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OPTIMIZATION OF POLYPHENOL, FLAVONOID AND TANNIN EXTRACTION CONDITIONS FROM *Pouzolzia zeylanica* L. BENN USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

In this study, the extraction of phenolic compounds from Pouzolzia Zeylanica L. Benn was conducted by using pure water as a solvent. The optimal conditions for the extraction of three phenolic compounds such as polyphenols, flavonoids and tannins were determined by using response surface methodology (RSM). A central composite design (CCD) was applied to investigate the effects of three independent variables, namely the ratio of water-to-dried material (20:1 to 30:1, v/w), temperature (70 to 90°C) and time extraction (20 to 40 minutes). The dependent variables were total polyphenol content (TPC), total flavonoid content (TFC) and tannin content (TC). A second-order polynomial model was used for predicting the response. Optimized conditions for bioactive compounds extraction, water-to-dried material ratio, time and temperature extraction were 27 (v/w), 30 minutes and 81°C, respectively. The experimental values agreed with predicted values within a 95% confidence interval. Total polyphenol, flavonoid and tannin content extracted by these optimized conditions were achieved (921 mgGAE/100g dried material (DM), 563 mgQE/100g DM and 643 mgTAE/100g DM, respectively).

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1 INTRODUCTION

Pouzolzia zeylanica L. Benn is considered as a perennial herb, variation in size and habit; stem erect or prostrate, 15-30 cm long. Leaves are 2-3.8 cm in length, ovate or ovate-lanceolate, obtuse, acute or acuminate, entire. Plant contains flavone, flavonoids, tannin, carotene, carotenoids, ascorbic, tartaric, malic and pectic acids, gum, minerals and their salts (Ghani, 2003); quercetin, vitexin, isovitexin, phylanthin, metyl sterate and β-sitosterol-3-*O*-β-D-glucopyranoside (Thuy, 2007); β-sitosterol, daucosterol, oleanolic acid, epicatechin, α-amyrin, eugenyl-β-rutinoside, 2α,3α,19α-trihydroxyurs-12en-28-oic, scopolin, scutellarein-7-*O*-α-L-rhamnoside, scopoletin, quercetin, quercetin-3-*O*-β-D-glucoside, apigenin and 2α-hydroxyursolic (Fu *et al.*, 2012); leaf powder also contains carbohydrates, gums, reducing sugar, alkaloids, steroids, glycosides, tannins, flavonoids and saponins (Saha and Paul, 2012a). Leaves are anthelmintic and vulnerary; used as a cicatrizant for gangrenous ulcers, in syphilis and gonorrhea. Leaf juice is used as galactagogic. Poultice of the herb is applied to sores, boils and to relieve stom-

achache (Yusuf *et al.,* 2006). In the Nalbari district, Assam leaf and stem paste is applied locally once or twice daily for itching. Plant leaf and stem rolled with banana leaf, heated and squeezed, juice mixed with goat's milk, and taken once for dysentery and loose stools of infant (Bhattacharjya and Borah, 2008). In Eastern Ghats, Andhra Pradesh, Indian paste of crushed shoots applied as poultice to bone fractures (Ratnam and Raju, 2008). The plant *Pouzolzia indica* claimed to be useful in treating snake poison in the Indian system of medicine (Ahmed *et al*., 2010). In Vietnam, *Pouzolzia zeylanica* plant can be used as fresh or dried plant, decoction drunk to treat cough, pulmonary tuberculosis, sore throat, enteritis, dysentery (Chi, 2012).

Traditionally, *Pouzolzia zeylanica* plants are prepared as an infusion with water, to make a tea. If these infusions can be optimized in terms of their phenolics content such as polyphenol, flavonoid and tannin. They could have had potential as beverages with medicinal properties. Several *in vitro* researches have indicated ethanolic extracts of *Pouzolzia zeylanica* possessed antibacterial, antifungal and cytotoxic activities (Paul and Saha, 2012; Saha *et al*., 2012; Saha and Paul, 2012b); it had no oral acute toxicity at the oral dose of 10 g material powder/kg (Tien *et al.*, 2010). The quantity of phenolic compounds (*e.g*. polyphenol, flavonoid and tannin) along with other factors influences the quality of the infusion are important properties in beverages as one of the important attributes of food is their appearance. Therefore, it is important to have information on the effect of extraction time and temperature, solid to liquid ratio on the content of phenolics in *Pouzolzia zeylanica* extracts.

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

Folin-Ciocalteu, Folin-Denis reagents and quercetin, gallic acid, tannic acid were obtained from Sigma Chemical Co. (USA) and Merck Chemical Supplies (Germany). All the chemicals, including the solvents, were of analytical grade.

