Optimization of ultrasound-assisted extraction of bioactive compounds from coffee pulp using propylene glycol as a solvent and their antioxidant activities

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ABSTRACT

In the cosmetic and pharmaceutical industries, it has been increasingly popular to use alternative solvents in the extraction of bioactive compounds from plants. Coffee pulp, a by-product of coffee production, contains different phenolic compounds with antioxidant properties. The effects of polyols, amplitude, extraction time, solvent concentration, and liquid–solid ratio on total phenolic content (TPC) using ultrasound-assisted extraction (UAE) were examined by single-factor studies. Three main factors that impact TPC were selected to optimize the extraction conditions for total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), and their antioxidant activities using the Box-Behnken design. Different extraction methods were compared, the bioactive compounds were identified and quantified by liquid chromatography triple quadrupole mass spectrometer (LC-QQQ), and the cytotoxicity and cellular antioxidant activities of the extract were studied. According to the response model, the optimal conditions for the extraction of antioxidants from coffee pulp were as follows: extraction time of 7.65 min, liquid–solid ratio of 22.22 mL/g, and solvent concentration of 46.71 %. Under optimized conditions, the values of TPC, TFC, TTC, 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging assay, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging assay, and Ferric reducing antioxidant power assay (FRAP) were 9.29 ± 0.02 mg GAE/g sample, 58.82 ± 1.38 mg QE/g sample, 8.69 ± 0.25 mg TAE/g sample, 7.56 ± 0.27 mg TEAC/g sample, 13.59 ± 0.25 mg TEAC/g sample, and 10.90 ± 0.24 mg FeSO$_4$/g sample, respectively. Compared with other extraction conditions, UAE with propylene glycol extract (PG-UAE) was significantly higher in TPC, TFC, TTC, DPPH, ABTS, and FRAP response values than UAE with ethanol (EtOH-UAE), maceration with propylene glycol (PG-maceration), and maceration with ethanol (EtOH -maceration) ($p < 0.05$). Major bioactive compounds detected by LC-QQQ included chlorogenic acid, caffeine, and trigonelline. At higher concentrations starting from 5 mg/ml, PG-UAE extract showed higher cell viability than EtOH-UAE in both cytotoxicity and cellular antioxidant assays. The researcher expects that this new extraction technique developed in this work could produce a higher yield of bioactive compounds with higher biological activity. Therefore, they can be used as active ingredients in cosmetics (anti-aging products) and pharmaceutical applications (food supplements, treatment for oxidative stress-related diseases) with minimal use of chemicals and energy.

1. Introduction

As the world population ages, the consequences of the skin aging process are inevitable. Skin aging is a complex biological and physiological process that is contributed by intrinsic factors such as genetics, hormones, and other cellular metabolisms. Extrinsic factors include constant exposure to the sun, air pollution, and chemicals. Both factors are capable of inducing the formation of free radicals, resulting in skin damage [1]. In an era of population aging, this skin aging phenomenon is regarded as an issue that has a huge psychosocial impact, such as self-confidence, the desire to look young, etc. [2,3]. Therefore, with an increase in the desire of people to look young as well as social pressure, a better understanding of the skin aging process and effective therapeutic interventions demand greater attention. Among various interventions, antioxidants are substances that halt or prevent oxidative damage caused by free radicals to corresponding molecules or cells by

neutralizing free radicals. They exert their effects by forming protective skin barriers against UV rays, suppressing melanogenesis, stimulating collagen synthesis, decreasing skin roughness, and inhibiting the action of destructive enzymes and mutation [4]. Therefore, antioxidants have been increasing used orally or topically for skin aging interventions.

Nowadays, coffee, especially arabica coffee (*Coffea arabica*), has become one of the most important cash crops. Adam et al. (2020) reported that the manufacture of global coffee has been raised from 9.5 million tons in 2017–2018 to 10.2 million tons in 2018–2019 and arabica coffee *(C. arabica)* accounts for 60 % of the global coffee production [5]. After the wet production process, there are by-products of coffee fruit processing; coffee pulp is the main residue, accounting for 30 % of the total weight of dry coffee cherries [6]. Up to 9.4 million tons of coffee pulp are produced every year [7]. These coffee pulp residues are generally disposed of as agricultural wastes. Consequently, this might have a serious impact on the environment. However, coffee pulp contains different varieties of phenolic compounds such as chlorogenic acid [8], anthocyanin [9], flavonoids [10], etc. that have health benefits, including antioxidant effects [11]. The valorization of coffee pulp as functional ingredients in cosmetic and pharmaceutical applications has been an interesting strategy to prevent the adverse effects of coffee pulp disposal on the environment.

Ultrasound-assisted extraction is an extraction technique that uses ultrasound waves to separate the desired components from various plants. It has several benefits: higher extraction efficiency, consumes lower energy, extracts higher quality and higher yield than the conventional methods [12]. Recently, this technique has been used to extract phenolic compounds from grapes [13], *Deverra scoparia* flower [14], waste spent coffee ground [15], lycopene from tomato paste [16], bioactive compounds from *Jatropha curcas* seed [17], etc. As another concern in the extraction process, when bioactive compounds from plants are extracted with organic solvents, the organic solvents may need to be separated from the extract due to the safety concerns about the residues of these organic solvents in the extract. It is an energy and time-consuming process. To solve this problem, polyols such as propylene glycol and glycerin can be considered as alternative solvents for plant extracts in the cosmetic industry as they can be used for cosmetic formulation and are known as generally recognized as safe (GRAS) chemicals by the U.S. Food and Drug Administration (FDA).

Recently, some studies have examined the effects of ultrasound-assisted extraction of coffee pulp with different types of solvents such as water [18], ethanol [18], and their mixture [18,19]. However, the study of the effects of ultrasound-assisted extraction of coffee pulp with polyols on bioactive compounds and their antioxidant activities is still limited. Therefore, this study aims to: evaluate the effects of ultrasound-assisted extraction using polyols, optimize the conditions that affect bioactive compounds and antioxidant activities of coffee pulp using response surface methodology (RSM), compare ultrasound-assisted extraction method and conventional extraction method in terms of the bioactive compounds, antioxidant activities, and phenolic compositions using polyol and conventional solvent, and assess the cytotoxicity and cellular antioxidant activities of the extracts to be used as functional ingredients in cosmetic and pharmaceutical applications.

2. Materials and methods

2.1. Coffee cherries pulp preparation

Fresh coffee cherry pulp (*Coffea arabica*) was collected during the crop season (February 2021) from Baan Doi Chang, Mae Suai District, Chiang Rai, Thailand. The fresh coffee cherry pulp was dried at 60 °C by using a tray dryer for 72 h. Then, a grinder was used to grind the coffee pulp into a fine powder, which was stored at room temperature until further use.

2.2. Ultrasound-assisted extraction

The bioactive compounds from coffee pulp were extracted using the method described by [20] with minor modification. An ultrasonic processor device (VCX 130, Vibra cell, Sonics, USA) with a 6 mm probe and a constant 20 kHz was used for the extraction process of coffee pulp. During the extraction process, the probe was immersed 2 cm in the extraction medium. The sonication process was performed by using 50 % of the duty cycle, which was achieved by sonication for 20 s, followed by a rest period of 20 s [21]. After the extraction, the resultant mixture was separated using centrifugation at 2490xg for 15 min [15].

2.3. One-factor-at-a-time

The one-factor-at-a-time (OFAT) approach was used to select appropriate factors among five independent variables. The experimental factors and their levels via OFAT experiments were: extraction solvents (propylene glycol, glycerin, butylene glycol, and water), amplitude (20 %, 30 %, and 40 %), extraction time (5 min, 10 min, 15 min, 20 min, 25 min, and 30 min), solvent concentration (20 %, 40 %, 60 %, 80 %, and 100 %), and liquid–solid ratio (10:1, 15:1, 20:1, 25:1, and 30:1). One independent factor was varied while the other variables were kept constant. TPC was evaluated to select the independent variables that had a significant influence on the extraction efficacy.

2.4. Experiment design for optimization of extraction method

Based on the variance ranges of each independent factor obtained from the OFAT experiments, optimization of extraction conditions for the bioactive compounds and their antioxidant properties from the coffee pulp (CP) was carried out using RSM with the Box-Behnken experiment. Variables of extraction time (X_1, min), liquid–solid ratio (X_2), and solvent concentration (X_3, %) at three coded variation levels were analyzed as shown in Table 1. The eighteen experiments were conducted to establish models for TPC, TFC, TTC, DPPH, ABTS, and FRAP.

The variation of the response values (Y) versus the 3 variables was fitted into a response surface model and presented as the following equation:

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

where X_i and X_j values are variables affecting the dependent responses Y; β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic, and interaction terms, respectively.

2.5. Determination of total phenolic content

The Folin-Ciocalteu's method described by [22] with minor modifications was used to determine the total phenolic content. Briefly, 20 μL of the diluted sample was mixed with 20 μL of Folin-Ciocalteu's reagent. After adding 100 μL of 7.5 % Na_2CO_3 solution, the mixture was incubated for 30 min in the dark at room temperature to allow its reaction. Then, the absorbance of the mixture was determined at 765 nm. Gallic acid was used as a standard and the results were presented as mg of gallic acid equivalent (GAE) per gram of sample.

