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Comparative analysis of triterpenoids from *Mikania cordifolia* collected from four different locations

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The species Mikania cordifolia *is distributed across America and widely found throughout Brazilian territory, where is popularly used against snake bites. Methanolic and dichloromethanic extracts prepared from* M. cordifolia *Robinson collected from four different locations in Brazil were submitted to liquid-liquid extraction and the hexanic phase and residues obtained from this step were analyzed for triterpenoids by gas chromatography. The specimens from Ribeirão Preto-SP and São Carlos-SP showed similar triterpenoid composition: ζ-amyrin, lupeol, lupenone, ζ-amyrin acetate, η-amyrin acetate, lupeol acetate, taraxasterol acetate, campesterol and η-sitosterol. Besides these triterpenoids, the specimen from Campos de Jordão-SP presented 11-oxours-12-ene, 11-oxoolean-12-ene and taraxerol acetate, and from Monte Verde, epitaraxerol e taraxerol acetate. The triterpene friedelin could be found in specimens from Ribeirão Preto and São Carlos.*

Uniterms
- Asteraceae
- *Mikania*
- Triterpenoids

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INTRODUCTION

The species *Mikania cordifolia* Robinson (Asteraceae, Eupatorieae) is widely distributed in America and can be found throughout Brazilian territory. *M. cordifolia*, as well other *Mikania* species, is known popularly as "guaco" and has been used for treatment of respiratory problems (Caribe *et al*., 1991). *M. cordifolia* has an ancient use by rainforests inhabitants to treat snake bites and, due to this application, it has been called "snake vine" (Caribe *et al*., 1991; Mors *et al.,* 2000). Biological activities described for *M. cordifolia* include antitrichomonal, antitrypanosomal and insecticide activities (Arias *et al*., 1995; Serrano *et al*., 2000). The genus *Mikania* has been extensively studied and for *M. cordifolia,* it has been described the presence of diterpenes, sesquiterpenes and phenylpropanoids (Boeker *et al.*, 1987; Bohlmann *et al.*, 1978; Castro *et al.*,1989; D' Agostino *et al.*, 1990; Herz *et al.*, 1977).

In continuation to our study on chemistry of *Mikania* species (Chaves *et al.*, 2003; Fabbri *et al.*, 1997; Nascimento *et al.* 2004; Nascimento, Oliveira, 2001; Veneziani, Oliveira, 1999) and considering the importance of this genus in tradicional medicine, we have examined *M. cordifolia* extracts from four different locations in Brazil.

MATERIAL AND METHODS

Plant material

The specimens were collected from four different locations in Brazil (Table I) with two distinct vegetations and were identified by Dr. Roberto Lourenço Esteves (Departamento de Biologia Animal e Vegetal, Universidade Estadual do Rio de Janeiro, Brazil). Voucher specimens were deposited at the Herbarium Bradeanum (Universidade Estadual do Rio de Janeiro) and the Herbarium of FFCLRP (Universidade de São Paulo).

Extraction

Aerial parts of each specimen (600g) were dried, pulverized and macerated with methanol and dichloromethane. The resulting extracts were filtered, concentrated under reduced pressure and dissolved in methanol:water (9:1). These extracts were submitted to liquid-liquid extraction with *n*-hexane. The residue obtained after filtration (R) and the hexanic phase (HP) of each methanolic (ME) and dichloromethanic extract (DE) were submitted to a clean-up step.

Clean-up procedure

10 mg of residues (R) and the hexanic phases (HP) of each methanolic (ME) and dichloromethanic extract (DE) of each *M. cordifolia* specimen were percolated though a silica gel column (Alltec, silica gel 200 mg, 3 mL) with 10 mL of *n*-hexane and 10 mL of chloroform (Schinor *et al*., 2004).

Gas chromatography analysis

Chloroformic fractions were analyzed by high resolution gas chromatography (HRGC) on a Hewlett-Packard model 5890 Series II gas chromatograph with Flame Ionization Detector (FID) at 330 °C and a split injector (split ratio 1:50 at 260 °C). Two capillary columns were employed to perform the analysis: HP-1 (cross-linked methyl-silicone, 30 m x 0.25 mm x 0.25 σm) and HP-50 (cross-linked 50% phenyl-methyl-silicone 30 m x 0.25 mm x 0.25 σm). For HP-1, the temperature program was 250 °C held for 12 min, increased 6 °C/min to 280 °C, and held this temperature for 30 min and for HP-50, the program was initiated at 50 °C, increased 20 °C/min to 250 °C (held for 4 min), increased 15 °C/min to 280 °C (held for 18 min), increased 10 °C/min to 290 °C (held for 30 min). All the standard compounds used in these analyses were isolated from different plant material in our laboratory and identified by spectral data with the exception of campesterol, stigmasterol and η-sitosterol (Supelco Inc.). Cholesterol was used as reference standard (Schinor *et al*., 2004).

