Influence of temperature and time of exposure to temperature on the yield of the obtaining process of ethanolic and hydroethanolic extracts from *Syzygium malaccense* (L.) Merr. & L. M. Perry leaves

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Abstract

The present study aimed to obtain alcoholic and hydroalcoholic (70% v/v) extract of Syzygium malaccense (L.) Merr. & L. M. Perry (red jambo fruit tree) leaves and investigated the influence of temperature and time of exposure to temperature on the extraction yield. It was applied a 2x2 factorial design, considering temperature and time of exposure as independent variables with two levels each: 30 or 60 °C for temperature and 60 or 120 minutes for time. For both ethanolic and hydroethanolic extraction, higher process yields were observed at higher temperature and time (24.26% and 19.00%, respectively), however, no significant difference was noticed when increasing the time of exposure to temperature.

Keywords: maceration, plant extract, red jambo fruit tree leaves

1. Introduction

Studies on plant materials have been encouraged by technology sector (especially food and pharmaceutical industries) in the sense of better investigating not only products and compounds derived from plants already studied, but also discovering new compounds from plants not yet studied. Such interest, among many others, is related to the fact that some microorganisms have shown resistance to antimicrobials commonly applied for products conservation (PEREIRA et al., 2009). It is known that substances isolated from plant material represent a promising way to increase products shelf life (ASKARI et al.,2012), moreover the use of plants extracts lead to desirable qualities required by the consumers such as clean label and natural ingredients.
The Brazilian Amazon region has a wide diversity of plants and a large part of this biome (more than 80%) has yet to be studied regarding its medicinal properties (SILVA and FRANCO, 2010). Included in this biome there is the species *Syzygium malaccense* (L.) Merr. & L. M. Perry, whose fruit (red jambo) is highly appreciated.
The jambo tree belongs to the Myrtaceae family, which is of Asian origin and found mainly in India. It is a plant widely used in such region due to its ability to alleviate diseases that can come from bacteria, fungi and viruses. Furthermore, jambo fruits present compounds such as carotenoids, phenolics, flavonoids, proanthocyanidins, tannins and terpenoids which have been proven to have protective characteristics against aggressive agents such as microorganisms (ZHENG et al, 2011; NUNES, 2015; SOUZA 2016; GIBBERT et al., 2017; MELO, 2009).
According to Veggi (2009), during compounds extraction process, soluble and volatile solids are extracted through mass transfer, as the raw material keeps in contact with a solvent. The extraction process can take place by several methods, such as: pure and direct contact with the solvent, vaporization and supercritical fluids. Among the first class of methods, the maceration is a technic used to obtain extracts containing non-

volatile compounds. It is considered a simple operation since it consists in keeping the sample in direct contact with the solvent during a determined period of time (heating and/or agitation can be applied) (COPPA et al., 2017).

There is no specific solvent for each type of extraction in plant materials, in that way, it can be even necessary the use of combinations between solvents in order to achieve the desirable extraction yields. Among the most used solvents are methanol, ethanol, acetone, ethyl acetate and water (AZEVEDO, 2010). According to Veggi (2009), the use of organic solvents can leave residues and/or promote oxidation when the solvent is eliminated, in this sense, ethanol and water are the most used solvents for reasons of low toxicity, hygiene and abundance (ANDREO AND JORGE, 2006).

Besides the solvent, factor as temperature and mechanical action can also play a roll on the extraction process through maceration (VEGGI, 2009). However, considering that the yields of the extraction processes can be low, it is important that the raw material is easily disponible.

Taking into consideration the above mentioned and adding the fact that there is a gap in the literature regarding ethanolic and hydroethanolic extracts of leaves of *Syzygium malaccense* (L.) Merr. & L. M. Perry, the present study works towards (a) the obtainment of such extracts and (b) the investigation of the influence of relevant process variables (temperature and time of exposure to temperature) on the yield of the extraction (maceration) process.

2. Method

Plant material obtention

Syzygium malaccense (L.) Merr. & L. M. Perry (red jambo fruit tree) leaves were collected from trees located at Federal University of Rondônia Campus (latitude = -9.9002° S, longitude = -63.0342° W). The leaves were manually collected in the month of September (before flowering) during the morning period (around 7am). The selection of the leaves was based on the stage of complete maturation, that is, the leaves presented a homogeneous dark green color.

