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Bio-guided search of active indole alkaloids from *Tabernaemontana catharinensis*: Antitumour activity, toxicity *in silico* and molecular modelling studies

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ABSTRACT

Active plant metabolites have been used as prototype drugs. In this context, *Tabernaemontana catharinensis* (Apocynaceae) has been highlighted because of the presence of active indole alkaloids. Thus, this study aims the bio-guided search of *T. catharinensis* cytotoxic alkaloids. The chemical composition was identified by high-resolution mass spectrometry, and fractionation was performed by open column and preparative thin-layer chromatography, from plant stems. The enriched fractions were tested *in vitro* in tumour cells A375 (melanoma cell line) and A549 (adenocarcinomic human alveolar basal epithelial cells), and non-tumour Vero cells (African green monkey kidney epithelial cells). The alkaloids identified as active were submitted to *in silico* toxicity prediction by ADME-Tox and OSIRIS programs and, also, to molecular docking, using topoisomerase I (PDB ID: 1SC7) by iGEMDOCK. As a result, six sub-fractions were obtained, which were identified as containing 16-*epi*-affinine, 12-methoxy-*n*-methyl-voachalotine, affinisine, voachalotine, coronaridine hydroxyindoline and ibogamine, respectively. The affinisine-containing sub-fraction showed selective toxicity against A375, with an IC_{50} of 11.73 $\mu g\,mL^{-1}$, and no cytotoxicity against normal cells (Vero). From the *in silico* toxicity test results, all indole alkaloid compounds had a low toxicity risk. The molecular docking data provided structural models and binding affinities of the plant's indole alkaloids and topoisomerase I. In summary, this bio-guided search revealed that the indole alkaloids from *T. catharinensis* display selective cytotoxicity in A375 tumour cells and toxicity *in silico*. Particularly, affinisine might be a chemotherapeutic for A375 melanoma cells.

1. Introduction

Cancer incidence rates have increased in the last decades. According to the World Health Organisation (WHO), 9.6 million people worldwide are estimated to die from cancer in 2018. In 2020, 17,114, 1 million new cancer cases and 10 million cancer deaths worldwide are expected, and these values continue to grow [1]. Data from Brazil's National Institute of Cancer (INCA), estimates 600,000 new cases in 2018 [2].

Among the malignant tumours, lung cancer is the most common, and its worldwide incidence increases 2% per year. The total cumulative average survival over 5 years ranges from 13 to 21% in developed countries and between 7 and 10% in developing countries [3]. Cutaneous melanoma is a type of skin cancer that originates in melanocytes. Although skin cancer is the most frequent cancer in Brazil and accounts for 30% of all malignancies registered in the country, melanoma represents only 3% of malignant neoplasms, although it is the most serious, due to its high possibility of metastasis [2].

The discovery of new drugs from plants extracts has been highlighted in recent years [4]. Examples, such as the indole alkaloids, including vinblastine and vincristine, were isolated from *Catharanthus roseus*, and have been used in antineoplastic treatment [5,6].

Present in South America, *Tabernaemontana catharinensis* A. DC. (family Apocynaceae), is well-known for the presence of indole alkaloids. These secondary metabolites present diverse chemical structures [7]. For instance, from methanol bark, wood and root extract of *T. catharinensis*, coronaridine, voacangine and ibogaine (iboga-type) were identified, as well as affinisine and vobasine (corynanthean-type) [8]. Compounds, like coronaridine [9], 3-oxo-coronaridine and ibogamine

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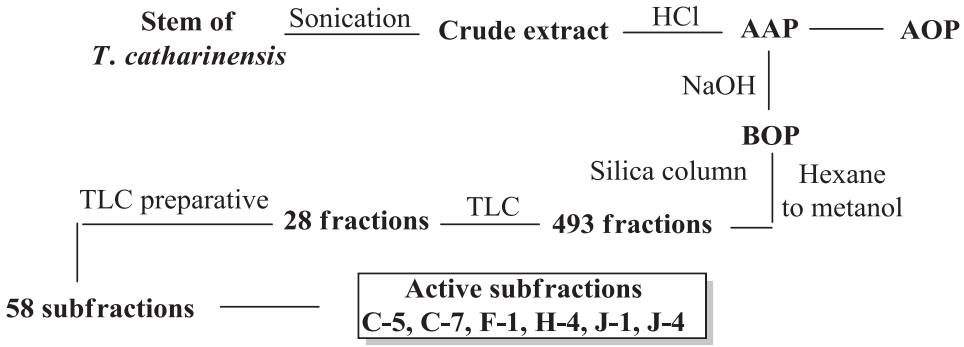

Scheme 1. Alkaloids extraction and fractionation of stem from *T. catharinensis*.

[10], have already been described as antitumour agents. Other activities have been defined for this plant's crude extracts and fractions, for instance, as an acetylcholinesterase inhibitor [11–13], antimicrobial [14,15], antioxidant [12,16], antiparasitic [17], antiophidic [18], anti-inflammatory [19], antimalarial [20,21], antibacterial [22] and antitumour agent [23–27]. Such properties are also related to the presence of indole alkaloids. Nevertheless, in most cases, these compounds are very cytotoxic, although, not selective, as demonstrated by Zhang et al. [28] for alkaloids isolated from *Tabernaemontana officinalis*.

