SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY
SOUTH. BRAZ. J. CHEM., Vol. 7, N° 8, 1999

QUANTITATIVE DETERMINATION OF FURANOCOUMARINS AND IDENTIFICATION OTHER CHEMICAL CONSTITUENTS OF RHIZOMES AND LEAVES FROM *DORSTENIA TUBICINA* AND COMMERCIAL SAMPLES

CLAUDIA A. L. CARDOSO[1,*], WAGNER VILEGAS[2] AND NELI K. HONDA[3]

[1]Universidade Estadual de Mato Grosso do Sul, Rua: Pereira Gomes, 355, 79400-000, Coxim, MS, Brazil.
[2]Instituto de Química - UNESP, Departamento de Química Orgânica, Caixa Postal 355, 14800-900, Araraquara, SP, Brazil.
[3]Universidade Federal de Mato Grosso do Sul, Departamento de Química, Caixa Postal 549, 79070-900, Campo Grande, MS, Brazil.

ABSTRACT

The chemical composition of the hexane extracts from the rhizomes and leaves of Dorstenia tubicina Ruiz et Pavon (Moraceae) and four commercial samples of Dorstenia was investigated by high resolution gas chromatography-flame ionization detector (HRGC-FID) and high resolution gas chromatographic-mass spectrometry (HRGC-MS). This is a rapid and sensitive method for the quantification of furanocoumarins and identification isoprene derivatives (triterpenes, steroids and vitamins) from apolar crude extracts of Dorstenia species.

RESUMO

A composição química dos extratos hexânicos das rizomas e folhas de Dorstenia tubicina Ruiz et Pavon (Moraceae) e quatro amostras comerciais de Dorstenia foram investigadas por cromatografia gasosa de alta resolução com detector de ionização de chama (HRGC-FID) e cromatografia gasosa de alta resolução com detector de massas (HRGC-MS). Este é um método rápido e sensível para a quantificação de furanocumarinas e identificação derivados isoprênicos (triterpenos, esteróides e vitaminas) de extratos apolares de espécies de Dorstenia.

KEYWORDS: *Dorstenia tubicina*, furanocoumarins, gas chromatography-mass spectrometry, steroids, triterpenes, vitamins

DOI: 10.48141/SBJCHEM.v7.n8.1999.52_1999.pdf

INTRODUCTION

Preparations based on plants used in traditional medicine have been widely employed in Brazil as an alternative to pharmaceuticals. The cost of modern pharmaceuticals is probitive for the majority of the population[1].

The chemical investigation of medicinal plants often requires the examination of several related species of one genus. Traditional phytochemical procedures of isolation and identification can be very time-consuming and generaly require considerable amounts of plant material. In the case of closely related chemical species, however, classical phytochemical methods can be exchanged for an analytical approach, based on chromatographic analyses and identification of known compounds using standards and/or spectrometric data. The process of isolation and identification of minor compounds is often a very complex matter, because plants usually contain only one major class of compounds. To detect such minor compounds, there is a need for a rapid method for the detection of the substances in a complex mixture of other extractives. The genus *Dorstenia* (Moraceae) has 37 Brazilian species[2]. Most of them are known as "carapiá" or "figueirilha". Some of them are used in folk medicine, mainly for skin diseases and as antiophidics[3].

Furanocoumarins are the most abundant compounds in these plants. They have been used empirically for centuries in the treatment of depigmentations, specially in Egypt[4]. Derivatives of psoralen, such as bergapten, are used in therapeutic treatment of psoriasis (buvatherapia) and mycosis fungoides[5]. Some of these compounds can be related to the topical utilization of preparations containing *Dorstenia* rhizomes in the treatment of skin diseases such as psoriasis and vitiligo[6]. Furthermore, these compounds are responsible for the pleasant odour of these plants, that are mixed to pipe tobacco[7]. A number of methods has been described for the analysis of furanocoumarins, among them high performance liquid chromatography-ultraviolet (HPLC-UV) and gas chromatography coupled to a flame ionization detector (GC-FID)[6,8-10].

