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Optimization of the extraction process of antioxidant compositions from *Glycyrrhiza uralensis* using the response surface method

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This study aimed to optimise and evaluate the antioxidant activity of *Glycyrrhiza uralensis* root extract using a simple heating method. The optimisation of extraction conditions was conducted using the Response Surface Methodology (RSM) and a Box-Behnken design (BBD), focusing on variables such as temperature, ethanol concentration, extraction time, and solvent-to-material ratio. The total phenolic content (TPC) and total flavonoid content (TFC) were used as response variables. The optimal extraction conditions were determined to be a temperature of 58°C, ethanol concentration of 56%, a liquid-to-solid ratio of 30 mL/g, and an extraction time of 190 minutes, resulting in TPC and TFC values of 158.81 mg GAE/g and 122.15 mg QE/g, respectively. The extract exhibited considerable DPPH and hydroxyl radical scavenging effects. These findings suggest that the optimized extraction method not only simplifies the process but also enhances the scalability and cost-effectiveness of industrial applications.

Keywords: Antioxidant, Box-Behnken, *Glycyrrhiza uralensis*, Response surface method

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Introduction

Glycyrrhiza uralensis, also known as licorice, is a highly valued herbal medicine that has been widely used across Asia for centuries[1]. Its roots are renowned for their therapeutic properties, particularly in alleviating pain, reducing inflammation, and soothing coughs[2]. Over the years, extensive research has been conducted to explore the chemical composition of *G. uralensis* roots, revealing a rich array of bioactive compounds. Among these, flavonoids, saponins, and various other phenolic compounds stand out for their significant medicinal properties[1,3,4]. These compounds have been the subject of numerous studies, which have demonstrated their potent bioactivities, including anti-inflammatory, antioxidant, anti-cancer, and immunomodulatory effects[2]. Additional research has expanded the understanding of *G. uralensis*, highlighting other significant bioactive compounds such as glycyrrhizin, isoliquiritigenin, isoliquiritin, liquiritigenin, and liquiritin, known for their anti-inflammatory and nitric oxide inhibitory effects[4,5]. Furthermore, polysaccharides extracted from *G. uralensis* residues exhibit promising antidiabetic activities, particularly through inhibition of α-glucosidase[6]. Comparative studies have shown diverse biological activities across various plant parts, including leaves, stems, and seeds, traditionally viewed as agricultural waste, revealing antioxidant, anticancer, antiviral, and antidiabetic potentials[7]. Prenylated phenolic compounds have shown particular promise against osteoarthritis inflammation[8], and licorice sprouts possess noteworthy antioxidant and lipid-lowering properties, reinforcing their dietary and therapeutic potential[9]. Notably, *G. uralensis* components have demonstrated substantial protective and regenerative effects on muscle tissues, making it a candidate for treating muscle-related disorders[10]. Advanced analytical techniques have elucidated the intricate metabolomic and transcriptomic profiles of *G. uralensis*, emphasizing its isoflavonoid biosynthesis pathways and their significant pharmacological roles[11]. Investigations also underscore its substantial antioxidant, hepatoprotective, anticancer, immunomodulatory, and cardiovascular protective effects, reinforcing its comprehensive therapeutic profile[12,13]. Recent studies further validate the anti-inflammatory and immunomodulatory properties of *G. uralensis* in treating ulcerative colitis through

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modulating immune responses and key inflammatory signaling pathways[14].

