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Ultrasound-Assisted Extraction of Bioactive Compounds from Sete Capote leaves (Campomanesia Guazumifolia Cambess.)

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***Abstract*— In this work, the ultrasound-assisted solvent extraction was investigated to obtain bioactive extracts from Sete Capote (Campomanesia Guazumifolia Cambess.) leaves. The effects of temperature, ultrasound power and solvent/leaf mass ratio upon the extraction yield through an experimental design were evaluated. Furthermore, chemical characterization of the extracts by determination of antioxidant capacity, quantification of phenolic compounds and gas chromatography were also performed. By the results of the chromatographic analysis, it was observed that Lupeol was the predominant antioxidant compound. The advantage of using ultrasound was verified once the results conventional extraction by maceration showed lower results values.**

Keywords*— *antioxidant activity; ultrasonic extraction; phenolic compounds

I. INTRODUCTION

Leaves and fruits of diverse species of plants have been studied as bioactive compounds source [1, 2, 3, 4]. In the food field, researches aim to find natural products with antioxidant activity to be used as an alternative to synthetic antioxidants broadly used in the industry, which can be related to possible deleterious effects on health [2, 5]. In the pharmaceutical industry, antioxidants are of great value since they are important for reducing the risk of developing diseases such as diabetes, hypertension, neurological degenerative diseases and some cancers [6,7].

Among the antioxidants, the phenolic compounds draw attention due to their biological actions associated with the prevention of diseases and their curative potential [8-9]. The characteristic of health protectors presented by these compounds is derived from properties such as the action of capturing free radicals, the ability to chelate transition metals and reactivity as a donor of hydrogen and electrons [3].

In Brazil there is a variety of native plants that have not had yet their full properties evaluated, as an example is the Campomanesia guazumifolia (Cambess.) (Sete Capote), belonging to the family Myrtaceae. To this family also belongs plants that give very common fruits in Brazil as pitanga and jabuticaba, which are, like the tree of Sete Capote, important wild fruit trees with sweet and edible fruits. The family Myrtaceae belongs to the order Myrtales and is formed by about 150 genera and 3,600 species, being very common plants, distributed in regions of tropical and subtropical climate, in Brazil, with emphasis on the fauna of the Atlantic Forest [10, 11, 12].

C. guazumifolia is a tree between 6 and 10 m in height, with single leaves, whole, opposite and without spatulas. Its flowers are white, large and showy and its trunk presents layers of bark that detach with great facility [13].

Studying the family Myrtaceae is important considering that many of its species are popularly used for the treatment of various health problems such as gastrointestinal disorders, hemorrhagic states, and infectious diseases. In traditional medicine, the use of C. guazumifolia is common in treatments of hepatic diseases, and diarrhea, it is also reported that indigenous culture uses it as a tonic [14,15,16].

According to studies developed by Arruda [17] the crude ethanolic extract of the Sete Capote leaf is effective in protecting the gastric mucosa from lesions caused by ethanol ingestion, acting efficiently in gastric emptying, as verified by the tests performed in animals, which supports the popular use of the species. The same author [17] also carried out phytochemical studies on C. guazumifolia, verifying the presence of flavonoid glycosides, iridoids, steroids and/or triterpenoids, saponins, and tannins.

Fruits such as pitanga and jabuticaba, belonging to the family Myrtaceae, have been frequently studied in recent years due to their antioxidant properties [18, 19], but there are few studies related to the extraction of compounds from C. guazumifolia leaves.

In the industry, plants extractions are usually performed employing steam drag or organic solvent extraction, these methods, however, present drawbacks, such as the need for solvent separation, high energy consumption in the case of drag by steam, consumption of large volumes of solvents and long extraction time, up to 72 hours, which can affect the extracted components [20, 21, 22], for that reason, extraction alternatives have been sought.

An interesting alternative is ultrasound-assisted extraction, which according to Klein *et al*. [1] is considered to be effective in extracting bioactive compounds. This technique has the advantages of

consuming less solvent, lower temperature, extraction time and cost and high reproducibility [23,24].

The effect of ultrasound is due to the phenomenon of cavitation, caused by sonic waves, which increases mass transfer and mixture efficiency by increasing the molecular mobility leading to higher chemical reactions rates [25]. Higher concentrations of bioactive compounds in ultrasonic assisted extraction are reported in the works of Khan *et al*. [26] and Carrera *et al*. [27]. According to Danlami *et al*. [28], some factors involved in ultrasonic extraction may be responsible for the increase in the yield of the extract, among them extraction time, potency and solvent used. Ethanol is a solvent that has the advantages of low cost, low environmental impact and already has been used successfully to obtain extracts of leaves of Uvaia (Eugenia Pyriformis C.), mango peel and vegetables of the caatinga [1, 2, 29, 30].

Silva *et al*. [31] studied the antimicrobial and antioxidant activity of C. guazumifolia leaf extracts obtained by conventional extraction by maceration using the solvents hexane, ethyl acetate, and ethanol. Arruda [17] carried out morphoanatomical, phytochemical and biological activity studies in extracts of C. guazumifolia leaf, where the extracts were also obtained by means of conventional extraction by maceration, using ethanol and water as solvents. It is not from the knowledge of the authors any studies evaluating the effect of ultrasound on the solvent extraction of the leaves of C. guazumifolia.

In this context, the aim of this study was to investigate the ultrasonic-assisted extraction of leaves of C. guazumifolia, to evaluate the effect of temperature, ultrasound power and solvent volume per mass leaf ratio on extraction yield, and to determine the antioxidant capacity and to quantify phenolic compounds present extracts from the leaves.

II. Material and Methods

A. Chemicals

The reagent used in the extractions was ethyl alcohol (NEON), with 99.8% of purity. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 6-Hydroxy-2,5,7,8 - tetramethylchroman-2-carboxylic acid (Trolox) from Sigma-Aldrich and ethyl alcohol 99.8% (NEON) were used for the determination of the antioxidant activity. The quantification of phenolic compounds was performed using the Folin-Ciocalteau reagent (Dynamics), ethyl alcohol 99.8% (NEON), gallic acid (Vetec) and sodium carbonate (Dynamic).

