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Chemical constituents of *Mikania glomerata* Spreng and *Mikania laevigata* Sch. Bip. ex Baker

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The species *Mikania glomerata* and *Mikania laevigata*, which are commonly known as guaco, are medicinal plant species widely employed for the treatment of respiratory diseases. In traditional medicine, both species have a long history of use. Currently, the medical use of these plants is widespread because pre-clinical studies have demonstrated their hypo-allergenic, antiasthmatic and antiulcerogenic properties. In recent decades, many studies have been conducted with the aim of isolating and identifying the metabolites of guaco from distinct extracts. Overall, making a correlation between a particular extract and its metabolic profile is difficult, because the reports from the literature are extensive and scattered. The present work provides an overview of the metabolic profiles of the guaco species, including extraction procedures, yields, analytical methods and the therapeutic potential of the main guaco metabolites. This review will contribute to the field by providing the historical context of the guaco species, which will be useful for guiding the design of new studies.

Key words: *Mikania glomerata*, *Mikania laevigata*, guaco, review, metabolites, coumarin.

INTRODUCTION

The species *Mikania glomerata* Sprengel and *Mikania laevigata* Schultz Bip. ex Baker, commonly known as guaco, are native plants of South America that are widely employed for the treatment of several inflammatory and allergic conditions (Moraes and Monteiro, 2006; Teske and Tretine, 1997; Duke et al., 2009). Both species grow in the same region and have similar morphological and chemical characteristics (Neves and Sá, 1991; Ceolin et al., 2006; Bastos et al., 2011; Oliveira et al., 1986; Lima and Biasi, 2002; Lima, 2003; Ritter and Miotto, 2005). One main difference between these species is in their flowering periods, which is September for *M. laevigata* and January for *M. glomerata* (Gasparetto et al., 2010; Napimoga and Yatsuda, 2010). Humans use these plants without distinction.

The guaco species have long been used by rainforest inhabitants, who have an ancient tradition of using guaco for snake bites, fevers, stomach discomfort and rheumatism (Pereira et al., 1994; Napimoga and Yatsuda, 2010). In folk medicine, guaco leaves have numerous uses because of their tonic, antipyretic, balsamic, antiophitic, stimulant, orexigenic, antispasmodic, expectorant, antimalarial and other properties (Coimbra, 1942; Lucas, 1942; Neves and Sá, 1991; Ruppelt et al., 1991; Galvani and Barreneche, 1994; Alice et al., 1995; Cortez et al., 1999; Matos, 2000; Gasparetto et al., 2010; Napimoga and Yatsuda, 2010).

Pre-clinical studies have also demonstrated that the guaco species can promote broncho-dilative, anti-ulcerogenic, hypo-allergenic, antispasmodic, anti-inflammatory, analgesic, antiophidian, antiparasitic and monoamine oxidase inhibitor effects (Oliveira et al., 1985; Ruppelt et al., 1991; Block et al., 1998; Fierro et al., 1999; Aboy et al., 2002; Soares de Moura et al., 2002; Suyenaga et al., 2002; Amaral et al., 2003; Bighetti et al., 2005; Luize et al., 2005; Maiorano et al., 2005; Santos et al., 2006; Graca et al., 2007; Freitas et al., 2008). Due to guaco's important effects, pharmaceutical preparations,

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including syrup and oral solutions, are freely distributed through various government phytotherapy programs and thus are widely used by the population (SES Rio de Janeiro, 1996; SES Campinas, 2001; Ogava et al., 2003; SES Cuiabá, 2004; Pires and Borella, 2004; Guimarães et al., 2006; Oliveira et al., 2006; Silva et al., 2006; Taufner et al., 2006; Brasil, 2006; 2007; 2008a).

Therapeutic properties have been exhibited in whole guaco plants, but the pharmacological effects of guaco are generally attributed to the leaves. Phytochemical screens that were conducted in whole plants revealed the presence of alcohols, acids, aldehydes, esters, organic esters, terpenes, diterpenes, triterpenes, steroids, and other metabolites; some of these metabolites were also associated with the therapeutic effects of guaco species (Gasparetto et al., 2010).

CHEMICAL CONSTITUENTS OF *M. GLOMERATA* AND *M. LAEVIGATA*

Numerous studies have been conducted to evaluate the chemical composition of *M. glomerata* and *M. laevigata*. Through different extraction procedures and conditions, a variety of compounds have been found in distinct parts of these plants. The details of the drug : solvent ratio, extraction procedures and metabolites found in each extract of guaco species are shown in Table 1.

High quantities of metallic elements in the dried leaves of the guaco species have been found through quantitative analyses using voltammetry and atomic absorption methods. Based on the dried weight of the leaves, the following elements were found: copper (1.75 mg%), iron (6.82 mg%), zinc (3.48 mg%), cadmium (2.1 mg%) and lead (0.43 mg%) (Andrade et al., 2005; Mamani et al., 2005).

