



Phytochemical analysis of hydroalcoholic extract dry leaf *calycophyllum sp.* (Pau-mulato)

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1 INTRODUCTION

The importance of plants is unseen by people, through research or only by their own knowledge. Natives used and still use the plants for various daily activities, such as therapeutic activity (PAULA, 2006), treatment of diseases and complementary feeding (CATARINA, 2015). At the beginning of its exploration, when no phytochemical knowledge about plants was known, its pharmacological activity was related through the similarity between it and some anatomical part of the human body. Thus, studies and deductions about plants began at a time when the conditions for such a study were minimal.

In Brazil, the extraction of vegetables began soon after its discovery by the Portuguese in 1500, until then its use was by the natives. In 1503 the first shipment of *Caesalpinia echinata* (Pau-brasil) was taken to Portugal, this transport generated enormous profits for the Portuguese court, and thus began the extraction of vegetables in Brazil, without any kind of ecological awareness, aiming only at the portugal's maximum profit (MENDONÇA, 2007). With the increase in the movement of people with the objective of extracting the material at any cost, there was no concern in carrying out the extraction in a sustainable way, with this, the brazilwood is on the list of endangered species (AGOSTINHO, 2007; HOGAN, 2005). One concept that highlights this thought very well is: "It is development that meets current needs without compromising the ability of future generations to meet their needs" (WECD, 1987).

Because of this troubled exploration beginning that occurred early on, nowadays it is possible to perceive its results. Brazil has approximately 19% of the world's flora, but at the same time suffers from illegal exploitation, a fact that proves this is the Atlantic Forest, which is currently considered one *hotspot*, a place of conservation due to its high degree of deforestation and shelter of endemic



species, because it has less than 10% of the area it once occupied (GIULIETTI, 2005; HIROTA, 2005; BITENCOURT, 2014). Analyzing all these variables, for the accomplishment of this work, a plant was chosen that was not among the endangered list and consecrated within popular knowledge.

Popularly known by the name of mulatto stick, mulateiro and also monkey slide, its taxonomy is characterized in the kingdom Plantae, division Magnoliopsida, order Rubiales, family Rubiaceae, genus *Calycophyllum* (PERÚ, 2009). It is found throughout the Amazon region from Brazil to countries such as Bolivia, Peru, Ecuador, and Colombia. It can reach around 35 meters high and 1.5 meters in diameter, the leaves have a simple appearance, glabras, which means devoid of trichomas, approximately 15 centimeters long by 6 centimeters wide, with the base slightly weakened (SCHUM, 2004). An important feature of this plant is the ability to remain in lowland areas, which are constantly flooded and deficiency in the oxygen supply. This capacity is based on the fact that the tree has an efficient system of ecophysiological adaptation through the kaolin lenticelas that assist the plant in the intense gas exchange, thus helping the very low exchange of gases that flows in the roots of the plant (SOUSA, 2011).

In the popular use aspect of the plant, the mulatto stick is mainly used to treat discharge. Discharge in popular language is an inflammatory or infectious process that occurs mainly in women in the genital region, in which there is the production of a darker-colored cervical fluid (FERRACIN, 2005). In addition, according to Jean (2011) the discharge is related to the change in consistency, quantity or smell and also through chemical or phytic irritation by soaps for example.

In addition to treating discharge the pau-mulatto is widely used in folk medicine using all its parts, where, from its bark is made a plaster, of topical use, which has as function p treatment of wounds, cuts and burns. From its cortex is made a tea that has action against diabetes, ovarian diseases, in the stomach and intestine, the sap taken from the stem has antioxidant action, antiparasite, antibacterial, repellent and insecticide and the tea made with its bark também is used by indigenous peoples on their bodies after bathing and then stay in the sun until dry, believing that the use of this tea helps to combat the deleterious effects of the sun, parasites, age and fungal infections (MELO, 2015).

