SHORT COMMUNICATION

In vitro antiproliferative activity of alkaloids isolated from Tabernaemontana catharinensis A.DC (Apocynaceae)

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In this work, four alkaloids from the stem bark of T. catharinensis were isolated, namely: voacangine (1); ethyl apovincaminate (2); affinisine (3) and voachalotine (4). The alkaloids were tested in vitro for antiproliferative capacity in eight tumor cell lines: U251 (glioma), MCF-7 (breast), NCI-ADR/RES (drug resistant ovary), 786-0 (kidney), NCI-H460 (lung), HT-29 (colon), K562 (leukemia) and PC-3 (prostate) and a non-tumor keratinocyte cell line (HaCat). Antiproliferative activity was observed after 48 hours and results expressed as the concentration needed to induce 50% growth inhibition (GI50) in µM. The chemotherapy drug Doxorubicin was used as a standard. The alkaloid affinisine (3) was the most promising, showing moderate inhibition rates in addition to the cytotoxic and cytocidal effect against all strains tested. It also proved to be a very promising compound, showing high selectivity rates when compared to the non-tumor keratinocyte cell line (HaCat).

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1. Introduction

Cancer is a health problem worldwide compromising around 1 million new cases a year according to the World Health Organization (WHO) being the of non-melanoma, lung, colon, breast and prostate cancers the most frequent (Mattiuzzi and Lippi 2019). In this context, the development of new alternatives for cancer treatment remains necessary and challenging (de Miguel and Calvo 2019). Natural products still a promisor source of new options for cancer prevention and chemotherapy (Newman and Cragg 2020; Sauter 2020).

*Tabernaemontana catharinensis* is found in Bolivia, Paraguay, Argentina, Uruguay and in the Northeast, Midwest, Southeast and South regions of Brazil. In Rio Grande do Sul, it is popularly known as ‘cobrina’ and ‘forquilheira’. Crude extracts from the bark of the stem, roots and leaves have wide ethnopharmacological applications, from vermifuge to snakebite antidote, analgesic and anti-inflammatory (Van Beek et al. 1984; Matozinhos and Konno 2011).

In studies with extracts and fractions of the bark of the stem and roots of *T. catharinensis*, numerous pharmacological activities were reported, including antitumor activities. Such activities are due to the presence of indole nucleus alkaloids (Rosales et al. 2019a,b, Naidoo et al. 2021).

Due to the high incidence of cancer, which devastates the world population, and the cellular resistance to conventional chemotherapy, the objective of this work was to carry out the acid-basic fractionation of the extract from the trunk bark of the *T. catharinensis*, to isolate and identify the major alkaloids and testing them against eight hitherto unreported human tumor cell lines, U251 (glioma), MCF-7 (breast), NCI-ADR/RES (drug resistant ovary), 786-0 (kidney), NCI-H460 (lung), HT-29 (colon), K562 (leukemia) and PC-3 (prostate), in addition to a non-tumor cell line (keratinocyte, HaCat) in order to obtain a supposed chemotherapeutic agent.

2. Results and discussion

Chromatographic purification of ethyl acetate acidic fraction (AAcF) and basic ethyl acetate fraction (BAcF) resulted in four compounds (Figure 1): voacangine (1), ethyl apovincaminate (2), affinisine (3) and voachalotine (4). The spectroscopic data was compared with the ones reported in the literature (Pereira et al. 2008; Nemes et al. 2008).

The in vitro antiproliferative activity of the major alkaloids isolated from *T. catharinensis* was evaluated against a panel of human tumor and non-tumor cell lines using doxorubicin as a positive control of cell growth inhibition (Table S1, Figure S1 supplementary material). The effect was expressed as the required concentration to promote 50% of cell growth inhibition (GI50) and it was classified according to the NCI’s criteria based on the log GI50 values (Fouche et al. 2008). The selectivity index (SI) (Suffness and Pezzuto 1990; Bézivin et al. 2003; Oliveira et al. 2015; Bormio Nunes et al. 2019) was calculated (Table S1) evidencing how many times one sample was more active against tumor cells than against non-tumor cells.

The alkaloid affinisine (3) showed the best antiproliferative results moderately inhibiting glioblastoma growth (U251, GI50 = 9.5 μM), multi-drug resistant ovarian
adenocarcinoma (NCI-ADR/RES, GI<sub>50</sub> = 8.3 μM), renal adenocarcinoma (786-0, GI<sub>50</sub> = 8.3 μM) and colorectal adenocarcinoma (HT29, GI<sub>50</sub> = 9.3 μM) cell lines. For all these cell lines, the selectivity index (SI) calculated was at least 1.5, which corresponds to how much affinisine is more active against tumor cells without causing an effect on non-tumor cells (keratinocytes, HaCat).

While ethyl apovincaminate (2) and voachalotine (4) were inactive (GI<sub>50</sub> > 50 μM), voacangine (1) showed weak cytostatic effect against colorectal adenocarcinoma (HT29, GI<sub>50</sub> = 14.4 μM) cells (Table S1). These results suggested the influence of substituent groups on the basic skeleton of alkaloids 1, 2 and 4.

According to the literature, affinisine was able to inhibit both butyryl- and acetil- colinesterases (Marinho et al. 2016). Against different *Plasmodium falciparum* strains, affinisine was inactive (IC<sub>50</sub> > 300 ng.mL<sup>-1</sup>) (Federici et al. 2000). Bioguided fractionation of *T. catharinensis* stem extract afforded some fractions that significantly reduced in 50% the viability of A375 (melanoma cell line) and A549 tumor cells (adenocarcinomic human alveolar basal epithelial cells) (Rosales et al. 2019). Affinisine was identified as the main compound in the most active fraction. In addition, the *in silico* evaluation evidenced the binding affinities of affinisine to topoisomerase besides its low toxicity risk (Rosales et al. 2019). The alkaloid voacangine induced apoptosis in THP-1 (acute monocytic leukemia, IC<sub>50</sub> = 61.40 μM) cells after 18 hours of exposure (Figueiredo et al. 2010) in addition to cholinesterase inhibitory effects (Marinho et al. 2016).

Indole alkaloids constitute a class of different products with complex chemical structures and potent antiproliferation activity against drug-sensitive and drug-resistant cancer cell lines. Several indole alkaloids have already been used in clinical trials

Figure 1. Compounds 1–4 structure.
to treat a variety of cancers, including drug resistant cancers, revealing the potential of indole alkaloids as purported anticancer agents. The structure-activity relationship (SAR) also demonstrated that indole alkaloids exhibited excellent activity against drug-resistant cancer cell lines, which deserve further investigation (Xu and Xu 2020).

Due to the results of cell growth inhibition, selective cytostatic effect and low toxicity against healthy cells, additional in vivo evaluations will be necessary to confirm the efficiency and safety of affinisine (3) as a possible chemotherapeutic agent.

3. Experimental
See supplementary materials.

4. Conclusion
In the present study, it was possible to isolate the four major alkaloids from T. catharinvestem bark and in vitro test their antiproliferative activities. Considering the tested alkaloids voacangine (1), ethyl apovincaminate (2), affinisine (3), and voachalotline (4), affinisine (3) was the most promising one against glioma cell lines (GI50: 9.5 μM; SI: 1.6), ovary (GI50: 8.3 μM; SI: 1.8), kidney (GI50: 8.3 μM; SI: 1.8) and colon (GI50: 9.3 μM; SI: 1.6).

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