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EVALUATION OF ANTIBACTERIAL AND ANTISAP ACTIVITIES OF THE AERIAL PART EXTRACTS OF *PALICOUREA GUIANENSIS* (RUBIACEAE).

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Abstract

The incidence of bacterial and fungal infections that affect the health of human beings has had a dramatic increase in recent years and this has led to the search for new antibiotics and antifungal natural or synthetic, in order to fight these microorganisms. Among these stands *Candida albicans*, a polymorphic fungus associated with various infections, one of its virulence factors is secretion 10 isoenzymes, known as SAP for its acronym "secreted aspartic proteinase" which have become a target for the search for new antifungal drugs. To evaluate the antibacterial and antiSAP activities of extracts obtained from the aerial parts of *Palicourea guianensis* Aubl, it was carried the methodology listed below. From the dried aerial parts of *P. guianensis* were obtained fractions of different polarities by liquid-liquid extraction and the aqueous extract obtained was fractionated on DIAION HP20 column. The determination of the antibacterial activity was carried out by the agar diffusion method, against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus cereus*. The evaluation of the antiSAP activity (inhibition of secretion or hydrolytic activity of enzymes of aspartic type) was determined by spectrofluorometry techniques and electrophoresis. The extracts did not show antibacterial activity at the evaluated concentrations tested. For the AntiSAP activity, the highest percentage of inhibition was PgDiClmet followed in descending order by PgAcOet, PgMeOH, PgHex and PgAcu, observing in each extract, inhibition above 50%. The extract PgDiClmet contained: tannins, saponins and alkaloids identified chemical testing features. The corresponding fractions PgAcu-F29 and PgAcu-F36-44, showed results about 98%, being promising as inhibitors of SAP. The tested extracts not exhibit antibacterial activity against the bacterias used, however these extracts exhibit antiSAP activity, of which PgDiClmet and PgAcu extract can be a natural source of specific inhibitors of SAP.

Keywords: *Candida albicans*, electrophoresis SDS-PAGE, extracts, spectrofluorimetry.

Introduction

The incidence of fungal and bacterial infections affecting the health of human beings has had a dramatic increase in recent years and this has led to the search for new antibiotics and antifungal natural or synthetic, in order to fight these microorganisms; However, administration of these drugs in some patients is not recommended due to side effects that may occur in the case antibacterial penicillins and cephalosporins are known for causing hypersensitivity, allergies and bleeding; regarding antifungal compounds, they are used compounds as flucytosine, few tested azoles and polyenes, fundamentally Amphotericin B, which produce severe side effects; including, gastrointestinal disorders, headache and anemia, they also have a narrow spectrum and poor penetration in tissues [1]. *Candida albicans* is a polymorphic fungus associated with various infections, one of the virulence factors is a secretion of 10 isoenzymes which show high proteolytic activity [2-4] and are called Secreted Aspartic Proteases or SAP, these isoenzymes have become a target for the search for new antifungal drugs [5]. Therefore, in order to find compounds having inhibitory action on aspartic proteases secreted by *C. albicans* (antiSAP activity), in recent years they have been studied worldwide active components (both extracts and essential oils) of a significant number of plants, it has not been observed fungal resistance and the side effects they have on the patients are low impact, or no [6-8].

Among the plant families that were studied looking for antiSAP action they are: Asteraceae, Bignoniaceae, Lamiaceae, Melastomata ceae, Rubiaceae, Salicaceae, Sapindaceae.

The Rubiaceae family is one of the largest in dicots and is found in abundance in tropical regions around the world, reporting 45% of the world's total species distribution for Colombia, consisting of trees, shrubs or herbs; *Palicourea* is an important genre of this family, located from Mexico to Brazil, which is known to biosynthesize 39 alkaloids and a high number of cases, these secondary metabolites are responsible for their biological activities [9]. The plant *Palicourea guianensis* Aubl., Commonly known as “cafecillo”, belonging to the Rubiaceae family shows antecedents for the treatment of poisonous snake bites, in addition to having activity against *Staphylococcus aureus* and *Enterococcus feacalis* [10]. So this study starts in order to assess whether the *P. guianensis* plant presents or no antibacterial activity and antiSAP of *Candida albicans*.

Materials and methods

Extraction of plant material

P. guianensis leaves were collected at the Botanical Garden of the Universidad Tecnológica de Pereira, the April 24, 2009 at 10:23 am and a sample of this material was sent to the National Herbarium of Colombia, where he was identified by the Dr. Orlando Adolfo Jara Muñoz and recorded with the catalog number 519 790 COL.

