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Ultrasound-Assisted Extraction of Phenolic Compounds from Red Jute (*Corchorus olitorius* L.) Leaves Using Aqueous Ethanol

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ABSTRACT

Extraction of bioactive components from natural sources has recently gained interest due to their medicinal benefits. Jute or "saluyot," a fast-growing perennial herb abundant in the Philippines, is known to have high nutritive value and is a good source of phenolic compounds. This study focused on the optimization of the ultrasound-assisted extraction of the antioxidant phenolic compounds from red jute (Corchorus olitorius L.) using aqueous ethanol. Characterization of fresh jute leaf samples showed that jute leaf contains 84.52 ± 0.00% w/w moisture, 3.81 ± 0.98% w/w extractives, 11.67 ± 0.98% w/w total solids and total phenolic content (TPC) of 17.72 ± 0.36 mg gallic acid equivalent (GAE)/gram bone dried leaves (gdl). Screening of the five factors (extraction temperature, extraction time, particle size, solid to solvent ratio and ethanol concentration) showed that extraction temperature, extraction time and ethanol concentration significantly affected the TPC of the extracts. Likewise, the interaction of particle size and ethanol concentration (negative effect) is significant. The optimum conditions were found to be 50 oC extraction temperature, 20 min extraction time and 69.037% ethanol using suggested quadratic equations obtained through the models generated via Central Composite Design. Optimum TPC was 15.08 ± 0.03 mgGAE/gdl and optimum percent recovery was 85.11 ± 0.17%. Small percent differences (0.80% both) with respect to predicted suggest high desirability of the model equations. The high recovery of phenolic compounds with the aid of ultrasonication even at one extraction cycle is therefore proven to be significant, suggesting the potential of jute as source of phenolic compounds.

Keywords: *phenolic compounds, ultrasound-assisted extraction, aqueous ethanol, red jute, optimization*

INTRODUCTION

Prevalence of certain diseases which cause increasing mortality among humans lead to the development and recovery of bioactive compounds such as phenolic compounds. Phenolic compounds which are secondary metabolites consisting of at least one phenolic group, are widely distributed in plants like fruits and vegetables, and are known to possess antioxidant power and inhibiting effects on undesirable compounds (Özeker, 1999). Moreover, the bioactivities of phenolic compounds provide chemopreventive properties and anticarcinogenic and anti-inflammatory effects (Huang, Cai & Zhang, 2010).

Jute or "saluyot", which is known to have high nutritive value (DA-Cagayan de Oro, 2013), is a good source of phenolic compounds. Jute as a fast-growing perennial herb that can be harvested 30 days after transplanting, is abundant in the Philippines during the months of November to February (Plants for a Future, 2008). But places such as Southern Mindanao where rainfall distribution is uniform allow planting of jute anytime of the year. Therefore, availability of which will not be a problem for large scale application. Cabie (2012) reported that as of 2006, the 692 hectares of jute plantation in the Philippines was able to provide a total of 1,949 tons. On average, farmers can harvest in a season about 80,000 bundles of "saluyot" per hectare. This can be translated to a gross income of PhP 640,000.

In the list of Philippine Medicinal Plants (StuartXchange Org, 2015), there are two species of jute in the Philippines; red jute (*Corchorus olitorious L.*) and white jute (*Corchorus capsularis*). Leaves from red jute mainly contain rutin hydrate, ellagic acid and quercetin hydrate which can be attributed to its natural antioxidant property (Ali et al*.,* 2021). These bioactive phenolic compounds are also considered highly valuable products.

Various extraction techniques, such as solvent, microwave-assisted, ultrasound-assisted, super-critical fluid, ionic liquid, pressurized fluid, Soxhlet and solid-phase extractions can be applied to recover phenolic compounds in plants (De Monte et al., 2014; Santana, 2009). In this study, ultrasound-assisted extraction was employed because of its recorded enhanced extraction yield; decreased time, energy and solvent consumptions; minimal CO_2 emission (Chemat et al., 2016); and availability of equipment. Ultrasound, which has a frequency higher than audible range (20-20000 kHz), transmits a series of compression and rarefaction cycles through the medium thereby pulling the molecules apart and creating cavitation bubbles. The bubbles collapse and consequently generate shockwaves and rapid inter-particle collision that cause fragmentation in cellular structure of plants. This then facilitates the solubilization of bioactive components from the surface of the plant tissue into the extraction solvent (Kumar et al., 2021). In terms of the extraction solvent, water, methanol, ethanol and acetone or combinations of these have been employed. For food grade application of the extract, aqueous ethanol was applied on red jute leaves.

