

Research Article

Process Optimization of Microwave-assisted Aqueous Extraction for Phytochemicals and Antioxidant Activity of *Syzygium Cumini*

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Abstract

This study optimized microwave assisted aqueous extraction of phytochemicals from *Syzygium cumini* leaf powder using water as solvent. Response surface methodology with a three factor Box Behnken design was used to quantify the effects of microwave power, extraction time, and particle size. Factor ranges were 360 - 900 W, 8 - 20 min, and 100 - 500 μm . Microwave irradiation was applied in pulsed mode to limit boiling. Process performance was evaluated using total phenolic content, total flavonoid content, total tannin content, and antioxidant activity measured by DPPH and ABTS assays. Numerical optimization predicted an optimum at 900 W, 18.94 min, and 276.54 μm . At these conditions, predicted responses were 305.44 mg GAE/g ds for total phenolics, 70.12 mg RE/g ds for total flavonoids, and 83.35 mg TAE/g ds for total tannins. Predicted antioxidant activities were 5.51 mM TE/g ds for DPPH and 5.78 mM TE/g ds for ABTS. Experimental validation was conducted at the nearest practical settings of 900 W, 20 min, and 273 μm . Measured values were 305.55 ± 0.07 mg GAE/g ds, 70.54 ± 0.05 mg RE/g ds, and 81.99 ± 0.03 mg TAE/g ds for total phenolics, total flavonoids, and total tannins, respectively. DPPH and ABTS reached 5.47 ± 0.03 and 5.74 ± 0.04 mM TE/g ds, respectively. The close agreement between predicted and measured responses supports the use of RSM to define an implementable operating window for aqueous microwave extraction of *Syzygium cumini* leaves.

Keywords

Response Surface Methodology, Bioactive Compounds, Plant Extract Processing, Phenolic Compounds, Radical Scavenging Activity

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Received: 30 December 2025; Accepted: 30 January 2026; Published: 2 February 2026



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1. Introduction

Plant derived phytochemicals remain of strong interest for food and health related applications because they can deliver antioxidant functionality when efficiently recovered from plant matrices [1]. This interest also reflects the push to valorize underutilized botanical resources and agro-derived materials as sources of natural bioactives [2]. In food systems, antioxidant extracts are valuable because they can slow oxidation that damages color, flavor, and nutritional quality, and they can extend shelf life while meeting demand for plant based and clean label ingredients [3]. Beyond food preservation, antioxidant rich plant extracts are increasingly sought as natural additives and value-added ingredients in food systems. Their practical use depends on efficient, food compatible extraction that preserves redox active compounds and supports reproducible functional performance [4]. To translate this potential into useable ingredients, extraction must be effective, food compatible, and amenable to scale up.

Syzygium cumini is a medicinal tree with broad ethnomedicinal documentation and a growing phytochemical evidence base [4, 5]. Available studies indicate that the species contains diverse secondary metabolites, including phenolics and flavonoids, together with other classes such as triterpenes and saponins [4-7]. Extracts and isolated compounds have been reported to show antioxidant activity *in vitro* [4, 5, 7]. These attributes support the view that *Syzygium cumini* could serve as a source of multifunctional phytochemicals for antioxidant enriched ingredients, provided that extraction is engineered to maximize recovery while preserving redox activity.

Extraction is the key bottleneck that connects botanical composition to ingredient performance [8]. Conventional solid liquid extraction is simple, but it is often slow and yield limited because mass transfer is constrained by cell wall barriers and diffusion resistance [9]. Water based extraction is attractive for food applications because it is inherently food grade and avoids organic solvents [10]. Yet aqueous systems can be less efficient for some phenolic subclasses, and uncontrolled heating can degrade thermolabile compounds [11]. This is particularly important when extracts are intended to deliver consistent functional performance, including antioxidant capacity measured by *in vitro* assays. Process intensification is therefore needed to obtain reproducible, activity rich extracts under aqueous conditions.

Microwave-assisted aqueous extraction (MAAE) is a promising intensification strategy. Microwave heating is rapid and volumetric, which can accelerate diffusion, improve solvent penetration, and promote structural disruption of plant tissues [8, 12]. However, extraction performance depends strongly on process settings. Microwave power level (MP) controls the rate of energy input and heating intensity. Extraction time (ET) determines cumulative exposure and the extent of solute release [13, 14]. Particle size ratio (PS) governs surface area and diffusion distance, and it influences how uniformly the matrix heats and hydrates [13, 14]. These variables can interact, such that excessive power or prolonged exposure may compromise

polyphenol integrity, while low intensity or coarse particles can limit release and antioxidant performance [14]. A robust optimization approach is therefore required to maximize both phytochemical recovery and antioxidant activity. Recent reports [8, 13] summarize that MAAE performance is governed by coupled effects of energy delivery, solvent system, and matrix properties, and they stress the need for statistically designed optimization rather than one-factor testing.

Microwave-based extraction has been explored for *Syzygium cumini*, but most reports [6, 7] do not provide design rules for a strictly aqueous, food-grade process. Solvent systems and targets vary across studies [5-7] and particle size effects are often not reported, which limits transferability and scale-up. This leaves an engineering gap for a controlled MAAE optimization that quantifies the combined effects of microwave power, extraction time, and particle size on phytochemical indices and antioxidant activity.

Response surface methodology (RSM) provides a statistically grounded route to model and optimize such multivariable processes [15]. It captures main effects, curvature, and interactions while reducing experimental effort compared with one factor at a time testing [15]. Accordingly, this study applies RSM to optimize MAAE of phytochemicals from *Syzygium cumini* powder, with MP, ET, and PS as the key process variables. Process performance was evaluated using total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC), alongside antioxidant activity measured by the 1,1 diphenyl 2 picrylhydrazyl (DPPH) and 2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid) (ABTS) assays as *in vitro* chemical screening metrics of extract redox capacity. By linking MP, ET, and PS to phytochemical recovery and antioxidant activity, this study sought to define an optimized, food compatible MAAE window. The work supports ingredient-oriented valorization of *Syzygium cumini* leaves for food formulation and oxidative stability applications.

