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Short communication

Damage and drying modify the composition of *Mikania glomerata* and *Mikania laevigata* leaves

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ABSTRACT

This study compared the influence of mechanical damage and drying on the chemical composition of *Mikania glomerata* Spreng. and *Mikania laevigata* Sch.Bip. ex Baker, Asteraceae, leaves. Leaves were collected 1–24 h after damage. Oven-drying at 40°C and shade-drying at ambient temperature were compared to lyophilization. Samples were extracted in 70% ethanol and analyzed by ultra-high-performance liquid chromatography with mass spectrometry. Significant ($p < 0.05$) increases of caffeoylquinic acids were observed in damaged leaves of both species and coumarin decreased in *M. laevigata,* indicating stress. Although the final water content was similar, the drying method affected the leaf composition. In shade-dried leaves of *M. laevigata* coumarin decreased and the presence of umbelliferone was observed; caffeoylquinic acid contents increased for 288 h in in both species. Apparently, enzymes were inactivated after 6 h in oven drying, stabilizing their chemical composition, while shade drying allowed enzymatic and microbial activity to continue; illustrating the importance of post harvesting procedures on the quality of medicinal plants.

Introduction

The Asteraceae family presents a multitude of valuable medicinal plants including *Mikania glomerata* Spreng. and *M. laevigata* Sch.Bip. ex Baker. These species, known as "guaco" in Brazil, are frequently used for their anti-inflammatory, antimicrobial, bronchodilator and even antiophidic effects (Maiorano et al., 2005; Gasparetto et al., 2011; Collaço et al., 2012). Their dried leaves are more commonly employed and stocked, as fresh leaves are unstable due to their water content which permits the onset of enzymatic degradation and microbial development, leading to the loss of active compounds in medicinal plants (Melo et al., 2004; Costa et al., 2005; Radünz et al., 2014).

In spite of the importance of the drying process of medicinal plants, few studies have addressed this subject, dealing mainly with species containing volatile compounds (Melo et al., 2004). The maximum temperature of the drying process is naturally limited by the stability of the plant compounds and the structures wherein they are stored (Martinazzo et al., 2013). According to Costa et al. (2005) temperatures over 45 °C can damage plant structures and composition, however, Radünz (2004) dried *M. glomerata* and mint leaves (*Mentha* × *villosa* Huds.) concluded that 50 °C presented the best results for coumarin and volatile oil contents. The flow of air around the plant material also affects the efficiency of the drying process (Melo et al., 2004).

Lyophilization conserves the original plant composition better, due to lower temperatures, and is considered to effectively reduce microbial contamination by fast moisture removal. The contents of natural pigments and volatiles was higher in lyophilized leaves of *Melissa officinalis*. and *Urtica dioica* (Branisa et al., 2017) and basil (Di Cesare et al., 2003). However, lyophilization is not always feasible for large amounts of plant material, for technical or economic reasons. Furthermore, the ideal drying procedures must be determined for each plant species, as many variables are involved.

The present study aimed to compare the influence of mechanical damage and the drying process on the chemical composition of *M. glomerata* and *M. laevigata* leaves, as some changes in composition may be related to stress response mechanisms induced by harvesting and drying procedures. Furthermore, common drying procedures (oven and shade drying) were compared to lyophilization; to determine which drying method best maintains the original plant composition for these species.

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Material and methods

Adult plants, growing in the field of the Biology Institute, in Unicamp (latitude 22°49′11.12″S; longitude 47°04′15.50″W) were used for the damage and drying experiments. Vouchers were deposited in the UEC herbarium under the number 102046 for *Mikania laevigata* Sch.Bip. ex Baker and number 102047 for *M. glomerata* Spreng., Asteraceae. Healthy stems of approximately 40 cm were collected from both plants in the morning of the same day for the drying experiment, whereas for the damage experiment, cuttings of these plants were allowed to root in one l pots in the greenhouse, with automatic irrigation. Over six months passed between the collection of material for these experiments, allowing the plants in the field to stabilize.