After collected, the fresh leaves were kept in polyethylene bags and taken to the laboratory until they were cleaned to remove superficial dirt and damaged parts. Then, the weight of the fresh leaves was measured and the leaves were taken to incubator (LimaTec, BR) at 40 °C for 24 hours to be dried. After drying, the dried leaves were grinded in a blender (Arno, model Power Mix Plus LQ20, BR) for 5 minutes at high velocity. Part of the powder of dried grinded leaves was taken to material moisture analysis and the remaining was stored in glass recipient, hermetically closed and covered with aluminum foil at room temperature until extraction experiment time.

Moisture content analysis of plant material

Moisture analysis was carried out in triplicate for the fresh leaves (FL) and for the dried leaves (DL). The gravimetric method was applied to determine moisture content of the samples. The analysis was carried out using a forced-convection air-oven at 105 °C for 24 hours. The percentage moisture content (U%) calculation was given by Equation (1).

$$U\ (\%) = \frac{FL\ weight - DL\ weight}{initial\ sample\ weight}\ x\ 100 \qquad (1)$$

Extracts obtention and extraction yield

For extraction, 100 mL of absolute ethanol (Synth, BR) or ethanol solution in water (70% v/v) was added to an Erlenmeyer (250 mL) containing approximately grams of the dried leaves powder (DLP). The set was covered with aluminum foil and taken to a shaken water-bath to be heated at desired temperature (T) and time (t). The variables temperature and time of exposure to temperature were tested in two levels according to the 2x2 factorial design presented in Table 1. The higher level for (+) T and t were 60 °C and 120 minutes, respectively, in the time that the lower levels (-) were 30 °C and 60 minutes.

Table 1

2x2 factorial design matrix applied to investigate the effect of temperature (T) and time of exposure to temperature (t) on the extraction yield

Assay	T (°C)	t (min)	Yield (%)	Yield (%)	Mean yield (%)
1	-	-	y_{11}	y_{12}	$\bar{y}_1$
2	+	-	y_{21}	y_{32}	$\bar{y}_2$
3	-	+	y_{31}	y_{32}	$\bar{y}_3$
4	+	+	y_{41}	y_{42}	$\bar{y}_4$

After heating, the set was left to rest (incubation) at room temperature until the contact time of powder leaves and solvent completed 24 hours. Subsequently the incubating time, the mixture was put through a sieve to separate the powder and the filtered material was divided into three parts (each part in one Petri dish) and taken to ventilated drying oven (Marconi, BR) at 60 °C until the solvent (ethanol or ethanol solution) was removed and the dried leaves extract (DLE) was obtained.

The weight of DLE was measured, in analytical balance (Quimis, BR), for the calculation of extraction yield (Y) which was expressed as the percentage on the weight of DLE to the raw material as suggested by Franzen et al. (2018) and illustrated at Equation (2).

$$Y\ (\%) = \frac{DLE\ weight}{DLP\ weight}\ x\ 100 \qquad (2)$$

The extraction experiments were performed in duplicate so the experimental error could be estimated and thus, the statistical significance of the effects could be evaluated. According to Neto et al. (2010), only the effects whose estimates (obtained experimentally) are superior, in absolute value, to the product of the standard error (s_{effect}) by the point of the Student's t distribution can be considered statistically significant. In the present study it was applied a Student's t point value for an 95% confidence interval and 4 degrees of freedom ($t_4 = 2{,}776$).

The standard error (s_{effect}) was calculated through Equation (3):

$$s_{effect} = \sqrt{\frac{s^2}{2}} \tag{3}$$

Since the tests were performed twice (1 degree of freedom), in order to calculate the common standard error (s) with 4 degrees of freedom, an extent form of Equation (4) was applied, considering the mean of all estimates weighted by their respective degrees of freedom.

$$s^2 = \frac{\left((N_A - 1)\ x\ s_A^2\right) + \left((N_B - 1)\ x\ s_B^2\right)}{(N_A - 1) + (N_B - 1)} \tag{4}$$

where N_A and N_B are the number of elements at samples A and B respectively and s_A and s_B are the sample variance, calculated through Equation (5):

$$s_y^2 = \frac{1}{N-1}\sum\left(y_y - \bar{y}\right)^2 \tag{5}$$

Investigation of temperature and time of exposure to temperature on extraction yield