Traditionally, the extraction of these valuable compounds from *G. uralensis* has been carried out using various methods, with ultrasonic-assisted extraction being one of the more advanced and widely studied techniques. This method, while effective, can be complex and may require specialized equipment, which could limit its applicability in large-scale production settings[15,16]. Recognizing this challenge, the present study sought to optimize a more straightforward and accessible extraction process, focusing on the use of a simple heating method to extract the antioxidant components of *G. uralensis*. Response Surface Methodology (RSM) has emerged as an effective statistical tool for optimizing extraction conditions, significantly enhancing the yield and quality of extracted bioactive compounds[17,18]. Recent studies have successfully applied RSM to optimize the ultrasonic-assisted extraction conditions for isoliquiritigenin and glycyrrhizic acid from *G. uralensis*, achieving higher extraction efficiencies compared to conventional extraction methods[16,19]. Additionally, deep eutectic solvents combined with ultrasonic-assisted extraction have been optimized using RSM to efficiently extract multiple bioactive flavonoids and saponins, demonstrating the potential for greener, more sustainable extraction processes[19]. Other innovative approaches, such as supercritical CO_2 extraction and ionic liquid-based extraction methods, have also utilized RSM to enhance the recovery of bioactive constituents from licorice roots, confirming its versatility and effectiveness in extraction optimization[20-22]. Despite these advances, there remains limited research specifically focusing on the optimization of antioxidant extraction from *G. uralensis*, highlighting the need for further studies to fill this critical gap and better harness the antioxidant potential of this medicinal herb.

By carefully optimizing the extraction conditions, this study has successfully developed a method that not only preserves the integrity and efficacy of the antioxidant compounds but also offers practical advantages for large-scale production. The simplicity and efficiency of the heating method make it a viable alternative to more complex extraction techniques, particularly in industrial applications where scalability and cost-effectiveness are critical considerations. Furthermore, the optimized conditions identified in this study can be readily applied to the extraction of bioactive products from *G. uralensis* roots, paving the way for their widespread use in the pharmaceutical industry. This approach has the potential to significantly enhance the availability of these beneficial compounds, contributing to the development of new medicinal formulations that harness the therapeutic power of *G. uralensis*. The findings of this study not only advance our understanding of efficient extraction processes but also highlight the enduring relevance of traditional herbal medicines in modern healthcare.

Materials and Methods

Instruments and chemicals

UV-Vis absorbance was measured on a BioTek Synergy HTX multimode reader (Agilent, US). The incubator and the sonication bath were supplied by Daihan Scientific, Korea. Ethanol (EtOH), water, DMSO, and other inorganic chemicals were supplied by Daihan Scientific, Korea. Folin-Ciocalteu reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid (TCA), deoxyribose, thiobarbituricacid, catechin, acarbose and quercetin were supplied by Merck, Germany.

Plant material

G. uralensis roots were collected in July 2022 in Hanoi, Vietnam. The samples were identified by Dr Nguyen The Cuong, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher specimen of the plant was deposited in the Joint Vietnam-Russia Tropical Science and Technology Research Center with code number VR908716. The sample was cleaned under water taps, dried at 50-55°C, powdered, and preserved for further experiments.

Total polyphenol assay

The total phenolic contents (TPC) of the samples were evaluated using the Folin-Ciocalteu method[23]. The standard solutions were prepared with several concentrations of gallic acid (10-400 µg/mL). The analytes were prepared by dissolving the extracts in methanol at certain concentrations. Each 100 µL of the sample or standard solution was mixed with 900 µL of Folin – Ciocalteu 10% and 1000 µL of Na_2CO_3 6% and incubated for 15 min at 40°C. Its absorbance was measured with a UV–Vis spectrophotometer at 750 nm. The total

phenolic content was calculated as mg of gallic acid equivalent (mg GAE/g) by using the gallic acid calibration curve.

Total flavonoid assay

The total flavonoid contents (TFC) of the samples were examined using a previous method[23]. The calibration standards were prepared with different concentrations of quercetin (10-200 μg/mL). The analytes were prepared by the same process as the total phenolic assay. Mix 100 μL of $NaNO_2$ 5% to 500 μL sample or standard solution at room temperature for 5 min, then add 100 μL of $AlCl_3$ 10% and 500 μL NaOH 1M to the mixture. Keep the mixture at room temperature for 15 min. Its absorbance was measured with a UV–Vis spectrophotometer at 510 nm. The total flavonoid content was calculated as mg quercetin equivalent (mg QE/g) by using the quercetin calibration curve.

Antioxidant assays

The antioxidant activities of extracts and subfractions were evaluated by DPPH and hydroxyl radicals scavenging assays. DPPH radical-scavenging activity was conducted by modifying a previous method[24]. Briefly, each sample (20 μL) was mixed with 380 μL of DPPH in methanol and then dark incubated at 37°C for 20 minutes. The absorbance was measured at 517 nm. Ascorbic acid was used as a positive control.

