Phytochemical analysis of the leaves of the mussaenda alicia plant

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Eduardo Kauê Mota Pantoja
Laboratory of Pharmacognosy and Phytochemistry - Federal University of Amapá – UNIFAP

Segundo Matheus Da Silva Sardinha
Institutional affiliation of the second author

Ana Luzia Ferreira Farias
Federal Institute of Amapá – IFAP

Patrick de Castro Cantuária
Institute of Scientific and Technological Research of the State of Amapá – IEPA

Sheylla Susan Moreira da Silva de Almeida
Laboratory of Pharmacognosy and Phytochemistry - Federal University of Amapá – UNIFAP

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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 this practice should be encouraged, forming a promising and effective path for the discovery of new drugs. These new products can bring in foreign exchange, in addition to offering opportunities to generate employment along the production chain, not only in the urban area, but, above all, in the rural area, collaborating for the deconcentration of income and, therefore, for the interiorization of the development of the Amazon (SILVA, 2013).

The use of plant species for the treatment and cure of certain illnesses is a practice as old as human beings, which has been passed down from generation to generation in the treatment of numerous diseases, which makes it clear that knowledge about medicinal plants, it represents, “often, the only therapeutic resource of several communities and ethnic groups” (FIRMO et al., 2011, p. 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 with the aim of improving the health of the population, the MS started to invest in use of phytotherapy as a complement to the SUS. However, for this to happen properly and safely, trained professionals are indispensable who understand the chemistry, toxicology and pharmacology of medicinal plants, in addition to the active principle, taking into account popular knowledge (SANTOS, et al., 2011).

It should be noted that plants are complex living beings and, as such, have a formidable metabolism, which induces the production of a wide variety of chemical substances, such as: proteins, lipids, carbohydrates, in addition to the nucleic acids that are common to all living beings, which influence the growth, reproduction and maintenance of plants (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) bring pollinators closer together; tannins, sesquiterpene lactones, alkaloids and iridoids have unpleasant flavors and can be toxic and irritating to other organisms. Such substances work as food deterrents and protect plants against predators and pathogens (CAMPOS, et al., 2016).

The Rubiaceae family is the largest of the Gentianales order 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 has about 4,555 species. Psychotria L. is the largest genus of the family, represented worldwide by about 1,650 species. For Brazil, about 96 genera are considered, and for the Northeast region, 66 genera and 277 species are found, and the others (30 genera) are distributed in the other regions of the country (PEREIRA; BARBOSA, 2004).

Several species of Rubiaceae have economic value, being used in food, ornamental, 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 alkaloids that generate various drugs, which are still analyzed as chemotaxonomic markers of some subfamilies and genera. The number of products described, their structural variety and pharmacological activities make alkaloids, along with antibiotics, one of the most important groups among natural substances with therapeutic importance (SOUZA; MENDONÇA; SILVA, 2013).

This species differs from the others due to its dense branching, acute leaf apex and obtuse base, inflorescence with 27-45 flowers and corolla lobes with an acute apex, flowering from November to September and bearing fruit from February to September. Therefore, when choosing the plants to be researched, it is necessary to take into account the botanical and chemotaxonomic information, since the probability of finding bioactive substances, whether unpublished or already described in the literature, is much greater. Such less explored or unexplored sources of biodiversity are often linked to new chemical diversity (SANTOS; SANO, 2012).

Phytochemical research has shown that rubiaceae alkaloids are part of more than 10 distinct classes, with emphasis on isoquinolines, with 44 substances described; the quinilines, with 70 alkaloids; the indoles with 391 isolated compounds. It should be noted that indole alkaloids are key chemical markers of this family, as in addition to the aforementioned compounds, aglycones and heterosides of iridoids, anthraquinones, triterpene saponin, flavonoids, lignoids, terpenoids and phenolic derivatives have also been confirmed (MORAES, 2013).

Considering the chemical profile of the Rubiacea family, there are many species without any study, which prevents taxonomists from making family and subfamily divisions. 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, as these studies are presented as an important tool for the chemotaxonomic classification of species within genera. (FERREIRA JÚNIOR; VIEIRA, 2015).

The genus Mussaenda belonging to this family comprises 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 performed so far. 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 saponins of the triterpene type, in addition to some mono and triterpenes. The species Mussaenda alicea, botanical synonym Mussaenda erythrophylla rosea, is popularly known as mussaenda-rosa, mussaendarosa-arbustiva, mussaenda-arbustiva, and it was verified that the hydroalcoholic extract of M. alicia has a high antioxidant activity.

Given the above, this article aims to evaluate the phytochemical profile of the crude extract of M. erythrophylla rosea and its partitions through qualitative tests, as well as to correlate the observed antioxidant activity, the concentration of total phenolics.

2 METHODOLOGY

2.1 CHARACTERIZATION OF THE COLLECTION PLACE

On May 19, 2017, between 8 pm and 9 pm, the first collection of leaves from the tree located at Praça Floriano Peixoto in Macapá - AP - Brazil was carried out.

On May 27, 2017, around 3:30 pm, 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 specimens 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.

To assemble the specimen, presses, newspapers and cardboard were placed on top of each other.