The response of the temperature and time to exposure to the temperature on the extraction yield was statistically calculated, as described by Neto et al. (2010). The temperature main effect (T) was calculated through the mean of the temperature effects at the two levels of time, according to Equation (6).

$$T = \bar{y}_+ - \bar{y}_- = \left(\frac{\bar{y}_2 + \bar{y}_4}{2}\right) - \left(\frac{\bar{y}_1 + \bar{y}_3}{2}\right) \tag{6}$$

where $\underline{y_i}$ used to represent the mean response observed at the i-th assay.

To calculate the effect of time (t), the upper level corresponds to assays 3 and 4 and the lower level to tests 1 and 2. The time of exposure to temperature mains effect was calculated by Equation (7).

$$t = \bar{y}_+ - \bar{y}_+ = \left(\frac{\bar{y}_3 + \bar{y}_4}{2}\right) - \left(\frac{\bar{y}_1 + \bar{y}_2}{2}\right) \tag{7}$$

The interaction between temperature and time ($T\ x\ t$) was calculated applying Equation (8).

$$T\ x\ t = \left(\frac{\bar{y}_4 - \bar{y}_3}{2}\right) - \left(\frac{\bar{y}_2 - \bar{y}_1}{2}\right) = \left(\frac{\bar{y}_1 + \bar{y}_4}{2}\right) - \left(\frac{\bar{y}_2 + \bar{y}_3}{2}\right) \tag{8}$$

3. Results

Moisture content of plant material

The drying process is essential to remove moisture from the leaves since it provides an advantage in preserving present bioactive compounds, so factors such as time and temperature must should be taken into account in the process in order to do not cause the degradation of the compounds desired to be extracted. The moisture of the plant material is able to influence both the final product (extract) characteristic and extraction yield (CANABARRO et al., 2017). According to Castro et al. (2008) the determination of moisture is an important step to avoid different interpretations regarding the absolute mass of fresh leaves and the final extraction yield.

In the present work, an average of 70.43±0.68% moisture content was found for fresh leaves (FL) while dry leaves (DL) moisture content presented an average of 6.66±0.50%. Thus, the applied drying process reduced the raw material mass by 68.32%, in such a way that this mass is attributed to the water present in the fresh raw material. In order to obtain better results, reduce extraction time and promote greater solvent contact with bioactive compounds, it is extremely important to reduce leaf moisture.

According to Oliveira (2011), data regarding the influence of factors such as drying on the chemical composition of plants are scarce but the author also reports that the relationship between the drying procedure of plant material and the quality of the final product (extract) is known. Silva and Casali (2000) affirm that the drying process of the plant material must be carried out immediately after harvesting in order to minimize losses of biologically active substances associated with enzymatic degradation due to the presence of water. According to the authors, high levels of moisture also favour microbial development, thus, drying enables the storage of the product for longer periods.

It is important to emphasize that the raw material moisture values are relative, as they depend on the type of plant and the biome in which this sample is found. Brum (2010) found an average moisture content of 50.8±0.7% for Eucalyptus (*E. citriodora*) leaves while Santana et al. (2013) found a value of 12.33±0.12% for leaves of Mikanai glomerata (*Asteraceae*).

Extraction yield

The yield of maceration process for obtainment of alcoholic and hydroalcoholic extracts at temperature and time investigated are presented in Tables 2 and 3, respectively.

