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Article

Qualitative Determination of Indole Alkaloids of *Tabernaemontana fuchsiaefolia* (Apocynaceae)

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Este trabalho descreve um procedimento rápido e eficiente para a separação e identificação de alcalóides indólicos do extrato etanólico de *Tabernaemontana fuchsiaefolia* (Apocynaceae). As frações alcaloídicas obtidas dos extratos etanólicos (das folhas, das cascas do caule e das cascas das raízes) foram fracionadas e analisadas por cromatografia em camada delgada (CCD) e por cromatografia gasosa acoplada a espectrometria de massa (CG-EM). Foram identificados os alcalóides indólicos ibogamina, coronaridina, pseudoindoxil ibogaina, hidroxiindolenina voacangina, pseudoindoxil voacangina, tabernantina, catarantina, voacangina, 19-oxovoacangina, 10-hidroxicoronaridina, afinisina, 16-*epi*-afinina, voachalotina, ibogalina e conofaringina.

This paper describes a fast and efficient procedure to separate and identify indole alkaloids from the ethanolic extract of *Tabernaemontana fuchsiaefolia* (Apocynaceae). The alkaloidal fractions obtained from ethanolic extracts of leaves and stem barks and root barks were fractioned and analyzed by Thin-Layer Chromatography (TLC) and by Gas Chromatography coupled to Mass Spectrometry (GC-MS). The following indole alkaloids were identified: ibogamine, coronaridine, ibogaine pseudoindoxyl, voacangine hydroxyindolenine, voacangine pseudoindoxyl, tabernanthine, catharanthine, voacangine, 19-oxovoacangine, 10-hydroxycoronaridine, affinisine, 16-*epi*-affinine, voachalotine, ibogaline, and conopharyngine.

Keywords: *Tabernaemontana fuchsiaefolia*, indole alkaloids analyses, alkaloids, GC-MS, TLC

Introduction

The genus *Tabernaemontana* belongs to the Apocynaceae family. Several species of this genus are used in folk medicine against many diseases: diarrhoea, skin affections, warts, syphilis, Hansen's Disease, cancer and insect bites.[1] The main constituents of the *Tabernaemontana* genus are indole alkaloids, a class of substances with a wide range of pharmacological activities: cholinesterase inhibitors,[2] analgesic, anti-inflammatory, bactericidal, oestrogenic, and stimulant and depressant of the central nervous system (CNS).[3] Taxonomy of the Apocynaceae family is quite complex and there are difficulties in its classification and terminology because there are a great number of synonyms. Both generic names, *Tabernaemontana* and *Peschiera,* have been used in Brazil and are matter of discussion.[4]

Tabernaemontana fuchsiaefolia A. DC., known as "leiteira" (milk weed), is a common lactiferous tree that grows in pastures of the Brazilian States of São Paulo and northern Paraná.[5,6] In Brazil, this species is commonly used to treat malaria.[7]

Federici *et al*.[8] reported that the extracts of *Tabernaemontana fuchsiaefolia* stem and root barks have good *in vitro* activity against *Plasmodium falciparum*, the parasite responsible for malaria.[8] Casado *et al*.[9] showed that the crude extract obtained from *Tabernaemontana fuchsiaefolia* stem barks presented depressant activity in CNS and also provoked spontaneous stimulation in isolated rat womb.[9]

One of the problems related to the use of vegetal crude extracts is the variation of concentration of their active ingredient due to crop time, place and soil properties. Therefore, the use of an analytical technique both simple and quick is desirable for the identification and quantification of the indole alkaloids present in

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Tabernaemontana fuchsiaefolia crude extracts

Among the various techniques used for the rapid identification of indole alkaloids, gas chromatography coupled to the mass spectrometry (GC-MS) is very efficient because it allows the simultaneous separation and identification of trace alkaloids in a complex mixture.[10-14]

In the present study, we have used GC-MS to effect the analyses of alkaloid extracts from different parts of *Tabernaemontana fuchsiaefolia*.

Experimental

Plant material

Leaves, and stems and roots of *Tabernaemontana fuchsiaefolia* were collected on the Universidade Estadual de Maringá (UEM) campus in November 1999 and identified by Dr. Maria Conceição de Souza. A specimen of *Tabernaemontana fuchsiaefolia* (HUM no. Reg. 8,325) was deposited in the Biology Department herbarium of UEM.