In this study, the red jute leaves were characterized in term of moisture content, total solids and total extractives, and total phenolic content (TPC). Preliminary experiment with and without ultrasonication was conducted to evaluate the significance of assisting the solvent extraction with ultrasonication for an improved recovery of phenolic compounds from red jute leaves. Also, screening through parametric study was done to determine the significant factors, their main effects and interaction effects on the extraction of phenolic compounds. Factors assessed were ethanol concentration, solid-to-solvent ratio, extraction time, particle size and extraction temperature.

An optimization study was employed to improve the recovery of phenolics from red jute while minimizing the cost of the process. A statistical model for numerical optimization of the significant extraction factors using Response Surface Methodology (RSM) was developed. The model was then employed to predict the optimum conditions, which were further validated by experimentation. The results of the optimization can be studied for further isolation of the desired component by purification, provided that the

optimum conditions are proven to give appreciable amount. Lastly, feasibility study can be conducted to upscale the extraction and estimate profit from the process.

MATERIALS AND METHODS

Chemicals

Anhydrous sodium carbonate (99.5% purity), gallic acid monohydrate (99.5% purity) and Folin-Ciocalteau reagent (2 N) were purchased from Lobo Chemie Ltd. Technical grade ethanol (95% v/v) and distilled water were utilized in preparing ethanol solutions.

Feedstock collection and preparation

Fresh leaf samples of red jute (*Corchorus olitorius L.*) were obtained along the San Cristobal River near Parian, Calamba, Laguna. The leaves were dried at 50°C for at least 10 h using Memmert convection oven. Moisture contents of the feedstock and the resulting dried leaves were determined. The dried leaves were ground using NutriBullet food processor and sieved employing U.S.A standard sieves of mesh 20 and 60 to obtain a sample with an average particle size of 545 microns and mesh 80 and 120 to prepare a sample with an average particle size of 151 microns, as shown in **Figure 1.** Sieved samples were packaged using polyethylene zip locks and stored in the dark at room temperature.

Determination of total extractives and total solids

Total extractives refer to the total soluble components of the leaves while the dried residue gives the total solids. To virtually recover all the soluble components, appreciable amount of solvent was used for extraction based on the method of Aki et al. (2017). Ethanol concentration of 75% was utilized. A half gram of dried sample was added with 5 mL ethanol for 20 min under ultrasonication. Centrifugation using MRC centrifuge model SCEN-207 at 2200 rpm for 10 min was done to separate the residue from the decant. Five repetitions were done or until the sample is exhausted. The extracts were combined, and the resulting solution was analyzed for TPC in terms of gallic acid equivalent (GAE)/ gram bone dried leaves (gdl). Three trials were performed. To determine the total extractives and total solids, the residue was dried at 105°C. The moisture content of dried leaf sample was accounted on the calculation of total extractives. Total solids on the other hand is given by the final mass of the residue.

Determination of total phenolic content

The method utilized was based on the procedure of Singleton and Rossi (1965) as cited by Michalczyk et al. (2008). Gallic acid solutions with concentration of 10, 20, 40, 80, 160 and 200 ppm were made by obtaining corresponding volumes from the 2000 ppm (w/v) stock solution and diluting each to 50 mL using aqueous ethanol. Note that a separate standard curve was generated for each ethanol concentration used. To analyze the TPC, a half milliliter of both standard solutions and the samples were dispensed into vials and added with five milliliters sodium carbonate (Na_2CO_3) aqueous solution at a concentration of 75 g/L. Then, 2.5 mL of 10x diluted Folin-Ciocalteu's reagent was dispensed to the vials. The resulting solutions were diluted to 10 mL by adding two milliliter distilled water. The absorbance of each solution was then read at a wavelength of 760 nm for the generation of

