

Response surface optimization and antioxidant activity of total proanthocyanidins fraction from *Abutilon theophrasti* Medic. leaves

Chunlian Tian^{1,2}, Zehui Zhang¹, Hong Wang¹, Qiu Peng¹,
Yuru Guo¹, Cancan Cui¹ and Mingchun Liu^{1*}

¹Department of Animal Pharmacy, College of Animal Husbandry and Veterinary, Shenyang Agricultural University, No.120 Dongling Road Shenhe Dist, Shenyang Liaoning Prov., People's Republic of China

²Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education", Yantai University, 264005, Yantai Shandong Prov., People's Republic of China

Abstract: The extraction procedure and antioxidant activity were investigated for total proanthocyanidins extracts from *Abutilon theophrasti* Medic. leaves collected in August, September and October. The maximum extraction yield was achieved with 90% ethanol, 80°C of heating reflux temperature, 149.94 min of extraction time and 60(ml/g) of the ratio of solvent and material, which were optimized by Box-Behnken Design of response surface method. Spectrophotometric study displayed that total proanthocyanidins content was (0.44±0.02)% (0.52±0.01)% and (0.59±0.01)% for August, September and October samples, respectively. The proanthocyanidins extracts exhibited much stronger antioxidant activity to scavenge ABTS and DPPH free radicals, and reduce ferric power than the control synthetic antioxidant BHT. The present findings suggest that the proanthocyanidins extract from *Abutilon theophrasti* Medic. leaves was a very interesting candidate for the research and development of natural and healthy antioxidant for the pharmaceutical and food industries.

Keywords: *Abutilon theophrasti* Medic. total proanthocyanidins, extraction process optimization, response surface methodology, antioxidant activity.

INTRODUCTION

The demand of healthy and safe food has increased with the rising of the quality of human life, and plant-based natural antioxidants are playing a very important role. On the one hand, the natural antioxidant has the characteristics of low toxicity comparing to synthetic antioxidants such as "BHT", "BHA", "TBHQ", "DG" and so on (Chaouche *et al.*, 2015); on the other hand, the ability of removal free radicals *in vivo* and *in vitro* has caused the extensive concern of researcher in medicine and food industry (Lou, Hsu, Ho, 2014; Okoth, Chenia, Koorbanally, 2013; Rahman, Imran, Islam, 2013). The antioxidant activity of many natural plant extracts, such as proanthocyanidins, flavonoids, polyphenol, essential oil, curcuminoids and polysaccharides compounds have been reported (Gunaratne, 2013; Kim, 2010; Changwei, Tatsunori, Tran, Atul, Shinkichi, 2011; Olsovska, *et al.*, 2013; Pajak, *et al.*, 2014; Sarikurku, *et al.*, 2009; Sivasothy, *et al.*, 2013; Wu, *et al.*, 2014; Zhou, Chen, Zhang, Blanchard, 2014).

Abutilon theophrasti Medic. (*A. theophrasti*) are distributed all over tropics and sub tropics (Fu & Hong, 1993), and applied widely to treat many illnesses, such as swell, ulcer and inflammation in folk (Gu & Jiang, 2009; Liu *et al.*, 2010). The preliminary studies shows that potential biological activities constituents of *A. theophrasti* roots, stems, leaves, seeds and episperms

*Corresponding author: e-mail: liumingchun@syau.edu.cn

from China were phenols and flavonoids compounds, moreover, the study revealed that the biological activities components was much richer in *A. theophrasti* leaves, it indicated that *A. theophrasti* leaves may be a potential natural antioxidant (Sikorska & Matlawska, 2008; Tian, Wang, Sheng, & Zhao, 2012).

In recent years, there are a variety of evaluation methods used to assay antioxidant activities of natural extracts, such as superoxide radical, hydroxyl radical, nitrite scavenging, total phenolic index, total radical trapping antioxidant parameter, ferric-ion reducing antioxidant power (FRAP), 2, 2' - azino - bis (3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) (ABTS) and 2, 2 - diphenyl -1-picrylhydrazyl (DPPH) radicals scavenging assay methods (Ali, Bahri, Chaouachi, Boussaïd, Harzallah-Skhiri, 2014; Chaouche *et al.*, 2015; Fu, *et al.*, 2013; Ioannone, *et al.*, 2015; Wang *et al.*, 2013; Wu, *et al.*, 2014). The last three methods are widely used as evaluation methods of antioxidant activity for plant extracts. The Proanthocyanidins (PAs) of *Caryota ochlandra* fruit pericarp exhibited a better antioxidant activity, which was evaluated by IC₅₀ DPPH (142.86±1.53g/ml), IC₅₀ ABTS (80.51±0.41g/ml) and FRAP (373.09±5.02mg ascorbic acid equivalent/g dry weight) (Chen, *et al.*, 2014); Lahouar, *et al.* (2014) evaluated the antioxidant activity of four kinds of Tunisian barley by assay of FRAP, ABTS and DPPH radicals scavenging activity, and revealed that they are stronger natural antioxidants; the assay methods of FRAP, ABTS