The hydroxyl radical scavenging assay was measured based on quantification of the degradation product of 2-deoxyribose by condensation with thiobarbituric acid[25]. 50 μL of the test sample was incubated with 100 μL of the phosphate buffer 50 mM pH 7.8, 100 μL of deoxyribose 2.8 mM, and 100 μL of $Fe(NH_4)_2(SO_4)_2$ 500 μM for 1 h at 37°C. Next, 250 μL of trichloroacetic acid (10%, w/v) and 250 μL of thiobarbituric acid (1% w/v) were added, and the reaction mixture was boiled for 15 min in a water bath. The colour development was measured at 532 nm.

The IC_{50} values were measured based on experiments in several concentrations. The statistically significant difference in IC_{50} values was evaluated by one-way ANOVA and Tukey's HSD posthoc analysis.

Experimental Design

The optimal extraction conditions were determined using the RSM. A Box-Behnken Design (BBD) was applied to design the experimental setup using the software Design-Expert 12.0 (Stat-Ease, Inc., Minneapolis, US). The independent variables included extraction temperature (°C, X1), ethanol concentration (%, X2), extraction time (minutes, X3), and solvent-to-material ratio (mL/g, X4), with TPC and TFC as the response variable. Ethanol concentrations of 0 (distilled water), 48, and 96% were used as solvents, while the temperatures ranged from 30 to 80°C (the central point at 55°C), the solvent-to-material ratio varied between 10 and 50 (the central point at 30) and the extracting time was set from 60 to 240 minutes (the central point at 150 minutes). Each experiment was performed in triplicate, and the average values were recorded as the observed response.

Results and Discussion

Optimization of extraction conditions by the RSM

The extraction of TPC from *G. uralensis* roots was further refined using the RSM with a BBD to optimize the process. Four key variables, coded and detailed in Table 1, were carefully selected for this study, resulting in the design of 30 distinct experiments. The outcomes of these experiments, summarized in Table 2, were analyzed using analysis of variance (ANOVA) to evaluate the response TPC.

The quadratic model generated from this analysis proved to be highly significant, with a *p*-value of less than 0.001 and a model F-value of 274.82. Additionally, the lack of fit F-value of 0.4378 (p = 0.8752) indicated that the lack of fit was not statistically significant relative to the pure error, with an 87.52% probability that such a lack of fit could occur due to random noise. The predicted R^2 value of 0.9866 was found to be in reasonable agreement with the adjusted R^2 of 0.9925, further confirming the model's accuracy. The relationship between TPC and the four independent variables was expressed through a second-order polynomial equation:

$TPC = -141.59556 + 2.89285X_1 + 5.00591X_2 + 3.11197X_3 + 0.103835X_4 - 0.001540X_1X_2 -$

Table 1 — Codes and levels of the variables

Variables	Unit	Code levels		
		-1	0	1
Temperature (X_1)	°C	50	65	80
Ethanol concentration (X_2)	%	0	48	96
Time (X_3)	minutes	60	150	240
Liquid-to-solid ratio (X_4)	mL/g	10	30	50

Table 2 — Responses of TPC of the extracts to independent variables using Box-Behnken design

Variables				TPC	TFC
X_1:EtOH	X_2:Temp.	X_3:Ratio	X_4:Time	(mg GAE/g)	(mg QE/g)
0	30	30	150	42.1	30.13
96	30	30	150	85.85	70.43
0	80	30	150	57.84	46.59
96	80	30	150	94.2	72.4
48	55	10	60	121.97	85.77
48	55	50	60	150.95	128.9
48	55	10	240	130.66	119.36
48	55	50	240	153.31	118.32
0	55	30	60	68.51	61.74
96	55	30	60	116.13	85.65
0	55	30	240	71.75	52.85
96	55	30	240	111.85	74.71
48	30	10	150	89.24	75.82
48	80	10	150	125.36	88.39
48	30	50	150	134.79	120.46
48	80	50	150	123.53	114.46
0	55	10	150	54.39	50.46
96	55	10	150	97.14	68.01
0	55	50	150	76.24	62.88
96	55	50	150	116.13	92.02
48	30	30	60	114.58	88.07
48	80	30	60	131.89	93.03
48	30	30	240	119.5	101.86
48	80	30	240	134.37	96.44
48	55	30	150	146.04	106.71
48	55	30	150	150.82	103.27
48	55	30	150	144.47	107.75
48	55	30	150	152.79	116.44
48	55	30	150	143.85	131.38
48	55	30	150	147.37	126.14