RESULTS AND DISCUSSION

The residues (R) and hexanic phases (HP) were obtained in proportions ranging from 38% to 60% for methanolic extracts (ME) and from 76% to 91% for dichloromethanic extracts (DE). These great amounts of lipid material in addition to innumerous biological activities described for triterpenoids, such as antitumor, anti-inflammatory and antioxidant (Moghadasian, 2000; Osvena *et al*., 2004; Saleen *et al*., 2004; Sultana *et al*., 2003), made the research of triterpenes and steroids relevant. The analysis of triterpenoids in *Mikania cordifolia* is essential because this group of substances may be related to neutralization of *Bothrops jararaca* venom (Mors *et al.,* 2000).

In this present work, the results of triterpenoids analysis summarized in Tables II, III, IV and V revealed that the different specimens of *M. cordifolia*, independently of collecting site, are rich in triterpenoids. These compounds were identified by comparison of relative retention value (considering cholesterol value 1.0) and campesterol, stigmasterol, η-sitosterol, η-amyrin, lupenone, lupeol, friedelin, pseudotaraxasterol, taraxasterol, η-amyrin

TABLE I - Collection data of *Mikania cordifolia* specimens

Location	Coletor Number*	Deposit Number**	Altitude (m)	Vegetation	Date
Ribeirão Preto - SP	NPL-259	SPFR-05313	595	Cerrado	27 May 2000
São Carlos - SP	NPL-261	SPFR-05315	854	Cerrado	27 May 2000
Campos de Jordão - SP	NPL-264	SPFR-05314	1030	Forest	29 May 2000
Monte Verde - MG	NPL-266	SPFR-05316	947	Forest	29 May 2000

*Herbarium Bradeanum (Universidade Estadual do Rio de Janeiro); **Herbarium of the Faculdade de Filosofia Ciências e Letras de Ribeirão Preto/Universidade de São Paulo

TABLE II -Triterpenoid composition of *M. cordifolia* specimen collected from Ribeirão Preto

Compounds	DE-R (%)	DE-HP (%)	ME-R (%)	ME-HP (%)
Campesterol	0.37	0.97	-	-
Stigmasterol	2.15	5.85	-	0.33
η-Sitosterol	2.21	4.55	0.85	1.07
Epitaraxerol	-	-	-	-
η-Amyrin	2.31	5.04	-	0.31
ζ-Amyrin/Lupenone	1.59	4.85	2.45	2.75
Lupeol	2.93	9.96	2.10	1.87
Taraxerol acetate	-	-	-	-
Friedelin	3.17	2.10	3.53	3.07
Pseudotaraxasterol	0.38	0.27	4.21	2.67
Taraxasterol	2.60	3.34	-	1.66
11-oxours-12-ene	-	-	-	-
11-oxoolean-12-ene	-	-	-	-
η-Amyrin acetate	8.24	7.36	5.70	5.00
ζ-Amyrin acetate	0.88	0.68	1.26	1.39
Lupeol acetate	15.72	16.38	8.93	7.51
Taraxasterol acetate	28.01	2.12	4.78	4.14

DE: dichloromethanic extract; ME: methanolic extract; HP: hexanic phase; R: residue; -: traces.

TABLE III -Triterpenoid composition of *M. cordifolia* specimen collected from São Carlos

Compounds	DE-R (%)	DE-HP (%)	ME-R (%)	ME-HP (%)
Campesterol	0.93	0.68	0,.47	0.25
Stigmasterol	1.68	4.97	3.06	6.66
η-Sitosterol	2.18	3.58	2.55	4.44
Epitaraxerol	-	-	-	-
η-Amyrin	2.18	3.72	3.60	5.93
ζ-Amyrin/Lupenone	0.83	2.01	1.69	2.48
Lupeol	2.95	5.87	4.64	8.42
Taraxerol acetate	-	-	-	-
Friedelin	1.43	5.03	2.10	-
Pseudotaraxasterol	1.30	1.79	0.83	1.00
Taraxasterol	3.03	4.44	4.09	4.25
11-oxours-12-ene	-	-	0.15	-
11-oxoolean-12-ene	-	-	-	-
η-Amyrin acetate	13.06	14.30	13.79	11.39
ζ-Amyrin acetate	1.26	1.62	1.80	1.90
Lupeol acetate	13.94	12.41	19.60	16.04
Taraxasterol acetate	22.54	17.92	25.65	10.79

DE: dichloromethanic extract; ME: methanolic extract; HP: hexanic phase; R: residue; -: traces.