2.2 Sample preparation and extraction

Pouzolzia zeylanica plants were collected in March 2015 from An Giang University. They were harvested after one-and-a-half-month cultivation, with 20-30 cm in height. The plants were then cleaned with tap-water, sun dried until the final moisture content about 12%, cut into small pieces about 2-3 cm long, packaged and stored in dark at room temperature for further experiments.

The dried samples of *Pouzolzia zeylanica* were extracted with water using airtight extractor (model GPA CC1-181907, Didatec Technologie France, 2007). String rate was maintained at 90 rounds per minute (rpm). The extract samples were fixed a volume for 5 liters. The samples were extracted at temperature of (63, 70, 80, 90 and 97°C), in duration of (13, 20, 30, 40 and 47 min) and solution to solid ratio of (17:1, 20:1, 25:1, 30:1 and 33:1 v/w). The extracts were filtered by cloth and determined their volumes. After that, the extracts were filtered using Buchner funnel with Whatman's No 1 filter paper. The filtrate (crude extract) was diluted in ethanol at an appropriate ratio using for analysis.

2.3 Experimental design

In this study, response surface methodology (RSM) with central composite design (CCD) in form (2^3 + star) was used to investigate the effects of three independent variables: X_1 (extraction temperature), X_2 (extraction time) and X_3 (water-to-dried material ratio) on the extraction of TPC, TFC, and TC contents. The independent variables were coded at five levels ($-\alpha$, -1, 0, +1, $+\alpha$) and the complete design consisted of 20 experimental points, including six replications of the centre points.

2.4 Statistical analysis

Experimental data showed that the response variables were fitted to a quadratic polynomial model (Equation 1). The general form of the quadratic polynomial model was as follows:

$$Y = b_o + b_1 X_1 + b_2X_2 + b_3X_3 + b_{1.1}X_1^2 + b_{2.2}X_2^2 + b_{3.3}X_3^2 + b_{1.2}X_1X_2 + b_{1.3}X_1X_3 + b_{2.3}X_2X_3 \quad (1)$$

Where Y is the predicted response parameter, X_1 is extraction temperature, X_2 is extraction time and X_3 is water-to-dried material ratio; b_o is the mean value of response at the central point of the experiment; b_1, b_2 and b_3 are the linear coefficients, b_{11}, b_{22} and b_{33} the quadratic coefficients and b_{12}, b_{13} and b_{23} the interaction coefficients. Experimental design and statistical treatment of result were performed using STAGRAPHICS Plus 15.0 for Windows.

In order to control the influence of extraction conditions (extraction temperature, extraction time and water-to-dried material ratio) on the contents of each phenolic compound, ANOVA, with more classification criteria, using Fisher's least significant difference test and the significant differences at the 5% level, were calculated. The difference was considered as not significant when P-value> 0.05, significant when P-value $\leq$ 0.05, and highly significant for P-value $\leq$ 0.01 and extremely signif-

icant for P-value ≤ 0.0001. Turkey's test was also performed for pair-wise comparisons at the 5% level.

2.5 Determination of chemical composition of *Pouzolzia zeylanica* L. Benn

2.5.1 Total polyphenol content (mg GAE/100 g dried material)

Total polyphenol content was determined by Folin-Ciocalteu reagent method (Hossain *et al*., 2013). Each crude extract (0.2 mL) was taken in a test tube and added 10% Folin-Ciocalteu reagent (1.5 mL). Then all test tubes were kept in a dark place for 5 min. Finally, 5% Na_2CO_3 (1.5 mL) was added to solution and mixed well in a vortex. Again, all the test tubes were kept in the dark for 2 h. The absorbance was measured for all solution by using UV-spectrophotometer at constant wavelength 750 nm. Total polyphenol concentrations were quantified by calibration curve obtained from measuring the absorbance of a known concentration of gallic acid standard in ethanol (y = 0.0082x + 0.0595 and r^2 = 0.9996). The total polyphenol content (TPC), milligrams of gallic acid equivalents (GAE) per 100-gram dried material (DM), was calculated by the following formula:

$$\text{TPC} = \frac{[\text{A} - 0.0595] \text{ x DF x V x } 100}{0.0082 \text{ x W}}$$

Where A is the absorbance of the test samples; DF is the dilution factor; V is volume of the obtained extracts, in liter; W is the weight of material sample, in gram; 100 is factor for conversion from 1 gram to 100 grams.

