# Phytochemical prospection of manioc leaves (*Manihot esculenta* Crantz).

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**Mayra Araújo da Cunha Leite**
Pharmacognosy and Phytochemistry Laboratory - Universidade Federal do Amapá – UNIFAP (Federal University of Amapá)

**Alice Mara Rosário da Costa**

**Ana Luzia Ferreira Farias**
Pharmacognosy and Phytochemistry Laboratory - Universidade Federal do Amapá – UNIFAP (Federal University of Amapá)

**Patrick de Castro Cantuária**
Instituto de Pesquisa Científicas e Tecnológicas do Estado do Amapá – IEPA (Institute of Scientific and Technological Research of Amapá State)

**Sheylla Susan Moreira da Silva de Almeida**
Pharmacognosy and Phytochemistry Laboratory - Universidade Federal do Amapá – UNIFAP (Federal University of Amapá)

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## 1 INTRODUCTION

Cassava or manioc (Manihot esculenta Crantz), belonging to the Euphorbiaceae family, is a tropical, perennial and woody plant that stands out for being one of the most important crops in the world in its multiple aptitudes: food, fresh or industrialized. In Brazil, country considered the probable center of origin and diversification of the species, manioc is the main component of a typical dish of high consumption in the North Region, the Maniçoba (VIEIRA et al, 2011; SILVA et al, 2013; SILVA et al, 2018).

Morphologically, manioc can reach up to 4 meters high and has cylindrical and woody branches interspersed between nodes and internodes. Its leaves are simple, lobed, formed by leaf blade and petiole. The number of lobes varies from three to eleven. The petiole varies from five to forty centimeters in length and its color varies from green to purple. Normally this plant produces four to eight tuberous roots of variable size and shape according to environmental conditions and cultivation. Thus, it is inferred that its morphology is very assorted, indicating high interspecific hybridization (SANTOS, 2022; TOMICH et al., 2008).

Its adaptability and hardiness to adverse weather conditions, arid and nutritionally poor soils, low production cost and resistance to pests, insects and water stress give it productive success (TOMICH et al., 2008; SOARES et al., 2016; SANTOS, 2022). According to data from the Food and Agriculture Organization of the United Nations (FAO) Brazil is the fourth largest producer of manioc in the world being Nigeria the first, followed by Thailand and Indonesia.

Manioc is used as raw material for various industrialized products such as flour, starch, beverages, starch, sweets, typical dishes, packaging, pellets, biodegradable films, mining, glues, textile and pharmaceutical industries. The foliage is the most nutritious part of maniva, rich in iron, zinc, manganese, magnesium, calcium, carotenoids, vitamins B1, B2, and C, and is even useful in the production of animal feed. (SOARES et al., 2016; ONO, 2020; VIEIRA et al., 2011; EBERTZ; PALOMINO, 2017)

This species stands out as one of the most toxic kinogenic plants in existence. Cyanogenic plants are those that contain cyanogenic glycosides, which when hydrolyzed release cyanide, hydrocyanic acid

(HCN). Linamarin and lotaustralin are cyanogenic glycosides found in the cell vacuole of manioc. After tissue lysis, the cell vacuoles are ruptured, releasing the glycosides that, in contact with the enzyme linamarase, which cleaves linamarin and lotaustralin, form free HCN. Cyanide, in turn, blocks the entire respiratory chain, leading to death by hypoxia. Others responsible for the cytotoxic effects of its leaves are anti-nutritive units that pose a risk to its consumption before being properly prepared, such as oxalic acid, hydrocyanic acid, nitrates, cyanide, trypsin inhibitors, phytate, polyphenols, and saponins (SILVA, 2016; SANTOS, 2022; FERRARO et al., 2016).

Even with its high levels of toxicity, certain pharmaceutical applications can be attributed to its elements, in the case of lectin (glycoproteins that bind to certain carbohydrates forming complexes that inactivate functional membrane receptors, causing agglutination) its use is beginning to be glimpsed in the area of oncology, immunology, hematology, mycology and bacteriology (SILVA et al., 2010).

