Food Chemistry Advances 3 (2023) 100538

Contents lists available at ScienceDirect

Food Chemistry Advances

journal homepage: www.elsevier.com/locate/focha

Influence of agitation and solvent percentage on the extraction of phytochemical compound from *Asystasia gangetica*

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ARTICLE INFO

Keywords:
Asystasia gangetica
Phytochemical
Total flavonoids
Total phenolic
Antioxidant activity
Response surface methodology

ABSTRACT

This study aims to investigate the influence of agitation and solvent percentage on the phytochemical extraction from *Asystasia gangetica* through response surface methodology and to optimize the extraction condition. The experiment was conducted based on the central composite design with two variables: solvent percentage and agitation speed. The total phenolic compound (Folin–Ciocalteu method), total flavonoid compound (Dowd method), and antioxidant activity (modified DPPH free radical scavenging assay), were analyzed through the analysis of variance. All models were significant (p-value <0.05) with strong R^2 values (over 80 %), indicating satisfactory regression analysis. The optimum condition is achieved at 204 rpm agitation speed and 72 % solvent percentage with total phenolic, total flavonoid, and antioxidant activity at its maximum value of 0.2086 mg/ml, 0.2082 mg/ml, and 1.0494 nmol/ul, respectively. The influence of factors on each phytochemical varies, with both being significant for total flavonoid compound extraction and insignificant for antioxidant activity. Meanwhile, for the total phenolic compound, the solvent percentage is insignificant. Determining the significant factors during phytochemical extraction could be useful in tailoring the suitable range to maximize yield. The bioactive compounds and nutritional composition of *A. gangetica* could be used to develop functional foods and development of drugs to manage various disease.

1. Introduction

Asystasia gangetica belongs to the family Achantaceae and is a herbal plant scientifically proven as a wild edible vegetable (Odoh et al., 2022; Ngozi et al., 2023). *A. gangetica* is a well-known plant species for its outstanding use in medicinal industries and not to mention in food industries since the early centuries. *A. gangetica* is generally consumed as vegetables as it is or cooked as a main dish for daily diets. *A. gangetica* is a valuable source of nutrients, particularly in areas with limited food access and supply. The chemical composition investigation of *A. gangetica* presents that the plant consists of a high carbohydrate composition acting as a principal energy source with a great energy value. Apart from carbohydrates, *A. gangetica* is also rich in vitamins A, C, E, and folic acid, Thiamine, Riboflavin, niacin and pyridoxine in a small amount (Ngozi et al., 2023).

Research has proven that *A. gangetica* is rich in bioactive or phytochemical compounds (Altemimi et al., 2017; Fotsing et al., 2021). Phytochemical compounds are the secondary metabolites produced by plants whose function is more focused on support and defense (Barbaza et al., 2021). These metabolites are necessary for the human diet, serving as micronutrients and facilitating biochemical processes. Phytochemical compounds are known as antioxidants, anti-inflammatory, antiviral, anticancer, antimicrobial, and antifungal properties (Górniak et al., 2018; Mendoza & Silva, 2018; Gunjal, 2020). The antimicrobial activities of phytochemicals have been the keystone of numerous scientific uses in pharmaceuticals, food processing industries, alternative medication, and several natural health remedies (Ajayi & Fuchs, 2015). Antimicrobial activity has been demonstrated by *A. gangetica,* in which various extract concentrations suppressed the growth of several bacterial strains. Methanolic extracts of *A.gangetica* have been proven to inhibit the development of pathogenic microbial includes *Pseudomonas* sp., *Bacillus* sp., *Escherichia* sp., and *Salmonella* sp.

In food processing industries, antimicrobial agents are incorporated directly into food particles or packaging to inhibit the targeted microbial activities (Fadiji et al., 2023). Antimicrobials can also be used as food preservatives to control foodborne bacteria and suppress spoilage microorganisms (Giacometti et al., 2021). Due to its high amount and diverse components of bioactive compounds, *A. gangetica* can be

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https://doi.org/10.1016/j.focha.2023.100538
Received 30 June 2023; Received in revised form 15 November 2023; Accepted 18 November 2023
Available online 19 November 2023

potentially used as a flavor enhancer (Ajuru et al., 2021). In addition, phytochemicals are generally used to improve the sensory and shelf-life of food products. Phytochemicals are also applied to enhance the nutritional value of food products due to their high antioxidant properties (Kawatra et al., 2022). The phytochemical compounds present in *A. gangetica* include phenolics, flavonoids, alkaloids, terpenoids, glycosides, sugars, steroids, saponins, amino acids, carbohydrates, and tannins (Eriamiatoe et al., 2020; Moe & Lwin, 2020; Ajuru et al., 2021; Barbaza et al., 2021). The phytochemical compounds were extracted through either ethanolic or methanolic extract, and the phytochemical screening can be performed through several methods based on the compounds of interest.

