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Influence of factors and interactions in ultrasound-assisted extraction and conventional maceration on aqueous extract of *Psidium guajava* leaves

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ABSTRACT

Background: Extraction techniques of phenolic compounds (PC), such as conventional maceration (CM) has been associated with high consumption of organic solvents and time.

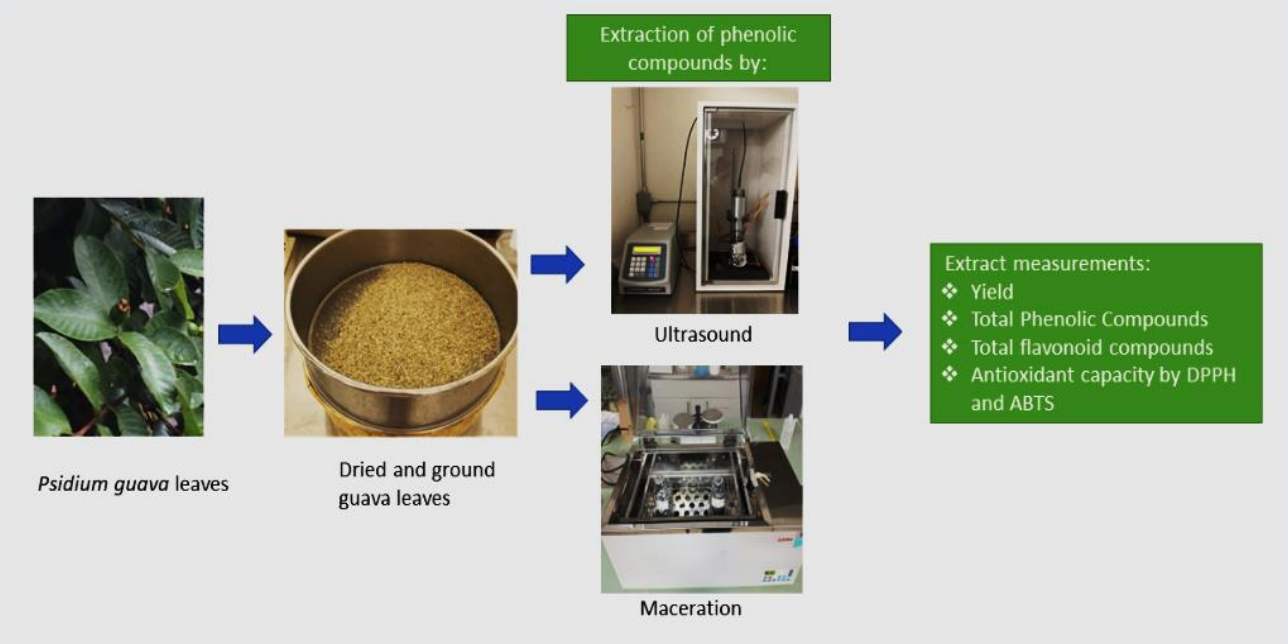
Objective: The objective of this study was to evaluate the effects and interactions of the variables involved in the aqueous extraction of phenolic compounds from *Psidium guajava* leaves by CM and ultrasound-assisted extraction (UAE).

Methods: A split-plot design was developed according to the extraction variables of interest. For CM was time, temperature, and dilution were considered and for UAE the wave amplitude was included. Response variables were considered the following parameters: yield, total phenolic compounds, total flavonoid compounds, antioxidant capacity measured as radical uptake by ABTS and DPPH.

Results: The results show that for UAE, the treatment of 1 g/ 50 mL, 50ºC temperature, 30% amplitude, and 20 min of extraction was associated with a higher extraction yield, reporting 125.84 mg of acid gallic equivalents/ g for PC, 381.18 mg quercetin equivalents/g for total flavonoids and 85.28% ABTS and DPPH 40.2% expressed as radical scavenging activity. On the other hand, the maceration method reported best extraction yield, using the treatment of 1 g/ 50 mL of dilution, 50ºC temperature and 10h of extraction time, achieving a total of 62.04 mg of gallic equivalents/ g for PC, 289 mg quercetin equivalents/g for total flavonoids, and 95.9% ABTS and DPPH 48% regarding radical scavenging activity.

Conclusion: The ultrasound process was more effective in extracting phenolic compounds, flavonoid compounds, and their extracts had the highest antioxidant capacity.

Keywords: *Psidium guajava*, phenolic compounds, extraction, ultrasound.



INTRODUCTION: Due to the persistence of degenerative chronic diseases, cancer and inadequate feeding, there is an increasing interest in both the study and use of natural bioactive components, such as polyphenols, found in natural sources such as fruits, vegetables, and nuts. Polyphenols are characterized by high antioxidant activity, modulatory action in the cellular oxidation process [1] and chronic inflammation [2]. There are numerous benefits in using bioactive compounds with antioxidant capacity in the food industry. Currently, synthetic antioxidants are the most commonly used. Consequently, these are submitted to a various analysis of safety and while the majority of these are considered safe, a few synthetic antioxidants are unsafe. For example, butylated hydroxyanisole commonly known as BHA, is a synthetic antioxidant found in the human diet, but there are very few studies that evaluate the relationship between human cancer and exposure, which has only been shown in rodent models [3].

