World Scientific Research Journal Volume 9 Issue 7, 2023
ISSN: 2472-3703 DOI: 10.6911/WSRJ.202307_9(7).0016

Definitive Screening Design for Optimizing the Ultrasonic Extraction Process of Hydroxysafflor Yellow A in Safflower

Chaoyue Wang, Yushun Yang, Minsheng Lou*
Jinhua Advance Research Institute, Jinhua, Zhejiang, China

Abstract

[Objective] To optimize the parameters in the ultrasonic extraction process of the safflower medicinal materials on the extraction rate of hydroxysafflor yellow A and to provide a reference for the production of hydroxysafflor yellow A. [Methods] Six method parameters in the ultrasonic extraction process of safflower were studied by the definitive screening design. The mass concentration of hydroxysafflor yellow A in the extract was defined as the evaluation index. Then a stepwise regression method was used to build the quantitative models between the evaluation index and the method parameters. The four most critical impact parameters and the optimal extraction method was defined. [Results] The liquid-to-material ratio, extraction time, methanol concentration and extraction power were determined as the four most critical method parameters. The optimized extraction process was as follows: the methanol concentration was 51.52%, the liquid-to-material ratio was 1:15, the extraction time was 70 min, and the extraction power was 450 W. Under this process condition, the mass concentration of hydroxysafflor yellow A was predicted to reach 0.650 mg/mL. The result of the verification experiment was close to the predicted value of the established model. [Conclusion] The definitive screening design was suitable for the optimization of the production process for traditional Chinese medicine. It could also help the less experienced R&D personnel quickly establish a reasonable extraction and purification process, and improve the efficiency of the experiment.

Keywords

Safflower; Hydroxysafflor yellow A; Definitive screening design.

1. INTRODUCTION

Safflower is the dried flower of *Carthamus tinctorius* L., which is an annual or biennial herb[1]. As a traditional Chinese medicine with the effect of activating blood circulation, dispersing blood stasis and relieving pain, safflower is mostly used in the treatment and prevention of blood stagnation, menstrual stasis, bruises, angina pectoris, kidney disease and coronary heart disease [2-6]. The significant therapeutic effects in modern diseases, especially in cardiovascular diseases, were observed in the clinical use of safflower [7,8]. It was found that the extract of safflower had significant efficacy in the treatment of diabetes [9]. It could not only suppress the increase of the blood glucose levels, but also improve the renal blood rheology, thus it could alleviate the pathology of diabetic nephropathy. Safflower yellow is a mixture of various water-soluble chalcone components with various pharmacological effects such as anti-inflammatory and anti-oxidative stress [10,11]. The content of hydroxysafflor yellow A was included as the one of the criteria for evaluating the quality of safflower in Chinese Pharmacopoeia 2020. Hydroxysafflor yellow A has rich pharmacological effects and is of great medicinal value in the cardiovascular system, central nervous system, hepatoprotection, asthma, improvement of lung function, improvement of kidney function, and cancer treatment [12-16].

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202307_9(7).0016

Due to the significant pharmacological effects and low toxic side effects, hydroxysafflor yellow A was one of the most promising new drugs in the clinical trails [17,18].

The extraction of hydroxysafflor yellow A from safflower was usually performed by the decoction, warm maceration, percolation and condensation reflux. Due to the poor thermal stability of hydroxysafflor yellow A, the high temperature of decoction and reflux method might cause the loss of hydroxysafflor yellow A. The percolation method, on the other hand, suffered from the time-consuming and labor-intensive solvent consumption. In contrast, the ultrasonic extraction exhibited the advantages of suitable temperature, easy operation, high recovery rate, and wide adaptation range [19]. However, many factors in the ultrasonic extraction process were unclear. The orthogonal design and response surface method optimization were two common methods used in the extraction experiments. There were still many factors to be explored that could cause a significant increase in the number of tests, thus could increase the cost and reduce the efficiency. The definitive screening design required only a small number of experiments to obtain the optimal conditions in most cases, and was therefore suitable for the experiments with a large number of uninvestigated parameters [20,21]. At present, the definitive screening design suggested good application results in pharmacy, chemistry, food, industry and other fields [22-25]. In this study, the definitive screening design was applied to optimize the factors on the yield of hydroxysafflor yellow A during the ultrasonic extraction of safflower. The result could provide the certain guidance for the extraction process of safflower in pharmacies, hospitals, and manufacturing industries. The information herein also played a key role in improving the utilization efficiency of the Chinese herbal medicine saffron and in reducing the actual production cost of hydroxysafflor yellow A.

