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Post-harvesting of *Solanum paniculatum* L. leaves. Part II: Antioxidant activity and chemical composition¹

Pós-colheita de folhas de *Solanum paniculatum* L. Parte II: Atividade antioxidante e composição química

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HIGHLIGHTS:

The aqueous extracts using dried leaves of 'jurubeba' have lower content of phenolic compounds in comparison to fresh leaves. The aqueous extracts prepared with the dried leaves of 'jurubeba' have lower total alkaloid content than those from fresh leaves. The drying time is reduced by 74.3% at air temperature of 50 °C compared to 40 °C under the speed of 0.4 m s^{-1} .

ABSTRACT: 'Jurubeba' (*Solanum paniculatum* L.) is a medicinal plant used in traditional medicine for liver problems, in addition to being used as a cholagogue, emmenagogue, healing agent, febrifuge, anti-inflammatory, antipyretic, tonic, decongestant, diuretic and against inappetence. Thus, the objective of this study was to evaluate the antioxidant activity, concentration of phenolic compounds and total alkaloids of aqueous extracts prepared from 'Jurubeba' leaves subjected to drying. 'Jurubeba' leaves were dried at different temperatures (40, 50, 60 and 70 °C) and velocities (0.4 and 0.8 m s⁻¹) of the drying air. Aqueous extracts of the leaves, subjected to drying, were evaluated by absorbance reading in a spectrophotometer. Increase in drying air temperature reduced the contents of total phenolics and total alkaloids of the leaf aqueous extracts, whereas increment in air velocity increased the concentration of these substances. Antioxidant activity was not influenced by the increase in air temperature at air velocity of 0.4 m s⁻¹, but under air velocity of 0.8 m s⁻¹ there was reduction with increasing temperature. Drying of 'Jurubeba' leaves can be carried out with temperature of 40 or 50 °C and drying air velocity of 0.4 m s⁻¹.

Key words: total alkaloids, phenolic compounds, aqueous extract, 'Jurubeba'

RESUMO: A jurubeba (*Solanum paniculatum* L.) é uma planta usada na medicina tradicional, para problemas hepáticos, além de ser empregada como colagoga, emenagoga, cicatrizante, febrífuga, anti-inflamatória, antipirética, tônica, descongestionante, diurética e na inapetência. Assim, objetivou-se com o presente estudo avaliar a atividade antioxidante, os teores de compostos fenólicos e de alcaloides totais dos extratos aquosos preparados a partir de folhas de jurubeba submetidas à secagem. As folhas de jurubeba foram secas em diferentes temperaturas (40, 50, 60 e 70 °C) e velocidades (0,4 e 0,8 m s⁻¹) do ar de secagem. Os extratos aquosos das folhas, submetidas a secagem, foram avaliados por meio de leitura de absorbância em espectrofotômetro. O aumento da temperatura do ar de secagem reduziu o teor de compostos fenólicos e alcaloides totais dos extratos aquosos das folhas, enquanto que o aumento da velocidade do ar aumentou o teor dessas substâncias, já a atividade antioxidante não foi influenciada pelo incremento da temperatura do ar na velocidade de 0,4 m s⁻¹, mas sob a velocidade do ar de 0,8 m s⁻¹ houve redução com o aumento da temperatura. A secagem das folhas de jurubeba pode ser realizada com temperatura de 40 ou 50 °C e velocidade do ar de secagem de 0,4 m s⁻¹.

Palavras-chave: alcaloides totais, compostos fenólicos, extrato aquoso, jurubeba



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Introduction

Solanum paniculatum L. ('Jurubeba') is a medicinal plant whose leaves, fruits and roots are used in traditional medicine against liver problems, besides being used as cholagogue, emmenagogue, healing agent, febrifuge, anti-inflammatory, antipyretic, tonic, decongestant, diuretic and against inappetence (Mesia-Vela et al., 2002; Lorenzi & Matos, 2008; Kaziyama et al., 2012; Grandi, 2014; Garlet et al., 2017).

