PharmacologyOnLine December 30, 2015

Newsletter • 2015 • vol.3 • 131-135

PHYTOCHEMICAL SCREENING, CYTOTOXIC AND THROMBOLYTIC ACTIVITY EVALUATION OF *MYRCIARIA STRIGIPES* O. BERG, *IPOMOEA ALBA* L. AND *SOLANUM CORDIFOLIUM* DUNAL LEAVES

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Abstract

The popular use of medicinal plants is an advantageous resource on the chemical-biological research in natural products area. *Ipomoea alba* L. (Convolvulaceae), *Solanum cordifolium* Dunal (Solanaceae) and *Myrciaria strigipes* O. Berg (Myrtaceae) are naturally occurring species in Brazil, used for snake bites treatments, liver disorders and abdominal pain, respectively. The aim of this study was to evaluate the chemical profile, the cytotoxicity and thrombolytic activity of ethanolic extracts of this species leaves. The chemical profile was characterized from the performance of phytochemical tests of metabolites classes identification. The cytotoxicity of the extracts was determined by brine shrimp lethality bioassay (*Artemia salina* Leach.), and the in vitro thrombolytic activity was evaluated through the extracts capacity to cause lysis in human blood clot. The phytochemical tests indicated the presence of alkaloids, flavonoids, steroids, tannins and coumarins in all three extracts. Saponins was detected in *I. alba* and *M. strigipes*, while naphthoquinones only in *M. strigipes*. *I. alba* and *S. cordifolium* extracts did not show cytotoxicity front A. salina, in contrast to M. strigipes extract (LD50 = 648.17 ppm). In thrombolytic test, *S. cordifolium* extract exhibited higher activity than *I. alba* and *M. strigipes* extracts (17.65, 10.60 and 3.88% lysis, respectively). This species extracts did not present promising thrombolytic activity. Only M. strigipes demonstrated cytotoxicity to A. salina, which evoke increased attention to the safety of this medicinal plant use.

Keywords: Thrombolytic activity; Medicinal plants; Phytochemical screening; Cytotoxicity activity

Introduction

The popular use of medicinal plants is an advantageous resource on the chemical-biological research in natural products area. The plant secondary metabolism is a large library of chemical compounds, whose structural diversity is continuously evolving and hence discovery [1]. In a preliminary investigation, natural products are subjected to in vitro assays for biological activity screening and chemical characterization tests. This makes it possible to infer which chemical classes may be involved to the biological response evaluated [2]. Historically, natural products have been a leading source of antithrombotic compounds (heparin, vitamin K antagonists, streptokinase, urokinase) [3]. Recent studies evaluate the in vitro thrombolytic activity of herbal extracts, aimed at finding new and promising natural compounds for this purpose [4-9].

Several discussions involving the clinical use of thrombolytic agents. Hypotension and cerebrovascular accident have been observed recurrently, after thrombolytic drugs administration. In addition, aged over 65, previous cardiopulmonary resuscitation and diabetes evoke the inhibition of this drugs use [10-13]. In sight of the clinical restrictions and therapeutic risks associates in thrombolytic therapy, there is a need to search for new molecules with this biological activity. Just as the industrially drugs, the natural products use for therapeutic purposes also requires care, considering their biosafety. In this regard, studies evaluate the plant extracts toxicity, in order to ensure the medicinal plants safe use [14-15].

Myrciaria strigipes O. Berg (Myrtaceae) is known as “cambucá-da-praia”, “ubanaxica”, “manaxica”, “cabeludinha-da-praia” and popurlarly used for cramps, edema and abdominal pain [16]. The *Myrciaria* genus has around 99 known species, which 21 are native in Brazil, including *M. strigipes*. These species are widespread in several Brazilian biomes as Amazon Forest, Caatinga, Cerrado, Atlantic Forest, Pampa and mainly cultivated in Rio de Janeiro, São Paulo, Minas Gerais and Espírito Santo [17]. *Ipomoea alba* L., popularly known as “dama-da-noite” and “boa-noite” in Brazil, belongs to Convolvulaceae family and it is a native species to tropical and subtropical regions of America, from Argentina to Florida. This specie is utilized in gardening due to its beauty, although also others purposes are related, such as treatment of paralysis and soft tissue swelling, snake bites and religious rituals [18-20]. *Solanum cordifolium* Dunal, known as “jurubeba” and “joá-manso”, is helpful for anemia and hepatic and digestive disorders [21]. This specie is a representative from Solanaceae family, which consists of approximately 3000 species and 90 genus, including *Solanum* genus that stands out with over 1.500 species [22]. *S. cordifolium* is native in Brazil and geographically distributed mainly in Rio de Janeiro, Minas Gerais and Espírito Santo [23].