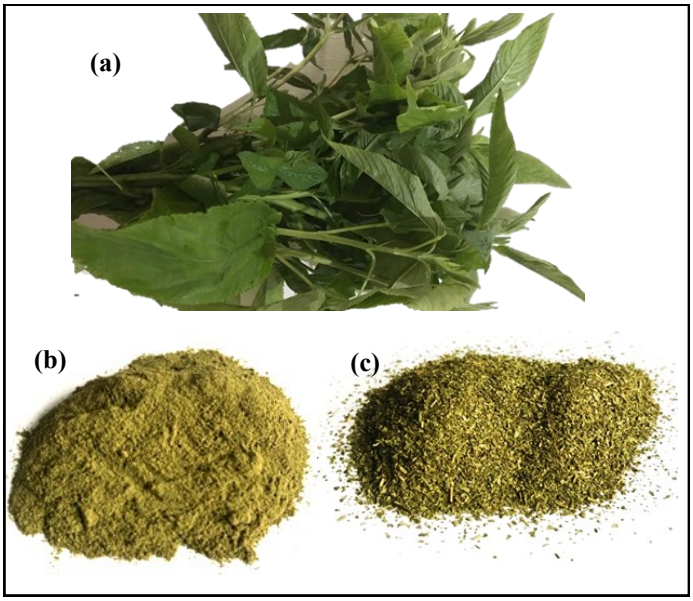

Figure 1. Red jute samples: (a) fresh leaves, and after size reduction: (b) 151 microns, and (c) 545 microns.

standard curve relating absorbance with the concentration. The TPC in Equation 1 was expressed in mgGAE/gdl.

$$TPC = \frac{Conc\ Diluted\ Decant\ (mgGAE/L) \times Dilution\ Vol.\ (L)}{Mass\ of\ bone\ dried\ leaves\ (g)} \times 100$$

Equation 1

Preliminary experiment

A two-level factorial considering varying extraction temperature (30^0C and 50^oC) with and without ultrasonication at constant particle size (151 microns), extraction time (20 min) and ethanol concentration (75%) was employed. Only one extraction cycle was done for this experiment and the corresponding TPC and percent recovery were obtained.

Experimental design

The factors considered for parametric study using 2^{k-1} factorial design were concentration of solvent (aqueous ethanol), solid-to-solvent ratio, particle size, extraction time and temperature. The concentration of the ethanol employed were at 25% and 75% with solid to solvent ratio of 5% and 20%. Average particle sizes were 151 and 545 microns. Extraction time was set to 20 min and 40 min. The working temperature of the water bath was maintained at 30^oC and 50^oC. Three replicates were considered for each combination of factors as suggested by Design Expert 11.

After identifying the significant factors, an optimization study was conducted with which runs were generated using Central-Composite Design (CCD) which included center levels of 40^oC extraction temperature, 30 min extraction time and 50% ethanol concentration. Also, particle size of 151 microns and solvent to solid ratio of 5:1 was considered for optimization experiment. All runs were performed in triplicate. **Table 1** shows the design matrix for the combination of factors in optimization.

Table 1. Experimental runs for the optimization study on the extraction of phenolic compounds using CCD.

RUN	C_{ETOH},	EXTRACTION TIME	EXTRACTION TEMP
	%v/v	(min)	(^{O}C)
1	75	40	30
2	50	20	40
3	50	30	40
4	25	40	30
5	25	20	50
6	25	40	50
7	75	20	30
8	25	30	40
9	50	30	30
10	25	20	30
11	50	40	40
12	75	30	40
13	50	30	50
14	75	20	50
15	75	40	50

Ultrasound-assisted extraction of phenolic compounds

A half gram of ground dried sample was weighed and transferred to 10 mL screw-capped vial. Two and a half milliliters to 10 mL of aqueous ethanol (25% - 75%) was added to the vials and the mixture was sonicated using an ultrasonic cleaner for 20-40 min at a temperature range of 30-50^{o}C, fixed power rating of 100W and water bath volume of two liters. An experimental set-up is shown in **Figure 2**. After leaching the desired components, the extract was separated by centrifugation at 3000 rpm for 10 min. No repetition of extraction was done for all experimental runs. Lastly, the extract was diluted to 50 mL mark of a volumetric flask with corresponding solvent or distilled water (Dent et al., 2015). The resulting solution was then analyzed for total phenolic content (TPC) in terms of mgGAE/gdl) and percent recovery of phenolic compounds, given by Equation 2.

$$\%\ recovery = \frac{TPC\ of\ Sample}{Theoretical\ TPC} \times 100$$

Equation 2

Experimental verification of optimum conditions

The predicted responses (optimum TPC, and recovery) based on the optimum conditions obtained from numerical optimization were verified in separate experiments. The same procedures were performed in triplicate. On the other hand, two blank experiments considering the level of each factor for optimal extraction but without ultrasonication were added to further compare unassisted extraction with extraction employing ultrasound.

Table 2. Compositional analysis of 100 g fresh red jute leaves.

