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Curcuma longa (Turmeric) Bioactive Extraction Using High Pressure Processing (HPP) for Enhanced Quality Content and Nutraceutical Properties

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Abstract

This study investigated the extraction of bioactive compounds from turmeric (*Curcuma longa*) using High-Pressure Processing (HPP) as an alternative to conventional methods, which often rely on chemical solvents and can degrade valuable compounds. The research tested different pressure levels (200, 400, and 600 MPa), extraction times (4, 6, and 10 minutes), and solid-to-liquid ratios (1:5, 1:10, and 1:15). The optimal results were achieved at 400 MPa, with a 6-minute extraction time and a 1:10 ratio, yielding the highest concentration of bioactive compounds at 0.42 mg/mL. Lower pressures required longer extraction times, while higher pressures caused some degradation of the compounds. The extracts demonstrated notable anti-inflammatory (0.42 μg/ml), antioxidant (0.82 mg/ml), and antimicrobial properties, highlighting their potential for functional applications. Future research should focus on scaling up this method and exploring its combination with other advanced extraction techniques for improved efficiency and stability.

1. Introduction

Chronic inflammation has emerged as a major contributor to numerous health issues, significantly impacting global morbidity and mortality rates. Recent research indicates that over 50% of deaths are linked to chronic inflammatory diseases, including ischemic heart disease, stroke, cancer, diabetes, and neurodegenerative disorders [1]. This shift in understanding highlights how the immune system and inflammatory processes are involved in a broad range of mental and physical health problems, rather than just a few specific conditions. Chronic inflammation can lead to long-term health complications, affecting various bodily systems and increasing the risk of developing serious diseases.

To combat chronic inflammation, there is a growing focus on developing anti-inflammatory treatments. Biopharmaceutical companies are creating biologics—complex compounds derived from living organisms—that target inflammatory pathways more effectively than traditional chemically synthesized medications. These biologics are essential for treating inflammatory disorders due to their ability to modulate the immune response [2]. Meanwhile, medicinal plants have gained attention for their potential therapeutic properties. Research into over 150,000 plant species has revealed promising natural products for treating various ailments, particularly those with anti-inflammatory effects.

Turmeric (*Curcuma longa*) is one such plant recognized for its anti-inflammatory properties, primarily due to its active compound, curcumin. Studies have shown that curcumin can interact with multiple molecular targets involved in inflammation, making it effective for treating conditions like arthritis, asthma, and inflammatory bowel disease. Historically, turmeric has been used in traditional medicine across Asia for a variety of ailments, and its therapeutic potential continues to be explored in modern research [3].

The main bioactive ingredient of *Curcuma longa*, or turmeric, curcumin, has shown strong antibacterial qualities against a variety of bacteria, fungi, and viruses. Research has demonstrated that curcumin has a wide range of antibacterial action, including against forms of bacteria resistant to antibiotics, including *Pseudomonas aeruginosa*, multidrug-resistant *Escherichia coli*, and methicillin-resistant *Staphylococcus aureus* (MRSA). Curcumin's antibacterial properties are ascribed to its capacity to perforate bacterial cell membranes, impede bacterial enzymes, and obstruct quorum sensing systems [4]. Additionally, curcumin has a potent inhibitory effect on enzymes that produce reactive oxygen, including lipoxygenase and cyclooxygenase.

Different extraction methods have been utilized to obtain bioactive compounds from turmeric. Traditional methods like steam distillation can degrade sensitive compounds due to high heat exposure. In contrast, High-Pressure Processing (HPP) is a promising technique that enhances the extraction efficiency of curcuminoids without damaging their quality. By applying high pressure for specific durations, HPP can effectively release beneficial compounds from turmeric, making it a valuable method for obtaining high-quality extracts [5]. Increased extraction efficiency is known to occur from HPP's ability to increase turmeric's release of curcuminoids. This study will be implementing the usage of HPP to obtain the bioactive contents of *Curcuma longa* successfully.

2. Methodology

This study investigated the extraction of bioactive compounds from turmeric (*Curcuma longa*) using the High-Pressure Processing (HPP) technique with the Hiperbaric 55 model at Universiti Putra Malaysia (UPM) under various conditions. After extraction, the bioactive compounds were separated through centrifugation with the Centrifuge MPW-380R, isolating the supernatants for functional analysis. The analysis focused on measuring Total Antioxidant, Total Phenolic Content, and Total Anti-Inflammatory properties in functional beverages. The goal was to compare results from different HPP conditions to optimize the concentration of bioactive compounds, ultimately enhancing the health benefits and development of functional beverages while identifying the best extraction conditions.

2.1 Materials

The research was conducted at the laboratory of Universiti Tun Hussein Onn Malaysia (UTHM) in the Pagoh campus. All solvents, chemicals, and reagents were prepared and supplied by the Material Laboratory and Upstream Bioprocess Laboratory at UTHM. The *Curcuma longa* used in this study was sourced from KIZA HERBS located in Temerloh, Pahang.

