Acta Physiologiae Plantarum (2021) 43:71
https://doi.org/10.1007/s11738-021-03241-1

ORIGINAL ARTICLE

Biomass production, essential oil's yield and composition of three genotypes of *Mikania laevigata* Sch. Bip. ex Baker

Maira Christina Marques Fonseca[1] · Mariane Borges Rodrigues de Ávila[2] · Ítalo Antônio Cotta Coutinho[3] · Rosana Gonçalves Rodrigues das Dôres[4] · Renata Maria Strozi Alves Meira[5] · Andréia Fonseca Silva[6]

Received: 5 August 2019 / Revised: 15 September 2020 / Accepted: 7 April 2021 / Published online: 17 April 2021

Abstract

Mikania laevigata leaves are used worldwide as herbal medicines. Our study investigates the production differences among three different genotypes of this herb: the genotypes' influence on the essential oil yield, chemical composition, and the plant tissues involved in the secretion of the chemical compounds produced by the leaves. The study performed the analysis during the summer and winter seasons. Plants were grown under an organic cultivation system in Oratórios, Minas Gerais, Brazil. Essential oils were extracted by hydrodistillation and analyzed by gas chromatography/mass spectrometer. The analysis highlighted substantial genetic variability among the genotypes. The genotype CENARGEN showed higher biomass production, oil yield, and a high concentration of major constituents detected by chromatographic analysis in essential oils, such as germacrene D and caryophyllene oxide. The season affected the chemical composition of the essential oils. Germacrene D and bicyclogermacrene were the major constituents in the winter-essential-oils; spatulenol and caryophyllene oxide were the major constituents in the summer-essential-oils.

Keywords Histochemistry · Environmental conditions · *Mikania laevigata* · Chemical profile

Introduction

Plants are a natural source of phytochemicals: chemical compounds that include active principles with biological activity in the human body (Atanasov et al. 2015; Farzaneh and Carvalho 2015). Brazil has the highest diversity of plants in the world, with an extensive range of species potentially useful in the treatment of several diseases (Dutra et al. 2016). *Mikania laevigata* Sch. Bip. ex Baker (Asterales, Asteraceae) is among the Brazilian plant species with medicinal properties (Napimoga and Yatsuda 2010). It is a climbing shrub that grows curling up in an anti-clockwise direction in some support, and has a cylindrical stem with longitudinal stria, with visible knots and opposite leaves in an ovate to oblong-lanceolate form (Oliveira et al. 1986). The species is native from Brazil and occurs in the phytogeographic domains of *Cerrado* (Brazilian Savannah) and Atlantic Forest (Ritter et al.

Communicated by A. Sojka.

✉ Mariane Borges Rodrigues de Ávila
avilanane@gmail.com

Maira Christina Marques Fonseca
mairacmf@yahoo.com.br

Ítalo Antônio Cotta Coutinho
italocoutinho@hotmail.com

Rosana Gonçalves Rodrigues das Dôres
rosanagrd@gmail.com

Renata Maria Strozi Alves Meira
renata.strozi.meira@gmail.com

Andréia Fonseca Silva
andreiasilva@epamig.br

[1] Empresa de Pesquisa Agropecuária de Minas Gerais-EPAMIG, Viçosa, MG 36.570-900, Brazil

[2] Departamento de Engenharia Agrícola, Universidade Federal de Viçosa, Viçosa, MG 36.570-900, Brazil

[3] Departamento de Biologia, Universidade Federal do Ceará, Fortaleza, CE 60440-900, Brazil

[4] Centro de Saúde, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brazil

[5] Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa, MG 36.570-900, Brazil

[6] Empresa de Pesquisa Agropecuária de Minas Gerais-EPAMIG, Belo Horizonte, MG 31170-495, Brazil

2015). It has been widely used in the ethnopharmacology (Gasparetto et al. 2010) as an antiseptic of the respiratory tract, expectorant, and antiasthmatic (Santos et al. 2006; Graça et al. 2007; Ricardo and Brandão 2018). The traditional uses of the plants should also include fever treatment, sudorific, antirheumatic, anti-inflammatory, cicatrizant, and bactericidal (Suyenaga et al. 2002; Duarte et al. 2004, 2007; Santos et al. 2006; Graça et al. 2007).

Although the development and mass production of chemically synthesized drugs revolutionized health care in the twentieth century, there are still countries that rely on traditional practitioners and herbal medicines (Benzie and Wachtel-Galor 2011). Such use of herbal medicines is not limited to developing countries as the public interest in natural therapies has significantly increased during the past two decades (Benzie and Wachtel-Galor 2011). As a result, the world market for herbal medicines is expanding (Dutra et al. 2016).

Brazilian biodiversity represents a precious resource for the discovery of new drugs. Even the same, the Brazilian participation in the global market for herbal medicines is still meager (Calixto 2000; Dutra et al. 2016). The volume of scientific research in this sector is also incipient (Dutra et al. 2016), and the development of innovations in the market of herbal medicines requires an assurance of quality, efficacy, and safety (Osman et al. 2018; Zago 2018).

Compounds of medicinal interest are secondary metabolites of the plants. Genetic, environmental, and physiological factors influence their production and concentration (Gobbo-Neto and Lopes 2007; Prinsloo and Nogemane 2018; Zandalinas et al. 2018). These elements of variability are the main hurdles to obtaining adequate raw material for the processing of herbal medicines and cosmetics in the industries (Aguiar et al. 2014). Thus, the importance of cultivation, harvesting, and drying must be pointed out, aiming at the standardization of the vegetable raw material supplied (Canter et al. 2005; Salgueiro et al. 2010; Aguiar et al. 2014; Govindaraghavan and Sucher 2015). The expanding herbal product market could drive overharvesting of plants collected from wild populations and thus threaten biodiversity (Benzie and Wachtel-Galor 2011). The same authors also advocate for the importance of the cultivation of medicinal plants.

The authentication of medicinal plants based on its DNA is an essential tool to ensure quality control (Desjardins 2008; Sucher and Carles 2008). The presence of genetic control on the production of a biosynthetic pathway does not guarantee the presence of metabolite produced by this pathway nor predicts its concentrations, due to complex interactions with the environment (Desjardins 2008). Therefore, it is crucial to study the genotypes and their respective chemical expression to guarantee the quality, efficacy, and safety of the raw material supplied to the industry (Sucher and Carles 2008; Benzie and Wachtel-Galor 2011; Govindaraghavan and Sucher 2015).

Aiming to solve the problems regarding the production of phytochemicals by *Mikania laevigata* leaves, used in the world market of herbal medicines, our study addressed the following questions: (1) Are there differences among three different genotypes of *Mikania laevigata* from the production standpoint?; if so, (2) do such differences influence the essential oil yield, and chemical composition?, (3) does the season interfere on the chemical composition and production?, and (4) what plant tissues are involved in the secretion of the chemical compounds produced by the leaves of *Mikania laevigata*?

Materials and methods

Plant material

The *Mikania laevigata* accessions (Fig. 1) were initially obtained from Embrapa Genetic Resources and Biotechnology (CENARGEN), the University of Campinas (CPQBA), and the University of Ribeirão Preto (UNAERP). Voucher specimens of the three accessions were deposited at the Herbarium of the Minas Gerais Agricultural Research Company (Fonseca MCM & Coutinho IAC, 1347, 03.IX.2013) (PAMG 57032, PAMG 57033 and PAMG 57031) and identified by an expert taxonomist from Minas Gerais Agricultural Research Company-EPAMIG.