COMPOSITION	FROM ACTUAL EXPERIMENT	FROM LITERATURES
Moisture Content, %	84.52 ± 0.004	86.35 ± 0.36 (Adeyeye et al., 2018)
Extractives, %	3.81 ± 0.98	--
Total solids, %	11.67 ± 0.98	--
TPC, mgGAE/g extract	81.93 ± 20.37	78 (İşeri et al., 2012) 5.00 ± 0.0020 (Ademiluyi et al., 2014)

Statistical analyses

Experimental results were analyzed using Design Expert 11. Analysis of variance (ANOVA) at 95% confidence level was employed to identify which factors have significant effect on the extraction as indicated by p-value <0.0500. Data were fitted to statistical models and the lack of fit was also calculated to verify validity of the model chosen. Diagnostic tests were done to detect any abnormalities with the data obtained. The difference of less than 0.2 between the adjusted R^2 and predicted R^2 indicates that model transformation is not necessary.

Figure 2. Extraction set-up using GT Sonic ultrasonic cleaner – 3L capacity.

RESULTS AND DISCUSSIONS

Composition of red jute

The compositional analysis of the red jute sample is shown in **Table 2**. The obtained moisture content of the sample was in agreement with the value presented by Adeyeye et al. (2018). Likewise, the measured TPC per gram of extract was higher but comparable to what was reported by İşeri et al. in 2012. But relative to the result obtained by Ademiluyi et al. (2014) with the same jute species, the jute used in this study has very high phenolic content per gram of extract. Deviations can be attributed to the differences in cultivation conditions as well as the different sources of the samples, cultural practices, post-harvest storage conditions and processing (Imeh & Khokar, 2002; Boyer & Liu, 2004 as cited by Henriquez et al., 2010). Because of these factors, the specific phenolic contents may also vary thus resulting to different density and mass of the extract. Notwithstanding, TPC in terms of mass of bone dried sample was also noted to easily calculate percent recovery. Maximum recoverable phenolic compounds determined using 75% ethanol at five extraction repetitions with the aid of ultrasonication was 17.72 ± 0.3638 mgGAE/gdl.

Effect of ultrasonication on phenolic content extraction

From the results, highest extraction of phenolic compounds without ultrasonication was 11.85 ± 0.20 mgGAE/gdL while highest extraction with ultrasonication was 14.03 ± 1.17 mgGAE/gdL. Based on the p-value of 0.0201 as provided ANOVA, the inclusion of ultrasonication can be

considered to significantly improve the extraction of phenolic compounds from red jute. This means that the ultrasonic waves were effective in disrupting the cells of jute leaves to allow better contact between the extracting solvent and metabolites involved in extraction and to enhance mass transfer across cell membranes (Hossain et al. (2011) as cited by Altemimi et al., 2015) Therefore, further experimentations can be proceeded to determine the significant factors for extraction utilizing ultrasonication.

Effect of significant parameters on phenolic content extraction

TPC measured ranged from 2.69 ± 0.35 to 15.54 ± 2.68 mgGAE/gdl. This clearly shows the differences in the conditions considered for extraction. Based on the corresponding p-values at 95% confidence interval, ANOVA in **Table 3** revealed the following significant factors: extraction temperature, extraction time, ethanol concentration, and the interaction of particle size and ethanol concentration.

Temperature has positive effect on the extraction of phenolic compounds. This was in agreement with the study of Spigno et al. (2007) regarding the extraction of phenolics from grape marc, which states that the increase in temperature enhances the solubility and diffusion coefficient of the desired solute. Therefore, liberation of phenolics from within the powdered leaf is hastened. However, temperature should be set below 60°C to avoid appreciable denaturation of phenolic compounds (Akowua et al., *2009*).

On the other hand, a negative effect of increasing the extraction time is observed. According to Chen et al. (2013), loss in phenolic compounds may occur at longer extraction time due to their possible oxidation, where the products are further polymerized into insoluble compound. Also, red jute is known to contain a significant amount of mucilaginous (Eskander, 2017) polysaccharide which is water-soluble. As extraction time is prolonged, more polysaccharide is extracted leading to the formation of gel (Bone and Mills, 2013). Consequently, the gel can trap some phenolic compounds extracted and hinder further extraction of which from the leaf particles, thus the decrease in extraction.

A positive effect is also illustrated by increasing ethanol concentration. This is due to higher solubility of phenolics in ethanol than in water. For instance, the solubility of gallic acid in ethanol is 23.732 ± 0.001 g/100 g whereas, its solubility in water is 1.072 ± 0.001 g/100 g only (Boas, 2017). Moreover, mucilage increases when the solvent

Table 3. Summary of ANOVA for the factorial model.

