Phytochemical Investigation and Cytotoxic Properties of Tabernaemontana catharinensis A. DC. Cultivated in Brazil

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ABSTRACT

Many laboratories of natural products have entered into their routines various biological assays simple and phytochemical study of plants extracts used popularly in medicine in order to select bioactive substances. Tabernaemontana catharinensis is a medicinal plant used in folk medicine with antidote for snakebites, to relieve toothache and as a vermifuge to eliminate warts. Phytochemical constituents and cytotoxic effect of the plant were investigated using the qualitative analysis and the brine shrimp lethality method, respectively. The phytochemistry of aqueous and hydroalcoholic extracts of T. catharinensis leaves and stem bark revealed the presence of alkaloids, flavonoids, phenols, tannins, sterols, saponins, catechins and coumarins. The aqueous extracts of this plant did not induce the lethality in the brine shrimp (Artemia salina Leach) bioassay (LC_{50} = 1057.90±0.48 and 1002.5±0.36 μg mL⁻¹ for leaves and stem bark, respectively); however, hydroalcoholic extracts shows cytotoxicity for this crustacean (LC_{50} = 629.35±0.12 and 504.18±0.15 μg mL⁻¹ for leaves and stem bark, respectively). In this study, it was observed that the toxicity to Artemia salina converged to the extracts containing the highest amount of bioactive compounds (hydroalcoholic extracts), other in vivo tests will be conducted to determine the exact mechanism of action involved in the toxicity described.

Key words: Tabernaemontana catharinensis, cytotoxicity, phytochemical constituents, hydroalcoholic extracts

INTRODUCTION

Plants of the genus Tabernaemontana, also known as Peschiera, family Apocynaceae, comprising about 44 species widely distributed in America and is potentially rich in alkaloids which are considered as chemical markers of this genus, thus contributing to the classification of its species (Chaturvedula et al., 2003; Pereira et al., 2008). Antileishmanial, trypanocidal, antiviral, antimicrobial, antitumoral, anti-inflammatory, antioxidant, analgesic and cardiotonic activities are well described for the Tabernaemontana species and presence of triterpenoids, steroids and several indole alkaloids can be responsible for these actions described (Van Beek et al., 1984; De Almeida et al., 2004; Pereira et al., 2008; Lim et al., 2009). Tabernaemontana catharinensis (common known as snake skin) is a native tree in the southern part of Brazil and in popularly medicine, it is used for its anti-inflammatory properties, to relieve toothache, eliminate warts and as antidote for snakebites (Leeuwenberg, 1994; Pereira et al., 2008).

Several researchers working with plant products are adding into their routine isolation, purification and structural elucidation, several simple biological assays in order to select and
monitor the phytochemical study of plant extracts in the search for substances bioactive (Ansari and Inamdar, 2010; Boligon et al., 2012). Among these bioassays is the brine shrimp toxicity (Artemia salina) which is characterized by being low cost, fast and does not require aseptic techniques (Mojica and Micor, 2007; Ramachandran et al., 2011). Numerous bioactive constituents have been obtained from medicinal herbs such as: flavonoids, phenolic compounds, alkaloids, steroids, tannins, saponins, catechins, among others; these chemical constituents are responsible for a particular pharmacological action on the human body (Janovik et al., 2011; Agrawal et al., 2011; Namrata et al., 2011; Gill et al., 2012; Das et al., 2012).

Considering the importance of the discovery of new therapeutic agents from plant products, the aim of this study was phytochemical investigation and evaluation of cytotoxic effect of the aqueous and hydroalcoholic extracts of T. catharinensis leaves, stem and bark.

MATERIALS AND METHODS

Plant collection: Leaves and stem bark of T. catharinensis were collected in Bossoroca (Rio Grande do Sul State of Brazil) in September of 2009 (coordinates 28º65'93" S and 55º01'27" W). A dried voucher specimen is preserved in the herbarium of the Department of Biology at Federal University of Santa Maria by register number SMBD 12355.

Preparation of extracts: The leaves (1580.76 g) and stem bark (1051.23 g) of the plant were dried at room temperature and powdered in a knife mill (0.86 μm). The powders were macerated separately at room temperature with ethanol 70% for seven days a week with daily shake-up. A portion of the ethanolic extracts were reserved and the other part was filtered and evaporated to remove ethanol to obtain the aqueous extracts remaining for stem, bark and leaves.

Phytochemical screening: Qualitative phytochemical screening analysis of the hydroalcoholic and aqueous extracts of the leaves and stem bark of T. catharinensis were carried out to identify the presence of various phytoconstituents (Moreira, 1979; Gill et al., 2012). The phytochemicals groups tested were: Alkaloids (Dragendorff, Bouchardat and Bertrand reactions), Flavonoids (Shinoda reaction), Phenols and Tannins (Ferric chloride 1%), Steroids (Liebermann-Burchard reaction), Saponins (Foam test), Catechins (Na,CO₃ reaction), Coumarins (NaOH/Ethanol, UV) and Reducing sugars (Benedict reaction).