Plants were grown under an organic cultivation system in Oratórios, MG (20° 25′ 49″ S; 42° 48′ 20″ W). The cuttings were placed in a substrate (Plantmax®) until cuttings naturally set their roots. Rooted cuttings were then transplanted at a spacing of 1 m within rows and 2 m between rows, and 5 kg of cow manure were added to each pit. After harvesting, the fresh weights of the plants were measured. Leaves and stems were weighed separately in a semi-analytical balance, and the results were expressed in kg/plant as adapted from de Castro et al. (2003).

Essential oil extraction and chromatographic analysis

The leaves were dried in a forced-air circulation oven (40 °C) up to constant weight. Essential oils were extracted by hydrodistillation using a Clevenger device, adapted to a round-bottomed two-liter flask as described by BRASIL (2010), with heating kept at the minimum temperature required to boil. The flask was loaded with 100 g of the fresh sample or the equivalent of 100 g for the previously homogenized dry samples. Distilled water was then added up until covering the material only at the beginning of the hydrodistillation process. The extraction time was set at six

Fig. 1 Organic cultivation of *Mikania laevigata* accessions

h, as calibrations based on preliminary tests, with three repetitions for each treatment. At one-hour intervals, after the beginning of the process, hydrolat samples (a mixture of water and oil) were taken, and all were grouped in a single sample. After the hydrolat was obtained, it was separated with pentane (10:1) in a 125 mL separation funnel, and the procedure was repeated three times.

Identification of the volatile constituents was performed using an Agilent HP-6890 gas chromatograph equipped with an HP-5975 Agilent mass selective detector and an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). The analysis was performed in the splitless injection mode, with the following temperatures: injector at 220 °C, the column at 60 °C, with the heating ramp from 3 °C min^{-1} up to the final temperature of 240 °C and detector 250 °C. Helium gas was used with drag traps at a flow rate of 1 mL min^{-1}. A sample of essential oils was dissolved in ethyl acetate (20 mg mL^{-1}) for the analysis. Retention indices (RI) were determined by injection of hydrocarbon standards and essential oil samples under the same conditions. The components of the oil were identified by comparison with the literature data (Adams 2007) and the Nist-11 spectral mass library profiles.

Anatomical study

Samples of leaves of the three accessions cultivated in Oratórios, as previously described, were fixed in a solution of formaldehyde-glutaraldehyde in phosphate buffer (Karnovsky 1965) for 24 h, rinsed three times in 0.1 M phosphate buffer solution at pH 7.2, dehydrated through a graded ethanol series from 10 to 95% and embedded in methylmethacrylate resin (Historesin, Leica), prepared according to the manufacturer. Cross and longitudinal sections with 3 or 5 µm thick were made in a rotatory microtome (Leica RM2155, Deerfield, IL, USA) and subsequently stained with toluidine blue at pH 4.4 (O'Brien and McCully 1981) for structural characterization. The slides were mounted in synthetic resin (Permount, Fisher Scientific, New Jersey, USA).

For the chemical characterization of the compounds present in the leaves, histochemical tests were conducted on either free-hand sections made from fixed material or sections made from methyl methacrylate embedded material as described above. Embedded sections were used in the histochemical tests to verify the presence of total proteins with xylidine Ponceau (O'Brien and McCully 1981); total polysaccharides with Periodic acid–Schiff reagent (O'Brien and McCully 1981); acid mucopolysaccharides with Alcian blue (Pearse 1980); and pectins/mucilage with Ruthenium red (Johansen 1940). Free-hand sections were used to verify the presence of total lipids with Sudan IV and Sudan black (Pearse 1980); essential oils and resin oils with Nadi reagent (David and Carde 1964); sesquiterpene lactones with sulfuric acid (Geissman and Griffin 1971); and rubber with oil red (Adler et al. 2014).

Observations and photographs were obtained using a light microscope (model AX70TRF; Olympus Optical, Tokyo, Japan) equipped with a U-Photo system and digital camera (AxioCam HRc; Carl Zeiss, Gottingen, Germany).

Statistical analyses

A subdivided plot scheme was used. The plots were composed by the periods of harvesting (summer and winter), and the subplots by the genotypes (CENARGEN, UNAERP, and CPQBA), in a randomized block design with seven replications. The data were submitted to analysis of one-way ANOVA, using the SAEG software 9.1, 2007 (UFV, Viçosa, Brazil), and the averages were compared by Tukey test at 5%

probability As considering the aim of the study the factors were analysed separately, even the interaction among them. The data of chromatographic analysis were expressed using descriptive statistics.

Results

Biomass production

The total production of biomass displayed a significant difference (Tukey 5%) among the accessions during the summer. The CENARGEN genotype (8.75 kg/plant) had the highest production, CPQBA (5.63 kg/plant) had the lowest production, and UNAERP (7.35 kg/plant) did not differ statistically from the others (Table 1). We observed no significant difference in fresh leaves production comparing the genotypes in each season. The CPQBA genotype had a stem production significantly lower than the other genotypes in both seasons. The comparison among biomass production during summer and winter highlighted the significant reduction of leaves and stems production in the CENARGEN genotype during the winter, and the lower amount of the stems in the UNAERP genotype, also during the winter.

Chromatographic analysis

CENARGEN (0.30%) and CPQBA (0.28%), genotypes presented significantly higher essential oil yield than UNAERP (0.19%) in the summer. In the winter, all the genotypes present significantly different oil yield, CPQBA (0.52%) followed by CENARGEN (0.43%) and UNAERP (0.32%). Besides, the summer-oil yield values of all genotypes were significantly different (Tukey 5%) from the winter-oil yield values.

Analyses of the composition of the essential oil revealed 21 main identified constituents (Table 2). However, the concentration of some of them varied significantly according to the season. Germacrene D and bicyclogermacrene were the major constituents in the winter-essential-oils. Spatulenol and caryophyllene oxide were the major constituents in the summer-essential-oils. The genotype CPQBA showed the highest concentration of germacrene D (40.25%), and UNAERP showed the highest concentration of bicyclogermacrene (19.66%). UNAERP showed the highest concentration of spatulenol (30.56%) and CENARGEN the most significant concentration of caryophyllene oxide (9.37%). Although the genotypes CENARGEN and UNAERP showed similar results regarding the chemical composition of their essential oils and total biomass production, the CENARGEN genotype displayed aa higher oil yield in both seasons, which could be an advantage from the commercial point of view.

Anatomical study

The anatomical characteristics that follow were the same for all accessions of *M. laevigata* studied, that is, CENARGEN, CPQBA, and UNAERP. The cross-sections of the leaves displayed a single-layered epidermis on both adaxial and abaxial sides (Fig. 2a). The abaxial epidermis displayed scattered secretory trichomes placed in a depression (Fig. 2b). Leaf clearings showed that such trichomes are uniseriate, filamentous, with a variable number of stalk cells and bear a larger apical cell turned downwards (curved trichome) in a way that it resembles a comma (Fig. 2b). Hypodermis was observed only on the adaxial side (Fig. 2a). Leaves presented dorsiventral mesophyll made up 2–3 layers of palisade parenchyma and 8–10 layers of spongy parenchyma (Fig. 2a, b), collateral vascular bundles and secretory ducts associated with the xylem, that is, on the adaxial side (Fig. 2a–d).