Screens of the essential oils obtained from the leaves of the guaco species demonstrated the presence of numerous compounds, such as α-acorenol, α-cadinol, α-copaene, α-humulene, α-muurolol, α-pinene, α-terpinol, β-pinene, β-farnesene, β-bourbonene, β-cubebene, β-elemene, β-caryophyllene, γ-elemene, (E)-β-ocimene, (E)-nerolidol, *p*-cymene, α, β, γ and ∆ cardinene, α and TAU-caudynol, epi-α-bisabolol, epi-α-muurolol, aromadendrene, bicyclogermacrene, caryophyllene oxid, citronellyl acetate, coumarin, cubebene, elemol, germacrene-B, germacrene-D, globulol, limonene, linalol, myrcene, nerolidol E, nonanal, sabinene, silvestrene, spathulenol, terpin 4-ol, *trans*-ocymene, *trans*-cariophyllene and 1,4-dimethoxybenzene (Radunz, 2004; Duarte et al., 2005; Rehder et al., 2006).

In hexanic and dichloromethane extracts, the most prevalent metabolites are the following: coumarin, *o*-coumaric acid, campesterol, terpenes, stigmasterol, lupeol, lupeol acetate, germacrene, β-sitosterol and peroxides (Oliveira et al., 1984; Vilegas et al., 1997a, b; Veneziani et al., 1999; Cabral et al., 2001; Schenkel et al., 2002; Contini et al., 2006). Analyses conducted using ethanolic extracts have mainly detected the presence of coumarin, *o*-coumaric acid, kaurenoic acid, benzoyl and cinnamoylgrandifloric acids (Cabral et al., 2001; Bertolucci et al., 2008; Bolina et al., 2009).

Of the preparations obtained using guaco, the aqueous leaf extracts are of interest, because they have been used as part of an ancient tradition in folk medicine (Napimoga and Yatsuda, 2010). Currently, the aqueous extract is widely used as a home remedy. However, studies evaluating its metabolic profile are scarce. Some studies have reported the presence of a few compounds, including coumarin, *o*-coumaric acid and syringaldehyde, in teas obtained through infusion and maceration (Cabral et al., 2001; Celeghini et al., 2001; Maiorano et al., 2005; Muceneeki et al., 2009).

The hydroalcoholic extracts are the most common preparations to be commercialized for therapeutic purposes; the majority of phytochemical assays have been conducted using this type of extract. Numerous compounds have been described, including the following: 1-ethoxy-1-phenylethanol, 2-5-cyclohexadiene-1,4-dione,2,6-bis, stigmasterol, 1-octadecene, 4-hydroxy-3,5-dimethoxybenzaldehyde, phytol, hexanoic acid, 8,11-octadecadienoic acid, 9,12,15-octadecatrienoic acid, 10,13-octadecadienoic acid, benzoylgrandifloric acid, caryophyllene oxide, cinnamoylgrandifloric acid, cupressenic acid, ethyl hexadecanoate, ethyl linoleoate, hexadecanoic acid, isobutiloxigrandifloric acid, isopropiloxigrandifloric acid, kaurenol, lupeol, lupeol acetate, octadecanoic acid, spathulenol and *trans*-caryophyllene (Oliveira et al., 1993; Biavatti et al., 2004; Santos, 2005; Yatsuda et al., 2005; Bertolucci et al., 2008; Alves et al., 2009; Bolina et al., 2009; Muceneeki et al., 2009).

Based on the quantitative studies using hydroalcoholic extracts, the most prevalent metabolites are also associated with the therapeutic effects of guaco, including coumarin (1,2-benzopyrone) (Biavatti et al., 2004; Bueno et al., 2009), *o*-coumaric acid (Santos, 2005), dihydrocoumarin (Alves et al., 2009), syringaldehyde (Muceneeki et al., 2009) and kaurenoic acid (Vilegas et al., 1997a, b; Yatsuda et al., 2005; Bertolucci et al., 2008). The chemical structures of these compounds are as shown in Figure 1.

THERAPEUTIC EFFECTS ASSOCIATED WITH THE MAIN GUACO METABOLITES

Several studies have evaluated the therapeutic potential of the main guaco metabolites. Pre-clinical studies have demonstrated numerous relevant properties of these substances that justify the therapeutic uses of the guaco species (Gasparetto et al., 2012).

In a mouse model of allergy-induced pneumonitis, a reduction in the influx of total leukocytes and eosinophils

Table 1. Details of the sample preparation, extraction procedures and metabolites found in each extract of guaco species