2. MATERIAL

2.1. Apparatus

The extraction was performed with XF-80 traditional Chinese medicine crusher (Changzhou Gaode Instrument Manufacturing Co., Ltd.), JA21002 electronic analytical balance (Zhejiang Nader Scientific Instruments Co., Ltd.), TS-10 Centrifugal Filter (Beijing Hengjiu Yuanjing Technology Development Co., Ltd.), and SB-800DT numerical ultrasonic cleaner (Hangzhou Zhongcan Technology Co., Ltd., Hangzhou, China). The analysis of the analytes were performed on the Agilent 1200 Series rapid resolution LC system.

2.2 Reagents and materials

Methanol (analytical-grade) was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd., and acetonitrile (chromatographic grade) was purchased from Tedia Company, Inc. The aqueous mobile phase used for the HPLC was the dually distilled water. Safflower was purchased from Quzhou People's Hospital and was identified by the Quzhou Institute of Pharmaceutical Inspection as the dried flowers of *Carthamus tinctorius* L. The standard substance hydroxysafflor yellow A was purchased from Chengdu Must Biological Technology Co., Ltd.

3. METHODS AND RESULTS

3.1. Analytical method

The analytical column was a Phenomenex Luna 5 μm C_{18} 100A column (150 mm×4.6 mm, i.d., 5 μm). The mobile phase was composed of 0.4% glacial acetic acid solution (A) and acetonitrile (B). A gradient elution was used as follows: 0~30 min, 2%B→70%B. The flow rate

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202307_9(7).0016

was 1 mL/min. The wavelength was detected at 403 nm and the column temperature was 30 °C. The injection volume was 20 μL.

3.2. Examination of linear relationship

Accurate 10.0 mg of hydroxysafflor yellow A was dissolved in 25 mL of 50% methanol under the ultrasonication, and 0.40 mg/mL of the standard solution was obtained. Afterwards, 1, 2, 4, 6, 8, 10 mL of the solution was respectively put in a 10 mL measuring bottle and diluted into 0.04, 0.08, 0.16, 0.24, 0.32, 0.40 mg/mL with methanol. The prepared solution was injected to HPLC and the peak areas of the samples at different concentrations were recorded. The linear curve of hydroxysafflor yellow A was obtained as Y=16621.25X-17.02 (R^2 = 0.99905). The results inferred a good linear relationship between the concentration and peak area of hydroxysafflor yellow A in the range of 40~400 μg/mL.

