The Optimization of the Anthraquinones Extraction from *Senna tora* Seeds Using Two-Factor Spherical Composite Experimental Design

Chaowalit Monton[1*], Sureewan Duangjit[2], Natawat Chankana[3], Laksana Charoenchai[1], Jirapornchai Suksaeree[1,4]

[1]The Herbal Medicinal Products Research and Development Center (cooperation between Rangsit University, Harbin Institute of Technology, and Heilongjiang University of Chinese Medicine), Faculty of Pharmacy, Rangsit University, Muang, Pathum Thani 12000, Thailand.
[2]Department of Pharmaceutical Chemistry and Technology, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Warinchamrab, Ubon Ratchathani 34190, Thailand.
[3]Sun-Herb Thai Chinese Manufacturing Plant, Faculty of Pharmacy, Rangsit University, Pathum Thani 12000, Thailand.
[4]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Rangsit University, Pathum Thani 12000, Thailand.
[*]Corresponding author: chaowalit@rsu.ac.th

Abstract

The objective of the study was to optimize the extraction of anthraquinones from *S. tora* seed using two-factor spherical composite design. The effect of ethanol concentration and herbal weight was simultaneously investigated to obtain the highest anthraquinones content. The extraction condition was based on spherical composite design. Five levels of each factor were varied. Ethanol concentration and herbal weight were varied from 40% to 90% and 1 to 9 g, respectively. The five responses including the content of aloe-emodin, rhein, emodin, chrysophanol, and physcion were determined using high performance liquid chromatography. The optimal condition was optimized using commercial computer software (Design-Expert). The accuracy was confirmed by extraction of *S. tora* seed using the optimal condition. Finally, an error of the estimation by computer software was calculated. Estimation of anthraquinones content extracted from *S. tora* seed provided from computer software showed that all five anthraquinones contemporary highly extracted when using low ethanol concentration with low herbal powder weight. The optimal condition providing a high content of anthraquinones was 43.1% ethanol and 1.0 g herbal weight. The experimental values of anthraquinones content provided from the optimal condition were 8.71 ppm aloe-emodin, 419.32 ppm rhein, 82.21 ppm emodin, 74.97 ppm chrysophanol, and 15.47 ppm physcion. The accuracy of this study was acceptable as the percent error less than 10. Computer software could be used for optimization of extraction of anthraquinones from *S. tora* seed with high accuracy.

Keywords**:** *Anthraquinones, Senna tora seed, computer software, high performance liquid chromatography*

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อหาค่าที่เหมาะสมของการสกัดแอนทราควิโนนจากเมล็ดชุมเห็ดไทย โดยใช้การออกแบบการทดลองแบบสเฟียริคอลคอมโพสิทแบบสองปัจจัย โดยศึกษาผลของความเข้มข้นของเอทานอลและน้ำหนักของผงสมุนไพรในคราวเดียวกัน เพื่อให้ได้ปริมาณแอนทราควิโนนสูงที่สุด สภาวะการสกัดใช้การออกแบบการทดลองแบบสเฟียริคอลคอมโพสิท แต่ละปัจจัยจะถูกปรับเปลี่ยน 5 ระดับ โดยที่ความเข้มข้นของเอทานอลและน้ำหนักผงสมุนไพรปรับเปลี่ยนจากร้อยละ 40 ถึงร้อยละ 90 และ 1 ถึง 9 กรัม ตามลำดับ จากนั้นวัดปริมาณสารแอนทราควิโนน 5 ชนิด ได้แก่ aloe-emodin, rhein, emodin, chrysophanol, และ physcion ด้วยโครมาโทกราฟีชนิดของเหลวสมรรถนะสูง การหาสภาวะที่เหมาะสมในการสกัดจะใช้ซอฟต์แวร์คอมพิวเตอร์ที่จำหน่ายในท้องตลาด (Design-Expert) สามารถยืนยันความถูกต้องโดยการใช้สภาวะที่ได้จากการทำนายเพื่อสกัดเมล็ดชุมเห็ดไทย และขั้นตอนสุดท้ายจะคำนวณความคลาดเคลื่อนของการทำนายที่ได้จากซอฟแวร์คอมพิวเตอร์ การทำนายปริมาณแอนทราควิโนนที่สกัดได้จากเมล็ดชุมเห็ดไทยด้วยโปรแกรมคอมพิวเตอร์ พบว่า แอนทราควิโนนทั้ง 5 ชนิดสกัดออกมาได้ในปริมาณสูงเมื่อใช้เอทานอลความเข้มข้นต่ำและผงสมุนไพรปริมาณต่ำ สภาวะที่เหมาะสมที่สามารถสกัดแอนทราควิโนนได้ในปริมาณสูงคือ เอทานอลความเข้มข้นร้อยละ 43.1 และผงสมุนไพรหนัก 1.0 กรัม สภาวะนี้จะสามารถสกัด aloe-emodin ได้ 8.71 ppm, rhein ได้ 419.32 ppm, emodin ได้ 82.21 ppm, chrysophanol ได้ 74.97 ppm, และ physcion ได้ 15.47 ppm ซึ่งความถูกต้องของการศึกษานี้สามารถยอมรับได้โดยให้ค่าความคลาดเคลื่อนต่ำกว่าร้อยละ 10 ซอฟต์แวร์คอมพิวเตอร์สามารถใช้เพื่อหาค่าที่เหมาะสมของการสกัดแอนทราควิโนนจากเมล็ดชุมเห็ดไทยและมีความถูกต้องสูง

