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Study of the Procedure for Bath Ultrasound-Assisted Extraction of Total Flavonoid from Lotus Seeds and Testing Some Biological Activities

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ABSTRACT

In this study, we investigated impact of single factor: solvent concentration ethanol, solvent pH, ultrasound time, solvent ratio: raw material and ultrasound temperature on the total flavonoid extraction from lotus seeds. Based on survey on factors affecting extraction conditions by the ultrasound bath method, all five surveyed factors were found to have strong influences on total flavonoid extraction process. From factorial design of experimental planning using surface response methodology (RSM), optimal conditions for extraction of total flavonoid from lotus seeds were found in extraction time (98.005 min); solvent concentration ethanol (86.160 %), pH solvent ethanol (4.546), bath ultrasound temperature (22.210°C) and solvent/material ratio (8.376 (v/w)). Under above optimum conditions, total extracted flavonoids was 0.812 (mg Catechin/g extract). Experimental results showed a high compatibility with estimated RSM model. Total flavonoid content obtained in the original liquid extract was 0.797 ± 0.011 (mg Catechin/g extract). The highest antioxidant activity was found to be from ethanol extract with IC_{50} value (0.058 ± 0.004 (µg/mL), in comparison with IC_{50} of ascorbic acid, 0.002 ± 0.001 (µg/mL). Moreover, 50 % of DPPH scavenging ability of liquid and condensed extract differed from each other.

Key words: Extract, Flavonoid, Lotus seed, *Nelumbo nucifera,* RMS.

INTRODUCTION

Nelumbo nucifera is an aquatic flowering plant. This perennial usually lives in lakes and ponds. Since ancient times, lotus flower has become familiar and close to Vietnamese's life as well as other countries such as India, China and Japan. Lotus flowers are not only play an important role in economic and mental areas but also spiritual aspects. All different parts of lotus can be used for preparing various delicious cuisine types and valuable traditional herbal medicine formulas (Vuong *et al.,* 2013). Lotus contains vast varieties of potential secondary compounds such as alkaloids, flavonoids, steroids, triterpenoids, glycosides and polyphenols, performing powerful antioxidant activities which are effective in improving human health (Marxen *et al.,* 2007). There were researches on benefits of consuming lotus seeds on human health, especially during the recent decade while people gained more and more awareness on protecting their health through functional foods (Fazekas *et al*., 2008). Up to now, lotus seeds have been proved to possess many pharmacological properties such as antioxidant (Xie *et al*., 2013; Vijayan and Tsou, 2010), anti-inflammatory (Jung *et al*., 2010), anti-memory loss (Liao and Lin, 2011; Liao *et al*., 2011) and anti-tumor activities (Poornima *et al.,* 2013). Scientists believe that significant biological or medicinal compounds in lotus seeds are naturally alkaloids and flavonoids (Chen *et al.,* 2007; Itoh *et al.,* 2011). Previous studies showed that lotus seeds were rich in flavonoids and alkaloids (Das *et al*., 1992; Moritz *et al.,* 2004; Li *et al.,* 2014) in which flavonoid is a large group containing approximately

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6000 compounds of secondary phytometabolites and the largest number of natural phenolic compounds (Paran and Michelmore, 1993). An investigation was conducted to study the antioxidant, anticancer and grafting activities of medicinal plants that Ahmed *et al.* (2016); Noorjahan and Saranya (2018) and Sharma *et al.* (2017).

Recent years, flavonoids have received considerable attentions because of their benefits of human's health, especially in preventing a number of chronic diseases, such as cardiovascular diseases, type II diabetes, neurodegenerative diseases and some types of cancer (Vijayan *et al*., 2010). Flavonoid study on lotus seeds conducted by Kredy *et al.* (2010)

has determined Flavonols in fresh lotus seeds *N. nucifera* Gaertn. (FSENN) and assays for their antioxidant activities.

