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Single factor optimisation of ultrasonic-assisted extraction for recovery of phenolic compounds and its antioxidant activity using one factor analysis

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Abstract

Bentong ginger (BG) or scientifically known as *Zingiber officinale* Roscoe, is a popular spice in Malaysia due to its unique pungency taste, spicy aroma, and high nutrition content. BG is classified as traditional medicine and contains abundant bioactive compounds, yet research on BG extraction using ultrasonic-assisted extraction to improve the release of thermosensitive compounds including gingerols is scarce and limited. In this study, the effects of three ultrasonic-assisted extraction (UAE) process factors, namely, amplitude (20–40%), sonication time (10–30 min), and solvent concentration (75–95%), were determined and optimised for maximum yield of phenolics and antioxidants from BG using one factor analysis approach-based Response Surface Methodology (RSM). The optimum extraction conditions for both total phenolic content (TPC) and antioxidant activity (DPPH radical scavenging) were as follows: amplitude of 30.34%, sonication time of 14 min, and solvent concentration of 84%. Regression equations for all experiments revealed that increasing process parameters to a certain level reduced the TPC yield and DPPH inhibition. TPC values vary from 1134.10 to 1313.39 mg GAE/L overall, while DPPH radical scavenging values are between 84.82 and 95.02%.

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1.0 Introduction

Ginger (*Zingiber officinale*) is one of the world's most valuable medicinal herbs, with Asia accounting for more than half of the global demand for ginger. Based on its remarkable medicinal properties and pleasant aroma, ginger is ranked among Malaysia's top ten potential herbs. Ginger exhibits biological activities such as antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, and cardiovascular protection (Mustafa, 2017). Gingerols homologs, and gingerol derivatives; shogaol and paradols are considered as the key pungent compounds that responsible for its spiciness and biological activities, despite the fact that more than 400 compounds have been discovered in ginger. Bentong ginger (BG), Malaysia patented ginger is being cultivated widely in high altitude in Bentong, Pahang exhibited high demand in both local and international markets. The taste of BG is spicier (high pungency taste) due to the high presence of phenolic compounds especially gingerols which contributed to many pharmacological benefits in human health. The amounts of bioactive compounds in BG were claimed to be significantly higher than in local ginger, which explains its higher price and market demand (Sharizan & Sahilah, 2021).

The amount of nutrients in ginger was believed to be affected by a number of factors, including the growing environment, agronomic application, ginger types, batches of samples, preservation techniques, extraction techniques, and storage conditions (Sharif et al., 2018). Sharizan & Sahilah (2021) revealed that the total phenolic content and antioxidant activity of BG extracted using methanol decreased as the harvesting time (month) increased. The highest total phenolic content with DPPH inhibition around 75.99% was

observed for BG harvested at 7 months. Moreover, Ghasemzadeh et al. (2016) demonstrated that increasing storage time (month) and temperature of ethanolic BG extracts causes a decrease in total phenolic content and DPPH inhibition.

Extraction is the most important step in recovering and purifying active ingredients from medicinal plants (Cha et al., 2020). Ultrasonic-assisted extraction (UAE) is an easy and economical technology that can be used to improve the extraction of active compounds, as a substitute for traditional extraction methods. UAE produces high-quality extracts due to its low operating temperature. UAE has several advantages, including reduced solvent use, energy consumption, and extraction time (Rehman et al., 2020), as well as the ability to overcome the disadvantages of utilising high temperature and pressure in thermo-labile extractions, such as ginger bioactive components (Syed Jaapar et al., 2017). Nevertheless, it has gained popularity in industrial applications because of the benefits of using less solvent with reduced time and, more crucially, being a versatile process that can be scaled up to an industrial level.

