Innovare
Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 4, 2015

Short Communication

TOXICOLOGICAL TESTING OF BARKS FROM *CROTON RHAMNIFOLIOIDES* (EUPHORBIACEAE*)*

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Received: 13 Dec 2014 Revised and Accepted: 05 Jan 2015

ABSTRACT

Objective: The present study aimed to study the phytochemical screening of stem bark of *C. rhamnifolioides* and evaluate the toxicological potential of *Artemia salina*.

Methods: Barks were subjected to methanol extraction at room temperature and, subsequently, a preliminary phytochemical study was conducted. The methanol extract at concentrations of 500, 250 and 125 mg/ml was investigated in the toxicological study through the *A. salina* method.

Results: The phytochemical screening showed the presence of secondary metabolites: alkaloids, triterpenes and flavonoids. The methanol extract showed a LC_{50} of 486.59 µg/ml, with a moderate toxicity, in which viable LC_{50} were 27.5, 82.5 and 100%, respectively.

Conclusion: This study may contribute to the knowledge of this species' toxicity, inform the public on the proper use of herbal and natural medicines and assist in the cataloging of information on plants used in popular medicine.

Keywords: *Croton rhamnifolioides*, Medicinal plants, Phytochemical, Toxicity.

The use of medicinal plants is a common habit in the population and currently, there is a growing interest in the use and trade of medicinal plants and herbal products in Brazil. According to the World Health Organization, millions of people use traditional medicine as their primary source of healing products, and sometimes it is the only source of health care [1].

Conventional and street markets are responsible for maintaining and propagating empirical knowledge through healers on the diversity of resources from medicinal plant species [2]. The healers' knowledge may provide important data for new scientific discoveries, which may lead to new knowledge on plants' therapeutic properties [3].

Croton rhamnifolioides is popularly known as "break-knife" or "white caatinga". It is generally found in Brazilian Northeast and it is popularly used in the treatment of stomach aches, upset stomach, vomits and diarrhea [4]. *Croton* species had their activities assessed and confirmed, such as for *Croton lechleri*, to which antioxidant, antimicrobial and antiviral activities have been discovered. Other plants with medicinal uses discovered were [5] *Croton zehntneri*, which has an anti-hermitical activity and cardiovascular effects [6, 7]; *Croton urucurana* is an anti-nociceptive [8]; *Croton campestris* is a molluscicide [9]; *Croton cuneatus* is an anti-inflammatory compound [10]; and *Croton lechleri* has mutagenic and antioxidant characteristics [11].

Although plants present many therapeutic uses known by the population, it is uncertain if they can be toxic both for humans and animals [12, 13]. Among the main problems caused by improper and prolonged use of medicinal species are allergic reactions and toxic effects on several organs [14]. Therefore, it is essential to inform the public on the proper use of plants and natural medicines.

The bioassay with *Artemia salina* is used as an indicator of toxicity of various chemicals substances, including plant extracts [15]. It has some advantages, such as quickness, practicality, [16] simplicity, low cost and it also uses a small amount of sample [17] while maintaining a good relation in *in vivo* tests, suggesting that it is a useful [16] and reliable method. [17] The lethality assays are widely used in toxicology testing and the median lethal concentration (LC_{50}) may thus be obtained. This consists in the necessary dosage to cause death in 50% of a sample under study. [18] Therefore, this study sought to perform phytochemical screening of stem bark *C. rhamnifolioides* and assess the toxicological potential over *A. salina*.

Barks (30g) of *C. rhamnifolioides* stem acquired in the public market of São José in Recife, Pernambuco state, Brazil, was washed in running water, cut in slices and subjected to a methanol extraction at room temperature for 48 hours. Then, the extract was filtered and rota evaporated at 40° C.

After obtaining the methanol extract of the stem bark of *C. rhamnifolioides*, a phytochemical study was carried out through a thin layer chromatography (TLC). In order to detect the presence of secondary metabolites (alkaloids, triterpenoids, flavonoids, coumarins and saponins), the following standards were used: alkaloids-catharanthus; triterpenes-lupeol; flavonoids-quercetin; coumarins-coumaric acid; and saponins – ziziphus. For developers, alkaloids-dragendoff; triterpenes-liberman; flavonoids-neu; coumarins-Koh-Etoh; and 10% Saponins-no developer was used [19], were used.

This bioassay used the methodology described by Meyer *et al.* [20] with adaptations. Encysted *A. salina* was placed in filtered seawater, so they were able hatch, and then they were placed for 48 hours under the action of an aerator. After hatching, larvae were checked for viability (normal motility) and placed in groups of 10 nauplii, each one containing 2.5 mL of filtered seawater. The extract was dissolved in seawater, creating a stock solution of twice the maximum concentration used in the experiment (1000 µg/ml). In tubes containing 2.5 ml of sea water and *A. salina* (n=10), 2.5 ml of the extract diluted on concentrations were added (1000, 500 and 250 µg/ml), so that each concentration could be decreased by half. The tubes at the end of the test showed concentrations of 500, 250 and 125 µg/ml, respectively. Each concentration was performed in quadruplicate. *A. salina* was exposed to the extract for 24 hours, and then the results were read. The vitality of *A. salina* was defined considering the movement of microcrustaceans.