Sample	Medicinal species	Drug : Solvent ratio (*w/v*)	Extraction procedure	Analytical method	Compound	Amount	Reference
Aqueous extract	*M. glomerata*	1:12	Reflux and maceration of fresh aerial parts with water or 1% NaOH	HPLC-DAD	Coumarin	Aqueous extract: 2.3% by refluxing and 1.69% by maceration; Basic aqueous solution: 2.4% by refluxing and 2% by maceration	Cabral et al. (2001)
Aqueous extract	*M. glomerata*	1:10	Infusion of dried leaves	HPLC-DAD	Coumarin	393.8 µg/ml	Celeghini et al. (2001)
Aqueous extract	*M. glomerata*	2:10	Infusion and maceration of dried or fresh leaves, roots and stems	TLC and HPLC-DAD	Coumarin and non-polar compounds	Qualitative analysis	Maiorano et al. (2005)
Aqueous extract	*M. glomerata* and *M. laevigata*	1:100	Infusion of leaves from different regions	HPLC-DAD	Coumarin and *o*-coumaric acid	*M. glomerata*: 45 µg/ml of coumarin and 35 µg/ml of *o*-coumaric acid. *M. laevigata*: 20.6 to 34.6 µg/ml of coumarin and 13.0 to 33.3 µg/ml of *o*-coumaric acid	Santos (2005)
Aqueous extract	*M. laevigata*	1:10	Decoction and maceration (microwave) of leaves	HPLC-DAD	Coumarin, *o*-coumaric acid and syringaldehyde	Decoction: 29.9 µg/ml of coumarin, 15.0 µg/ml of *o*-coumaric acid and 1.5 µg/ml of syringaldehyde. Microwave: 27.5 µg/ml of coumarin, 14.0 µg/ml of *o*-coumaric acid and 1.3 µg/ml of syringaldehyde	Muceneeki et al. (2009)
Hydroalcoholic extract	*M. glomerata*	1:1	Percolation of leaves. Hexanic, ethanolic, chloroformic and ethyl acetate fractions were obtained	TLC	Kaurenoic acid, cinnamoylgrandifloric acid, stigmasterol and coumarin	Qualitative analysis	Oliveira et al. (1993)
Hydroalcoholic extract	*M. glomerata*	1:10	Maceration by sonication of dried leaves and stems	HPLC-DAD	Coumarin	Stems: 0.59 to 1.16 mg/g; Leaves: 4.05 to 7.74 mg/g	Pereira et al. (1998)
Hydroalcoholic extract	*M. glomerata*	1:10	Maceration by sonication and percolation of leaves	HPLC-DAD	Coumarin	Maceration: 15.2 to 656.1 µg/ml; Percolation: 17.3 to 562 µg/ml	Celeghini et al. (1999)
Hydroalcoholic extract	*M. glomerata*	1.5:10	Reflux and percolation of dried leaves	HPLC-DAD	Coumarin	Ethanol 50%: 780.0 µg/ml by percolation and 630.0 µg/ml by reflux; Ethanol 96%: 720.0 µg/ml by percolation and 570.0 µg/ml by reflux	Aboy et al. (2000)
Hydroalcoholic extract	*M. glomerata*	1:10	Maceration, maceration by sonication and supercritical fluid of leaves	TLC and HPLC-DAD	Coumarin	Maceration: 696.4 µg/ml; Ultra-sound maceration: 656.2 µg/ml; Supercritical fluid. The extracts were not analyzed using HPLC because they presented a high content of chlorophyll.	Celeghini et al. (2001)
Hydroalcoholic extract	*M. glomerata*	Tincture: 2:10 and fluid extract: 1:1	Percolation of powder from leaves	First derivative spectrophotometry	Coumarin	3790.0 µg/ml by fluid extract and 1100.0 µg/ml by tincture	Osorio (2004)

Table 1. Contd.

Hydroalcoholic extract	*M. glomerata*	Not specified	Maceration of fresh leaves. The final extract was fractionated in dichloromethane, and the two phases (aqueous and organic) were evaluated.	GC-FID and GC-MS	Coumarin, *o*-coumaric acid, dihydrocoumarin, phytol, hexanoic acid, ethyl hexadecanoate, dimethoxybenzaldehyde, ethyl linoleoate, kaurenol, kaurenoic acid and isomers	GC-FID: Coumarin 11.4% *w/w;* GC-MS: Qualitative analysis	Soares de Moura et al. (2002)
Hydroalcoholic extract	*M. laevigata*	1:1 and 1:10	Maceration and percolation of dried leaves	HPLC-DAD	Coumarin	Percolation: 1:1 *w/v*: 1010.0 µg/ml by ethanol 36%; 1580.0 µg/ml by ethanol 60%; 1770.0 µg/ml by ethanol 70%. Percolation: 1:10 *w/v*: 270.0 µg/ml. Maceration (7 days, ethanol 70%, 1:1 *w/v*): 1480.0 µg/ml. Maceration (50 °C, ethanol 70%, 1:1 *w/v*): 2450.0 µg/ml	Biavatti et al. (2004)
Hydroalcoholic extract	*M. glomerata and M. laevigata*	1:2	Percolation of leaves from different regions	TLC and HPLC-DAD	Coumarin, *o*-coumaric acid	Coumarin: 840.0 to 1580.0 µg/ml; *o*-coumaric acid: 570.0 to 1730.0 µg/ml.	Santos (2005)
Hydroalcoholic extract	*M. laevigata*	0.1:25	Reflux/powder from leaves	TLC	Coumarin, *o*-coumaric acid	Official method for the identification of coumarin and *o*-coumaric acid	Brasil (2005)
Hydroalcoholic extract	*M. laevigata*	0.1:25	Reflux/powder from leaves	HPLC-DAD	Coumarin	Official assay	Brasil (2005)
Hydroalcoholic extract	*M. glomerata*	1:1	Percolation of irradiated aerial parts and powder from aerial parts	HPLC-DAD	Coumarin and o-coumaric acid	The content of coumarin increased with gamma ray irradiation (3.5 and 5.0 KGy; by Celsius-137 source), while the content of o-coumaric acid decreased.	Peregrino and Leitão (2005)
Hydroalcoholic extract	*M. laevigata*	2:30	Maceration of dried leaves and powder from leaves	TLC and GC-MS	Coumarins, terpenes and organic acids	Qualitative analysis	Bighetti et al. (2005)
Hydroalcoholic extract	*M. laevigata*	1:2	Maceration of dried leaves. The extract was partitioned using water/CHCL3	GC-MS	Coumarin	3.83% (relative percentage)	Graca et al. (2007)

Table 1. Contd.

