CHEMICAL CONSTITUENTS FROM *Tabernaemontana catharinensis* ROOT BARK: A BRIEF NMR REVIEW OF INDOLE ALKALOIDS AND *IN VITRO* CYTOTOXICITY

Paulo Sérgio Pereira*, Suzelei de Castro França, Paulo Vinicius Anderson de Oliveira, Camila Moniz de Souza Breves e Sarazete Izidia Vaz Pereira
Unidade de Biotecnologia, Universidade de Ribeirão Preto, Av. Costábile Romano, 2201, 14096-900 Ribeirão Preto – SP, Brasil
Suely Vilela Sampaio e Auro Nomizo
Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, 14040-903 Ribeirão Preto – SP, Brasil
Diones Aparecida Dias
Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, 14040-903 Ribeirão Preto – SP, Brasil

This work describes the isolation and structural determination of pharmacological compounds present in the bark of roots of *Tabernaemontana catharinensis* (Apocynaceae). Among the 27 substances detected 12 were identified as terpenoid-indole alkaloids, 2 steroids and 13 pentacyclic triterpenes. Structures were outlined based on HMQC, COSY, DEPT, ^{13}C, and ^{1}H NMR data and MS. Spectral data of indole alkaloids were reviewed. An *in vitro* screening of the extracts and isolated compounds was carried out. Compounds ibogamine (**5**), 3-oxo-coronaridine (**9**) and 12-methoxy-4-methylvoachalotine (MMV) demonstrated effective cytotoxicity towards SKBR-3 breast adenocarcinoma and C-8161 human melanoma tumor cell lines.

Keywords: *Tabernaemontana catharinensis*; indole alkaloids; cytotoxicity.

INTRODUCTION

As part of a phytochemical study of the genus *Tabernaemontana* the species *T. catharinensis* was investigated. The genus *Tabernaemontana* pertains to the family Apocynaceae. Recently, 99 species from this genus were reported of which 44 occur in America. The species identified in Brazil belong to the section *Peschiera* and *Bonafousia*. *T. catharinensis* is the only species known from Argentina and Paraguay occurring also in Brazil and Bolivia[1].

Tabernaemontana species are well known for their terpenoid-indole alkaloid contents with approximately 240 structurally different bases already described. These types of compounds are responsible for many pharmacological activities such as: antileishmanial; antibacterial; antitumoral; hypoglycaemic, analgesic and cardiotonic action[2]. The section *Peschiera* contains primarily indole alkaloids of the type corynathean and heynean[3]. Besides indole alkaloids the presence of several pentacyclic triterpenoids was also evidenced in *Tabernaemontana*[4-6].

In a previous publication we reported on the presence of the indole alkaloids[7]. In continuation of our studies on this species the present communication report the isolation and identification of the biosynthetically interesting indole alkaloids, steroids and triterpenoids from root bark extracts.

RESULTS AND DISCUSSION

The root bark was extracted with ethanol and the non-polar compounds were separated with hexane. EtOAc and *n*-BuOH extracts were submitted to acid-base extraction for separation of alkaloids.

In this work, the ethyl acetate basic fraction of the root bark of *T. catharinensis* yielded twelve indole alkaloids: coronaridine (**1**), coronaridine hydroxyindolenine (**2**), voacangine (**3**), voacangine hydroxyindolenine (**4**), ibogamine (**5**), heyneanine (**6**), voacristine (**7**), voacristine hydroxyindolenine (**8**), 3-oxo-coronaridine (**9**), 3-hydroxycoronaridine hydroxyindolenine (**10**), vobasine (**11**) and voachalotine (**12**). This is the first report on the presence of the alkaloids **7**, **8**, **9** and **12** in *T. catharinensis* while **10** has never been described. Besides, EH extract yielded ten known triterpenes: 20(30)-taraxasten-3β-yl (**13**), iso-lupeyl (**14**), bauerenyl (**15**), β-amyrin (**16**), pseudotaraxastenyl (**17**), α-amyrin (**18**), iso-ursenyl (**19**), lupeyl (**20**) and 20(30)-ursen-3β-yl (**21**) acetates; and two steroids: stigmasterol (**22**) and sitosterol (**23**). Three known pentacyclic triterpenes were isolated from neutral fraction EAc2: betulinic acid (**25**), oleanolic acid (**26**) and ursolic acid (**27**). From the isolated triterpenes identified, the **13**, **15**, **17**, **19** and **21** are described for the first time in *T. catharinensis* and **24** is a new compound.

The reversed-phase ion-pair HPLC system was found to be highly selective in the separation of various *Tabernaemontana* alkaloids[8]. The alkaloids **2-5**, **7** and **8** were separated by reversed-phase ion-pair HPLC.

