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Effect of drying temperature on yield and phytochemical quality of essential oil extracted from *Mikania laevigata* (Guaco) leaves

Efeito da temperatura de secagem sobre o rendimento e qualidade fitoquímica de óleo essencial extraído de folhas de *Mikania laevigata* (Guaco)

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Maira Christina Marques Fonseca
Doutora em Fitotecnia pela Universidade Federal de Viçosa (UFV)
Pesquisadora - Empresa de Pesquisa Agropecuária de Minas Gerais
Endereço: Vila Gianeti, casa 46. Campus da UFV, Viçosa, MG, Brasil. CEP: 36570-900 E-mail: mairacmf@yahoo.com.br

Cláudia Lúcia de Oliveira Pinto
Doutora em Microbiologia Agrícola pela Universidade Federal de Viçosa (UFV)
Pesquisadora aposentada - Empresa de Pesquisa Agropecuária de Minas Gerais Endereço: Rua dos Estudantes, 120, apto 202, Centro, Viçosa, MG, Brasil. CEP: 36570-081 E-mail: caudia.epamig@gmail.com

Adilson Sartoratto
Doutor em Química pela Universidade Estadual de Campinas (Unicamp)
Pesquisador do Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas da Unicamp
Endereço: Rua Alexandre Cazelatto, 999 - Vila Betel, Paulínia, SP, Brasil. CEP: 13148-218 E-mail: adilson@cpqba.unicamp.br

Túlio I. Machado
Mestrando em Biologia pela Universidade Federal de Viçosa (UFV) Endereço: Av. Peter Henry Rolfs, s/n - Campus Universitário, Viçosa, MG, Brasil. CEP: 36570-900
E-mail: tulioiglesias96@gmail.com

Estermary P. Bitencourt
Farmacêutica pela Univiçosa – Centro Universitário e Viçosa
Endereço: Av. Maria de Paula Santana, 3815 - Silvestre, Viçosa, MG, Brasil, CEP: 36576-340 E-mail: estermarypb@hotmail.com

Yonara P. Neves
Doutora em Fitotecnia pela Universidade Federal de Viçosa (UFV)
Pesquisadora da Empresa de Pesquisa Agropecuária de Minas Gerais
Endereço;Vila Gianeti, casa 46. Campus da UFV, Viçosa, MG, Brasil. CEP: 36570-900
E-mail: yonarapoltronieri@hotmail.com

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Maria Regina de M. Souza
Doutora em Fitotecnia pela Universidade Federal de Viçosa (UFV)
Pesquisadora da Empresa de Pesquisa Agropecuária de Minas
Endereço:Vila Gianeti, casa 46. Campus da UFV, Viçosa, MG, Brasil. CEP: 36570-900
E-mail: reginas.epamig@gmail.com

Sérgio Maurício L. Donzeles
Doutor em Engenharia Agrícola pela Universidade Federal de Viçosa.
Pesquisador da Empresa de Pesquisa Agropecuária de Minas Gerais
Endereço:Vila Gianetti, Casa 46, Campus da UFV, Viçosa, MG, Brasil. CEP: 36570-900
E-mail: slopes@epamig.br

Andréia F. Silva
Mestre em Botânica pela Universidade Federal de Viçosa.
Pesquisadora da Empresa de Pesquisa Agropecuária de Minas
Endereço:Av. José Cândido da Silveira, 1.647, União, Belo Horizonte, MG, Brasil. CEP: 31170-495
E-mail: andreiasilva@epamig.br

Maria Aparecida N. Sediyama
Doutora em Fitotecnia pela Universidade Federal de Viçosa (UFV)
Pesquisadora aposentada da Empresa de Pesquisa Agropecuária de Minas
Endereço:Rua Vinícius de Moraes, 157, Bairro de Fátima, Viçosa, MG, Brasil. CEP: 36572-164
E-mail: mariasediyama@gmail.com

