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Variation in contents of active components and antibacterial activity in different parts of *Lonicera japonica* Thunb

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Abstract

Background: *Lonicera japonica* Thunb has been comprehensively used as a traditional Chinese medicine which contains a variety of medicinally active substances.

Objectives: The active constituents in various parts of *L. japonica* Thunb (leaves [LE], flowers [FL], and green buds [GB]) were studied to rational and efficient use of this medicinal plant.

Methods: UV–vis spectrophotometry was used for the determination of the total flavonoids; gas chromatography–mass spectrometric method was employed for the analysis of volatile components; and high-performance liquid chromatography technology was applied to detect nine bioactive components including chlorogenic acid, neochlorogenic acid, isochlorogenic acid C, caffeic acid, luteolin, galuteolin, hyperoside, rutin, and quercetin in different parts of *L. japonica* Thunb. The antimicrobial effects of LE, FL, and GB were further measured using agar plate drilling method.

Results: The content of the total flavonoids in different portions of *L. japonica* Thunb has significant difference, and the content with the decreasing sequence is found in GB, LE, and FL. The contents of main volatile components were highest in FL, followed by GB and LE. Besides, the contents of galuteolin, luteolin, rutin, and isochlorogenic acid C were higher in LE than those in FL and GB, and neochlorogenic acid content was similar in LE and FL. The antimicrobial effects showed that LE had the best inhibitory activity on *Staphylococcus aureus* (SA) and extended spectrum β-lactamases SA.

Conclusions: The research in this study provided valuable evidences for the reasonable and efficient utilization of *L. japonica* Thunb.

Keywords: antibiosis; flavonoids; *Lonicera japonica* Thunb; volatile ingredients

Lonicera japonica Thunb is a common medicinal plant, which plays a significant role in traditional Chinese medicine formula for treating exogenous wind-heat, sores, epidemic febrile diseases, carbuncles, and some other infectious diseases [1]. The modern pharmacological researches show that *L. japonica* possesses a wide range of pharmacological activities, such as antibacterial, anti-inflammatory, antiviral, anti-endotoxin, and hypolipidemic activities [2–7]. Additionally, it is also used in health products, cosmetics, and soft drinks for its specific activities [8–11]. Therefore, many

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scholars have drawn great attention on *L. japonica* Thunb. *L. japonica* Thunb contains a variety of medicinally active ingredients, mainly including organic acids, flavonoids, iridoids, saponins, and volatile oils [12–18].

Among them, flavonoids are the main constituents which have a vital role in the treatment of liver injury, cardiovascular disease, and cerebrovascular disease [19, 20]. Moreover, volatile components exist in the aerial parts of *L. japonica* Thunb and used in medicine, cosmetics, spices, and other industries [21–23]. In addition, organic acid is another significant component of *L. japonica* Thunb [24]. Chlorogenic acid, as well as galuteolin, is the main antimicrobial component which is considered as the criterion for evaluating the quality of *L. japonica* Thunb [25].

However, the conventional medical part of this plant is flower (FL) [26]. In China, the output of leaves (LE) was about nine times more than FL [27] but they are considered as a useless part of the plant and discarded by herbal medicine market which might lead to a huge loss of potential medical resources.

In this study, we focused on the active ingredients in different parts of *L. japonica* Thunb. Through the preliminary experiments, it was found that the contents of luteoside and galuteolin in LE were greater than those in FL [28, 29]. To get more comprehensive data, rational exploitation, and utilization of this medicine, UV–vis, high-performance liquid chromatography (HPLC), and gas chromatography–mass spectrometry (GC-MS) measurements were used to determine the contents of active components. Additionally, the antimicrobial effect of contents were also investigated and compared using agar plate drilling method.

Materials and methods

Neochlorogenic acid, isochlorogenic acid C, and hyperoside were purchased from Vic's Biological Technology Co., Ltd. (Sichuan, China). Rutin was purchased from Aladdin Industrial Corporation (Shanghai, China). Luteolin, quercetin, chlorogenic acid, and caffeic acid were provided by National Institutes for Food and Drug Control (Beijing, China). Galuteolin was separated and identified from LE of *L. japonica* Thunb in our laboratory (over 98% purity) [30]. HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany) and water was supplied by a Millipore Water Purification System (Millipore, Bedford, MA, USA). Other chemicals were all of analytical reagent grade.

The strains including *Staphylococcus aureus* (SA, ATCC25923), extended spectrum β-lactamases SA (ESBL-SA), *Escherichia coli* (EC), and ESBL-EC were obtained from Huaihe Hospital affiliated to Henan University (Kaifeng, China).

The LE, GB, and FL were collected in June from Medicinal Plant Garden, Henan University (Kaifeng, China). All voucher specimens, which were authenticated as *L. japonica* Thunb LE, GB, and FL by Professor Qin Li (School of Pharmacy of Henan University), were dried in oven at 40ºC until the weight remained constant. The dried plant material was ground into powder and passed through a 40-mesh sieve.

The UV–vis absorption spectra was carried out on a TU-1900 spectrophotometer (Puxi Analytic Instrument Ltd., Beijing, China) equipped with 1.0 cm quartz cells. The HPLC analyses were measured on an Agilent 1200 HPLC system (Agilent Technologies, MA, USA), equipped with a quaternary pump, an autosampler, a column heater–cooler, and a diode array detector (DAD). The separation was performed on a COSMOSIL 5C18-PAQ (4.6 mm i.d. 250 mm, 5 μm). The GC-MS analyses were carried out using "Agilent 7890 A" gas chromatograph coupled with 7000B triple quadrupole mass spectrometer (Agilent Technologies).

