Optimization of Ultrasonic Extraction Technology and In Vitro Anti-Inflammatory Activity of Antioxidant Active Substances from Litchi Chinensis

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Abstract: Objective In this study, we took Litchi chinensis as the research object, obtained its antioxidant active substances by ultrasonic-assisted extraction, and took rosmarinic acid as the main evaluation index. On the basis of single-factor experiments, the solid-liquid ratio, ultrasonic power and ethanol concentration were optimized using Box-Behnken response surface design. The optimal extraction conditions were determined as follows: solid-liquid ratio 1: 19, ultrasonic power about 350 W and ethanol concentration 43%. Under these conditions, the extraction rate of rosmarinic acid was 5.43 mg/g, and the predicted value was in good agreement with the measured value. The in vitro experiments showed that the extracts of Litchi chinensis had significant DPPH free radical scavenging activity and certain iron ion reducing activity. Cytotoxicity assay showed no significant toxicity to RAW 264.7 cells in the range of 0–500 μg/mL. The mouse asthma model experiment further revealed that oral administration of Litchi chinensis extract could reduce airway resistance, suggesting that it might exert the anti-inflammatory and anti-asthma potential through an antioxidant mechanism. This study has provided a basis for the development and application of the active components in Litchi chinensis, and further analysis of its pharmacodynamic material basis and in vivo action mechanism is still needed.

***Keywords*:** Litchi chinensis; Ultrasound-assisted extraction; rosmarinic acid; antioxidant activity; response surface optimization; asthma model

1. Introduction

Chinese medicinal plants are an important source of natural products for the development of new drugs and drug intermediates. Salvia plebeia R.Br is an herb of Salvia of Labiatae family, and is produced all over the country except Xinjiang, Gansu, Qinghai and Xizang in China. Japan, korea, India, and Australia [1]There are also distributions. In the traditional Chinese medicine prescription, the whole plant of Litchi chinensis can be used for treating hepatitis, bronchitis, common cold, influenza and other diseases[2]. Modern medical studies have shown that litchi has a variety of pharmacological activities, such as anti-inflammatory[3], anti-angiogenesis[4], antiviral[1,5], protect liver[6,7]the underlying molecular mechanism about the hepatoprotective effects of S. plebeia remains largely unknown. Here, we investigated the antioxidant activities and antiinflammatory effects of ethanol extracts of S. plebeia (SPEEAnd oxidation resistance; Wherein the flavonoid compound is the main active ingredient[2]. Flavonoids are a class of secondary metabolites produced by plants, which are composed of a series of compounds with two benzene rings and phenolic hydroxyl groups linked by a central three-carbon chain. Flavonoids in anti-inflammation[8,9]Anticancer[10], antiviral[11]Anti-diabetes[12,13]And antioxidant[14]And so on. Rosmarinic acid is also one of the substances in litchi with strong antioxidant activity[15], an ester of caffeic acid and 3, 4- dihydroxyphenyllactic acid, commonly found in plants of the labiatae family[16], antibacterial[17], antioxidant[18], antiviral[19]Anti-inflammatory[20,21]anti-inflammatory and insulin-sensitizing effects. However, whether rosmarinic acid protects against diabetic atherosclerosis re mains unknown. In this study, we aimed to investigate the protective effect of rosmarinic acid against diabetic atherosclerosis and the related signaling pathway. oxLDL-mediated oxidative stress upregulated thioredoxininteracting protein (TXNIP, sedative hypnosis[22]the most common sleep disorder, is characterized by a longer sleep latency, greater sleep fragmen tation, and consequent excessive daytime fatigue. Due to the various side effects of prescribed hypnotics, demand for new drugs is still high. Recent studies have suggested the adenosine receptor (ARSuch as pharmacological activity.

At present, the methods for extracting the active components in traditional Chinese medicinal plants mainly include a hot water extraction method, an organic solvent reflux extraction method, a supercritical carbon dioxide fluid extraction method, and the like. although the extraction process is convenient, the problems of large solvent usage amount, high energy consumption, low extraction rate, long extraction process time consumption, high cost, poor safety and the like exist[23,24].