Table 1
Independent variables at three coded variation levels for the Box–Behnken design.

Independent Variable	Label	Levels −1	0	1
Extraction time (min)	X_1	5	10	15
Liquid-solid ratio	X_2	10	20	30
Solvent concentration (%)	X_3	20	50	80

2.6. Determination of total flavonoid content

The method described by [23] with slight modifications was used to assess the total flavonoid content of coffee pulp extract. 20 μL of the diluted sample was mixed with 15 μL of 5 % $NaNO_2$ and kept in the dark for 5 min. After 15 μL of 10 % $AlCl_3$ solution was added to the reaction, it was incubated for 6 min at room temperature. The reaction was mixed with 100 μL of 1 M NaOH solution and kept for a further 10 min. The absorbance of the mixture was determined at 510 nm. Quercetin was used as a standard and the results were presented as mg of quercetin equivalent (QE) per gram of sample.

2.7. Determination of total tannin content

The method prescribed by [23] was slightly modified to determine total tannin content. Briefly, 25 μL of Folin-Ciocalteu's reagent was added to 20 μL of the diluted sample. After 50 μL of 35 % Na_2CO_3 solution was added to the reaction, the mixture was incubated at room temperature for 30 min. The absorbance of the mixture was measured at 700 nm. Tannic acid was used as a standard and the values of TTC were presented as mg of tannic acid equivalent (TAE) per gram of sample.

2.8. Antioxidant activities

2.8.1. 1,1-Diphenyl-2-picryl-hydrazil (DPPH) radical scavenging assay

A protocol reported by [22] was slightly modified to evaluate the DPPH radical scavenging assay. Briefly, 20 μL of the diluted samples was added to 135 μL of 0.1 mM DPPH solution and kept in a dark place at room temperature for 30 min. The absorbance of the mixture was determined at 517 nm. Trolox was used as a standard and the values were presented as mg of Trolox equivalent antioxidant capacity (TEAC) per gram of sample. The radical scavenging activity of the DPPH assay was evaluated as follows:

DPPH radical scavenging activity (%) = [(absorbance of control – absorbance of sample)/ absorbance of control] × 100.

2.8.2. 2,2′-Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging assay

The method stated by [22] was modified to evaluate the ABTS assay. 7 mM ABTS and 2.45 mM potassium persulfate were mixed to prepare $ABTS^{\bullet+}$ stock solution and incubated in the dark at room temperature for 16 h. The working solution was prepared by mixing 5 mL of stock solution of $ABTS^{\bullet+}$ and 75 mL of deionized water. 160 μL of $ABTS^{\bullet+}$ working solution was mixed with 10 μL of the diluted sample or Trolox (as a standard solution) and incubated in the dark at room temperature for 30 min. The absorbance of the mixture was determined at 743 nm. Trolox was used as a standard and the values were represented as mg of Trolox equivalent antioxidant capacity (TEAC) per gram of sample. The radical scavenging activity of the ABTS assay was calculated as follows:

ABTS radical scavenging activity (%) = [(absorbance of control – absorbance of sample)/ absorbance of control] × 100.

2.8.3. Ferric reducing antioxidant power assay (FRAP)

A previous protocol stated by [24] with minor modifications was used to evaluate the FRAP assay. The FRAP reagent was prepared by mixing 30 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution in the ratio of 10:1:1. The FRAP solution was freshly prepared before use. 10 μL of the diluted sample was mixed with 180 μL of the FRAP solution and kept at room temperature for 4 min. The absorbance of the mixture was determined at 593 nm. $FeSO_4$ was used as a standard and the values of the FRAP assay were presented as mg of $FeSO_4$ per gram of sample.

2.9. Validation of the model

According to the response model, a verification experiment was evaluated using the optimal extraction conditions of BBD to check the accuracy of the response model.

2.10. Comparison of UAE with conventional method and conventional solvent

A comparative study was carried out between UAE and a conventional method (maceration) using different solvents (propylene glycol or ethanol). The bioactive compounds (TPC, TFC, and TTC) and antioxidant activities (DPPH, ABTS, and FRAP) of these conditions were determined.

2.10.1. Ultrasound-assisted extraction with a polyol and a conventional solvent

Under optimal conditions for extraction obtained from BBD design, PG-UAE and EtOH-UAE were performed according to the protocol stated above. The mixtures were centrifuged, and the supernatants were collected for the analysis of the bioactive compounds and their antioxidant activities.

2.10.2. Maceration with a polyol and a conventional solvent

Maceration of coffee pulp was accomplished according to the method reported by [25] with slight modification. In brief, 1 g of coffee pulp was macerated in 46.71 % w/v of each solvent (propylene glycol or ethanol), with the liquid–solid ratio of 22.22 mL/g, using an incubator shaker at the speed of 100 rpm and 25 °C for 24 h. The mixtures were centrifuged, and the supernatants were collected for the analysis of the bioactive compounds and their antioxidant activities.

2.11. Identification and quantitation of bioactive compounds by LC-QQQ

The bioactive compounds in coffee pulp samples were determined according to a protocol stated by [26] with minor modification. A Nexera X2 UHPLC system (Shimadzu, Kyoto, Japan) coupled to an LCMS-8060 triple quadrupole mass spectrometer (QQQ) (Shimadzu, Kyoto, Japan) was operated in both positive and negative ionization modes. LabSolutions software (Shimadzu, Kyoto, Japan) was used to analyze the data of LC-QQQ. After that, the bioactive ingredients of the samples were substantiated by contrasting the precursor ions (m/z), product ions (m/z), and retention time (RT, min) as presented in Table 3. Bioactive compounds were quantified by peak areas of samples with the bioactive compound standards and the contents were presented as mg per gram of sample.

2.12. Cell culture

The NIH/3T3 fibroblasts (ATCC®CRL-1658[TM]) were purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 1 % penicillin/streptomycin solution, and maintained at 37 °C in a humidified incubator with 5 % CO_2 (Binder, model CB210, Germany). The cell passages from 17 to 25 were used in this study. All experiments in cell culture were carried out in triplicate.

2.12.1. Determination of appropriate hydrogen peroxide (H_2O_2) concentration

The assay was operated using a protocol stated by [27] with minor modifications. Hydrogen peroxide was used to induce oxidative stress in the NIH/3T3 fibroblasts at different concentrations (100 μM to 1000 μM) and culture medium was used as the control for 1 h. Then, the culture medium was removed and replaced with a serum-free culture medium and freshly prepared MTT solution. The cells were further incubated at 37 °C for 4 h. After discarding the medium containing MTT from each well, dimethyl sulfoxide (DMSO) solution was used to dissolve the remaining MTT-formazan crystals. The plate was incubated

Table 2
Box–Behnken design with predicted and actual responses of TPC, TFC, TTC, DPPH, ABTS, and FRAP.

Run	Extraction time (X_1, min) Actual	Coded	Liquid-solid ratio (X_2) Actual	Coded	Solvent concentration (X_3, %) Actual	Coded	TPC (mg GAE/g) Predicted	Actual	TFC (mg QE/g) Predicted	Actual	TTC (mg TAE/g) Predicted	Actual	DPPH (mg TEAC/g) Predicted	Actual	ABTS (mg TEAC/g) Predicted	Actual	FRAP (mg FeSO$_4$/g) Predicted	Actual
1	5	−1	10	−1	50	0	6.51	6.58	42.29	38.89	6.27	6.33	4.24	4.58	7.20	7.10	6.53	6.94
2	15	1	10	−1	50	0	5.68	5.63	37.74	31.27	5.64	5.59	4.30	4.17	6.40	6.24	5.83	5.93
3	5	−1	30	1	50	0	8.84	8.89	37.86	44.34	8.90	8.95	7.82	7.95	14.27	14.43	9.28	9.18
4	15	1	30	1	50	0	10.06	9.98	37.94	41.34	8.93	8.86	8.53	8.19	15.59	15.68	10.08	9.68
5	5	−1	20	0	20	−1	6.74	6.79	25.82	22.93	6.83	6.68	4.94	4.54	9.90	9.75	6.06	6.11
6	15	1	20	0	20	−1	6.67	6.85	21.09	21.27	6.02	5.98	4.93	4.99	9.34	9.26	6.02	6.38
7	5	−1	20	0	80	1	7.27	7.10	26.82	26.63	7.09	7.13	5.29	5.23	10.25	10.33	7.68	7.32
8	15	1	20	0	80	1	7.73	7.68	27.08	29.97	7.31	7.46	6.08	6.48	11.32	11.47	7.83	7.78
9	10	0	10	−1	20	−1	4.91	4.78	13.02	19.31	4.41	4.50	2.81	2.87	4.75	4.99	4.85	4.39
10	10	0	30	1	20	−1	7.78	7.67	19.69	16.10	7.11	7.21	7.52	7.79	12.77	12.76	6.87	6.92
11	10	0	10	−1	80	1	5.22	5.32	25.30	28.88	4.93	4.82	4.37	4.10	5.80	5.81	5.09	5.04
12	10	0	30	1	80	1	9.06	9.19	14.40	8.11	8.14	8.05	7.46	7.40	14.04	13.80	10.07	10.52
13	10	0	20	0	50	0	9.23	9.33	58.86	60.39	8.05	8.40	7.27	7.56	12.81	12.68	10.38	10.85
14	10	0	20	0	50	0	9.23	9.14	58.86	61.15	8.05	8.04	7.27	7.68	12.81	13.39	10.38	10.65
15	10	0	20	0	50	0	9.23	9.52	58.86	53.74	8.05	8.26	7.27	7.56	12.81	12.81	10.38	10.04
16	10	0	20	0	50	0	9.23	9.31	58.86	54.13	8.05	7.75	7.27	6.68	12.81	12.57	10.38	10.41
17	10	0	20	0	50	0	9.23	9.05	58.86	62.99	8.05	7.91	7.27	6.60	12.81	12.45	10.38	10.21
18	10	0	20	0	50	0	9.23	9.01	58.86	60.78	8.05	7.94	7.27	7.56	12.81	12.98	10.38	10.14

Table 3
LC-QQQ parameters of bioactive compounds for analysis of coffee pulp extracts.