Hydroalcoholic extract	*M. glomerata and M. laevigata*	1:5	Maceration of powder from dried leaves. The extract was concentrated under reduced pressure and lyophilized. Hexane fractions were obtained, and the residue was used to prepare ethyl acetate fractions.	TLC and GC-MS	Coumarin[1], dihydrocoumarin[2], spathulenol[3], hexadecanoic acid[4], cupressenic acid[5], kaurenol[6], Kaurenoic acid[7], isopropiloxigrandifloric acid[8], 2-5-ciclohexadiene-1,4-dione,2,6-bis9, 1-octadecene[10], octadecanoic acid[11], diterpenic acid[12], caryophyllene oxide[13], 10,13-octadecadienoic acid[14], 9,12 -octadecadienoic acid[15], ester diterpenic[16], isobutiloxigrandifloric acid[17], and *trans*-cariofileno[18]	Relative percentage: 1 (1.43 to 40.08); 2 (1.75 to 1.93); 3 (2.50 to 6.17); 4 (5.06 to 12.17); 5 (1.83 to 27.99); 6 (1.83 to 4.46); 7 (4.92 to 52.47); 8 (0.45 to 3.75); 9 (3.68); 10 (35.65); 11 (3.78); 12 (0.65 to 22.60); 13 (2.84); 14 (6.99); 15 (2.29 to 10.28); 16 (2.99); 17 (0.4 to 0.59) 18 (2.44)	Yatsuda et al. (2005)
Hydroalcoholic extract	*M. glomerata*	0.5: 20	Maceration of dried powder from leaves	GC-FID	Coumarin	1330.0 µg/ml	Bueno et al. (2009)
Hydroalcoholic extract	*M. glomerata*	1:5	Percolation of dried leaves and tinctures from local markets	TCL and HPLC-DAD	Coumarin	Leaves: not detected to 0.71%; Tinctures 0.072% to 0.176%	Alvarenga et al. (2009)
Hydroalcoholic extract	*M. glomerata*	1:10	Maceration of fresh or dried leaves. Several different proportions of water/ethanol were tested using dried leaves to optimize the process of extraction	HPLC-DAD	Coumarin	Fresh and dried leaves: 690 µg/ml. Leaves dried in a stove: 900 µg/ml. Different proportions of ethanol: 0%: 150 µg/ml; 10%: 210 µg/ml; 20%: 280 µg/ml; 30%: 230 µg/ml; 40%: 230 µg/ml; 50%: 260 µg/ml; 60%: 310 µg/ml; 70%: 470 µg/ml; 80%: 180 µg/ml; 90%: 40 µg/ml; 94%: 50 µg/ml	Rocha et al. (2008)
Hydroalcoholic extract	*M. laevigata*	Not specified	Percolation of dried leaves. A degradation study was conducted by diluting the extract in 1 M HCL, 1% H2O2, 1 M NaOH, water, and heating the extract under reflux for 12 h	HPLC-DAD	Coumarin, o-coumaric acid and syringaldehyde	Hydroalcoholic extract: coumarin: 13.0 µg/ml; o-coumaric acid: 8.6 µg/ml, syringaldehyde: 1.3 µg/ml. Stress study: coumarin was stable in all conditions; o-coumaric acid was 100% degraded in acid media; syringaldehyde was 51% degraded at high temperature and 100% degraded in oxidative stress conditions	Muceneeki et al. (2009)

Table 1. Contd.

