Research, Society and Development, v. 9, n. 10, e3629108635, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.8635

Aspidosperma excelsum and its pharmacological potential: i*n silico* studies of pharmacokinetic prediction, toxicological and biological activity

Aspidosperma excelsum e seu potencial farmacológico: estudos *in silico* de predição farmacocinética, atividade toxicológica e biológica

Aspidosperma excelsum y su potencial farmacológico: estudios *in silico* de predicción farmacocinética, actividad toxicológica y biológica

Received: 09/20/2020 | Reviewed: 09/26/2020 | Accept: 09/28/2020 | Published: 09/30/2020

Juliana Correa-Barbosa
ORCID: https://orcid.org/0000-0002-8814-3190
Universidade Federal do Pará, Brasil
E-mail: correabjuliana@gmail.com
Milena Cristina Martins da Silva
ORCID: https://orcid.org/0000-0002-5434-4242
Universidade Federal do Pará, Brasil
E-mail: milenacristina8080@hotmail.com
Sandro Percário
ORCID: https://orcid.org/0000-0002-9528-0361
Universidade Federal do Pará, Brasil
E-mail: percario@ufpa.br
Davi do Socorro Barros Brasil
ORCID: https://orcid.org/0000-0002-1461-7306
Universidade Federal do Pará, Brasil
E-mail: davibb@ufpa.br
Maria Fâni Dolabela
ORCID: https://orcid.org/0000-0003-0804-5804
Universidade Federal do Pará, Brasil
E-mail: fanidolabela20@gmail.com
Valdicley Vieira Vale
ORCID: https://orcid.org/0000-0001-6570-4875
Universidade Federal do Pará, Brasil
E-mail: valdicleyvale@gmail.com

Abstract

Based on ethnobotanical studies, the *Aspidosperma excelsum* was selected due to its highest claim of popular use for malaria and febrile diseases treatments. This species is rich in secondary metabolites as alkaloids and therefore, the aim of this study was to evaluate the pharmacokinetic, toxicological and biological activity of alkaloids isolated from *Aspidosperma excelsum* by *in silico* studies. All substances already isolated from this species were submitted to predictive studies of biological, toxicological and pharmacokinetic activities. Predictive studies of biological activities did not attribute the antimalarial activity to pure substances. However, other activities were found, such as: action on central nervous system and antineoplastic activity. In pharmacokinetic terms, many substances showed an inhibitory action on cytochrome P450 (CYP) and many adverse reactions, highlighting actions on the CNS. Also, several alkaloids, being nitrogenous substances, presented mutagenic or genotoxic activities. Thus, it is demonstrated the species potential for biological activities not yet studied, as well as the importance of investigating its pharmacokinetic and toxicological properties, justifying the accomplishment of the present study.

Keywords: Medicinal plants; Prediction; Malaria; Alkaloids.

Resumo

A partir de estudos etnobotânicos, selecionou-se a espécie *Aspidosperma excelsum*, que apresenta maior alegação de uso popular no tratamento de malária e doenças febris. Esta espécie é rica em metabólitos secundários, dentre eles os alcaloides e por isso, o objetivo do presente estudo foi avaliar o potencial farmacocinético, toxicológico e atividade biológica de alcaloides isolados de *Aspidosperma excelsum* por estudos *in silico*. Todas as substâncias já isoladas desta espécie foram submetidas a estudos preditivos de atividade biológica, toxicológica e farmacocinética. Estudos preditivos de atividades biológicas não atribuíram esta atividade antimalárica às substâncias puras. Porém, outras atividades foram encontradas, tais como: ação no sistema nervoso central e antineoplásico. Em termos farmacocinéticos, muitas substâncias mostraram ação inibitória sobre o citocromo P450 (CYP). Além de muitas reações adversas, destacando-se ações sobre o SNC. Quanto à toxicidade, vários alcaloides, por serem substâncias nitrogenadas, apresentaram atividade mutagênica ou genotóxica. Assim, demonstra-se o potencial desta espécie para atividades biológicas ainda não estudadas, bem como a importância da investigação de suas propriedades farmacocinéticas e toxicológicas, justificando a realização do presente estudo.

Palavras-chave: Plantas medicinais; Predições, Malária; Alcaloides.

Resumen

De los estudios etnobotánicos, se seleccionó la especie *Aspidosperma excelsum*, que tiene el mayor reclamo de uso popular en el tratamiento de la malaria y enfermedades febriles. Esta especie es rica en metabolitos secundarios, entre ellos alcaloides, por lo que el objetivo del presente estudio fue evaluar la actividad farmacocinética, toxicológica y biológica de alcaloides aislados de Aspidosperma excelsum mediante estudios in silico. Todas las sustancias ya aisladas de esta especie fueron sometidas a estudios predictivos de actividad biológica, toxicológica y farmacocinética. Los estudios predictivos de actividades biológicas no han atribuido esta actividad antipalúdica a sustancias puras. Sin embargo, se encontraron otras actividades, tales como: acción sobre el sistema nervioso central y antineoplásico. En términos farmacocinéticos, muchas sustancias mostraron una acción inhibidora sobre el citocromo P450 (CYP). Además de muchas reacciones adversas, destacan las acciones sobre el SNC. En cuanto a la toxicidad, varios alcaloides, al ser sustancias nitrogenadas, mostraron actividad mutagénica o genotóxica. Así, demuestra el potencial de esta especie para actividades biológicas aún no estudiadas, así como la importancia de investigar sus propiedades farmacocinéticas y toxicológicas, justificando la realización de este estudio.

Palabras clave: Plantas medicinales; Predicciones; Malaria; Alcaloides.

1. Introduction

The use of plants for medicinal purposes - treatment, cure and prevention of diseases - is one of the oldest forms of human medicinal practice (Veiga-Jr & Pinto, 2005). The use of plants, for medicinal purposes in Brazil, started with the indigenous people, who had knowledge about Brazilian flora. The shamans were responsible for improving their use and passing on knowledge to new generations (Martins, et al., 2000; Sousa, 1971).

The arrival of African slaves to Brazil contributed, without any doubt, to the expansion of the number of species, as they brought plants used both in religious ceremonies and for medicinal purposes. Europeans absorbed the knowledge about the care of pathologies that were typical of the American continent (Martins, et al., 2000; Sousa, 1971). Therefore, the use of medicinal plants to treat diseases in Brazil is ingrained to European, African and native cultures, resulting in a multicultural production, which for a long time was the main form of cure used by the rural population. (Coelho, 1989).

With the development in the pharmaceutical industry, many practices, involving plants, have been replaced by industrialized medicines. However, due to geographical isolation and

difficulties in accessing medicines, the Amazon population has maintained a large part of its medicinal culture. Although much important information has been lost, plants continue to play an important role in the treatment of pathologies and the searching for therapeutic alternatives.

In the USA, 42% of the population used medicinal plants at least once in 1996. This percentage is about 33.8% higher than in 1990, when the same survey was conducted (Eisenberg, 1998).

Germany consumes half of plant extracts marketed across Europe (about $ 3.5 billion of the $ 7 billion total, or $ 42.90 per capita, in 1997 values). In this country, medicinal plants are used to treat colds (66%), flu (38%), digestive or intestinal tract diseases (25%), headaches (25%), insomnia (25%), stomach ulcer (36%), nervousness (21%), bronchitis (15%), skin diseases (15%), fatigue and exhaustion (12%; Calixto, 2000).

Within the ethnobotanical uses of Amazonian plants, the use for malaria and febrile diseases stands out, as it is a disease that affects the region constantly. Milliken (1997) reports that several botanical families are used in traditional medicine, for the treatment of febrile diseases and malaria, in North America and South America. Alkaloids have been isolated from many species used to treat malaria. Some of these belong to families; Rubiaceae, Apocynacea among others.