คำสำคัญ: *แอนทราควิโนน เมล็ดชุมเห็ดไทย ซอฟต์แวร์คอมพิวเตอร์ โครมาโทกราฟีชนิดของเหลวสมรรถนะสูง*

---

1. Introduction

Anthraquinones (9,10-dioxoanthracenes) established an important naturally-occurring compound. Anthraquinones promoted several medical applications; laxatives, antimicrobial and antiinflammation (Malik and Müller, 2016). *Senna tora* (L.) Roxb. is a plant in the family of Fabaceae. Some examples of major anthraquinones found in *S. tora* seeds are shown in Figure 1. Several solvent systems are used to extract crude from *S. tora* seed containing anthraquinones such as methanol (Choi et al., 1997; Choi et al., 1998; Jang et al., 2007; Kim et al., 2004; Sui-Ming et al., 1989), and 60% ethanol (Zhu et al., 2008). Nowadays, environmental consideration of extraction solvent is generally accepted. Ethanol and water are widely used in the green extraction of natural products. Thus, optimization of the concentration of ethanol aqueous solution and extraction condition is an important manner to obtain a high content of natural active compound.

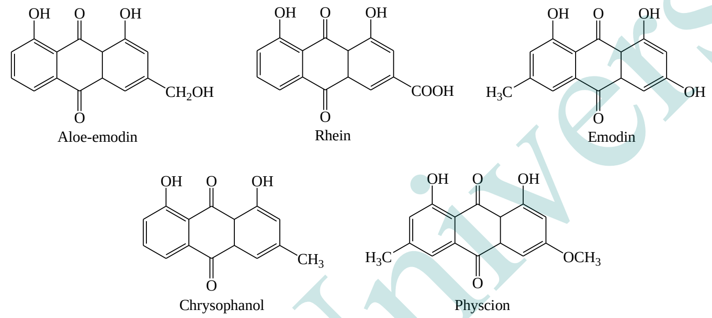


Figure 1 Chemical structure of some anthraquinones.

Recently, modern experimental design showed superior benefits compared to the traditional one at time trialing: less time and financial consuming and less drug substance use. Interaction effects can be identified, and surface response can be characterized (Gibson, 2016). Design-Expert® is such a type of user-friendly software that succeeds in the optimization of extraction of a chemical constituent from many plants such as phenolic compound from wheat (Liyana-Pathirana and Shahidi, 2005), puerarin and daidzein from dried roots of *Pueraria thomsonii* Benth. (Liu et al., 2011), iridoids from roots and rhizomes of *Gentiana rigescens* (Pan et al., 2015), a polysaccharide from *Sargassum thunbergii* (Yuan et al., 2015), phenolic compound and flavonoids from *Melaleuca bracteata* leaves (Hou et al., 2016), anthocyanin from *Ixora siamensis* (Mat Nor and Arof, 2016).

2. Objective

The objective of the study was to optimize the extraction of anthraquinones from *S. tora* seeds using two-factor spherical composite design to obtain the highest amount of five anthraquinones i.e. aloe-emodin, rhein, emodin, chrysophanol, and physcion. Two factors – ethanol concentration and herbal weight - were simultaneously investigated. Furthermore, the accuracy of the predicted value of software was also confirmed by the experiment.