The food industry and consumers are leaning towards the reduction of synthetic additives and their replacement by natural alternatives, since they are

obtained from renewable and sustainable sources, promoting the reduction of agro-industrial waste and diminishing the use of chemical products and solvents. Thus, the search for natural compounds, extraction efficiency, preservation, and bioavailability are part of the modern challenges in food investigation.

Fruits and leaves provide antioxidants, such is the case of guava (*Psidium guajava*). Guava leaves are commonly underutilized or treated as agro-industrial waste. Several authors have reported the presence of bioactive compounds in guava leaves and have been used traditionally to treat diverse illnesses. Hence, guava leaves can be treated as a functional food. According to the classification and regulation of functional foods (FF), a functional food is a natural or processed food that contains biologically active compounds, which, in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, to promote optimal health and reduce the risk of chronic/viral diseases and manage their symptoms [4]. The bioactive compounds contained in guava leaves include meroterpenoids, triterpenes and flavonoids. Bioactive compounds with antioxidant capacity such as polyphenols, are the major bioactive compound found in guava leaves as compared to the fruit and flower [5].

P. guajava leaves have diverse biological activities, such as antioxidant activity and antidiabetic activity [6] due to the presence of polysaccharides. They exhibit antitumorigenic activity through the inhibition of pathways related to the progression of cancer, such as the PI·K-Akt signaling pathway [7]. The antiproliferative and antiestrogenic activity is demonstrated through tumor inhibition due to the presence of meroterpenoid guajadial [8], anti-inflammatory activity through inhibition of PGE2, COX-2, NO, iNOS, ERK1/2, leukocyte cell migration, and suppression of edema [9]. Lipid-lowering activity, antihypertensive activity [10], and hepatoprotective activity [11] have been noted.

Several techniques have been used through the years to extract these important components from leaves. Therefore, the search for new methodologies underpins the implementation of assisted methods such as ultrasound-assisted extraction, which are scalable at industrial level in comparison to conventional methods, such as maceration.

Ultrasound-assisted extraction (UAE) is based on the propagation of mechanical waves formed by a set of cycles, defined by the combination of high and low pressures called compressions and rarefactions, which in turn cause the formation of bubbles that implode on the surface of the plant matrix, generating an output of the compounds of interest [12]. The main advantage of this process is the shorter process time and better temperature management.

Conventional maceration (CM) is a solid-liquid extraction technique, generally combined with temperature, which allows the extraction of the components. However, it has been reported that prolonged high-temperature exposure can lead to a greater amount of denaturation of thermolabile compounds. The main limitations of this technique are the long extraction time, solvent, and energy consumption [13]. Nowadays, this technique is highly used because it is not necessary to have sophisticated equipment and it is accessible to most researchers. While each technique has its advantages and disadvantages, the main goal of the chosen method is the achievement of high yields of the compounds and the preservation of their chemical structures. [14]. However, one of the first barriers faced by researchers is understanding the determining factors and their interactions for the efficiency of extraction and preservation of the compounds according to the chosen method.

The objective of this study was to compare the extraction efficiency and antioxidant activity of phenolic compounds from *Psidium guajava* leaves. The aqueous extracts were obtained by the UAE method and conventional maceration, based on different

combinations of the variables involved, such as amplitude, temperature, time, and dilution in the case of UAE. In regard to regarding CM, the variables of interest were time, temperature, and dilution.

MATERIALS AND METHODS

Materials: *Psidium guajava* leaves were collected from at the experimental site "Los cañones" of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias in 2018. The variety "Hidrozac" was selected. The samples were dried in the shade for 2 weeks and then ground with Thermomix Vorvek T31 equipment. The ground material was sieved in a 0.8 mm diameter mesh and stored in dark hermetic containers at 25ºC.

Split-plot experiment design: Using a split-plot design for both the methods, UAE case A and CM case B, the variables were arranged as shown in Tables 1 and 2.

Conventional Maceration Extraction: Each treatment condition was applied. All treatments were prepared and macerated using a thermostatic bath (Julabo, Model SW22) stirring at 250rpm, for an appropriate time, temperature, and dilution in each of the treatments. At the end of the maceration time, the treatments were centrifuged at 1000 rpm (CRM GLOBE, Centrificient IV IM, Model TD5M-WS) for 5 min. The supernatant was stored in 50 mL Falcon tubes covered in aluminum foil and was frozen at -25ºC until further analysis.

Ultrasound-Assisted Extraction: Each treatment condition was applied. All treatments were prepared and extracted using an ultrasonic device (Vibra Cell, Model VC505) coupled with a temperature regulator and for the set time, dilution, the amplitude of the wave, and temperature in each of the treatments. At the end of the extraction time, the treatments were centrifuged at 1000rpm for 5 min. The supernatant was stored in 50 mL Falcon tubes covered in aluminum foil and was frozen at -25ºC until further analysis.

Table 1. Ultrasound assisted extraction plot combinations.

Treatment	Time (min)	Percentage of Amplitude	Dilution g of leaves/ mL H_2O	Temperature (ºC)
T1	10	30	1/50	30
T2	10	30	1/50	50
T3	10	30	1/100	30
T4	10	30	1/100	50
T5	10	50	1/50	30
T6	10	50	1/50	50
T7	10	50	1/100	30
T8	10	50	1/100	50
T9	20	30	1/50	30
T10	20	30	1/50	50
T11	20	30	1/100	30
T12	20	30	1/100	50
T13	20	50	1/50	30
T14	20	50	1/50	50
T15	20	50	1/100	30
T16	20	50	1/100	50

A total of 16 treatments, with 3 replicates in each treatment.