2.5.2 Total flavonoid content (mg QE/100 g DM)

Aluminum chloride colorimetric method was used for flavonoids determination (Eswari *et al*., 2013; Mandal *et al*., 2013). About 1 mL of the crude extracts/standard of different concentration solution was mixed with 3 mL ethanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1M sodium acetate and 5.8 mL of distilled water. It remained at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with spectrophotometer against blank. The calibration curve was prepared by diluting quercetin in ethanol (y = 0.0054 x + 0.0026 and r^2 = 0.9995). The total flavonoid content (TFC), milligrams of quercetin equivalents (QE) per 100-gram dried material (DM), was calculated by the following formula:

$$\text{TFC} = \frac{[\text{A} - 0.0026] \text{ x DF x V x } 100}{0.0054 \text{ x W}}$$

Where A is the absorbance of the test samples; DF is the dilution factor; V is volume of the obtained extracts, in liter; W is the weight of material sample, in gram; 100 is factor for conversion from 1 gram to 100 grams.

2.5.3 Tannin content (mg TAE/100 g DM)

Tannin content was determined by Folin-Denis method (Laitonjam *et al.,* 2013). Each crude extract (0.5 mL) and distilled water (0.5 mL) were taken in a test tube. Finally, the samples were treated with 0.5 mL of freshly prepared Folin-Denis reagent and 20% sodium carbonate (2 mL) was added, shaken well, warmed on boiling water-bath for 1 min and cooled to room temperature. Absorbance of the coloured complex was measured at 700 nm. Tannin concentration was quantified based on the calibration curve of tannic acid in ethanol (y = 0.0098x + 0.0478 and r^2 = 0.9996). The tannin content (TC), milligrams of tannic acid equivalents (TAE) per 100-gram dried material (DM), was calculated by the following formula:

$$\text{TC} = \frac{[\text{A} - 0.0478] \text{ x DF x V x } 100}{0.0098 \text{ xW}}$$

Where A is the absorbance of the test samples; DF is the dilution factor; V is volume of the obtained extracts, in litre; W is the weight of material sample, in gram; 100 is factor for conversion from 1 gram to 100 grams.

3 RESULTS AND DISCUSSION

3.1 Effect of the extraction parameters on total polyphenol content (TPC)

The results of ANOVA analysis (Table 1) showed that the linear, quadratic and interaction factors of extraction temperature, time and water-to-dried material ratio had effect on total polyphenol content from obtained extract with reliability 95%. The linear, quadratic and interaction factors of temperature and water-to-dried material ratio were extremely significant for P-value ≤ 0.0001; the interaction factors of temperature and time, and the quadratic factors of time were highly significant (P-value ≤ 0.01); the linear factor of time and interaction factor of time and water-to-dried material ratio were significant (P-value ≤ 0.05).

Table 1: ANOVA for the quadratic model of total polyphenol content (mg GAE/100g DM)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-ratio*	*P-value*
X_1: Extraction Temperature	44130.6	1	44130.6	394.78	0.0000
X_2: Extraction Time	1731.53	1	1731.53	15.49	0.0110
X_3: Water-to-dried material ratio	23948.4	1	23948.4	214.23	0.0000
X_1X_1	65656.1	1	65656.1	587.33	0.0000
X_1X_2	7582.96	1	7582.96	67.83	0.0004
X_1X_3	13521.9	1	13521.9	120.96	0.0001
X_2X_2	2812.22	1	2812.22	25.16	0.0041
X_2X_3	859.051	1	859.051	7.68	0.0393
X_3X_3	50306.2	1	50306.2	450.02	0.0000
Lack-of-fit	2765.15	5	553.031	4.95	0.0520
Pure error	558.933	5	111.787	-	-
Total (corr.)	200783.	19	-	-	-
R-squared			0.9834		
R-squared (adjusted for d.f.)			0.9685		

The coefficient of determination (R^2) of the predicted models in this response was 0.9834 and P-value for Lack of fit was 0.05. These values would give a relative good fit to the mathematic model in Equation 2.

$$TPC\ (mg\ GAE/100g\ DM) = -4653.53 + 102.36\ X_1 + 28.96\ X_2 + 54.54\ X_3 - 0.675\ X_1^2 - 0.308\ X_1X_2 + 0.822\ X_1X_3 - 0.139\ X_2^2 + 0.207\ X_2X_3 - 2.363X_3^2 \quad (2)$$

Where Y is the predicted TPC (%), X_1 is extraction temperature, X_2 is extraction time and X_3 is water-to-dried material ratio.

