Original Article

International Journal of Research in Pharmaceutical Sciences

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Optimization of phenolic extraction from *Amomum compactum* fruits using simplex centroid design

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Article History:

Received on: 11 Nov 2020
Revised on: 12 Dec 2020
Accepted on: 16 Dec 2020

Keywords:

Amomum compactum Sol. Ex Maton,
extract yield,
phenolic extraction,
simplex centroid design,
extraction efficiency

Abstract

Cardamom (*Amomum compactum* Sol. Ex Maton) is a medicinal plant that belongs to the family *Zingiberaceae*. Phenolic compound detected in Cardamom fruit that responsible for several pharmacological activities. Optimization used the simplex centroid design for the yield and phenolic extractions of Cardamom fruit through maceration to optimize the mixing of water, acetone, methanol, and ethanol. The extraction yield is determined by weighing the extracted material compared to the whole sample. Total phenolic content was measured by a spectrophotometric method using Folin-Ciocalteu reagent. The results show that the best for relating extract yield is the linear model, while the quadratic model is the best to connect total phenolic content response. Water (100%) solvent extraction on Cardamom fruits obtained the highest extract yield (10.52%) and the lowest extract yield from 100% ethanol. The extraction of phenolic compounds with a mixture of water (50%)/ethanol (50%) resulted in maximum total phenolic content (168.98 mg GAE/g). However, the 100% ethanol of solvent extraction noted the minimum phenolic content (93.15 mg GAE/g). The results show that we should carefully choose the solvent mixture extraction to achieve the extract yield and phenolic extraction goals. This study first reported an optimization study on phenolic compounds extracted from Cardamom fruit.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11iSPL4.4603

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INTRODUCTION

Fruit and vegetable-rich diets are associated with a beneficial function in many human diseases, and the phenolic content of plants is linked to these benefits (Rio *et al.*, 2013). Evidently, because of their beneficial health effects, phenolic compounds have attracted interest in recent years. Phenolic compounds have been extensively studied among the secondary metabolites of different plant species due to their neuroprotection, antioxidant, anti-hyperglycemic, osteoprotection, immunomodulation and anti-inflammatory effects in humans (Chang *et al.*, 2021). Furthermore, various fruits have various phenolic profiles (Nguyen *et al.*, 2019). Their possible positions in human health vary, therefore.

Amomum compactum Sol. Ex Maton (Cardamom), a common medicinal plant in the family Zingiberaceae, has been widely cultivated for its fruit to produce spice and herb. Cardamom fruits have been used for several applications in medicines, food, and culinary. Indeed, Cardamom fruits intake is associated with a variety of health benefits, including anticancer, antioxidant, antibacterial, antidiabetic, insecticidal and gastro-protective activities (Ashokkumar *et al.*, 2020). Besides being a source of essential oils, cardamom fruits are also among the most common sources of phenolic compounds (Paul and Bhattacharjee, 2018). Phenolics of Cardamom fruits are medicinally crucial because of their antioxidant (Bhatti *et al.*, 2010), antibacterial (Garg *et al.*, 2016), and antimutagenic (Saeed *et al.*, 2014) properties.

Different factors, including particle size, temperature, time, solvent polarity, and solvent concentration, usually influence plant compounds extraction from the plant material (Qomaliyah *et al.*, 2019). Besides that, the solvents of various polarities, different phytochemicals are extracted depending on the chemical composition, as no single solvent can be useful in extracting all the phytochemical compounds present in the plant material. Therefore, the use of a solvent mixture, which can be binary, ternary, or even multi-component combination, is also convenient. In this method, synergistic effects may be observed between solvents, which can yield in the phytochemical's extraction with various compound characteristics. Studies show that the phenolic yield in plant material has an essential impact on solvent polarity combinations (Panzella *et al.*, 2020). Moreover, the value of that is already known; solvent optimization to obtain extracts rich in phenolic compounds from Cardamom fruit is not yet found in the literature.

Optimization of extraction of essential oil compounds (Suttiarporn *et al.*, 2020) from Cardamom fruit has been widely documented, but little attention has been paid to the use of solvent mixtures to optimize phenolic yield. Therefore, this study aimed to determine the effect of different solvent mixtures on the Cardamom fruit maceration process and assess its impact on the yield of extraction and total phenolic content.