As revealed by cross-sections of the leaf blade, the midrib is composed of 4–5 collateral vascular bundles (Fig. 2a, e): three major central bundles and 1–2 smaller bundles placed at the sides of the three major bundles (Fig. 2e). When the midrib displayed two smaller bundles, then one was placed on each side. Both adaxial and abaxial sides of the midrib displayed secretory ducts, associated with the xylem, and with the phloem or near to it, respectively (Fig. 2e). Four-six layers of angular collenchyma (Fig. 2e) were present at the midrib. The ducts were lined with a single-layered secretory epithelium, which is also true for the ducts found elsewhere on the leaf blade.

Table 1 Genotypes' biomass production during summer and winter

GENOTYPES	Total production (kg/plant)		Leaves production (kg/plant)		Stems production (kg/plant)	
	Summer	Winter	Summer	Winter	Summer	Winter
CENARGEN	8.75 aA	6.58 aB	4.21 aA	3.17 aB	4.54 aA	3.40 aB
UNAERP	7.35 abA	5.41 aB	3.55 aA	2.61 aA	3.81 aA	2.80 aB
CPQBA	5.63 bA	4.81 aA	3.40 aA	2.90 aA	2.24 bA	1.91 bA
CV (%)	20.53		19.47		21.85	

The same small letters indicate that biomass production did not differ between genotypes. The same capital letters indicate that biomass production did not differ between seasons by the Tukey test ($p \leq 0.05$)

Table 2 Chemical composition of *M. laevigata* genotypes' essential oils in different harvesting seasons. Oratórios, 2013

Chemical composition (Relative percentage)	Genotypes					
	CENARGEN		CPQBA		UNAERP	
	Harvest					
	W	S	W	S	W	S
α-pinene	5.90	1.49	10.34	1.72	2.14	–
Sabinene	–	–	0.39	–	–	–
β-pinene	1.58	–	3.09	–	0.86	–
β-myrene	0.68	–	1.66	–	1.93	–
Limonene	–	–	0.67	–	–	–
Delta-elemene	0.77	0.86	0.90	0.99	0.86	–
α-copaene	1.25	1.09	1.14	1.14	0.54	–
β-cube	0.70	–	0.54	–	0.82	–
Trans-caryophyllene	9.01	9.17	8.01	9.58	8.76	7.69
α-humulene	1.20	1.11	1.08	1.21	1.31	1.05
Germacrene D	37.00	17.28	40.25	22.54	35.26	16.74
Bicyclogermacrene	15.67	5.74	16.04	7.71	19.66	6.07
α-bulnesene	0.53	–	–	–	–	–
Delta-cadinene	2.17	1.49	2.02	1.52	2.80	1.58
Germacrene B	2.20	2.59	1.93	2.67	2.33	2.52
Spathulenol	8.94	26.61	4.92	23.53	7.16	30.56
Caryophyllene oxide	3.04	9.37	0.81	8.68	1.01	9.19
Humulene epoxide II	–	–	–	–	–	1.44
Epi-α-muurolol (tau-muurolol)	1.33	1.87	1.05	1.55	2.03	2.22
α-cadinol	2.26	3.91	1.78	3.11	3.35	4.45
Germacra-4(15), 5,10 (14)-trien-1-α-ol	0.84	2.68	0.99	1.82	0.73	2.74

W winter, *S* summer

The histochemical test of the secretory trichomes displayed positive results only to polysaccharides (Fig. 3a, b) and sesquiterpene± lactones (Fig. 3c, d), identified using PAS and sulphuric acid, respectively. The secretory epithelial cells lining the ducts discharged several compounds into the duct lumen. The secretion within the duct lumen is composed of a mixture of compounds. The histochemical tests highlighted the presence of Oil/resins (Fig. 3e, f), sesquiterpene lactones (Fig. 3g), hydrochloric acid (Fig. 3h), and rubber (Fig. 3i, j). On the other hand, the histochemical tests did not display the presence of total proteins, acid mucopolysaccharides, pectins, and total lipids.

Discussion

Biomass production

Plant production depends on a complex network of interactions of various biotic and abiotic factors (Desjardins 2008). Several studies on various types of plant products describe the interaction of many factors on the plants' yield, such as temperature (Gobbo-Neto and Lopes 2007), seasonality (Prinsloo and Nogemane 2018), and solar radiation (de Castro et al. 2003; Gobbo-Neto and Lopes 2007). The most intense sun's radiation and longer photoperiod in this season explain the most significant summer production of biomass of the CENARGEN and UNAERP genotypes (Fonseca et al. 2006). The result might be due to the higher photosynthetic rates and, consequently, higher mass accumulation (Wullschleger et al. 1996).

Although, the high intensity of solar radiation and high temperatures can cause oxidative damage to the plants affecting photosynthesis and accumulation of mass negatively. CPQBA genotype did not differ in the winter biomass production from the summer's production. This genotype may have a different adaptation to the modifications of temperature and sun radiation. de Castro et al. (2003) also claimed the variation of the biomass production of *Mikania glomera* in different photoperiods. Raposo et al. (2017) corroborated these observations, as studying the influence of solar radiation on the production of *Mikania laevigata* in the Lower Amazon River under shade.

The production of secondary metabolites can occur concurrently with the biomass accumulation according to the adaptation of each genotype to the edaphoclimatic factors

