



Process Optimization of Ultrasound-Driven Extraction of Phytochemicals from Litchi (Litchi Chinensis) Peel through Response Surface Analysis

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Abstract

This research focuses on a pulsed-mode ultrasound-assisted extraction (PUAE) technique aimed at increasing the extraction of phenolic compounds from litchi (*Litchi chinensis* Sonn cv. Shahi) peel. Initially, one factor at a time (OFAT) experiments were performed to determine the optimum particle size and solvent concentration, resulting <250 µm particle size and 50% ethanol solution as the greatest extraction potential. The optimization of three key process parameters ultrasound power, stirring speed, and sonication duration was performed using response surface-based experiments (RSM) integrated with an experimental design named Box–Behnken (BBD). The effects of these variables were evaluated based on five responses: total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity (AOX), total condensed tannin (TCT) and ferric reducing antioxidant power (FRAP). The developed regression models had high R² values (over 0.87) alongside non-significant lack-of-fit, ensuring reliability. Most responses were dominated by significant linear effects of ultrasound power and sonication time, while stirring rpm was largely inconsequential. The conditions reached were set at ultrasound power of 599.99 W, stirring of 654.99 rpm, and 15.00 min sonication time which produced values of 97.25 ± 0.96 mg GAE/g TPC, 90.03 ± 1.59 mg QE/g TFC, 85.78 ± 0.06% AOX, 24.69 mg TE/g FRAP, and 64.00 ± 1.02 mg CE/g TCT with an overall desirability of 0.94. High pressure liquid chromatography (HPLC) of the extract was analysed to established the presence of several major phenolic constituents. The study affirms PUAE as a green, efficient, and novel method for valorising shahi litchi peel for nutraceutical and functional food applications.



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Introduction

Litchi (*Litchi chinensis*), a non-climacteric, subtropical, arillate fruit of high commercial importance, from Sapindaceae family, is indigenous to Southern China. The fruit is valued for its sweet taste, translucent pulpy aril and characteristic bright-reddish pericarp. Globally, China is the highest producer of litchi, followed by India, with both countries contributing approximately 91% of the total world production.¹ The "Shahi" variety of litchi, predominantly cultivated in the Bihar region of India, is distinguished by its characteristic aroma and exceptional taste. It is regarded as one of the most flavourful and premium litchi cultivars due to its superior sensory attributes. In "Shahi" litchi, the seed has a weight proportion of around 10-20%, while the peel (pericarp) constitutes around 15%. The edible portion, or aril, accounts for approximately 65–75% of the fruit's weight.² Litchi aril and juice is rich in vitamin C and antioxidants and recent research had shown that the litchi seed contains starch, protein, fiber, and minerals, with potential benefits including antioxidant, anti-inflammatory, and hypoglycemic effects.³ Litchi peel is often regarded as a by-product; however, recent researches have highlighted its rich composition including polyphenols, flavonoids, terpenoids, etc. These compounds have significant bioactivity potential, and other health-beneficial properties.²

Litchi peel contains abundant polyphenolic compounds, with total phenolic content ranging from 9.37 to 30.06 mg of GAE/g. The total flavonoid content varies between 7.12 and 23.46 mg CE/g. The total anthocyanin content lies between 1.77 and 20.94 mg in terms of cyanidin-3-glucoside equivalents, while the total proanthocyanidin content ranges from 1.71 to 7.46 mg of epicatechin. It is good in treating many ailments including cough, hypertension, heart damage, oxidative damages etc.⁴ Given these attributes, the selective and efficient recovery of bioactive constituents from litchi peel holds significant importance.^{4,5}

Response Surface Methodology (RSM) is a well utilized statistical tool for modelling and optimizing processes by establishing relationships between independent variables and response variables.⁶ The Box Behnken Design (BBD) is a commonly employed experimental design within RSM, which

facilitates the effective estimation of coefficients in an empirical model.⁷ This design strategically selects experimental points from a three-level factorial framework, thereby reducing the number of required experiments compared to full factorial or central composite designs. Due to its cost-effectiveness and improved efficiency, BBD is preferred for many optimization studies.⁸ Additionally, center points are incorporated to assess model curvature, process performance, and experimental variability.