2. Materials and Methods

2.1. Materials

Fresh leaves of *Syzygium cumini* were collected in Ngaoundere, Adamawa Region, Cameroon. Botanical identification was confirmed by a taxonomist at the Botanical Survey, Department of Science, University of Ngaoundere. The leaves were dried at 40 °C for 24 h in a forced convection oven (UF110, Memmert GmbH, Schwabach, Germany) and then milled using a hammer mill (FitzMill L1A, Fitzpatrick, Waterloo, ON, Canada). The resulting powder was separated into particle size fractions by sieving through stainless steel sieves with nominal apertures of 100, 250, and 400 µm using a mechanical sieve shaker (BK-TS200, Biobase, Jinan, China). Each fraction was packed in airtight polyethylene bags and stored at room temperature until extraction. All analytical

grade reagents and chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (China).

2.2. Microwave-Assisted Aqueous Extraction

MAAE was conducted using a domestic microwave unit (H30MOMS9HG, Hisense, Qingdao, China). The powder fractions specified in the experimental design were combined with distilled water at a 1:20 (w/v) solid to liquid ratio. Irradiation was applied at the design selected power levels and exposure times, using a pulsed sequence of 5 s on and 15 s off to minimize boiling [16]. The treated mixtures were centrifuged (BKC-TH21, Biobase, Jinan, China) for 15 min at 8000 rpm and then filtered. The resulting extracts were stored at -29°C until analysis.

2.3. Experimental Design

RSM was applied to quantify the effects of key aqueous extraction variables on phytochemical recovery and antioxidant capacity of *Syzygium cumini* leaf powder. A three level, three factor Box Behnken design (BBD) was used to identify operating conditions that maximize compositional responses and antioxidant performance. The independent variables were MP, ET, and PS, denoted as X_1 , X_2 , and X_3 , respectively. Factor levels were selected from preliminary trials and are reported in Table 1.

Table 1. Coded and actual factor levels for the Box Behnken design.

Independent variables	Symbols	Coded levels		
	X	-1	0	1
MP	X_1	360	630	900
ET	X_2	8	14	20
PS	X_3	100	300	500

MP -Microwave power (W), ET-Extraction time (min), and PS-Particle size (μm)

The independent variables were transformed into coded values using the following equation:

$$x_i = \frac{X_i - X_{cp}}{\Delta X_i}; i = 1, 2, 3 \quad (1)$$

Where x_i represents the coded value of factor i , X_i is the corresponding actual value, X_{cp} denotes the centre point, and ΔX_i is the increment between coded levels 0 and 1.

The responses included phytochemical indices TPC, TFC and TTC, together with antioxidant capacity evaluated by

DPPH, and ABTS assays. The experimental data were subjected to multiple regression analysis and fitted to a second order polynomial model.

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j \quad (2)$$

Where Y is the predicted response, β_0 is the intercept, β_i are the linear coefficients, β_{ii} are the quadratic coefficients, and β_{ij} are the interaction coefficients, with X_i and X_j representing the independent variables.

2.4. Analytical Methods

2.4.1. Total Phenolic Content Determination

TPC was determined according to Tchabo, Ma [17] using a Folin Ciocalteu colorimetric procedure with minor adjustments. A 0.1 ml aliquot of the filtered supernatant was mixed with 7.9 ml deionized water and 0.5 ml Folin Ciocalteu reagent. After 1 to 8 min, 1.5 ml sodium carbonate solution (20 % w/v) was added, and the mixture was incubated in a water bath at $40 \pm 2^{\circ}\text{C}$ for 30 min. Absorbance was measured at 765 nm. TPC was expressed as mg gallic acid equivalents per g of dry solids.

2.4.2. Total Flavonoid Content Determination

TFC was determined using the aluminium chloride colorimetric assay [17]. A 1.0 ml aliquot of the filtered extract was placed in a 10 ml volumetric flask, followed by 4.0 ml deionized water and 0.3 ml sodium nitrite (5% w/v). After 5 min, 0.3 ml aluminium chloride (10% w/v) was added, and 1 min later 2.0 ml sodium hydroxide (1 M) was introduced. The solution was made up to 10 ml with deionized water, mixed well, and absorbance was measured at 510 nm using a UV visible spectrophotometer. TFC was expressed as mg rutin equivalents per g of dry solids.

2.4.3. Total Tannin Content Determination

TTC was quantified following Haile and Kang [18] using a Folin–Ciocalteu colorimetric assay with minor procedural adjustments. Briefly, 0.1 mL of the extract was mixed with 7.5 ml of distilled water and 0.5 ml of Folin–Ciocalteu reagent. Subsequently, 1.0 ml of sodium carbonate solution (35% w/v) was added, and the volume was adjusted to 10 ml with distilled water. The reaction mixture was incubated at ambient temperature for 30 min, after which absorbance was recorded at 700 nm. TTC was expressed as mg tannic acid equivalents per g of dry solids.

2.4.4. DPPH Determination

DPPH radical scavenging capacity of the extract was determined according to Tchabo, Ma [19]. Briefly, 1.0 ml of the extract was mixed with 6.0 ml of DPPH solution in methanol

(60 mM). The mixture was incubated for 30 min at room temperature in the dark, and absorbance was measured at 517 nm. DPPH activity was expressed as mM Trolox equivalents per g of dry solids.

2.4.5. ABTS Determination

ABTS radical scavenging capacity of the extract was determined according to Tchabo, Ma [19]. Briefly, 0.125 ml of extract was mixed with 5.0 ml of ABTS working solution prepared by reacting ABTS (2.45 mM) with 140 mM of ammonium persulfate and incubating the mixture in the dark for 16 h. The reaction mixture was then kept for 15 min at room temperature, and absorbance was measured at 734 nm. ABTS activity was expressed as mM Trolox equivalents per g of dry solids.

2.5. Statistical Analysis

All extraction trials and analytical determinations were performed thrice, and results were reported as mean values. Box

Behnken response surface models were generated and evaluated using Design Expert v13 (Stat Ease Inc., Minneapolis, MN, USA). Treatment effects were examined by ANOVA, and mean difference were assessed using Tukey's test at $p < 0.05$ in OriginPro 2025 (OriginLab Corp., Northampton, MA, USA).

3. Results and Discussion

3.1. Experimental Outcomes and Model Adequacy

The experimental data were obtained using a three-factor BBD comprising 15 experimental runs, including three replicate trials to assess pure error and confirm repeatability (Table 2).

Table 2. Experimental design matrix and response for phytochemical contents and antioxidant activities of *Syzygium cumini* leaf extract.