Besides furanocoumarins, terpenoids also occur in the *Dorstenia* genus[3,6]. It has also been suggested that the terpenoidal constituents might be related to the antiophidical activity of some *Dorstenia* species[11-12]. However, as most of terpenoids lack a chromophore group, their analysis by spectrophotometry poses a problem to the analysis of crude *Dorstenia* extracts.

GC-FID and high resolution gas chromatography - mass spectrometry (HRGC-MS) have proved to be a valuable tool for separation and identification of mixtures of apolar furanocoumarins and terpenoids[6]. Consequently, they are an useful alternative for the investigation either of new species of this genus or for the analysis of samples found in local commerce.

Dorstenia tubicina is an herbaceous plant used in the city of Campo Grande, Mato Grosso do Sul State, Brazil for the treatment of antiophidics and vitiligo. There are no phytochemical or pharmacological studies reported on *D. tubicina* in the literature.

In this work we report the identification of the components present in the hexane extracts of rhizomes and leaves of *D. tubicina* and in four commercial samples obtained in the local commerce of Campo Grande and quantification of furanocoumarins using GC-FID and HRGC-MS.

EXPERIMENTAL

Plant material. The plant *Dorstenia tubicina* Ruiz et Pavon was collected in Aquidauana, Mato Grosso do Sul, Brazil in 1996 and identified by Dr. Jose Pedro Carauta from Jardim Botânico do Rio de Janeiro, Brazil. A voucher specimen is kept in the Herbarium of the University.

The commercial samples were purchased in the local commerce in Campo Grande, MS. Standard substances were obtained from a collection of our laboratory.

Sample extraction. D. *tubicina* (rhizomes and leaves) and the four commercial samples were separatedly dried at 37°C for 1 day. A quantity of 1g of each sample was powdered and extracted with 30 mL hexane (maceration in sonic bath for 30 min). The solutions were filtrared with a filter paper. The solvents were evaporated under vacuum. One milligramas of each extract was redissolved with 1 mL of hexane, filtered with a Millex filter of 0.45 μm and directly analysed by HRGC-FID with standards and also by HRGC-MS. Samples were extracted in triplicate.

Extract characterization. GC analyses were performed with a VARIAN 3400 gas chromatograph equipped with a capillary fused sílica LM-5 (15m x 0.2 mm i.d., film thickness 0.5 μm) and with a flame ionization detector (FID). H_2 was used as carrier gas at a flow rate 0.8 mL/min and the injection split ratio was 1:20. The injection temperature was 280°C. Column temperature was programmed 150-240 °C, linear increase 10 °C/min, 240°-280 °C , linear increase 5 °C/min, held for 20 min. The detector temperature was 300 °C . Samples of 1 μL were injected with a 10 μL Hamilton syringe.

The HRGC-MS analysis were performed on an SHIMADZU QP 5000, with electron impact ionization (70eV), coupled to an SHIMADZU GC-17B gas chromatograph in the same column and temperature program described above. Helium was used as carrier gas at a flow rate 0.8 mL/min. The MS scan range was 45-550 u. Samples of 1 μL were injected with a 10 μL Hamilton syringe.

Determination of the GC-FID detection limit. The GC-FID detection limit was determined by injecting in triplicate 6 solutions of known concentrations (1.0 μL each injection), and lowering the concentration of the sample until the detection of a peak with twice the height of base noise line; the corresponding

concentration was considered being the minimal amount detectable by GC-FID for those substances.

Calibration curves. Estimation of the content of psoralen and bergapten in plant material was performed by external calibration. The compounds were dissolved separately in analytical grade chloroform in order to obtain the stock solutions which were appropriately diluted to concentrations ranging from 1-100 μg/mL of compounds. Aliquots (1.0 μL) for seven dilutions of each standard were analysed by GC-FID. Each determination was carried out in triplicate.