B. Sete Capote leaves characterization

Sete Capote leaves (Campomanesia guazumifolia Cambess.) were collected in a private property in the city of Serranopolis do Iguaçu-PR. The material was collected at random points and the plants were dried in the shade for 14 days at ambient temperature (20 ± 5ºC). Afterward, it was milled in a blender machine and the resulting particles classified by sieving, using sieves of 7, 9, 12, 16, 24 and 32 mesh. Each granulometric class was separated and stored.

The humidity of the ground leaves was determined by the gravimetric method. Approximately 2 g of leaves were weighed in Petri dish and taken to the oven at 105°C for about 24 h, until constant mass. They were then removed from the oven and placed in the desiccator containing silica gel for cooling to room temperature and weighed again to constant mass. The percentage of moisture was obtained by means of the mass difference between the beginning and end of the procedure. The analysis was performed in triplicate.

For the determination of the chemical composition, a gas chromatograph equipped with a linear quadrupole mass spectrometer model 5975C (Agilent), an HP-5MS column, 5% diphenyl, 95% dimethylpolysiloxane (30 m x 0.25 mm) and detector ionization flame (FID) was employed. The extract analysis was performed by injecting 1.0 µl of the sample, previously diluted with dichloromethane, at a split ratio of 1:50, the injector temperature of 280°C. The carrier gas employed was Helium, at a rate of 1.0 mL/min, with oven programmed for an initial temperature of 40°C (2 min), up to 280°C at a rate of 5°C/min, where it was kept for 20 min. The identification of the compounds detected in the chromatograms of the samples was performed using the mass spectral database NIST11 and AMDIS program (*Automated mass spectral deconvolution and identification system*).

C. Extraction methods

The ultrasound-assisted extraction of the Sete Capote leaves extractions were performed in an ultrasonic Eco-sonics (Ultronique) with a frequency of 20 kHz, maximum ultrasonic power of 450 W with a titanium microtip probe of 4 mm diameter coupled.

An experimental design 2^3, with triplicate at the central point, was used to evaluate the effect of the variables, which were ultrasound power, ratio of solvent volume per leaf mass (v/m) and temperature, being the response the yield of extraction. The volume of solvent in each extraction was 50 mL and the experiments were carried out in a jacketed reactor to control the temperature from a thermostatic bath model MA-184 (MARCONI).

The factors evaluated in the experimental design, as well as their levels and codifications, are presented in Table 1, where the power corresponds to the percentage of the maximum ultrasound power, 450 W. The experimental conditions were defined based on the work of Klein *et al*. [1] and considering the operational conditions in order to guarantee a wide variation between the points.

Table 1. Experimental design for the ultrasound assisted extraction.

Power (%)	Ratio solvent/leaf (ml/g)	Temperature (°C)
20 (-1)	10 (-1)	40 (-1)
50 (0)	15 (0)	50 (0)
80 (+1)	20 (+1)	60 (+1)

The time of each extraction was of 10 min, as well as used in the works of Khoigani *et al.* [32] and Dary *et al*. [33], the extracts were subsequently filtered on filter paper and taken to the oven (50°C) for complete evaporation of the solvent. Yields were calculated by the ratio of dry extract mass to leaf mass used.

In order to evaluate the effect of ultrasound on extractions, conventional extraction by maceration was also performed. The plant material was mixed with 50 ml of ethyl alcohol in erlenmeyer and placed in an incubator with orbital agitation (TECHNAL TE-421) at a temperature of 60°C and constant stirring of 150 rpm. The condition of temperature and solvent volume ratio per leaf mass corresponding to the highest yield of ultrasonic-assisted extraction was employed. Following, the extract was filtered on filter paper and taken to the oven (50°C) for complete removal of the solvent and the mass of dried extract obtained was determined.

The antioxidant activity was also determined for this sample and the phenolic compounds were quantified by means of the same methods used for the extracts obtained by ultrasound.

D. Determination of antioxidant activity

The determination of the antioxidant activity was performed by the DPPH method and was based on the work of Enujiugha *et al*. [34]. A solution (≈0.03 $mg_{extract}$/mL) was prepared with each of the extracts. For this purpose, the samples were dissolved in 50 mL of ethyl alcohol (S1) and 180 µL of that extract solution was diluted to 10 mL in ethyl alcohol (S2). Subsequently, 0.2 mL of the extract solution was placed to react with 3.8 mL of the DPPH radical solution (0.1 µM in ethanol). The mixture was vigorously stirred for 1 min in a vortex (Kasvi, K45-2810) and allowed to stand at ambient temperature in the dark for 30 min.

Trolox standard solutions (25 to 1000 µM in ethanol) were also prepared. Then the absorbance for the sample and the Trolox solutions were measured using a spectrophotometer (UV-Vis 1800, Shimadzu) at 517 nm with ethanol sample as the blank. The antioxidant capacity of each sample was expressed in microles curve of Trolox equivalent (TE) per milligram of extract.

E. Quantification of phenolic compounds

Quantification of phenolic compounds was performed using the Folin-Ciocalteau reagent, which involves the reduction of the reagent by the phenolic compounds of the samples with the formation of a blue complex whose intensity increases linearly at 760 nm and was based on the procedure presented by Singleton and Rossi [35].

