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Preliminary study of the extract of the barks *of Licania macrophylla* Benth: phytochemicals and toxicological aspects

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ABSTRACT: The objective of this research was to evaluate the phytochemical, physicochemical and toxicity profile of ethanol crude extract of *L. macrophylla*in relation to *Artemiasalina* L. The Phytochemical analysis of the ethanol crude extract of the barks and stem and physicochemical calculation were performed by methods found in the Brazilian Pharmacopoeia. Phytochemical analysis detected saponins, organic acids, reducing sugars, tannins, anthraquinones, and depsidedepsidone. In relation to the physicochemical parameters, it showed pH = 4.64, due to the occurrence of saponins, organic acids and tannins; lipid = 0.55% indicates that the material presents few lipids of low molecular weight; humidity = 12.09% ± 0.12, relates to a small amount of water, indispensable for non occurrence of development of microorganisms or enzymatic degradation. Waste by incineration is within the pharmacognostic standards of 8.30% ± 0.54. The crude extract is practically nontoxic with LC_{50} = 1253µg/mL, since the mortality rate and LC_{50} should be higher than 1000µg/mL to be considered nontoxic. Phytochemical analysis have confirmed, in part, the use of species for phytotherapic purposes, however, the type of treatment and packaging can influence the determination of secondary metabolites. The adopted physicochemical parameters have shown that the species is free of decomposersagents.

Keywords: herbal medicine, *L. macrophilla* Benth, toxicity, physicochemical.

Estudo preliminar do extrato etanólico das cascas de *Licania macrophylla* Benth: aspectos fitoquímico e toxicológico

RESUMO: O objetivo desta pesquisa foi avaliar o perfil fitoquímico, físico-químico da espécie vegetal e determinar a toxicidade do extrato bruto etanólico de *L. macrophylla* Benth frente às larvas de *Artemia salina* L. A análise fitoquímica foi realizado por meio do extrato bruto etanólico das cascas do caule e a determinação físico-química foi realizada de acordo com a Farmacopeia Brasileira. As análises fitoquímicas detectaram a presença de saponinas, ácidos orgânicos, açúcares redutores, taninos, antraquinonas, depsídios e depsidonas. Em relação aos parâmetros físico-químicos a planta apresentou pH=4,64, pela ocorrência de saponinas, ácidos orgânicos e taninos; Lipídeos= 0,55% indica que o material vegetal apresenta poucos lipídios de baixo peso molecular; Umidade= 12,09%±0,12, o que relaciona a pouca quantidade de água, fator indispensável para a não ocorrência de desenvolvimento de microrganismo ou degradação enzimática. Os resíduos por incineração (cinzas) da espécie se encontra dentro dos padrões farmacognósticos de 8,30% ± 0,54. O extrato bruto segundo o teste de toxicidade é atóxico com CL_{50}=1253µg/mL, isto é, confirma-se a relação estabelecida entre a taxa de mortalidade e CL_{50} deve ser superior a 1000µg/mL para serem considerados atóxicos. As análises fitoquímicas confirmaram em parte a utilização da espécie para fins fitoterápicos, porém a forma de tratamento e acondicionamento pode influenciar na determinação de metabólitos secundários. Os parâmetros físico-químicos adotados mostraram que espécie encontra-se livre de agentes decompositores.

Palavras-chave: fitoquímica, *L. macrophilla* Benth, toxicidade, físico-química.

1. Introduction

Medicinal plants were used for many years as the first preventive measure in treatment and cure of diseases (CARVALHO et al., 2010). From popular and ethnicities use there were known and discovered several species with pharmacological potential, thus, the efficacy of the use of the plant contribute to the relevance and realization of the associated therapeutic properties (HOCAYEN et al., 2012). Herbal medicine (Phytotherapy) has emerged as a medical option well accepted and accessible to people of the world, and in the case of Brazil it is pleasant to the needs of hundreds of local municipalities in primary health care (MELO et al., 2007).