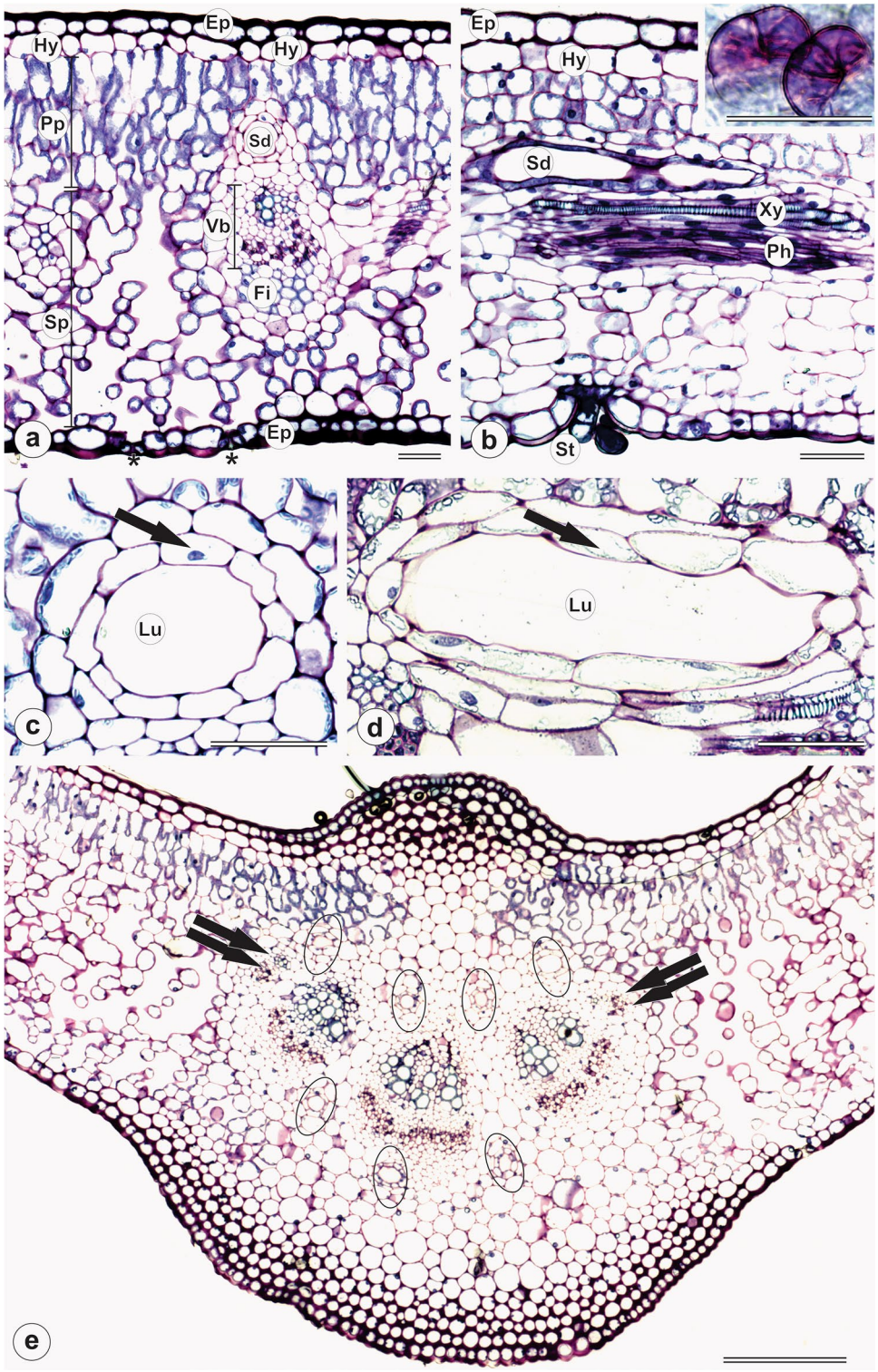

Fig. 2 Leaf anatomy of *Mikania laevigata* as observed in the cross (**a**, **c** and **e**) and longitudinal (**b** and **d**) sections. **a**, **b**. **a** Hypostomatic leaf (asterisk indicates stomata) with the single-layered epidermis (Ep) subtended by a single-layered hypodermis (Hy) on the adaxial side. Uniseriate secretory trichomes (St) with the apical cells turned downwards similar to a comma (type II trichome), are found on the abaxial side. Dorsiventral mesophyll with palisade parenchyma (Pp) on the adaxial and spongy parenchyma (Sp) on the abaxial side. The secretory duct (Sd) is found above xylem (Xy), while below the phloem (Ph), there is a bundle of fibers (Fi). **b** Note the detail of type II trichome on a leaf clearing at the top right. **c**, **d** Secretory ducts are formed by a single-layered epithelium (arrow) that is lined with a lumen (Lu). **e** Midrib vascularized by three major collateral vascular bundles and two smaller bundles (double arrow). Notice the presence of collenchyma (Co) on both adaxial and abaxial side and secretory ducts near xylem and phloem (circles). Bars = 50 µm

(Pereira and De Andrade 1998; Pereira et al. 2016). Thus, it is possible to cultivate medicinal plants choosing the best genotype fitting a specific condition of production to increase uniformity and predictability of metabolites production with an optimized yield (Canter et al. 2005; Govindaraghavan and Sucher 2015).

Chromatographic analysis

In the case of medicinal plants, it is desirable to maintain the quantity and quality of the active ingredient to guarantee the constant biological effect (Benzie and Wachtel-Galor 2011). The influence of genetic and environmental variations in

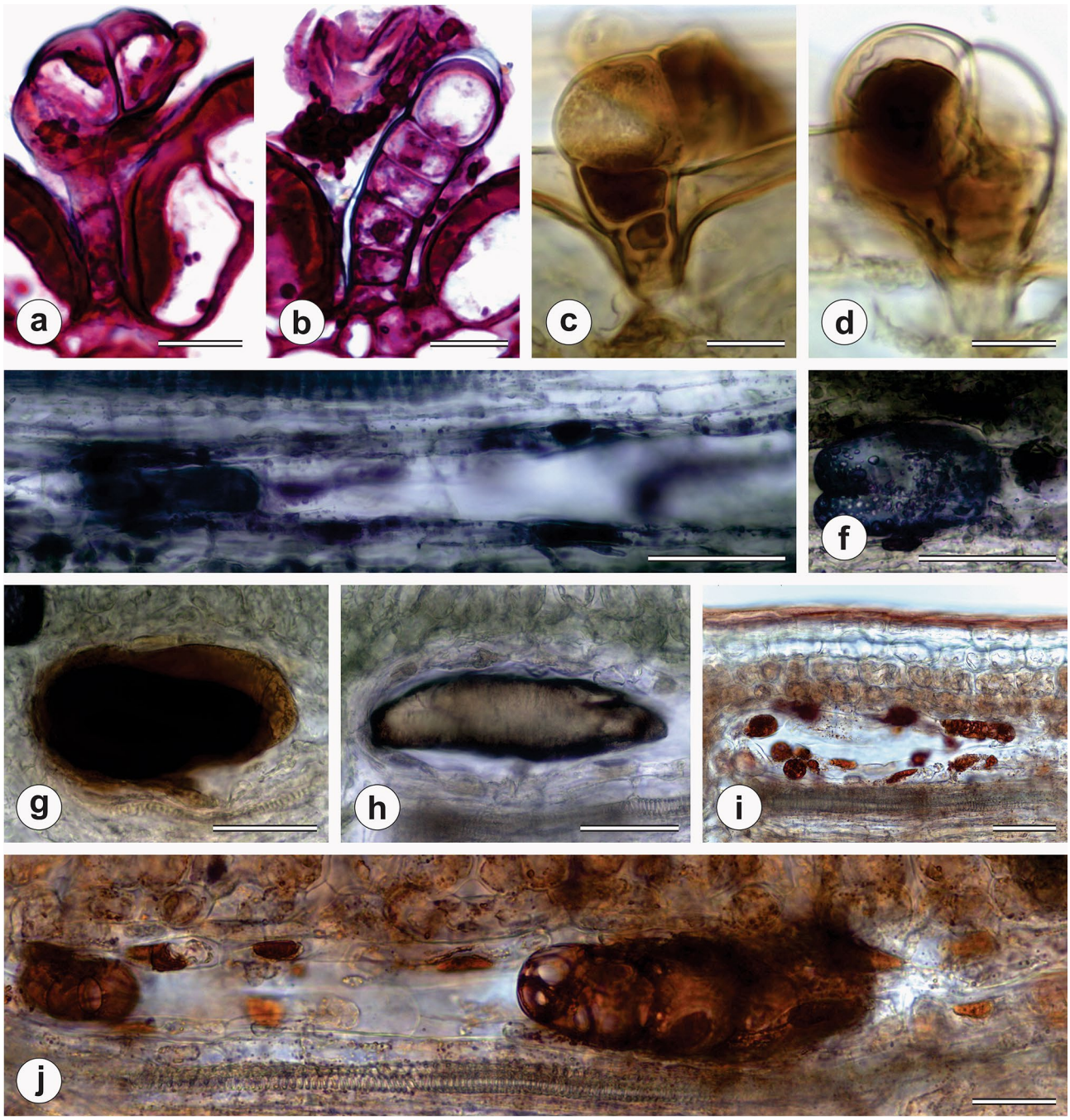

Fig. 3 Results of the histochemical tests performed on the leaves of *Mikania laevigata*. **a**, **b** Positive results for total polysaccharides (PAS) note the purple/magenta color. **c**, **d** The dark brown color shows the reaction for sesquiterpene lactones (with sulphuric acid) in secretory trichomes found on the abaxial leaf side. **e**, **f** The blueish/purple color demonstrates the positive results in the secretory ducts for essential oils/resins (with Nadi reagent). **g**, **h** The dark brown color shows a positive reaction to sesquiterpene lactones, with sulfuric acid and hydrochloric acid, respectively. **i**, **j** The red color using the oil red reagent demonstrates the presence of rubber. Bars = 30 µm

the yield and composition of the essential oils is well documented (Pereira and De Andrade 1998; Gobbo-Neto and Lopes 2007; Benso et al. 2015; Juliani 2016) and may have implications on their biological effects (Desjardins 2008).