In this analysis, a solution (≈0.05 $mg_{extract}$/mL) was prepared with each of the extracts. The samples were dissolved in 50 ml of ethyl alcohol and 0.36 ml of that extract solution was diluted to 10 mL in ethyl alcohol. For the reaction, an aliquot of 0.3 mL of the extract solution in ethanol was added to a test tube with 2.5 mL of 10% aqueous Folin-Ciocalteau reagent and 2.0 mL of sodium carbonate 7.5%. The mixture was incubated for 5 minutes in a 50°C water bath and subsequently, the absorbance was measured at 760 nm (UV-Vis 1800, Shimadzu). The blank was obtained by replacing the sample volume with water, maintaining the same amounts of Folin-Ciocalteu reagent and sodium carbonate solution. A standard curve was prepared with gallic acid (5-80 µg/mL) and the results expressed in µg equivalents of gallic acid (EGA) per mg of extract. Each assay was performed in triplicate.

III. Results and Discussion

A. Particle size and moisture content

With the granulometry analysis of the Sete Capote leaves after being ground, it was possible to verify that most of the material was retained in the fraction 24/32 mesh, representing 42.15% of the total sample mass analyzed, meaning that the average diameter of the largest part of the particles is 0.605 mm, and the samples in this size range were used in the following extractions experiments.

The samples analyzed had an average moisture content of 7.73 ± 0.68%. The verified humidity is relatively low, considering that the sample underwent drying in the shade and room temperature (20 ± 5ºC). The biomass drying is a very important step for most the extraction processes of compounds present in medicinal, aromatic and spice species since the reduction of the moisture is important for the stabilization of the plant metabolism, immobilizing the enzymatic activity that degrades the active principles present in plant matter [36-37].

B. Experimental design for extraction of Sete Capote leaves

The results obtained in the ultrasonic assisted extraction, using the same tensile time in all the experiments, are presented in Table 2, where the yields are shown according to the conditions determined by the experimental design.

From the data presented in Table 2, it is verified that the lowest yield, 1.08%, was reached in the minimum conditions of the variables (20% of maximum ultrasound power, 40°C of temperature and 10 ml/g of solvent/solid ratio). In the maximum values of the variables (ultrasound power 80%, a temperature of 60°C and ratio of 20 ml/g), the highest yield was obtained, the maximum yield was achieved, 4.11%.

Table 2. Operating conditions and yields of ultrasonic assisted and conventional extraction.

Sample	Power (%)	Temperature (^{o}C)	Solvent/solid ratio (ml/g)	Yield (%)
1	20	40	10	1.08
2	80	40	10	1.89
3	20	60	10	1.31
4	80	60	10	2.17
6	20	40	20	1.15
8	80	40	20	2.86
9	20	60	20	2.42
10	80	60	20	4.11
5,7,11	50	50	15	2.06 ± 0.27[a]
Maceration	-	60	20	2.29

[a] triplicate average.

The results presented in Table 2 confirm the advantages of ultrasonic-assisted extraction with respect to conventional by maceration previously cited [25], indicating that there is an increase in mass transfer due to the phenomenon of cavitation, caused by sonic waves since the extraction yield was up to 79% higher when assisted by ultrasound.

It is observed that when temperature and power are fixed, there is a yield increase with the increase of the solvent/solid ratio, the same effect is beheld when temperature and solvent/solid ratio are fixed and the power is increased and when solvent/solid ratio and power are fixed and the temperature is increased, in all cases there is enhancement in the yield, which indicates that all conditions had a positive effect on yield. It is also noticed that the increase in yield is more significant when the varied condition is the ultrasound power, indicating that this variable has a higher influence.

In Figure 1 the Pareto Graph is shown and it is possible to verify that the variables power of the ultrasound, ratio, temperature and the interactions between the variables are significant at the significance level of 5%, with the exception of the interaction between ultrasound power and solvent/solid ratio, being the most significant variable the ultrasound power. It is notable, as was previously observed, that all the variables have a positive effect, in other words, the yield is enhanced by the increase of the evaluated variables in the studied conditions.

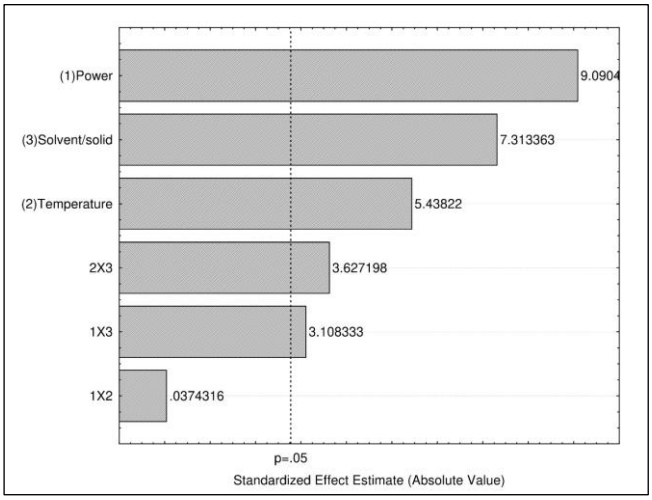

Figure 1. Pareto Graph.

As assessed by Klein *et al*. [1] and Wen *et al*. [38], the increase in yield of the extraction of compounds in vegetable matter is positive with the increase in the solvent/solid ratio, since it follows the principles of mass transfer, where higher concentration gradients are obtained when the ratio between the quantity of solvent and mass of sample used is increased. The influence of ultrasound power applied on the yield of plant extracts was also studied by Klein *et al*. [1] and Wen *et al*. [38] which reported the same trending, the yield was shown to be directly proportional to the power applied in the extraction. The effect of ultrasound is due to the cavitation phenomenon, which is higher as higher energy is applied, ie, the higher the power the greater the formation and implosion of the cavitation bubbles that cause the rupture of the cell walls and facilitate the penetration of the solvent, consequently, there is enhancement on the extraction rate.

The positive effect of temperature is probably due to the increase in the permeability of cell walls that can be weakened with increasing temperature, which facilitates the diffusion process of the extract [39].