2.2 Collection and Preparation of Sample

The High Processing Pressure (HPP) technique was used to extract the raw materials (*Curcuma longa*) from KIZA HERBS in Temerloh, Pahang. The materials were first cleaned, any foreign objects were taken out, and then they were allowed to dry at room temperature. Prior to being sliced and allowed to dry in an oven set at 50°C for three days, the original weight of the cleaned materials was determined. Three days later, the samples were gathered, and a weighing scale was used to determine the moisture content of the dried materials. After that, the sample was crushed into a fine powder using a PX-MFC90D hammer grinder. The samples were then put into the 80 mL bottles in accordance with **Table 1**.

***Table 1*:** Samples Set Conditions including *Curcuma longa* in varying weights (5 g, 7.27 g, and 13.33 g).

Weight (g)		5 g			7.27 g			13.33 g		
Extraction Time (mins)		4	6	10	4	6	10	4	6	10
Pressure (MPa)	200	√	√	√	√	√	√	√	√	√
	400	√	√	√	√	√	√	√	√	√
	600	√	√	√	√	√	√	√	√	√
Total		27 Bottles								

An overview of the experimental setups listed in **Table 2**.

***Table 2*:** Overview of the experimental setups in HPP**.**

Overview of the experimental setups in HPP	
Material	*Curcuma longa*
Sample Solid Weight (g)	5 g, 7.27 g, 13.33 g
Extraction Pressure (MPa)	200 MPa, 400 MPa, 600 MPa
Extraction Time (min)	4 min, 6 min, 10 min

2.3 Extraction of *Curcuma longa* using HPP

Curcuma longa was extracted utilising the High-Pressure Processing (HPP) technique. The conditions listed in Table 1 were followed while introducing the sample bottles into the HPP machine, and Table 3 details the HPP's parameters. After that, *Curcuma longa* was centrifuged using a Centrifuge MPW-380R machine. The centrifugation settings for the samples were 5,000 rpm, 10 min, and 8 °C. The liquid supernatant was at the top of the two layers, while the solid pellet was at the bottom. Total vitamin C, total phenolic content, total flavonoid content, and anti-inflammatory, antioxidant, and antibacterial properties were all analysed using the liquid supernatant that was extracted from the pellet.

2.4 Determination of Total Phenolic Content (TPC) for Nutritional Analysis

The Folin-Ciocalteu Reagent was used in the Lowry Assay procedure to determine the samples' Total Phenolic Content (TPC). Initially, 2 mL of Folin-Ciocalteu Reagent were combined with 400 µL of the extracted *Curcuma longa* samples, and the mixture was left to react for four minutes at room temperature. After adding 1.6 mL of alkaline $Na_2CO_3NA\_2CO\_3Na_2CO_3$ the solution was left in a dark place for two hours. At 760 nm, the absorbance was determined with a UV-Vis spectrophotometer. Gallic acid was used as the reference component in a standard curve that was created by preparing a series of solutions with concentrations of 50, 100, 150, 200, and 250 µg/mL. 5 mL of diluted Folin-Ciocalteu (F-C) reagent, diluted 1:10 with water, and 4 mL of sodium carbonate solution (75 g/L) were combined with 1 mL of each gallic acid solution. After 30 minutes of reaction time, the mixes' absorbance at 765 nm was measured. The Total Phenolic Content (TPC) was calculated using Eq.1.

$$\text{Concentration (TPC)} = ((\text{Abs})-0.355)/0.8838 \text{ (graph gradient)} \quad \text{(Eq.1)}$$

2.5 Determination of Total Flavonoids Content (TFC) for Nutritional Analysis

To measure the total flavonoids in the *Curcuma longa* extract, 4 mL of 4 M aqueous NaOH was added to 2 mL of the extract in a test tube, resulting in a yellow precipitate that indicated the presence of flavonoids. To confirm this, a few drops of concentrated HCl were added, which turned the yellow solution colorless. This reaction demonstrated that the yellow precipitate formed with NaOH was a common qualitative test for flavonoids, known for their antioxidant and pharmacological properties.

The process for determining total flavonoid content (TFC) began with preparing standard solutions of quercetin. A stock solution of quercetin (1 mg/mL) was made by dissolving 10 mg of quercetin in 10 mL of methanol, which was then diluted to create standard solutions at concentrations of 10, 20, 40, 60, 80, and 100 µg/mL. Next, 1 mL of each quercetin standard solution was placed in separate test tubes, to which 4 mL of distilled water and 0.3 mL of 5% sodium nitrite ($NaNO_2$) were added and mixed thoroughly. After letting the mixtures stand for 5 minutes, 0.3 mL of 10% aluminum chloride ($AlCl_3$) was added and allowed to sit for another 6 minutes. Then, 2 mL of 1 M sodium hydroxide (NaOH) was added to complete the reaction, and the final volume was adjusted to 10 mL with distilled water. The absorbance of each solution was measured at 510 nm using a UV-Vis spectrophotometer, with a blank sample prepared without quercetin for reference. A standard curve was created by plotting absorbance values against quercetin concentrations.