RESUMO
Mikania laevigata Sch. Bip. ex Baker, Asteraceae, comumente conhecida como guaco, é uma espécie medicinal nativa do Brasil. Dentre os pontos críticos do processamento pós-colheita de espécies medicinais, a temperatura de secagem deve ser considerada, pois pode interferir no rendimento e na qualidade fitoquímica do material vegetal e, consequentemente, na ação terapêutica. O efeito da temperatura de secagem foi avaliado no rendimento e na qualidade fitoquímica do óleo essencial extraído das folhas de *M. laevigata*. O cultivo foi realizado em sistema orgânico, utilizando o genótipo selecionado (Cenargen) para esta região. As folhas foram colhidas e imediatamente submetidas ao processo de secagem a 40, 50 e 60 ° C. Os óleos essenciais foram extraídos por hidrodestilação em aparelho de Clevenger e os constituintes químicos identificados por cromatografia gasosa acoplada ao espectrofotômetro de massa (CG-MS). Houve redução no rendimento de óleo essencial ($p < 0,05$) com o aumento da temperatura de secagem. Entretanto, a 60 ºC, houve maior concentração de cumarina, à qual é atribuída a ação broncodilatadora e expectorante. Portanto, recomendamos secar as folhas de *M. laevigata* a 60 ºC para obter o maior teor de cumarina e garantir o efeito terapêutico broncodilatador e expectorante.

Palavras chave: constituintes químicos, plantas medicinais, pos-colheita, *Mikania*.

ABSTRACT
Mikania laevigata Sch. Bip. ex Baker, Asteraceae, commonly known as the guaco, is medicinal species native to Brazil. Among the critical points of post-harvest processing of medicinal species, the drying temperature must be considered, because its can interfere in the yield and phytochemical quality of plant material, and, consequently, in the therapeutic action. The effect of the drying

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temperature was evaluated on the yield and phytochemical quality of the essential oil extracted from *M. laevigata* leaves. The cultivation was carried out in an organic system and using the select genotype (Cenargen) for this region. The leaves were harvested and immediately submitted to drying process at 40, 50 and 60 °C. The essential oils were extracted by hydrodistillation in Clevenger apparatus and the chemical constituents was evaluated using gas chromatography coupled to the mass spectrophotometer (CG-MS). There was a reduction in the essential oil yield ($p < 0.05$) with increasing drying temperature. However, at 60 ºC there was a higher concentration of coumarin to which the bronchodilator and expectorant action is attributed. Therefore, we recommended dry the *M. laevigata* leaves at 60 ºC to obtain the highest coumarin content and guarantee the bronchodilator and expectorant therapeutic effect.

Key words: Chemical constituents; medicinal plants; postharvest; *Mikania*.

1 INTRODUCTION

Mikania laevigata Sch. Bip. ex Baker, Asteraceae, also known as guaco, it is a perennial species from Brazil (Mikania, 2020). It is considered as an medicinal herb due to its therapeutic properties, such as antiallergic (FIERRO et al., 1999; SANTOS, 2005), antimicrobial (YATSUDA et al., 2005; MOREIRA et al., 2016), analgesic, anti-inflammatory (RUPPELT et al., 1991), anti-hemorrhagic (MOURÃO et al., 2014), anxiolytic, antioxidant (SANTANA et al., 2014) and anti-diarrheal (SALGADO et al., 2005). This species stands out due to its properties as bronchodilator and expectorant (MINISTÉRIO Da SAÚDE, 2018). It is also used in folk medicine for health treatment, such as asthma, bronchitis, chronic lung diseases, cough, and rheumatism (LUCAS, 1941; OLIVEIRA et al., 1994, MINISTÉRIO DA SAÚDE, 2018). Thereby, in Brazil, *M. laevigata* it is listed in National List of Medicinal Plants of Interest to the Unified Health System (RENISUS) as part of governmental program to guide researches and studies about medicinal species (Carvalho, 2011). In the Brazilian Pharmacopoeia, the use of leaves of *M. laevigata* is recommended as expectorant and to ease coughs due to balsamic effect of its leaves (ANVISA, 2018).

M. laevigata presents cinnamic acid derivatives from bioactive compounds (cumarina, ácido o-cumárico), also kurene type diterpenes (kaurenoic acid, benzoylgrandifluoric acid, cinaminoyl-grandi hydrofluoric acid) (SANTOS et al., 2006; BERTOLUCCI et al., 2013). Among these, coumarin stands out (chemical species marker), which it is assigned aromatic and pharmacological characteristics (CZELUSNIAK et al., 2012). The coumarin is mainly present in the leaves and because of its biological proprieties; it is recognized in treatment of respiratory diseases like asthma and bronchitis (FREITAS et al., 2008; ALVES et al., 2009; CZELUSNIAK et al., 2012).

