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Lobelia chinensis: chemical constituents and anticancer activity perspective

CHEN Mei-Wan[1#], CHEN Wen-Rong[2#], ZHANG Jin-Ming[1], LONG Xiao-Ying[2], WANG Yi-Tao[1 *]

[1] *State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macau 999078, China;*
[2] *School of Traditional Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou 510006, China*

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[ABSTRACT]

Research has demonstrated that many chemical constituents dominated by piperidine alkaloids and flavonoids, such as lobelanidine, lobeline, and lobelanine, have been obtained from *Lobelia chinensis* Lour. Experimental studies and clinical applications have also indicated that *L. chinensis* possesses a number of pharmacological activities (e.g., diuretic, choleretic, breathing excitement, anti-venom, anti-bacterial, and anticancer). This paper focuses on the properties, chemical constituents, and anticancer activity of *L. chinensis* to clarify the connection among them, and identify the active anticancer compounds. This work serves as the foundation for further research and development of *L. chinensis*.

[KEY WORDS] *Lobelia chinensis*; Chemical constituents; Anticancer activity

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Introduction

Lobelia chinensis Lour. (*L. chinensis*) is a plant of the Campanulaceae family that is commonly known as ji-jie-suo, xi-mi-cao, and she-she-cao. It has been widely used as an antidote, diuretic, and hemostat in traditional Chinese medicine for decades [1-2]. This plant is dispersed widely throughout East Asia, including China, Korea, and Japan [1]. The increasing research interests have afforded a variety of chemical compounds from this plant, including piperidine alkaloids, such as lobeline, norlobelanine, and lobelanine; coumarins, such as 6, 7-dimethoxycoumarin, 5-hydroxy-7-methoxycoumarin, and 5,7-dimethoxy-6-hydroxy-coumarin; and terpenoids, including phytol, phytenal, cycloeucalenol, and 24-methylene-cycloartanol. Based on these chemical constituents, *L. chinensis* exhibits various biological properties, including anti-bacterial, anti-venom, and anticancer properties. Furthermore, it has been reported to have potential anti-viral and anti-inflammatory activity [3]. In this article, the recent studies on the metabolites and the anticancer activity profiles of *L. chinensis* are summarized to expand its use in clinical applications. As far as is known, *L. chinensis* has been mainly used to treat liver, gastric, intestinal, and breast tumors.

Character of *L. chinensis*

L. chinensis twines around itself when growing. It has yellow roots that are 1−2 mm in diameter, and a light yellow-brown surface. The stem is grey-green with branches and joints; the brown-green leaves grow toward one another. The shape of the plant is long and narrow, wrinkling and showing sparse and short teeth on the edge. It has small, light purple-red flowers with a single, tiny, long peduncle growing under the axillary, five cracks on the bottom of the corolla, and white hair within. The smell of *L. chinensis* is weak, but special, which is irritating, a little sweet and hot [4].

Chemical Ingredients of *L. chinensis*

The main constituents of *L. chinensis* are piperidine alkaloids and flavonoids; other components include terpenes, alkynes, coumarins, and amino acids [2]. The main piperidine

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[*Corresponding author] WANG Yi-Tao: Prof., Tel/Fax: 853-839746 91, 853-28841358; E-mail: ytwang@umac.mo
[#]Co-first authors
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alkaloids contributing to the anticancer effects are dominated by norlobelanine (**1**), lobeline (**2**), lobelanidine (**3**), lobelanine (**4**), radicamine A (**5**), and radicamine B (**6**) [1], which are shown in Fig. 1. In addition, the flavonoids are comprised of apigenin (**7**), luteolin (**8**), quercetin (**9**), linarin (**10**), and luteolin 3', 4'-dimethylether-7-*O*-*β*-D-glucoside (**11**) (Fig. 2) [5-6]. Among the flavonoids, apigenin and luteolin have been demonstrated as antitumor effective compounds by Cai *et al* [7] and Zhang *et al* [8]. The terpenoids in it mainly include cycloeucalenol, 24-methylenecycloartanol and *β*-amyrin. Some ingredients like lobetyolin (**12**) and lobetyolinin (**13**) are considered to be typical polyacetylene constituents of the Campanulaceae (Fig. 3) [9-10]. In addition, coumarins can be found in *L. chinensis*, such as 6, 7-dimethoxycoumarin (**14**), 5, 7-dimethoxy-6-hydroxycoumarin (**15**), 5-hydroxy-7-methoxycoumarin (**16**), and 5-hydroxy-6, 7-methoxycoumarin (**17**) (Fig. 4).

