

## Evaluation of antimicrobial activity of three *Aspidosperma* species

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### Abstract

Several species of the genus *Aspidosperma* are used in folk medicine as a potential agent against malaria, leishmaniasis, antimicrobial and inflammatory process. For this study we selected the species *Aspidosperma tomentosum*, *A. macrocarpum* and *A. pyriformium* commonly known as “peroba”. The aim of this study was to evaluate the antimicrobial activity of extracts and fractions of these species against strains of Gram positive and Gram negative bacteria and yeasts. We used ethanol extracts of root bark, stem bark, stem and root of *A. tomentosum*, *A. macrocarpum* were used ethanolic extracts of the twigs and stems, leaves, stem bark and stem, and the fractions obtained from crude extract of the stem. For the species *A. pyriformium* were used crude ethanol extract of the fruit, flower, root, root bark, bark of the wood and timber, and the fractions of crude ethanol extract of the wood. The antimicrobial activity was observed on strains of Gram positive (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6623), Gram negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 15442) and *Candida* species (*Candida albicans* ATCC 10231, *C. parapsilosis* ATCC 22019 and *C. tropicalis*), by broth microdilution test for determining the minimum inhibitory concentration (MIC) and minimal bactericidal and fungicidal concentration (MBC/MFC). The extracts and fractions of *A. macrocarpum* showed no activity against the tested bacteria (MIC > 1000 µg/mL) and ethyl acetate fraction (MIC 250 µg/mL) and the alkaloidal fraction (MIC 500 µg/mL) of the stem showed weak activity for *C. parapsilosis*. The crude extract of root bark of *A. tomentosum* showed a weak antibacterial activity in *S. aureus* and *B. subtilis* (MIC 1000 and 500 µg/mL, respectively) and showed no activity against yeasts (MIC > 1000 µg/mL). For the species *A. pyriformium* only the alkaloidal fraction of the wood showed a moderate activity with MIC of 125 and 250 µg/mL in *S. aureus* and *B. subtilis*, and the MBC was 1000 µg/mL for both bacteria. This fraction also showed a weak activity against *C. parapsilosis* and *C. tropicalis* (MIC 500 µg/mL). Although there are ethnopharmacological reports about the popular use of some species of *Aspidosperma* as antimicrobial, as this study found no significant activity for this action in the extracts and fractions of *A. tomentosum*, *A. macrocarpum* and *A. pyriformium* against the tested microorganisms. Tests for verification of the action potential of these extracts on protozoa are being conducted.

Key words: *Aspidosperma tomentosum*, *Aspidosperma macrocarpum*, *Aspidosperma pyriformium*, antimicrobial activity

## Introduction

The plant kingdom is responsible for the biggest part of chemical diversity known and reported in the literature (1).

It is estimated that 25% of modern medicine comes directly or indirectly from plants, and one third of the most prescribed and sold medicine in the world were developed from natural products (2, 3).

The species of the genus *Aspidosperma* belong to the family *Apocynaceae* and are restricted of Americas, they are found between Mexico and Argentina. Besides the good wood provided by the trees of *Aspidosperma* species, the barks are used as infusion by folk medicine from Amazonia (4, 5).

For this study we selected the species *Aspidosperma tomentosum* Mart., *A. macrocarpum* Mart., and *A. pyriforme* Mart., species commonly known as “peroba” in the most of Brazilian regions and as “carapanaúba” in Amazon region (6). Other denominations are used by folk medicine such as “peroba-do-campo” for species of *A. tomentosum* Mart., “pau-pereira” or “guatambu” for *A. macrocarpum* Mart. and “pereiro” or “pereiro-vermelho” for *A. pyriforme* Mart. species, in the “caatinga” region this species may reach 8 meters high (7).

When chemical constitution of *Apocynaceae* family is concerned one important characteristic is that the species of *Aspidosperma* present as indolic alkaloids chemotaxonomy markers, mainly the monoterpene considered as a group of molecules with great medicative potential (8, 9, 10).

