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Response surface optimization on the total phenolic content and antioxidant activities of Sabah Snake Grass (*Clinacanthus nutans)* leaves Peleg kinetic modelling extract

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Abstract

Sabah Snake Grass (*Clinacanthus nutans*) is interesting traditional medicine because of pharmacology characteristics that contain rich in phenolic content and antioxidant. The aim of study was to determine the optimum yield and exhaustive time extraction using Peleg's model. Qualitative and quantification test for detection of orientin and vitexin using method of high performance liquid chromatography (HPLC). Based on the results obtained, the optimum concentration for orientin (0.72 ± 0.002 mg/g) and vitexin (2.10 ± 0.13 mg/g) were observed at 18 h of extraction ($t_{exhaustive}$). The optimum extraction parameters for optimum recovery of phenolic content and antioxidant activities from the leaves of *Clinachantus nutans* were determined using response surface methodology (RSM). The total phenolic content (TPC) was analyzed using the Folin-Ciocalteu method and antioxidant activities were evaluated through 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging activity and the ferric reducing antioxidant power (FRAP) assay. A central composite rotatable design (CCRD) was employed to investigate the effects of ultrasonic frequency (X1: 25 - 40 kHz), temperature (X2: 40 - 80ºC) and solid-to-liquid ratio (X3: 10 - 30 ml/g) on the recovery of TPC (Y1) and antioxidant activities of DPPH (Y2) and FRAP (Y3). The optimal parameter achieved based on combination of extraction parameters: X1 = 25 kHz sonication capacity, X2 = 80ºC temperature and X3 = 30 g/ml of solid-liquid ratio. These optimum conditions yielded TPC of (25.09 mg GAE/g), DPPH (66.85%), and FRAP (9.44 µmol TE/g). The optimum values of TPC and DPPH from this study are comparable with green tea (benchmark). Our results revealed that distilled water may be a good choice for extracting antioxidant activity of *Clinacanthus mutans*. Concentration of orientin and vitexin compounds were extracted during optimization exhibited lower than the finding from Peleg model. Prolonged extraction at high temperature during optimization may degrade flavonoid and phenolic acid compounds. However, the concentration of extracted compound (orientin and vitexin) from the optimum parameters had produced high in phenolic content and antioxidant activity. In conclusion, the application of Peleg model was able to determine the extraction exhaustive time at the maximum extract yield. In addition, this study proved that the application both models (RSM and Peleg) have been successfully be able to provide the optimum extraction parameters with high total phenolic content and antioxidant activity.

Introduction

Herbal plant such as Sabah snake grass or scientifically known as *Clinacanthus nutans* is widely grown in tropical Asia including Malaysia, Thailand, Indonesia and China (Yuann *et al*., 2012). Normally, this plant is a small shrub that can be found in north East Asia area primarily in Malaysia and Thailand (Tuntiwachwuttikul *et al*., 2004). Sabah snake grass leave has diverse and potential medicinal uses in traditional herbal medicine for treating variety of diseases such as diabetes mellitus, fever, diarrhea and dysuria (Png *et al*., 2012).

Clinacanthus nutans, belonging to the family of *Acanthaceae* and it is known in Malaysia as Sabah snake grass 'Belalai Gajah'. There are two type of *Clinacanthus* which is Clinachantus nutans and *Clinachanthus siamensis*. This two herb plant has

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similar appearances but different size and shape of leaves (Shim *et al*., 2013). In Malaysia, it has traditionally been used to treat diseases and its potential to be used as natural nutraceuticals for cancer prevention and treatment (Roosita *et al*., 2008). Sabah snake grass is a medicinal plants in Thailand and commonly is known as 'Phaya Yo' or 'Phaya Plong Thong', which has been used in treatment of skin rashes, insects and snake bites, fever, herpes simplex virus (HSV), and varicella- zoster virus (VSZ) lesion (Sakdarat, 2009). Meanwhile, Sabah snake grass is also utilized in Indonesia as a traditional medicine and it is known by local as 'Dandang Gendis' for treating diabetes mellitus and intestinal inflammation (Sakdarat, 2009).