Table 2

Yield of obtainment process of jambo tree (Syzygium malaccense (L.) Merr. & L. M. Perry) leaves ethanolic extract

Assay	Temperature (°C)	Heating time (min)	Yield (%)	Yield (%)	Mean yield (%)
1	30	60	14.91	15.34	15.12
2	60	60	20.16	23.80	21.98
3	30	120	18.09	20.16	19.13
4	60	120	25.35	23.20	24.27

Table 3

Yield of obtainment process of jambo tree (Syzygium malaccense (L.) Merr. & L. M. Perry) leaves hydroethanolic extract

Assay	Temperature (°C)	Heating time (min)	Yield (%)	Yield (%)	Mean yield (%)
1	30	60	11.16	11.31	11.23
2	60	60	17.79	16.77	17.28
3	30	120	12.77	10.80	11.79
4	60	120	18.80	19.20	19.00

Basto (2011) studied ethanolic extracts obtained from 25 different species of fruit trees' leaves including *Aceima smeiithii* leaves (known with the popular name mini-jambo). Such extraction presented a yield of 12.7% for a sample of 10 grams and a period of contact with the solvent of merely 5 minutes, due to the use of polytron. Another example of the yield of the process of obtaining ethanolic extract from leaves of the same family, the study of Chaibub et al. (2013), investigated leaves of *S. odoratissima* and reported a yield of 32.5%, which was associated to better process efficiency due the using of a rotaevaporator.

Rodrigues et al. (2011) studied the yield of ethanolic extracts, obtained through maceration of leaves from different plants of Lamiaceae family. The authors reported a yield of 20.96% for extract of *P. barbatus* and 41.34% of extract of *P. ornatus*. The greater data observed by the authors compared to those found in the present study may be related to (a) the type of solvent, specially concerning to the hydroethanolic extract, since the authors used P. A. ethanol; (b) plant material: solvent ratio employed, as the authors use 20 grams of leaves for every 100 mL of solvent and (c) the authors report greater removal of water content during drying (on average 90.12 ± 8, 22%). All these factors can promote a better interaction of the active ingredient with the solvent if the principles show affinity with the solvent used.

Silva et al. (2013) produced hydroethanolic extract of guava leaves. In their study, the authors reduced the moisture content of the leaves by drying in an oven at 40 °C for seven days and macerated the ground dried leaves in a 70% ethanol solution in water, also for 7 days. The authors do not report the proportion of solvent used but report that they observed a yield of 16.42%, a value that is within the range presented in the present study (Table 3).

Venancio et al. (2020) obtained ethanolic extract of leaves of the *cupuaçu* tree through maceration. The authors observed an extraction yield varying from 1.46% and 1.54%, nonetheless they considered the obtained yield to be low, attributing the results to the use of ethanol instead of methanol as solvent due to its lower polarity and consequent better interaction with the components of the plant material.

From the data presented in Tables 2 and 3, it can be observed that the yield of the obtainment processes of ethanolic and hydroethanolic extracts of jambo tree (*Syzygium malaccense (L.)* Merr. & L. M. Perry) leaves was affected by both temperature (assays 1 and 3 *versus* assays 2 and 4) and time of exposure to temperature (assays 1 and 2 *versus* assays 3 and 4).

Regarding to ethanolic extract, temperature effect is more pronounced at the lower level of time exposure, i. e., for 60 minutes of exposure to temperature, the extraction yield increased 6.86% (going from 15.12% to 21.98% at 30 and 60 °C, respectively) while a yield increasement of 5.15% was observed for 120 minutes of exposure to temperature (going from 19.13% to 24.27% at 30 and 60 °C, respectively). The effect of the time of exposure to temperature was also noticed to be more pronounced at the lower level of temperature: at 30 °C, the changing in time of exposure to temperature (from 60 minutes to 120 minutes) resulted in an extraction yield 4,0% (going from 15.12% to 19.13%) while at 60 °C, the same changing in time of exposure to temperature resulted in an extraction yield 2.29% (going from 21.98% to 24.27%).

Concerning the hydroethanolic extraction, the temperature effect was noticed to be more pronounced when applying 120 minutes of exposure, that is, it showed a 7.21% higher yield (going from 11.79% to 19.00% at 30 and 60 °C, respectively). In contrast, when the 60 min time of exposure was applied, the extraction yield increased 6.04% (going from 11.23 to 17.28% at 30 and 60 °C, respectively). Related to the effect of time of exposure, it was noticed to be more evident (1.72% higher yield) at 60°C (going from 17.28% to 19.00% in 60 and 120 min respectively) when compared to 30 °C, in which yield increased 0.55%.

