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# PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT POTENTIAL AND TOXICITY OF THE CRUDE ETHANOLIC EXTRACT OF *Piper aduncum*

**Letícia Assis Vieira de Azevedo Caputo[1], Ana Luzia Ferreira Farias[2], Patrick de Castro Cantuária[3] and Sheylla Susan Moreira da Silva de Almeida*[4]**

[1]Laboratory of Pharmacognosy and Phytochemistry, Federal University of Amapá. 0000-0001-5604-5644. Macapá, AP, Brazil.
[2]Laboratory of Pharmacognosy and Phytochemistry, Federal University of Amapá. 0000-0002-5338-6598. Macapá, AP, Brazil.
[3]Herbarium Amapaense, Institute of Scientific and Technological Research of the State of Amapá. 0000-0002-3676-7866. Macapá, AP, Brazil.
[4]Laboratory of Pharmacognosy and Phytochemistry, Federal University of Amapá. 0000-0002-7687-8288. Macapá, AP, Brazil.

***Corresponding Author: Sheylla Susan Moreira da Silva de Almeida**
Laboratory of Pharmacognosy and Phytochemistry, Federal University of Amapá. 0000-0001-5604-5644. Macapá, AP, Brazil.

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## ABSTRACT
The species *Piper aduncum*, known as Pimenta de Macaco (Monkey Pepper), is used by the Macapá riverside population as an antiseptic, a coagulant for haemorrhages and for the treatment of ulcers and intestinal infections. There are reports of its use also as astringent, antifídico, diuretic and tonic of the uterus. Thus, the present work aims to evaluate the phytochemical profile, toxicity and antioxidants of the leaf of *Piper aduncum* species. The identification of secondary metabolites was performed through qualitative colorimetric tests. The antioxidant activity was evaluated by the sequestration method of the 2,2-diphenyl-1-picrylhydrazyl radical, while the cytotoxic activity was evaluated using *Artemia salina*. The results revealed the presence of phenols and tannins, organic acids, reducing sugars, saponins, anthraquinones, and alkaloids. The crude ethanoic extract (CEE) presented potent antioxidant activity ($IC_{50}$de 150,163 µg.mL-$^{1}$), and high cytotoxic activity against *A. salina*, with LC50 of 792.83 µg.mL-$^{1}$.

**KEYWORDS:** Long pepper, natural products, Amazonian plant.

## INTRODUCTION
According to the World Health Organization, research on medicinal plants is of great benefit as it encourages the discovery of herbal medicines together with its various benefits, as well as highlighting the risks they can cause. In addition, more than 65% of the world's population mainly uses plants in the treatment of various disorders.[1.2]

The Piperaceae family is pantropical with species distributed throughout the Americas from Mexico to southwestern Argentina.[3] In this family, there are shrub, herbaceous and arboreal plants of more than 3 m. The stem is articulated and knotted; the leaves are whole, dorsiventral, alternate, rarely opposite or verticillate, petiolate with stipules.[4]

*Piper* is the largest genus of the Piperaceae family with more than 700 species, of which about 170 grow natively in Brazil.[3] In addition to *Piper nigrun*, which is the most popular and widely used as a condiment, many other species have pharmacological, insecticidal, or other economic uses.[5]

The species *Piper aduncum*, belonging to the family Piperaceae is a plant of economic interest for the Amazon and that can be used in pest control. This species produces an essential oil called dilapiol, whose insecticidal effect was described by Maia et al. (1998).[6] Several studies have shown that this plant, besides the medicinal importance, as anti-inflammatory, anti-hemorrhagic, astringent, diuretic and others, also presents insecticidal, bactericidal and fungicidal activity.[6-12]

The essential oil of *P. aduncum* was tested against the fungus *Clinipellis perniciosa*, known as "Vassoura de Bruxa" (witch-broom), responsible for a pathogenic attack on cacao and cupuaçu. At the concentration of 50 to 100 ppm inhibited 100% growth and germination of this fungus.[13] Thus, the present work aims to evaluate

the phytochemical profile, cytotoxic and antioxidant activity of the leaves of *Piper aduncum* L.

## MATERIALS AND METHODS

### Plant Material

The plants were collected in the municipality of Laranjal do Jari, located in the southern region of Amapá, in a rural area near BR156. Tall, mature plants with a well-developed stem were removed from the root, from 3 pm to 4 pm.

### Samples preparation and obtaining the crude ethanoic extract (CEE)

The plant material was dried for 2 weeks in the open air, without sunlight. After drying, the leaves were crushed in a blender. The crushed material (300g) was subjected to the hot reflow method, followed by filtration and rotary evaporator concentration, to obtain CEE.