This paper describes the phytochemical study and the cytotoxicity and thrombolytic activity evaluation of ethanolic extracts from *I. alba*, *S. cordifolium* and *M. strigipes* leaves, aiming to contribute to the chemical-biological knowledge of these species.

Methods

Collection and identification of plant material and preparation of ethanolic extracts

I. alba, *M. strigipes* and *S. cordifolium* leaves were collected in Governador Valadares (MG), São Mateus (ES) and Vila Velha (ES), Brazil, respectively. The plant material collected was identified and a voucher specimen of *I. alba* has been deposited in the herbarium at the Vale do Rio Doce University (Univale) under the identification number 744, and a voucher specimens of *M. strigipes* and *S. cordifolium* has been deposited in the VIES Herbarium at the Federal University of Espírito Santo under identification number 25.038 and 12.357-1, respectively. The collected leaves were separately dried for about 72 hours in an oven at 38-40 °C. The dried plant materials were ground into coarse powder and separately extracted by passive maceration with ethanol. It was then filtered, and the filtrate was then concentrated using a rotary evaporator. The three extracts obtained were preserved in a refrigerator (2 to 5 °C) until used in subsequent chemical and biological assays.

Preliminary phytochemical screening

The ethanolic extracts were subjected to preliminary phytochemical screening to identify major classes of secondary metabolites. For each class of metabolite there are specific reactions that indicate their presence from change or formation of color, foam, fluorescence or precipitate. These tests were evaluated the presence of flavonoids, triterpenes, steroids, naphthoquinones, saponins, alkaloids, tannins, coumarins and anthraquinone heterosides [24-25].

Artemia salina lethality assay

The cytotoxicity of the extracts was evaluated on *A. salina*, according to the methodology proposed by Meyer et al (1982) [26]. *A. salina* encysted eggs were incubated for 48 hours in saline solution

(36 g/L) at 28 °C, under constant light and aeration, for hatching of larvae in a nauplii state. Then the larvae were distributed into tubes containing extracts at different concentrations (250, 500, and 1000 ppm). Potassium dichromate (250, 500, and 1000 ppm) and extracts vehicle (dimethyl sulfoxide, DMSO, 1% v/v) were employed as positive and negative control, respectively. Survivors larvae were counted after 24 hours. The lethality percentage was calculated for each concentration and then the 50% lethal dose (LD50) values were determined by linear regression. The assay was performed in triplicate.

In vitro thrombolytic activity

The in vitro thrombolytic activity of the extracts was evaluated according to the methodology proposed by Prasad et al. (2006) [27]. Small portions of human venous blood (500 µL) contained in microcentrifuge tubes were incubated at 37 °C for 45 minutes. After formation of clot, the remaining fluid was removed without disrupting the clot formed and each microcentrifuge tube was weighed to determine the clot weight. After the addition of 150 uL of thrombolytic agent, the tubes were incubated at 37 °C for 90 minutes and the resulting fluid clot lysis was carefully aspirated. Then, the clot was again weighed and the percentage of clot lysis was calculated. The extracts were evaluated at concentration of 1,0 mg/mL solubilized in propylene glycol 10% v/v. As thrombolytic activity control it was used streptokinase 100.000 UI, solubilized in distilled water and the thrombolytic activity of vehicles was evaluated too. The assay was performed in quadruplicate. The significance of the percentage of clot lysis promoted by plants extracts and streptokinase when compared with their vehicles was tested by the Student t test with independent samples, using IBM SPSS Statistics 20 program ($p<0.05$). This assay was approved by the Ethics Committee at the Federal University of Espírito Santo, Brazil, under number 148.873.

Results

Preliminary phytochemical screening

Phytochemical tests revealed the presence of alkaloids, flavonoids, steroids, tannins, and coumarins in all three plants studied. Saponins was detected in *I. alba* and *M. strigipes*, while naphthoquinones only in *M. strigipes*.