Regression equation for evaluation total polyphenol content showed that the linear coefficients of temperature, time and water-to-dried material ratio factors, and interaction coefficients of temperature and water-to-dried material ratio, time and water-to-dried material ratio had developed proportional to polyphenolic content. However, the quadratic coefficient of temperature, time and water-to-dried material factors, interaction coefficient of temperature and time had relative in inverse ratio to polyphenol content.

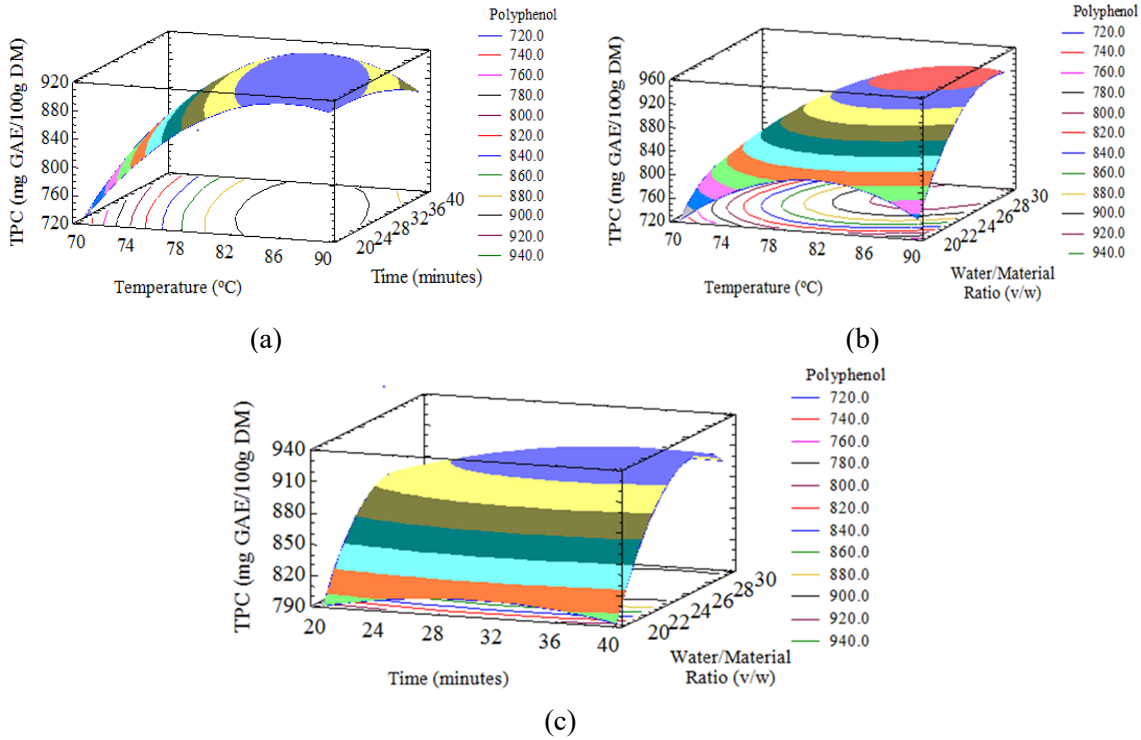

Fig. 1: Total polyphenol content (TPC) surface plots. The three-dimensional graphs were plotted between independent variables while the remaining independent variable was kept at its zero level

The response surface plots shown in Figure 1 given by their shapes, inform the significance of each experimental parameter. It can be noticed from Figure 1 (a) and (b) that temperature had a positive quadratic effect on TPC since it increased with temperature increase to reach an optimum of 86.04°C. The study results of Son and Tu (2009), reported an increase in total polyphenolic content in increasing temperature about 80-90°C for polyphenol extraction from dust green tea. The enhancing capacity of the temperature parameter on the extraction efficiency of phenolic compounds was reported by many authors (Spigno and Faveri, 2007; Spigno *et al*., 2007; Rajha *et al*., 2012). It ameliorates the mass transfer, improves the solubilization of the solutes in the solvent and reduces the surface tension and viscosity (Ramos *et al*., 2002). Nevertheless, beyond a certain value the denaturation of the phenolic compounds can occur. Regarding the duration of the extraction process, short (Bonilla *et al*., 1999; Pinelo *et al*., 2005; Yilmaz and Toledo, 2006) and long extraction periods can be found in the literatures (Jayaprakasha *et al*., 2001; Pinelo *et al*., 2005). In Figure 1 (c) showed a negative quadratic effect on the TPC, there is a slightly increase in TPC by increasing of time to reach an optimum (29.45 min). The short time of extraction could be avoided the degradation of phenolic compounds, because during short time, the temperature enhanced the extraction process, but with relatively longer time for extraction, the effect is inverted, and the phenolic compounds are threatened by oxidation or degradation (Yilmaz and Toledo, 2006). Figure 1 (b) and (c) showed water-to-dried material ratio from 26-29 (v/w) well extraction of polyphenolic and reach an optimum of 27.79 (v/w). Roughly, high amount of solvent will create a chance for solute was contacted with solvent. Thus, the solutions can be better transferred from material to solvent (Cacace and Mazza, 2003). The optimal conditions for extraction of total polyphenol content were found to be at temperature of 86.04°C, extraction time of 29.45 min and extraction water-to-dried material of 27.79 (v/w). Under these optimized conditions, the highest level of total polyphenol content was obtained (934.553 mg GAE/100g DM).