Table 2. Conventional maceration plot combinations.

Treatment	Time (h)	Dilution g of leaves/ mL of H_2O	Temperature (ºC)
T1	2	1/50	30
T2	2	1/50	50
T3	2	1/100	30
T4	2	1/100	50
T5	10	1/50	30
T6	10	1/50	50
T7	10	1/100	30
T8	10	1/100	50

A total of 8 treatments, with 4 replicates in each treatment.

Total phenolic content (TPC): Total phenolic content was quantified using the methodology of Makkar et al. [15], with minimum modifications. A volume of 250 µL of the extract was added to 250 µL of Folin Ciocalteu's phenol reagent and 250µL of sodium carbonate. The mixture was homogenized and incubated at 40°C for 30min. Next, 2mL of distilled water was added to the mixture and the absorbance was measured at 750nm.

Total flavonoid content (TFC): Quantification of flavonoids was carried out using the methodology proposed by Liu et al. [16]. A volume of 150µL of each sample and 150µL of sodium nitrite (5%) were mixed. Then, 150µL of aluminum chloride at 10% was added along with 1mL of sodium hydroxide 0.1M to the mixture. The resulting mixture was homogenized, and the absorbance was recorded at 510nm.

ABTS: The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation–based assay (ABTS•+) was measured according to the methodology of Re et al. [17]. A stock solution containing 2:1 volumes of 7 mM ABTS solution and 2.45 mM of potassium persulfate was rested from 12 to 16 h at 22°C and was adjusted with absolute ethanol until it showed an absorbance value of 0.700nm, according to the original methodology. After placing the measuring cell in the spectrophotometer, 50 µL of each diluted infusion (1:2) and 950 µL of the above resulting solution were added. After 1 min, the absorbance was measured at 734 nm. In this case, the results were expressed as a percentage of radical uptake inhibition, as per the calibration curve prepared using the same standard.

DPPH: The 2,2-Diphenyl-1-picrylhydrazyl (DPPH•) free radical assay was based on the method described by Randhir and Shetty [18] with slight modifications. Briefly, 50µL of each sample were mixed with 2.95mM of DPPH· solution 60µM (DPPH·+ methanol) to obtain a total volume of 3mL. The samples were homogenized for 10 sec and left for 30 min in a dark area, and afterwards the

absorbance was measured at 517 nm. Results were expressed in the percentage of radical uptake inhibition, as per the calibration curve prepared with the same standard.

Percentage of radical uptake: The percentage of uptake was calculated by the formula proposed by Kulišić et al. [19], as follows, *% uptake = (initial A - final A / initial A).* The antioxidant activity of the different compounds is measured based on the degree of decolorization that they cause in the solution of the monocation radical ABTS-+ or DPPH- radical. The decolorization assay is based on the ability of the test substance to capture the unpaired electron of the radical or to release a proton.

Statistical analysis: The statistical analysis was performed using JMP software V15. The significant differences among the mean values of different characteristics were determined using the Tukey test for multiple comparisons, statistical significance was set at $P<0.05$. Data were reported as the mean ± standard errors and graphics were performed with GraphPad Prism software V9.0.1.

RESULTS AND DISCUSSION

Total phenolic content (TPC) and Total flavonoid content (TFC): UAE requires the management of important variables of the extraction such as time, temperature, concentration, and solvent used, which are directly related to extraction efficiency and yield [20]. CM is a simple technique, but also requires the management of time, temperature, solvent and dilution to be effective for the extraction of bioactive compounds [14]. This study is focused on the variables and the interactions that occurred in each of the methods used. Figure 1, is presented at a big scale with the factors in the model, expressed as a log of utility. This result shows the importance of each factor and how these variables are involved in the results of the assays applied, indicating that the factors studied are contributing to both models. The split-plot design allowed us to understand all factors and their interactions. In Figure 2, it is observed that some of the factors are enhanced when they are combined and can be useful in maximizing the performance of both methods.

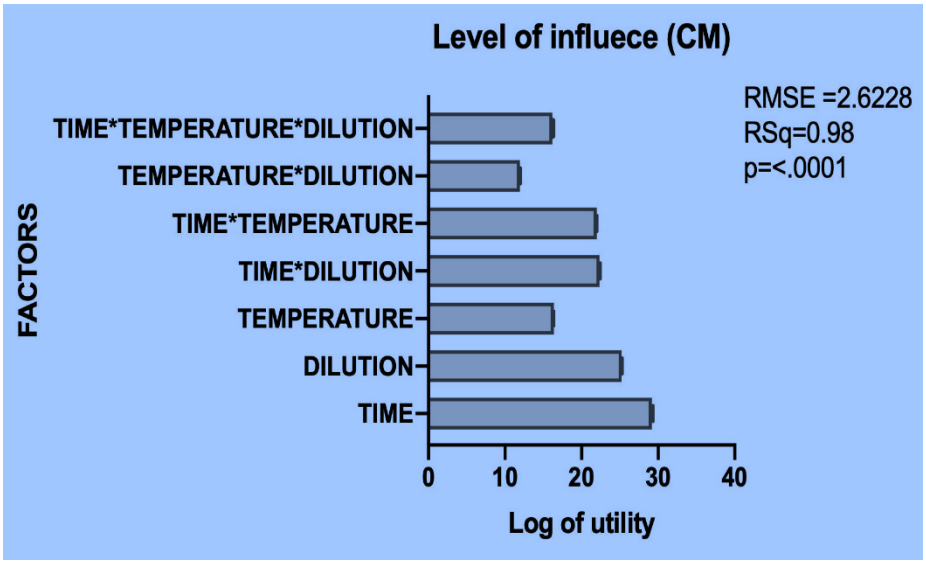


Figure 1. Level of influence of the factors and interactions of conventional maceration (CM).