Therefore, the search for active compounds of plants capable of being used as antitumour compounds remains a focus of research. This work conducted a bio-guided search of active compounds in sub-fractions of *T. catharinensis* against A375 (melanoma cell line) and A549 (adenocarcinomic human alveolar basal epithelial cells). The results were compared with tests in Vero cells (from African green monkey kidney epithelial cells), to evaluate the selectivity of the action. Thus, this report on the bio-guided search for indole alkaloids facilitates the identification of active compounds without the onerous isolation work. The *in silico* toxicity evaluation (genotoxic, in reproductive-system, cardiotoxic and irritant effects) of the indole alkaloids complete the study.

2. Materials and methods

2.1. Chemicals

All the reagents and solvents were of high-performance liquid chromatography or analytical grade and purchased from Merck® (Germany). Dragendorff reagent was obtained from the mixture of bismuth nitrate, potassium iodide, acetic acid and water. Silica gel GF254 (10–40 μm) prepared for thin-layer chromatography (TLC), and silica gels 0.063–0.200 and 0.040–0.063 mm, for column chromatography, were obtained from Merck®. Dulbecco's modified Eagle's medium (DMEM), 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulphoxide (DMSO) were acquired from Sigma–Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Cultilab Lab, Inc. (Campinas, Brazil). Ultrapure water was prepared by a Milli-Q water purification system (Millipore, France).

2.2. Plant material

The stems (6 kg) of *T. catharinensis* were collected in Ijuí, Rio Grande do Sul, Brazil, in November 2017 (28°26′06.4″S, 53°56′15.7″W), and a voucher specimen has been identified by Felipe Gonzatti and deposited in the Caxias do Sul University Herbarium (HUCS 34038-34057/g1669) at Caxias do Sul, Rio Grande do Sul. Brazil.

2.3. Tabernaemontana catharinensis extracts and fractionation

After the removal of inflorescences, the stems were dried in a greenhouse with forced air circulation at 30 °C for 7 days. The dry material was ground in a grinder mill (Willey TE 650, Tecnal®, SP, Brazil) and the product (420 g) was extracted by ultrasound (Sonics Vibra-Cell™ VC 505–750 ultrasonic processor, Newtown, USA) using ethanol as extraction solvent (10 mL g^{-1}), at 40% amplitude and 500 W power, for 30 min. After the solution was filtered, the solvent was rotary evaporated under reduced pressure. The remaining solid was extracted using an acid–base process [29]. Briefly, the crude extract was dissolved in acidic water (100 mL, 0.1% HCl), which was extracted with dichloromethane (3 × 50 mL), giving the acid organic phase (AOP). The remaining aqueous phase was neutralised with 10% NaOH until attaining pH 11.1 and re-extracted with dichloromethane (3 × 50 mL), generating the basic organic phase (BOP). The BOP solvent was removed under reduced pressure, yielding 3.15 g, which was fractionated in an open chromatographic column (25 cm) using silica gel 60 (0.063–0.200 mm) and the following mobile phase solvent sequence: hexane; hexane:chloroform (1:1 v/v); hexane:chloroform (1:3 v/v); hexane:chloroform (1:6 v/v); hexane:chloroform (1:9 v/v); hexane:chloroform (1:19 v/v); chloroform; chloroform:methanol (49:1 v/v); chloroform:methanol (7:3 v/v); chloroform:methanol (1:32 v/v), and methanol, which generated 493 fractions (approximately 150 mL each). The process was monitored by TLC and revealed with Dragendorff reagent. Preparative TLC was performed, using a glass, silica gel-coated TLC plate (20 × 20 cm), with fluorescent indicator F254 and chloroform:methanol (95:5 v/v), as the mobile phase. Scheme 1 summarises the fractionation performed.

2.4. Chemical characterisation

The dried sub-fractions were dissolved in a solution of 50% (v/v) chromatographic grade acetonitrile (Tedia, Fairfield, OH, USA) and 50% (v/v) deionised water, in which 0.1% formic acid was added, for analysis in positive electrospray ionisation, ESI(+), mode. The individual solutions were infused directly into the ESI source via a syringe pump (Harvard Apparatus, Hamilton, Reno, Nevada), at a flow rate of 180 μL min^{-1}. The ESI(+)-mass spectrometry (ESI(+)MS) and tandem ESI(+)MS/MS profiles were acquired using a hybrid high-resolution and high-accuracy (5 μL L^{-1}) micrOTOF-Q mass spectrometer (Bruker Scientific®, Billerica, USA) under the following conditions: capillary and cone voltages were set to +3500 and +40 V, respectively, with a de-solvation temperature of 200 °C. The collision-induced dissociations energy (CID) for the ESI(+)MS/MS was optimised for each component. Diagnostic ions in the different fractions were identified by the comparison of their dissociation patterns, exact mass and isotopic ratio, with compounds identified in previous studies. For data acquisition and processing, time-of-flight (TOF) control and data analysis software (Bruker Scientific®) were used. The data were collected in the 70–1000 *m/z* range, at the speed of two scans per second, providing 50,000 FWHM resolution, at 200 *m/z*. No important ions were observed below 180 *m/z* and above 750 *m/z*, so the ESI(+)-MS data is shown in the 180–750 *m/z* range.

Table 1
Identification of major compounds of sub-fraction.