Due to their strong antimicrobial and antioxidant properties, phenolics are classified as major bioactive compounds in various plant extracts. These properties help prevent the degradation and rancidity of fat and lipid-based foods and reduce microbial accumulation in foods (Ajayi & Fusch, 2015). Flavonoid is of polyphenol class, having low molecular weight with numerous derivatives. Flavonoids possess antioxidant and anti-inflammatory properties, frequently used against viruses and bacteria. The influence of flavonoids is also noticed in cancer and diabetes studies. Studies on different plant materials suggest that antimicrobial, antifungal, and antiparasitic properties are also demonstrated by flavonoids (Barbaza et al., 2021).

The extraction of phytochemicals from plant species has been the subject of interest for researchers owing to their immense significance in various applications; however, extracting them as part of phytochemical studies presents challenges and barriers. Extraction of active compounds from plants requires proper extraction methods and procedures that result in extracts and fractions rich in phytochemical components. Scientists have devised several extraction procedures for phytochemical components to ensure the potency and effectiveness of the extracts to serve their functions. The basic parameters affecting the quality of the extracted phytoconstituents include the solvents used to extract plant material, the plant part of interest, and the extraction procedures (Fotsing et al., 2021). Different extraction procedures influence the quantity and secondary metabolites being produced. Other factors include extraction time, temperature, solvent nature, concentration, and polarity (Altemimi et al., 2017; Mendoza & Silva, 2018). External factors such as development stage, plant components, fertilization, and soil pH, as well as climatic elements, for example, the availability of water and intensity of light, have been shown to have a major impact on phytochemical content and profile (Borges et al., 2018).

Although past studies have discussed the effects of parameters on the phytochemical extraction of *A. gangetica*, the application of the response surface methodology (RSM) during the extraction is yet to be employed to the best of our knowledge. RSM is a set of statistical approaches for planning experiments, generating models, assessing effect variables, and determining optimal conditions for desired results. RSM has been frequently employed in medium optimization with minimal trials to find and quantify the numerous relationships between different factors. The central composite design (CCD) is a useful design model in RSM for fitting second-order quadratic polynomials in the optimization process. Optimization of one variable at a time demands multiple experiments, which are timely and costly; hence, a proper optimization method is crucial. Therefore, the present study aims to investigate the influence of agitation and solvent percentage on the phytochemical extraction of *Asystasia gangetica* through RSM and to optimize the extraction condition. Determining the optimum condition for the phytochemical extraction is useful for obtaining maximum extracts and reducing the experimental period and reagents used during the experiments.

2. Experimental

2.1. Collection and preparation of plant sample

A. Gangetica was collected from the backyard and roadside area in the Universiti Malaysia Pahang Al-Sultan Abdullah (UMPSA) Gambang. The sample was deposited in the FTKKP laboratory, UMPSA. The collected sample was washed and dried for three days in an oven at 40 °C (Barbaza et al., 2021). The dried plant was then ground using a grinder and sieved before being kept in a tight container.

2.2. Experimental setup for central composite design

A central composite design (CCD) with two variables (agitation and solvent percentage) and five levels (agitation: 160 to 240 rpm; solvent percentage: 60 to 80 %) was utilized to obtain the optimum condition for the phytochemical extraction. The preliminary experiment was performed beforehand to select the two most significant factors affecting the phytochemical extraction with agitation, type of solvent, solvent percentage, extraction times, and temperature as chosen parameters. The result from the preliminary experiment shows that solvent percentage and agitation speed were the two most contributing factors and, therefore, were selected to be optimized, with total phenolic compound (TPC), total flavonoid compound (TFC), and antioxidant activity (AA) as responses. Thirteen experimental runs with randomized conditions (Table 1) were performed, including center points (200 rpm agitation speed, 70 % solvent), in which the order of the running tests was strictly randomized to eliminate potential bias (Dzulkefli & Zainol, 2018). The experiment was conducted in triplicate to ensure the validity of the experimental statistical procedures.

The experimental outputs were recorded in the Design-Expert software and were analyzed using the analysis of variance (ANOVA) to verify the optimum condition for the phytochemical extraction. ANOVA was used to evaluate the validity of the model statistically and was fitted to a second-order model to associate the independent variable with the response variable (Zainol et al., 2022). Eq. (1) represents the coded model for the quadratic equation.

$$\gamma = \beta_o \sum_{i=1}^{\eta} \beta_i \mathscr{X}_i + \sum_{i=1}^{\eta} \beta_{ii} \mathscr{X}_i^2 + \sum \sum_{i<j} \beta_{ij} \mathscr{X}_i \mathscr{X}_j + \varepsilon \quad (1)$$

where γ is the response (measured variables), β_o is the constant coefficient, β_i, β_{ii}, and β_{ij} are the linear coefficient, quadratic coefficient and interaction coefficient effect, respectively, $\mathscr{X}_i$and $\mathscr{X}_i^2$ are the independent variables, η is the number of variables studied, and ε is the error. A validation experiment was performed based on its predicted optimum condition to validate the model. Response surface plots were used to depict the interaction effects of the independent variable on the dependent variable.