3.3. Single-factor test

Based on the literature review, the single-factor test was used to investigate the soaking time, extraction solvent concentration, liquid to material ratio, ultrasonic extraction temperature, ultrasonic extraction time, and ultrasonic extraction power in the ultrasonic extraction process of safflower. The yield of hydroxysafflor yellow A was used as the index for comprehensive evaluation. The design levels of each factor were shown in Table 1. About 10.0 g of sieved dried safflower powder was weighed for each single-factor test, and the effect of the factors on the ultrasonic extraction of hydroxysafflor yellow A from safflower was investigated according to the design in Table 1. The effect of the soaking time was shown in Figure 1(a). The length of the soaking time affected the extraction rate of hydroxysafflor yellow A to some extent. When the soaking time extended from 20 min to 240 min, the hydroxysafflor yellow A content increased at first, became steady, and then decreased. The concentration of hydroxysafflor yellow A was relatively higher between 50 min and 90 min of soaking time. In consideration of the time cost factor, the soaking time of 50 min was selected as appropriate. The concentrations of the solvents affected the solubility forces, which made it important for the dissolution of the internal components of safflower. As shown in Figure 1(b), when the methanol concentration increased from 10% to 80%, the hydroxysafflor yellow A content showed a trend of first growth and then decrease. The highest point was found at 50% of methanol concentration. The amount of the solvent was closely related to the content of the target product. In a certain range, the more solvent used, the higher dissolution rate of the target product and the extraction rate there would be. However, the excessive solvent usage would bring the problems such as cost increase, resource waste and environmental pollution. Therefore, determining an appropriate liquid-to-material ratio played a very critical role in the whole safflower extraction process. As shown in Figure 1(c), the maximum concentration was reached at the material-to-liquidratio of 1:20. In consideration of the adequacy of the reaction time and the reduction of losses, 60 min was the most suitable extraction time. The extraction temperature directly affected the diffusion rate of the solvent of the target extract. The selection of a reasonable extraction temperature not only improved the extraction efficiency, but also reduced the energy consumption. The concentration of hydroxysafflor yellow A reached its maximum value at 50 ℃ as shown in Figure 1(d). The extension of the ultrasonic extraction time could effectively increase the amount of the dissolved components. However, an overlong time also damaged the active ingredients and increased the time costs. Therefore, it was also crucial to explore a suitable extraction time for the process. The results of the extraction time were shown in Figure 1(e). The ultrasonic power affected the cavitation intensity of the sonicator, and the enhancement of the cavitation strengthened the penetration and transmission of the plant cell contents through the cell membranes. The extraction rate of the target product was maximized at 400 W for the ultrasonicator power as shown in Figure 1(f).

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202307_9(7).0016

Table 1. The level design table of the single-factor test

Examining factors	Levels	Fixed Factors
Soaking time	20 min, 30 min, 40 min, 50 min, 60 min, 90 min, 120 min, 240 min	Methanol concentration 30%; material-to-liquid ratio 1:10; extraction temperature 50℃; extraction time 30 min; extraction power 100 W
Methanol concentration	10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%	Soaking time 30 min; material-to-liquid ratio 1:10; extraction temperature 50℃; extraction time 30 min; extraction power 100 W
Material-to-liquid ratio	1:5, 1:10, 1:15, 1:20, 1:25, 1:30	Soaking time 30 min; methanol concentration 30%; extraction temperature 50℃; extraction time 30 min; extraction power 100 W
Extraction temperature	40, 50, 60, 70, 80 ℃	Soaking time 30 min; methanol concentration 30%; material-to-liquid ratio 1:10; extraction time 30 min; extraction power 100 W
Extraction time	10, 30, 60, 90, 120 min	Soaking time 30 min; methanol concentration 30%; material-to-liquid ratio 1:10; extraction temperature 50℃; extraction power 100 W
Extraction power	50, 100, 200, 300, 400, 500 W	Soaking time 30 min; methanol concentration 30%; material to liquid ratio 1:10; extraction temperature 50℃; extraction time 30 min