SOURCE	SUM OF SQUARES	df	MEAN SQUARE	F-value	p-value	REMARKS
Model	580.13	8	72.52	12.24	< 0.0001	s
a-Temp	35.39	1	35.39	5.98	0.0195	s
b-Extraction	35.98	1	35.98	6.08	0.0186	s
C-Particle	0.2676	1	0.2676	0.0452	0.8329	ns
E-EtOH Concentra-	427.97	1	427.97	72.25	< 0.0001	s
Ab	19.75	1	19.75	3.33	0.0762	ns
aE	20.52	1	20.52	3.46	0.0709	ns
bC	16.02	1	16.02	2.70	0.1088	ns
CE	41.52	1	41.52	7.01	0.0120	s
Residual	213.24	36	5.92			
Lack of Fit	23.53	7	3.36	0.5140	0.8165	ns
Pure Error	189.71	29	6.54			
Cor Total	793.37	44				

s = significant; ns = not significant

becomes more aqueous because of favored extraction of the polysaccharide content in lower concentration of ethanol -water solutions (Yan, 2017). The polysaccharide contains uronic acid, a hydrocolloid which surpasses the viscosity of other food hydrocolloids such as guar gum and locust bean gum (Yamazaki et al., 2009).

Lastly, the interaction of particle size and ethanol concentration has a negative effect. It was observed that the difference in recovery provided at varying particle sizes becomes more prominent when concentration is higher. This was due to favored extraction of phenolic compounds at higher concentration of ethanol especially at greater surface area given by smaller particle size (Bacani et al., 2015).

Coded equations and optimized conditions for phenolic content extraction

The statistical model generated is best fitted to quadratic form based on small sequential p-value of 0.0015. Also, the small difference (<0.2) between the predicted and adjusted R^2 indicates the reliability of the model and that no transformation is necessary. The coded equations for TPC and percent recovery in terms of temperature (A), extraction time (B) and ethanol concentration (C) are then given by Equations 3 and 4, respectively.