3. Materials and methods

3.1 Materials

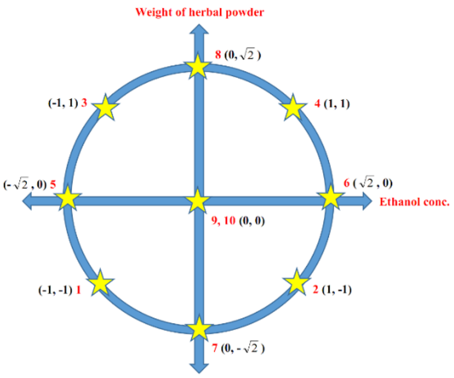
Aloe-emodin, rhein, and emodin were purchased from Sigma-Aldrich, USA. Chrysophanol and physcion were purchased from Chengdu Biopurify Phytochemicals Ltd., China. Absolute ethanol was purchased from QRëC, New Zealand. Methanol (HPLC grade) was purchased from Honeywell-Burdick & Jackson, USA. Orthophosphoric acid (85%) was purchased from Carlo-Erba, France. Reverse osmosis and ultrapure water were produced by Puris-Expe water system, Korea.

3.2 Plant sample

S. tora seeds were collected from Buached District, Surin Province, Thailand. It was dried in a hot air oven at 50 °C for 24 h, pulverized, and passed through a 20-mesh sieve.

3.3 Optimization procedure

The extraction condition, i.e. ethanol concentration (X_1) and herbal weight to 100 mL solvent (X_2), was based on spherical composite design. Five levels of each factor were varied. X_1 and X_2 were varied from 40% to 90% and 1 to 9 g, respectively. Ten extraction conditions (8 non-center points and 2 center points) were used to extract the *S. tora* seeds (Figure 2). The five responses including the content of aloe-emodin (Y_1), rhein (Y_2), emodin (Y_3), chrysophanol (Y_4), and physcion (Y_5) were monitored.



Condition	X_1	Ethanol conc. (%)	X_2	Weight of herbal powder (g)
1	-1	47.3	-1	2.2
2	1	82.7	-1	2.2
3	-1	47.3	1	7.8
4	1	82.7	1	7.8
5	$-\sqrt{2}$	40	0	5.0
6	$\sqrt{2}$	90	0	5.0
7	0	65	$-\sqrt{2}$	1.0
8	0	65	$\sqrt{2}$	9.0
9	0	65	0	5.0
10	0	65	0	5.0

Figure 2 Two-factor spherical composite experimental design.

According to extraction method, 100 mL ethanol aqueous solution was added to *S. tora* seed powder. The obtained mixture was shaken (level 5) in a water bath (Memmert, Germany) at 65.1 °C for 78.5 min. The obtained solution was immediately filtered through a 0.45-µm pore-size syringe filter and injected into HPLC instrument for analysis of anthraquinones content. The content of individual anthraquinones was calculated based on the standard calibration curve of each anthraquinone. Then, the content of anthraquinones was calculated based on a dried weight basis of *S. tora* seed powder in a unit of ppm.

The optimal condition was estimated using Design-Expert® 10.0.3 software (Stat-Ease Inc., USA) to obtain the simultaneously high content of anthraquinones. The optimal condition from the software was confirmed again by the experiment. The accuracy of the predicted values by the experiment was reported as % error (below equation).

$$\%\text{Error} = \frac{\text{experimental value} - \text{predicted value}}{\text{experimental value}} \times 100$$

3.4 HPLC condition

Anthraquinones content was analyzed using HPLC instrument equipped with a photodiode array detector, an autosampler, and an autoinjector (Agilent 1260 infinity, Agilent Technologies, USA). A Luna C18(2) column (250×4.60 mm i.d., 5 µm) was used for separation. The injection volume was 50 µL. The column temperature was controlled at 25 °C. The gradient system comprised methanol and 0.01% orthophosphoric acid aqueous solution with a flow rate of 1 mL/min (Table 1). The detection wavelength was 254 nm.

Table 1 Gradient system of mobile phase using for anthraquinones analysis.