3.2 Effect of the extraction parameters on total flavonoid content (TFC)

Similarly, the results of ANOVA analysis (Table 2) showed that the linear, quadratic and interaction factors of temperature, time and water-to-material ratio had effect on total flavonoid content from obtained extract with reliability 95%. In there, the linear and quadratic factors of extraction time and water-to-dried material, quadratic factor of extraction temperature, interaction of factor of temperature and water-to-material ratio were extremely significant for P-value ≤ 0.0001; the linear factor of temperature was highly significant for P-value ≤ 0.01; the interaction factor of temperature and time, and interaction factor of time and water-to-dried material ratio were significant for P-value ≤ 0.05.

Table 2: ANOVA for the quadratic model of total flavonoid content (mg QE/100g DM)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
X_1: Extraction Temperature	429.429	1	429.429	16.98	0.0092
X_2: Extraction Time	3373.25	1	3373.25	133.40	0.0001
X_3: Water-to-dried material ratio	17779.2	1	17779.2	703.11	0.0000
X_1X_1	73162.0	1	73162.0	2893.30	0.0000
X_1X_2	179.551	1	179.551	7.10	0.0446
X_1X_3	4767.76	1	4767.76	188.55	0.0000
X_2X_2	6512.85	1	6512.85	257.56	0.0000
X_2X_3	308.761	1	308.761	12.21	0.0174
X_3X_3	21947.2	1	21947.2	867.93	0.0000
Lack-of-fit	538.469	5	107.694	4.26	0.0689
Pure error	126.433	5	25.2867	-	-
Total (corr.)	117395.	19	-	-	-
R-squared			0.9943		
R-squared (adjusted for d.f.)			0.9892		

The coefficient of determination (R^2) of the predicted models in this response was 0.9943 and P-value for Lack of fit was 0.0689. These values would give a relative good fit to the mathematic model in Equation 3.

$$TFC\ (mg\ QE/100g\ DM) = -4076.34 + 99.814\,X_1 + 4.287\,X_2 + 42.477X_3 - 0.712\,X_1^2 + 0.047\,X_1X_2 + 0.488\,X_1X_3 - 0.213\,X_2^2 + 0.124\,X_2X_3 - 1.56\,X_3^2 \quad (3)$$

Where Y is the predicted TPC (%), X_1 is extraction temperature, X_2 is extraction time and X_3 is water-to-dried material ratio.

Regression equation for evaluation total flavonoid content showed that the linear coefficients of temperature, time and water-to-dried material ratio factors, and interaction coefficients of temperature and time, temperature and water-to-dried material ratio, and time and water-to-dried material ratio that developed proportional to flavonoid content. However, the quadratic coefficient of temperature, time and water-to-dried material factors showed an inverse correlation with the flavonoid contents.

Flavonoids extraction was reported to be affected by many parameters such as time, temperature, solvent concentrate, solid to liquid ratio and extraction cycles (Silva *et al*., 2007; Liu *et al*., 2009; Zhu *et al*., 2011). Herein, temperature had a positive quadratic effect on flavonoid content in Figure 2 (a) and (b). Temperature increase led to flavonoid content increase to reach an optimum of 80.27°C. Some authors showed the effect of temperature on flavonoids extraction. Sheng *et al.* (2013) explained the better liberation of bioactive compounds from plant cells by the decrease of solvent viscosity and the increase of molecular movement with temperature elevation. However, as the extraction temperature was elevated higher than the optimal temperature, the total flavonoid content could be decreased. The bioactive compounds are always sensitive at high temperature, so that extraction at high temperature and longer time, the bioactive compounds will be decomposed (Son and Tu, 2009).

Time had a negative quadratic effect in Figure 2 (c), the TFC yield increase for 22-28 minutes then decrease, probably due to the decomposition phenomenon observed with relatively extended extraction time (Sheng *et al.,* 2013). The optimal extraction time was reached 26.98 minutes.