$0.000745X_1X_3 - 0.000435X_1X_4 - 0.023690X_2X_3 - 0.000271X_2X_4 – 0.000879X_3X_4 - 0.023809X_1^2 - 0.035547X_2^2 - 0.017899X_3^2 - 0.000085X_4^2$

Similarly, the RSM approach was applied to evaluate the extraction of TFC. The modified quadratic model for TFC also showed a significant p-value of less than 0.001 with a model F-value of 25.10. The lack of fit for this model was not significant, with a lack of fit F-value of 0.45 (p = 0.8690), indicating an 86.90% chance that this lack of fit could be attributed to noise. The R^2 value of 0.9388and the adjusted R^2 of 0.9014 support the validity of the model. The relationship between TFC and the four independent variables was also captured in a second-order polynomial equation:

$$TFC = -102.47628 + 2.20810X_1 + 3.45897X_2 + 1.90780X_3 + 0.266345X_4 - 0.003019X_1X_2 + 0.003018X_1X_3 - 0.009285X_2X_3 - 0.001153X_2X_4 - 0.006135X_3X_4 - 0.019504X_1^2 - 0.025859X_2^2$$

The result of ANOVA revealed that X_1 (temperature), X_2 (ethanol concentration), X_3 (time), X_4 (solid-to-liquid ratio), X_1^2(temperature × temperature), X_2^2(ethanol concentration × ethanol concentration) were significant model terms. In detail, these factors could deeply affect the TPC and TFC of the extraction product. The comprehensive analysis using RSM and BBD provided a deep understanding of how various factors influenced the extraction efficiency of both TPC and TFC from *G. uralensis* roots. These optimized conditions and models can now serve as a foundation for further refining the extraction processes, potentially leading to more effective and scalable methods for harnessing the medicinal properties of *G. uralensis*.

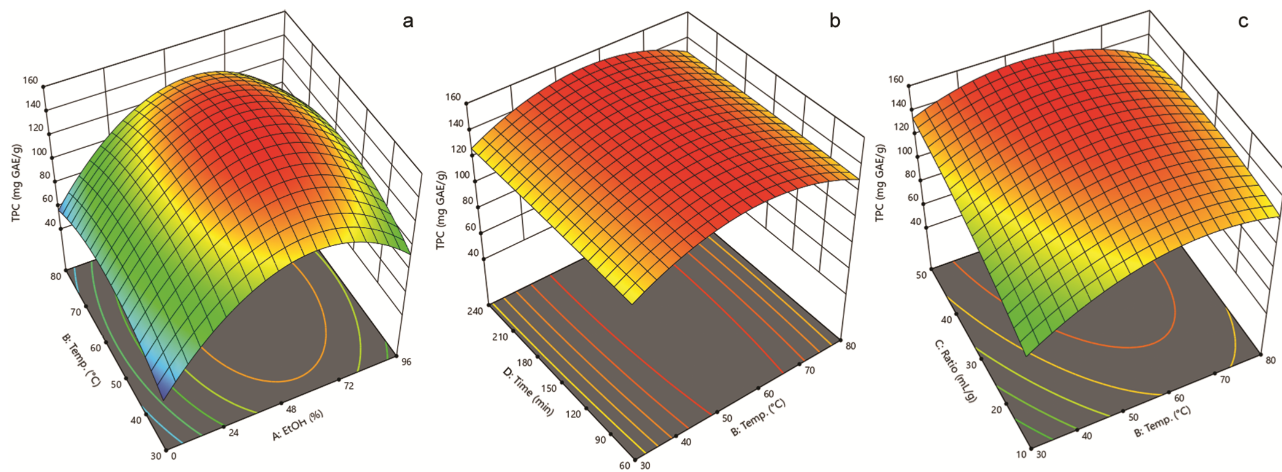

Fig. 1 — Response surfaces a) temperature vs ethanol concentration, b) temperature vs time, and c) temperature vs liquid-to-solid ratio to total phenolic contents of *G. uralensis* roots extracts.