The collected material was allowed to dry at room temperature for 8 days. Following this was dried in oven at 50 ° C for 2 days, finally dried leaves were ground and were stored. 600 g of aerial parts of *P. guianensis* were taken and were subjected to extraction with methanol, by ultrasound Fisher Scientific, model FS60H for 20 min, the obtained extract was fractionated by liquid-liquid extractions with water and adding the following solvents: n-hexane, dichloromethane and ethyl acetate; for obtaining the respective extracts plus the aqueous residue (PgMeOH, PgHex, PgDiClmet, PgAcOet and PgAcu), with which the qualitative determination of secondary metabolites was performed and the activities were evaluated at concentrations of 4 mg / mL and 2 mg / mL for antibacterial activity and 0.5 mg / mL for antiSAP activity, all dissolved in DMSO 30%. The aqueous extract was fractionated on DIAION HP20 column to obtain 17 fractions.

Qualitative determination of secondary metabolites

For this determination were carried out qualitative characterization tests [11], to determine the presence of alkaloids, phenols, tannins, steroids, triterpenes, sap onins, coumarins and flavonoids.

Determination of the antibacterial activity

Bacteria *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 11778), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) were used, and the antibacterial activity was evaluated per agar diffusion. Antibiotics were used as positive controls, which were ampicillin sodium (100 and 250 mg / mL), anhydrous ampicillin (100 ug / mL) and trimethoprim (100 ug / mL) (concentrations determined by performing a sensitivity testing); negative control for the solvent in which the solutions were diluted to evaluate and finally the control bacterial growth was used.

Determination of antiSAP activity

Growth of *C. albicans*. A strain of *C. albicans* (ATCC 10231) was used for the production of SAP, performing a 1X YNB broth

culture, supplemented with 0.1% BSA (Bovine Serum Albumin) and 0.5% glucose; at a concentration of 1 CFU / ml of medium, pH 7.5 and 28 ° C for a period of 48 h. Pepstatin A compound (10 uM) was used as control of inhibition of proteases.

Fluorimetric technique

Measuring the proteolytic capacity of SAP was performed based on the technique described by Arias and Marulanda 2010 [12]. The assay is based on the ability of fluorexon to form a complex non-fluorescent transition with Cu +2; which is displaced by amino acids released in proteolysis of BSA caused by SAP, when the compound lose its link with copper recovers its fluorescence [12-13], it was monitored and the proteolytic activity of the enzyme. The procedure consisted of adding 50 µL in a well of the fluorexon-copper complex (CuSO4 5,0 µM: Fluorexon 5,5 µM 1:1), 25 µL of culture medium and 25 µL of each sample to evaluate respectively
(PgMeOH, PgHex, PgDiClmet, PgAcOet, PgAcu and PgAcu extract fractions), finally the volume was adjusted to 250 µL PBS with 5 µM buffer at pH 5.0 . To measure the signal produced was used a Varian fluorometer at a wavelength of 525 nm emission and 486 nm excitation. Percent inhibition was determined by comparison to the positive control, which was treated similarly using the sample pepstatin A (10 mM) instead of extract (equation 1). As a negative control for inhibition, a mixture of proteases (SIGMA) was used in dilution 1/100000 further assays were performed using extracts only to check the influence of these on the fluorescent compound, to the same conditions for these samples and positive control. The conditions used in the fluorometer are shown in Table 1.

$$\% I = \frac{m_{ex} - m_{SAP}}{m_{Peps} - m_{SAP}} \times 100 \quad (1)$$

Where:
% I is the percent inhibition.
m_{ex} is linearization slope of the graph of the extract.
m_{SAP} is the slope of the linearization chart SAP.
m_{Peps} is linearization slope of the graph of SAP + pepstatin A.

With the parameters outlined above; Measurements were made by varying the concentration of the substrate and observing the change in the initial velocity (vo), represented by the slope. Considering that the reaction rate is related to the concentration of the substrate, a nonlinear regression allowing to calculate the necessary constants is used. The results obtained in the fluorometer were treated by computer software (Cary Eclipse) and the statistical treatment of the data collected was carried out in the GraphPadPrism 5.0 program.