Runs	Independent variables			Responses				
	MP (W)	ET (min)	PS (µm)	Phytochemicals			Antioxidant activities	
	X_1	X_2	X_3	TPC (mg GAE/g ds)	TFC (mg RE/g ds)	TTC (mg TAE/g ds)	DPPH (mM TE/g ds)	ABTS (mM TE/g ds)
1	630	20	100	279.01	65.00	78.43	4.93	5.03
2	360	14	100	261.77	59.05	80.14	4.77	4.69
3	360	14	500	230.22	40.82	64.76	3.72	4.09
4	630	20	500	270.54	55.87	70.01	4.27	5.16
5 ^a	630	14	300	283.25	61.06	78.69	5.01	5.61
6	900	14	100	297.63	64.74	83.35	5.23	5.41
7	900	14	500	275.20	63.97	83.15	4.96	5.21
8 ^a	630	14	300	281.38	62.06	81.36	4.96	5.41
9	360	8	300	238.14	49.79	67.73	4.18	4.23
10 ^a	630	14	300	282.33	62.03	79.57	4.99	5.56
11	360	20	300	243.82	49.48	73.89	4.15	4.31
12	900	20	300	304.78	70.38	81.50	5.42	5.72
13	900	8	300	261.38	53.87	77.34	4.60	4.62
14	630	8	100	262.52	56.87	73.25	4.32	4.92
15	630	8	500	233.89	50.48	65.53	3.81	3.88

^aCentral points (used to determine the experimental error).

MP-Microwave power, ET-Extraction time, PS-Particle size, TPC-total phenolic content, TFC-total flavonoid content, TTC-total tannin content, DPPH-1,1 diphenyl 2 picrylhydrazyl, and ABTS-2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid)

The wide response ranges observed throughout the experimental domain suggest that the measured variables are strongly influenced by the selected MAAE operating conditions. TPC ranged from 230.22 to 304.78 mg GAE per g ds, TFC from 40.82 to 70.38 mg RE per g ds, and TTC from 64.76 to 83.35 mg TAE per g ds. Antioxidant activities varied from 3.72 to 5.42 mM TE per g ds for DPPH and from 3.88 to 5.72 mM TE per g ds for ABTS. The highest overall phytochemical and antioxidant outputs were obtained in run 12 (900 W, 20 min, and 300 μ m), yielding the maximum TPC, TFC, DPPH and ABTS. In addition, TTC reached its maximum in run 6 (900 W, 14 min, and 100 μ m), indicating that tannin recovery exhibited a dis-

tinct response pattern relative to the other endpoints. The lowest response levels were generally associated with coarser particles and short extraction time. Run 3 at 360 W, 14 min, and 500 μ m gave the minimum TPC, TFC, and TTC, while run 15 at 630 W, 8 min, and 500 μ m produced the minimum ABTS and near minimum DPPH. These trends indicate that, within the studied domain, extraction performance depended on both sufficient process energy and mass transfer accessibility, and that optima were response dependent. Consequently, the experimental data were subjected to hierarchical polynomial modeling, and model adequacy was assessed using *p* values, lack-of-fit tests, and predictive metrics (Table 3).

Table 3. Model adequacy summary.

Source	Model p-value	Lack of Fit p-value	Adjusted R ²	Predicted R ²	Remarks
TPC					
Linear	0.0004	0.0054	0.7442	0.6664	
2FI	0.3345	0.0054	0.7646	0.6905	
Quadratic	0.0007	0.0635	0.9841	0.9125	Suggested
Cubic	0.0635		0.9983		Aliased
TFC					
Linear	0.0015	0.0133	0.6692	0.5014	
2FI	0.0218	0.0277	0.8552	0.8037	
Quadratic	0.0027	0.1906	0.9836	0.9168	Suggested
Cubic	0.1906		0.9946		Aliased
TTC					
Linear	0.0075	0.0818	0.5531	0.3378	
2FI	0.3925	0.0782	0.5686	0.0651	
Quadratic	0.0009	0.7449	0.9691	0.9141	Suggested
Cubic	0.7449		0.9538		Aliased
DPPH					
Linear	0.0029	0.0052	0.626	0.4772	
2FI	0.3752	0.005	0.6438	0.3643	
Quadratic	< 0.0001	0.1336	0.9896	0.9453	Suggested
Cubic	0.1336		0.9976		Aliased
ABTS					
Linear	0.0195	0.0462	0.4637	0.3224	
2FI	0.3711	0.0447	0.491	0.3963	
Quadratic	0.0001	0.8749	0.9836	0.9666	Suggested
Cubic	0.8749		0.9692		Aliased

TPC-total phenolic content, TFC-total flavonoid content, TTC-total tannin content, DPPH-1,1 diphenyl 2 picrylhydrazyl, ABTS-2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid), and 2FI- two factor interaction

Across all responses, the fitted quadratic model was statistically significant, with sequential p values spanning 0.0027 to below 0.0001, indicating that incorporating quadratic terms improve the fit to the experimental data beyond what linear and two-factor interaction models could achieve. Across all responses, the sequential p -values for the quadratic model ranged from 0.0027 to 0.0001. This indicates that inclusion of second-order terms significantly improved model adequacy and captured curvature that was not explained by the linear or two-factor interaction models [20]. Although simpler models showed statistical significance, omission of curvature terms can result in systematic lack of fit when the underlying response surface is nonlinear [15, 21]. These findings therefore support the selection of the quadratic model. Moreover, the quadratic models also provided the best balance between goodness of fit and predictive ability [15]. Adjusted R^2 values ranged from 0.969 to 0.990, while predicted R^2 values varied between 0.915 and 0.967, indicating that most of the response variability was explained within the studied factor space [22]. The differences between adjusted and predicted R^2 were small, ranging from 0.013 to 0.057, which reflects stable predictive performance across the experimental domain [21]. In addition, lack-of-fit tests were not significant for any quadratic model, with p -values between 0.064 and 0.875. This indicates the absence of systematic deviation between experimental observations and model predictions [23]. By comparison, linear and

two-factor interaction models generally exhibited lower adjusted R^2 values and showed significant lack of fit for several responses. These results demonstrate that simpler models were unable to adequately describe the experimental trends. Although cubic models occasionally yielded slightly higher adjusted R^2 values, they were aliased within the BBD. This was due to an insufficient number and distribution of experimental points to uniquely estimate all higher-order terms [24]. Under such conditions, some cubic effects become confounded and their coefficients cannot be meaningfully interpreted. Based on these considerations, the quadratic model was retained for response surface analysis and optimization of microwave-assisted aqueous extraction of *Syzygium cumini*.