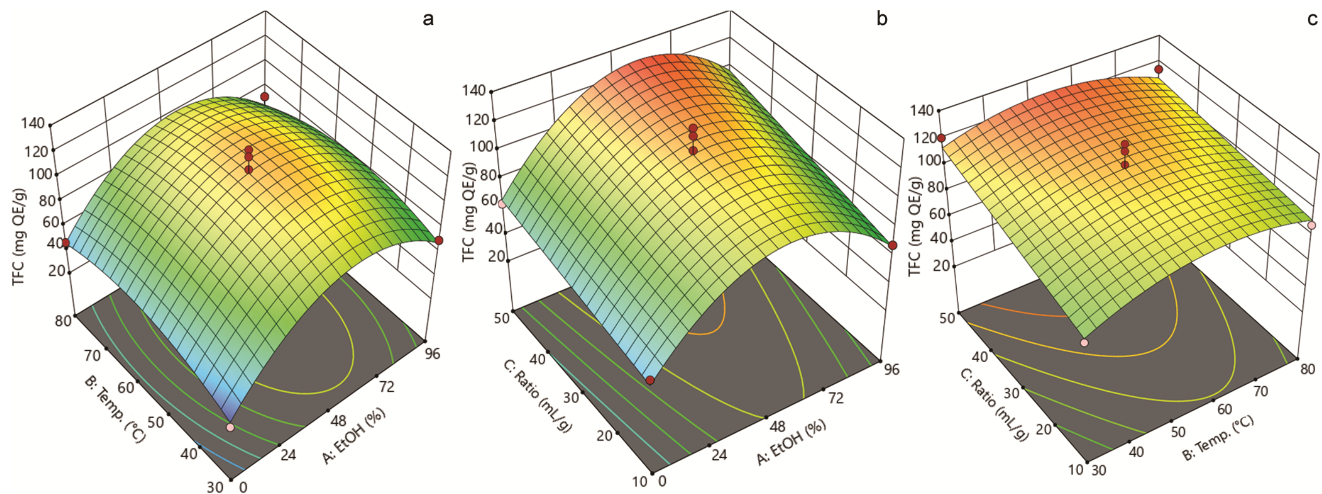

Fig. 2 — Response surfaces a) temperature vs ethanol concentration, (b) temperature vs time, and (c) temperature vs liquid-to-solid ratio to total flavonoid contents of *G. uralensis* roots extracts.

Fig. 1 and Fig. 2 present three-dimensional response surface plots, illustrating how different extraction parameters influence the TPC and TFC of the extracts. The plots show that extending the extraction time and increasing the liquid-to-solid ratio can enhance both TPC and TFC in the final product. Additionally, the extraction temperature and ethanol concentration must be elevated to specific levels to maximize the yield of phenolic compounds. Using the RSM and calculations performed with Design-Expert software, the optimal conditions for extracting phenolics and flavonoids from *G. uralensis* root were identified. The ideal extraction parameters were determined to be a temperature of 57.2°C, an ethanol concentration of 55.9%, a liquid-to-solid ratio of 30.0 mL/g, and an extraction time of 189.6 minutes. Under these conditions, the predicted optimal TPC and TFC values were 150.30 mg GAE/g and 115.25 mg QE/g, respectively.

To validate these findings, an actual extraction was conducted under slightly adjusted conditions: a temperature of 58°C, an ethanol concentration of 56%, a liquid-to-solid ratio of 30 mL/g, and an extraction time of 190 minutes. The results from this confirmation test closely matched the predicted values, with the actual TPC measured at 158.81 mg GAE/g and the TFC at 122.15 mg QE/g (based on triplicate tests). These actual results closely aligned with the calculated predictions, demonstrating the effectiveness and accuracy of the optimized extraction conditions. The study's findings underscore the importance of precise control over extraction

Table 3 — Free radicals scavenging activities of *G. uralensis* root extract in the optimal extracting condition.