In Malaysia, this herb is commonly consumed by people in rural area as it is believed to contain high nutrients and rich in antioxidants. The extract of this plant have great antioxidant potential for repairing the oxidative damaged (Pannangpetch *et al*., 2007). Previously, Sabah snake grass has been phytochemically and chemically isolated of stigmasterol, lupeol, b-sitosterol,six known C-glycosyl flavones, vitexin, isovitexin, shaftoside, isomollupentin-7-O-b-glucopyranoside, orientin, isoorientin, five sulfur-containing glycosides, two glycoglycerolipids, a mixture of nine cerebrosides and a monoacylmonogalatosylglycerol. Only the two glycoglycerolipids have been shown to exhibit antiviral activity. Their potential is strengthened by the existence of several flavonoid phytochemical compounds in leaf extract such as C-glycosyl flavones; shaftoside, isomollupentin 7-O-*β*-glucopyranoside, orientin, isoorientin, vitexin and isovitexin (Teshim *et al*., 1997). They possess important biological activities including antimicrobial activity (isoorientin, vitexin), hepatoprotective activity (isoorientin), and antioxidant activity (isovitexin) (Zhang *et al*., 2008). The flavonoid phytochemicals are natural antioxidants regarded as a powerful antioxidant and has gained increasing attention as a potential protector of various human diseases such as certain cardiovascular diseases and cancer (Ren *et al*., 2003). The availability of phenolic compounds in Sabah snake grass leaves as antioxidant agent is confirmed. However, high extraction production of bioactive compounds from plant is need to be attained as it can reduce the economic cost. Many parameters have been established to influence the extraction efficacy, such as extraction methods, particle size, solvent type, solvent concentration, solvent-to-solid ratio, extraction temperature, extraction time and pH (Silva, 2007). Response surface methodology (RSM) is an effective tool for optimizing complex processes (Liu *et al*., 2010). It has been successfully demonstrated that RSM can be used as a tool in optimizing the extraction of flavonoid compounds from many medicinal plants (Liu *et al*., 2010). Therefore, this is the important herbal plant in determination the level of flavonoid phytochemical content in Sabah snake grass leave which has the potential in protecting various diseases. To the best of our knowledge, no other studies have been undertaken related in optimizing the extraction of phenolic compounds from Sabah snake grass leaves using RSM. For that reason, this study was aimed to: 1) determine the main important bio-active constituents (mainly flavonoids) such as orientin and vitexin using high performance liquid chromatography (HPLC); 2) determine the optimum yield of flavonoids and exhaustive time extraction using Peleg's mathematical model and 3) determine the optimum operational parameters (e. g. sonication frequency, temperature and solid-to-liquid ratio) for optimum recovery of phenolic content and antioxidant activities using response surface methodology (RSM).

Materials and methods

Plant material

Sabah snake grass fresh leaves (2.5 kg) were collected from Sendayan, Seremban, Malaysia. Voucher specimens SBID 019/12 was prepared and authenticated by botanist from Forest Research Institute Malaysia (FRIM). Young leaves (1 month-old) was selected prior to extraction (Fazil *et al*., 2016). The samples were dried in an oven at 40°C (Chelyn *et al*., 2014; Zubairi *et al*., 2014b) for 48 h then ground and sifted for homogenization. The samples were kept in bottles prior to extraction process and stored at room temperature (25°C).

Chemicals and reagents

Chemical standards vitexin and orientin, methanol, glacial acetic acid, Folin–Ciocalteu phenol reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma (USA). Sodium carbonate was purchased from (Systerm, Selangor, Malaysia). 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) was purchased from Fluka, Switzerland and ferric chloride ($FeCl_3 \cdot 6H_2O$) was purchased from John Kollin Corporation, UK. All the reagents used in the HPLC analyses were of HPLC grade.

Normal soaking solid-liquid extraction process

Approximately 30 g of dried samples were

wrapped with muslin bag and the extraction was carried out in 500 ml beakers (solid-to-liquid ratio of 1: 10 (w/v)) for 72 h (Zubairi *et al*., 2014c; Zubairi *et al*., 2014e; Nasir and Bohari, 2015). Next, every 2 h interval, 1 ml of extract was collected using a micropipette and weighed for the extract concentration measurement (mg/g) (n = 3). The volume (ml) of the remaining extracts were re-measured for mass balance. After the extraction process completed, the extract was then centrifuged at speed 4000 rpm for 10 min and filtered through a Whatman No. 1 filter paper. The removal of any unwanted debris from the extract was then collected in an amber reagent bottle. The filtrate was subsequently used for the determination of flavonoid compounds by using HPLC.

Kinetic model of normal soaking solid-liquid extraction

The Peleg's model is mathematical modelling and a useful engineering tool that able to predict the optimize concentration extract in a minimal time of experimental (Turhan *et al*., 2002; Othman *et al*., 2017; Hashim *et al*., 2018). Two constant K in this model with mathematic equation y = mx + c show that K_1 is value for y-intercept and K_2 is the gradient value. The Peleg's model is shown as:

$$C(t) = \frac{C_o + t}{K_1 + K_2 \times t} \quad (1)$$

Where C(t) is the extract concentration (mg/g) at t (min), C_o is the concentration at t = 0 (mg/g), K_1 is the constant value for Peleg 1 (min.g/ml) and K_2 is the constant value for Peleg 2 (ml/g). Concentration (C_o) at t = 0 is assumed as 0. So, the linear Equation (2) is derived as:

$$t/[\, C(t) - C_o] = K_1 + K_2 \times t \quad (2)$$

Peleg 1 constant (K_1) relates to rate of concentration (B_o) at a specific time process ($t = t_o$). In Equation (3):

$$B_o = 1/K_1 \quad (3)$$

The Peleg capacity constant K_2 relates to maximum extraction. When $t \rightarrow \infty$ (extraction reached between the dissolved substance in a sample of bulk volume of the extract). Equation (4) gives relation between the equilibrium of extract concentration and constant of K_2

$$C|t\rightarrow\infty = Ce = 1/K_1 \quad (4)$$

Identification of flavonoids compounds via HPLC analysis

Determine flavonoids compounds were performed using a HPLC system (Shimadzu). The chromatographic separation was performed using a XBridge C18 at 30˚C. The solvent system consisted of 2 solvents: A; 0.1% acetic acid and B; MeOH. The flow rate and injection volume were adjusted at 10 µl and 0.8 ml/min, respectively. The detection was monitored at 280 nm (Zubairi *et al*., 2014a; Zubairi *et al*., 2014d). Individual orientin and vitexin contents were determined by the peak area using response factor method:

$$\text{Response Factor (RF)} = \frac{\text{Peak standard area (µv*s}}{\text{Standard concentration (mg/g)}} \quad (5)$$

$$\text{Sample Concentration (mg/g)} = \frac{\text{Peak sample area (µv*s)}}{\text{RF (µv*s mg/g}^{-1})} \quad (6)$$