Pulsed-mode sonication (PUAE) is an advanced modification of conventional ultrasound-assisted extraction (UAE), wherein ultrasonic energy is applied intermittently in an on-off cycle rather than continuously.⁹ This pulsed approach offers several advantages over the continuous mode, including enhanced equipment longevity, reduced thermal degradation of polyphenols, lower energy consumption, and improved extraction efficiency due to the dynamic, non-equilibrium conditions it creates.¹⁰ The periodic interruption of ultrasound waves minimizes excessive cavitation damage while promoting superior mass transfer, making PUAE as a better extraction technique for thermolabile and sensitive bioactive compounds. There are scanty literatures on PUAE of litchi peel, hence the study finds a novelty for PUAE in litchi peel bioactive compounds extraction. Beyond the extraction technique itself, the efficiency of bioactive compound recovery is significantly influenced by solvent concentration and the particle size of peel powder. Solvent extraction efficiency is primarily influenced by the solvent's solubility and diffusion properties, which govern mass transfer dynamics and overall yield. Additionally, particle size plays a crucial role, as finer particles provide a larger exposed surface area, facilitating improved solvent penetration and enhanced extraction efficiency.¹¹ Optimizing these parameters is crucial for maximizing bioactive compound extraction while maintaining process efficiency and selectivity. The influence of ultrasound power, extraction time, and rotor speed on PUAE of litchi peel remains insufficiently explored. This study investigates the effects of extraction conditions on total phenol content (TPC), total condensed tannin (TCT), total flavonoid content (TFC), DPPH radical scavenging activity (AOX) and ferric reducing antioxidant power (FRAP). Furthermore, optimization of process parameters is done using RSM.

Materials and Methods

Litchi (*Litchi chinensis*) fruits were obtained from the orchards of ICAR-NRC on Litchi, Muzaffarpur, Bihar, India. The peels were separated, washed and sundried (<10% moisture content), followed by grinding and separation into three different size fractions viz. >355 µm, 250-355 µm and <250 µm using different sieves. The dried and powdered litchi pericarps were employed for subsequent extraction experiments. To assess the effect of size fraction on extractability, pericarp powders of varying size fractions were subjected to ultrasonic extraction using a probe-type sonicator (NuwavPromaster; NuTech) operated at 600 W for 5 minutes with 50% ethanol, maintaining a feed-solvent ratio of 1:40. The resulting extracts were centrifuged and filtered prior to biochemical analyses. Furthermore, different solvents and their concentration were examined by extracting 1 g of dried pericarp (<250 µm) in 40 mL of each solvent and their aqueous mixtures. The solvents tested included water, methanol, ethanol, and their 50% aqueous solutions.

Extraction of Bioactive Compounds

PUAE of dried pericarp powder was carried out in a probe type ultrasound system (NuwavPromaster; NuTech) at a frequency of 40 kHz, using a probe having a diameter of 6 mm, inserted to a total depth of 2 mm, inside the sample. The solvent-to-feed proportion was maintained at 40:1, using 50% aqueous ethanol as an extraction solvent in all the experiments. Ultrasonic power was adjusted between 200 and 600 W, with sonication durations ranging from 5 to 15 minutes. The extraction setup was equipped with a magnetic stirring mechanism, and the rotor speed was varied from 400 to 800 RPM during the process. The ranges of independent variables (Appendix-A) were fixed using findings from prior experimentations and researches.

Determination of Response Variables

Total Polyphenol Content Determination

The polyphenol present in extract was measured using the protocol suggested by Singleton and Rossi *et al.*¹² with minor changes. Folin-Ciocalteu reagent in a basic media using sodium carbonate (Na₂CO₃) was

used for the analysis. TPC was represented in terms of the gallic acid equivalence (mg GAE/g).

Total Flavonoid Content Determination

The complete flavonoid content of extract was measured as per the modified procedure of Vuong *et al.*¹³ The absorbance measured at 510 nm after a time of 15 min was used for the evaluation of TFC in quercetin equivalence (mg QE/g).

DPPH based Antioxidant activity (AOX)

The AOX was evaluated using a method adapted from Barros *et al.*¹⁴ The decrease in DPPH radical concentration was quantified at an absorbance of 517 nm. Calculation for the AOX was done using the Eq. 1.

$$AOX = [(A_0 - A_1)/A_0] \times 100 \quad \dots(1)$$

Here, A₀ denotes the absorbance measured for the DPPH solution, while A₁ shows sample's absorbance.

FRAP Determination

The assay prepared using FRAP reagent was employed to evaluate the antioxidant activity using Benzie and Strain *et al.*¹⁵ protocol. FRAP reagent along with TPTZ was used for the analysis. The antioxidant activity was evaluated at an absorbance of 593 nm in terms of Trolox equivalence (TE) per gram of sample.

Total Condensed Tannin

The condensed tannin in peel extracts was evaluated by the method of Broadhurst and Jones *et al.*¹⁶ which is also known as vanillin-HCL method. In this method, the spectral reading was recorded at 500 nm and expressed in terms of catechin equivalents (mg CE) per gram of dry sample weight.