Sample	DPPH (IC_{50}, μg/mL)	Hydroxyl (IC_{50}, μg/mL)
G. uralensis root extract	18.56±1.14	38.32±3.87
Ascorbic acid *	35.21±1.73	-
Catechin**	-	36.44±2.87

*, ** positive controls

variables and provide a robust framework for optimizing the extraction of bioactive compounds from *G. uralensis*, which can be applied in larger-scale production settings.

Antioxidant activities

Free radicals are unstable molecules or atoms that contain one or more unpaired electrons, which make them highly reactive. Due to their instability, free radicals can readily interact with and damage nearby stable molecules. Under normal physiological conditions, the body maintains a delicate balance between the production of reactive free radicals and the body's endogenous antioxidant defence mechanisms. This balance is crucial for protecting cells and tissues from oxidative damage. However, when this equilibrium is disrupted, it can lead to oxidative stress, a condition that results in cellular damage. This damage can affect vital biomolecules such as DNA and proteins, potentially leading to cell death. Over time, oxidative stress is implicated in the development of various diseases, including diabetes, cardiovascular diseases, inflammation, and cancer[26]. In this study, the antioxidant properties of *G. uralensis* root extract were thoroughly evaluated under optimal conditions, focusing on its ability to scavenge free radicals. The extract's effectiveness was assessed using two well-established assays: the DPPH radical scavenging assay and the hydroxyl radical scavenging assay. Table 3 revealed that the G. *uralensis* root extract exhibited a particularly strong DPPH radical scavenging effect, with an IC50 value of 18.56 μg/mL. This result is significantly lower than the IC_{50} value of 35.21 μg/mL observed for ascorbic acid, a widely recognised antioxidant and the positive control in this study. This indicates that the *G. uralensis* root extract is more potent in neutralizing DPPH radicals than ascorbic acid. Moreover, the extract also demonstrated substantial scavenging activity against hydroxyl radicals, with an IC50 value of 38.32 μg/mL. This value is very close to that of catechin, another positive control known for its strong antioxidant properties, which had an IC_{50} value of 36.44 μg/mL. These findings suggest that the *G. uralensis* root extract is highly effective in combating oxidative stress by neutralizing harmful free radicals, such as DPPH and hydroxyl radicals. The strong antioxidant activity of the *G. uralensis* root extract, as demonstrated by its low IC_{50} values in both assays, highlights its potential therapeutic benefits. The extract could be particularly valuable in the development of natural antioxidant supplements or pharmaceuticals aimed at preventing or mitigating oxidative damage, which is implicated in a variety of chronic diseases, including cardiovascular diseases, cancer, and inflammatory conditions. The results of this study underscore the importance of further research into *G. uralensis* and its bioactive compounds, as they may offer significant health benefits through their potent antioxidant effects.

Conclusion

In this study, a simplified heating method for extracting antioxidant compounds from *G. uralensis* root was successfully optimised, demonstrating significant improvements in both extraction efficiency and practicality compared to more complex methods. Through the application of RSM and BBD, the optimal extraction conditions were identified as a temperature of 58°C, ethanol concentration of 56%, a liquid-to-solid ratio of 30 mL/g, and an extraction time of 190 minutes. These conditions resulted in high TPC and TFC, with actual values closely matching the predicted optimal outcomes. The antioxidant activity of the extract was assessed using DPPH and hydroxyl radical scavenging assays, revealing superior performance in scavenging DPPH radicals compared to ascorbic acid and comparable activity against hydroxyl radicals to catechin. These results underscore the potent antioxidant potential of *G. uralensis* root extract, which could be harnessed for developing natural antioxidant supplements and pharmaceuticals. The simplified extraction method not only facilitates large-scale production but also offers a cost-effective alternative to more sophisticated techniques. The findings highlight the enduring relevance of traditional herbal medicines in modern health applications and provide a robust foundation for

further research into the therapeutic benefits of *G. uralensis* and its bioactive compounds.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1 Liu Y, Chen Y, Wang D, Gao X, Guo H, *et al.*, Studies on chemical constituents on roots of *Glycyrrhiza uralensis*, *Chin J Pharm Anal*, 2011, **31** (7), 1251-1255.

