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Phytochemical analysis of the leaves of the *Mussaenda alicia* plant

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1 INTRODUCTION

Studies in the area of natural products have been developed with the biomonitoring of their plant extracts, a fact that improves research on bioactive substances of economic interest. Therefore, there is a demand for scientific studies involving plants with biological activity, so that such practice should be encouraged, forming a promising and effective path for the discovery of new drugs. These new products can bring foreign exchange, besides offering opportunities for job creation along the production chain, not only in the urban area, but, above all, in the rural area, contributing to the deconcentration of income and, therefore, to the internalization of the development of the Amazon (SILVA, 2013).

The use of plant species for the treatment and cure of certain diseases is a practice as old as the human being, who seeing being passed from generation to generation in the treatment of numerous diseases, which is evident that knowledge about medicinal plants represents, "often, the only therapeutic resource of various communities and ethnic groups" (FIRMO et al., 2011, 92).

In Brazil, the Ministry of Health (MS) determines to prioritize the investigation of medicinal plants and the implementation of phytotherapy as an official practice of medicine, because in view of the country's biodiversity and in order to improve the health of the population, the Ministry of Health began to invest in the use of phytotherapy as a complement to the SUS. However, for this to happen adequately and safely, it is indispensable that trained professionals understand the chemistry, toxicology and pharmacology of medicinal plants, in addition to the active ingredient, taking into account popular knowledge (SANTOS, et al., 2011).

It is emphasized that plants are complex living beings and, as such, have a formidable metabolism, which induces the production of a wide variety of chemicalsubstances, such as: proteins, lipids, carbohydrates, in addition to nucleic acids that are common to all living beings, which influence the growth, reproduction and maintenance of vegetables (SILVA, 2009).

However, a high number of chemical compounds produced by plants have other purposes, such as: pigments (flavonoids, anthocyanins and betalains) and essential oils (monoterpenes, sesquiterpenes and phenylpropanoids) approximate pollinators; tannins, sesquiterpene lactone, alkaloids and iridiums present unpleasant flavors and can be toxic and irritating to other organisms. These substances work as food deterrents and protect plants from predators and pathogens (CAMPOS, et al., 2016).

The *Rubiaceae family* is the largest of the *order Gentianales* and involves about 640 genera and approximately 10,700 species, classified into four subfamilies (*Cinchonoideae, Ixoroideae, Antirheoideae and Rubioideae*) and 44 tribes, essentially tropical, being considered the fourth largest family of Angiosperms. Several genera are endemic to the neotropical region, which exhibits about 4,555 species. *Psychotria* L. is the largest genus in the family, represented worldwide by about 1,650 species. For Brazil, about 96 genera are considered, and for the Northeast region, there are 66 genera and 277 species, and the other (30 genera) are distributed in the other regions of the country (PEREIRA; BARBOSA, 2004).

Several species of *Rubiaceae up* to economic value, being used in the food, ornamental aspect, as well as in the pharmaceutical industry, as medicinal and/or toxic. It is noteworthy that the rubiaceae family stands out for the production of bioactive alkaloides that generate several drugs, which are still analyzed as chemotaxonomic markers of some subfamilies and genera. The amount of products described, their structural variety and pharmacological activities make alkaloides, along with antibiotics, one of the most important groups among natural substances with therapeutic importance (SOUZA; MARIE; SILVA, 2013).

This species differs from the other by dense branching, acute leaf apex and obtuse base, inflorescence with 27-45 flowers and corolla lobes with acute apex, blooming from November to September and, fruiting in the months of February to September. Therefore, choosingthe plants to be researched makes it necessary to take into account botanical and chemotaxonomic information, because the probability of finding bioactive substances are unpublished or already described in the literature is much higher. Such less exploited or unexplored sources of biodiversity are frequently related to the new chemical diversity (SANTOS; SANO, 2012).

Phytochemical research has shown that rubiaceal alkaloides are part of more than 10 distinct classes, especially in isoquinonicos, with 44 substances described; quinycinics, with 70 alkaloides; indolic compounds with 391 isolated compounds. It is emphasized that indole alkaloids are fundamental chemical markers of this family, because in addition to the compounds mentioned, aglycones and heterosides of iridoids, anthraquinoes, triterpenic saponin, flavonoids, lignoids, terpenoids and phenolic derivatives were still confirmed (MORAES, 2013).

Considering the chemical profile of the *Rubiacea* family, they have many species without any study, which prevents taxonomists from performing divisions in the family and subfamily. Thus, the in-depth knowledge of *the Rubiaceae family*, of great metabolic diversity and pronounced pharmacological potential can open perspectives for the chemistry, pharmacology and chemotaxonomy of this family, because these studies are an important tool for the chemotaxonomic classification of species within genera (FERREIRA JÚNIOR; VIEIRA, 2015).

The genus *Mussaenda* belonging to this family covers about 200 species, which are native to Tropical Africa, Asia and the Pacific Islands. Many of these species are used as ornamental shrubs, due to the fact that their showy and colorful bracts are similar to white sepals, bordered (white with pink margins), pink or red with yellow flowers, in addition to their pleasant fragrance (FERREIRA JÚNIOR; VIEIRA, 2015).