In the post-harvest care with the plant raw material for the manufacture of herbal medicines, drying and storage are essential to obtain a quality product. If not properly carried out, drying may compromise the content of the constituents of the plant material (Martinazzo et al., 2013).

The drying process of medicinal plants has been well studied, but most studies evaluate only the effect of different drying air methods/conditions on the drying kinetics of these products (Radünz et al., 2011; Prates et al., 2012; Alves et al., 2017; Martins et al., 2018). These studies of characterization of the drying curves of medicinal plants without a joint analysis of the effect of drying on the quality of these products do not provide sufficient information for decision-making on how to dry medicinal plants.

In this context, studies evaluating the effects of drying on the quality and yield of secondary metabolites of medicinal plants have intensified in the last two decades (Dias et al., 2011; Oliveira et al., 2011; Lemos et al., 2012; Rocha et al., 2012; 2014; Martinazzo et al., 2013; Gasparin et al., 2014).

In view of the above, the objective of this study was to evaluate the antioxidant activity and contents of phenolic compounds and total alkaloids of aqueous extracts prepared from 'Jurubeba' leaves subjected to different drying air conditions.

MATERIAL AND METHODS

The collected plant material came from the cultivation of 150 'Jurubeba' plants, which were grown from seedlings and transplanted in September 2016, in an experimental area located at the Faculty of Agricultural Sciences - FCA, Federal University of Grande Dourados (UFGD), in Dourados, MS, Brazil. An exsiccate is deposited in the DDMS Herbarium of UFGD, under number 5553.

For planting and along the development of the crop, all the cultural practices (soil tillage and correction, control of weeds and insects) were carried out and soil moisture was maintained in the experimental area with a drip irrigation system, to promote adequate development of the crop.

'Jurubeba' leaves were collected selectively, between June and July 2017, choosing material without injuries or with incidence of diseases, in order to ensure the homogeneity of the physical and chemical characteristics of the product. The plant material was collected in the morning, avoiding collecting material after rainfall or with dew remnants on the surface.

After collection, the plant material was sent to the Laboratory of Pre-Processing and Storage of Agricultural Products/FCA, where a sample was separated to determine the initial moisture content of 'Jurubeba' leaves using the gravimetric method, in an oven at 103 ± 1 °C, for 24 h,

in triplicate (ASABE, 2010). Each triplicate contained approximately 7 g of leaves, which had an initial moisture content of approximately 2.76 (decimal, d.b.). The rest of the sample was immediately dried in an experimental fixed-bed dryer, as described by Goneli et al. (2016). Drying was performed for various drying air conditions (Table 1), until the leaves reached the final moisture content of approximately 0.11 (decimal, d.b.). A sample of approximately 25.6 g of leaves per dryer tray was used for each drying air condition.

The study was conducted in a completely randomized design, in a 4 x 2 factorial scheme, and the treatments consisted of four temperatures and two drying air velocities. For each drying air condition, a portion of dry leaves of *Solanum paniculatum* was used to prepare three samples of aqueous extract (repetitions) and each repetition of each treatment was analyzed for the contents of phenolic compounds, contents of alkaloids and antioxidant activity.

Aqueous extracts of 'Jurubeba' leaves were prepared and analyzed in the laboratories of the Center for Studies in Natural Resources of the State University of Mato Grosso do Sul, campus of Dourados, MS, Brazil.

The aqueous extract of 'Jurubeba' leaves, dried under different drying air conditions, was prepared according to the recommendation contained in the monograph on *Solanum paniculatum* (Brasil, 2015), which mentions that the whole plant, that is, any part of the plant, can be used in the form of infusion and that a cup of tea can be administered three to four times a day.