The water-to-dried material ratio had a positive quadratic effect on flavonoid content. It is noticed from Figure 2 (b) and (c) that the flavonoid content increased in increasing water-to-dried material ratio to reach an optimum of 27.23 (v/w).

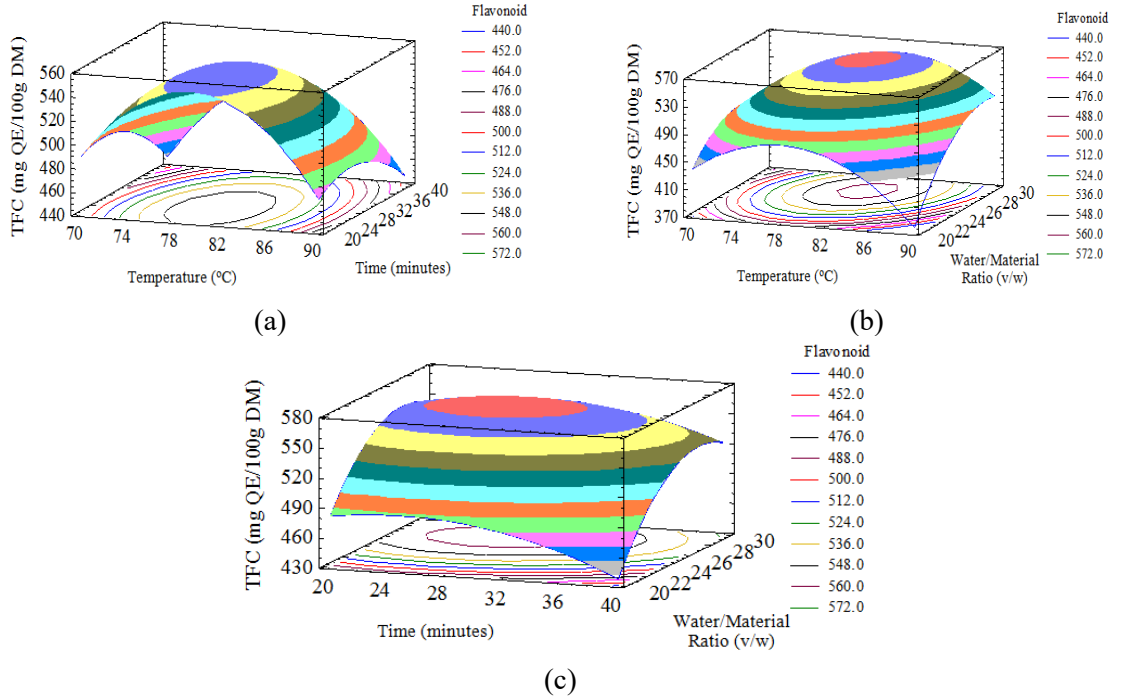

Fig. 2: Total flavonoid content (TFC) surface plots. The three-dimensional graphs were plotted between independent variables while the remaining independent variable was kept at its zero level

The optimum conditions for extraction of total flavonoid content were found to be at extraction temperature of 80.27°C, extraction time of 26.98 min and extraction water-to-dried material of 27.23 (v/w). Under these optimized conditions, the highest content of total flavonoids was found (566.039 mg QE/100g DM).

3.3 Effect of the extraction parameters on tannin content (TC)

Similarly, the results of ANOVA analysis (Table 3) showed that the linear, quadratic and interaction factors of temperature, time and water-to-dried material ratio had effect on tannin content from obtained extract with reliability 95%. In there, the

quadratic factor of extraction temperature was extremely significant for P-value ≤ 0.0001; the linear factors of temperature and water-to-dried material ratio, interaction factors of time and water-to-dried material ratio, quadratic factors of time and water-to-dried material were highly significant for P-value ≤ 0.01; the linear factor of time, interaction of temperature and time factors, temperature and water-to-dried material ratio were significant for P-value ≤ 0.05.