When analyzing the families mentioned, it is observed that several of them have alkaloids as their main metabolite, as for example: Rubiaceae (Cardoso, et al., 2008), Apocynaceae (Pereira, et al., 2007) and Rutaceae (Waterman, 1975). It is worth mentioning that, several studies have demonstrated the antimalarial activity of alkaloids (Frederich, et al., 2008).

Aiming to facilitate new studies with alkaloids isolated from plants, the objective of this work was to evaluate the alkaloids pharmacokinetic, toxicological and biological activity potential by *in silico* method.

2. Material and Methods

This work used the scientific model of comparison for diversification in the predictions of pharmacokinetic, toxicological, physical-chemical and biological activity, in which the designed molecules were compared with others in the databases of the programs used (Pereira, et al., 2018).

This study initially carried out a survey of ethnobotanical studies, carried out in the Amazon, which reported the use of medicinal plants for the treatment of malaria. The most cited plants were selected and researched whether chemical studies and antimalarial activity assessment had been carried out. At the end of these analyzes, two species were selected to carry out the studies *in silico*, *Apidosperma ntitidum* and *A. excelsum*. The following programs were used in the study: ChemSketch version C20E41 (COPYRIGHT © 1994-2015), for the design of chemical structures; PreADMET (version 2.0, Preadmet 2020), for pharmacokinetic and toxicological predictions (Preadmet, 2020); PASS online - Prediction of activity spectra for substances (Way2Drug.com © 2011 version 2.0 2016), for the prediction of biological activity spectra, Windows 2010 operating system (Filimonov, et al., 2014); the online servers Mcule property calculator (Mcule - 2020) and Chemicalize (Chemcalize, 2020) to estimate the physical and chemical characteristics of the compounds.

ADMEtox calculations were performed by comparison with a similar substance, following the rule of Lipinski or "rule of five" and similar leader, in which a molecule must present values for 4 parameters multiple of 5: log P ≤ 5, Molecular Mass ≤ 500; Hydrogen bond acceptors ≤ 10, and hydrogen bond donors ≤ 5 (Lipinski, 2004).

In pharmacokinetic studies, skin permeability (high permeability: < 0.1; Low permeability: > 0.1) and intestinal absorption (Human Intestinal Absorption = HIA) were evaluated, considering the following parameters: HIA 0 - 20% (low absorption), 20 - 70% (moderate absorption) and > 70% (high absorption; Yee, 1997); Permeability in Caco2 and MDCK cells - High permeability > 70 nm/sec, medium permeability 4-70 nm/sec and low permeability < 4 nm/sec (Yazdanian, et al., 1998).b Regarding the distribution of substances, the following references were used: strongly linked to albumin > 90% and moderate to weak binding to albumin < 90% (Preadmet, 2020). The capacity for crossing the blood-brain barrier (BBB) was classified as: it freely crosses the BBB > 2.0, moderately crosses the BBB 2.0 - 0.1 and crosses in a reduced way or does not cross < 0.1 (Ajay, et al., 1999).

The following criteria were used to evaluate the results of metabolism: if the substances undergo phase 1 metabolism, if they inhibit any CYP and which CYP were inhibited. Substances that inhibit 2 or more CYP, especially CYP3A4 and CYP2C9, can interfere with the metabolism of a large number of drugs and other substances, which may contribute to increase their toxicity. For substances that inhibit only one CYP, there may be a reduction in the number of drugs which have pharmacokinetic interaction with it. Non-inhibitory and non-CYP-inducing substances are considered ideal substances, as they do not interfere in the metabolism of other drugs.

The mutagenicity prediction was carried out by the Ames test, using several strains of *Salmonella typhimuri*um (TA98, TA100 and TA1535). The tested variable was the mutagenic agent ability to cause reversion in growth in a media free of histidine (Ames, et al., 1975). The results of the Ames Test were considered positive when occurred a mutation reversion in one or more bacteria, negative when no mutation reversion was observed. When there was a false positive, that is, there was no mutation reversion in any clone of the bacterium and even then, the program classified it as mutagenic, the result was not considered.

The prediction of carcinogenic potential of the compounds in rodents (Rodent Carcinogenicity), was performed based on data from the National Toxicology Program (NTP) and FDA (Food and Drug Administration). The results were expressed in: (+) carcinogenic and (-) non-carcinogenic.

The assessment of toxicity criteria in marine organisms used the following parameters: algal toxicity - toxic < 1 mg/L and non-toxic > 1 mg/L (Costa, et al., 2008); toxicity in Daphnia sp - toxic < 0.22 µg/mL and non-toxic > 0.22 µg/mL (Guilhermino, et al., 2000); toxicity in Medaka fish - very toxic < 1 mg/L, toxic between 1-10 mg/L, harmful between 10-100 mg/L and non-toxic > 100 mg/L (Zucker & Hazard, 1985).

The biological activity of the substances was assessed by the similarity with more than 250,000 biologically active substances, including drugs and toxic compounds. As an evaluation criterion, the Ap (Activity Probability) of 0.7 was adopted, that is, 70% (Filimonov, et al., 2014).

3. Results and Discussion

In an ethnobotanical survey carried out in São Felix do Xingu (State of Pará, Brazil) and Projeto de Colonização do Machadinho (State of Rondônia, Brazil), 10 species were used to treat febrile diseases and/or malaria (Table 1; Brandão, et al., 1992).

An ethnobotanical study with 7 indigenous tribes and the Luso-Brazilian population (State of Roraima, Brazil), showed 99 species, belonging to 82 genera and 41 families. The species used in Roraima are described in Table 1 (Milliken, 1997).

Another ethnobotanical study, carried out on the banks of the Rio Negro (State of Amazonas, Brazil), found some species used to treat febrile diseases (Table 1; Santos, et al., 2005). A study in the traditional community of Barão de Igarapé Miri observed the use of different plants to treat febrile diseases, however some species (mentioned by the popular name) were not classified in botanical terms (Table 1; Pinto & Barbosa, 2009).

Table 1. Ethnobotanical surveys from different regions of the Brazilian Amazon.

Botanical Family	Species/Study		
	Brandão, et al., 1992	**Milliken, 1997**	**Other studies**
Apocynaceae	*Aspidosperma nitidum* Benth.	*A.excelsum* Benth	*A.excelsum* Benth[1,2]
	Geissospermum sericeum Benth.	*A. nitidum* Benth	*A. nitidum* Benth[1]
		G. sericeum (Sagot) Benth & Hook	*H. articulatus* (Vahl). Woods[1]
		Himatanthus articulates (Vahl). Woodson	
Curcubitaceae	*Momordica charantia* L.	*Momordica charantia* L.	Not reported
Gentianacea	*Lisianthus speciosus* Cham.	*Irlbachia alata* (Aubl.) Macs ssp. alata	Not reported
Labiatae	*Leonolis nepetaefolia* R.Br.	*Plectranthus barbatus* Andrews	*Rosmarinus officinalis* L[2]
Lecytidaceae	*Berthollethia excelsa* Humb. et Bonpl	Without claim of use	Without claim of use
Leguminosae	*Cassia ocidentalis* L.	*Acosmuim* sp	Without claim of use
		Andira surinamensis (Bondt) Splitg ex Pulle	
		Bauhnia ungulata L	
		Bauhnia sp	
		Caesalpinia ferrea Mart ex Tul.	
		Hymenaea courbaril L.	
		Inga ssp	
		Pithecellobuim (Albizia) sp	

