population to treat malaria, where the disease occurs endemically. The bark decoction is the preparation most commonly used. However, few information about

the administration and the preparation of extracts were described. Species found in other regions of the country, such as *A. subincanum* and *A. tomentosum*, are less studied with regard to their ethnopharmacological and biological aspects, although they have been investigated in order to evaluate their potential as antimalarial.

With the exploration of Cerrado for agriculture and cattle raising, a drastic fragmentation of what is left of the biome has occurred. There is a low level of protection afforded to the Cerrado and it currently stands at less than 3% of its total area. This Brazilian biome is considering a hotspot with a high level of endemic species. Considering the cited aspects, it is necessary to study the species found in Cerrado and the unexplored potential of the species in question. *A. subincanum* and *A. tomentosum* are found in Cerrado region, the latter one being endemic.

The traditional uses described for bark extracts of *A. subincanum* and *A. tomentosum* were not studied yet. Other activities not associated to traditional use included anticholinesterase, antimicrobial and antimalarial activity of the ethanolic extract of *A. subincanum* were reported. The indole alkaloids from *Aspidosperma* have shown relevant activity against *Plasmodium falciparum*, *in vitro*. *In vivo* tests of alkaloid-enriched extracts need to be done to evaluate their efficacy. Other important aspect concerning the mechanism-based studies. This may lead to phytotherapies against malaria in Latin America where the disease still is an important public health problem. Others activities, such as against Alzheimer disease and anti-inflammatory non-opioid analgesic properties, should be better investigated considering the good experimental results obtained. Despite the *Aspidosperma* genus is endemic in Latin America, in Brazil there is not any marketed drug or dietary supplement containing *Aspidosperma* species even though the popular bottles can be found in the local markets. However, in other countries some homeopathic medicines (India and Pakistan) as well as dietary supplements (Belgium, France and German) are commercially available.

The syntheses of these alkaloids are difficult considering the many asymmetric centers and the structural complexity although many efforts have been done to obtain more efficient and less toxic derivatives. Biotechnological tools may provide new approaches to obtain these alkaloids in larger

quantities. Therefore it is very important to understand the biosynthetic pathway. Nevertheless, the proposed biosynthetic pathways of MIA identified in *A. subincanum* and *A. tomentosum* need to be more investigated.

Fig. 11 - *Aspidosperma* species: ethnopharmacological use, pharmacological studies, gaps and future suggestions

9- Conclusions

Aspidosperma subincanum and *Aspidosperma tomentosum* are used by the population of Brazil to treat many diseases. The biological activities described for the extracts of both species are in agreement with their ethnopharmacological use, such as against malaria and for the treatment of diabetes and other illnesses. Further studies are needed to evaluate the mechanisms of action and the pharmacological profile of the isolated alkaloids as well as their toxicity. In addition, other biological activities which were suggested by ethnopharmacological data have not been investigated yet. It would be an interesting area to search for

new bioactive compounds, including the development of effective, safe, and low cost phytomedicines to treat endemic diseases as for example, malaria. Some biosynthetic pathways have been proposed for ellipticine and related alkaloids. However, because of a lack of experimental support, their biosynthetic pathways remain an interesting enigma to be solved. The identification of indole alkaloids from *Aspidosperma* using modern spectroscopic techniques is a challenge because for many of them NMR assignments are either not available or incomplete. On the other hand, both *Aspidosperma* species are sources of lead compounds, which can be used for developing new drugs. Different routes were used to achieve the total synthesis of some of their alkaloids, new derivatives and analogues.

In conclusion, the information presented in this review could help to develop new insights for research into *Aspidosperma subincanum* and *Aspidosperma tomentosum* as well as their related indole alkaloids, aiming for a rational exploration of them. This may contribute either to the development of new products for medicinal use or to socio-economic improvement.

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Authors' contributions

VLA, LP and KF designed the research. AFS collaborated with the botanical aspects. PRVC, CGS, JCDL and VLA analyzed the data and wrote the manuscript. The authors read and approved the manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest.

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