Bioactive Compounds	Retention Time (RT, min)	Ionization Mode of the Analytes	Precursor Ion (m/z, [M − H]$^-$)	Product Ion (m/z)
Chlorogenic acid	3.705	Negative	325.95	191.05
Caffeine	3.633	Positive	195.00	138.15, 110.10
Trigonelline	0.921	Positive	138.10	92.10, 94.05, 78.05

at room temperature for 5 min and the absorbance was measured at 570 nm using a microplate reader. Cell viability (%) was evaluated using the following equation:

$$\% \text{ Cell viability} = (A/B) \times 100$$

where A is the absorbance of cells treated with H$_2$O$_2$ and B is the absorbance of the control.

The concentration that reduced cell viability by 60 % was chosen to evaluate the cytoprotective effect of coffee pulp extracts.

2.12.2. Cytotoxicity assay

The MTT assay was used to evaluate the cytotoxicity [28]. The NIH/3T3 fibroblasts were plated in 96-well plates at a concentration of 15,000 cells/well and maintained in the incubator until they occupied more than 80 % of the confluence. After the old culture medium was removed, cells were treated with serum-free culture medium (control), samples, and ascorbic acid at different concentrations. Then, they were incubated for 24 h. The medium was removed and replaced with a fresh serum-free culture medium and freshly prepared MTT solution and further incubated for 4 h. After discarding the medium containing MTT from each well, dimethyl sulfoxide (DMSO) solution was used to dissolve the remaining MTT-formazan crystals. The plate was incubated for 5 min and the absorbance was measured at 570 nm using a microplate reader [29]. Cell viability (%) was evaluated using the following equation:

$$\% \text{ Cell viability} = (A/B) \times 100$$

where A is the absorbance of the sample and B is the absorbance of the control.

2.12.3. Cytoprotective effect of coffee pulp extracts on NIH/3T3 fibroblasts against hydrogen peroxide-induced oxidative stress

The assay was evaluated using the method stated by [29] with slight modifications. The NIH/3T3 fibroblasts were seeded in 96-well plates at a concentration of 15,000 cells/well and incubated until they occupied more than 80 % of the confluence. After the old culture medium was removed, cells were treated with serum-free culture medium (control), samples, and ascorbic acid at different concentrations and incubated for 24 h. The samples were substituted with a fresh serum-free culture medium containing 400 μM hydrogen peroxide and incubated for 1 h. Then, the medium was removed and replaced with a fresh serum-free culture medium and freshly prepared MTT solution and further incubated for 4 h. After discarding the medium containing MTT from each well, dimethyl sulfoxide (DMSO) solution was used to dissolve the remaining MTT-formazan crystals. The plate was incubated for 5 min and the absorbance was measured at 570 nm using a microplate reader. Cell viability (%) was determined using the following equation:

$$\% \text{ Cell viability} = (C/D) \times 100$$

where C is the absorbance of the sample treated with hydrogen peroxide and D is the absorbance of the control.

2.13. Statistical analysis

All experiments in this study, except RSM, were evaluated in triplicate. The statistical analysis of the data obtained from RSM with the Box–Behnken experiment was carried out with the R software using the rsm package. One-sample *t*-test was used to determine the difference between the actual value and the predicted value during the validation process. The comparison of bioactive compounds and antioxidant activities in the OFAT experiments and between extraction methods was evaluated using one-way ANOVA. The pairwise comparison between groups was examined using Fisher's least significant difference (LSD) test. The statistical significance level was considered at *p < 0.05*. All values were expressed as the mean ± standard deviation.

3. Result and discussion

3.1. One-factor-at-a-time

3.1.1. Effect of extraction solvents

As the polarity of solvents can determine the extraction efficiency of bioactive compounds from raw materials, the polarity of solvents should be compatible with the polarity of targeted bioactive molecules [30]. To evaluate the effect of different extraction solvents on the TPC of the coffee pulp, the experiments were carried out using different solvents (propylene glycol, glycerin, butylene glycol, and water) under certain conditions: 30 % of amplitude, 20:1 of liquid–solid ratio, 15 min duration, and 60 % w/v of solvent concentration at room temperature. After centrifugation, the characteristics of the resultant extracts are exhibited in the liquid form with the intense straw color and characteristic coffee brew odor. The TPC in each resultant extract was determined. The results are presented in Fig. 1. Samples with aqueous-propylene glycol yielded the highest phenolic content of 8.91 ± 0.40 mg GAE/g sample, followed by samples with aqueous-glycerin (8.64 ± 0.40 mg GAE/g sample) and they were not significantly different *(p ≥ 0.05)*. However, these values were significantly different from samples with aqueous-butylene glycol (7.44 ± 0.52 mg GAE/g sample) and samples with water (6.88 ± 0.07 mg GAE/g sample) *(p < 0.05)*. A previous study in which phenolic compounds were extracted from the fruits of *Terminalia chebula* reported that an aqueous-propylene glycol mixture compared to ethanol yielded higher phenolic compounds [31]. The recovery of phenolic compounds in different plants depends on the polarity of extraction solvents and the solubility of these compounds in the solvents [32]. The yield of phenolic compounds can be enhanced in solvents with

high polarity [33]. A study of phenolic compound extracted from orange peel reported that the number of hydroxyl groups (–OH) in the solvent affected the yield of phenolic compounds [34]. The solvents with a higher number of –OH groups had a higher viscosity than those with a lower number of –OH groups. This can limit the transfer of active compounds and reduce the yield of extraction content [34]. In this study, the result of the extraction process using propylene glycol showed the highest yield value. Therefore, aqueous-propylene glycol system has been chosen to proceed with further experiments.

3.1.2. Effect of the amplitude of the ultrasonic wave

In the ultrasound system with a sonication probe, amplitude plays a significant role in the extraction process as it determines the number of compression cycles of ultrasonic [35]. Under certain conditions of 60 % w/v of propylene glycol, 15 min of duration, and 20:1 of liquid–solid ratio, extraction of coffee pulp was carried out using various amplitudes of 20 %, 30 %, and 40 % at room temperature. Total phenolic content was determined. The results of the effect of amplitude variation on phenolic compounds of coffee pulp through ultrasound-assisted extraction are presented in Fig. 1. TPC increased with an increase in the percent amplitude, and a significant increase in TPC was observed between samples using 40 % and 30 % amplitude of the ultrasonic probe processor as compared to 20 % amplitude *(p < 0.05)*. The result showed a positive correlation between the response and the percent of amplitude. This could be due to the improved interaction between the solvent and solid at high amplitude [36]. Previous studies stated that bubble collapse becomes forceful at high amplitude as the bubble size is directly proportionate to the amplitude of ultrasonic waves [36–38]. Microfractures and micro-cavitation are formed in plant tissues due to strong shear forces caused by the bubble collapse, resulting in disruption of cell walls [36,39,40]. The amplitude of the ultrasonic probe processor at 40 % that yielded the optimal value has been chosen to continue further experiments.