As if that were not enough, the mulatto stick still has an application in the industry. Its wood is used in the reforestation of riparian forest areas. It is considered of good quality and has a very long service life, being quite resistant to fungi and other types of pests, so it is used in frames, frames, floors, plywood and even in shipbuilding (CASTILHO, 2013). After this review of the plant, its importance in the timber, pharmaceutical and popular medicinal use is evident.

Therefore, it is important to know what are the main classes of secondary metabolites that are found in the leaf of the mulatto stick to have a basis when performing phytochemical tests. According to Magrini (2015) antioxidant agents, phenolic compounds and unsaturated compounds were found in the extract. In addition, lactonas, anthraquinone, flavonoids, reducing and free sugars, iridoids, condensed



tannins and saponins were found in the hydroalcoholic extract, and the presence of alkaloids was not detected (PERU, 2009).

One or more electrons of unpaired atoms along with organic or inorganic molecules can be titled as free radicals, which have very specific characteristics at the time when their meia-life is short-lived, quite untenable and reactive, this gives them the ability to form compounds that weaken the body's systems. (MAIN, 1999). For this type of mechanism antioxidants are used. An antioxidant needs to present in its chemical structure regions that are suppliers of electrons or hydrogen atoms, being possible to stop the oxidative process that is responsible for some degenerative diseases and the skin's own aging (MAGRINI, 2016). A very good type of antioxidant in plants are phenolic compounds. They may appear in the simple form or polymers, but in their structure will always present aromatic and hydroxyl aneis that gives them antioxidant power, there are about five thousand phenolic compounds in nature, in resveratrol, quercetin, coffee acid and flavonols were found antitumor activities, this makes clear the importance of knowing types phenolic sands that each plant has (ANGELO; JORGE, 2007; SILVA, 2014).

Another compound not so frequent in plants are lactone, even in bibliographic reviews it is not much cited. Lactone sesquiterpenca has 15 carbons in its composition, with three conjugated cyclic chains and their organic functions, ether and ketone (MARIA *et al.*, 2009). There are some classifications for lactone, among them the sesquiterpenes that are produced by the plants according to the incidence of ultraviolet rays, the higher the incidence was that day, the higher the production by the plant, having the anti-inflammatory property (GOBBO-NETO; LOPES, 2007). In addition, antiulcerogenic properties were also found since they increase the concentration of prostaglandins in the gastric mucosa (MARIA *et al.*, 2009).

Another secondary metabolite present in the mulatto wood and perhaps the most frequent in vegetables is flavonoid. They are polyphenols synthesized from the phenylpropanoid route and are chemically classified as substances consisting of a common nucleus of cromanona phenyl with relocation of a or more hydroxyls, its main characteristic in chemical stanza is related to the presence of the flavium nucleus, which is characterized by three phenolic rings (PETERS, 2008; SILVA, 2014). It also works as an antioxidant, but its compounds are most widely used adjuncts to conceptual treatments. Its main applications are focused on the tumor, cardiovascular, hepatic, renal area.

Flavonoids act as adjunct to chemotherapy and radiotherapy treatment, because these procedures greatly weaken the patient, mainly through the formation of free radicals that age good cells, so it acts by capturing free radicals and killing most good living cells. Another application of flavonoids is in cardiovascular parte which, administered in certain amounts, consists in the reduction of LDL, characterized as bad cholesterol, and in the inhibition of platelet agragation, reducing the risks of



developing atherosclerosis. In addition, it has immunosuppressive characteristics, that is, it can be used after transplants to weaken the patient's immunological system and prevent the body from rejecting the organ and nephroprotective, avoiding tubular damage and interstitial inflammation. As if that were not enough, it still acts as a protector of the liver, an organ responsible for the main chemical transformations that occur in the body, through the antioxidative liver capacity. Drinking wine in moderate doses for years helps prevent diseases such as cancer, rheumatism, Alzheimer, Parkinson's among other age-related pathologies (BEHLING; SENDÃO, 2004; MAMEDE, 2004;).