RESULTS AND DISCUSSION

In order to optimize the analyses for furanocoumarins and isoprene derivatives, several chromatographic runs were made by GC-FID under different conditions. At the conditions presented, furanocoumarins are eluted first (t_R among 4.0-7.0 min), the monoterpenic furanocoumarin DT is eluted with t_R 16-17 min and isoprene derivatives (triterpenes, steroids, vitamins) are eluted between 13.0-22.0 min (Fig.1-3,Table I).

The co-injection of authentic standards were also used to confirm the presence of psoralen, bergapten, α-sitosterol, stigmasterol, α- amyrin, β-amyrin, α- amyrin acetate, β-amyrin acetate, α-tocopherol, vitamin E. The presence of DT and isoprenoids (not fully identified) were deduced on the basis of matching with the NIST 62500 data bank (with 62235 compounds) and also by their MS-fragmentation pattern compared to the literature[6,13-18].

The calibration curve was linear in the range 10-100 (R=0,9999) μg/mL for psoralen and 5-90 μg/mL (R=0,9997)for bergapten. The limits of detection for psoralen 0.1 μg/mL and for bergapten 0.3 μg/mL.

Table II shows the contents of furanocoumarins in the *Dorstenia* species.

The HRGC-FID and HRGC-MS profile of the hexane extracts of *Dorstenia tubicina* showed some differences between the chemical composition of the rhizomes and leaves. The major compounds in the rhizomes are the furanocoumarins, while isoprene derivatives predominate in the leaves. Furanocoumarin DT is present only in trace amounts in the rhizomes. A common characteristic observed in the rhizomes and leaves was the presence of almost equal amounts of α- amyrin acetate and β-amyrin acetate. Sitosterol is present in significative amounts only in the leaves, where the major constituent is vitamin E. Some minor compounds were lost because of their noisy mass reports.

The chemical composition of *D. tubicina* herein examined showed to be similar to other *Dorstenia* species, in which the major compounds are the furanocoumarins[3, 6-7, 13-14].

Table I. Retention Times of the Components

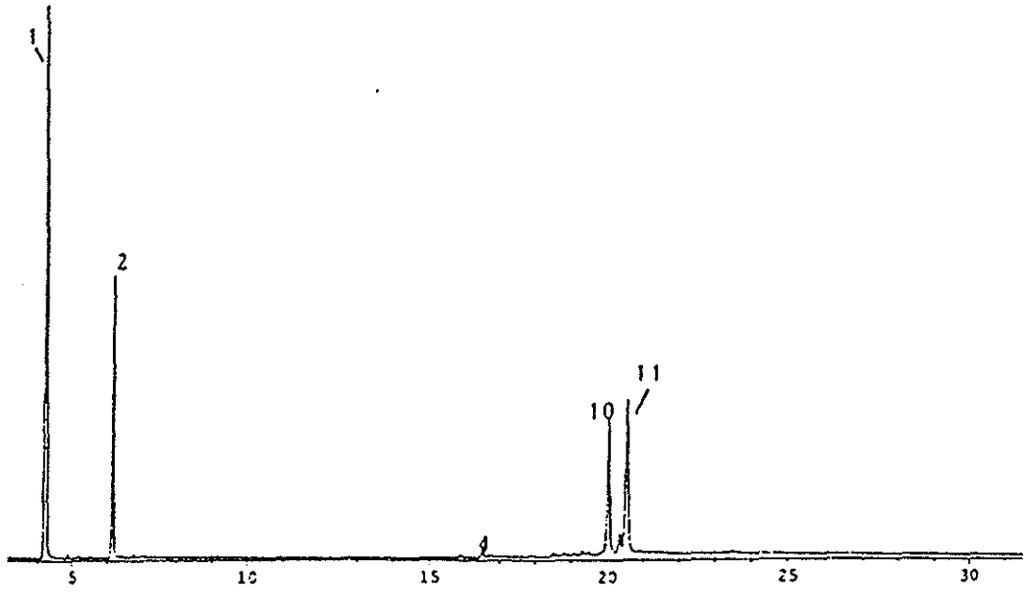
Peak	*Compound*	*MW*	t_r*(min)*
1	Psoralen	186	4.15
2	Bergapten	216	6.25
3	α-tocopherol	416	15.52
4	DT	368	16.55
5	Vitamin E	430	16.67
6	Stigmasterol	412	17.99
7	α-sitosterol	414	18.51
8	β-amyrin	426	18.75
9	α-amyrin	426	19.41
10	β-amyrin acetate	468	20.62
11	α-amyrin acetate	468	21.21