TABLE IV -Triterpenoid composition of *M. cordifolia* specimen collected from Campos de Jordão

Compounds	DE-R (%)	DE-HP (%)	ME-R (%)	ME-HP (%)
Campesterol	0.87	0.32	0.41	0.27
Stigmasterol	2.47	2.96	3.36	2.57
η-Sitosterol	3.09	3.82	3.70	3.14
Epitaraxerol	-	-	-	-
η-Amyrin	5.66	7.95	10.45	10.35
ζ-Amyrin/Lupenone	1.63	2.61	2.19	3.02
Lupeol	2.49	4.62	2.55	4.48
Taraxerol acetate	-	0.47	0.42	0.79
Friedelin	-	-	-	-
Pseudotaraxasterol	-	0.62	-	0.45
Taraxasterol	2.57	2.30	4.63	3.04
11-oxours-12-ene	-	-	-	0.03
11-oxoolean-12-ene	-	-	-	0.03
η-Amyrin acetate	4.52	10.25	11.04	7.81
ζ-Amyrin acetate	0.31	1.71	1.91	1.00
Lupeol acetate	1.44	2.94	1.64	1.27
Taraxasterol acetate	15.68	20.67	23.18	15.72

DE: dichloromethanic extract; ME: methanolic extract; HP: hexanic phase; R: residue; -: traces.

TABLE V - Triterpenoid composition of *M. cordifolia* specimen collected from Monte Verde

Compounds	DE-R (%)	DE-HP (%)	ME-R (%)	ME-HP (%)
Campesterol	0.14	-	0.50	0.43
Stigmasterol	0.89	0.35	2.61	1.70
η-Sitosterol	2.47	-	6.96	4.01
Epitaraxerol	-	-	0.24	-
η-Amyrin	3.64	14.55	10.47	22.70
ζ-Amyrin/Lupenone	5.64	8.93	14.15	13.05
Lupeol	5.39	19.40	13.40	29.39
Taraxerol acetate	-	-	0.11	-
Friedelin	-	-	-	-
Pseudotaraxasterol	0.50		0.15	0.07
Taraxasterol	0.35	0.14	0.42	0.71
11-oxours-12-ene	-	-	-	-
11-oxoolean-12-ene	-	-	-	-
η-Amyrin acetate	19.09	18.84	11.56	0.85
ζ-Amyrin acetate	0.67	0.67	-	-
Lupeol acetate	15.71	6.62	16.79	1.05
Taraxasterol acetate	1.09	0.22	1.17	0.92

DE: dichloromethanic extract; ME: methanolic extract; HP: hexanic phase; R: residue; -: traces.

acetate, η-amyrin acetate and lupeol acetate were confirmed by co-injection of standards.

The steroids campesterol, stigmasterol, η-sitosterol, taraxasterol, pseudotaraxasterol and taraxasterol acetate were found in the extracts of all *M. cordifolia* specimens analysed. Epitaraxerol was just detected in Monte Verde specimen and taraxerol acetate in Monte Verde and Campos de Jordão specimens, both from forest vegetation.

Major triterpenoids found in the extracts are lupeol acetate, η-amyrin acetate and taraxasterol acetate (except for Monte Verde specimen). Friedelin was only observed