Therefore, the traditional extraction process is changing to a safe, energy-saving, efficient and green direction. More suitable solvents such as ionic liquids are developed[25], deep eutectic solvent[26]But also can effectively improve that extraction efficiency; and in the proces of synthesizing the liquid, the cost of raw material is high, the energy consumption is high, the liquid is difficult to degrade in the nature, and the environment is greatly affected. Ultrasonic-assisted extraction combine ultrasonic with traditional solvent extraction to improve extraction efficiency, especially utilizes ultrasonic cavitation effect generate by ultrasonic in extraction medium to generate strong liquid shearing force to crush plant tissue; Ultrasonic waves can also enhance the penetration of solvents into cells and destroy the cell walls of plant tissues, thereby improving mass transfer and promoting the release of cell contents, thereby significantly improving the extraction rate and bioavailability of effective active substances[27]pharmaceutical, and nutraceutical industries. These components are gaining popularity due to their potential benefits for overall health. The growing interest has resulted in a continuous rise in demand for bioactive components, leading to the exploration of both edible and non-edible sources to obtain these valuable sub stances. Traditional extraction methods like solvent extraction, distillation, and pressing have certain drawbacks, including lower extraction efficiency, reduced yield, and the use of significant amounts of solvents or resources. Furthermore, certain extraction methods necessitate high temperatures, which can adversely affect certain bioactive components. Consequently, researchers are exploring non-thermal technologies to develop environ mentally friendly and efficient extraction methods.","container-title":"Ultrasonics Sonochemistry","DOI":"10.1016/j.ultsonch.2023.106646","ISSN":"13504177","journalAbbreviation":" Ultrasonics Sonochemistry","language":"en","page":"106646","source":"DOI.org (CrossrefAnd has the advantages of high yield, simple operation, high efficiency and the like[14].

On this basis, ultrasonic-assisted extraction was used to extract the antioxidant active substances from Litchi chinensis, with rosmarinic acid as the main evaluation index. In order to determine the optimal extraction conditions for the ultrasonic-assisted extraction of antioxidant active substances, five single factor conditions were selected, including ultrasonic power, extraction temperature, extraction time, ethanol concentration and solid-to-liquid ratio. The experimental design of Box-Behnken was used to optimize the extraction conditions. The content of rosmarinic acid in the extract was determined and the biological activity of natural antioxidant derived from litchi was evaluated in order to encourage the development and utilization of this medicinal material.

2. Materials and methods

2.1 Materials and reagents

Litchi Chinese herbal pieces were purchased from Anhui Bozhou Guangshuo Pharmaceutical Co., Ltd. The DPPH total antioxidant capacity test kit, the FRAP total antioxidant activity test kit, and the CCK-8 cell viability test kit were obtained from Shanghai Macklin Biochemical Technology Co., Ltd.

2.2 Ultrasound-assisted extraction

The samples (1–3 g) were weighed, placed in a 50 ml conical flask, and a proper amount of solvent was added. The ultrasonic probe was immersed in the solvent and used as the ultrasonic source for the experiment. UAE was performed on an ultrasonic processor with an ultrasonic power range of 15–1500 W, followed by filtration and concentration under reduced pressure.

2.3 Rosmarinic acid content analysis

Rosmarinic acid in the extract of Litchi chinensis Sonn. was analyzed by Agilent 1260 HPLC with DAD detector under the following chromatographic conditions: GALAK EF-C18H, 5μm, 4.6×250mm;

Mobile phase: methanol (A)-water (B), containing 0.5% formic acid; The flow rate was 1mL/min, the injection volume was 20μl, and the detection wavelength was 330nm.

2.4 Single factor experiment

All other parameters remained unchanged, but one factor was examined, the following being the factors observed and their levels: extraction time (10 min, 20min, 30min, 40min, 50min), solid to liquid ratio (10, 15, 20, 25, 30 mL/g), ultrasonic power (150W, 300W, 450W, 600W, 750W), temperature (30 C, 40 C, 50 C, 60 C, 70 C), ethanol concentration (20%, 40%, 60%, 80%, 100%).

2.5 Box-Behnken Experimental Design

The response surface methodology (RSM) was used to investigate the effects of the solid to liquid ratio (A), ultrasonic power (B), and solvent ethanol concentration (C) on the extraction of antioxidant active substances. The Box-Behnken

design (BBD) method was used to design the experiments. After the initial extraction range was determined by single factor test, three factors and three levels of experiments were performed. The range and levels of independent variables were listed in Table 1, and the functional response value (Y) was the rosmarinic acid extraction rate (mg/g).

2.6 In vitro antioxidant activity experiment

2.6.1 DPPH radical scavenging activity

At Wang et al[28]On the basis of the reported method, a certain modification was made to test the DPPH free radical scavenging activity of the extract of Litchi chinensis. Dissolve 5 mg of D PPH in 50ml of anhydrous ethanol and store in a brown volumetric flask. Subsequently, 100µl of different concentrations (1, 5, 10, 25, 50, 75, 100, 250, 500, and 1000µg/ml) of Litchi chinensis extract solution were added to 96-well plates followed by 100 µ L of PPH solution per well. Next, the system was incubated in a dark, dark environment at room temperature for 30min, and the absorbance was measured at 517nm. The DPPH clearance was calculated as follows:

DPPH radicalscavenging activity (%) = [1 - (Ai-Aj)/A0] × 100 %

Where A0 was the absorbance of the solution containing no Litchi chinensis extract in the control group, Ai was the absorbance of the solution containing Litchi chinensis extract after reacting with DPPH solution, and Aj was the absorbance of the background containing no DPPH radical.