The functional activity of a natural product has stimulated the popular and institutional interest in strengthening the use of medicinal plants in its several pharmaceutical ways, defined by herbal medicine, without the use of isolated compounds. In Brazil, during the 80's, begins the interest of inserting herbal medicines in the Unified Health System (SUS - in Portuguese), however, only in mid-2006 it was approved by the National Policy on Traditional Medicine and Herbal Medicines by Decree 5833, 22nd June 2006 (BRASIL, 2006 apud KUNZ, 2007).

In the Chrysobalanaceae family there are 18 genera and presents 531 species, commonly distributed in tropical and subtropicas areas (HEMSING; ROMERO, 2010).

Several genera included *Atuna, Kostermanthus, Licania, Magnistipula, Maranthes*and three *Parinari*, which reach 30m height and upper limbs in its top emerging in the rainforest. The flower, by difference, is compared, although almost every genus is characterized by fundamental uniformity of inflorescence and floral structure (ZUQUE et al., 2004). Its leaves are characterized by a simple and alternate shape, pentamerous flowers, free petals, superior ovary and ovaries and erect ovules. In its composition there is composition of flavonoids, triterpenes, diterpenes, steroids and tannins (YAKANDAWALA et al., 2001; LOCATELLI, 2009).

In African continent, traditional population widely uses some species of Chrysobalanaceae family for treatment of epilepsy, malaria, dysentery and toothache. The *Licania macrophylla* Benth is popularly known as "anauera" or "anuera", and in Brazil, specifically in the Amazon region, the species is used in the prophylaxis of parasitic amoebic and dysentery disorders (MEDEIROS et al., 2012).

Secondary metabolites comprise a rich and diverse class of compounds intended to provide functional activities or properties of a given plant. However, the quality of plant material does not guarantee itself the safety and efficacy of the final product, since the photochemical analysis is guided of qualitative tests for the detection of metabolites classes. Then, the physicochemical analysis associated with pharmacological ensures, partly, the effectiveness through pre-clinical and clinical tests of the recommended effects (SIMÕES et al., 2010).

In the methanol extract of the stem barks and flowers of *L. macrophylla* Benth, were isolated and determined structures of flavanol (-)-4'-O-methyl-*epi*-gallocatechin-3'-O-α-L-rhamnoside, licanol, along with nine compounds known and identified as (-)-4'-O-methyl-epi gallocatechin, pheophytin A, 132-hydroxy-(132-S)-pheophytin A, pheophytin B, sitosterol, stigmasterol, sitosterol-β-O-glucoside, alcohol betulinic and oleanolic acid (MEDEIROS et al., 2012).

The toxicological analysis of *Artemia salina* L. is a preliminary bioassay that allows to estimate the evaluation of the toxicity of the extract, through the lethal concentration (LC_{50}) of the fraction or active components facing to a marine organism. It is an essential test to determine the biological activity potential of the compound or extract (NASCIMENTO et al., 2008). This work aimed to evaluate the phytochemical and physicohemical profile of the plant species and determine the toxicity of the ethanol crude extract of *L. macrophylla* Benth in relation to larvae of *Artemia salina* L.

2. Material and Methods

Collection and identification of vegetal material

The vegetal material was collected in the Fazendinha Environmental Protection Area, in Macapá. The species was identified by Prof. Dr. Wellgliane Pararício, at the Herbarium of the Federal University of Amapá-UNIFAP.

Obtaining extracts

The stem bark of the species was separated for drying at room temperature and powdered in a cutting mill at the Bioprospecting and Atomic Absorption Lab - UNIFAP. After drying and pulverizing, the vegetal material was placed in a round-bottomed flask with ethanol 96°GL in proportion 1:2 (w/v), comprising an extraction period of 4 days until exhaustion. Subsequently, the extract was concentrated on a rotary evaporator under reduced pressure and placed in an erlenmeyer in a desiccator.

Physicochemical analyzes

The adopted physicochemical parameters in this study were: pH, Waste by Incineration (Ash), Lipids and Humidity. They were carried out according to standards of the Adolfo Lutz Institute (2008) and Brazilian Pharmacopoeia (2010).