Ruvanthika *et al.* (2017) conducted research on evaluation of natural compounds in lotus seeds and seed coats of *N. nucifera*. Total phenolic content in the seed coats and seeds were 93.450 mg Gallic Acid Equivalents and 10.5 mg/g. Total flavonoid content was 295.312 mg/g quercetin in lotus seed coats and 28.125 mg/g in lotus seeds. Tannin content was 508.7 mg/g in seed coats and 69.637 mg/g in seeds. In 2018, Jiang *et al.* investigated chemical composition of embryos of *N. nucifera* lotus seeds, which detected 4 new flavon C-glycoside.

MATERIALS AND METHODS

Materials

White lotus seeds (*Nelumbo nucifera*) used in this study were purchased from lotus farmers in inner and suburban of Thua Thien Hue province. Lotus seeds are peeled, rinsed under tap water and dried at 50°C to < 10% moisture. Lotus seeds are then finely grinded and passed through 1mm sieve to obtain lotus powder, which was kept in polyethylene (PE) bags in an air tight plastic containers at room temperature to avoid light and moist for future experiments. Catechin was from Sigma.

Methods

Experimental design

Ultrasound-assisted extraction flavonoids from lotus seed: Total flavonoids were extracted from lotus seeds (d = 1mm) by bath ultrasound-assisted extraction with (1) ethanol: water (v/v) at 30; 50; 70; and 90 % (v/v); (2) pH 3; 4; 5; 6; 7; (3) ultrasonic treating time at 30; 60; 90; 120 and 150 minutes; (4) solvent: material ratio at 5:1; 10:1; 15:1; 20:1; 25:1 and 30:1 (v/w); and (5) ultrasonic treating temperature at 10; 30; 50; 70 and 90 °C, respectively. Highest results of previous experiments are applied for subsequent experiments. After single-factor survey, all 5 factors that had highest impacts on total flavonoid content in lotus seeds are selected. Experiments were designed according to Surface response methodology (RMS) and results were analysed by Design Expert 10 software.

Determination of total flavonoid content

Total flavonoid content was determined as described in Chang *et al.* (2002). Ethanol (70%) (Merck, Germany) is used to prepare serial dilutions of 6.26; 12.5; 25, 50; 100; 200 and 300 µg/mL standard flavonoid solutions from Catechin (Sigma lot: # WXBC3261V); while 10% $AlCl_3$ solution and 1M CH_3COOK solution were prepared in water. A volume of 0.5 mL of Catechin solution was added to 1.5 mL of ethanol in 5 minutes. Then, 0.1 mL of 10% $AlCl_3$ was added followed by 6 minute incubation. Finally, 0.1 mL of CH_3COOK 1M and 2.8 mL of distilled water were added to the mixture. After 45 minute incubation at room temperature, sample absorbance was measured by using the UV - Vis spectrophotometer (U2900 Hitachi, Japan) at 415 nm to build standard curve. Lotus flavonoid samples were conducted similar to Catechin. The experiment was triplicated.

Extraction yield of flavonoid was calculated as below:

$$F = \frac{c \times v}{m}$$

Where,

F: extraction yield of flavonoids (mg Catechin/g extract); c: the value of x from standard curve with Catechin (mg/mL), V: volume of extract (mL); m: weight of sample V (g).

Determination of antioxidant activity

The antioxidant activities of liquid extract and condensed extract were measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method as described in Vuong *et al.* (2013). An amount of 1 ml sample was added to test tube, followed by 1 mL of DPPH of 0.2 mM and 30 minute incubation in the darkness. Absorbance was measured at 517 nm. The ascorbic acid was used as control. Condensed extraction were prepared serial dilutions of 0.05; 0.67; 0.1; 0.2; 0.5 and 1 mg/mL; liquid extract solution was diluted with ratios of 1; 1/2; 1/5; 1/10; 1/15 and 1/20. DPPH solution 0.2 mM was prepared in 70 % ethanol and ascorbic acid was prepared as of 0.05; 0.67; 0.1; 0.2; 0.5 and 1 mg/mL.

DPPH free radical scavenging abilities were calculated as:

$$\%SC = \frac{OD_c - OD_m}{OD_c} \times 100$$

Where: OD_m: optical density of sample after deducting blank (without DPPH); OD_c: optical density of blank sample after deducting blank (without DPPH).

