



Study on Optimization of Extraction Process of Saponins from *Bulbus Lilii* and Their Antidepressant Effects

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Lilium brownii is used for dreaminess, absent-mindedness. The extraction of polysaccharide and saponin constituents in *Bulbus Lilii* have been extensively studied, but relatively little attention has been paid to the process. The objective of the study is to investigate the extraction process of saponins from *Bulbus Lilii* and their antidepressant effects in mice. Orthogonal experiment was used to optimize the concentration and amount of the extraction solvent, as well as the extraction time of *Bulbus Lilii* saponins, meanwhile, immobility time of mice in the tail suspension and forced swimming tests and antagonism of reserpine-induced hypothermia were observed. As results optimal process for the extraction of saponins from *Bulbus Lilii* was 1 time heat reflux extraction with 70 % ethanol for 3 h; compared with the control group, each *Bulbus Lilii* saponin experimental group could significantly shorten the immobility time of mice in the tail suspension and forced swimming tests and could effectively antagonize reserpine-induced hypothermia in mice as well. It is concluded that *Bulbus Lilii* saponins have antidepressant effects.

Keywords: *Bulbus Lilii* saponins, Orthogonal experiment, Forced swimming.

INTRODUCTION

Bulbus Lilii is the fleshy bulb scale of *Lilium brownii* F.E. Brown var. *viridulum* Baker in the family Liliaceae. It is sweet in taste, cold in nature and is used for deficiency of yin, irritating dry cough, overstrain cough, hemoptysis, dysphoria, palpitation, insomnia, dreaminess, absent-mindedness, etc. It works especially well for tuberculosis, as well as nourishment and relief of bronchitis and is thus reputed as a "good vegetable and medicine"^{1,2}. *Bulbus Lilii* mainly contains steroidal saponins, phenolic glycerol glucosides, polysaccharides, amino acids, phospholipids as well as trace elements and other constituents³. Although Chinese scholars have extensively studied the extraction of polysaccharide and saponin constituents in *Bulbus Lilii*. But relatively little attention has been paid to the process⁴⁻⁷. In this experiment, extraction process of saponins in *Bulbus Lilii* was studied by orthogonal design and the antidepressant effect and mechanism of *Bulbus Lilii* saponins were preliminarily investigated.

EXPERIMENTAL

Bulbus Lilii was purchased from the market, which was identified as the fleshy underground bulbs of *Lilium brownii*

F. E. Brown var. *viridulum* Baker in family Liliaceae. Fluoxetine hydrochloride tablets (Changzhou Siyao Pharmaceutical Co., Ltd.), reserpine injection (Tianjin Jinyao Amino Acid Co., Ltd.), diosgenin reference substance (purchased from the National Institute for the Control of Pharmaceutical and Biological Products).

Kunming mice, half male and half female, weighing 18-22 g, acclimatized for a period of 4 days, fed and watered ad libitum. All experimental procedures were approved by the Animal Research Ethics Committee.

UV-756 UV-visible spectrophotometer, R series rotary evaporator, electric heated thermostatic water bath.

Optimization of extraction process of saponins from *Bulbus Lilii*

Design of extraction process: 10 g of *Bulbus Lilii* was crushed into coarse powder and extracted under reflux with different volume fractions of ethanol in accordance with the conditions in Table-1, the filtrates were combined, filtered and ethanol was recovered, the residue was then dissolved and extracted sequentially with diethyl ether and *n*-butanol. The extracts were evaporated and concentrated in a water bath to a dry paste, then weighed.

Saponin content was determined by spectrophotometry with diosgenin as the reference substance based on the vanillin-perchloric acid chromogenic reaction of steroidal saponin compounds and the extraction yield of total saponins of *Bulbus Lili* was calculated.