Several species of genus *Aspidosperma* are used in folk medicine as a potential agent against malaria, leishmaniasis, antimicrobial, antiinflammatory process (uterus and ovary), rheumatism, against cancer, stomach diseases, diabetes, cholesterol, hypertension and erectile dysfunction (10, 11, 12, 13, 14).

Based on these ethnopharmacology information some studies about biological action of *Aspidosperma* species were reported, as an exam-

ple, antibacterial action of *A. ramiflorum* extract which demonstrated a good activity on *Bacillus subtilis* and *Staphylococcus aureus* and of *A. pyriforme* and *A. olivaceum* extracts which present moderate action on *B. subtilis* (15).

The purpose of this study was to evaluate the antimicrobial activity of extracts and fractions of species *A. tomentosum* Mart., *A. macrocarpum* Mart., and *A. pyriforme* Mart against strains of Gram positive and Gram negative bacteria and yeasts.

## Methods

### Plants collection

The root and stem barks of *A. tomentosum* species were collected in May 2004; twigs, stems, stem bark and leaves of *A. macrocarpum* were collected in November 2007, both in Planaltina city - GO - Brazil. The fruit, flower, root, root bark of the wood and bark of the plant species *A. pyriforme* were collected in October 2001 in São José da Tapera - AL - Brazil. The species were identified by the botanist Dr. J. E. de Paula, the University of Brasilia (UnB), where a voucher specimen of each specimen is deposited. Voucher specimen: *A. tomentosum* n. JEP 3732 (UnB); *A. macrocarpum*: n. JEP3767 (UnB); *A. pyriforme* - n. JEP 3686 (UnB).

### Plant extract and fractionation

The material collected from three species of *Aspidosperma* (*A. tomentosum*, *A. macrocarpum* and *A. pyriforme*) was dried in a circulating air hothouse at medium temperature of 45 °C, for 72 h and grinded in knife mill Tecnal Marconi mod. TE 048, and stored in a dark and dry place to be used in the extract preparation.

### *Aspidosperma tomentosum* extraction

The powder of stem barks (3.6 kg), root barks (2.4 kg), stem (3.0 kg) and root (2.0 kg) of *A. tomentosum* Mart., was submitted to extraction in perco-

lator with 95% ethanol at environmental temperature (27 °C), in three cycles of 72 h each, the ethanolic solution was concentrated under reduced pressure at 40 °C in rota-evaporator unit to remove the solvent, providing respectively the crude ethanolic extract of *A. tomentosum*: root bark (AT1 – 230.60 g), stem bark (AT2 – 316.50 g), stem (AT3 – 320.40 g), and root (AT4 – 250.80 g). In this study for *A. tomentosum* species only crude extract was tested.

#### *Aspidosperma macrocarpum* extraction

Percolation with 95% ethanol was done, in three cycles of 72 h each, with powder of stem barks (4.0 kg), leaves (1.6 kg), stems (3.5 kg) and branches (1.2 kg) of *A. macrocarpum*, the procedure was conducted at environmental temperature (27 °C). After this, the ethanolic solution was concentrated under reduced pressure at 40 °C in rota-evaporator unit, providing the crude ethanolic extract of *A. macrocarpum*: of branches and stems (AM1 – 187.10 g), leaves (AM2 – 436.00 g), stem barks (AM3 – 507.00 g), and stem (AM4 – 560.00 g).

From the ethanolic crude extract of stem (AM4) of *A. macrocarpum* liquid-liquid partition was made. After the extract dissolution in methanol/water (3:2) the partition with hexane was made, from this procedure the hexanic fraction (AM4 – 7.8 g) was obtained. The fraction methanol/water (3:2) (AM4F.M. – 14.5 g) was also used for the antimicrobial, and from this fraction more three partitions were made: in chloroform, ethyl acetate, and butanol, providing the following fractions; chloroformic fraction (AM4F.C. – 52.15 g), ethyl acetate fraction (AM4F.A. – 2.7 g), and butanolic fraction (AM4F.B. – 28.7g).