$$\text{Concentration (TFC)}=((\text{Abs})-0.0234)/0.7918 \text{ (graph gradient)} \quad \text{(Eq.2)}$$

2.6 Determination of Total Vitamin C Content (TVC) for Nutritional Analysis

To determine the concentration of vitamin C in *Curcuma longa* extract using the colorimetric method with 2,6-dichlorophenolindophenol (DCPIP), a standard curve was first created. This involved preparing a series of standard ascorbic acid solutions with known concentrations (e.g., 1 mg/mL, 2 mg/mL, 3 mg/mL). For each concentration, 1 mL of the standard solution was placed in separate test tubes, followed by the addition of 1 mL of DCPIP solution. After mixing thoroughly, the reaction was allowed to proceed for a few minutes, and the absorbance of each solution was measured at 520 nm using a spectrophotometer. The absorbance values were

then plotted against the corresponding ascorbic acid concentrations to generate a standard curve, which served as a reference for determining vitamin C concentration in the sample.

For analyzing the *Curcuma longa* extract, 1 mL of the extract was added to a clean test tube along with 1 mL of DCPIP solution. The mixture was thoroughly mixed and allowed to react for a few minutes, similar to the standard solutions. The absorbance of this solution was measured at 520 nm using the spectrophotometer. By comparing the absorbance of the extract to the standard curve, the concentration of vitamin C in the extract was determined using Eq.3.

$$\text{Concentration (TVC)}=((\text{Abs})+0.0344)/0.251 \text{ (graph gradient)} \quad \text{(Eq.3)}$$

2.7 Determination of Total Antioxidant (TAO) for Functional Properties

The total antioxidant activity is measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method. The process starts by weighing the sample and dissolving it in ethanol to create a stock solution at a concentration of 1 mg/mL, using a vortex mixer. From this stock solution, four different concentrations (125, 250, 500, and 1000 µg/mL) are prepared through serial dilution. In each test tube, 2 mL of 0.1 M DPPH solution is added along with 2 mL of the sample solution. The mixtures are thoroughly mixed to ensure uniformity and then incubated in a dark room for 30 minutes to allow the reaction to take place. A blank control is prepared by mixing 2 mL of absolute ethanol with 2 mL of 0.1 mM DPPH solution. After incubation, a color change from purple to yellow indicates the presence of antioxidant activity in the sample. The absorbance of each mixture is then measured at 517 nm using a UV-Vis spectrophotometer and the concentration is calculated using formula Eq.4.

$$\text{Concentration (TAO)}=((\text{Abs})-0.736)/0.68 \text{ (graph gradient)} \quad \text{(Eq.4)}$$

2.8 Determination of Total Anti-Inflammatory (TAI) for Functional Properties

The total anti-inflammatory testing procedure for *Curcuma longa* began with diluting the sample extract and standard stock solutions to various concentrations (125, 250, 500, and 1000 µg/mL) using a 0.1 M phosphate buffer solution (pH 7.4). For each concentration, 0.02 mL of the sample extract or standard solution was mixed with 4.78 mL of phosphate-buffered saline (pH 6.4) and 0.2 mL of 1% BSA solution. The contents of the test tubes were thoroughly mixed using a vortex mixer. The test tubes were then incubated at 37°C in a water bath for 15 minutes. Following the initial incubation, the test tubes were heated to 70°C for 5 minutes and then allowed to cool to room temperature. Finally, the absorbance of the mixtures was measured at 660 nm using a UV-Vis spectrophotometer, with a phosphate buffer solution without the sample used as the control.

$$\text{Concentration (TAI)}=((\text{Abs})+0.0588)/0.689 \text{ (graph gradient)} \quad \text{(Eq.5)}$$

2.9 Determination of Total Anti-Inflammatory (TAI) for Functional Properties

The Agar Well Diffusion Method is commonly used to evaluate the antimicrobial properties of plant extracts, including *Curcuma longa*. The process begins by preparing a stock solution of the extract, made by dissolving a measured amount of dried *Curcuma longa* powder in a solvent like water, ethanol, or methanol. This solution is filtered to remove impurities and stored at 4°C for stability. Next, agar plates are created by mixing 15-20 grams of agar powder with 1 liter of distilled water, heating the mixture to dissolve the agar, and then sterilizing it. The sterile agar is poured into Petri dishes to form a 1-2 mm thick layer and allowed to solidify at room temperature.

Once solidified, the agar plates are inoculated with a test microorganism, such as *Escherichia coli*, ensuring even coverage. A specific volume of the *Curcuma longa* extract (typically 5-10 µL) is added to wells punched into the agar. The plates are incubated at around 37°C for about 24 hours to allow microbial growth and interaction with the extract. After incubation, the zone of inhibition around each well is measured; this area indicates where microbial growth was prevented due to the antimicrobial activity of the extract. Larger zones signify stronger antimicrobial properties.