Sub-fraction	Precursor Ion *m/z*	Fragmentation ms/ms (%)	Diff ppm	Isotopic ratio (mSigma)	Identification	References
C-5	325.1918	307.1924 (3.23), 265.1703 (8.86), 152.1068 (12.02), 122.0987 (0.22)	2.2	19.0	16-*epi*-affinine	[12,49]
C-7	281.2005	182.1003 (22.54), 158.0937 (28.79), 144.0814 (54.92), 122.0952 (52.75)	2.6	15.1	Ibogamine	[50]
F-1	411.2290	200.1070 (100), 180.0996 (13.81)	0.1	36.5	12-methoxy-*n*b-methyl-voachalotine	[12,51]
H-4	309.1983	291.1854 (17.79), 170.0958 (19.03), 158.0963 (25.16), 138.0908 (42.00)	4.4	17.2	Affinisine	[12,49,50]
J-1	355.1982	353.1835 (6.70), 337.1874 (84.95)	9.6	6.9	Coronaridine hydroxyindoline	[10,12]
J-4	367.2016	337.2024 (3.25), 305.1633 (8.55), 236.1401 (1.62), 170.0952 (86.58)	0.0	0.0	Voachalotine	[10,12]

2.5. Determination of cytotoxic activity

The A375, A549 and Vero cell lines were cultured in DMEM supplemented media with antibiotics (1% penicillin and streptomycin) and 10% FBS (Gibco BRL/Life Technologies, Carlsbad, CA, USA) at 5% CO_2 and 37 °C. Based on the protocol of Alley [30], the cytotoxic activity of fractions was assessed as follows: the cells were seeded in 96-well flat-bottom microplates, at a density of 7×10^4 cell mL^{-1} for the Vero cell line, 1×10^5 cell mL^{-1} for the A375 cell line, and 1.25×10^5 cell mL^{-1} for the A549 cell line with 10% FBS in DMEM. After cell attachment, serial dilutions of the fractions, with increasing sub-fraction concentrations (10, 25 and 50 μg mL^{-1}) at 37 °C in the culture medium were added to the cells for 24 h. Aliquots were removed and assayed by the MTT method. The MTT solution was removed after 2 h of incubation and the formazan crystals dissolved by adding 100 μL DMSO to each well, followed by agitation on a rotary shaker for 30 min, protected from light. Absorption was determined at 570 nm (SpectraMax® 190, Molecular Devices, San Jose, CA, USA) and the results were expressed as percentage viability of the negative control (cells that did not receive treatment). At experiments were performed for each cell line, and the mean and standard deviation (SD) of the IC_{50} (%) values (dose causing 50% cell survival) were determined [31].

2.6. Toxicity in silico

To identify possible toxic effects, the predictive toxicity of the indole alkaloids present in the sub-fractions was examined. The risks of damage, such as genotoxic damage, endocrine disruption, irritation and inhibition of the hERG gene (the human ether-go-go-related gene), were analysed. The data were generated online using the ADME-Tox web server software (ACD/Labs, ACD/Percepta Platform, version 12.01, Toronto, Canada; www.acdlabs.com). The compounds, 16-*epi*-affinine, ibogamine, 12-methoxy-*n*b-methyl-voachalotine, affinisine, coronaridine hydroxyindoline and voachalotine, were submitted to drug similarity evaluation and drug profiles, comparing them with a reference substance, using OSIRIS Property Explorer (http://www.organic-chemistry.org/prog/peo).

2.7. Molecular modelling studies

The molecular modelling study was done, in triplicate, for validation of the method. All calculations were performed using density functional theory B3LYP/6-311G* calculations in the gas phase (Spartan'08 for Windows, Wavefunction, Inc., Irvine, USA). The geometry of the indole alkaloids, 16-*epi*-affinine, ibogamine, 12-methoxy-*n*-methyl-voachalotine, affinisine, coronaridine hydroxyindoline and voachalotine, were fully optimised, and then submitted to systematic conformational analysis, with the torsion angle increment set to 30° in the range 0–360°. The lowest energy conformer for each compound was saved in pdb. format, to use in performing the docking studies.

The three-dimensional structure of Topo I was downloaded from the Protein Data Bank (PDB), before performing the docking studies. Protein was prepared by removing the water molecules and adding polar hydrogens, using Autodock Tools 1.5.6 [32]. All docking studies were performed using iGEMDOCK software at drug screening accuracy settings [33], in which the individual binding poses of each compound were assessed and submitted to dock in the active site of Topo I. The genetic algorithm parameters were set at a population size of 200 with 70 generations and 3 solutions, with GEMDOCK scoring function of hydrophobic and electrostatic (1:1 preference).

2.8. Statistical analysis

Data are expressed as the mean ± SD of each group. Significant differences among the treatment groups, in triplicate, were determined by analysis of variance, followed by Tukey's multiple comparisons post