In post-harvest processing, several factors can influence the phytochemistry quality and the essential oil yield (CANTWELL; REID, 1994; GOBBO-NETO; LOPES, 2007). Among these

factors, drying is a critical process for conservation and maintaining the quality of medicinal properties, which is essential for the pharmaceutical industry in the production of herbal medicines according to the standards of quality and therapeutic efficacy. Therefore, drying is a critical process for bioactive compounds conservation and achieving recommended yield of bioactive compounds (CORRÊA et al., 2004; LORENZI and MATOS, 2008). It should be noted that the drying method, the velocity, and the air temperature may influence the content of the active principle of medicinal plants (MELO et al., 2004). Wherefore, the influence of drying temperature of medicinal species on the yield and the phytochemical quality of essential oils are important for establishing conditions that preserve the chemical constituents of therapeutic interest and the economic value added to the vegetal material. The objective of this study was to verify the effect of drying temperature on the yield and the phytochemical quality of essential oils extracted from M. laevigata (Cenargen genotype) leaves, grown in an organic system.

2 MATERIAL AND METHODS

2.1 CULTIVATION OF *M. laevigata*

The research was carried out at the Piranga Valley Experimental Field of the Minas Gerais Agricultural Research Company (EPAMIG), in Oratórios, Minas Gerais, Brazil. The geographical coordinates of the municipality are: latitude 20°30” S, 43°00” W and the altitude was 500 m, with the following meteorological variables: average annual maximum temperature of 21.8 °C and annual minimum of 19.5 °C and precipitation average annual rainfall of 1.250 mm. The predominant climatic type in the place is the Cwa, humid tropical, the Aw, semi-humid of hot summers, being the original vegetation constituted of subcaducifolia tropical forest, according to Köppen's classification (Cunha et al., 2000). The soil was classified as sandy loam-clay, with the following chemical characteristics: pH (water 1: 2.5) = 6.2; P (Mehlich) = 49.8 mg dm^{-3}; P (remainder) = 40 mg L^{-1}; K = 220 mg dm^{-3}; Ca = 2.6 cmol dm^{-3}; Mg = 1.3 cmol dm^{-3}; Al = 0 cmol dm^{-3}; H + Al = 2.8 cmol dm^{-3}; SB = 4.5 cmol dm^{-3}; t = 4.5 cmol dm^{-3}; m = 0%; T = 7.3 cmol dm^{-3}; V = 61%; Organic matter = 2.63 dag kg^{-1}.

Fertilization was carried out, according to the soil analysis, using cattle manure, with the following chemical characteristics (%): N (1.4), P (0.39), K (0.88), Ca (1.54), Mg (0.27), S (0.23), CO (10.45); C/N (7.46). The drip irrigation system was used and for the control of spontaneous plants, manual weeding was performed when necessary.

Seedlings of *M. laevigata* (Cenargen genotype) were purchased from Embrapa Genetic Resources. The planting and cultivation in an organic system, in 16 x 32 m area and using the 2 x 1

m spacing. Branches were harvested from 40 useful plants in the central part of the production area. The harvest was carried out in August of 2019 at morning period. Immediately after harvesting, the leaves were detached from the branches, selected and dried at temperatures of 40, 50 and 60 ºC in an oven with forced air circulation until reaching constant weight. Subsequently, 30 samples (100 g) of dry leaves were separated, packaged in polyethylene bags, sealed and conditioned in kraftpaper bag until the extraction of essential oils.

2.2 PLANT MATERIAL

Samples of *Mikania laevigata* Sch. Bip. ex Baker, Asteraceae (Genotype Cenargen-Embapa), were collected in Oratórios, Minas Gerais, Brazil, and authenticated by Andréia Fonseca Silva in Minas Gerais Agricultural Research Company (EPAMIG). A voucher specimen (PAMG 57032) is deposited at the Herbarium PAMG of EPAMIG, Belo Horizonte, Minas Gerais, Brazil.

2.3 EXTRACTION OF ESSENTIAL OIL, CALCULATION OF YIELD AND STORAGE

The extraction of essential oil from the leaves using the modified Clevenger apparatus adapted to a round-bottomed flask with a capacity of 2000 mL. In each extraction, 500 mL of distilled water and 100 g of leaves were added to the flask, beginning the hydrodistillation process. The extraction was carried out by dragging the essential oil through water vapor, for 4 hours. After extraction, the oil yield was calculated by the difference between the final weight of the bottle containing the oil and the initial weight of the bottle, without oil, obtaining the yield for 100 g of leaves. The oil samples were stored at 4 °C in an amber glass bottle with a screw cap, until chromatographic analysis.