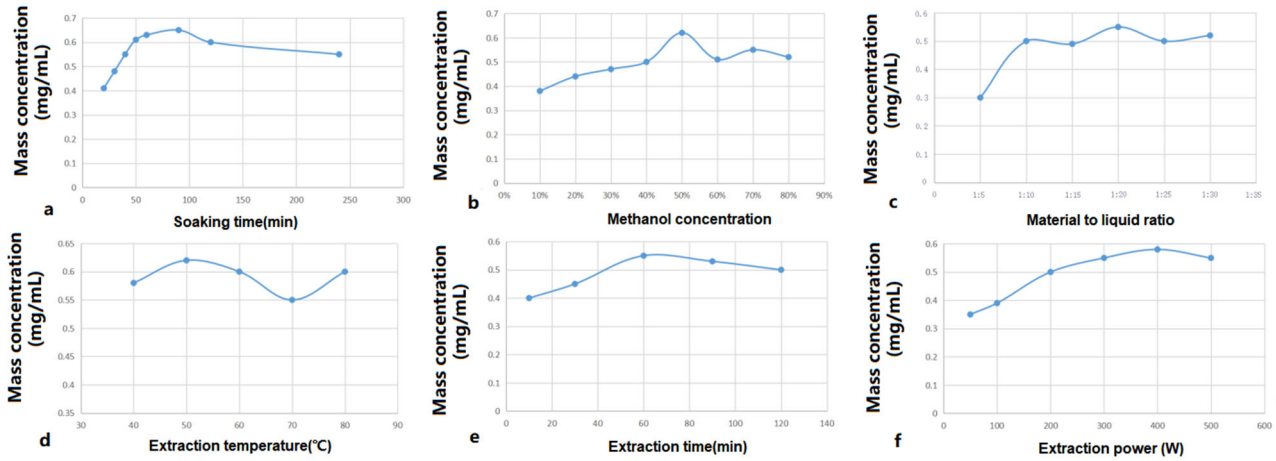


Figure 1. The influence of various factors on the concentration of hydroxysafflor yellow

3.4. Definitive screening design

A large number of factors was considered in the ultrasound-assisted extraction process of safflower. The definitive screening design allowed the significant trial size reduction. Based on the results of the single-factor test, the definitive screening design was used to further investigate the uncertain process parameters including the soaking time, extraction solvent concentration, liquid-to-material ratio, ultrasonic extraction temperature, ultrasonic extraction time, and ultrasonic extraction power. The experimental design was performed with the JMP software (SAS, USA). The center point experiment was independently repeated three times for a total 15 sets of experiments. The high and low levels of each factor were designed according to the results in Table 2. The process evaluation index was the mass concentration of hydroxysafflor yellow A.

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202307_9(7).0016

Table 2. Parameters and their levels of the definitive screening designed experiments

Parameters	Units	Coding value		
		-1	0	1
Soaking time	min	40	50	60
Methanol concentration		40%	50%	60%
Material to liquid ratio		1:15	1:20	1:25
Extraction temperature	℃	40	50	60
Extraction time	min	50	60	70
Extraction power	W	350	400	450

3.4. Data processing and model validation

The quantitative model between the process evaluation index (the mass concentration of hydroxysafflor yellow A) and each method parameter was constructed as Equation (1).

$$Y = a_0 + \sum_{i=1}^{9} a_i X_i + \sum_{i=1}^{9} a_{ii} X_i^2 + \sum_{i=1}^{8} \sum_{j=i+1}^{9} a_{ij} X_i X_j \quad (1)$$

In the above equation, a_0 was a constant; a_i, a_{ii} and a_{ij} were the regression coefficients of the primary, secondary and interaction terms, respectively; X_i and X_j were the parameters; Y was the evaluation index (mass concentration of hydroxysafflor yellow A) of this study. The model was simplified by the corrected red pool information content criterion (AICc) combined with the stepwise advance method.

The quantitative model of the mass concentration of hydroxysafflor yellow A and the method parameters were obtained according to the experimental results in Table 3. The regression coefficients and ANOVA results of the model were shown in Table 4. The R^2 value was greater than 0.75 and the *P* value was less than 0.0001, which indicated that the linear model was sufficient to fit the relationship between the process evaluation index and each parameter in the ultrasonic extraction process. The small difference between the model (R^2 = 0.9763) and the corrected coefficient of determination (R_{adj}^2 = 0.9585) further indicated that the model fit was reliable and covered most of the variation. As shown in Figure 2, the red solid line was the fitted line, while the horizontal dashed line was the mean value of the actual mass concentration of hydroxysafflor yellow A. Accordingly, the red shading was the 95% confidence interval. As shown in Figure 3, the four most critical factors were identified in order as the material-to-liquid ratio, ultrasonic extraction time, methanol concentration, and ultrasonic extraction power. The *P*-values corresponding to the regression coefficients of these four parameters were all less than 0.05, which indicated that these parameters were a significant for the extraction rate of hydroxysafflor yellow A. The maximum willingness of mass concentration of hydroxysafflor yellow A was illustrated in Figure 4. There was some inconsistency between the trend of the material-to-liquid ratio and the trend of the single factor examination. The possible reason might be that there was an obvious interaction between the material-liquid ratio and the extraction time, and the change of the extraction time might affect the examination of the material-liquid ratio. In Figure 4, the methanol concentration was 0.152, the material-to-liquid ratio was -1, the extraction time was 1, and the extraction power was 1. The methanol concentration of 51.52%, material-to-liquid ratio of 1:15, extraction time of 70 min, and extraction power of 450 W were obtained by the conversion and rounding. The simulated value of hydroxysafflor yellow A was calculated as 0.650 mg/mL. The validation experiments based on the optimal process conditions were derived from the proposed software model. The results