2.3. Extraction procedure

The extraction procedure followed the conventional method using different solvent percentages. In this study, methanol was used as a

Table 1
Experimental setup for phytochemical extraction generated by the Design-Expert software.

Std	Run	Agitation (Rpm)	Solvent percent (%)
1	1	180	65
2	3	220	65
3	8	180	75
4	4	220	75
5	10	160	70
6	11	240	70
7	9	200	60
8	7	200	80
9	6	200	70
10	12	200	70
11	5	200	70
12	13	200	70
13	2	200	70

solvent throughout the whole study. The extraction of the phytochemical compounds was determined by the polarity of the solvents. Generally, polar solvents such as methanol and water are employed to extract polar compounds (Abubakar & Haque, 2020). The extraction was carried out according to the setup generated by the Design-expert software, as tabulated in Table 1. Briefly, 5 g of powdered plant samples was mixed with methanol and distilled water at room temperature. The solvent percentage was prepared by mixing the methanol with distilled water to make a 100 ml volume. The mixture was then kept in an incubator shaker at 80 °C for 5 h. The sample was then centrifuged at 5800 ppm for 15 min before being filtered with a Whatman filter paper. The same procedure was repeated using different agitation speeds and methanol percentages following the setups in Table 1. All extracts were kept in the freezer at 4 °C until further use. In this study, controlled variables such as temperature (80 °C) and extraction times (5 h) were kept constant during the whole experiment to eliminate potential sources of variability in the results. The experiments were conducted in a well-ventilated area to minimize exposure to fumes or vapors, and personal protective gear such as safety goggles and gloves were used during the experiment to protect against chemical spills and contact.

2.4. Determination of TPC

TPC was determined by the Folin-Ciocalteu method mentioned by Li et al. (2016). A mixture of 2.5 ml Folin–Ciocalteu reagent and 0.5 ml sample was prepared and rested for 5 min before being added with 2 ml of sodium carbonate. The mixture was kept in the dark for 1 h before being measured with a spectrophotometer at 750 nm. The gallic acid solution was utilized as a standard to produce a calibration curve.

2.5. Determination of TFC

The TFC was measured by the Dowd method, as described by Shirin and Prakash (2010). A mixture of 1 ml sample and 0.3 ml of 10 % aluminium trichloride solution was prepared and rested for 5 min. Subsequently, 2 ml of 1 M of sodium hydroxide was added and rested for another 6 min before being added with 4.4 ml distilled water. The mixture was measured with a spectrophotometer at 450 nm. Quercetin was used as the standard calibration curve.

2.6. Determination of AA

The AA extract was measured through a modified DPPH free radical scavenging assay method (Nile & Park, 2015). The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a rapid and reasonable method for measuring the antioxidant properties using the free radicals to assess the potentiality of a substance to serve as a free-radical scavenger (FRS) (Baliyan et al., 2022). A mixture of 1 ml sample and 2 ml 0.1 M DPPH methanolic solution was produced and incubated for 30 min in the dark at room temperature. The mixture was then monitored through a spectrophotometer at 366 nm using quercetin as standard.

2.7. Experimental validation

A validation experiment was pursued to verify the optimum condition suggested by the Design-Expert software. The error analysis was utilized to verify the optimum conditions between the predicted and actual data.