MATERIALS AND METHODS

Samples preparations

Cardamom fruits were collected from a local farmer in Bogor, West Java, Indonesia (6°43'30.4"S; 106°41'40.6"E; altitude of 1267 m) and identified in Tropical Biopharmaca Research Center, Bogor Agricultural University, Indonesia (BMK0472052020). Before being milled into powder and prepared for future use, the samples were sun-dried for five days to a constant weight. Analytical grades are both solvents (water, acetone, methanol, and ethanol) and reagents used and supplied by both Sigma-Aldrich (Sigma Co., USA) and Merck. Equipment, such as the weighing balance, was well-calibrated before use according to the manufacturers' requirements.

Experimental design

The simplex centroid design under mixture method, provided by Design-Expert program (version 11.0), was used to assess the influence of the solvent composition (a mixture of water (A), acetone (B), methanol (C), and ethanol (D)) on the phenolic extraction from Cardamom fruits. The component levels (water, acetone, methanol, and ethanol) were 0 and 100 percent (Table 1). The program then produced fifteen experimental runs, which reflect a mixed percentage of solvent composition by different portions.

Extraction procedure and determination of extract yield and total phenolic content

Extracts were made by the maceration method in a 100 ml extraction unit containing the selected solvents (water, acetone, methanol, and ethanol), based on the simplex centroid design as presented in Table 2. Briefly, 10 g of sample mixtures were extracted with 100 ml of a solvent mixture. Under light cover, the samples were stirred at 140 rpm for 30 min. Then, the sample mixtures were macerated for 24 h in the darkroom. The solution was filtered using Whatman No. 4 filter paper. The extraction process was repeated, and solutions were collected. Finally, each sample's solutions were concentrated in rotary vacuum evaporator (HAHNVAPOR, Korea) to give the actual extract yield. The extracts were collected and weighed to determine the percentage of extraction yield.

The extracts obtained were examined for total phenolic content. Total phenolic extracts were quantitated with the Folin-Ciocalteu reagent adapted from previously reported method (Khumaida *et al.*, 2019). Gallic acid has been used as an external standard. Briefly, extract sample (10 ml) was blended with 10 ml of 10% Folin-Ciocalteu, 160 ml of distilled water, and 20 ml of 10% Na_2CO_3. Mixtures were then incubated for 30 min at room temperature. Finally, absorption samples or standards were eventually measured using the microplate reader (Epoch BioTek, USA) at 750 nm. Results expressed as gallic acid equivalent (mg GAE/g extract).

Statistical data analysis

The data response from Cardamom fruits extraction experiments were statistically analyzed using Design-Expert software version 11.0 (Stat-Ease Inc., Minneapolis, USA).

RESULTS AND DISCUSSION

Optimization of Cardamom fruits extract

Extract yield response of solvent optimization of Cardamom fruits extraction are presented in Table 2. Extract yield ranged from 2.77 to 10.52% in actual value and varied from 3.01 to 9.96% in predicted value. The maximum extract yield (10,52%) was provided by 100% water composition (component A) of the experiment run 11. Run 14 indicates an extract yield of 8,68%, consisting of 100% acetone composition (component B), but the solvent's use might not be seen as cost-efficient compared to a water solvent.

Table 1: Simplex centroid design factors and their levels

Component	Name	Level (%)	
		Low	High
A	water	0	100
B	acetone	0	100
C	methanol	0	100
D	ethanol	0	100

Extract phenolic content of Cardamom fruits extraction ranged from 77.87 to 168.98 mg GAE/g in actual value, while in predicted value varied from 80.61 to 155.33 mg GAE/g extract (Table 2). The experimental Run 7 was a mixture of water (50%) and ethanol (50%) (components A and D) that gave the highest total phenolic content (168.98 mg GAE/g), even though the mixture of acetone (50%) and methanol (50%) (component B and C) under Run 9 also provided relatively good phenolic content of 139.91 33 mg GAE/g but the use of the two solvents cannot be considered a cost-efficiency.