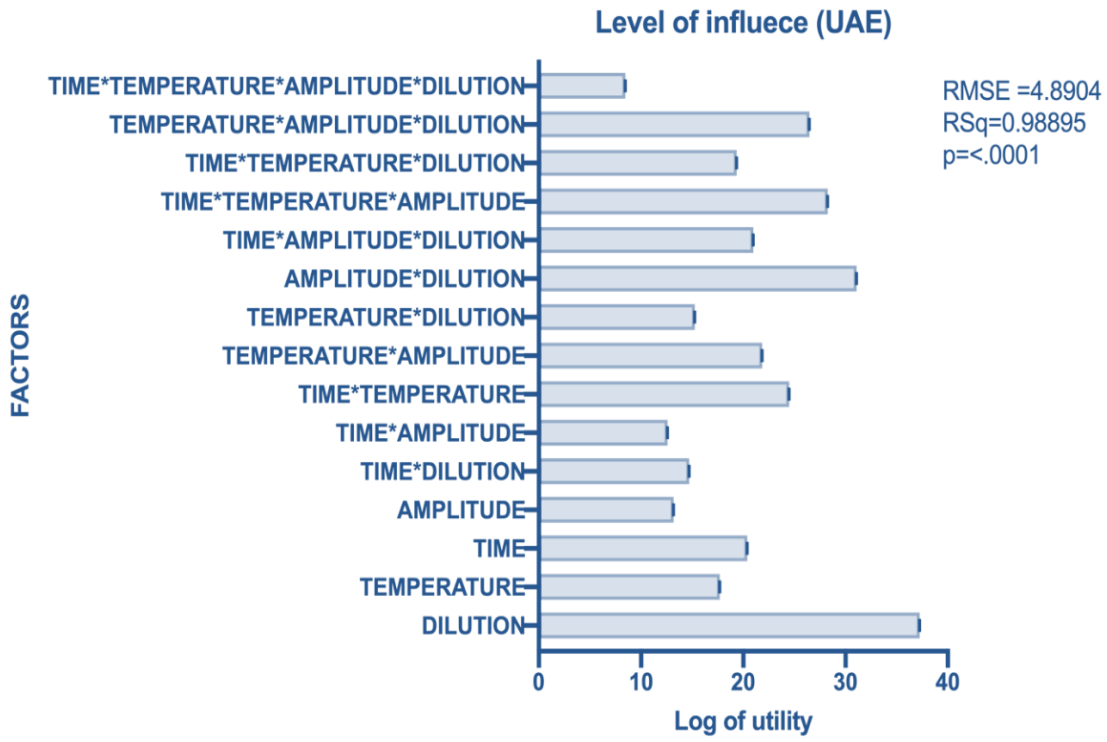


Figure 2. Level of influence of the factors and interactions of the ultrasound-assisted extraction (UAE).

In this case, the level of influence represents a useful analysis for determining the most significant effects on each factor and its interactions, as well as determine the effect size of the whole model.

A significant difference can be observed in phenolic compounds (Figure 3) between the treatments and methods in terms of extraction yield. The UAE technique has the highest extraction yield (125.84 mg GAE/ g), two times higher than the highest extraction yield in CM (62.04 mg GAE/ g). These treatments have a dilution of 1g/50mL and were applied at a higher temperature of 50ºC. Regarding time selection, extended times result in better performance at 10h for CM and 20 min for UAE.

In the process of UAE, the extraction yield is enhanced due to the collapse of the cavitation bubbles on the surface of the matrix, causing interference on the cell's wall material, initiating the process of mass transference to the solvent; Thus, increasing the material extracted [21].

Results in Figure 3 show that for UAE, T10 and T14, do not show statistical differences (p=0.08) and were the best treatments in yield of extraction. The only difference among treatments is the amplitude of the wave, 30% for T10 and 50% for T14. From the results, it appears that both amplitude and percentages are adequate to improve the yield of extraction. However, in comparison to the other factors, the amplitude is not the most determinant. On the other hand, T8 and T3 had the lowest rate of extraction. Regarding CM results, T5 was the best combination for CM, which showed a yield 85% higher than the lowest combination, a notorious discrepancy between best treatments, UAE 51% higher than CM.