Time (min)	Methanol (%v/v)	0.01% Orthophosphoric acid (%v/v)
0	70	30
12	70	30
14	90	10
22	90	10
23	70	30
25	70	30

4. Results

The HPLC chromatogram of *S. tora* seed extract is shown in Figure 3 in which five identified peaks at retention time of 10.280, 16.073, 19.273, 20.293, and 22.553 min were performed by aloe-emodin, rhein, emodin, chrysophanol, and physcion, respectively. In addition, the content of anthraquinones obtained from model conditions is shown in Table 2. According to the content of individual anthraquinone, rhein and aloe-emodin had the highest and lowest content, respectively.

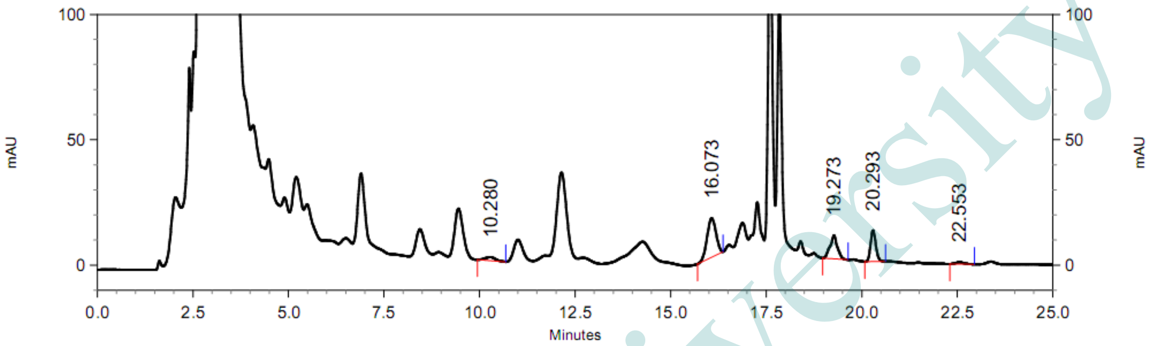


Figure 3 HPLC chromatogram of *S. tora* seed extract obtained from the optimal condition. The identified peaks were aloe-emodin, rhein, emodin, chrysophanol, and physcion, respectively.

Table 2 Content of anthraquinones obtained from model conditions.

Condition	Content (ppm)				
	Aloe-emodin	Rhein	Emodin	Chrysophanol	Physcion
1	5.88±0.45	335.76±6.64	51.13±2.55	61.97±1.55	10.84±0.30
2	0.00±0.00	13.56±0.77	29.79±1.18	14.35±0.91	8.83±0.54
3	3.67±0.17	202.79±3.86	33.30±0.64	47.42±0.49	7.04±0.04
4	0.00±0.00	14.09±0.38	18.54±0.04	13.71±0.06	6.10±0.08
5	5.76±0.28	418.55±14.57	40.23±1.75	64.27±2.78	7.86±1.37
6	0.00±0.00	0.00±0.00	21.14±0.80	8.84±0.09	5.70±0.78
7	3.18±0.50	124.70±2.77	110.25±35.49	41.00±0.35	20.72±3.76
8	0.96±0.13	126.26±1.70	34.09±0.57	44.95±0.14	7.71±0.27
9	1.35±0.02	126.25±1.04	37.88±0.32	41.02±1.44	8.44±0.29
10	0.90±0.09	99.20±0.99	33.12±0.77	34.39±0.12	8.41±0.07

The contour plots and response surfaces of optimal model condition for all anthraquinones obtained from computer software are shown in Figure 4 and 5, respectively. According to the effects of ethanol concentration, the lower ethanol concentration was, the higher content of anthraquinone was extracted. In the case of herbal weight, using a low amount of herbal powder, high content of anthraquinones was extracted. This study revealed that the patterns of surface responses and contour plots of the model condition of aloe-emodin, rhein, and chrysophanol were similar. In addition, emodin was similar to physcion.