Experimental design

The extraction parameters were optimized using response surface methodology (RSM). A three-level factorial central composite design (CCRD) was developed to obtain an optimize extraction method for determination of total phenolic and antioxidant capacity in Sabah snake grass extract. The overall design was consisted of 20 experiments, included 8 factorial points, 6 axial points and 6 centre points. Effect of the level of the three parameter were obtained based on three dimensional plots and their respective contour plots. Based on the Peng *et al*. (2012), simultaneous interaction effect of three parameter on the responds were studied from those three dimensional plots. The optimization extraction process was carried out by using sonication tab (POWER SONIC420; P: 200 watts) instead of using normal soaking extraction method as to facilitate the secretion and diffusion of bio-active constituents (mainly flavonoids). Three selected independent variables were selected and determined based on the preliminary and previous studies, namely frequency sonication (X1: 25 - 40 kHz) (Melecchi *et al*., 2006), temperature (X2: 40 - 80ºC) (Goldsmith *et al*., 2014) and solid-to-liquid ratio (X3: 10 - 30 ml/g) (Tan *et al*., 2011) on the total phenolic content (Y1), antioxidant capacity of DPPH (Y2) and FRAP (Y3). Coded and uncoded levels of the independent variable and the experimental design were given in Table 1. The response optimizer was applied for both graphical and numerical optimizations to obtain optimum conditions and predicted values for the response variables.

Table 1: Coded and uncoded levels of independent variables used in the response surface methodology

Independent variables	Symbol	Level		
		Low (-1)	Middle (0)	High (+1)
Ultrasonic frequency (kHz)	X1	25	30	40
Temperature (°C)	X2	40	60	80
Solid-to-liquid ratio (g/ml)	X3	10	20	30

Determination of Total Phenolic Content (TPC)

TPCs of the extracts were determined according to the method by Musa *et al*. (2011). 0.2 ml extract and gallic acid was placed in a separate 10 ml vials, followed by the addition of 0.4 ml water and 0.5 ml diluted Folin–Ciocalteu reagent. The mixtures were swirled and allowed to stand for 5 min before mixing 1 ml of sodium carbonate (7.5% w/v). The solutions were allowed to stand for 2 h at room temperature and later the absorbance was observed at 765 nm using UV-visible spectrophotometer (Zubairi and Jaeis, 2014).

2,2-Diphenyl-1-pycryl-hydrazyl(DPPH) free radical scavenging activity

DPPH free radical scavenging activity was measured by the method of (Xu and Chang, 2007). Ferric reducing antioxidant power (FRAP) was estimated according to adapted procedure of Musa *et al*. (2011) with minor modifications. FRAP reagent was modified on HCl preparation (40 mM HCl; 20 mM FeCl3 6H2O in the ratio of 10:1:1). The free radical scavenging activity was calculated according to the following equation:

$$\text{Scavenging activity (\%)} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100 \quad (7)$$

$\text{Abs}_{\text{Control}}$ = Absorbance of DPPH solution without sample

$\text{Abs}_{\text{Sample}}$ = Absorbance of DPPH solution with sample

Ferric Reducing Antioxidant Power (FRAP)

The antioxidant capacity of each sample was estimated according to adapted procedure of Musa *et al*. (2011) with minor modifications. FRAP reagent was prepared as using 300 mM acetate buffer, pH 3.6 [3.1 g sodium acetate trihydrate, plus 16-ml glacial acetic acid made up to 1: l with distilled water]; 10 mM TPTZ (2,4,6-tri(2-pyridyl)-striazine), in 40 mM HCl; and 20 mM $FeCl_3$ $6H_2O$ in the ratio of 10:1:1 to give the working reagent. For the analysis, 100 µL of sample extracts was added to the fresh FRAP reagent (1 ml) and incubated for 30 min. After 30 min of incubation, the absorbance was measured at 595 nm. The result was expressed as milligrams of Trolox equivalents per 1 g of fresh sample (mg TE/g of FW).

Model verification

The experimental and predicted values of TPC were compared in order to determine the validity of the model. To verify those results, experimental runs were carried out in replicate under the selected optimised conditions.

Statistical analysis

Statistical analysis was conducted with SPSS 23.0 software (SAS Institute, USA). A value of $p<0.05$ was considered statistically significant. Fitness to RSM model between theoretical and practical values of the predicted dependent variable was determined by Student's t-test (ANOVA). Total phenolic and antioxidant capacity of the Sabah snake grass were expressed as means ± standard deviations (SD). The Design Expert (Version 6.0.10, Stat-Ease Inc., Minneapolis) statistical software was employed to design the CCRD and analyse the experimental data in RSM.