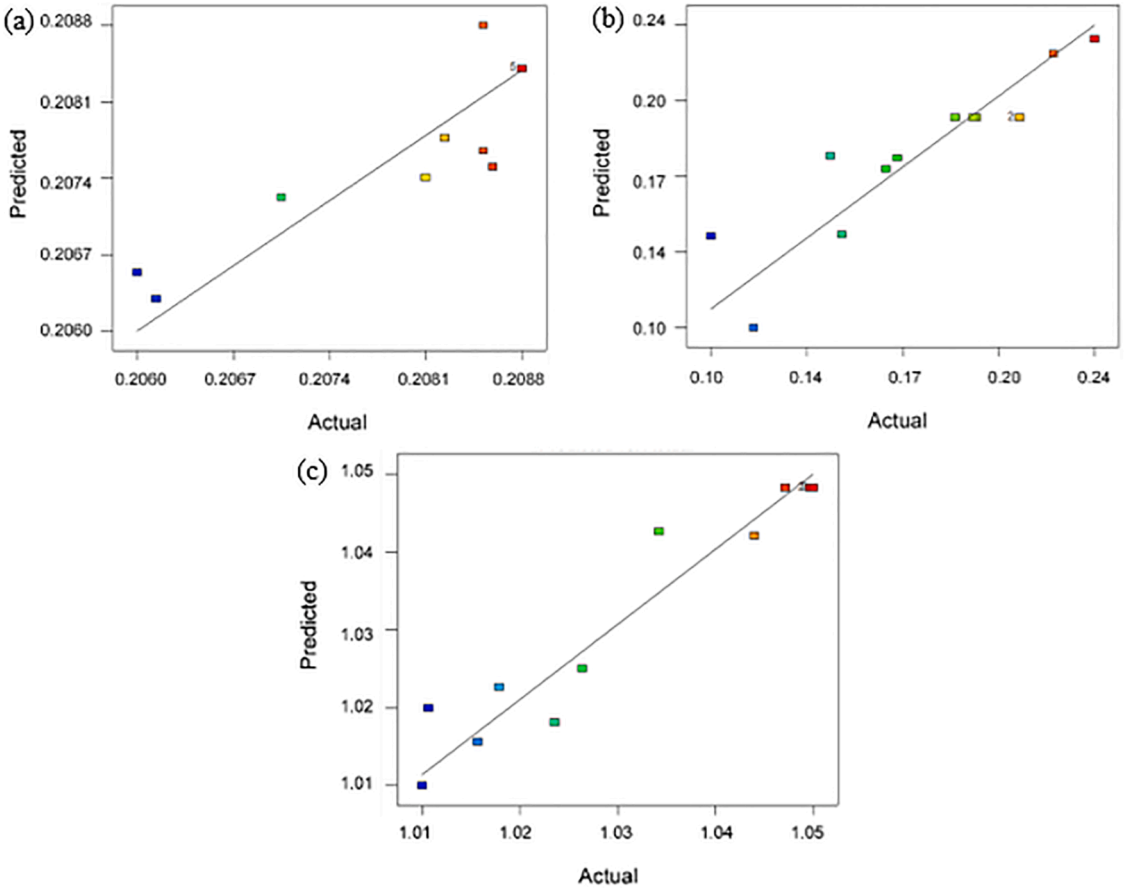

Fig. 1. Correlation of actual and predicted values by the model for (a) TPC, (b) TFC, and (c) AA.

3. Results and discussions

3.1. Phytochemical concentration

Fig. 1 shows the predicted against actual TPC, TFC, and AA values. A linear distribution is seen, implying that the model is well-fitted. The normal probability plot presents that the residual follows a normal distribution, producing an approximately straight line. A linear straight line displays a good normal probability; an S-shaped plot shows a bad normal probability plot. In addition, good residuals against the predicted plot should be randomly scattered instead of in a megaphone shape (Chun et al., 2015).

The highest percentage residual for TPC, TFC, and AA were 0.07 %, 2.24 %, and 0.7 %, respectively. These small residual percentages implied that the obtained values were acceptable, and the occurrence of errors could be due to the nature of the extraction process and minor systematic errors. The residual represents the relationship between the predicted and actual values, with values closer to 0 representing a good fit and is strongly correlated. The highest TPC value of 0.2084 was obtained in experimental conditions of 200 rpm agitation speed and 70 % solvent percentage. Meanwhile, the highest TFC value of 0.2243 was achieved in experimental conditions of 220 rpm agitation speed and 75 % methanol percentage. For AA, the highest value of 1.053 was obtained at a center point of 200 rpm agitation speed and 70 % methanol.

3.2. Analysis of variance (ANOVA)

The use of ANOVA in statistical analysis is to determine the coefficient of the model and evaluate the significance of the selected variables. ANOVA is also used to determine the relevance of the selected range (Ismail et al., 2014; Chun et al., 2015; Samad & Zainol, 2017). In this study, ANOVA was performed to evaluate the variables and their interaction. The statistical significance of the regression equation was evaluated using *F*-values; meanwhile, the significance of each coefficient was determined by *p*-values (Jamaluddin et al., 2014). A *p*-value of less than 0.05 implies that the model terms are significant (Ismail et al., 2014). The lack of fit examined the model fitting accuracy of the actual data and predicted models (Umar et al., 2022). The lack of it is contrary to the whole-model test, in which the lack of fit tests evaluates the significance of any left-out element of the model; meanwhile, the whole-model test indicates the significance of all terms included in the model. A significant lack of fit presents that the run is well replicated with a small variance (Mohd Sharif et al., 2017).

Table 2 displays the significance of the model evaluated by ANOVA for TPC, TFC, and AA concentration. The *F*-value of the model for TPC is 5.77, implying that the model is significant, with only a 2.00 % chance that the value could be attributed to noise. The *p*-value of the model is less than 0.05; hence, the model is significant and could represent the process. A small *p*-value and large *F*-value indicate that the independent variables present a significant effect on the respective response variables. Agitation is seen as a significant factor for TPC concentration, shown by its *p*-value of lower than 0.05. Likewise, both quadratic effect of A^2 and B^2 exhibits a significant *p*-value. The quadratic terms can better capture the nonlinear relationship between variables and hence improve the accuracy of the prediction. Meanwhile, both solvent percentage and AB interaction are insignificant, presented by the large *p*-value. The obtained R^2 value of 0.8047 shows that the model can be applied to navigate the phytochemical extraction design since the R^2 value of more than 0.8 is a valid number for biological processes (Olmez, 2009).