Hydroalcoholic extract	*M. laevigata*	Not specified	Maceration of dried leaves	GC-MS	Dihydrocoumarin, coumarin, spathylenol, phytol, ent-beyer-15-en-19-oic-acid, lupeol and lupeol acetate	Relative percentage: dihydrocoumarin (32.3%), coumarin (36.92%), spathylenol (4.48%), phytol (2.4%), ent-beyer-15-en-19-oic-acid (3.32%), lupeol (5.91%) and lupeol acetate (3.34%)	Alves et al. (2009)
Ethanolic extract	*M. glomerata*	1:12	Reflux and reflux at room temperature of aerial parts (dried or fresh) collected from different regions	HPLC-DAD	Coumarin	Dried aerial parts: Reflux: 0.09 to 1.59%; Reflux at room temperature: 0.03 to 1.35%; Fresh aerial parts: Reflux: 1.9 to 2.7%; Reflux at room temperature: 1.9%	Cabral et al. (2001)
Ethanolic extract	*M. glomerata and M. laevigata*	1:60	Maceration by sonication of dried powder from leaves	HPLC-DAD	Coumarin (COU), o-coumaric acid (OCA), kaurenoic acid (KAU), benzoylgrandifloric acid (BA) and cinnamoylgrandifloric acid (CA)	*M. glomerata*: COU: not detected; OCA: not detected; BA: 0.14 to 0.17%; CA: 0.05 to 0.06%; KAU: 0.65 to 0.85%. *M. laevigata*: COU: 0.28 to 0.56%; OCA: <0.045%; BA: 0.29 to 0.41%; CA: 0.17 to 0.26%; KAU: 0.30 to 0.48%	Bertolucci et al. (2008)
Ethanolic extract	*M. glomerata and M. laevigata*	0.1: 25	Percolation (qualitative analysis) and reflux (quantitative analysis) of dried leaves	TLC and HPLC	Coumarin, steroids, triterpenes and flavonic heterosides	Qualitative analysis: coumarin, steroids, triterpenes and flavonic heterosides; Reflux: coumarin: 0.3 to 0.43%	Bolina et al. (2009)
Methanolic extract	*M. glomerata*	Not specified	Maceration of leaves	HPLC-DAD	Coumarin	0.28 to 0.51% (w/w)	Radunz (2004)
Hexanic extract	*M. glomerata and M. laevigata*	1:7.5	Percolation of dried powder from aerial parts	Melting point, NMR, TLC, IR and MS	Coumarin, kaurenoic acid, cinnamoylgrandifloric acid and stigmaterol	Qualitative analysis	Oliveira et al. (1984)
Hexanic extract	*M. glomerata*	1:10	Maceration of powder from dried leaves	GC-FID	Coumarin and kaurenoic acid	Calculated based on dry weight: coumarin 4.4 mg/g; kaurenoic acid: 2.0 mg/g	Vilegas et al. (1997a)
Hexanic extract	*M. glomerata*	1:10 and 5:200	Maceration (A), maceration by sonication (B), soxhlet (C), CO2 supercritical state (D) and hexane by supercritical state (E) of dried leaves	GC-FID and GC-MS	GC-FID: kaurenoic acid and coumarin(quantitative); GC-MS: coumarin, lupeol, kaurenoic acid, kaurene diterpene, sesquiterpenes, lupeol acetate, 11-methylbutanoic acid and germacrene (qualitative)	Coumarin (dry weight basis); A: 4.5 mg/g; B: 2.5 mg/g; C: Not detected; D: 0.3 mg/g; E: 5.0 mg/g; Kaurenoic acid (dry weight basis) A: 1.9 mg/g; B: 2.5 mg/g; C: 1.9 mg/g; D: Not detected; E: 2.0 mg/g	Vilegas et al. (1997b)

Table 1. Contd.

Hexanic extract	*M. glomerata*	Not specified	Maceration of powder from dried branches and leaves	TLC	Branches:friedelin, ent-kaur-16(17)-en-19-oic acid, ent-15β-benzoyloxy kaur-16(17)-en-19-oic acid, grandiforic acid, 17-hydroxy-ent-kaur-15(16)-en 19-oic acid; Leaves: coumarin, o-coumaric acid, stigmasterol, β-si tosterol, ent-15b-isobutyryloxykaur-16(17)-en-19-oic	Qualitative analysis	Veneziani et al. (1999)
Hexanic extract	*M. glomerata*	1:12	Reflux and reflux at room temperature of dried aerial parts from different regions	HPLC-DAD	Coumarin	Reflux: 0.07%; Reflux at room temperature: 0.02%	Cabral et al. (2001)
Hexanic extract	*M. glomerata*	1:10	Maceration of leaves cultivated by cutting or micropropagation	GC-FID	Coumarin and kaurenoic acid	Qualitative analysis	Contini et al. (2006)
Dichloromethanic extract	*M. glomerata*	Not specified	Maceration by sonication of powder from lyophilized cells	TLC, CLAE preparative, NMR, CG-FID	Campesterol, stigmasterol, β-sitosterol and coumarin	Qualitative analysis	Santos et al. (1999)
Dichloromethanic and chloroformic extracts	*M. laevigata*	1:1 or 1:2	Maceration of fresh and crushed material	TLC	Peroxides	Weak positive reaction	Schenkel et al. (2002)
Syrup	*M. glomerata*	-	Liquid-liquid with chloroform	UV-VIS	Coumarin	74.2 µg/ml	Silva et al. (2008)
Syrup	*M. glomerata*	-	Liquid-liquid with dichloromethane	HPLC-DAD	Coumarin	26.2 to 52.4 µg/ml	Rocha et al. (2008)
Syrup	*M. glomerata*	-	Liquid-liquid with ethyl acetate	GC-FID	Coumarin	143.0 µg/ml	Bueno et al. (2009)
Syrup	*M. glomerata*	-	Samples were direclty diluted in methanol /water (80:20 v/v)	UV-VIS	Coumarin	1280.0 to 1400.0 µg/ml	Amaral et al. (2009)
Syrup and oral solution	*M. glomerata* alone or associated with other plant extracts	-	Samples were directly diluted in a 1:1 v/v of ultrapure water and then in a 60:40 v/v acetonitrile/water solution	HPLC-MS	Coumarin, dihydrocoumarin, syringaldehyde, kaurenoic acid and o-coumaric acid	Coumarin: 2.3 to 280.4 µg/ml; o-coumaric acid: 0.01 to 22.2 µg/ml; kaurenoic acid: 3.1 to 130.0 µg/ml; dihydrocoumarin: 0.03 to 1.5 µg/ml and syringaldehyde: 0.02 to 1.2 µg/ml	Gasparetto et al. (2011a)
Syrup and oral solution	*M. glomerata* alone or associated with other plant extracts	-	Samples were directly diluted to a 1:1 v/v in a 65:30:5 v/v/v water/methanol/acetonitrile solution	HPLC-DAD	Coumarin, dihydrocoumarin, syringaldehyde and o-coumaric acid	Coumarin: 2.3 to 281.0 µg/ml; o-coumaric acid: traces to 23.7 µg/ml; dihydrocoumarin: not detected to 1.5 µg/ml; and syringaldehyde: not detected to 1.2 µg/ml	Gasparetto et al. (2011b)