3.1.3. Effect of time of extraction

Extraction time forms an essential part to be considered in a plant extraction procedure, as it saves time, cost, and energy [41]. Under certain conditions of 60 % w/v of propylene glycol, 40 % of amplitude, and 20:1 of liquid–solid ratio, the extraction of coffee pulp was performed using various durations of 5 min, 10 min, 15 min, 20 min, 25 min, and 30 min at room temperature, and the content of phenolic compounds was determined. The results of the effect of time variation on ultrasound-assisted extraction of coffee pulp are shown in Fig. 1.
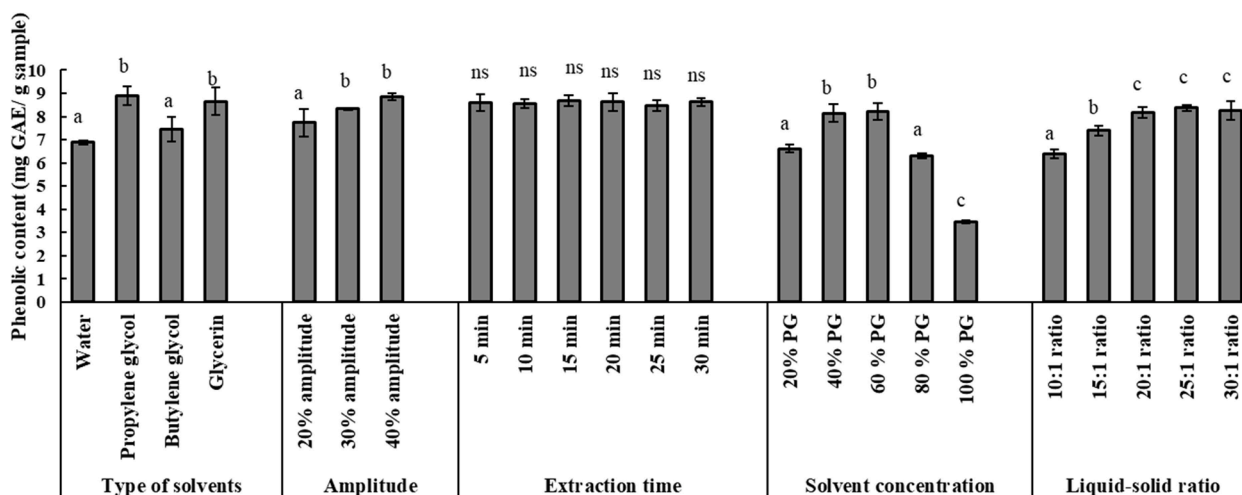

Fig. 1. Effect of variable conditions on total phenolic contents through the ultrasound-assisted extraction of coffee pulp. Values are expressed as mean ± standard deviation *(n = 3)*. ns indicates no significant difference *(p ≥ 0.05)*. Values with different superscript letters indicate the statistically significant difference *(p < 0.05)* within group.

Variation in duration of extraction did not produce a significant difference in the TPC of coffee pulp $(p \geq 0.05)$. Samples extracted for 15 min yielded the highest value of TPC (8.67 ± 0.23 mg GAE/g sample), while samples extracted for 5 min produced the lowest yield value of TPC (8.45 ± 0.23 mg GAE/g sample). However, these values were not significantly different $(p \geq 0.05)$. Although prolonged exposure to time can improve the yield of the bioactive compounds by enhancing the diffusion of these compounds from the plant into the solvent [36], it can also cause a high temperature that might degrade bioactive compounds [42]. Therefore, the duration of the extraction process that saves time and energy consumption, 5 min, has been chosen for further experiments.

3.1.4. Effect of solvent concentration

In the aqueous-cosolvent system, variation in the composition of solvent concentration can enhance the solubility of bioactive compounds [43]. Extraction of coffee pulp was carried out using various concentrations of propylene glycol, 20 %, 40 %, 60 %, 80 %, and 100 % w/v under certain conditions: 40 % amplitude, 5 min duration, and 20:1 of liquid–solid ratio at room temperature. Total phenolic content was evaluated. The results of the effect of solvent concentration on the TPC of coffee pulp through the ultrasound-assisted extraction are presented in Fig. 1. Samples with 40 % and 60 % w/v aqueous-propylene glycol provided the highest phenolic yield values (8.15 ± 0.39 mg GAE/g sample and 8.20 ± 0.36 mg GAE/g sample, respectively). These values are significantly different from those of samples with 20 %, 80 %, and 100 % w/v aqueous-propylene glycol $(p < 0.05)$. Based on the principle of like dissolves like [44], 40 % and 60 % w/v aqueous-propylene glycol might have the most similar polarity for phenolic compounds. Hence, for subsequent experiments, 40 % w/v of aqueous-propylene glycol has been chosen in order to reduce costs related to the extraction process by minimizing the solvent used.

3.1.5. Effect of liquid–solid ratio

The yield of phenolic compounds is considerably impacted by the liquid–solid ratio [45]. Extraction of coffee pulp was done using various liquid–solid ratios of 1:10, 1:15, 1:20, 1:25, and 1:30 under certain conditions: 40 % w/v of propylene glycol, 40 % of amplitude, 5 min duration, and room temperature. Total phenolic content was determined. The results of the effect of different liquid–solid ratios on the TPC of coffee pulp through ultrasound-assisted extraction are shown in Fig. 1. The yield value of TPC significantly increased with an increase in liquid–solid ratio until it reached a 20:1 of liquid–solid ratio $(p < 0.05)$. However, a further increase in liquid–solid ratio from 20:1 to 30:1 did not significantly increase the TPC. This finding could be due to the dissolution equilibrium. Generally, higher solvent-solid ratio will result in greater concentration difference and this can enhance mass transfer and promote solute dissolution [46]. However, once the dissolution process has reached its equilibrium, extraction efficiency cannot be improved by a further increase in the liquid–solid ratio [46,47].

3.2. Optimization of extraction method using RSM

According to the results of OFAT experiments, an aqueous-propylene glycol and 40 % amplitude of probe sonicator produced the highest yield of phenolic compounds from the coffee pulp. Optimization of conditions for bioactive compound extraction and antioxidant activities using RSM was performed with variables of extraction time (X_1, min), liquid–solid ratio (X_2), and solvent concentration (X_3, %) at three variation levels as shown in Table 1. The results of TPC, TFC, TTC, and antioxidant activities are given in Table 2. The TPC value of coffee pulp extract ranged from 4.78 mg GAE/g sample to 9.98 mg GAE/g sample, and the TFC value of coffee pulp extract varied from 8.11 mg QE/g sample to 62.99 mg QE/g sample. TTC of coffee pulp extract ranged from 4.50 mg TAE/g sample to 8.95 mg TAE/g sample. For antioxidant activities of coffee pulp extract, the DPPH values ranged from 2.87 mg TEAC/g sample to

8.19 mg TEAC/g sample. Meanwhile, the ABTS values differed from 4.99 mg TEAC/g sample to 15.68 mg TEAC/g sample, and the FRAP values varied from 4.39 mg FeSO$_4$/g sample to 10.85 mg FeSO$_4$/g sample.

3.2.1. Fitting the models and statistical analysis

The results in Table 2 were analyzed by multiple regression fitting and quadratic multinomial equations that expressed the relationship between the three variables and TPC, TFC, TTC, DPPH, ABTS, and FRAP were obtained as follows:

$$\begin{aligned}
\mathrm{Y(TPC)} = {} & 9.225 + 0.099X_1 + 1.677X_2 + 0.399X_3 + 0.511(X_1 * X_2) \\
& + 0.131(X_1 * X_3) + 0.242(X_2 * X_3) - 0.545X_1^2 - 0.911X_2^2 \\
& - 1.575X_3^2
\end{aligned} \tag{2}$$

$$\begin{aligned}
\mathrm{Y(TFC)} = {} & 58.862 - 1.117X_1 - 1.058X_2 + 1.748X_3 + 1.156(X_1 * X_2) \\
& + 1.251(X_1 * X_3) - 4.391(X_2 * X_3) - 6.402X_1^2 - 13.503X_2^2 \\
& - 27.258X_3^2
\end{aligned} \tag{3}$$

$$\begin{aligned}
\mathrm{Y(TTC)} = {} & 8.049 - 0.149X_1 + 1.479X_2 + 0.385X_3 + 0.163(X_1 * X_2) \\
& + 0.257(X_1 * X_3) + 0.129(X_2 * X_3) + 0.024X_1^2 - 0.641X_2^2 \\
& - 1.260X_3^2
\end{aligned} \tag{4}$$

$$\begin{aligned}
\mathrm{Y(DPPH)} = {} & 7.273 + 0.191X_1 + 1.951X_2 + 0.376X_3 + 0.161(X_1 * X_2) \\
& + 0.200(X_1 * X_3) - 0.404(X_2 * X_3) - 0.640X_1^2 - 0.410X_2^2 \\
& - 1.323X_3^2
\end{aligned} \tag{5}$$

$$\begin{aligned}
\mathrm{Y(ABTS)} = {} & 12.812 + 0.130X_1 + 4.066X_2 + 0.582X_3 + 0.530(X_1 * X_2) \\
& + 0.406(X_1 * X_3) + 0.053(X_2 * X_3) - 0.544X_1^2 - 1.405X_2^2 \\
& - 2.067X_3^2
\end{aligned} \tag{6}$$

$$\begin{aligned}
\mathrm{Y(FRAP)} = {} & 10.384 + 0.026X_1 + 1.751X_2 + 0.858X_3 + 0.375(X_1 * X_2) \\
& + 0.048(X_1 * X_3) + 0.739(X_2 * X_3) - 1.136X_1^2 - 1.316X_2^2 \\
& - 2.350X_3^2
\end{aligned} \tag{7}$$

where Y refers to the response values of TPC, TFC, TTC, DPPH, ABTS, and FRAP of the extract, X_1, X_2, and X_3 are extraction time, liquid–solid ratio, and solvent concentration, respectively.