		Senna obtusifolia (L) Irwin & Barneby	
		S. occidentalis (L) Link	
		Senna spp	
Moraceae	*Dastenia* SP	*Cecropia* spp	Without claim of use
		Ficus SP	
Nictaginaceae	*Boerhavia hirsuta* Willd.	Without claim of use	Without claim of use
Rhamnaceae	*Zizuphus* SP	*Ampelozizyphus amazonicus* Ducke	Without claim of use
Rutaceae	*Ezembeckia febrifuga* A. Juss	*Citrus aurantufolia* (Christm.) Swingli	*Citrus limon* L.[2]
Verbanaceae	*Stachypheta cayennensis* (Rich)	*Lippia schomburgkiana* Schum	Without claim of use
	Vahl.	*Stachytarpheta cayennensis* (Rich) Wahl	
Meliaceae	Without claim of use	*Cedrela odorata* L	*Cedrela odorata* L[2]
Myrtaceae	Without claim of use	*Eucalyptus citriodora* Hook	*Eucalyptus* sp
		Psidium sp	
Piperaceae	Without claim of use	*Peperomia* spp	Piper sp[2]
		Pothomorphe peltata (L) Meq.	
Portulacaceae	Without claim of use	*Portulaca* SP	*Portulaca pilosa*[2]

1-Santos, et al., 2005; 2- Pinto, 2009. Source: Authors

When we correlated the results of 4 studies, we noticed that certain families were mentioned in at least 3 studies: Apocynaceae, Labiatae, Rutaceae and Rhamnaceae (Table 1). The Apocynaceae and Rutaceae families are rich in alkaloids. In the species evaluation, the most mentioned were *Aspidosperma excelsum*, *A. nitidum* and *Himatanthus articulathus* (Table 1). Based on this assumption, *A. excelsum* and *A. nitidum* were used in this study.

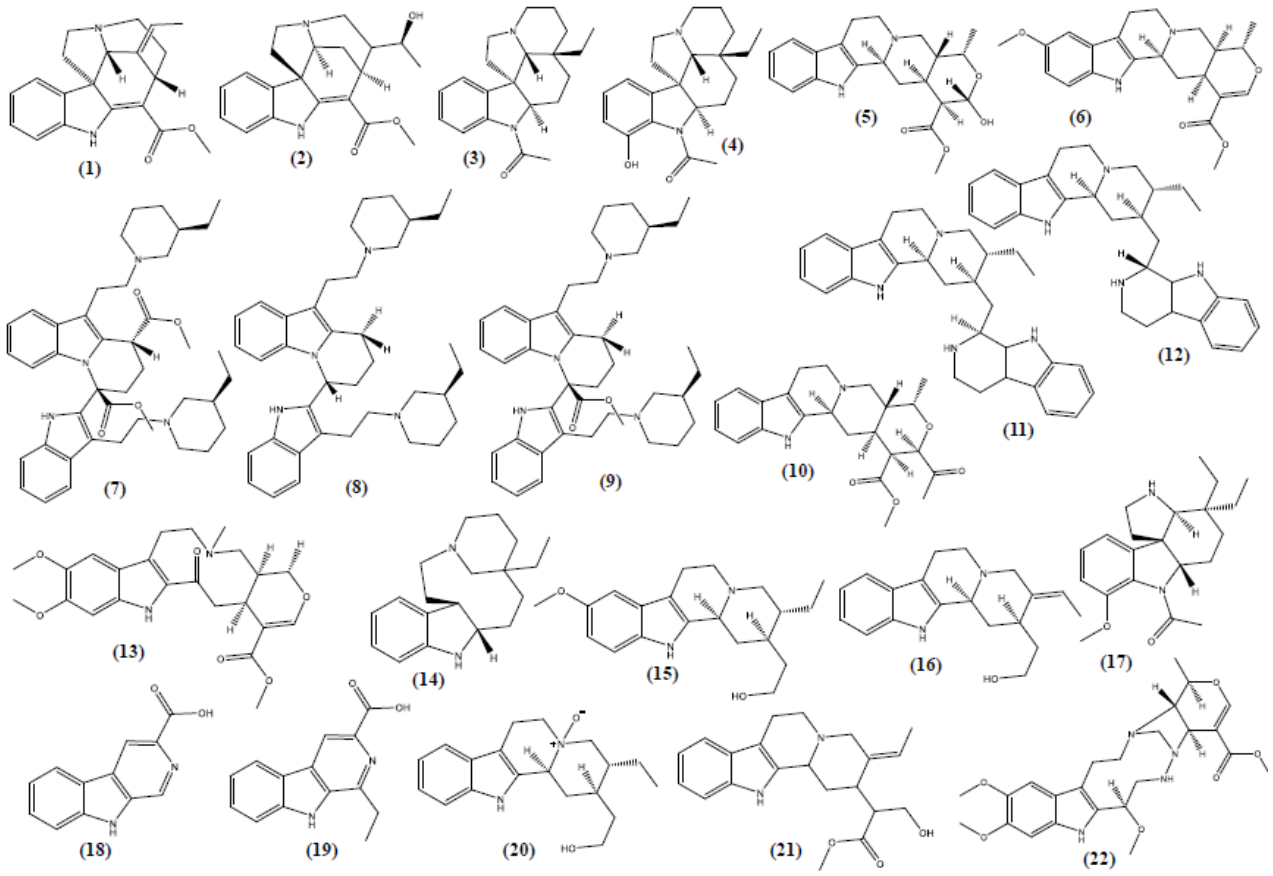
Bark extracts of *A. excelsum* were subjected to cytochemical studies, leading to the isolation of the following alkaloids: 11-methoxytubotaiwine (1), compactinervin (2), N-desmethyl aspidospermidine (3), O-desmethyl aspidospermidine (4), aricin (5), yohimbine (6), tetrahydro-secamine (7), 16-demethoxy-carboxyl-tetrahydro-secamine (8), dide-methoxy-carboxyl-tetrahydro-secamine (9), O-acetyl-yohimbine (10), R-ocrylphuanine (11), S-ocrylifuanine (12) and excelsinine (13; Figure 1; Marques, 1988).

Phytochemical studies of *A. nitidum* extract led to the isolation of 10-methoxy-dihydro-corinanteol (14), corinanteol (15; Arndt, et al., 1967), aspidospermine (16), quebrachamine (17), yohimbine (6; Marques, 1996), harman carboxylic acid (18; Pereira, 2006), 3-ethyl-harman-carboxylic acid (19; Nascimento, et al., 2009), dihydro-corinanteol (20), dehydro -siriquin (21; Nascimento, et al., 2008) and braznitidumina (22; Figure 1; Pereira, 2006).

Extracts from shells of *A. excelsum* were submitted to pytochemical studies, leading to the isolation of the following alkaloids: 11-methoxytubotaiwine (1), compactinervin (2), N-demethyl aspidospermidine (3), O-demethyl aspidospermidine (4), aricine (5), yohimbine (6), tetrahydro-secamine (7), 16-desmethoxy-carboxyl-tetrahydro-secamine (8), didesmethoxy-carboxyl-tetrahydro-secamine (9), O-acetyl-yohimbine (10), R-ocrylifuanine (11), S-ocrylifuanine (12) and excelsinin (13; Figure 1; Marques, 1988).

Phytochemical studies of the extract of *A. nitidum* led to the isolation of 10-methoxy-dihydro-corinanteol (14), corinanteol (15; Arndt, et al., 1967), aspidospermine (16), quebrachamine (17), yohimbine (6; Marques, 1996), harman carboxylic acid (18; Pereira, 2006), 3-ethyl-harman-carboxylic acid (19; Nascimento, et al., 2009), dihydro-corinanteol (20), des-hydrositsiriquine (21; Nascimento et al., 2008) and braznitidumine (22; Figura 1; Pereira, 2006).