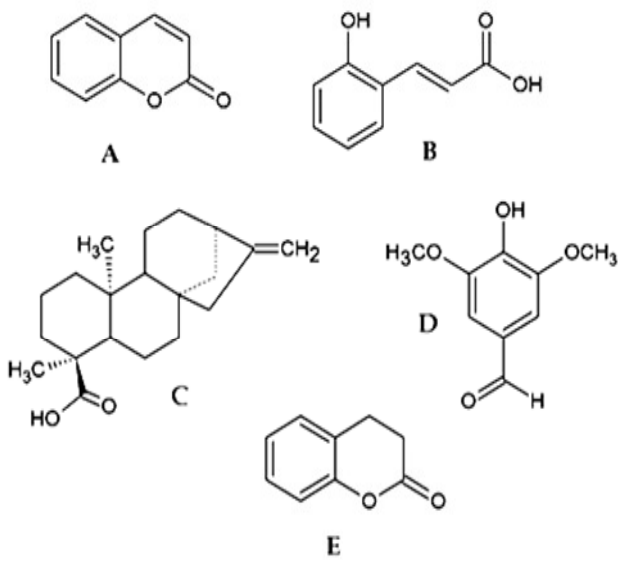

Figure 1. Chemical structures of (A) coumarin, (B) *o*-coumaric acid, (C) kaurenoic acid, (D) syringaldehyde, and (E) dihydrocoumarin.

in the lung tissue was observed upon treatment with coumarin and *o*-coumaric acid (Santos et al., 2006). Anti-inflammatory and antioxidant activities have been described for dihydrocoumarin, which is one of the major compounds in hydroalcoholic extracts (Hoult and Paya, 1996; Alves et al., 2009; Gu and Xue, 2010). Syringaldehyde has been shown to have moderate antioxidant activity (Bortolomeazzi et al., 2007), and it contributes to the anti-inflammatory properties of the guaco extracts by the dose-dependent inhibition of cyclooxygenase-2 activity (IC_{50} = 3.5 µg/ml) (Farah et al., 1992; Stanikunaite et al., 2009).

Kaurenoic acid has been demonstrated to have strong anti-inflammatory activities in lipopolysaccharide-induced RAW264.7 macrophages by the dose-dependent inhibition of the synthesis of nitric oxide (IC_{50} = 51.73 µM), the release of prostaglandin E_2 (IC_{50} = 106.09 µM), and the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (Choi et al., 2011). The anti-inflammatory effect of kaurenoic acid on acetic acid-induced colitis in rats was also observed in animals that received 100 mg/kg kaurenoic acid by rectal and oral routes (> 40% reduction in the gross damage score and > 30% reduction in the wet weight of damaged colon tissue) (Paiva et al., 2002).

For the carrageenan-induced paw edema of mice, kaurenoic acid at 50 mg/kg reduced 34.4% of the swelling that occurred 5 h after the induction of inflammation (Choi et al., 2011). At a concentration of 160 µM, kaurenoic acid significantly decreased the contraction of rat uterine muscle that was pre-contracted with oxytocin (*E*max = 83%) and acetylcholine (*E*max = 91%) (Cunha et al., 2003). At 10 µM or higher concentration, kaurenoic acid also had concentration-dependent activity on vascular smooth muscle (endothelium-intact or denuded rat aortic rings) that was pre-contracted with phenylephrine and potassium chloride (Tirapelli et al., 2002, 2004).

Despite the therapeutic relevance of the described metabolites, the benefits of guaco have been attributed mainly to the presence of the coumarin (1,2-benzopirone), which is considered to be the main biomarker of guaco (Hoult and Paya, 1996; Castro, 2002; Pedroso et al., 2008; Brasil, 2008b). In Brazil, the daily uptake (0.5 to 5 mg) of coumarin has been approved by regulatory agencies (Brasil, 2008b), but the recommended doses vary according to the therapy (Lacy et al., 2004).

