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TOXIC AND ANTIMICROBIAL POTENTIAL FROM PIPER ADUNCUM CRUDE EXTRACT

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ABSTRACT

Piper aduncum is used for many communities as medicinal plant to treat and to cure different diseases, including gastrointestinal disorders. The aim of this study was to evaluate the anti-microbial and toxic potential of aqueous crude extract from leaves of *Piper aduncum,* and to approximate the effect in laboratory tests to what is possibly observed with the tea use. Healthy leaves of *Piper aduncum* were used to prepare the aqueous crude extract in three concentrations (20 g/L, 10 g/L and 5 g/L). It was demonstrated that the *P. aduncum* infusion has no effect as antimicrobial, and it did not have any toxic effect against blood cells, but potential toxic effect on cell development.

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INTRODUCTION

Piper aduncum (Piperaceae) is a shrub, found in borders of gallery forests and wet places, widespread in Central America, Mexico, Antilles and South America. In Brazil it is commonly known as monkey pepper and aperta-ruão and grows naturally in the Amazon and Atlantic Forest (Lentz et al. 1998; Baldoqui et al. 1999; Saralegui 2004; Lago et al. 2004). This specie is used in traditional medicine on urinary infections, disease of skin, leucorrhea and gastrointestinal disorders, abdominal pain and inhibition of viral and bacterial growth (ANEJA, 2010). These effects can be assigned to its compounds - monoterpenes, alkaloids, sesquiterpenes and others (Vieira 2011). But it is known that these compounds possibly depend of the geographic area of collection that could influence the biological properties (Gutiérrez 2016). *Piper aduncum*is used in Brazil, in the northeast region as infusion and has anti-inflammatory, antibacterial and cytotoxic activity (Navickiene et al. 2006; De Carvalho Castro, 2016). Medicinal plants are widely used by Brazilian population, but scientific data are insufficient (Dutra, 2009; Silva, et al.; 2012), much of ethnobotanical studies uses information from medicinal plants users in communities with botanical and/ or pharmacological data from these populations. Brazil has almost 19% of the world's flora, Amazon forest has great diversity, and almost 99% of the medicinal plants do not have its efficacy and pharmacological safety proven (Fão et al. 2102; Alex Tales et al., 2013). The popular use of *Piper aduncum* is through tea leaf for the therapeutics claims previously cited. Therefore, in order to reproduce its use, in this study the infusion of the plant was chosen to approximate the effect in laboratory tests to what is possibly observed with the tea use. Thus, the aim of this study is to evaluate the anti-microbial and toxic potential of aqueous crude extract from leaves of *Piper aduncum,* which is used against gastrointestinal disorders.

MATERIALS AND METHODS

Plant material collection: Leaves of *Piper aduncum*were collected from April to June of 2016, in São Raimundo Farm (-10.263553, -48.310664), in Palmas/Tocantins. The specimen was identified by Rodney Viana, in the herbarium of Federal University of Tocantins, at Núcleo de EstudosAmbientais (NEAMB), with a voucher specimen HTO:11.134.

Aqueous crude extract preparation: Healthy leaves of *Piper aduncum*were used to prepare the crude extract according Lubian

(2010) with modifications and protocols of Brazilian Sanitary Agency. The leaves were dried and crushed and prepared by infusion, as the traditional preparation, in three concentrations, 20 g of leaves in 1L of mineral water and dilutions of 10 g/L and 5 g/L. The leaves remained in infusion for 15 minutes in water at 100°C. The crude extracts were filtered two times and kept in dark bottles at 4° C.

Cytotoxic Evaluation: This experiment followed methodology recommended by Fiskejö (1993) and Meneguetti (2014), based on meristems germination alterations. Healthy onions, (*Allium cepa*), with similar size and the same origin and not sprouted, were used. Three *A. cepa* bulbs were partially submerged in 50 ml of each crude extract concentration and control for germination at 25° C for 72h. Mineral water was used as negative control. The three largest roots were measured with a digital pachymeter (Starret 799) 72 h after germination of the control group, in a total of 9 roots per assay. Three replicates were made and the tests were repeated two times.