Results and discussion

Flavonoid compounds

RP-HPLC was performed for determination of flavonoid compounds in Sabah snake grass extract which was orientin and vitexin. In this study, water (a polar solvent) was used as an extraction solvent because it is non-toxic and environmentally-friendly solvent for natural product extractions (Tan *et al*., 2014). Besides, it was important to determine the optimal conditions for extracting flavonoids as to mimic the green tea concoction preparation since this plant has been widely used as tea too for therapeutic application. The result showed that orientin and vitexin compounds were successfully detected in Sabah snake grass leave extract. It was identified by comparing the retention times of flavonoid compounds standard with the peak obtained from the extract via external standard method (Figure 1). Based on the chromatographic profile, orientin and vitexin compounds that had been extracted at the 18th hour had a retention time of 28.233 ± 0.02 min and 28.62 ± 0.04 min respectively.

Orientin and vitexin compounds are water-soluble flavonoid and known as polar molecule.

In this study, C18 column was used during HPLC analysis and it is non-polar properties. Separation of sample component was done based on hydrophobic interaction between compound and stationary phase which involves the adsorption of hydrophobic (nonpolar) compounds onto a hydrophobic stationary phase in a polar mobile phase, resulting in more polar compounds eluting first (Dobes *et al*., 2013). Sample separation is based on differences in the hydrophobic nature of the part of the molecule near the surface. Using polar solvent as an extraction solvent also assisted in production of flavonoid compound in Sabah snake grass extract. This study had shown that the concentration of orientin compound in Sabah snake grass extract was significant lower (0.720 ± 0.022 mg/g) as compared to vitexin compound which was (2.095 ± 0.131 mg/g) (Table 2). The concentration value for interval sampling every 2 h (extraction time 72 h) was subsequently used for the determination of exhaustive yield and time of extraction via Peleg model.

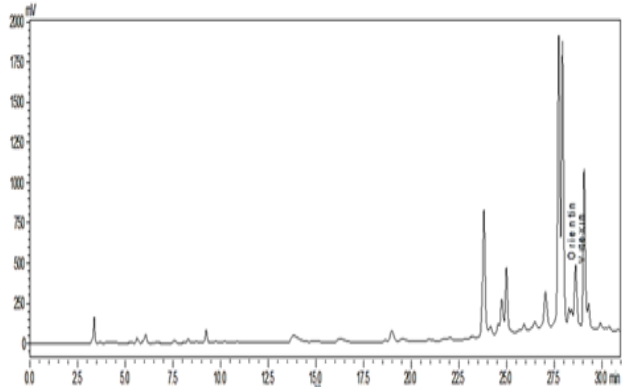


Figure 1: HPLC-UV/DAD chromatogram of Sabah snake grass leaves water extracts containing orientin and vitexin bio-active constituents

Table 2: Orientin and vitexin concentrations in Sabah snake grass water extract

	Orientin	Vitexin
Concentration (mg/g)	0.72 ± 0.02[a]	2.10 ± 0.13[b]

Mean value [a-b]. Different alphabet letters indicate significant difference (p<0.05)

Yield of extract and exhaustive time of extraction

The Peleg's model is mathematical modelling and a useful engineering tool that able to predict the optimize concentration extract in a short time, reducing energy and the used of solvent. The Peleg's model is an empirical model which is commonly used and an easy mathematical modelling. Figure 2(a) illustrates the plotted kinetic mass (min/mg) versus time of extraction (min). The graph plotted to determine the yield/concentration (K_1) of each compound (orientin and vitexin). Extraction rate (mg/min) and the maximum extraction yield were studied based on the K_1 and K_2 constant. The constant K_1 (y-intercept) is referred to mass/concentration meanwhile the constant K_2 is related to the gradient value via the transformation kinetics curves. The lower K_1, the higher the extraction yield/concentration (Turhan *et al*., 2002). The logarithm graph in Figure 2(b) was plotted to determine the exhaustive time of extraction ($t_{exhaustive}$) based on the K_2 value.

Based on the Table 3 data, the calculation of Peleg model from sample showed that the orientin compound had lower extraction rate (3.25 ± 0.45 mg/min) as compared to compound vitexin (3.58 ± 0.35 mg/min). According to Raymond (2007), the reaction rate of extraction of a substance will be decreased if the concentration is increasing. However, the maximum extraction yield for orientin compound (133.97 ± 2.76 mg) was significantly (p<0.05) higher than the vitexin compound (76.55 ± 1.48 mg). The exhaustive time to produce the highest concentration of orientin compound extracted from Sabah snake grass's leaves was too long (17.72 ± 0.71 h) as compared to vitexin compound (7.56 ± 0.72 h) (p<0.05). However, 18 h of extraction has been chosen in optimization study due to the exhaustive concentration of orientin compound were successfully attained as well as maintaining the exhaustive concentration of vitexin. Extraction for 7 h may not be enough to increase the yield of extract for vitexin compound. A study from Melecchi *et al*. (2006) showed that the prolonged time of extraction may able to increase the yield of extraction. Therefore, time extraction for 18 h has been chosen to ensure that both bio-active compounds can be extracted at higher rate.