3.2. Impact of Microwave-Assisted Aqueous Extraction Parameters on Phytochemical Content

3.2.1. Impact on total Phenol Content

The quadratic model for TPC was significant ($p < 0.0001$) and demonstrated satisfactory adequacy, with a non-significant lack of fit ($p > 0.05$) and low residual error (SD 2.86; CV 1.07%). The high adjusted R^2 (0.98), predicted R^2 (0.91), and adequate precision (31.27) confirm that the fitted surface captures TPC variation across the experimental domain (Table 4).

Table 4. Analysis of variance and regression coefficients of the quadratic model for phytochemical and antioxidant responses of *Syzygium cumini* leaf extract.

Source	DF	TPC (mg GAE/g ds)		TFC (mg RE/g ds)		TTC (mg TAE/g ds)		DPPH (mM TE/g ds)		ABTS (mM TE/g ds)	
		CE	p-Value	CE	p-Value	CE	p-Value	CE	p-Value	CE	p-Value
Model	9	282.32	< 0.0001***	61.72	< 0.0001***	79.87	0.0002**	4.99	< 0.0001***	5.53	< 0.0001***
<i>Linear</i>											
X_1 (MP)	1	20.63	< 0.0001***	6.73	< 0.0001***	4.85	< 0.0001***	0.42	< 0.0001***	0.46	< 0.0001***
X_2 (ET)	1	12.78	< 0.0001***	3.71	0.0001**	2.50	0.0014*	0.23	< 0.0001***	0.32	< 0.0001***
X_3 (PS)	1	-11.38	< 0.0001***	-4.32	< 0.0001***	-3.96	0.0002**	-0.31	< 0.0001***	-0.21	0.0005**
<i>Interaction</i>											
X_1X_2	1	9.43	0.0012*	4.21	0.0004**	-0.50	0.4101	0.21	0.0005**	0.26	0.0011*
X_1X_3	1	2.28	0.1715	4.37	0.0003**	3.79	0.0010*	0.20	0.0007**	0.10	0.0465*
X_2X_3	1	5.04	0.0168*	-0.69	0.2258	-0.18	0.7659	-0.04	0.2141	0.29	0.0006**
<i>Quadratic</i>											
X_1^2	1	-7.79	0.0034*	-2.87	0.0026*	0.64	0.3173	-0.03	0.3121	-0.35	0.0003**

Source	DF	TPC (mg GAE/g ds)		TFC (mg RE/g ds)		TTC (mg TAE/g ds)		DPPH (mM TE/g ds)		ABTS (mM TE/g ds)	
		CE	p-Value	CE	p-Value	CE	p-Value	CE	p-Value	CE	p-Value
X_2^2	1	-12.50	0.0004**	-2.96	0.0022*	-5.40	0.0002**	-0.37	< 0.0001***	-0.45	< 0.0001***
X_3^2	1	-8.33	0.0025*	-1.70	0.0217*	-2.67	0.0058*	-0.29	0.0001**	-0.32	0.0004**
Residual	5										
Lack of fit	3		0.0635		0.1906		0.7449		0.1336		0.8749
SD		2.86		0.99		1.11		0.05		0.08	
CV %		1.07		1.72		1.47		1.14		1.54	
Adj-R ²		0.98		0.98		0.97		0.99		0.98	
Pred-R ²		0.91		0.92		0.91		0.95		0.97	
AP		31.27		35.54		19.41		39.88		29.50	

Total phenolic content (TPC), Total flavonoid content (TFC), Total tannin content (TTC), 1,1 diphenyl 2 picrylhydrazyl (DPPH), 2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid) (ABTS), CE (coefficient estimate), SS (sum of squares), DF (degree of freedom), Standard deviation (SD), Coefficient of variation (CV), Adjusted R-squared (Adj-R²), Predicted R-squared (Pred-R²), Adequate precision (AP), Microwave power (MP), Extraction time (ET), and Particle size (PS). Asterisks (*, ** and ***) indicate 5%, 0.1% and 0.01% significant levels, respectively.

MP and ET exerted significant positive linear effects on TPC (both $p < 0.0001$), whereas PS had a significant negative effect ($p < 0.0001$) (Table 4). These directions indicate that increasing energy delivery and contact time improves the release of the broad phenolic pool, primarily by accelerating internal heating of the hydrated matrix, promoting microstructural opening, and sustaining concentration gradients that drive diffusion [12]. The negative PS effect reflects reduced geometric resistance at smaller sizes, where surface area is higher and diffusion paths are shorter, so phenolics that are distributed across vacuolar, cytosolic, and cell wall associated domains can transfer more efficiently into the aqueous phase [25]. Curvature was evidenced by significant negative quadratic terms for X_1^2 ($p < 0.05$), X_2^2 ($p < 0.001$), and X_3^2 ($p < 0.05$) (Table 4). These terms show diminishing returns as the factors approach their upper levels. This trend is consistent with depletion of the readily extractable phenolic pool, together with increasing losses from oxidative conversion, renewed association with insoluble matrix fractions, and greater thermal heterogeneity at high energy input [11]. Very fine particles can also increase slurry packing and effective viscosity, which can reduce local solvent renewal and limit additional release despite larger nominal area [26]. Interaction analysis

clarifies how these effects combine. The positive $X_1 * X_2$ term ($p < 0.05$) indicates synergy, where longer residence time allows microwave induced disruption to translate into greater net transfer before curvature dominates, as reflected by the rising surface in Figure 1a. The positive $X_2 * X_3$ interaction ($p < 0.05$) indicates that extended time partly compensates for coarser particles by allowing diffusion to proceed further in larger fragments [27], as reflected by the response surface pattern in Figure 1b. In contrast, the $X_1 * X_3$ interaction was not significant ($p > 0.05$), suggesting that, within the tested window, the positive power effect on TPC is broadly retained across particle sizes (Table 4).

3.2.2. Impact on Total Flavonoid Content

The quadratic model for TFC was highly significant ($p < 0.0001$) and showed adequate fit, as supported by a non-significant lack of fit ($p > 0.05$), low residual dispersion (SD of 0.99; CV of 1.72%), and strong adequacy metrics (adjusted R² of 0.98, predicted R² of 0.92, adequate precision of 35.54) (Table 4). At the linear level, MP increased TFC ($p < 0.0001$) and ET also increased TFC ($p < 0.001$), while PS decreased TFC ($p < 0.0001$) (Table 4).