The *F*-value of the model for TFC is 6.45, suggesting that the model is significant, with only a 1.49 % chance that the value could be this large due to noise. The model is significant, presented by the small *p*-value of 0.0149, thereby could represent the process. The concentration of TFC is seen to be significantly affected by the model terms A, B, and A^2 presented by the *p*-value of less than 0.05. Meanwhile, AB interaction and B^2 were insignificant to the process, illustrated by the high *p*-value. The R^2 value for the model is 0.8216, implying that the model is significant. The *F*-value of the model for AA is 18.92, demonstrating that the model is significant, with only a 0.06 % chance that the value could be this large due to noise. The *p*-value of the model is relatively low, which indicates that the model is significant and could present the extraction process. Both agitation and solvent percentage exhibit an insignificant effect on AA concentration, proven by its *p*-value of more than 0.05. Meanwhile, AB interaction and both quadratic effects of A^2 and B^2 are significant for AA, demonstrated by the small *p*-value. The R^2 value of 0.9311 is closer to 1, showing the high statistical significance of the model and that the model is accepted and could represent the extraction process (Karazhiyan et al., 2011). The significant lack of fit values for all three TPC, TFC, and AA models shows that the run is well replicated in

Table 2
ANOVA tables for TPC, TFC, and AA.

	Source	Sum of squares	Mean square	*F* Value	*p*-value	R^2
TPC	Model	7.02E−06	1.4E−06	5.77	0.0200	0.8047
	A	1.57E−06	1.57E−06	6.45	0.0386	
	B	9E−07	9E−07	3.70	0.0960	
	AB	6.75E−07	6.75E−07	2.77	0.1398	
	A^2	2.6E−06	2.6E−06	10.67	0.0137	
	B^2	2.38E−06	2.38E−06	9.76	0.0168	
	Residual	1.705E−006	2.436E−007			
	Lack of fit	1.705E−006	5.683E−007	5.046E+005	<0.0001	
TFC	Model	0.014778	0.002956	6.45	0.0149	0.8216
	A	0.004341	0.004341	9.47	0.0179	
	B	0.005786	0.005786	12.62	0.0093	
	AB	6.35E−05	6.35E−05	0.14	0.7209	
	A^2	0.004502	0.004502	9.82	0.0165	
	B^2	0.000104	0.000104	0.23	0.6485	
	Residual	3.210E−003	4.585E−004			
	Lack of fit	2.828E−003	9.428E−004	9.89	0.0254	
AA	Model	0.003651	0.00073	18.92	0.0006	0.9311
	A	2.08E−05	2.08E−05	0.54	0.4863	
	B	3.11E−05	3.11E−05	0.81	0.3990	
	AB	0.00053	0.00053	13.73	0.0076	
	A^2	0.001488	0.001488	38.55	0.0004	
	B^2	0.002409	0.002409	62.43	<0.0001	
	Residual	2.701E−004	3.859E−005			
	Lack of fit	2.629E−004	8.763E−005	48.28	0.0013	

A = agitation, *B* = solvent percentage.

the experiment.

The correlation between the main variables and the TPC, TFC, and AA concentration can be defined by the quadratic equations in coded terms as presented by Eqs. (2), (3), and (4), respectively. The equations demonstrate the model correlating the interaction between input and output variables with A and B representing agitation speed and solvent percentage. Meanwhile, AB presents the interaction between main variables A and B.

$$TPC = 0.2084 + 0.0004A + 0.0003B + 0.0004AB - 0.0003A^2 - 0.0003B^2 \quad (2)$$

$$TFC = 0.1962 + 0.0190A + 0.0220B + 0.0040AB - 0.0140A^2 - 0.0021B^2 \quad (3)$$

$$AA = 1.0513 + 0.0013A + 0.0016B - 0.0115AB - 0.0081A^2 - 0.0103B^2 \quad (4)$$

3.3. Effect of independent factors on phytochemical extract

The effect of two independent variables on the phytochemical extraction is portrayed in Fig. 2. The effect of agitation on TPC and TFC concentration as presented in Fig. 2(a) and (c) present that the concentration of extracts increases with agitation speed. A similar observation was obtained by Muhammad et al. (2014) in which the increase of agitation speed from 50 to 300 rpm significantly increased the TFC concentration extracted from *Averrhoa bilimbi* by 47 %. A high agitation speed increases the mass transfer coefficient and enhances the convective mass transfer, resulting in larger extraction yields (Tagliazucchi et al., 2010). Elhag et al. (2018) reported that agitation speed is a