3. Results and Discussion

3.1 Sample Extraction

Curcuma longa samples were effectively extracted using High Pressure Processing (HPP), employing filtered water as the extraction medium. Prior to the HPP extraction, the solid phase appeared homogeneous, while the liquid phase exhibited a clear separation into two distinct layers, as evidenced by visual color observation. Following the HPP extraction, three distinct phases emerged: a dark brown liquid phase at the top, a middle phase that was whitish and contained a mixture of liquid and solid, and a bottom phase that was predominantly solid with some liquid content as in **Figure 1**.

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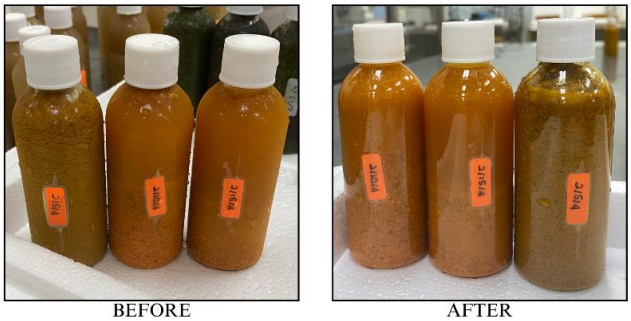

***Fig. 1*: *Curcuma longa* sample before and after HPP.**

3.2 Quantification of Nutritional Content of Extracted Content

3.2.1 Total Phenolic Content

Table 3 presents the total phenolic content (TPC) for turmeric under various High-Pressure Processing (HPP) operating conditions. In the case of turmeric, at 200 MPa, 1:5 S/L ratio, 4 minutes, the TPC concentration is at its maximum (235.000 µg/ml), whereas at 200 MPa, 1:15 S/L ratio, 4 minutes, it is at its lowest (24.022 µg/ml). Although they do not perform better than 200 MPa at the ideal 1:5 ratio, higher pressures (400 and 600 MPa) appear to favor extraction for longer durations at 1:10 and 1:15 ratios.

***Table 3*: TPC concentrations (µg/ml) for turmeric (*Curcuma longa*) at different HPP operating conditions.**

Pressure (MPa)	S/L Ratio (w/v)	Time (minutes)	Concentration (µg/ml)
200	1:5	4	235.000 *
		6	232.120
		10	211.413
	1:10	4	45.489
		6	195.598
		10	200.326
	1:15	4	24.022 ^
		6	89.511
		10	121.793
400	1:5	4	85.761
		6	139.891
		10	129.728
	1:10	4	106.793
		6	76.141
		10	218.587
	1:15	4	53.478
		6	89.185
		10	122.446
600	1:5	4	172.065
		6	173.370
		10	159.348
	1:10	4	36.359
		6	223.261
		10	161.196
	1:15	4	34.511
		6	94.511
		10	84.511

(^) minimum values of TPC; (*) maximum values of TPC

3.2.2 Total Flavonoids Content

Table 4 shows the total flavonoid concentration (TFC) in turmeric (*Curcuma longa*) under various High-Pressure Processing (HPP) conditions, including pressures of 200, 400, and 600 MPa, solid-to-liquid (S/L) ratios of 1:5, 1:10, and 1:15, and treatment times of 4, 6, and 10 minutes. The highest TFC concentration measured was

0.958 µg/mL at 400 MPa with a S/L ratio of 1:5 and a treatment time of 6 minutes. The lowest concentration was 0.609 µg/mL at 200 MPa with a S/L ratio of 1:10 and a treatment time of 4 minutes.

The results suggest that increasing pressure generally enhances TFC concentrations, peaking at 400 MPa, but concentrations start to decline at 600 MPa. Thus, the optimal conditions for maximizing TFC in turmeric are a pressure of 400 MPa, a S/L ratio of 1:5, and a processing time of 6 minutes.

***Table 4*:** TFC concentrations (µg/ml) for turmeric (*Curcuma longa*) at different HPP operating condition.

Pressure (MPa)	S/L Ratio (w/v)	Time (minutes)	Concentration (µg/ml)
200	1:5	4	0.805
		6	0.844
		10	0.903
	1:10	4	0.609
		6	0.709
		10	0.723
	1:15	4	0.608 ^
		6	0.851
		10	0.753
400	1:5	4	0.949
		6	0.958 *
		10	0.892
	1:10	4	0.681
		6	0.656
		10	0.657
	1:15	4	0.736
		6	0.842
		10	0.746
600	1:5	4	0.678
		6	0.658
		10	0.627
	1:10	4	0.772
		6	0.867
		10	0.660
	1:15	4	0.678
		6	0.769
		10	0.776

(^) minimum values of TFC; (*) maximum values of TFC

3.2.3 Total Vitamin C Content

The data in **Table 5** shows that the extraction of Total Volatile Compounds (TVC) from turmeric (*Curcuma longa*) is significantly affected by pressure, solid-to-liquid ratio (S/L), and extraction time. The maximum TVC concentration reached 8.380 µg/mL at 400 MPa with a 1:10 S/L ratio after 6 minutes, while the minimum concentration was 3.838 µg/mL at the same pressure but after only 4 minutes. The 1:10 ratio consistently yielded the highest concentrations across different extraction times, indicating more efficient extraction due to better solvent penetration. In contrast, the 1:5 and 1:15 ratios resulted in lower TVC levels, suggesting that insufficient solvent or excess solid material limits extraction efficiency. Overall, longer extraction times generally improved TVC concentrations, particularly at the 1:10 ratio and higher pressures.