In the summer, all genotypes displayed a lower oil yield than in the winter. In the summer, in Oratórios, the temperatures are much higher than in the winter. The high temperatures increase the chemical reactions interfering in oil

stability and contribute to the free radicals formation that stimulates the oxidation process (Turek and Stintzing 2013).

Weather parameters influence the oil yield and composition of essential oils in aromatic plants (Gobbo-Neto and Lopes 2007; Gasparetto et al. 2010). The increase in maximum temperature during the summer can reduce oil content. However, it can also increase the concentration of some chemical constituents due to influencing modulations of the plant's metabolic pathways by photosynthetic carbon production, leading to the group of sesquiterpenoids (Sangwan et al. 2001). The weather parameters during winter can also affect these metabolic pathways favoring specific constituents (Sangwan et al. 2001; de Castro et al. 2003; Gobbo-Neto and Lopes 2007).

In *Mikania*'s essential oils composition, germacrene D, bicyclogermacrene, caryophyllene oxide, and spathulenol have been reported as the major compounds (Limberger et al. 2001; Ferreira and De Oliveira 2010; Guimarães et al. 2012). These components are related to some biological activities, such as intense antimicrobial activity (Betoni et al. 2006; Duarte et al. 2007; Matawali et al. 2016) and vigorous activity against *Candida albicans* (Duarte et al. 2005). According to Judzentiene et al. (2016), the essential oil of *Eupatorium cannabium* L., rich in germacrene D and spathulenol, presented high levels of antioxidant activity.

Besides, Gasparetto et al. (2012b) quoted germacrene D, bicyclogermacrene, caryophyllene oxide, and spathulenol, α-humulene, germacrene B as major constituents involved in *Mikania*'s biological effects. Guimarães et al. (2012) studied *Mikania glauca*'s essential oil from two seasons, winter and spring. Their chromatography analysis demonstrated the predominance of monoterpene hydrocarbons in the essential oil and the influence of season on components concentrations. Other studies with the genus *Mikania*, of similar genotypes, have described similar chemical profile (Rehder et al. 2006), with sesquiterpenes production (Yatsuda et al. 2005; Rufatto et al. 2013).

Moreover, the genotype adaptation to weather variations of each region of production will influence the concentration of chemical constituents in essential oils (Chatterjee et al. 2015). In this study, the CENARGEN genotype had high oil yield in both periods of harvest: summer and winter, and presented high content of components detected in the chromatographic analyses even with a reduction in leaf and stem production in the winter. In the final analysis, this genotype displayed a better adaptation to this region of cultivation Zona da Mata, Minas Gerais, Southeast of Brazil, concerning biomass production and essential oils quality and yield.

As depicted by literature, the different species and genotypes of *Mikania* spp. have a wide variety of chemical composition and yields of essential oils and extracts (Limberger et al. 2001; Rehder et al. 2006; Gasparetto et al. 2010; Czelusniak et al. 2012). Coumarin is a chemical marker of *Mikania*, which has been associate with the therapeutic effect of *Mikania*'s extract (Gasparetto et al. 2012a). Although, it is essential to emphasize that the complex arrangement of molecules and the synergism between all groups of bioactive substances interfere in the therapeutic effect and on its mechanisms of action (Harris 2002; Betoni et al. 2006; Zoubiri and Baaliouamer 2014; Kaulmann and Bohn 2016). The selection of the correct genotype adapted to the producing region and the quantification of the active compound guarantee the quality of raw material and the safety of therapeutic effect (Scalzo et al. 2005; Chatterjee et al. 2015).

Anatomical study

As observed in *M. laevigata*, a single-layered epidermis on both adaxial and abaxial sides have also been found for other Asteraceae species (Bartoli et al. 2011; Oliveira et al. 2013; Kromer et al. 2016; Raman et al. 2018; Bezerra et al. 2018) and seems to be a common characteristic for this family. Other studies have reported the presence of a single-layered epidermis for *M. laevigata* (Oliveira et al. 1986; Budel et al. 2009; Costa et al. 2018) and other species of *Mikania* (Amorin et al. 2014; Almeida et al. 2017). Other authors also claim the presence of a hypodermis only on the adaxial leaf of *M. laevigata* (Oliveira et al. 1986; Milan et al. 2006; Budel et al. 2009). The position of the hypodermis is quite a distinctive characters when compared to other species of *Mikania,* which lack such features, such as *Mikania lanuginosa* DC (Amorin et al. 2014), *Mikania campanulata* Gardner, *Mikania cordifolia* (L.f.) Willd., *Mikania glomerata* Spreng., *Mikania hastato-cordata* Malme, *Mikania micropterа* DC., and *Mikania sessilifolia* DC (Almeida et al. 2017). However, *Mikania glomerata* presents the same anatomical feature (Milan et al. 2006; Almeida et al. 2017).

Dorsiventral mesophyll, collateral vascular bundles and secretory ducts associated with the xylem have also been reported for *M. laevigata* (Oliveira et al. 1986; Milan et al. 2006; Budel et al. 2009; Costa et al. 2018). As the number of palisade/spongy parenchyma layers vary among Asteraceae species (Simon et al. 2002; Milan et al. 2006; Bezerra et al. 2018), such characteristics along, with the presence of a hypodermis on the adaxial side of the leaf blade, could be used as an excellent diagnostic feature in the identification of *M. laevigata* aiding in the quality control of dried leaves sold at markets. It is interesting to notice that our study evaluated three different accessions of *M. laevigata* (CENARGEN, CPQBA, and UNAERP), and all accessions displayed the same characteristics described above, again confirming the potential of using such characteristics as an additional taxonomic tool. However, *M. laevigata* and *M. glomerata* are challenging to distinguish, based on morphoanatomical characters (Gasparetto et al. 2010; Costa et al. 2018).

Several authors reported curved secretory trichomes, type II, scattered on the abaxial epidermis for several species of *Mikania* spp. (Castro et al. 1997; Milan et al. 2006; Budel et al. 2009; Gasparetto et al. 2010; Amorin et al. 2014; Almeida et al. 2017). However, some authors mention that the apical cell varied from a spherical to a spatulate shape (Almeida et al. 2017) while in our study, only a spherical shape was observed. Several types of trichome are found in Asteraceae, and they are crucial for the taxonomy of the family (Anderson and Weberg 1974; Anderson et al. 1979; Ascensão and Pais 1987; Castro et al. 1997; Oliveira et al. 2013; Dos Santos et al. 2015; Rojas-Leal et al. 2018).

The number of vascular bundles in the midrib seems to be promising distinctive character among *Mikania* species. For instance, the number of vascular bundles at the midrib is fixed at six for *M. lanuginosa* (Amorin et al. 2014). However, once again, such characteristic overlaps when comparing *M. glomerata,* and *M. laevigata* as the number of vascular bundles for both species varies from 3 to 5 when considering other studies (Milan et al. 2006; Costa et al. 2018) as well as ours. The major difference is found in *M. laevigata*, as it may present up to eight vascular bundles (Gasparetto et al. 2010).