Experimental Design

BBD having 3 levels and five centre points was applied for finding optimum sonication parameters to maximize the bioactive compound's recovery with high antioxidant potential. The independent factors considered were ultrasound power (X₁), stirring speed (X₂), and sonication time (X₃), as

designed using a software named Design Expert with a version of 11.1.2.0. Each variable was studied at three levels, both in coded and actual forms (Appendix-A). A second order polynomial fit of the generated experimental data was conducted with the generalized quadratic equation for the response prediction as given below:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad \dots(2)$$

High Pressure Liquid Chromatography (HPLC) Analysis of Extract

Chromatographic analysis was performed using a binary HPLC system (Shimadzu, Kyoto, Japan). The system was equipped with LC-20AD pumps, a manual injector (Rheodyne 7725i), and a Prominence photodiode array detector (SPD-M20A). A reverse phase chromatography with C18 type column having dimension of 250 × 4.6 mm, with the packed material particle size of 5 µm was utilized in the study. The analytical procedure was based on a previously reported method by Mradu *et al.*¹⁷ 10 mg of each reference compound were dissolved in methanol to create standard solutions, which were then diluted to 50–200 mg/L. Prior to injection, 0.22 µm membranes were used to filter both standards and samples. The mobile phase consisted of first solvent as deionized water (Solvent A) and second one as acetonitrile having 0.02% TFA (Solvent B), operated at 1.0 mL min⁻¹ under a gradient program. Phenolic acids and flavonoids were detected at 280 nm using a photodiode array detector, with system

pressure maintained below 400 kgf cm⁻².

Statistical Analysis

The statistical evaluation was conducted using a Design expert software of version 11.1.2.0. The coefficient of determination (R²), projected R², adjusted R², p-value of the representative model, and lack of fit p value were among the statistical metrics used to assess the model's adequacy. To guarantee dependability, every experiment was carried out in triplicate. Using Origin Pro 8.5.0, ANOVA and post hoc test were conducted to establish statistical significance.

Results

Variation in Extractability with Solvent Concentration and Particle Size

Effects of solvent concentration, as well as particle size on TPC, TFC and AOX were examined initially followed by RSM (Table 1, 2). Solvent concentration significantly (p<0.05) affected extractability of bioactive polyphenols and flavonoids with high antioxidant properties. Aqueous ethanol (50%) and aqueous methanol (50%) yielded the highest TPC, TFC, and AOX, indicating their superior extraction capability. Water showed moderate extraction efficiency, while pure ethanol exhibited the lowest values across all parameters. Methanol performed better than ethanol but was less effective than its aqueous counterpart. The enhanced extraction with aqueous ethanol and methanol (50%) suggests their ability to dissolve both polar and semi-polar compounds, optimizing phenolic and flavonoid recovery.¹⁸

Table 1: Influence of Solvent Concentration on extractability of Litchi peel bioactive

Solvent Concentration	TPC (mg GAE/g)	TFC (mg QE/g)	AOX (%)
Water	76.02±1.11 ^b	40.41±0.53 ^c	61.19±1.82 ^b
Ethanol	39.67±0.82 ^d	20.40±0.44 ^c	42.19±1.08 ^d
Methanol	69.17±1.19 ^c	31.35±0.77 ^d	54.46±1.18 ^c
Aq. Ethanol (50%)	84.32±2.25 ^a	92.40±5.22 ^a	89.02±0.87 ^a
Aq. Methanol (50%)	84.84±0.96 ^a	75.30±5.13 ^b	89.15±0.67 ^a

The extractability of bioactive compounds from Litchi peel powder was significantly influenced by particle size (Table 2). The smallest particle size (<250 µm) yielded the highest TPC, TFC, AOX, FRAP and TCT. The medium-sized particles (250–355 µm) showed moderate extraction efficiency, while the largest particles (>355 µm) had the lowest bioactive compound recovery.

Table 2: Effect of particle size on extractability of litchi peel bioactive compounds

Particle Size	TPC (mg GAE/g)	TFC (mg QE/g)	AOX (%)	FRAP (mg TE/g)	TCT (mg CE/g)
>355 μm	56.73 $\pm$ 2.25 ^c	42.86 $\pm$ 0.73 ^c	78.77 $\pm$ 1.47 ^b	16.04 $\pm$ 0.10 ^c	47.24 $\pm$ 0.03 ^b
250-355 μm	73.29 $\pm$ 0.63 ^b	49.01 $\pm$ 0.35 ^b	85.97 $\pm$ 0.80 ^a	20.58 $\pm$ 0.06 ^b	75.64 $\pm$ 0.06 ^a
<250 μm	78.84 $\pm$ 1.22 ^a	54.83 $\pm$ 1.12 ^a	88.30 $\pm$ 0.06 ^a	23.19 $\pm$ 0.00 ^a	86.46 $\pm$ 0.10 ^a

Effects of Extraction Parameters on TPC

The effect of ultrasound power on the total phenolic content (TPC) of litchi peel is summarized in Appendix-B, with values ranging from 76.24 to 97.77 mg GAE/g dry weight. Regression analysis (Table 3) shows that the linear terms of ultrasound power and sonication time had a statistically significant effect on TPC ($p < 0.05$), with ultrasound power exhibiting a highly significant and positive influence ($p < 0.01$). Quadratic terms and interaction effects were found to be non-significant ($p > 0.05$). With an R^2 of 0.90, a

second-order polynomial equation (Eq. 3) provided a good match to the experimental data. Because the divergence between the adjusted and predicted R^2 was less than 0.25, the model equation was shown to be reasonably predictable. A negligible lack of fit indicated the model's dependability. According to the regression analysis, response surface plots can be visually analysed (Fig. 1(a)). Overall, the results demonstrated that greater phenolic extract was obtained with higher ultrasonic power and sonication time.