Higher polar compounds are generally readily extracted by water, whereas strongly hydroxylated phenolic compounds such as catechins are more soluble in alcohols such as ethanol and methanol (Loarce *et al.*, 2020). As a pure solvent, acetone was inefficient in recovering the compounds of interest. Thus, considering that non-polar solvents, such as acetone, chloroform, and ethyl acetate, have a greater affinity with low-polarity compounds (Wakeel *et al.*, 2019), it is believed that some of these phytochemical compounds have been detected in the Cardamom fruits extracts analyzed. On the other hand, the combination of various solvents has created fascinating synergistic results. The highest yield obtained from polar solvents in this study can suggest that some of the Cardamom components contain several polar metabolites, resulting in high outcomes. Polyphenol, such as phenolics and flavonoids, are reported as essential polar compounds in cardamom fruits (Deepa *et al.*, 2013). Besides, the yield of non-polar solvents in this work is relatively low so that they are no better than polar solvents. A relatively high phenolic yield was provided by experimental runs involving the mixture of non-polar and polar solvents, especially methanol, which may indicate the influence of polar solvents and the affinity of Cardamom fruit components with non-polar solvents (Amma *et al.*, 2015). For phenolic compounds, extraction well recommended polar solvents such as methanol (Zhang *et al.*, 2020), but they were not well-preferred when the extract intended for human and medicinal purposes. Hence, this study preferred polar solvents such as water and ethanol.

Analysis of variance (ANOVA)

The ANOVA of extract yield and total phenolic content responses of optimization of Cardamom fruit extraction is presented in Table 3. ANOVA of the findings showed significant for extract yield and not significant for total phenolic content of Cardamom fruits affected by mixture solvent extraction at $p < 0.05$. Water/ethanol mixture (AD component) is a vital model term and has powerful effects on phenolic yield based on high F-value and low p-value. The determination coefficient (R^2) of extract yield and total phenolic content were 0.6849 and 0.7797, respectively. These analyses indicated the reliability of the data distribution in the experiment. The Adeq Precision of extract yield and total phenolic content were 10.3849 and 5.4189, respectively. This result showed Adeq Precision exceeding four. Therefore, this study indicates an adequate signal encouraging model use (Araromi *et al.*, 2017).

The linear model generated describing the impact of interactions between the A (water), B (acetone), C (methanol), and D (ethanol) on the extract yield and total phenolic content is defined as the coded value in equation 1 and 2, respectively. In extract yield response, the value of the equations' coefficients was 9.96, 6.59, 5.51, and 3.01 obtained for water, acetone, methanol, and ethanol, respectively. This analysis indicates that the solvents have positive effects on the extract yield of Cardamom fruits. Water > acetone > methanol > ethanol is shown in order of impact and this is consistent with extract yield 10.52%, 8.68%, 5.97%, and 2.77% (Table 2) experimental results achieved for the 100% composition of water, acetone, methanol, and ethanol, respectively. For total phenolic response, the coefficients 104.71, 80.61, 125.16, and 96.63 achieved for water, acetone, methanol, and ethanol, respectively, indicate that the solvents have positive results

Table 2: Matrix of experimental design and responses for extraction yield and total phenolic content in Cardamom fruits extracts by simplex centroid design

Run	Components composition (%)				Extract yield (%)		Total phenolic content (mg GAE/g)	
	Water (A)	Acetone (B)	Methanol (C)	Ethanol (D)	Practical	Predicted	Practical	Predicted
1	0.00	0.00	50.00	50.00	3.98	4.26	98.06	88.88
2	50.00	0.00	50.00	0.00	7.87	7.74	118.61	102.68
3	0.00	0.00	0.00	100.00	2.77	3.01	93.15	96.63
4	0.00	50.00	0.00	50.00	4.38	4.80	101.48	97.53
5	50.00	50.00	0.00	0.00	5.13	8.27	114.17	103.48
6	0.00	0.00	100.00	0.00	5.97	5.51	121.11	125.16
7	50.00	0.00	0.00	50.00	7.33	6.49	168.98	155.33
8	0.00	33.33	33.33	33.33	4.51	5.04	110.46	108.66
9	0.00	50.00	50.00	0.00	5.13	6.05	139.91	133.68
10	25.00	25.00	25.00	25.00	5.79	6.27	115.19	119.51
11	100.00	0.00	0.00	0.00	10.52	9.96	99.54	104.71
12	33.33	33.33	33.33	0.00	7.88	7.35	103.15	116.54
13	33.33	33.33	0.00	33.33	7.33	6.52	118.8	127.05
14	0.00	100	0.00	0.00	8.68	6.59	77.87	80.61
15	33.33	0.00	33.33	33.33	6.75	6.16	97.87	117.90