Human erythrocyte osmotic fragility: The erythrocyte osmotic fragility is a standard test to study the membrane stability, by determining the erythrocyte ability to hemolysis in hypotonic salt solution (Suess et al. 1948). Since the consumption of phytotherapeutic products has increased worldwide, the evaluation of erythrocyte osmotic fragility during exposure to these products should be evaluated (Willcox 2004). This assay was previously approved by an Institutional Ethics Committee N° 066/2013. Blood sample (4 ml) was collected by intravenous puncture in evacuated tubes with EDTA from healthy volunteers. Assay tubes (Eppendorf®) containing 1mL of blood and 1 mL of crude extract at the 3 concentrations (20 g/L, 10 g/L and 5 g/L) and 1 mL of NaCl 0,9% and 1 mL of blood was used as control, these tubes were pre-incubated at 37°C for 1 h, then the tubes were centrifuged at 1500 rpm for 10 minutes, the supernatant was removed and the samples were washed with NaCl 0,9% and centrifuged at 1500 rpm for 10 minutes. 1 mL of NaCl 0,9% was added in the sample, then homogenized and transferred 50 µL to assay tubes of with saline solution in different concentration (0,12%, 0,24%, 0,48%, 0,60%, 0,72% and 0,9%) and pre-incubated at 37° C for 1 hour. The lysis of erythrocytes was followed by measuring absorbance of the supernatant at 540 nm. Three replicates were made and the tests were repeated three times.

Phytochemical screening: Phytochemical screening was performed using standard procedures proposed by Matos (1988), as qualitative tests, and the determinations were given by precipitate reactions or color change. Three replicates were made for the following tests: Organic acids, tannins, catechins, flavonoids, cardiac glicosides, sesquiterpenlactones and others lactones, azulenes, carotenoids, saponins, alkaloids, anthraquinones, steroids and triterpenoids.

Antimocrobial activity: The crude extracts antimicrobial activity was evaluated by the agar wells diffusion technique according to Rios (1988) against *Sthaphylococcus aureus* ATCC 25923*, Escherichia coli* ATCC 25922*, Salmonella typhimurium* ATCC 14028 e *Shigella flexnen* ATCC 12022, entropathogenic bacteria. The microbial suspensions used for inoculation were prepared at 10^5 CFU by diluting fresh cultures at McFarland 0,5 density. Microorganisms suspensions were plated in Mueller-Hinton agar, after dry, five 6 mm holes were perforated and 50 µL of the extracts concentration (20 g/L, 10 g/L, 5 g/L) were added to the wells, distillated water was used as negative control and 30 mcg of chloramphenicol as positive control. The dishes were incubated at 37° C for 24 h. The antibacterial activity of each extract was expressed by measuring the diameter of zone of growth inhibition in mm using a digital pachymeter. The tests were performed in triplicates.

Statistical analyses: Roots growth and elongation data were used to calculate the Relative Growth Index (RGI) and Germination Index (GI) according to Young's method (2012). These indices were obtained by the following equations: RGI = RLS/RLC. Where RLS is the radicle length of the sample and RLC is the radicle length of the control. GI% = (RLS x GSS)/ (RLC x GLC) x 100. Where GSS is the number of germinate roots in the sample and GLC is the number of germinate roots control. The erythrocyte osmotic fragility test data were compared with Tukey's test 99%.

RESULTS AND DISCUSSION

Piper aduncum is a widespread species in Tocantins and it is used for medicinal purpose. This study was designed to reproduce *in vitro* the popular use of *Piper aduncum* infusion. *Piper aduncum* crude aqueous extracts concentrations (20 g/L, 10 g/L and 5 g/L) did not show significant difference to control at erythrocyte osmotic fragility (Fig. 1).

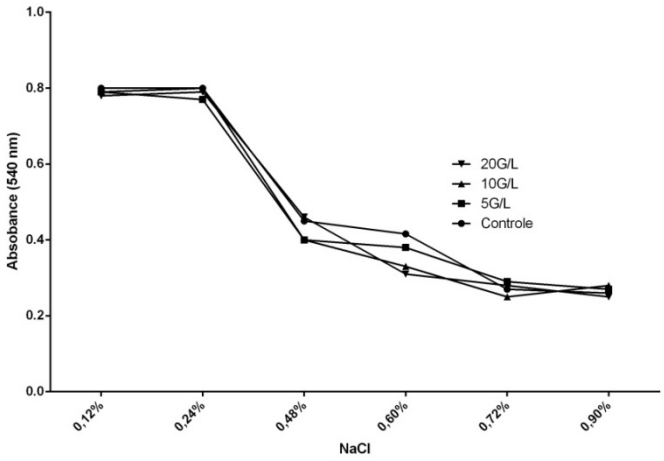

Figure 1 Osmotic fragility of blood samples treated with different concentrations of *Piper aduncum* aqueous crude extracts and sodium chloride solution (0,9% NaCl) as control.