The aqueous extracts were prepared using 1 g of leaf dried and scraped in 150 mL of water at 98 °C and kept in contact for 15 min in a closed container. The aqueous extracts of fresh leaves were prepared using a mass of fresh plant material equivalent to the mass of dry matter of the plant material already dehydrated in the drying process described, i.e., approximately 3.4 g of fresh 'Jurubeba' leaves.

All aqueous extracts obtained were subjected to analysis to determine the contents of phenolic compounds (Djeridane et al., 2006), total alkaloids (Oliveira et al., 2006) and antioxidant activity (Kumaran & Karunakaran, 2006).

To determine the content of phenolic compounds, 1.5 mL of aqueous solution sodium carbonate at 20%, 0.5 mL of Folin-Ciocalteu reagent (1/10 v/v) and 1 mL distilled water were added to every 100 μL of each extract, from the plant material subjected to different drying conditions and from the fresh plant material, and each sample was allowed to react for 30 min. Then, reading was taken in a spectrophotometer at 760 nm wavelength (Djeridane et al., 2006). The same procedure was performed for the blank, but replacing 100 μL of sample with 100 μL of distilled water.

The concentration of phenolic compounds was calculated by preparing an analytical curve, using gallic acid as standard,

Table 1. Time (h) required for 'Jurubeba' leaves to reach the average moisture content of approximately 0.11 (decimal, d.b.) under different drying air conditions

Velocity	Temperature (°C)			
(m s ⁻¹)	40	50	60	70
0.40	12.33	3.17	2.00	0.75
0.80	10.33	2.67	1.58	0.67

at concentrations from 5 to 500 μg mL⁻¹. The experimental procedure applied to the standard was the same as that used for the samples. With data obtained from the standard, the absorbance curve was plotted as a function of gallic acid concentration and a linear regression was fitted (a = -0.018; b = 0.0016; R² = 0.997), which was used to calculate the phenolic compound content of the samples. The results were expressed in mg of gallic acid per g of lyophilized extract. All analyses were performed in triplicate.

To determine the total alkaloid content, a 4-mL aliquot (triplicate) was collected from each aqueous extract sample and acidified to pH 2-2.5 with 1 N HCl. Then, 4 mL of the acidified solution was transferred from each repetition to test tubes and 0.2 mL of Dragendorff reagent was added to each tube. All tubes were then centrifuged at 2400 rpm for 30 min and, after centrifugation, the supernatant was discarded and the residue was treated with 0.1 mL of absolute ethyl alcohol. Subsequently, 0.2 mL of sodium sulfite at 1% was added and the tubes were centrifuged again at 2400 rpm for 30 min. Then, the supernatant was discarded and the residue was treated with 0.2 mL of concentrated nitric acid. After the described procedures, the resulting content of each repetition was transferred to 5-mL volumetric flasks and the volume was completed with distilled water. Finally, 0.5 mL of this solution was removed and 2.5 mL of thiourea at 3% were added. For each sample, the spectrophotometer reading was performed at 435 nm wavelength. Blank was obtained by mixing nitric acid and thiourea (Oliveira et al., 2006).

The concentration of alkaloids was calculated by preparing an analytical curve, using berberine as standard, at concentrations from 1.39 to 7.02 μg mL⁻¹. With data obtained from the standard, the absorbance curve was plotted as a function of berberine concentration and a linear regression was fitted (a = 0.0338; b = 0.0972; R² = 0.9823), which was used to calculate the alkaloid content in the samples. The results were expressed in mg of berberine per g of lyophilized extract. The analyses were performed in triplicate.

The antioxidant activities of the aqueous extracts were evaluated using the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl). Antioxidant activity was determined in a room protected from light, at temperature of 25 \pm 1 °C, and all tests were performed in triplicate.