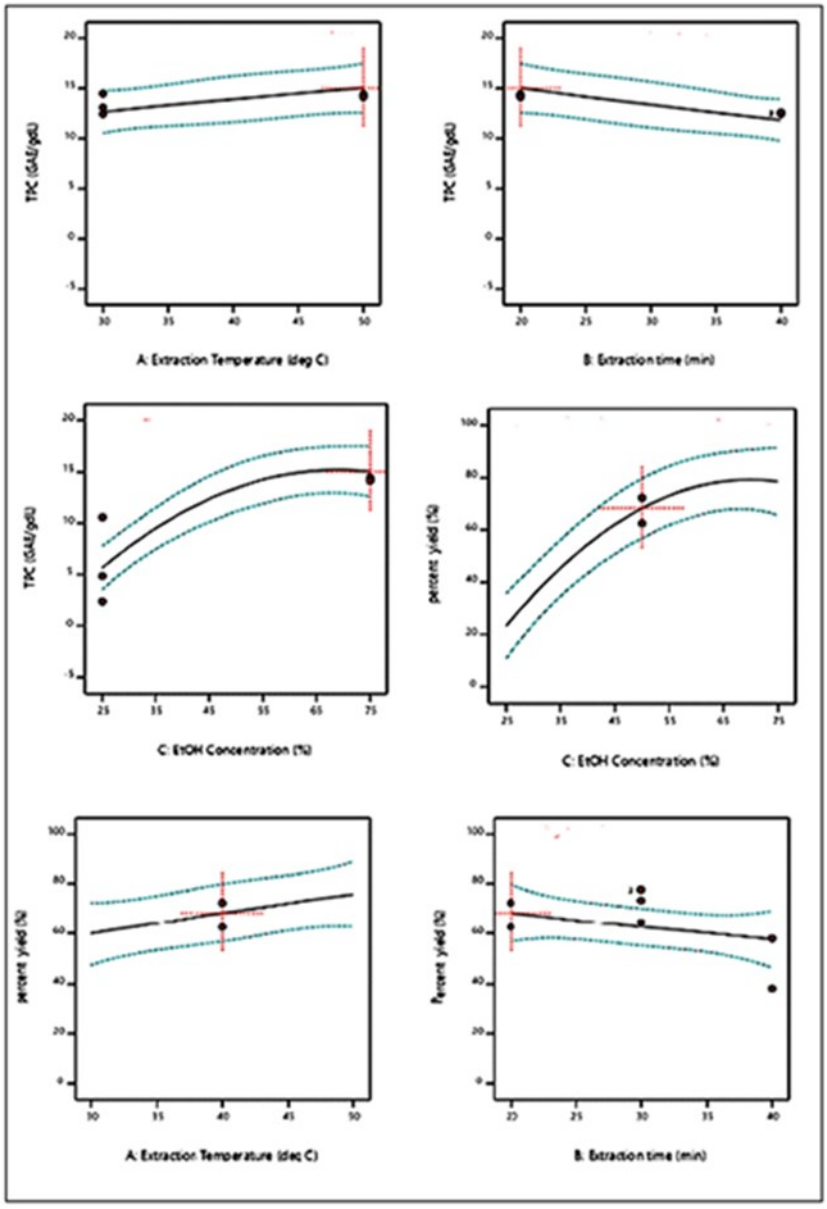

Figure 3. Effects of the main factors on TPC and percent recovery using RSM.

$$TPC = 11.11 + 1.08A - 0.9301B + 4.56C - 0.3224AB - 0.2109AC - 0.3417BC - 0.0894A^2 + 0.0647B^2 - 3.08C^2$$ **Equation 3**

$$\%\ recovery = 62.70 + 6.08A - 5.25B + 25.74C - 1.82AB - 1.19AC - 1.93C - 0.5046A^2 + 0.3652B^2 - 17.37C^2$$ **Equation 4**

Both the models generated give an R^2 of 0.8657, which indicates high desirability in describing empirical results relating the three main significant factors with TPC and percent recovery. Moreover, the models are found significant at p-value of <0.0001. Likewise, lack of fit is not significant. Therefore, the models can be used in obtaining optimum conditions by numerical optimization.

Figure 3 shows the three main factors on both TPC and percent recovery. Curvature of responses can also be observed. This implies that optimal conditions are near the high values of extraction temperature (50°C) and ethanol concentration (75%) and near the low value (20 min) of extraction time.

Figure 4 shows the optimum conditions based on color coding. Estimating the values from the contour plots gives a range of 45- 75% for ethanol concentration. Meanwhile, the darkest red portions of the plots show that the optimum Values for extraction temperature and extraction time as 50°C and 20 min respectively. However, these conclusions will only be valid within the range considered. Consequently, further optimization beyond the ranges considered would be recommended.

An increase in temperature and decrease in extraction time will most likely increase both the TPC and recovery. As mentioned earlier, increasing the temperature enhances solubility and diffusion coefficient of the desired solute (Akowua *et al.*, 2009). However, appreciable denaturation may occur when temperature reaches 60°C. To avoid it, temperature should be lesser, or the extraction should be shortened. For instance, a similar study of Yingngam, Monschein & Brantner in 2014 on formosum leaves resulted to higher optimum extraction temperature and lesser optimum time of 65°C and 15 min, respectively.

Using numerical optimization, the optimum combination of factors for the extraction of phenolics and percent recovery were determined to be 50°C extraction temperature, 20 min extraction time and 69.04% ethanol at a high desirability of 0.843. The best combination of factors as suggested by the software has a predicted TPC of 15.2 and percent recovery of 85.80%.

The results of the experimental verification for the optimum extraction conditions are shown in **Table 4**. The TPC measured from actual experimentation was 15.08 ± 0.03 GAE/gdl. This gave a percent