and DPPH radicals scavenging activity were used for analysis of the antioxidant activity of the phenolic acids and flavonoids in the seeds and sprouts of mung beans, radish, broccoli and sunflower (Pajak, *et al.*, 2014). Therefore, the objectives of this paper were (a) to investigate extraction process of procyanidines from *A. theophrasti* leaves by Box-Behnken Design of response surface method; (b) to measure the content of procyanidines fraction from *A. theophrasti* leaves collected in August, September and October 2014; and (c) to evaluate of the antioxidant activity of extracts collected in three months.

MATERIALS AND METHODS

Apparatus and reagents

The total proanthocyanidin concentration and antioxidant activity were assayed by T6 ultraviolet and visible spectrophotometer (Beijing Puxi Tongyong Inc., China). Standard of catechin acid (HPLC purity < 98.0%) were purchased from National Institutes for Food and Drug Control (Shenyang, China). ABTS, DPPH and 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). All analytical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

Samples and processing

A. theophrasti leaves were gathered in Jilin province of China in August, September and October 2014 (No.1408, 1409 and 1410), and were authenticated by professor Shaofan Du of Shenyang Agricultural University according to Compendium of Materia Medica. The voucher specimens KT/JL/CH/AT/08/14, KT/JL/CH/AT/09/14 and KT/JL/CH/AT/10/14 were deposited at Department of Animal Pharmacy, College of Animal Husbandry and Veterinary, Shenyang Agricultural University for future reference. *A. theophrasti* leaves were dried in a shady and dry place under the outdoor temperature during 30 days after washed by flowing water, and ground to finely powder.

Experimental design

Single factor experiments

According to the results of preliminary experiments, four major influence factors, including concentration of ethanol solution, extraction temperature, extraction time and ratio of solvent to material, were investigated for response surface experiments. The levels optimization for the above four factors was studied by single factor experiment with the range of levels for concentration of ethanol solution 20-100 %, reflux extraction temperature 20-100°C, extraction time 30-150 min and ratio of solvent to material 20-60ml/g and the reflux extraction times were two cycles. When one influence factor was evaluated, the levels of other three factors were the middle ones, and the extraction yield of total proanthocyanidins was chose as index.

Optimization of extraction conditions by box-behnken design

Three levels of each factor were confirmed by the single factor design, and then a four variables (X_1 , concentration of ethanol solution; X_2 , extraction temperature; X_3 , extraction time; X_4 , ratio of solvent to material) and three levels Box-Behnken design (BBD) was introduced to optimize the extraction process with the yield of total proanthocyanidins extract (TPCE) as dependent variable. As shown in table 1, the BBD was consisted of 24 factorial points and five replicates of central points in the experiment.

Determination of total proanthocyanidins concentration

Total proanthocyanidins concentration (TPCC) was measured using vanillin-sulfuric acid assay method (Gunaratne, 2013) with some modification. The extracts solution with different concentration (1ml) was mixed to 2.5ml of 3% vanillin in methanol (w/v) and 2.5ml of 30% sulfuric acid/methanol solution with methanol as reagent blank. The absorbance was determined at 500 nm after darkly incubation for 20 min in 30°C, and TPCC was expressed as mg (+)-catechin equivalents (CE) per g dry weight of the medicinal materials (mg CE/g DW).

Antioxidant activity assay

FRAP, ABTS and DPPH radicals scavenging activities of TPCE from *A. theophrasti* leaves were assayed as the method of Tian *et al.* (2018), and all determinations were performed in triplicate.

STATISTICAL ANALYSIS

All measurement results were three replicate and analyzed by Design Expert 8.0.6 software (State, Inc., Minneapolis, USA) and SPSS 17.0 (SPSS 17.0 for WINDOWS; SPSS Inc., Chicago, IL). In all statistical analyses, p values < 0.05 were regarded as statistically significant and p values < 0.01 as very significance.