Table 3: Regression coefficients of the fitted second-order model for the responses of litchi peel extract and the corresponding ANOVA results of the obtained data.

Terms	Regression coefficients				
	TPC (mg GAE/g)	TFC (mg QE/g)	AOX (%)	FRAP (mg TE/g)	TCT (mg CE/g)
Intercept					
β_0	+89.11	+86.87	+82.64	+23.24	+54.52
Linear					
β_1	+5.52	-4.44	+1.46	+0.70	+2.22
β_2	-2.00	+0.26	+0.179	+0.12	+0.85
β_3	+4.33	+5.86	+2.98	+1.51	+7.10
Quadratic					
β_{11}	+0.79	+2.90	-0.22	-0.089	+0.27
β_{22}	+0.41	-2.27	+0.018	-0.12	+0.71
β_{33}	-1.05	-3.59	-0.81	-0.81	+2.14
Interaction					
β_{12}	+0.98	-1.98	-0.51	-0.040	+2.33
β_{13}	-1.85	+2.83	-0.096	+0.087	+0.73
β_{23}	+2.33	+0.91	-0.28	+0.18	+0.40
R^2	0.9045	0.8913	0.8779	0.9260	0.9146
R^2 (adjusted)	0.7817	0.7516	0.7210	0.8309	0.8048
R^2 (Predicted)	0.6800	0.2638	0.7274	0.6743	0.6657
CV (%)	2.99	3.76	1.65	2.36	4.59
P- value (Regression)	0.0077	0.0116	0.0167	0.0033	0.0053
P- value (lack of fit)	0.8991	0.57	0.9757	0.8076	0.8516

$$\text{TPC} = 89.10 + 5.52X_1 + 4.33X_3 \quad \dots(3)$$

Effect of Extraction Parameter on TFC

The influence of independent factors on the total flavonoid content (TFC) of Shahi litchi peel is presented in Appendix-B, with values ranging from 72.23 to 94.30 mg QE/g dry weight. Regression analysis (Table 2) demonstrated that the linear impact of ultrasound power and sonication time significantly influenced TFC ($p < 0.05$), with sonication time exhibiting a particularly strong and positive effect ($p < 0.01$). In contrast, quadratic and interaction terms were not statistically significant ($p > 0.05$), shows that the relationship between the independent variables and TFC was primarily linear within the studied range. The same effect can be visualized through the response surface curves (Fig. 1(b)). Based on the statistical significance of the regression coefficients, the influence of process variables on TFC is described by Equation (4).

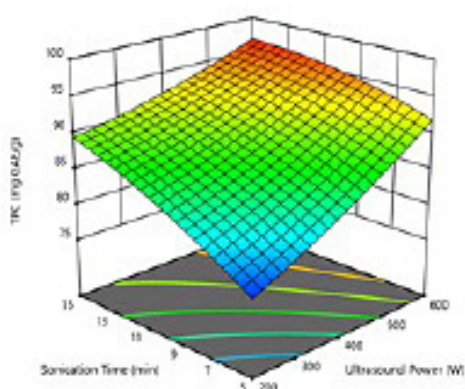
$$\text{TFC} = 86.87 - 4.44X_1 + 5.86X_3 \quad \dots(4)$$

Both the projected R² and the low corrected R² for flavonoids indicated a greater block effect. Excellent model precision and experimental consistency are indicated by a CV of 3.76%.

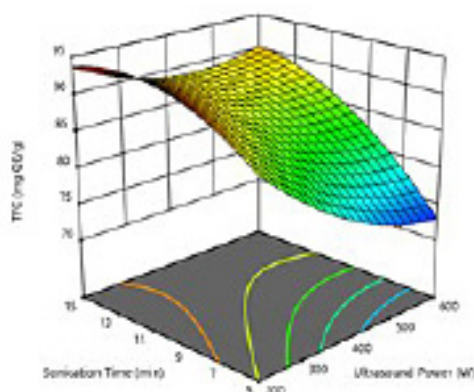
CV= coefficient of variation

Effects of Extraction Parameters on AOX

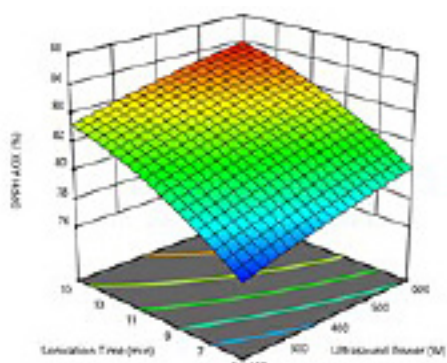
The impact of process variables on the AOX of Shahi litchi peel is summarized in Appendix-B, with values ranging from 76.96 % to 86.05 %. Regression analysis (Table 3) revealed that linear term of ultrasound power and sonication time was significant ($p < 0.05$), with sonication time exerting a particularly strong positive influence ($p < 0.01$). The non-significance of quadratic and interaction terms indicated a predominantly linear relationship within the studied range. The model showed a very good fitting of data with R² of 0.87 and a minimal difference (< 0.2) between adjusted and predicted R², confirming model reliability.