The antioxidant test with the free radical DPPH was carried out at the concentrations of 1, 10, 100, 500, 1000 $\mu g \ mL^{-1}$ of each sample, employing a prepared solution of DPPH at 0.004% in methanol. 3000 μL of the DPPH solution were added to every 100 μL of the study sample. Spectrophotometer readings at 517 nm wavelength were performed after 30 min of reaction (Kumaran & Karunakaran, 2006). Radical scavenging activity was calculated according to Eq. 1:

%reduction DPPH =
$$\frac{\text{Abs. Blank} - \text{Abs. Sample}}{\text{Abs. Blank}} 100$$
 (1)

where:

Abs - absorbance.

The results were presented in minimum inhibitory concentration (IC_{50}). IC_{50} expresses the minimum concentration

of antioxidant required to reduce by 50% the initial concentration of DPPH. From the absorbances obtained with the different dilutions of the sample, the % reduction of DPPH was plotted on the Y-axis and the concentration of extracts (μg mL⁻¹) was plotted on the X-axis to obtain the concentration of the sample with capacity to reduce DPPH by 50%.

The contents of phenolic compounds, total alkaloids and antioxidant activities of aqueous extracts prepared from 'Jurubeba' leaves subjected to different drying air conditions were analyzed by linear regression. For the fitted linear regressions (y = b.x + a), the significance of the slope of the 'b' line was analyzed by t-test.

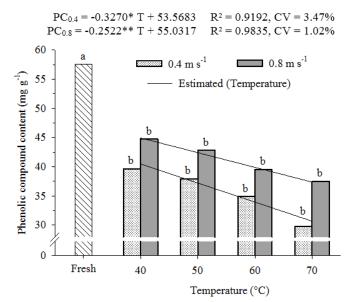
Data from aqueous extracts of 'Jurubeba' leaves (content of phenolic compounds, content of total alkaloids and antioxidant activity) were subjected to Student's t-test, to make comparisons between the aqueous extracts of the fresh leaves with each aqueous extract of the leaves subjected to different drying air conditions.

RESULTS AND DISCUSSION

The phenolic compound content of the aqueous extracts of 'Jurubeba' leaves, dried with drying air velocity of 0.4 m s⁻¹, reduced from 40.49 to 30.68 mg g⁻¹ as the air temperature increased from 40 to 70 °C (Figure 1), that is, a reduction of 24.23%. For the aqueous extracts of leaves dried at drying air velocity of 0.8 m s⁻¹, the content of phenolic compounds decreased from 44.94 to 37.38 mg g⁻¹ with the increase in air temperature, which corresponds to a reduction of 16.83%.

There was the effect of drying air temperature, evidenced in the linear regression equations to estimate the phenolic compound content of the aqueous extracts of 'Jurubeba' leaves dried at drying air velocities of 0.4 and 0.8 m s⁻¹ (Figure 1).

Behavior similar to that observed in the present study was found by Niamnuy et al. (2013) when drying leaves of



**, * - Significant at $p \le 0.01$ and $p \le 0.05$ by t-test, respectively; Means followed by the same letter do not differ statistically by t-test (p $\le 0.01)$

Figure 1. Phenolic compound content of aqueous extracts of fresh and dried 'Jurubeba' leaves as a function of drying air temperature and velocity

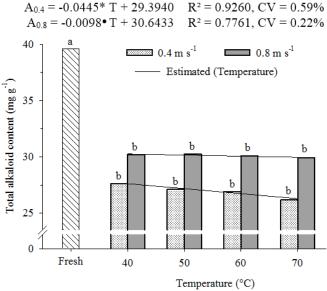
Centella asiatica (L.) using different drying methods, including hot-air drying, combined infrared-hot air drying and low-pressure superheated steam drying, each of these methods at different drying air temperatures (50, 60 and 70 °C). The authors verified that the increase in drying air temperature, regardless of the method used, resulted in greater degradation of phenolic compounds.

The fact that the phenolic compound content is higher in the aqueous extracts of leaves dried at drying air velocity of 0.8 m s⁻¹, for all temperatures, can be attributed to the shorter drying time compared to the air velocity of 0.4 m s⁻¹ (Table 1). Consequently, there was shorter time of exposure to the drying air, enabling less thermal and/or oxidative degradation of phenolic compounds, since the shorter time of exposure to the drying air corresponds to a shorter time exposed to the presence of oxygen and a lower thermal load during drying.