2.2 EXPERIMENTAL PROCEDURE

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

2.2.1 Obtaining raw extracts

a) The leaves from the first collection were washed and dried at room temperature for 5 days and subsequently dried at 45±1° 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 from the first collection was manually crushed on May 24, 2017 and the material from the second collection on June 7, 2017.

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

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

d) On the 8th and 12th of June, the evaporation route of the filtrates was carried out

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

2.2.2 Phytochemical screening

According to Barbosa (2004) the methodology for carrying out the phytochemical studies was as follows:

3 CONCLUSION

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

The phytochemical study of the crude alcoholic extract of Mussaenda Alicia leaves showed positive results for the presence of secondary metabolites such as saponins, reducing sugars, alkaloids, purines, azulenes, depside and depsidones and coumarin derivatives. Negative results were also observed for organic acids, polysaccharides, proteins and amino acids, phenols and tannins, flavonoids, cardiac glycosides, catechins, sesquiterpenolactones, steroids and triterpenoids, carotenoids and anthraquinones. (Table 1). However, these results do not necessarily state that there is presence or absence, since there are divergences about extraction techniques, collection period and carelessness associated with the legitimacy of the plant material (MARQUES et al. 2012)

Table 1 – Data from the phytochemical analysis of secondary metabolites.

Secondary metabolite	**Results**
Saponins	+
Organic acids	-
Reducing sugars	+
polysaccharides	-
Proteins and amino acids	-
phenols and tannins	-
flavonoids	-
alkaloids	+
Purines	+
cardiac glycosides	-
Catechins	-
sesquiterpenolatones	-
Steroids and Triterpenoids	-
Azulenos	+
Carotenoids	-
Depsides and Depsidones	+
coumarin derivatives	+
anthraquinones	-

+ Presence; - 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 to be the active principle of various plant extracts and that, in order to obtain a positive result in relation to this secondary metabolite, it is necessary that, in aqueous solution, the permanence of foam in abundance. In the research by Vieira et al., (2001) saponins are normally glycosidic triterpenoids constituted by aglycone (sapogenol) linked to one or more sugar units.

The importance of this class of metabolites is a result of industrialization, and it is possible to find it in the food, textile, cosmetics and mainly pharmacological sectors, in view of the antiplatelet, hypocholesterolemic, antitumor, immunoadjuvant, anti-inflammatory, antibacterial, insecticide, fungicide and leishmanicidal (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 an increase in their excretion, and, therefore, part of the cholesterol in the bloodstream would be diverted to supply its deficiency in the bile.

Saponins are important for the action of plant drugs, especially those traditionally used as expectorants 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 the respiratory fluid and reduce its viscosity. Another possibility would be related to its originating surface tension, lower viscosity and greater ease of expulsion of mucus. The diuretic activity is attributed to irritation of the renal epithelium caused by saponins. However, in research carried out by DINIZ (2006), triterpene saponins reduced the urinary flow in rats.

The probable mechanism would be the increase in water reabsorption in the renal tubules, since an increase in the activity of renal ATPases was verified. Other outstanding uses are as an adjuvant to increase drug absorption by increasing solubility or interfering with absorption mechanisms, and as an adjuvant to increase the immune response (CASTEJON, 2011).

According to Demiate et al. (2002) reducing sugars are efficient carbohydrates in the reduction of cationic ions, such as copper and iron, in alkaline solutions and are characterized by composing groups of aldehydes or free ketones in their structure. 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.

The sugars in leaves influence the metabolic state of photosynthesis and/or the ability to translocate these to reserve tissues (ZIELINSKI et al. 2009) and in circumstances involving water deficit, as it causes an increase in sucrose synthesis that collaborates with the osmotic regulation without inhibiting 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, earning the title of chemopreventives for having prophylactic actions such as inhibiting cancer cells and acting as a muscle relaxant, helping the excretion of kidney stones.

Purines have a central biosynthetic 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 its products (IMP, AMP and GMP) precede cytokinins, ureides, compounds involved in the symbiotic fixation/storage of nitrogen in leguminous plants 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) depsides and depsidones are polyketides 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 throughout a plant. Because they have a lactonic ring, when extraction occurs in an 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, starting to consider their addition to food as adulteration (SIMOES, 2012).

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

Coumarins containing dihydroxylated groups in ortho position such as fraxethin 10 (7,8-dihydroxy-6-methoxycoumarin), esculetin 11 (6,7-dihydroxycoumarin and 4-methylesculetin 12 (6 ,7-dihydroxy-4-methylcoumarin) are powerful inhibitors of lipid peroxidation, in addition to eliminating the superoxide radical anion and chelating iron ions. These properties make them substances of interest as antioxidants, with possible application in the prevention of diseases caused by free radicals (SIMOES, 2012).

Through the phytochemical tests carried out, it was possible to identify the presence of some classes of secondary metabolites, such as saponins, reducing sugars, purines, coumarin derivatives, depside and depsidones, as determined by the objective of this study. Classes that are of interest to pharmacology, thus allowing the discussion of the scientific knowledge of the Mussaenda Alicia plant.

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