Again et al. (2022) study the effect of ultrasonic assisted extraction process parameters including temperature (30–60 ℃), time (10–30 min) and ultrasonic intensity (1768–5304.10 W/cm^2) on the recovery of bioactive compound from BG in terms of crude yield, total phenolic content, ferric reducing-antioxidant power (FRAP) and antioxidant activity (DPPH radical scavenging). The effectiveness of the extraction process is generally determined by a variety of variables. Process modelling techniques like response surface methodology (RSM) are needed to optimise extraction effectively. RSM is a helpful method for classifying the influence of independent variables on the outcomes of a chemical process. It employs statistical and mathematical methods to ascertain the relationship between independent and response variables (Kashyap et al., 2021). However, research on the optimisation of BG extraction techniques for high recovery of bioactive compounds such as phenolic compounds and antioxidant properties using RSM is still limited. Thus, further investigation is needed to determine the best parameter for BG extraction using UAE.

In this study, UAE techniques were employed for the extraction of bioactive compounds from BG. The effect of the amplitude (20–40%), sonication time (10–30 min), and solvent concentration (75–95%) (Dirar et al., 2019) towards the extraction on total phenolic content (TPC) and their antioxidant activity been studied with the aid of RSM. The one factor analysis approach was employed to determine the optimum condition of extraction parameters for the responses.

2.0 Methodology

2.1 Materials

Fresh Bentong ginger (BG) was procured from Pahang Agriculture Department, Malaysia. The samples were stored in −4 ℃ until further process. All of the chemicals and reagent used in this study were of commercial grade. The chemicals used were ethanol (95% purity), methanol (99.4% purity), Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH•) solid and sodium carbonate which were purchased from Sigma-Aldrich.

2.2 Sample preparation

BG samples were rinsed thoroughly, and the peel was removed. The ginger pulp was then diced into uniform dimensions of 1 mm ×1 mm × 1 mm each, and the samples were dried for 24 hours in a hot air oven dryer at 40 °C. Afterwards, the samples were ground with a powder grinding machine (Akiro, Malaysia, 2500 W) at 36000 rpm with 300 mesh fineness. The grinding process was done for 3 minutes using 20 g of samples per run until it turned into fine particles.

2.3 Sample extraction

The extraction of BG powder was carried out by using an ultrasonic processor at 700 W and 20 kHz (QSONICA/Q700 Sonicator). Extraction was carried out by mixing 5 g of powdered ginger with 100 mL of ethanol and distilled water mixture solution of various concentrations while providing thorough contact in a mixing unit at room temperature (25 ± 2 °C) (Kou et al., 2018). Then, the extracts were centrifuged at 4000 rpm over 30 min at 25 °C and the supernatants were separated by decantation and kept at −4 °C until the analysis. Total phenolic content and DPPH radical scavenging activity were determined. All measurements were carried out in triplicate.

2.4 TPC determination

TPC was determined using the Folin-Ciocalteu method, as described by Azman et al., (2014). The calibration of Folin-Ciocalteu method was performed

using gallic acid as a standard. 10 mL of Folin Ciocalteu (FC) reagent was diluted with 90 mL of distilled water to obtain 10% of FC reagent. Then, 100 mL of distilled water was used to dilute 30.8 g sodium carbonate solid to prepare 30.8 % of sodium carbonate solution. The mixture's final concentration (v/v) was: 10 µL of samples, 50 µL of 10% of FC reagent, and 150 µL of 30.8% of sodium carbonate solution. The mixture was finally diluted with 790 µL of distilled water, shaken, and incubated in the dark for one hour. The absorbance at 765 nm was measured using a UV-Vis Spectrophotometer (Thermoscientific Genesys 50, Singapore) against water as a blank. The results were expressed as mg of gallic acid equivalents/L sample (mg GAE/ L sample).