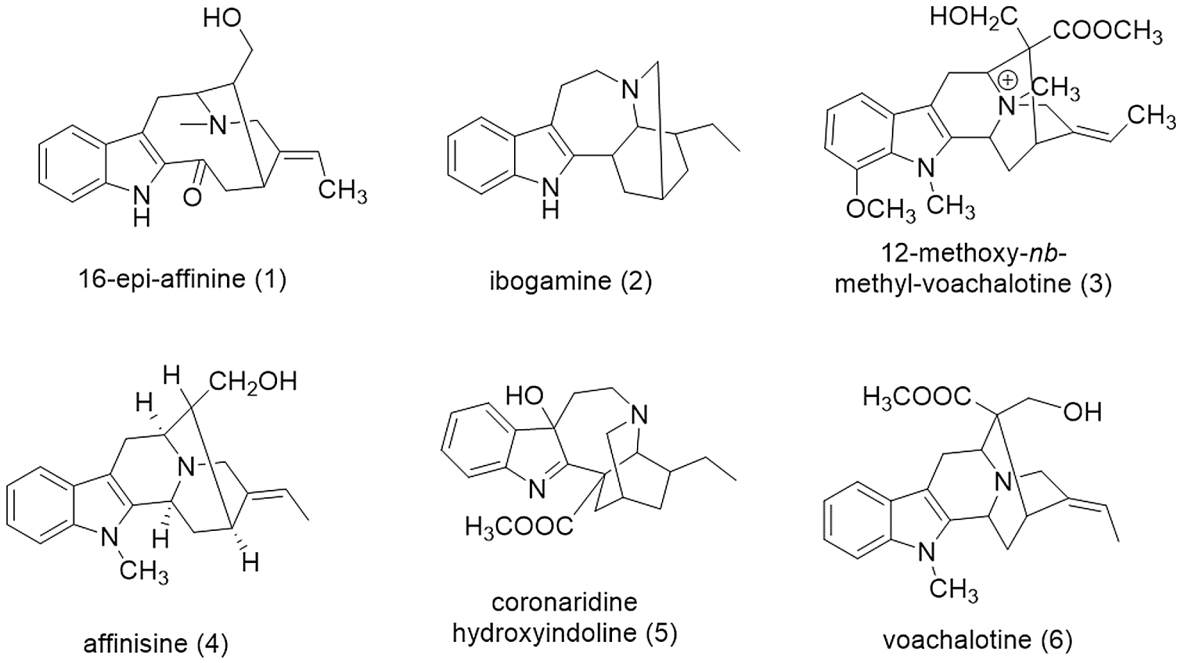

Fig. 1. Indole alkaloids identified in sub-fractions of *Tabernaemontana catharinensis.*

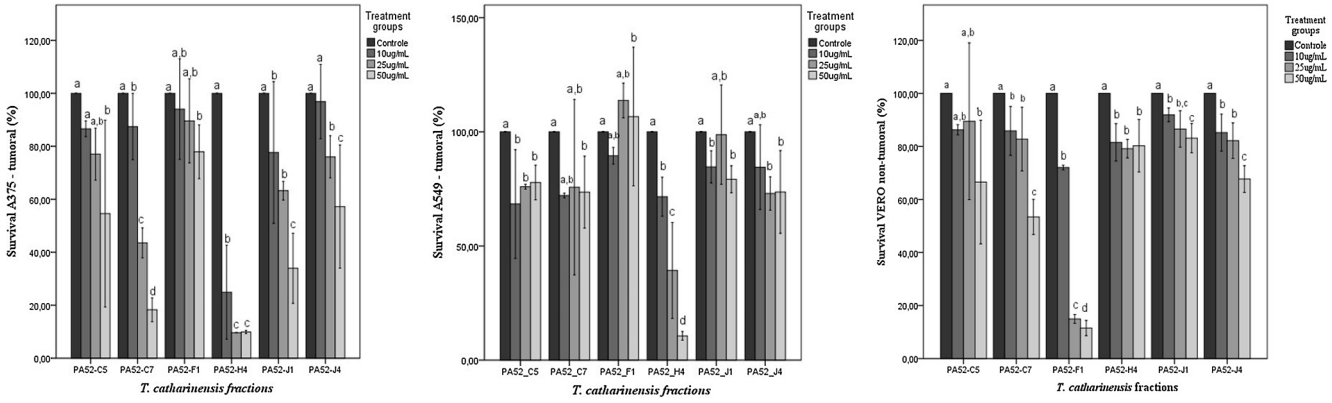

Fig. 2. Effect of alkaloid sub-fraction of *Tabernaemontana catharinensis* in different concentrations on the proliferation of A549, A375 and Vero cells. Anova followed by Tukey test expresses values in means ± SD and statistical analyses. Different letters represent statistical significance among groups ($p < 0.05$).

Table 2
Cell viability IC_{50} of *Tabernaemontana catharinensis* subfractions using different cell lines.

Cell viability IC_{50}[a]			
Entry	A549	A375	VERO
C-5	133.62 ± 74.15 μg·mL^{-1}	60.05 ± 3.33 μg·mL^{-1}	86.26 ± 25.33 μg·mL^{-1}
C-7	82.8 ± 11.91 μg·mL^{-1}	28.39 ± 0.23 μg·mL^{-1}	58.42 ± 3.89 μg·mL^{-1}
F-1	98.11 ± 14.89 μg·mL^{-1}	138.54 ± 3.69 μg·mL^{-1}	21.03 ± 0.14 μg·mL^{-1}
H-4	58.67 ± 6.14 μg·mL^{-1}	11.73 ± 36.25 μg·mL^{-1}	148.19 ± 4.77 μg·mL^{-1}
J-1	216.13 ± 69.02 μg·mL^{-1}	36.25 ± 0.78 μg·mL^{-1}	155.38 ± 27.69 μg·mL^{-1}
J-4	64.49 ± 16.68 μg·mL^{-1}	56.96 ± 15.01 μg·mL^{-1}	93.86 ± 20.53 μg·mL^{-1}

[a] Cell viability was assessed by (MTT) assay and dose–response curves calculated the IC_{50} values (dose causing 50% cell survival) for each sub-fraction and treatment.

hoc test. These analyses were conducted using SPSS 24.0 (Armonk, NY, USA) at $p < 0.05$.

3. Results and discussion

Plant metabolites have been highlighted as possible antineoplastic agents, with particular emphasis on the indole alkaloids. *Tabernaemontana catharinensis* (family Apocynaceae) is rich in indole alkaloids of the iboga and corynanthean types [7], which have several bioactivities, such as acetylcholinesterase inhibition [11–13], and anti-inflammatory [19] and antitumoral activities [23–28]. Thus, the current study undertook a bio-guided search for active (against A375 and A549 tumour cells) and selectively cytotoxic compounds from this plant.