The analysis of the results was conducted using R software on quadratic multinomial regression models with response values of TPC, TFC, TTC, DPPH, ABTS, and FRAP as shown in Table 4. The results indicated that all models had significant values $(p < 0.05)$, with F values of 121.51, 14.51, 65.48, 20.37, 185.80, and 45.06, respectively. The coefficient of determination (R^2) of TPC, TFC, TTC, DPPH, ABTS, and FRAP fitting models was greater than 0.90, indicating that the predicted and experimental data were more than 90 % consistent [48]. The adjusted R square ($adjR^2$) of TPC, TFC, TTC, DPPH, ABTS, and FRAP models was greater than 0.85. This suggests that there were only a few percent of significant differences between the predicted values and the adjusted values, which verified the reliability of the model [41]. Moreover, the lack of fit was insignificant $(p \geq 0.05)$, suggesting that the models were appropriate.

3.2.2. Effects of independent variables on bioactive compounds and antioxidant activities of coffee pulp extract

The effects of extraction time (X_1, min), liquid–solid ratio (X_2), and solvent concentration (X_3, %) on TPC, TFC, TTC, DPPH, ABTS, and FRAP values of coffee pulp extracts resulting from 18 experimental runs were fitted by quadratic regression equations, as presented in Equation 2–7. The estimated coefficients and the results were expressed in Table 5. Moreover, the interactions among extraction time, liquid–solid ratio, and solvent concentration and their effects on the TPC, TFC, TTC, DPPH,

Table 4

Analysis of variance of TPC, TFC, TTC, DPPH, ABTS, and FRAP for BBD using R software.

Response value	Source	Sum Square	Df	Mean Square	F value	Pr (>F)
	Model	43.726	9	4.858	121.51	1.37E-07
	Linear	23.863	3	7.954	198.947	7.49E-08
	Interaction	1.351	3	0.451	11.267	0.003
	Quadratic	18.512	3	6.171	154.33	2.03E-07
TPC	Residuals	0.320	8	0.040		
	Lack of fit	0.137	3	0.046	1.255	0.3834
	Pure error	0.183	5	0.037		
	Total	44.046	17			
	R square	0.993				
	Adj R square	0.985				
	Model	4985.9	9	553.99	14.51	4.73E-04
	Linear	43.4	3	14.45	0.379	0.771
	Interaction	88.7	3	29.58	0.775	0.540
	Quadratic	4853.8	3	1617.95	42.385	2.95E-05
TFC	Residuals	305.4	8	38.17		
	Lack of fit	228.5	3	76.17	4.955	0.059
	Pure error	76.9	5	15.37		
	Total	5291.3	17			
	R square	0.942				
	Adj R square	0.877				
	Model	28.868	9	3.208	65.48	1.56E-06
	Linear	18.864	3	6.288	128.360	4.19E-07
	Interaction	0.438	3	0.146	2.982	0.096
	Quadratic	9.566	3	3.189	65.091	5.81E-06
TTC	Residuals	0.392	8	0.049		
	Lack of fit	0.099	3	0.033	0.562	0.663
	Pure error	0.293	5	0.059		
	Total	29.260	17			
	R square	0.987				
	Adj R square	0.972				
	Model	44.601	9	4.956	20.37	1.36E-04
	Linear	31.865	3	10.622	43.664	2.64E-05
	Interaction	0.916	3	0.305	1.255	0.353
	Quadratic	11.820	3	3.940	16.197	0.001
DPPH	Residuals	1.946	8	0.243		
	Lack of fit	0.742	3	0.247	1.027	0.455
	Pure error	1.204	5	0.241		
	Total	46.547	17			
	R square	0.958				
	Adj R square	0.911				
	Model	170.210	9	18.912	185.80	2.55E-08
	Linear	135.102	3	45.034	442.365	3.17E-09
	Interaction	1.784	3	0.595	5.843	0.021
	Quadratic	33.323	3	11.108	109.111	7.90E-07
ABTS	Residuals	0.814	8	0.102		
	Lack of fit	0.250	3	0.083	0.740	5.72E-01
	Pure error	0.564	5	0.113		
	Total	171.024	17			
	R square	0.995				
	Adj R square	0.990				
	Model	77.602	9	8.622	45.06	6.67E-06
	Linear	30.410	3	10.137	52.976	

Table 4 (*continued*)

Response value	Source	Sum Square	Df	Mean Square	F value	Pr (>F)
						1.27E-05
	Interaction	2.758	3	0.920	4.806	0.034
	Quadratic	44.434	3	14.811	77.408	2.99E-06
FRAP	Residuals	1.531	8	0.191		
	Lack of fit	1.036	3	0.345	3.491	1.06E-01
	Pure error	0.495	5	0.099		
	Total	79.133	17			
	R square	0.981				
	Adj R square	0.959				

ABTS, and FRAP values are plotted in Fig. 2.

Among the linear variables, the effect of the liquid–solid ratio (X_2) was positively significant in all responses except TFC $(p < 0.05)$, while the effect of solvent concentration (X_3, %) was positively significant in all responses except TFC and DPPH assays $(p < 0.05)$. The effect of extraction time (X_1, min) was not significant in all responses. In terms of interactions, the interaction of extraction time and liquid–solid ratio (X_1X_2) was positively significant in TPC and ABTS assays $(p < 0.05)$. Meanwhile, the interaction of extraction time and solvent concentration (X_1X_3) was positively significant in TTC and ABTS assay $(p < 0.05)$ and the interaction of liquid–solid ratio and solvent concentration (X_2X_3) was positively significant in TPC and FRAP assays $(p < 0.05)$. In the quadratic terms, solvent concentration (X_3^2) was negatively significant in all responses $(p < 0.05)$ and the liquid–solid ratio (X_2^2) was also negatively significant in all responses except the DPPH assay $(p < 0.05)$. The quadratic term of extraction time (X_1^2) was negatively significant in TPC, DPPH, ABTS, and FRAP assays $(p < 0.05)$.

In terms of TPC, TFC, and FRAP assays, their values increased as the liquid–solid ratio increased. However, once they had reached their optimal condition, a further increase in the liquid–solid ratio did not produce an increase in their values. Moreover, their values increased with an increase in solvent concentration, but with a further increase in concentration beyond the optimal condition, there was a gradual decline in these values. However, their values did not change significantly with an increase in the duration of extraction. Regarding these results, a previous study of microwave-assisted extraction of coffee pulp using a water–ethanol mixture reported that TPC increased with an increase in liquid–solid ratio and decreased with an increase in solvent concentration [19]. TFC findings were supported by a previous study of the UAE of phenolic compounds from spent coffee grounds in which TFC increased with an increase in liquid–solid ratio until it reached the ratio of 20:1, and a further increase in liquid–solid ratio resulted in a decrease in TFC [15]. Furthermore, a previous study of microwave-assisted extraction of *Lithocarpus polystachyus* Rehd. stated that the FRAP value initially increased with the increase in liquid–solid ratio and then decreased when the liquid–solid ratio passed beyond its optimal condition [49].

In terms of TTC, DPPH, and ABTS assays, their values increased with an increase in the liquid–solid ratio, and the effect of extraction time and the effect of solvent concentration shared a similar pattern with TPC, TFC, and FRAP. In terms of TTC, these findings were in accordance with a previous study of UAE of phenolic compounds from rhizomes of *Rheum moorcroftianum* in which TTC increased with an increase in the liquid–solid ratio [50]. In addition, DPPH activity findings were supported by a previous study of UAE of phenolic compounds from rhizomes of *R. moorcroftianum* in which DPPH capacity increased with an increase in the liquid–solid ratio [50]. In terms of ABTS, a previous study of UAE of phenolic compounds from rhizomes of *R. moorcroftianum* reported that ABTS capacity increased with an increase in liquid–solid ratio until it reached its dissolution equilibrium and a further increase in the liquid–solid ratio led to a decreasing trend [50]. Among three variables, the

Table 5

Regression analysis of TPC, TFC, TTC, DPPH, ABTS, and FRAP for BBD using R software.

