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Phytochemical Study, Chemicalphysical Analysis and Toxicological Testing of Stem Bark of *Dalbergia monetaria* L. f.

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ELDM and SSMDSDA planned all experiments. Authors ELDM and SSMDSDA supported the study of physical and chemical composition and quality of fillet. Authors ELDM, SSMDSDA and RDSR wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Assessing the phytochemical profile, physicochemical and toxicity of crude ethanolic extract of *Dalbergia monetaria* L. f. against *Artemia salina* L.
Study Design: The study aimed to determine the major classes of secondary metabolites of plant species *Dalbergia monetaria* through technical and classical methodologies.

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Place and Duration of Study: Laboratory of Pharmacognosy and Phytochemistry of Pharmacy Course, between July 2012 and March 2013.
Methodology: The phytochemical screening of the crude ethanolic extract from the stem bark was performed according to the methodology described by Brazilian Pharmacopoeia (2010). The physicochemical analyses were performed by the Institute Aldolf Lutz (2008) and methods found in the Brazilian Pharmacopoeia. The toxicology test followed the method decribed in the literature.
Results: The phytochemical analysis detected reducing sugars, saponin, phenols and tannins. The physicochemical parameters, the pH, indicated the presence of a potentially acidic substance; the moisture content of = 6.81% which relates to, showed little amount of water present, an indispensable factor for non-occurrence of microorganism development or enzymatic degradation. The ash obtained by incineration was 5.65%.
Conclusion: The phytochemical analysis confirmed, in part, the use of the plant species for therapeutic purposes, but the form of treatment and conditioning can influence the determination of secondary metabolites. The physicochemical parameters evaluated have shown that the species is free from decomposing agents.

Keywords: Medicinal plants; secondary metabolites; verônica; crude extract ethanolic.

1. INTRODUCTION

Brazil has one of the highest diversity of plant species on the planet [1]. This variety has a pontential to the creation and updating of drugs applied to numerous pathology therapy [2]. Given the importance of the therapeutic potential of medicinal plants, the studies and research are of high value in the understanding their pharmacological activity and toxicological properties [3].

Dalbergia monetaria L.f. is a species of the Fabaceae family [4,5]; is a climbing shrub, presenting leaves with 3-5 oval leaflets, acuminata, concolor. It has white flowers arranged in racemes paniculados and fruit-type pod glabra [6]. It is known popularly as “veronica” and it occurs naturally in the states of the Amazon region, northern Brazil [6].

In traditional medicine, the use of *Dalbergia monetaria* bark is directed to the treatment of anemia, gastric disorders and liverwort detoxification. Also, it is used for treatment of vaginal discharge, in regulating the menstrual cycle and in recovery post-natal of the vaginal muscles [6].

The main metabolite described in the literature for the genus *Dalbergia* is the proanthocyanidins, from *D. monetary* bark [7]. What are the not hydrolyzable tannins, with up to three condensed units [6]. The proanthocyanidins (catechin) inhibit histidine decarboxylase, which are linked with the production of histamine; catechins act as important antiulcerogenic promoters. On the other hand, most nerve ulcers, occur by the inhibition of histamine decarboxylase, thus releasing large amount of histamine in the stomach, thus catechins can intermediate this process [6].

Among the other metabolites found, one can cite the carotenoids glicosylicos isolated from an alcoholic extract of the *Dalbergia latifolia* seeds [8]. In the leaves of *Dalbergia coromandeliana* P. it was found the apioglycoside of prunetin [9].

The *Artemia salina* microcrustacean is used as a low-cost option for studies of toxicity and possible pharmacological properties [10]. Due their sensitivity, in which the feed occurs through filtration, where the food is taken into the digestive tract by toracopodes, so the filtration rate reduces with the accumulation of particles which will affect your heartbeat [11].

This type of toxicological testing has been used to indicate lethality of plant extracts [12]. According to the World Health Organization (WHO), substances showing LC50 less than 1000 ppm in *Artemia salina*, are declared toxic [11].

The present research aimed to carry out investigation, phytochemical physical chemistry, through ash tests, pH and humidity and toxicological activity of meoh extract of bark of *Dalbergia monetaria* L. f.

2. MATERIALS AND METHODS

2.1 Extracts Preparation

The plant material was collected at Fazendinha, district of the city of Macapá, on July 2, 2012.

The bark of the stem of the plant species were separated and dried at room temperature and ground in a Wiley mill. After drying and grinding, the botanical material was placed in a round-bottomed flask with ethanol 96ºGL in the ratio 1:2 (m/v). The extraction period lasted 4 days. Subsequently, the extract was concentrated in a rotoevaporator under reduced pressure and stored in an erlenmeyer flask inside a desiccator.

2.2 Physicochemical Analysis

The physicochemical parameters analyzed in this research were: pH, ash by incineration and moisture, according to the procedures of the Institute Aldolf Lutz [13] and methods found in the Brazilian Pharmacopoeia [14].