The RSM (response surface methodology) equation, obtained by using the significant variables only, is given by Equation (1). The employed model was evaluated by the f-test and from the analysis of variance (ANOVA), the calculated f-value (31.42) was significantly higher than the critical f-value (6.16). As a consequence, the proposed regression model is statistically valid and can adequately represent the experimental data in the studied range of the parameters. Besides, the accuracy of the regression model for the experimental data can be also observed based on the coefficient of determination (R2) of 0.98. Thus, both the f-values and R2 value indicate that the yields of the ultrasonic assisted extraction can be well described as a function of the linear terms of ultrasound power, volume of solvent per leaf mass ratio and temperature (see Equation (1)), in the investigated conditions range.

$$\text{Yield } (\%) = 2.5006 - 0.0005 * P - 0.0379 * T - 0.22267 * R + 0.001442 * P * R + 0.0050 * T * R \quad (1)$$

Where P is the ultrasound power (%), T is temperature (ºC) and R is the solvent/solid ratio (ml/g).

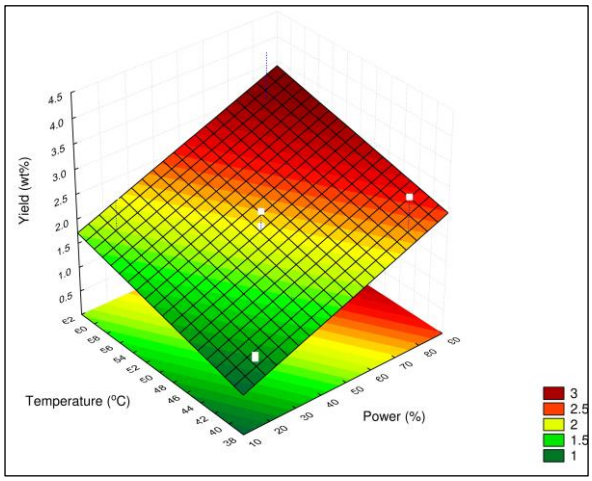

Figure 2. Response surface of yield value as a function of ultrasound power and temperature.

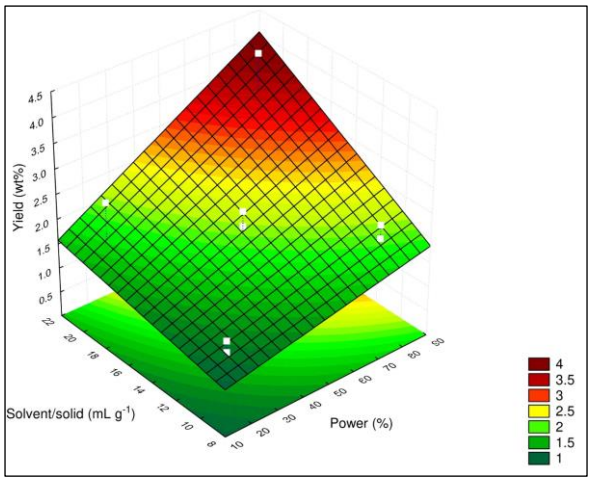

Figure 3. Response surface of yield value as a function of ultrasound power and solvent volume/leaf mass ratio.

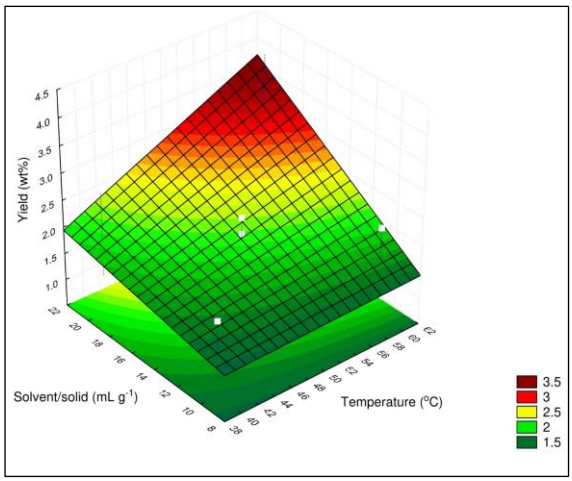

Figure 4. Response surface of yield value as a function of solvent volume/leaf mass ratio and temperature.

In Figure 2, 3 and 4 are presented the response surfaces of yield value of ultrasonic-assisted extraction as a function of ultrasound power, solvent/solid ratio, and temperature. It can be observed in these three figures that the regions with the best results are located in the conditions of higher temperature, ultrasound power, and solvent/solid ratio, being the higher yield of 4.11% verified at these conditions. The authors did not find in the literature any other studies on the extraction of bioactive compounds from the Sete Capote leaves and therefore, results could not be compared.

C. Chemical Composition

The chemical composition of the Sete Capote leaf extract, referring to the central point of the experimental design (conditions used in samples 5, 7 and 11), was determined by means of gas chromatography analysis, and is presented in Table 3.

In Table 3 it is possible to identify which antioxidant compounds are present and to verify that those that are present in larger quantities are the cyclolanostanol methylene, sitosterol, amirin and lupeol, tritoperoid compounds which, according to Silva *et al*. [39], have a significant anti-inflammatory capacity. Sitosterol and amirin, for instance, are pentacyclic triterpenoids that have significant anti-inflammatory activity, and lupeol, besides having considerable anti-inflammatory activity, has been constantly studied in alternative treatments for pancreatic cancer, neuroblastoma and pulmonary adenocarcinoma [41]. Hence, it can be highlighted that the use of the leaves of C. guazumifolia in traditional medicine has scientific support.

Table 3. Chemical composition of the extracts obtained by gas chromatography.