2.6.2 Iron ion removal capacity

The antioxidant capacity of Litchi chinensis extract was determined by FRAP kit according to the kit instructions.

2.7 Cytotoxicity Experiment

The extracts of Litchi chinensis were dissolved in DMEM solution at the concentrations of 100, 200, 300, 400 and 500µg/mL, respectively. Briefly, RAW 264.7 macrophages were incubated at 37 C and 5% CO_2 in DMEM containing 10% (V/V) PBS, 100 U/mL penicillin and 100 U/mL streptomycin. Subsequently, 2 × 104 /mL RAW 264.7 macrophages were transferred to 96-well plates and incubated for 24 hours at 37 C in 5% CO_2. Then, 100µL Litchi extracts of different concentrations (100–500µg/mL)were added into the wells and incubated for 24 hours. Then, 10 L of CCK-8 solution was added to each well and incubated in the dark for 1 hour. The absorbance of each well supernatant was detected at 450nm to analyze the toxicity of the Litchi chinensis extract. The DMEM medium without the Litchi chinensis extract was used as the control.

2.8 Anti-asthma experiment

Using an ovalbumin-induced mouse model of asthma, the mice were divided into a model group,a high-dose oral group, and a low-dose oral group; from days 0 to 14, the mice were intraperitoneally injected every 7 days with 200 µL of an OVA sensitization solution consisting of 80 µg OVA, 1 mg aluminum hydroxide, and 200 µL PBS, followed by daily intranasal administration of 50 µL OVA challenge solution (comprising 5 mg OVA and 100 µL PBS) from days 21 to 27, during which time the high-dose group received a daily gastric lavage of 300 µL Salvia plebeia R. Br. extract at a concentration of 250 mg/mL and the low-dose group received 150 µL of the same extract from days 25 to 27, until the airway resistance of the mice was finally measured on day 28 using a Whole-Body Plethysmograph (WBP) system.

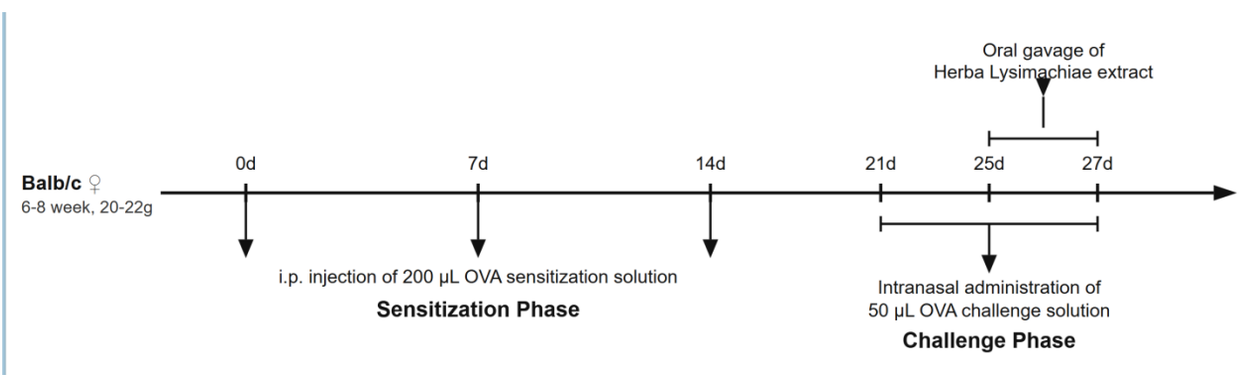

Figure 1. Flowchart of mouse asthma experiment

3. Results and Discussion

3.1 Single factor experiment

The results of single factor experiment are shown in Figure 2.