The standard curve was developed with percentages of DPPH inhibitions, obtaining at different concentrations. From there, calculating the value of IC_{50} based on the standard curve equation ((y) = ax + b) with y = 50 % to find x (x is the value of IC_{50} which need to find) (Marxen *et al.,* 2007).

In vitro cancer cell line culture

Cancer cell lines were cultured as a monolayer in DMEM culture medium (Dulbecco's Modified Eagle Medium) with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES and 1.0 mM sodium pyruvate, adding 10 % fetal bovine serum-FBS (Gibco, Invitrogen). Cells were transplanted after 3-5 days at a ratio (1:3) and kept in CO_2 incubators at 37°C, 5 % CO_2.

Cytotoxic assay was confirmed by the National Cancer Institute (NCI) as a standard test to select and detect substances that can inhibit growth of cancer cells or kill them *in vitro*. The assay was carried out based on method of Monks (1991) and was conducted to determine total cellular protein content based on the optical density (OD) measured when the protein components of the cell were dyed with Sulforhodamine B (SRB, Sigma-Aldrich, USA). Measured OD values were proportional to the amount of SRB attached to the protein molecule, thus the more cells give (the more the protein) the larger the OD value. SRB assay was performed on KB (human carcinoma in the mouth), LNCaP

(human prostate carcinoma), SK-LU-1 (human lung carcinoma), SK-Mel-2 (human melanoma), HepG2 (human hepatocellular carcinoma), MCF7 (human breast carcinoma), HL-60 (human leukemia), SW 480 (human colorectal carcinoma), MKN7 (human gastric adenocarcinoma) and Hela (human cervical carcinoma) cell lines.

Reagent (10 µL) diluted in 10 % DMSO (in sterile distilled water, Merck, Germany) was fed into the wells of the 96-well disk (Corning, USA) for a selective concentration of 100 µg/mL. The active reagents were determined IC_{50} by using serial dilution of of 100, 20, 4 and 0.8 µg/mL. Then, trypsinized cancer cells at appropriate concentrations were added into the wells (180 µL medium), incubating in an incubator for 48 hours.

Another 96-well tray without reagents but with cancer cells (180 µL medium) was prepared in 3 columns for control on day 0. After 1 hour, the control plate on day 0 would be fixed with Trichloroacetic acid (TCA, Sigma-Aldrich, USA).

After the development stage in the CO_2 incubator, the cells were fixed to the bottom of the wells with TCA during 60 minutes, dyed with SRB for 30 minutes at 37°C. Discard the SRB and the tested wells were washed 3 times with 5 % acetic acid and allowed to dry in air at room temperature.

Finally, using 10 m M tris (hydroxymethyl) aminomethane solution to dissolve the amount of SRB that has been attached and dyed the protein molecules, put it on a gently shaker plate for 10 minutes and using the ELISA Plate Reader (Bio-Rad) to read the result of the color content of the SRB dye through the absorption spectrum at 515 nm. The percentage inhibiting cell growth in the presence of the test substance would be determined through the following formula:

$$\% \text{ Alive cells} = \frac{[OD_{reagent} - OD_{days0}]}{[OD_{negetive\ control} - OD_{days0}]} \times 100$$

$$\% \text{ Inhibited cells} = 100 - \% \text{ alive cells}$$

The assay was carried out in triplicated. Ellipticine (Sigma-Aldrich, USA) was used as positive control at concentrations of 10; 2; 0.4 and 0.08 µg/mL. DMSO 10 % was used as a negative control. The value of IC_{50} would be determined by using TableCurve 2Dv4 software (System software Inc., San Jose, California, USA). According to the standard National Cancer Institute (NCI), the residue was considered to have good activity with $IC_{50} \leq 20$ µg/mL, while the pure substance was considered to have good activity when $IC_{50} \leq 5$ µM (Hughes *et al.*, 2011).

Statistical analysis

The data were analyzed with Excel 2010, Design Expert 10 and IBM SPSS Statistics 20.