Peak [1]	t_R [2]	Compound Name [3]	%A [4]
1	31.848	Not determined	1.80
2	31.994	Tetramethyl Hexadecene	0.24
3	32.358	Tetramethyl Hexadecenol	0.32
4	32.473	Not determined	0.07
5	32.712	Tetramedyl Hexadecenol	0.62
6	44.200	Cyclanolanthanol Methylene	16.22
7	44.637	Ledeno	1.74
8 a 11	44.824 a 47.394	Not determined	23.43
12	48.549	Sitosterol	15.23
13	49.642	Amirin	5.77
14 a 16	51.224 a 52.389	Not determined	2.28
17	53.211	Amirin	10.02
18	53.794	Lupeol	19.57
19 a 22	55.178 a 61.151	Not determined	2.11
23	62.399	Vitamin E	0.86

[1]Peak number by the elution order in the column. [2]t_R = Retention time of the compound in the column in minutes. [3]Most common name of the identified compound. 4% A = Percentage of normalized area which indicates the relative distribution of the compounds in the sample.

The author Li *et al*. [42], also identified in the fruit of the jamelão (Eugenia jambolana Lam), a species belonging to the family Myrtaceae, compounds present in the Sete Capote leaves, such as Sistosterol, the same was reported in the studies of Sarges *et al*. [43], on the stem of another plant of the same family, Eugenia prostrates, identifying the presence of amirin and lupeol.

D. Antioxidant activity - Ability to sequester free radicals

The ability to sequester free radicals relative to the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was initially chosen because it is a simple and fast methodology [44]. The antioxidant potential of each sample, expressed in Trolox equivalent (TE) curve micromoles per milligram extract, together with the respective deviation are presented in Table 4.

Table 4 - Determination of the ability to sequester free radicals (DPPH)

Sample	Antioxidant Potential ($\mu mol_{Trolox}/mg_{Extract}$)
1	5.83 ± 0.15^{b}
2	5.00 ± 0.12^{c}
3	5.80 ± 0.30^{b}
4	2.79 ± 0.07^{f}
6	6.49 ± 0.24^{a}
8	3.37 ± 0.16^{de}
9	3.70 ± 0.03^{d}
10	4.77 ± 0.14^{c}
5, 7, 11	4.54 ± 0.16^{c}
Maceration	3.03 ± 0.22^{ef}

The same letter in the same column indicates that there is no significant difference in the confidence level of 5%.

In this method, the antioxidant substances present in the extract react with the DPPH which is a stable radical and converts it to 2,2-diphenyl-1-picrylhydrazine, thus the higher the DPPH consumption the higher the antioxidant power of the sample [45]. The ultrasound power, as shown in Figure 1, is the most significant variable in the extraction process, thus, Table 4 shows that, in general, there is an inversely proportional relationship between this variable and the antioxidant capacity, being that the samples submitted to extractions with the lowest power (20%), presented higher antioxidant capacity, as is the case of samples 6, 1 and 3. As evaluated by Chew *et al*. [46], the antioxidant capacity of a given material does not depend exclusively on the amount of antioxidants present in the extract, and it is important to consider the type of structure and the interactions formed between them. Considering the high efficiency of mass transfer in ultrasonic-assisted extractions, it is plausible to consider that when using higher ultrasound power in bioactive extraction, other components may be transferred from the biomass to the liquid phase, negatively affecting the capacity antioxidant.

The temperature is also a variable of considerable influence on the extraction of the bioactive compounds present in the vegetal matter, and although the increase of the temperature favors the extraction whereas increases the solubility of the solute, as well as the coefficient of diffusion, as shown in Table 4, there is a general reduction of oxidant activity, possibly due to the thermal degradation of the phenolic compounds, thus reducing the antioxidant capacity of the extract [47, 48].

The extract obtained by means of the maceration technique, at 60ºC and agitation of 150 rpm, had lower antioxidant potential when compared to most of the extracts that were obtained by ultrasonic assisted extraction. It is worthy to mention that maceration extraction was performed at the highest conditions evaluated in this study, and as it was previously mentioned, degradation of the antioxidant constituents can occur in the corresponding conditions.

The antioxidant potential of Sete Capote leaf was also determined by Arruda [17], using the DPPH method, where the extracts of the biomass obtained by soxhlet presented an antioxidant activity of 25.5 µg/mL, this being a value from the range of results obtained in this study, which was 16.84 µg.mL-1 up to 42.62 µg/mL. Sobeh *et al*. [49] evaluated the antioxidant potential of the leaf of Jambo-pink leaf (Syzygium Jambos), a tree of Myrtaceae family, and determined the value of 5.7 ± 0.45 µg/mL, showing that the Sete Capote leaf has antioxidant potential superior to some plants of the same family.

E. Phenolic Compounds

The results obtained in the determination of total phenols by the Folin-Ciocalteau method as gallic acid equivalents (EGA) per gram of the sample are presented in Table 5.

Table 5 - Total phenol content.

Sample	$\mu g_{EGA}/mg_{extract}$
1	525.24 ± 13.45^{dc}
2	574.42 ± 13.78^{b}
3	626.57 ± 8.65^{a}
4	546.16 ± 9.88^{cb}
6	577.94 ± 17.28^{b}
8	388.61 ± 9.97^{e}
9	373.72 ± 3.31^{e}
10	325.91 ± 8.97^{f}
11	509.51 ± 6.19^{d}
Maceration	300.96 ± 16.50^{f}

The same letter in the same column indicates that there is no significant difference in the confidence level of 5%.

From the obtained results it was possible to confirm the presence and quantification of the total phenol content in the samples, and, in general, it is possible to observe that there is a direct correlation with the antioxidant potential, since samples 6, 1 and 3, are the ones with higher levels of phenols, as well as for the

antioxidant potential. Furthermore, the samples submitted to higher operating conditions at the time of extraction had lower phenolic contents, as was the case for the antioxidant potential, which is also possible due to the degradation of their constituents.

The correlation between total phenols and antioxidant activity reported in the literature is contradictory. While Benvenuti *et al*. [50] and Prado *et al*. [51] observed a significant correlation, while Imeh and Khokbar [52] did not observe direct correlation.

The phenol content found for the extract obtained by maceration was lower than the others, maintaining the pattern observed in the previous analyzes, thus reaffirming the advantages of the use of ultrasonic-assisted technique in the extraction of compounds from the Sete Capote leaf.