The presence of characteristic secondary metabolites highlights manuka as a candidate for further research. Condensed tannins, as well as saponins and flavonoids, are markers of interesting pharmacological actions, such as body stimulants, anti-inflammatory and antioxidant (BUSTOS et al., 2012).

Although there are several ways to use manioc leaves, their applications are very restricted to animal feed, leaving aside all the potential that this species can offer. It is noteworthy that one of the technological innovations of manioc is its use to combat herbivorous pests, where a methane extract of its organic compounds was able to reduce or even eliminate insect populations that attacked plantations (SANTOS et al., 2013).

It is worth remembering that theoretical bases described in the literature, describe manioc leaves as waste or production waste in crops and plantations, ruling out its employment and bioavailability in new technological applications such as preparation of insecticides, development of alternative ethane fuels and even in the treatment of industrial effluents by its presence of tannins, which act as flocculants agents. Thus, it is evident the need for further studies and methodologies for the absolute use, reduction of production and cultivation waste, as well as the dissemination of new scientific applications discovered in research on the species cited.

The objective of this research is the extraction of the active principles from the leaves of Manihot esculentaas well as its phytochemical analysis in the prospection of its secondary metabolites in search of its medicinal use in the treatment of symptoms of human diseases, developing new methodologies for the evolution of its possible uses.

## 2 METHODOLOGY

The methodology for the realization and obtainment of the ethanolic extract of maniva was done in a series of steps, from obtaining the raw material (leaf portions) to the final phytochemical and cytotoxic tests, passing through physical and chemical instances.

The collection of maniva leaves occurred in the Jardim Felicidade neighborhood, in the northern part of the municipality of Macapá, Amapá State. Leaves of the same age and with the same nutritional nature (same color, size, visual aspect, without traces of herbivory, magnesium or phosphorus deficiency) were selected. Then, the collection site was cataloged through geographic location by GPS, 0º5.491'N Latitude and 51º2.943'0 Longitude. Afterwards, the natural drying process was initiated by placing the leaves on newspapers, spread out in a nine square meter room avoiding direct exposure to sunlight for one week. During the day they were left with natural ventilation and during the night there was no incidence of any artificial light source, aiming to avoid processes that could compromise the samples.

After one week, some leaves were curled on the apical and lateral portions, while others did not present this aspect. The samples, in their entirety, were taken to the greenhouse in newspaper envelopes, where they remained at an average temperature of 60 ºC, with 30 minute intervals to check if the leaves were already dry. The process was repeated several times over a period of three days.

After the collection, it was weighed on a 100kg scale, obtaining a total gross weight of 3.8kg. With this the refined weight, without water or humidity, was measured in a semi-analytical scale, resulting in 575.6 grams (enough for the beginning of rotavaporization and phytochemical analysis). It is worth mentioning that the total mass was not properly utilized, part of the material was compromised by fungus action, while others were outside the standard selection profile of the cataloged leaves. All the shredded material was collected and forwarded to a precision scale, where the entire sample was weighed.

In the following process a plastic container with a lid, with a capacity of more than two and a half liters, was used, and all the plant material was added, with two liters of 96% ethyl alcohol, for the adsorption of the secondary metabolites, organic compounds and active principles of the sample. For the ratio of matter to alcohol, three hundred grams to one liter of alcohol was made. This preparation was kept at room temperature and away from direct sunlight, and stirred every 24 hours.

The extract was obtained using the rotaevaporation method, which consists of a two-phase system of physical transformations. In the first phase, the crude extract is placed in a flask, which will be heated to an average temperature of 60 degrees Celsius, under rotation and in a water bath, passing the gases that come out of the extract to another system, through a glass tube. In the second phase, the vaporized is condensed, thanks to a pressure reduction imposed by a vacuum pump coupled to the rotary evaporator system that, in turn, will be adding water at low temperatures in an ascending circular column. This process allows the water to exchange heat with the gases that were evaporated in the previous phase, causing the change to the liquid state, which is collected by another flask coupled below the condensing system. To obtain the refined extract, the rotaevaporation process must be done in triplicate. After all this process, the mixture becomes extremely concentrated and in a smaller quantity, being necessary to use the concentrate of the triplicates together (10.66 grams) to prepare the solid principle and perform the phytochemical tests.