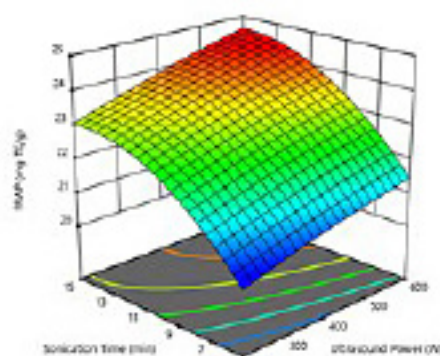
(a)



(b)



(d)



(c)

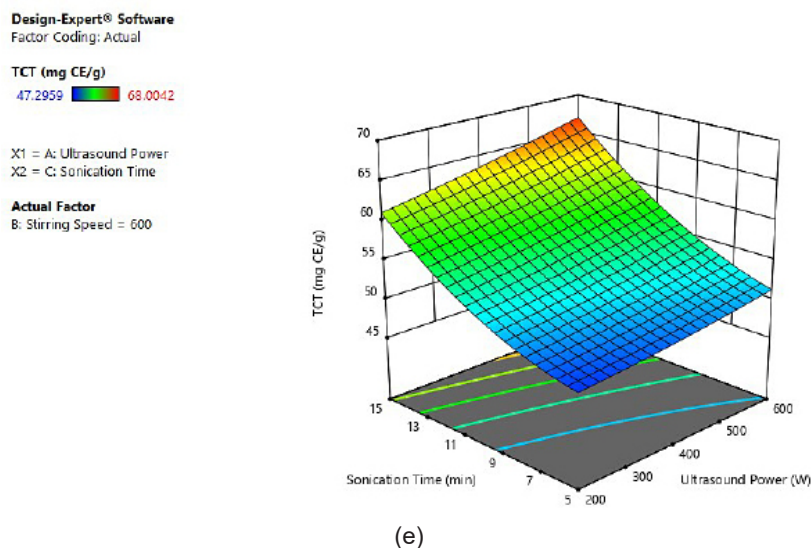


Fig. 1: Surface plots representing the effect of sonication power and time on TPC (a), TFC (b), AOX (c), FRAP (d), and TCT (e) of litchi peel extract

$$\text{DPPH AOX} = 82.64 + 1.45X_1 + 2.98X_3 \quad \dots(5)$$

Effects of Sonication on FRAP

The effect of process variables on the FRAP of litchi peel is given in Appendix-B, which showed a range of values from 20.44 to 24.40 mg TE/g sample for FRAP. Regression analysis (Table 3) represented that sonication power and time had significant linear impact ($p < 0.05$). Sonication time exerted a strong positive linear ($p < 0.01$) and quadratic influence ($p > 0.05$). There was mostly a linear association within the examined range, while all other quadratic and interaction terms were not significant ($p > 0.05$). The model demonstrated good fit with an R^2 of 0.92 and a difference (< 0.2) between adjusted and predicted R^2 , confirming models' reasonable reliability. The model equation for FRAP is following:

$$\text{FRAP} = 23.24 + 0.70X_1 + 1.51X_3 - 0.81X_3^2 \quad \dots(6)$$

From the response surface plot (Fig. 1(d)), it is evident that sonication time exerts a more pronounced influence on FRAP values compared to the other variables, indicating its critical role in enhancing the ferric reducing antioxidant potential of Shahi litchi peel extract.

Impact of Sonication on Condensed Tannins

Condensed tannins, a predominant class of polyphenolic compounds in litchi peel, are recognized as major contributors to its antioxidant activity. Structurally, they are polymers of flavan-3-ol units, primarily catechin/epicatechin, and are abundantly distributed in plant-derived tissues such as peels, seeds, and barks.¹⁹ Owing to their potent antioxidant activity, the efficient extraction of TCT is considered highly desirable for maximizing the functional value of plant-derived extracts.²⁰

The observed values for TCT ranged from 47.29 to 68.00 mg catechin equivalents (CE)/g sample (Appendix B). Regression analysis (Table 3) indicated that the linear effects of sonication time and power were statistically significant ($p < 0.05$), with sonication time exhibiting a particularly strong and positive effect ($p < 0.01$). Quadratic terms as well as interaction terms were insignificant ($p > 0.05$), suggesting that the relationship between the variables and TCT content was primarily linear within the experimental range. The fitted model exhibited a high coefficient of determination ($R^2 = 0.91$), with a minimal difference (< 0.2) between predicted and adjusted R^2 values, confirming the model's robustness and predictive adequacy. The

reduced quadratic (Eq. 5) for TCT, incorporating only significant terms, is expressed as follows.

$$\text{TCT} = 54.52 + 2.22X_1 + 7.10X_3 \quad \dots(7)$$

Optimization of Pulsed Mode Ultrasound-based Extraction Parameters

RSM was used to optimize the bioactive compound's recovery as well as their antioxidative potential from Litchi peel. The experimental values obtained under different ultrasound parameters are presented in (Appendix-B). Table 3 summarises the regression coefficients (in coded form) obtained from ANOVA as well as important statistical markers including model F-values, R² adjusted R² CV, and lack-of-fit p-values.