RESULTS

As presented in table 1, 29 experiments in the design matrix were performed with the yield of proanthocyanidins as index and the data were analyzed statistically by multiple regression analysis using the Design Expert software (version 8.0.6) to get the following proanthocyanidins final equation in terms of coded factors:

$$Y = 0.49 + 0.058X_1 + 0.0075X_2 + 0.016X_3 + 0.0075X_4 - 0.0025X_1X_2 - 0.005X_1X_3 - 0.005X_1X_4 - 0.0075X_2X_3 - 0.0025X_2X_4 - 0.005X_3X_4 - 0.003X_1^2 + 0.002X_2^2 - 0.0005X_3^2 + 0.007X_4^2$$

The optimal extraction parameters of proanthocyanidins obtained from Design-Expert software were list as follows: concentration of ethanol solution, 90%; extraction temperature, 80°C; extraction time, 149.94 min

and ratio of solvent to material, 60ml/g. Considering the operating convenience, extraction time was adjusted to 150 min, and triple experiments were performed under the revised conditions and the yield (0.521%) was almost at the same as the predicted result (0.515%).

The determined coefficient and the adjusted coefficient were 0.9754 and 0.9508, respectively. The model had good significance with the lower p -value ($p < 0.0001$) and variation (C.V.) at 1.85. The linear coefficients and quadratic term coefficients were very significant ($p < 0.0001$) for the concentration of ethanol solution (X_1) and reflux extraction time (X_3), and significant ($p < 0.05$) for reflux extraction temperature (X_2) and ratio of solvent to material (X_4).

As shown in table 2, the yields of proanthocyanidins were $(0.44 \pm 0.02)\%$, $(0.52 \pm 0.01)\%$ and $(0.59 \pm 0.01)\%$ for the samples collected in August, September and October 2014, respectively.

Comparing to the control of BHT ($IC_{50 \text{ ABTS}}$, 2.29 $\mu\text{g/ml}$), the $IC_{50 \text{ ABTS}}$ value of August sample was the lowest (0.16 $\mu\text{g/ml}$), and 0.22 and 0.23 $\mu\text{g/ml}$ for September and October (table 2), which were lower about 10 times to BHT.

DPPH radical scavenging abilities of August sample was the best with IC_{50} value (2.37 $\mu\text{g/ml}$), and 2.79 and 2.81 $\mu\text{g/ml}$ for the September and October, which were about 3 times lower than the control of BHT (IC_{50} , 8.10 $\mu\text{g/ml}$).

Similarly to DPPH and ABTS assays, August extract exhibited a strong reduction power with 2.34 mmol $\text{Fe}^{2+}/100 \mu\text{g/ml}$ than BHT with 0.92 mmol $\text{Fe}^{2+}/100 \mu\text{g/ml}$ (table 2), and September and October extracts were less efficient than the August extract with 1.83 and 1.73 mmol $\text{Fe}^{2+}/100 \mu\text{g/ml}$, which were all much higher than the control BHT.

Pearson test was used to evaluate the correlation between antioxidant activity and TPCC, and the correlations were shown in table 3. The coefficient of associations was from 0.903 to 0.945 for TPCC (%) and the antioxidant assays.

DISCUSSION

The optimization of single factor experiments

In this part, concentration of ethanol solution, reflux extraction temperature, reflux extraction time and ratio of solvent to material were investigated, and the effect of any one factor was investigated on the yield of proanthocyanidins extract with the other three factors at the middle level.

Effect of concentration of ethanol solution

The concentration of ethanol solution is an important

factor of proanthocyanidins extraction. In this research, the concentration of ethanol solution was set at 20, 40, 60, 80 and 100%, respectively.

As shown X_1 line in fig. 1, the yield of proanthocyanidins improved with the increase of ethanol concentration, which might be due to the polarity of proanthocyanidins close to ethanol solvent. After 80%, a higher concentration of ethanol resulted in a slightly increased yield of proanthocyanidins than 60% to 80% ethanol, which indicated that the yield of proanthocyanidins was influenced significantly by the lower concentration of ethanol.