Table 3: Yield of extract and exhaustive time of Sabah snake grasses leaves extraction containing orientin and vitexin in 72 h of extraction

	Orientin	Vitexin
Extraction rate (mg/min)	3.25 ± 0.45	3.58 ± 0.35
Maximum yield extraction (mg)	133.97 ± 2.76[a]	76.55 ± 1.48[b]
Time exhaustive extraction (h)	17.72 ± 0.71[a]	7.56 ± 0.72[b]

Mean value [a-b]. Different alphabet letters in same row indicate significant difference (p<0.05)

Response surface optimization of processing parameter

Fitting the models

To optimize the ultrasound-assisted extraction (UAE) on the total phenolic content and antioxidants content from Sabah snake grass extracts, a central composite rotatable design (CCRD) was designed and employed. A fixed extraction time (18 h) was chosen based on the Peleg model calculation. In this study, the lower and upper values for the factors were set at +alpha (+α = 1.682) and –alpha (-1.682) and thus all the factor levels was chosen within the limits that were desirable and practical. The experimental values of total phenolic content (TPC) and total antioxidant activities of Sabah snake grass extracts under various experimental conditions were presented in Table 4. The results showed that TPC, DPPH and FRAP of Sabah snake grass leaves ranged from (4.74 - 25.10 mg GAE/g), (13.33 - 65.24%) and (0.91-10.44 µmol TE/g), respectively. The statistic results reported that 2 factor interactions (2FI) model were the most suitable with TPC and FRAP data. Meanwhile, DPPH data was suggested to use quadratic model. The result suggested that the solid-liquid ratio had the greatest impact on the extraction of TPC, DPPH and FRAP from Sabah snake grass leaves. The regression equation coefficient for TPC, the linear parameters (X1, X3) and interaction parameters (X1X3, X2X3) were significant at the level of $p<0.05$. For antioxidant capacity which is DPPH, the linear parameters (X1, X3) and interaction parameters (X1X2, X1X3) were significant at the level of $p<0.05$ and quadratic parameter X12 was significant at the level of $p<0.05$. In the FRAP, (X3) and (X1X2, X1X3, X2X3) were significant at the level of $p<0.05$. Analysis of variance (ANOVA) was performed to evaluate the quality of the fitted model. The ANOVA of the regression model showed that the model was significant ($p<0.05$) to all the response variables. Model fitting was decided by the coefficient of determination (R^2) and significant of lack-of-fit. The closer the R^2 value to unity, the better the experimental model fits the actual data Melecchi *et al*. (2006). The R^2 of the models for TPC, DPPH and FRAP were 0.8104, 0.9289 and 0.7908, respectively. The lack of fit-value for the TPC, DPPH and FRAP model were considered insignificant ($p>0.05$). The value of lack of fit ($p>0.05$) indicated the reliability of the model.

Table 4. Experimental design and values of the observed response surface optimization
*replication at centre point

Run	Independent variables			Response variables		
	Ultrasonic frequency (kHz)	Temperature (°C)	Solid-to-liquid ratio (g/ml)	TPC (mg GAE/g)	Antioxidant capacity	
					DPPH (%)	FRAP (µmol TE/g)
	X_1	X_2	X_3	Y_1	Y_2	Y_3
1	19.89(1.681)	20.00 (0)	60.00 (0)	11.95	58.67	2.93
2	40.00(1)	10.00 (-1)	80.00 (1)	4.32	25.08	1.3
3*	32.50 (0)	20.00 (0)	60.00 (0)	9.71	33.01	3.80
4*	32.50 (0)	20.00 (0)	60.00 (0)	9.50	36.77	3.99
5*	32.50 (0)	20.00 (0)	60.00 (0)	8.90	31.66	2.67
6	25.00 (-1)	10.00 (-1)	80.00 (1)	5.10	27.59	2.15
7	32.50 (0)	36.82 (1.682)	60.00 (0)	12.00	49.91	3.29
8*	32.50 (0)	20.00 (0)	60.00 (0)	6.00	36.91	2.58
9	32.50 (0)	20.00 (0)	26.36 (-1.682)	9.23	32.3	3.23
10	32.50 (0)	20.00 (0)	93.64 (1.682)	8.79	35.87	2.75
11*	32.50 (0)	20.00 (0)	60.00 (0)	9.99	41.32	3.29
12	40.00 (1)	30.00 (1)	40.00 (-1)	9.34	45.10	2.00
13	32.50 (0)	3.18 (-1.682)	60.00 (0)	4.74	13.33	0.91
14	25.00 (-1)	30.00 (1)	80.00 (1)	25.10	65.24	10.44
15	45.11 (1.682)	20.00 (0)	60.00 (0)	10.54	42.40	2.82
16	25.00 (-1)	10.00 (-1)	40.00 (-1)	9.379	33.36	4.10
17	40.00 (1)	10.00 (-1)	40.00 (-1)	10.16	37.91	4.66
18	40.00 (1)	30.00 (1)	80.00 (1)	9.46	31.35	3.23
19*	32.50 (0)	20.00 (0)	60.00 (0)	9.79	32.50	3.70
20	25.00 (-1)	30.00 (1)	40.00 (-1)	13.47	49.21	2.03