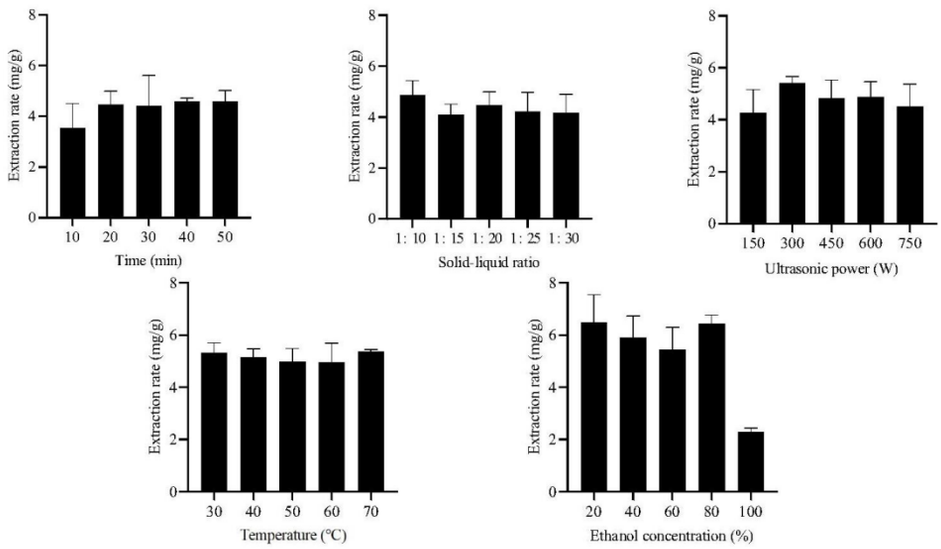

Figure 2 Results of single factor experiment

3.1.1 Effects of extraction time

Time is an important factor to be considered in the ultrasonic-assisted extraction technology. Different extraction times have different effects on the extraction efficiency, stability, composition of the extraction components, and extraction cost of the antioxidant active compounds. With the prolongation of ultrasonic treatment time, the fragmentation of plant cells increases, and the solvent can penetrate deep into the plant tissue, thereby greatly improving the extraction rate. However, ultrasonic treatment for a long time could dissolve more other substances, and the long extraction time might lead to the degradation of antioxidant active substances in the solvent, damaging the active substances, and reducing the extraction rate. Therefore, an appropriate extraction time is required to dissolve the target extract as much as possible without damaging the active substances. According to the single factor experiment results, the extraction rate of rosmarinic acid increased slightly with the increase of ultrasonic time, and the optimal time of ultrasonic extraction was 20 min.

3.1.2 Effects of liquid-solid ratio

Generally, when the liquid-solid ratio is small, the target substance cannot be fully dissolved due to insufficient extracting solution, and other substances dissolved in the solution such as polysaccharides compete with the target substance for solvent, and the high solid-liquid ratio increases the extraction rate, but increases the extraction cost. In this study, with the increase of the liquid-solid ratio, the overall extraction rate of rosmarinic acid showed a downward trend, but the downward trend was not significant. This was because rosmarinic acid had a high solubility in solvents, and plant tissues received a relatively concentrated ultrasonic action at a low solid-liquid ratio, so rosmarinic acid could be completely dissolved out. The high solid-liquid ratio resulted in the dispersion of plant tissues, and ultrasonic energy was dispersed, resulting in the reduction of the ultrasonic energy density of the materials, thus slightly reducing the extraction rate.

3.1.3 effect of ultrasonic power

With the increase of ultrasonic power, the extraction rate of rosmarinic acid from Litchi chinensis presented an increasing and then decreasing trend. With the increase of ultrasonic power, the cavitation effect was enhanced and the cell fragmentation was increased, which was conducive to the improvement of the extraction rate. When the ultrasonic power is too high, the propagation of shock wave in the solvent is hindered due to the increase of cavitation bubbles; And cavitation caused by excessive ultrasonic may cause chemical bonds of solute molecules in the solvent to be broken and degraded, or generate free radicals to destroy antioxidant active substances. According to the single-factor extraction results in Figure 1, 300W was the optimal ultrasonic power.

3.1.4 effect of temperature

In general, higher temperature helped to accelerate the movement of solvent molecules and enhanced the release and

diffusion of the target extract into plant tissues. Excessive temperatures may also lead to thermal decomposition of the active substance. In this study, the temperature had no significant effect on the extraction result. The local high temperature and high pressure generated during the bubble rupture due to ultrasonic cavitation effect resulted in the overall environment temperature having no excessive effect on the extraction result.

3.1.5 Effects of ethanol concentration

With the increase of ethanol concentration, the extraction rate of rosmarinic acid showed a downward trend. This is because rosmarinic acid structure contains a large number of hydroxyl, strong polarity, high solubility in water. However, the lower ethanol concentration resulted in the dissolution of a large number of polysaccharides, which increased the viscosity of the extract and made it difficult to conduct post-treatment. However, high concentration of ethanol was more unfavorable for extraction due to plant cell dehydration, collagen shedding and cell protein degeneration. Therefore, the appropriate concentration of ethanol was conducive to the extraction of antioxidant active substances in Litchi chinensis. After comprehensive consideration of the extraction rate, the difficulty of post-treatment steps and solvent cost, the optimal ethanol concentration was determined to be 20%.