The secretory trichomes displayed positive results only to polysaccharides and lactones. This result is on the counterhand of Costa et al. (2018), who claimed positive results for lipids. However, the authors did not mention any control test, neither for Sudan Red, nor Nile Blue reagents, suggesting that probably other lipophilic compounds, but not lipids have been stained instead. Besides, the study did not present ant picture of histochemical analyses on the trichomes. The presence of lipidic substances was reported for *M. glomerata* (Milan et al. 2006). A more complex mixture of compounds was observed to be secreted by other types of trichomes in Asteraceae (Ascensão and Pais 1987; Pagni et al. 2004; Andreucci et al. 2008). As proved by our study, when comparing the secretory trichomes of *M. laevigata* to *M. glomerata*, the chemical nature of the exudates of secretory trichomes is promising as an additional tool to the taxonomy. However, few studies investigated the chemical nature of secretory trichomes in *Mikania*. Usually, the secretory ducts are the major concern of the investigations.

Our results show that the *Mikania* medicinal metabolites are produced by the secretory epithelial cells lining the ducts, which discharge their secretion into the lumen. Such metabolites are made up of a mixture of compounds the secretory epithelium cells, as well as the secretion within the lumen, displayed positive results to oil/resins, lactones, and rubber. The histochemical tests for total proteins, total polysaccharides, acid mucopolysaccharides, pectins, and total lipids turned negative results. However, positive results for lipids, and also for phenolic compounds, were reported by Costa et al. (2018). This paper is the first report of rubber in secretory ducts of *M. laevigata*. Although the literature reports that coumarins are the predominant compound in *Mikania* tinctures (Alvarenga et al. 2009), the medicinal properties of *Mikania* may be the result of such a mixture of compounds identified by the histochemical tests instead of coumarins only. It is worth mentioning that the exudates from the leaf secretory trichomes could also contribute to the compounds that show medicinal properties as lactones were also found in their secretion.

Conclusions

This work pointed out the existence of substantial genetic variability among the genotypes. The genotype CENARGEN showed major biomass production, oil yield and a high concentration of major constituents detected by chromatographic analysis in essential oils. The season affected the essential oils chemical composition. Germacrene D and bicyclogermacrene were the major constituents in the winter-essential-oils, and spatulenol and caryophyllene oxide were the major constituents in the summer-essential-oils. The total summer-biomass production was higher than winter biomass production. The anatomical analysis pointed out secretory trichomes and secretory ducts in the leaf. The secretory trichomes highlighted the exclusive presence of polysaccharides and sesquiterpene lactones.

Author contribution statement All authors contributed to the study conception and design. Material preparation and data collection were performed by MCMF, ÍACC, RGRD, RMSAM and AFS. Analyzed the data: MCMF, MBRÁ and ÍACC. The first draft of the manuscript was written by MBRÁ and ÍACC and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgements We thank the Brazilian Agencies: Minas Gerais's Research Foundation FAPEMIG) [CAG-APQ-04676-10] and National Council for Scientific and Technological Development (CNPq) [561136/2010-6] for their financial support. RMSAM thanks CNPq for her productivity grant (DEB #307762/2016-5). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES) Finance Code 001.

Declarations

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this paper.

References

Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectrometry. Allured publishing corporation, Carol Stream

Adler PB, Salguero-gómez R, Compagnoni A et al (2014) Functional traits explain variation in plant life history strategies. Proc Natl Acad Sci 111:740–745. https://doi.org/10.1073/pnas.1410430111

Aguiar JLN, Albert ALM, Moreira JC, Leite PC (2014) RAPD-PCR na identificação molecular de plantas medicinais regulamentadas pelo Sistema Único de Saúde do Brasil. Vigilância Sanitária em Debate. https://doi.org/10.3395/2317-269x.00193

Almeida VP, Hirt AA, Raeski PA et al (2017) Comparative morphoanatomical analysis of Mikania species. Braz J Pharmacogn 27:9–19. https://doi.org/10.1016/j.bjp.2016.05.002

Alvarenga FCR, Garcia EDF, Bastos EMAF et al (2009) Avaliação da qualidade de amostras comerciais de folhas e tinturas de guaco. Braz J Pharmacogn 19:442–448. https://doi.org/10.1590/S0102-695X2009000300018

Amorin M, de Paula JP, da Silva RZ et al (2014) Pharmacobotanical study of the leaf and stem of *Mikania lanuginosa* for its quality control. Rev Bras Farmacogn 24:531–537. https://doi.org/10.1016/j.bjp.2014.10.002

Anderson L, Weberg P (1974) The anatomy and taxonomyh of Vanclevea (Asteraceae). Gt Basin Nat 34:151–160

Anderson LC, Hartman RL, Stuessy TF (1979) Morphology, anatomy, and taxonomic relationships of *Otopappus australis* (Asteraceae). Syst Bot 4:44. https://doi.org/10.2307/2418664

Andreucci AC, Ciccarelli D, Desideri I, Pagni AM (2008) Glandular hairs and secretory ducts of *Matricaria chamomilla* (Asteraceae): morphology and histochemistry. Ann Bot Fenn 45:11–18

Ascensão L, Pais M (1987) Glandular trichomes of *Artemisia campestris* (ssp. Maritima): ontogeny and histochemistry of the secretory product. Bot Gaz 148:221–227

Atanasov AG, Waltenberger B, Pferschy-Wenzig EM et al (2015) Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv 33:1582–1614. https://doi.org/10.1016/j.biotechadv.2015.08.001

Bartoli A, Galati BG, Tortosa RD (2011) Anatomical studies of the secretory structures: glandular trichomes and ducts, in *Grindelia pulchella* Dunal (Astereae, Asteraceae). Flora Morphol Distrib Funct Ecol Plants 206:1063–1068. https://doi.org/10.1016/j.flora.2011.07.012

Benso B, de Alencar S, Rosalen P et al (2015) Antibacterial activity of essential oils and their isolated constituents against cariogenic bacteria: a systematic review. Molecules 20:7329–7358. https://doi.org/10.3390/molecules20047329

Benzie IFF, Wachtel-Galor S (2011) An introduction to its history, usage, regulation, current trends, and research needs. In: Benzie IFF, Wachtel-Galor S (eds) Herbal medicine, 2nd edn. CRC Press/Taylor & Francis, Boca Raton, pp 1–11

Betoni JEC, Mantovani RP, Barbosa LN et al (2006) Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. Mem Inst Oswaldo Cruz 101:387–390. https://doi.org/10.1590/S0074-02762006000400007

Bezerra LDA, Mangabeira PAO, de Oliveira RA et al (2018) Leaf blade structure of *Verbesina macrophylla* (Cass.) F. S. Blake (Asteraceae): ontogeny, duct secretion mechanism and essential oil composition. Plant Biol 20:433–443. https://doi.org/10.1111/plb.12700

Brasil, Agencia Nacional de Vigilância Sanitária (2010) Farmacopéia Brasileira, 5th edn, vol 1. http://www.anvisa.gov.br/hotsite/cd_farmacopeia/pdf/volume1.pdf

Budel JM, Duarte MR, Kosciuv I et al (2009) Contribuição ao estudo farmacognóstico de *Mikania laevigata* Sch. Bip. ex Baker (guaco), visando o controle de qualidade da matéria-prima. Braz J Pharmacogn 19:545–552. https://doi.org/10.1590/S0102-695X2009000400006