The leaves of the Jambo tree (Syzygium Jambos) and the pitangueira (Eugenia uniflora L.), both from the same family of Sete Capote, Myrtaceae, had their total phenol content determined at 466 $\mu g_{EGA}/mg_{extract}$ and 229.38 $\mu g_{EGA}/mg_{extract}$, respectively, approaching the values presented in Table 5 [49-53].

In general, the extracts evaluated presented high levels of phenolic compounds, when compared to data from other species described in the literature, such as pecan nests (118 $\mu g_{EGA}/mg_{extract}$) and Brazilian plants with great proven medicinal properties such as amêndoa-brava (397.9 $\mu g_{EGA}/mg_{extract}$), caneleiro (483.63 $\mu g_{EGA}/mg_{extract}$), capitão (439.38 $\mu g_{EGA}/mg_{extract}$), and pau-terra-da-folha-grande (394.9 $\mu g_{EGA}/mg_{extract}$) [45- 51].

IV. Conclusion

It was verified analyzing the results of the experimental design that the variables temperature, ultrasound power and solvent volume/leaf mass (v/m) were significant effects on the yield of extraction of Sete Capote leaves, showing positive effect, achieving the maximum yield, 4.11%, in the higher variable values evaluated (temperature 60°C, volume solvent/leaf mass ratio 20 and ultrasound power 80%). The general second-order model was the best representative of the kinetics data for the ultrasonic-assisted extraction.

It was observed that the extracts obtained have a considerable antioxidant capacity and the highest antioxidant potential observed was 6.49 ± 0.24 $\mu mol_{Trolox}/mg_{Extract}$ which was reached in the sample of lower yield, driving us to believe that the high ultrasound power, temperature and amount of solvent led to sampling degradation, thus, for an efficient oxidizing agent the use of milder conditions should be employed.

The presence of phenolic compounds was also confirmed and was in the range of 626.57 ± 8.65 and 373.72 ± 3.31 $\mu g_{EGA}/mg_{extract}$ respectively, values considered significant when compared to other species described in the literature.

With the verification of the chemical composition by the means of gas chromatography it was possible to identify the main antioxidant compounds present in the extract, which were Cyclolanostanol Methylene, Sistosterol, Amirin and Lupeol, all recognized for their anti-inflammatory action, which supports the medicinal use of the Sete Capote plant.

The conventional extraction method by maceration was carried out at the best conditions identified in the experimental design step and the advantage of using ultrasound was demonstrated, considering that the conventional extraction presented lower values of yield (2.29%), antioxidant potential (3.03 ± 0.22 $\mu mol_{Trolox}/mg_{Extract}$) and phenolic compounds (300.96 ± 16.50 $\mu g_{EGA}/mg_{extract}$).

The results reported in the present work encourage the continuity of studies evaluating antioxidant action of Sete Capote leaves as well as the optimization of the processes of extraction of bioactive compounds.

V. Acknowledgemenst

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VI. References

[1] Klein E. J.; Santos, K. A.; Palú, F.; Vieira, M. G. A.; Silva, E. A. da. (2018) Use of supercritical CO2 and ultrasound-assisted extractions to obtain α/β-amyrin-rich extracts from uvaia leaves (Eugenia pyriformis Cambess). The Journal of Supercritical Fluids, 137: 1-8.

[2] Júnior, N. O. R.; Fernandez, L. G.; Castro, R. D. de; Silva, L. C.; Gaulberto, S. A.; Pereira, M. L. A.; Silva, M. V. da. (2011) Compostos bioativos e atividade antioxidante de extratos brutos de espécies vegetais da caatinga. Brazilian Journal of Food Technology, 14(1): 50-57.

[3] Santos, M. A. I.; Simão, A. A.; Marques, T. R.; Sackz, A. A.; Corrêa, A. D. (2016) Efeito de diferentes métodos de extração sobre a atividade antioxidante e o perfil de compostos fenólicos da folha de mandioca. Brazilian Journal of Food Technology, 19, e2015067.

[4] Soares, M.; Welter, L.; Kuskoski, L. G.; Fett, R. (2008) Compostos fenólicos e atividade antioxidante da casca de uvas niágara e isabel. Revista Brasileira de Fruticultura, 30(1): 59-64.

[5] Nascimento, J. C.; Lage, L. F. O.; Camargos, C. R. D.; Amaral, A. C.; Costa, L. M.; Sousa, A. N. de; Oliveira, F. Q. (2011) Determinação da atividade antioxidante pelo método DPPH e doseamento de flavonóides totais em extratos de folhas da Bauhinia variegata L. Revista Brasileira de Farmácia, 92(4): 327-332.

[6] Aruoma, O. I. (2002) Neuroprotection by dietary antioxidants: new age of research. Die Nahrung, 46(6): 381-382.

[7] Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M. T.; Mazur, M.; Telser, J. (2007) Free radicals and antioxidants in normal physiological functions and

human disease. The International Journal of Biochemistry & Cell Biology, 39(1): 44-84.

[8] Vuong, Q.; Goldsmith, C.; Dang, T.; Nguyen, V.; Bhuyan, D.; Sadeqzadeh, E.; Scarlett, C.; Bowyer, M. (2014) Optimisation of ultrasound-assisted extraction conditions for phenolic content and antioxidant capacity from Euphorbia tirucalli using response surface methodology. Antioxidants, 3(3): 604-617.

[9] Bhuyan, D. J., Vuong, Q. V., Chalmers, A. C., van Altena, I. A., Bowyer, M. C., & Scarlett, C. J. (2016). Development of the ultrasonic conditions as an advanced technique for extraction of phenolic compounds from Eucalyptus robusta. Separation Science and Technology, 52(1): 100–112.

[10] Cronquist, A. (1981) An integrate system of classification of flowering plants. Columbia University Press: New York, USA.

