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Phytochemical study of *Mikania pseudohoffmanianna* G. M. Barroso ex W. C. Holmes

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This work describes the fractionation of methanol and dichloromethane extracts of aerial parts from the Mikania pseudohoffmanniana *G. M. Barroso ex W. C. Holmes. The phytochemical study of extracts led to isolation and the identification of 16 known compounds, including: steroids: campesterol, stigmasterol and β-sitosterol, diterpenes:* ent-*15β*-E-*cinnamoyloxy-kaur-16-en-19-oic acid,* ent-*15β*-Z-*cinnamoyloxy-kaur-16-en-19-oic acid and* ent-*kaur-16-en-19-oic acid, triterpenes: α-amyrin, β-amyrin, α-amyrin acetate, β-amyrin acetate, lupeol, lupeol acetate and friedelin, coumarin: scopoletin, flavonoid: quercetin and caffeoyl quinic acid derivative: 4,5-di-*O-*[E]-caffeoyl quinic acid.*

Uniterms
- *Mikania pseudohoffmanianna*
- Asteraceae
- Terpenoids
- Flavonoids
- Quinic acid derivative

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INTRODUCTION

The genus *Mikania* (family Asteraceae, tribe Eupatoriae, subtribe Mikaniinae) is widespread in Brazil, 150 of its 300 species occurring in this country (King, Robinson, 1987). Some species of the genus known as guaco are traditional medicine used against a variety of diseases (Holetz *et al.*, 2002).

Mikania pseudohoffmanniana G. M. Barroso ex W. Holmes is an endemic vine found in Brazil in Minas Gerais, Paraná and São Paulo. A great variety of constituents, the taxonomic complexity and biological applications justify the phytochemical study of species in the family (King; Robinson, 1987). We have been investigating the chemical composition of Brazilian guaco belonging to the genus *Mikania*. As part of this work we report here the isolation and structural determination of some constituents from the aerial parts of *Mikania pseudohoffmanniana*.

MATERIAL AND METHODS

General

The IR spectra were recorded on a Nicolet Protégé-460 spectrophotometer in the 4000-600 cm^{-1} range in the form of KBr pellets. ^{1}H (300 and 400 MHz) and ^{13}C NMR (75 and 100 MHz) spectra were obtained on a Bruker DPX 300 or 400 in $CDCl_3$, Methanol-d_4 or DMSO-d_6 with TMS as internal standard. ESI-MS and ESI-MS/MS spectra were obtained on a Micromass Quattro LC spectrometer.

GC analyses

HRGC analyses were performed on a Hewlett-Packard 5890 model Serie-II gas chromatograph equipped with a 30 m x 0,25 mm d. i., column coated (0,25 μm film thickness) with cross-linked poly-

methyseloxone (HP-1) and 30 m x 0,25 mm d. i., column coated (0,25 µm film thickness) with cross-linked 50% phenyl-methyseloxone (HP-50). Samples were introduced using the split mode (Split ratio 1:60) a 260 ºC. Hydrogen was used as carrier gas at an average linear velocity 42 cm/s at 250 ºC. Temperature FID was 300 ºC. For HP-50 the column temperature was 280 ºC (isotherm) and for HP-1 the column temperature program was 250 ºC held for 12 min, increased at 6 ºC/min to 280 ºC, and held this temperature for 20 min. The inject volume was 1 µL. Data were processed on a Hewlett-Packard 3395 model. The certified standard of sterols (stigmasterol, campesterol an β-sitosterol) employed in HRGC analysis were purchased from Supelco Inc., while the triterpenes (α-amyrin, β-amyrin, α-amyrin acetate, β-amyrin acetate, lupeol, lupeol acetate and friedelin) used as standards were isolated from different plant material in our laboratory and identified by spectral data of ^{1}H and ^{13}C NMR measurements.

Plant Material

Mikania pseudohoffmanniana G.M. Barroso ex W.C. Holmes was collected at Campos do Jordão-SP, Brazil, in May 2000, and was identified by Prof. Dr. Roberto Lourenço Esteves (Departamento de Biologia Animal e Vegetal da Universidade Federal do Rio de Janeiro). A voucher sample (NPL-269 and NPL-276) was deposited in the herbarium of the Departament of Biology, FFCLRP-USP and was used for the authentication of the species.