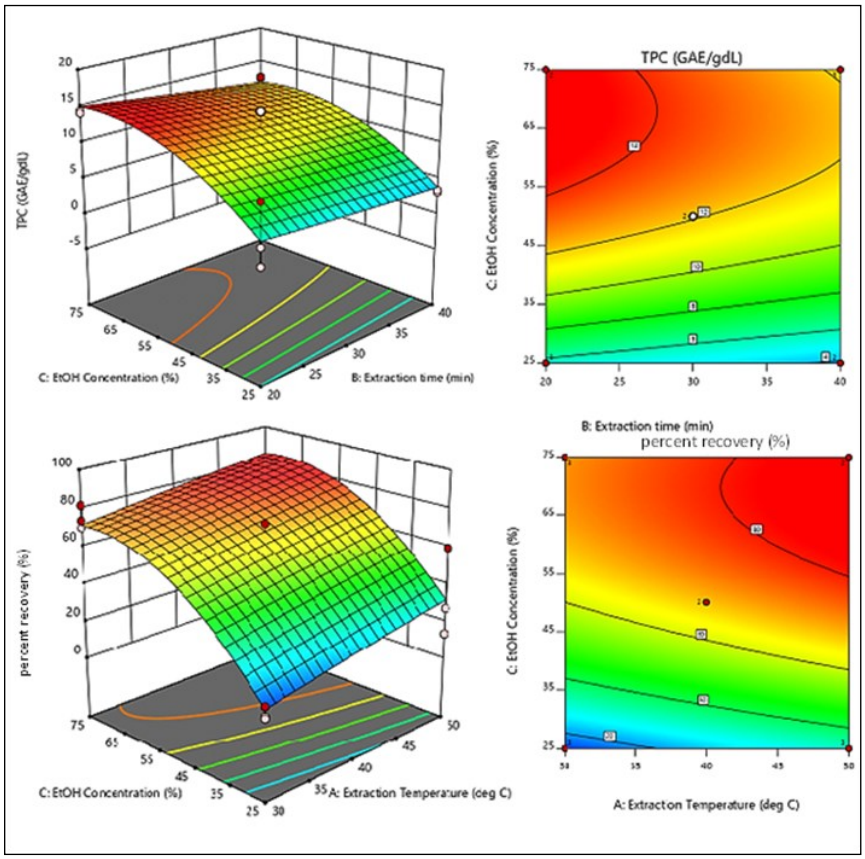

Figure 4. Contour and 3D plots showing the effect of ethanol concentration and a) extraction time on TPC at 50°C; and b) extraction temperature on percent recovery at 20 min extraction time.

Table 4. Comparison of predicted and actual optimum TPC and percent recovery through experimental verification

TPC (mgGAE/gdL)		PERCENT RECOVERY (%)	
Predicted	Actual	Predicted	Actual
15.2	15.08± 0.0307	85.797	85.11

difference of 0.80% with respect to the predicted value. This implies high desirability of the model employed. On the other hand, actual percent recovery was 85.11% which gives a percent difference of 0.80% with respect to the predicted recovery. This implies a very good agreement of the model with actual results. From the results of parametric study, it could be noted that the highest TPC (15.54 ± 2.68 mgGAE/gdl) and percent recovery (87.72 ± 15.12) obtained were higher than the design values and verified values. However, this extraction will need 300% more solvent for a small additional recovered of 2.51%. Therefore, it would be more practical to choose the design optimum conditions.

Table 5. List of some studies that optimized the ultrasound-assisted extraction of phenolic compounds using aqueous ethanol.

DRIED PRECURSOR	OPTIMAL EXTRACTION CONDITIONS				TPC (mgGAE/g – wet basis)	REFERENCE
	SOLVENT-SOLID RATIO (mL/g)	C_{ETOH}, %v/v	TIME, (min)	TEMP (^{O}C)		
argel leaves	--	39.14	37.07	60	[a] 73.02	Mohamed Ahmed et al., 2020
grape seeds	--	53.15	29.03	56.03	5.44	Ghafoor et al., 2009
perilla leaves	--	56.44	55.46	53.68	48.85	Li et al., 2016
formosum leaves	--	50.33	15	65	40 ±1.00	Yingngam et al*., 2014*
perilla leaves	22.15	76.58	53.84	52.75	63.11	Cui et al., 2017
turmeric	--	82	64	32	47.32	Şahin, 2018
shantung maple leaves	15.31	66.21	30	60	75.94	Yang et al., 2017
red jute	48.80	70.92	37.20	68.06	13.38	Biswas et al., 2022
[b] black glutinous rice	--	70	5	--	[a] 136.17	Pariyarath et al., 2018
Vietnamese brown seaweed	100	60	60	60	[a] 9.07 ±0.49	Hassan et al., 2021
black locust flower	--	60	30	59	25.4	Gajic et al., 2019
coffee shells	--	55	9	--	[a] 41.16 ±0.020	Solano et al., 2022
avocado seed	--	41.2	65.1	49	145.87	Monzón et al., 2021
avocado peels	--	49.5	61.8	50.9	124.62	
Chloromolaena odorara leaves	43	57	35	--	111.77	Sirijeerachai et al., 2019
[b] walnut shells	--	43.71	10	0	92.96±1.47	Wang et al., 2020
[b] orange peels	--	45	35	-	30.42±1.5	Razola-Día et al., 2020
Ajuga herb	35	41	50	60	3.552	Zhang et al., 2019
bitter melon	--	59	14	--	18.73	Lee & Yoon, 2021
Okra leaves	40	60	30	70	13.21	Olawuyi et al., 2020
Moringa Oleif-era	5	70	20	42	276.64	Benarima et al., 2020

[a] per gram dried extract; [b] used ultrasonic probe

In comparison with a similar study of Biswas *et. al* in 2022 as shown in **Table 5**, the result of this study had almost the same TPC, 13.82 ± 0.03 mg GAE/gdl in wet basis. The optimum ethanol concentrations of the two studies can also be concluded the same but higher temperature and extraction time was obtained by Biswas et al. (2022). The large deviation in optimum temperature can be attributed to the different ranges of values considered. As mentioned earlier, exploring higher temperature is recommended. Large volume of solvent was also used by Biswas et al. *(2022).* This can provide more water molecules which favor extraction of mucilage while entrapping the phenolics. Consequently, longer time is necessary for extraction. This is then compensated by the positive effect of temperature. Thus, different optimum conditions are expected to be obtained.