Table 3
Physicochemical and pharmacokinetics properties and *in vitro* toxicity of molecules calculated using admetSAR software.

Entry	Molecule	Blood brain barrier	Blood brain barrier probability	Human intestinal absorption	Human intestinal absorption probability	CYP interaction and substrate probability	hERG inhibitor (Cardiac toxicity)
1	16-*epi*-affinine	BBB +	0.9035	HIA +	0.9965	CYP 3A4 64%	Weak
2	Ibogamine	BBB +	0.9873	HIA +	0.9970	CYP 3A4 66% CYP 2D6 53% CYP 1A2 68%	Weak
3	12-methoxy-*nb*-methyl-voachalotine	BBB +	0.8064	HIA-	0.9493	CYP 3A4 64%	Weak
4	affinisine	BBB +	0.9794	HIA +	0.9366	CYP 1A2 57% CYP 2D6 62%	Weak
5	Coronaridine hydroxyindoline	BBB +	0.8213	HIA +	0.9067	CYP 3A4 69% CYP 2D6 52%	Weak
6	Voachalotine	BBB +	0.9369	HIA +	0.8792	CYP 3A4 53% CYP 1A2 51%	Weak

In this work, the first step was the plant stem ultrasound-assisted extraction, which resulted in 8% yield (plant dry mass basis). The ultrasonic waves are mechanical vibrations with frequencies greater than 20 kHz, which break the cellular structure, releasing the intracellular compounds. Also, from the cavitation process, the solvent easily permeates the system, by increasing the mass transfer rate between the plant and the solvent [34]. As the next step, an acid–base extraction was used to obtain fractions rich in alkaloids, which resulted in a dry plant yield of 0.75% (Scheme 1). In a study, were implemented the same technique for generating methanolic extracts of indole alkaloids from *Mitragyna speciosa* leaves, with about 0.1% yield [35]. Such low amounts of active compounds in plants discourage the use of bioactive extracts or isolated molecules as drugs. However, from bio-guided evaluations, it is possible to identify prototypes, which, for example, may be synthesized, for future studies.

In sequence, an open column and preparative TLC complemented the procedure, to obtain enriched fractions. From the 493 fractions acquired, those sharing similarities were combined, yielding 28 fractions, of which, eight contained alkaloids. From these eight, a preparative TLC was performed, resulting in 58 sub-fractions, which were analysed by HRMS–ESI(+), yielding six alkaloid-enriched sub-fractions (termed C-5, C-7, F-1, H-4, J-1 and J-4), which were used for cytotoxicity tests.

Identification of the chemical composition of plant extracts/fractions is challenging because of the complex constitution. HRMS–ESI has been highlighted for simultaneous chemical characterisation of plant metabolites in extracts [36]. The exact mass, fragmentation pathway and isotopic ratio are used for the quantification of flavonoids [37,38] and alkaloids [12], among other classes of compounds in complex mixtures. Nicola et al. (2013) indicated the presence of 16-*epi*-affinine, 12-methoxy-*n*-methyl-voachalotine and affinisine in fractions of *T. catharinensis*, obtained by the same analytical technique [12]. The current research tested six sub-fractions pre-determined as containing alkaloids, among which the sub-fraction C-5 showed the ion with 325.1918 *m/z*, corresponding to 16-*epi*-affinine, confirmed by the isotopic ratio and fragmentation pathway. Meanwhile, the sub-fraction C-7 was characterised by the presence of ibogamine (281.2005 *m/z*), and 12-methoxy-*n*-methyl-voachalotine (411.2290 *m/z*) was identified in F-1. In H-4, J-1 and J-4, the main compounds were affinisine (309.1983 *m/z*), coronaridine hydroxyindoline (355.1982 *m/z*) and voachalotine (367.2016 *m/z*), respectively (Table 1 and Fig. 1).

For highly reliable identification of target compound by ESI–Q–TOF, the accepted accuracy threshold for confirmation of elemental compositions was established as ≤ 10 ppm [39]. Figs. S1.1–S1.6 are in supplementary material and show the full mass spectrum HRMS-ESI(+) for all sub-fractions, highlighting the exact *m/z*, isotopic ratio, as well as the fragmentation of the main compounds.

The sub-fractions, C-5, C-7, F-1, H-4, J-1 and J-4, which showed different indole alkaloids were investigated through *in vitro* cytotoxic analysis. Fig. 2 presents the results of the viability of Vero, A375 and A549 cells treated with *T. catharinensis* sub-fractions at 10, 25 and 50 μg mL^{-1} (w/v).

In general, the treated A375 cells demonstrated survival rates above 50%, except when challenged with H-4, which was toxic at all concentrations tested and caused more than 80% cell death. A549 cells presented over 60% survival, with a progressive death trend for C-7, H-4 and J-4 (dose-dependent). In comparison, C-5 and J-1 showed, respectively, a 27 and 23% higher mortality rate at the lowest dose tested. The sub-fractions C-7 and H-4 were cytotoxic against A375 cells, with IC_{50} 28.39 and 11.73 μg mL^{-1}, respectively, but neither was cytotoxic against A549 cells (Table 2). For H-4, in which the main compound was affinisine, low cytotoxicity (IC_{50} 148.19 μg mL^{-1}) against Vero cells was evident, indicating the selectivity of this compound. Considering that the sub-fractions were not pure, the data obtained may have been due to synergistic action. However, according to the difference in activity presented by the sub-fractions, this bio-guided study indicates the

Table 4
ADMET properties and prediction cardiac toxicity of molecules calculated using Osiris Property Explorer and admetSAR software's.