***Table 5*:** TVC concentrations (µg/ml) for turmeric (*Curcuma longa*) at different HPP operating conditions.

Pressure (MPa)	S/L Ratio (w/v)	Time (minutes)	Concentration (µg/ml)
200	1:5	4	5.934
		6	6.621
		10	6.890
	1:10	4	4.006
		6	6.689
		10	8.123
	1:15	4	3.512 ^
		6	5.332
		10	5.488

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Pressure (MPa)	S/L Ratio (w/v)	Time (minutes)	Concentration (µg/ml)
400	1:5	4	7.159
		6	6.721
		10	7.448
	1:10	4	3.838
		6	8.380 *
		10	8.025
	1:15	4	4.006
		6	5.824
		10	8.494
600	1:5	4	5.613
		6	5.386
		10	5.986
	1:10	4	4.071
		6	6.340
		10	7.701
	1:15	4	4.902
		6	6.183
		10	5.539

(^) minimum values of TVC; (*) maximum values of TVC

3.3 Quantification of Nutraceutical Content of Extracted Content

3.3.1 Total Antioxidant

Table 6 shows the Total Antioxidant (TAO) concentrations of turmeric (*Curcuma longa*) under different high-pressure processing (HPP) conditions, including pressure, solid-to-liquid (S/L) ratio, and processing time. At 200 MPa, the highest TAO concentration was 0.815 mg/mL at a 1:10 S/L ratio after 4 minutes. At 400 MPa, TAO levels slightly increased, peaking at 0.726 mg/mL with a 1:15 ratio and 4 minutes. The highest TAO concentration of 0.790 mg/mL was observed at 600 MPa, particularly at a 1:10 S/L ratio after 4 minutes. Overall, lower S/L ratios (1:10 or 1:15) produced higher antioxidant levels compared to a 1:5 ratio across all pressures.

***Table 6*:** TAO concentrations (µg/ml) for turmeric (*Curcuma longa*) at different HPP operating condition**.**

Pressure (MPa)	S/L Ratio (w/v)	Time (minutes)	Concentration (mg/ml)
200	1:5	4	0.091 ^
		6	0.168
		10	0.021
	1:10	4	0.815 *
		6	0.707
		10	0.274
	1:15	4	0.726
		6	0.536
		10	0.756
400	1:5	4	0.240
		6	0.174
		10	0.267
	1:10	4	0.722
		6	0.354
		10	0.354
	1:15	4	0.726
		6	0.480
		10	0.688
600	1:5	4	0.691
		6	0.685
		10	0.418
	1:10	4	0.790
		6	0.672
		10	0.354
	1:15	4	0.586

Pressure (MPa)	S/L Ratio (w/v)	Time (minutes)	Concentration (mg/ml)
		6	0.471
		10	0.403

(^) minimum values of TAO; (*) maximum values of TAO

3.3.2 Total Anti-Inflammatory

Table 7 presents the Total Anti-Inflammatory (TAI) concentrations of turmeric (*Curcuma longa*) under various high-pressure processing (HPP) conditions, showing clear trends based on pressure, solid-to-liquid (S/L) ratios, and processing times. At 200 MPa, the highest TAI concentration of 0.318 µg/mL was found at a 1:5 S/L ratio after 10 minutes, with lower concentrations at higher S/L ratios. At 400 MPa, the peak concentration of 0.423 µg/mL occurred at a 1:10 ratio after 6 minutes, indicating optimal extraction under these conditions. At 600 MPa, TAI concentrations were generally highest, with a maximum of 0.318 µg/mL again at a 1:5 ratio and 10 minutes, similar to the result at 200 MPa. Overall, the 1:5 S/L ratio typically produced the highest TAI concentrations, while the 1:15 ratio yielded the lowest, suggesting that lower dilution improves extraction efficiency.