2.5 DPPH radical scavenging activity determination

BG sample extracts were analysed by using DPPH (2,2-diphenyl-1-picrylhydrazyl) following the method of Azman et al., (2014) with minor modifications where 0.2 mM of DPPH reagent was used by diluting 0.0016 g of DPPH solid into 200 mL methanol and the sample was added to DPPH reagent at the ratio of 25:97.5 (v/v). The reagent was stored for 24 h in the dark Schott bottle wrapped with aluminium foil before being used. After that, a cuvette was filled with 25 µL of sample followed by 975 µL of DPPH reagent. The mixture solution was then shaken vigorously for 10 s before being stored in a dark place at room temperature for 4 h. The absorbance was measured at 517 nm using UV-Vis Spectrophotometer (Thermoscientific Genesys 50, Singapore). Eq. (1) was used to calculate the antioxidant activity:

$$DPPH\ radical\ scavenging\ (\%) = \frac{A_b - A_{bs}}{A_b} \times 100\% \quad (1)$$

where A_b = absorbance of blank DPPH, A_{bs} = absorbance of blank DPPH and ginger extract samples.

2.6 Experimental design

One factor analysis design was used to carried out the experiment to evaluate the effects of three independent variables on the total phenolic content and antioxidant activity, including amplitude (20–40%), sonication time (10–30 min) and solvent concentration (75–95%). Table 1 shows the experimental table built in one factor analysis design of response surface methodology (RSM) with five levels of numeric factors. The experiment will be carried out starting by manipulating the amplitude of ultrasonic probe followed by sonication time and solvent concentration.

Table 1: Values of coded and real values of three independent values

Coded values		−2	−1	0	+1	+2
Real values	Amplitude (%)	20	25	30	35	40
	Sonication time (min)	10	15	20	25	30
	Solvent concentration (%)	75	80	85	90	95

For amplitude, the sonication time and solvent concentration kept constant at 20 min and 80% ethanol concentration, respectively while for sonication time, the solvent concentration was kept constant at 80% ethanol concentration but the value for amplitude will be selected based on the previous experiment for amplitude and the same goes to solvent concentration where both value for amplitude and sonication will be selected based on the previous experiment. The suggested optimised values later were used to do a confirmation run to validate the accuracy and the percentage error will be calculated according to Eq. (2) (Trujillo-Mayol et al., 2019).

$$Percentage\ error\ (\%) = \left|\frac{EV - PV}{PV}\right| \times 100\% \quad (2)$$

where EV is the experimental value and PV is the predicted value.

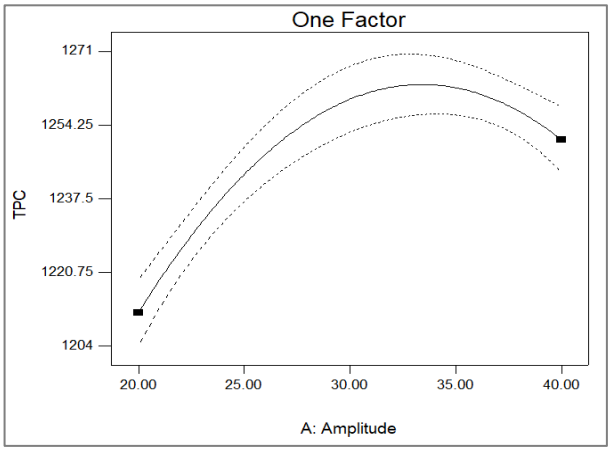
2.7 Statistical analysis

All of the analyses were done in triplicate in this study, and the results were expressed as mean value ± standard deviation (SD). Microsoft Excel and Design Expert (version 7.1.3, Stat-Ease, Inc., Minneapolis, MN) tools were used to conduct the statistical analysis.