Table 3: ANOVA for the quadratic model of tannin content (mg TAE/100g DM)

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
X_1: Extraction Temperature	2394.15	1	2394.15	17.36	0.0088
X_2: Extraction Time	1456.02	1	1456.02	10.56	0.0227
X_3: Water-to-dried material ratio	3095.76	1	3095.76	22.45	0.0052
X_1X_1	25103.9	1	25103.9	182.07	0.0000
X_1X_2	1326.13	1	1326.13	9.62	0.0268
X_1X_3	1501.52	1	1501.52	10.89	0.0215
X_2X_2	12725.7	1	12725.7	92.29	0.0002
X_2X_3	3793.21	1	3793.21	27.51	0.0033
X_3X_3	4927.27	1	4927.27	35.74	0.0019
Lack-of-fit	236.737	5	47.3474	0.34	0.8672
Pure error	689.413	5	137.883	-	-
Total (corr.)	51240.5	19	-	-	-
R-squared			0.9819		
R-squared (adjusted for d.f.)			0.9657		

The coefficient of determination (R^2) of the predicted models in this response was 0.9819 and P-value for Lack of fit was 0.8672. These values would give a relative good fit to the mathematic model in Equation 4.

$$TC\ (mgTAE/100g\ DM) = -4157.0 + 78.816\,X_1 + 40.0497\,X_2 + 74.977\,X_3 - 0.417X_1^2 - 0.129X_1X_2 - 0.274\,X_1X_3 - 0.297\,X_2^2 - 0.435X_2X_3 - 0.739X_3^2 \quad (4)$$

Where Y is the predicted TPC (%), X_1 is extraction temperature, X_2 is extraction time and X_3 is water-to-dried material ratio.

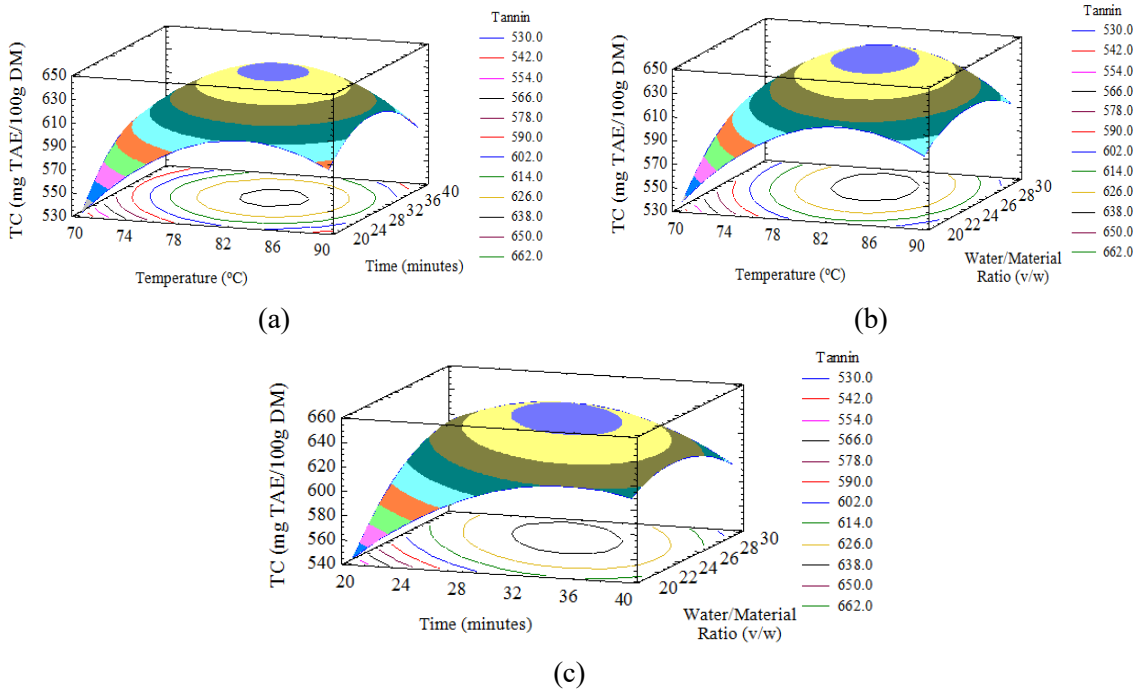

Fig. 3: Tannin content (TC) surface plots. The three-dimensional graphs were plotted between independent variables while the remaining independent variable was kept at its zero level

Regression equation for evaluation tannin content showed that the linear coefficients of temperature, time and water-to-dried material ratio factors that developed were proportional to the tannin content.

However, the quadratic coefficient of temperature, time and water-to-dried material factors and interaction coefficients of temperature and time, temperature and water-to-dried material ratio, time and water-to-dried material ratio showed an inverse correlation with the tannin content.

As showed in Figure 3 (a), (b) and (c), temperature, time and water-to-dried material ratio had positive quadratic effects on the tannin content. Tannin content increased in increasing time to reach its optimal value after 30.21 minutes, later on, a decrease was obtained.