Results and Discussion

Development of Catechin standard curve equation

Catechin serial dilutions of 6.26; 12.5; 25, 50; 100; 200 and 300 µg/mL were prepared in 70 % ethanol. Catechin in the mixture with 10 % $AlCl_3$ and 1M CH_3COOK formed colorful complex, whose absorbances were measured at 415 nm on UV-Vis machine (U2900 Hitachi, Japan). It was revealed that OD values obtained from different Catechin concentrations showing great correlation ($R^2 = 0.967$) and the obtained linear equation was $y = 0.004x + 0.142$ (Fig1).

Optimization of total flavonoid extraction from lotus seeds by RSM surface response methodology

In study, In order to find out the optimum conditions for the whole extraction process, after investigating effects of single-factor on total flavonoid extraction, optimization of extraction procedure was processed by RSM surface response methodology by Design Expert 10 software (Stat-Ease Inc, Minneapolis, USA to predict optimal problem by the "expected function" to pick out values of the five factors at which the total flavonoid content was highest by applying regression analysis of experimental data to obtain a quadratic polynomial model showing total flavonoid content. ANOVA was used to evaluate the results.

Five single factors were used as 5 variables (time, solvent concentration, pH solvent, temperature and solvent: material ratio) namely from X_1 to X_5 to design prediction of optimization function Y, representing for total flavonoid content. Their upper and lower levels are shown in Table 1. Experimental design for RSM factorial design with 29 condition combinations together with their responses were presented in Table 2.

Table 2 indicated that total flavonoid content obtained from lotus seeds ranged from 0.146 ± 0.029 mg Catechin/g extract to 0.880 ± 0.071 mg Catechin/g extract and were input into Design Expert 10. Statistical analysis coefficients and SE coefficients in Table 3 showed high significance levels of all variables where most values with $p < 0.05$.

According to above notation, three linear factors (X_1, X_2, X_5) and six second-order values (X_1X_2, X_1X_5, X_3X_4, X_3X_5, X_1^2, X_3^2) participating in the model were shown in the confidence level, depend on 95 %, except for factors X_3, X_4 and 9 interact (X_1X_3, X_1X_4, X_2X_3, X_2X_4, X_2X_5, X_4X_5, X_2^2, X_4^2, X_5^2) which had $p > 0.05$ therefore, these values were not meaningful.

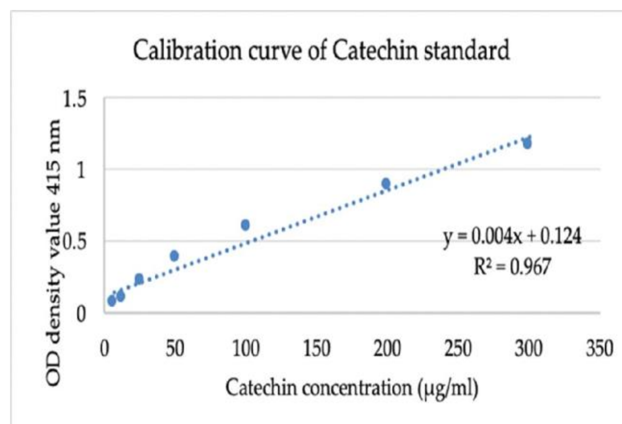

Fig 1: Calibration curve of catechin standard.

Table 1: Factors and the levels of experimental design.

Factor	Variable	Lower level	Base level	Upper level	Interval
A: Time	X_1	30.60	90	120.150	30
B: Concentration	X_2	10.30	50	70.90	20
C: pH solvent	X_3	3.40	5	6.70	1
D: Temperature	X_4	10.30	50	70.90	20
E: Ratio of solvent to raw material	X_5	5.10	15	20.25	5

Table 2: Experimental design for matrix of five factors affecting total flavonoid extraction from lotus seeds.