Calixto JB (2000) Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines. Brazi J Med Biol Res 33:179–189. https://doi.org/10.1590/S0100-879X2000000200004

Canter PH, Thomas H, Ernst E (2005) Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. Trends Biotechnol 23:180–185. https://doi.org/10.1016/j.tibtech.2005.02.002

Castro MM, Leitão-Filho HF, Monteiro WR (1997) Utilização de estruturas secretoras na identificação dos gêneros de Asteraceae de uma vegetação de cerrado. Rev Bras Botânica 20:163–174. https://doi.org/10.1590/S0100-84041997000200007

Chatterjee B, Pancholi J, Kevalia J, Kothari V (2015) Significance of molecular markers and DNA based technology in research and standardization of medicinal plants : a review. Int J Res Med 4:5–16

Costa VCDO, Borghi AA, Mayer JLS, Sawaya ACHF (2018) Comparison of the morphology, anatomy, and chemical profile of *Mikania glomerata* and *Mikania laevigata*. Planta Med 84:191–200. https://doi.org/10.1055/s-0043-119226

Czelusniak KE, Brocco A, Pereira DF, Freitas GBL (2012) Farmacobotânica, fitoquímica e farmacologia do Guaco: Revisão considerando *Mikania glomerata* Sprengel e *Mikania laevigata* Schulyz Bip. ex Baker. Rev Bras Plantas Med 14:400–409. https://doi.org/10.1590/S1516-05722012000200022

David R, Carde J-D (1964) Coloration différentielle des inclusions lipidiques et terpéniques des pseudophylles du Pin maritime au moyen du réactif nadi. Comptes Rendus l'Académie des Sci Paris 258:1338–1340

de Zago MSL (2018) Vinte e dois anos de pesquisa sobre plantas medicinais: uma análise cienciométrica. Tecnia 3:157–173

de Castro EM, Pinto JEBP, Alvarenga AA et al (2003) Crescimento e anatomia foliar de plantas jovens de *Mikania glomerata* Sprengel (Guaco) submetidas a diferentes fotoperíodos. Cienc e Agrotecnologia 27:1293–1300. https://doi.org/10.1590/S1413-70542003000600013

de Guimarães LGL, Nelson DL, de Souza JA et al (2012) Chemical analyses of the essential oils from leaves of *Mikania glauca* Mart. ex Baker. J Essent Oil Res 24:599–604. https://doi.org/10.1080/10412905.2012.742789

Desjardins AE (2008) Natural product chemistry meets genetics: when is a genotype a chemotype? J Agric Food Chem 56:7587–7592. https://doi.org/10.1021/jf801239j

dos Santos SC, Krueger CL, Junior AA et al (2006) LC Characterisation of guaco medicinal extracts, *Mikania laevigata* and *M. glomerata*, and their effects on allergic pneumonitis. Planta Med 72:679–684. https://doi.org/10.1055/s-2006-931577

Dos Santos VLP, Franco CRC, Amano E et al (2015) Anatomical investigations of *Piper amalago* (Jaborandi-manso) for the quality control. Braz J Pharmacogn 25:85–91. https://doi.org/10.1016/j.bjp.2015.03.001

Duarte MCT, Figueira GM, Pereira B et al (2004) Atividade antimicrobiana de extratos hidroalcólicos de espécies da coleção de plantas medicinais CPQBA/UNICAMP. Rev Bras Farmacogn 14:06–08

Duarte MCT, Figueira GM, Sartoratto A et al (2005) Anti-Candida activity of Brazilian medicinal plants. J Ethnopharmacol 97:305–311. https://doi.org/10.1016/j.jep.2004.11.016

Duarte MCT, Leme EE, Delarmelina C et al (2007) Activity of essential oils from Brazilian medicinal plants on Escherichia coli. J Ethnopharmacol 111:197–201. https://doi.org/10.1016/j.jep.2006.11.034

Dutra RC, Campos MM, Santos ARS, Calixto JB (2016) Medicinal plants in Brazil: pharmacological studies, drug discovery, challenges and perspectives. Pharmacol Res 112:4–29. https://doi.org/10.1016/j.phrs.2016.01.021

Farzaneh V, Carvalho IS (2015) A review of the health benefit potentials of herbal plant infusions and their mechanism of actions. Ind Crop Prod 65:247–258. https://doi.org/10.1016/j.indcrop.2014.10.057

Ferreira FP, De Oliveira DCR (2010) New constituents from *Mikania laevigata* Shultz Bip. ex Baker. Tetrahedron Lett 51:6856–6859. https://doi.org/10.1016/j.tetlet.2010.10.102

Fonseca JM, Rushing JW, Rajapakse NC et al (2006) Potential implications of medicinal plant production in controlled environments: the case of feverfew (*Tanacetum parthenium*). HortScience 41:531–535

Gasparetto JC, Campos FR, Budel JM, Pontarolo R (2010) *Mikania glomerata* Spreng. e *M. laevigata* Sch. Bip. ex Baker, Asteraceae: Estudos agronômicos, genéticos, morfoanatômicos, químicos, farmacológicos, toxicológicos e uso nos programas de fitoterapia do Brasil. Braz J Pharmacogn 20:627–640. https://doi.org/10.1590/S0102-695X2010000400025

Gasparetto JC, Francisco TMG De, Pontarolo R (2012a) Chemical constituents of *Mikania glomerata* Spreng and M*ikania laevigata* Sch. Bip. ex Baker. In: William Acree. Toxicity and drug testing. Intech, Rijeka, pp 297–320

Gasparetto JC, Roberto Pontarolo, Francisco TMG de, Campos FR (2012b) *Mikania glomerata* and *M. laevigata*: clinical and toxicological advances. In: Toxicity and drug testing. p 64

Geissman TA, Griffin TS (1971) Sesquiterpene lactones: acid-catalyzed color reactions as an aid in structure determination. Phytochemistry 10:2475–2485

Gobbo-Neto L, Lopes NP (2007) Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quim Nova 30:374–381. https://doi.org/10.1590/S0100-40422007000200026

Govindaraghavan S, Sucher NJ (2015) Quality assessment of medicinal herbs and their extracts: criteria and prerequisites for consistent safety and efficacy of herbal medicines. Epilepsy Behav 52:363–371. https://doi.org/10.1016/j.yebeh.2015.03.004

Graça C, Baggio CH, Freitas CS et al (2007) In vivo assessment of safety and mechanisms underlying in vitro relaxation induced by *Mikania laevigata* Schultz Bip. ex Baker in the rat trachea. J Ethnopharmacol 112:430–439. https://doi.org/10.1016/j.jep.2007.03.026

Harris R (2002) Synergism in the essential oil world. Int J Aromather 12:179–186. https://doi.org/10.1016/S0962-4562(02)00083-8

Johansen DA (1940) Plant microtechnique. Mcgraw-Hill, New York

Judzentiene A, Garjonyte R, Budiene J (2016) Variability, toxicity, and antioxidant activity of *Eupatorium cannabinum* (hemp agrimony) essential oils. Pharm Biol 54:945–953. https://doi.org/10.3109/13880209.2015.1078384

Juliani HR (2016) Physical and chemical properties, composition, and biological activity of essential oils of philippine medicinal plants. J Med Act Plants 5:28–35. https://doi.org/10.7275/R5P84933

Karnovsky MJ (1965) A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J Cell Biol 27:137–138