Parameter	TPC				TFC				TTC			
	Estimated	Std. Error	t value	Pr(>|t|)	Estimated	Std. Error	t value	Pr(>|t|)	Estimated	Std. Error	t value	Pr(>|t|)
intercept	9.227	0.082	113.015	4.20E-14 ***	58.862	2.522	23.337	1.21E-08 ***	8.049	0.090	89.083	2.81E-13 ***
X_1	0.099	0.071	1.397	0.200	−1.117	2.184	−0.511	0.623	−0.149	0.078	−1.906	0.093
X_2	1.677	0.071	23.728	1.06E-08 ***	−1.058	2.184	−0.484	0.641	1.479	0.078	18.899	6.35E-08 ***
X_3	0.399	0.071	5.645	4.84E-04***	1.748	2.184	0.800	0.447	0.385	0.078	4.926	1.15E-03 **
$X_1:X_2$	0.511	0.100	5.117	9.11E-04 ***	1.156	3.089	0.374	0.718	0.163	0.111	1.477	0.178
$X_1:X_3$	0.131	0.100	1.311	0.226	1.251	3.089	0.405	0.696	0.257	0.111	2.323	0.049 *
$X_2:X_3$	0.243	0.100	2.428	0.041 *	−4.392	3.089	−1.422	0.193	0.129	0.111	1.169	0.276
X_1^2	−0.545	0.096	−5.693	4.58E-04 ***	−6.402	2.958	−2.165	0.062	0.024	0.106	0.226	0.827
X_2^2	−0.911	0.096	−9.513	1.23E-05 ***	−13.503	2.958	−4.565	1.83E-03 **	−0.641	0.106	−6.048	3.07E-04 ***
X_3^2	−1.575	0.096	−16.456	1.88E-07 ***	−27.258	2.958	−9.216	1.56E-05 ***	−1.260	0.106	−11.896	2.29E-06 ***

Parameter	DPPH				ABTS				FRAP			
	Estimated	Std. Error	t value	Pr(>|t|)	Estimated	Std. Error	t value	Pr(>|t|)	Estimated	Std. Error	t value	Pr(>|t|)
intercept	7.273	0.201	36.120	3.78E-10 ***	12.812	0.130	98.360	1.28E-13 ***	10.384	0.179	58.146	8.50E-12 ***
X_1	0.191	0.174	1.098	0.304	0.130	0.113	1.152	0.282	0.026	0.155	0.170	0.870
X_2	1.951	0.174	11.187	3.65E-06 ***	4.066	0.113	36.044	3.85E-10 ***	1.751	0.155	11.319	3.34E-06 ***
X_3	0.376	0.174	2.155	0.063	0.582	0.113	5.159	8.65E-04 ***	0.858	0.155	5.548	5.42E-04 ***
$X_1:X_2$	0.161	0.247	0.654	0.531	0.528	0.160	3.310	0.011 *	0.375	0.219	1.715	0.125
$X_1:X_3$	0.200	0.247	0.811	0.441	0.406	0.160	2.543	0.035*	0.048	0.219	0.220	0.831
$X_2:X_3$	−0.404	0.247	−1.637	0.140	0.053	0.160	0.331	0.749	0.739	0.219	3.381	9.63E-03**
X_1^2	−0.640	0.236	−2.710	0.027 *	−0.544	0.153	−3.560	7.40E-03 **	−1.136	0.209	−5.425	6.28E-04 ***
X_2^2	−0.410	0.236	−1.737	0.121	−1.404	0.153	−9.198	1.58E-05 ***	−1.316	0.209	−6.284	2.37E-04 ***
X_3^2	−1.323	0.236	−5.606	5.07E-04 ***	−2.066	0.153	−13.529	8.55E-07 ***	−2.350	0.209	−11.224	3.56E-06 ***

Significant codes: '***' $p < 0.001$, '**' $p < 0.01$, '*' $p < 0.05$.
Significant codes: '***' $p < 0.001$, '**' $p < 0.01$, '*' $p < 0.05$.

effect of the liquid–solid ratio was found to be stronger than that of the remaining variables in all parameters.

3.3. Validation of the model

According to the response model, the optimal three independent variables were found as follows: extraction time of 7.65 min, the liquid–solid ratio of 22.22 mL/g, and solvent concentration of 46.71 %. Using these optimal extraction conditions, a verification experiment was evaluated to check the accuracy of the response model. The predicted values and the results of actual experiments are shown in Table 6. It was found that all the results of the verification experiment were not significantly different from the prediction ($p \geq 0.05$) except the result of the FRAP assay, in which the actual value was statistically and significantly higher than the predicted value ($p = 0.045$). However, the actual value was only a 6.15 % increase as compared with the predicted value. Therefore, these results pointed out that the response model was reliable.

3.4. Comparison of UAE with conventional method and conventional solvent

Using optimal conditions of the response model, the dried coffee pulp was extracted under the conditions of PG-UAE, EtOH-UAE, PG-maceration, and EtOH-maceration. The corresponding results with

significant levels are presented in Table 7. PG-UAE was significantly higher than the other three extraction conditions in all response values, including TPC, TFC, TTC, DPPH, ABTS, and FRAP ($p < 0.05$). Apart from the DPPH value, PG-maceration had the second-highest efficiency in all the remaining parameters, and EtOH-UAE had the second-highest value in DPPH activity. Meanwhile, EtOH-maceration produced the lowest yield value of TPC, TTC, DPPH, ABTS, and FRAP activity, and EtOH-UAE yielded the lowest TFC. These results indicated that using propylene glycol instead of ethanol as an extraction solvent yielded significantly higher bioactive compounds, and antioxidant activities of the plants either by UAE or maceration. Comparing UAE and maceration using the same extraction solvent, UAE yielded higher values in all parameters and it required a much shorter time. This finding was consistent with a previous study in which UAE was the most efficient method compared with maceration and Soxhlet extraction [51]. In addition, a previous study of the extraction of phenolic compounds from *Terminalia chebula* Retz. supported our findings that extract in an aqueous-propylene glycol system showed higher antioxidant activity than aqueous–ethanol system [43]. The high efficiency of UAE was explained by mechanical effects and cavitation caused by ultrasound that destroyed the cell wall and promoted the release of active compounds [52,53].
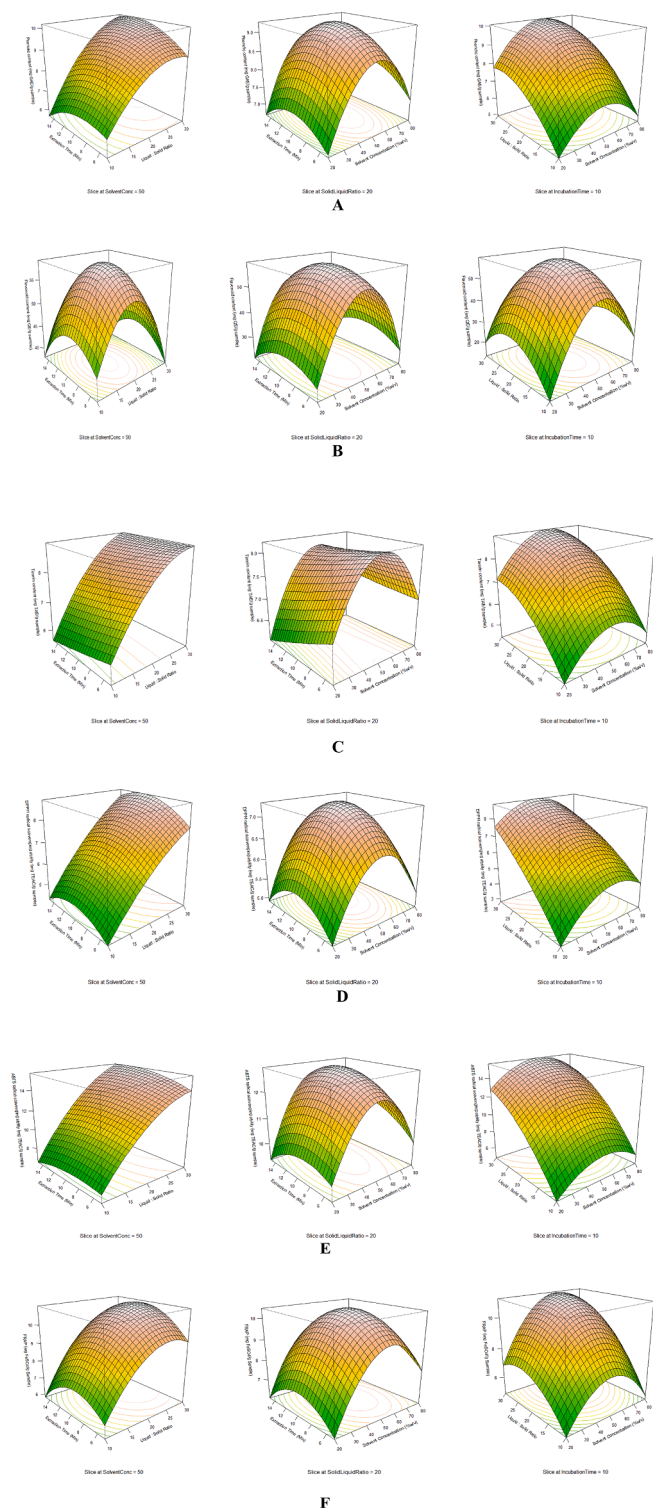
Fig. 2. The three-dimensional response surface plots for the effects of extraction time, liquid–solid ratio, and solvent concentration on (A) TPC, (B) TFC, (C) TTC, (D) DPPH, (E) ABTS, and (F) FRAP.

3.5. Identification and quantitation of bioactive compounds by LC-QQQ

Phenolic compounds have different pharmacological actions due to variations in their chemical structure [25]. Therefore, it is necessary to characterize the type of phenolic compounds in the extract. In both PG-UAE and EtOH-UAE samples, several bioactive compounds including chlorogenic acid, caffeine, and trigonelline were detected as presented

in Fig. 3. In both extracts, the predominant phenolic compound was trigonelline, followed by caffeine, and chlorogenic acid. However, the content of trigonelline was significantly higher in EtOH-UAE than in PG-UAE $(p < 0.05)$, while a significantly higher content of chlorogenic acid was detected in the PG-UAE than in EtOH-UAE $(p < 0.05)$. The other compound was not significantly different $(p \geq 0.05)$. The antioxidant activity of coffee pulp extract might be contributed by the action of these compounds. Bioactive compounds in coffee, such as caffeine, trigonelline, and chlorogenic acid, have several skin benefits. They can retard aging process due to ultraviolet radiation, prevent skin photo-damage by acting as antioxidants [54–56], and can promote hair growth by inhibiting 5α-reductase enzymes [57]. This suggests that coffee pulp extract might convey these potential benefits.