All the alkaloids were found to be known tertiary alkaloids isolated from *Tabernaemontana* species. Of the 12 alkaloids isolated 10 are of the ibogan class and the other 2 of the corynanthean class. They were identified by comparison with published data (UV, IR, ^{1}H NMR, ^{13}C NMR and MS)[3,5,9-14].

Structures of compounds **1-10**, were elucidated on the basis of the ^{13}C NMR chemical shifts (Table 1) and comparison with the signals attributed to ibogamine (**5**).

The relative configurations of C-14, C-16 e C-21 of iboga type alkaloids remain identical because of the bonds between C(14)-C(3)-N(4)[15]. The presence of a methoxy group at C-10, in the indole ring induce significant dowfield shifts of the resonance signal of alkaloids **3** and **7** ($\Delta\delta = + 35$ ppm) and $\Delta\delta = + 39$ ppm of hydroxyindolenine alkaloids **4** and **8**. Moreover this substituent cause upfield of C-o

*e-mail: ppereira@unaerp.br

Table 1. ^{13}C NMR spectra data of indole alkaloids (CDCl$_3$, TMS)

Carbon	1	2	3	4	5	6	7	8	9	10	11	12
2	136.5	189.3	137.3	186.8	141.6	136.9	136.6	186.1	135.6	186.8	134.1	137.2
3	51.5	48.7	51.6	48.6	49.8	51.3	52.3	47.8	172.9	61.1	190.1	47.8
5	53.1	49.0	52.6	49.0	54.1	52.2	51.4	48.0	42.6	43.5	57.2	53.6
6	22.0	33.8	22.2	34.1	20.4	22.9	21.4	33.1	20.9	30.4	20.3	22.3
7	110.2	88.3	109.9	88.3	108.8	109.6	109.4	87.8	109.1	88.2	120.4	105.0
8	128.7	142.6	129.0	144.4	129.5	128.4	128.7	143.7	127.7	141.2	128.5	126.1
9	118.4	126.7	100.7	107.9	117.7	118.3	100.6	108.0	118.2	127.0	120.8	118.3
10	119.1	120.8	154.0	159.1	118.9	119.3	154.0	159.3	119.4	121.0	120.4	118.8
11	121.8	129.1	111.8	113.7	120.8	122.1	112.1	114.0	122.2	129.5	126.7	121.0
12	110.3	121.4	111.1	121.3	110.1	110.5	111.3	121.5	110.5	121.6	111.7	108.7
13	135.4	151.3	130.5	144.8	134.6	135.6	130.7	144.7	133.8	150.9	136.3	138.4
14	27.3	27.0	26.9	27.0	26.2	26.7	26.6	26.4	30.8	29.1	43.0	28.3
15	32.0	32.0	31.6	32.0	31.8	21.4	22.9	22.9	35.3	37.5	30.4	30.3
16	55.0	58.7	55.7	58.5	41.0	54.1	55.9	55.7	55.5	53.4	46.6	53.3
17	36.4	34.8	36.4	34.5	33.9	36.8	36.8	35.2	35.7	35.3	-	63.1
18	11.6	11.5	11.5	11.5	11.8	20.3	20.3	20.3	11.2	11.2	12.3	12.7
19	26.7	26.5	26.4	26.5	27.6	71.3	71.2	71.3	27.5	27.3	120.8	116.0
20	39.1	37.5	38.9	37.5	41.7	39.4	39.4	38.2	38.1	38.0	135.8	136.4
21	57.4	58.3	57.5	58.4	57.6	59.7	59.6	60.1	56.0	53.3	51.8	55.9
22	175.7	173.6	175.4	173.9	-	174.8	174.6	173.0	175.8	171.6	171.2	176.4
CO$_2$CH$_3$	52.5	53.1	52.6	53.1	-	52.9	52.9	53.5	52.9	53.4	50.3	52.1
N$_1$-CH$_3$	-	-	-	-	-	-	-	-	-	-	-	29.1
N$_4$-CH$_3$	-	-	-	-	-	-	-	-	-	-	42.3	-
OCH$_3$	-	-	56.0	55.7	-	-	54.0	57.4	-	-	-	-