During the damage experiment, leaves of thirty individual plants of each species were damaged with tweezers; ten individuals of each species were not damaged and their leaves immediately collected and frozen in liquid nitrogen (control). The leaves of ten more individuals were collected 1, 12 and 24 h after damage; immediately placed in liquid nitrogen and stored at −40 °C until lyophilization.

For the drying experiment, lyophilization was compared to oven-drying at 40 °C with forced ventilation and shade-drying at ambient temperature (approximately 25 °C). Aliquots of leaves of both species were immediately weighed and frozen in liquid nitrogen (control). The remaining leaves of both species were randomly assigned to one of the methods and weighed initially. Oven-dried leaves were collected after 6, 12, 18, 24, 30, 36, 42 and 48 h. Shade-dried leaves were placed on benches and allowed to dry naturally, collecting after 48, 96, 144, 192, 240, 288 and 336 h. The collected samples were weighed and immediately frozen, lyophilized, to determine the residual water content, then stored in closed polypropylene tubes (50 ml) at room temperature in the dark until extraction.

All lyophilized plant material was ground manually using a mortar and pistil, while frozen with liquid nitrogen. Preliminary tests were carried out to determine the proportion of leaves to solvent to assure an exhaustive extraction using 66.6 mg of each sample in 1 ml of 70% ethanol and water solution, in an ultrasonic bath, for 30 min, then centrifuged at 13,000× *g* for 5 min. An aliquot of 100 μl of the supernatant solution was placed in a vial, adding another 100 μl 70% ethanol and water solution was added, followed by 800 μl of purified water. An aliquot of 4 μl of each final solution was analyzed by ultra-high-performance liquid chromatography with mass spectrometry (UHPLC-MS), according to the method described in (Melo and Sawaya, 2015). Commercial standards were used for identification and quantification, except for grandifloric acid which was a donated isolated compound.

The areas of the main peaks (ions and retention time), in both negative and positive ion modes, were extracted from the UHPLC–MS chromatograms for each sample and were submitted to ANOVA, followed by the Fisher LSD test, using Graphpad Prism software, version 6.01 (GraphPad Software, La Jolla California USA). Data were considered significantly different when $p < 0.05$. For the damage experiment there were ten biological replicates per treatment. For the drying experiment there were three for each collection time.

Results and discussion

Representative coumarins, terpenes and phenolic compounds were evaluated. Their structures, retention times, *m/z* of precursor and product ions are in Supplementary Table 1.

In the damage experiment (Supplementary Fig. 1), *M. glomerata* extracts presented significant increase of both chlorogenic and dicaffeoylquinic acids after 48 h. There was an initial reduction in kaurenoic acid after 1 and 12 h which returned to the original level after 24 h. Low concentrations of coumarin were found in this specie and decreased further after 1 and 12 h. For *M. laevigata*, coumarin also decreased in the first hour but returned to the original level after 12 h. Both chlorogenic and dicaffeoylquinic acids increased significantly after 12 h and 24 h, whereas kaurenoic acid decreased.

The increase in chlorogenic and dicaffeoylquinic acids is a known reactions to damage (Gouvea et al., 2012), although both species increased the production of phenolic compounds at different speeds. The reduction in coumarin may be related to the use of a common precursor (cinnamic acid) to this end. The reduction of kaurenoic acid in both species, although related to a different biosynthetic pathway may be an indirect effect of carbon allocation to defense mechanisms and the production of phenolic compounds.

The effect of the drying process on the concentration of coumarin, *o*-coumaric acid, umbelliferone, chlorogenic acid, dicaffeoylquinic acid, kaurenoic acid and grandifloric acid was determined in relation to a control of the original lyophilized leaves of *M. laevigata* and *M. glomerata*: Both species lost water a similar rate, however the shade-dried leaves of *M. laevigata* lost water more slowly at first, possibly due to the thicker waxy-cuticular layer on the leaves (Supplementary Fig. 2). Lyophilization dried the leaves more efficiently, *M. laevigata* leaves lost 76.7 % (m/m) and *M. glomerata* lost 80.4 % (m/m).