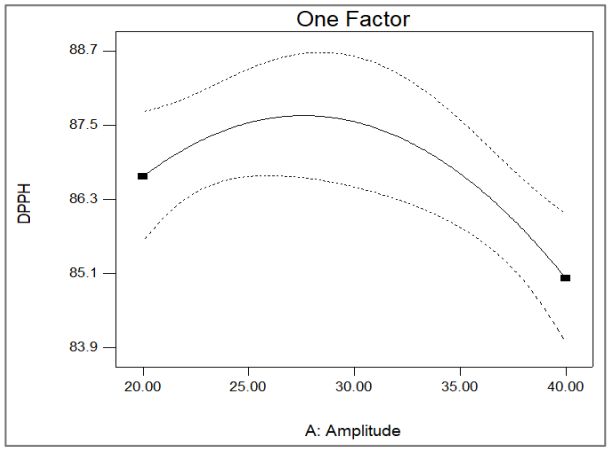
3.0 Results and discussion

3.1 Effect of amplitude on TPC and DPPH radical scavenging

The effect of different amplitude (20, 25, 30, 35, and 40%) was evaluated while keeping the other factors constant, such as 80% of ethanol concentration and the extraction time was set at 20 min. Fig. 1(i) and (ii) illustrate the effect of amplitude on TPC and DPPH radical scavenging, respectively. The dotted lines represent the 95% confidence band on the mean prediction at any given amplitude. The plot for both responses increases when the amplitude increases and the highest value of TPC and DPPH inhibition was observed at 33% and 29%, respectively. This phenomenon can be explained as the number of compression and rarefaction cycles of ultrasonic waves



(i)



(ii)

Fig. 1: One factor plot of amplitude for (i) TPC and (ii) DPPH radical scavenging

rises as the amplitude increases. Ultrasonic waves generate cavitation, or the formation of bubbles in the liquid that can explosively collapse. Consequently, the cell wall structure is disrupted, allowing the phenolic compound to diffuse more easily into the solvent (Yancheshmeh et al., 2022). However, the value of TPC decreased when the amplitude exceeds 33% and the value of DPPH inhibition decreased when the amplitude exceeds 29%. TPC reduced at greater amplitudes due to ultrasonic chemical effects, which resulted in the generation of free radicals within cavitation bubbles. These free radicals reduce the phenolic components in the extract, lowering the total phenols, instead of the Folin-Ciocalteu reagent used in TPC analysis (Kashyap et al., 2021). According to Eq. (3) and Eq. (4), increasing amplitude reduces both responses as indicated by the negative coefficient except linear effect of amplitude for TPC.

$$TPC = 1260.12 + 19.67A - 28.87A^2 \quad (3)$$

$$DPPH = 87.55 - 0.82A - 1.71A^2 \quad (4)$$

where A is amplitude.

Table 2: Summary for amplitude optimisation using RSM (Design Expert software)

Name	Goal	Lower limit	Upper limit	Suggested optimum
Amplitude (%)	is in range	20	40	30.34
TPC (mg GAE/L)	maximise	1211.51	1268.22	1260.84
DPPH (%)	maximise	84.82	87.53	87.52

Table 3: Confirmatory analysis for amplitude experiment

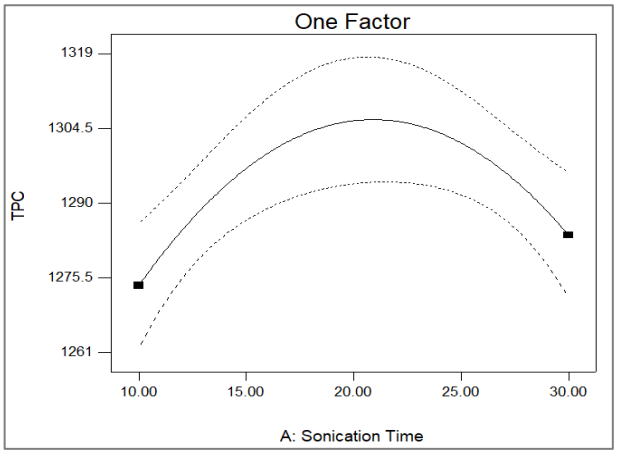
Responses	Predicted	Experimental	Percentage error (%)
TPC (mg GAE/L)	1260.84	1254.37	0.51
DPPH (%)	87.52	85.41	2.41

The value of the TPC will eventually decrease as amplitude increase because the quadratic effect of amplitude has a coefficient value greater than the linear effect.