Table 3: ANOVA for the linear model of extract yield and quadratic model of total phenolic content

Source	Extract yield (linear model)					Total phenolic content (quadratic model)				
	Sum of squares	DF	Mean square	F-value	P-value	Sum of squares	DF	Mean square	F-value	P-value
Model	40.12	3	13.37	7.97	0.0042*	5048.07	9	560.90	1.97	0.2362
Linear mixture	40.12	3	13.37	7.97	0.0042*	638.93	3	212.98	0.75	0.5690
AB	-	-	-	-	-	103.13	1	103.13	0.36	0.5738
AC	-	-	-	-	-	132.47	1	132.47	0.46	0.5258
AD	-	-	-	-	-	2635.29	1	2635.29	9.24	0.0288*
BC	-	-	-	-	-	836.10	1	836.10	2.93	0.1475
BD	-	-	-	-	-	70.04	1	70.04	0.25	0.6412
CD	-	-	-	-	-	427.53	1	427.53	1.50	0.2754
Residual	18.46	11	1.68		-	1426.03	5	285.21	-	-
Cor total	58.58	14	-	-	-	6474.09	14	-	-	-

*Significant at 95% confidence interval

Table 4: Numerical optimization solution for yield extract and total phenolic content

Water	Acetone	Methanol	Ethanol	Yield extract	Total phenolic content	Desirability
0.724	0.000	0.000	0.276	8.045	146.132	0.714

