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Application of two-level factorial design in optimization of maceration extraction of *Curcuma longa* curcuminoids extracts

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ABSTRACT

A two-level factorial design was used to identify extraction factors that could improve *Curcuma longa* rhizome extraction yield (EY) and curcuminoid content (CC). These extraction factors included solid/liquid ratio (A: 0.10–0.20 g/mL), ethanol concentration (B: 50–70%), and time maceration (C: 3–6 h). Under the prevailing circumstances, the yield of extract and CC varied from 2.86% to 20.90% and from 1.80% to 21.59%, respectively. The findings indicated that all three parameters were statistically significant ($P < 0.05$) in contributing to increase EY. The increase in CC was significantly influenced by ethanol concentration alone. Therefore, these findings suggest that adjusting the solid/liquid ratio, ethanol concentration, and maceration time can enhance the efficiency of curcuminoid extraction from *C. longa* rhizomes.

1. INTRODUCTION

Curcuminoids are a group of phenolic compounds that serve as the primary bioactive markers in the rhizomes of turmeric, *Curcuma longa* L., which belongs to the *Zingiberaceae* family [1]. Curcuminoids include curcumin, demethoxycurcumin, and bis-demethoxycurcumin. Among these, curcumin is the most abundant and extensively studied component because of its significant therapeutic potential [2]. These compounds vivid yellow hue not only distinguishe the rhizome but also found widespread use in the culinary arts as a natural food coloring agent [3]. Beyond their esthetic contribution to gastronomy, curcuminoids have garnered attention in the scientific community due to their diverse pharmacological activities. Studies have provided evidence for their efficacy in treating a spectrum of diseases, including treat neurological disorders [4], Alzheimer's disease [5], and neuropathic pain [6]. In addition, they exhibit potent anticancer [7], antidiabetic [8], antibiofilms [9], and antioxidant [10,11] properties, making them a versatile tool in the pharmacological and nutraceutical industries.

The process of extracting compounds from plant matrices is a sophisticated operation that has a profound impact on the quality and yield of the target compounds. As such, there is a critical need to refine the extraction methods to maximize efficiency and cost-effectiveness. While various factors such as solvents choice [12], extraction methods, duration [13], and the solid-to-liquid ratio [14] play a pivotal role in determining the success of the extraction process, evolving methodologies are reshaping the landscape of extraction technology. Although traditional extraction techniques such as maceration [15] and Soxhlet extraction [2] have laid the groundwork for curcuminoid extraction, they are increasingly being supplanted by modern techniques that leverage advances in technology. These newer methods, including microwave-assisted extraction [16], enzyme-assisted extraction [17], ultrasound-assisted extraction [18], and supercritical liquid extraction [19], promise enhanced efficiency, although often at a greater cost. Despite these advancements, the economic implications mean that traditional methods remain in widespread use, particularly in settings with limited resources.

The traditional single-variable method is the most frequently used technique to examine the impact of various extraction conditions. However, in the current context, there is a need for information on multiple factors to optimize extraction, necessitating the use of an experimental design (ED). ED is a strategic method utilized to assess the impact of various elements on a specific procedure, as well as to understand the interplay among these elements and their influence on the procedure's outcomes. The benefits of using ED encompass the identification of optimal outcomes within the design framework and the analysis of factor interrelations. Efficiency is achieved by conducting a reduced number of experiments, which in turn conserves resources such as time, finances, and labor, thereby simplifying the experimental interpretation process [20]. The two-level factorial design, a method introduced recently, is employed to analyze the interaction among different parameters in the extraction process [21].

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Researchers have employed various ED methodologies to systematically optimize the extraction of curcuminoid compounds. Paulucci *et al*. adopted the response surface methodology to fine-tune parameters such as time, agitation speed, drug-to-solvent ratio, ethanolic strength, and extraction temperature in dynamic maceration to optimize curcumin extraction [15]. Other researchers have studied the solid-to-solvent ratio, temperature, particle size, mixing time, and solvent using maceration with response surface methodology [22] as parameter extraction methods for curcuminoids obtained from *C. longa* rhizomes. In the existing literature, we have discovered that curcuminoid extraction can be conducted through maceration using an ED method. However, we did not come across no studies have employed a two-level factorial design to enhance the extraction of curcuminoid compounds from *C. longa* rhizomes. The previous research has demonstrated that various solid-to-liquid ratios, ethanol concentrations, and extraction times can be independently utilized to achieve satisfactory outcomes when attempting to extract curcuminoid compounds [2,15,22-26]. To optimize the extraction process, we employed a two-level factorial design to determine the most effective combination of solid/liquid ratio, ethanol concentration, and maceration time for the extraction of curcuminoids from turmeric rhizomes.

