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Keywords: Enzyme-ultrasound extraction; bioactive compound; antioxidant; anti-inflammatory; antidiabetic



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## Article

# Bioactivity Enhancement of *Curcuma longa* Extract via Optimized Enzyme-Assisted Ultrasonic Extraction

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**Abstract:** To enhance the levels of bioactive compounds in turmeric extract, we optimized the enzyme-assisted ultrasonication extraction conditions employing a Central Composite Design within Response Surface Methodology. By varying the  $\alpha$ -amylase enzyme concentration (1.5%, 5%, or 8.5%) and ultrasonication time (10, 20, or 100 min), the conditions were optimized based on the levels of the response variables (total phenolic and flavonoid content, 2,2-diphenyl-1-picrylhydrazyl [DPPH] radical scavenging activity, and Trolox equivalent antioxidant capacity [TEAC]). The optimal conditions, comprising 8.14% enzyme concentration and 100 min of ultrasonication, achieved total phenolic content of 184.2 mg GAE/g, total flavonoid content of 197.1 mg QE/g, DPPH radical scavenging activity of 96.9 mM TE/g, and TEAC of 84.7 mM TE/g. Following treatment with the optimized extract, lipopolysaccharide-induced nitric oxide production as well as cell viability were evaluated. The results showed a NO inhibition rate over 90% at the optimized extract of 200  $\mu$ g/mL, with minimal impact on cell survival. The optimized extract exhibited greater antidiabetic potential in the  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition assays and the greater inhibition of angiotensin-converting enzyme than the water- and solvent-derived extracts. These findings provide insight into the use of optimized extraction methods to extract higher levels of bioactive compounds from turmeric.

**Keywords:** Enzyme-ultrasound extraction; bioactive compound; antioxidant; anti-inflammatory; antidiabetic.

## 1. Introduction

Turmeric (*Curcuma longa*), a resilient perennial herb indigenous to India, is used as a spice, traditional herb, and food additive [1]. The turmeric rhizome contains major bioactives with anti-inflammatory potential [2]. These include curcuminoid compounds, which comprise primary polyphenols such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin [3]. Given its anti-inflammatory properties, curcumin offers therapeutic potential for treating inflammatory diseases, thus supporting a holistic health-management strategy [4]. The curcuminoids in turmeric, and curcumin in particular, are widely reported to have antidiabetic potential, via multiple mechanisms [31,32]. In diabetic animals, curcumin supplementation reduces levels of blood glucose and glycosylated hemoglobin and of the anti-inflammatory markers such as IL-6, TNF- $\alpha$ , and MCP-1 in diabetic animals [33]. The administration of large doses of curcumin or turmeric reduces blood pressure by promoting eNOS production, boosting antioxidant capacity by restoring glutathione levels, and reducing excess reactive oxygen species levels [34]. Curcumin and turmeric extract enhance nitric oxide bioavailability, promote endothelial vasorelaxation, resulting in the production of antioxidant enzymes [35].

The selection of effective extraction methods is central in preserving the bioactivity of the extract [5]. Common extraction techniques such as maceration, microwave-assisted extraction, and Soxhlet extraction exhibit various drawbacks, including low extraction yields, prolonged processing periods, excessive solvent consumption, and the need to use high temperatures; therefore, better extraction technology that is more economical and environmentally sustainable is required [6]. Enzyme-assisted extraction utilizes catalysts to break down the cellular matrix and release metabolites [7]. Combining enzymatic extraction and ultrasonication offers synergistic benefits in terms of green extraction technology [8]. Using this approach, extraction can be accelerated, enhancing the release of bioactives and providing a cost-effective and ecofriendly method for improving extraction efficiency [9]. Optimization of extraction conditions (such as enzyme concentration and ultrasonication time) based on experimental results would be essential to meet this target.

To address this need, we extracted turmeric bioactives using an enzyme-assisted ultrasonication extraction method. Response surface methodology (RSM) was applied to optimize the conditions with respect to the levels of the bioactives extracted and on their antioxidant capacities. The optimized extract was further analyzed on cytotoxicity, NO production, as well as their anti-inflammatory activity and antidiabetic potential.