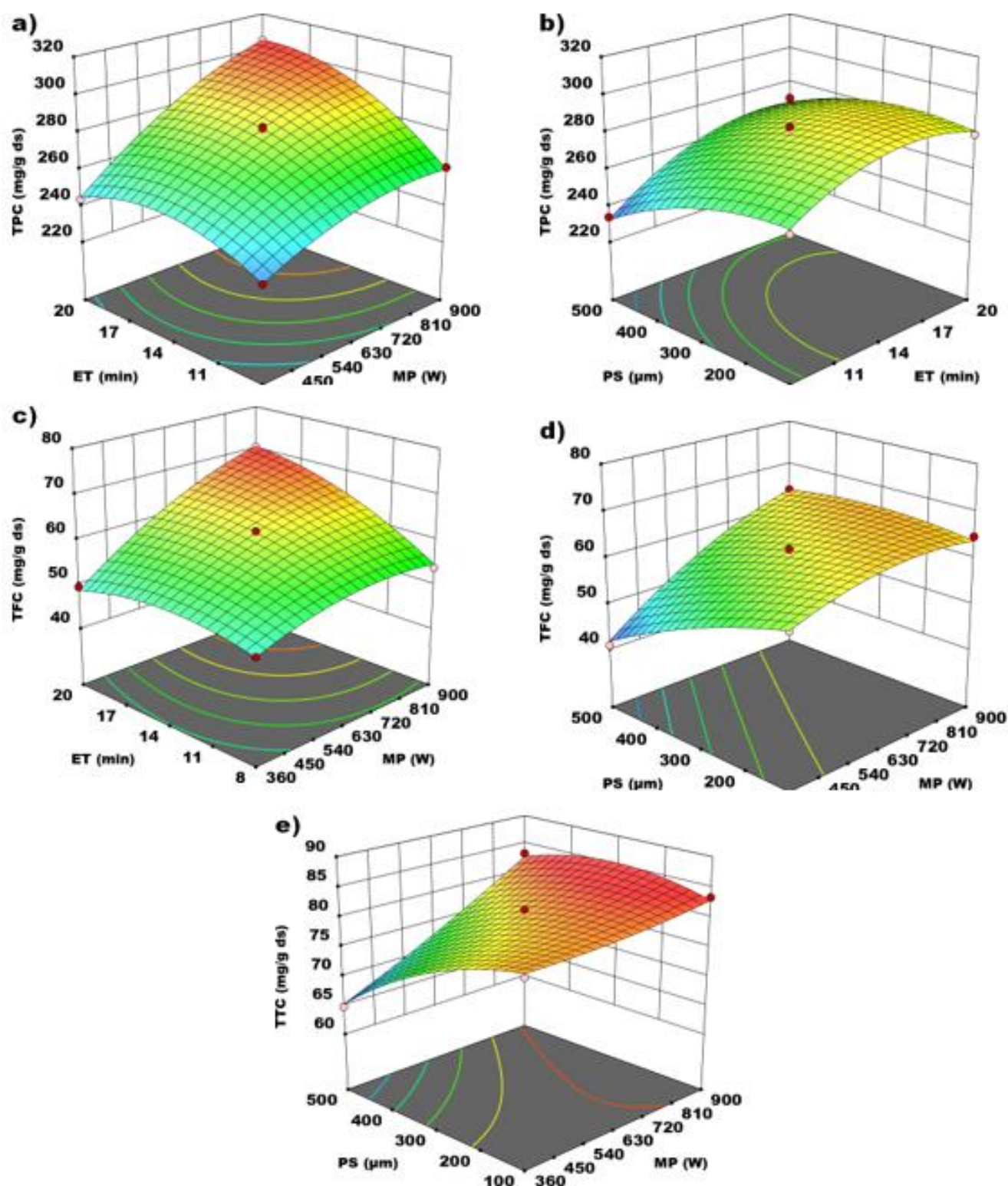


Figure 1. 3D surface plots for the interactive effect of microwave power, extraction time and particle size on the phytochemicals of *Syzygium cumini* leaf extract.

(a) TPC-Total phenolic content as function of MP-microwave power and ET-extraction time, (b) TPC-Total phenolic content as function of ET-extraction time and PS-particle size, (c) TFC-Total flavonoid content as function of MP-microwave power and ET-extraction time, (d) TFC-Total flavonoid content as function of MP-microwave power and PS-particle size, and (e) TTC-Total tannin content as function of MP-microwave power and PS-particle size.

Although flavonoids are included in the total phenolic pool, their response is often more constrained by localization and

molecular architecture [28]. Many phenolics occur as glycosides or esterified forms. They can also be compartmentalized or weakly associated with cell wall components. As a result, recovery depends on diffusion time and on the extent of microstructural disruption that opens access pathways and releases bound forms [29]. The positive power effect therefore reflects the role of energy density in weakening physical barriers and improving solvent access to flavonoid rich domains [12]. In contrast, the negative PS effect indicates that larger particles increase intraparticle resistance, which restricts mass transfer and limits flavonoid release [30]. Furthermore, the significant negative quadratic terms for X_1^2 , X_2^2 , and X_3^2 (all $p < 0.05$) indicate pronounced curvature and earlier saturation of TFC relative to TPC (Table 4). This behavior reflects a balance between improved release and increasing loss or conversion of structurally sensitive flavonoids as conditions become more severe [31]. Oxidation and heat driven rearrangements can lower the fraction that remains reactive in the colorimetric assay [31]. Interaction terms further differentiate the process controls. $X_1 * X_2$ and $X_1 * X_3$ were both positive and highly significant (both $p < 0.001$), whereas $X_2 * X_3$ was not significant ($p > 0.05$) (Table 4). The MP $\times$ ET synergy implies that time is required for the structural changes induced by microwave exposure to result in cumulative solute migration [32], as illustrated in Figure 1c. The significant MP $\times$ PS interaction indicates that the effect of power depends on particle size. Finer particles show a stronger response because shorter diffusion paths and smaller internal gradients allow released flavonoids to leave the matrix more readily [25], which is reflected by the response surface shape in Figure 1d. In contrast, the non-significant ET $\times$ PS term suggests that extending time alone is insufficient to overcome particle size constraints for flavonoids [30], which supports the interpretation that accessibility created by energy input is the dominant limiting step for this response within the studied range.