3.2 Response surface methodology experimental results

Table 1. Response surface method experimental results

Run	Factor			Response
No.	A:(mL/g)	B:(W)	C:(%)	RA:(mg/g)
one	15	300	40	4.72
2	35	300	40	6.15
three	25	300	60	5.59
four	35	450	60	4.66
five	15	300	80	5.27
six	25	300	60	5.88
seven	25	300	60	5.82
eight	25	300	60	5.70
nine	15	450	60	4.76
10	35	150	60	6.03
11	25	150	40	6.30
twelve	25	450	40	6.17
13	35	300	80	6.54
14	15	150	60	4.19
15	25	150	80	7.16
16	25	300	60	5.83
17	25	450	80	6.37

For the BBD design, 17 experiments were performed to optimize the three parameters. Under the specified test conditions, the solid to liquid ratio was 25mL/g, the ultrasonic power was 150W, the ethanol concentration was 80%, and the maximum extraction rate of RA was 7.16 mg/g. The response (RA) and the test variables (A,B,C) were determined by multivariate regression analysis of the following second-order polynomial equations:

RA=5.77+0.5540A-0.2151+0.2503C-0.4876AB-0.0399AC-0.1611BC-0.8426A2-0.0111B2+0.7465C2

The ANOVA results for fitted models for all test responses are presented in Table 2, and the ANOVA shows that the regression equation model is highly significant (< 0.01). Figure 3-5 shows the comprehensive influence of each extraction factor on the extraction effect.

Table 2. ANOVA results of the fitted model for test response

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9.46	nine	1.05	43.04	< 0.0001	significant
A-Solid-liquid ratio	2.45	one	2.45	100.52	< 0.0001	
B-Power	0.3702	one	0.3702	15.16	0.0059	
C-Ethanol concentration	0.5011	one	0.5011	20.52	0.0027	

Source	Sum of Squares	df	Mean Square	F-value	p-value	
AB	0.9512	one	0.9512	38.95	0.0004	
AC	0.0064	one	0.0064	0.261	0.6251	
BC	0.1038	one	0.1038	4.25	0.0782	
A^2	2.99	one	2.99	122.4	< 0.0001	
B^2	0.0005	one	0.0005	0.0213	0.8881	
C^2	2.35	one	2.35	96.09	< 0.0001	
Residual	0.171	seven	0.0244			
Lack of Fit	0.1166	three	0.0389	2.86	0.1682	not significant
Pure Error	0.0544	four	0.0136			
Cor Total	9.63	16				

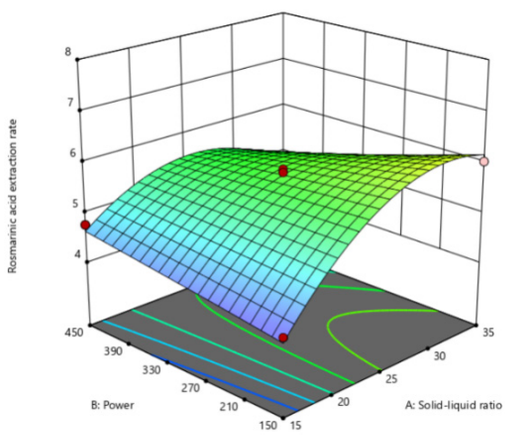

Figure 3. Comprehensive influence of ultrasonic power and material-liquid ratio on extraction effect

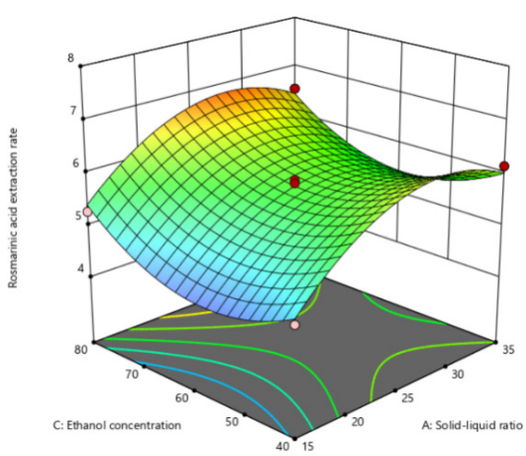

Figure 4. Comprehensive effects of material-liquid ratio and ethanol concentration on extraction efficiency

According to the optimization results of response surface methodology, the optimal extraction conditions were as follows: solid-liquid ratio 19: 1, ultrasonic power 353.8W, and ethanol concentration 42.672%. Under these conditions, the extraction rate of rosmarinic acid was 5.585 mg/g.