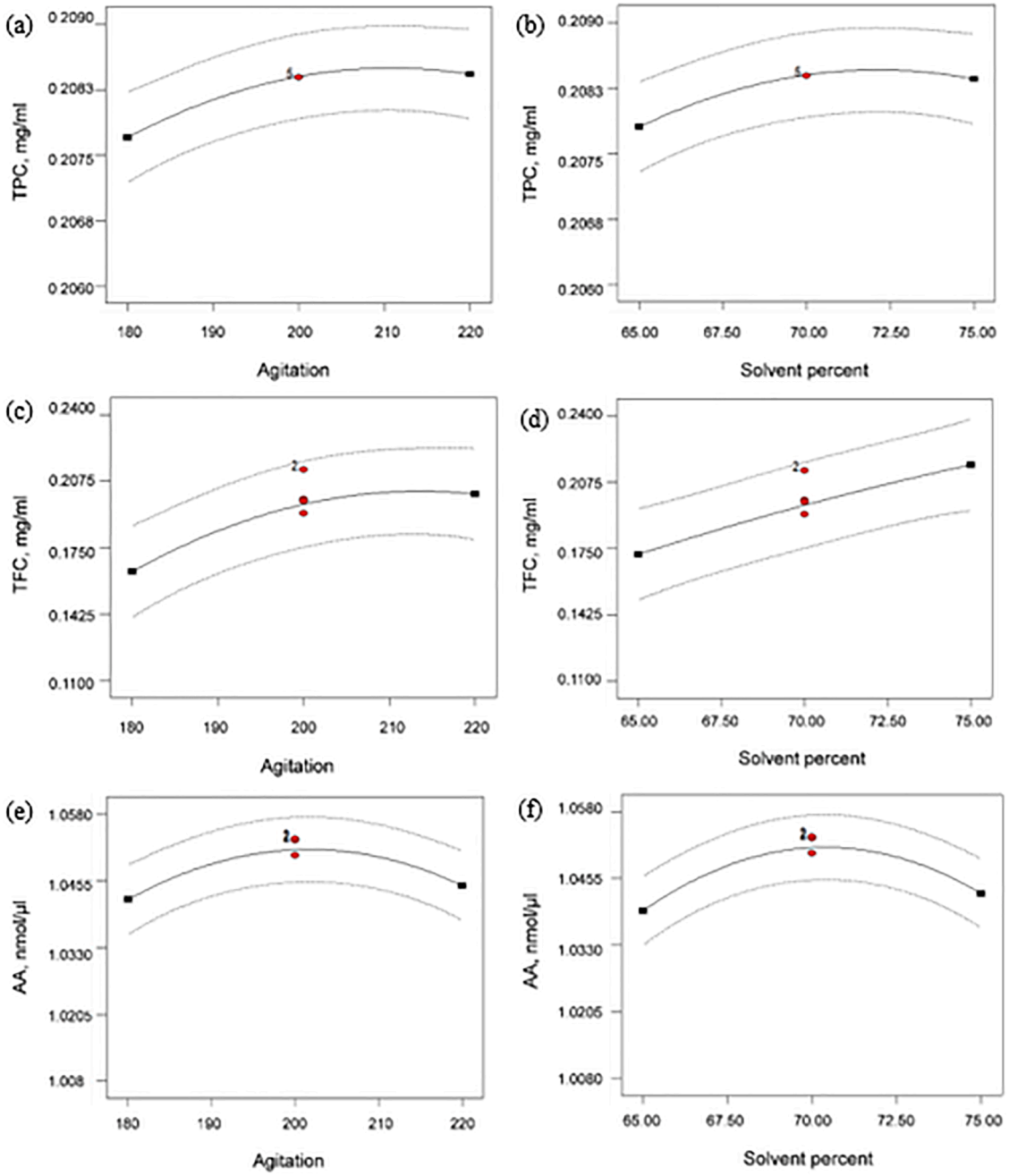

Fig. 2. Effect of agitation and solvent percentage on (a,b) TPC, (c,d) TFC, and (e,f) AA concentration.

significant factor influencing the extraction of saponins from *Eurycoma longifolia* roots, in which increased agitation speeds increased saponin yields.

As shown in Fig. 2(b) and (d), the TPC and TFC concentrations improved with increasing solvent percent. Similar to this finding, Do et al. (2014) stated that a higher TPC concentration was obtained when *Limnophila aromatica* was extracted at 75 % methanolic percentage than other percentages. This is because water extracts contain more non-phenolic compounds, such as carbohydrates, than other extracts. The complex synthesis of some phenolic chemicals soluble in ethanol, acetone, and methanol might also explain this situation. These phenolic compounds may contain more phenol groups or larger molecular weights than the phenolics in the water extract. The effect of agitation and solvent percentages on AA (Fig. 2(e) and (f)) exhibits a varied trend from that of TPC and TFC. The highest AA value was obtained at the center point of 200 rpm agitation speed and 70 % methanol percentage. The AA concentration increases with agitation speed and solvent percentage and presents a reverse trend after reaching the center point. Excessive agitation speed could result in greater mechanical forces or hydrodynamic shear stresses, hence reducing yields (Samad & Zainol, 2017).