To obtain the ash content, 3 g of plant material was taken and transferred to a porcelain crucible, previously calcined under 450℃ in a muffle furnace. After cooling, the recipient was weighted. The plant material was submitted to ashing and it was subsequently cooled in Aaron for 30 min and weighted on an analytical balance. This procedure was performed in triplicate and after the calculation was done using the following equation:

$$\text{ashes percent m/m} = \frac{100xN}{P}$$

N = Mass of ash (g)
P = Mass of the sample (g)
m/m = mass per mass

In the determination of pH, 10 g of the sample was weighted into a beaker and added 100 ml of distilled water. Determining the pH unit, and was performed readings of pH 4.0 and 7.0 caps. The procedure took place in 10:40 the 18:50, under the temperature of 27.2℃.

The analyses of the moisture was done in triplicate, and the procedure were as follows: 2 g was of sample was taken into a porcelain crucible, previously weighted. The material was heated by 3 h and subsequently cooled in the desiccator; after reaching room temperature, was weighted again. The operation was redone until the weight has been constant. Then, the following calculation was employed, and the average of the moisture content was obtained.

$$\text{humidity percent m/m} = \frac{100xN}{P}$$

N = number of grams of moisture (loss of mass in g)

P = number of grams of the sample

m/m = mass per mass

2.3 Phytochemical Study and Sample Preparation for Toxicological Testing

The phytochemical screening of the extract obtained was performed using specific developers, according to the methodology proposed by Brazilian Pharmacopeia [14], for organic acids, reducing sugars, saponins, polysaccharides, phenols and tannins, flavonoids, alkaloids, purine, steroids and triterpenoids, depiside and depsidone, anthraquinones and catechins.

2.4 Cytotoxicity Assay Front *Artemia salina*

The cytotoxicity assay employing *Artemia salina* was accomplished based on the methodology described by Araujo et al., [15] and Lobo et al. [16]. Initially, 250 mL of synthetic sea salt solution (35.5 g/L) were prepared and added to 25 mg of hatching eggs of *A. salina.* The solution was exposed to artificial light during a period of 24 hours to larvae hatch (metanauplius), and then the metanauplios separated and placed in a dark environment for 24 hours period. The mother solution was prepared with 62.5 mg of the crude extract of the stem bark, added to 28 ml of synthetic sea salt solution and 2 ml of dimethylsulfoxide (DMSO) to facilitate the solubilization of the crude extract of the stem bark.

At the end of the period in dark, the nauplios was selected and divided into 7 groups of 10 subjects in each test tube to which mother solution was added at the rate (3000 µL to 100 µL). The total volume was adjusted to 5 ml with synthetic sea salt solution, and the final solutions had the concentrations ranging from 1250 µg/mL to 1 µg/mL; the groups were assigned according to their respective concentration and all tests were performed in triplicate.

3. RESULTS AND DISCUSSION

Preliminary phytochemical analysis of the stem bark crude ethanolic extract of *D. monetaria* L. f. revealed the presence of secondary metabolites as follows: reducing sugars, saponin, phenols and tannins. Table 1 shows the obtained results.

Table 1. Preliminary phytochemical screening analysis of the stem bark crude ethanolic extract of the bark of *D. monetaria* L. f.

Metabolite class	Results
Organic acids	-
Reducing sugars	+
Alkaloids	-
Depsids and depsidones	-
Steroids and triterpenoids	-
Phenols and tannins	+
Flavonoids	-
Polysaccharides	-
Purines	-
Saponins	+

+ Presence; - Absence

Reducing sugars are used as aggregating flavor in the food industry. It is known the anti-inflammatory activity of saponins for species *Aesculus hippocastanum* L. (chestnut-from-India) and *Glycyrrhiza glabra* L. (alcaçuz) [17]. In the species of *Glycyrrhiza glabra* L. and *Gymnema sylvestre* (Retz), the antiviral activity of saponins can be observed [17].

The use of medicinal plants, abundant in tannins, is applied by traditional use on stomach disorders therapy, such as gastritis and ulcer, kidney disorders and inflammatory development [18]. The pharmacological activity of the tannins is expected to be due to: 1) complexation with metal ions (iron, manganese, vanadium, copper, aluminum, calcium, etc.); 2) antioxidant activity and scavenging free radicals; and 3) complexing ability with other molecules, including such macromolecules as proteins and polysaccharides [18].

The protection delivered by the tannins in the treatment of wounds, burns and inflammations is through the production a protective layer (tannin-protein complex and/or polysaccharide) over the injured dermis or mucosa, thus, helping in the healing spontaneous progress (epithelial regeneration and formation of vessels) [18]. This is likely to occur in the use of *D. monetaria* in the treatment of gastric disorders, where its use is widespread in the region.