2. Materials and Methods

2.1. Materials

Turmeric rhizome was acquired from a local market in Jindo (Republic of Korea). Chemicals including catechin, gallic acid, Griess reagent,  $\alpha$ -amylase enzyme (30 U/mg),  $\alpha$ -glucosidase, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Folin-Ciocalteu's phenol, acetic acid, and aluminum chloride, were procured from Sigma-Aldrich (St. Louis, MO, USA). Biological materials such as fetal bovine serum, penicillin/streptomycin, and Minimum Essential Medium were sourced from Welgene (Gyeongsan, Republic of Korea).

2.2. Optimization Process

2.2.1. Enzyme-Assisted Ultrasonication

Enzymatic extraction was performed as previously described [10], with some modification. Turmeric powder (10 g) was mixed with 65 mL of distilled water and 25 mL of 0.2 M sodium acetate buffer (pH 5). The mixture was treated with different concentrations of  $\alpha$ -amylase enzyme (10 U/mL), generated from RSM-CCD design, followed by incubation for 2 h at 35 °C. After incubation, the mixture was centrifuged at 3000  $\times$ g for 15 min. To facilitate enzyme extraction and deactivation, the resulting supernatant was incubated in a water bath at 65 °C for 2 h, followed by filtration and drying in oven at 80 °C for 16 h. The resulting enzyme-treated powder (5 g) was dissolved in 100 mL of 80% ethanol and subjected to ultrasonic treatment using an ultrasonic cleaner (UC-20, JeioTech, Republic of Korea) for different sonication times, according to the RSM design. The sonicated mixture was then incubated with shaking at 130 rpm for 2 h at 35 °C, then centrifuged at 3000  $\times$ g for 15 min. After rotary evaporating the solvent under vacuum at 40 °C, the concentrated powder obtained was reconstituted in 10 mL of distilled water for further analysis.

2.2.2. Optimization via RSM

RSM modeling was performed using Design Expert 8.0.6 (Stat-Ease, Minneapolis, MN, USA). Enzyme concentration ( $X_1$ : 1.5%, 5%, and 8.5%) and ultrasonication time ( $X_2$ : 10, 20, and 100 min) were used as the independent variables to optimize extract bioactivity, in terms of total flavonoid content (TFC) and total phenolic content (TPC) as well as Trolox equivalent antioxidant capacity (TEAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity [11]. The variables were ranged based on prior findings [12]. The levels of the independent variables were coded -1, 0, or 1 (low, medium, and high, respectively) (Table 1).

Table 1. Independent variables and their coded values used in RSM-CCD design.

Independent variables	Symbol	Coded levels		
		-1	0	+1
Enzyme concentration (%)	X1	1.5	5	8.5

Ultrasonication time (min)	X2	10	20	100
Each analysis was performed in triplicate for 13 experimental extraction processes. The optimal extraction conditions were validated by examining the absolute residual error (%), which is based on the observed and predicted values, as follows:.				

Absolute residual error (%) = (Observed value – Predicted value / Observed value) × 100 (1)

2.3. Analysis

2.3.1. Bioactive Component Levels and Antioxidant Activity

Total phenolic content (TPC) was accessed using a previously established method [13]. Briefly, turmeric extract (20 µL), 500 µL of 10% (w/v) Folin–Ciocalteu reagent, and 20% sodium carbonate (w/v) were combined together. The solution was thoroughly mixed and incubated under the dark at 25±2°C for 2 h. Subsequently, absorbance was measured at 765 nm using a spectrophotometer (EMCLAB Instruments, Duisburg, Germany), using gallic acid as a standard.

Total flavonoid content (TFC) was determined using the colorimetric assay method [14]. Each sample extract (100 µL) was mixed with 75 µL of 5% sodium nitrite and 1.25 mL of distilled water. The mixture was stirred and allowed to react for 6 min at 25±2°C. Then, 150 µL of 10% aluminum chloride (w/v) was added and the solution was left to react for an additional 5 min. Subsequently, 0.5 mL of 1 mol/L sodium hydroxide was added to the mixture. Absorbance was determined at 510 nm using a spectrophotometer (EMCLAB Instruments), using quercetin as a standard.

The DPPH free radical scavenging activity of the extracted samples was examined via colorimetric method, as previously described [15]. Initially, 50 µL of the sample extract (w/v) was mixed with 1950 µL of 0.1 mM DPPH solution. The resulting mixture was vortexed to ensure thorough mixing, then incubated in the dark for 30 min at 25±2°C. After incubation, absorbance was detected at 517 nm using a spectrophotometer (EMCLAB Instruments), using Trolox as a standard.

