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

A.A. Boligon and M.L. Athayde
Department of Industrial Pharmacy, Federal University of Santa Maria, Santa Maria, RS, Brazil

Corresponding Author: A.A. Boligon, Department of Industrial Pharmacy, Federal University of Santa Maria, Santa Maria, RS, Brazil

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 28°65'93" S and 55°01'27" 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_2CO_3 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). LD_{50} were determined using the Probit analysis method (Finny, 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. catharinensis* 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. catharinensis* a quaternary base alkaloid which proved to be able to inhibit the lethal activity of 2 LD_{50} 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. catharinensis* 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. catharinensis* extracts were evaluated by brine shrimp lethality bioassay (Table 2) in view of the fact that *Artemia salina* larvae have

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

	T. catharinensis			
	Leaves		Stem bark	
Phytoconstituents	Hydroalcoholic	Aqueous	Hydroalcoholic	Aqueous
Alkaloids	+++	+	+++	++
Flavonoids	+	-	+	-
Phenols and tannins	++	+	+++	++
Steroids	++	+	+	-
Saponins	++	-	++	-
Catechins	+	-	+++	+
Coumarins	-	-	++	-
Reducing sugars	-	ND	-	ND

-: Absent, Presence; +++: Strong, ++: Average, +: Weak, No: Not determined

Table 2: Cytotoxic activities of the *T. catharinensis* extracts against *Artemia salina* larvae

T. catharinensis	Extracts	LC_{50}±SD (μg mL^{-1})
Leaves	Aqueous	1057.90±0.48
	Hydroalcoholic	629.35±0.12
Stem bark	Aqueous	1002.50±0.36
	Hydroalcoholic	504.18±0.15

Results are expressed as Mean±SD of three determinations

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 (LC_{50} value <1000 μg mL^{-1}) and non-toxic (LC_{50} value >1000 μg mL^{-1}), aqueous tested extracts of *T. catharinensis* may be considered non-toxic for this crustacean (LC_{50} = 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 (LC_{50} = 629.35±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.

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REFERENCES

Adinortey, M.B., J.K. Sarfo, E.T. Quayson, A. Weremfo, C.A. Adinortey, W. Ekloh and J. Ocran, 2012. Phytochemical screening, proximate and mineral composition of *Launaea taraxacifolia* leaves. J. Med. Plant, 6: 171-179.

Agrawal, B., S. Das and A. Pandey, 2011. *Boerhaavia diffusa* Linn: A review on its phytochemical and pharmacological profile. Asian J. Applied Sci., 4: 663-684.

Ansari, J.A. and N.N. Inamdar, 2010. The promise of traditional medicines. Int. J. Pharmacol., 6: 808-812.

Batina, M.D.F.C., A.C.O. Cintra, E.L.G. Veronese, M.A.S. Lavrador and J.R. Giglio *et al.*, 2000. Inhibition of the lethal and myotoxic activities of *Crotalus durissus* terrificus venom by *Tabernaemontana catharinensis*: Identification of one of the active components. Planta Med., 66: 424-428.

Boligon, A.A., T.F. De Brum, J.K. Frolhich, A.L.F. Froeder and M.L. Athayde, 2012. HPLC/DAD profile and determination of total phenolics, flavonoids, tannins and alkaloids contents of *Scutia buxifolia* Reissek stem bark. Res. J. Phytochem., 6: 84-91.

Chaturvedula, V.S.P., S. Sprague, J.K. Schilling and D.G. Kingston, 2003. New cytotoxic indole alkaloids from *Tabernaemontana calcarea* from the Madagascar rainforest. J. Nat. Prod., 66: 528-531.

Das, J., A. Chowdhury, S.K. Biswas, U.K. Karmakar, S.R. Sharif, S.Z. Raihan and M. Abdul Muhit, 2012. Cytotoxicity and antifungal activities of ethanolic and chloroform extracts of *Cucumis sativus* Linn (cucurbitaceae) leaves and stems. Res. J. Phytochem., 6: 25-30.

De Almeida, L., A.C.O. Cintra, E.L.G. Veronese, A. Nomizo and J.J. Franco *et al.*, 2004. Anticrotalic and antitumoral activities of gel filtration fractions of aqueous extract from *Tabernaemontana catharinensis* (Apocynaceae). Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol., 137: 19-27.