Figure 1. Substances isolated from *A. nitidum* and *A. excelsum.*



1- 11-methoxytubotaiwine, 2- compactinervin, 3- N-demethyl aspidospermidine, 4- O-demethyl aspidospermidine, 5- aricine, 6- yohimbine, 7- tetrahydro-secamine, 8- 16-desmethoxy-carboxyl-tetrahydro-secamine, 9- didesmethoxy-carboxyl-tetrahydro-secamine, 10- O-acetyl-yohimbine, 11- R-ocrylifuanine, 12- S-ocrylifuanine, 13- excelsinin, 14- 10-methoxy-dihydro-corinanteol, 15- corinanteol, 16- aspidospermine, 17- quebrachamine, 18- harman carboxylic acid, 19- 3-ethyl-harman-carboxylic acid, 20- dihydro-corinanteol, 21- des-hydrositsiriquine and 22- braznitidumine. Source: Authors.

The hydroalcoholic extract from the shell of *A. excelsum* and their fractions were tested in a *P. falciparum* clone, the results were evaluated at different exposure times (24, 48 and 72h). The was active at all the different exposure times, with the greatest activity after 24h of exposure (IC_{50} = 5.2 µg/mL; 12 µg/mL and 16 µg/mL). The alkaloids rich fraction showed less activity than the extract (IC_{50} = 37.2 µg/mL; 11 µg/mL and 30 µg/mL). The methanolic fraction was very active in *P. falciparum* in all subjects (IC_{50} = 0.04 µg/mL; 0.014 µg/mL; 0.01 µg/mL). Chromatographic studies suggest that the major alkaloid of this fraction is yohimbine, and the authors claims this metabolite may be the responsible for antimalarial activity (Gomes, 2011).

The antimalarial activity of *A. nitidum* was assessed. The ethanol extract (IC_{50} 3.6 ± 0.37 µg/mL), fraction of alkaloids (IC_{50} 2.32 ± 0.19 µg/mL) and fraction of neutrals (IC_{50}

3.34 ± 0.31 µg/mL) proved to be active. The ethanolic extract presented as a major constituent an alkaloid with a chromophore suggestive of β-carbolinic, while the alkaloid and neutral fractions presented as major constituent an alkaloid with an aspidospermine nucleus (Martins, 2012).

The *A. excelsum* and *A. nitidum* are considered synonymous and have shown very divergent results in terms of antiplasmodic activity and major component of their ethanolic extracts. In this work, the same species were considered.

Regarding pharmacokinetic aspects, it is known that most drugs are absorbed, mainly, by the intestine due to its greater absorption surface compared with the stomach, in addition to presenting a mucous layer that lines the cells. In this predictive study, we observed that alkaloids must be absorbed, mainly, in the intestine. In alkaloids with high water solubility (Log P < 1; molecules 18 and 19), their diffusion probably occurs through cell barriers with the participation of proteins (active transport). Whereas, in alkaloids with a balance between water solubility: liposolubility (Log P > 2 < 5, molecules 1, 2, 3, 4, 5, 6, 10, 13, 14, 15, 16, 17, 20 and 21), the diffusion probably occurs due to passive transport (Table 2).

Percutaneous permeability is favored by low molecular weight, increased liposolubility and high basicity (Silva, et al., 2010; Swart, et al., 2005). The present alkaloids, generally, are weak bases and have a balance between their fat-soluble: water-soluble character (Log P> 0 and <5). Therefore, percutaneous permeability is low (Table 2).

When talking about drug interactions, pharmacokinetic interactions are the most relevant, occurring in any phase of the ADME (absorption, distribution, metabolism and excretion). Regarding distribution, the main drug interaction is competition for the albumin binding site. When using two drugs that bind strongly to albumin, an increase in free fraction percentage of one of the drugs may occur. Alkaloids, due to their basic character, are likely to bind to alpha-1 glycoproetin acid, with the majority of alkaloids being moderately to weakly bound to this plasma protein. The main chemical bonds involved in the drug-receptor interactions are ionic, electrostatic, hydrophobic and hydrogen bonds, and induced dipole. Only alkaloids 7, 8, 17, 18 and 19 are highly bound to plasma protein (Table 2). The presence of free electron pairs can induce this bond, both of carboxylic and ester groups, present in the molecules, as of nitrogen itself.

Another important drug interaction occurs when one drug alters the metabolism of a second drug. Some drugs induce the expression of cytochrome P450 (CYP) enzymes, accelerating the metabolism of other drugs which use this enzyme in their detoxification process. Other drugs prevent the metabolism of others. The program used in this study

predicts the inhibitory potential of alkaloids on different CYPs. The alkaloids 7, 8, 18 and 19 can inhibit CYP2C9, while alkaloids 7, 9 and 17 possibly inhibit CYP2C19. The alkaloids 6, 7, 8, 12, 14 and 22 probably inhibit CYP3A4 (Table 2).

Among all substances, the number 7 (tetrahydro-secamine) deserves special attention, since it can inhibit the 3 CYPs (Table 2). When we chemically evaluate the cimetidine, which also inhibits several CYP-450 enzymes, we realize it has 4 hydrogen acceptor groups, which are capable of making hydrogen bonds with protonated enzyme residues, it also has 3 donor groups of hydrogen. The tetrahydro-secamine has 6 hydrogen accepting groups and 1 donor group, which can possibly do this type of interaction. Another important fact is the presence of nitrogen in cimetidine and alkaloids, which can bind strongly to the ion of CYPs, however the confirmation of this data must be done by more accurate studies, such as docking calculations, free energy and molecular dynamics because these are dependent on molecules configuration.

The structure 17 (quebrachamine) has a low molecular weight (= 282 Da) and high liposolubility (oil-water partition coefficient = 5.22), this explains its wide biological activity in predictive studies. In chemical terms, atropine, an alkaloid used to treat various pathologies, is a tropane alkaloid with a molecular weight similar to alkaloid 17 (289 Da), however it has a lower oil-water partition coefficient (1.8) and so its effect is mainly peripheral, its refutes our hypothesis that the broad activity of the alkaloid 17 on the CNS is related to high oil-water partition coefficient (high liposolubility).

Table 2. Predictive studies of the pharmacokinetic aspects of alkaloids isolated from *A. excelsum* and *A. nitidum.*

Pharmacokinetic aspects	Alkaloids
Absorption	
- Stomach	None
- Intestine	All the alkaloids
- Percutaneous permeability	Low absorption: all of them
Distribution	
- Plasma protein binding	
High (>85%)	7, 8, 17, 18 e 19
Moderate to low	1,2,3,4,5,6,9,10,11,12,13,14,15,16,20,21 e 22
- Blood-brain barrier	
Low: <0,1	22
Moderate: 0,1-2,0	1,2,3,4,5,6,13,16,18,19
High: >2,0	7,8,9,10,11,12,14,15,17,20 e
Metabolism	
CYP2C9 (Inhibitor)	7, 8, 18 e 19
CYP2C19 (Inhibitor)	7, 9 e 17
CYP3A4 (Inhibitor)	6,7,8,12,14 e 22

1- 11-methoxytubotaiwine, 2- compactinervin, 3- N-demethyl aspidospermidine, 4- O-demethyl aspidospermidine, 5- aricine, 6- yohimbine, 7- tetrahydro-secamine, 8- 16-desmethoxy-carboxyl-tetrahydro-secamine, 9- didesmethoxy-carboxyl-tetrahydro-secamine, 10- O-acetyl-yohimbine, 11- R-ocrylifuanine, 12- S-ocrylifuanine, 13- excelsinin, 14- 10-methoxy-dihydro-corinanteol, 15- corinanteol, 16- aspidospermine, 17-quebrachamine, 18- harman carboxylic acid, 19- 3-ethyl-harman-carboxylic acid, 20- dihydro-corinanteol, 21-des-hydrositsiriquine and 22- braznitidumine. Source: Authors.

The alkaloids isolated from these species, in general, are indole alkaloids, with a molecular weight less than 300 Da (Pereira, 2007) and beta-carbolinic alkaloids with a higher molecular weight than indole alkaloids.