The ethanolic crude extract of stem (AM4) of *A. macrocarpum* was submitted to acid/basis extraction to obtain alkaloids (16). In this extraction 10 g of ethanolic crude extract was used, 892 mg of alkaloidal fraction was obtained (AM4F.ALC.). The precipitant of organic fraction (AM4F.O.1. – 5.00 g) was separated to be used in antimicrobial tests, the

supernatant of organic fraction and the aqueous fraction (AM4F.AQ – 3.50 g.) all of them obtained from alkaloids extraction of ethanolic crude extract (AM4) of *A. Macrocarpum* stem. The solvents were removed of the fractions through rota-evaporation under reduced pressure at 40 °C.

#### *Aspidosperma pyrifolium* extraction

The ethanolic crude extracts of *A. pyrifolium* were obtained from stem bark (3.0 kg) (AP3 – 150.00 g), stem (2.8 kg) (AP4 – 150.00 g), root bark (1.0 kg) (AP9 – 70.00 g), root (1.5 kg) (AP12 – 80.00 g), flowers (0.2 kg) (AP11 – 10.00 g) and from the fruit (0.50 kg) (AP10 – 25.00 g). The process for obtaining these extracts was performed in a Soxhlet apparatus, in 95% ethanol, for 72 h, the ethanolic solution was concentrated under reduced pressure at 40 °C in rota-evaporator unit.

The ethanolic crude extract of the stem bark of *A. pyrifolium* was dissolved in methanol (300 mL) and water (450 mL), of mixtures (hydromethanol fraction) resulting was performed an ethyl acetate partition. The ethyl acetate fraction (AP1 – 91.00 g) and hydromethanol (AP2 – 55 g) obtained were concentrated under reduced pressure in rota-evaporator at 40 °C.

To fractionate the ethanolic crude extract of the stem (AP4) of *A. pyrifolium* first a liquid-liquid partition, the ethanolic crude extract (AP4) was solubilized in water and submitted to the ethyl acetate and butanol, from this process the following fractions were obtained: ethyl acetate (AP5 – 89,00 g), butanolic (AP6 – 10.00 g) and aqueous (AP7 – 50.00 g). From the fraction AP5 (40.00 g) the extraction for alkaloid was performed (16). After the solubilization in chloroform and extraction with HCl 0.1 N, the following fractions were obtained: the organic one (AP5-FO – 21.50 g) and the acid fraction (AP8 – 19.20 g) from the extraction for alkaloids. After the basification with Na<sub>2</sub>CO<sub>3</sub> and after new extraction in chloroform the following fractions were obtained: alkaloidal (AP5.ALC. – 4.30 g) and the aqueous (AP5.AQ. – 17.00 g) from *A. pyrifolium*

stem.

All the extracts and fractions described above were tested for antimicrobial activity reported in this study.

### **Microorganisms used and growth conditions**

The antimicrobial activity was observed on strains of Gram positive (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6623), Gram negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 15442) and *Candida* species (*Candida albicans* ATCC 10231, *C. parapsilosis* ATCC 22019 and *C. tropicalis*). The bacteria were grown in nutrient broth (Difco Laboratories, Detroit, MI) at 37 °C and maintained on nutrient agar slants at 4 °C. The yeast grown maintained on Sabourand-dextrose agar (Merck SA, São Paulo, Brazil).

### **Antimicrobial susceptibility testing**

The minimum inhibitory concentrations (MICs) of all extracts, fractions and reference antibiotics (tetracycline, vancomycin, penicillin, and nistatin - Sigma) were determined by microdilution techniques in Mueller-Hinton broth (Merck) for bacteria and RPM-1640 medium (Sigma) for yeast (17). Inoculate was prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard (10<sup>8</sup> colony-forming units [CFU]/mL) and diluted 1:10 for the broth microdilution procedure. Microtiter trays were incubated at 37 °C and the MICs were recorded after 24 h of incubation. Two susceptibility endpoints were recorded for each isolated. The MIC was defined as the lowest concentration of compounds at which the microorganism tested does not demonstrate visible growth. MBC (Minimum Bactericidal Concentration) and MFC (Minimum Fungicidal Concentration) was defined as the lowest concentration yielding negative subcultures or only one colony (17).