Piperidine alkaloids isolated from L. chinensis

Given the physicochemical properties of alkaloids, several useful methods can be used to extract alkaloids from *L. chinensis,* such as solvent extraction and direct extraction. Kuang *et al* [5] chose reflux extraction to obtain the constituents of *L. chinensis* after comparing the extraction efficiencies of ultrasonic extraction, soaking, percolation, and reflux extraction. The results were obtained through an orthogonal

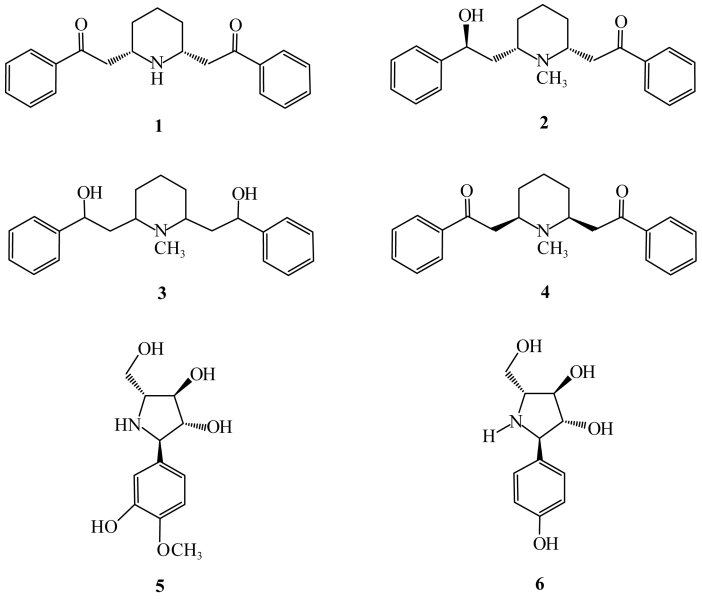


Fig. 1 Structures of the alkaloids isolated from *L. chinensis*: norlobelanine (1), lobeline (2), lobelanidine (3), lobelanine (4), radicamine A (5) and radicamine B (6)

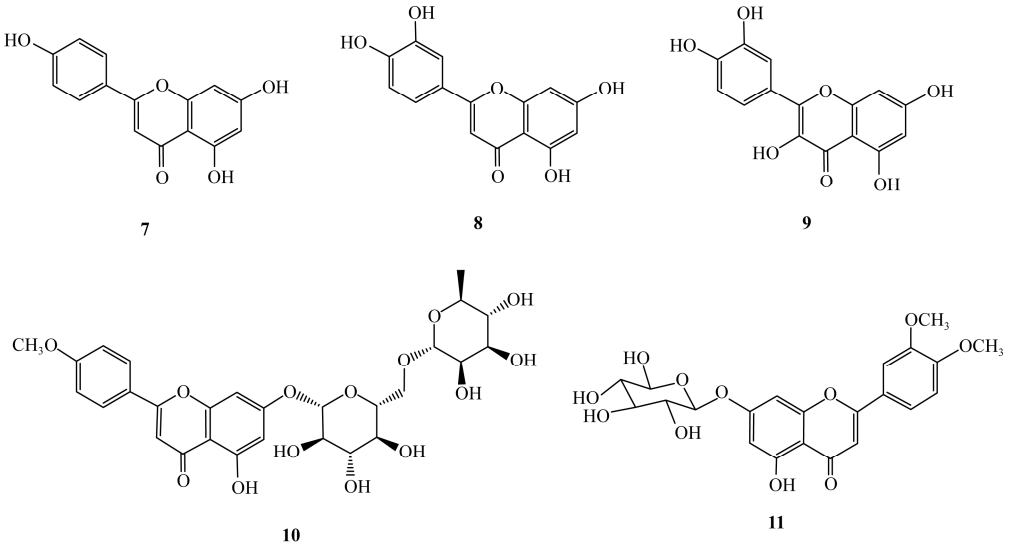
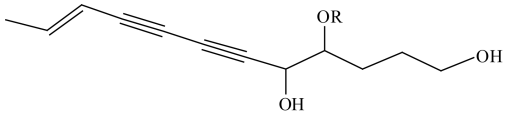