Furthermore, phenolic content is linked to DPPH free radical scavenging capacity due to phenolics' ability to donate hydrogen and stable radical intermediates (Kaur et al., 2021). According to the one factor analysis via design expert software, the suggested optimum value for amplitude was encoded at 30.34% with the predicted value of TPC at 1260.84 mg GAE/L and DPPH at 87.52% as shown in Table 2. The optimum value of amplitude reported in this study relatively lower than the value reported by Contreras-López et al., (2020). The optimum value of amplitude from this experiment will be used for confirmatory and subsequent analysis. However, the value will be rounded to 30% because the ultrasonic processor can only accept whole numbers. The error obtained was 0.51% with 2.41% for TPC and DPPH as shown in Table 3. The error between experimental and predicted data is less than 10% in which considered low error (Jamshaid & Ahmed, 2022).

3.2 Effect of sonication time on TPC and DPPH

The sonication time had a positive influence on phenolic compound extraction from dried BG, resulting in a gradual increase in TPC. As shown in Fig. 2(i), the plot is almost symmetrical in shape. The dotted lines represent the 95% confidence band on the mean prediction at any given sonication time. The cavitation processes and thermal impacts of ultrasound waves, which promote cell wall breakdown and a surge in phenolic compound mass transfer rate, may be linked to TPC augmentation as sonication time increases.



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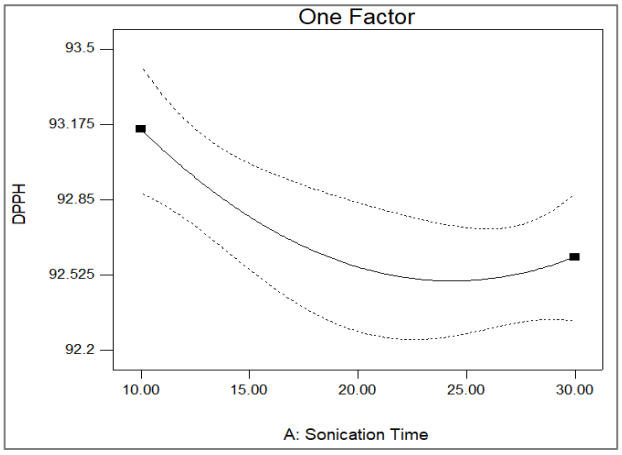


(ii)

Fig. 2: One factor plot of sonication time for (i) TPC and (ii) DPPH radical scavenging

Furthermore, the diffusion of phenolic compounds from the tissue to the solvent was facilitated by a prolonged contact time between the sample and the solvent (Yancheshmeh et al., 2022). The steady increase in TPC value of the extract with time, according to Hassan et al. (2021), can be attributed to the fact that phenolic and other active components are still attached inside the cell matrix during the initial phases of extraction, necessitating more time to detach. The highest TPC was at 20 min with value at 1313.39 mg GAE/L.

However, in the case of DPPH radical scavenging, the results were found to be in reverse direction of the TPC, where different sonication time had a negative effect on DPPH inhibition (Eq. (5) and Eq. (6)).

$$TPC = 1305.97 + 4.91B - 27.08B^2 \quad (5)$$

$$DPPH = 94.56 - 0.28B + 0.32B^2 \quad (6)$$

where B is sonication time.

DPPH inhibition decreased from 93.22% to 92.5 % with increasing time. The varying extraction times, on the other hand, resulted in no significant differences in DPPH radical scavenging. The phenolic compounds in extracts at 10 min were the most effective at scavenging free radicals. Our findings are in agreement with Zain et al. (2020) who revealed the negative effect of sonication time on DPPH inhibition.