Cytotoxicity assay (Brine Shrimp lethality bioassay): Shrimp eggs were purchased from a local Pet Shop (Santa Maria, RS, Brazil). The eggs were hatched in a glass flask containing 1 liter of artificial seawater (Meyer et al., 1982). The flask was aerated with the aid of an air pump and kept in a water bath at 29-30°C. A bright light source was left on and the nauplii hatched within 24 h. Each sample to be tested was dissolved in DMSO and subsequent diluted serially (10, 5, 1, 0.1 and 0.01 mg mL⁻¹) in seawater. Ten nauplii were collected with a Pasteur pipette and added to each set of tubes containing the samples. A check count was performed and the number of alive nauplii after 24 h was noted. The negative control was saline solution and the positive control was potassium dichromate solution (20 ppm). LD₅₀ were determined using the Probit analysis method (Finn, 1971).

Statistical analysis: The results of cytotoxic effect were presented as mean of triplicate and Standard Deviation (SD).
RESULTS AND DISCUSSION

Hydroalcoholic extracts of the leaves and stem bark of *T. catherinensis* showed the presence of alkaloids, flavonoids, phenols, tannins, sterols, saponins and catechins. Coumarin was not found in the hydroalcoholic extract leaves and reducing sugars are not present in the hydroalcoholic extract of both parts of the plant. On the other hand, the aqueous extracts of the plant revealed the presence of alkaloids, phenols, tannins, steroids and catechins (Table 1).

The phytochemical screening is an important step in the chemical and pharmacological study of a medicinal plant. It may suggest possible pharmacological effects of its extracts or fractions in comparison of identified phytochemicals groups, highlighting a close relationship with its main therapeutic uses (Jana and Shekhawat, 2010; Adinortey et al., 2012). Thereby, results of phytochemical screening are in accordance with previously published work, Batina et al. (2000) isolated from *T. catherinensis* a quaternary base alkaloid which proved to be able to inhibit the lethal activity of 2 LD₅₀ of *Crotalus durissus terrificus* venom, the presence of alkaloids can also be related with anticancer and antileishmanial activities described for the specie (Van Beek et al., 1984). The presence of flavonoids and phenols may have related to the *T. catherinensis* use as antioxidant and antimycobacterial (Pereira et al., 2005). Additionally, steroids were identified for this specie and this group of substances may promote antitumoral, anti-inflammatory, analgesic properties (Pereira et al., 2008; Gill et al., 2011).

The *Artemia salina* lethality assay has been demonstrated to be an effective, robust and rapid assay method for primary screening of extracts and compounds for potential cytotoxic activities (Meyer et al., 1982; Monira et al., 2012). Cytotoxicity of *T. catherinensis* extracts were evaluated by brine shrimp lethality bioassay (Table 2) in view of the fact that *Artemia salina* larvae have

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Hydroalcoholic</th>
<th>Aqueous</th>
<th>Hydroalcoholic</th>
<th>Aqueous</th>
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</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Phenols and tannins</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>Steroids</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Saponins</td>
<td>++</td>
<td>-</td>
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<td>Catechins</td>
<td>+</td>
<td>-</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>Coumarins</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
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<tr>
<td>Reducing sugars</td>
<td>-</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
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*: Absent, Presence; +++: Strong, ++: Average, +: Weak, No: Not determined

<table>
<thead>
<tr>
<th><em>T. catherinensis</em></th>
<th>Extracts</th>
<th>LC₅₀±SD (µg mL⁻¹)</th>
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<tr>
<td>Leaves</td>
<td>Aqueous</td>
<td>1057.50±0.48</td>
</tr>
<tr>
<td></td>
<td>Hydroalcoholic</td>
<td>629.35±0.12</td>
</tr>
<tr>
<td>Stem bark</td>
<td>Aqueous</td>
<td>1002.50±0.36</td>
</tr>
<tr>
<td></td>
<td>Hydroalcoholic</td>
<td>504.18±0.15</td>
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Results are expressed as Means±SD of three determinations

Table 1: Phytochemical analysis of the hydroalcoholic and aqueous extracts of *T. catherinensis* leaves and stem bark

Table 2: Cytotoxic activities of the *T. catherinensis* extracts against *Artemia salina* larvae
been used as a target organism to detect bioactive compounds in plant extracts (Sam, 1993). Accordingly to Meyer et al. (1982), who classified crude extracts and pure substances into toxic (LC₅₀ value <1000 μg mL⁻¹) and non-toxic (LC₅₀ value >1000 μg mL⁻¹), aqueous tested extracts of T. catharinensis may be considered non-toxic for this crustacean (LC₅₀ = 1057.90±0.48 and 1002.5±0.36 μg mL⁻¹ for leaves and stem bark, respectively). However, the hydroalcoholic extracts were classified with toxic (LC₅₀ = 828.35±0.12 and 504.18±0.15 μg mL⁻¹ for leaves and stem bark, respectively) (Table 2). These toxicological data, can be correlated with tests acute oral toxicity in animals (Parra et al., 2001). Das et al. (2012) using the brine shrimp bioassay demonstrated also toxicity for the hydroalcoholic extract of Cucumis sativus.

CONCLUSION

It was concluded that the hydroalcoholic extracts of T. catharinensis leaves and stem bark had cytotoxic activity. The present study in vitro of the plant was a preliminary investigation for future research work. So, further phytochemical and pharmacological studies on T. catharinensis are strongly recommended to elucidate the extract chemical compounds and mechanisms involved.

ACKNOWLEDGMENT

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REFERENCES