The TEAC assay was conducted as previously described [13]. Initially, a solution of ABTS (7.4 mM) and potassium persulfate (2.6 mM) was prepared at an equal ratio and placed in the dark at 25±2°C for at least 16 h to enable formation of ABTS radical cations (ABTS<sup>+</sup>). After diluting with 100% methanol to achieve an absorbance of 0.7 ± 0.01 at 734 nm, 2960 µL of the diluted ABTS<sup>+</sup> solution was mixed with 40 µL of the extract. The reaction was done within 7 min and absorbance was read by a microplate reader (EMCLAB Instruments), using Trolox as a standard.

2.3.2. Bioactivity

2.3.2.1. Anti-Inflammatory Effects

RAW 264.7 cells were grown in Minimum Essential Medium (MEM), supplemented with 10% FBS and 1% penicillin/streptomycin in a humidified incubator at 37 °C with 5% CO<sub>2</sub>.

To determine the cytotoxicity of the extract and the concentration that maximizes cell viability, an MTT assay was conducted as previously described [16]. The MTT stock solution was prepared using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide diluted with PBS (5 mg/mL) and was filtered through a 0.2 µm membrane filter. The RAW 264.7 cells were seeded at a density of 2 × 10<sup>4</sup> cells/well and incubated for 24 h at 37 °C. After incubation, the culture medium was removed, and the cells were treated with varying concentrations of the turmeric extract. MTT stock solution (10%, 100 µL) was then added to the cells and was removed by suction after 4 h. The remaining formazan crystals were dissolved in 100 µL of dimethylsulfoxide, and absorbance was determined at 595 nm using an EPOCH 2 microplate reader (Biotek, Winooski, VT, USA). Absorbance is presented relative to that of the control group.

For nitric oxide analysis, raw 264.7 cells were cultured in MEM containing 10% FBS at 37 °C in a 48-well plate at 1 × 10<sup>5</sup> cells/well in an incubator with 5% CO<sub>2</sub>. The nitric oxide assay was conducted as previously described [17], with slight modifications. The cell culture medium was then treated with various concentrations of the optimized extract along with 1 µg/mL lipopolysaccharide (LPS) for 24 h. After incubation, 50 µL of the supernatant from each well was mixed with an equal volume of Griess reagent and allowed to react for 10 min. Absorbance was examined at 540 nm using a



microplate reader (EMCLAB). Sodium nitrate was utilized as a standard to determine the nitric oxide content of each sample.

### 2.3.2.2. Antidiabetic Effects

The antidiabetic potential of the optimized extract was accessed by comparing those of the ethanol- and water-derived extracts by analyzing their inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase activity [18]. First, a mixture of 50  $\mu$ L of the  $\alpha$ -amylase (25U/mL) and 50  $\mu$ L of each extract were placed in a 96-well plate and were kept at 25 °C for 10 min. Next, 1% starch solution (50  $\mu$ L) in 0.02 M sodium phosphate buffer (pH 6.9), was combined to the mixture and incubated for 10 min at 25 °C. The reaction was stopped with 100  $\mu$ L of the dinitrosalicylic acid by steaming the plate for 5 min, and cooling for 20 min. The absorbance was then measured at 540 nm using an EPOCH 2 microplate reader (Biotek) for calculation of inhibition (%) using the following equation:

$$\text{Inhibition (\%)} = (\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}) / \text{Absorbance}_{\text{control}} \times 100 \quad (2)$$

To analyze  $\alpha$ -glucosidase inhibitory activity, a 96-well plate was prepared with 10  $\mu$ L of  $\alpha$ -glucosidase enzyme solution (0.5 U/mL), 20  $\mu$ L of the sample at various concentrations, and 50  $\mu$ L of phosphate buffer (50 mM, pH 6.8), followed by incubation for 5 min at 37 °C. Subsequently, 20  $\mu$ L of a 1 mM p-nitrophenyl- $\alpha$ -D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.9) was added, then the mixture was incubated for 30 min at 37 °C. After incubation, 50  $\mu$ L of 0.1 M sodium carbonate was added, absorbance was recorded at 405 nm, and inhibition was calculated as described in equation (2). The logit method was applied to obtain the IC<sub>50</sub> values of both  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory effect.