Appendix-C presents the predicted and experimental values of the independent variables and corresponding responses. In all cases, the relative error remained below 10%, indicating the adequacy and predictive reliability of the developed models. Elevated ultrasound power and extended sonication time contributed significantly to enhanced extraction efficiency, whereas stirring speed exhibited no substantial influence on the responses. This might be due to dominant cavitation effect which subdues the

effect of stirrer rotation for extraction. The optimal values of bioactive compounds are comparable with those mentioned in the literature by Cano-Gómez *et al.*⁴ This optimized condition, in conjunction with previously determined solvent parameters, yielded maximal recovery of bio-actives from Litchi peel. The optimal conditions were ultrasound power of 599.99 W, stirring speed of 654.99 rpm, and sonication time of 15.00 min, resulting in TPC, TFC, AOX, FRAP, and TCT values of 97.25 ± 0.96 mg GAE/g, 90.03 ± 1.59 mg QE/g, 85.78 ± 0.06 AOX (%), 24.69 mg TE/g, and 64.00 ± 1.02 mg CE/g, respectively. The overall desirability of the optimized extraction condition was 0.94.

HPLC Analysis of Litchi Peel Extract

Calibration curves were constructed for the following standards: Gallic acid ($y = 56715x$, $R^2 = 0.9998$), Catechin ($y = 11600x$, $R^2 = 0.99$), Caffeic acid ($y = 95201x$, $R^2 = 0.99$), Ferulic acid ($y = 88715x$, $R^2 = 0.99$), and Quercetin ($y = 32909x$, $R^2 = 0.99$). Peel extract showed the presence of all targeted phenolic compounds, including gallic acid (53.74 ± 2.55 mg/L), catechin (45.98 ± 1.81 mg/L), caffeic acid (16.16 ± 0.59 mg/L), ferulic acid (21.73 ± 1.48 mg/L), and quercetin (9.48 ± 0.74 mg/L) (Fig. 2).

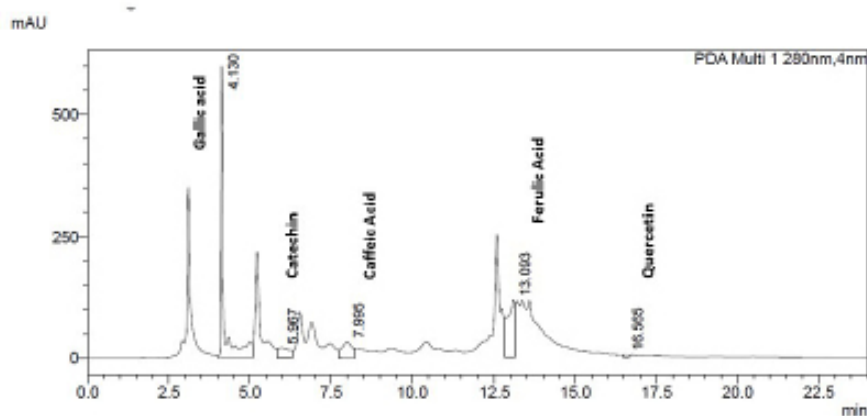


Fig. 2: HPLC chromatogram of sample

Discussion

Water effectively extracted phenolic compounds but demonstrated limited efficiency for flavonoids due to their preferential solubility in alcoholic solvents.²¹ The differential extraction efficiency correlates with flavonoid polarity characteristics.

Polar flavonoids exhibit higher solubility in aqueous and pure alcoholic media, whereas nonpolar variants (iso-flavones, flavanones, flavones, flavonols) demonstrate enhanced affinity for lower-polarity solvents (chloroform, dichloromethane, diethyl ether, ethyl acetate).²² The observed moderate flavonoid

extraction efficiency with water can be attributed to its high polarity, which constrains solubilisation of nonpolar and weakly polar flavonoid constituents. Conversely, the presence of ethanol or methanol in aqueous mixtures enhances solubility by balancing polarity, thereby improving extraction efficiency. Ethanol alone, due to its lower polarity, was less effective at extracting hydrophilic phenolic and antioxidants,²³ while methanol, being more polar, performed better but remained inferior to its aqueous mixture.²⁴ Similar studies were reported by^{25,26} for *Limnophila aromatic* and *Polygonatum sibiricum* respectively.