Effect of ultrasonic frequency, temperature and solid-to-liquid ratio

The herbal extraction process along with sonication is important to enhanced efficiency and shortening time of extraction time in order to increase the yield of extract (Melecchi *et al*., 2016). Figure 3 show the schematic diagram of vegetal tissue where during the extraction process, two type of physical mechanism are involved once the wall are broken: 1) diffusion through the cell walls and 2) washing out (rinsing) the cell content (Vinatoru, 2001; Zubairi *et al*., 2016). This sonification can be easily destroyed the leaves cell wall as compared to other parts of the plant (e.g. stem and roots) due its morphologically thin and weak structures. For that reason, TPC was influenced by the ultrasonic frequency significantly with the negative linear effect and some part of interaction (X1X3 and X2X3). The predicted response surface showed that the effect of ultrasonic frequency and temperature on TPC at constant solid-to-liquid ratio (20 g/ml) exhibited in linear plane formed (Figure 4a). As the ultrasonic frequency was decreasing from 40 kHz to 25 kHz, the TPC was increasing to 13.20 mg GAE/g. Similarly, the value of DPPH (48.91%) and FRAP (5.20 µmol TE/g) were the highest at the lowest frequency which was 25 kHz (Figure 4b and 4c). Increasing extraction of TPC, DPPH and FRAP could be attributed by the high release of energy at medium frequency whereby sufficient enough to increase the extraction substances.

TPC increased with the increment of solid-to-liquid ratio up to 30 g/ml, meanwhile DPPH also increased from 33.42% to 45.82% (Figure 4d and 4e). According to Campos *et al*. (2013), the increment of TPC which was in line with the increase of solid-to-liquid ratio was due to the principle of mass transfer. Concentration gradient that exists between the solvent and solid driving force of the mass transfer from the solid and this situation becomes better when the solid-liquid ratio of greater use.

The interaction relationship (X2X3) of TPC and FRAP showed significant positive linear effect (p<0.05). Figure 4f and 4g illustrates the interaction effect between the temperature and solid-liquid ratio TPC and FRAP at fixed frequency 32.5 kHz. Increasing in solid-liquid ratio (10 - 30 g/ml) and temperature (40 - 80ºC) caused the elevation value of TPC and FRAP. In general, increasing the temperature beyond certain values may encourage possible concurrent decomposition of energy bonding in phenolic compounds and hence increases the phenolic yield in extract (Spigno *et al*., 2007). It was suggested that the interaction between high temperature and solid-liquid ratio is required to obtain the highest phenolic compound extracted from Sabah snake grass's leaves.

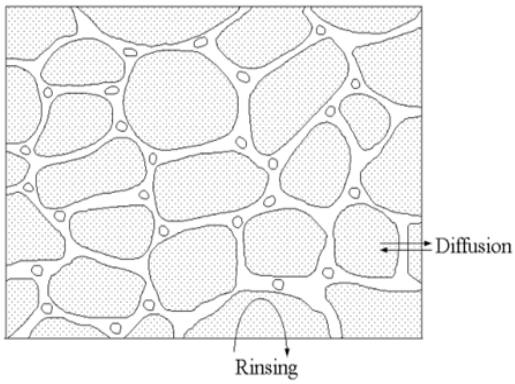
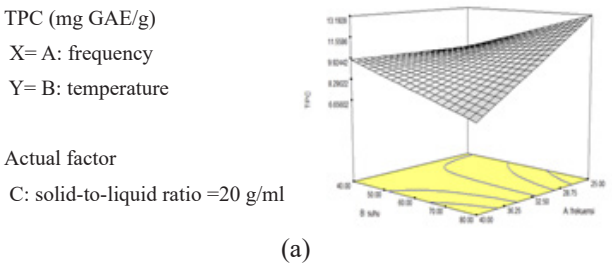
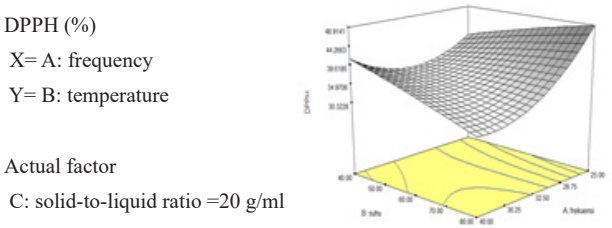


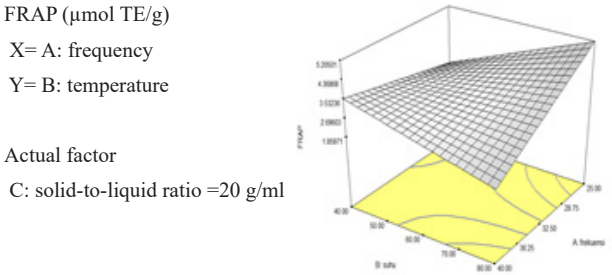
Figure 3: Schematic diagram of vegetal cell structures during the normal soaking extraction process with 2 different mechanisms involved: 1) Rinsing and 2) Difussion (Zubairi et al., 2016)