3.6. Cell culture

3.6.1. Determination of appropriate hydrogen peroxide concentration

When cells are treated with H_2O_2, many cells are killed by H_2O_2-induced oxidative stress through necrosis or apoptosis depending on the concentration of H_2O_2 [58]. At a lower H_2O_2 concentration, cells undergo apoptosis, while at a higher H_2O_2 concentration, cells will experience necrosis [59]. Therefore, it is essential to evaluate the appropriate concentration of H_2O_2 to be used for treating the cultured cells. In this study, dose–response experiments of H_2O_2 are assessed to determine the appropriate concentration to induce oxidative stress in the NIH/3T3 fibroblasts. The results of dose–response experiments of H_2O_2 are presented in Fig. 4. According to the results, H_2O_2 at all concentrations had a significant decrease in cell viability compared to the control $(p < 0.05)$. After incubation with different concentrations of H_2O_2 for 1 h, the cell viability of 100 μM, 200 μM, and 300 μM H_2O_2 was higher than 70 %. Meanwhile, the cell viability of 400 μM, 500 μM, and 1000 μM of H_2O_2 decreased to 61.70 ± 2.70 %, 62.83 ± 2.87 %, and 55.66 ± 4.44 %, respectively. Therefore, the minimal concentration of H_2O_2 that yielded cell viability close to 60 % (400 μM) is selected to be used for further procedures.

3.6.2. Cytotoxicity assay

Dose-response experiments were performed to evaluate the maximal non-toxic concentration of coffee pulp extracts and ascorbic acid. The results of the cytotoxicity assay of ascorbic acid, PG-UAE, and EtOH-UAE are presented in Fig. 5. Two concentrations of ascorbic acid, 0.001 mg/ml and 0.01 mg/ml were considered as non-cytotoxic concentrations for the NIH/3T3 fibroblast cells with cell viability of 108.33 ± 4.69 % and 80.20 ± 5.03 %, respectively. The concentration of ascorbic acid at 0.1 mg/ml was found to suppress cell survival with cell viability of 38.11 ± 1.73 %. Higher doses of ascorbic acid are known to be cytotoxic to the cells as they cause metabolic stress that leads to cell apoptosis [60]. Apart from the concentration of 25 mg/ml of both extract samples, which reduced the cell viability of the NIH/3T3 fibroblasts below 70 %, all the remaining concentrations of 1 mg/ml, 5 mg/ml, 7.5 mg/ml, and 10 mg/ml of both extract samples were considered as non-cytotoxic because their cell viability was higher than 80 % [29]. Comparing the cell viability between PG-UAE and EtOH-UAE, at higher concentrations starting from 5 mg/ml, PG-UAE showed higher cell viability than EtOH-UAE. This could be explained by a previous study that stated that propylene glycol was less toxic than ethanol in cell culture [61].

3.6.3. Cytoprotective effect of coffee pulp extracts on NIH/3T3 fibroblasts against hydrogen peroxide-induced oxidative stress

The cytoprotective effect of coffee pulp extracts was evaluated by the assay in which cellular oxidative stress was induced by H_2O_2 as a pro-oxidant; it caused cell death by inducing DNA damage [62]. The results of the cytoprotective effect of coffee pulp extracts on 400 μM H_2O_2-induced oxidative stress in the NIH/3T3 fibroblasts are presented in Fig. 6. The viability of the cells treated with H_2O_2 was found to

Table 6
The results of validation of the model for TPC, TFC, TTC, DPPH, ABTS, and FRAP.

Conditions	Extraction time (X_1)	Liquid-solid ratio (X_2)	Solvent concentration (X_3)	TPC (mg GAE/g sample)	TFC (mg QE/g sample)	TTC (mg TAE/g sample)	DPPH (mg TEAC/g sample)	ABTS (mg TEAC/g sample)	FRAP (mg FeSO$_4$/g sample)
Predicted	7.65 min	22.22 mL/g	46.71 %	9.27	56.61	8.36	7.40	13.34	10.27
Actual				9.29 ± 0.02 [ns]	58.82 ± 1.38 [ns]	8.69 ± 0.26 [ns]	7.56 ± 0.27 [ns]	13.59 ± 0.25 [ns]	10.90 ± 0.24*

Values are expressed as mean $\pm$ standard deviation (n = 3). * indicates the statistically significant difference within the same column ($p < 0.05$). ns indicates no significant difference ($p \geq 0.05$) within the same column.

Table 7
The comparison of TPC, TFC, TTC, DPPH, ABTS, and FRAP between PG-UAE, EtOH-UAE, PG-Maceration, and EtOH-Maceration.

Extraction methods	Extraction time (X_1)	Liquid-solid ratio (X_2)	Solvent concentration (X_3)	TPC (mg GAE/g sample)	TFC (mg QE/g sample)	TTC (mg TAE/g sample)	DPPH (mg TEAC/g sample)	ABTS (mg TEAC/g sample)	FRAP (mg FeSO$_4$/g sample)
PG-UAE	7.65 min	22.22 mL/g	46.71 %	9.29 ± 0.02^a	58.82 ± 1.38^a	8.69 ± 0.25^a	7.56 ± 0.27^a	13.59 ± 0.25^a	10.90 ± 0.24^a
EtOH-UAE				7.49 ± 0.04^b	33.78 ± 0.38^b	7.11 ± 0.15^b	6.59 ± 0.13^b	10.39 ± 0.51^b	8.12 ± 0.09^b
PG-Maceration	24 h	22.22 mL/g	46.71 %	8.50 ± 0.14^c	51.72 ± 1.94^c	7.77 ± 0.12^c	5.63 ± 0.10^c	11.20 ± 0.24^c	9.90 ± 0.07^c
EtOH-Maceration				7.19 ± 0.05^b	45.20 ± 0.83^d	6.47 ± 0.26^d	4.66 ± 0.10^d	9.21 ± 0.04^d	7.64 ± 0.14^d

Values are expressed as mean $\pm$ standard deviation (n = 3). Values within the same column with different superscript letters indicate a statistically significant difference ($p < 0.05$).
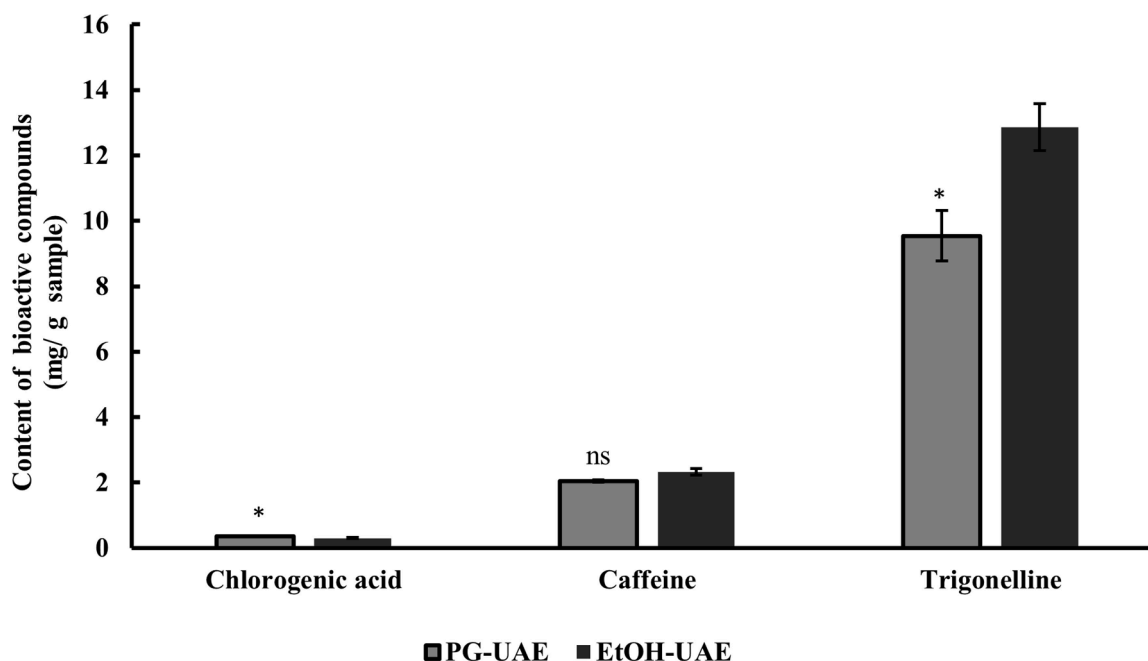

Fig. 3. Identification and quantification of bioactive compounds in the coffee pulp extract by LC-QQQ. ns indicates no significant difference ($p \geq 0.05$). * indicates a significant difference ($p < 0.05$) as compared to EtOH-UAE.