Electrophoresis SDS-polyacrylamide gel (SDS-PAGE)

For protein electrophoresis using polyacrylamide gels (PAGE: polyacrylamide gel electrophoresis) in the presence of the detergent anionic sodium dodecyl sulfate (SDS), it is a technique known as SDS-PAGE. A resolution gel 10% acrylamide and a concentration gel 4% were used for that. The electrophoresis was run with a change in potential difference starting at 80 V for 30 minutes, then it increased to 100 V for 45 minutes and finally 120 V the last 45 minutes. The gel was stained with a solution of brilliant blue of Commassie. Preconditions of incubation of yeasts were conducted according to the methodology specified above in Materials and Methods, for the growth of *Candida albicans:* the yeasts were initially incubated in 1X YNB broth culture, supplemented with 0.1 % BSA (Bovine Serum Albumin) and 0.5% glucose; at a concentration of 1 CFU / ml of medium, pH 7.5 and 28 ° C for a period of 48 h.
This technique seeks to assess qualitatively both the presence or absence of SAP and its proteolytic activity on the BSA substrate; for qualitative determination was evaluated degradation of bovine serum albumin (BSA) and the presence of a characteristic band corresponding to SAP, the presence of the protease determines the non-inhibition of this enzyme [12]. Therefore, this activity is evidenced by observing the presence of substrate (BSA) and a decrease in band intensity of SAP.

Results

Qualitative determination of secondary metabolites

The results obtained are presented in Table 2. These were expressed as follows: no presence (-) limited presence (+), mild presence (++), moderate presence (+++) abundant presence (++++).

Antibacterial activity

The results showed that both PgMeOH extract, as the extracts PgHex, PgDiClmet, PgAcOet and PgAcu and also the PgAcu extract fractions showed no activity against bacteria tested. The results are presented in Table 3.

Evaluation of extracts against antiSAP activity
Fluorometric determination

In Figure 1 the behavior of the proteolytic activity is

presented in the middle where it grows *C. albicans* against the extract PgMeOH and compared with the behavior in the presence of inhibitor pepstatin A. As shown, the PgMeOH extract shows a similar behavior to the control (Pestatina A) as evidenced by a decrease in the fluorescence signal and thus in an inhibition of protease activity, demonstrating the potential of this extract as inhibitor. Furthermore, in Figure 1 the signal of production of proteases is observed, confirming the induction thereof in culture. The extracts were evaluated by fluorometry technique, showing similar characteristics PgMeOH extract; high percentages of inhibition obtained for PgDiClmet (96.26%), followed in descending order by PgAcOet, PgMeOH, PgHex and PgAcu, where percentages of inhibition near or above 60% were observed, assuming a greater inhibition by compounds with medium polarity. According to data obtained (see Table 4), the PgAcu extract fractions showed a percentage of inhibition greater than 50%, as the mother extract, where protruded PgAcu-18 corresponding to an inhibition rate of 92.44 and also PgAcu-F29 and PgAcu-F36-44 with a result about 98%, which make them promising as inhibitors of SAP.

Electrophoretic determination

In Figures 2 and 3, the electrophoretic profile of the medium where it grows *C. albicans* is observed after treated with extracts and fractions evaluated of *P. guianensis*, also the positive and negative controls. According to Figure 2 inhibition of proteolysis seen in the presence of all the extracts except the PgMeOH extract and the disappearance of the band of proteases in the presence of pepstatin and dichloromethane extract was observed. In samples treated with the control, during an incubation period of 26 hours, the band corresponding to treatment with pepstatin A (P), showed complete inhibition of SAP; since only he presented a band corresponding to BSA and no characteristic band of SAP. By contrast in the negative control for inhibition which was performed by placing the yeast incubated after 26h, without any inhibitor you can observe the presence of SAP and absence of BSA. In Figure 3, the electrophoretic pattern of the medium where *C. albicans* grows is showed after treated with the fractions of the aqueous extract of *P. guianensis*. In it can be seen that after the guided fractionation, fractions that showed inhibition were PgAcu-F8-F9 PgAcu, PgAcu-F10-13 and PgAcu-F18, from which protrude the PgAcu-9 and PgAcu-18 which presented the SAP band accompanied by a faint band of BSA, indicating a small decrease in the production of protease or its enzymatic activity. The other fractions did not show inhibition of SAP. Because in Figure 3 only the characteristic protease band was observed and there was no presence of substrate, this indicated that the BSA was completely consumed in the production of the enzyme by the yeast *C. albicans*. It should be emphasized that the active fractions differ by electrophoresis of the fractions that showed activity by fluorometric assay.