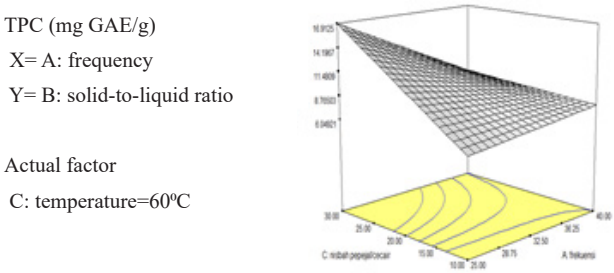
(a)



(b)



(c)



(d)

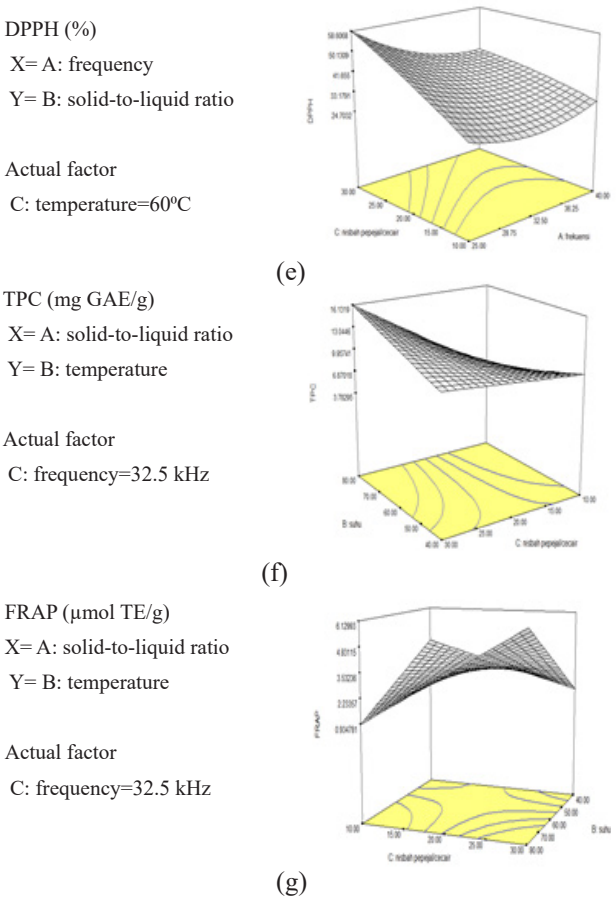


Figure 4: Response surface plot on the effects of ultrasonic frequency and temperature on (a) TPC (b) DPPH (c) FRAP; effects of ultrasonic frequency and solid-to-liquid ratio on (d) TPC (e) DPPH; effects of temperature and solid-liquid ratio on (f) TPC (g) FRAP of Sabah snake grass extracts

Optimum extraction processing parameters

Determination of optimum extraction processing parameters of Sabah snake grass leaves was based on the highest desirability value. Table 5a presented the optimum conditions for TPC, DPPH and FRAP, and its predicted and experimental values. Based on Balachandran *et al*. (2006) findings, the value of TPV, DPPH and FRAP were increased due to its mechanical effect on cell walls evidence by scanning electron microscopy (SEM) which has extended the rupturing of the vegetal walls. In fact, more extracts containing those specific bio-active constituents were increased profoundly. For that reason, the optimum conditions were found to be: ultrasonic frequency (25 kHz), temperature (80ºC) and solid-liquid ratio (30 g/ml). Under these optimal conditions, the model predicted a maximum response of TPC (21.34 mg GAE/g), DPPH (64.69%) and FRAP (8.64 µmol TE/g) of Sabah snake grass leaves extract.

Verification of predictive model

Table 5b showed that the experimental results were much closed to the predicted results. This indicated that there was a high fit degree between the values observed in experiment and the value predicted from the regression model. Hence, the response surface modelling could be applied effectively to predict the maximum yield extraction of total phenolic contents and antioxidant capacity from Sabah snake grass leaves.

Table 5: (a) The predicted result of processing optimum parameter from snake grass leave extracts, (b) Comparison between the predicted and experimental result for TPC, DPPH and FRAP at the optimum conditions and (c) Comparison between optimal values for TPC, DPPH and FRAP with other normal soaking extraction process.