Effect of the reflux extraction temperature

As presented in X_2 line in fig. 1, the effect of reflux extraction temperature (20, 40, 60, 80 and 100°C) on extraction yield of proanthocyanidins was studied, and the yield significantly increased with the temperature increasing from 40 to 60°C, while increased slowly from 60 to 100°C, which can be explained by the thermal motion of proanthocyanidins molecule, namely, more higher dissolving out from the raw materials more higher extraction temperature.

Effect of the reflux extraction time

In this work, reflux extraction time was set at 30, 60, 90, 120 and 150 min, respectively. According to X_3 line in fig. 1, the yield of proanthocyanidins increased with the increase of reflux extraction time from 30 to 120 min, and reached the peak value (0.21%). The results indicated that the yield of proanthocyanidins enhanced with the increasing of the interaction time of the raw materials and solvent. However, a longer extraction time than 120 min presented a negative effect on the yield of proanthocyanidins, which may be the increasing of dissolution of the impurities.

Effect of the ratio of solvent to material

Suitable ratio of solvent to material was not only an ideal extraction condition, but also an effective method to save cost of experiment material such as reagents, water and power. In this research, ratio of solvent to material was set at 20, 30, 40, 50 and 60 ml/g in X_4 line in fig. 1, and the yield of proanthocyanidins increased significantly from 20 to 40ml/g, while increased slowly from 40 to 60ml/g. On the whole, the yield of proanthocyanidins has been less affected by the ratio of solvent to material than other three factors, and the yield was ranged only from 0.11% to 0.18% in the range of 20 to 60ml/g.

According to the results of single factor experiments, three levels of the four factors were adopted for the RSM experiments as follows: the concentration of ethanol solution (80, 90, 100%), the reflux extraction temperature (80, 90, 100°C), the reflux extraction time (90, 120, 150 min) and the ratio of solvent to material (40, 50, 60).

Table 1: The Box-Behnken design matrix and response values for TPCE yield.

Run	Variable levels				Response 1(R1)
	X ₁	X ₂	X ₃	X ₄	
1	80	90	120	60	0.44
2	90	90	150	40	0.50
3	90	90	120	50	0.49
4	90	80	90	50	0.45
5	90	80	120	60	0.50
6	100	90	150	50	0.55
7	90	90	90	40	0.47
8	90	90	120	50	0.49
9	80	90	150	50	0.45
10	100	80	120	50	0.54
11	90	80	150	50	0.51
12	90	100	150	50	0.50
13	90	90	120	50	0.48
14	90	90	90	60	0.50
15	80	100	120	50	0.44
16	100	100	120	50	0.56
17	80	80	120	50	0.41
18	100	90	120	40	0.54
19	90	100	90	50	0.47
20	80	90	90	50	0.41
21	90	80	120	40	0.48
22	90	100	120	40	0.50
23	80	90	120	40	0.42
24	90	90	150	60	0.51
25	90	90	120	50	0.48
26	90	90	120	50	0.49
27	100	90	120	60	0.54
28	90	100	120	60	0.51
29	100	90	90	50	0.53

Table 2: Yield of total proanthocyanidins extracts, IC₅₀ values for evaluated antioxidant assays and EC₅₀ values reducing power of total proanthocyanidins extracts from *A. theophrasti* leaves.

No.	Yield of extracts (%)	IC ₅₀ ABTS (µg/ml)	IC ₅₀ DPPH (µg/ml)	FRAP (mmol Fe ²⁺ /100µg/ml)
Sample 1	0.44±0.02	0.16	2.37	2.34
Sample 2	0.52±0.01	0.22	2.79	1.83
Sample 3	0.59±0.01	0.23	2.81	1.73
BHT	-	2.29	8.10	0.92

IC₅₀, inhibition concentration 50%; Sample 1, 2 and 3 were collected in August, September and October 2014.

Table 3: Correlation coefficients between assays

	ABTS	DPPH	FRAP	TPCC(%)
ABTS	1	0.996	-1.000*	0.938
DPPH	-	1	-0.994	0.903
FRAP	-	-	1	-0.945
Yield of extracts (%)	-	-	-	1

**Significant at $p < 0.01$; *Significant at $p < 0.05$.

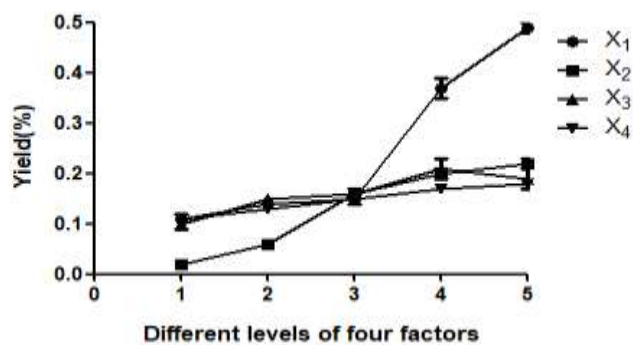


Fig. 1: Effect of the concentration of ethanol solution (X_1), the reflux extraction temperature (X_2), the reflux extraction time (X_3) and the ratio of solvent to material (X_4) on the extraction yield of total proanthocyanidins.