The same tendency of tannin augmentation was observed with temperature and water-to-dried material ratio increase, until they reached 80.96ºC and 26.79 (v/w) respectively. Tannin extraction from bark was patented to be preferably conducted at high temperatures, between 90°C and 100°C (Connoly, 1993).

The optimum conditions for extraction of tannin content were found to be at extraction temperature, time and water-to-dried material are 80.96ºC, 30.21 min and 26.79 (v/w) respectively. Under these optimized conditions, the experimental maximum amount of tannin content was 643.127 mg TAE/100g DM.

3.4 Multiple response optimization

The simultaneous optimization of multiple responses is a main concern for industrial applications (Tsai *et al.,* 2010) especially that the energy cost of the process in significantly diminished when extraction parameters are optimized (Spigno *et al*., 2007). The response variables TPC, TFC and TC were optimized separately, therefore allowing the targeting of a certain class of compounds only by varying the extraction parameters. Yet, the desirability function in the RSM was utilized to reveal the combination of the parameters (temperature, time and water-to-dried material ratio) capable of simultaneously maximizing all the response (TPC, TFC and TC). The overplay plot (Figure 4) shows the outlines superposition of all the studied responses and the simultaneous optimum for all responses is showed by the black spot (Figure 4 a, b and c).

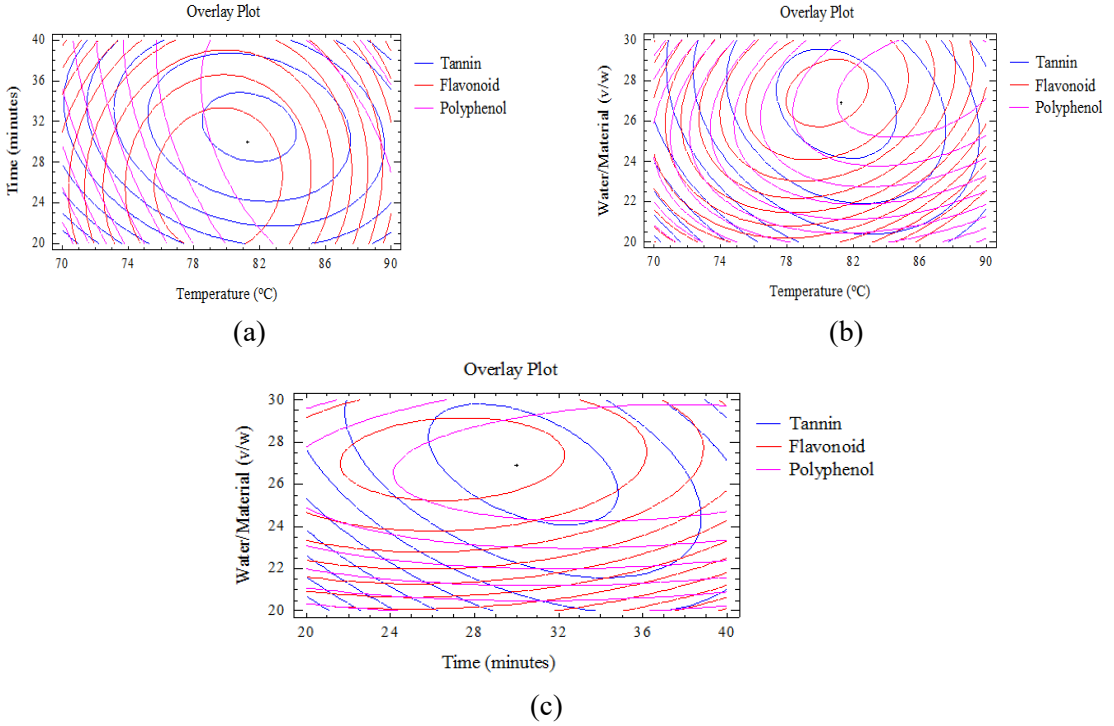

Fig. 4: Overplay plots. It was plotted between independent variables while the remaining independent variable was kept at its zero level

4 CONCLUSIONS

Response Surface Methodology was revealed accurate in predicting models and optimizing several extraction conditions for the simultaneous maximization of many parameters such as temperature, thus minimizing the degradation process. A potential alternative was proposed for an industrial solid-liquid extraction process of phenolic compounds from *Pouzolzia zeylanica* plant. The optimal conditions for extraction of phenolic compounds were found to be at extraction temperature, time and

water-to-dried material ratio are 81°C, 30 minutes and 27 (v/w), respectively. Under these optimized conditions, the highest content of TPC, TFC and TC were found (921 mg GAE/100g DM, 563 mg QE/100g DM and 643 mg TAE/100g DM, respectively).

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