2.4 ANALYSIS AND IDENTIFICATION OF VOLATILE CHEMICAL CONSTITUENTS

The identification and content of volatile constituents were carried out in an Agilent gas chromatograph, model HP-6890, equipped with an Agilent mass selective detector, model HP-5975 and an HP-5MS capillary column (30 m x 0.25 mm x 0.25 µm). The splitless injection mode was used, in the following temperature conditions: injector at 220 °C, column at 60 °C, with heating ramp of 3 °C/min^{-1} and final temperature of 240 °C and detector at 250 °C. Helium was used as carrier gas at a flow rate of 1 mL/min^{-1}. A sample of essential oil was dissolved in ethyl acetate (20 mg/mL^{-1}) for analysis. The identification of the analytes was carried out by comparing the retention indices (IR) obtained by the injection of hydrocarbon standards (C-8 to C-24), with the equipment's database (NIST-11 library) and with data from literature (Adams, 2007).

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2.6 STATISTICAL ANALYSIS OF OIL YIELD

The Tuckey test was used to compare the averages at 5% probability, with the aid of the SAEG statistical analysis program. (RIBEIRO Jr, 2001).

3 RESULTS AND DISCUSSION

3.1 YIELD OF ESSENTIAL OIL EXTRACTED FROM M. *Laevigata* LEAVES

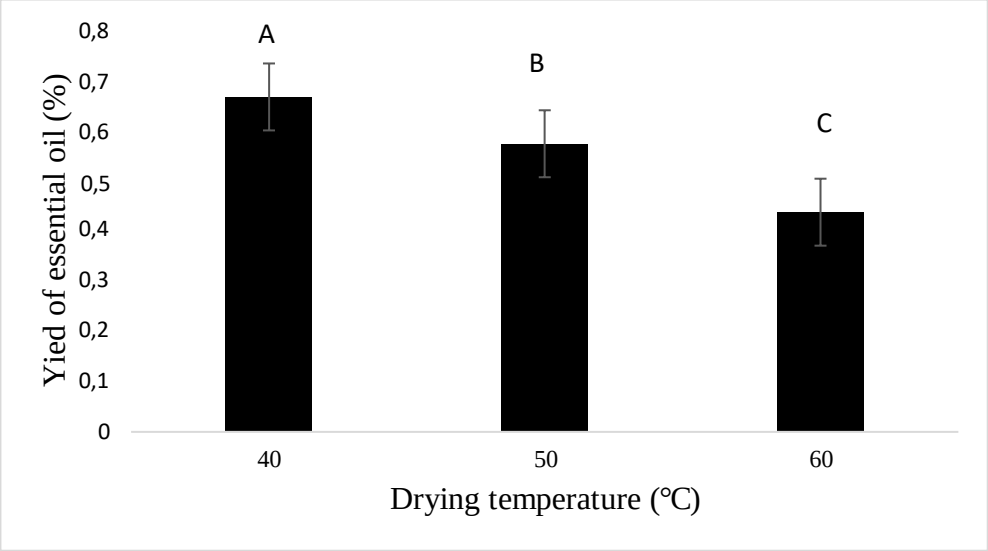
There was a significant difference ($p<0,001$) on essential oil yield due to the increase in the drying temperature of the *M. laevigata* leaves at 40, 50 and 60 ºC (Figure 1). With increasing temperature, there was a loss in essential oil yield of 20,37%, extracted from leaves subjected to drying at 50 ºC compared to the yield of leaves subjected to drying at 40 ºC and 35,36% compared to the yield of leaves subjected to drying at 60 ºC.

According to Oliveira et al. (1999), guaco essential oil is produced, mainly, in the secretory structures interior or glandular channels that is located in the parenchymal tissue of the leaves, not in fragile structures, such as glandular hairs, trichomes and epidermal glands (Rocha et al., 2014). However, as *M. laevigata* leaves were triturated immediately before the extraction process, these structures probably ruptured and, consequently, there was more volatilization of essential oil constituents, resulting in the yield reduction. (Figure 1).

Our results are similar to those observed in other studies on medicinal species drying. Soares et al. (2007) found higher extractive yields of essential oils from basil leaves submitted to drying at 40 ºC, however, higher yield of linalool in leaves subjected to drying between 50 to 60 ºC. Santos Júnior et al (2020) observed that the application of 45 ° C drying temperature promote the best yield for *Alpinia zerumbet*. Figiel et al. (2010) and Szumny et al. (2010) studying the influence of drying methods on the volatile compounds reported that the use of high temperatures promoted a decrease

in the essential oil yield, which is associated with the volatilization of some chemical constituents during the drying process.