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202307_9(7).0016

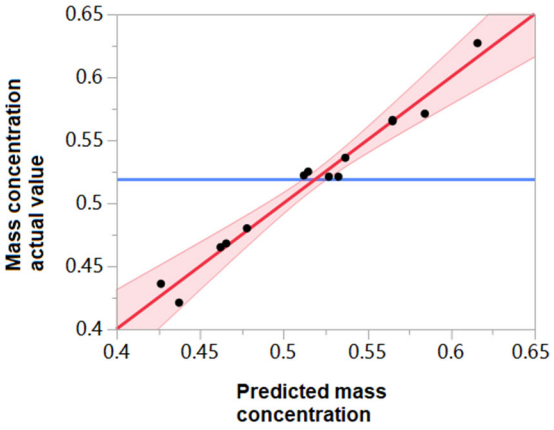
were 0.645 mg/mL, 0.648 mg/mL and 0.677 mg/mL with the average value of 0.656 mg/mL for three replicate experiments. The SD value was calculated as 0.01443, therefore the tested value was close to the predicted 0.650 mg/mLfrom the regression equation. Additionally, the percentage deviation between the predicted and real values was only 0.923%.

Table 3. The conditions and results of the definitive screening designed experiments

No.	Method parameters						Mass concentration (mg/mL)
	Soaking time(min)	Methanol concentration	Material-to-liquid ratio	Extraction temperature(℃)	Extraction time(min)	Extraction power (W)	
1	0	1	1	1	1	1	0.522
2	0	0	0	0	0	0	0.566
3	-1	-1	1	0	1	-1	0.421
4	1	1	-1	0	-1	1	0.536
5	0	-1	-1	-1	-1	-1	0.465
6	-1	-1	-1	1	0	1	0.521
7	0	0	0	0	0	0	0.565
8	1	0	-1	1	1	-1	0.627
9	-1	0	1	-1	-1	1	0.521
10	1	1	1	-1	0	-1	0.468
11	-1	1	-1	-1	1	0	0.571
12	0	0	0	0	0	0	0.565
13	1	-1	0	-1	1	1	0.525
14	1	-1	1	1	-1	0	0.436
15	-1	1	0	1	-1	-1	0.480

Table 4. Regression coefficients and the ANOVA results of the models

Model	Mass concentration of the hydroxysafflor yellow A	
	Regression coefficient	*P*
Intercept distance	0.56541	—
Methanol concentration	0.0209	0.00043
Material to liquid ratio	-0.0352	0.00001
Ultrasonic extraction time	0.0228	0.00024
Ultrasonic extraction power	0.0164	0.00199
Methanol concentration×methanol concentration	-0.0688	0.00001
Material to liquid ratio×ultrasonic extraction time	-0.0092	0.07126
R^2	0.9763	
R_{adj}^2	0.9585	
P-Value	<0.0001	



ISSN: 2472-3703 DOI: 10.6911/WSRJ.202307_9(7).0016

Figure 2. The relationship curve between the predicted value and actual value of the mass concentration of hydroxysafflor yellow A