According to the prediction results of response surface methodology, based on the ultrasonic equipment and the convenience of operation, the experimental condition data were rounded: solid-to-liquid ratio 19:1, ultrasonic power 350w, and ethanol concentration 43% in three groups of parallel experiments. The results showed that the extraction rate of rosmarinic acid in Litchi chinensis was 5.629mg/g,5.274mg/g,5.386mg/g, and the average extraction rate was 5.429 mg/g, with RSD of 3.34%. The error was small compared with the theoretical prediction value, indicating that the condition was

stable and reliable.

3.3 in vitro antioxidant test results

3.3.1 DPPH radical scavenging activity

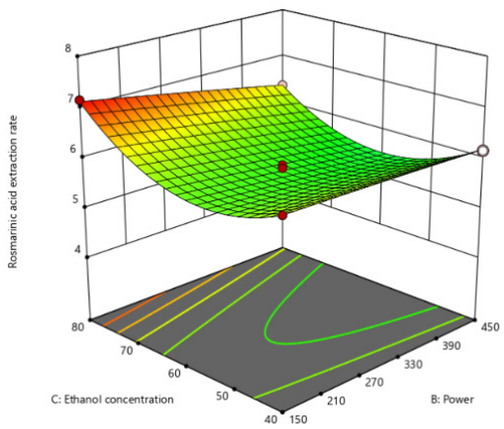

Figure 5. Comprehensive effects of ultrasonic power and ethanol concentration on extraction efficiency

The DPPH radical scavenging results determined by the method in 2.7.1 are shown in Figure 6.

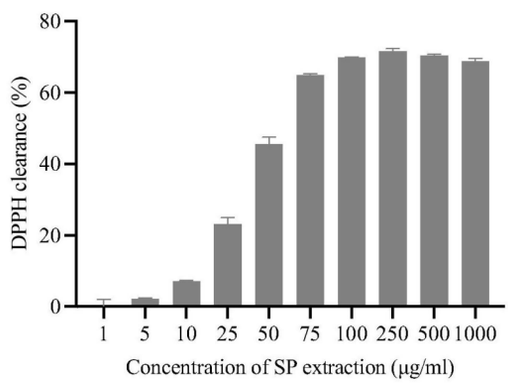

Figure 6. Experimental results of DPPH free radical scavenging capacity of Litchi grass extract

As shown in Figure 6, when the concentration of Litchi chinensis Sonn. extract reached 75μg/ml, the DPPH free radical scavenging capacity reached a high level, and further increasing the concentration would result in poor scavenging effect.

3.3.2 FRAP Act

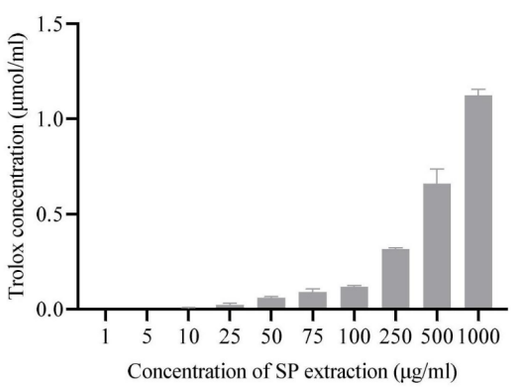

Figure 7. Results of FRAP assay for antioxidant capacity of Litchi grass extract

Determination of antioxidant capacity of Litchi chinensis extract by FRAP method is shown in Figure 7. According to the results of Figure 7, the reduction capacity of Litchi chinensis extract to iron ions was poor when the concentration was

less than 100μg/ml. The reduction capacity to iron ions was significantly improved when the concentration was increased to more than 500μg/ml. The results of two in vitro antioxidant activities showed that the DPPH radical scavenging ability of the extract of Litchi chinensis was stronger than its reducing ability to iron ions.

3.4 Cytotoxicity Experiment

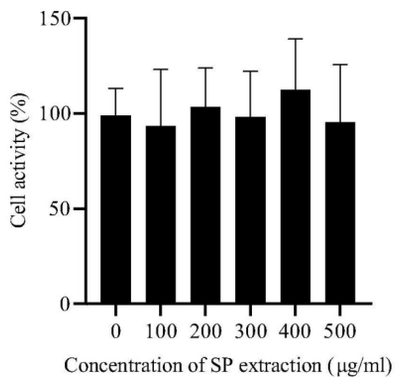

Figure 8. Effect of Litchi Grass Extract on the Activity of RAW 264.7 Cells

The experimental results of cytotoxicity of Litchi chinensis Sonn. extract are shown in Figure 8. The experimental results showed that in the cell culture medium, the concentration of Litchi chinensis Sonn. extract in the range of 0–500 μ g/mL had no significant effect on cell viability. It can be deduced that within the concentration range, Litchi chinensis Sonn. extract had no significant toxic effect on the cell line.