[11] Barroso, G. M. (1984) Sistemática de angiospermas do Brasil, v. 2.; Imprensa Universitária da UFV: Viçosa, BR.

[12] Joly, A. B. (1985) Botânica: introdução à taxonomia vegetal, 7Ed.; Nacional: São Paulo, BR.

[13] Lorenzi, H. (2002) Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas do Brasil. 4Ed.; Instituto Plantarum: Nova Odessa, BR.

[14] Brandão, M. (1991) Plantas medicamentosas do cerrado mineiro. Informe Agropecuário, 15(168): 15-20.

[15] Dorigoni, P. A.; ghedini, P. C.; Fróes, L.; Baptista, K. C.; Ethur, A.B. M.; Baldisseroto, B.; Bürger, M. E.; Almeida, C. E.; Lopes, A. M. V.; Záchia, R. A. (2001) Levantamento de dados sobre plantas medicinais de uso popular no munícipio de São João o Polêsine, RS, Brasil, I – relação entre enfermidades e espécies utilizadas. Revista Brasileira de Plantas Medicinais, 4(1): 69-79.

[16] Brack, P; Kinupp, V. F.; Sobral, M. E. G. (2007) Levantamento preliminar de espécies frutíferas de árvores e arbustos nativos com uso atual ou potencial do Rio Grande do Sul. Revista Brasileira de Agroecologia, 2(1): 1769-1772.

[17] Arruda, M. F.C. (2013) Estudos morfoanatômicos, fitoquímicos e de atividade biológicas de Campomanesia guazumifolia (Cambess.) O. Berg, Myrtaceae. 95 p. Dissertação (Mestrado em Ciências Farmacêuticas) – Setor de Ciências da Saúde, Universidade Federal do Paraná, Curitiba.

[18] Luzia, D.; Bertanha, B.; Jorge, N. (2010) Sementes de pitanga (Eugenia uniflora L.): potencial antioxidante e perfil de ácidos graxos. Revista do Instituto Adolfo Lutz, 69(2): 175-80.

[19] Peres, M. T. L. P. *et al*. (2013) Phytotoxic and antioxidante activity of seven native fruits of Brazil. Acta Botanica Brasilica, 27(4): 836-846.

[20] Díaz-Reinoso, B.; Moure, A.; Domínguez, H.; Parajó, J. C. (2006) Supercritical CO 2 extraction and purification of compounds with antioxidant activity. Journal of Agricultural and Food Chemistry, 54: 2441–2469.

[21] Cristofoli, N. L., Lima, C. A. R., Vieira, M. M. C., Andrade, K. S., & Ferreira, S. R. S. (2018). Antioxidant and antimicrobial potential of cajazeira leaves (Spondias mombin) extracts. Separation Science and Technology, 1–11.

[22] Chen, C., Chen, T., Liu, Y., Zou, D., You, J., & Li, Y. (2014). Rapid Screening, Identification, Separation, and Purification of Four Bioactive Compounds fromSwertia mussotiiFranch. Separation Science and Technology, 50(4), 604–610.

[23] Joana Gil-Chávez, G.; Villa, J.A.; Fernando AyalaZavala, J.; Basilio Heredia, J.; Sepulveda, D.; Yahia, E. M.; González-Aguilar, G.A. (2013) Technologies for extraction and production of bioactive compounds to be used as nutraceuticals and food ingredients: an overview. Comprehensive Reviews in Food Science and Food Safety, 12: 5–23

[24] Luque-Garcí a, J.; Luque De Castro, M. (2003) Ultrasound: a powerful tool for leaching. TrAC Trends in Analytical Chemistry, 22: 41–47.

[25] Price, G. J. (1992) Introduction to Sonochemistry. In: Price, G. J. Current Trends in Sonochemistry. Cambridge: The Royal Society of Chemistry, 1-7.

[26] Khan, M. K.; Abert-vian, M.; Fabiano-Tixer, A. S.; Dangles, O.; Chemat, F. (2010) Ultrassond-assisted extraction of polyphenols (flavonone glycosides) from orange (Citrus sinensis L.) peel. Food Chemistry, 119(2): 851-858.

[27] Carrera, C.; Rodríguez, A. R.; Palma, M.; Barroso, C. G. (2012) Ultrasound assisted extraction of phenolic compounds from grapes. Analytica Chimica Acta, 732:100-104.

[28] Danlami, J. M. *et al*. (2014) A comparative study of various oil extraction techniques from pants. Reviews in Chemical Engineering, 30(6): 605-626.

[29] Matos, J. E. X. de; Silva, F. J. A. da; Vieira, P. B. (2008) Solventes para extração do líquido da castanha de caju (LCC) e compatibilidade ambiental. Revista Tecnologia, 29(1): 101-109.

[30] Guerra, A. P.; Garcia, V.; Silva, C. (2016) Otimização da extração de compostos fenólicos da casca de manga (Tommy atkins) utilizando processo assistido por ultrassom. E-xacta, 9(1): 103-110.

[31] Silva, E. R. S.; Aliatti, L. N. S.; Brait, D. R. H.; Queiroz, E. M.; Batitoste, M.; Negrão, F.J.; Cardoso, C. A. L. (2014) Determinação das atividades antimicrobiana e antioxidante dos teores de fenóis e flavonoides em extratos orgânicos de campomanesia guazunifolia, In: 54º Congresso Brasileiro de Química: Natal, BR.

[32] Khoigani, S. R.; Rajaei, A.; Goli, S. A. H. (2017) Optimization of ultrasound-assisted extraction for the maximum quantity and quality of phenolics from Stachys lavandulifolia. Separation Science and Technology, 52(11): 1815-1825.

[33] Dary, C.; Baghdikian, B.; Kim, S.; Mabrouki, F.; Hul, S.; Jabbour, F.; Ollivier, E.; Bun, S. (2017) Optimization of ultrasound-assisted extraction of bioactive alkaloids from Stephania cambodica using response surface methodology. Comptes Rendus Chimie, 20(11-12): 996-1005.