## 3 CONCLUSION

The phytochemical analysis showed positive results for four classes of secondary metabolites: saponins, phenols and tannins, steroids and triterpenoids, and coumarin derivatives, described in Table 1.

Table 1: Phytochemical tests of the hydroalcoholic extract of Manihot esculenta.

| Phytochemical tests | Results |
|---|---|
| Saponins | + |
| Organic acids | - |
| Reducing sugars | - |
| Polysaccharides | - |
| Proteins and amino acids | - |
| Phenols and Tannins | + |
| Flavonoids | - |
| Purines | - |
| Catechins | - |
| Steroids & triterpenoids | + |
| Depsides and Depsidones | - |
| Coumarin Derivatives | + |
| Anthraquinones | - |

Parameters: Positive: (+); Negative: (-).

Saponins are glycosides of the secondary plant metabolism, characterized by the formation of foam when subjected to agitation, besides having detergent, surfactant, and emulsifier properties. They are compounds formed by a hydrophilic part, which interacts with water, and a lipophilic part, which has a strong attraction to lipids. Their laboratory identification can be done with blood hemolysis tests (in vitro), or even by a simple dilution of the extract in water and stirring the mixture for a few minutes, leaving it resting soon after the process. A much discussed action about saponins, is their hemolytic capacity of erythrocytes, the latter being justified by the ability of the glycoside to combine with the cholesterol molecules present in the erythrocyte membrane, disturbing the internal-external balance, promoting the rupture of the cell, with consequent release of hemoglobin. In addition, they are known for their anti-inflammatory, healing, antibacterial, antifungal, antiparasitic, diuretic, and expectorant properties. In plants, it can play protective roles against herbivory by insects or other beings that may feed on the plant (DE OLIVEIRA et al., 2008; BARBOSA et al., 2021; MARQUES et al., 2021).

Due to their reducing properties and their chemical structure, phenolic acids are recognized for their antioxidant activity, since they are able to neutralize free radicals and act in the chelation of transition metals. This property provides protection against various diseases caused by oxidative stress (CARVALHO; GOSMANN; SCHENKEL, 2007; SOUSA, 2007). Merlin and collaborators (2017) in a study on phenolic compounds in plants also reported the antidepressant and anti-inflammatory activity of this substance. In cassava stands out the presence of condensed tannins or catechins, which develop antinutritive role and cause bitter taste and irritability of insect mucous membranes, preventing it from being consumed (MONTEIRO et al., 2005).

In the tests were also detected triterpenes, compounds with potent antibacterial, antiviral, fungicidal, analgesic, antipyretic, hepatoprotective and anti-inflammatory activity, which undergo several decarboxylations giving rise to steroids. These, in turn, also have numerous pharmacological activities highlighting the decrease in blood cholesterol levels and the decrease in the development of cardiovascular problems (RODRIGUES, 2010; COELHO 2021; RODRIGUES 2018;

Coumarins are heterosides that exhibit numerous properties. Among them is the anticoagulant action of dicumarol, a substance derived from 4-hydroxy-coumarin, which was discovered during the investigation of a hemorrhagic disease in cattle fed fermented yellow clover (Melilotus officinalis Lam). From this drug a class of anticoagulant drugs was developed, from which several others, such as warfarin, were derived. Furthermore, immunosuppressive, antispasmodic, antibacterial, vasodilatory, antithrombotic, and antioxidant activities from coumarins are also described in the literature.. (KUSTER; ROCHA, 2007)

The phytochemical analysis allowed the identification of groups that favor its use in the most diverse productive segments as a cheap and easy-to-acquire product that can be used as an agricultural input. Therefore, the multifaceted nature of this plant stands out, as well as its richness, bioavailability in the northern region of Brazil and abundance in the Amazon rainforest, as well as other states of the country, which facilitates research in the most diverse fields and increases the pool of national technological material. There are few reports and studies about manioc within the current literature, but it has a great biological potential in the development of insecticides, pest control, plantation aid with rotation and food supplementation of animals involved in agriculture. In the pharmaceutical industry, new formulations can be considered due to the quantity and variety of metabolites present in this plant.

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