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Short communication

Qualitative determination of indole alkaloids, triterpenoids and steroids of *Tabernaemontana hilariana*

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Abstract

This paper reports the separation and identification of indole alkaloids, steroids and triterpenoids from the ethanolic extracts of *Tabernaemontana hilariana* (Apocynaceae). The alkaloidal fractions from the ethanolic extracts obtained (root barks, green fruits, ripe fruits and seeds) were fractionated and analysed by thin-layer chromatography, capillary gas chromatography–flame ionization detection (cGC–FID) as well as by high-resolution gas chromatography–mass spectrometry (HRGC–MS). 3-Hydroxycoronaridine, ibogamine, coronaridine pseudoindoxyl, coronaridine, catharanthine, voacangine hydroxyindolenine, voacangine pseudoindoxyl, tabernanthine, tetraphyllicine, 3-hydroxyvoacangine, voacangine, isovoacangine and 3-oxocoronaridine were identified. The insoluble fraction of ethanolic extracts obtained from the root barks and green fruits were analysed and ten aliphatic constituents were also identified by cGC–FID and HRGC–MS.

Keywords: *Tabernaemontana hilariana*; Plant materials; Indoles; Alkaloids; Triterpenoids; Steroids

1. Introduction

The genus *Tabernaemontana* belongs to the family Apocynaceae and comprises about 100 species. They occur in tropical as well as subtropical parts of the world. *Tabernaemontana* spp. are famous for their indole alkaloid contents [1]. Many species have been or are still used in traditional medicine [2]. Since the first isolation of the pure alkaloid from this genus in 1939, more than 300 different indole alkaloids were isolated from the *Tabernaemontana* species and some of them possess useful pharmacological properties, such as antiparasitic, hallucinogenic and antimicrobial activities [1–3]. The genus is under revision by Leeuwenberg and has a large number of synonyms [1].

Tabernaemontana hilariana Muell Arg. (Apocynaceae) is a small tree that grows wild in State São Paulo, Brazil. The leaves are lethal to cattle [4]. The presence of toxic plants in pastures is responsible for 5% of the cattle deaths in the Paraná State, Brazil [5]. The identification of the constituents of this plant is important not only from a pharmacological point of view but also for the chemotaxonomy of the Apocynaceae family [3].

Analysis and identification of complex mixtures

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containing known indole alkaloids is frequently carried out by thin-layer chromatography (TLC), through comparison of R_F values in different solvent systems, and by comparison of specific color reactions of the components of the mixture with reference compounds [6].

Among the available techniques for the identification of indole alkaloids, triterpenoids and steroids, capillary gas chromatography–flame ionization detection (GC–FID) has been shown to be a very efficient system in separating such mixtures due to its speed and resolution [7,8]. Another advantage of this technique is the easy coupling to a mass spectrometer leading to the knowledge of new and/or minor compounds of a mixture without the need for laborious isolation procedures.

We have focused the present work on the analysis of the constituents from four parts of *Tabernaemontana hilariana* using TLC, capillary GC and HRGC–MS.

2. Experimental

2.1. Plant material and extraction of the alkaloids

Green fruits, ripe fruits, seeds and root barks of *T. hilariana* were collected at Boa Esperança, SP, Brazil, by Dr. Gilberto Pozetti in 1988. A specimen has been kept at our Institute. A 100-g amount of each part was separately dried for 24 h in an oven (40°C) powdered and extracted (Soxhlet) for 24 h with 500 ml of 96% ethanol. The solvents were evaporated under vacuum. A 200-mg amount of each extract was submitted to the usual acid–base extraction giving the crude alkaloidal fractions as well as insoluble fractions [9].

2.2. Clean-up of the extracts

Samples of each of the five crude alkaloidal (10 mg) were fractionated on silica cartridges [Sep-pak Classic, Millipore, 690 mg, 55–105 μm, eluted with (1) 15 ml $CHCl_3$ (F1), (2) 15 ml $CHCl_3$–CH_3OH (9:1, v/v) (F2), (3) 20 ml CH_3OH (F3)]. The solvents were evaporated under a nitrogen stream and 1 mg of each sample was redissolved in 5 ml of chloroform for analysis. The F_1, F_2 and F_3 fractions were analysed by thin-layer chromatography (TLC). F_1 and F_2 were also analysed by GC and HRGC–MS.