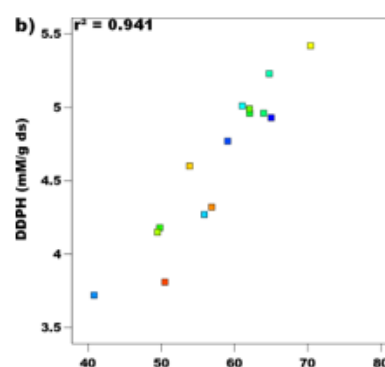
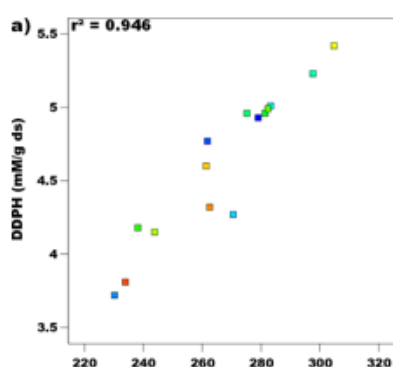
3.2.3. Impact on Total Tannin Content

The quadratic model for total tannin content was significant ($p < 0.001$) and adequate across the design space, as indicated

by a non-significant lack of fit ($p > 0.05$), low error (SD of 1.11; CV of 1.47%), and satisfactory predictive statistics (adjusted R^2 of 0.97, predicted R^2 of 0.91, adequate precision of 19.41) (Table 4). MP had a strong positive linear effect on TTC ($p < 0.0001$) and ET also increased TTC ($p < 0.05$), whereas PS decreased TTC ($p < 0.001$) (Table 4). This pattern is consistent with tannins being comparatively high molecular mass polyphenols with strong affinity for cell wall polysaccharides and proteins, so their release requires both effective matrix opening and sufficient time for desorption and diffusion [33]. The negative PS effect again reflects longer internal pathways and reduced solvent access in coarser material [25, 34]. Curvature, however, was not uniform across factors. The quadratic term for X_1^2 was not significant ($p > 0.05$), indicating an approximately linear power response within the tested range, whereas X_2^2 ($p < 0.0001$) and X_3^2 ($p < 0.001$) showed significant negative quadratic effects (Table 4). These latter terms indicate reduced marginal gains at very long extraction times and at extreme powder fineness. This can be attributed to competing processes, including tannin self-association, renewed complexation with insoluble macromolecules, and oxidative coupling that decreases the extractable fraction [35, 36]. Interaction effects were selective. $X_1 * X_2$ and $X_2 * X_3$ were not significant (both $p > 0.05$), whereas $X_1 * X_3$ was positive and significant ($p < 0.05$) (Table 4). This $X_1 * X_3$ effect indicates that higher MP is particularly beneficial when PS is larger, since stronger internal heating improves solvent penetration and promotes micro fracturing that reduce intraparticle resistance in coarse fragments [12, 37], as depicted by the response surface trend in Figure 1e. For finer particles, accessibility is already high, so increasing power yields smaller incremental gains and curvature with respect to power remains weak.

3.2.4. Impact on DPPH Radical Scavenging Activity

The quadratic model for DPPH was highly significant ($p < 0.0001$) with a non-significant lack of fit ($p > 0.05$), indicating satisfactory representation of the response within the tested factor space (Table 4).



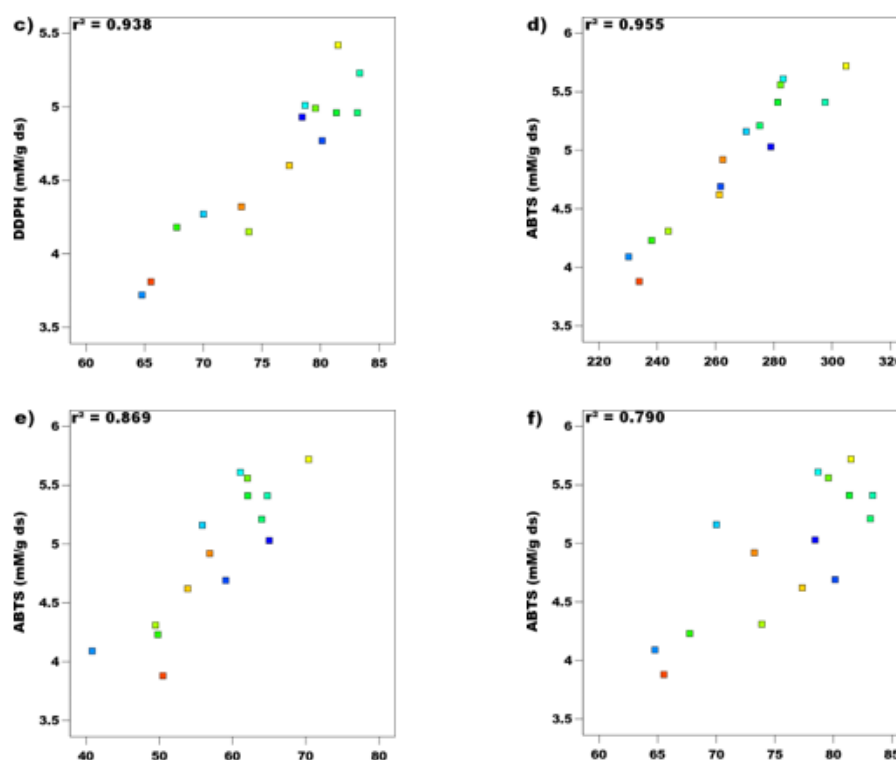


Figure 2. Pairwise relationships between antioxidant activity and phytochemicals *Syzygium cumini* leaf extract.

(a) DPPH-1,1 diphenyl 2 picrylhydrazyl vs TPC-Total phenolic content, (b) DPPH-1,1 diphenyl 2 picrylhydrazyl vs TFC-Total flavonoid content, (c) DPPH-1,1 diphenyl 2 picrylhydrazyl vs TTC-Total tannin content, (d) ABTS-2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid) vs TPC-Total phenolic content, (e) ABTS-2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid) vs TFC-Total flavonoid content, and (f) ABTS-2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid) vs TTC-Total tannin content.