2. MATERIALS AND METHODS

2.1. Plant Material

Dried *C. longa* rhizome powder was obtained from the Tropical Biopharmaca Research Center in Bogor, West Java, Indonesia. Rhizomes were subjected to a series of preparatory procedures, including washing, drying, and grinding. Initially, rhizomes were thoroughly washed with water and subsequently cut into smaller pieces. Following this, the cleaned and cut rhizomes were dried in an oven for 2 days and overnight at a temperature of 45°C. Once dried, rhizomes were ground to a particle size of 80 mesh.

2.2. Experimental Extraction

The extraction of curcuminoid compounds from *C. longa* rhizomes was optimized using a two-level factorial design. The design employed two variable levels, as listed in Table 1. These variables comprise independent factors such as the solid/liquid ratio (ranging from 0.10 to 0.20 g/mL), ethanol concentration (50–70%), and time maceration (3–6 h). Dried *C. longa* rhizome powder (15 g) was placed in a conical flask with aqueous ethanol at the concentrations chosen based on the ED [Table 1]. The solid/liquid ratio and time were selected based on ED. Extraction was performed using the maceration method in a water bath shaker (Memmert, WNE14, Germany). The extract was then filtered and the filtrate was concentrated using a rotary evaporator to obtain the extraction yield (EY).

2.3. Curcuminoid Analysis

The extracted samples were analyzed using high-performance liquid chromatography (HPLC) [27]. The stationary phase used was a C18 compound, while the mobile phase was acetonitrile and 2% acetic acid with a column diameter length of 25 × 4.6 mm, a flow rate of 1 mL/min, a wavelength of 425 nm, and a UV detector. Samples were weighed as much as 0.05 g and dissolved in methanol in a 50-mL volumetric flask until the line. The solution was diluted by a factor of ×50. The diluted solution was then filtered with 0.45-µm Whatman filter paper, sonicated for 30 min, and put into an HPLC vial. A total of 20 µL of the sample solution was injected into the HPLC column using curcuminoid standards at a concentration of 0.5 ppm. The curcuminoid content (CC) was determined by comparing the standard area with the sample as a percentage.

Table 1: Levels of factors utilized in a two-level factorial design.

Factor	Notation	Factor levels	
		Low (−)	High (+)
Solid/liquid ratio (g/mL)	A	0.10	0.20
Ethanol concentration (%)	B	50	70
Time maceration (h)	C	3	6

2.4. Statistical Analysis

Design Expert Version 22.0 software (Minneapolis, USA) and Microsoft Excel Version 16.79 were employed to analyze the design, corresponding results, and statistical evaluation.

3. RESULTS AND DISCUSSION

3.1. Selection of Extraction Factors for Two-Level Factorial Design

The efficiency and effectiveness of curcuminoid extraction from *C. longa* rhizomes can be influenced by several factors including the solid-to-solvent ratio, ethanol concentration, and duration of the extraction process. The previous studies have demonstrated a broad variation in the extraction factor range. Maceration, also referred to as solid-liquid extraction or "soaking," is a commonly utilized and well-known method for extracting solvents from solid substances [25]. A study conducted by Yulianto *et al*. obtained a higher EY of curcumin using a solid-to-liquid ratio of 0.1 g/mL [23]. Popuri and Pagala found that ethanol and an 8:1 solvent-to-solid ratio yielded the highest curcumin extraction efficiency from *C. longa* after 1 h [24]. Similarly, several studies have reported that ethanol is the preferred organic solvent for curcumin extraction [2,15,22]. In addition, the concentration of ethanol in the solvent combination plays a significant role in determining the effectiveness of the curcuminoid extraction [26]. Given the varying extraction parameters reported in the literature, a range of parameters was deliberately chosen and assessed in this investigation, namely, the solid/liquid ratio (A: 0.10–0.20 g/mL), ethanol concentration (B: 50–70 %), and maceration time (C: 3–6 h) [Table 1]. The results pertaining to the EYs and CC responses are listed in Table 1.