In silico studies suggest monoterpene indole alkaloids, except quebrachamine (17), had different biological activities in the Nervous System (Table 3).

A chemical characteristic that interferes with BBB crossing is the molecular weight. Compounds with molecular weight less than 500 Da cross this barrier better (Lipinski, 2004; Lipinski, et al., 2001).

Among the activities in CNS, it seems antidepressant effect deserves greater attention, since several alkaloids interfere with the levels of serotonin in synaptic cleft (1, 11, 12, 14, 15,

17 and 20) and/or have an antidepressant effect (15 and 17; Table 3). It appears that substances with few groups of donors (maximum 5) and acceptors (maximum 10) of hydrogen can interfere with the reception of serotonin, this characteristic is observed in the alkaloids 11, 12, 14, 15, 17, fluoxetine (donor 1; acceptor 5), sertraline (donor 1, acceptor 1) and imipramine (donor 1, acceptor 2), similar to these drugs, alkaloids are nitrogenous substances, and it seems that nitrogen participates in the mechanism of action of drugs (Romeiro, et al., 2003). However, pharmacological studies are necessary to validate these predictive results.

In the cardiovascular system, two main activities stand out: antihypertensive and cardiac tonic. The antihypertensive effect may be related to the diuretic effect (3, 16 and 19) and vasodilation (5, 6, 10 and 13; Table 3). Diuretics which act in the loop of Henle (furosemide) and in the distal tubule (hydrochlorothiazide) interfere in the process of reabsorption of electrolytes, especially sodium. These drugs have high polarity (Log p < 2), similar fact was observed in alkaloids with diuretic activity (16 and 19; Table 3).

The Study demonstrated yohimbine activity on erectile dysfunction. In general, drugs used in erectile dysfunction have vasodilating properties. The alkaloids 5, 6, 10 and 13 have moderate water solubility (Log P approximately 2), this characteristic is observed in sildenafil (log P = 1.5), nifedipine (Log P = 2.2) and verapamil (Log P = 3.9). Furthermore, it is already known yohimbine acts directly in blocking α-1-adrenergic receptors, decreasing the synthesis of IP3 and releasing Ca^{2+} causing muscle relaxation (Hodges & Sparks, 2013). Studies also suggest that yohimbine acts in the muscle relaxation of human cavernous tissue by non-adrenergic pathway, probably activating the nitrergic soluble guanylate cyclase (NO-SGC) via and KATP (Freitas, et al., 2009).

The vinblastine and vincristine are alkaloids that contain 2 indole nuclei and are used as antineoplastics. These alkaloids inhibit the polymerization of tubillins, preventing the formation of mitotic spindle. When comparing some chemical aspects of these drugs to the alkaloids of this study (only 1 indole group) we observe that molecular weight, solubility coefficient, among others are different. Therefore, more specific studies such as doking, molecular dynamics and free energy studies are needed to elucidate the mechanism of action of these alkaloids.

An interesting fact called the attention: few molecules have antiparasitic/antileishmania activity. The structures do not showed antimalarial activity in predictive studies, however several *in vitro* studies using *P. falciparum* (Dolabela, et al., 2015; Chierrito, et al., 2014; Oliveira, et al., 2009; Frederich, et al., 2008; Mitaine-Offer, et al., 2002)

and *in vivo* with *P. berghei* (Coutinho, et al., 2013; Bero, et al., 2009) has validated this popular use. These *in silico* studies are predictive and can be used in the screening of substances, however, the performance of an antimalarial evaluation study is essential to validate the use of plants and their derivatives.

Table 3. *In silico* studies to predict biological activities of alkaloids isolated from *A. excelsum* and *A. nitidum.*

Biological Activities	Alkaloids
Autonomic and Central Nervous System	
General anesthetic	5 e 6
Non-selective adrenergic agonist (α2 e β2)	17 e 18
Non-selective adrenergic agonist (α5 e 6; β4 a 5)	17 e 18
Opioid receptor antagonist (κ)	4
β-adrenergic receptor antagonist	6, 15 e 21
Antidepressant	15 e 17
Antiparkinsonian	2
Antipsychotic	7, 8, 9 e 17
Neuromuscular block	3, 4, 13, 16 e 17
Cognitive disorders	4 e 19
Spasmolytic	5, 14, 15 e 17
5-HT release	1, 11, 12, 14, 15, 17 e 20
Renal Cardiovascular System	
Antihypertensive	1
ATPase Transport Inhibitor - Diuretic	3, 16 e 19
Vasodilator	5, 6, 10 e 13
Antidiuretic	5 e 6
Inotropic	17
Cardiovascular tonic	3 e 17
Chemotherapy	
Antineoplasic	3, 4, 13, 16 e 22
Antineoplastic: non-Hodgking lymphoma	3
Apoptosis (induction)	2 e 12
Antiprotozoal	9

Leishmanicide	6 e 10
Hepatitis B	20
Diverse	
Analgesic	2, 14 e 15
Anti-inflammatory	7
Angiogenesis (induction)	6, 15 e 21
Anti-eczantema	7, 8, 9 e 19
Anti-hypoxia	15 e 17
Tonic	3 e 16
Respiratory Tonic	3 e 15

Obs.: It was active when the predictive effect (pa) was greater than 70%.
1- 11-methoxytubotaiwine, 2- compactinervin, 3- N-demethyl aspidospermidine, 4- O-demethyl aspidospermidine, 5- aricine, 6- yohimbine, 7- tetrahydro-secamine, 8- 16-desmethoxy-carboxyl-tetrahydro-secamine, 9- didesmethoxy-carboxyl-tetrahydro-secamine, 10- O-acetyl-yohimbine, 11- R-ocrylifuanine, 12- S-ocrylifuanine, 13- excelsinin, 14- 10-methoxy-dihydro-corinanteol, 15-corinanteol, 16- aspidospermine, 17- quebrachamine, 18- harman carboxylic acid, 19- 3-ethyl-harman-carboxylic acid, 20- dihydro-corinanteol, 21- des-hydrositsiriquine and 22- braznitidumine. Source: Authors.

‘In addition to *in silico* studies for pharmacokinetic aspects and prediction of biological activities, predictive studies are capable to predict possible adverse reactions (Pass online), mutagenicity and genotoxicity (Preadmet, 2020; Yamashida, et al., 2000). The alkaloids 7, 8, 9 and 17 have possibly activity on euphoria and as antipsychotic. When we compare these molecules with antipsychotic drugs, we perceive the essential presence of nitrogen in the chain as it increases the number of hydrogen acceptors, also the low molecular weight seems to be involved in this process (Table 4).

The antipsychotic effect has been related to the blockade of dopamine receptors in the nigrostriatal and mesolimbic region. However, many antipsychotics have low specificity, binding to different sites (Gorenstein & Scavone, 1999), it is responsible for several adverse drug reactions (ADR). An ADR that can occur is cardiac arrhythmia, alkaloids 7, 8 and 9, probably, can cause this ADR (Table 4).

Another possible ADR is hypotension, in the case of alkaloids 5, 6 and 10, they probably have a vasodilator effect. While alkaloids 3 and 16, in predictive studies, had a diuretic effect. The alkaloids 6, 15, 21 can be blockers of beta-adrenergic receptors (Tables 3 and 4). Therefore, this possible ADR may be related to the biological activity.

It is known that alkaloids from vinca such as vinblastine and vincristine are neurotoxic, and they are indole alkaloids from Apocynaceae. In this study alkaloids 6, 15 and 20, also terpene indole alkaloids, presented in the *in silico* study the possibility of neurotoxicity (Table 4), however, as in vinca alkaloids, this effect can be dose-dependent and reversible (Chabner, et al., 2012).