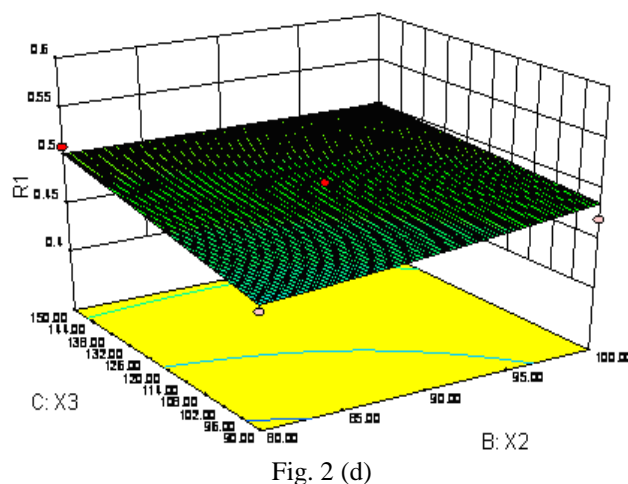


Fig. 2 (d)

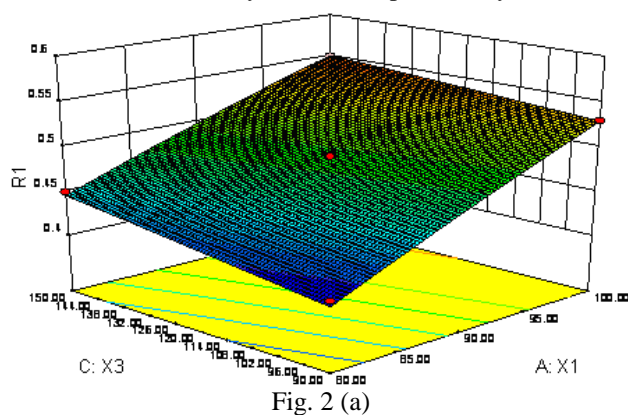


Fig. 2 (a)

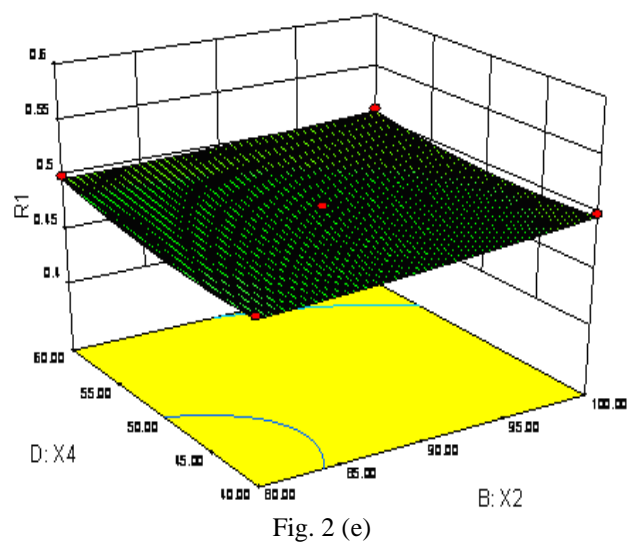


Fig. 2 (e)

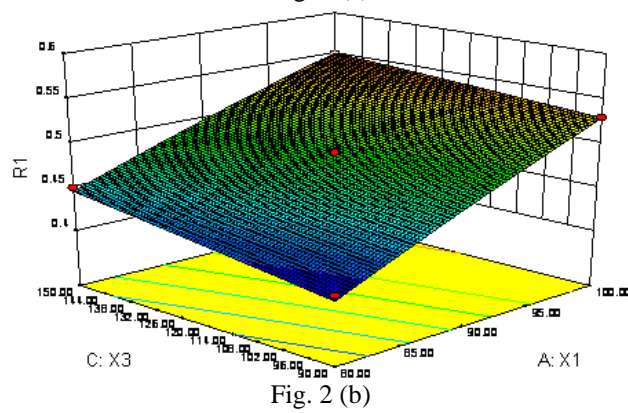


Fig. 2 (b)