***Table 7*:** TAI concentrations (µg/ml) for turmeric (*Curcuma longa*) at different HPP operating conditions**.**

Pressure (MPa)	S/L Ratio (w/v)	Time (minutes)	Concentration (µg/ml)
200	1:5	4	0.124
		6	0.123
		10	0.318
	1:10	4	0.090
		6	0.109
		10	0.093
	1:15	4	0.087 ^
		6	0.096
		10	0.103
400	1:5	4	0.098
		6	0.104
		10	0.100
	1:10	4	0.106
		6	0.423 *
		10	0.107
	1:15	4	0.091
		6	0.107
		10	0.103
600	1:5	4	0.135
		6	0.188
		10	0.318
	1:10	4	0.103
		6	0.133
		10	0.120
	1:15	4	0.125
		6	0.101
		10	0.240

(^) minimum values of TAI; (*) maximum values of TAI

3.3.3 Total Antimicrobial

The purpose of this study was to investigate the antibacterial properties of *Curcuma longa*, extracted using High-Pressure Processing (HPP), against clinically relevant strains of Escherichia coli. The extracted turmeric sample, produced under optimal conditions (400 MPa, 1:10 w/v, 6 minutes), was used for microbiological testing as Sample 2, with nutrient agar as the growth medium. The antimicrobial properties of turmeric have been widely studied, particularly against various bacterial strains, including E. coli.

As shown in **Figure 2**, the positive control, Chloramphenicol, demonstrated a strong antibacterial effect by preventing bacterial growth around it. In contrast, Sample 1 showed limited antimicrobial effectiveness, likely due to contamination from solid turmeric compounds on the sample paper. However, Sample 2 displayed a

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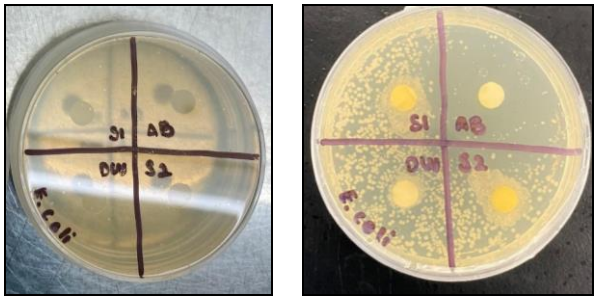
positive result by inhibiting bacterial growth, indicating that the turmeric extract contains antibacterial properties.



Before After

***Fig. 2*:** Before and after picture of antimicrobial testing on turmeric extract.

3.4 Effect of Pressure

The effect of pressure during High Pressure Processing (HPP) was evaluated at levels of 200 MPa, 400 MPa, and 600 MPa, with extraction times of 4, 6, and 10 minutes as in **Figure 3**. At 200 MPa, total phenolic content (TPC) peaked at 235.000 µg/mL with a solid-to-liquid (S/L) ratio of 1:5 after 4 minutes, but TPC levels declined with longer processing times, likely due to breakdown of phenolic compounds. Higher S/L ratios resulted in much lower TPC concentrations, with the lowest at 24.022 µg/mL for a 1:15 ratio after 4 minutes. At 400 MPa, TPC levels were generally lower than at 200 MPa for the same S/L ratio, but longer processing times improved extraction efficiency, particularly at a 1:10 ratio, where TPC reached 218.587 µg/mL after 10 minutes. At 600 MPa, TPC concentrations were more stable but did not exceed those at 200 MPa; the highest value was 223.261 µg/mL at a 1:10 ratio after 6 minutes. The results indicate that while moderate pressure and longer extraction times can enhance TPC extraction, high dilution remains inefficient even at increased pressures.

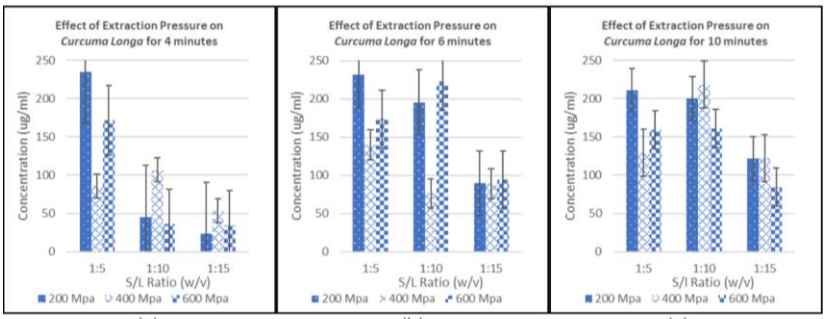


***Fig. 3*:** The effect of pressure on Total Phenolic Content at different S/L ratio for the extraction time of (a) 4 min (b) 6 min (c) 10 min.

The presence of oxygen in the extraction media during HPP treatment might lead to oxidative stress. High pressures have the ability to speed up oxidation-promoting processes, which may result in the production of reactive oxygen species (ROS). These ROS have the ability to react with phenolic chemicals, leading to polymerisation or oxidative destruction [6]. Through a process called polymerisation, smaller phenolic molecules join forces to create larger, more intricate structures that might not have the same biological activity as their parent substances. Consequently, extended exposure to high pressure may accidentally lower the concentration and quality of phenolic compounds, even though HPP can significantly improve extraction efficiency [7].