DT=5-[3-(4,5-Dihydro-5,5-dimethyl-4-oxo-2-furanyl)-butoxy]-7H-furo[3-2-g][1] benzopyran-7-one.

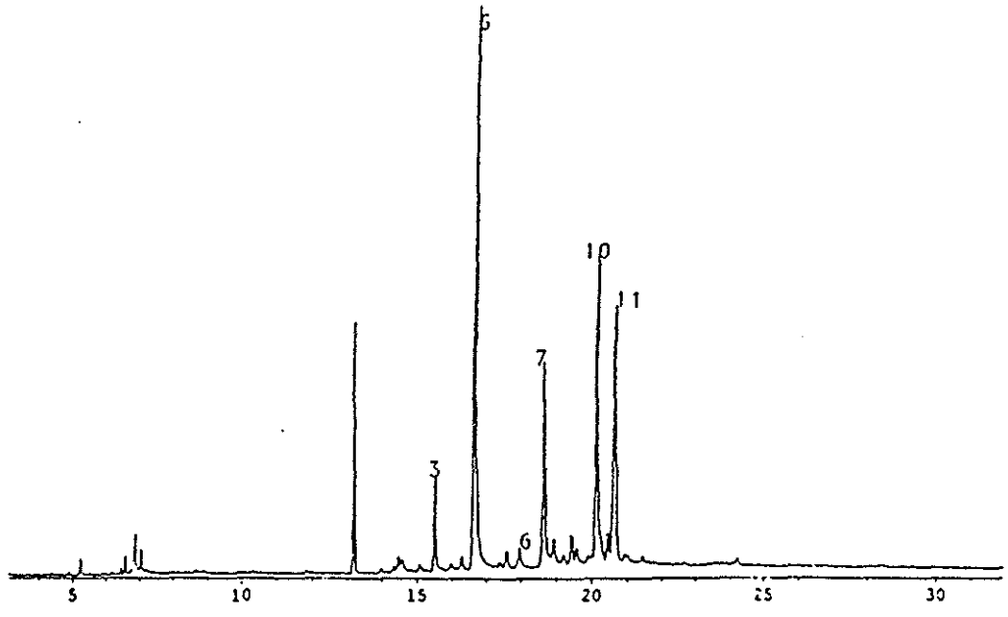
Table II. Contents (μg/g) of Furanocoumarins in *Dorstenia* Species (dry weight) and in Commercial Samples.

Peak	*D.tubicina Rhizomes*	*D.tubicina Leaves*	*Sample 1*	*Sample 2*	*Sample 3*	*Sample 4*
1	3650	-	3355	3590	1790	1335
2	1770	-	1205	1870	3495	-