### 2.3.2.3. Angiotensin-I Converting Enzyme (ACE) Inhibition

ACE inhibition assays were conducted as previously described [19,20], with modifications. ACE enzyme at 25 mU/mL, from rabbit lung, was incubated with 8.3 mM hippuryl-L-histidyl-L-leucine (HHL) in a buffered solution (50 mM sodium borate containing 0.3 M NaCl, pH 8.3). Subsequently, 50  $\mu$ L of each extract was mixed with 50  $\mu$ L ACE solution, and the mixture was incubated for 10 min at 37 °C. Thereafter, 150  $\mu$ L of 8.3 mM HHL substrate solution was added to the mixture, which was again incubated for 30 min at 37 °C. The reaction was terminated by adding 250  $\mu$ L of 1.0 M HCl, followed by addition of 0.5 mL ethyl acetate for extraction. After centrifuging (4000  $\times g$ , 15 min at 25 °C), the concentration was carried out using a rotary evaporator 50 °C for 10 min. The resulting dried sample was dissolved in 1 mL of distilled water, and absorbance was determined at 228 nm using an EPOCH 2 microplate reader (Biotek).

### 2.4. Statistical Analysis

Each experiment was conducted in triplicate. All results are demonstrated as the mean  $\pm$  SD. ANOVA was performed to determine the significance of the differences between samples. GraphPad Prism 10.3.1 (GraphPad, San Diego, CA, USA) and XLSTAT 2021.2 (Addinsoft, Paris, France) were applied.

## 3. Results and Discussion

### 3.1. RSM Design Matrix and Model Fitting

To optimize the extraction conditions, model fitting was performed using the relevant model-response parameters, namely sum of squares, df, mean squares, the  $F$  and  $p$  values, standard deviation,  $R^2$ , adjusted  $R^2$ , and lack of fit (Table 2). A robust model is characterized by a significant  $F$ -value,  $p < 0.05$ , and an insignificant lack-of-fit value [21]. The fitted model was significant, with a high  $F$ -value; all of the response variables exhibited significance ( $p < 0.0001$ ).  $R^2$  and adequate precision are key metrics for accessing model fitness. The closer the  $R^2$  value is to 1, the more accurately the model fits. Here,  $R^2$  was greater than 0.98 in all cases, substantially exceeding the acceptable threshold of 0.8, thus revealing that the model is reliable and that the independent and response variables are strongly related [22]. The adequate precision for all variables exceeded the minimum acceptable ratio of 4, ranging from 24.53 to 78.21, depending on the response variable. This suggests a robust signal-to-

noise ratio and shows that the adjusted model possesses sufficient signal for navigating the design space [23].

Table 2. ANOVA results for the response variables.

Source	TPC	TFC	DPPH radical scavenging activity	TEAC
Sum of squares (model)	12128.44	2625.34	3485.41	4402.28
Degrees of freedom	5	5	5	5
Mean squares (model)	2425.69	2625.34	697.08	880.46
F (model)	122.75	895.20	474.73	93.35
p (model)	<0.0001	<0.0001	<0.0001	<0.0001
Standard deviation	4.45	1.71	1.21	3.07
R <sup>2</sup>	0.9887	0.9984	0.9971	0.9852
Adequate precision	28.80	78.21	59.81	24.53
Lack of fit	51.12	11.33	6.28	38.82

TPC, total phenolic content; TFC, total flavonoid content; DPPH; 2,2-diphenyl-1-picrylhydrazyl; TEAC, Trolox equivalent antioxidant capacity.