significantly decrease to 61.46 ± 1.50 % compared to the control ($p < 0.05$). Cell viability was significantly increased to 76.65 ± 1.57 % after treating cells with ascorbic acid at 0.01 mg/ml before oxidative stress was induced in the NIH/3T3 fibroblasts by H$_2$O$_2$. All concentrations of the PG-UAE extract and EtOH-UAE extract showed a statistically significant increase in cell viability compared to the H$_2$O$_2$ treatment ($p < 0.05$). Comparing the antioxidant efficacy between PG-UAE extract and EtOH-UAE extract of the same concentration, PG-UAE extract exhibited higher cell viability compared to EtOH-UAE extract. These results are in agreement with the *in vitro* antioxidant assays and results of the content of bioactive compounds of both extracts. Based on the results of *in vitro*

and cellular antioxidant assays, it can be concluded that chlorogenic acid may contribute to the antioxidant activities of coffee pulp extract, as coffee pulp extract using propylene glycol yielded higher antioxidant activity in both *in vitro* and cellular antioxidant assays and it had a higher content of chlorogenic acid. According to Cozzi et al. (1995), the antioxidant activity of these extracts was contributed by phenolic compounds and chlorogenic acid acting as free radical scavenging agents to prevent oxidative damage caused by H$_2$O$_2$ [63]. A recent study of cell viability of H$_2$O$_2$-induced oxidative stress on the NIH/3T3 fibroblasts of coffee cherry extracts using ethyl acetate and ethanol showed that the coffee cherry extracts were able to protect the cells and
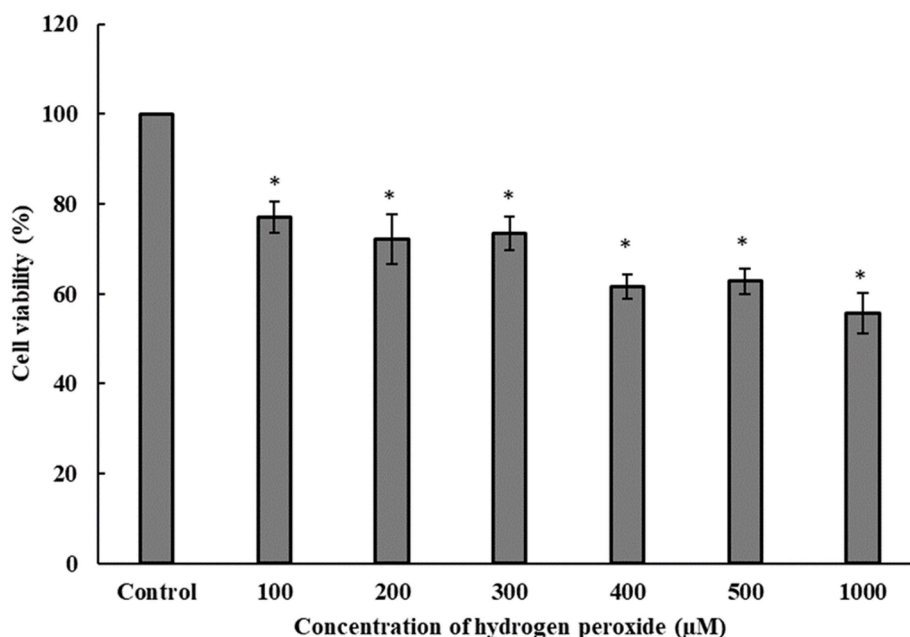
Fig. 4. Cell viability of the NIH/3T3 fibroblasts treated with various concentrations of H_2O_2 for 1 h. * indicates a significant difference compared with the control ($p < 0.05$).
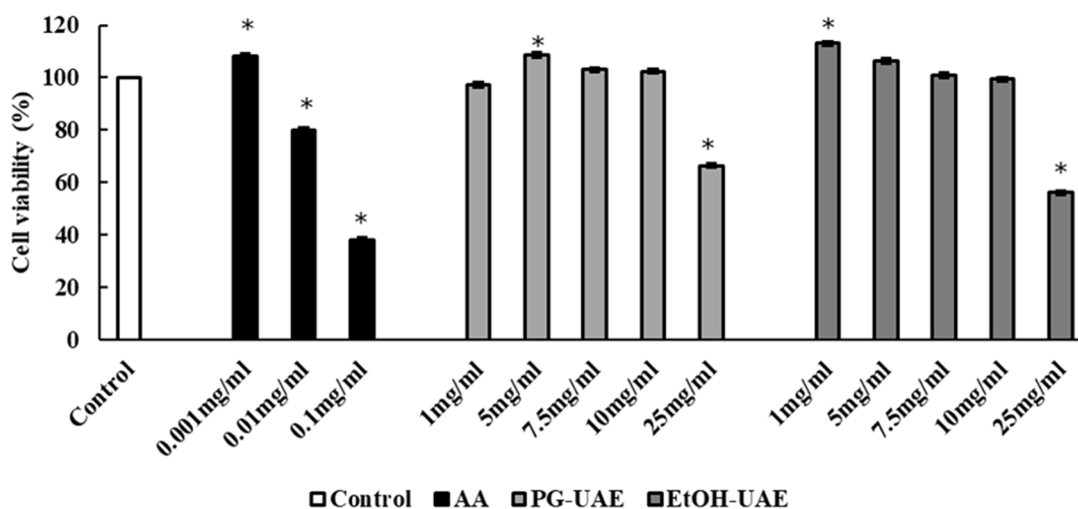

Fig. 5. Cytotoxicity assay of ascorbic acid (AA), PG-UAE, and EtOH-UAE at different concentrations, * indicates the significant difference from the control ($p < 0.05$).

showed an increase in cell viability compared to the cells treated with H_2O_2 [64].

4. Conclusion

Using BBD in RSM, this study was performed to optimize ultrasound-assisted extraction parameters of coffee pulp. Among the various polyols, propylene glycol extract produced the highest total phenolic content. According to the response model, the three optimal conditions for extraction of phenolic compounds from coffee pulp were extraction time of 7.65 min, liquid–solid ratio of 22.22 mL/g, and solvent concentration of 46.71 %. The liquid–solid ratio was found to be the factor affecting the extraction of coffee pulp samples in all parameters. The results of the validation procedure testified that the response model was reliable. Among four different extraction conditions, PG-UAE yielded significantly higher values in all responses, including TPC, TFC, TTC, DPPH, ABTS, and FRAP. In the analysis of bioactive compounds using LC-QQQ,

chlorogenic acid, caffeine, and trigonelline were predominant in both PG-UAE and EtOH-UAE extracts. In both cell cytotoxicity and cellular antioxidant assays, at higher concentrations starting from 5 mg/ml, PG-UAE demonstrated higher cell viability and cellular antioxidant activity than EtOH-UAE. Hence, ultrasound-assisted extraction of coffee pulp with propylene glycol could be a novel extraction technique that can yield higher bioactive compounds with higher antioxidant activities, which can be used as active ingredients in cosmetics (anti-aging products) and pharmaceutical applications (food supplements, anti-cancer drugs) while minimizing chemical and energy consumption.

CRediT authorship contribution statement

Hla Myo: Formal analysis, Investigation, Visualization, Writing – original draft. **Nuntawat Khat-udomkiri:** Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – review & editing, Project administration, Funding acquisition.
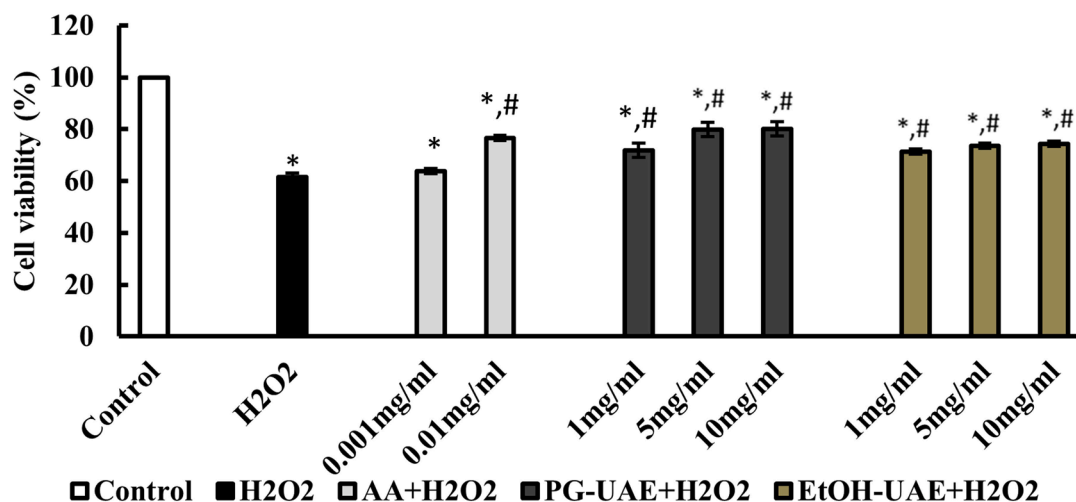
Fig. 6. Cellular antioxidant assay of ascorbic acid (AA), PG-UAE, and EtOH-UAE. * indicates the significant difference from the control *(p < 0.05)*, # indicates the significant difference from the H_2O_2 group *(p < 0.05)*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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