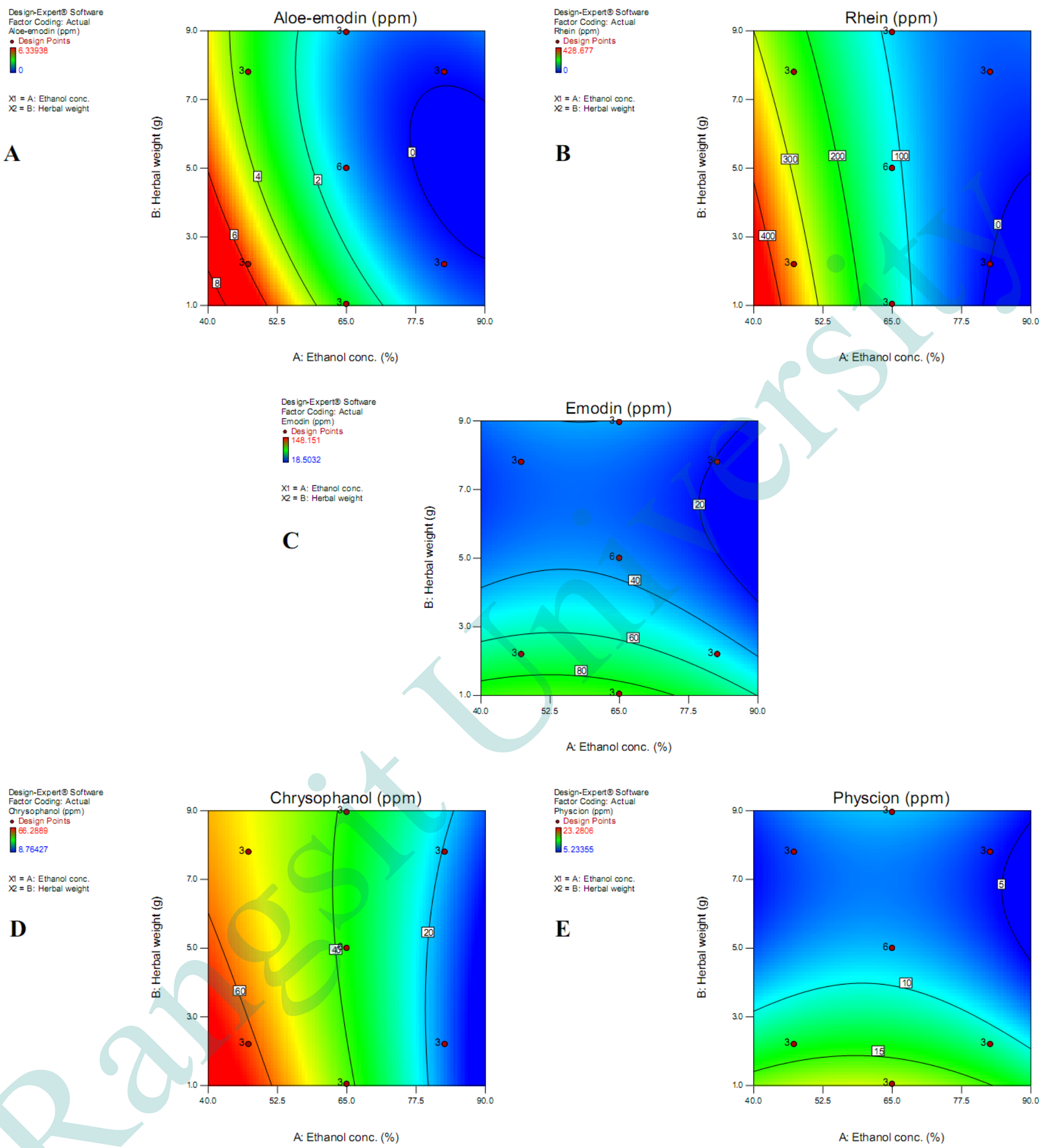


Figure 4 Contour plots of the model condition of (A) aloe-emodin, (B) rhein, (C) emodin, (D) chrysophanol, and (E) physcion.