Entry	Druglikeness	DrugScore	Mutagenic	Tumorigenic	Reproductive effective	Irritant	Carcinogenicity
1	3.8133	0.83	None	None	None	None	None
2	4.0805	0.71	None	None	None	None	None
3	−0.68862	0.56	None	None	None	None	None
4	3.038	0.84	None	None	None	None	None
5	2.2942	0.81	None	None	None	None	None
6	1.2548	0.75	None	None	None	None	None

Table 5
Central pharmacological interactions (H-bonding and Van der Walls) and Docking results of molecules using IGemdock software.

Entry	Total Energy (Kcal mol^{-1})	VDW (Kcal mol^{-1})	H-Bonding (Kcal mol^{-1})	Electrostatic (Kcal mol^{-1})
1	−70.12	−55.15	−14.97	0
2	−64.76	−53.36	−11.39	0
3	−85.71	−34.97	−50.46	−0.28
4	−70.92	−42.91	−28.01	0
5	−79.83	−47.64	−32.20	0
6	−75.38	−61.14	−14.24	0

most active alkaloid.

Indole alkaloids, such as ibogamine, 3-oxocoronaridine and 12-methoxy-4-methylvoachalotine, have been described as cytotoxic against human melanoma (C-8161) with IC_{50} 13.0, 63.4 and 0.1 mg mL^{-1}, respectively [10]. Moreover, the coronaridine extracted from *T. catharinensis* root, showed a cytotoxic IC_{50} of 54.47 μg mL^{-1}, in the laryngeal carcinoma cell line (Hep-2) compared to other alkaloids [9]. Similarly, fractions obtained from the aqueous extract of the same plant, presented potent antitumour activity *in vitro*, with IC_{50} 50.4 mg mL^{-1} in human breast cancer cells (SKBR-3) [40].

In a recent study, the monoterpenoid indole alkaloids from the bark of *Voacanga africana* exhibited cytotoxicity against A375 cells, with IC_{50} of 5.0–19.5 μg mL^{-1} [41]. Also, cytotoxicity in the same cell line was shown for the alkaloids, kunstleramide (IC_{50} 64.65 μg mL^{-1}) [42] and velutinam (IC_{50} 25.60 μg mL^{-1}) [43].

In silico toxicity assessments have increased exponentially in recent decades, supporting the understanding of the activity of chemical compounds [44]. Thus, here the 16-*epi*-affinine (**1**), ibogamine (**2**), 12-methoxy-*n*-methyl-voachalotine (**3**), affinisine (**4**), coronaridine hydroxyindoline (**5**) and voachalotine (**6**) were evaluated for ADME-Tox properties and screened by ACD/TOX (ACD Labs software), which provides fragments that could lead to possible toxic effects.

The toxic effects were assessed by the probability of producing positive results in the Ames test, the endocrine disruptor test, the hERG activation and irritant effects. The molecules (**1–6**) showed a low probability of causing toxic effects in all the parameters evaluated (Table 3). All compounds showed a good likelihood of crossing the intestinal human cells and blood–brain barrier. The cytochrome P450 (CYP) enzymes constitute a family of mono-oxygenases that catalyse the oxidative metabolism of a diverse variety of xenobiotics, herbicides, pesticides, environmental pollutants and industrial compounds because of their broad substrate specificity. Regulation of CYP activity via inhibition or induction by xenobiotics and other drugs is frequently the origin of drug interactions [45]. Computerised predictions of interactions between the alkaloids and CYP suggested a high probability of coronaridine hydroxyindoline (**5**) to interact with CYP3A4, indicating this molecule may be metabolised by this enzyme. CYP3A4 is the enzyme most frequently involved in drug interactions, so some bioactive compounds that show high interaction with this protein have their activity interrupted. Although the *in silico* toxicity prediction studies suggested some level of CYP3A4 interaction, the hERG inactivation, strongly related to this CYP protein function, presented a weak effect, for all compounds studied.

OSIRIS software was used for calculating the fragment-based toxicity risks of all compounds. The data (Table 4) suggested that all indole alkaloids tested may be non-genotoxic, non-reproductive-system toxic, non-cardiotoxic and lack irritant effects. Furthermore, considering the drug design concepts of drug-likeness and drug score indices, it was found that 16-*epi*-affinine (**1**), affinisine (**4**) and ibogamine (**2**) had great drug-likeness, with values of 3.8, 3.0 and 4.0, respectively. Affinisine (**4**) showed the greatest drug score (0.84). These indices indicated these compounds showed similarity to the balance of molecular properties and structural features of known drugs.

The initial evaluation of ADME-Tox properties can minimise the time and cost of screening and testing, by identifying the strongest candidates for development and rejecting those with a low probability of success [46]. To investigate the ADME-Tox pharmacokinetic properties, Singh et al. (2016) tested 100 alkaloids *in silico*, including betanin, brucine, canthin-6-one, castanospermine, cryptolepine, harmine, echitamine, ellipticine, harmaline, harmalol, vincristine, vinpocetine, vinblastine, vinorelbine and voacangine. The authors found 18 compounds met all the ADME-Tox descriptors and obeyed the Lipinski's rule, of which only one was an indole alkaloid (canthin-6-one). In addition, the compounds tested showed interaction with the enzyme CYP2D6. Like the indole alkaloids evaluated in this study, canthin-6-one does not have high toxicity [47].