Isolation of alkaloids

The stem bark alkaloidal extract (1.0 g) was fractioned on a neutral alumina chromatographic column eluted with CH_2Cl_2, and a mixture of EtOAc and MeOH in an increasing order of polarity. The fractions obtained were combined based on TLC analysis. Fraction A was eluted with CH_2Cl_2 (124.1 mg) and submitted to open column chromatography on neutral alumina by using hexane, CH_2Cl_2, and mixtures of EtOAc and MeOH with increasing order of polarity. Fractionation resulted in the isolation of **1** (15.0 mg), **2** (40.5 mg), and **3** (7.0 mg). Fraction B (71.3 mg) was eluted with CH_2Cl_2/EtOAc (80:20, v/v) and purified by silica gel PTLC with $CHCl_3$/MeOH (90:10, v/v, atm NH_3) as an eluant to yield substance **4** (24 mg). Fraction C (25.7 mg) was eluted with CH_2Cl_2/EtOAc (1:1, v/v) and afforded substance **5** (15 mg) after recrystallization from hexane/ CH_2Cl_2 (1:1, v/v).

*Coronaridine (**1**)*

Amorphous solid, ^{1}H NMR ($CDCl_3$, 300 MHz), δ 0.90 (3H, t, *J* 7.5 Hz, H-18), 1.13 (1H, m, H-15$_a$), 1.33 (1H, m, H-20), 1.45 (1H, m, H-19$_a$), 1.55 (1H, m, H-19$_b$), 1.74 (1H, m, H-15$_b$), 1.88 (1H, m, H-14), 1.91 (1H, m, H-17$_a$), 2.57 (1H, ddd, *J* 2.4, 5.7 and 14.1 Hz, H-17$_b$), 2.80 (1H, d, *J* 8.7 Hz, H-3$_a$), 2.90 (1H, dd, *J* 3.9 and 8.7 Hz, H-3$_b$), 3.01 (1H, m, H-5$_a$), 3.15 (1H, m, H-5$_b$), 3.21 (1H, m, H-6$_a$), 3.39 (1H, m, H-6$_b$), 3.56 (1H, bs, H-21), 3.71 (3H, s, CO_2Me), 7.08 (1H, ddd, *J* 1.2, 7.2 and 7.5 Hz, H-10), 7.14 (1H, ddd, *J* 1.2, 6.3 and 7.2 Hz, H-11), 7.24 (1H, dd, *J* 1.2 and 6.3 Hz, H-12), 7.47 (1H, dd, *J* 1.2 and 7.5 Hz, H-9), 7.81 (1H, bs, N-H). ^{13}C NMR ($CDCl_3$, 75.5 MHz), δ 11.5 (C-18), 22.0 (C-6), 26.6 (C-19), 27.3 (C-14), 31.9 (C-15), 36.4 (C-17), 39.1 (C-20), 51.5 (C-3), 52.5 (CO_2Me), 53.1 (C-5), 55.0 (C-16), 57.4 (C-21), 110.4 (C-12), 110.4 (C-7), 118.5 (C-9), 119.3 (C-10), 122.0 (C-11), 128.9 (C-8), 135.5 (C-13), 136.7 (C-2), 175.9 (CO_2Me).

*Voacangine (**2**)*

Crystalline needles, ^{1}H NMR ($CDCl_3$, 300 MHz), δ 0.90 (3H, t, *J* 7.5 Hz, H-18), 1.12 (1H, m, H-15$_a$), 1.32 (1H, m, H-20), 1.44 (1H, m, H-19$_a$), 1.55 (1H, m, H-19$_b$), 1.73 (1H, m, H-15$_b$), 1.87 (1H, m, H-14), 1.90 (1H, m, H-17$_a$), 2.57 (1H, ddd, *J* 2.4, 5.7 and 14.1 Hz, H-17$_b$), 2.80 (1H, d, *J* 8.4 Hz, H-3$_a$), 2.90 (1H, m, H-3$_b$), 2.98 (1H, m, H-5$_a$), 3.13 (1H, m, H-5$_b$), 3.22 (1H, m, H-6$_a$), 3.37 (1H, m, H-6$_b$), 3.54 (1H, bs, H-21), 3.71 (3H, s, CO_2Me), 3.81 (3H, s, OMe), 6.80 (1H, dd, *J* 2.4 and 8.7 Hz, H-11), 6.92 (1H, d, *J* 2.4 Hz, H-9), 7.13 (1H, d, *J* 8.7 Hz, H-12), 7.67 (1H, bs, N-H). ^{13}C NMR ($CDCl_3$, 75.5 MHz), δ 11.5 (C-18), 22.1 (C-6), 26.6 (C-19), 27.2 (C-14), 31.9 (C-15), 36.5 (C-17), 38.0 (C-20), 51.5 (C-3), 52.5 (CO_2Me), 53.1 (C-5), 55.1 (C-16),