Phytochemical study and sample preparation for toxicological testing

A phytochemical screening of the obtained extract was conducted with the use of specific revealing reagents, according to the methodology proposed by the Brazilian Pharmacopoeia (2010)and Macêdo (2005) for organic acids, reducing sugars, saponins, polysaccharides, phenols and tannins, flavonoids, alkaloids, purines, steroids and triterpenes, and depsides and depsidone, anthraquinones and catechins.

The cytotoxicity assay of *Artemia salina* was based on the technique of Araujo et al., (2010) and Lôboet al., (2010) with some modifications. Initially, it was prepared 250 mL of synthetic sea salt solution (35.5 g/L) for incubation of 25mg of eggs of *A. salina*, which were exposed to artificial light for 24h for larvae hatching (metanauplius), then, the metanauplius were separated and placed in the dark environment for a period of 24h. The mother solution was prepared containing 62.5 mg of the crude extract from the stem barks, added 28 mL of synthetic sea salt solution and 2 mL of dimethylsulfoxide (DMSO) to facilitate the solubilization.

Subsequently, at the end of the dark period they were selected and divided into 7 groups with 10 subjects in each test tube, and in each group it was added an aliquot with draw al from mother solution (3125, 2500, 1250, 625, 250, 25 to 2.5 μL) in which the volume was completed to 5 mL with the synthetic sea salt solution, and the final solutions had concentrations varying from 1250, 1000, 500, 250, 100, 10 to 1 μg/mL, thereby, the groups were designated according to their respective concentration and all tests were performed in triplicate.

3. Results

The preliminary phytochemical analysis of the ethanolic extract of the stem barks of *L. macrophylla* Benth revealed the presence of secondary metabolites: saponins, organic acids, reducing sugars, tannins, anthraquinones, depsides and depsidones. The results can be observed in Table 1.

Table 1. Preliminary phytochemical analysis of ethanol crude extract of stem barks of *L. macrophylla* Benth.

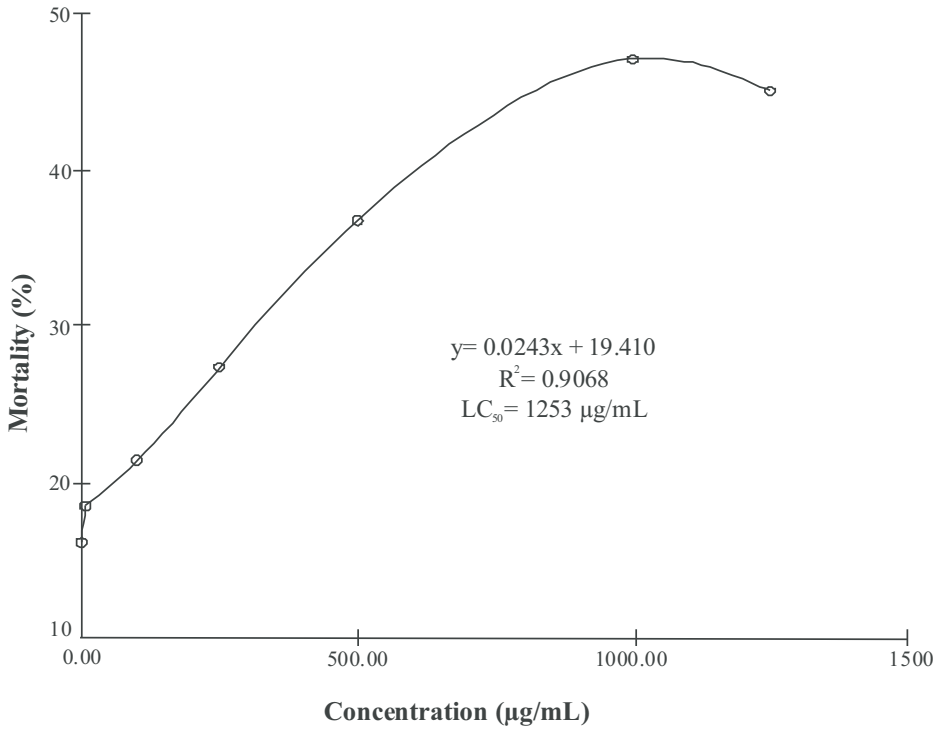
MetaboliteClass	Result
Saponins	+
OrganicAcids	+
ReducingSugars	+
Polysaccharides	-
Tannins	+
Flavonoids	-
Alkaloids	-
Purines	-
Catechins	-
SteroidsandTriterpenes	-
DepsidesandDepsidones	+
Anthraquinones	+