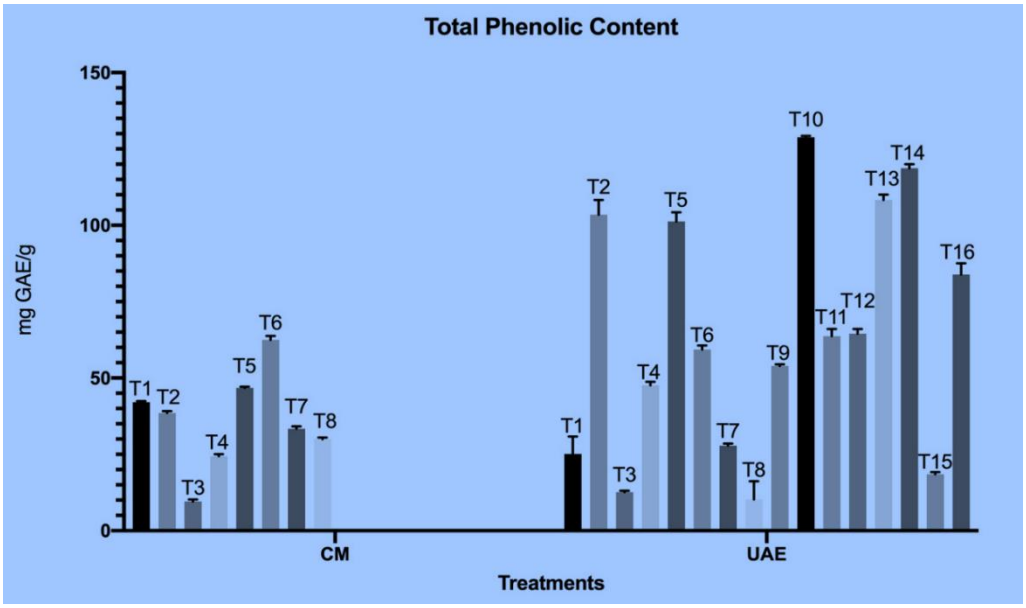


Figure 3. The yield of extraction of total phenolic compounds in conventional maceration (CM) and ultrasound-assisted extraction methods (UAE).

Flavonoids are a critical component of guava leaves [22]. A high concentration of these components was measured in the aqueous solution of both methods (Figure 4). UAE showed the highest concentration of flavonoids, 381.5 mg EQ/ g for T14, and 356.9 mg EQ/ g for T10, with no significant statistical difference between them. CM had a 2.31% higher yield of flavonoid extraction, 390.52 mg EQ/ g when T2 condition was used. Our results showed a higher yield of extraction, as compared to the study done by Camarena-Tello et al. [23] using CM from guava leaves extraction. An extraction efficiency can be achieved with CM and UAE. These methodologies can be highly effective to extract flavonoids. The antioxidant activity of aqueous extracts results is presented as a percentage of radical uptake.

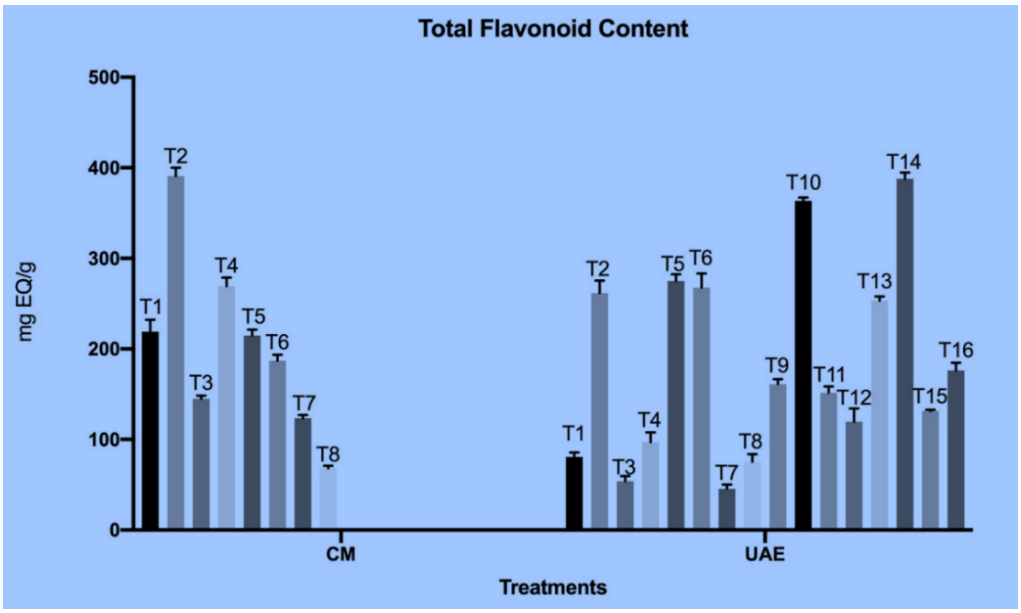


Figure 4. The yield of extraction of total flavonoid compounds in conventional maceration (CM) and ultrasound-assisted extraction (UAE) methods.

Percentage of radical uptake: A great yield was found in both extraction methods. Depending on the treatment, a higher percentage of radical uptake can be achieved. In UAE, T2 showed 93.4% of radical uptake. On the other hand, in UAE, T15 showed the lowest radical uptake, 13.2%. The method of extraction is one of the central factors to obtain high-quality natural antioxidants, as well as the proper selection of all factors and combinations, which can determine the level of extraction of those biocomponents. CM method showed the highest radical uptake, reaching 95.9%, while the lowest for CM was 43.8% using T3 condition. Although CM had a lower phenol content than UAE, this method had a higher flavonoid content. It appears that CM is a simple and an effective method to prevent antioxidant degradation and demonstrates a good antioxidant capacity. Results can be observed in Figures 5 and 6. To prevent degradation in the UAE method, factors must be controlled. It is desirable that the chosen method have benefits, such as shorter extraction time, decreased solvent consumption, an increase in the extraction yield, enhanced quality of the extracts, and most importantly, preserve the integrity of biocomponents.