Another compound found was tannins, more specifically the condensed tannins. Tannins are macromolecules belonging to the group of phenolic compounds with the ability to complex with large molecules, such as proteins, and can be found in several vegetables from different families. (BATTESTIN; MATSUDA; MACEDO, 2004; PAIVA *et al.*, 2002). The condensed tannins are formed by catechins or leucoanthocyanin, having a very well arranged stanza, being resistant to breakage through water, but solubilized in aqueous organic (BATTESTIN; MATSUDA; MACEDO, 2004). Condensed tannins have proven a function in inhibiting viral replication, which includes the HIV virus, in which it has properties that are capable of even neutralizing transcriptase reverse virus, in addition, acts as repellent due to its high toxic content to insects, causing reactions that limit their digestibility and consequently death. It is still possible to use it as an inhibitor of the growth of bacteria and fungi, with the presence of tannins, some species of bacteria such as *Staphylococcus sp* and fungus *Fomes sp* are extremely sensitive to low concentrations of the compound (MONTEIRO *et al.*, 2005).

Another compound that is part of the secondary metabolism of many plants are saponins, with the ability to make foam, being considered surfactants. They were obtained from oil, however the depletion, price increase, environmental issues and by increasing legislation regarding its use, its acquisition from oil no longer became a viable business, it was at that moment when compounds of natural origin that performed similar functions began to be used, so saponins gained value in the industry, being characterized as biosurfactants (NITSCHKE; PASTORE, 2002). They have the specialties of dispersion of phases, emulsifier, sparkling and wetting, being used more in various areas of the industry such as cosmetics, fertilizers and pharmaceutical, being the most used class that of lipopeptides (BARROS; PEAR TREE; PASTORE, 2008). Some of its biological properties are related to an analgesic and anti-inflammatory character, related to the presence of equinolic acid (BORELLA *et al.*, 2006). Due to the presence of glyphosate, which is a phosphorus organo herbicide, saponin gains a character of high toxicity, being irritating to the skin and eyes, resembling the spray of pepper, because of this, can be used as a pesticide (JUNIOR, 2002). According to Aguiar (2005), saponins may be related to cholesterol lowering. Iridoides, another class of substances found, are part of terpenes, that is, they come from isoprenes. Terpenes are hydrocarbons found in animals and plants, with molecular conformation in



(C₅H₈)_n, being formed from the head-tail union of molecules isoprenos, which are also the main components of essential oils (BERGAMASCHI, 2006). Iridoids are found abundantly in dicotyledonous angiosperms, presenting defense function and mainly food disuasiveness due to bitter taste, being more easily found in glycosylated form (VENDRUSCOLO, 2014). Among the biological activities described for iridoides, it can be fungitoxic, antineoplastic, antiplastic, antimicrobial, insecticide, hepatotoxic, anti-inflammatory and immunomodulatory (SILVA *et al.*, 1998).

Reducing sugars in plants are used for formation and emission of vegetative and floristic shoots. Long-chain sugars can be converted into smaller chain sugars through hydrolysis reactions. The different properties of reducing sugars differentiate them from the common sugar, being possible to see their reductions when, for example, a fruit darkens when left in contact with air, due to its oxidation reactions. Because of this, reducing sugars are used differently in the food industry, especially with regard to the rapid dissolution of sugar when placed in the mouth (OETTERER, 2005; OLIVEIRA *et al.*, 2013).

Thus, the respective work aimed at pharmacognostic characterization and phytochemical analysis of the crude hydroalcoholic extract of *Calycophyllum sp* to determine the presence of secondary metabolites and correlate with its probable effects of the use of pau-mulatto in folk medicine.