Discussion

No antibacterial activity of extracts of *P. guianensis* were presented, evaluated against bacteria at concentrations of 2000 µg/mL y 4000 µg/mL (see Table 3), previous works by Suffredini et al [10] and Benitez Stashenko [15] found that the extracts obtained from this Rubiaceae, have activity against *S. aureus* and *E. feacalis*; however extraction processes used by these authors were different and the concentrations of the extracts evaluated were 10 times higher than those reported in this study, so the minimum inhibitory concentration should be above the treated in this research.

Chemical testing (Table 2) showed that the PgDiClmet extract containing compounds such as tannins, saponins and alkaloids, of which it is presumed that the tannins are primarily responsible for the antiSAP activity, because they are present in all extracts. This activity has been demonstrated for ellagic acid, a compound which is highly distributed in these plants and is part of the main components of the hydrolyzable tannins [16]. Flavonoids, alkaloids and saponins, may also possess activity antiSAP or cause a synergistic effect that increases the activity, as demonstrated in studies of different biological activities related to these compounds [17]. The high activity of the extract PgDiClmet, which was undoubtedly higher, could be associated to the synergistic effect, possibly due to the presence of several components like tannins and alkaloids. In studies of the *Miconia myriantha* from Melastomataceae family, which contains compounds like matucinol-7-O- [4 ', 6' '- O- (S) - hexahidroxidifenoil] -β-D-glucopyranoside and ellagic acid, SAP inhibition was presented with an IC_{50} de 8,4 µM y 10,5 µM, respectively [16] therefore, it is presumed that ellagic acid derivatives present in the extracts and fractions, could be responsible for this activity. Studies by Zhang et al [18] showed that ethanol extracts of Nauclea orientalis, another plant of the Rubiaceae family, it possess antiSAP activity with IC_{50} of 3 µg/mL, this extract as PgDiClmet, has lots of tannins, so they checked its importance in this

activity with an assay in which all tannins are eliminated resulting in a decreased IC_{50} to 16 µg/mL. Other phenolic compounds with such activity are: 3-Geranyl-2,4,6-trihydroxybenzophenone, a phenolic ketone and xanthones, 1,3,5,7-Tetrahydroxy-8 isoprenylxanthone and 1,3,5-trihydroxy-8 - isoprenylxanthone [19]. Extracts with apparently similar content, ie also containing tannins and alkaloids as the PgHex extract showed less inhibition compared to the PgDiClmet extract, this might be due to the nature of the solvent used for extraction, since the hexane is a solvent with lower polarity compared with dichloromethane, which are obtained in the same type of compounds with different characteristics such as long carbon chain and high molecular weight which may alter its behavior. Besides the presence of compounds such as triterpenoids and steroids which might cause differences in inhibition results observed. The electrophoretic result shows the decrease in protease production due to inhibitor action, demonstrating a decrease in band intensity; the inhibitory activity of pepstatin A was also verified against aspartic protease [20]. For the samples evaluated with extracts, were observed both bands present, both the BSA as SAP, which indicated that despite the production of SAP, this was partially inhibited, and observing the band of the BSA substrate , this being fainter in the extract PgMeOH; that is, there was an increase in the protease production and hence less inhibition (being a crude extract) while the PgDiClmet, a strong band for BSA was observed and a faint band for SAP, showing highest inhibition, which coincide with the fluorometric assay; However, electrophoresis is used only as qualitative parameter and it indicated only the presence or absence of protease; that is, if it was produced or not, the enzyme in the performed culture. The results of electrophoretic runs, does not match that found in the fluorimetric technique, this is mainly due to the electrophoresis showed only the presence or absence of SAP in the gel, indicating that fractions with positive results (PgAcu-F8-F9 PgAcu, PgAcu-F10-13 and PgAcu-F18) appear to inhibit protein biosynthesis like the mother extract and the fractions with a high percentage inhibition of SAP (PgAcu-F29 and PgAcu-F36-44) studied by fluorimetric technique inhibited the proteolytic activity of the enzyme, since, in gel electrophoresis not have the characteristic band of BSA indicating inhibition, ie not affect the production of SAP. According to the data, extract fractions PgAcu, PgAcu-F29 and PgAcu-F36-44 are promising as inhibitors of SAP. As expected then in these extracts predominate substances as tannins, alkaloids, flavonoids and saponins; the absorption maxima of the chromatograms made to these fractions range from 230 nm, 270 nm, 280 nm and 320 nm, confirming the presence of metabolites biologically active [21]. Overall, extracts of P. guianensis plant not possess antibacterial activity against *E. coli, B. cereus, S. aureus* and *P. aerouginosa* at concentrations of 2000 µg/mL. However antiSAP exhibit activity with inhibition percentages above 60% comparing with the specific inhibitor pepstatin A, determined by means of the techniques used, the most outstanding were the PgDiClmet and the PgAcu and extracts. Both extracts contain secondary metabolites studied in phytochemical for its wide range of biological activities; for this reason, it is expected that compounds like tannins, flavonoids, saponins and alkaloids are responsible for the activity of extracts obtained. Also, it was shown that the fractions of the PgAcu extract, possess antiSAP activity; for PgAcu-F6 + 7 PgAcu-F8-F18 PgAcu, PgAcu-F19, F20-PgAcu, PgAcu-F22-27, PgAcu-F28, F29-PgAcu, PgAcu-F30 + 31 PgAcu-F33-34 and PgAcu -F36-44 the inhibition is observed at the level of the enzymatic reaction by the positive results by fluorometric technique and for PgAcu-F8-F9 PgAcu, PgAcu-F10-13 and PgAcu-F18 was determined through electrophoresis that inhibit protease production in low proportion. Notably the PgAcu-18 fraction showed activity by both enzymatic determinations both electrophoretic and fluorimetric.