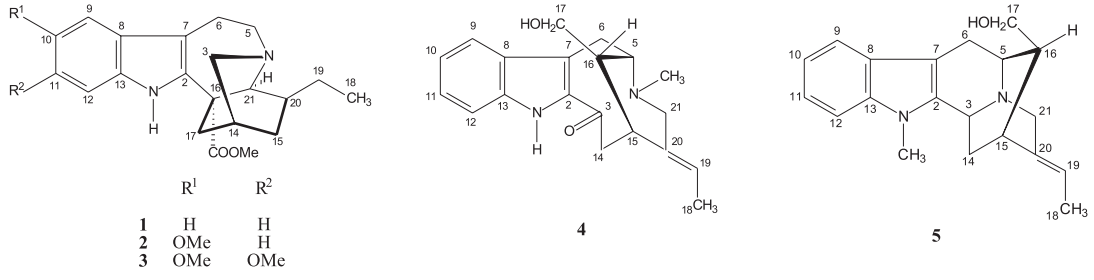

Figure 1. Structures of alkaloids isolated from *Tabernaemontana fuchsiaefolia* stem bark.

55.7 (OMe), 57.5 (C-21), 110.2 (C-7), 100.8 (C-9), 111.1 (C-12), 111.9 (C-11), 129.3 (C-8), 130.6 (C-13), 137.6 (C-2), 154.2 (C-10), 175.9 (CO_2Me).

*Conopharyngine (**3**)*

Crystals, ^{1}H NMR ($CDCl_3$, 300 MHz), δ 0.89 (3H, t, *J* 7.5 Hz, H-18), 1.12 (1H, m, H-15$_a$), 1.31 (1H, m, H-20), 1.44 (1H, m, H-19$_a$), 1.54 (1H, m, H-19$_b$), 1.73 (1H, m, H-15$_b$), 1.87 (1H, m, H-14), 1.90 (1H, m, H-17$_a$), 2.54 (1H, ddd, *J* 2.4, 5.7 and 14.1 Hz, H-17$_b$), 2.81 (1H, d, *J* 8.4 Hz, H-3$_a$), 2.90 (1H, m, H-3$_b$), 2.97 (1H, m, H-5$_a$), 3.11 (1H, m, H-5$_b$), 3.21 (1H, m, H-6$_a$), 3.37 (1H, m, H-6$_b$), 3.53 (1H, bs, H-21), 3.71 (3H, s, CO_2Me), 3.89 (3H, s, 10-OMe), 3.92 (3H, s, 11-OMe), 6.78 (1H, s, H-12), 6.90 (1H, s, H-9), 7.59 (1H, bs, N-H).

*16-Epi-affinine (**4**)*

Light yellow oil, ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.71 (3H, dd, *J* 2.1 and 6.9 Hz, H-18), 1.96 (1H, m, H-16), 2.57 (3H, s, N-4-Me), 2.68 (2H, m, H-14), 3.03 (1H, d, *J* 13.8 Hz, H-21$_a$), 3.11 (1H, m, H-15), 3.30 (1H, m, H-5), 3.34 (1H, m, H-6$_a$), 3.50 (1H, m, H-6$_b$), 3.64 (2H, m, H-17), 3.71 (1H, bd, *J* 13.8 Hz, H-21$_b$), 5.47 (1H, bq, *J* 6.9 Hz, H-19), 7.18 (1H, ddd, *J* 1.2, 6.3 and 7.5 Hz, H-10), 7.38 (1H, m, H-11), 7.39 (1H, m, H-12), 7.72 (1H, dd, *J* 0.9 and 7.5 Hz, H-9), 9.12 (1H, s, N-1-H). ^{13}C NMR ($CDCl_3$, 75.5 MHz), δ 12.0 (C-18), 19.1 (C-6), 31.8 (C-15), 37.8 (C-16), 41.9 (N-Me),.43.5 (C-14), 52.0 (C-21), 57.3 (C-5), 68.1 (C-17), 112.2 (C-12), 120.5 (C-7), 120.6 (C-10), 120.8 (C-9), 121.1 (C-19), 126.9 (C-11), 128.5 (C-8), 135.2 (C-2), 135.6 (C-20), 136.4 (C-13), 191.3 (C-3).