2 METHODOLOGY

Using the Barbosa *et al* methodology. (2001), the material was collected in the city of Macapá, on the campus of the Federal University of Amapá (Lat.-0.0067570, Long.-51,0786180) and part of the material used for the preparation of exsicates for further cataloging. The rest of the material was then placed to dry in a place with shade, without direct contact with ultraviolet rays to prevent the proliferation of fungi and then in the Pharmacognosy and Phytochemistry laboratory of the Federal University of Amapá, taken to stucco inside envelopes at 45°C, after performed the drying of the material, the same, was crushed. Then the crushed material was stored in a glass pot where hydrated ethyl alcohol 92.8° INPM was added for a period of 72 hours and then filtered and concentrated in an evaporator route to obtain the extract, and this process was performed 3 times and left to evaporate the remaining alcohol at temperature Controlled. Finally, with the obtaining of the extract, phytochemical tests were performed in order to detect the presence of secondary metabolites.

3 CONCLUSION

Phytochemical tests were performed, the presence of saponin metabolites, organic acids, phenols and tannins, purines, azulenos, carotenoids, depsides and depsidons were detected as shown in Table 1.



Table 1- Results of phytochemical tests

Phytochemical Tests	Positive	Negative
Saponin	+	
Organic acids	+	
Reducing sugars		-
Polysaccharides		-
Proteins and amino acids		-
Phenols and tannins	+	
Flavonoids		-
Alkaloids		-
Purines	+	
Cardiac glycosides		-
Catechins		-
Sesquiterpenolactones		-
Steroids and Triterpenoids		-
Azulenones	+	
Carotenoids	+	
Deposides and depositions	+	
Coumarin derivatives		-
Anthraquinone		-

(+) positive (-) negative for the presence of the metabolite

Source: Results obtained from the manual provided by UFPA (BARBOSA, 2004)

The first metabolite with positive presence detected were saponins which is considered a steroidal glycoside or also polycyclic terpenes. Its main characteristic is that in its structure it has an amphiphilic character, that is, one part lipophilic (triterpene or steroid) and the other hydrophilic (sugars) (CASTEJON, 2011). Due to its amphiphilic character it interacts with the membrane phospholipids of fungi and bacteria giving it a character that is described in its popular uses, which is the antifungal and antibacterial character.

Among its biological properties it is possible to highlight its anti-inflammatory and hypocholesterolemic actions. In its anti-inflammatory characteristic, saponins are able to inhibit the levels of TNF α and PGE2 presented by the model of air bag induced by carrageenan this factor that is probably associated with its use for inflammation in the treatment of discharges. With regard to their hypocholesterolemic potential, saponins greatly reduce the adhering of a specific type of leukocyte, the monocyte, in endothelial cells thus, the formation of fat plaques within veins and arteries has



reduced probability (GOULART, 2006). Thus demonstrating its potential for treatment for diabetes, as this reduces the presence of blood sugar.

They can be found more easily in emulsion and gel formulations, which are the most accepted in the pharmaceutical industry. They are used in medications taking advantage of their venotonic and anti-edematous capacity, giving the blood vessel the ability to decrease capillary permeability and increase the resistance of the vessel wall, thus assisting in the good blood circulation at the site of application and consequently decrease in the area of the ede (PINTO, 2013).

Detected second, organic acids are characterized by the presence of a carboxylic group at the tip of the chain. This group presents itself in the form of a double bond between carbon and oxygen, called carbonyl and a simple bond between carbon and a hydroxyl, conferring acid character for the molecule (PINTO, 2002).

They are mainly used in the composition of antioxidants. These can be divided into oxidizing agent classes. In general, antioxidants are responsible in doing the detoxification of the body. They have the characteristic of performing electronic exchange, that is, sharing or donating electrons to certain compounds, thereby inactive or decreasing the toxicity potential related to that molecule (RAMALHO, 2006). Thus probably the effect on the treatment of discharge and its use for skin improvement, anti-aging and as UV protection is given by its presence. The main organic acids of pharmaceutical importance are tartaric, malic, citric and silícico. These substances are mainly present in the composition of skin rejuvenation products, shampoos and foods.

Third, phenolic compounds are metabolites that act as antioxidants because of their chemical structure. They are formed by the benzene ring with hydroxyls directly associated with the cyclic structure. It is a vast group and can be divided into polyphenols (flavonoids) and simple phenols (non-flavonoids) (MAMEDE, 2004). Tannins can be classified into two very distinct groups, but both have a common characteristic for both types, which is the presence of the polyhydrophenol molecule (DEGÁSPARI, 2004).