Figure 1. Yield of essential oils extracted from leaves of *M. laevigata* submitted to drying in an oven with air circulation at 40, 50 and 60 ºC.



3.2 IDENTIFICATION OF CHEMICAL CONSTITUENTS IN ESSENTIAL OIL EXTRACTED OF *M. laevigata* leaves

The chemical constituents of essential oil extracted from guaco leaves dried at 40, 50, and 60 ºC were identified (Table 1). The majoritary compounds were alpha-pinene, trans-caryophyllene, germacrene D and bicyclogermacrene. Germacrene D was the constituent with the highest concentration in the essential oil extracted from *M. laevigata* leaves at all drying temperatures evaluated (Table 1) and its reduction was observed with increasing temperature (Table 1). This result is similar to that found by Oliveira et al. (1999). The coumarin concentration varied with the increase in the drying temperature of the leaves, and a higher content was observed in leaves dried at 60 ºC (Table 1). Our results are similar to those observed by Pereira et al. (2000) who also found higher coumarin yield in dried plants at a higher temperature (50 ºC) compared to dried leaves at 35 and 25 ºC. Radünz et al. (2012) observed that the coumarin concentration was higher when the leaves were subjected to drying temperatures above 40 ºC.

Thus, in order to obtain higher concentrations of coumarin, the drying of guaco leaves must be carried out at 60 ºC.

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Table 1. Identified analytes and their content in essential oils extracted from *M. laevigata* leaves submitted to drying in an oven with forced air circulation at 40, 50 and 60 ºC.

t_R (min)	IR	Identification	Drying temperature (°C)		
			40	50	60
5,38	932	alpha-pipene	6.08	3.40	2.61
6.39	971	sabinene	0.26	0.19	-
6.49	975	beta-pinene	2.05	1.25	0.68
6.85	989	beta-mircene	0.65	0.41	0.26
8.05	1027	limonene	0.29	0.21	-
20.30	1335	delta-elemene	0.83	0.83	1.12
21.87	1373	alpha-copaene	1.73	1.80	1.77
22.24	1382	beta-bourbonene	0.30	0.33	0.29
22.48	1387	beta-cubebene	0.92	0.93	0.71
22.56	1389	beta-elemene	0.45	0.52	0.87
23.69	1417	trans-cariofilene	12.80	12.87	13.18
24.22	1430	Coumarin	0.26	0.24	0.40
25.02	1450	alpha-humulene	1.52	1.57	1.60
26.29	1482	germacrene D	44.40	40.92	36.85
26.83	1495	biciclogermacrene	15.20	14.35	12.93
26.93	1497	alfa-muurolene	0.27	0.29	0.35
27.10	1502	alfa-bulnesene	0.60	0.51	0.34
27.46	1511	gama-cadinene	0.37	0.41	0.53
27.82	1521	delta-cadinene	1.36	1.55	2.37
29.88	1574	espatulenol	3.34	6.20	8.55
30.07	1579	karyophylene oxide	1.68	3.02	4.04
32.73	1650	alfa-cadinol	0.71	0.91	1.20
33.87	1681	germacra-4(15),5,10(14)-trien-1-alfa-ol	0.35	0.64	0.79

4 CONCLUSIONS

The yield of essential oil extracted of *M. glomerata* (Cenargen genotype) leaves reduces with increasing of drying temperature. However, a higher coumarin content was observed at higher temperature. Considering that coumarin is the chemical constituent of greatest therapeutic interest in *M. laevigata*, drying at 60 ºC is indicated by the drying method employed.

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AUTHORS' CONTRIBUTIONS

MCMF: Supervision, Funding acquisition, Conceptualization, Methodology, Investigation, Writing, Review and Editing. MANS: Methodology, Investigation, Review and Editing. CLOP: Investigation, Writing, Review and Editing. AS: Methodology, CG-MS analysis and interpretation of data. TIM: Investigation, Writing and Review. EPB: Methodology, Investigation. YPN: statistical analysis of experimental data. MRMS: Investigation and Review. SMLD: Methodology, Investigation, Review and Editing. AFS: contributed in collecting plant sample and identification, confection of herbarium, deposited at the Herbarium, Writing - Review & Editing;

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