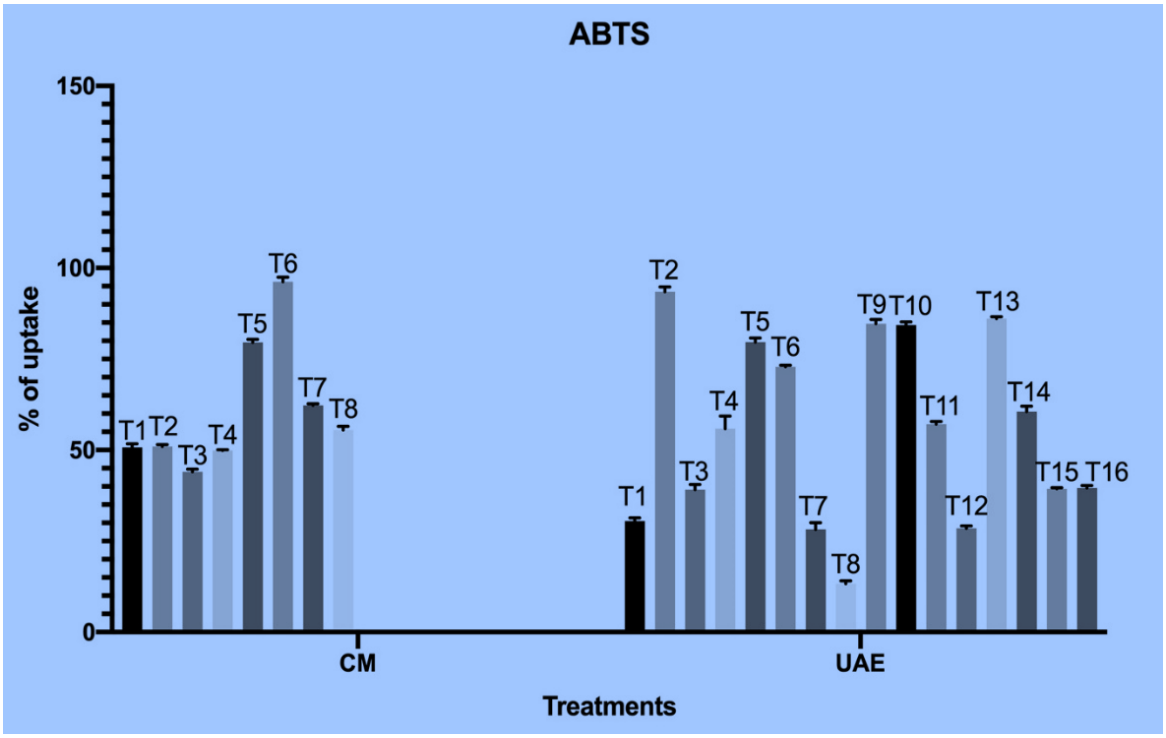


Figure 5. Percentage of uptake ABTS in conventional maceration (CM) and ultrasound-assisted extraction methods (UAE).

On the other hand, it is important to mention that in both methods of extraction a lower percentage of radical uptake in the DPPH assay was found. ABTS assay measures the activity of hydrophilic and lipophilic compounds. DPPH can only be dissolved in an organic medium, so it preferentially measures the antioxidant capacity of low-polar compounds [24].