*Affinisine (**5**)*

Crystalline needles, ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.65 (3H, dd, *J* 2.1 and 6.9 Hz, H-18), 1.67 (1H, m, H-14$_a$), 1.81 (1H, m, H-16), 2.08 (1H, ddd, *J* 2.4, 10.2 and 11.4 Hz, H-14$_b$), 2.63 (1H, dd, *J* 1.2 and 15.6 Hz, H-6$_a$), 2.79 (1H, dd, *J* 1.2 and 6.0 Hz, H-5), 2.83 (1H, m, H-15), 3.06 (1H, dd, *J* 4.8 and 15.6 Hz, H-6$_b$), 3.52 (1H, dd, *J* 8.4 and 10.5 Hz, H-17$_a$), 3.60 (1H, dd, *J* 8.1 and 10.5 Hz, H-17$_b$), 3.62 (2H, m, H-21), 3.64 (3H, s, N-1-Me), 4.21 (1H, dd, *J* 2.7 and 9.9 Hz, H-3), 5.42 (1H, bq, *J* 6.9 Hz, H-19), 7.09 (1H, ddd, *J* 1.2, 7.5 and 8.4 Hz, H-10), 7.18 (1H, ddd, *J* 1.2, 8.1 and 8.4 Hz, H-11), 7.29 (1H, bd, *J* 8.1 Hz, H-12), 7.48 (1H, bd, *J* 7.5 Hz, H-9). ^{13}C NMR ($CDCl_3$, 75.5 MHz), δ 12.7 (C-18), 27.0 (C-6), 29.2 (N-Me), 27.5 (C-15), 32.9 (C-14), 44.3 (C-16), 49.4 (C-3), 54.1 (C-5), 56.4 (C-21), 65.1 (C-17), 103.7 (C-7), 108.8 (C-12), 118.2 (C-9), 116.6 (C-19), 118.9 (C-10), 120.9 (C-11), 127.5 (C-8), 136.4 (C-20), 137.4 (C-13), 139.8 (C-2).

Sample preparation

Samples of each of the three crude alkaloidal extracts (50 mg) were fractioned on a silica gel (70-230 mesh, 2 g) column (ø = 9mm), eluted with (*i*) 100 mL of $CHCl_3$: MeOH (4:1, v/v) (F1), (*ii*) 20 mL of $CHCl_3$: MeOH (1:1, v/v) (F2) and (*iii*) 20 mL of MeOH (F3). The solvents were evaporated under vacuum and each sample was dissolved in $CHCl_3$ (1 mg mL^{-1}) for analysis. Fractions F1, F2, and F3 were analyzed by thin-layer chromatography (TLC). Only F1 was analyzed by GC-MS.[15,16] Five indole alkaloids (coronaridine, voacangine, voachalotine, 16-*epi*-affinine, and affinisine) isolated from the stem bark of *Tabernaemontana fuchsiaefolia* were used as standards in this study.

Thin-Layer Chromatography

F1, F2, F3 and the five indole alkaloid standards were analyzed by TLC plates (0.20 mm thickness, silica gel Machery-Nagel) with toluene-ethanol-ammonia (aq.) (95:5:5, v/v/v) as an eluant.[15,16] The plates were developed separately under UV light (254; 366 nm) and with Dragendorff's reagent, iodoplatinate, ferric chloride-perchloric acid (FCPA), and ceric sulphate-sulfuric acid (CSSA). The plates were subsequently heated with a hot-air blower until the characteristic coloured spots were visible.[17]

Gas Chromatography-Mass Spectrometry

GC-MS analysis was performed on a Shimadzu QP 2000A mass-selective detector with electron impact ionization (70 eV). The column used was a SE-30 (60 m x 0.25 mm i.d. and 0.25 μm phase thickness). Helium was used as a carrier gas at 0.7 mL min^{-1}. The injection temperature was 270 ºC. The column temperature was programmed to rise from 100 to 225 ºC at 15 ºC min^{-1}, from 225 to 260 ºC at 2.5 ºC min^{-1}, and from 260 to 300 ºC at 15 ºC min^{-1}.