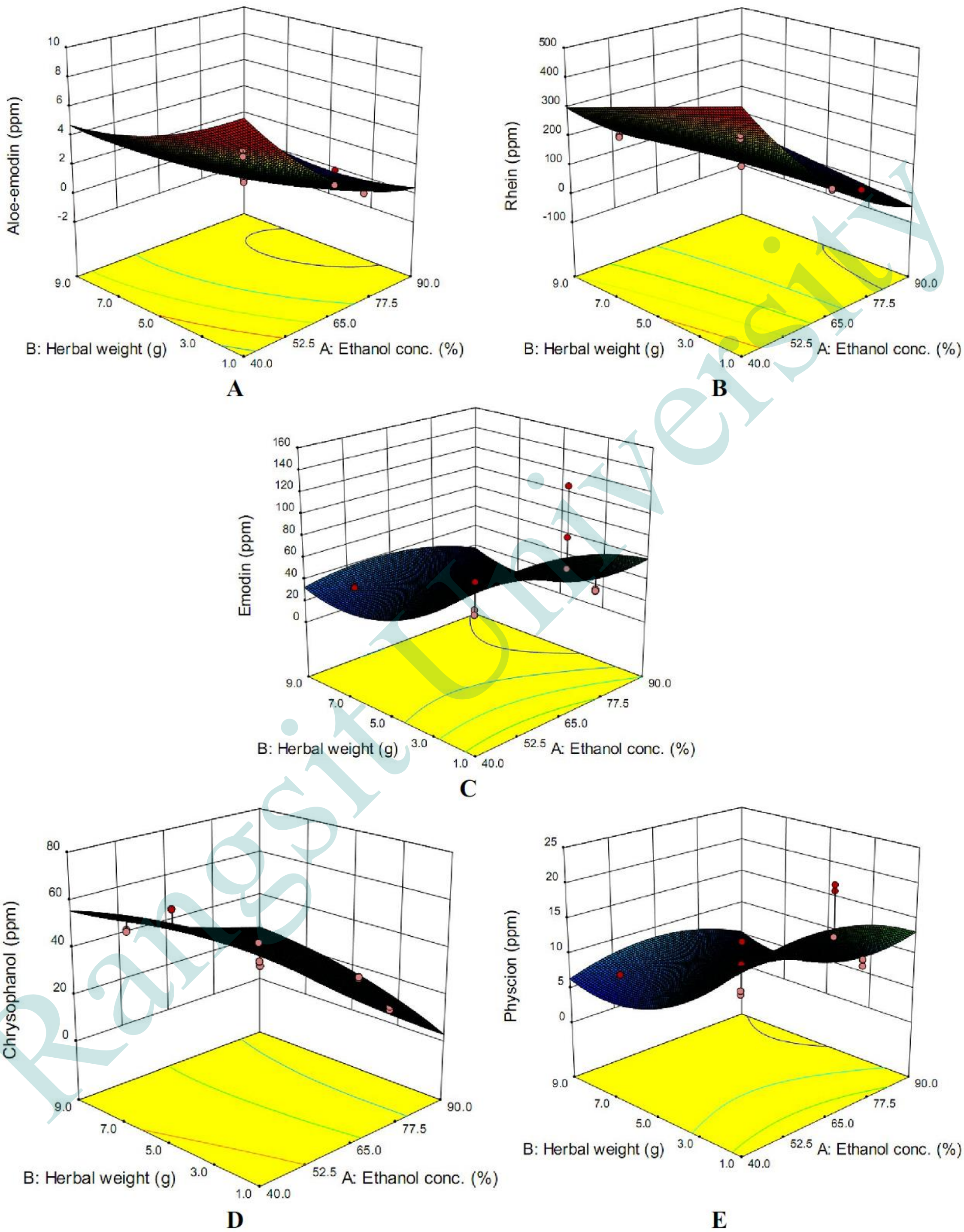


Figure 5 Response surfaces of the model condition of (A) aloe-emodin, (B) rhein, (C) emodin, (D) chrysophanol, and (E) physcion.

The optimal condition achieved when using 43.1% ethanol and a herbal weight of 1.0 g with a slightly high desirability of 0.813. The maximum anthraquinones were obtained under this condition. The predicted value and experimental value with a percent error of each anthraquinone provided from computer software are shown in Table 3.

Table 3 Predicted value, experimental value, and %error of optimal condition.

Compounds	Predicted value (ppm)	Experimental value (ppm)	%Error
Aloe-emodin	8.01	8.71±0.27	8.09
Rhein	428.68	419.32±18.47	-2.23
Emodin	90.06	82.21±15.48	-9.55
Chrysophanol	69.89	74.97±7.30	6.77
Physcion	16.88	15.47±0.20	-9.10