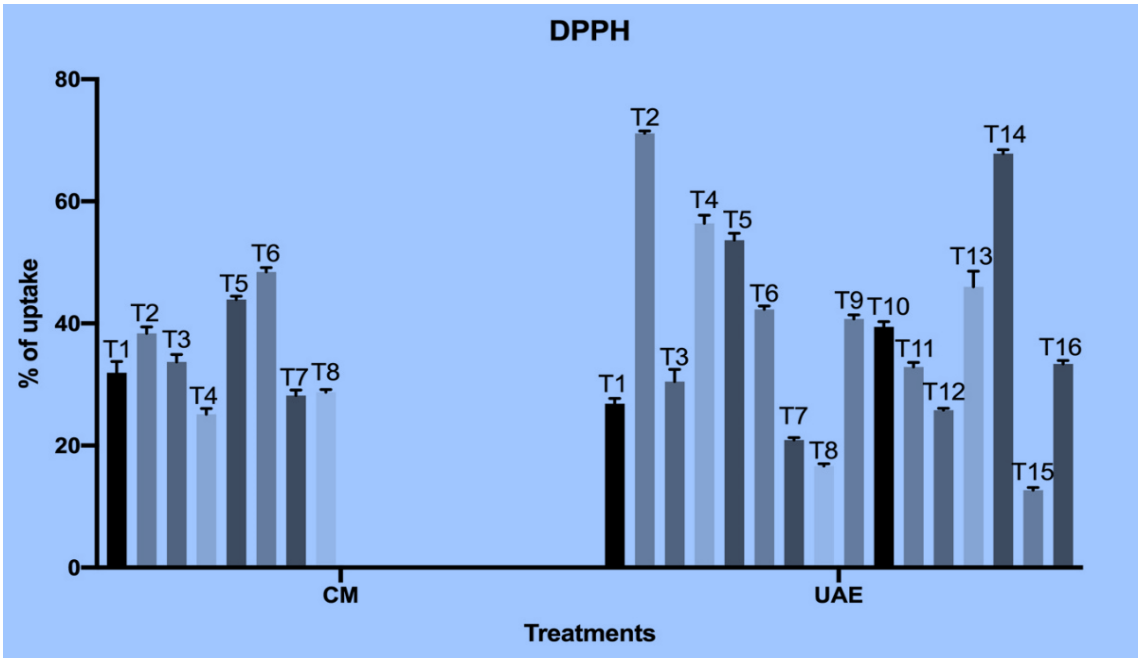


Figure 6. Percentage of uptake DPPH in conventional maceration (CM) and ultrasound-assisted extraction (UAE) methods.

EVALUATION OF FACTORS AND EFFECTS

Effect of amplitude of wave (AW): The amplitude of the wave is an important factor, as it has been shown that an increase in cycles favors the extraction yield, due to an increase in the number of compressions and rarefactions. [25]. In this study, we observed that a range between 30% and 50% amplitude enhances the yield of extraction. However, the amplitude was important, but not a determinant in comparison to temperature, time, and dilution.

Results in Table 3 showed that there are significant differences between both AW conditions used. The 50% AW is highly associated with an increased yield of extraction, these results agree with Goula [26], who reports that optimum operating conditions were at 60% of AW. It seems that higher AW can interfere with antioxidant activity in aqueous water extract. It has been reported that a solvent with low vapor pressure is preferred in UAE, as the collapse of cavitation bubbles is more intense, in comparison to solvents with high vapor pressure [27], as vapor depends on the temperature applied. In this study, the results of 30% of AW appear to protect antioxidant activity in the aqueous extracts. In addition, high AW can lead to rapid deterioration of the ultrasonic transducer, resulting in liquid agitation, instead of cavitation and low transmission of the ultrasound through the liquid media [20]. The AW should be increased when high viscosity liquids are used, such as oils [28].

Table 3. Evaluation of amplitude in ultrasound-assisted extraction (UAE) method.

			30%	50%
Assay	**p-value**	**SD**	**Mean**	**Mean**
Total phenols mg GAE/ g	0.0030*	0.99825063	61.6813	66.2196
Flavonoids mg QE/ g	<.001*	3.2292639	160.955	199.4077
ABTS (%)	<.0001*	0.35655662	58.7887	52.4578
DPPH (%)	<.0001*	0.26539839	43.5289	45.6691

Even though AW resulted significative, a better antioxidant activity was observed when the AW was 30% at temperature of 50ºC. This remarks on the importance of the other factors and possible interactions when choosing the percentage of AW.

Effect of dilution and concentration: Dilution was the most important factor that contributes to a higher level of extraction (Figures 1 and 2). Results in Tables 4 and 5 showed that in both methods of extraction, a minor dilution was associated with a higher concentration of biocomponents extracted. Our results showed that 1 g/ 50 mL enhanced the yield of extraction and antioxidant activity up to 52.51% (mean) in the UAE method and 35.48% (mean) in the CM method, for each assay. Dilution is a key factor to achieve the highest yield and plays an important role in antioxidant activity.

Table 4. Evaluation of dilution in ultrasound-assisted extraction (UAE) method.

Assay	p-value	SD	Dilution	
			1 g/ 50 mL	1 g/ 100 mL
			Mean	Mean
Total phenols mg GAE/ g	<.0001*	0.9982506	87.1937	40.7072
Flavonoids mg QE/ g	<.0001*	3.2292639	254.591	105.841
ABTS (%)	<.0001*	0.3565566	73.6154	37.6311
DPPH (%)	<.0001*	0.2653983	59.1247	30.0733

Table 5. Evaluation of dilution in conventional maceration (CM) method**.**

Assay	p-value	SD	Dilution	
			1 g/ 50 mL	1 g/ 100 mL
			Mean	Mean
Total phenols mg GAE/ g	<.0001*	0.6556905	46.45	24.19
Flavonoids mg QE/ g	<.0001*	1.8314365	253.51	152.211
ABTS (%)	<.0001*	0.2320818	69.314	52.7035
DPPH (%)	<.0001*	0.52664905	40.6991	28.7937

It must be considered that the concentration directly affects the polarity of the solvent when CM is used, while absorption ultrasound depends on the dielectric constant of the solvent and increases with the water content [29]. In this case, the aqueous extract facilitates the extraction of polar components, such as flavonoids. These polar components have many unsubstituted hydroxyl groups or sugars and are very soluble in water in comparison to other solvents such as ethanol, methanol, and acetone.

Effect of time: Time is another variable that affects the extraction efficiency of phenols from the matrix of interest. In Table 6, significant differences between the mean values were observed regarding UAE, where 20 min is the time associated with a higher extraction yield and radical uptake, reporting up to 40.3% total phenols, 34.5% total flavonoids, and 13.44% in the case of ABTS and 9.08% in the case of DPPH, these are higher results in comparison to those obtained when applying 10 min of extraction time. Other authors have reported that 20 min is the best time to extract bioactive components without degradation when UAE was used. [25]. CM showed that 10 h is a better time selection to extract total phenol compounds preserving the antioxidant activity. However, to improve the extraction of flavonoids, it may be better to shorten the extraction time, as observed in Table 7. In this case, 2 h of time of extraction presented the highest yield of extraction. It is observed that a longer extraction time could diminish flavonoid integrity, which was observed when there was 10 h of extraction. The total phenols confer an adequate antioxidant activity in both treatments. It is important to consider that selection of time must be in harmony with other variables, such as temperature.