3.2. Influence of Extraction Factors on EYs

The results of the experiment indicated that various factors, such as solid/liquid ratio, ethanol concentration, and maceration time, had a statistically significant impact ($P < 0.05$) on achieving a greater EY from *C. longa* [Table 2]. These three factors contributed 35.73%, 8.30%, and 30.61%, respectively, to the achievement of a greater EY [Table 3]. Consequently, the ethanol concentration is more important than the solid/liquid ratio and maceration time because it has a stronger influence on the EY, as indicated by its higher percentage contribution to the fluctuations in the measured factor. EY decreased from 17.34 to 9.21% as the solid/liquid ratio increased from 0.1 to 0.2 g/mL [Table 4]. However, the ethanol concentration influenced the EY, with an increase in ethanol concentration (50–70%) resulting in an EY elevation from 9.44 to 17.11% [Table 4]. When the maceration time increased from 3 to 6 h, EY decreased from 14.99 to 11.56% [Table 4]. As shown in Figure 1, increasing the ethanol concentration improved

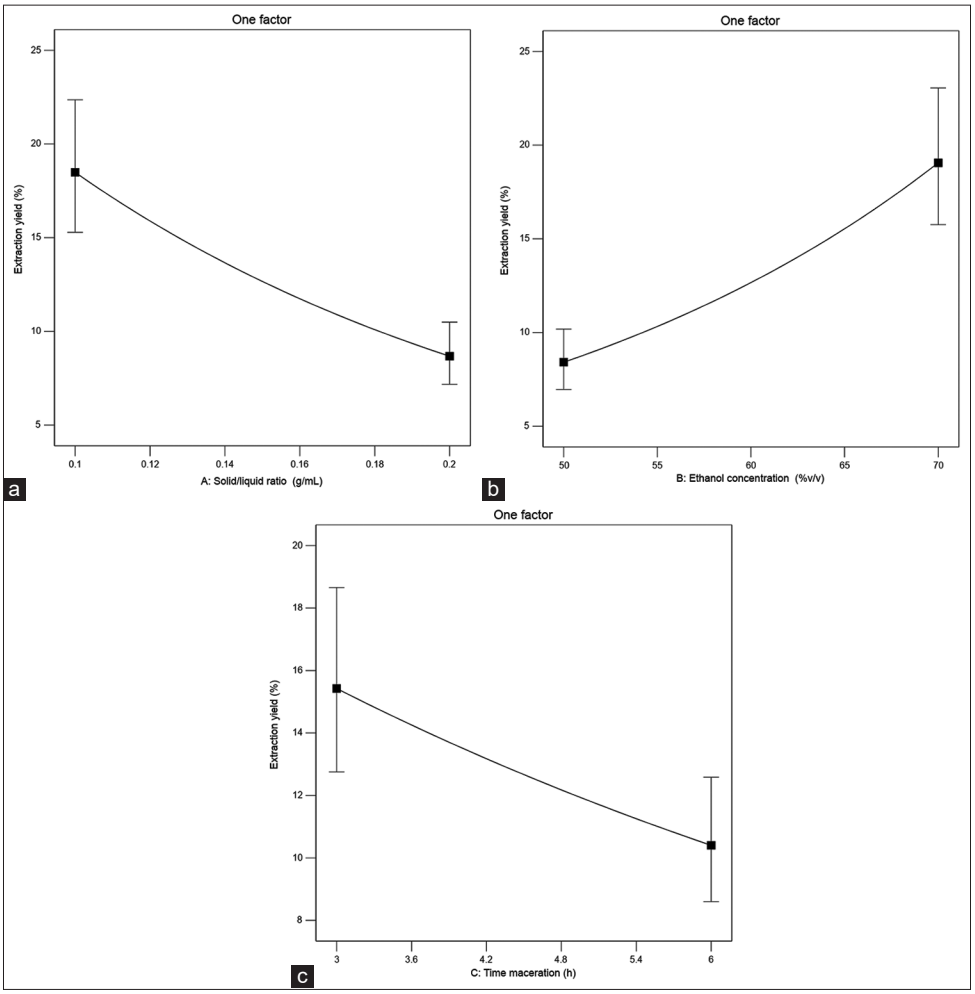

Figure 1: Effect of solid/liquid ratio (a), ethanol concentration (b), and time maceration (c) on the extraction yield of *Curcuma longa* rhizome.