Artemia salina lethality assay

The ethanolic extracts from *I. alba* and *S. cordifolium* leaves did not show cytotoxicity on *A. salina* (LD50>1000 ppm), as opposed to *M. strigipes* leaves ethanolic extract (LD50 648.17 ppm).

In vitro thrombolytic activity

The *S. cordifolium* extract exhibited higher activity than *I. alba* and *M. strigipes* extracts (17.65, 10.60 e 3.88% lysis, respectively). The highest percentage of lysis was achieved by streptokinase, whose value was 50,12%. *S. cordifolium* extract and streptokinase showed significant differences ($p <0,001$ and $p = 0.003$, respectively) when compared with the respective vehicles used for the solubilization. All results are presented in Table 01.

Discussion

The knowledge related to the presence of certain chemical classes in plant species facilitates the choice of the chromatographic process and isolation, as well as guides the possible biological assays [28]. Another application of the results obtained in the phytochemical screening is associated with the definition of chemical markers for quality control of these species [29]. Studies show that the chemical composition of plant species can be changed, according to climatic factors (circadian cycle and seasonality), herbivory, or even the human action [30], which can influence directly the therapeutic action promoted by using of medicinal plant.

Among the three species, *M. strigipes* shows a greater varietmy of chemical classes. The presence of naphthoquinones may be related to the cytotoxicity performed by this extract in the *A. salina* bioassay. This chemical class is known for its toxicity, because of their bioactive properties, such as antitumor, microbicides trypanocidal activities [31].

The *A. salina* toxicity assay is widely used for predicting important pharmacological activities such as enzyme inhibition, ion channels interference and antimicrobial, cytotoxic and anti-*Trypanosoma cruzi* activities [32-34]. In this respect, extracts that have higher toxicity on *A. salina* can be most promising for these activities. However, plant species which expressing toxicity on *A. salina* may also present risks to human health [35-36]. Among the species evaluated in this study, only *M. strigipes* showed cytotoxicity to the *A. salina* larvae, which arouses greater attention to the safety use of this medicinal plant and leads new studies to investigate the bioactive potential of this extract.

Approximately 29.6% of all deaths worldwide in 2010 were due to cardiovascular diseases, among them there is the ischaemic heart disease that was most responsible for this percentage [37]. In the ischemic diseases treatment an alternative for the restoration

of blood flow is the thrombolysis promoted by drugs [10]. The thrombolytic drugs arsenal is still limited when compared to other therapeutic classes, such as the antiplatelet agents used in cardiovascular diseases [38]. Thus, research in order to discover new thrombolytic agents are needed in attempt to expand the list of drugs in this pharmacological class. The three plant species studied showed clots lysis ability of weak to moderate, and only *S. cordifolium* extract had statistical difference when compared to the vehicle. Similar results with other plant species have been described by several researchers. Khatun et al in 2014 [13] evaluated the thrombolytic activity of *Cestrum diurnum*, reaching 8.78% as a result of clots lysis. Hussain et al 2014 [10] studying the thrombolytic activity of four medicinal plants available in Bangladesh found results ranging from 15.1% to 21.26% of clots lysis. On the other hand, the six plants studied by Prasad et al 2007 [15] two, *Bacopa monnieri* and *Fagonia Arabica*, showed promising clots lysis capacity, 41.8% and 75.6%, respectively, which then proves the existence of plant molecules with such a bioactive property. The results found in this paper contribute to the chemical-biological knowledge of *Myrciaria strigipes*, *Ipomoea alba* and *Solanum cordifolium*. In view of the literature, this study is the first report to assess the thrombolytic activity of these plant species. The bioassay with larvae of *A. salina* allowed to infer that *M. strigipes* species has cytotoxic potential, which arouses attention to the secure use of this medicinal plant.

Acknowledgments

UFES and Postgraduate Program in Pharmaceutical Science – UFES (PPGCFAR).

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Table 1. Thrombolytic activity of *I. alba*, *S. cordifolium* and *M. strigipes* extracts

Extracts, Control and Vehicles	***Clot lysis (%)***	***p* value (compared to vehicle)**
Ipomoea alba	10.60 ± 2,62	0.116
Solanum cordifolium	17.65 ± 3,88	0.003
Myrciaria strigipes	9.54 ± 1,97	0.247
Streptoquinase	50.12 ± 9,23	<0.001
Water	6.42 ± 6,22	-
Propylenoglycol 10%	8.10 ± 0,73	-