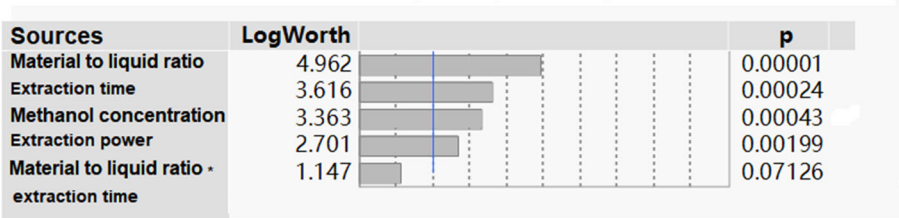


Figure 3. Pareto chart of standardized effects following response transformation

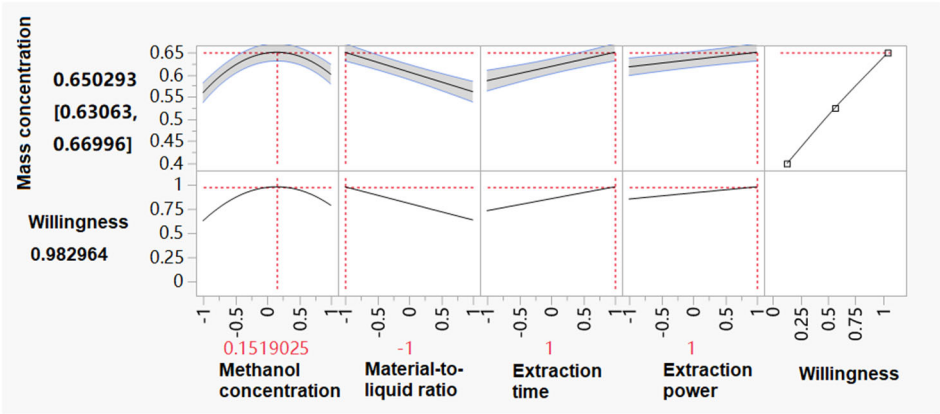


Figure 4. The Maximum willingness of the mass concentration of hydroxysafflor yellow A

4. DISCUSSION

In this paper, by applying the definitive screening design, a mathematical model was established between the mass concentration of hydroxysafflor yellow A in the extract and six method parameters. The most suitable solution for the ultrasonic extraction process of safflower was determined, and the four key method parameters in the process were also identified in order as the material-to-liquid ratio, extraction time, methanol concentration and extraction power. Among them, the material-to-liquid ratio was the most critical factor for the affection of the ultrasonic extraction process. Therefore, in the actual operation process, such as hospital pharmacy decoction, industrial production, and food production, the parameter material-to-liquid ratio should be strictly controlled. The study confirmed that the definitive screening design could achieve rapid modeling for a large number of method parameters, while only 2k+1 trials (k was the number of factors) were required for realizing the optimization. As a result, the costly additional tests to resolve uncertainty in the initial results of standard screening designs could be avoided. This research experiment was suitable for the optimization of the Chinese medicine production process. More importantly, the standard procedure could help the less experienced R&D personnel to quickly establish a reasonable extraction and purification protocol, thus to improve the experimental efficiency. For the practitioners of the experimental design or industrial process design, they need to keep abreast of the developments in experimental design and to choose specific test methods for different practical situations to ensure the maximum economic benefits with the minimum costs.

5. CONCLUSION

In this study, a definitive screening design was applied to optimize the ultrasonic extraction process of hydroxysafflor yellow A from safflower. The quantitative mathematical model between the evaluation index and the method parameters was established with multiple regression analysis. The four most critical method parameters were clarified in order as the

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202307_9(7).0016

material-to-liquid ratio, ultrasonic extraction time, methanol concentration, and ultrasonic extraction power. The optimum extraction process was determined as follows: liquid-to-material ratio 1:15, ultrasonic extraction time 70 min, methanol concentration 51.52%, and ultrasonic extraction power 450 W. After the methodological verification, the established ultrasonic extraction process of safflower could effectively reduce the loss of hydroxysafflor yellow A, and also improve the production efficiency. The information in this work provided an important guidance for the actual industrial production.

ACKNOWLEDGMENTS

This paper was supported by Jinhua Science and Technology Bureau (2021-4-308) and Scientific Research Project of Jinhua Advanced Research Institute (GYY202104).