Results and Discussion

Fractionation of *T. fuchsiaefolia* alkaloidal stem bark extracts resulted in five indole alkaloids which had already been reported for this same species: coronaridine (**1**), voacangine (**2**), conopharyngine (**3**), 16-*epi*-affinine (**4**) and affinisine (**5**). The indole alkaloids were identified by the usual spectrometric methods (^{1}H NMR, ^{13}C NMR, and MS) and also by comparison of their NMR data with those in literature.[18-20]

All F1 alkaloidal fractions were analyzed by TLC using the isolated alkaloids as standards. Coronaridine (**1**), voacangine (**2**), conopharyngine (**3**), and 16-*epi*-affinine

(**4**) could be identified by their R_f values and colours. TLC of fractions F2 and F3 showed the presence of polar constituents and they were not analyzed by GC-MS [15,16]

GC-MS analysis of F1 fractions with standards confirmed the presence of indole alkaloids coronaridine (**1**), voacangine (**2**), conopharyngine (**3**), 16-epi-affinine (**4**) and affinisine (**5**). The presence of other ten indole alkaloids was evidenced by comparison with the NBS database from the GC-MS equipment itself and by comparison and analysis of each fragmentation pattern with data in the literature.[21,22] The alkaloids identified in the alkaloidal extracts from *T. fuchsiaefolia* belong to the *ibogan* class (*coronaridine-group* and *pseudoindoxyl-group*) and to the *corynanthean* class (*akuammidine-group* and *vobasine-group*). Table 1 shows GC-MS experimental data, retention time, $M^{+\cdot}$, and main fragments (their respective relative intensities) for indole alkaloids of fraction F1 from *T. fuchsiaefolia*.

Table 1. CG-MS experimental data, retention time, $M^{+\cdot}$ and main fragments *m/z* (relative intensity) for indole alkaloids of F1 fraction from *T. fuchsiaefolia*

Peak	Alkaloids	MF	t_r (min)	$[M]^{+\cdot}$	Main Fragments *m/z*
1	Ibogamine[a]	$C_{19}H_{24}N_2$	38.01	280(45)	195(31), 156(16), 154(12), 149(31), 136(100), 135(65), 130 (14), 122(39)
2	Coronaridine[a,b] (**1**)	$C_{21}H_{26}N_2O_2$	39.43	338(100)	323(28), 309(6), 256(13), 214(27), 136(92), 135(28), 124(46), 122(36)
3	Ibogaine pseudoindoxyl[a]	$C_{20}H_{26}N_2O_3$	42.44	326(100)	207(42), 189(25),176(26), 150(76), 138(57), 122(91)
4	Voacangine hydroxyindolenine[a]	$C_{22}H_{28}N_2O_4$	43.20	384(100)	369(22), 368(27), 367(66), 225(12), 207(47), 122(45)
5	Voacangine pseudoindoxyl[a]	$C_{22}H_{28}N_2O_4$	46.00	384(100)	325(23), 247(16), 209(36), 208(20), 175(16), 138(57), 122(67)
6	Tabernanthine[a]	$C_{20}H_{26}N_2O$	46.30	310(41)	295(9), 281(4), 25(41), 186(13), 149(35), 136(100), 122(44)
7	Catharanthine[a]	$C_{21}H_{24}N_2O_2$	46.54	336(84)	335(75), 321(31), 277(30), 182(100), 168(50)
8	Voacangine[a,b] (**2**)	$C_{22}H_{28}N_2O_3$	47.58	368(100)	283(14), 253(16), 244(20), 184(30), 136(88), 135(36), 124(36), 122(37)
9	19-Oxovoacangine[a]	$C_{22}H_{26}N_2O_4$	47.85	382(48)	339(3), 244(16), 184(16), 160(23), 150(10), 136(100)
10	10-Hydroxycoronaridine[a]	$C_{21}H_{26}N_2O_3$	48.05	354(100)	339(19), 269(10), 230(22),170(23), 136(93),124(41), 122(50)
11	Affinisine[a,b] (**5**)	$C_{20}H_{24}N_2O$	48.36	308(96)	307(66), 291(7), 277(33), 263(8), 249(7), 183(100), 182(83)
12	16-*Epi*-affinine[a,b] (**4**)	$C_{20}H_{24}N_2O_2$	48.75	324(1)	307(15), 306(35), 153(12), 152(100), 151(13), 148(14), 135(18), 122(14)
13	Voachalotine[a]	$C_{22}H_{26}N_2O_3$	50.29	366(100)	365(16), 307(62), 263(2), 183(18), 182(11)
14	Ibogaline[a]	$C_{21}H_{28}N_2O_2$	52.81	340(100)	339(6), 255(27), 170(15), 149(37), 136(60), 135(48), 122(35)
15	Conopharyngine[a,b] (**3**)	$C_{23}H_{30}N_2O_4$	53.50	398(100)	313(7), 274(13), 208(14), 148(20), 136(97), 135(25), 124(32), 122(40)

MF: Molecular Formula; t_r: Retention Time; [a]Alkaloid identified by GC-MS analysis; [b]Alkaloid standard.