The antioxidant activity of some phenolic acids has been described [19]. This is due especially to the reducing property and their chemical structure, which allow a significant role in neutralizing or sequestrate free radicals and chelate with transition metals, acting both in the initial period and in the development of oxidative process [20].

Table 2 contains the results analyses of the physicochemical parameters of the *D. monetaria* L. f. bark of the stem.

Table 2. Physicochemical characterization of the *D. monetaria* L. f. bark of the stem

Parameters	Results
Ph	5.35
Humidity (% m/m)	6.81%
Ash (% m/m)	5.65%

The pH is an indicator of possible chemical changes of the vegetable, distinguish which substances will be obtained, according to the polarity and chemical character, and attest if the acid can be harmful to microorganisms [21].

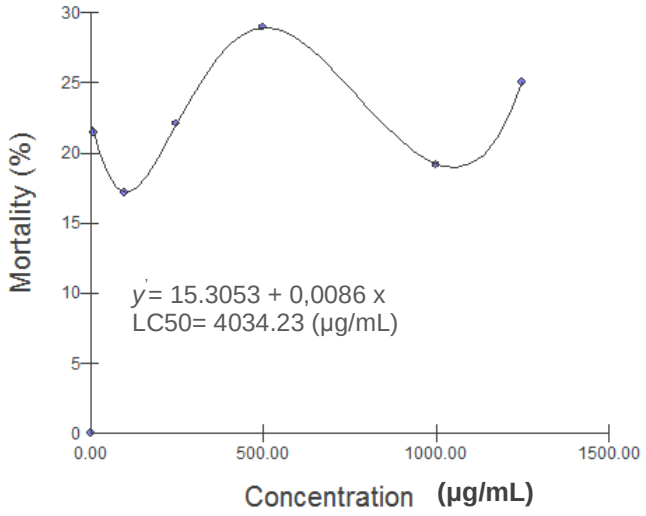
The pH value of 5.35 for the *D. monetaria* L. f., characterized the presence of potentially acidic substances such as secondary metabolites, phenols and saponins espumidicas, or by the absence of basic compounds (alkaloids).

The ash test is intended to indicate the content of inorganic impurities present in organic matter [14]. These inorganic substances can be represented by Mg, Ca, and Fe. Large amounts of ash (in some cases derived from the environment), may indicate alteration or pollution [22].

In this experiment the ach content of the plant material was 5.65%. The recommended limit, according to the Brazilian 2010 Pharmacopoeia, is 15% [14]. Hence the plant material is in compliance with the requirement and probably presents low amounts of inorganic impurities.

The emergence of microorganisms and hydrolysis on the extract components can be due to excessive moisture [22]. The proposed limit of moisture to plant drugs is 14% [23]. The bark botanical material presented 6.81% of water content. Moisture content found predicts the botanical material is protected attacks by microorganisms and enzymatic actions responsible, in part, by the transformation of its natural properties.

Many toxicological tests with plant extracts developed with *A. salina* are found in the literature, because of their speed and accessibility [15]. In Graphic 1, it is demonstrated the mortality as a function of extract concentrations for the dilutions does extract the *Dalbergia monetaria* obtained from the present study.



Graphic 1. Results of the *D. monetaria* L. f. ethanolic extract cytotoxicity test using larvae of *A. salina*

According to the toxicity test of the crude extract, *D. monetaria* L. f. has low toxicity, since the LC50 was estimated as 4034.23 µg/ml. Statistically, the *F* value (1.3686) is significant and thus the mortality rate increases with the decrease in determining the LC50.

Therefore, the plant extract is considered as non-toxic. It should be noted that the correspondence between mortality and LC50 presented by the extract on the larvae of *A. salina* L. is more than 1000 µg/mL [23].

This experiment has shown correspondence with other biological activities, such as antitumor and activity against *Trypanosoma cruzi*, when there are high toxicity to *A. salina* [24]. Soon, the extract of *D. monetaria* presents this potential. This experiment is reputable for toxicological knowledge of possible biological activities.

4. CONCLUSION

According to the results presented in this work, the phytochemical analysis showed the presence reducing sugars, phenols and tannins and saponins. Reducing sugars are used as flavor adding agents, in the food industry. The anti-inflammatory activity of saponins is well known for the species *Aesculus hippocastanum* l. Phenols and tannins can be used as astringent helping in the treatment of wounds, burns and inflammation, thus improving the spontaneous healing progress. The presence of those secondary metabolites that have biological activity reported in the literature correlate with the alleged plant use by the population. The physico-chemical parameters analyzed demonstrated that the plant material does not suffer decay per share agents. About the toxicology bioassay conducted with the ethanolic extract using the larvae of *A. salina* L., it was found that the plant extract presents low-toxicity, indicating that there is a certain degree of security with the use of the population as cleaning bath and tea use. The crude extract presents potential for medicinal purposes and is consistent with its use by popular medicine.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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