No	Expression					Y Total
	X_1 Time(min)	X_2 Concentration(%)	X_3 pH	X_4 Temperature(°C)	X_5 Ratio of Solvent: Raw material(v/w)	flavonotid content (mg Catechin/g extract)
1	30	90	3	90	20:1	0.459 ± 0.002
2	150	10	3	90	10:1	0.743 ± 0.007
3	30	10	2	10	5:1	0.717 ± 0.003
4	90	50	5	50	15:1	0.387 ± 0.053
5	30	90	3	10	5:1	0.761 ± 0.054
6	30	90	7	30	25:1	0.146 ± 0.029
7	30	90	7	90	5:1	0.546 ± 0.002
8	30	10	3	10	10:1	0.326 ± 0.002
9	150	10	7	10	5:1	0.687 ± 0.001
10	30	10	3	90	5:1	0.474 ± 0.006
11	150	10	3	10	5:1	0.663 ± 0.002
12	90	50	5	50	15:1	0.384 ± 0.068
13	150	90	7	90	25:1	0.652 ± 0.002
14	150	90	7	90	5:1	0.615 ± 0.013
15	150	10	7	90	5:1	0.515 ± 0.024
16	60	90	5	10	25:1	0.484 ± 0.026
17	150	10	7	10	25:1	0.591 ± 0.013
18	90	50	5	50	15:1	0.487 ± 0.028
19	150	50	5	90	25:1	0.232 ± 0.006
20	150	90	3	90	5:1	0.880 ± 0.071
21	150	90	3	10	5:1	0.734 ± 0.013
22	30	10	7	90	5:1	0.368 ± 0.016
23	30	90	7	10	5:1	0.684 ± 0.003
24	150	90	3	10	25:1	0.442 ± 0.024
25	150	90	7	10	5:1	0.502 ± 0.007
26	90	10	3	50	25:1	0.271 ± 0.064
27	30	10	5	50	25:1	0.164 ± 0.004
28	30	50	3	10	25:1	0.335 ± 0.015
29	30	10	7	90	25:1	0.287 ± 0.005

Regression definition (Table 3). Three linear regressions are X_1, X_2, X_5 and six quadratic X_1X_2, X_1X_5, X_3X_4, X_3X_5, X_1^2, X_3^2 possessed confident level over 95 %. Variables X_3, X_4 and nine interactions X_1X_3, X_1X_4, X_2X_3, X_2X_4, X_2X_5, X_4X_5, X_2^2, X_4^2, X_5^2 having > *0.05* did not have significant linear regression impact.

The final regression equation indicating the relationship between total flavonoid content and independent variables in quadratic model was shown as below:

$$Y = 0.42 + 0.13X_1 + 0.097X_2 - 0.16X_5 - 0.054X_1X_2 + 0.093X_1X_5 - 0.048X_3X_4 + 0.08X_3X_5 - 0.5X_1^2 + 0.2X_3^2$$

Where, Y: Predicted total flavonoid content

Value of the Fisher test model (F = 10.140) and low probability value ($p = 0.0087$) in Table 4 indicated that the set regression equation has high statistical significance. On the other hand, the model's suitability was checked through the correlation coefficient of determination R^2.

According to Guan and Yao (2008), a suitable model having R^2 value above the lowest acceptable value of 80 %. R^2 value of this model was 97.590 %; R^2 - (adj) was 87.970 % and all p values showed statistically significant levels. This indicated that the model constructed based on the selected variables was appropriate and showed quite high compatibility between experimental and predictive values.

Table 3: Significance levels of regression coefficients.

Factor	Coef	SE Coef
β%	0.420	0.036
X_1	0.130	0.023
X_2	0.097	0.020
X_3	0.011	0.021
X_4	9.380E-003	0.018
X_5	-0.160	0.026
X_1X_2	-0.054	0.018
X_1X_3	-0.023	0.018
X_1X_4	0.027	0.017
X_1X_5	0.093	0.027
X_2X_3	-0.039	0.016
X_2X_4	0.029	0.018
X_2X_5	0.030	0.021
X_3X_4	-0.048	0.017
X_3X_5	0.081	0.019
X_4X_5	0.014	0.020
X_1^2	-0.500	0.096
X_2^2	0.150	0.099
X_3^2	0.200	0.075
X_4^2	0.270	0.110
X_5^2	-0.070	0.053

Moreover, the relevance of this model was also assessed through the F value of Lack of fit. The correlation model is good, needing a fit between actual and theoretical data, therefore, the obtained model was predicted when the Lack of fit test (nonconformity) was not statistical. The results in Table 3 showed that both values of F (1.260) and *p (0.472) > 0.05*, thus, they did not have statistically significant. Therefore, the suitability of the model was significant (Table 4).