5. Discussion

A type of extraction solvent was found to be an important factor affecting the efficiency of extraction. Several publications reported that 50-80% ethanol was the most suitable solvent for extraction of anthraquinones from plants. The 50%, 60%, 70%, and 80% ethanol were the most suitable solvents for extraction of anthraquinones from *Morinda* sp. (Temiyaputra et al., 2008), *Heterophyllaea pustulata* Hook f. (Vázquez et al., 2015), *Polygoni multiflori* (Jiao and Zuo, 2009), and *Rheum palmatum* L. (Wang et al., 2008), respectively. According to the solubility, anthraquinones were well soluble in hot water and dilute alcohol (Troy and Beringer, 2006). Our results showed that aloe-emodin, rhein, and chrysophanol were soluble well in 40% ethanol, of which the concentration was lower than those in previous reports. Emodin and physcion were highly extracted at a low to medium ethanol concentration (approximately 40-65% ethanol), which was close to the ethanol concentrations previously reported. The similarity of contour plots and response surfaces between aloe-emodin, rhein, and chrysophanol, or between emodin and physcion, could be described by the similarity of their chemical structures. Aloe-emodin, rhein, and chrysophanol, had no substitution groups at C-6 while they had hydroxymethyl, carboxyl, and methyl group, respectively. In the case of emodin and physcion, they both had a methyl group at the C-6 position. A slight difference between these two compounds was found to be at the C-3 position. Emodin contained a hydroxyl group, but physcion had a methoxy group. However, the hydroxyl group, in terms of its structure, was comparable to the methoxy group.

Our results showed that, using a low herbal weight, high solvent-to-solid ratio, a higher content of anthraquinones was extracted when compared to the lower solvent-to-solid ratio. This phenomenon was found in the extractions in other research (Pinelo et al., 2005; Tan, Tan, and Ho, 2011; Wong, Tan, and Ho, 2013). According to the mass transfer principle, the concentration gradient between herbal powder matrix and bulk of the solvent is an important factor affecting the mass transfer. The higher solvent-to-solid ratio was, the higher amount of extracted compound was obtained. However, from an economical point of view, higher solvent consumption caused a higher production cost. Thus, this factor should be carefully optimized (Meireles, 2009). In addition, using the low ethanol concentration with low herbal weight (or high solvent-to-solid ratio) performed a positive effect on the extraction of anthraquinones from *S. tora* seeds.

The error of the estimation of computer software was less than 10% of all anthraquinone types. This result indicated that the optimization of extraction of anthraquinones from *S. tora* seeds by using the computer software was successful with sufficiently high accuracy. However, the limitation of the extraction method used in this work was not an exhaustive extraction. The content of anthraquinones might not be a total content of each anthraquinone in this plant.

6. Conclusions

The extraction condition of *S. tora* seeds (i.e. ethanol concentration and herbal weight) was optimized to obtain a simultaneously high content of anthraquinones: aloe-emodin, rhein, emodin, chrysophanol, and physcion. The optimal condition obtained from the computer software was 43.1% ethanol and a 1.0 g herbal weight. The extraction using the optimal condition provided 8.71 ppm aloe-emodin, 419.32 ppm rhein, 82.21 ppm emodin, 74.97 ppm chrysophanol, and 15.47 ppm physcion. The prediction value of the content of each anthraquinone was close to the experimental value with a low percent error (less than 10). In conclusion, computer software could be utilized for an optimization of extraction of anthraquinones from *S. tora* seeds with a high percent accuracy.

7. Acknowledgements

The authors would like to thank the Faculty of Pharmacy, Rangsit University for facilities and financial support.

8. References

Choi, J. S., Lee, H. J., Park, K. Y., Ha, J. O., & Kang, S.-S. (1997). *In vitro* antimutagenic effects of anthraquinone aglycones and naphthopyrone glycosides from *Cassia tora*. *Planta Med, 63*(1), 11-14.

Choi, J. S., Lee, H. J., Park, K. Y., & Jung, G. O. (1998). *In vitro* antimutagenic effects of alaternin and isorubrofusarin gentiobioside from roasted *Cassia tora*. *Nat Prod Sci, 4*(2), 100-104.

Gibson, M. (2016). *Pharmaceutical Preformulation and Formulation: A Practical Guide from Candidate Drug Selection to Commercial Dosage Form* (2nd ed. Vol. 199). New York: Informa Healthcare USA, Inc.

Hou, W., Zhang, W., Chen, G., & Luo, Y. (2016). Optimization of extraction conditions for maximal phenolic, flavonoid and antioxidant activity from *Melaleuca bracteata* leaves using the response surface methodology. *PLOS ONE*, 1-16.

Jang, D. S., Lee, G. Y., Kim, Y. S., Lee, Y. M., Kim, C. S., Yoo, J. L., & Kim, J. S. (2007). Anthraquinones from the seeds of *Cassia tora* with inhibitory activity on protein glycation and aldose reductase. *Biol Pharm Bull, 30*(11), 2207-2210.