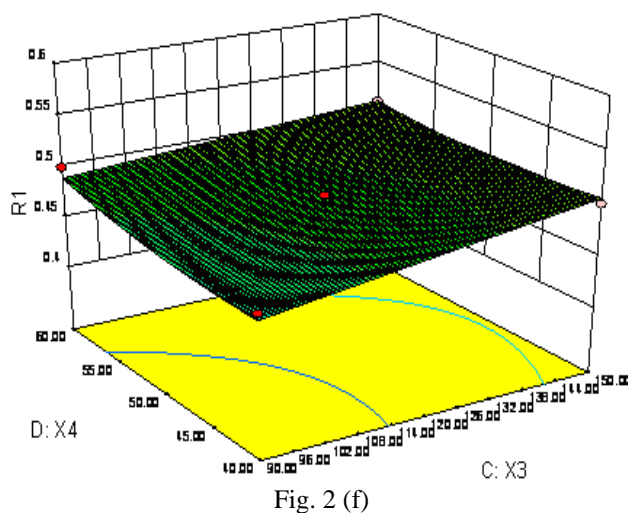


Fig. 2 (f)

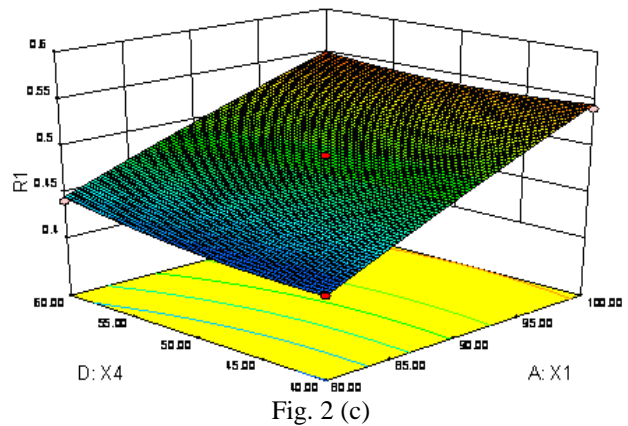


Fig. 2 (c)

Fig. 2: (a-f) Response surface plots of the concentration of ethanol solution (X_1 ,%), the reflux extraction temperature (X_2 , $^{\circ}$ C), the reflux extraction time (X_3 , min) and the ratio of solvent to material (X_4 , ml/g) on proanthocyanidins yield.

Optimization of extraction conditions by BBD

Model fitting and statistical analysis

The determined coefficient ($R^2=0.9754$) indicated that only 2.46% of total variants cannot be explained by the model, and the adjusted R^2 (0.9508) suggested a better correlation between the experimental values and predicted values. The lower p -value ($p<0.0001$) and variation(C.V.) at 1.85 indicated that the model can represent accurately the actual relationship between parameters and response.

The linear coefficients and quadratic term coefficients of concentration of ethanol solution (X_1) and reflux extraction time (X_3) were very significant ($p<0.0001$), and the coefficients of reflux extraction temperature (X_2) and ratio of solvent to material (X_4) were significant ($p<0.05$). However, no significant effect was observed in interaction factors ($p>0.05$). In addition, concentration of ethanol solution and reflux extraction time were two most significant factors influenced the proanthocyanidins yield, and then reflux extraction temperature and ratio of solvent to material.

Graphical interpretation and optimization of procedure

Fig. 2 was the 3D response surface curves showing the effects of the concentration of ethanol solution, reflux extraction temperature, reflux extraction time and the ratio of solvent to material on the yield of TPCE, and each figure provided the effects of two factors on the yield of TPCE. From fig. 2a to 2c, the yield of TPCE increased with increasing of the concentration of ethanol solution and one of other three factors. But further increasing any two factors of reflux extraction temperature, reflux extraction time and the ratio of solvent to material would not increase significantly the extraction yield of TPCE (fig. 2d to 2e).

TPCC of samples collected from various seasons

The results in the table 2 indicated that the TPCC was connected with the season, and TPCC was the highest in October and the lowest in August. This suggests that harvested time was important for extraction proanthocyanidins.

