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Artigo

Differences in secondary metabolites from leaf extracts of *Mikania glomerata* Sprengel obtained by micropropagation and cuttings

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RESUMO: "Diferenças nos metabólitos secundários acumulados nos extratos das folhas de *Mikania glomerata* Sprengel obtidos por micropropagação e estaquia". A análise realizada por CG dos extratos diclorometânicos das folhas de *Mikania glomerata* Sprengel mostrou que a planta propagada por estaca produziu cumarina e ácido caurenóico, enquanto que o material micropropagado acumulou apenas cumarina.

Unitermos: *Mikania glomerata*, Asteraceae, micropropagação, estaquia, ácido caurenóico.

ABSTRACT: GC analysis of the dichloromethane extracts obtained from cultivated specimens of *Mikania glomerata* Sprengel possibilited to verify that cuttings technique led to production of kaurenoic acid and coumarin while the same results have not been observed by propagation process using *in vitro* techniques.

Keywords: *Mikania glomerata*, Asteraceae, micropropagation, cuttings, kaurenoic acid.

INTRODUCTION

Mikania glomerata Sprengel (Asteraceae) is a vine commonly found in the forests of Argentina, Brazil and Paraguai and is popularly known as "guaco", "guaco trepador", "erva de cobra", "cipó – caatinga" and "coração de Jesus" (Gupta, 1995). Its leaf infusions and syrups are widely used in folk medicine for the treatment of respiratory tract diseases (Teske; Tentini, 1997; Pereira et al., 2004; Soares et al., 2006). *Mikania glomerata* shows many well-known pharmacological activities, among them antimicrobial (Pessini et al., 2003; Amaral et al., 2003; Santos et al., 2003; Duarte et al., 2004), anti-inflammatory (Falcão et al., 2005) and antidiarrhoeal (Salgado et al., 2005). The major constituents of the plants are kauran-type diterpenes (Veneziani; Oliveira, 1999) with various biological activities, including antimicrobial activity (Ghisalberti, 1997). Vilegas et al. (1997) reported that the biological activity *of Mikania glomerata* extracts is due to the presence of the compounds coumarin and kaurenoic acid.

Recently, there has been an increased interest in developing technical subsides for the commercial production of "guaco" (*Mikania glomerata* Sprengel) by propagation processes (Negrelle, 2001; Pereira et al., 1999) offering a viable tool for mass multiplication and germplasm conservation of rare, endangered and threatened medicinal plants.

The purpose of this study was to investigate the differences in chemical accumulation between leaf extracts of *Mikania glomerata* obtained by micropropagation and cuttings.

MATERIAL AND METHODS

Plant material

Cultivated specimens of *Mikania glomerata* Sprengel (Compositae) were obtained by propagation by cuttings and *in vitro* techniques processes as previously described by Pereira et al. (1998) and Pereira et al. (1999). *Mikania glomerata* Sprengel was collected in October 1994 from a single plant growing in the experimental area of the Universidade de Ribeirão Preto, São Paulo state, Brazil. A voucher specimen is deposited at the herbarium HPMU (Universidade de Ribeirão Preto; accession number 0024). Leaf materials were maintained in a greenhouse for two years and no morphological differences were observed.

Extraction of the plant material and clean-up of the extract

Five 0.2 g samples of leaves obtained by cutting

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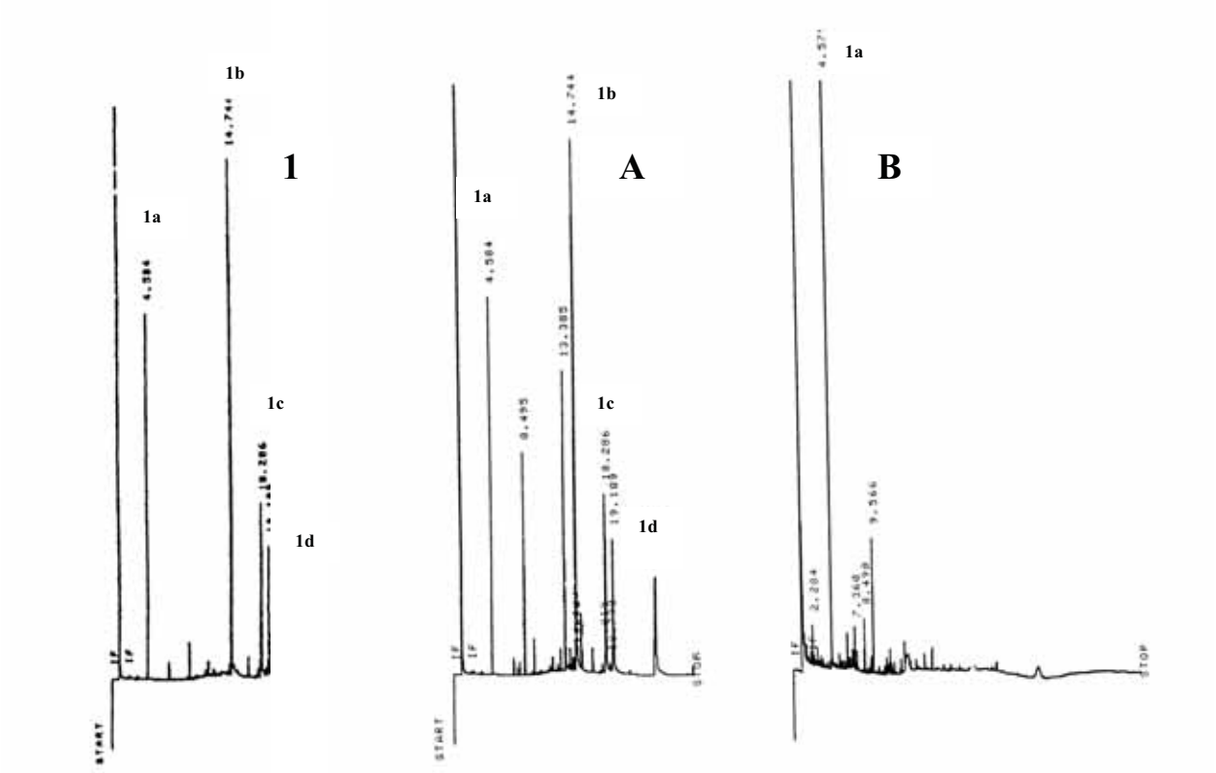