The median lethal concentration (LC_{50}) of the extracts of the bark of *C. rhamnifolioides* was determined using the OriginPro 8 software (OriginLab, USA).

The results from the phytochemical study using a methanolic extract of bark of *C. rhamnifolioides* are shown in table 1.

Table 1: Phytochemical prospection of the methanol extract of the bark of *C. rhamnifolioides*

Classes of metabolites	Results
Alkaloids	+++
Triterpenes	+++
Flavonoids	+
Coumarins	_
Saponins	_

(+++)=strongly positive; (++)=Weakly positive; (+) Positive; (-) Negative.

The extract showed presence of alkaloids, flavonoids and triterpenes, and the absence of coumarins and saponins.

Randau (2004) [4], in a similar study using the methanol extract of the stem of *C. rhamnifolioides*, also reported the presence of alkaloids, flavonoids and triterpenes and the absence of coumarins. Santos (2013) [21], in studies using aerial parts (leaves and stems) of *C. rhamnifolioides*, detected the presence of alkaloids, flavonoids and triterpenes and the absence of saponin.

Among the classes of chemical compounds found in plants, there are alkaloids. It is estimated that this class has more than 4.000 compounds, which accounts for 15-20% of all known natural products [22, 23]. They are well known due to significant pharmacological activities, such as morphine and tubocurarine, being an active principle of currently relevant anesthetics [24].

Flavonoids are a class of polyphenols, which is present in relative abundance on vegetables' secondary metabolites. These compounds also have a pharmacological importance resulting from certain properties attributed to representatives of this class, such as anticarcinogenic, anti-inflammatory, anti allergic, antiulcer, antiviral actions, among others properties [25].

Regarding steroids or triterpenes, they are essential or volatile oils. The therapeutic interest in this class of secondary compounds is justified by the importance of cardiotonics glycosides, which are part of this group, and by the interest on sitosterol, stigmasterol and espirostanicas saponins, which serve as raw material primarily for the production of contraceptives, steroids and anti-inflammatories [26].

The methanol extract from the bark of *C. rhamnifolioides* was tested at concentrations 500, 250 and 125 µg/ml, showing a viable *A. saline* of 27.5, 82.5 and 100%, respectively, as demonstrated in fig. 1.

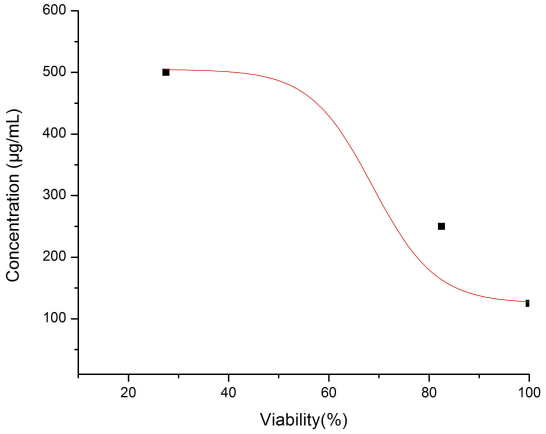

Fig. 1: Toxicological potential of the methanol extract of the bark of *C. rhamnifolioides* on *A. salina*

The results showed that methanol extract has a moderate toxicity with a LC_{50} of 486.59 µg/ml.

According to NGUTA *et al.* (2011) [27], extracts with values of LC_{50} less than 100 µg/ml show high toxicity; LC_{50} between 100 and 500 µg/ml show moderate toxicity; LC_{50} between 500 and 1000 µg/ml show low toxicity; and LC_{50} above 1, 000 µg/ml are considered nontoxic. BUSSMANN *et al.* (2011) [28] stated that extracts with values of LC_{50} less than 249 µg/ml have a high toxicity, LC_{50} between 250 and 499 µg/ml showed a moderate toxicity, LC_{50} between 500 and 1, 000 µg/ml had a mild toxicity and LC_{50} above 1, 000 µg/ml are considered nontoxic.

Thus, the methanol extract of the stem bark of *C. rhamnifolioides* showed a moderate toxicity against *A. salina.*

The use of medicinal plants by the population has shown, over the years that certain species may have adverse reactions and toxic effects on humans and animals [29]. In Brazil, the use of medicinal plants is widespread and, in most cases, the choice for a therapy based on medicinal plants has always been made without a medical guidance [30]. Researches show that many of these plants have potentially dangerous substances and, therefore, should be carefully used, bewaring their toxicological risks [29].

The fact that *C. rhamnifolioides* species have shown a moderate toxicity and highlights the need for more studies aiming at an expanded knowledge on this plant used by people for the treatment of stomach aches, upset stomach, vomiting and diarrhea. More Studies are needed in order to investigate how this species acts, what are its toxic and side effects and if it offers risk to the population when used for long-term periods.

The preliminary phytochemical screening of the methanol extract from the bark of *C. rhamnifolioides* showed the presence of classes of secondary chemical compounds, such as alkaloids, triterpenes and flavonoids, potentially active in biological models. The methanol extract showed a moderate toxicity, revealing the need for further studies, so that that knowledge on this plant, widely used by population, may be expanded.

ACKNOWLEDGEMENT

Acknowledge the financial support received from CAPES / UFPE

CONFLICT OF INTERESTS

Declared None

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