Fig. 2 Structures of the flavonoids isolated from *Lobelia chinensis*: apigenin (7), luteolin (8), quercetin (9), linarin (10) and luteolin 3', 4'-dimethylether-7-*O*-*β*-D-glucoside (11)

experimental design. The best conditions for extraction involved using 90% ethanol, eight times the volume of *whole herb* with refluxing for 1 h twice. In this way, the amount of 17.10 mg·g^{-1} total alkaloids could be extracted.

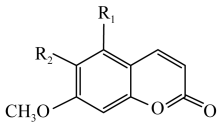
Flavonoids extracted from L. chinensis

Several methods have been applied to extract flavonoids of *L. chinensis.* Huang *et al* [11] optimized the extraction parameters of flavonoid glycosides from *L. chinensis* by ultrosonic-assisted extraction. The optimum extraction conditions were as follows: ethanol concentration of 50%，extraction duration of 35 min，extraction temperature of 50 °C, and solid to liquid ratio of 1 : 50, obtaining 4.21 mg·g^{-1} flavonoid glycosides under these conditions. Subsequently, Huang *et al* [12] studied the ultrasonic-assisted semi-bionic extraction method for the flavonoid glycosides from Herba *L. chinensis*. This method combines holistic medicine and molecular medicine to simulate the progress and circumstances in which oral medicines are taken and absorbed in the gastrointestinal tract, whose aim is to extract and reserve active ingredients of raw materials as far as possible. The content of 4.70 mg·g^{-1} flavonoid glycosides was found by orthogonal design under the optimal conditions as follows: 70% (*V/V*) of ethanol-water as the extracting solvent, 1 : 40 g·mL^{-1} of solid to liquid ratio,70w of ultrasonic power, and 25 min of ultrasonic time. Jiang *et al* [13] used 70% ethanol (12 × *V/W* herbal weight) to extract for three times, 1 h per time, and combined various chromatographic techniques, including (silica gel, polyamide, Sephadex LH-20, ODS, and MCI) and re-crystallization methods to separate nine flavonoids, including apigenin, luteolin, diosmetin, chrysoeriol, hesperidin, loteolin-7-*O*-*β*-D-glucoside, apigenin-7-*O*-*β*-D-glucoside, linarin, and diosmin.



12 lobetyolin R = glc
13 lobetyolinin R = glc^{6}-^{1}glc

Fig. 3 Structures of polyacetylenes isolated from *L. chinensis*: lobetyolin (12) and lobetyolinin (13)



	R_1	R_2
14	H	OCH_3
15	OCH_3	OH
16	OH	H
17	OH	OCH_3

Fig. 4 Chemical structures of coumarins isolated from *L. chinensis:* 6, 7-dimethoxycoumarin (14), 5, 7-dimethoxy-6-hydroxycoumarin (15), 5-hydroxy-7-methoxycoumarin (16) and 6 ,7-dimethoxy-5-hydroxycoumarin (17)

Terpenes and coumarins extracted from L. chinensis

Isolation and purification of *L. chinensis* were carried out by Deng *et al* [14] using silica gel, Sephadex LH-20, and preparative HPLC. Four terpenes including phytol, cycloeucalenol Ⅰ, cycloeucalenol Ⅱ, 24-methylenecycloartanol were obtained. Han *et al* [10] obtained three coumarin compounds (Fig. 4) from 80% ethanol extract of *L. chinensis* by column chromate. Additionally, Chen *et al* [15] also isolated these three coumarins from 95% ethanol extract of full plant of *L. chinensis.*

Antitumor Activity

Contemporary studies have revealed that a decoction of *L. chinensis* provides diuretic, choleretic, excited breathing, anti-central nervous system effects, improved hyperlipidemia and vascular endothelial protection, anti-proliferation of arterial smooth muscle cells, anti-venom, anti-bacterial, and anticancer properties. The decoction is also used to treat abdominal edema, various swellings, snakebites, for atherosclerosis, for vascular disease from renal hypertension, and for tumors, where it shows particular therapeutic effects. Both the decoction and the alkaloids of *L. chinensis* show antitumor activity, creating an area for pharmaceutical research.