2 Yin L, Guan E, Zhang Y, Shu Z, Wang B, *et al.*, Chemical profile and anti-inflammatory activity of total flavonoids from *Glycyrrhiza uralensis* Fisch, *Iran J Pharm Res*, 2018, **17**(2), 726-734.

3 Tanaka K, Ina A, Hayashi K and Komatsu K, Comparison of chemical constituents in *Glycyrrhiza uralensis* from various sources using a multivariate statistical approach, *J Tradit Med*, 2010, **27**, 210-216, doi: 10.11339/jtm.27.210.

4 Shang Z, Tian Y, Yi Y, Li K, Qiao X, *et al.*, Comparative bioactivity evaluation and chemical profiling of different parts of the medicinal plant *Glycyrrhiza uralensis*, *J Pharm Biomed Anal*, 2022, **215**, 114793, doi: 10.1016/j.jpba.2022.114793.

5 Tanemoto R, Okuyama T, Matsuo H, Okumura T, Ikeya Y, *et al.*, The constituents of licorice (*Glycyrrhiza uralensis*) differentially suppress nitric oxide production in interleukin-1β-treated hepatocytes, *Biochem Biophys Rep*, 2015, **2**, 153-159, doi: 10.1016/j.bbrep.2015.06.004.

6 Guo D, Yin X, Wu D, Chen J and Ye X, Natural polysaccharides from *Glycyrrhiza uralensis* residues with typical glucan structure showing inhibition on α-glucosidase activities, *Int J Biol Macromol*, 2023, **224**, 776-785, doi: 10.1016/j.ijbiomac.2022.10.165.

7 Park Y-S, Kang S-M, Kim Y-J and Lee I-J, Exploring the dietary and therapeutic potential of licorice (*Glycyrrhiza uralensis* Fisch.) sprouts, *J Ethnopharmacol*, 2024, **328**, 118101, doi: 10.1016/j.jep.2024.118101.

8 Zhao L, Chen X, Shao X, Wang Z, Du Y, *et al.*, Prenylated phenolic compounds from licorice (*Glycyrrhiza uralensis*) and their anti-inflammatory activity against osteoarthritis, *Food Funct*, 2022, **13**(2), 795-805, doi: 10.1039/D1FO03659A.

9 Zhong R, Wen C, Qiu Y, Shen X, Sun Z, *et al.*, Anti-inflammatory and immunomodulatory effects of *Glycyrrhiza uralensis* fisch. On ulcerative colitis in rats: Role of nucleotide-binding oligomerization domain 2/receptor-interacting protein 2/nuclear factor-kappa B signaling pathway, *J Ethnopharmacol*, 2025, **344**, 119457, doi: 10.1016/j.jep.2025.119457.

10 Kim J H, Huh Y-C, Hur M, Park W T, Moon Y-H, *et al.*, Inhibition of soluble epoxide hydrolase activity by components of *Glycyrrhiza uralensis*, *Int J Mol Sci*, 2023, **24**(7), 6485, doi: 10.3390/ijms24076485.

11 Lu Y, Ding Z, Zhang D, Zhu F and Gao B, Integrated metabolomic and transcriptomic analysis reveals the pharmacological effects and differential mechanisms of isoflavone biosynthesis in four species of *Glycyrrhiza*, *Int J Mol Sci*, 2025, **26**(6), 2539, doi: 10.3390/ijms26062539.

12 Kim J, Lee J, Kang J-S, Shim H, Kang D, *et al.*, Contributions of interspecific hybrids to genetic variability in *Glycyrrhiza uralensis* and *G. Glabra*, *Sci Rep*, 2025, **15**(1), 8764, doi: 10.1038/s41598-025-92115-4.

13 Liu H, Cui J, Zhang L, Chang G and Wang W, Screening of anti chronic nonbacterial prostatitis activity of different extractions of the aerial part of *Glycyrrhiza uralensis* and network pharmacology research, *Biomed Rep*, 2021, **15**(6), 99, doi: 10.3390/ijms26062539.