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References

1. Rodríguez, C., Santos, R., Lemos, J., et al., Effects of Antifungal Agents in SAP Activity of *Candida albicans* Isolates. Mycopathologia 2010;169(2):91-98.
2. da Costa, K.R., Ferreira, J.C., Komesu, M.C., et al., *Candida albicans* and *Candida tropicalis* in oral candidosis: quantitative analysis, exoenzyme activity, and antifungal drug sensitivity. Mycopathologia 2009;167(2):73–79.
3. Gropp, K., Schild, L., Schindler, S., et al., The yeast *Candida albicans* evades human complement attack by secretion of aspartic proteases. Mol Immunol 2009;47(2-3):465-475.
4. Aoki, W., Kitahara, N., Miura, N., et al., Comprehensive characterization of secreted aspartic proteases encoded by a virulence gene family in *Candida albicans*. J Biochem 2011;150(4):431-438.
5. Santos, A.L.S., Braga-Silva, L.A., Aspartic Protease Inhibitors: Effective Drugs against the Human Fungal Pathogen *Candida albicans.* Mini Rev Med Chem 2013;13(1):155-162.

6. Adwan, G., Salameh, Y., Adwan, K., Effect of ethanolic extract of *Ecballium elaterium* against *Staphylococcus aureus* and *Candida albicans*. Asian Pac J Trop Biomed 2011;1(6):456-460.
7. Naeini, A., Khosravi, A., Chitsaz, M., et al., Anti-*Candida albicans* activity of some Iranian plants used in traditional medicine. Med Mycol 2009;19(3):168-172.
8. Höfling, J.F., Mardegan, R.C., Anibal, P.C., et al., Evaluation of antifungal activity of medicinal plant extracts against oral *Candida albicans* and proteinases. Mycopathologia 2011;172(2):117-124.
9. Niño, J., Narváez, D., Mosquera, O. et al., Antibacterial, antifungal and cytotoxic activities of eight Asteraceae and two Rubiaceae plants from Colombian biodiversity. Braz J Microbiol 2006;37(4):566-570.
10. Suffredini, I., Barradas, M., Varella, A. et al., Antibacterial activity of brazilian amazon plant extracts. Braz J Infect Dis 2006;10(6):400-402.
11. Hinojosa, J., Gutiérrez, M., Siller, F., et al., Screening fitoquímico y capacidad antiinflamatoria de hojas de *Tithonia tubaeformis*. Biotecnia 2013;15(2):53-60.
12. Arias, C., Marulanda, J., Estandarización y aplicación de un método espectrofluorométrico para la identificación de inhibidores de la proteasa aspártica (SAP) de *Candida albicans*, a partir de extracto de plantas de la familia Melastomataceae y Rubiaceae. [Thesis]. Pereira: Universidad Tecnológica de Pereira. Escuela de Tecnología Química; 2010.
13. Dean, K., Kleyn, G., Renaudet, O., et al., A green fluorescent chemosensor for amino acids provides a versatile high-throughput screening (HTS) assay for proteases. Bioorg Med Chem Lett 2003;13(10):1653-1656.
14. Campbell, M.K., Farrell, S.O., Bioquímica. 5th ed. Thomson Brooks Cole; 2006.
15. Benítez, N., Stashenko, E., Validación antibiótica de plantas medicinales del noroeste de Colombia contra *Staphylococcus aureus*. Bol Latinoam Caribe Plant Med Aromat 2009;8(2):145-150.
16. Li, X., Jacob, M., Pasco, D., et al., Phenolic compounds from *Miconia myriantha* inhibiting *Candida* aspartic proteases. J Nat Prod 2001;64(10):1282-1285.
17. Brown, J., Huang, G., Haley-Zitlin, V. et al., Antibacterial Effects of Grape Extracts on *Helicobacter pylori*. Appl Environ Microbiol 2009;75(3):848-852.
18. Zhang, Z., ElSohly, H., Jacob, M., et al., New Indole Alkaloids from the Bark of *Nauclea orientalis*. J Nat Prod 2001;64(8):1001-1005.
19. Polya, G., Protein and Non-Protein Protease Inhibitors from Plants. Atta-ur-Rahman (Ed.) Stud Nat Prod Chem 2003;29(1):567-641
20. Cadicamo, C., Mortier, J., Wolber, G., et al., Design, synthesis, inhibition studies, and molecular modeling of pepstatin analogues addressing different secreted aspartic proteinases of *Candida albicans*. Biochem Pharmacol 2013;85(7):881–887.
21. Domínguez, X., Métodos de identificación fitoquímica. 1st ed. México: LIMUSA; 1973.