($\Delta\delta$ = -10 and -17 ppm of **3** and **7**; $\Delta\delta$ = -8 and –10 ppm of **4** and **8**), downfield of C-p ($\Delta\delta$ = -5 ppm of **3** and **7**) and no change of C-m resonance shifts. Carbomethoxy substituent at C-16 shielded it about 13.0-14.7 ppm in compounds **1**, **3** and **6** in comparison with the same site in structure **5**. Interaction of a hydroxyl group at C-19 caused upfield shift of C-15 signal ($\Delta\delta$ = – 10 ppm) and downfield of C-20 and C-21 resonance signals ($\Delta\delta$ = + 0,5 ppm and $\Delta\delta$ = + 2 ppm), respectively. This effect also suggests a hydrogen bond between N-4 and that hydroxy substituent. The shifts of the carbons C-15, C-20 and C-21, very close to those reported by Perera *et al*.[9], clearly confirm the configuration of 19S for compounds **6**, **7** and **8**. In the isolated alkaloids ^{13}C NMR resonance signals near at 88 ppm and 187 ppm are indicative of the presence of a hydroxy group at C-7 of structures **2**, **4**, **8** and **10**, shielding C-7 and deshielding C-2, respectively. Moreover, comparing the nearly identical shift pattern of structures **10** and **2,** the presence of a hydroxy group attached to the C-3 of compound **10** is denoted by the downfield shift of C-3 by 12 ppm and the interactions of this group causing downfield shifts of C-14, C-15, C-17 and C-20. Moreover, the hydroxy group at C-3 must present the S configuration due to the cyclic conformation of isoquinuclidine group allowing a non-ligand interaction between the OH at C-3 and C-15. The deshielding of C-15 by 5.5 ppm and shielding of C-21 by 5.0 ppm reinforce this point and confirm this compound to possess structure **10**.

Biogenetic correlation shows that indole alkaloids of corynanthe type containing a non re-arranged monoterpene unit, as compounds **11** and **12**, retain the α configuration for H-15. Furthermore, the link C(5)-C(16)-C(15) only is allowed if H-3 has the α configuration[15]. The carbonyl and the methoxy group of compound **11** resonate at downfield of 2,7 and 1,8 ppm, respectively due to the proximity of those groups indicating the S configuration for C-16. ^{13}C NMR spectra data of those a-acylindole alkaloids also denote the typical anisotropic effect of indole on the carbonyl of the ester group[16].

Alkaloids of the sarpagine type are formed by an indole ring fused to a quinuclidine system, which confers a relative rigidity to the structure allowing a more detailed analysis of the stereo effects of substituents. Upfield resonance of C-17 shows that the CH$_2$OH group is in the position endo in relation to the indole system, inducing a hydrogen-hydrogen non-bonded γ–*gauche* type interaction with the C-14 that is eclipsed by the C-6[16].

Fractionation of EAc2 (neutral fraction) resulted in the purification of pentacyclic triterpenes: betulinic acid (**25**), oleanolic acid (**26**) and ursolic acid (**27**). On the basis of chemical shifts from the ^{13}C NMR spectra, isolated triterpenes of the oleanane, ursane and lupane type were identified[17].

The presence of lupane moiety in the triterpenoid **24** was recognised by chemical shifts and multiplicity of all ^{13}C NMR signals, deduced by the comparative analysis of the proton noise decoupled and DEPT spectra. The chemical shifts of H-3 (4.45-4.50 ppm) and carbon atoms C-3 (80.9 ppm), C-2 (24.1 ppm) and C-4 (38.3 ppm) of this compound compared with those of triterpenoids 3β-O-acetyllupeol indicated the presence of an acyl group at the oxygen atom of C-3[18]. The ^{1}H NMR spectrum displayed a singlet for two ethylenic protons in a 1,1-disubstituted double bond (4.67 ppm), this is confirmed by carbon atoms chemical shifts at 110.0 and 150.2 ppm. Chemical shift at 75.6 ppm and the absence of a multiplet at 3.50 ppm indicate the hydroxyl group in the C-19, the DEPT spectrum confirm that this signal is the quaternary carbon. The recognition of this presence of this basic skeleton in the natural esters pointed to the esterification of the hydroxyl function at C-3 with different acyl groups. The acidic methanolysis of the ester yielded a basic skeleton as the alcoholic portion and a mixture of the methyl esters as the acidic portion. The mixture of these methyl esters was recognized by GC/MS analysis. The base peak at *m/z* 74 in the mass spectra of the methyl esters can be rationalized by McLafferty rearrangement of the molecular ion to produce olefin fragment at *m/z* 196. The molecular ion of the main methyl ester was detected at *m/z* 270, together common mass fragments; suggest that it has an unsaturated long chain with 14 methylenic groups.

The chemical investigation in this work can help the

chemotaxonomic identification of *Tabernaemontana* species such as: *T. affinis* Muell. Arg., *T. affinis* var. *lanceolata* Muell. Arg., *T. australis* Muell. Arg., *T. acuminata* Muell. Arg., *T. Hybrida* Hand.-Maz., *T. salicifolia* Hand.-Maz., *Peschiera albidiflora* Miers and *T. hilariana* Muell. Arg., which are supposed to be heterotypical synonymous of *T. catharinensis* A.DC.[1].

Cytotoxic activities of extracts and compounds were evaluated on SKBR-3 and C-8161 human tumor cell lines (Table 2). Both extracts and compounds showed considerable cytotoxic activity against tumor cell lines. We also observed that compound **9** at moderate or high concentrations shows cytotoxic activity against SKBR-3 breast adenocarcinoma and C-8161 melanoma tumor cells. Observations previously showed *T. calcarea* extracts are a source of indole alkaloids that exhibit selective cytotoxicity against the A2780 ovarian cancer cell lines[19].