3.2. Effects of Extraction Conditions on Bioactive Component Levels and Antioxidant Activity

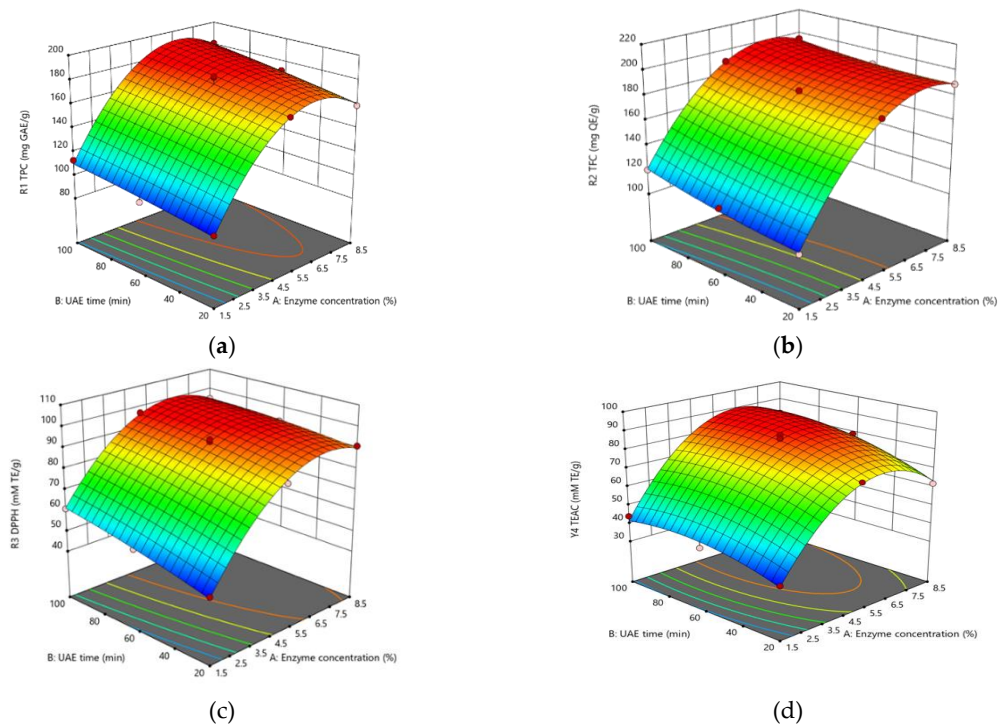
Depending on the enzyme concentration and ultrasonication times used, TPC ranged from 98.1 to 183.6 mg GAE/g, TFC from 102 to 195 mg QE/g, DPPH radical scavenging activity from 48.9 to 97.1 mM TE/g, and TEAC from 35.1 to 88.6 mM TE/g (Table 3). TPC and TFC increased with increasing enzyme concentrations and longer ultrasonication times (Figure 1a, b) [24]. The highest TPC (183.6 mg GAE/g) was achieved at 5% enzyme concentration with 60 min of ultrasonication, and the highest TFC (195.0 mg QE/g) at 8.5% enzyme concentration with 10 min of extraction time. These findings suggest that enzyme concentration and ultrasonication time substantially affect the extraction of turmeric bioactives, consistent with prior findings [25]. DPPH radical scavenging activity increased with increasing enzyme concentration and declining ultrasonication time (Figure 1c), with the greatest activity (97.1 mM TE/g DPPH) obtained at 5% enzyme concentration with 10 min of ultrasonication. The highest TEAC (88.6 mM TE/g) was described in the experimental run of X<sub>1</sub> (5%) and X<sub>2</sub> (60 min).

Table 3. Experimental determination of the effects of the independent variables on the response variables, based on the Central Composite Design.

Independent variables			Response Variables			
Run	X <sub>1</sub> (%)	X <sub>2</sub> (min)	TPC *** (mg GAE/g)	TFC *** (mg QE/g)	DPPH radical scavenging activity *** (mM TE/g)	TEAC *** (mM TE/g)
1	5	20	168.1 ± 0.4j	181.9 ± 0.3i	85.6 ± 0.7i	74.2 ± 1.3j
2	5	60	174.4 ± 0.3g	181.9 ± 0.3c	94.2 ± 0.1e	81.2 ± 0.7g
3	8.5	20	159.8 ± 0.2h	189.9 ± 0.5g	91.7 ± 0.1g	62.1 ± 0.2h
4	1.5	60	100.9 ± 0.4bcd	112.9 ± 0.5a	55.8 ± 0.2b	40.0 ± 0.3de
5	5	10	180.8 ± 0.6i	191.6 ± 0.3h	97.1 ± 0.2h	83.1 ± 1.6i
6	5	60	174.4 ± 0.3e	184.0 ± 0.4c	93.2 ± 0.1d	86.7 ± 0.7f
7	8.5	10	181.3 ± 0.6f	195.0 ± 0.7f	95.6 ± 0.7f	82.6 ± 0.7k
8	5	60	183.6 ± 0.7cd	182.1 ± 0.4b	94.9 ± 0.5a	86.7 ± 1.7d
9	8.5	60	172.4 ± 0.2e	189.2 ± 0.5e	93.2 ± 0.5c	79.5 ± 0.3e
10	1.5	20	113.3 ± 0.2d	120.4 ± 0.6d	61.2 ± 0.4d	44.9 ± 0.3b
11	5	60	174.4 ± 0.4ab	181.9 ± 0.2e	92.4 ± 0.4bc	86.4 ± 0.9c
12	5	60	182.8 ± 0.2abc	184.0 ± 0.6e	92.8 ± 0.4d	88.6 ± 0.2b
13	1.5	20	98.1 ± 0.4a	102.7 ± 0.7d	48.9 ± 0.5de	35.1 ± 0.5a