Average of three determinations; standard deviation < 5%
(-) = Not detected

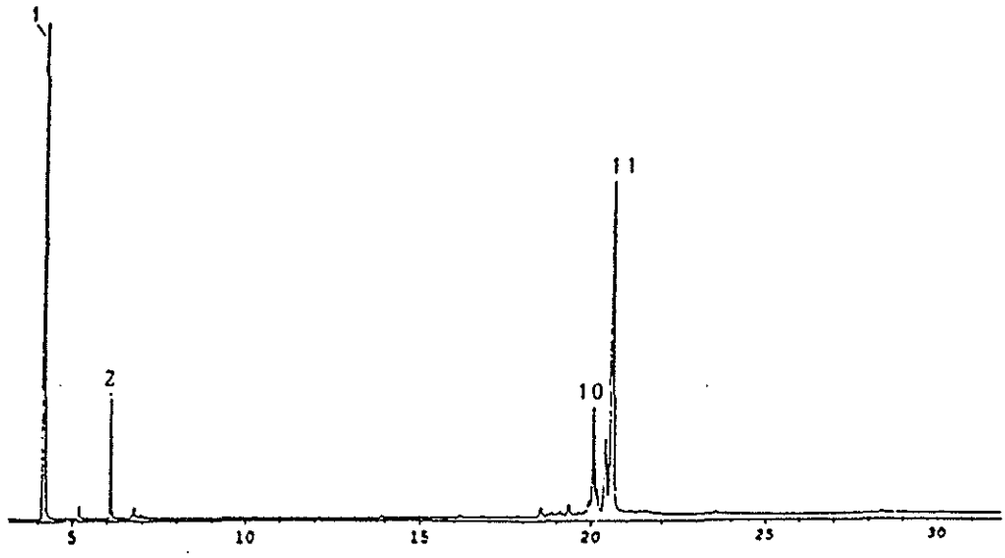


(a)
Time (min)

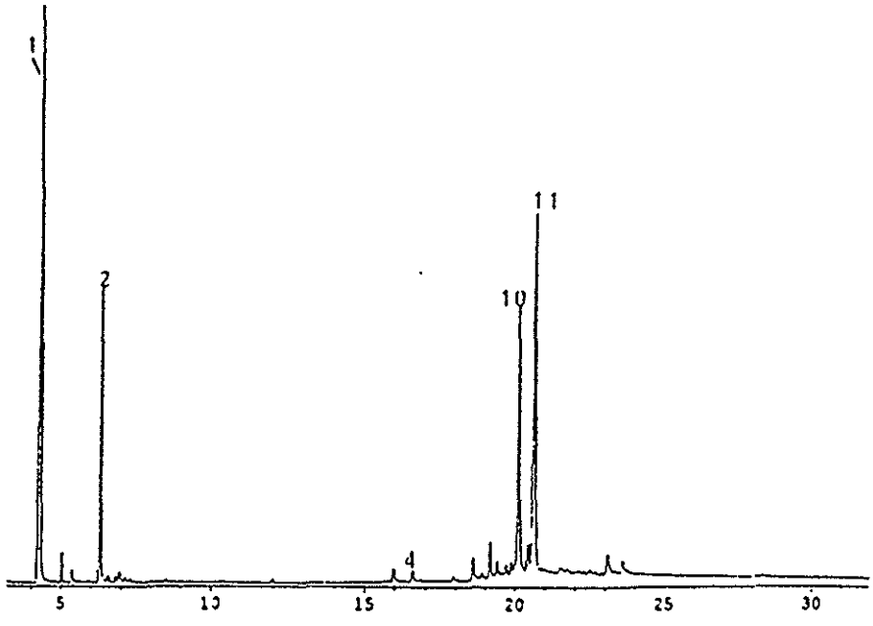


(b)
Time (min)

Figure 1. Gas Chromatographic (GC) analysis of hexane extract from *Dorstenia tubicina*. (a)rhizomes and (b) leaves.



(1)
Time (min)



(2)
Time (min)

Figure 2. Gas Chromatographic (GC) analysis of hexane extract from commercial samples (1 and 2).