Table 2. Characterization of the *in vitro* cytotoxic-related anti-tumor effects of the crude extract and compounds

Compounds	% Citotoxicity			
	SKBR-3 cell lines		C-8161 cells lines	
	0.1 mg/mL	1 mg/mL	0.1 mg/mL	1 mg/mL
MMV	12.3	22.7	0.1	15.6
5	NT	NT	13.0	70.0
9	43.9	71.5	63.4	79.7
EAc2	20.1	71.4	3.3	17.8
EAc3	8.0	21.1	54.1	58.5
EAc4	9.1	57.3	16.3	42.5
EB2	8.3	30.8	5.4	24.3
EB3	NT	NT	13.8	35.8
EB4	1.7	8.14	12.7	32.1
EALA	NT	NT	14.6	43.6
EALB	32.3	85.6	8.9	24.4
MTT	3.5	10.8	1.6	10.5

Cytotoxicity activity of the extract or isolated compounds was analyzed by MTT assay as described in materials and methods. MMV = 12-Methoxy-4-methylvoachalotine[7,22]. NT = no tested. Values represent the average of triplicate samples. Data are representative of at least three independent experiments.

Recent work has demonstrated that betulinic acid is cytotoxic against other non-melanoma (neuroectodermal and malignant brain tumor) human tumor varieties[20].

In this work we confirm previously observation that *T. catharinensis* root bark extract contains compounds with tumor cytotoxic activity. We also suggest the compounds isolated and reported by first time here are at least in part responsible for this property. Importantly our results shows that compounds have cytotoxic activity in both tumor cell lines tested. The compounds at the concentration tested in this work against human tumor cell lines do not shown significant cytotoxic activity on pheripheral blood mononuclear cells (data not shown). This observations is in agreement with observations that lupane type triterpenes as betulinic acid presents selective cytotoxicity against tumor cells and favorable therapeutic index, even at doses up to 500 mg/kg body weight, betulinic acid is a very promising new chemotherapeutic agent for the treatment of cancer[20,21]. The triterpenes cytotoxic activity mechanisms on tumor cells remains unclear. Betulinic acid appears to function by means of inducing apoptosis in cells irrespective of their p53 status[20]. Further experiments are necessary to check the apoptosis induction property of compounds **5** and **9**.

EXPERIMENTAL

General

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300 and 75 MHz, respectively. Mass spectra were determined at 70 eV using a direct inlet system. HPLC was performed on a Shimadzu LC-10AD instrument with diode array detector.

Plant material and isolation procedure

Roots of *T. catharinensis* A.DC. were collected in Assis, SP. Voucher specimen were deposited at the herbarium (SPFR) of the Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, USP (Reg. No. 02940).

Dried root bark (950 g) was extracted three times through maceration with ethanol for ten days. Crude extract (150 g) was submitted to partition with hexane, ethyl acetate and *n*-butanol. After evaporation of the solvent the following extracts were obtained: hexane, EtOAc and *n*-BuOH extracts.

The EtOAc and *n*-BuOH extracts were further submitted to acid-base partition (Figure 1). Basic fractions EAc 3 and EB 3 were successively fractionated on silica gel 60 column using hexane:Me_2CO or $CHCl_3$:MeOH. Same fractions of EB 3 were purified by HPLC using Supelcosil™ LC-18 column (250 x 10 mm, 5 μm) under isocratic condition (MeOH:*n*-heptanesulfonic acid, 0.05 M, pH 5.7; 7:3). The detection was monitored at 280, 313 and 350 nm and mobile phase flux was 2.0 mL/min. Neutral Fraction (EAc 2) was fractionated on silica gel 60 column using hexane:acetone (8:2, 7:3 and 1:1). Fractions eluted with hexane:acetone (8:2) were purified on reverse-phase HPLC using Supelcosil™ LC-18 column (250 x 10 mm) and MeOH:NaH_2PO_4 (0.05 M, pH 4.0) (85:15) as mobile phase. The flow rate was 3.0 mL/min and detection was at 210 nm. Chromatographic system is equipped with diode array detector. Indole alkaloids **1** to **12** were obtained in this procedure.
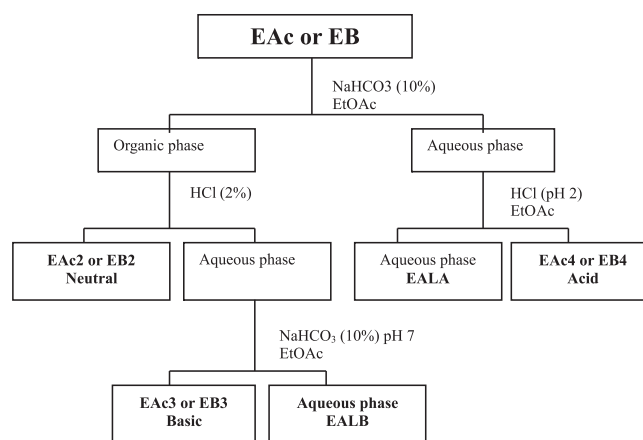