REFERENCES

[1] Safflower (Carthamus tinctorius), in: Encycl. Dict. Genet. Genomics Proteomics, John Wiley & Sons, Inc., 2004. https://doi.org/10.1002/0471684228.egp11120.

[2] C.H. Park, M.J. Kim, C.Y. Yang, T. Yokozawa, Y.S. Shin, Safflower seed extract synergizes the therapeutic effect of cisplatin and reduces cisplatin-induced nephrotoxicity in human colorectal carcinoma RKO cells and RKO-transplanted mice, 13 (2019) 328–334. https://doi.org/10.5582/ddt.2019.01086.

[3] Q. Meng, H. Liu, H. Wu, C. Tang, X. Fu, X. Fang, Y. Xu, B. Chen, Y. Xie, Q. Liu, A Network Pharmacology Study to Explore the Underlying Mechanism of Safflower (Carthamus tinctorius L .) in the Treatment of Coronary Heart Disease, 2022 (2022).

[4] A. Alaiye, E. Kaya, C. Cingi, D. Burukog, An Experimental Comparison of the Analgesic and Anti-Inflammatory Effects of Safflower Oil, Benzydamine HCl, and Naproxen Sodium ¨, 00 (2020) 1–8. https://doi.org/10.1089/jmf.2019.0157.

[5] Y. Chen, M. Li, J. Wen, X. Pan, Z. Deng, J. Chen, G. Chen, L. Yu, Y. Tang, G. Li, X. Xie, C. Peng, Pharmacological Activities of Safflower Yellow and Its Clinical Applications, 2022 (2022).

[6] P.H. Lu, C.Y. Kuo, C.C. Chan, L.K. Wang, M.L. Chen, I.S. Tzeng, F.M. Tsai, Safflower extract inhibits adp-induced human platelet aggregation, Plants. 10 (2021). https://doi.org/10.3390/plants10061192.

[7] B. Xian, R. Wang, H. Jiang, Y. Zhou, J. Yan, X. Huang, J. Chen, Q. Wu, C. Chen, Z. Xi, C. Ren, J. Pei, Biomedicine & Pharmacotherapy Comprehensive review of two groups of flavonoids in Carthamus, Biomed. Pharmacother. 153 (2022) 113462. https://doi.org/10.1016/j.biopha.2022.113462.

[8] X. Zhou, L. Tang, Y. Xu, G. Zhou, Z. Wang, Towards a better understanding of medicinal uses of Carthamus tinctorius L . in traditional Chinese medicine : A phytochemical and pharmacological review, J. Ethnopharmacol. 151 (2014) 27–43. https://doi.org/10.1016/j.jep.2013.10.050.

[9] R. Higa, M. Kurtz, M.B. Mazzucco, D. Musikant, V. White, A. Jawerbaum, Folic acid and safflower oil supplementation interacts and protects embryos from maternal diabetes-induced damage, 18 (2012) 253–264. https://doi.org/10.1093/molehr/gar080.

[10] L. Wang, B.O.A. Botchway, X. Liu, A.R. Vaz, The Repression of the HMGB1-TLR4-NF- κ B Signaling Pathway by Safflower Yellow May Improve Spinal Cord Injury, 15 (2021) 1–9. https://doi.org/10.3389/fnins.2021.803885.

[11] T. Bacchetti, C. Morresi, L. Bellachioma, G. Ferretti, Antioxidant and Pro-Oxidant Properties of Carthamus Tinctorius , Hydroxy Safflor Yellow A , and Safflor Yellow A, (2020) 1–10.

ISSN: 2472-3703 DOI: 10.6911/WSRJ.202307_9(7).0016

[12] X. Yi, J. Wang, W. Lin, L. Chen, H. Li, T. Lan, Z. Li, Hydroxysafflower yellow A alleviates HK-2 cells injury in cold hypoxia / reoxygenation via mitochondrial apoptosis, Transpl. Immunol. 74 (2022) 101610. https://doi.org/10.1016/j.trim.2022.101610.