Level	Factor		
	A (Ethanol concentration %)	B (Extraction time h)	C (Extraction time)
1	70	1	1
2	80	2	2
3	90	3	3

Study on antidepressant activity of *Bulbus Lili* saponins

Animal grouping and administration: The mice were randomized into five groups, namely the blank control group (distilled water), lily saponin low-, medium- and high-dose groups (50, 100 and 150 mg/kg) and fluoxetine group (20 mg/kg), administration volume was all 0.2 mL/kg. The mice in each group were intragastrically administered once daily at 8 a.m. morning for 7 consecutive days.

Tail suspension test in mice: By Steru *et al.*⁸ procedure, 7 d after administration, the mice were suspended in the tail suspension device upside down and the sight of adjacent mouse was blocked by a board. Cumulative immobility time of mice in the treatment groups and control group within 6 min were recorded.

Forced swimming test in mice: Referring to Porsolt *et al.*⁹ method, 90 min after the last administration, the mice were placed in a 20 cm diameter bucket filled with water to a depth of 15 cm and maintained at 25 °C. After a period of time, activity of mice decreased due to despair, preconditioning time was 2 min and the immobility time within the last 5 min was recorded.

Reserpine reversal test: Referring to Askew *et al.*¹⁰ method, 4 mg/kg reserpine was intraperitoneally injected to the mice at the last administration, after 2 h, rectal temperature was measured and differences in the change of rectal temperature between treatment groups, control group and normal control group were compared.

RESULTS AND DISCUSSION

As can be seen from the experimental results in Table-2, the range (R) value of influencing factors differed from each other, their sizes in proper order were, the order of factors influencing total saponins was B > A > C and the optimal extraction conditions were obtained as B₃A₁C₁. Taking into account the extraction rate and actual production situation of total saponins of *Bulbus Lili*, the optimal extraction conditions of *Bulbus Lili* saponins were confirmed as B₃A₁C₁, *i.e.* 1 time heat reflux extraction with 70% ethanol for 3 h.

Tail suspension test: The immobility state presented in the tail suspension model of mice also reflects the despair behavior of animals, different dose groups of *Bulbus Lili* saponins could significantly shorten the immobility time of mice in the tail suspension test in a dose dependent manner, Fig. 1.

Experiment no.	A	B	C	Saponin extraction yield (%)
1	1	1	1	0.56
2	1	2	2	0.87
3	1	3	3	1.34
4	2	1	2	0.98
5	2	2	3	0.61
6	2	3	1	1.13
7	3	1	3	0.49
8	3	2	1	0.79
9	3	3	2	0.55
k ₁	2.77	2.03	2.48	
k ₂	2.72	2.27	2.40	
k ₃	1.83	3.02	2.44	
K ₁	0.92	0.68	0.83	
K ₂	0.91	0.76	0.80	
K ₃	0.61	1.01	0.81	
R	0.31	0.33	0.02	

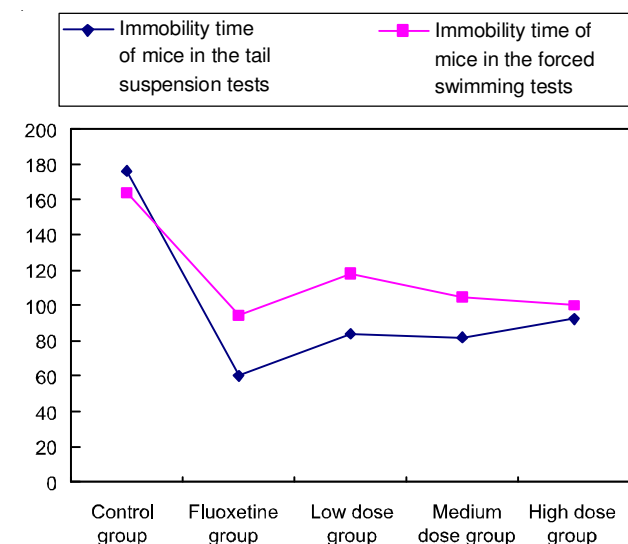


Fig. 1. Results for the effect of *Bulbus Lili* saponins on tail suspension & forced swimming tests

Forced swimming test: Fig. 1 shows that each experimental group could all significantly shorten the immobility time of mice in the forced swimming test compared with the control group, indicating that saponins of *Bulbus Lili* had antidepressant effects.