14 Lee E J, Shaikh S, Ahmad K, Ahmad S S, Lim J H, *et al.*, Isolation and characterization of compounds from *Glycyrrhiza uralensis* as therapeutic agents for the muscle disorders, *Int J Mol Sci*, 2021, **22**(2), 876, doi: 10.3390/ijms22020876.

15 Liang Y, Wen Q, Yang Z, Li C and Li F, Optimization of ultrasound-assisted extraction of glycyrrhizic acid and glabridin from *Glycyrrhiza glabra* L. using response surface methodology and development of a green analytical method, *Sustain Chem Pharm*, 2024, **37**, 101385, doi: 10.1016/j.scp.2023.101385.

16 Simayi Z, Aierken W, Rozi P, Ababaikeri G, Bo C, *et al.*, Optimization of ultrasound-assisted extraction, structural, functional, and antioxidant properties of *Glycyrrhiza uralensis* seed protein, *Process Biochem*, 2023, **124**, 1-12, doi: 10.1016/j.procbio.2022.11.001.

17 Bezerra M A, Santelli R E, Oliveira E P, Villar L S and Escaleira L A, Response surface methodology (RSM) as a tool for optimization in analytical chemistry, *Talanta*, 2008, **76**(5), 965-977, doi: 10.1016/j.talanta.2008.05.019.

18 Weremfo A, Abassah-Oppong S, Adulley F, Dabie K and Seidu-Larry S, Response surface methodology as a tool to optimize the extraction of bioactive compounds from plant sources, *J Sci Food Agric*, 2023, **103**(1), 26-36, doi: 10.1002/jsfa.12121.

19 Yu P, Li Q, Feng Y, Ma S, Chen Y, *et al.*, Extraction and analysis of six effective components in *Glycyrrhiza uralensis* Fisch by deep eutectic solvents (DES) combined with quantitative analysis of multi-components by single marker (QAMS) method, *Molecules*, 2021, **26**(5), 1310, doi: 10.3390/molecules26051310.

20 Hedayati A and Ghoreishi S M, Supercritical carbon dioxide extraction of glycyrrhizic acid from licorice plant root using binary entrainer: Experimental optimization via response surface methodology, *J Supercrit Fluids*, 2015, **100**, 209-217, doi: 10.1016/j.supflu.2015.03.005.

21 Xing C, Cui W-Q, Zhang Y, Zou X-S, Hao J-Y, *et al.*, Ultrasound-assisted deep eutectic solvents extraction of glabridin and isoliquiritigenin from *Glycyrrhiza glabra*: Optimization, extraction mechanism and *in vitro* bioactivities, *Ultrason Sonochem*, 2022, **83**, 105946, doi: 10.1016/j.ultsonch.2022.105946.

22 Hao J, Liu J, Zhang L, Jing Y and Ji Y, A Study of the ionic liquid-based ultrasonic-assisted extraction of isoliquiritigenin from *Glycyrrhiza uralensis*, *Biomed Res Int*, 2020, **2020**(1), 7102046, doi: 10.1155/2020/7102046.

23 Fattahi S, Zabihi E, Abedian Z, Pourbagher R, Motevalizadeh A A, *et al.*, Total phenolic and flavonoid contents of aqueous extract of stinging nettle and *in vitro* antiproliferative effect on Hela and BT-474 cell lines, *Int J Mol Cell Med*, 2014, **3**(2), 102-107.

24 Brand Williams W, Cuvelier M E and Berset C, Use of a free radical method to evaluate antioxidant activity, *LWT - Food Sci Technol*, 1995, **28**(1), 25-30, doi: 10.1016/S0023-6438(95)80008-5.

25 Thuong P T, Su N D, Ngoc T M, Hung T M, Dang N H, *et al.*, Antioxidant activity and principles of Vietnam bitter tea Ilex kudingcha, *Food Chem*, 2009, **113**(1), 139-145, doi: 10.1016/j.foodchem.2008.07.041.

26 Arulselvan P, Fard M T, Tan W S, Gothai S, Fakurazi S, *et al.*, Role of antioxidants and natural products in inflammation, *Oxid Med Cell Longev*, 2016, **2016**, 5276130, doi: 10.1155/2016/5276130.