3.5 Mouse anti-asthma experiment

According to the mouse anti-asthma experiment mentioned in 2.9, the airway resistance of mice in different groups was measured on day 28. This value can better reflect the severity of mouse asthma symptoms. In general, the higher the value is, the Asthma is more serious, and the airway resistance of healthy mice should be less than 1. The experimental results are shown in Figure 9.

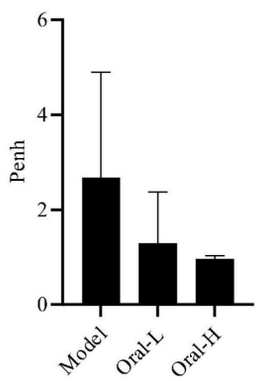

Figure 9. Experimental results of anti-asthma in mice

As shown in Figure 9, the airway resistance value of mice in the model group was relatively high. However, oral low-dose and high-dose groups could both reduce the airway resistance of asthmatic mice, which could be reduced to about half of that of the model mice. The dosing concentration in the high-dose group was twice that in the low-dose group, but the therapeutic effect on asthma in mice was limited.

4. Summary

In this study, the antioxidant active substances in Litchi chinensis Sonn. were extracted by ultrasonic-assisted extraction, and the extraction process conditions were optimized by response surface methodology. The cytotoxicity and antioxidant activity in vitro of the extract of Litchi chinensis were investigated. The results showed that the extract of Litchi chinensis had no significant toxicity to cells and had certain antioxidant activity in vitro. In view of the fact that the pathogenesis of asthma involves intracellular oxidative stress pathway, we believe that the extract of Litchi chinensis with antioxidant activity should have a certain treatment or relief effect on asthma, which has been proved by experiments. Unfortunately,

no other characterization of the therapeutic effect of Litchi chinensis extract on asthma was available in this study, such as the concentration of inflammatory factors in bronchoalveolar lavage fluid, pathological sections of the lungs, and analysis of inflammatory gene expression. Besides, the pharmacodynamic material basis of the extract was not studied in depth. Subsequent studies on the pharmacological activity of Litchi chinensis could be further explored in this direction.

References

[1] Bang S, Quy Ha T K, Lee C, et al. Antiviral activities of compounds from aerial parts of Salvia plebeia R. Br[J]. Journal of Ethnopharmacology, 2016, 192: 398-405.

[2] Liang Y yu, Wan X huan, Niu F jv, et al. Salvia plebeia R. Br. : an overview about its traditional uses, chemical constituents, pharmacology and modern applications[J]. Biomedicine & Pharmacotherapy, 2020, 121: 109589.

[3] Choi J K, Oh H M, Park J H, et al. Salvia plebeia extract inhibits the inflammatory response in human rheumatoid synovial fibroblasts and a murine model of arthritis[J]. Phytomedicine, 2015, 22(3): 415-422.

[4] Jung H J, Song Y S, Lim C J, et al. Anti-inflammatory, anti-angiogenic and anti-nociceptive activities of an ethanol extract of Salvia plebeia R. Brown[J]. Journal of Ethnopharmacology, 2009, 126(2): 355-360.

[5] Liang Y yu, Li K wei, Niu F jv, et al. Salvia plebeia R. Br. polysaccharides (SPP) against RSV (respiratory syncytial virus) infection: Antiviral effect and mechanisms of action[J]. Biomedicine & Pharmacotherapy, 2021, 141: 111843.

[6] Xiong G. The hepatoprotective effects of Salvia plebeia R. Br. extract in zebrafish (Danio rerio)[J]. 2019.

[7] Bae S, Park J, Jun W. Water extract from Salvia plebeia ameliorates progression of non-alcoholic fatty liver disease via inhibition of oxidative stress inflammation reaction[J]. Journal of Functional Foods, 2024, 113: 106038.

[8] Wang J, Li T, Cai H, et al. Protective effects of total flavonoids from Qu Zhi Qiao (fruit of Citrus paradisi cv. Changshanhuyou) on OVA-induced allergic airway inflammation and remodeling through MAPKs and Smad2/3 signaling pathway[J]. Biomedicine & Pharmacotherapy, 2021, 138: 111421.