It is also noticeable in **Table 5** that the study almost had the same conditions for optimum TPC yield as that of the work of Benarima et al. (2020) on *Moringa oleifera*. This study did not optimize the solvent-solid ratio but also used 5 mL ethanol/gdl. Benarima et al. (2020) on the other hand, did not optimize the ethanol concentration but used 70% v/v which was only 1.39% different with respect to the optimum in this study. Lastly, both resulted to 20 min optimum extraction time. The similarities could be attributed to the mucilaginous property of both leaves. In conclusion, high concentration of ethanol should be used for mucilaginous precursor while minimizing the solvent-solid ratio. Consequently, lesser cost of solvent will be achieved. Likewise, less energy will be consumed as lower temperature will be needed for extraction.

In addition to this, TPC and percent recovery at the optimum extraction conditions without the aid of ultrasonication (as blank) were also determined. The blanks had an average TPC of 7.58 ± 0.61mg/gdl and an average recovery of 42.79 ± 3.47%. This gave a percent difference of 49.73% and 51.54% respectively with respect to actual TPC and percent recovery measured when ultrasonication was employed. These large deviations imply that the application of ultrasound using ultrasonic bath can greatly improve the extraction of phenolic compounds from red jute. This is consistent with the results from preliminary experiment. Nevertheless, ultrasonication can be further improved if ultrasonic probe will be used rather than water bath. In fact, some of the listed studies in Table 5 included ultrasonication frequency as one of the factors optimized, since the ultrasonic intensity provided by the probe is more powerful and is adjustable. From the ultrasound-assisted extraction conducted by Han et al. (2018) on walnut shells, TPC yield with respect to ultrasonic bath method was almost doubled when ultrasonic probe was used.

CONCLUSION

Preliminary experiment proved the significance of assisting the solvent extraction with ultrasonication. Three significant main factors affecting the extraction of phenolic compounds from red jute leaves using ultrasound-assisted extraction were identified: extraction temperature, extraction time and ethanol concentration. The positive effect of temperature means that the solubility and diffusivity of desired solute increase with temperature. On the other hand, the negative effect of increasing extraction time was due to the possible oxidation and polymerization of phenolic compounds. Lastly, the positive effect of increasing ethanol concentration can be attributed to the greater solubility of phenolics in ethanol than in water

Optimizing the effects of the significant factors on the extraction using RSM, a significant model equation with high R^2 of 0.87 and insignificant lack of fit indicates high desirability in describing empirical results. Using numerical optimization, optimum condition where identified as 50°C extraction temperature, 20 min extraction time and 69.04% ethanol with high desirability of 0.843, predicted TPC of 15.2 and percent recovery of 85.80%. However, the optimum conditions may vary because of the mucilaginous property of red jute leaves. Though solvent-solid ratio was not varied, high ratio can lead to longer time of extraction due to increased recovery of mucilage that entraps phenolic compounds. Consequently, higher temperature will be needed to liberate the phenolic compounds from the gel formed.

Experimental verification was employed, giving a TPC of 15.08 ± 0.03 mg GAE/gdl (dry basis) or 13.82 ± 0.03 mg GAE/gdl (wet basis) and percent recovery of 85.11%. The very small difference in values implies high accuracy of the models in

predicting actual TPC and percent recovery. Overall, ultrasound-assisted extraction could be a potential method of recovering significant amount of phenolics from red jute leaves while minimizing solvent input and energy consumption by understanding the properties of the leaves.

RECOMMENDATIONS

The results of the study can serve as a basis in conducting feasibility study to design a profitable process of recovering phenolic compounds from red jute leaves in a large-scale operation. In doing so, it is recommended that drying be done under the sun. The centrifugation operation can also be omitted by coupling ultrasonication with filtration. These will then help minimize the capital and production costs. The use of ultrasonic probe instead of ultrasonic bath extraction method can also be considered to improve the extraction. Likewise, addition and optimization of isolation steps of main phenolic contents such as rutin hydrate, quercetin hydrate and ellagic acid can be considered to ensure maximized profit when applied in food and drug industries.

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