+ Present; - Absent.

The determined values in physicochemical analysis of stem bark of *L. macrophylla* Benth are shown in Table 2. The crude extract, according to the toxicity test, is nontoxic, because the LC_{50} calculated was 1253µg/mL, statistically the value *F* (59.3935) is significant with (p=0.0011), thereby, increases the mortality rate due to reduction in the determination of the LC_{50} value. **Graphic 1** expresses the relation between mortality and extract concentrations for dilution.

Table 2. Physicochemical characterization of the stem barks of *L. macrophylla* Benth.

Parameters	Result
pH	4.64
Humidity (% m/m)	12.09 ± 0.12
Ash (% m/m)	8.30± 0.54
Lipids (% m/m)	0.55



Graphic 1. Toxicity test of the extract of *L. macrophylla* retated to *A. salina* L. in different concentrations.

4. Discussion

The saponins present anthelmintic, antiviral and anti-inflammatory activities, the anthraquinones stand out as allelopathic, laxative, antibacterial and antifungal properties, the presence of these secondary metabolites corroborates, in part, with the population use of the plant.

Saponines, in general, presents amphiphilic character and capability of complex to form steroids, proteins and membrane phospholipids, behaviors that determines the number of biological activities for this compound, as described in the literature, such as hemolytic, ichtyotoxic, molluscicide, anthelmintic and spermicidal (SIMÕES et al., 2010; OLIVEIRA; RAMOS; ALMEIDA, 2013). The mechanism of action purposed for some saponines is in the capability of break the plasma membrane of microorganisms, resulting in extravasation of cellular content and, finally, death. Another action of saponines is their use in human diet for reduction of serum levels, because this compound interacts with cholesterol in their complexation and oxidation (KAISER et. al., 2010). In that way, it reinforces the use of this species by traditional communities and points for a more detailed study of the isolated and purified active principles.

Organic acids presents antifungal activity, as described in literature (SIMÕES, 2010), are largely used in food industry as additives, can act since as antimicrobial agents until antioxidants (FIORUCCI; SOARES; CAVALHEIRO, 2002; OLIVEIRA; RAMOS; ALMEIDA, 2013).

Plants have the ability of cumulate organic acids in their vacuoles; this can be evidenced in citric fruit juices, due to the presence citric acid. These acids are not restricted to the fruits and can appear also in many plants leaves. Organic acids have bacteriostatic power and bactericidal gram-negative, *in-vitro*, provided acid molecules find themselves ionized (IHEJIRIKA, 2011; KUMAR et al., 2011). These compounds were detected in ethanolic leaves' brute extract, what justifies, in part, the use of the vegetal species for these activities.

Consumption of foods and fruits rich in reducing sugars and proteins bring health benefit, because these compounds are biologically active because they have antioxidant and antimutagenic activity (SHIBAO; BASTO, 2011). Oxidation of free radicals and compounds foreign to body occur in the glycoside bond of sugars in which are reduced, these compounds are readily excreted from the body without help from carriers, decreasing cellular activity without causing stress or premature cellular aging (SILVA et al., 2003; QUADROS et al., 2010).

Reducing sugars are characterized by the end of carbon chain,bycarbon atoms not spatially prevented to react, that is, carbons that are not evolved in glycoside bonds, for instance, fructose and galactose. In the other hand, saccharose is an antioxidant but not a reducing sugar. Utilization of fruits of species are considerable source of nutrients and energy for the maintenance of daily activities (CANIATO et al., 2004).