Figure 1. *Flux diagram for fractionation of EAc and EB extracts of T. catharinensis*

Coronaridine (**1**) (229 mg): ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.32 (*s*, NH), 7.45 (*dl*, J = 6.6 Hz, H-9), 7.21 (*dl*, J = 6.6 Hz, H-12), 7.11 (*ddd*, J = 6.1 and 1.1 Hz, H-1), 7.04 (*ddd*, J = 6.6 and 1.1 Hz, H-10), 3.67 (*s*, CO_2CH_3), 0.90(*t*, J = 7.2 Hz, H-18). ^{13}C NMR (75 MHz, $CDCl_3$): Table 1. MS (70 eV) *m/z* (rel.int.): 338 [M+] (100), 254 (3), 214 (18), 148 (9), 136 (59), 130 (14).

Coronaridine hydroxyindolenine (**2**) (26 mg): ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44 (*d*, J=7Hz, H-10), 7.17-7.38 (*m*, H-9,11,12), 3.79 (*s*,

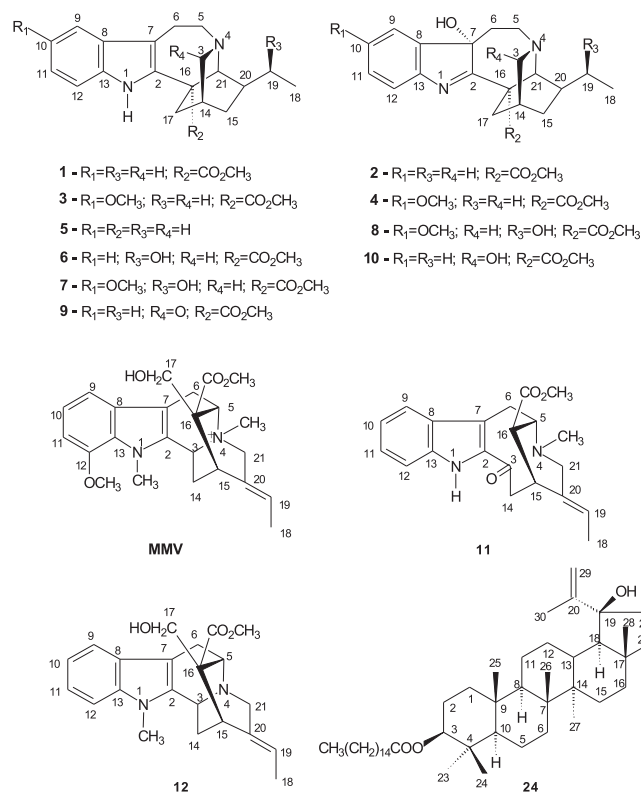**Figure 2.** *Structure of indole alkaloids (1-12 and MMV) and triterpene methyl ester (24) isolated of the root bark of T. catharinensis*

H-21), 3.68 (*s*, CO$_2$CH$_3$), 3.5 (*m*, H-5'), 2.95 (*ddd*, J=15.4 and 2 Hz, H-5), 2.70 (*m*, H-17', 3), 2.48 (*ddd*, J=14.4 and 3Hz, H-17), 1.97 (*m*, H-6'), 1.90 (*m*, H-14), 1.86 (*m*, H-6), 1.78 (*m*, H-15'), 1.42 (*m*, H-20, 19, 19'), 1.10 (*m*, H-15), 0.86 (*t*, J=7.5 Hz, H-18). ^{13}C NMR (75 MHz, CDCl$_3$): Table 1. MS (70 eV) *m/z* (rel.int.): 354 [M$^+$], 337, 295, 170, 160, 59.

Voacangine (**3**) (15.5 mg): ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.90 (*s*, NH), 7.13 (*d*, *J* = 8.6 Hz, H-12), 6.91 (*d*, *J* = 2.6 Hz, H-9), 6.80 ppm (*dd*, *J* = 8.6 and 2.6 Hz, H-11), 3.85 (*s*, OCH$_3$), 3.73 (*s*, CO$_2$CH$_3$), 0.90 (*t*, J = 7 Hz, H-18). ^{13}C NMR (75 MHz, CDCl$_3$): Table 1.