(a)

Ultrasonic frequenscy (kHz)	Temperature (ºC)	Solid-to-liquid ratio (g/ml)	TPC(mg GAE/g)	DPPH (%)	FRAP (µmol TE/g)	Desirability
25.00	80.00	30	21.34	64.69	8.64	0.870

(b)

Variables	Predicted values	Experimental values
TPC	21.34 mg GAE/g	25.09 mg GAE/g
DPPH	64.69%	66.85%
FRAP	8.64 µmol TE/g	9.44 µmol TE/g

(c)

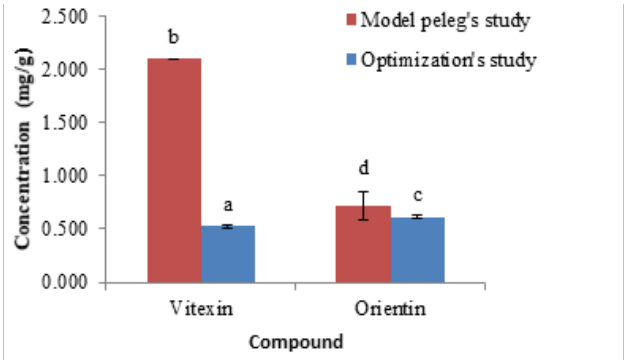
	Water extraction			Solvent extraction
	Current study (optimized parameters)	Al-Obaidi & Sahib *et al.* (2015)	Yashin *et al*. (2011)	Ghasemzadeh *et al*. (2014) (normal soaking extraction)
	Sabah snake grass	Green tea	Green tea	Sabah snake grass
TPC	25.09 mg GAE/g	23.00 mg GAE/g	N/A	11.32 mg GAE/g
DPPH	66.85%	69.00%	N/A	51.3%
FRAP	9.44 µmol TE/g	N/A	571.00 µmol TE/g	N/A

Comparison between optimal values for TPC, DPPH and FRAP with previous studies

Based on the optimal value of TPC, DPPH and FRAP (Table 5c), comparison has been made between the Sabah snake grass leaf extract and green tea extract using the same solvent extraction (distilled water). Green tea leaves contain the highest amount of total polyphenol and phenolic compounds and known as natural antioxidants (Tanizawa *et al*., 1984). Therefore, green tea extract has been chosen as a benchmark for this study. Based on the study carried out by Al-Obaidi *et al*. (2015), the phenolic content and antioxidant capacities (TPC and DPPH) of water extracts green tea leaves were almost comparable to this study. The green tea was extracted in boiling water for 3 h and prior to antioxidant. Meanwhile, in this study, the optimized parameters involved (ultrasonic frequency (25 kHz), temperature (80ºC) and solid-liquid ratio (30 g/ml)) underwent for 18 h of extraction time and it was suggested that the exhaustive extraction time for this study has possibly increased the recovery of antioxidant capacity (DPPH) which is equivalent with the antioxidant content in green tea. However, as stated by Yashin *et al*. (2011), the highest value of FRAP had been significantly different in green tea compared to this study. The low value of FRAP in Sabah snake grass extract may due to the high temperature used during the extraction process. On the contrary, Ghasemzadeh *et al*. (2014) reported that the TPC and DPPH of Sabah snake grass leaves extract using organic solvent were 11.32 mg GAE/g and 51.3% respectively which were lower than the results obtained. Thus, it showed that phenolic content depends strongly with the polarity solvent used, where fractional polar (water) attract more phenolic fraction compared to less polar (methanol) solvents (Hayouni *et al*., 2007).

Comparison of flavonoids concentration between Peleg model and response surface optimization

Figure 5 illustrates the concentration of flavonoid compounds (orientin and vitexin) extracted from Sabah snake grass leaves obtained from Peleg model and optimization studies. Optimization study showed that concentration of flavonoid compounds were significantly lower (p<0.05) than the Peleg model study. Peleg model study (2.01 ± 0.130 mg/g) was reported yielded higher concentration of vitexin compound compared to the optimization study (0.52 ± 0.002 mg/g). Concentration value of orientin compound from Peleg model study (0.72 ± 0.022 mg/g) exhibited slightly higher than the optimization study (0.62 ± 0.014 mg/g). It could be concluded that using different extraction method has affected the concentration value of flavonoid compound. Prolonged extraction at high temperature may cause degradation production of flavonoid and phenolic compounds (Nuutila *et al*., 2002). However, the concentration of extracted compound (orientin and vitexin) from the optimum parameters had produced high in phenolic content and antioxidant activity (e.g. DPPH) even though the both important phenolic contents were compromised as compared to the Peleg model kinetic studies.



Mean value [a-d]. Different alphabet letters indicate significant difference (p<0.05)

Figure 5: Concentration value of flavonoid compounds in Sabah snake grass leaves extracted via optimization and Peleg model studies

Conclusion

The present study confirmed that orientin and vitexin compounds had been detected in Sabah snake grass's leaves by using water extraction (time extraction of 72 h). The kinetic model (Peleg's model) showed exhaustive time of extraction (18 h) for Sabah snake grass extract have been chosen for the response surface optimization to identify the best processing parameters with respect to maximum yield of extract. The RSM was successfully employed to optimize the extraction and several experimental parameters have been evaluated. The chosen processing parameters such as ultrasonic frequency, extraction temperature and solid-to-liquid ratio have given a good effect on the extraction rate of TPC, DPPH and FRAP. The optimal parameter achieved based on combination of extraction parameters are as follows: X1 = 25 kHz ultrasonic frequency, X2 = 80ºC temperature and X3 = 30 g/ml of solid-to-liquid and yielded TPC, DPPH and FRAP of 25.09 mg GAE/g, 66.85% and 9.44 µmol TE/g respectively. The application of both models (RSM and Peleg) have been effectively be able to provide the optimum extraction parameters with high total phenolic content and antioxidant activity.

Acknowledgments

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