Kaulmann A, Bohn T (2016) Bioactivity of polyphenols: preventive and adjuvant strategies toward reducing inflammatory bowel diseases—promises, perspectives, and pitfalls. Oxid Med Cell Longev 2016:1–29. https://doi.org/10.1155/2016/9346470

Kromer K, Kreitschitz A, Kleinteich T et al (2016) Oil secretory system in vegetative organs of three Arnica taxa: essential oil synthesis, distribution and accumulation. Plant Cell Physiol 57:1020–1037. https://doi.org/10.1093/pcp/pcw040

Limberger RP, Aboy AL, Bassani VL et al (2001) Essential oils from four Mikania species (Asteraceae). J Essent Oil Res 13:225–228. https://doi.org/10.1080/10412905.2001.9699676

Matawali A, Chin LP, Eng HS, Gansau JA (2016) Antibacterial and phytochemical investigations of Mikania micrantha H. B. K. (Asteraceae) from Sabah, Malaysia. Trans Sci Technol 3:244–250

Milan P, Hayashi AH, Appezzato-da-Glória B (2006) Comparative leaf morphology and anatomy of three Asteraceae species. Braz Arch Biol Technol 49:135–144. https://doi.org/10.1590/s1516-89132006000100016

Napimoga MH, Yatsuda R (2010) Scientific evidence for *Mikania laevigata* and *Mikania glomerata* as a pharmacological tool. J Pharm Pharmacol 62:809–820. https://doi.org/10.1211/jpp.62.07.0001

O'Brien TP, McCully ME (1981) The study of plant structure principles and selected methods. Termarcarphi Ptey. Ltd., Melbourne

Oliveira F, Akisue G, Akisue M, Jorge L (1986) Morfodiagnose das folhas e das partes reprodutivas de *Mikania laevigata* Schultz Bip. ex Baker. Rev Bras Farmacogn 1:20–34

Oliveira T, Bombo AB, Appezzato-Da-Glória B (2013) Anatomy of vegetative organs with an emphasis on the secretory structures of two species of Aldama (Asteraceae–Heliantheae). Botany 91:335–342. https://doi.org/10.1139/cjb-2012-0271

Osman AG, Haider S, Chittiboyina AG, Khan IA (2018) Utility of alkaloids as chemical and biomarkers for quality, efficacy, and safety assessment of botanical ingredients. Phytomedicine. https://doi.org/10.1016/j.phymed.2018.03.064

Pagni A, Olarndo R, Masini A, Ciccarelli D (2004) Secretory structures of Santolina ligustica Arrigoni (Asteraceae), an Italian endemic species. Isr J Plant Sci 51:185–192. https://doi.org/10.1560/ggyh-4404-qc86-krhv

Pearse AGE (1980) Histochemistry, theoretical and applied. Churchill, London

Pereira PADP, De Andrade JB (1998) Fontes, reatividade e quantificação de metanol e etanol na atmosfera. Quim Nova 21:744–754. https://doi.org/10.1590/S0100-40421998000600015

Pereira A, Montanari Junior I, da Silva D et al (2016) Effect of accessions and environment conditions on coumarin, O-coumaric and kaurenoic acids levels of *Mikania laevigata*. Planta Med 82:1431–1437. https://doi.org/10.1055/s-0042-108339

Prinsloo G, Nogemane N (2018) The effects of season and water availability on chemical composition, secondary metabolites and biological activity in plants. Phytochem Rev 17:889–902. https://doi.org/10.1007/s11101-018-9567-z

Raman V, Takeda IJM, Almeida VP et al (2018) Foliar anatomy and microscopy of six Brazilian species of Baccharis (Asteraceae). Microsc Res Tech 81:832–842. https://doi.org/10.1002/jemt.23045

Raposo JDA, Nevesa FG, de Aguiar WA et al (2017) Cultivation of Guaco (*Mikania laevigata* Sch. Bip. ex Baker) in the Lower Amazon River and monitoring of coumarin, its principal active constituent. Rev Fitos 10:227–237. https://doi.org/10.5935/2446-4775.20160018

Rehder VLG, Sartoratto A, Rodrigues MVN (2006) Essential oils composition from leaves, inflorescences and seeds of *Mikania laevigata* Schultz Bip. ex Baker and *Mikania glomerata* Sprengel. Rev Bras Plantas Med 8:116–118

Ricardo LM, Brandão MGL (2018) *Mikania glomerata* Spreng. & *Mikania laevigata* Sch. Bip. ex Baker. In: Albuquerque U, Patil UMÁ (eds) Medicinal and Aromatic Plants of the World, 5th edn. Springer, Dordrecht, pp 337–344

Ritter MR, Liro RM, Roque N, Nakajima JN, Souza-Buturi FO, Oliveira CT (2015) *Mikania* in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. http://reflora.jbrj.gov.br/jabot/floradobrasil/FB5378. Accessed 2 Nov 2019

Rojas-Leal A, Terrazas T, Villaseñor J (2018) Foliar architecture of some members of the tribe Senecioneae (Asteraceae) with a key for identification of the Mexican genera. Phytotaxa 364:136. https://doi.org/10.11646/phytotaxa.364.2.2

Rufatto LC, Finimundy TC, Roesch-Ely M, Moura S (2013) *Mikania laevigata*: chemical characterization and selective cytotoxic activity of extracts on tumor cell lines. Phytomedicine 20:883–889. https://doi.org/10.1016/j.phymed.2013.03.016

Salgueiro L, Martins AP, Correia H (2010) Raw materials: the importance of quality and safety. A review. Flavour Fragr J 25:253–271. https://doi.org/10.1002/ffj.1973

Sangwan NS, Farooqi AHA, Shabih F, Sangwan RS (2001) Regulation of essential oil production in plant. Plant Growth Regul 34:3–21

Scalzo J, Politi A, Pellegrini N et al (2005) Plant genotype affects total antioxidant capacity and phenolic contents in fruit. Nutrition 21:207–213. https://doi.org/10.1016/j.nut.2004.03.025

Simon P, Katinas L, Arambarri A (2002) Secretory structures in *Tagetes minuta* (Asteraceae, Helenieae). Bol la Soc Argetina Bot 37:181–191

Sucher NJ, Carles MC (2008) Genome-based approaches to the authentication of medicinal plants. Planta Med 74:603–623. https://doi.org/10.1055/s-2008-1074517

Suyenaga ES, Reche E, Farias FM et al (2002) Antiinflammatory investigation of some species of Mikania. Phyther Res 16:519–523. https://doi.org/10.1002/ptr.908

Turek C, Stintzing FC (2013) Stability of essential oils: a review. Compr Rev Food Sci Food Saf 12:40–53. https://doi.org/10.1111/1541-4337.12006

Wullschleger SD, Sanderson MA, McLaughlin SB, Biradar DP, Rayburn AL (1996) Photosynthetic rates and ploidy levels among populations of switchgrass. Crop Sci 36:306–312

Yatsuda R, Rosalen PL, Cury JA et al (2005) Effects of Mikania genus plants on growth and cell adherence of mutans streptococci. J Ethnopharmacol 97:183–189. https://doi.org/10.1016/j.jep.2004.09.042

Zandalinas SI, Mittler R, Balfagón D et al (2018) Plant adaptations to the combination of drought and high temperatures. Physiol Plant 162:2–12. https://doi.org/10.1111/ppl.12540

Zoubiri S, Baaliouamer A (2014) Potentiality of plants as source of insecticide principles. J Saudi Chem Soc 18:925–938. https://doi.org/10.1016/j.jscs.2011.11.015