Figure 1. GC analyses of the dichloromethane extract from callus obtained from *M. glomerata* Sprengel cuttings (**A**) and leaf explants (**B**). **1**: reference compounds used in GC analyses: **1a**: coumarin (rt = 4.584); **1b**: *ent*-kaur-16-en-19-oic acid (rt = 14.744); **1c**: 15β-hydroxy-*ent*-kaur-16-en-19-oic-acid (rt = 18.286); **1d**: 17-hydroxy-*ent*-isokaur-15(16)-en-19-oic acid (rt=19.189).

(A) or micropropagated leaves (B) were macerated with hexane (2 ml; room temperature) during 10 min of sonication. The extract was filtered and eluted through a glass column (1 cm i.d) containing 0.2 g of silica gel (0.063 – 0.200 mm, Merck, Darmstadt, Germany) in the bottom and 0.04 g of active charcoal (Reagen, Rio de Janeiro, Brazil) on the top. The column was then eluted consecutively with 2 ml of hexane and 3 ml of dichloromethane. The fractions were combined and evaporated under N_2 flux, at room temperature until dryness. These extracts were diluted in 1 ml of a dichloromethane solution (0.5 mg/ml) of internal standard (goyazensolide).

GC experimental procedure

The GC analysis was performed using a Hewlett-Packard 5890 series II instrument equipped with a 30 m x 0.25 μ.m i.d. column coated with methyl silicone (0.25 μm film thickness; HP-1 column, supplied by Hewlett-Packard). 1 μL samples were injected using the split mode (1:60 split ratio) with the detector temperature at 300 °C and the column temperature programmed from 105 °C to 200 °C at 13 °C/min, from 200 °C to 240 °C at 6 °C/min (remaining at 240 °C for 20 min.) and from 240 °C to 280 °C at 10 °C/min (remaining at 280 °C for 10 min.). Hydrogen was used as the carrier gas at an average linear velocity of 39 cm/s. Data were processed using a Hewlett-Packard HP 3395 integrator. Each determination was carried out at least in triplicate.

RESULTS AND DISCUSSION

Previous investigation of vegetative propagation by cuttings experiments showed that *M. glomerata* presented positive results for coumarins, steroids, triterpenoids, saponins and volatile acids (Lima, 2002).

In the present study, GC analysis of leaves of *Mikania glomerata* Sprengel obtained by cutting technique presented coumarin and kaurenoic acid as the major compounds (Figure 1A), as also observed in other wild *M. glomerata* (Celeghini et al., 2001; Vilegas et al., 1997).

Otherwise, Santos et al. (1999) showed that the phytochemical analysis of the dichloromethane extract from callus obtained from *Mikania glomerata* Sprengel leaf explants led to the isolation of a mixture of the steroids campesterol, stigmasterol and β-sitosterol besides

coumarin. The kaurenoic acid derivative (diterpene) usually found in large amounts in this specie has not been detected (Figure 1B).

According to Pereira et al. (1999), the greatest difficulty in establishing this micropropagation protocol was the high levels of contamination by fungi and bacteria, thus requiring the cultivation of over 5000 explants and a 14 months period to achieve plantlet disinfestation. The mutagenic potential of numerous antibiotics has already been demonstrated, and could be related to the decrease of kaurenoic acid.

On the basis of these data, we can suggest that the efforts for complete elimination of both endogenous and exogenous microorganisms using different classes of antibiotics certainly altered the plant metabolism, resulting in the discrepancy observed in the chemical composition of this micropropagation process.

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