All values are presented as mean ± SD (n=3). \*\*\* *p* < 0.0001. Different small letters after the values within a column identify runs that were significantly different. X<sub>1</sub>, enzyme concentration; X<sub>2</sub> ultrasonication time; TPC,

Total phenolic content; TFC, total flavonoid content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TEAC, Trolox equivalent antioxidant capacity; TE, Trolox equivalent; GAE, gallic acid equivalent; QE, quercetin equivalent.



**Figure 1.** Response surface plots of the Response Surface Methodology Central Composite Design (RSM–CCD) analysis of turmeric bioactives extraction. (a) Total Phenolic Content (TPC), (b) Total Flavonoid Content (TFC), (c) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and (d) Trolox equivalent antioxidant capacity (TEAC), with respect to ultrasonication time and enzyme concentration.

3.3. Optimization of Extraction Conditions and Process Validation

The optimized extraction conditions, determined using the CCD method, were an 8.14% enzyme concentration with 100 min of ultrasonication. By aiming to maximize the response variables, the optimized conditions were obtained. The optimal extraction conditions were validated experimentally and the results were compared with the values predicted via RSM model (Table 4). The predicted values for the dependent variables were as follows: TPC at 184.2 mg GAE/g, TFC at 197.1 mg QE/g, DPPH radical scavenging activity at, 97.1 mM TE/g, and TEAC at 84.8 mM TE/g. The experimentally obtained values were 184.6 mg GAE/g for TPC, 198.2 mg QE/g for TFC, 97.1 mM TE/g for DPPH, and 84.7 mM TE/g for TEAC, all of which were consistent with the predicted values. The absolute residual errors for all variables were less than 1%, confirming the accuracy of the predictive model (Table 4).

**Table 4.** Validation of the predictive model.

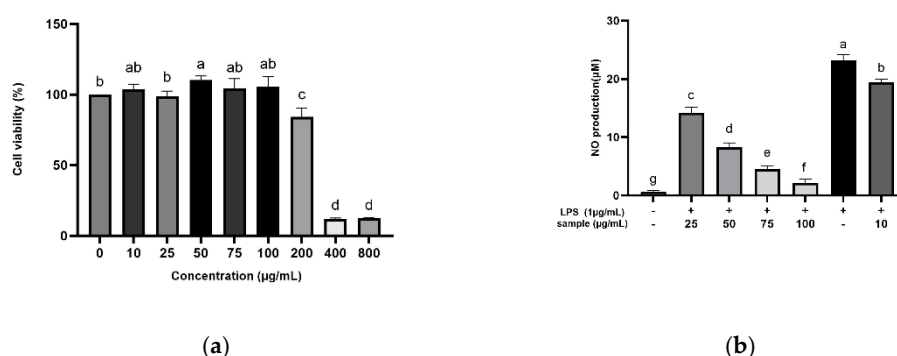
Response	Objective	Predicted value	Experimental value	Absolute residual error (%)
TPC (mg GAE/g)	Maximum	184.2	184.6 ± 0.1	0.20
TFC (mg QE/g)	Maximum	197.1	198.2 ± 0.1	0.50
DPPH radical scavenging activity (mM TE/g)	Maximum	96.9	97.1 ± 0.5	0.19
TEAC (Mm TE/g)	Maximum	84.7	84.8 ± 0.3	0.13

Results are presented as the mean ± SD (n=3). TPC, total phenolic content; TFC, total flavonoid content; DPPH; 2,2 diphenyl-1-picrylhydrazyl; TEAC; Trolox equivalent antioxidant capacity; TE, Trolox equivalent; GAE, gallic acid equivalent; QE, quercetin equivalent.