### **Results**

The crude ethanolic extract of root bark of *A. tomentosum* (AT1) showed a weak antibacterial activity of *S. aureus* and *B. subtilis* (MIC 1000 and 500 µg/mL, respectively) (Table 1) and no activity against yeasts (MIC >1000 µg/mL). For the species *A. pyriformium* only the alkaloidal fraction (AP5.ALC) of the wood showed moderate activity with MIC of 125 and 250 µg/mL in *S. aureus* and *B. subtilis* (Table 1), and the MBC was 1000 µg/mL for both bacteria. This fraction (AP5.ALC) also showed a weak activity against *C. parapsilosis* and *C. tropicalis* (MIC 500 µg/mL) (Table 2). The extracts and fractions of *A. macrocarpum* showed no activity against the tested bacteria (MIC >1000 µg/mL), and ethyl acetate fraction (AM4F.A) (MIC 250 µg/mL) and the alkaloidal fraction (AM4F. ALC) (MIC 500 µg/mL) of the stem showed weak activity for *C. parapsilosis* (Table 2). The other extracts and fractions tested did not show activity against the tests (MIC >1000 µg/mL).

see Table 1.

see Table 2.

### **Discussion**

The antimicrobial activity of extracts and fractions of *Aspidosperma* species tested against Gram positive and Gram negative bacteria and *Candida* species in this study did not present significant results. Although results from the literature describe the use of *Aspidosperma* species from Suriname with antimicrobial as the one observed for alkaloid type secamine and the indolic alkaloid dihydrocorynantheol isolated from stem barks of *A. marcgravianum* Woodson active against Gram positive bacteria and the activity of indolic alkaloids aspidoscarpine, reserpine and reserpiline against *C. albicans*, *Aspergillus niger*, *P. aeruginosa* and *E. coli* (18).

The alkaloids isolated from the root barks of *A. excelsum* Benth present activity against Gram positive bacteria *B. subtilis*. However its were inactive against Gram negative bacteria. Among the active alkaloidal structures type secamine reported in the study two new ones were described 16-hydroxytetrahydrosecamine and 16-hydroxy,16-



demethoxycarbonyltetrahydro secamine (19).

The antibacterial activity was also reported for the ethanolic extract obtained from the wood *A. polyneuron* known as “peroba-rosa” which presented strong action against *Proteus mirabilis*. The microbiological assay was performed through the diffusion method in solid medium through plaque cavity. A qualitative analysis of this extract presented positive result for presence of phenols and total alkaloids (20).

The ethanolic extract from root, stem and leaf of *A. polyneuron* presented antifungal activity on *Cladosporium herbarum* through the method biorrelation on plates of thin layer chromatography (21). Another study reported the presence of a new alkaloid isolated from ethanolic extract of *A. Polyneuron* roots, it was identified as 2,7-dihydroxiquibrachamina (22).

The antibacterial action of methanolic extract and fractions obtained after acid/basis and the pure substance obtained from the stem bark of *A. ramiflorum* species were evaluated through the microdilution in broth Mueller-Hinton technique. The methanolic extract demonstrated moderate activity against *B. subtilis* (MIC: 250 µg/mL) and *S. aureus* (MIC: 500 µg/mL), and they were inactive against *E. coli* and *P. aeruginosa* (MIC: > 1000 µg/mL). While the fraction denominated IV, obtained from chloroform fraction of acid/basis extraction presented a good activity against *B. subtilis* and *S. aureus* (MIC: 15.6 µg/mL) and moderated action against *E. coli* and *P. aeruginosa* (MIC: 250 µg/mL). The pure substances denominated ramiflorines A and B (bis-indolic alkaloids), both, presented good activity against *S. aureus* (MIC: 25 µg/mL) and *Enterococcus faecalis* (MIC: 50 µg/mL) (23).