Table 2. Indole alkaloid contents of ethanolic extracts from various parts of *Tabernaemontana fuchsiaefolia* (this work) and in literature data[8,24,25]

Alkaloids	Ethanolic Extracts (F1)[a]			Literature		
	Sb	L	Rb	Isolation Method[b]	Plant Part[c]	Ref.
Ibogamine	++	+	+++	acid	Rb	8
Coronaridine (**1**)	++	++	+++	acid	Sb,Rb, S	8
Ibogaine pseudoindoxyl	+	-	-			
Voacangine hydroxyindolenine	-	+	-	basic	Sb	24
Voacangine pseudoindoxyl	-	++	-			
Tabernanthine	++	+++	+	acid	Sb	25
Catharanthine	+	-	-			
Voacangine (**2**)	+++	+++	++	acid, basic	Sb, S	8, 24
19-Oxovoacangine	-	+	-			
10-Hydroxycoronaridine	-	+	-			
Affinisine (**5**)	+++	-	-	acid, basic	Sb	8,24
16-*Epi*-affinine (**4**)	++	-	-	acid, basic	Sb	8,24
Voachalotine	+	-	+	acid, basic	Sb	8,24
Ibogaline	++	-	-			
Conopharyngine (**3**)	++	+	-	acid	Sb	8
Perivine	-	-	-	acid, basic	Sb	8,24
Vobasine	-	-	-	acid	Sb	8
Heyneanine	-	-	-	acid	Sb	8
Voacristine	-	-	-	acid	Sb	8
3-Hydroxycoronaridine	-	-	-	acid	Rb	8
Vobasinol	-	-	-	acid	Rb	8

Relative alkaloid abundance: major (+++), intermediary (++), minor (+), Not detected (-). [a]Alkaloids detected by GC-MS in ethanolic extract of *T. fuchsiaefolia* obtained by neutral method; [b]Alkaloid isolation method used; [c] plant part used: stem bark (Sb), leaves (L), root bark (Rb), and seeds (S).

Table 2 shows the indole alkaloid contents in alkaloidal extracts obtained from various parts of *T. fuchsiaefolia* and some literature data. The GC-MS chromatographic profile of these fractions shows that the stem bark extracts have the largest number and the largest concentrations of alkaloids. The *corynanthean* alkaloid class (*akuammidine-group* and *vobasine-group*) was not detected in the leaves. Only voachalotine, an alkaloid belonging to the *corynanthean* class (*akuammidine-group*), was detected, but in low concentration, in root bark extracts. Two alkaloids from this same class, 16-*epi*-affinine (the *vobasine-group*) and affinisine (*akuammidine-group*), were detected in stem bark extracts in higher concentration. Alkaloids with the *ibogan* skeleton, such as coronaridine, voacangine, ibogamine, and tabernanthine were present in all extracts analyzed, of which voacangine was the most abundant one. Coronaridine was more abundant in roots than in other parts analyzed. According to Delorenzi,[23] coronaridine, which is the main indole alkaloid, of the chloroform fraction obtained from the ethanolic bark extract of *Peschiera australis*, is responsible for its action against *Leishmania amazonensis*. The indole alkaloid voachalotine, which is the chemosystematic marker of this species[1], was detected in stem and root bark extracts.

Conclusions

Gas chromatography coupled to mass spectrometry (GC-MS) proved to be a valuable tool for the analysis of *T. fuchsiaefolia* monomeric indole alkaloids. A total of fifteen indole alkaloids could be quickly and easily identified in alkaloidal extracts from different parts of *T. fuchsiaefolia*. GC-MS retention time and standard addition provided the identity of five indole alkaloids present in the extracts. Mass spectrometry analysis confirmed the presence of alkaloids coronaridine, voacangine, voachalotine, 16-*epi*-affinine, and affinisine, and indicated the presence of ten other alkaloids. However, this method is limited to the analysis of less polar components, unless a derivation of the most polar components is provided.[14]

Perivine, vobasine, vobasinol, heyneanine, voacristine, and 3-hydroxycoronaridine previously reported in *T. fucshiaefolia,* were not detected in this study probably due to their low concentration at collecting time or the isolation method used. We have used the neutral method (ethanol) to prepare the extracts for GC-MS analysis, while the alkaloids[8,24,25] reported were obtained by either acid or basic methods.

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