Therefore, it shows that these extracts did not have deleterious effects on erythrocytes membranes. Barros (2016) demonstrates that essential oil of *Piper aduncum* has a dual action, protective and disruptive on red blood cells depending of the concentration. Similarly, Sousa (2008) observed that essential oil of *P. aduncum* has high safety margin with minimal toxic effect under blood cells. The *Allium cepa* roots evaluation is a secure method to assess toxicity and cytotoxity of many compounds, including plants extracts, on eukaryotic cells. The germination and elongation of the roots are evaluated on this technique (Muñoz-Solarte et al. 2013). In this study the roots growth evaluation was according Young (2012), that RGI, root growth index, was used to determine the toxic effect: Inhibition of root elongation (I): 0.0 <X>0.8; No significant effects (NSE): 0.8≤X≥1,2; and stimulation of root elongation (S):X>1.2, where X is the RGI value. As shown on Table 1, the three concentrations of aqueous crude extracts of *P. aduncum* inhibited the root growth presenting a toxic potential.

Table 1 Root Growth Index (RGI) / Germination Index (GI) of *Allium cepa* and toxicity categories of *Piper aduncum* aqueous extract concentrations.

	TIME 1		TIME 2	
Concentration	RGI	GI %	RGI	GI %
NC	1	100	1	100
20g/L	0 (I)	0	0 (I)	0
10g/L	0.185 (I)	18.53	0.146 (I)	8.039
5g/L	0.202 (I)	14.141	0.383 (I)	99.96

NC: Negative control (mineral water). Toxicity categories: I - inhibition; NSE - no significant effects; S: stimulation

Martins (2016) observed that crude aqueous extracts of *Belluciagrossularioides* had a significant inhibitory effect on *A. cepa*roots. Therefore, it can be observed that aqueous crude extract of *Piper aduncum*does not have toxic effect on cell membranes, but has toxic potential on cell replication. Phytochemical screening of *P. aduncum* infusion presented saponins and sesquiterpenlactones and others lactones. Saponins have irritating action because they form

complexes with cholesterol from membranes of intestinal mucosa cells; exfoliation occurs with loss of function and decrease the absorption area (Simões, 2017). And this laxative effect can act positively or negatively in gastrointestinal symptoms. Vásquez (2015) demonstrated that ethanolic extract of *Piper aduncum* presented many metabolites: alkaloids, flavonoids, tanins, phenols, saponins and quinones. Secondary metabolites have limited distribution in nature, and are not produced in all conditions. Some are produced for appreciated reasons like defense against predators, growth and plant development, protect against UV radiation (Dewick, 2002; Pereira, 2016). The traditional medicine uses *P. aduncum* leaf infusion as antibacterial. According to Abreu (2015), essential oil and ethanolic extract of *Piper aduncum*sube sp. *Ossanum* had some antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*even on resistant urophatogenic strains. However, in this study, aqueous crude extract of *P. aduncum* did not inhibited the entero-pathogenic bacteria growth. This finding shows that *P. aduncum* infusion has no effect against these etiological agents of gastrointestinal disorders. These data are important because the use of natural products is associated to non-risk and health benefits. Alex Tales (2013) demonstrated that information about the phytotherapic medicine from medicinal plants showed high percentage of divergence confronted with pharmacological tests, and there are few studies on plants considered medicinal in the poorest regions of Brazil.

CONCLUSION

The infusions of *Piper aduncum* leaves, collected in this specific area, did not have toxic effect on blood cells membranes, though they had toxic potential on cells development, and its use could be safe depending of the daily intake of plant infusion. But its use as antimicrobial for gastrointestinal disorders does not show any effect.

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