According to another study performed with ethanolic crude extract of several species of plants collected in Atlantic forest region in São Paulo – SP - Brazil, just the extract of *A. ramiflorum* branches and leaves presented weak activity against *E. coli* (inhibition zone: 2.5 e 1.4 mm, for branch and leaf, respectively). The microbiological assay was performed through the diffusion method in solid medium

through plaque cavity. The extract demonstrate itself inactive for *S. aureus* and *C. albicans*, as well the extract from *A. Olivaceum* species did not present activity (24).

Antifungal properties were also reported for *A. ramiflorum* alkaloids. The ramiflorine, a substance was more active demonstrating a good antifungal activity against *Cryptococcus neoformans* (MIC: 3.12 – 12.5 µg/mL) (25). Study performed with ethanolic crude extract of *A. pyricolum* and *A. olivaceum* (MIC: 125 e 250 µg/mL, respectively) demonstrated a moderate antibacterial activity against *B. subtilis* (15).

The minimum inhibitory concentration demonstrated for the ethanolic extract of root barks of *A. tomentosum* (AT1) was weak for *S. aureus* (1000 µg/mL) and *B. subtilis* (1000 µg/mL), considered inactive for Gram negative bacteria which were tested (Table 1). The extract of *A. tomentosum* tested did not present activity for *Candida* species.

According to the literature reports the ethanolic extracts of *A. tomentosum* demonstrated activity on trypomastigote forms of *Trypanosoma cruzi* (14). Good results were reported for antiproliferative action of the terpenic fraction of dichloromethane extract obtained from aerial parts of *A. tomentosum* on lineages of human cells: MCF7 (breast) e NCI460 (lung) (26).

The alkaloidal fraction (AP5.ALC) obtained from stem of *A. pyrifolium* specie demonstrated moderate activity for *S. aureus* and *B. subtilis* (MIC: 125 e 250 µg/mL, respectively) (Table 1), and weak activity against *C. parapsilosis* (500 µg/mL) and *C. tropicalis* (500 µg/mL) (Table 2).

However the insecticide activity against *Plutella xylostella* larvae was reported for ethanolic extracts of stem barks, fruit and root of *A. pyrifolium* with mortality rate of 51.716% for the barks extracts, 13.320% for root extract and 11,73% for fruit extract. Sub-fractions obtained from ethanolic extract of stem bark presented 100% of mortality on *P. xylostella* larvae. The insecticide activity of these sub-fractions was related for the presence of indolic

monoterpenoids alkaloids: aspidofractine, 15-demetoxipirifoline e N-formilaspidofractina isolated from *A. pyriformium* (27).

A study demonstrated low antiplasmodial action of aspidolimidina alkaloid from *A. pyriformium* Mart, and cytotoxicity due to the tetrahydrofurane ring presence (28).

The antimicrobial action of *A. macrocarpum* specie was observed only for the ethyl acetate fraction (AM4F.A) (MIC 250 µg/mL) and alkaloidal fraction (AM4F. ALC) (MIC 500 µg/mL) obtained from stem ethanolic extract.

According to literature data the extract obtained from the leaves of *A. macrocarpum* presented activity against amastigote form of *T. cruzi* (IC<sub>50</sub>: 59.212 %) (29). The extract obtained from root stem of *A. macrocarpum* demonstrated activity on *Plasmodium falciparum* (IC<sub>50</sub>: 4.9 g/mL) (30).

*Aspidosperma* species have been chemically investigated and special emphasis is given to endolic alkaloids. According to the literature the search for new bioactive substances of *Aspidosperma* specie represent great scientific interest, as the structural diversity of indolic alkaloids present in all the species of this genus.

Among other species of *Aspidosperma* which presented phytochemical study, can be listed as chemical constituents of *A. illustre*, where it were isolated two indolic monoterpenes alkaloids, β-ioimbine and 1,2-dihidroaspidospermidine besides the triterpenes molecules (5). Indolic alkaloids type elipticine and N-metiltetra-hidroelipticine were isolated from *A. vargasii* and the aspidocarpine compound of *A. desmanthum* (31). Two new endolic alkaloids with plumerano skeleton were obtained from methanolic extract from stem barks and seeds of *A. spruceanum* (32). A new alkaloid isolated from ethanolic extract of *A. polyneuron* roots was identified as 2,7-dihidroxi quebrachamine (22). From stem barks of *A. pyriformium* the 15-demetoxipirifoline, aspidofractine and N-formilaspidofractine (33) were isolated.