Table 6. Evaluation of time in ultrasound-assisted extraction (UAE) method.

			10 min	20 min
Assay	**p-value**	**SD**	Mean	Mean
Total phenols mg GAE/ g	<.0001*	0.99825063	48.1565	79.7444
Flavonoids mg QE/ g	<.0001*	3.2292639	142.773	217.659
ABTS (%)	<.0001*	0.35655662	51.6293	59.6172
DPPH (%)	<.0001*	0.26539839	42.4806	46.7175

Table 7. Evaluation of time in conventional maceration (CM) method.

			2 h	10 h
Assay	**p-value**	**SD**	Mean	Mean
Total phenols mg GAE/ g	<.0001*	0.65569050	27.6976	42.9510
Flavonoids mg QE/ g	<.0001*	1.8314365	255.955	149.76779
ABTS (%)	<.0001*	0.23208182	48.7316	73.3193
DPPH (%)	<.0001*	0.52664905	31.8357	37.6571

Effect of Temperature: Results presented in Tables 8 and 9 showed significant differences between the two temperature levels. This study shows that the selection of temperature is key to obtaining both a higher extraction yield and a high antioxidant activity. When the use of temperature of 30°C and 50°C was compared, the following differences were obtained: 1.54% more radical absorption for ABTS and 10.39% for DPPH using EUA, and 6.58% and 1 .35% using CM at temperature of 50 °C as compared to 30 °C. This means that using temperature of

50 °C was obtained a high extraction of components preserving the antioxidant activity of the biocompounds. Furthermore, this finding can be observed in other studies that used similar temperatures. Oroian et al. [21] reported the best extraction yield at 58ºC, while others conclude that an increase in temperatures improves extraction yield [30]. In Table 8, when the temperature increased from 30 °C to 50ºC, it promotes 33.45% and 31.56% the extraction of total phenols and total flavonoids, respectively for UAE. A similar result for CM is shown in Table 9, increasing 18% and 24% the extraction of total phenols and total flavonoids.

Table 8. Evaluation of temperature in ultrasound-assisted extraction methods (UAE).

Assay	p-value	SD	30 ºC	50 ºC
			Mean	Mean
Total phenols mg GAE/ g	<.0001*	0.99825063	51.1182	76.7827
Flavonoids mg QE/ g	<.0001 *	3.2292639	146.447	213.985
ABTS (%)	0.0932	0.35655662	55.187	56.0595
DPPH (%)	<.0001 *	0.26539839	42.1506	47.0475

Table 9. Evaluation of temperature in conventional maceration (CM) method.

Assay	p-value	SD	30 ºC	50 ºC
			Mean	Mean
Total phenols mg GAE/ g	<.0001*	0.65569050	31.9544	38.6943
Flavonoids mg QE/ g	<.0001 *	1.8314365	175.336	230.386
ABTS (%)	<.0001*	0.23208182	58.9921	63.0588
DPPH (%)	<.0001 *	0.52664905	34.5305	35.0024

CONCLUSION: Both methods of extraction were efficient in extracting bioactive compounds. The selection of method depends on the infrastructure and time availability. The study provided valuable information regarding how different factors and combinations can be determined to maximize the yield of extraction and antioxidant activity. In the case of UAE, it was observed that amplitude, dilution, temperature and time play an important role in enhancing extraction efficiency. In reference to dilution, a lower solid/liquid ratio is associated with a higher yield. The higher temperature is considered one of the variables that largely determines the number of components extracted, but this increase should guarantee the integrity of the compounds. To evaluate the integrity of the compounds, it is imperative to perform radical scavenging assays. The best UAE conditions were 20 min of extraction time, temperature of 50 ºC, dilution of 1 g of leaves in 50 mL of water and

wave amplitude at 50% or 30%. On the other hand, conventional maceration demonstrated that selection of conditions is a key factor to improve the level of extraction, choosing the best CM conditions can allow the extraction of a large number of compounds, similar to methodologies considered more current. Therefore, we conclude that the main limitation to extract a high amount of biocompounds is not only due to technique, but also to the conditions and management of the variables involved. Ultimately, it was found that the best conditions for CM were 20 min of extraction time, temperature of 50 ºC and dilution of 1 g of leaves in 50 mL of water. In regard to guava leaves, we believe that guava leaves should be used as functional food. They contain an important concentration of bioactive compounds and ahigh antioxidant capacity capable to reduce free radicals. It can be used to produce functional foods, as it is a diverse source, affordable, and can withstand long periods of storage when properly handled.

List of Abbreviations: PC, phenolic compounds; FF, Functional; CM, conventional maceration; UAE, ultrasound assisted extraction; TPC, total phenolic content; TFC, total flavonoid content, GAE/ g, gallic acid equivalent; EQ/g, quercetin equivalent; AW, amplitude of wave; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation–based assay; DPPH: The 2,2-Diphenyl-1-picrylhydrazyl (DPPH•+) free radical assay.

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