Table 4: Summary for sonication time optimisation using RSM (Design Expert software)

Name	Goal	Lower limit	Upper limit	Suggested optimum
Sonication time (min)	is in range	10	30	14
TPC (mg GAE/L)	maximise	1273.82	1313.39	1293.21
DPPH (%)	maximise	92.5	93.22	92.84

Table 5: Confirmatory analysis for sonication time

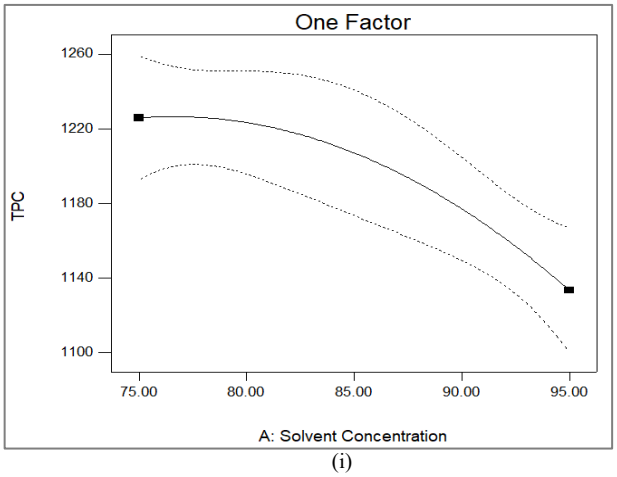
Responses	Predicted	Experimental	Percentage error (%)
TPC (mg GAE/L)	1293.21	1293.60	0.03
DPPH (%)	92.84	93.90	1.14

Prolonged sonication time led to a reduction in both the phenolic content and DPPH antioxidant activity. According to Yancheshmeh et al. (2022), the lower osmotic pressure at the end of the process was accompanied by slower extraction of bioactive compounds into the solvent, which may have resulted in a reduction in TPC recovery. Long-term extraction leads thermally labile phenolic chemicals to degrade, resulting in low TPC (Chen et al., 2020). Moreover, the reduction in TPC and DPPH inhibition could be due to compound oxidation caused by prolonged exposure to environmental conditions like temperature, light, and oxygen (Zain et al., 2020). As a result, to extract higher phenolic procedures, a longer extraction time was not required.

The optimum sonication time for both responses in this study was 14 min, as shown in Table 4, and the percentage error for both responses was within the acceptable range (Table 5). Previously, Murphy et al. (2020) reported a sonication time of around 11 min with a TPC value of 1039.64 mg GAE)/g for non-BG, whereas the optimum sonication time reported by Again et al. (2022) was 25 min with TPC and DPPH inhibition of 704.10 mg GAE/g and 88.80% for BG, respectively. As a result, it has been shown that the process techniques and ginger types affect the outcome of the result.

3.3 Effect of solvent concentration on TPC and DPPH

Solvent extractions are the most widely utilised to extract bioactive compounds from natural products



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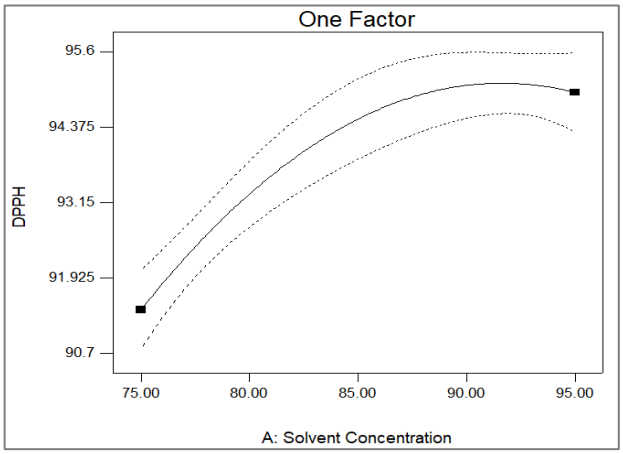

(ii)

Fig. 3: One factor plot of solvent concentration for (i) TPC and (ii) DPPH radical scavenging

because of simplicity, effectiveness, and large production capacity. Ethanol, water and their combination classified as green solvents and they are considered to be Generally Recognised as Safe (GRAS) solvent for food grade (Trujillo-Mayol et al., 2019). The effect of a solvent mixture of ethanol and water on the TPC and DPPH antioxidant activity of ginger extracts was determined in this study by varying the solvent concentration from 75% to 95% while keeping the amplitude and time constant at 30% and 14 min, respectively.