### Preliminary phytochemical analysis

With the dry extract of the plant the phytochemical tests were performed, in a total of 17 test were found 7 types of secondary metabolites. The phytochemical analysis was performed aiming the identification of the main secondary metabolites present in the CEE of the leaves of *P. aduncum*. 10 phytochemical tests (flavonoids, organic acids, phenols and tannins, steroids and triterpenoids, depsides, and depsidones, alkaloids, reducing sugars, saponins, resins and anthraquinones) were performed according to the methodology described by Barbosa (2001).[14]

### Antioxidant activity

The quantitative evaluation of the antioxidant activity was based on the methodology proposed by Sousa et al. (2007)[15], Lopes-Lutz et al. (2008)[16] and Andrade et al. (2012)[17] against the consumption of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with some adaptations to laboratory conditions. For the evaluation, 2.7 mL of the stock solution of DPPH was added in a test tube, followed by the addition of 0.3 mL of the essential oil solution. The blank was prepared, by a mixture of 2.7 mL of methanol and 0.3 mL of a methanolic solution of each concentration of the CEE evaluated. After 30 minutes, spectrophotometer readings (Biospectro SP-22) at the wavelength of 517 nm were performed. The assay was performed in triplicate and the calculation of the percentage of antioxidant activity (% AA) was calculated with the following equation[15]:

$(AA\%) = 100 - \{[(Abs_{sample} - Abs_{white}) \times 100] / Abs_{control}\}$

% AA = percentage of antioxidant activity

$Abs_{sample}$ = Sample Absorbance

$Abs_{white}$ = Absorbance of white

$Abs_{control}$ = Control Absorbance

### Cytotoxic activity with *Artemia salina* LEACH

The *A. salina* Leach cytotoxicity assay was based on the technique of Araujo et al. (2010)[18] and Lôbo et al. (2010)[19], with adaptations. An aqueous solution of synthetic sea salt (35.5 gL-1) was prepared for incubation of 25 mg of *A. salina* eggs, in which they were placed in the dark for 24 h for hatching of the larvae (nauplii), then the nauplii were exposed to artificial light in a period of 24 h to reach methanuplion stage. The mother solution was prepared to contain 54 mg of CEE, 22.5 mL of the solution of synthetic sea salt and 4.5 mL of 5% dimethylsufoxide (DMSO) to facilitate the solubilization of the solution.

The methanophores were selected and divided into 7 groups with 10 individuals in each test tube. Each group received aliquots of the stock solution (2500, 1875, 1250, 625, 250, 135 µL), which were then filled to 5 mL with synthetic sea salt solution to give final solutions with the following concentrations 1000, 750, 500, 250, 100, 50 $\mu g.mL^{-1}$. The tests were performed in triplicates. After 24 hours were counted the number of dead. The lethal concentration causing 50% mortality in the population ($LC_{50}$) was analyzed by the determination by Probit analysis using SPSS® software.

## RESULTS AND DISCUSSION

Preliminary results of the phytochemical study of CEE from leaves of *Piper aduncum* L showed the presence of phenols and tannins, organic acids, reducing sugars, saponins, anthraquinones and alkaloids (**Table 1**).

**Table 1: Results of CEE phytochemical prospecting of *Piper aduncum* L. leaves.**

| Class of secondary metabolites | Results |
|---|---|
| Phenols | + |
| Flavonoids | - |
| Tannins | + |
| Steroids and triterpenoids | - |
| Organic acids | + |
| Reducing sugars | + |
| Depsides and depsidones | - |
| Saponins | + |
| Resins | - |
| Anthraquinones | + |
| Alkaloids | + |

Parameters: (+)present, (-)absent

The phytochemical test was positive for the alkaloids, which according to Henriques (2010)[20] are organic nitrogen compounds that present relevant pharmacological activities, and they can be classified biogenetically, being the main classes: tropic alkaloids, indolic, pyrrolizidine, steroidal and methylxanthines. For Bacchi (2010)[21] the alkaloids inhibit the acetylcholine actions in autonomic effectors innervated by the parasympathetic pathway, which means that its action is antagonistic. This mechanism of action is mainly related to antimuscarinic effects that are correlated with its antispasmodic action on the gastrointestinal tract, and its frequent use in gastrointestinal disorders and peptic ulcers. To this metabolite, the literature has also associated effects on the sympathetic/noradrenergic pathway, for example, yohimbine has a hypotensive and

vasodilating effect on the peripheral circulation, it has also been used in cases of male impotence.[22,23]