Many alkaloids, in the mutagenicity prediction, showed positive results in the Ames test (1, 2, 8, 9, 10, 11, 12, 14, 15,17, 18, 19, 20 and 22). In alkaloid 9, the prediction results suggest that it is mutagenic and carcinogenic to rats and mice. The alkaloids 11, 12 and 15 probably have a mutagenic and carcinogenic effect on mice. Whereas, the alkaloids 14, 17, 20 and 22 may cause mutagenic and carcinogenic effects for rats (Table 4).

Table 4. Prediction of adverse reactions and toxicological events of alkaloids isolated from *A. excelsum* and *A. nitidum.*

Adverse reaction	Alkaloids
Gastrointestinal system	
Emetic	11 e 12
Gastrointestinal bleeding	18, 19 e 20
Peptic ulcer	5
Splenomegaly	18
Central Nervous System	
Sedation	5 e 10
Tremor	1 e 8
Ataxia	15
Euphoria	4, 7, 8, 9 e 17
Neurotoxicity	6, 15 e 20
Optic neuropathy	3, 6, 10 e 16
Coma	3
Metabolic changes	
Metabolic acidosis	18
Hyperglycemia	18
Hyperthermia	6
Hyperuricemia	18
Renal cardiovascular system	

Heart block	14
Hypotension	2,3,5,6,7,10, 14, 15, 16, 21 e 22
Myocarditis	4,7,9 e 17
Tachycardia	17
QT prolonged interval	7,8 e 9
Thrombophlebitis	2 e 16
Urinary retention	3
Respiratory problems	
Apnea	1 e 8
Dyspnea	5
Bronchoconstriction	2, 3 e 6
Respiratory impairment	16
Respiratory failure	4
Ophthalmic problems	
Glaucoma	10 e 15
Mydriasis	11, 12 e 21
Toxicological events	**Alkaloids**
Positive Ames Test	1, 2, 3, 5, 8, 9, 11, 12, 14, 15, 17, 18, 19, 20 e 22
Carcinogenic in rats	9, 14, 17, 20 e 22
Carcinogenic in mice	9, 11, 12, 15 e 21

1- 11-methoxytubotaiwine, 2- compactinervin, 3- N-demethyl aspidospermidine, 4- O-demethyl aspidospermidine, 5- aricine, 6- yohimbine, 7- tetrahydro-secamine, 8- 16-desmethoxy-carboxyl-tetrahydro-secamine, 9- didesmethoxy-carboxyl-tetrahydro-secamine, 10- O-acetyl-yohimbine, 11- R-ocrylifuanine, 12- S-ocrylifuanine, 13- excelsinin, 14- 10-methoxy-dihydro-corinanteol, 15-corinanteol, 16- aspidospermine, 17- quebrachamine, 18- harman carboxylic acid, 19- 3-ethyl-harman-carboxylic acid, 20- dihydro-corinanteol, 21- des-hydrositsiriquine and 22- braznitidumine. Source: Authors.

In general, alkaloids show adverse reactions already described for other substances in the class. We emphasize that these studies are predictive and can assist in the establishment of experimental protocols. In this context, genotoxicity and carcinogenicity tests are suggested.

The nitro compounds are one of the most important groups among the various mutagenic substances that have reactive groups, because during metabolism they can be protonated. These can react with O_2, generating intracellular oxidative stress, causing DNA damage (Chadfield & Hinton, 2004). However, the mechanisms involved in alkaloid mutagenicity and carcinogenicity need to be further studied.

4. Conclusion

The species belonging to the genus Aspidosperma have been widely submitted to assays for evaluating their antimalarial activity *in vitro* (Dolabela, et al., 2015; Chierito, et al., 2014; Meneguetti, et al., 2014; Paula, et al., 2014; Coutinho, et al., 2013; Dolabela, et al., 2012; Andrade-Neto, et al., 2007) and *in vivo* (Chierito, et al., 2014; Coutinho, et al., 2013; Silva, et al., 2012). Most studies evaluated extracts and their fractions, and a few isolated alkaloids. The results were promising in most cases and the fractionation apparently contributes to antimalarial activity. However, this fractionation also can contribute to cytotoxicity, genotoxicity, carcinogenicity and mutagenicity.

It is important to remember that more than 200 alkaloids have been isolated from Aspidosperma, most of them have not been evaluated yet for antimalarial activity. Therefore, thinking about a strategy to select the alkaloids to be prioritized in this study is very important.

Phytochemical studies of these species are not always easy, since, in most cases, the content of alkaloids is very low in the plant. This requires a large amount of the plants bark. Another difficulty is the diversity of alkaloids in each species, and isomers are often detected what makes it difficult to isolate these metabolites.

A very valuable strategy is the *in silico* study to predict activity, possible adverse reactions and toxic effects (cytotoxicity, carcinogenicity, genotoxicity and mutagenicity).

In order to develop a study strategy for these alkaloids, the following methods were used: ADMEtox and PASS *online*. Although *in vivo* and *in vitro* studies demonstrate the antimalaria activity of some indole and beta-carboline alkaloids, the antimalaria activity was not detected by these methods. This occurs due to these programs cross chemical structures, evaluating the chemical similarity, without considering their stereochemistry, perhaps the programs do not have in their database the alkaloids described with this antimalaria activity. Furthermore, this work presents a series of biological activities, in various systems, to which these alkaloids can be subjected to *in vitro* and *in vivo* tests with greater chances of positive results.

New *in silico* studies of these alkaloids are important and the steps could be: 1st - survey of the species belonging to this genus; 2nd research of phytochemical studies for each species: identification of alkaloids; 3rd realization of *in silico* studies for more robust calculations to validate biological activity; 4th relate the data obtained to the literature (biological activity, pharmacodynamic, pharmacokinetic and toxicological aspects); 5th

selection of the most promising species; 6th obtainment of plant material, phytochemical studies, evaluation of activity and toxicity.

This better planning could provide reduced research cost, reduced time to obtain a promising substance and greater chance of success. The authors did not find studies of this kind with this experimental design.

Therefore, we suggest in vitro and in vivo studies of the species for diseases of the central nervous system and other targets mentioned in this work, as we believe that this plant, rich in alkaloids, is extremely promising.

Acknowledgments

The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES (Brasília, Brazil) and the National Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq for the creation of a scholarship to carry out this work.

Referências

Ajay, A., Bermis, G. W., & Murkco, M. A. (1999). Designing libraries with CNS activity. *J Med Chem.*, 42 (24), 4942-4951. doi:10.1021/jm990017w.

Ames, B. N., Mccann, J., & Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/Mammalian-microsome mutagenicity test. *Mutat. Res*., 31 (6), 347-364. doi:10.1016/0165-1161(75)90046-1.

Andrade-Neto, V. F., Pohlit, A. M., Pinto, A. C., Silva, E. C., Nogueira, K. L., Melo, M. R. S., Henrique, M. C., Amorim, R. C. N., Silva, L. F., Costa, M. R. F., Nunomura, R. C. S., Nunomura, S. M., Alecrim, W. D., Alecrin, M. G., Chaves, F. C. M. & Vieira, P. P. R. (2007). *In vitro* inhibition of Plasmodium falciparum by substances isolated fromamazonian anmalarial plants. *Mem. Inst. Oswaldo Cruz.*, 102 (3), 359-365. doi:10.1590/S0074-02762007000300016.

Arndt, R. R., Brown, S. H., Ling, N. C., Roller, P., Carl, D., Ferrhra, J. M., Gilbert, F. B., Mimnda, E. C., & Flore, S. E. (1967). Alkaloid studies-LVIII. The alkaloids of six aspidosperma species. *Phytochemistry*., 6, 1653-1658. doi:10.1016/S0031-9422(00)82898-8.

Bero, J., Fréderich, M., & Quetin-Leclercq, J. (2009). Antimalarial compounds Isolated from plants used in traditional medicine. *J. Pharm. Pharmacol*., 61, 1401-1433. doi: 10.1211/jpp/61.11.0001.