[13] L. Wang, X. Cui, J. He, S. Duan, C. Liu, Phytomedicine Hydroxysafflor yellows alleviate thrombosis and acetaminophen-induced toxicity in vivo by enhancing blood circulation and poison excretion, Phytomedicine. 87 (2021) 153579. https://doi.org/10.1016/j.phymed.2021.153579.

[14] M. Jiang, L. Zhou, N. Xu, Q. An, Hydroxysaf fl or yellow A inhibited lipopolysaccharide- induced non-small cell lung cancer cell proliferation , migration , and invasion by suppressing the PI3K / AKT / mTOR and ERK / MAPK signaling pathways, 10 (2019) 1319–1333. https://doi.org/10.1111/1759-7714.13019.

[15] Y. Sun, D. Xu, Z. Qin, P. Wang, B. Hu, J. Yu, Y. Zhao, B. Cai, Y. Chen, M. Lu, J. Liu, X. Liu, Protective cerebrovascular e ff ects of hydroxysa ffl or yellow A (HSYA) on ischemic stroke, 818 (2018) 604–609. https://doi.org/10.1016/j.ejphar.2017.11.033.

[16] F. Rossi, J. Ye, S. Lu, M. Wang, W. Ge, H. Liu, Y. Qi, J. Fu, Corrigendum : Hydroxysaf fl or Yellow A Protects Against Myocardial Ischemia / Reperfusion Injury via Suppressing NLRP3 In fl ammasome and Activating Autophagy, 12 (2021) 671318. https://doi.org/10.3389/fphar.2020.01170.

[17] X. Bai, W. Wang, R. Fu, S. Yue, H. Gao, Y. Chen, Y. Tang, Therapeutic Potential of Hydroxysaf fl or Yellow A on Cardio- Cerebrovascular Diseases, 11 (2020) 1–14. https://doi.org/10.3389/fphar.2020.01265.

[18] Y. Sun, G. Yanming, L. Jinxin, X. Lamei, M. Fan, H. Qian, Y. Li, L. Wang, Hydroxysafflor Yellow A - An Important Natural Pigment for Treating Metabolic Diseases Hydroxysafflor Yellow A - An Important Natural Pigment for, Food Rev. Int. 00 (2021) 1–15. https://doi.org/10.1080/87559129.2021.2013256.

[19] M. Musterman, P. Placeholder, M. Musterman, Extraction and determination of flavonoids in Carthamus tinctorius, 1133 (2018) 1129–1133.

[20] A.R. Vazquez, P. Goos, E.D. Schoen, pt e us cr, Technometrics. 0 (2019) 000. https://doi.org/10.1080/00401706.2019.1566095.

[21] B. Jones, J.S. Hunter, D.C. Montgomery, B. Jones, J.S. Hunter, D.C. Montgomery, Partial replication of definitive screening designs, Qual. Eng. 0 (2019) 1–6. https://doi.org/10.1080/08982112.2019.1680847.

[22] M.H. Alhelali, S.D. Georgiou, S. Stylianou, Screening designs based on weighing matrices with added two-level categorical factors, J. Qual. Technol. 0 (2019) 1–12. https://doi.org/10.1080/00224065.2019.1571341.

[23] N. Nguyen, T. Pham, M.P. Vuong, AC, Chemom. Intell. Lab. Syst. (2018). https://doi.org/10.1016/j.chemolab.2018.06.006.

[24] J. Zhao, W. Li, H. Qu, G. Tian, Y. Wei, Application of definitive screening design to quantify the effects of process parameters on key granule characteristics and optimize operating parameters in pulsed-spray fluid-bed granulation, Particuology. (2018). https://doi.org/10.1016/j.partic.2018.03.007.

[25] H. Favre, A.C. Neto, An application of definitive screening designs (DSDs) to a food product optimization and adaptations to jones & nachtsheim methodology for fitting DSD models, Food Qual. Prefer. 88 (2021) 104106. https://doi.org/10.1016/j.foodqual.2020.104106.