Extraction and fractionation

Dried and powdered whole *M. pseudohoffmanniana* plants (1.3 kg) were extracted exhaustively at room temperature with dichloromethane and methanol in successive phases. Solvents were evaporated under reduced pressure and furnished 22.0 g and 40.0 g, respectively. The dichloromethane extract was chromatographed over Si gel H eluting with hexane, and gradually increasing the polarity with EtOAc and MeOH. Thirteen fractions were collected and monitored by TLC. Fractions 1 (2.62 g) and 2 (7.59 g) were analyzed by GC and it was identified a mixture of stigmasterol, α-amyrin and its acetate, β-amyrin acetate, lupeol and its acetate fraction 1 furnished β-sitosterol and fraction 2 furnished β-amyrin and friedelin. The fraction 3 (5.15 g) was chromatographed over Si gel 60 eluting with hexane, and gradually increasing the polarity with EtOAc and MeOH. Fourteen fractions were collected and monitored by TLC. The fraction 3 (35.0 mg) was subjected to prep. TCL (silica-gel) eluted in dichloromethane:acetone (19:1) gave a mixture of 3.0 mg of *ent*-15β-*E*-cinnamoyloxy-kaur-16-en-19-oic and *ent*-15β-*Z*-cinnamoyloxy-kaur-16-en-19-oic acids. The fraction 5 (54.0 mg) was submitted to prep. TCL (silica gel) eluted in dichloromethane:acetone (7:3) gave *ent*-kaur-16-en-19-oic acid (2 mg). The crude methanolic extract underwent a partition by utilizing MeOH:H_2O (9:1), hexane, chloroform and buthanol. That procedure yields a precipitate (1.4 g) and the hexane (0.4 g), $CHCl_3$ (5.8 g) and buthanolic (10.5 g) fractions. The precipitate and hexane fraction were analyzed by GC and a mixture of stigmasterol, α-amyrin and its acetate, β-amyrin and its acetate, lupeol and its acetate were identified in both. The hexane fraction contained campesterol, friedelin and *ent*-kaur-16-en-19-oic acid. The chloroformic fraction was chromatographed over Si gel 60 eluting with hexane, and gradually increasing the polarity with EtOAc and MeOH. Thirteen fractions were collected and monitored by TLC. The fraction 7 (76.0 mg) was subject to prep. TCL (silica gel) eluted in dichloromethane:acetone (8:2) gave scopoletin (3.0 mg). A part of the buthanolic fraction (3.5 g) from the crude methanolic extract was chromatographed on a Sephadex LH – 20 column using methanol as eluent, and 10 fractions were collected. Fraction 6 gave 4,5-di-*O*-[*E*]-caffeoyl quinic acid (7.0 mg) and fraction 14 gave of quercetin (8.0 mg).

RESULTS AND DISCUSSION

Phytochemical study of *Mikania pseudohoffmanniana* led to possible the identification of 16 compounds. Triterpenes (α-amyrin, β-amyrin, α-amyrin acetate, β-amyrin acetate, lupeol, lupeol acetate and friedelin) and steroids (campesterol, stigmasterol and bð-sitosterol) are all described in literature and were identified by ^{1}H NMR data and HRGC. Identification of the steroids and triterpenes was carried out by comparison of relative retention times with those of references compounds. Acid diterpenes of kaurenes bulk (*ent*-15β-*E*-cinnamoyloxy-kaur-16-en-19-oic, *ent*-15β-*Z*-cinnamoyloxy-kaur-16-en-19-oic and *ent*-kaur-16-en-19-oic acids) were identified by IR, ^{1}H and ^{13}C NMR spectra and the data were compared with thoses reported in the literature (Silverstein *et al.*, 1994; VichnewskiI *et al.*, 1977; Reed *et al.*, 1993; Velandia *et al.*, 1998; Ohno *et al.*, 1979; Yamasaki *et al.*, 1976). Scopoletin coumarin was identified by IR, ^{1}H and ^{13}C NMR spectra and mass espectometry and the data were compared with the ones found in literature (Dean, *et al.*, 1967; Nascimento, Oliveira, 2001). The

derivative of caffeoyl quinic acid: 4,5-di-*O*-[*E*]-caffeoyl quinic acid and flavonoid: quercetin were identified by IR, ^{1}H and ^{13}C NMR spectra and mass espectometry and the data were compared with thoses reported in the literature (Pauli *et al.*, 1998; Merfort, 1992; Sánchez-Rabaneda, *et al.,* 2003; Santos, 2004; Harborne*, et al*., 1986; Fabre, *et al*.; 2001; Erlend, Dag, 2002).

These classes of substances identified and isolated from the species *Mikania pseudohoffmanniana* fit into the profile displayed by the *Mikania* genus. All these substances have been described in the literature and most are widely distributed in the Asteraceae family and they also present studies in relation to some biologic activities such as antimicrobial and trypanocidal ones (Alves, *et al*., 1995; Holetz, *et al*., 2002). The bronchodilatador activity of *Mikania* species is related to the presence of coumarin and the antimicrobial activity is attributed to the presence of diterpene of the kaurenes type (Moura, *et al*., 2001; Lentz, *et al*., 1998).

As to chemosystematics the chemical profile of *Mikania pseudohoffmanniana* presented in this work is similar to that of other Brazilian *Mikania* species. Since this is the first study of this plant that has ever been carried out this is a valuable contribution to the knowledge of its chemical profile.

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RESUMO

Estudo fitoquímico de *Mikania pseudohoffmanianna* G. M. Barroso ex W. C. Holmes

O fracionamento dos extratos diclorometânico e metanólico das partes aéreas de Mikania pseudo-hoffmanniana *G. M. Barroso ex W. C. Holmes resultou na identificação de 16 substâncias: os esteróides: campesterol, estigmasterol e* β*-sitosterol; os diterpenos: ácido ent-15*β*-E-cinamoiloxi-caur-16-en-19-óico, ácido ent-15*β*-Z-cinamoiloxi-caur-16-en-19-óico e ácido ent-caur-16-en-19-óico; os triterpenos:* α*-amirina,* β*-amirina, acetato de* α*-amirina, acetato de* β*-amirina, lupeol, acetato de lupeol e friedelina; a cumarina: escopoletina; o flavonóide: quercetina e o derivado do ácido cafeoilquínico: 4,5-di-*O-[E]*-cafeoilquínico.*

UNITERMOS: Mikania pseudohoffmanianna. Asteraceae. *Terpenóides. Flavonóide. Derivado do ácido quínico.*

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