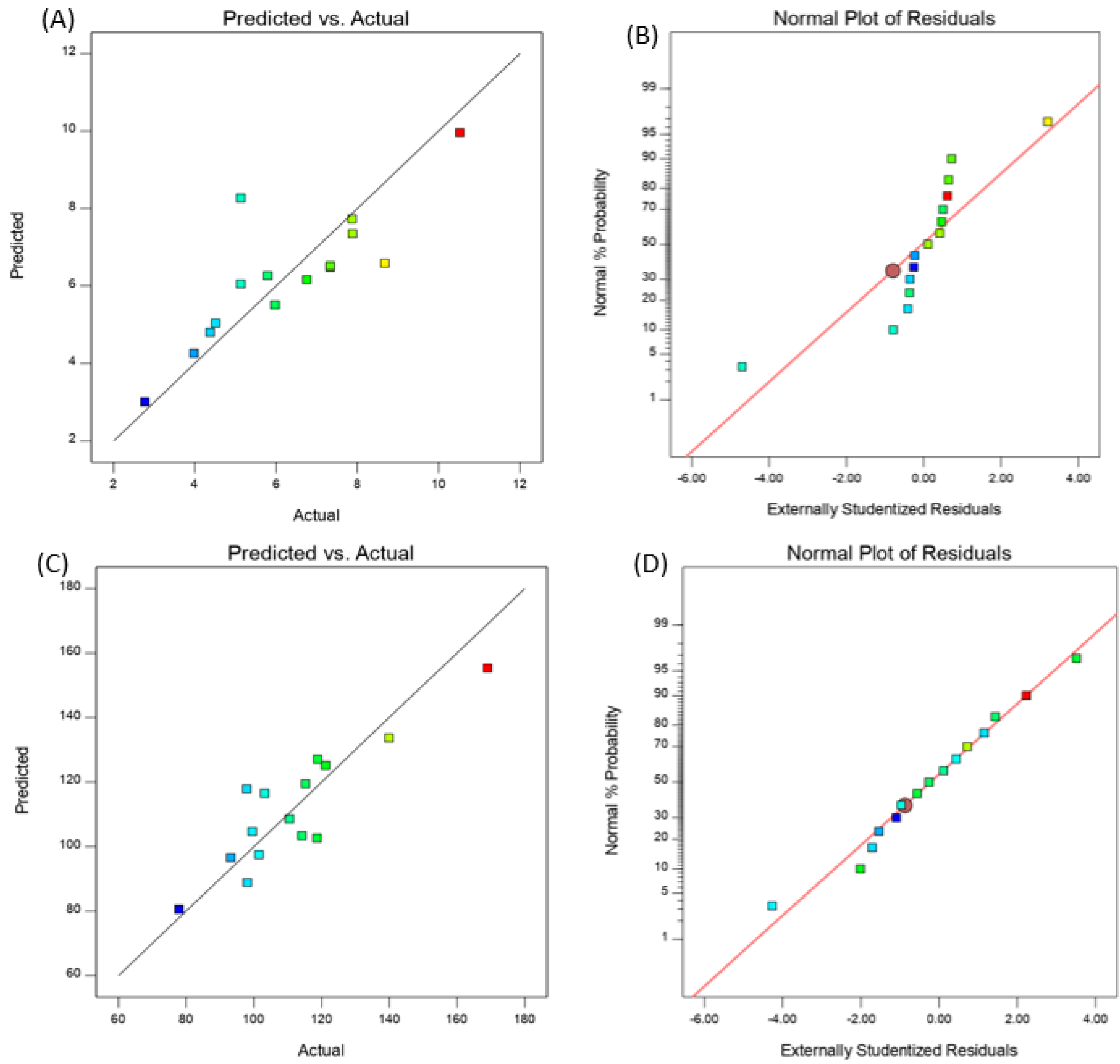

Figure 1: Comparison of actual values with predicted values for extract yield (A) and normal plot of residual for extractyield (B) and total phenolic content (C) and total phenolic content (D)

on the total phenolic content of Cardamom fruits.

The order of impact given by methanol > water > ethanol > acetone, consistent with the total phenolic yield of 121.11 mg GAE/g, 99.54 mg GAE/g, 93.15 mg GAE/g, and 77.87 mg GAE/g obtained from experimental methods (Table 2) of 100% composition methanol, water, ethanol, and acetone. Moreover, the mixtures of water/methanol, water/ethanol, acetone/methanol, and acetone/ethanol indicate positive impacts of 43.26, 218.66, 123.16 and 35.65 on the total phenolic yield of Cardamom fruits.

On the other hand, the mixtures of water/methanol and methanol/ethanol show negative impact of -49.02 and -88.07 on the total phenolic yield of Cardamom fruits. That means that the use of any solvent mixture is not favourably promoted towards the statistical of the phenolic yield of cardamom fruits, which is generally supported experimentally (Martínez-Ramos *et al.*, 2020).

$$\text{Yield (extract)} = 9.96A + 6.59B + 5.51C + 3.01D \quad (1)$$

$$\begin{aligned} \text{Yield (total phenolic)} &= 104.71A + 80.61B \\ &+125.16C + 96.63D + 43.26AB - 49.02AC \\ &+218.66AD + 123.16BC + 35.65BD - 88.07CD \end{aligned} \quad (2)$$

where A = water, B = acetone, C = methanol, and D = ethanol

The relation between real and expected plots of values (Figure 1A and Figure 1C respectively for extract