Coumarin, an anticoagulant and antithrombotic agent, can be used to reduce the swelling caused by lymphatic and venous vessel problems. When coumarin is administered to the rat duodenum (100 mg/kg), it can produce antiulcerogenic activity by inhibiting the secretion of acids, which is mediated by the parasympathetic system (Bighetti et al., 2005).

The activation of macrophages and cells of the immune system by coumarin have also been described (Hoult and Paya, 1996; Lacy et al., 2004). In rodents, this substance decreases the swelling caused by thermal damage; in humans, coumarin reduced the lymphedema of patients who have elephantiasis or who have had a mastectomy (Hoult and Paya, 1996). In cancer therapy, coumarin is used as an adjuvant in melanoma therapy (Thornes et al., 1994); it might also have applications in the treatment of metastatic prostate cancer by stabilizing the levels of the prostate specific antigen (PSA) (Lacy et al., 2004). Treatments combining coumarin and cimetidine (1200 mg/day) led to a reduction in the metastasis of carcinomas without toxic side effects (Thornes et al., 1982).

Coumarin induced a concentration-dependent relaxation in guinea pig tracheae that were pre-contracted with histamine (EC_{50} = 35.0 µg/ml) or carbachol (EC_{50} = 33.4 µg/ml) (Ramanitrahasimbola et al., 2005). However, coumarin was less effective in the guinea pig tracheae (EC_{50} = 130.8 µg/ml) and endothelium-denuded tracheae (EC_{50} = 153.4 µg/ml) that were pre-contracted with potassium chloride. Coumarin combined with theophylline had a significant additive relaxation effect on the pre-contracted tracheae, and this effect was not blocked by propranolol. These results indicate that the bronchodilator effect of coumarin is partly due to an endothelium-dependent tracheal relaxation and a non-specific tracheal relaxation (Ramanitrahasimbola et al., 2005).

PROCEDURES FOR COUMARIN EXTRACTION

Coumarin is considered to be the main metabolite of guaco. To obtain the maximum yield of coumarin, a variety

of procedures are performed using different plant parts, solvents and conditions.

The procedures for coumarin extraction are frequently discussed in the literature; however, there is no consensus regarding the most efficient system. To facilitate new investigations, information such as the drug: solvent ratio, the sample preparation, the metabolites found in each extract and the amounts of these metabolites are summarized in Table 1.

Cabral et al. (2001) demonstrated that the most suitable and economically profitable method of obtaining the highest yield of coumarin was to reflux the fresh aerial parts of plants that were collected from regions of higher altitudes and temperatures. The authors also indicated that the extraction of 10 g of fresh plant tissue 3 times with 40 ml of a 1% (*w/v*) NaOH solution (refluxing or at room temperature) allowed a simple and complete recovery of coumarin for a low ecological cost (Cabral et al., 2001).

Aqueous extracts obtained by microwave and decoction (10% *w/v*, dried leaves) presented at least twice the level of coumarin as compared to the hydroalcoholic extracts obtained by percolation (Muceneeki et al., 2009). However, this information is contradictory to the results of other investigations, which found that hydroalcoholic solutions had a greater capacity for coumarin extraction (Celeghini et al., 1999, 2001; Rocha et al., 2008; Bueno et al., 2009).

In summary, the maximum yields of coumarin can be achieved on a small scale by macerating the dried leaves of guaco by sonication at 40°C (Celeghini et al., 2001). This system has been demonstrated as the most efficient for coumarin extraction, because it requires less time and presents the same or better efficiency as compared to other techniques (Celeghini et al., 2001). A hydroalcoholic solution at 70:30 *v/v* ratio was the most economical solvent that allowed a profitable recovery of coumarin (Rocha et al., 2008; Bueno et al., 2009). Regarding the drug:solvent ratio, the greatest amount of soluble solids and coumarin content was obtained at 1.5:10 *w/v* (Aboy et al., 2000).

Finally, the geographic origins and seasonality of the guaco species are crucial to obtaining desirable levels of coumarin (Pereira et al., 2000, Gasparetto et al., 2012). The highest levels of coumarin have been obtained using leaves from young plants (100 days old) that were cultivated from cuttings and grown with organic fertilizers (humus) under full sunlight (16 h light period) in regions of high altitudes and temperatures (Pereira et al., 1998; Cabral et al., 2001; Castro et al., 2006; Souza et al., 2007; Contini et al., 2006). Plants collected during the early evening of December and July also demonstrated the highest levels of coumarin (Pereira et al., 2000). Another organic solvent, hexane, has demonstrated a superior capacity for coumarin extraction over ethanol and methanol (Vilegas et al., 1997a, b), which were inadequate for this purpose (Table 1).

ANALYTICAL PROCEDURES

Several analytical procedures have been described for the qualitative and quantitative analysis of the main guaco metabolites in extracts and preparations. These procedures include thin layer chromatography (TLC), ultraviolet spectroscopy (UV-VIS), gas chromatography with a flame ionization detector (GC-FID), gas chromatography coupled with mass spectrometry (GC-MS), and high performance liquid chromatography with diode array detector (HPLC-DAD) and mass spectrometry (HPLC-MS) analyser.