The docking procedure aimed to identify individual poses of components of *T. catharinensis* sub-fractions that may bind to the Topo I active site. The iGEMDOCK software was used to suggest a model of

Table 6
Central pharmacological interactions (Van der Waals, H Bonding, and electrostatic) of compounds and amino acid residues and DNA base involved in the indenosoisoquinoline M38 binding site of TOP-1 with normal and applying the Residues Consensus Analysis.

	H-Bond			Van de Waals				
Entry	TGP[a] 11	ARG 364	ASP 533	TGP[a] 11	ARG 364	LYS 532	ASP 533	THR 718
1	−5.4	−3.4	−5.0	−35.5	−5.7	−2.4	−5.6	−1.3
2	−11.4	0	0	−32.2	−6.4	−2.2	−6.2	−2.6
3	−41.4	−5.6	−3.5	−12.6	−1.3	−4.3	−7.0	−7.8
4	−21.4	−1.0	−5.6	−18.2	−7.2	−8.9	−5.8	−2.1
5	−25.3	−7.0	0	−30.0	−8.1	−2.7	−4.6	−2.5
6	−11.1	0	−3.1	−38.1	−5.7	−5.8	−3.1	−5.2

[a] Confirmed by residues consensus analysis.

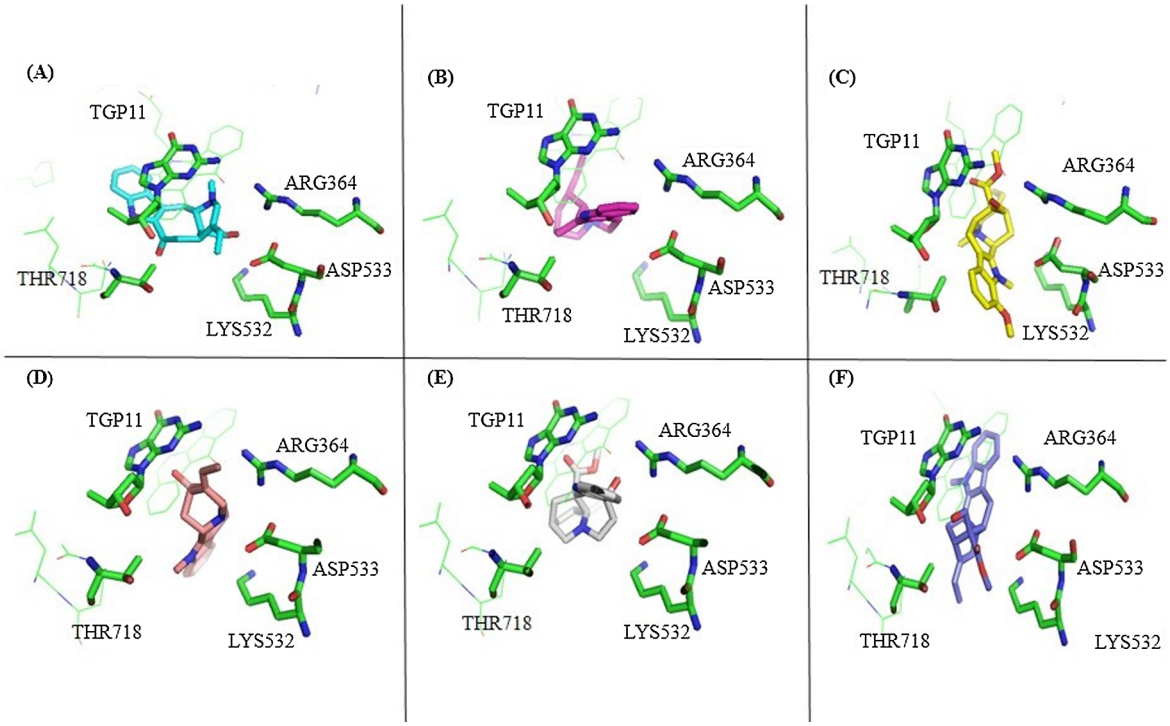

Fig. 3. Binding of indole alkaloids in the active site of Topoisomerase I (PDB: 1SC7). Graphic visualization obtained using PyMOL. (A) 16-*epi*-affinine, (B) iboagamine, (C) 12-methoxy-*n*b-methyl-voachalotine, (D) affinisine, (E) coronaridine hydroxyindoline and (F) voachalotine.