A 50-mg amount of the insoluble fractions obtained from the ethanolic extracts of root barks and green fruits were extracted with 10 ml of hexane in order to selectively extract compounds of low polarity appropriate for GC analysis, including low-functionalized steroids and triterpenoids, thus eliminating highly polar compounds. The hexane-soluble fractions (H1) were filtrated and solvents were evaporated under a nitrogen stream. A 1-mg amount of each fraction was redissolved in 5 ml of chloroform and analyzed by HRGC–FID and HRGC–MS.

Standards indole alkaloids, steroids and triterpenoids were obtained from a collection at our laboratory.

2.3. Thin-layer chromatography

F1, F2, F3 and several indole alkaloid standards were analysed by laboratory-made TLC plates (0.1 mm thickness, silica gel G Merck, Art. 7731) eluted with toluene–ethanol–ammonia (95:5:5, v/v/v). The plates were separately developed 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 [6].

2.4. Gas chromatography

GC analysis were performed in a Varian 3400 gas chromatograph equipped with a capillary fused-silica LM-5 (15 m×0.2 mm I.D., film thickness 0.2 μm) and with an FID system. H_2 was used as carrier gas at a flow-rate 0.8 ml/min. The injection split ratio was 1:20. The injection temperature was 250°C. The column temperature was programmed to rise from 150 to 280°C at 15°C/min; this temperature was held for 20 min. The detector temperature was 290°C. Samples of 1 μl were injected with a 10-μl Hamilton syringe.

2.5. Gas chromatography–mass spectrometry

The HRGC–MS analysis were performed on a

Shimadzu QP 5000 mass-selective detector, with electron impact ionization (70 eV), coupled to a Shimadzu GC-17B gas chromatograph with the column and temperature programme as described above. Helium was used as carrier gas at a flow-rate of 0.6 ml/min and the MS scan range was 50–550 u.

3. Results and discussion

Coronaridine (R_F=68), voacangine (R_F=52) and ibogamine (R_F=59) could be unequivocally identified by their R_F values and colours when compared to the standards and literature data [6]. From this procedure we observed that F3 retained only polar compounds (R_F<0.2), that could not be injected into the gas chromatograph and thus were not identified. The F1 and F2 fractions were further analysed by HRGC–FID and HRGC–MS.

The coinjection of authentic standards and HRGC–MS analysis was used to identify ibogamine, coronaridine, tabernanthine, isovoacangine, catharanthine, tetraphyllicine, voacangine, campesterol, stigmasterol, sitosterol, β-amyrin, α-amyrin, lupeol, taraxasterol, β-amyrin acetate, α-amyrin acetate and lupeol acetate. The presence of the six other indole alkaloids was deduced by matching with the NIST 62 500 data bank (with 62 235 compounds) and also by their MS fragmentation pattern as compared with the literature [10].

Table 1 shows the retention times of indole alkaloids, steroids and triterpenoids found in *T. hilariana*. The chromatographic profile shows three distinct regions of elution: from 10.04 min to 15.10 min only the elution of the alkaloids occurs; from 16.45 min to 17.60 min the steroids are eluted and from 17.85 min to 20.86 min the elution of the triterpenoids occurs. Thus the chromatographic conditions employed afforded a baseline resolution and allowed the separation of this complex mixture of 23 constituents in less than 21 min.

Table 2 shows the compounds present in F1 and F2 in each of the four crude alkaloidal samples. The HRGC–MS profile of these fractions showed some similarities between the chemical pattern of the ripe fruits, green fruits, seed and root barks relative to the alkaloidal fractions. Coronaridine is present in considerable amounts in all of the parts analysed.

Table 1
Retention times of indole alkaloids, steroids and triterpenoids from *T. hilariana*

Peak	Compounds	M_r	t_R (min)
1	3-Hydroxycoronaridine[a]	354	10.04
2	Ibogamine[a,b]	280	10.15
3	Coronaridine pseudoindoxil[1]	354	10.30
4	Coronaridine[a,b]	338	11.16
5	Catharanthine[a,b]	336	11.48
6	Voacangine hydroxyindolenine[a]	384	12.20
7	Voacangine pseudoindoxil[a]	384	13.14
8	Tabernanthine[a,b]	310	13.32
9	Tetraphillicine[a,b]	308	13.71
10	3-Hydroxyvoacangine[1]	384	13.82
11	Voacangine [a,b]	368	13.89
12	Isovoacangine[a,b]	368	14.91
13	3-Oxocoronaridine[a]	352	15.10
14	Campesterol[a,b]	400	16.45
15	Stigmasterol[a,b]	412	16.95
16	Sitosterol[a,b]	414	17.60
17	β-Amyrin[a,b]	426	17.85
18	Taraxasterol[a,b]	426	18.00
19	α-Amyrin[a,b]	426	18.41
20	Lupeol[a,b]	426	18.85
21	β-Amyrin acetate[a,b]	468	19.55
22	α-Amyrin acetate[a,b]	468	20.11
23	Lupeol acetate[a,b]	468	20.86

[a] Structure confirmed by MS.
[b] Standard compounds.