Voacangine Hydroxyindolenine (**4**) (22 mg): ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.37 (*d*, J=8.5Hz, H-12), 7.26 (*s*, NH), 6.90 (*d*, J=2.5Hz, H-9), 6.81 (*dd*, J=8.5 and 2.5 Hz, H-11), 3.81 (*s*, OCH$_3$), 3.70 (*s*, CO$_2$CH$_3$), 0.86 (*t*, J = 7 Hz, H-18). ^{13}C NMR (75 MHz, CDCl$_3$): Table 1. MS (70 eV) *m/z* (rel.int.): 384 [M$^+$], 325, 209, 138, 109, 59.

Ibogamine (**5**) (60 mg): ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.75 (*sl*, NH), 7.44 (*m*, H-9); 7.19 (*m*, H-12), 7.07 (*m*, H-10, H-11), 0.89 (*t*, J = 7.15 Hz, H-18). ^{13}C NMR (75 MHz, CDCl$_3$): Table 1. MS (70 eV) *m/z* (rel.int.): 280 [M$^+$] (53), 195 (29), 168 (10), 156 (16), 148 (16), 136 (100).

Heyneanine (**6**) (94 mg): ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.31 (*s*, NH), 7.46 (*dl*, J=7,5Hz, H-9), 7.25 (*dl*, J=7.5Hz, H-12), 7.14 (*ddd*, J=7.5 e 1.5Hz, H-11), 6.97 (*ddd*, J=7.5 and 1.5Hz, H-10), 4.17 (*dq*, J=6.5 and 2Hz, H-19), 3.88 (*s*, H-21), 3.72 (*s*, COOMe), 1.16 (*d*, J=6.5Hz, H-18). ^{13}C NMR (75 MHz, CDCl$_3$): Table 1. MS (70 eV) *m/z* (rel.int.): 354 [M$^+$] (54), 338 (4), 309 (11), 253 (6), 214 (33), 167 (22), 152 (42), 130 (23), 96 (27), 51(100).

Voacristine (**7**) (17.4 mg): ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.17 (*s*, NH), 7.14 (*d*, J=8.6 Hz, H-12), 6.90 (*d*, J=2.6 Hz, H-9), 6.79 (*dd*, J=8.6 and 2.6 Hz, H-11), 3.83 (*s*, OCH$_3$), 3.71 (*s*, CO$_2$CH$_3$), 1.10 (*d*, J=6.4 Hz, H-18). ^{13}C NMR (75 MHz, CDCl$_3$): Table 1. MS (70 eV) *m/z* (rel.int.): 368 [M^{+1} - OH] (100), 325 (1), 244 (15), 160 (20), 148 (11), 136 (61), 122 (20).

Voacristine hydroxyindolenine (**8**) (8.7 mg): ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.36 (*d*, J = 8.3 Hz, H-12), 6.91 (*d*, *J* = 3.0 Hz, H-9), 6.82 (*dd*, *J* = 8.3 e 2.6 Hz, H-11). ^{13}C NMR (75 MHz, CDCl$_3$): Table 1.

3-Oxo-coronaridine (**9**) (35 mg): ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.14 (*s*, NH), 7.47 (*d*, H-7,5, H-9), 7.22 (*d*, 7.5, H-12), 7.10 (*ddd*, J=7.5 and 1.5 Hz, H-10), 7.09 (*ddd*, J=7.5 and 1.5 Hz, H-11), 3.70 (*s*, CO$_2$CH$_3$), 0.93 (*t*, J=7.5 Hz, H-18). ^{13}C NMR (75 MHz, CDCl$_3$): Table 1.

3-Hydroxycoronaridine hydroxyindolenine (**10**) (5.2 mg): ^{1}H NMR (300 MHz, CDCl$_3$): shifts not resolved. ^{13}C NMR (75 MHz, CDCl$_3$): Table 1.

Vobasine (**11**) (56 mg): ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.02 (*s*, NH), 7.71 (*d*, J=9.0 Hz, H-9), 7.33 (*d*, J=3.4 Hz, H-12), 7.15 (*m*, H-10 and H-11), 5.45 (*q*, J=6.0 Hz, H-19), 2.65 (*s*, NCH$_3$), 2.61 (*s*, CO$_2$CH$_3$), 1.71(*dd*, J=6.8 and 1.8 Hz, H-18). ^{13}C NMR (75 MHz, CDCl$_3$): Table 1. MS (70 eV) *m/z* (rel.int.): 352 [M$^+$], 167, 122, 59.

Voachalotine (**12**) (52.4 mg): ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.8-6.9 (*m*, H-9, H-10, H-11, H-12), 5.34 (*m*, H-19), 3.73 (*s*, CO$_2$CH$_3$), 3.62 (*s*, NCH$_3$), 1.63 (*d*, J=6.6 Hz, H-18). ^{13}C NMR (75 MHz, CDCl$_3$): Table 1.