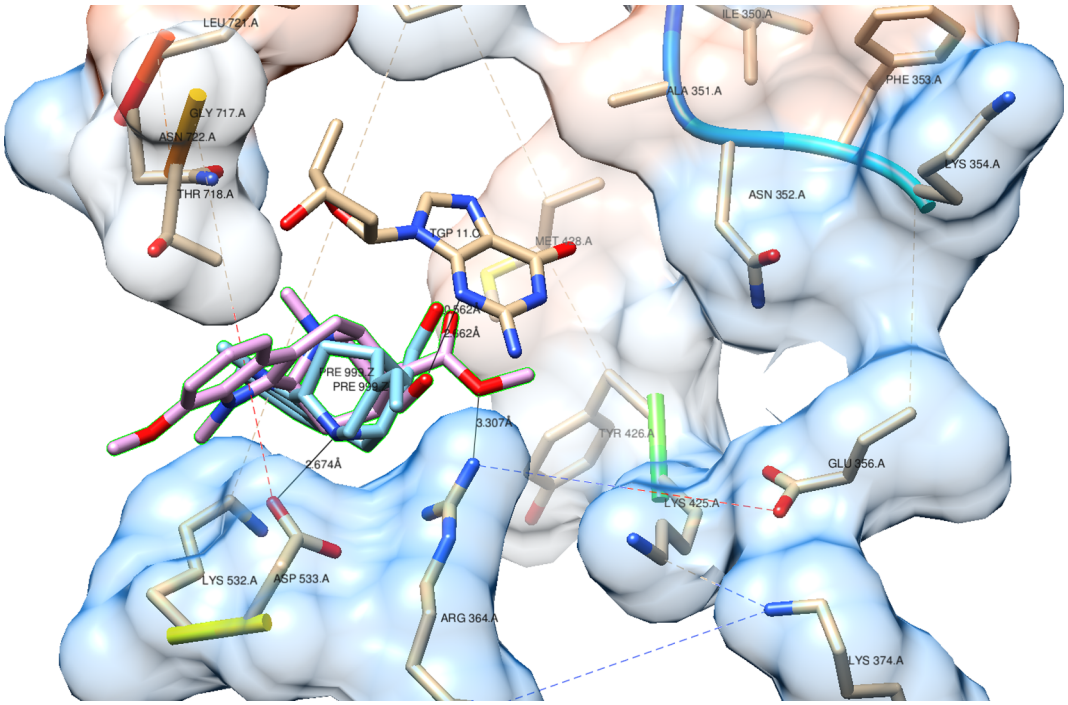

Fig. 4. Binding of indole alkaloids 1 and 3 and main H-bond interactions with the active site of Topoisomerase I (PDB: 1SC7). Graphic visualization obtained using Chimera 1.10.1, hidrophobic surface, transparency of 80%, dot size 1.0, probe radius 1.4.

ligand–protein interactions and energy-based scoring function, aiming to understand the pharmacological mechanism of action. Topo I has been identified as the potential target of several antineoplastic drugs because of its involvement in cell replication and proliferation, and this protein complex is expressed more in cancer cells than normal cells [47]. Molecules with similar structures have already been shown to be Topo I inhibitory agents [48]. The empirical scoring function is the estimated total of Van der Waals, H-bonds and electrostatic energy. In this work, the compounds showed mainly Van der Waals and H-bond interactions of molecules with the indenoisoquinoline M38 site of Topo I (Table 5).

The best pose ligands showed affinities with decreasing total energy values for compound **3** > **5** > **6** > **4** > **1** ≅ **2**, respectively. Only compound (**3**), 12-methoxy-*n*-methyl-voachalotine, showed a slight

electrostatic interaction with the protein site studied. The main interactions values were −34.97 for Van der Waals and −50.46 for H-bonding and the total energy was −85.71 kcal mol^{-1}. All the ligand–amino acid residue interactions are shown in Table 6.

In the present study, the indole alkaloids fit into the indenoisoquinoline M38 binding pocket present in Topo I and showed interactions with TGP11 (guanine DNA base), and ARG364, LYS532, ASP533 and THR718 amino acid residues (Fig. 3). Considering the example of compounds **1** and **3**, the H-bond molecular distance of 2.6 Å of the hydroxyl moieties of both compounds with the nitrogen atom of the pyrimidinone ring from guanine (GTP11)-fused rings, was noted (Fig. 4). All the molecules exhibited Van der Waals interactions with ARG364 and four compounds with H-bonding interactions, excluding ibogamine (**2**) and voachalotine (**6**) (Table 6). This ARG364 residue is the main amino acid involved in interactions with the indenoisoquinoline M38 binding pocket of Topo I and the bindings generated between ligands and this arginine may be used to validate the results obtained. After applying the post-screening *s* consensus residue analysis, TGP11 was detected as the main molecule involved in this local ligand–receptor binding (with Z-score −1.93 and −1.65 for Van der Waals and H-bonding, respectively, and WPharma 1.00), for all compounds studied. The results obtained corroborate those documented in other studies with alkaloids. The β-carboline ring fits well into the M38 binding pocket present in Topo 1, and the substituents exhibited interactions with TGP11 and hydrophobic interactions with ARG364 and THR718 amino acid residues [48].

These results complement the prediction obtained by ACD/Labs software and may be used to suggest the theoretical, qualitative results of alkaloids safety. These *in silico* predictions not only consider the quantitative dose-response results but are indicative of the potential toxic effects, and complement the experimental toxicity assay performed in this work.

4. Conclusions

In this study, six sub-fractions (C-5, C-7, F-1, H-4, J-1 and J-4) rich in indole alkaloids from *T. catharinensis* were obtained by ultrasound-assisted extraction, open chromatographic column and preparative TLC. The indole alkaloids identified in the sub-fractions were 16-*epi*-affinine, 12-methoxy-*n*-methyl-voachalotine, affinisine, voachalotine, coronaridine hydroxyindoline and ibogamine. From these, H-4, which contained affinisine as the main compound, was the most active against A375 cells, lucking cytotoxicity against the normal Vero cell line (selectivity).

It was hypothesised that indole alkaloid constituents of the plant might be attributed to its Topo I interaction. The theoretical models may be used to assist in the investigation of the pharmacological properties of *T. catharinensis*. The *in silico* prediction assessments suggested all the indole alkaloids showed low risk to cause toxicity, which may indicate the relative safety of this plant.

In summary, these results imply that affinisine may be a potential anticancer agent. However, there is a need for further studies with the compound, in addition to testing the molecule in pure form, identifying and confirming its properties, mode of action, and performing pharmacokinetic and pharmacodynamics tests.

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Conflict of interest

No conflict of interest declared.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.12.016.

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