Model dispersion was low, with SD of 0.05 and CV of 1.14%, and adequacy statistics were strong, with adjusted R^2 of 0.99, predicted R^2 of 0.95, and adequate precision of 39.88, supporting reliable interpretation of factor effects. Across the design space, DPPH was closely aligned with the phytochemical indices, showing strong relationships with TPC ($r^2 = 0.946$, Figure 2a), TFC ($r^2 = 0.941$, Figure 2b), and TTC ($r^2 = 0.938$, Figure 2c). These high r^2 values indicate that most of variation in DPPH can be attributable to changes in the extracted phenolic, flavonoid, and tannin pools [38, 39], highlighting that factor effects act mainly through phytochemical enrichment and preservation of redox-active structures [40]. All three linear terms were significant (Table 4), with MP (CE 0.42, $p < 0.0001$) and ET (CE 0.23, $p < 0.0001$) increasing DPPH, whereas PS (CE -0.31 , $p < 0.0001$) decreased it. These trends indicate that higher power and longer extraction promote accumulation of phytochemicals contributing to DPPH, whereas larger particles restrict recovery due to diffusion limitations [41, 42]. Interaction effects further highlight how variable combinations impact DPPH. Both $X_1 * X_2$ (CE = 0.21, $p < 0.001$) and $X_1 * X_3$ (CE = 0.20, $p < 0.001$) were significant, while $X_2 * X_3$ was not ($p > 0.05$) (Table 4). The MP $\times$ ET interaction, illustrated in Figure 3a, shows that longer ET amplifies the effect of MP by allowing cumulative release and accumulation of DPPH-active solutes. The MP $\times$

PS interaction in Figure 3b, indicates that smaller particles increase the microwave response by reducing internal resistance and increasing exposure of antioxidant-rich domains, supporting greater transfer of the phytochemical pool [12, 25, 37]. Curvature was dominated by significant negative quadratic effects of X_2^2 (CE -0.37 , $p < 0.0001$) and X_3^2 (CE -0.29 , $p < 0.001$), whereas X_1^2 was not significant ($p > 0.05$) (Table 4). This suggests that the effect of MP increases across the range, but longer ET and smaller PS reach saturation [41], reflecting exhaustion of the easily extractable fraction, so each increment adds less DPPH-active phytochemical [43].

3.2.5. Impact on ABTS Radical Cation Scavenging Activity

The quadratic model for ABTS was highly significant ($p < 0.0001$) with a non-significant lack of fit ($p > 0.05$), confirming adequate model structure (Table 4). Residual variation remained low (SD = 0.08, CV = 1.54%), and model adequacy statistics indicated strong explanatory and predictive performance ($R^2 = 0.98$, predicted $R^2 = 0.97$, and adequate precision = 29.50). The ABTS response displayed a differentiated attribution pattern (Figure 2), with very strong correlation to TPC (Figure 2d, $r^2 = 0.955$), weaker correlation with TFC (Figure 2e, $r^2 = 0.869$), and the weakest with TTC (Figure 2f, $r^2 = 0.790$). This suggests that ABTS is primarily driven by

total phenolics, while flavonoids contribute partially and tannins account for a smaller fraction, supporting the view that ABTS changes mainly reflect conditions that enrich or deplete TPC [44]. All linear effects were significant (Table 4), with ABTS increasing under higher MP (CE = 0.46, $p < 0.0001$) and longer ET (CE = 0.32, $p < 0.0001$), and decreasing with larger PS (CE = -0.21, $p < 0.001$). As stated above the ABTS tracks TPC most closely (Figure 2d), and the positive MP and ET effects reflect net enrichment of the phenolic pool, while coarser particles limit its transfer to the liquid phase [45]. Interaction behavior was more developed for ABTS than for DPPH. $X_1 * X_2$ was significant and positive (CE 0.26, $p < 0.05$), $X_1 * X_3$ was also significant (CE 0.10, $p < 0.05$), and $X_2 * X_3$ was strongly positive (CE 0.29, $p < 0.001$) (Table 4). These interactions indicate that ABTS benefits most when MP and ET are increased together, and when diffusion

constraints from PS are offset either by finer particles or longer ET [46]. The response surfaces in Figure 3c and 3d highlight these dependencies, showing that longer ET can partly compensate for larger PS, consistent with time-dependent enrichment of soluble phenolics [47]. All quadratic terms for ABTS were negative and significant, with X_1^2 (CE -0.35, $p < 0.001$), X_2^2 (CE -0.45, $p < 0.0001$), and X_3^2 (CE -0.32, $p < 0.001$) (Table 4), confirming pronounced curvature and indicates an interior optimum. Given the strong ABTS-TPC relationship in Figure 2d, this curvature indicates that at higher severity the marginal contribution of newly extracted phenolics to ABTS quenching decreases [46], while a larger fraction of the phenolic pool shifts toward less reactive forms [48], which together reduce incremental gains despite continued extraction [49].