Antitumor activity of L. chinensis decoction

The decoction of *L. chinensis* can efficiently restrain liver cancer growth, enhance the immunity of mice, and modulate the expression of P53 and C-erbB-2 in the liver cancer organization, all of which contribute to preventing tumors in the body, as well as curing them [16]. Liu *et al*. investigated the anti-cancer effects of *L. chinensis* on H22 hepatocellar carcinoma-bearing mice, by adjusting P27 and survivin expression [17]. Similar to 5-fluorouracil, the positive control, the *L. chinensis* decoction exhibited stronger P27 and weaker surviving expression. Compared with the control group, the decoction group had significantly different results ($P < 0.05$), however, when comparing the decoction and 5-fluorouracil groups, the difference was insignificant ($P > 0.05$). The *L. chinensis* decoction reduced the size of the H22 liver tumors, achieving an anti-tumor rate of 33.98%, notably lower than the control group ($P < 0.05$) [17]. Based on its efficacy, *L. chinensis* shows activity related to the mechanism of the expression of P27 and survivin. Moreover, Liu *et al* [17] evaluated the anticancer effects on tumor size and anti-tumor rate of *L. chinensis* decoction, which indicated a reduction in tumor weights in 39.48% of mice with significant difference compared to control ($P < 0.05$).

L. chinensis can also cause the tumor cell to die by influencing the Ca^{2+} information in the tumor cell. Gao *et al* [18] conducted a study on the human liver tumor cell $HepG_2$, measuring the concentration of free Ca^{2+} in the $HepG_2$ cell plasma before and after delivering the *L. chinensis.* They determined that *L. chinensis* can increase the concentration of free Ca^{2+} in the liver tumor cells. Further studies have revealed that the altered equilibrium of Ca^{2+} concentration will

activate the endonuclease and break the DNA, follow the DNA ladder, and change the morphology of the tumor cells causing the cells to die. Other research on *L. chinensis* examined the influence of free Ca^{2+} concentration in HeLa cells, and its cell proliferation inhibition mechanism showing that *L. chinensis* did not demonstrate a clear performance in restraining the proliferation of HeLa cells, but did increase the free Ca^{2+} concentration in the cells by promoting the release of Ca^{2+} stored and the internal flow of Ca^{2+} outside the cells [19].

Cytotoxic activity of alkaloids in vitro

With increasing concentration and time, the anti-growth rate of *L. chinensis* alkaloids on BC-38 gastric cancer cells increases. At a concentration of 300 $mg \cdot L^{-1}$, the restraining rate in BC-38 cells reached 85.6%, at 16 h of administration, the restraining rate reaches 90.3% [20]. The altered equilibrium between the endothelin (ET) of artery endothelial cells and the expression of endothelial nitric oxide synthase (ecNOS) may be the main factor for artery endothelial injuries during early atherosclerosis. A comparison between the alkaloids of *L. chinensis* and the total saponins of *Paris chinensis* in terms of their influence on the expression of ET and ecNOS found that both can restrain the synthesis and release of ET, but the alkaloids are better than the *Paris chinensis* saponins in promoting the synthesis and release of ecNOS [21]. Wang *et al.* [22] concluded that alkaloids can restrain the proliferation of VSMC led by ET-1 in a concentration-dependent manner.