Table 1. Instrumental parameters for the Cary Eclipse fluorometer.

PARAMETER	PROGRAMMING
Excitation wavelength (nm)	486
Emission Wavelength (nm)	525
Excitation and emission slit (nm)	5
PMT detector voltage (V)	400 (Low)
Excitation Filter	Automatic
Filter Issue	Open
Analysis Time (min)	20
Data collection cycles (min)	0,5

Table 2. Characterization assays of secondary metabolites.

Assay	Metabolite	Extract				
		PgMeOH	PgHex	PgDiClmet	PgAcOet	PgAcu
Dragendorff reagent	alkaloids	-	++	-	-	-
Mayer reagent	alkaloids	-	++	++	-	-
Ferric Chloride	phenols	-	-	-	+++	+++
Try Gelatin-NaCl	tannins	-	+++	+++	++	++
Liebermann-Burchard	Steroids and triterpenes	+	+	-	-	-
Assay for saponins	saponins	+	++	+++	++++	++
Assay for coumarins	coumarins	++	-	++	+++	+++
Assay for flavonoids	flavonoids	-	+	-	++	++
Bromine water assay	condensed tannins	-	+++	+++	+	+

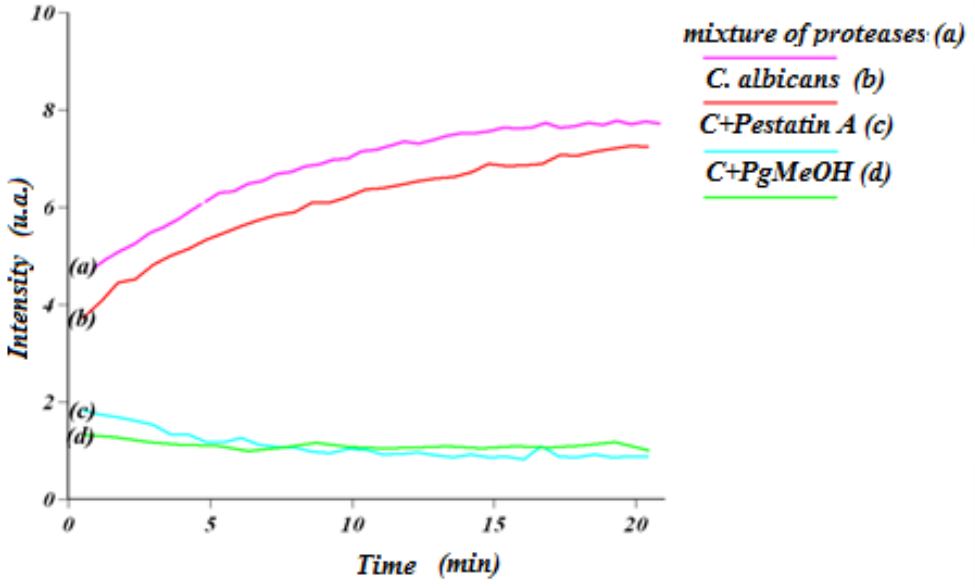

Figure 1. Graphic inhibition of the proteolytic activity of SAP of *C. albicans* by the fluorometric technique.