TLC has been described as the most economical technique for the qualitative assessment of coumarin, *o*-coumaric acid, syringaldehyde, terpenes, organic acids, steroids, peroxides and other substances in guaco extracts (Oliveira et al., 1984, 1993; Veneziani et al., 1999; Celeghini et al., 2001; Schenkel et al., 2002; Bighetti et al., 2005; Maiorano et al., 2005; Santos, 2005; Yatsuda et al., 2005; Alvarenga et al., 2009; Bolina et al., 2009). Different techniques have been applied for quantitative assessment, each with distinct advantages and disadvantages.

First derivative spectrophotometry is a low-cost and simple technique that is useful for the determination of coumarin in guaco extracts. However, this technique has low selectivity, and several steps of sample pre-treatment are needed prior to analysis, rendering the system laborious and time-consuming (Osorio, 2004). Ultraviolet analysis has also been used to monitor coumarin directly in phytomedicines without sample pre-treatment. However, this method uses only one specific wavelength (275 nm), and matrix interferences are expected, because preservatives and other syrup constituents have luminous absorption at the same wavelength (Amaral et al., 2009).

Chromatographic systems, such as HPLC-DAD, HPLC-MS, GC-FID and GC-MS, have been described as the most suitable techniques for the determination of guaco metabolites. In particular, these systems present high sensitivity, reproducibility and capacity for the separation of guaco metabolites into different matrices. Despite the potential for high selectivity in using these techniques, some methods monitor only a specific number of metabolites. In other cases, the methods are targeted exclusively for qualitative assessments (Pereira et al., 1998; Celeghini et al., 1999; Santos et al., 1999; Aboy et al., 2000; Cabral et al., 2001; Celeghini et al., 2001; Soares de Moura et al., 2002; Biavatti et al., 2004; Osorio, 2004; Radunz, 2004; Duarte et al., 2005; Maiorano et al., 2005; Contini et al., 2006; Graca et al., 2007; Rocha et al., 2008; Silva et al., 2008; Alvarenga et al., 2009; Bolina et al., 2009; Bueno et al., 2009).

GC analyses have been applied in the screening of essential oils (Radunz, 2004; Duarte et al., 2005; Rehder et al., 2006). However, the quantitative assessment of guaco metabolites using GC is uncommon, because

several steps of sample preparation are required before injection. This process makes the assay laborious, time-consuming and harmful to the environment. In additional, most of the results obtained with GC analysis are not accurate because they are expressed as a relative percentage (Vilegas et al., 1997b; Soares de Moura et al., 2002; Yatsuda et al., 2005; Rehder et al., 2006; Graca et al., 2007; Alves et al., 2009).

The methods based on HPLC systems have been described as the most suitable for the routine analysis of guaco metabolites. HPLC methods are sensitive, reproducible and selective, features that are useful for complex matrices such as extracts and pharmaceutical preparations. Low-cost analysis can be achieved through the use of HPLC because most of the developed methods employ popular columns (e.g., octadecilsilane) and use simple mobile phases composed of methanol/water or acetonitrile/water (Pereira et al., 1998; Celeghini et al., 1999; Aboy et al., 2000; Cabral et al., 2001; Celeghini et al., 2001; Biavatti et al., 2004; Brasil, 2005; Maiorano et al., 2005; Peregrino and Leitão, 2005; Santos, 2005; Bertolucci et al., 2008; Rocha et al., 2008; Alvarenga et al., 2009; Bolina et al., 2009; Muceneeki et al., 2009; Gasparetto et al., 2011a, 2011b). From the methods applied for the quantitative assessment of guaco metabolites, only a few were fully validated according to worldwide regulations (Bertolucci et al., 2008; Muceneeki et al., 2009; Gasparetto et al., 2011a, 2011b). The use of validated methods is crucial for guaranteeing reliable results that are suitable for the intended purpose.

CONCLUSION

This review highlights the importance of *M. glomerata* and *M. laevigata* (guaco) as medicinal herbs. Both species present analogous chemical profiles and morphological characteristics, thus requiring a full anatomical analysis to distinguish between the two. Many studies have been conducted with the aim of obtaining the maximum yield of coumarin, as this substance has been described as the most prevalent metabolite in the guaco species. The resulting data demonstrate that the maximum yields of coumarin can be achieved by macerating leaves (1.5:10 *w/v*) in a 70:30 *v/v* hydroalcoholic solution using sonication at 40°C. To obtain the maximum yield of coumarin, researchers should also consider the use of leaves from young plants (100 days old) that are obtained in regions of high altitudes and temperatures, cultivated from cuttings, and grown with organic fertilizers (humus) under full sunlight (16 h light period). Although, the extraction of coumarin has been extensively investigated, additional studies are needed to evaluate the differences in coumarin levels between dried and fresh leaves, because these data are contradictory in the literature. The pharmacological effects of guaco should not be attributed solely to coumarin, because guaco species possess major compounds that have therapeutic relevance, as found through *in vitro* and *in vivo* studies. Thus, more-conclusive investigations should be conducted to understand the real mechanisms of the guaco's effects. By conducting further studies, it will be possible to standardize the most effective extractive process for therapeutic purposes. The main goal of these studies should be to ascertain the benefits and safe uses of guaco.

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