The chemical composition varied significantly throughout both drying procedures. The concentration of coumarin, the best-known active compound of both species, was initially much higher in *M. laevigata* (266 μg/ml) than *M. glomerata* (7.2 μg/ml). Both oven-drying and shade-drying reduced coumarin contents of *M. laevigata* leaves (Fig. 1A), shade-drying reduced *M. glomerata* coumarin content (Fig. 1D). *o*-Coumaric acid was only detected in *M. laevigata* leaves. Its concentration varied during the drying process (being both precursor and degradation product of coumarin) and was significantly higher at the end of both shade and oven-drying (Fig. 1C). Umbelliferone was initially detected in low concentrations only in *M. laevigata* leaves and its concentration decreased through oven drying. However, in shade-dried *M. laevigata* and *M. glomerata* leaves, umbelliferone concentration increased significantly, possibly by the oxidation of coumarin (Fig. 1D and E). Although several coumarins are biosynthetically related, coumarin itself does not possess anticoagulant activity, while umbelliferone does (Jain and Joshi, 2012). Therefore, one must bear in mind that badly dried plant material could increase the undesirable anticoagulant activity.

Apparently, in *M. laevigata,* the phenylpropanoid pathway is directed mainly toward the biosynthesis of coumarin via *o*-coumaric acid, whereas in *M. glomerata* this pathway is directed mainly toward the biosynthesis of caffeoylquinic acids via *p*-coumaric acid. Both chlorogenic and dicaffeoylquinic acids were initially in higher concentrations in *M. glomerata* (Fig. 2B and D). However, during the drying procedure, leaf tissues responded to stress, forming caffeoylquinic acids in both species. This increase is similar to the damage response in plants observed in the first experiment indicating that enzymes involved in this pathway could still be active after 6 h in the oven at 40 °C or up to 192 h during shade drying (Fig. 2).

The diterpenes quantified herein are biosynthesized through an unrelated pathway. Kaurenoic acid was detected in both species, with a slightly higher concentration in *M. glomerata*, and remained relatively stable throughout both drying procedures. A slight increase in the first days of shade drying and at 6 h oven drying may be related to senescence retarding reactions in the plant material, through the biosynthesis of gibberellin (Skutnik et al., 2001; Trindade, 2012). The increase of grandifloric acid may be related to