Hexane extract was fractionated on a silica gel 60 column and purified by HPLC using Supelcosil™ LC-18 column (250 x 10 mm, 5 μm) under isocratic condition (MeOH as mobile phase), the absorbance was 210 nm and mobile phase flux was 3.0 mL/min. Fractionation of hexane extracts from the root bark of *T. catharinensis* resulted in the separation of fractions containing triterpenes (EH 1.4 and EH 1.7) and steroids (EH 1.10). Further purification of EH 1.4 by reversed phase HPLC, produced pooled fractions: EH 1.4.1 (R$_t$= 18.5-21 min), EH 1.4.2 (R$_t$= 21.5-23 min), EH 1.4.3 (R$_t$= 26-32 min) and EH 1.4.4 (R$_t$= 32-34 min).

Fractions EH 1.4.1 (**15**, **22**) (27 mg); EH 1.4.2 (**21**) (23 mg); EH 1.4.3 (**14**, **17**, **18**, **19**, **20**) (54.7 mg); EH 1.4.4 (**16**, **18**, **19**) (49.8 mg); EH 1.10 (**22**, **23**) (106.2 mg). ^{13}C NMR (75 MHz, CDCl$_3$): 154.5 (C-20), 107.1 (C-30) (**14**); 149.9 (C-20), 109.4 (C-29) (**15**); 116.2 (C-7), 145.4 (C-8) (**16**); 121.6 (C-12), 145.1 (C-13) (**17**); 118.8 (C-19), 139.8 (C-20) (**18**); 124.3 (C-12), 139.6 (C-13) (**19**); 159.1 (C-14), 116.4 (C-15) (**20**); 150.9 (C-20), 109.3 (C-29) (**21**); 153.2 (C-20), 107.0 (C-30) (**22**); 140.7 (C-5), 121.6 (C-6) (**22**); 140.7 (C-5), 121.6 (C-6), 138.2 (C-22), 129.2 (C-23) (**23**).

Fraction EH 1.7 was fractionated on a silica gel 60 column using hexane:EtOAc afforded 45.9 mg of the triterpenoid **24**. ^{1}H NMR (300 MHz, CDCl$_3$): δ 4.67 (*s*, H-29), 4.47 (*m*, H-3), 2.28 (*t*, J=7.5 Hz, H-2'a,b), 2.10-1.20 (*m*), 1.64 (*s*, H-30), 1.15-0.82 (*s*, CH$_3$). ^{13}C NMR (75 MHz, CDCl$_3$): 39.0 (C-1), 24.1(C-2), 80.9(C-3), 38.3(C-4), 56.3(C-5), 18.5(C-6), 35.5(C-7), 40.7(C-8), 50.9(C-9), 37.4(C-10), 21.9(C-11), 25.0(C-12), 38.3(C-13), 42.6(C-14), 27.9(C-15), 35.2(C-16), 50.4(C-17), 49.8(C-18), 75.6(C-19), 150.2(C-20), 32.3(C-21), 39.5(C-22), 28.3(C-23), 15.8(C-24), 16.6(C-25), 16.9(C-26), 14.5(C-27), 19.1(C-28), 110.0(C-29), 20.3(C-30), 174.0(C-1´), 35.2(C-2´), 25.5(C-3´), 29(C-4´ to C-13´), 31.6(C-14´), 21.9(C-15´), 14.5(C-16´).

Acidic methanolysis of **24**. The acidic methanolysis was performed according Sobrinho *et al.*[18]. Compound **24** (45.9 mg) was refluxed for 6 h with methanol (5 mL) and HCl (1 mL). After cooling, the solution was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried with anhydrous Na_2SO_4 and concentrated under vacuum, furnishing a 3-hydroxyl compound and a mixture of methyl esters, after chromatography on a silica gel column. Analysis of methyl esters mixture was performed by GC/MS. EIMS of main methyl ester (70 eV), *m/z* (rel.int.): 270 (M^+, 1), 239 (1), 227 (2), 199 (1), 143 (6), 129 (3), 101 (4), 97 (4), 88 (4), 87 (53), 83 (6), 75 (18), 74 (100).

Tumor cell lines culture

The SKBR-3 human breast adenocarcinoma and C-8161 human melanoma tumor cell lines was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The tumor cell lines were cultured and maintained in RPMI 1640 medium supplemented with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate, 10% fetal bovine serum, 100 units/ml penicillin and 100 µg/mL streptomycin. All cell lines were maintained at 37 °C in an atmosphere of 5% CO_2 and 95% air with more than 95% humidity.