In conclusion, this regression equation could be used to theoretically predict total flavonoid content of liquid extraction from lotus seeds.

Response surface graphs (Fig 2) showed correlations between factors such as time, solvent concentration, solvent pH, ultrasound temperature, ratio of solvent to material to total flavonoid content from lotus seeds liquid extraction. As long as increase factors (extracting time, sonicating intensity, solvent: material ratio), flavonoid content climbed up to a peak then plummeted.

Regression predicted optimal flavonoid content would be archived – around 0.812 mg Catechin/g extract at Time (98.005 minutes); solvent concentration (86 %), pH (4.546), ultrasonic bath temperature (22.210 °C) and ratio of solvent/ material (8.376 (v/w) (Fig 3).

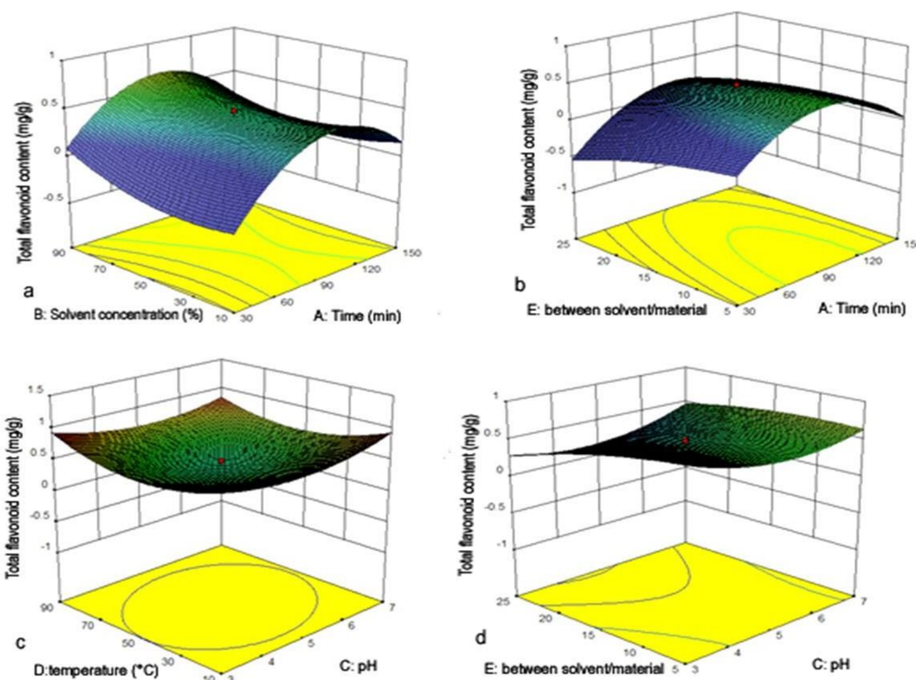

Fig 2: Total flavonoid content response surfaces were plotted in 3D to determine ranges of conditional value for optimal responses.

Table 4: Analysis of variance (ANOVA) of the regression equation for total flavonoid extraction in lotus seeds.

Source	Sum of squares	Degrees of freedom	Mean square	F Value	P Value
Model	0.800	20	0.040	10.140	0.009
Lack-of-Fit	0.013	3	4.322E – 003	1.260	0.472
Pure Error	6.873E – 003	2	3.436E – 003		
Total	0.830	25			

R - Sq =97.590% ; R^2 - (adj) = 87.970%.