Fig. 3 shows that the TPC value of the extract decreased with increasing ethanol concentration, meanwhile the DPPH inhibition increased with increasing concentration, indicating that the antioxidant activity of ginger extract increased with increasing concentration which is also in agreement with Eq. (7) and Eq. (8).

$$TPC = 1207.09 - 46.26C - 27.41C^2 \quad (7)$$

$$DPPH = 94.51 + 1.76C - 1.33C^2 \quad (8)$$

where C is solvent (ethanol) concentration.

Table 6: Summary for solvent concentration optimisation using RSM (Design Expert software)

Name	Goal	Lower limit	Upper limit	Suggested optimum
Solvent concentration (%)	is in range	75	95	84
TPC (mg GAE/L)	maximise	1134.10	1233.67	1209.98
DPPH (%)	maximise	90.95	95.02	94.39

Table 7: Confirmatory analysis for solvent concentration experiment

Responses	Predicted	Experimental	Percentage error (%)
TPC (mg GAE/L)	1209.98	1208.87	0.09
DPPH (%)	94.39	95.46	1.13

The dotted lines represent the 95% confidence band on the mean prediction at any given solvent concentration. TPC curve began to fall off when the ethanol concentration exceeds 76.5%. According to prior study, the polarity of solvent will reduce when the concentration of ethanol high in the binary solvent system of water-ethanol, which influences phenolic component extraction (Azman et al., 2014; Kou et al., 2018).

In contrast to TPC, Fig. 3(ii) shows that the DPPH inhibition was at its peak when ethanol concentration was 95%. This variation may be attributed to the chemical nature of the extract's bioactive constituents, and a synergy of phenolic compounds with each other and other antioxidant-containing compounds such as flavonoids in the extract could contribute to an increase in antioxidant activity at the highest ethanol concentration (Alonso-Carrillo et al., 2017). The ethanol concentration of 84% was the optimum value for both TPC and DPPH of ginger extract (Table 6). The predicted results matched the experimental data well, as seen in Table 7. Our findings are consistent with those of Murphy et al. (2020), who discovered that the optimum ethanol concentration is 86%.

4.0 Conclusions

An environmentally friendly approach, ultrasonic-assisted extraction (UAE), was utilised to obtain an antioxidant-rich extract from Bentong ginger (BG) in this work. Three extraction parameters including amplitude, sonication time, and solvent (ethanol) concentration were optimised to obtain maximum yield of total phenolic content and DPPH antioxidant activity

of BG. RSM employing one factor analysis was successfully used to evaluate the effect of extraction parameters and to predict the optimum conditions for the UAE. The optimum condition for three parameters towards both responses were found to be amplitude of 30.34%, sonication time of 14 min and ethanol concentration of 84%. The TPC value obtained under optimum condition of amplitude, sonication and solvent concentration were 1260.84 mg GAE/L, 1293.21 mg GAE/L and 1209.98 mg GAE/L, respectively whereas for DPPH inhibition were 87.52, 92.84, and 94.39%, respectively. Higher antioxidant activity was observed in this study, implying that experiment optimisation techniques should be used to increase bioactive compound recovery. The results revealed that there were no significant differences in TPC and DPPH inhibition obtained from BG extracts between predicted and experimental values. This study discovered that BG extract contains valuable polyphenol compounds as well as high antioxidant activity, which would have broad prospects and substantial economic benefits. Future studies should be conducted on the therapeutic potential of BG extracts.

Author contribution statement

Wan Nor Sofiana Wan Ghazali: Investigation, Writing- Original draft preparation. **Sarmilaah Dewi Subramaniam:** Investigation. **Sureena Abdullah:** Formal analysis. **Nurul Aini Mohd Azman:** Validation, Writing- Reviewing and Editing

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.

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