Phenolic acids possess a very large variety of actions in the body. Your metabolism is related to the esterification process. The stomach does not have esterases capable of degrading the molecule, so it passes practically intact through the first part of the gastrointestinal tract, and those from food when they reach the duodenum and jejunum are absorbed almost entirely by the body (BASTOS, 2009) probably conferring the action of relief of stomach pains and cramps as descrito in the literature.

Fourth, purine is a type of alkaloid, also known as false alkaloid. It is named because it is not derived from the common alkaloid route, because it is derived from a nitrogen base (ROSA, 2016).

Like true alkaloides, purines also develop very important functions in vegetables. In addition to participating in dna formation and being a vehicle for the synthesis of various compounds in the cell, it



still plays important roles in protecting against predapain and animal feed. In addition, it is also the basis of several consecrated drugs used in various parts of the body (BARREIRO; FRAGA, 2014)

Fifth, azulene is an isomer organic compound of naphthalene that has intense blue color, being different for a chemical compound with basic characteristics. It was first discovered in the millolyof a plant of the same genus of wormwood (ALMEIDA; MARTINEZ; PINTO, 2017).

A widely used derivative is camazuleno. This composition has the ability to inhibit cyclooxygenase-2, the main enzyme that catalysis of the reaction of aaquidonic acid in prostaglandins and thromboxanes (AMARAL, 2014). These characteristics find give this compound anti-inflammatory, antifungal characteristics. In addition, it has analgesic, photoprotective and stimulating properties

for tissue regeneration (DE REZENDE, 2015) its presence of strong evidence of various plant actions, as described in the literature.

In the sixth, they are pigment-like chemicals. They are part of the family of compounds found abundantly in nature, mainly in fungi, bacteria, algae, plants and animals, ranging from yellow to red. Carotenoides have liposoluble characteristics and oxidisable molecules (ALVARENGA; DA SILVA; SYLOS, 2015).

They have a chemical structure formed by tetraterpenois, having as main characteristic the ability to absorb visible light at different wavelengths. In nature it is possible to find more than 900 different types of carotenoides, this variation is caused by the fact that molecules undergo processes of isomerization, hydrogenation, cycling, migration of double bonds, dehydrogenation among others (DA SILVA, 2017).

Carotenoides can be employed in the food industry to intensify food coloring in order to make them more attractive in the eyes of consumers. Another very interesting application is in poultry and aquaculture, this compound is used in poultry feeding in order to leave the egg yolk with the tone more intense yellow. In the pharmaceutical industry, carotene derivatives are used in tanning of people and in the protection of visual systems, avoiding degeneration by age (FERREIRA, 2014). Thus, carotenoides corroborate a lot for the use of the plant in the industry, besides being one of the probable reasons for its use in ethical cosm andby indigenous peoples on the skin to protect against UV rays and to delay aging.

In seventh place, phenolic compounds are found in lichens and superior vegetables obtained through the biosynthetic route of acetate polymalonate. In addition, natural xanthones and depsidonas are obtained from different routes (MOTA, 2015).

The classification of these compounds is varied due to their precursors which can also be obtained by different routes, but even with different ways of obtaining this compound, it is possible to relate it with antiviral, antitumor, antipyretica, antiviral and analgesic properties. For plants it has photoprotective and



antiherbivory function (DE ALMEIDA *et al.*, 2015).

Therefore, the presence of secondary metabolites shows that the plant may have a strong use in the industry, and for the treatment of diseases since the presence of these compounds indicates such activities already described in the literature being popular use. With isso, further study is necessary about the crude extract of *Calycophyllum sp.* to more accurately determine its uses.



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Digitar a referência na margem esquerda usando-se espaço simples (um) entre as linhas e espaço duplo para separar as referências entre si. A referência bibliográfica poderá ser eletronicamente associada, citada de acordo com o exemplo abaixo:

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