Table 2: *P*-value of extraction yields and curcuminoids content againts factors.

Factors	*P*-value for responses	
	Extraction yields (%)	Curcuminoids (%)
A-Solid/liquid ratio (g/mL)	0.0028	0.0899
B-Ethanol concentration (%)	0.0019	0.0005
C-Time maceration (h)	0.0444	0.2369

Table 3: Percentage contribution of factors on extraction yields and curcuminoids responses.

Factors	Contribution (%)	
	Extraction yields	Curcuminoids
A-Solid/liquid ratio (g/mL)	30.61	4.45
B-Ethanol concentration (%)	35.73	66.03
C-Time maceration (h)	8.30	1.82
AB	7.52	7.08
AC	0.02	5.96
BC	10.07	0.17
ABC	0.06	0.37

Table 4: Design matrix, yield, and curcuminoid responses of a two-level factorial design.

Run	Factors			Responses	
	A	B	C	Yield	Curcuminoids
	(g/mL)	(%v/v)	(h)	(%)	(%)
1	0.2	70	6	14.05	16.37
2	0.2	50	6	2.86	1.80
3	0.15	60	4.5	14.68	9.57
4	0.15	60	4.5	13.19	12.03
5	0.2	50	3	6.21	1.11
6	0.15	60	4.5	16.30	18.50
7	0.1	50	3	20.29	6.53
8	0.1	70	6	20.90	9.53
9	0.1	50	6	8.41	2.66
10	0.15	60	4.5	16.78	7.33
11	0.1	70	3	19.76	21.59
12	0.2	70	3	13.72	16.14

A: Solid/liquid ratio (g/mL); B: Ethanol concentration (%); and C: Time maceration (h).

the EY, while increasing the solids-to-liquids ratio and maceration time had the opposite effect. The findings of this study concur with