[34] Enujiugha, V. N.; Justina, Y. T.; Sunday A. M.; Aderonke I. O. (2012) DPPH Radical Scavenging Capacity of Phenolic Extracts from African Yam Bean (Sphenostylis stenocarpa). Food and Nutrition Sciences, 3(1): 7-13.

[35] Singleton, V. L.; Rossi, J. A. (1965) American Journal of Enology and Viticulture, 16: 144.

[36] Corrêa, C. J. R.; Ming, L. C.; Scheffer, M. C. (1991) Cultivo de plantas medicinais, condimentares e aromáticas, 1Ed.; EMA TER-Paraná: Curitiba, BR.

[37] Von Hewrtwig, L. F. (1991) Plantas aromáticas e medicinais: plantio, colheita, secagem, comercialização, 2Ed.; Ícone: São Paulo, BR.

[38] Wen, C.; Zhang, J.; Zhang, H. Dzah, S. C.; Zandile, M.; Duan, Y.; Ma, H.; Luo, X. (2018) Advances in ultrasound assisted extraction of bioactive compounds from cash crops - A review. Sonoquímica Ultrassônica, 48: 538-549.

[39] Silva, C.; Garcia, V. A. dos S.; Franciscato, L. M. S. dos S. (2016) Extração assistida por ultrassom de compostos bioativos das cascas de lichia (Litchi Chinensis Sonn.). Revista Ciências Exatas e Naturais, 18(1): 81-96.

[40] Costa, H. N. R da; Santos, M. C. dos; Alcântara, A. F. de; Silva, M. C.; França, R. C.; Pilô-Veloso, D. (2008) Constituintes químicos e atividade antiedematogênica de Peltodon radicans (Lamiaceae). Química Nova, 31(4): 744-750.

[41] Gabriel, G. H.; Nepomuceno, L. L.; Pimenta, V. de S. C.; Araújo, E. G. de. (2017) Agentes antineoplásicos para o tratamento do osteossarcoma. Enciclopédia Biosfera, 14(26): 13-28.

[42] Li, Y.; Xu, J.; Yuan, C.; Ma, H.; Liu, T.; Liu, F.; Seeram, N. P.; Mu, Y.; Huang, X.; Li, L. (2017) Chemical composition and anti-hyperglycaemic effects of triterpenoid enriched Eugenia jambolana Lam. berry extract. Journal of Functional Foods, 28: 1-10.

[43] Sarges, F. N.; Monteiro, P. H. B.; Silva, E. S.; Guilhon, G. M. S. P.; Zoghbi, M. G. B.; Santos, L. S.; Arruda, M. S. P.; Silva, M. N. (2010) Estudo químico do caule de Eugenia protenta (Myrtaceae). Sociedade Brasileira de Química (SBQ).

[44] Oliveira, G. L. S. (2015) Determinação da capacidade antioxidante de produtos naturais in vitro pelo método do DPPH: estudo de revisão. Revista Brasileira de Plantas Medicinais, 17(1): 36-44.

[45] Sousa, C. M. M.; Silva, H. R.; Vieira, G. M.; Ayres, M. C. C.; Costa, C. L. S.; Araújo, D. S.; Cavalcante, L. C. D.; Barros, E. D. S.; Araújo, P. B. M.; Brandão, M. S.; Chaves, M. H. (2007) Fenóis totais e atividade antioxidante de cinco plantas medicinais. Química Nova, 30(2): 351-355.

[46] Chew, K. K.; Khoo, M. Z.; Ng, S. Y.; Thoo, Y. Y.; Wan Aida, W. M.; Ho, C. W. (2011) Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of Orthosiphon stamineus extracts. International Food Research Journal, 18(4): 1427-1435.

[47] Vongsangnak, W.; Gua, J.; Chauvatcharin, S.; Zhong, J. J. (2004) Towards efficient extraction of notoginseng saponins from cultured cells of Panax notoginseng. Biochemical Engineering Journal, 18(2): 115-120.

[48] Spigno, G.; Tramelli, L.; Faveri, D. M. D. (2007) Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. Journal of Food Engineering, 81(1): 200-208.

[49] Sobeh, M.; Esmatb, A.; Petrukc, G.; Abdelfattahd, M. A. O.; Dmirieha, M.; Montic, D. M.; Abdel-Naime, A. B.; Winka, M. (2018) Phenolic compounds from Syzygium jambos (Myrtaceae) exhibit distinct antioxidant and hepatoprotective activities in vivo. Journal of Functional Foods, 41: 223-231.

[50] Benvenuti, S.; Pellati, F.; Melegari, M.; Bertelli, D. (2004) Polyphenols, Anthocyanins, Ascorbic Acid, and Radical Scavenging Activity of Rubus, Ribes and Aronia. Food Chemistry and Toxicology, 69(3): 164-169.

[51] Prado, A. C. P do; Aragão, A. M.; Fett, R.; Block, J. M. (2009) Compostos Fenólicos e atividade antioxidante de extratos da noz-pecã [carya illinoinensis (Wangenh.) C. Koch]. Brazilian Journal of Food Technology, 12(4): 323-332.

[52] Imeh, U.; Khokbar, S. (2002) Distribution of conjugated and free phenols in fruits: Antioxidant activity and cultivar variations. Journal of Agricultural and Food Chemistry, 50(22): 6301-6306.

[53] Lorenzo, J. M.; Vargas, F. C.; Strozzi, I.; Pateiro, M.; Furtado, M. M.; Sant'ana, A. S.; Rocchetti, G.; Barba, F. J.; Dominguez, R.; Lucini, L.; Sobral, P. J. A. (2018) Influence of pitanga leaf extracts on lipid and protein oxidation of pork burger during shelf-life. Food Research International, 114: 47-54.