The improved extraction with smaller particle sizes can be attributed to the increased surface area and reduced diffusion resistance, which enhanced the penetration of solvents and enhanced bioactive compounds release.²⁷ Smaller particles allow better interaction between the solvent and plant matrix, leading to higher phenolic and flavonoid recovery.²⁸ Additionally, reduced particle size decreases mass transfer limitations, promoting efficient diffusion of antioxidants such as tannins.²⁹ Similar findings were reported by^{30,31} for groundnut and grape respectively.

The observed effect of sonication time and power on TPC can be explained by the mechanism of acoustic cavitation. Higher ultrasound power increases the frequency and strength of cavitation bubbles, generating intense micro-jets and shockwaves that effectively rupture plant cell walls and promote the release of phenolic compounds.³² This mechanical disruption increased the release of intracellular phenolic compounds into the solvent, thereby increasing extraction yield.³³ Similarly, Prolonged sonication enhances cavitation intensity, leading to the formation of numerous microbubbles that exert greater mechanical stress on cell structures, thereby facilitating increased rupture and release of bioactive polyphenols.³⁴ Conversely, the stirring speed applied through an internal stirrer within the ultrasonic bath to facilitate uniform distribution of acoustic energy did not exert a statistically significant effect on TPC. This outcome can be ascribed to the predominant influence of acoustic cavitation in ultrasound-assisted extraction (UAE), wherein mechanical agitation serves a supplementary rather than a principal function in enhancing mass transfer.³⁵ Similar findings were reported by³⁶ for soy protein extraction by ultrasound.

In case of flavonoid extraction, increasing ultrasound power enhanced solvent penetration and expanded the effective surface area for extraction, leading to improved release of soluble flavonoids from the litchi peel matrix.⁹ The rise in TFC with longer sonication time may be due to effective cell disruption and mass transfer, without inducing thermal or oxidative degradation of flavonoids.³⁷ The non-significant interaction effects are likely due to major flavonoids compound present in litchi peel are quercetin, rutin, and kaempferol. These compounds have structural stability and moderate polarity,³⁸ which favour linear responses to ultrasound power and sonication time. The A study by Kumar & Rao *et al.*,⁹ reported better flavonoids recovery with enhancement in sonication time. A research by Vo *et al.*³⁹ found that increase in ultrasound power enhances the total flavonoids recovery in Passion Fruit Peels.

In case of antioxidant activity (AOX), a positive linear effect of ultrasound power represented that higher ultrasound power intensifies cavitation effects, promoting significant disruption of cell walls and facilitating the diffusion of radical-scavenging into the solvent phase.³⁵ DPPH antioxidant activity increased with longer sonication times until it reached an optimal point, at which point it declined, possibly as a result of the damaging effects of high power sonication.³⁴ Similar findings were reported for higher DPPH radical scavenging activity capacity with increase in ultrasound power and sonication time for araticum peel and defatted hemp by Arruda *et al.*⁴⁰ and Teh and Birch *et al.*⁴¹ The increase in FRAP value with the rise in ultrasound power may be attribute to the intensification of energy input improves solvent penetration and mass transfer, thereby increasing the extraction of compounds capable of donating electrons in the FRAP.⁴² Similar findings were reported by Rout *et al.*⁴³ for oregano leaf and Kobus *et al.*⁴⁴ for *Sorbus intermedia*.

The enhanced recovery of TCT with increasing ultrasound power and sonication time may be attributed to the structural characteristics of flavan-3-ols, such as catechin/ epicatechin, which comprise the core units of proanthocyanidins. These compounds are often localized within the vacuoles or bound to the cell wall matrix via hydrogen bonding and hydrophobic interactions.⁴⁵ Acoustic cavitation generated by ultrasonication disrupts these cellular structures and weakens non-covalent interactions,

promoting the release of both monomeric and oligomeric tannins.⁴⁶ Longer sonication time further increases the exposure of these macromolecules to solvent interaction, allowing for more complete solubilization of proanthocyanidins without inducing degradation.⁴⁷ The absence of a significant effect from stirring speed supports the notion that diffusion-limited, matrix-bound phenolics such as condensed tannins are not easily mobilized by bulk fluid motion alone,⁴⁸ but require the localized, high-energy micro-environment provided by cavitation collapse. Similar findings were reported by Sivakumar *et al.*⁴⁹ Rifna and Dwivedi *et al.*⁵⁰ for Avaram bark and pomegranate peel respectively.

The created models were shown to be statistically significant ($p < 0.05$) by the F-values for all responses (Table 3). Elevated R^2 values indicated strong model accuracy and reliability. The proximity between R^2 and adjusted R^2 values further affirmed the robustness of the models and the consistency between predicted and observed data. Additionally, coefficients of variation (CV) remained below 5% across all responses, indicating high experimental precision and reproducibility. The lack-of-fit p-values were non-significant ($p > 0.05$), confirming that the variability in the response data was primarily due to pure error rather than inadequacies in the model structure, thus validating the RSM approach. The HPLC analysis of the extract showed the presence of major phenolic acids and flavonoids in litchi peel which are highly antioxidative in nature, thus contributed to their bioactivity. Litchi peel showed the highest concentration of gallic acid, followed by catechin.