Table 3. Results of the measurement of the inhibition zones (mm) obtained for assessing the antibacterial activity of the extracts of P. guianensis against different bacteria tested.

E. coli							***P. aeruginosa***						
Concentration	4000 µg/mL			2000 µg/mL			Concentration	4000 µg/mL			2000 µg/mL		
Extract	1	2	3	1	2	3	Extract	1	2	3	1	2	3
PgMeOH	0	0	0	0	0	0	PgMeOH	0	0	0	0	0	0
PgAcu	0	0	0	0	0	0	PgAcu	0	0	0	0	0	0
PgDiClmet	0	0	0	0	0	0	PgDiClmet	0	0	0	0	0	0
PgAcOet	0	0	0	0	0	0	PgAcOet	0	0	0	0	0	0
PgHex	0	0	0	0	0	0	PgHex	0	0	0	0	0	0
CONTROL FOR ASSAYS							**CONTROL FOR ASSAYS**						
Trim[a] 100 µg/mL	2	2	22	2	2	22	Amp Na[b] 100 µg/mL	1	1	20	3	2	24
	2	2		2	2			8	8		2	2	
S. aureus							*B. cereus*						
Concentration	4000 µg/mL			2000 µg/mL			Concentration	4000 µg/mL			2000 µg/mL		
Extract	1	2	3	1	2	3	**Extract**	1	2	3	1	2	3
PgMeOH	0	0	0	0	0	0	**PgMeOH**	0	0	0	0	0	0
PgAcu	0	0	0	0	0	0	**PgAcu**	0	0	0	0	0	0
PgDiClmet	0	0	0	0	0	0	**PgDiClmet**	0	0	0	0	0	0
PgAcOet	0	0	0	0	0	0	**PgAcOet**	0	0	0	0	0	0
PgHex	0	0	0	0	0	0	**PgHex**	0	0	0	0	0	0
CONTROL FOR ASSAYS							**CONTROL FOR ASSAYS**						
Amp Anh[c] 100 µg/mL	3	3	36	3	3	32	Amp Na 250 µg/mL	1	1	14	1	1	14
	6	4		2	0			4	2		6	4	

[a]Trim = Trimetoprim
[b]Amp Na = Ampicillin sodium
[c]Amp Anh = Ampicillin Anhydrous

Table 4. SAP inhibition percentages obtained by fluorimetric determination.

Extract	%I	Extract	%I
PgMeOH	67,38	**PgAcu-19**	66,58
PgHex	61,79	**PgAcu-20**	77,18
PgDiClmet	96,26	**PgAcu-22->27**	52,91
PgAcOet	68,54	**PgAcu-28**	70,52
PgAcu	59,64	**PgAcu-29**	98,03
PgAcu-6+7	92,44	**PgAcu-30+31**	93,16
PgAcu-8	53,13	**PgAcu-33->34**	95,22
PgAcu-18	92,44	**PgAcu-36->44**	98,5