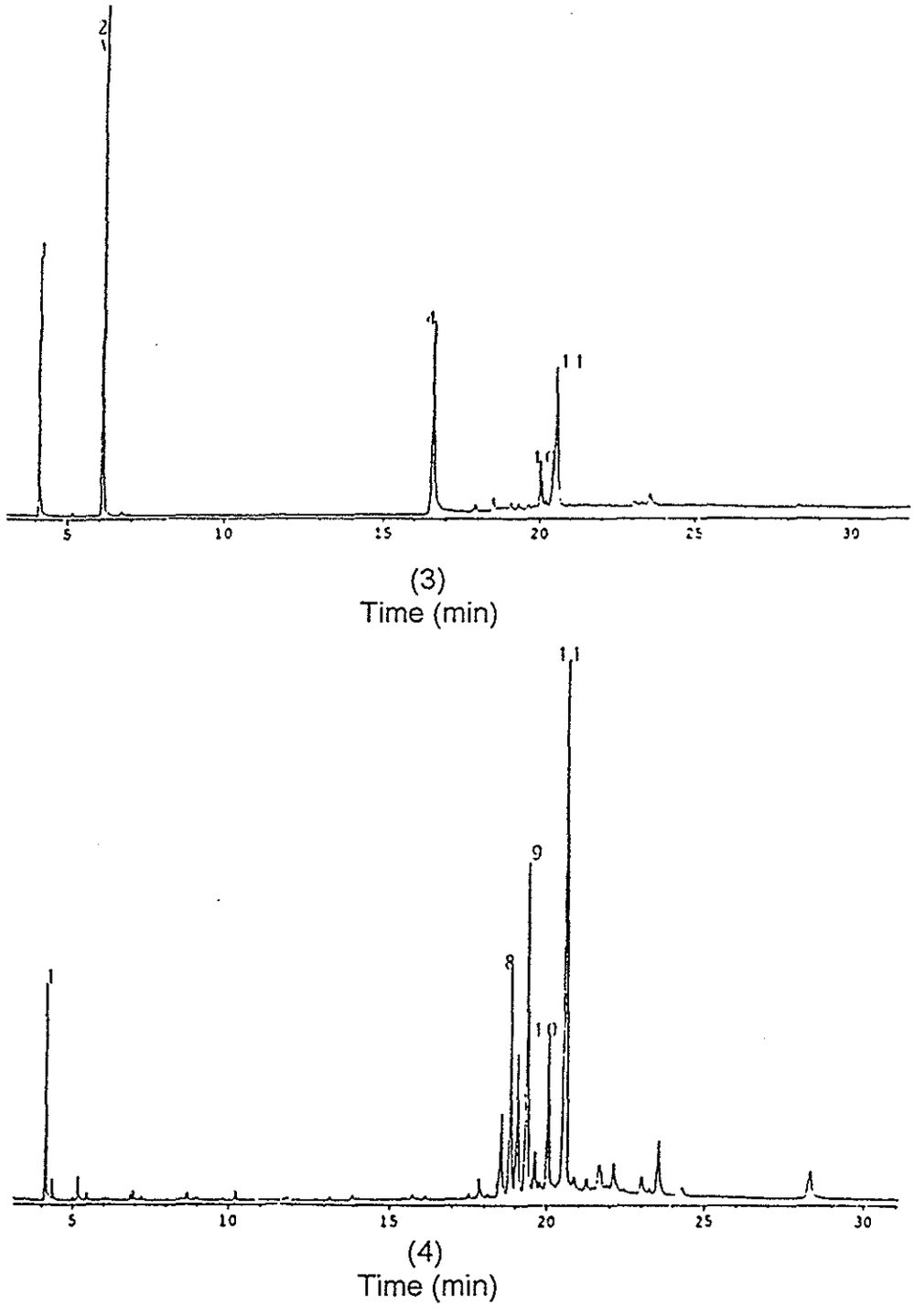


Figure 3. Gas chromatographic (GC) analysis of hexane extract from commercial samples (3 and 4).