3.4. Effect of interaction factors on phytochemical extraction

The contour plot, as displayed in Fig. 3, illustrated the interaction between the variables and response. The figure visualized the interaction between agitation speed and solvent percent against TPC, TFC, and AA concentration. The plot exhibited that the optimum point is located within the experimental region. The elliptical contour plot shown by all responses proved a significant interaction between agitation and solvent percentage.

Both flavonoid and phenolic are essential antioxidant compounds responsible for deactivating free radicals. This is due to their ability to donate hydrogen atoms to free radicals as well as in scavenging free radicals (Aryal et al., 2019). Several studies have reported a proportional relationship between TPC and TFC with antioxidant capacity. Aryal et al. (2019) reported a linear correlation between TPC and TFC on DPPH activity, which suggests that both phytochemical compounds are extremely accountable for the antioxidant activity of the plant extracts.

3.5. Optimum condition and model validation

The optimum condition for the phytochemical extraction was chosen based on the analysis of the numerical optimization method of the Design-Expert software, with the desirability value closest to 1. Both agitation speed and solvent percentage were set within the range, whereas the TPC, TFC, and AA were put to the maximum to achieve maximum desirability. Based on the analysis, the conditions at which 204 rpm agitation speed and 72 % solvent percentage is the most desired condition with the highest desirability value of 0.930. The desirability value closer to 1 indicates that the design is appropriate and can be used. At this selected condition, the TPC, TFC, and AA values could go up to 0.2086, 0.2082, and 1.05, respectively.

A validation experiment was conducted in triplicate according to the recommended optimum condition to verify the predicted value generated by the software. The percentage of error obtained for TPC, TFC, and AA were 1.15 %, 4.51 %, and 0.34 %, respectively. The obtained error values were in an acceptable range, and the values followed the rule of thumb of an adequate error percentage of less than 30 %. The actual TPC, TFC, and AA values at the suggested optimum conditions were 0.2110, 0.1988, and 1.0530 mg/ml each. Therefore, the recommended optimum point is an acceptable condition for phytochemical extraction.