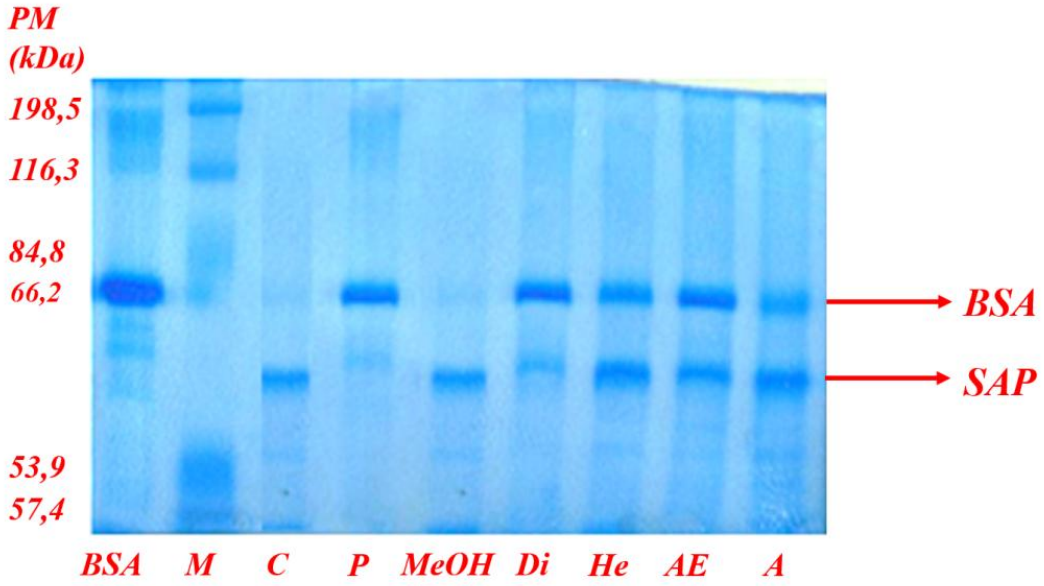

Figure 2. Electrophoresis of the extracts; BSA (culture medium with 0.1% BSA), M (molecular weight marker), C (*C. albicans* induced), P (*C. albicans* + pespstatina A), MeOH (*C. albicans* + PgMeOH), Di (*C. albicans* + PgDiClmet), He (*C. albicans* + PgHex), AE (*C. albicans* + PgAcOet) and A (*C. albicans* + PgAcu)

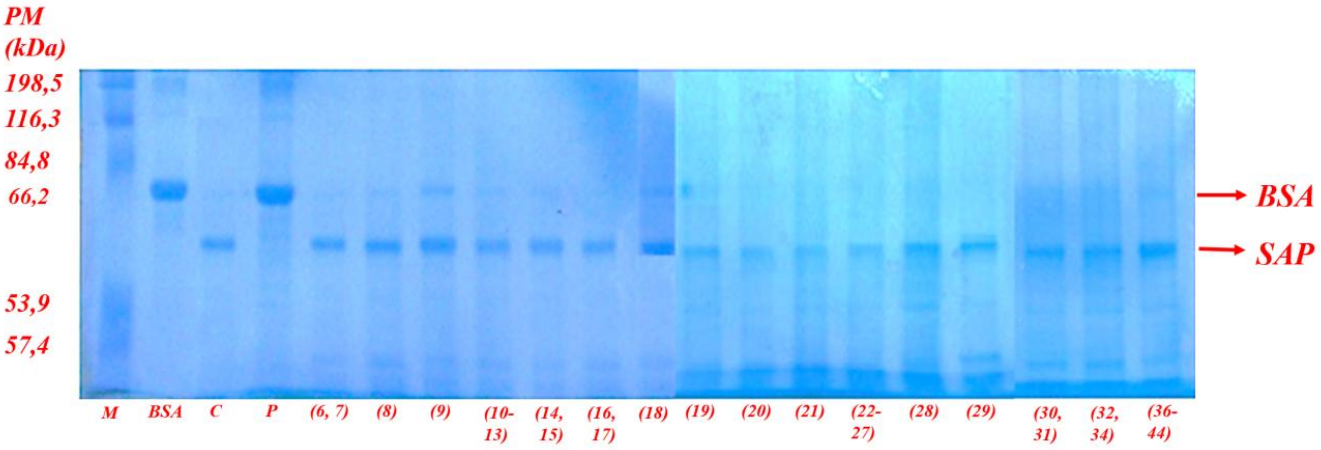

Figure 3. Electrophoresis of the aqueous extract fractions; M (molecular weight marker), BSA (BSA culture medium at 0, 1%), C (*C. albicans*), P (*C. albicans* + pespstatina A), (6 + 7) (*C. albicans* + PgAcu fraction 6 + 7), (8) (*C. albicans* + fraction of PgAcu 8), (9) (*C. albicans* + fraction PgAcu 9) (10-13) (*C. albicans* + fraction PgAcu 10-13) , (14 + 15) (*C. albicans* + fraction PgAcu 14 + 15), (16 + 17) (*C. albicans* + fraction PgAcu 16 + 17), (18) (*C. albicans* + fraction PgAcu 18), (19) (*C. albicans* + fraction PgAcu 19) (20) (*C. albicans* + fraction PgAcu 20) (21) (*C. albicans* + fraction PgAcu 21) (22-27) (*C. albicans* + fraction PgAcu 22 -27) (28) (*C. albicans* + fraction PgAcu 28) (29) (*C. albicans* + fraction PgAcu 29), (30 + 31) (*C. albicans* + fraction PgAcu 30 + 31), (32- 34) (*C. albicans* + fraction PgAcu 32-34) and (36-44) (*C. albicans* + fraction PgAcu 36-44).