Conclusion

Based on the results of this study, pulsed mode sonication proved effective in enhancing the bioactive compounds recovery from Shahi litchi peel. The extraction process was optimized using BBD under RSM, which efficiently identified the best conditions for maximizing TPC, TFC, AOX, FRAP, and TCT. The optimum conditions were found to be: ultrasound power of 599.99 W, sonication time of 15.00 min, and stirring speed of 654.99 rpm. The developed regression models showed high reliability with R^2 values ranging from 0.87 to 0.92 and relative errors less than 10 %, which represents better alignment between the predicted and experimental values. Linear effects of sonication time and power

were significant ($p < 0.05$), while stirring speed had no significant influence. The superiority of PUAE was attributed to efficient acoustic cavitation, which enhanced mass transfer and compound release without excessive thermal degradation. The use of aqueous alcoholic solvents and reduced particle size further improved extractability. The extracted compounds possess potential applicability in the development of phytopharmaceutical or herbal therapeutic formulations for industry, thereby offering scope for value addition, entrepreneurship development, and future research advancements. This green extraction protocol offers a sustainable and efficient approach for valorising litchi peel waste and holds potential for broader application in the recovery of bioactive compounds from plant-based food processing residues.

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This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Information Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

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Not Applicable

Author Contributions

- **Ankit kumar:** Conceptualization, Methodology Design, Experimental Execution Manuscript Writing.
- **Chaman Kumar:** Conducted Optimization Trials Using Design Expert Software, Statistical Analysis of Results, and Preparation of

Graphical Outputs and Tables.

- **Pavuluri Srinivasa Rao:** Supervision, Technical Guidance on Ultrasound Extraction Optimization And Statistical Modelling.
- **Sunil Kumar:** Assisted in sample Preparation, and Laboratory Coordination.
- **Bhagya Vijayan:** Assisted in Manuscript Writing and Formatting.
- **Abhay Kumar:** Technical Guidance on Evaluation Of Bioactivity
- **Ipsita Samal:** Contributed in Final Document Preparation.
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Appendix –A

**Independent variables along-with the factor levels
in Box-Behnken Design**

Independent variable	Factor level		
	-1	0	1
Ultrasound Power (W)	200	400	600
Stirring Speed (rpm)	400	600	800
Sonication time (min)	5	10	15

Appendix-B

Litchi peel's response variables as derived from a Box-Behnken design of experimental variables

Run Order	Run	Ultrasound Power(X1)	Stirring Speed(X2)	Sonication Time(X3)	TPC	TFC	DPPH AOX	FRAP	TCT
		W	RPM	min	mg GAE/g	mg QE/g	%	mg TE/g	mg CE/g
14	1	400	600	10	94.29	86.37	84.97	23.99	57.37
9	2	400	400	5	88.78	75.32	78.58	20.92	49.72
6	3	600	600	5	92.66	73.65	79.81	21.27	52.18
4	4	600	800	10	94.20	83.00	83.62	24.10	59.76
15	5	400	600	10	85.39	82.20	83.44	23.34	52.18
12	6	400	800	15	92.80	88.50	84.55	24.04	65.78
16	7	400	600	10	87.72	85.46	80.73	23.50	53.83
11	8	400	400	15	92.64	87.95	84.63	23.64	62.94
17	9	400	600	10	88.29	89.62	82.98	22.26	51.08
5	10	200	600	5	76.24	89.72	76.96	20.44	47.29
2	11	600	400	10	95.76	84.64	84.41	23.77	53.75
13	12	400	600	10	89.82	90.67	81.07	23.09	58.09
7	13	200	600	15	88.73	93.05	83.58	23.22	60.20
3	14	200	800	10	82.88	94.30	81.48	22.38	52.56
8	15	600	600	15	97.77	88.29	86.05	24.40	68.00
1	16	200	400	10	88.37	88.04	80.22	21.88	55.87
10	17	400	800	5	79.63	72.23	79.61	20.62	50.97

Appendix-C

Optimization of extraction conditions for maximum bioactive recovery from litchi peel

Factors	Units	Predicted value	Input value	Relative error (%)
Ultrasound power	W	599.99	600	0
Stirring speed	rpm	654.99	650	0.76
Sonication time	min	15	15	0
Responses	Units	Predicted value	Experimental value	Relative error (%)
TPC	mg GAE/g	97.25	93.82	3.53
TFC	mg QE/g	90.03	87.49	2.82
DPPH	AOX (%)	85.78	82.77	3.51
FRAP	mg TE/g	24.69	23.51	4.79
TCT	mg CE/g	64.00	66.59	4.04