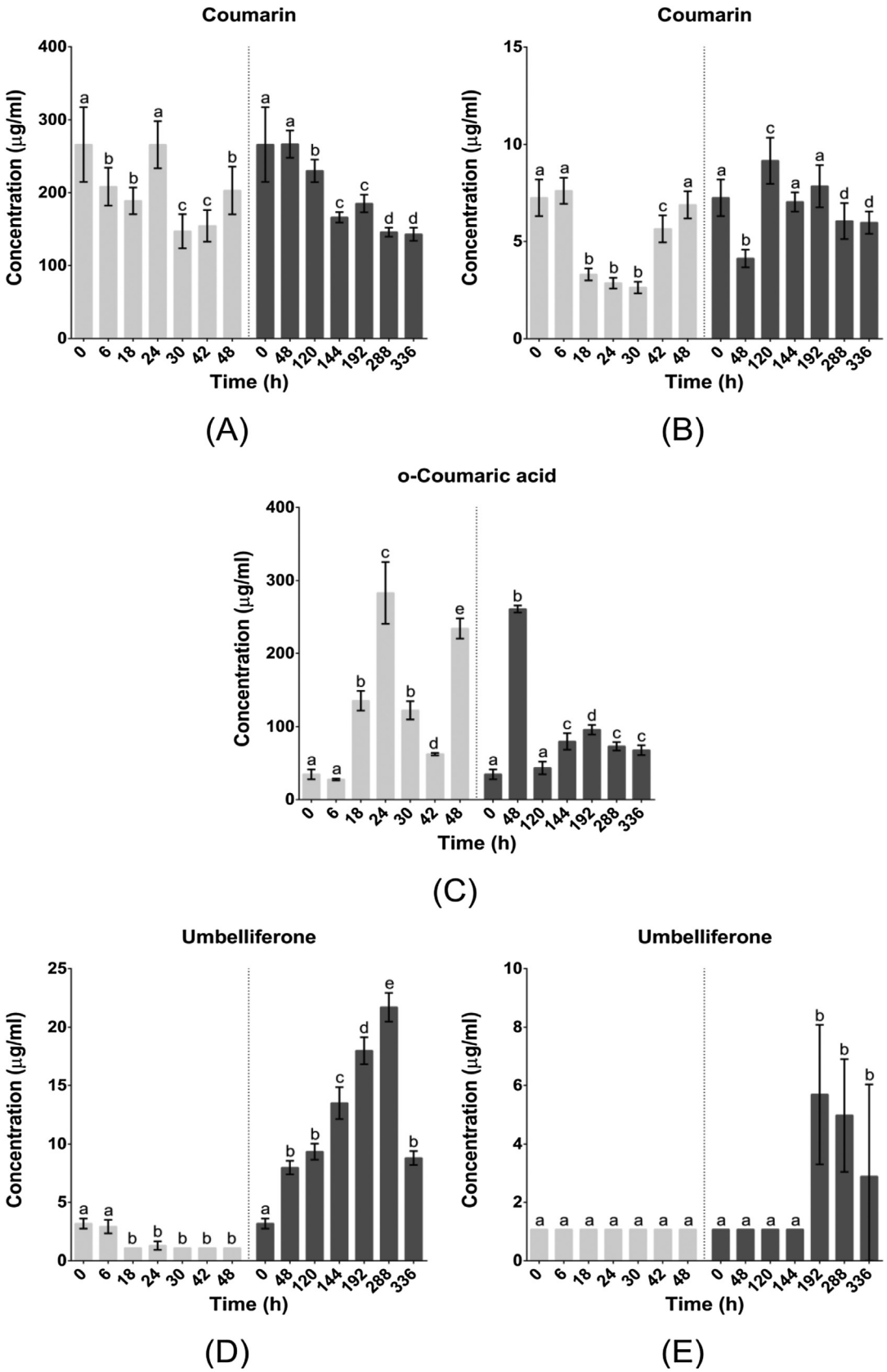

Fig. 1. Concentration (µg/ml) of coumarin (A) *o*-coumaric acid (C) and umbelliferone (D) in leaf extracts of *Mikania laevigata;* Concentration (µg/ml) of coumarin (B) and umbelliferone (E) in leaf extracts of *M. glomerata*; dried in the oven (■) or shade (■). Different letters within the same graph indicate significant differences ($p < 0.05$) n = 3.