### 3.4. Bioactivity

#### 3.4.1. Anti-Inflammatory Potential

The anti-inflammatory potential of the optimized turmeric extract was investigated via MTT assay (Figure 2). At levels up to 100  $\mu\text{g/mL}$ , the optimized extract exhibited no cytotoxic effects on RAW 264.7 cell viability (Figure 2a), consistent with prior findings for curcumin and curcumin diglutamic acid [26]. These findings indicate that 100  $\mu\text{g/mL}$  is the maximum turmeric extract concentration that can be used without damaging cell viability and integrity. The optimized extract's inhibitory effect on LPS-induced nitric oxide production by RAW 264.7 cells was examined (Figure 2b). LPS-treated cells exhibited higher nitric oxide levels, peaking at 23  $\mu\text{M}$ , compared with 0.6  $\mu\text{M}$  in the control. Nitric oxide production was significantly lower at higher concentrations of the optimized extract, declining from 14  $\mu\text{M}$  nitric oxide at 25  $\mu\text{g/mL}$  of the extract to 2  $\mu\text{M}$  at 100  $\mu\text{g/mL}$ , consistent with prior findings [27].

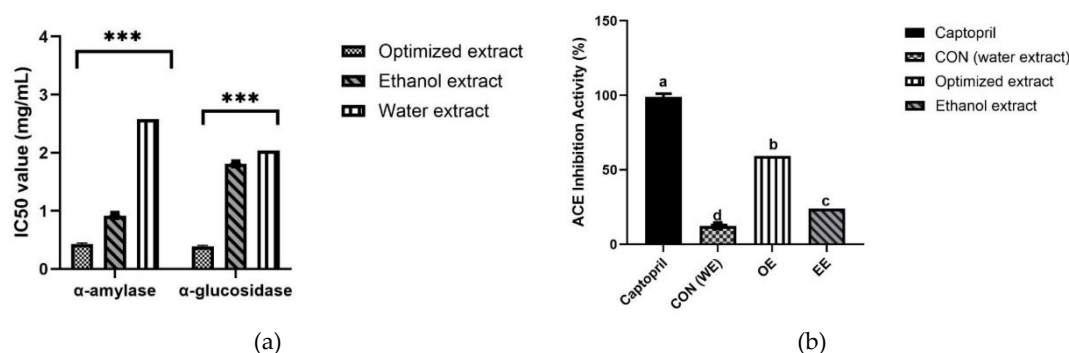


**Figure 2.** Effects of the optimized turmeric extract on Raw 264.7 cell viability (a) and on lipopolysaccharide (LPS)-induced nitric oxide (NO) production (b). Different small letters indicate significant differences (Duncan's test).

#### 3.4.2. Antidiabetic Potential

The antidiabetic potential of the turmeric extracts obtained using the optimized approach, using water, or using ethanol, was evaluated in terms of  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity (Figure 3a). For  $\alpha$ -amylase, the optimized extract achieved 76.3% inhibition ( $\text{IC}_{50}$ , 0.43  $\text{mg/mL}$ ), whereas the standard drug acarbose achieved 98.1% inhibition ( $\text{IC}_{50}$ , 0.2  $\text{mg/mL}$ ). The ethanol-derived extract achieved 58.3% inhibition ( $\text{IC}_{50}$ , 0.92  $\text{mg/mL}$ ), whereas the water-derived extract achieved the lowest inhibition, of 25.2%, with an  $\text{IC}_{50}$  of 2.58  $\text{mg/mL}$ .

The  $\alpha$ -glucosidase inhibition assay revealed similar results, with the optimized extract achieving the highest inhibition, of 78.9% ( $\text{IC}_{50}$ , 0.39  $\text{mg/mL}$ ); the ethanol-derived extract achieved 43.11% inhibition ( $\text{IC}_{50}$ , 1.81  $\text{mg/mL}$ ) and the water-derived extract achieved 28.07% inhibition ( $\text{IC}_{50}$ , 2.04  $\text{mg/mL}$ ).



**Figure 3.** Effects of the optimized turmeric extract on antidiabetic potential (a) and on angiotensin-I converting enzyme (ACE) inhibitory activity (b). CON, control. Different small letters indicate significant differences (Duncan's test); \*\*\*,  $p < 0.0001$ .