Influence of temperature (T) and time of exposure to temperature (t) on extraction yield

Since variations in extraction yield values can be observed when applying different temperatures and time of exposure to temperature, it can be said that there is an interaction between those investigated variables, i.e., the effect of a variable may depend on the level of the other variable, considering that, in the absence of interaction between such variables, the values for a changing in time of exposure at a given temperature and vice versa should be equal (except for experimental error).

For the ethanolic and hydroethanolic extraction, the interaction effect ($T\ x\ t$) calculated were, respectively, -0.85% and 0.58%. In the case of ethanolic extraction, the negative interaction means that, for a constant temperature, when the time of exposure to temperature is increased, the extraction yield decrease -0.85%, on average. On the other side, in the case of hydroethanolic extraction, an increase at the time of exposure to temperature leads to an increase of 0.58% on the extraction yield, on average.

Although it is evident that a variable influence on the extraction yield depends on the level of the other variable, it is relevant to know the individual influence of the variable (T or t), that is, the mains effect. So, the main effect of temperature and time of exposure to temperature variables on the process of obtainment of alcoholic and hydroalcoholic extracts are presented in Tables 4 and 5, respectively.

Table 4

Main effect of temperature (T) and time to exposure to temperature (t) on the process of obtainment of ethanolic extract of jambo tree (Syzygium malaccense (L.) Merr. & L. M. Perry) leaves

Variable	Effect (%)
Temperature (T)	6.00
time (t)	3.14
$T\ x\ t$	-0,85

Table 5

Main effect of temperature (T) and time to exposure to temperature (t) on the process of obtainment of hydroethanolic extract of jambo tree (Syzygium malaccense (L.) Merr. & L. M. Perry) leaves

Variable	Effect (%)
Temperature (T)	6.63
time (t)	1.14
$T \; x \; t$	0.58

From Tables 4 and 5 it can be observed that temperature main effect is greater than time of exposure to temperature for both ethanolic and hydroethanolic extraction. As presented values are positive (+), it can be affirmed that the increase in temperature as well as the increase of the time of exposure to temperature (when considering each variable at a time) resulted in the extraction yield gain (by the percentage presented). However, it was considered important to analyze if the estimated effects differ statistically from zero.

Table 6 present the minimum values (at Product column) that an effect must present in other to be considered statistically significant.

Table 6

Absolute values for considering the effects T and t statistically significant on the process of obtainment of hydroethanolic extract of jambo tree (Syzygium malaccense (L.) Merr. & L. M. Perry) leaves, at 95% confidence interval and 4 degrees of freedom

Extraction	s_{effect}	Student´s t point, t_4	Product
Ethanolic	1.182	2.776	3.281
Hydroethanolic	0.566	2.776	1.571

From Table 6, between the studied variable, only the effect of temperature (T) can be considered statistically significant since for both extractions such variable presented an effect greater than the product of the standard error (s_{effect}) by the point of the Student's t distribution (6.00 > 3.281, for ethanolic extraction and 6.63 > 1,571, for hydroethanolic extraction).

5. Conclusion

Studies related to the development and applicability of plant extracts have been motivated in order to search for natural products, like plants extracts, that can help in efforts against the use of chemical products as preservatives in food and medicines. Obtaining ethanolic and hydroethanolic extracts from leaves of the jambo tree by maceration is a viable process with regard to the simplicity of the process and the high availability of the raw material. The present study suggested the application of a heating step (at mild temperatures) prior to the maceration process, with the aim of investigating an alternative to add advantages to the maceration process in terms of higher process yield.

When investigating the effect of the interaction between the studied variables (temperature and time) for ethanolic extraction, it was found that there is an antagonism, that is, the increase in one factor weakens the other factor under study, but the opposite was observed for hydroethanolic extraction. Moreover, it was verified that the temperature application time (60 min or 120 min) did not have a significant effect on the yield both of ethanolic and hydroethanolic extractions, suggesting that the shorter temperature application time may be more viable for economic reasons. The use of organic solvents in aqueous solution, as is the case of the solution used in this study (70% v/v ethanol), can favor the process in the sense of economic and safety (less volatility and toxicity).

For the extracts obtained, it is suggested to carry out a study to identify/categorize the chemical compounds present, since it is known that compounds present in the leaves of the red jambo fruit have biological activities and, consequently, can play roles as natural preservatives.

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