3.5 Effect of Time

At 200 MPa pressure and a 1:5 solid-to-liquid (S/L) ratio in **Figure 4**, the highest total phenolic content (TPC) concentration reached 235.000 µg/mL after 4 minutes, but TPC levels declined to 232.120 µg/mL and 211.413 µg/mL with longer processing times, suggesting that prolonged exposure may lead to the breakdown of phenolic compounds. At a 1:10 S/L ratio, TPC increased from 45.489 µg/mL at 4 minutes to 200.326 µg/mL at 10 minutes, indicating that longer extraction durations can enhance yields, though not as effectively as the ideal 1:5 ratio. TPC values at a 1:15 S/L ratio remained low, with a minimum of 24.022 µg/mL at 4 minutes and only reaching 121.793 µg/mL at 10 minutes, reflecting poor extraction from highly diluted mixtures.

At 400 MPa and a 1:5 S/L ratio, TPC concentrations were lower than at 200 MPa, ranging from 85.761 µg/mL at 4 minutes to a maximum of 139.891 µg/mL at 6 minutes before declining again, suggesting that higher pressures may degrade sensitive phenolic compounds over time. For the 1:10 S/L ratio, TPC peaked at 218.587 µg/mL after 10 minutes, highlighting the benefits of extended processing under moderate pressure conditions. At 600 MPa, TPC concentrations stabilized but did not exceed those observed at lower pressures; for example, with a 1:5 S/L ratio, TPC reached a maximum of 173.370 µg/mL after 6 minutes before decreasing.

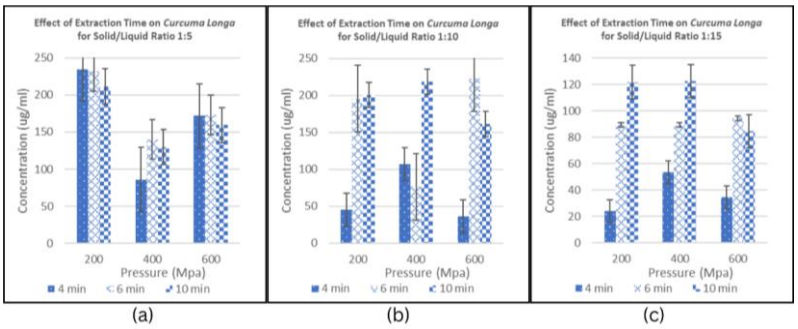


***Fig. 4*:** The effect of extraction time on TPC at different Pressure for the S/L ratio of (a) 1:5 (b) 1:10 (c) 1:15

The relationship between extraction time and Total Phenolic Content (TPC) is critical in optimizing the extraction process for phenolic compounds. The provided analysis results indicate that TPC can vary significantly with changes in extraction time. The effect of extraction time in HPP were carried out at 4, 6, and 10 minutes for various pressure and solid/liquid ratio. TPC typically rises with extraction time until a certain point. This initial increase can be explained by the phenolic compounds increased solvent exposure, which increases their solubility and diffusion into the extraction medium . As the solvent enters the plant matrix and starts to efficiently dissolve phenolic chemicals, for example, research has indicated that TPC can rise dramatically in the initial minutes of extraction [10].

3.6 Effect of Solid To Liquid Ratio

The solid-to-liquid (S/L) ratio significantly affects the Total Phenolic Content (TPC) in turmeric (*Curcuma longa*) extraction, observing Figure 5. At 200 MPa, the 1:5 S/L ratio consistently yields the highest TPC, peaking at 235.0 µg/mL after 4 minutes, although it decreases over time while remaining the highest among all ratios. The 1:10 ratio starts lower at 45.5 µg/mL at 4 minutes but increases to 200.3 µg/mL by 10 minutes, still not reaching the levels of the 1:5 ratio. The 1:15 ratio produces the lowest TPC values overall. At 400 MPa, the 1:5 ratio shows variable TPC readings of 85.8 µg/mL at 4 minutes, 139.9 µg/mL at 6 minutes, and 129.7 µg/mL at 10 minutes, while the 1:10 ratio increases from 106.8 µg/mL at 4 minutes to 218.6 µg/mL at 10 minutes.

The 1:15 ratio remains low with TPC values of 53.5 µg/mL at 4 minutes and 122.4 µg/mL at 10 minutes. At 600 MPa, the 1:5 ratio provides stable results with TPC values of 172.1 µg/mL at 4 minutes and 173.4 µg/mL at 6 minutes, dropping to 159.3 µg/mL at 10 minutes. The 1:10 ratio begins low at 36.4 µg/mL at 4 minutes but rises to 223.3 µg/mL at 6 minutes before decreasing again at 10 minutes, while the 1:15 ratio continues to show the lowest TPC values across all conditions.