Niamnuy et al. (2013) verified that, when *Centella asiatica* (L.) leaves were dried by low-pressure superheated steam, the material had higher content of phenolic compounds than when other drying methods were used; the authors attributed this result to the fact that this drying method has oxygen-free atmosphere, hence reducing the oxidation of the compound.

Also in Figure 1, it can be noted that the aqueous extracts of 'Jurubeba' leaves, regardless of the air conditions under which the leaves were dried, showed average contents of phenolic compounds statistically lower than that of the aqueous extract prepared from fresh leaves, which had phenolic compound content of 57.57 mg g⁻¹.

According to the regression equations to estimate the total alkaloid content of the aqueous extracts of 'Jurubeba' (Figure 2), for the drying air velocity of 0.8 m s⁻¹, the aqueous extracts prepared from the material dried at the different drying air temperatures showed a significant difference (p \leq 0.10) in total alkaloid content as a function of the increase in air temperature. For the drying air velocity of 0.4 m s⁻¹, the total alkaloid content of the aqueous extracts was statistically (p \leq 0.05) influenced by the drying air temperature.



*, • - Significant at $p \le 0.05$ and $p \le 0.10$ by t-test, respectively. Means followed by the same letter do not differ by t-test ($p \le 0.01$)

Figure 2. Total alkaloid content (expressed as berberine sulfate equivalent g⁻¹ extract) of fresh and dried 'Jurubeba' leaves as a function of drying air temperature and velocity

The total alkaloid content of the aqueous extracts prepared from 'Jurubeba' leaves, subjected to drying, decreased from 27.61 to 26.28 mg g⁻¹ as the drying air temperature increased from 40 to 70 °C under air velocity of 0.4 m s⁻¹, that is, a reduction of 4.82% (Figure 2), whereas for the drying air velocity of 0.8 m s⁻¹ it decreased from 30.25 to 29.96 mg g⁻¹, which corresponds to a reduction of 0.96%.

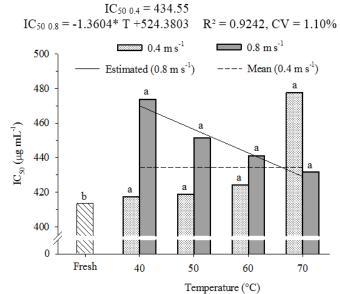
Aqueous extracts prepared with fresh leaves of 'Jurubeba' had total alkaloid content (39.60 mg g^{-1}) statistically higher than that of aqueous extracts prepared from dry leaves, regardless of the drying air conditions (Figure 2).

The total alkaloid content of aqueous extracts prepared from leaves subjected to drying with air velocity of 0.8 m s⁻¹ was higher than that of aqueous extracts prepared from the material dried under drying air velocity of 0.4 m s⁻¹ (Figure 2). This behavior can probably be attributed to the thermal load to which 'Jurubeba' leaves were exposed during drying with air velocity of 0.4 m s⁻¹, because they remained exposed to heated air for a longer time to reach the desired moisture content (Table 1), which led to reduction in the content of total alkaloids due to the thermal degradation of these compounds.

Reduction in the alkaloid content in 'Jurubeba' leaves is important in cases where there is constant consumption of this species, since high toxicity resulting from this class of secondary metabolites has been reported (Hyacienth & Almeida, 2015; Anwar et al., 2018).

Antioxidant activity expressed in IC_{50} represents the necessary concentration of the antioxidant to reduce the DPPH radical by 50%, and the lower the IC_{50} value, the higher the antioxidant activity of the material (Negri et al., 2009).