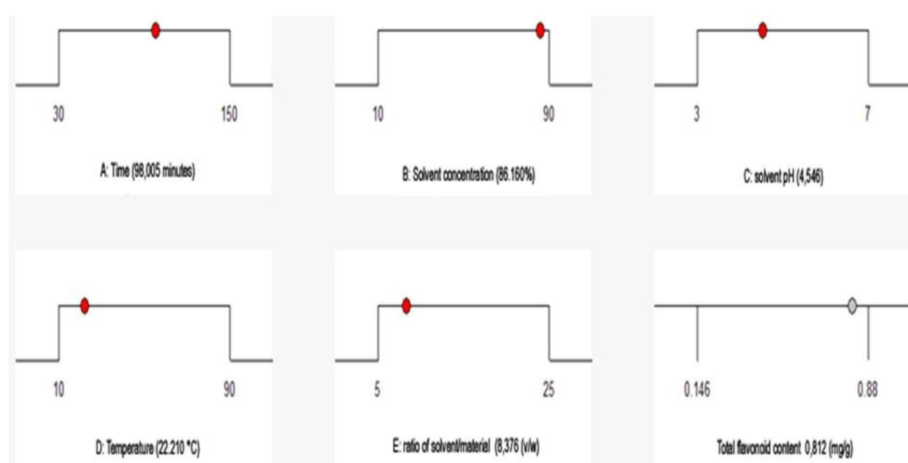

Fig 3: Expected function and optimal conditions for total flavonoid content to confirm whether predicting model fit with real experiments, flavonoid extraction was carried out at proposed optimum condition in triplicated.

From the obtained liquid extract, we proceed determine the total flavonoid content in the extract. The result is show total flavonoid content obtained in real experiment was 0.797 ± 0.011 (mg Catechin/g extract), lower than the theoretical prediction by Design Expert 10 (0.812 (mg Catechin/g extract). This discrepancy may be because of experimental manipulation.

Biological activities of ethanol liquid and dried extract from lotus seeds

Antioxidant activity

Liquid extract was concentrated by the vacuum evaporation system (EI-VAP Advantage G3, Heidolph - Germany). Total

Table 5: Flavonoid content and DPPH free radical scavenging activity of lotus seed extracts in ethanol.

Concentration of extraction (mg/mL)	Total flavonoid content (mg Catechin/g extract)	% free radical scavenging activity SC
1	1.540 ± 0.014	90.060 ± 1.029
0.500	1.046 ± 0.012	85.200 ± 0.242
0.200	0.536 ± 0.122	72.030 ± 0.208
0.100	0.312 ± 0.131	62.850 ± 0.284
0.067	0.189 ± 0.034	55.450 ± 0.360
0.050	0.146 ± 0.029	39.280 ± 0.157
$LSD_{0.05}$	0.070	0.040

Table 6: IC_{50} values of lotus seed extract and ascorbic acid control.

Sample name	Equation	R^2	IC_{50} (µg/mL)
Extraction	y = 15.513 ln(x) + 94.132	0.920	0.058 ± 0.004
Acid ascorbic	y = 26.678 ln(x) + 212.400	0.983	0.002 ± 0.001

Table 7: Carcinogenic effects of lotus seed extract.

Concentration	Inhibitions of lotus seed extract on cell lines (%)									
(µg/mL)	LNCaP	HepG2	MCF7	Hela	SK-LU-1	MKN7	SW 480	KB	SK-Mel2	HL-60
100	85.410	83.840	87.360	87.770	83.900	75.440	80.130	78.580	79.100	81.880
20	31.670	20.000	25.180	22.870	19.500	20.670	25.630	16.590	19.960	30.070
4	9.930	2.070	5.800	5.720	7.980	10.220	11.050	2.530	5.870	0.450
0.8	-9.680	-3.710	-1.010	-5.410	-6.450	-1.910	2.110	-2.610	-2.740	-2.160
IC_{50}	37.330± 3.950	54.110± 5.580	47.550± 4.410	48.020± 3.660	52.410± 5.370	57.450± 5.020	50.490± 3.740	61.060± 6.530	56.450± 2.310	45.990± 4.280
Concentration	Inhibitions of Ellipticine on cell lines (%)									
(µg/mL)	LNCaP	HepG2	MCF7	Hela	SK-LU-1	MKN7	SW 480	KB	SK-Mel2	HL-60
10	95.140	97.200	107.250	99.140	100.210	100.060	86.130	92.870	92.640	101.250
2	76.680	71.880	71.370	76.050	81.800	82.510	75.870	72.080	73.930	80.940
0.4	48.220	49.870	51.080	51.670	55.310	50.210	50.040	50.190	49.310	51.270
0.08	23.100	21.080	21.140	23.230	21.770	22.020	25.720	24.450	21.460	20.620
IC_{50}	0.450± 0.010	0.490± 0.020	0.480± 0.040	0.410± 0.020	0.360± 0.020	0.400± 0.040	0.440± 0.050	0.450± 0.030	0.480± 0.050	0.410± 0.040