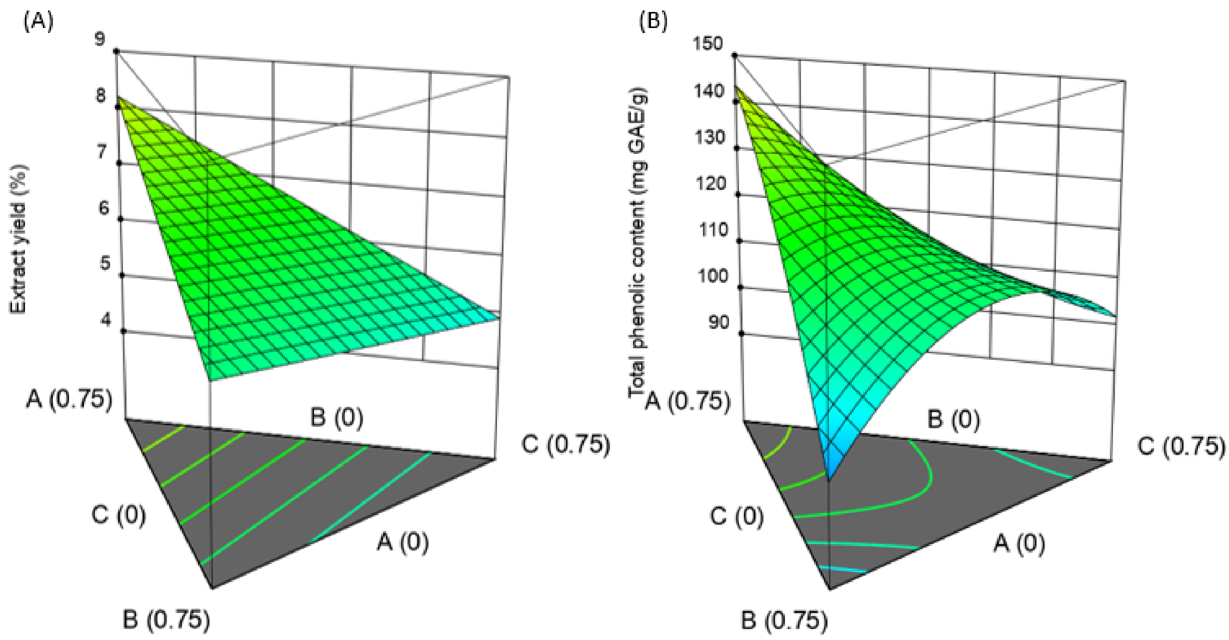

Figure 2: Response surface plots on the impact of solvent mixtures on extract yield (A) and total phenolic content (B) of Cardamom fruits extraction

yield and total phenolic contents) is relatively low, as shown in the proximity of these points to the usual line of origin. Figure 1B (extract yield) and Figure 1D (total phenolic content) showed less stacking of the values, but there is a large disjoint above the 90% normal likelihood. Figure 1 (A-D) demonstrates the suitability of models for extract yield and total phenolic responses of Cardamom fruits extraction.

Optimization solution of extract yield and total phenolic content of Cardamom fruits

The experimental set of variables tested the best numerical optimizing solution is typically chosen based on the desirability of 1.00 as desirable as the case may be (Khalafyan *et al.*, 2019). The maximum yield of extract and total phenolic content was obtained from 100% water (10.52%) and a mixture of water-ethanol (168.98 mg GAE/g). The optimum solution was obtained at 71% desirability, which gives 8.045% for extract yield and 146.132 mg GAE/g for total phenolic content with 72% water and 28% ethanol solvents their optimum volume (Table 4).

Graphical interaction of the solvent mixture on the extraction of Cardamom fruits

Figure 2A demonstrates the response surface plot for the effect of the interaction of water (A), acetone (B), and methanol (C) on extract yield while retaining ethanol at 25%. On the other hand, the interaction between water (A), acetone (B), and methanol (C) on the total phenolic content, while the ethanol is 25%, is shown in Figure 2B. The curvature existence of all surface plots in Figure 2 shows the reciprocal interactions between the solvents examined for extract yields and total phenolic content of Cardamom fruits extraction.

CONCLUSIONS

The optimization of the mixing of four solvents (water, acetone, methanol, and ethanol) for the extraction of the phenolic from Cardamom fruits using the maceration method is reported for the first time in this work. The optimization experiments were based on the simplex centroid design, which indicates that 100% water and a mixture of water-ethanol (50%-50%) is better suited for maximum extract yield (10.52%) and total phenolic content (168.98 mg GAE/g), respectively, of Cardamom fruits extraction. The software's proposed optimization approach shows that the best possible result is obtained from mixtures of 72% water and 28% ethanol, with the desirability of 71%. This research has shown the ability to extract phenolic from cardamom fruits with suitable solvents.

Conflict of interest

The authors declare that they have no conflict of interest

Funding support

This study was funded by the PDUPT research program of IPB University (4026/IT3.L1/PN/2020).

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