Cytotoxic activity of flavonoids in vitro

In recent years, the flavonoid compounds luteolin and apigenin have shown remarkable performance in antitumor activity [23-24]. Luteolin exhibited cancer cells block effects *in vitro* and *in vivo*, involving in protection from carcinogenic stimuli, inhibition of tumor cell proliferation, induction of cell cycle arrest and induction of apoptosis *via* intrinsic and extrinsic signaling pathways [25]. Furthermore, luteolin can cause many kinds of tumor cells to die by strongly activating the TNF-α and TRAIL [26]. TNF-α is powerful in promoting apoptosis after administering the colorectal cancer cell line COLO205, as well as HCT116 and HeLa cells of cervical cancer with luteolin [27]. Xiao *et al* [28] studied the effects of luteolin on HO-8910PM *in vitro* and explored its possible mechanisms, revealing that luteolin can suppress ERK2, decrease the secretion of MMP-9, and further affect the anti-attack activity of ovarian tumor cells, or stop the procession of transfer in ovarian tumor cells. After administration of luteolin (5−20 $\mu mol \cdot L^{-1}$), the apoptosis of liver tumor cells CBRH7919tk/GCV was determined by flow cytometry and the PI staining method. The results revealed dense yellow-green debris in the cell nucleus and cytoplasm, or yellow-green chromatin densely collected in the internal side of the nuclear membrane, in addition, the cell membrane was bubble-like and bulging, and small bodies were apoptotic. An increase in the drug concentration enhanced the drug therapeutic efficacy through apoptosis [29]. Wang *et al* [30] delivered the MTS to investigate the anti-proliferative effects of luteolin on A549, HeLa, MCF-7, Caco2, AGS, MGC-803, and HepG2 cells. Their study revealed that 10 $\mu mol \cdot L^{-1}$ of luteolin produced little anti-proliferation activity in the cells, but luteolin was shown to enhance the anti-activity of bexarotene in HeLa cell proliferation, even when the concentration of bexarotene was quite low (0.1 $\mu mol \cdot L^{-1}$). This finding reveals that low concentration of luteolin has little toxic effect on the cancer cell line, but it can sensitize chemotherapeutic drugs like bexarotene in various cancer cell lines.

Antitumor activity of herbal extraction

Liang *et al* [31] observed the inhibition effect of a plant preparation containing *L. chinensis* on the tumor growth in S180 sarcoma transplanted mice. The results revealed that this plant product can apparently restrain the tumor growth *in vivo.* Recently, the cytotoxic activity of *L. chinensis* has received growing attention. Shao and co-workers [16] explored the cytotoxic activity of *L. chinensis* extract on C-erbB-2 and P53 of H22 rat liver tumor cells lines. Liu *et al* [17] studied the cytotoxic activity of a *L. chinensis* extract on H22 liver tumor cells lines of rats and investigated the effect on the expression of P27 and survivin. Moreover, Gao and co-workers [19] evaluated the anti-Hela cells effects of *L. chinensis* extracts on the influence of cardinal on cytoplasmatic free Ca^{2+} density. With co-incubation of 7.5 $mg \cdot mL^{-1}$ extraction solution for 5d, a conclusion was drawn that it could lead to the death of liver tumor cells by promoting intracellular Ca^{2+} release and extracellular Ca^{2+} withdrawal. The Ca^{2+} pathway has been demonstrated to relate to apoptosis. Thus, increasing intracellular Ca^{2+} concentration may be the mechanism for promoting tumor cell apoptosis. In addition, some researchers also explored the alkaloids and flavonoids from *L. chinensis* which mainly contribute to the cytotoxicity. So far, a number of *in vitro* experiments about the total alkaloids have been reported, but the mechanism of action has been explored only rarely [32]. Furthermore, 10−30 $\mu mol \cdot L^{-1}$ concentration of apigenin and luteolin showed cytotoxic activity by anti-proliferation of tumor cells and the stopping of the cell growth cycle [33].

The anticancer activity of *L. chinensis* was mainly explored *in vitro*. However, research has revealed its anticancer effects by molecular docking method based on targeting proteins [34]. Kaempferol-3-*O*-β-glucoside in *L. chinensis* was screened out as the effective compound on Bcl-xl protein to promote apoptosis.

Conclusions

L. chinensis contains ingredients that possess anticancer activity. An examination of the extraction and therapeutic constituents of *L. chinensis* in terms of the anticancer activity will affect its comprehensive utilization and exploitation. Understanding the chemical constituents and the corresponding extractive methods are the premise for future studies of *L. chinensis*. Indeed, defining the technology and methods of extraction

is essential for future research. Based on recent studies of *L. chinensis*, it is known that *L. chinensis* has anticancer activity, however, whether it produces such activity on other kinds of tumor cells, and the details of its anticancer mechanism are not yet well understood and require further exploration.

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