### 3.4.3. ACE-Inhibitory Potential

The ACE-inhibitory activity of the optimized extract, water-derived extract, and ethanol-derived extracts was compared with that of captopril, an ACE inhibitor (Figure 3b). The optimized extract achieved significantly greater inhibition (59.35%) than the ethanol- and water-derived extracts (24% and 12.41%, respectively). Captopril achieved the highest inhibition (98.81%), followed by the optimized extract (59.34%), the ethanol-derived extract (24.1%), and the water-derived extract (12.42%). The optimized extract achieved better inhibitory activity than the other extracts, indicating its superior antihypertensive potential.

### 3.5. Discussion

This study targeted to optimize turmeric extraction conditions, revealing that increasing the concentration of the extraction enzymes and the ultrasonication times improved the release of bioactives and the antioxidant capacity of the extract. These findings indicate that enzyme-assisted ultrasonic extraction, which increases the concentration and bioavailability of the active ingredients, and particularly of curcuminoids, may enhance the many benefits offered by curcumin and turmeric. Similarly, prior findings have suggested that extending the ultrasonication time improves the extraction efficiency of bioactives [28]. Longer ultrasonication times enhance plant cell wall breakdown and facilitate the release of flavonoids and phenolic compounds [23]. Similarly, higher enzyme concentrations promote cell wall degradation, making the target compounds more accessible and increasing their extraction yields. The combined application of enzymatic and ultrasonic extraction synergistically enhanced extraction efficiency, thus enhancing antioxidant capacity. By optimizing the  $\alpha$ -amylase concentration and ultrasonication time, this integrated approach offers a promising green extraction method for turmeric. Process validation revealed the effectiveness of the RSM approach and the optimal extraction conditions for maximizing bioactive component levels in the turmeric extracts. The predicted and experimentally obtained values were similar, indicating the accuracy of the optimized model [13].

Based on the cytotoxicity assay, this optimized turmeric extract can be used at up to 100  $\mu\text{g/mL}$  without reducing cell viability or integrity. The optimized extract effectively suppressed LPS-induced nitric oxide production, suggesting its anti-inflammatory potential. The presence in the optimized extract of curcumin, which exhibits anti-inflammatory activity, may explain this result. In LPS-induced macrophage cells, curcumin prevented cytotoxicity and reduced nitric oxide production [29]. Owing to its ability to reduce nitric oxide generation, turmeric extract can potentially reduce inflammation associated with diseases such as arthritis, cardiovascular disease, and neurological disorders.

The potential antidiabetic effects of the optimized turmeric extract were examined by analyzing its inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase activity. These enzymes, which regulate blood glucose levels, offer potential as therapeutic targets in managing diabetes. In both inhibitory assays, the optimized extract achieved lower  $\text{IC}_{50}$  values than the other types of extracts, suggesting greater inhibitory capacity. The optimized turmeric extract can thus effectively prevent the breakdown of starch and glucose polymers by  $\alpha$ -amylase and  $\alpha$ -glucosidase. The superior performance of the optimized extract can be attributed to the greater concentrations and bioactivity levels of its active compounds, making it more effective at inhibiting the target enzymes than the ethanol- or water-derived extracts. This is consistent with prior findings that inhibitory activity was lowest for a water-derived extract and highest for an ultrasound-derived extract [30].

ACE is essential for controlling blood pressure and prevent hypertension [36]. The antihypertensive potential of turmeric extracts has been revealed both in vitro and in vivo [36]. Curcuminoids, including curcumin, monodemethoxycurcumin, and bide methoxycurcumin, are the major bioactive components in turmeric, exhibiting antihyperglycemic, antihypertensive, anti-inflammatory, and anticancer effects [36]. Based on our findings, the optimized extract achieved better inhibition of ACE than the other types of extracts, suggesting its greater potential in managing blood pressure.

## 4. Conclusions

The conditions for enzyme-assisted ultrasonication extraction were successfully optimized. The response variables, in respect of the levels of bioactives and the antioxidant activity of the extract,

varied significantly with enzyme concentration and ultrasonication time. The RSM model performed reliably, and the optimization results were validated experimentally. These findings highlight the improvements in bioactivity that the optimized extract achieves over conventionally derived extracts. Based on these findings, the optimized extract exhibits substantial anti-inflammatory, antihypertensive, and antidiabetic potential. These findings provide valuable insight into the application of turmeric extracts with improved functional properties. These findings reveal the potential of this enhanced extraction approach for improving the bioactivity of turmeric extracts, thus supporting the development of foods with improved functional properties.

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