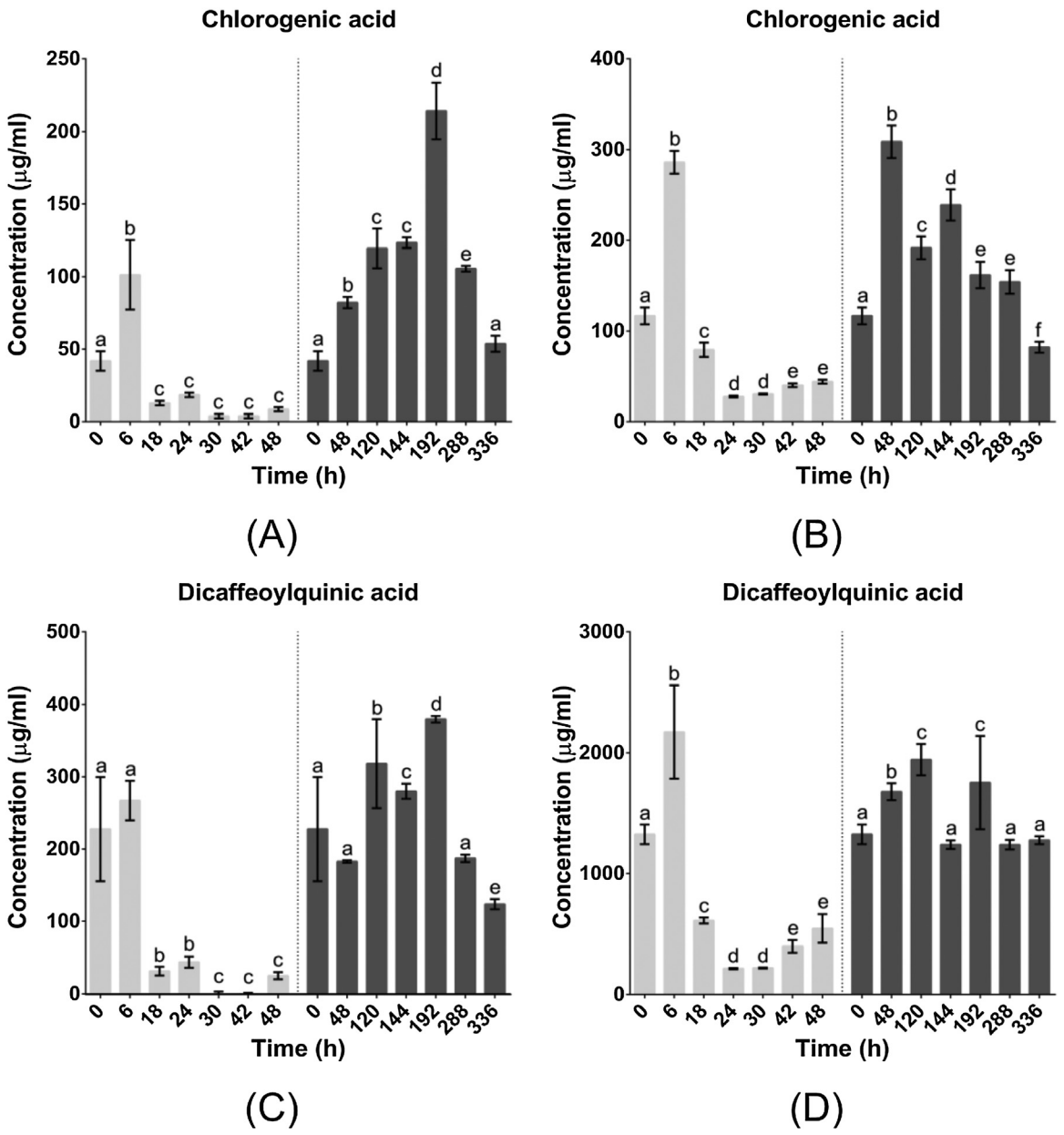

Fig. 2. Chlorogenic acid and dicaffeoylquinic acid concentration (µg/ml) in leaf extracts of *Mikania laevigata* (A) and (C) and *M. glomerata* (B) and (D); dried in the oven (■) or shade (■). Different letters within the same graph indicate significant differences ($p < 0.05$) n = 3.

the same reactions, or may be related to the oxidation of kaurenoic acid (Supplementary Fig. 3).

In spite of the lower water content in the shade-dried leaves after 2 weeks, oven drying at 40 °C preserved the composition of the leaves more closely to the lyophilization process, although the results of enzymatic activity were still detected for up to 6 h. Shade-drying resulted in greater degradation of coumarin and increase of umbelliferone.

Conclusions

Both species react to damage reducing coumarin synthesis and increasing caffeoylquinic acids in the first 6 h. In rooted plants this tends to equilibrate after 24–48 h. During the drying procedure, the harvested leaves suffer hydric and damage stress, adjusting their metabolism to react to these factors. They are also subject to microbial degradation as long as the water contents allow. Therefore, drying procedures that quickly inactivate enzymes and reduce water content are preferable. Oven drying at 40 °C preserved the composition of the leaves more closely to the lyophilization process; shade drying resulted in an increase of umbelliferone, which is not recommendable. Therefore, for these species, lyophilization or oven drying is preferable.

Authors' contributions

AAB contributed in the collection of plant samples, execution of the drying test and laboratory work, chromatographic analysis, data analysis and elaboration of the work. CLA has contributed to the development of assembling and obtaining the data related to the damage test. ACHFS contributed to the supervision of laboratory work, translated the article into the English language and contributed to the critical reading of the manuscript. All authors read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bjp.2019.08.006.

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