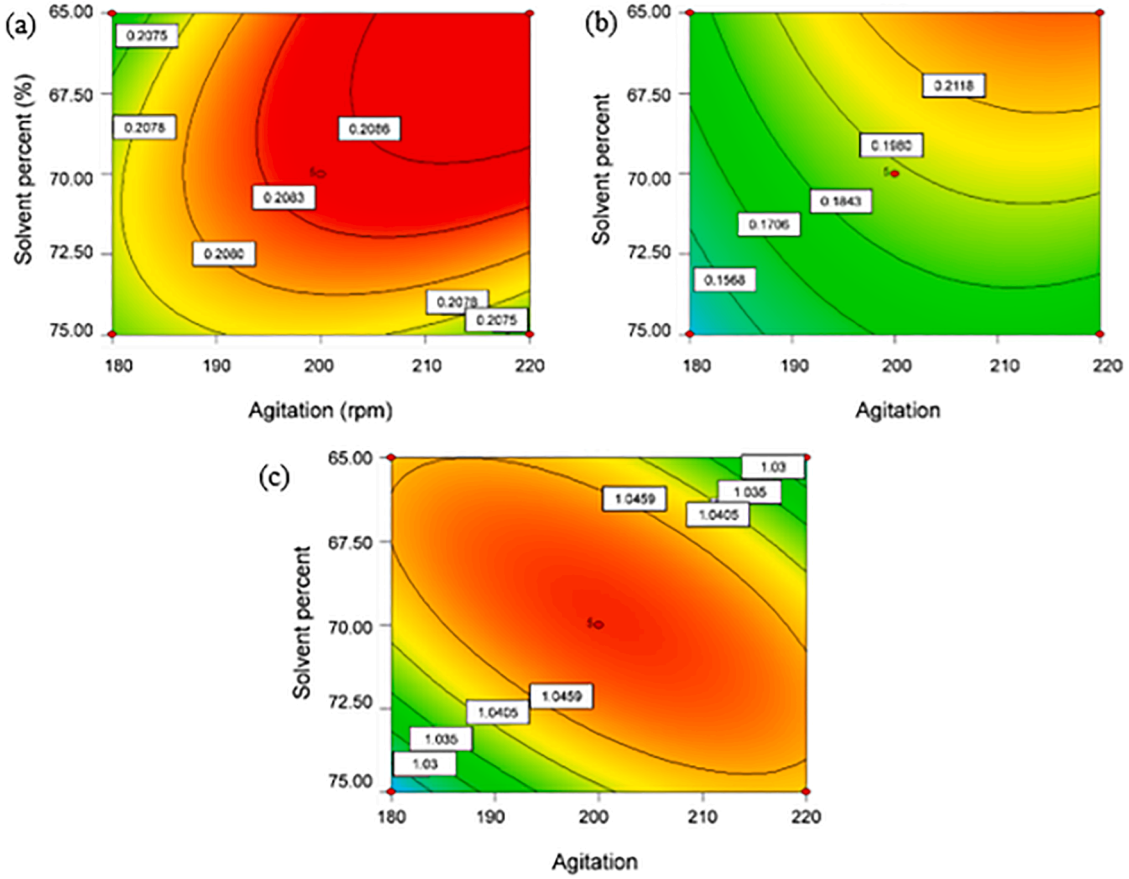

Fig. 3. Interaction of agitation and solvent percentage presented in a contour plot for (a) TPC, (b) TFC, and (c) AA.

However, separating the optimum condition for each compound could result in more yield. This is due to the different parameter range requirements for each response. For example, if we separate the maximum value for each response, the optimum condition could be varied. From the experimental data, the maximum TFC and AA were obtained in an experimental condition of 200 rpm agitation speed and 70 % solvent percentage. Meanwhile, the maximum value of TFC was obtained at 200 rpm agitation speed and 80 % solvent percentage. Therefore, it is important to acknowledge the significant process parameters for each response to maximize the yield further.

The varied optimum condition for each phytochemical implies that a compromise was made by using a single set of optimum conditions for all compounds, potentially leading to suboptimal yields for some individual compounds, which presents its limitation. However, given the small error percentage between the experimented and predicted values obtained during the validation experiment, the universal optimum condition that maximizes all phytochemical compounds could, therefore, be used to represent the whole process.

In comparison with other studies, Kim et al. (2022) have also applied RSM to evaluate the optimum condition for flavonoid extraction from *Daphne genkwa* with temperature (4 to 45 °C), agitation speed (50 to 250 rpm), and extraction times (12 to 48 h) as parameters. All factors were significant to the extraction process, with an agitation speed of 150 rpm being the optimized speed. The difference in the optimized agitation speed value obtained from this study could be due to the chosen plant sample being extracted in the study. Basir et al. (2020) also employed RSM to optimize TPC and AA extraction from *A. gangetica* through several extraction methods. The optimum TPC and AA were recorded at 2.73 mg GAE/g and 59.75 %, respectively. The high TPC value obtained by Basir et al. (2020) compared to the current study might be due to the selection of different extraction methods, in which the optimization experiment was pursued on the combination of cold-maceration and ultrasonic-assisted extraction (UAE) method. The methanolic extraction of *A. gangetica,* as reported by Barbaza et al. (2021), presents that the plant extract consists of 85.48 μg/mg flavonoids. Meanwhile, the TPC and TFC values of *A. gangetica* through ethanolic extraction, obtained by Janakiraman et al. (2021), were 139.68 and 237.77 mg GAE/g, respectively. The variation in TPC and TFC values as compared to the current study is possibly due to the amount of plant powder used during the experiment, in which more plant powder could result in a greater amount of TPC and TFC. Therefore, with such variations in the TPC and TFC obtained from several studies, recognizing and identifying the significant process parameters is indeed useful in scaling up the phytochemical extracts. Determining optimum extraction conditions could further maximize the phytochemical extraction since the extraction can be performed at their corresponding optimum process parameters.

4. Conclusion

The influence of agitation and solvent percentage on the phytochemical extraction of *A. gangetica* was determined in this study, with the factors being significant for TPC extraction. Both factors were insignificant for AA, and as for TFC, the solvent percentage was insignificant. The optimum phytochemical extraction condition was obtained at 204 rpm agitation speed and 72 % solvent percentage with TPC, TFC, and AA at their maximum value of 0.2086, 0.2082, and 1.0494 mg/ml, respectively. The findings of this study could be used to increase phytochemical extraction yields for future use in food and pharmaceutical industries. It is recommended to consider other significant factors affecting the extraction to obtain maximized extracts from the plant. Future research is recommended to implement alternative extraction methods, investigate more parameters, and development of the extracted compounds into marketable products.

CRediT authorship contribution statement

Norazwina Zainol: Methodology, Data curation, Resources, Supervision, Project administration, Conceptualization, Funding acquisition. **Nor Hazwani Aziz:** Writing – original draft, Writing – review & editing. **Amir Syarifudin Baharudin:** Writing – original draft, Investigation.

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.

Acknowledgments

The authors would like to thank the Ministry of Higher Education for providing financial support under Fundamental Research Grant Scheme (FRGS) no. FRGS/1/2023/TK05/UMP/02/7 (University reference RDU230130) and Universiti Malaysia Pahang Al-Sultan Abdullah for laboratory facilities.

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