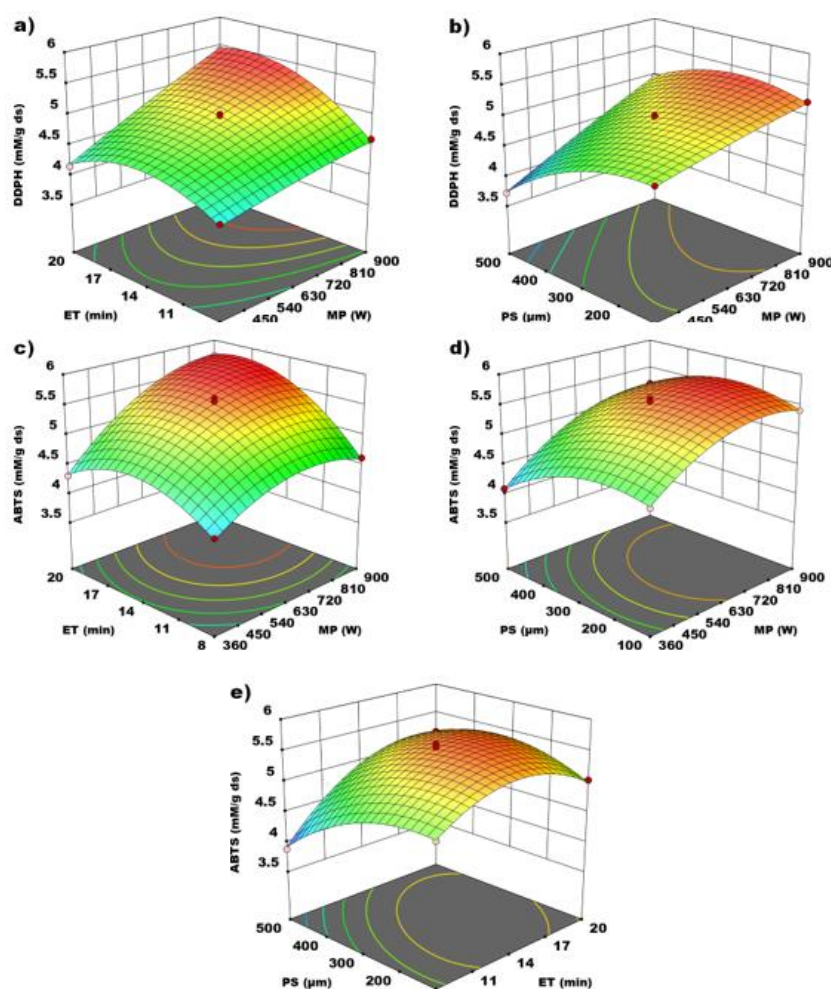


Figure 3. 3D surface plots for the interactive effect of microwave power, extraction time and particle size on the antioxidant activity of *Syzygium cumini* leaf extract.

(a) DPPH-1,1 diphenyl 2 picrylhydrazyl as function of MP-microwave power and of ET-extraction time, (a) DPPH-1,1 diphenyl 2 picrylhydrazyl as function of MP-microwave power and PS-particle size, (c) ABTS-2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid) as function of MP-microwave power and ET-extraction time, (d) ABTS-2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid) as function of MP-microwave power and PS-particle size, and ABTS-2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid) as function of ET-extraction time and PS-particle size.

3.3. Optimal MAAE Conditions for *Syzygium cumini* Phytochemicals and Antioxidants

Desirability-based optimization was performed by prioritizing TPC, TFC, and antioxidant activities (DPPH and ABTS) with the highest importance, while TTC was assigned moderate importance to balance bioactivity with potential antinutritional effects [50, 51]. The model predicted optimum conditions at MP 900 W, ET 18.94 min, and PS 276.54 μm , with predicted responses of TPC 305.44 mg GAE/g ds, TFC 70.12 mg RE/g ds, TTC 83.35 mg TAE/g ds, DPPH 5.51 mM TE/g ds, and ABTS 5.78 mM TE/g ds. For practical validation, ET and PS were rounded to 20 min and 273 μm , respectively, while maintaining MP at 900 W, in accordance with equipment setting resolution. Experimental results closely matched the predicted values, and at the 95% confidence level the experimental means fell within the model prediction intervals, confirming the accuracy of the quadratic model. The measured TPC, TFC, and TTC were 305.55 ± 0.07 mg GAE/g ds, 70.54 ± 0.05 mg RE/g ds, and 81.99 ± 0.03 mg TAE/g ds, respectively, while DPPH and ABTS reached 5.47 ± 0.03 mM TE/g ds and 5.74 ± 0.04 mM TE/g ds. These results indicate that the optimized conditions significantly improve both extractable phytochemical content and antioxidant capacity, thereby supporting the predictive reliability of the model. Overall, the selected operating window provides high phytochemical yield and strong antioxidant activity, thereby confirming the robustness of the optimization outcome. From an engineering standpoint, the optimum reflects a combination of high MP, moderate ET, and intermediate PS. High power ensures rapid volumetric heating and efficient matrix disruption, while the moderate extraction time allows sufficient diffusion of solubilized compounds without excessive thermal degradation [37, 46]. The intermediate PS increases the exposed surface area compared with coarser particles but avoids overly fine powders that could compact and hinder solvent penetration [25, 52]. Taken together, these conditions favor maximal recovery of bioactive compounds while preserving their redox-active properties, in agreement with the observed TPC–TFC–antioxidant trends.

4. Conclusion

This work applied response surface methodology to engineer a food grade microwave assisted aqueous extraction of *Syzygium cumini* leaf powder. The goal was to quantify how microwave power, extraction time, and particle size control phytochemical yield and antioxidant capacity, then identify an operating window that balances these targets. Across the design space, the three phytochemical indices showed a shared transport pattern. Longer extraction time increased recovery, while coarser material lowered it due to stronger intraparticle resistance and longer diffusion paths. Total phenolics behaved

as a broad pool that responds strongly to energy input and contact time. Gains then weakened near the upper settings as the readily extractable fraction becomes progressively depleted and quality losses become more influential. Total flavonoids showed earlier saturation and stronger curvature, which is consistent with a more sensitive fraction that is more easily altered during severe processing. Total tannins were more dependent on time and on how power couples with particle size. This agrees with their stronger affinity for macromolecular components and their slower release from the plant matrix. Antioxidant capacity followed the same processing levers. DPPH and ABTS increased with higher power and longer time, and decreased with coarser particles. DPPH tracked the phytochemical indices closely and was strengthened when power was paired with sufficient time and reduced internal resistance. ABTS showed a more selective weighting toward total phenolics and a richer interaction structure. This indicates that the largest gains require coordinated increases in energy delivery and contact time while diffusion constraints are reduced or offset. Multi response desirability optimization prioritized total phenolics, total flavonoids, and both antioxidant assays, while giving total tannins moderate importance to maintain bioactivity without pushing the extract toward excessive tannin load. The model predicted an optimum at 900 W, 18.94 min, and 276.54 μm . Validation at 900 W, 20 min, and 273 μm gave experimental responses that fell within the model prediction intervals at the 95 percent confidence level. This agreement demonstrates a robust operating window for scaled food processing, where energy input and particle engineering are tuned together to maximize extract potency while preserving antioxidant quality.

Abbreviations

MP	Microwave Power
ET	Extraction Time
PS	Particle Size
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
TTC	Total Tannin Content
DPPH	1,1 Diphenyl 2 Picrylhydrazyl
ABTS	2,2 Azino bis (3 Ethylbenzothiazoline 6 Sulfonic Acid)

Author Contributions

William Tchabo: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft

Germaine Yadang: Formal Analysis, Investigation, Writing – review & editing

Spéro Ulrich Koba Edikou: Visualization, Writing – review & editing

Ibrahima Kaba: Formal Analysis, Visualization

Sékou Kouyaté: Data curation, Software

Durand Dah-Nouvlessounon: Methodology, Writing – review & editing

Joseph Dossou: Conceptualization, Supervision

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sector.

Data Availability Statement

The data is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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