Jiao, Y., & Zuo, Y. (2009). Ultrasonic extraction and HPLC determination of anthraquinones, aloe-emodine, emodine, rheine, chrysophanol and physcione, in roots of *Polygoni multiflori*. *Phytochem Anal, 20*(4), 272-278.

Kim, Y. M., Lee, C. H., Kim, H. G., & Lee, H. S. (2004). Anthraquinones isolated from *Cassia tora* (Leguminosae) seed show an antifungal property against phytopathogenic fungi. *J Agric Food Chem, 52*(1), 6096−6100.

Liu, Y. K., Yan, E., Zhan, H. Y., & Zhang, Z.-Q. (2011). Response surface optimization of microwave-assisted extraction for HPLC-fluorescence determination of puerarin and daidzein in *Radix Puerariae thomsonii*. *J Pharm Anal, 1*(1), 13-19.

Liyana-Pathirana, C., & Shahidi, F. (2005). Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chem, 93*(1), 47-56.

Malik, E. M., & Müller, C. E. (2016). Anthraquinones as pharmacological tools and drugs. *Med Res Rev, 36*(4), 705-748.

Mat Nor, N. A., & Arof, A. K. (2016). Optimization of extraction parameters for anthocyanin from *Ixora siamensis* by response surface methodology. *Opt Quant Electron, 48*(3), 204.

Meireles, M. A. A. (2009). *Extracting Bioactive Compounds for Food Products: Theory and Applications*. New York: CRC Press.

Pan, Y., Zhang, J., Shen, T., Zuo, Z.-T., Jin, H., Wang, Y.-Z., & Li, W.-Y. (2015). Optimization of ultrasonic extraction by response surface methodology combined with ultrafast liquid chromatography–ultraviolet method for determination of four iridoids in *Gentiana rigescens*. *J Food Drug Anal, 23*(3), 529-537.

Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J., & Núñez, M. J. (2005). Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. *J Agric Food Chem, 53*(6), 2111-2117.

Sui-Ming, W., Wong, M. M., Seligmann, O., & Wagner, H. (1989). Anthraquinone glycosides from the seeds of *Cassia tora*. *Phytochemistry, 28*(1), 211-214.

Tan, P. W., Tan, C. P., & Ho, C. W. (2011). Antioxidant properties: Effects of solid-to-solvent ratio on antioxidant compounds and capacities of Pegaga (*Centella asiatica*). *Int Food Res J, 18*, 557-562.

Temiyaputra, W., Suebsanga, T., Yajom, K., Piyaworanon, S., & Leksawasdi, N. (2008). Influences of anthraquinone extraction techniques from *Morinda* sp. on extraction efficiency. *Kasetsart J: Nat Sci, 42*(-), 118-126.

Troy, D. B., & Beringer, P. (2006). *Remington: The Science and Practice of Pharmacy* (21st ed.). Maryland: Lippincott Williams & Wilkins.

Vázquez, M. F. B., Comini, L. R., Martini, R. E., Montoya, S. C. N., Bottini, S., & Cabrera, J. L. (2015). Ultrasonic-assisted extraction of anthraquinones from *Heterophyllaea pustulata* Hook f. (Rubiaceae) using ethanol–water mixtures. *Ind Crops Prod, 69*(-), 278-283.

Wang, L., Li, D., Bao, C., You, J., Wang, Z., Shi, Y., & Zhang, H. (2008). Ultrasonic extraction and separation of anthraquinones from *Rheum palmatum* L. *Ultrason Sonochem, 15*(5), 738-746.

Wong, B. Y., Tan, C. P., & Ho, C. W. (2013). Effect of solid-to-solvent ratio on phenolic content and antioxidant capacities of “Dukung Anak” (*Phyllanthus niruri*). *Int Food Res J, 20*(1), 325-330.

Yuan, X., Zeng, Y., Nie, K., Luo, D., & Wang, Z. (2015). Extraction optimization, characterization and bioactivities of a major polysaccharide from *Sargassum thunbergii*. *PLOS ONE*, 1-11.

Zhu, L., Yu, S., Zeng, X., Fu, X., & Zhao, M. (2008). Preparative separation and purification of five anthraquinones from *Cassia tora* L. by high-speed counter-current chromatography. *Sep Purif Technol, 63*(3), 665-669.