Reserpine hypothermia antagonism test: As can be seen from Table-3, each experimental group could effectively antagonize reserpine-induced hypothermia in mice, which showed certain dose-effect relationship.

Group	Dose (mg/kg)	Mouse body temperature (°C)
Blank control group		36.8 ± 0.87**
Reserpine group		32.5 ± 0.68
Fluoxetine group	20	33.5 ± 0.82*
	50	33.6 ± 1.24**
<i>Bulbus Lili</i> saponin group	100	33.8 ± 1.37**
	150	34.7 ± 1.59**

Comparison with the reserpine group, *P < 0.05, **P < 0.01

The medicinal value of *Bulbus Lili* has been recognized very early in China. China's classic "Shen Nong's Herbal Classic" recorded that "*Bulbus Lili*, sweet in taste and neutral in nature, treats pathogenic qi, abdominal distension and cardiodynia, can induce diuresis, relax the bowels, tonify middle-jiao and qi, grows in the mountains and valleys." As a representative both medicinal and edible plant, physicians and scholars through the ages have done abundant researches on the efficacy and clinical applications of *Bulbus Lili*. In this paper, extraction process of saponins from *Bulbus Lili* was optimized, the results showed that: 1 time heat reflux extraction with 70 % ethanol for 3 h could effectively improve the extraction yield of *Lilium brownii* saponins and reduce their losses.

Depression is a mental disorder, which is basically characterized by persistent emotional downturn. It is often accompanied by slow behaviour, mental retardation and a variety of somatic symptoms. The cause of depression is relatively complex, despite the extensive researches done by Chinese and foreign scholars, its pathogenesis and mechanism are yet not clear. In terms of possible pathogenic causes, depression is generally divided into primary depression and secondary depression.

In 1958, Kline for the first time gave the depressed patients iproniazid, which showed a good antidepressant effect; since then, the era of treatment of depressive illness using chemical drugs began¹¹. Researchers at China and abroad have researched and developed various categories of drugs such as tricyclic antidepressants (TCAs), heterocyclic antidepressants (HCAs), novel reversible monoamine oxidase inhibitors (RIMAs), selective 5-serotonin reuptake inhibitors (SSRIs), selective norepinephrine reuptake inhibitors (NaRIs), 5-serotonin and norepinephrine reuptake inhibitors (SNaRIs), as well as noreadrenergic and specific 5-serotonergic antidepressants (NaSSAs) for the diagnosis and treatment of depressive illness¹¹.

According to statistics, in 2000, annual sales of antidepressants reached \$13.4 billion, accounting for 4.2 % of the global share of sales, ranking No. 3; sales growth rate was 18 %, ranking No. 5.

While the antidepressants have stepped into the golden period of development, we must see that a variety of adverse reactions caused by taking antidepressants have seriously affected and restricted its development, for example, TCAs cause cardiac toxicity such as postural hypotension, arrhythmia, atrioventricular block, heart failure, myocardial infarction; patients taking SSRIs have adverse reactions such as insomnia, impotence, nausea, constipation, hyperhidrosis, dry mouth, fatigue, drowsiness, *etc.*, the search of Chinese medicines and natural medicines which have effective antidepressant effects with small toxic and side effects has become the focus of researches on antidepressant diseases.

This experimental results showed that compared with the control group, each *Bulbus Lili* saponin experimental group could all significantly shorten the immobility time of mice in the tail suspension test and forced swimming test and could effectively antagonize reserpine-induced hypothermia in mice as well, preliminarily demonstrating the inhibitory effect of *Bulbus Lili* saponins on depressive illness in mice, its mechanism of action required more studies.

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