Study related with antiprotozoal activity are also

described in ethnopharmacological reports and are proved in studies performed with extracts and alkaloids isolated from *Aspidosperma* species, especially activity against *P. falciparum* and trypanosomatids. An example, the active alkaloids *A. ramiflorum* against *Leishmania braziliensis* and *L. amazonensis* (23, 34). Other *Aspidosperma* species reported in the literature against malaria are *A. quebracho-blanco* zschlechdt., *A. polyneuron* Muell., *A. album* (Vahl) Benoist, *A. discolor* DC., *A. excelsum* Benth., *A. nitidum* Benth (35, 36).

Due to the great variety and chemical peculiarity of compounds structures, especially alkaloids, found among species of *Aspidosperma* genus, it can justify the gamma of biological activities described for these plants used by the traditional medicine (10).

## Conclusions

Although there are ethnopharmacological reports about the utilization of some species of *Aspidosperma* with antimicrobial activity, this study found no significant activity for the extracts and fractions of *A. tomentosum*, *A. macrocarpum* and *A. pyriformium* against the tested microorganisms. Despite the great quantity of studies reported in the literature about the chemical isolation of indolic alkaloids of *Aspidosperma* species, new chemical structure are being identified proving the chemical diversity, and the gamma of therapeutical application of *Aspidosperma* species. Tests for verification of the potential activity of these extracts on protozoa are being conducted.