The chromatographic pattern of the commercial samples 1 and 2 are similar to that of *D. tubicina*, with psoralen as the major compound. Sample 3 also presents these furanocoumarins, but bergapten predominates over psoralen. The monoterpenic furanocoumarin 4 was found only in samples 2 and 3. On the other hand, sample nr. 4 presented small amounts of psoralen and no bergapten, besides other isoprene derivatives in the range of 13.0-22.0 min, among which we could identify α- amyrin and β-amyrin. It is worth noting that only samples 2 and 3 were sold as the intact rhizome, while samples 1 and 4 were sold as a powder. Indeed, in sample 4 we could verify the presence of small parts of the rhizomes as well as of the leaves. Such type of addulteration is frequent and is done in order to increase the weight of the herb.

The sensitivity of the GC-FID and HRGC-MS for both furanocoumarins and isoprene derivatives allowed the fast chemical characterization of the constituents present in this yet uninvestigated *Dorstenia* specie without the necessity of isolating the individual known substances.

The chromatographic fingerprints of the authentic *Dorstenia tubicina* have permited the identification of the constituents from commercial samples and also the detection of possible adulterations.

ACKNOWLEDGMENTS

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for a fellowship to C.A.L.C. and the Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq) for a grant to W.V.

REFERENCES

1. L.C. Di Stasi, C. A. Hiruma, E. M. Guimarães and C. M. Santos, *Fitoterapia 6*, 529 (1994).
2. M. Pio Correa, "Dicionário de plantas úteis do Brasil a das exóticas cultivadas", Vol.2, Ministério da Agricultura, Rio de Janeiro, 1926, p.161.
3. W. Vilegas, J.H.Y Vilegas and G.L. Pozetti, *Rev. Cienc. Farm.14*, 133 (1994).
4. D. Chouchi and D. Barth, *J. Chromatogr. 672* , 177 (1994).
5. D. A. Averbeck and E. Moustacchi, *Phytochem. Phytobiol. 31*, 475 (1980).
6. J.H.Y. Vilegas, F.M. Lanças, W. Vilegas and G.L Pozetti, *Phytochemical Analysis 4* 230 (1993).
7. W. Vilegas, J.H.Y. Vilegas and G.L. Pozetti, *Rev. Latinoamer. Quim. 23,* 133 (1994).

8. F.R. Stermitz and R.D. Thomas, *J. Chromatogr. 77* , 431 (1973).
9. H.J. Thompson and S.A. Brown, *J. Chromatogr. 314* , 323 (1984).
10. T. Wawrzynowicz and M. Waksmundzka-Hajnos, *J. Liq. Chomatogr. 13*, 3925 (1990).
11. W. B. Mors, M.C. Nascimento, J.P. Parente, G. da Silva Melo, P.A. Suarez- Kurtz, *Toxicon.27*, 1003 (1989).
12. N.A. Pereira, B.M.R. Pereira, M.C. Nascimento, J.P. Parente and W.B. Mors, *Planta. Med.60*, 99 (1994).
13. R.M. Kuster, R.R. Bernardo, A.J.R. Silva, J.P. Parente and W.B. Mors *Phytochemistry 36* , 221 (1994).
14. J.H.Y. Vilegas, W. Vilegas, C.F. Camilot, and G.L. Pozetti, *Rev. Cienc. Farm. 12* 89 (1990).
15. S. Matsunaga, R. Tanaka and M. Akagi, *Phytochemistry 27*, 535 (1988).
16. R.C. Heupel, *Phytochemistry 24* ,2929 (1985).
17. M. Hirohara, Y. Yasuoka, Y. Arai, K. Shiojima, H. Ageta and H.C. Chang *Phytochemistry 45* , 1023 (1997).
18. H. Budzikiewicz, J.M. Wilson and C. Djerassi, *J. Am. Chem. Soc. 85*, 3688(1963).

The SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY (ISSN: 2674-6891; 0104-5431) is an open-access journal since 1993. Journal DOI: 10.48141/SBJCHEM.
http://www.sbjchem.com.
This text was introduced in this file in 2021 for compliance reasons.