Ibogamine and voacangine are also found in all the parts, but in lower concentrations than coronaridine. It is also interesting to note that ibogamine elutes only in fraction F2. Voacangine hydroxyindolenine, tetraphyllicine and 3-hydroxyvoacangine were found only in the root barks, while catharanthine was detected only in the green fruits. We also observed that the alkaloid composition of the green fruits is somewhat more complex than that of the ripe fruits.

Previous work with a sample of *T. hilariana* collected in 1984 led to the identification of 3-hydroxycoronaridine, ibogamine, coronaridine, voacangine, voacangine hydroxyindolenine and 3-(2-oxopropyl)coronaridine from root barks and 3-hydroxycoronaridine, coronaridine, voacangine, voacangine hydroxyindolenine, voacangine pseudoindoxyl from green fruits [11]. In the present work a sample collected in 1988 was used and the following alkaloids were identified: 3-hydroxycoronaridine, ibogamine, coronaridine, voacangine, voacangine hydroxyindolenine, besides 3-hydroxyvoacangine,

Table 2
Indole alkaloids contents of various parts of *Tabernaemontana hilariana*

Alkaloids	Ethanolic extracts							
	Fruits				Seed		Root barks	
	Green		Ripe					
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
3-Hydroxycoronaridine	−	−	−	−	−	−	+++	−
Ibogamine	−	++	−	++	−	++	−	+++
Cornaridina pseudoindoxil	−	+	−	−	−	++	−	−
Coronaridine	+++	+	+++	+	+++	+	+++	+
Catharanthine	+	+	−	−	−	−	+	−
Voacangine hydroxyindolenine	+	−	−	−	−	−	−	+
Voacangine pseudoindoxil	+	+	−	+	−	++	−	−
Tabernanthine	−	−	−	−	−	+	−	−
Tetraphillicine	−	−	−	−	−	−	−	+
3-Hydroxyvoacangine	−	−	−	−	−	−	−	+
Voacangine	++	+	+	−	+	−	++	+
Isovoacangine	−	−	−	−	−	−	−	+
3-Oxocoronaridine	+	−		−	−	−	+	

−:Absent
Relative abundance: +++: major component; ++: intermediary concentration; +: minor component

tetraphyllicine, isovoacangine and 3-oxocoronaridine in the root barks. In the green fruits we found coronaridine, voacangine and ibogamine, besides voacangine pseudoindoxyl, coronaridine pseudoindoxyl, catharanthine and 3-oxocoronaridine. Voacangine hydroxyindolenine and 3-(2-oxopropyl)coronaridine, a suspect artifact [12], were not found this time in the green fruits.

Table 3 shows the aliphatic compounds present in H1 fractions from the root barks and green fruits. The H1 fractions showed some similarities between the chemical pattern of root barks and green fruits. β-amyrin acetate is the major component in both parts, followed by α-amyrin, β-amyrin and α-amyrin acetate. Lupeol was found only in the green fruits, while campesterol and taraxasterol were detected as minor constituents only in the root barks.

This approach has proven to be an excellent alternative to the classical phytochemical analysis when combined with spectroscopic identification for the analysis of crude extracts of plants belonging to a chemically known genus such as *Tabernaemontana*. A total of twenty-three known alkaloids, steroids and triterpenoids from several parts of *T. hilariana* were rapidly separated and easily identified without the need for long, tedious and complex isolation steps and spectrometric identification of the compounds. The procedure adopted in this paper limited the scope of the analysis to the less polar constituents. Concerning the indole alkaloids from green fruits and root barks, there were a few differences between the composition of the plants collected in 1984 from that collected in 1988.

Table 3
Triterpenoid and steroid contents from *Tabernaemontana hilariana*

Compounds	Ethanolic extracts	
	Root barks H1	Green fruits H1
Campesterol	+	−
Stigmasterol	+	+
Sitosterol	+	++
α-Amyrin	++	++
Taraxasterol	+	−
β-Amyrin	++	+
Lupeol	−	+
β-Amyrin acetate	++	++
α-Amyrin acetate	++	++
Lupeol acetate	+	++

−: Absent.
Relative abundance: +++: major component; ++: intermediary concentration; +: minor component.

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