Antioxidant activity

The antioxidant activity was inversely proportional to TPCC, which may be due to the difference of structures and properties of the chemical compositions in the three extracts. In a word, the three extracts all exhibited higher antioxidant activity than the control synthetic antioxidant BHT, and will be very interesting candidates for the research and development of natural and healthy antioxidant for the pharmaceutical and food industries.

Pearson correlation analysis

As shown in table 3, the TPCC (%) was highly correlated to the antioxidant assays with r value ranged from 0.903 to 0.945. The highest correlation coefficient was 0.945 for

TPCC and FRAP, and the lowest was 0.903 between TPCC and DPPH assay, which indicated that proanthocyanidins compounds contributed to the antioxidant activity.

Table 3 also listed the results of correlations of the antioxidant activity assay. The highest significant correlation ($p>0.05$) was 1.000 between FRAP and ABTS assays, and the correlation coefficient was much higher between DPPH and ABTS, FRAP and DPPH with r value 0.996 and 0.994. This implied that the correlation coefficient was much better in the three antioxidant assays for evaluating the antioxidant activity of proanthocyanidins extracts.

CONCLUSION

This is the first study focused on extraction procedure, content determination and antioxidant activity of TPCE from *A. theophrasti* leaves. The extraction procedure of TPCE was optimized by single factor test and RSM with four-variable, three-level and the optimum extraction procedure was 90% ethanol solution, 80°C, 149.94 min of extracting time and 60 (ml/g) of the ratio of solvent and material. The antioxidant activity of TPCE was determined successfully by ABTS radical scavenging activity, DPPH radical scavenging activity and FRAP assays and it is revealed that TPCE exhibits better antioxidant activity. The experiment results will be useful for further research and development of new, healthy and nature antioxidant for food and pharmaceutical industries.

ACKNOWLEDGEMENTS

The project was supported by National Natural Science Foundation of China (Grant No.31402252) and Youth Foundation of Shenyang Agricultural University (Grant No.20131001).

REFERENCES

- Ali IBEH, Bahri R, Chaouachi M, Boussaid M and Harzallah-Skhiri F (2014). Phenolic content, antioxidant and allelopathic activities of various extracts of *Thymus numidicus* Poir. organs. *Ind. Crops Prod.*, **62**: 188-195.
- Chaouche TM, Haddouchi F, Atik-Bekara F, Ksouri R, Azzi R, Boucherit Z, Tefiani C and Lariat R (2015). Antioxidant, haemolytic activities and HPLC-DAD-ESI-MSⁿ characterization of phenolic compounds from root bark of *Juniperus oxycedrus* subsp. *oxycedrus*. *Ind. Crops Prod.*, **64**: 182-187.
- Changwei A, Tatsunori H, Tran DK, Atul U and Shinkichi T (2011). Antioxidant phenolic compounds from *Smilax sebeana* Miq. *LWT-Food Sci. Technol.*, **44**(7): 1681-1686.