Brandão, M. G. L., Carvalho, L. H., & Krettli, A. U. (1992). Antimaláricos de uso popular na Amazônia. *Ciênc. Hoje*., 13(78), 9-11.

Calixto, J. B. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phototherapeutic agents). *Braz. J. Med. Biol. Res.*, 33 (2), 179-189. doi:10.1590/S0100-879X2000000200004.

Cardoso, C. L., Silva, D. H. L, Yung, C. M., Castro-Gamboa, I., & Bolzani, V. S. (2008). Indole monoterpene alkaloids from *Chimarrhis turbinata* DC Prodr: a contribuition to the chemotaxonomic studies of the Rubiaceae family. *Rev. Bras. Farmacogn.*, 18 (1), 26-28. doi:10.1590/S0102-695X2008000100007.

Chadfield, M. S., & Hinton, M. H. (2004). *In vitro* activity of nitrofuran derivatives on growth and morphology of *Salmonella entérica* serotype Enteritidis. *J. App. Microbiol.*, 96, 1002–1012. doi:10.1111/j.1365-2672.2004.02225.x.

Chabner, B. A., Bertino, J., Cleary, J., Ortiz, T., Lane, A., Supko, J. G., & Ryan, D. (2012). Agentes citotóxicos. In*:* Brunton, L. L., Chabner, B. A., & Knollmann, B. C. *As bases faramcológicas da Terapêutica de Goodman e Gilman* (pp. 1677-1729). Porto Alegre, Brasil: AMGH.

Chemicalize. (2020). *Calculate properties instantly, search chemical data, and draw molecules online.* Retrieved july 6, 2020, from https://chemicalize.com/.

Chierrito, T. P. C., Aguiar, A. C. C., Andrade, I. M., Ceravolo, I. P., Gonçalves, R. A. C., Oliveira, A. J. B., & Krettli, A. U. (2014). Anti-malarial activity of indole alkaloids isolated from *Aspidosperma olivaceum*. *Malar. J.,* 13, 142. doi:10.1186/1475-2875-13-142.

Coelho, S. R. (1989). Levantamento de plantas medicinais em comunidades de Rio Novo do Sul, Iconha, Itapemirim e Cachoeiro de Itapemirim. *I Encontro sobre plantas medicinais do Espírito Santo*, 1. Rio Novo do Sul, Brasil, 13-27. Retrieved from https://biblioteca.incaper.es.gov.br/digital/bitstream/123456789/3507/1/plantasmedicinaisderionovodosul-rozeli.pdf.

Costa, C. R., Olivi, P., Botta, C. M. R., & Espindola, E. L. G. (2008). A toxicidade em ambientes aquáticos: discussão e métodos de avaliação. *Quim Nova*., 3 (7), 1820-1830. doi:10.21577/0100-4042.20170558.

Coutinho, J. P., Aguiar, A. C., Santos, P. A., Lima, J. C., Rocha, M. G. L, Zani, C. L., Alves, T. M. A., Santana, A. E. G., Pereira, M. M., & Kretli, A. U. (2013). *Aspidosperma* (Apocynaceae) plant cytotoxicity and activity towards malaria parasites. Part I: *Aspidosperma nitidum* (Benth) used as a remedy to treat fever and malaria in the Amazon. *Mem. Inst. Oswaldo Cruz.,* 108 (8), 974-982. doi:10.1590/0074-0276130246.

Dolabela, M. F., Póvoa, M. M., Brandão, G. C., Rocha, F. D., Soares, L. F., Paula, R. C., & Oliveira, A. B. (2015). Aspidosperma species as sources of anti-malarials: uleine is the major anti-malarial indole alkaloid from *Aspidosperma parvifolium* (Apocynaceae). *Malar J.*, 14, 498. doi:10.1186/s12936-015-0997-4.

Eisenberg, D. M., Davis, R. B., Ettner, S. L., & Appel, S. (1998). Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. *JAMA.*, 280 (18), 1569-1575. doi:10.1001/jama.280.18.1569.

Filimonov, D. A., Lagunin, A. A., Gloriozova, T. A., Rudik, A. V., Druzhilovskii, D. S., Pogodin, P. V., & Poroikov, V. V. (2014). Prediction of the biological activity spectra of organic compounds using the PASS online web resource. *Chemistry of Heterocyclic Compounds*, 50 (3), 444-457. doi:10.1007/s10593-014-1496-1.

Frederich, M., Tits, M., & Angenot, L. (2008). Potential antimalarial activity of indole alkaloids. *Trans R Soc Trop Med Hyg,*. 102 (1), 11-19. doi:10.1016/j.trstmh.2007.10.002.

Freitas, F. C., Nascimento, N. R. F., Cerqueira, J. B. G., Morais, M. E. A., Pegadas, R. P., & Gonzaga-Silva, L.F. (2009). Yohimbine relaxes the human corpus cavernosum through a non-adrenergic mechanism involving the activation of K^+ ATP-dependent channels. *Int. J. Impot. Res.,* 21 (6), 356-361. doi:10.1038/ijir.2009.41.

Guilhermino, L., Diamantino, T., Silva, M. C., & Soares, A. M. V. M. (2000). Acute toxicity test with Daphnia magna: Na alternative to mammals in the Prescreening of Chemical Toxicity?. *Ecotoxicol Environ Saf*., 46 (3), 57-362. doi:10.1006/eesa.2000.1916.

Gomes, L. F. S. (2011). *Abordagem fitoquímica, determinação da atividade antiplasmódica in vitro e avaliação da toxicidade preliminar da toxicidade do extrato hidroetanólico das cascas A. excelsum* Benth (Apocynaceae). Dissertação (Mestrado), Belém: PPGCF-ICS-UFPA. Retrieved from http://repositorio.ufpa.br/jspui/handle/2011/5622.

Gorenstein, C., Scavone, C. (1999). Avanços em psicofarmacologia - mecanismos de ação de psicofármacos hoje. *Rev Bras Psiquiatr.,* 21 (1), 64-73. Retrieved from https://www.scielo.br/scielo.php?pid=S151644461999000100012&script=sci_abstract&tlng=pt.

Hodges, G. J., & Sparks, P. A. (2013). Contributions of endothelial nitric oxide synthase, noradrenaline, and neuropeptide Y to local warming-induced cutaneous vasodilatation in men. *Microvasc. res.*, 90, 128-134. doi:10.1016/j.mvr.2013.08.011.

Lipinski, C. A. (2004). Lead- and drug-like compounds: the rule of five revolution. *Drug Discovery today: Technologies.*, 1 (4), 337-341. doi:10.1016/j.ddtec.2004.11.007.

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Fenney, P. J. (2001). Experimental and computational approache to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev*., 46 (1-3), 3-26. doi:10.1016/S0169-409X(96)00423-1.

Marques, M. F. S., Kato, L., Leitao-Filho, H. F., & Reis, F. A. M. (1996). Indole alkaloids from *Aspidosperma ramiflorum*. *Phytochemistry.* 41 (3), 963-967. doi:10.1016/0031-9422(95)00660-5.

Martins, E. R., Castro, D. M., Castellani, D. C. & Dias, J. E. (2000). *Plantas medicinais*. Viçosa, Brasil: Editora UFV.

Martins, M. T. (2012). *Estudo farmacognóstico, fitoquímico e atividades biológicas* de *Aspidosperma nitidum* Benth. Ex Mull. Arg. Dissertação (Mestrado), Belém: PPGCF- ICS-UFPA.

Mcule. (2020). Property calculator. Retrieved from https://mcule.com/apps/property-calculator/.