flavonoid content and antioxidant activities were performed as previously described (Table 5 and 6).

Total flavonoid content obtained in condensed extract was 1.540 ± 0.014 mg Catechin/g extract, which was much higher than the liquid extract (0.797 ± 0.011 mg Catechin/g extract). Linear regression equation obtained from lotus seed extract was: y = 15.513 ln (x) + 94.132, with a correlation coefficient $R^2 = 0.920$. IC_{50}, the amount exhibiting 50 % of DPPH, of flavonoid was 0.058 ± 0.004 µg/mL, which was 29 times lower than that of Ascorbic acid 0.002 ± 0.001 µg/mL.

Cytotoxic activity assay

Lotus extract exhibited highest inhibition on LNCaP cell line with IC_{50} around 37.330 ± 3.950 µg/ml, next to HL-60 with IC_{50} 45.990 ± 4.280 µg/ml. Lowest inhibition amongst testes cell lines was found on KB cell line with IC_{50} 61.060 ± 6.530 µg/mL.

From the liquid extract, we diluted it to different concentrations to test the toxic activity on some cancer cell lines. The results in Table 7 showed that the extract has activity on the cancer cell lines tested with IC_{50} = 37.330 – 61.060 µg/mL. Positive ellipticine controls were stable in experiments (Table 7).

CONCLUSION

In conclusion, the optimal linear regression equation for Y as expected total flavonoid content in lotus seeds will be calculated as:

$$Y = 0.42 + 0.13X_1 + 0.097X_2 - 0.16X_5 - 0.,054X_1X_2 + 0.093X_1X_5 - 0.048X_3X_4 + 0.08X_3X_5 - 0.5X_1^2 + 0.2X_3^2$$

Y: expected total flavonoids in lotus seeds

Calculated optimal conditions for obtaining highest total flavonoid content, predicted to be 0.812 mg Catechin/ g extract, were: Time (98 .005 minutes); solvent concentration (86 %), p H (4 . 546) , tank ultrasonic temperature (22.210 °C) and solvent/material ratio (8.376 (v/w)). Confirming experiments yielded 0.797 ± 0.011 mg Catechin/g extract and in liquid extract is 1.540 ± 0.014 mg Catechin/g extract.

Antioxidant activitiy of condensed extract was found as 0.058 ± 0.004 (µg/mL), 29 times lower than that of ascorbic acid with IC_{50} 0.002 ± 0.001 µg/mL. IC_{50} of *in vitro* cytotoxicity activities against cancer cell lines were 7.330 – 61.060 µg/mL. Highest inhibition were found on cancer cell lines with proposed mechanisms as KB (human carcinoma in the mouth); LNCaP (human prostate carcinoma); SK-LU-1 (human lung carcinoma); SK-Mel-2 (human melanoma); HepG2 (human hepatocellular carcinoma); MCF7 (human breast carcinoma); HL-60 (human leukemia); SW 480 (human colorectal carcinoma); MKN7 (human gastric adenocarcinoma) and Hela (human cervixcal carcinoma) cell lines.

From obtained results, lotus seeds were found to be potential for antioxidant and anticancer phytochemistry source. Further experiments should be carried out in the future to detect bioactive compounds and their working mechanisms underlying those activities.

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Competing interests

Authors have declared that no competing interests exist.

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