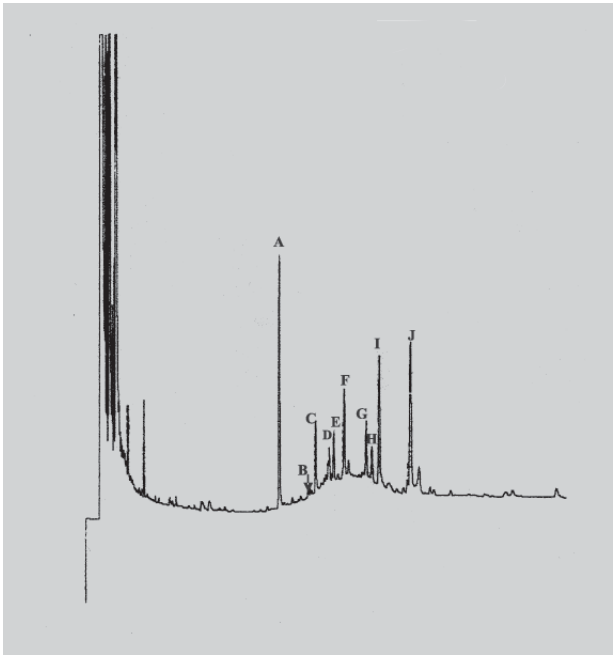

FIGURE 1 - GC chromatogram of triterpenoids from *M. cordifolia* from São Carlos– SP (DE-HP) using capilary colunm HP-1. (**A**) cholesterol (retention time: 14.182 min.); (**B**) campesterol; (**C**) stigmasterol; (**D**) η-sitosterol; (**E**) η-amyrin; (**F**) ζ-amyrin/lupeol; (**G**) η-amyrin acetate; (**H**) taraxasterol; (**I**) ζ-amyrin acetate/lupeol acetate; (**J**) taraxasterol acetate.

in specimens collected from São Carlos and Ribeirão Preto, where the vegetation is cerrado (Brazilian savanna).

The use of two different capillary colunms ruled out the possibility of peak coincidence (Figure 1 and 2), but the coincidence of ζ-amyrin and lupenone was excluded by analysis of the NMR ^{13}C spectral data (Olea, Roque, 1990), where it was just observed the lupenone presence.

CONCLUSIONS

The present work is the first study for triterpenoid chemistry in *M. cordifolia* collected from different locations and showed that there are no significant qualitative differences related to the presence of triterpenes and steroids. It can be concluded that all collected specimens of *M. cordifolia* presented similar constitution of triterpenoids, despite some possible differences in proportions.

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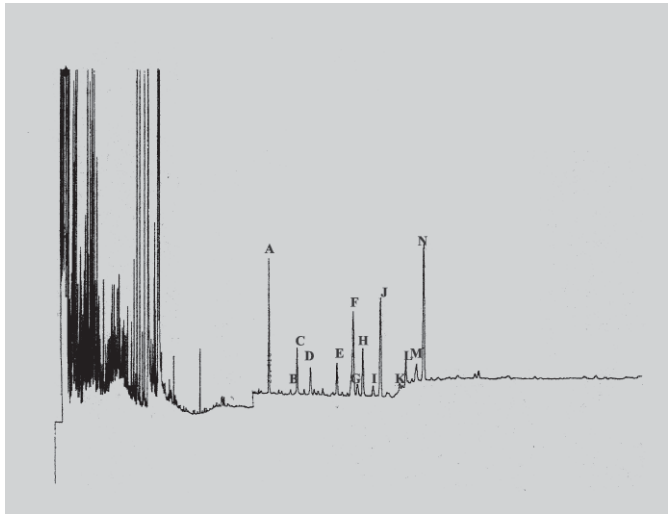

FIGURE 2 - GC chromatogram of triterpenoids from *M. cordifolia* from São Carlos– SP (DE-HP) using capilary colunm HP-50. (**A**) cholesterol (retention time: 14.182 min.); (**B**) campesterol; (**C**) stigmasterol; (**D**) η-sitosterol; (**E**) η-amyrin; (**F**) η-amyrin acetate; (**G**) ζ-amyrin/ lupenone; (**H**) lupeol; (**I**) ζ-amyrin acetate; (**J**) lupeol acetate; (**K**) pseudotaraxasterol; (**L**) taraxasterol; (**M**) friedelin; (**N**) taraxasterol acetate.

RESUMO

Análise comparativa de triterpenóides de *Mikania cordifolia* coletada em quatro locais diferentes

A espécie Mikania cordifolia *distribui-se por toda a América e é amplamente encontrada em quase todo o território brasileiro, onde é utilizada popularmente contra mordidas de serpentes. Extratos metanólicos e diclorometânicos preparados a partir e* M. cordifolia *Robinson coletadas em quatro locais diferentes do Brasil foram submetidos à extração líquido-líquido e os extratos hexânicos e resíduos obtidos nesta etapa foram analisados para a pesquisa de triterpenóides por cromatografia em fase gasosa. Os espécimes coletados em Ribeirão Preto-SP e São Carlos-SP apresentaram os triterpenóides η-amirina, lupeol, lupenona, acetato de ζ-amirina, acetato de η-amirina, acetato de lupeol, acetato de taraxasterol, campesterol e η-sitosterol na suas composições. Além destes trierpenóides, o espécime de Campos de Jordão-SP apresentou 11-oxours-12-eno, 11-oxoolean-12-eno e acetato de taraxerol e, o de Monte verde-MG, epitaraxerol e acetato de taraxerol. A*

friedelina foi observada apenas nas amostras de Ribeirão Preto-SP e São Carlos-SP.

UNITERMOS: *Asteraceae*. Mikania. *Triterpenóides*.

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