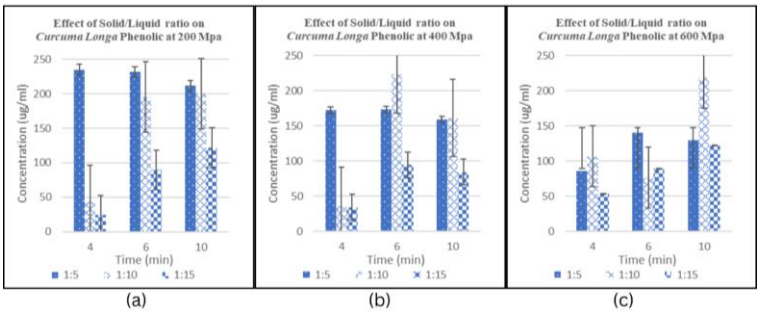


***Fig. 5*:** The effect of solid to liquid on TPC at different Time for the extraction Pressure of (a) 200Mpa (b) 400Mpa (c) 600Mpa

In high-pressure processing (HPP), the concentration gradient between the solvent and the solute (phenolic compounds) is controlled by the S/L ratio, which has a major impact on the extraction process. A steeper concentration gradient produced by a greater solid-to-solvent ratio (e.g., 1:5) speeds up the diffusion of phenolic

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chemicals from the turmeric matrix into the solvent. The mass transfer hypothesis supports this idea, which states that phenolic compounds flow into the solvent more quickly and effectively when there is a larger gradient [8].

Furthermore, the probability of phenolic molecules coming into contact with solvent molecules rises with the amount of solvent added, enhancing extraction efficiency even more. A number of variables, such as the particular plant material, the extraction technique (such as high hydrostatic pressure or solvent type), temperature, and extraction duration, can greatly affect the ideal S/L ratio [9].

The relationship between the solid-to-liquid (S/L) ratio and total phenolic content (TPC) yield is not linear. Once equilibrium is established between the phenolic compounds in the turmeric matrix and the solvent, adding more solvent does not lead to proportional increases in extraction yield. This effect is demonstrated in studies showing that TPC levels tend to plateau beyond certain S/L ratios [10]

3.7 Overall Table for Highest Values with Optimal Conditions

The Table 8 shows that the parameters that produce the highest concentrations of different phytochemicals may be used to determine the most balanced and efficient extraction conditions for *Curcuma longa*. A pressure of 400 MPa, a solid-liquid ratio of 1:10, and an extraction duration of 6 minutes were determined to be the ideal parameters.

Table 8: The Optimum Operating Conditions for Each Parameter.

	Highest Concentration	Pressure (MPa)	Parameter Solid-liquid Ratio (w/v) %	Extraction Time (Minutes)
TPC	235.00 ug/mL	200	1:05	4
TFC	0.96 mg/mL	400	1:05	6
TVC	8.49 ug/mL	400	1:15	10
TAO	0.82 mg/mL	200	1:10	4
TAI	0.42 mg/mL	400	1:10	6

This selection is supported by the high yield of various phytochemicals obtained under specific extraction conditions, particularly at 400 MPa. With a solid-to-liquid ratio of 1:5 and an extraction time of 6 minutes, the total flavonoid content (TFC) reaches 0.96 mg/mL, while the total anthocyanin content (TAI) peaks at 0.42 mg/mL with a 1:10 ratio. Higher pressure and an appropriate solid-to-liquid ratio facilitate more effective extraction of phytochemicals, even though the total phenolic content (TPC) is highest at lower pressures (200 MPa). The efficiency of the 6-minute extraction time is particularly beneficial for industrial applications where time is crucial, as it minimizes the risk of degradation of sensitive compounds. Additionally, the 1:10 solid-to-liquid ratio strikes an optimal balance between reducing solvent usage and enhancing extraction efficiency, ensuring sufficient solvent is available to extract the desired compounds without overly diluting them, which could lower the concentration and effectiveness of the final extract [8]. Selecting a pressure of 400 MPa, a solid-liquid ratio of 1:10, and an extraction time of 6 minutes optimizes the extraction process for *Curcuma longa*. These conditions not only maximize the yield of essential phytochemicals but also ensure efficiency and sustainability, making them well-suited for both research and commercial purposes.

4. Conclusion

The research demonstrated the effective extraction of *Curcuma longa* using high-pressure processing (HPP) under various conditions, including different pressure levels, solid-to-liquid (S/L) ratios, and processing times. Optimized HPP facilitated the disruption of plant cell walls, enhancing the release of bioactive compounds such as phenolics, flavonoids, and curcuminoids while preserving their structural integrity. The nutritional analysis revealed high levels of key bioactive components, with maximum total phenolic content (TPC) achieved at 200 MPa with a 1:5 S/L ratio for 4 minutes, and the best total flavonoid content (TFC) obtained at 400 MPa with a 1:5 S/L ratio for 6 minutes.

The functional characteristics of the extracts indicated strong bioactive potential, showing high antioxidant activity in the DPPH assay, demonstrated anti-inflammatory properties in the BSA denaturation assay, and notable antibacterial effects in the agar well diffusion technique. Additionally, the study explored how HPP parameters influenced extraction efficiency and nutraceutical activity, finding that higher pressures and shorter times preserved more bioactive components. The optimal parameters identified were 400 MPa, a 1:10 S/L ratio, and a processing time of 6 minutes, maximizing extraction efficiency while maintaining bioactivity. Overall, the study concluded that HPP is an effective and environmentally friendly method for extracting and preserving the nutritional and functional qualities of *Curcuma longa*.

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