This class has few phytochemical studies carried out until present. However, Zhao et al., (apud NASCIMENTO; SOARES; VALVERDE, 2017), investigating the natural products of *the species Mussaenda pubescens, identified* substances belonging to the class of triterpene saponins, in addition to some mono and triterpenes. The species *Mussaenda alicea*, botanical synomy botânica *Mussaenda erythrophylla rosea*, is popularly known *as mussaenda-rosa, mussaendarosa-arbustiva, mussaendarosa-arbustiva*, and it is verified that the hydroalcoholic extract *of M. alicia* has a high antioxidant activity.

In view of the above, this article presents as objective to evaluate the phytochemical profile of *the crude extract of M. erythrophylla rosea* and its partitions by qualitative tests, as well as to correlate the observed antioxidant activity, the concentration of total phenolics.

2 METHODOLOGY

2.1 CHARACTERIZATION OF THE COLLECTION SITE

On May 19, 2017, between 20 and 21h, the first collection of tree leaves located in Floriano Peixoto Square in Macapá - AP - Brazil was performed.

On May 27, 2017, around 3:30 p.m., the second collection was carried out in a private plantation, located on Avenida Pedro Lazarino in Macapá - AP - Brazil.

Five branches were used to make the exsicates to be sent for identification by specialists in the Herbarium Amapaense (HAMAB) of the Institute of Scientific and Technological Research of the State of Amapá, in Macapá - AP - Brazil.

Presses, newspapers and cardboards were used to assemble the exsicate, which were placed overlapping with each other.

2.2 EXPERIMENTAL PROCEDURE

For the composition of the phytochemical study, the extract was obtained according to the steps described below:

Obtaining the gross extracts

a) The leaves of the first collection were washed and dried at room temperature for 5 days and subsequently dried at 45±1º C, in a recirculating air oven for 30 minutes; The leaves from the second collection were taken directly to the greenhouse on June 7, 2017, as they did not require washing.

b) The botanical material of the first collection was manually crushed on May 24, 2017 and the material of the second collection, on June 7, 2017.

c) The extraction method used was macerated, where 327 g of plant material was placed together with 3L of alcohol 92.8°c, at room temperature, with occasional agitations, in a glass container closed for 3 days and then filtration.

The process was repeated 3 times, changing only the amount of alcohol, which became 2L in the last two times, due to the decrease in the volume of plant material.

d) On June 8 and 12, the evaporation route of the filtrates was made

e) Thus obtaining the alcoholic crude extract of the leaves of *Mussaenda alicia* (117.02g).

Phytochemical screening

According to Barbosa (2004) the methodology for the implementation of phytochemical studies occurred as follows:

3 CONCLUSION

From 327g of plant matter, 117.02g of crude alcoholic extract was obtained, which presented black and pasty appearance.

The phytochemical study of the raw alcoholic extract of *the leaves of Mussaenda Alicia* showed positive results for the presence of secondary metabolites such as saponins, reducing sugars, alkaloids, purines, azulenos, depsidios and depsidons and coumarin derivatives, Negative results were also observed for organic acids, polysaccharides, proteins and amino acids, phenols and tannins, flavonoids, cardiac glycosides, catechins, sesquiterpenolacones, steroids and triterpenoides, carotenoides and anthraquiones. (Table 1). However, these results do not state that there is a presence or absence, since there are divergences about extraction techniques, collection period and oversights associated with the legitimacy of plant material (MARQUES et al. 2012)

Table 1 - Data from phytochemical analysis of secondary metabolites.

Secondary metabolite	Result
Saponins	+
Organic acids	-
Reducing sugars	+
Polysaccharides	-
Proteins and amino acids	-
Phenols and tannins	-
Flavonoids	-
Alkaloids	+
Purines	+
Cardiac glycosides	-
Catechins	-
Sesquiterpenolatones	-
Steroids and Triterpenoids	-
Azulens	+
Carotenoides	-
Depsidia and Depsidones	+
Coumarin derivatives	+
Anthraquinonas	-

+ Presence; He's out of absence.
Source: Eduardo Kauê Mota Pantoja

Generally, secondary metabolites that occur in low concentration do not directly participate in plant growth and development. The production and storage of these metabolites comprise the defense against attacks by herbivores and pathogens and varies according to variations in climatic conditions such as water availability, radiation, temperature and light conditions.

According to Cartejon (2011) there are numerous lines of research that confirm that saponins are found as an active ingredient of various plant extracts and that to obtain a positive result in relation to this secondary metabolite it is necessary that, in aqueous solution, it occurs to the permanence of foam in abundance. In the research by Vieira et al., (2001) saponins are usually glycoid triterpenoids consisting of aglycone (sapogenol) linked to one or more units of sugar.

The importance of this class of metabolites is the result of industrialization, being possible to find it in food, textile, cosmetic and mainly pharmacological sectors, in view of the action of antiplatelet, hypocholesterolemic, antitumor, immunoadjuvant, anti-inflammatory, antibacterial, insecticide, fungicide and leishmanicide (COSTA, 2014).