Finny, D., 1971. Probit Analysis. Cambridge University Press, Cambridge.

Gill, N.S., J. Bajwa, K. Dhiman, P. Sharma and S. Sood *et al.*, 2011. Evaluation of therapeutic potential of traditionally consumed *Cucumis melo* seeds. Asian J. Plant Sci., 10: 86-91.

Gill, N.S., R. Kaur, R. Arora and M. Bali, 2012. Phytochemical investigation of *Caesalpinia crista* seed extract for their therapeutic potential. Res. J. Med. Plant, 6: 100-107.

Jana, S. and G.S. Shekhawat, 2010. Phytochemical analysis and antibacterial screening of *in vivo* and *in vitro* extracts of Indian medicinal herb: *Anethum graveolens*. Res. J. Med. Plant., 4: 206-212.

Janovik, V., A.A. Boligon, R.V. Bandeira and M.L. Athayde, 2011. HPLC/DAD analysis, determination of total phenolic and flavonoid contents and antioxidant activity from the leaves of *Cariniana domestica* (Mart) miers. Res. J. Phytochem., 5: 209-215.

Leeuwenberg, A.J.M., 1994. A Revision of Tabernaemontana. The New World Species and Stemmadenia. Vol. 2, The Royal Botanic Gardens, Kew, UK.

Lim, K.H., N.F. Thomas, Z. Abdullah and T.S. Kam, 2009. Seco-tabersonine alkaloids from *Tabernaemontana corymbosa*. Phytochemistry, 70: 424-429.

Meyer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. McLaughlin, 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med., 45: 31-34.

Mojica, E.R.E. and R.L. Micor, 2007. Bioactivity study of *Barringtonia asiatica* (Linnaeus) Kurz. seed aqueous extract in *Artemia salina*. Int. J. Bot., 3: 325-328.

Monira, S., A. Haque, A. Muhit, N.C. Sarker, A.H.M.K. Alam, A.A. Rahman and P. Khondkar, 2012. Antimicrobial, antioxidant and cytotoxic properties of *Hypsizygus tessulatus* cultivated in Bangladesh. Res. J. Med. Plant, 6 : 300-308.

Moreira, E.A., 1979. Contribution to the phytochemical study of *Lobelia hassleri* A. ZAHLB e *Lobelia stellfeldii* R. braga companulaceae. Tribuna Farmaceutica, 47: 13-39.

Namrata, L. Kumar, D. Ghosh, S.C. Dwivedi and B. Singh, 2011. Wild edible plants of Uttarakhand Himalaya: A potential nutraceutical source. Res. J. Med. Plant, 5: 670-684.

Parra, A.L., R.S. Yhebra, I.G. Sardinas and L.I. Buela, 2001. Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD_{50} value) in mice, to determine oral acute toxicity of plant extracts. Phytomedical, 8: 395-400.

Pereira, C.G., P.F. Leal, D.N. Sato and M.A.A. Meireles, 2005. Antioxidant and antimycobacterial activities of *Tabernaemontana catharinensis* extracts obtained by Supercritical CO_2^+ cosolvent. J. Med. Foods, 8: 533-538.

Pereira, P.S., S.C. Franca, P.V.A. Oliveira, C.M.S. Breves and S.I.V. Pereira *et al.*, 2008. Chemical constituents from *Tabernaemontana catharinensis* root bark: A brief NMR review of indole alkaloids and *in vitro* cytotoxicity. Quim. Nova, 31: 20-24.

Ramachandran, S., M. Vamsikrishna, K.V. Gowthami, B. Heera and M.D. Dhanaraju, 2011. Assessment of cytotoxic activity of *Agave cantula* using brine shrimp (*Artemia salina*) lethality bioassay. Asian J. Sci. Res., 4: 90-94.

Sam, T.W., 1993. Toxicity Using the Brine Shrimp *Artemia salina*. In: Bioactive Natural Products: Detection, Isolation and Structural Determination, Colegate, S.M and R.J. Molyneux (Eds.). CRC Press, USA., pp: 441.

Van Beek, T.A., R. Verpoorte, A.B. Svendsen, A.J.M. Leeuwenberg and N.G. Bisset, 1984. *Tabernaemontana* L. (Apocynaceae): A review of its taxonomy, phytochemistry, ethnobotany and pharmacology. J. Ethnopharmacol., 10: 1-156.