- Chen XX, Feng HL, Ding YM, Chai WM, Xiang ZH, Shi Y and Chen QX (2014). Structure characterization of proanthocyanidins from *Caryota ochlandra* Hance and their bioactivities. *Food Chem.*, **155**: 1-8.
- Fu CD and Hong YF (1993). Research on chemical composition of *Abutilon theophrasti* Medic. *Foreign Med. Sci.*, **15**(2): 4-7.
- Fu R, Zhang YT, Guo YR, Huang QL, Peng T, Xu Y, Tang L and Chen F (2013). Antioxidant and anti-inflammatory activities of the phenolic extracts of *Sapium sebiferum* (L.) Roxb. leaves. *J. Ethnopharmacol.*, **147**(2): 517-524.
- Gu GY and Jiang Y (2009). Research on chemical composition and pharmacological action of *Abutilon indicum* and *Abutilon*. *Modern Pharm. Clinic*, **24**(6): 338-340.
- Gunaratne A, Wu K, Li DQ, Bentota A, Corke H and Cai YZ (2013). Antioxidant activity and nutritional quality of traditional red-grained rice varieties containing proanthocyanidins. *Food Chem.*, **138**(2-3): 1153-1161.
- Ioannone F, Di CD, M Mattia, Gregorio De, Sergi M, Serafini M and Sacchetti G (2015). Flavanols, proanthocyanidins and antioxidant activity changes during cocoa (*Theobroma cacao* L.) roasting as affected by temperature and time of processing. *Food Chem.*, **174**(1): 256-262.
- Kim JS, Kwon YS, Chun WJ, Kim TY, Sun JH, Yu CY and Kim MJ (2010). *Rhus verniciflua* Stokes flavonoid extracts have anti-oxidant, anti-microbial and α -glucosidase inhibitory effect. *Food Chem.*, **120**(2): 539-543.
- Lahouar L, Arem AE, Ghrairi F, Chahdoura H, Salem HB, Felah ME and Achour L (2014). Phytochemical content and antioxidant properties of diverse varieties of whole barley (*Hordeum vulgare* L.) grown in Tunisia. *Food Chem.*, **145**: 578-583.
- Liu H, Ni SF, Kang JH, Luo RF, Wu YF, Cui YT and Li ZX (2010). The pharmaceutical research situation of plants of *Abutilon*. *Northwest Pharm. J.*, **25**(1): 68-69.
- Lou SN, Hsu YS and Ho CT (2014). Flavonoid compositions and antioxidant activity of calamondin extracts prepared using different solvents. *J. Food Drug Anal.*, **22**(3):290-295.
- Sarikurkcü C, Arisoy K, Tepe B, Cakir A, Abali G and Mete E (2009). Studies on the antioxidant activity of essential oil and different solvent extracts of *Vitex agnus castus* L. fruits from Turkey. *Food Chem. Toxicol.*, **47**(10): 2479-2483.
- Sikorska M and Matlawska I (2008). Polyphenolic compounds from *Abutilon grandiflorum* leaves. *Acta Pol. Pharm.*, **65**(4): 467-471.
- Sivasothy Y, Sulaiman SF, Ooi KL, Ibrahim H and Awang K (2013). Antioxidant and antibacterial activities of flavonoids and curcuminoids from *Zingiber spectabile* Griff. *Food Control*, **30**(2): 714-720.
- Tian CL, Wang M, Sheng CH and Zhao CJ (2012). Accuracy mass screening and identification of phenolic compounds from the five parts of *Abutilon theophrasti* Medic. by reverse phase high performance liquid chromatography - electrospray ionization - quadrupoles - time of flight - mass spectrometry. *J. Sep. Sci.*, **35**: 763-772.
- Chunlian Tian, Peng Zhang, Caixia Yang, Xiang Gao Hong Wang, Yuru Guo and Mingchun Liu (2018). Extraction process, component analysis, and in vitro antioxidant, antibacterial and anti-inflammatory activities of total flavonoids extracts from *Abutilon theophrasti* Medic. leaves. *Mediat. Inflamm.*, **2**: 1-17.
- Okoth DA, Chenia HY and Koorbanally NA (2013). Antibacterial and antioxidant activities of flavonoids from *Lannea alata* (Engl.) Engl. (Anacardiaceae). *Phytochem. Lett.*, **6**(3): 476-481.
- Olsovská J, Kamenik Z, Cejka P, Jurkova M and Mikyska A (2013). Ultra-high-performance liquid chromatography profiling method for chemical screening of proanthocyanidins in Czech hops. *Talanta*, **116**: 919- 926.
- Pajak P, Socha R, Galkowska D, Roznowski J and Fortuna T (2014). Phenolic profile and antioxidant activity in selected seeds and sprouts. *Food Chem.*, **143**: 300-306.
- Rahman MA, Imran TB and Islam S (2013). Antioxidative, antimicrobial and cytotoxic effects of the phenolics of *Leea indica* leaf extract. *Saudi J. Biol. Sci.*, **20**(3): 213-225.
- Wang LJ, Yang XS, Qin PY, Shan F and Ren GX (2013). Flavonoid composition, antibacterial and antioxidant properties of tartary buckwheat bran extract. *Ind. Crops Prod.*, **49**: 312-317.
- Wojdyło A, Oszmianski J and Czemerys R (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem.*, **105**(3): 940-949.
- Wu GH, Hu T, Li ZY, Huang ZL and Jiang JG (2014). *In vitro* antioxidant activities of the polysaccharides from *Pleurotus tuber-regium* (Fr.) Sing. *Food Chem.*, **148**: 351-356.
- Zhou ZK, Chen XS, Zhang M and Blanchard C (2014). Phenolics, flavonoids, proanthocyanidin and antioxidant activity of brown rice with different pericarp colors following storage. *J. Stored Prod. Res.*, **59**: 120-125.