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## References

1. Viegas Júnior C, Bolzani VS, Barreiro EJ. Os produtos Naturais e a Química Medicinal Moderna. *Quim Nova* 2006; 29(2): 326-337.
2. Shu Y. Recent natural products based drug development. A pharmaceutical industry perspective. *J Nat Prod* 1998; 61:1053-1071.
3. Calixto JB. Biodiversidade como fonte de medicamentos. *Rev Soc Bras Prog Cien* 2003; 3:37-9.
4. Pereira MM, Jácome RLRP, Alcântara AFC, Alves RB, Raslan DS. Alcalóides indólicos isolados de espécies do gênero *Aspidosperma* (Apocynaceae). *Quim Nova* 2007; 30(4):970-983.
5. Barbosa LF, Mathias L, Braz-Filho R, Vieira IJ. Chemical Constituents from *Aspidosperma illustre* (Apocynaceae). *J Brazil Chem Soc.* 2010; 21(8):1434-1438.
6. Henrique CM, Nunomora SM, Pohlit AM. Alcalóides indólicos de cascas de *Aspidosperma vargasii* e *A. desmanthum*. *Quim Nova* 2010; 33(2):284-287.
7. Lorenzi H. *Árvóres brasileiras: manual de identificação e cultivo de plantas arbóreas do Brasil*. 2 ed. Nova Odessa, SP: Instituto Plantarum. 2002.
8. Nunes DS. Contribuição ao estudo químico do gênero *Aspidosperma*. *Aspidosperma pruinosum* Markgraf. 1980. 177 p. Dissertação, Campinas.
9. Pereira MM, Alcântara AFC, Piló-Veloso D, Raslan DS. NMR structural analysis of Braznitidumine: A new indole alkaloidal with 1,2,9-triazabicyclo [7.2.1] system, isolated from *Aspidosperma nitidum* (Apocynaceae). *J Braz Chem Soc.* 2006;17(7): 1274-1280.
10. Oliveira VB, Freitas MSM, Mathias L, Braz-Filho R, Vieira IJC. Atividade biológica e alcaloides indólicos do gênero *Aspidosperma* (Apocynaceae): uma revisão. *Braz J Med Plan* 2009; 11(1): 92-99.
11. Lino RC, Garrote CFD. Isolamento dos alcalóides indólicos presentes na casca do caule de *Aspidosperma subincanum* Mart., para obtenção de padrões com finalidade de desenvolvimento de metodologia para doseamento com marcadores de matéria-prima vegetal. *Rev Eletr Farm.* 2005; (2)2:107-109.
12. Campos RA, Lima Júnior RCP, UCHOA DEA, Silveira ER, Santos FA, Rao VSN. Pro-erectile effects of an alkaloidal rich fraction from *Aspidosperma ulei* root bark in mice. *J Ethnopharmacol* 2006; 104:240-244.
13. Barbosa LF, Mathias L, Braz-Filho R, Vieira IJ. Chemical Constituents from *Aspidosperma illustre* (Apocynaceae). *J Brazil Chem Soc* 2010; 21(8):1434-1438.
14. Marcondes ABS, Melo LVL, Ribeiro RV, et al. Levantamento etnobotânico de plantas utilizadas como anti-hiperlipêmica e anorexígenas pela população de Nova Xavantina-MT, Brasil. *Rev Bras Farmacogn* 2010; 20 (4):549-562.
15. Oliveira AJB, Koike L, Reis FAM, et al. Preliminary Studies on the Antibacterial Activity of Ethanol Crude Extracts and Alkaloids from Species of *Aspidosperma*. *Pharm Biol* 2009; 47:1085-1089.
16. Marinho AF. Desenvolvimento e Validação de Método Analítico para Quantificação da Warifteína em Extratos de *Cissampelos Sympdialis* Eichl (Milona). Tese de Doutorado. LTF, PPGNSB, UFPB, João Pessoa, PB, 2008.
17. CLSI. 7. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts: third edition (M27-A3). Wayne, PA: CLSI; 2008.
18. Verpoorte R, Ruigrok CLM, Baerheim Svendsen A. Medicinal Plants of Surinam II: Antimicrobial Active Alkaloids from *Aspidosperma marcgravianum* *Planta Med* 1982; 46(11):149-152.
19. Verpoorte R, Kos-Kuyck E, Tjin A Tsoi A, Ruigrok CLM, Baerheim Svendsen A. Medicinal Plants of Surinam III: Antimicrobially Active Alkaloids from *Aspidosperma excelsum*. *Planta Med* 1983; 48(8):283-289.
20. Granato D, Nunes DS, Mattos PP, et al. Chemical and Biological Evaluation of Rejects from the Wood Industry. *Brazil Arch Biolog Technol* 2005; 48:237-241.
21. Ferreira DT, Silva Jr JV, Soeira LS, et al. Avaliação da atividade antifúngica dos extratos etanólicos de raiz, caule e folha de *Aspidosperma polyneuron*. XI Encontro de Química da Região Sul (XI SBQSUL) 2003, Pelotas, RS, Brasil, resumos: QO-83.
22. Santos TA, Ferreira DT, Pinto JP, Faccione M, Braz-Filho R. New alkaloid from *Aspidosperma polyneuron* roots. *Nat Prod Commun* 2008; 3(0):1-4.
23. Tanaka JCA, Silva CC, Oliveira AJB, Nakamura CV, Dias Filho BP. Antileishmanial activity of indole alkaloids from *Aspidosperma ramiflorum*. *Braz J Med Biol Res* 2006; 39:387-391.
24. Agripino Dg, Lima MEL, Silva MR, et al. Screening of Brazilian Plants for Antimicrobial and DNA-Damaging Activities. I. Atlantic Rain Forest – Ecological Station Juréia-Itatins. *Biota Neotropica* 2004; 4(2):1-15.
25. Souza ACM, Hasimoto LKS, Silva MRR, et al. Propriedades antifúngicas dos alcalóides de *Aspidosperma ramiflorum*. In: 29a Reunião Anual da Sociedade Brasileira de Química 2006. Águas de Lindóia, São Paulo, Brasil. Resumos. São Paulo: ADALTECH.
26. Kohn LK, Pizão PE, Foglio MA, et al. Antiproliferative activity of crude extract and fractions obtained from *Aspidosperma tomentosum* Mart. *Rev Bras PI Med* 2006; 8:110-115.
27. Trindade RCP, Silva PP, Araújo-Júnior JX, Lima IS, Paula JE, Sant'ana EG. Mortality of *Plutella xylostella* larvae treated with *Aspidosperma pyriformis* ethanol extracts. *Pesq Agropec Bras* 2008; 43(12): 1813-1816.
28. Miataine-Offer AC, Sauvain M, Valentin A, Callapa, J, Mallié M, Zèches-Hanrot M. Antiplasmodial activity of *Aspidosperma* indole alkaloids. *Phytomed* 2002; 9:142-145.
29. Mesquita ML, Desrivot J, Bories C, Fournet A, Paula JE, Grellier P. Antileishmanial and trypanocidal activity of Brazilian Cerrado plants. *Mem Inst Oswaldo Cruz* 2005; 100(7):783-787.
30. Mesquita ML, Grellier P, Mambu L, Paula JE, Espindola LS. In vitro antiplasmodial activity of Brazilian Cerrado plants used as traditional remedies. *J Ethnopharmacol* 2007; 110:165-170.
31. Henrique CM, Nunomora SM, Pohlit AM. Alcalóides indólicos de cascas de *Aspidosperma vargasii* e *A. desmanthum*. *Quim Nova* 2010; 33(2):284-287.
32. Oliveira VB, Vieira IJC, Braz-Filho R, et al. Spruceanumines A and B, Novel Plumeran indole alkaloids from *Aspidosperma spruceanum* (Apocynaceae). *J Braz Chem Soc* 2009; 20(4): 753-759.
33. Araújo Júnior JX de, Antheaume C, Trindade RCP, Schmitt M, Bourguignon JJ, Sant'ana AEG. Isolation and characterisation of the monoterpenoid indole alkaloids of *Aspidosperma pyriformis*. *Phytochem Rev* 2007; 6:183-188.
34. Ferreira ICP, Lonardoni MVC, Machado GMC. Anti-leishmanial activity of alkaloidal extract from *Aspidosperma ramiflorum*. *Mem Inst Oswaldo Cruz* 2004; 99(3): 325-327.