Meneguetti, D. U. O., Cunha, R. M., Lima, R. A., Oliviera, F. A. S., Medeiros, D. S. S., Passarini, G. M., Medeiros, P. S. M., Militão, J. S. L. T., & Facundo, V. A. (2014). Antimalarial ethnopharmacology in the Brazilian Amazon. *Rev. Ciênc. Farm. Básica Apl.*, 35 (4), 577-587. Retrieved from https://rcfba.fcfar.unesp.br/index.php/ojs/article/view/86.

Milliken, W. (1997) Traditional anti-malarial medicine in Roraima, Brazil. *Econ Bot.*, 51 (3), 212-237. Retrieved from https://link.springer.com/article/10.1007/BF02862091.

Mitaine-Offer, A. C., Sauvain, M., Valentin, A., Callapa, J., Mallie, M., & Zeches-Hanrot, M. (2002). Antiplasmodial activity of *Aspidosperma* indole alkaloids. *Phytomedicine.*, 9 (2), 142-145. doi:10.1078/0944-7113-00094.

Nascimento, P. C., Araújo, R. M., & Silveira, E. R. (2009). Aplicação da CLAE na análise fitoquímica de *Aspidosperma nitidum*. Fortaleza: 32° Reunião anual da sociedade brasileira de química. Retrieved from http://www.sbq.org.br/32ra/.

Nascimento, P. C., & Silveira, E.R. (2008). Alcalóides indólicos de *Aspidosperma nitidum.* Aguas de Lindoia: *Reunião anual da sociedade brasileira de química.* Retrieved from http://www.sbq.org.br/31ra/.

Oliveira, A. B., Dolabela, M. F., Braga, F.C., Jácome, R. L. R. P., Varotti, F., & Póvoa, M. M. (2009). Plant-derived antimalarial agents: new leads and efficient phythomedicines. Part I. Alkaloids. *An. Acad. Bras. Ciênc.*, 81 (4), 715-740. Doi:10.1590/S0001-37652009000400011.

Pereira, A. S., Shitsuka, D. M., Pereira, F. J., & Shitsuka, R. (2018). *Metodologia da pesquisa científica.* Santa Maria: UFSM, NTE. Retrieved from https://repositorio.ufsm.br/bitstream/handle/1/15824/Lic_Computacao_Metodologia-Pesquisa-Cientifica.pdf.

Pereira, M. M., Alcântara, A. F. C., Piló-Veloso, D., & Raslan, D. S. (2006). NMR structural analysis of Braznitidumine: A new Índole Alkaloid with 1,2,9- Triazabicyclo[7.2.1]System, Isolated from *Aspidosperma nitidum* (Apocynaceae). *J. Braz. Chem. Soc.*, 17 (7), 1274-1280. doi:10.1590/S0103-50532006000700012.

Paula, R. C., Dolabela, M. F., & Oliveira, A. B. (2014). *Aspidosperma* species as sources of antimalarials. Part III. Review of traditional use and antimalarial activity. *Planta Med.*, 80 (5), 378-386. doi:10.1055/s-0034-1368168.

Pereira, M. M., Jácome, R. L. R. P., Alcantara, A. F. C., Alves, R. B., & Raslan, D. S. (2007). Alcalóides indólicos isolados de espécies do gênero Aspidosperma (Apocynaceae). *Quim. Nova.*, 30 (4), 970-983. doi:10.1590/S0100-40422007000400037.

Pinto, L. N., & Barbosa, W. L. R. (2009). Etnofarmácia no municipio de Igarapé Miri-Pa. In: BARBOSA WLR (org.), *Etnofarmácia: fitoterapia popular e ciência farmacêutica* (pp 49-139). Belém, Brasil: NUMA/UFPA.

Preadmet. (2020). ADME TOX calculation. Retrieved july 6, 2020, from http://preadmet.bmdrc.kr.

Romeiro, L. A. S., Fraga, C. A. M., & Barreiro E. J. (2003). Novas estratégias para o tratamento da depressão: uma visão da química medicinal. *Quim. nova.*, 26 (3), 347-358. doi:10.1590/S0100-40422003000300012.

Santos, M. A. S., Kahwage, C. C., Coelho-Ferreira, M. R., & Sampaio, N. A. (2005). Medicinas Tradicionais no Vale do Rio Negro (Amazonas, Brasil). Observações ale do Rio Negro (Amazonas, Brasil). Observações sobre Etnofarmacologia e o Uso da Planta Saracura-Mirá (*Ampelozizyphus amazonicus*): Atividade Farmacológica e/ou Eficácia Simbólica. *Bol. Mus. Para. Emílio Goeldi, sér. Ciências Humanas*., 1 (1), 137-147. Retrieved from https://repositorio.museugoeldi.br/bitstream/mgoeldi/507/1/B%20MPEG%20C%20Hum%201%281%29%202005%20SANTOS.pdf.

Silva, J. A., Apolinário, A. C., Souza, M. S. R., Damasceno, B. P. G. L., & Medeiros, A. C. D. (2010). Administração cutânea de fármacos: desafios e estratégias para o desenvolvimento de formulações transdérmicas. *Rev Ciênc Farm Básica Apl.*, 31 (3), 125-131. Retrieved from file:///C:/Users/Valdicley/Downloads/357-Article%20Text-1081-1-10-20190919%20(1).pdf.

Silva, L. F. R., Montoia, A., Amorim, R. C., Melo, M. R., Henrique, M.C., Nunomura, S. M., Costa, M. R., Andrade-Neto, V. F., Costa, D. S., Dantas, G., Lavrado, J., Moreira, R., Paulo, A., Pinto, A. C., Tadei, W. P., Zacardi, R. S., Eberlin, M. N., & Pohlit, A. M. (2012). Comparative *in vitro* and *in vivo* antimalarial activity of the índole alkaloids ellipticine, olivacine, cryptolepine and a synthetic cryptolepine analog. *Phytomedicine.*, 20, 71-76. doi:10.1016/j.phymed.2012.09.008.

Sousa, G. S. (1971). *Tratado descritivo do Brasil em 1587*. São Paulo, Brasil: Companhia Ed. Nacional e Ed. da USP.

Swart, H., Breytenbach, J. C., Hadgraft, J., & Plessis, J. (2005). Synthesis and transdermal penetration of NSAID glycoside esters. *Int. J. pharm.*, 301, 71-79. doi:10.1016/j.ijpharm.2005.05.030.

Veiga-Jr., V. F., Pinto, A. C., & Maciel, M. A. M. (2005). Plantas medicinais: cura segura?. *Quim. Nova*., 28 (3), 519-528. doi:10.1590/S0100-40422005000300026.

Waterman, P. G. (1975). New combinations in *Zanthoxylum* L. *Taxon.*, 24, 361-366. doi:10.2307/1218347.

Yamashita, S., Furubayashi, T., Kataoka, M., Sakane, T., Sezaki, H., & Tokuda, H. (2000). Optimized conditions for prediction of intestinal drug permeability using Caco-2 cells. *Eur. J. Pharm.*, 10, 195-204. doi:10.1016/s0928-0987(00)00076-2

Yazdanian, M., Glynn, S. L., Wright, J. L., & Hawi, A. (1998). Correlating partitioning and Caco-2 cell permeability of structurally diverse small molecular weight compounds. *Pharm Res.*, 15 (9), 1490-1494. Retrieved from https://link.springer.com/article/10.1023/A:1011930411574.

Zucker, E. (1985). *Hazard Evaluation Division Standard Evaluation Procedure: Acute toxicity test for freshwater Fish*. Washington, USA: USEPA. Retrieved from https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB86129277.xhtml.

Percentage of contribution of each author in the manuscript

Juliana Correa-Barbosa – 20%

Milena Cristina Martins da Silva – 20%

Sandro Percário – 10%

Davi do Socorro Barros Brasil – 10%

Maria Fâni Dolabela – 20%

Valdicley Vieira Vale – 20%