The tannins presented activity in the treatment of diarrhea, rheumatism, burns, and renal and urinary system problems (SIMÕES et al., 2010). The presence of tannins in the plants is associated with the biological role of chemical defense against microorganism, and vertebrate or invertebrate herbivores attacks. It's believed that their activities are due to, by part, the ability to form complexes with other molecules including macromolecules, as proteins and polysaccharides (OLIVEIRA; RAMOS; ALMEIDA, 2013).

Depsides and depsidones have been recognized for presenting antioxidant, antiviral, antitumor, analgesical and antipyretic activities. This class of secondary metabolic gives a bitter taste in various species of plants in that they are present, a property related also to organic acids (MENDES et al., 2011).

Anthraquinones and some isolated substances, like aloine, are used as laxative, and usually have toxic properties, for which is attributed the chemical defense function, related to alleopathic activity, that is, production and excretion of substances to environment capable of inhibit the growing of other germinative species, act against termites and raise the value of commercial woods that have such secondary metabolic (SIMÕES, 2010).

There is absence of studies records for the species aphrodisiac activity, because, according to Medeiros et al., (2012), there are reports of use of stem bark by indigenous communities as a sexual stimulant.

In study of Gomes et al. (2006) on the chemical composition of leaves of species *L. macrophylla*Benth from the Combu Island – Pa region, specifically of ethanol crude extrate, detected the positive presence for flavonoids saponnins, phenols, steroids and triterpenoids. In comparison to results found, it's observed equivalence in results. However, the presence of flavonoids in the branches was not observed, because the non detection may be associated to the low concentration of this metabolite in the extract, what suggests an future study for the quantification and structural elucidation of the metabolites found.

Studies of Medeiros et al. (2012) using metanol crude extracts of barks of *L. macrophylla*Benth collected in Forest Reserve of Amapá State's Scientifical and Technological Research Institute, in Porto Grande, demonstrated activity against bacteria multiresistant to drugs, assigning to (-)-4'-O-methyl-*epi*-gallocatechin-3'-O-α-L-rhamnoside, a flavonoid never before described in scientific literature.

The pH 4.64 value of *L. macrophylla* Benth, characterizes the presence of potentially acidic substances. The presence of secondary metabolites as saponins, organic acids and tannins tend to lower the pH of the species, since the presence of a carboxyl group on aglycone or the sugar chain increases the release of ions H^+ the reaction medium (SIMÕES et al., 2010).

The maximum limit recommended of humidity to plant drugs is 14% (SIMÕES et al., 2010*apud*HUBINGER et al., 2009) and, thus, it appears that the botanical powder material presented result of 12.09±0.12 less than satisfactory. The found humidity content enables us to predict that the botanical material is protected from microorganisms attack and enzymatic actions that may modify its natural properties.

The waste by incineration (ash) is within the pharmacognostic standards of 8.30±0.54%, important data, as they are non-volatile inorganic materials. Another important data on purity and quality, since the adulteration of vegetable drugs, especially sprayed, can be carried out with mineral material, such as sand, and other impurities (PESSINI et al., 2003).

Lipids are highly energetic organic compounds, contain essential fatty acids to the body and act as carriers of fat soluble vitamins. The found value was 0.551% extraction, but the residues obtained in the extraction are not only lipids, but of all compounds under the conditions of the determination, can be extracted by the solvent, thus, it is necessary a more rigorous analysis for the qualitative identification and, therefore, its quantification (RODRIGUES et al., 2010; RAMOS et al., 2010).

It is observed by the adjusted coefficient of determination (R^2) of 90.68%, that the rate of survival or mortality is explained by the concentration, and other factors must act as predictors of its increase. It is confirmed that the relation between mortality and LC_{50} presented by the plant extract on the larvae of *A. salina* L.must be higher than 1000µg/mL to be considered nontoxic (NASCIMENTO et al., 2008; ARAUJO et al., 2010).

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