From an in vitro assay to verify the effect of saponins present in yerba mate with bile acids and cholesterol, FERREIRA et al. (1997 apud CASTEJON, 2011) concluded that there is a decrease in these acids and increased excretion, and therefore part of the cholesterol in the bloodstream would be diverted to supply its deficiency in bile.

Saponins are important for the action of plant drugs, especially those traditionally used as expectorant and diuretics. However, the mechanism of action of these drugs is not well understood. Some authors argue that irritation in the respiratory tract would increase the volume of respiratory fluid and reduce

its viscosity. Another possibility would be related to its superficial tension originating, lower viscosity and greater ease of mucus expulsion. Diuretic activity is attributed to irritation of the renal epithelium caused by saponins. However, in a study conducted by DINIZ (2006), triterpene saponins reduced urinary flow in rats.

The likely mechanism would be increased water resorption in the recurrent tubules, since there was an increase in the activity of the atpases. Other posted jobs are as adjuvants to increase the absorption of medicines by increasing solubility or interference in absorption mechanisms and as an adjunct to increase the immune response (CASTEJON, 2011).

According to Demiate et al., reducing sugars are efficient carbohydrates in the reduction of cationic ions, such as copper and iron, in alkaline solutions and are characterized by compute in their structure clusters of aldehydes or free ketonas. These sugars are represented by monosaccharides, such as glucose and fructose; and certain types of disaccharides, such as maltose, derived from glucose, and lactose formed from galactose and glucose.

Leaf sugars influence the metabolic state of photosynthesis and/or the translocation capacity of these to the reserve tissues (ZIELINSKI et al. 2009) and in circumstances involving water deficit, as it causes an increase in sucrose synthesis that collaborates with osphototic regulation without the inhibition of photosynthesis (ROSÁRIO; ALMEIDA, 2016)

Alkaloids are natural compounds that can form from amino acids or terpenes and sterols and constitute a heterogeneous group, having a complex structure. In its chain are present carbon, hydrogen and nitrogen that mostly form an oxygenated heterocyclic ring (CABRAL; PITA, 2015)

According to the America Dietetic Association (2014 apud SILVA, 2005) the definition of alkaloid is based on a substance identified in vegetables, and when consumed in correct amounts, it has devices for the maintenance of human metabolism, gaining the title of chemopreventive because they have prophylactic actions such as inhibiting cancer cells and acting as myorelaxant, aiding the excretion of kidney stones.

Purines have a central biosynthesis pathway in the metabolism of any organism being precursors of DNA, RNA and important cofactors such as Coenzyme A, FAD, NAD and NADP. In plants this pathway is of great relevance, since their products (IMP, AMP and GMP) are precedents of cytokinins, uredids, compounds involved in symbiotic fixation/nitrogen storage in legumes and alkaloids such as theobromine and caffeine (FEITOSA et al. 2005).

According to Marque et al. (2011) Azulene is an isomer of naphthalene that has some pharmacological properties. Classified as an essential oil, it is present in plants and can serve as an anti-inflammatory action, such as chamomile for example.

According to Medeiros (2010 apud ROSARIO; ALMEIDA, 2016) depsidonas and depsidonas are policetides that derive from the dehydration and formation of a cyclic chain of orselinic acid. These metabolites are related to anti-inflammatory and antibiotic effects and are therefore being studied so that their synthesis can be used for pharmacological purposes.

With regard to coumarin derivatives, they are found everywhere in a plant. Because they have a lactating ring, when extraction occurs in alkaline medium, this ring opens, which results in obtaining substances in the form of water soluble salts. However, studies have revealed that such substances can be toxic to be consumed, considering their addition to food as adulteration (SIMOES, 2012).

In the pharmacological area, the derivatives of 4-hydroxycoumarin, the first drug with anticoagulant action of dicumarol, which derive other drugs such as warfarin among others, stand out. In addition, coumarins also present vasodilator activity, indicated for the treatment of male impotence. Several other studies have already evidenced important pharmacological activity presented by xanthones that have inhibitory action of the enzyme monoaminooxidade, linked to the treatment of depressive conditions (SIMOES, 2012).

Coumarins containing dihydroxylated groups in orthoposition such as frax etin 10 (7,8-dihydroxi-6-methoxy-cumarin), esculetin 11 (6,7-hydrox i-coumarin and 4 -methylettagedin12 (6,7-dihydroxyl-4-methylcoumarin) are powerful inhibitors of lipid peroxidation, in addition to eliminating superoxide radical anion and squeeaking iron ions. These properties make them substances of interest as antioxidants, of possible application in the prevention of diseases caused by free radicals (SIMOES, 2012).

Through phytochemical tests performed, it was possible to identify the presence of some classes of secondary metabolites, such as saponins, reducing sugars, purines, coumarin derivatives, depsidios and depsidonas, as determined by the objective of this study. Classes that are interested in pharmacology, thus allowing the discussion of scientific knowledge of the plant *Mussaenda Alicia.*

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