Tumor cytotoxic activity

Tumor cells cytotoxic activity of substances was assessed by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) as previosly described[19]. Briefly, tumor cells cultured in appropriate flasks and maintained in continuously exponential growth were detached with 0.05% trypsin, 0.02% EDTA in calcium-free phosphate buffered saline (PBS) and washed 3 times with RPMI medium at 1000 rpm/15 min/10 °C. Cells were placed in 96-well plates at a density of 1×105 cells per well. After 24 h of culture, the medium was removed and fresh medium, with or without different concentrations of indicated extracts/compounds or control drug Methotrexate (10–0.01 mg/mL), was added to the wells and incubated for 24 h. At the end of treatment period, 10 µL of MTT (10 mg/mL) was added to wells, and cells were incubated for a further 4 h. Finally, 50 µL of 20% sodium dodecyl sulfate solution was supplied to each well. Formazan crystals were dissolved at 37 ºC overnight. The absorbance of each well was read on a microplate reader at a wavelength of 540 nm. Cytotoxic rate was calculated as follows: % of cytotoxicity of compounds = 1 - Absorbance drug treated/Absorbance control x l00.

REFERENCES

1. Leeuwenberg, A. J. M.; *A revision of Tabernaemontana. The new world species and Stemmadenia*, The Royal Botanic Gardens: Kew, 1994, Vol. 2.
2. van Beek, T. A.; van Gessel, M. A. J. T. In *Alkaloids: chemical and biological perspectives*; Pelletier, S. W.,Ed: Wiley and Sons: New York, 1988**,** Vol. 6
3. Zhu, J. P.; Guggisberg, A.; Kalt-Hadamowski, M.; Hesse, M.; *Pl. Syst. Evol.* **1990**, *172*, 13.
4. Matos, F. J. A.; Braz Filho, R.; Gottlieb, O. R.; Machado, F.W.L.; Madruga, M. I. L. M.; *Phytochemistry* **1976**, *15*, 551.
5. Nielsen, H. B.; Hazell, A.; Hazell, R.; Ghia, F.; Torssell, K. B. G.; *Phytochemistry* **1994**, *37*, 1729.
6. Achenbach, H.; Waibel, R.; Zwanzger, M.; *Phytochemistry* **1994**, *37*, 1737.
7. Pereira, P. S.; Sampaio, S. V.; França, S. C.; Dias, D. A.; *Acta Horticulturae* **1999**, *501*, 171.
8. Perera, P.; van Beek, T.A.; Verpoorte, R.; *J. Chromatogr.* **1984**, *285*, 214.
9. Perera, P.; Samuelsson, G.; van Beek, T. A.; Verpoorte, R.; *Planta Med.* **1983**, *47*, 148.
10. Wenkert, E.; Cochran, D. W.; Gottlieb, H. E.; Hagaman, E. W.; Braz Filho, R.; Matos, F. J. A.; Madruga, M. I. L. M.; *Helv. Chim. Acta* **1976**, *59*, 2437.
11. Achenbach, H.; Raffelsberger, B.; *Z. Naturforsch., B: Chem. Sci.* **1980**, *35*, 219.
12. Monnerat, C. S.; de Souza, J. J.; Mathias, L.; Braz-Filho, R.; Vieira, I. J. C.; *J. Braz. Chem. Soc.* **2005**, *16*, 1331.
13. Zocoler, M. A.; de Oliveira, A. J. B.; Sarragiotto, M. H.; Grzesiuk, V. L.; Vidotti, G. J.; *J. Braz. Chem. Soc.* **2005**, *16*, 1372.
14. Medeiros, W. L. B.; Vieira, I. J. C.; Mathias, L.; Braz-Filho, R.; Schripsema, J.; *J. Braz. Chem. Soc.* **2001**, *12*, 368.
15. van Beek, T. A.; Verpoorte, R.; Svendsen, A.B.; Leeuwenberg, A. J. M.; Bisset, N. J.; *J. Ethnopharmacol.* **1984**, *10*, 1.
16. Ahond, A.; Bui, A. M.; Potier, P.; Hagaman, E. W.; Wenkert, E.; *J. Org. Chem.* **1976**, *41*, 1878.
17. Mahato, S. B.; Kundu, A. P.; *Phytochemistry* **1994**, *37*, 1517.
18. Sobrinho, D. C.; Hauptli, M. B.; Appolinário, E. V.; Kollenz, C. L. M.; Carvalho, M. G.; Braz Filho, R.; *J. Braz. Chem. Soc.* **1991**, *2*, 15.
19. Prakash, C. V. S.; Sprague, S.; Schilling, J. K.; Kingston, D. G. I.; *J. Nat. Prod.* **2003**, *66*, 528.
20. Zuco, V.; Supino, R.; Righetti, S. C.; Cleris, L.; Marchesi, E.; Gambacorti-Passerini, C.; Formelli, F.; *Cancer Lett.* **2002**, *175*, 17.
21. Cichewicz, R. H.; Kouzi, S. A.; *Med. Res. Rev.* **2004**, *24*, 90.
22. de Almeida, L.; Cintra, A. C. O.; Veronese, E. L.G.; Nomizo, A.; Franco, J. J.; Arantes, E. C.; Giglio, J. R.; Sampaio, S. V.; *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2004**, *137*, 19.