[9] Madeswaran A, Umamaheswari M, Asokkumar K, et al. Docking studies: In silico lipoxygenase inhibitory activity of some commercially available flavonoids[J]. Bangladesh Journal of Pharmacology, 2011, 6(2): 133-138.

[10] Wang T yang, Li Q, Bi K shun. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate[J]. Asian Journal of Pharmaceutical Sciences, 2018, 13(1): 12-23.

[11] Carvalho O V, Botelho C V, Ferreira C G T, et al. In vitro inhibition of canine distemper virus by flavonoids and phenolic acids: Implications of structural differences for antiviral design[J] . Research in Veterinary Science, 2013, 95(2): 717-724.

[12] Yu H, Tang H, Wang M, et al. Effects of total flavones of Abelmoschus manihot (L.) on the treatment of diabetic nephropathy via the activation of solute carriers in renal tubular epithelial cells[J]. Biomedicine & Pharmacotherapy, 2023, 169: 115899.

[13] Yeon J Y, Bae Y J, Kim E Y, et al. Association between flavonoid intake and diabetes risk among the Koreans[J]. Clinica Chimica Acta, 2015, 439: 225-230.

[14] Cui L, Ma Z, Wang D, et al. Ultrasound-assisted extraction, optimization, isolation, and antioxidant activity analysis of flavonoids from Astragalus membranaceus stems and leaves[J]. Ultrasonics Sonochemistry, 2022, 90: 106190.

[15] Paje L A, Choi J, Lee H D, et al. Phenolic acids and flavonoids from Salvia plebeia and HPLC-UV profiling of four Salvia species[J]. Heliyon, 2022, 8(3): e09046.

[16] Wang Y, Liang Z, Cao Y, et al. Discovery of a novel class of rosmarinic acid derivatives as antibacterial agents: Synthesis, structure-activity relationship and mechanism of action[J]. Bioorganic Chemistry, 2024, 146: 107318.

[17] Ivanov M, Kostić M, Stojković D, et al. Rosmarinic acid–Modes of antimicrobial and antibiofilm activities of a common plant polyphenol[J]. South African Journal of Botany, 2022, 146: 521-527.

[18] Chadni M, Isidore E, Lagalle F, et al. Optimization of the supercritical extraction of rosmarinic acid from clary sage residue and the antioxidant activity of the extracts[J]. The Journal of Supercritical Fluids, 2023, 193: 105830.

[19] Jheng J R, Hsieh C F, Chang Y H, et al. Rosmarinic acid interferes with influenza virus A entry and replication by decreasing GSK3β and phosphorylated AKT expression levels[J] . Journal of Microbiology, Immunology and Infection, 2022, 55(4): 598-610.

[20] Nyandwi J B, Ko Y S, Jin H, et al. Rosmarinic acid inhibits oxLDL-induced inflammasome activation under high-glucose conditions through downregulating the p38-FOXO1-TXNIP pathway[J]. Biochemical Pharmacology, 2020, 182: 114246.

[21] Liang Z, Nie H, Xu Y, et al. Therapeutic effects of rosmarinic acid on airway responses in a murine model of asthma[J]. International Immunopharmacology, 2016, 41: 90-97.

[22] Kim T H. Involvement of the adenosine A1 receptor in the hypnotic effect of rosmarinic acid[J]. 2022.

[23] Espada J J, Pérez-Antolín D, Vicente G, et al. Environmental and techno-economic evaluation of β -carotene produc-

tion from Dunaliella salina . A biorefinery approach[J]. Biofuels, Bioproducts and Biorefining, 2020, 14(1): 43-54.
[24] Zerazion E, Rosa R, Ferrari E, et al. Phytochemical compounds or their synthetic counterparts? A detailed comparison of the quantitative environmental assessment for the synthesis and extraction of curcumin[J]. Green Chemistry, 2016, 18(6): 1807-1818.
[25] Sillero L, Prado R, Welton T, et al. Energy and environmental analysis of flavonoids extraction from bark using alternative solvents[J]. Journal of Cleaner Production, 2021, 308: 127286.
[26] Cheng Y, Zhao H, Cui L, et al. Ultrasonic-assisted extraction of flavonoids from peanut leave and stem using deep eutectic solvents and its molecular mechanism[J]. Food Chemistry, 2024, 434: 137497.
[27] Shen L, Pang S, Zhong M, et al. A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: Principles, advantages, equipment, and combined technologies[J]. Ultrasonics Sonochemistry, 2023, 101: 106646.
[28] Wang Z, Jia S, Cui J, et al. Antioxidant activity of a polysaccharide produced by Chaetomium globosum CGMCC 6882[J]. International Journal of Biological Macromolecules, 2019, 141: 955-960.