The values of antioxidant activity, expressed in IC_{50} , of the aqueous extracts prepared from 'Jurubeba' leaves subjected to drying with air velocity of 0.4 m s⁻¹ did not show a defined behavior to allow a significant linear equation to be fitted to represent the phenomenon (Figure 3), thus obtaining an



* - Significant at $p \le 0.05$ by t-test. Means followed by the same letter do not differ by t-test $(n \le 0.01)$

Figure 3. Antioxidant activity of aqueous extracts of fresh and dried 'Jurubeba' leaves as a function of drying air temperature and velocity

mean IC $_{50}$ value of 434.55 μg mL $^{\text{-}1}$ for the 50% reduction of DPPH.

With respect to leaves dried under air velocity of $0.4~m~s^{-1}$, $417.45~\mu g~m L^{-1}$ of the infusion of 'Jurubeba' leaves dried at 40~ °C were necessary for the 50% reduction in DPPH and, for the infusion prepared with leaves dried at air temperature of 70~ °C, $477.74~\mu g~m L^{-1}$ were necessary to perform the same reaction (Figure 3), which corresponds to 14.44% in the capacity for reduction.

Negri et al. (2009), evaluating the antioxidant activity of branches and leaves of *Maytenus ilicifolia* (espinheira santa), dried at different air temperatures (40, 50, 60, 70 and 90 °C), found that the material dried under air temperature of 40 °C showed higher antioxidant activity, and 4.02 μ g mL⁻¹ were necessary for the 50% reduction in DPPH.

The antioxidant activity of the aqueous extracts of 'Jurubeba' leaves dried under temperature of 40 °C and drying air velocity of 0.4 m s⁻¹ (Figure 3) corroborates the behavior observed for phenolic compound content (Figure 1), which was higher under air temperature of 40 °C. This was already expected, given the trend in the positive relationship between higher phenolic compound content and higher antioxidant activity.

For the aqueous extracts of 'Jurubeba' leaves dried at air velocity of 0.8 m s⁻¹, antioxidant activity increased with the increase in drying air temperature, and 469.96 μg mL⁻¹ were necessary to reduce by 50% the DPPH of the infusion prepared with material dried at 40 °C, while 429.15 μg mL⁻¹ were necessary for the infusion of material dried at 70 °C (Figure 3), which corresponds to a 9.51% increase in the capacity for reduction.

The antioxidant activity of the aqueous extract prepared from fresh leaves of 'Jurubeba' is statistically higher than that of aqueous extracts prepared from the leaves subjected to drying, and the infusion prepared with fresh leaves required 413.56 $\mu g \ m L^{-1}$ to reduce 50% of the DPPH (Figure 3). In general, it is verified that leaves dried at air velocity of 0.4 m s⁻¹ showed better results of antioxidant activity, especially when drying air temperatures of 40 and 50 °C were used.

According to Figures 1, 2 and 3, considering the drying air condition that promotes the highest levels of phenolic compounds and antioxidant activity and low levels of total alkaloids, it can be inferred that drying with temperature of 40 °C and air velocity of 0.4 m s⁻¹ leads to the best results for the analyzed variables mentioned above. By extrapolating this line of reasoning, it can also be inferred that drying at temperature of 50 °C under air velocity of 0.4 m s⁻¹ also promotes good results in relation to the variables analyzed, with the advantage of a 74.3% reduction in drying time of the plant material (from 12.33 to 3.17 h).

Conclusions

- 1. Increase in drying air temperature reduced the contents of phenolic compounds and total alkaloids of the aqueous extracts of 'Jurubeba' leaves, while the increase in air velocity led to higher contents.
- 2. The antioxidant activity of aqueous extracts of 'Jurubeba' leaves was not influenced by the increase in air temperature

- at the velocity of 0.4 m s $^{-1}$, but under air velocity of 0.8 m s $^{-1}$ there was a reduction in antioxidant activity as the temperature increased.
- 3. In general, 'Jurubeba' leaves can be dried with temperature of 40 or 50 °C and drying air velocity of 0.4 m s⁻¹.

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