Another secondary metabolite found in the CEE extract of *Piper aduncum* L. leaves were phenols and tannins. According to the literature[24], phenols are commonly used as flavorings, because many are constituents of volatile oils. They also have antioxidant properties, so that they can act by delaying the development of pathologies caused by oxidative reactions. Its expectorant action was first identified with the use of doguaiacol, which generated guaifenesin, a commercially used expectorant.[24] Tannins are characterized primarily by their ability to complex with other molecules, including macromolecules such as proteins and polysaccharides. Its effect on the healing process of wounds, burns, peptic ulcers is directly related to the formation of the tannin/protein complex, which generates a protective layer on the injured area, allowing the physiological process of healing to occur.[25] A potent antifungal action is associated with tannins, it is assumed that a process of enzymatic inhibition and complexation of the tannins to the membrane of the fungal cell occurs, thus compromising the cellular metabolism, and can thus be characterized as a fungistatic and/or fungicidal agent.[26]

The saponins also exhibit expectorant activity, but the mechanism is not well elucidated, possibly increasing the volume of the respiratory fluid, hydrating the bronchial secretion and, thus, facilitating the expulsion/expectoration of the mucus. These substances also have the ability to complex with steroids, which is why their antifungal properties.[27,28]

In relation to the antioxidant potential, the CEE of *P. aducum* showed a significant increase of the antioxidant activity according to the concentration increase, reaching the maximum value of 74.76% (**Table 2**) in the highest concentration (250 µg.mL$^{-1}$).

**Table 2: Antioxidant activity CEE of the *Piper aduncum*.**

| Ethanol extract concentrations (µg.mL-$^{1}$) | % AA |
|---|---|
| 7.81 | 13.61± 0.19 |
| 15.62 | 15.15±0.63 |
| 31.25 | 19±1.16 |
| 62.5 | 28.41±0.82 |
| 125 | 44.83±2.86 |
| 250 | 74.76±0.52 |

The amount of CEE tested required to decrease the initial concentration of DPPH by 50% was 150.163 µg.mL-1 coefficient of correlation (R2) of 0.9994. According to Ramalho and Jorge (2006)[29], natural antioxidants encompass a wide class of chemical compounds, which can act on different lipid oxidation media, donating protons, such as phenolic compounds, and/or chelating with oxidation reaction catalysts (synergistic), such as citric and ascorbic acids.

Studies by Pasos (2010)[30] showed that the ethanolic extract of *P. aducum* presented potent antioxidant activity, with 25.3% remaining DPPH, by the decoction extraction method when compared to the BHT control (24.69% remnant DPPH). According to the author, the antioxidant potential may be related to the chemical composition of the extract, which has secondary metabolites such as sesquiterpenes such as nerolidiol and α-caryophyllene.

The toxicity test with *A. salina* L. is a biological assay that consists in evaluating the susceptibility of this microcrustacean to the exposure of a certain constituent such as extracts, chemicals, pesticides, among others.[31] Amarante (2011)[32] classified both organic extracts and aqueous extracts as nontoxic if the $LC_{50}$ is above 1000 µg.mL$^{-1}$, those with low toxicity if the LC50 is higher than 500 µg.mL$^{-1}$, with moderate toxicity if the LC50 is between 100 to 500 µg.mL$^{-1}$ and very toxic if $CL_{50}$ is less than 100 µg.mL$^{-1}$.

**Table 3** shows the mean mortality performed in the 24 h period of the cytotoxic activity of the *P. aducum* CEE. The results showed that the CEE of *P. aducum* presented moderate toxicity to *A. salina*, with $CL_{50}$ 792.83 µg.mL$^{-1}$ and a correlation coefficient of ($R^2$ 0.8910).

**Table 3: Percentage of mortality of *A. salina* larvae at different concentrations of CEE of *Piper aduncum*.**

| Concentrations (µg.mL$^{-1}$) | Mortality % |
|---|---|
| Control | 0 |
| 50 | 0 |
| 100 | 0.2 |
| 250 | 2.33 |
| 500 | 11.058 |
| 750 | 27.81 |
| 1000 | 85.7 |

Studies carried out by Muller (2011)[33] showed that the ethanolic extract of *P. aducum* showed high toxicity to *A. salina*, with $LC_{50}$ of 48, 2 µg.mL$^{-1}$. However, *P. aducum* presented low toxicity, with LC50 of 792.83 µg.mL$^{-1}$. According to Ferreira et al. (2017)[34], toxic plants produce secondary metabolites which, by inhalation, ingestion or contact, can cause pathological changes in humans and animals, and in some cases can lead to serious disturbances in the body and even death.

## CONCLUSION

The CEE of the leaves of *Piper aduncum* presented as main chemical constituents the following classes of secondary metabolites: phenols and tannins, organic acids, reducing sugars, saponins, anthraquinones and alkaloids. Regarding the biological activities, the extract presented potent antioxidant activity, and low cytotoxic activity against *A. salina*.

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