35. Bourdy G, Oporto P, Gimenez A, Deharo E. A search for natural bioactive compounds in Bolivia through a multidisciplinary approach Part VI. Evaluation of the antimalarial activity of plants used by Isoceño-Guaraní Indians. *J Ethnopharmacol* 2004; 93:269-277.

36. Botsaris AS. Plants used traditionally to treat malaria in Brazil: the archives of Flora medicinal. *J Ethnobiol Ethnomedicine* 2007; 3(18):1-8.

Samples	Minimum Inhibitory Concentrations – MIC ( $\mu\text{g/mL}$ )			
	<i>S.aureus</i>	<i>B.subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
AT1	1000	500	>1000	>1000
AP5.ALC	125	250	>1000	>1000
Penicillin	0.019	-	-	-
Vancomycin	-	0.18	-	-
Tetracycline	-	-	1.57	3.15

Table 1. Minimum Inhibitory Concentrations of *A. tomentosum* and of *A. pyriformium* in bacteria Gram positive and Gram negative.

AT1: crude ethanolic extract of root bark of *A. tomentosum*.

AP5.ALC: alkaloidal fraction of wood of *A. pyriformium*.

Samples	Minimum Inhibitory Concentrations – MIC ( $\mu\text{g/mL}$ )		
	<i>C. albicans</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>
AM4.F.A	>1000	250	1000
AM4.F.ALC	>1000	500	500
AP5.ALC	>1000	500	500
Nystatin	1.56	1.56	3.12

Table 2. Minimum Inhibitory Concentrations of the fraction of *A. macrocarpum* and of *A. pyriformium* in *Candida* species.

AM4.F.A: ethyl acetate fraction of stem of *A. macrocarpum*.

AM4.F.ALC: alkaloidal fraction of stem of *A. macrocarpum*.

AP5.ALC: alkaloidal fraction of wood of *A. pyriformium*