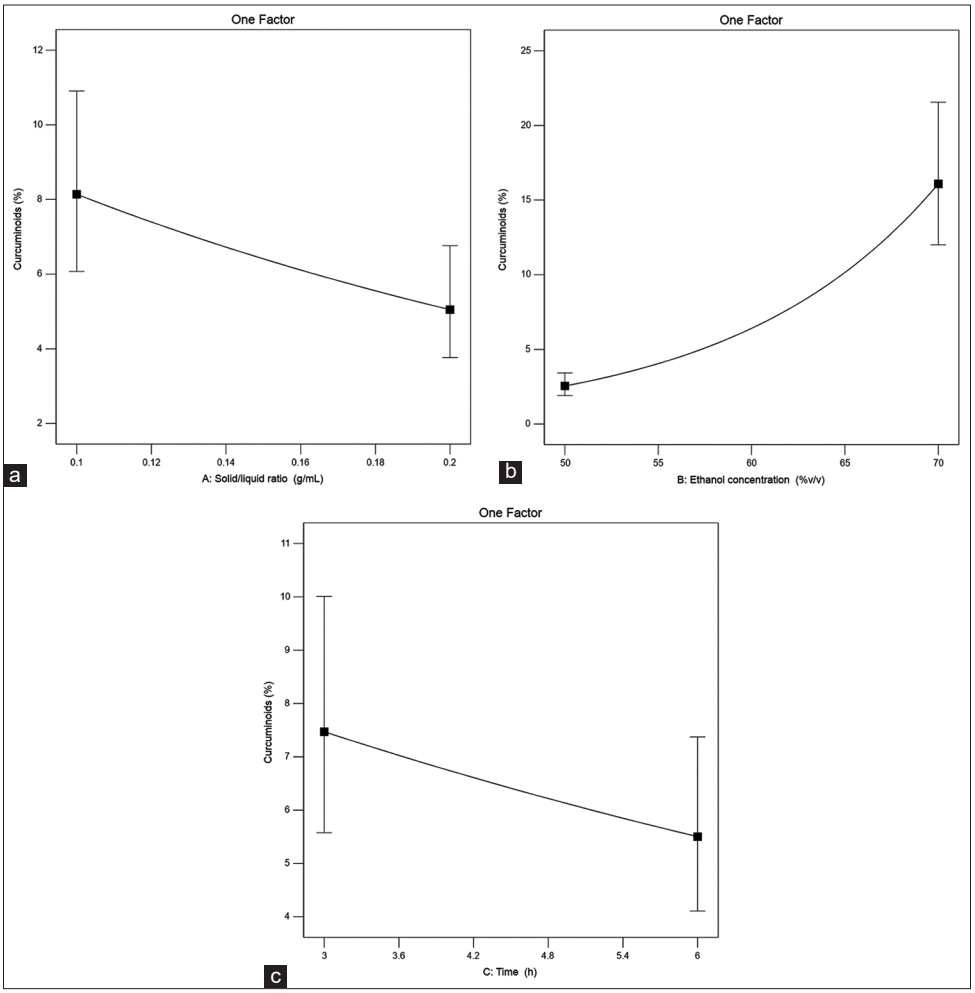

Figure 2: Effect of solid/liquid ratio (a), ethanol concentration (b), and time maceration (c) on the curcuminoids content of *Curcuma longa* rhizome.

those of previous investigations, which have demonstrated that the utilization of ethanol as a solvent for EYs in *C. longa* rhizome results in a greater quantity of extract compared to other organic solvents, such as chloroform and ethyl acetate [24].

3.3. Influence of Extraction Factors on CC

The extraction of curcuminoids from *C. longa* rhizomes was significantly ($P < 0.05$) influenced by ethanol concentration compared to the solid/liquid ratio and maceration time [Table 2]. This factor contributed to a 66.03% increase in the CC, as demonstrated in Table 3. The concentration of curcuminoids decreased from 10.08 to 8.86% and from 11.34 to 7.59% as the solid/liquid ratio (0.1–0.2 g/mL) and time maceration (3–6 h) factors were increased, respectively [Table 4]. In contrast, ethanol concentration had a significant impact on the extraction of curcuminoids. Specifically, an increase in ethanol concentration within the range of 50-70% resulted in a notable increase in CC from 3.02% to 15.91%, as indicated in Table 4. The CC decreased with an increase in the solid/liquid ratio and maceration period [Figure 2]. The increase in the CC with increase ethanol concentration is shown in Figure 2. The findings of this study demonstrated a higher concentration of curcuminoids compared to the previous investigations that employed 25% ethanol in the maceration method, which resulted in a CC of 0.84% in *C. longa* [28]. An ultrasound-assisted approach using ethanol at 35°C for 1 h resulted in a curcumin yield of 72%, which was higher than that obtained using methanol, acetone, and ethyl acetate [2]. The present study and relevant literature have identified various factors that affect the extraction of curcuminoids in turmeric rhizomes, such as the solvent, solid to solvent ratio, duration of extraction, and extraction method employed. Recent advancements in curcuminoid extraction research have highlighted the efficacy of ultrasonic-assisted extraction, which increases curcumin yields at the lower temperatures and with less ethanol, thus offering an energy-efficient alternative [29]. Concurrently, the use of ethanol modified by subcritical solvent extraction was found to enhance the solubility and recovery of curcuminoids from turmeric [28], indicating that these innovative techniques could lead to more sustainable and effective extraction methods, warranting further investigation for industrial application optimization.

4. CONCLUSIONS

The implementation of the two-level factorial design was effectively carried out, leading to the identification of significant factors that contribute to an increased yield and higher content of curcuminoids from *C. longa* rhizome extractions through maceration. It was observed that ethanol concentration and solid-to-liquid ratio were pivotal in optimizing the EY from *C. longa* rhizomes. In contrast, variations in the solid-to-liquid ratio and the length of maceration time did not exhibit a significant effect on the yield of curcuminoids. Notably, ethanol concentration has been identified as a decisive factor

influencing the efficacy of the curcuminoid extraction. These findings underscore the necessity for further investigative experiments to refine and optimize the extraction parameters, aiming to maximize the recovery of curcuminoid compounds and improve the overall effectiveness of the maceration method.

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6. AUTHOR CONTRIBUTIONS

All authors have made a significant contribution to the work reported, which includes, but is not limited to, their involvement in the conception, study design, execution, acquisition of data, analysis, and interpretation as well as their participation in drafting, revising, and critically reviewing the article. They have also given final approval for the version to be published and agreed on the journal to which the article has been submitted. Moreover, they acknowledge accountabilities for all aspects of their work.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

12. PUBLISHER'S NOTE

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