Determination of total flavonoids

Of note, 2 g powder of different parts of *L. japonica* Thunb was refluxed three times at 80°C using 100 mL of 80% aqueous methanol (v/v) for 3 h. The extracting solution was merged for vacuum rotatory evaporation at 35°C and completely dried in an atmospheric oven. Then the crude extract solutions were obtained and diluted with methanol to 50 mL. All extracts were stored in refrigerator and filtered before analysis.

Total flavonoids were determined according to the methods in Pharmacopoeia of the People's Republic of China, using rutin as the standard [25]. In a 10-mL Eppendorf tube, 0.2 mL of extract, 0.5 mL of 5% $NaNO_2$, 0.5 mL of 10% $AlCl_3$, 4.0 mL 4% NaOH, and 4.8 mL distilled water were added sequentially every 5 min. The absorbance was measured at 510 nm. Accuracy was evaluated by analyzing the standard solution six times. Then, the relative standard deviation (RSD) of the absorbance of the standard compounds was calculated. To confirm the repeatability, five different sample solutions prepared from the same sample were analyzed and RSD was used to indicate the change. For stability studies, each of the above sample solutions was analyzed at 10, 20, 30, 40, 50, and 60 min, respectively. Recovery test was used to evaluate the accuracy of the method. The ratio of detection quantity (actual)

to scalar quantity (theoretical) was used to calculate the recovery rate.

Determination of volatile oil using GC-MS

One gram of powder was immersed with ether for 12 h to obtain the crude extract solutions. Then the extracts were stored at 4°C until use.

Using helium as a carrier gas, the components were separated at a constant flow rate of 1.0 mL/min for 45 min. The 5 μL sample extract was injected. The initial temperature was set at 100°C, whereas the injector temperature was set at 280°C. Throughout the process, temperature flow was set at the speed of increasing 10°C/min.

The mass ionization was carried out using electron ionization mode at +70 eV and acquired *m*/*z* from 30 to 650.

Determination of nine analytes using HPLC

The samples prepared as 2.3 were filtered through a Millipore filter (0.45 μm) before analysis. The nine analytes were measured according to our previous work [31]. A mixture of solvent A (0.5% aqueous acetic acid; v/v) and solvent B (acetonitrile) was used as the mobile phase at a flow rate of 1.0 mL/min. The gradient elution program was 0–10 min, 12–20% B; 10–20 min, 20% B; and 20–40 min, 20–30% B. The column temperature was kept at 25°C; the detection wavelength was 327 nm; and the injection volume was 10 μL.

In vitro antibacterial activity study

The antimicrobial activity was tested by agar well diffusion method against both gram-positive and gram-negative bacteria. Each strain was grown in sterile nutrient broth at 37°C for 24 h and stored at 4°C before use. Twenty milliliters of sterilized agar medium were poured into each petri dish. Four holes were drilled in the agar medium orderly of each dish after medium was solidified. Plant extracts (50 μL) dissolved in sterile water at 1 g/mL concentration were added in the holes, and the same volume (50 μL) of sterile water was used as a negative control. Approximately 50 μL of suspension (10^6 cfu/mL) of the test microorganisms was smeared uniformly onto the medium. The inoculated plates were incubated for 24 h at 37°C. Each sample was assayed in triplicate (1 disk/plate). After incubation, the diameters (mm) of the inhibition zone were measured.

Results

Total flavonoids in L. japonica Thunb LE, GB, and FL

The total flavonoids concentration was calculated from a calibration curve using rutin as the standard. A series of rutin standard solutions were prepared in methanol in the range of 10–70 μg/mL to obtain the linear response ($Y = 0.00329 + 0.0122X$, $r = 0.9995$, where Y is the absorbance at 510 nm and X is the concentration of total flavonoids [μg/mL]). The precision, repeatability, and stability were presented as RSD of 1.42, 2.13, and 1.79%, respectively. The recovery of the total flavonoids was 98.32%. All data demonstrated that the established method is accurate enough for the determination of total flavonoids in *L. japonica* Thunb LE, GB, and FL.

Qualitative evaluations were performed on *L. japonica* Thunb samples of LE, GB, and FL, which are shown in **Table 1**. The contents of total flavonoids in LE, GB, and FL were 6.68, 7.38, and 6.35%, respectively. It can be seen that the content of total flavonoids in LE of *L. japonica* Thunb is lower than that in GB; however, it is comparable with that in FL, which is consistent with the reports in literatures [28].

Nine analytes in LE, GB, and FL

From the above results, it can be seen that there was difference in total flavonoids in different part of *L. japonica* Thunb. However, it is not clear which flavonoid or component plays the determinative role. Therefore, nine analytes that commonly appeared in this plant were used for further comparison. The nine bioactive components including chlorogenic acid, neochlorogenic acid, isochlorogenic acid C, caffeic acid, luteolin, galuteolin, hyperoside, rutin, and quercetin were determined in different parts of *L. japonica* Thunb. The typical chromatography of standards and leaf sample are shown in **Figure 1**. Their contents are summarized in **Table 2**. First, the content of isochlorogenic acid C

Table 1. Total flavonoids in *L. japonica* Thunb leaves (LE), green buds (GB), and flowers (FL) ($n = 3$)

	LE*	GB	FL
Content/%	6.68	7.38	6.35
RSD %	0.63	0.41	1.42

*The LE were collected in June 2015 with FL and GB.

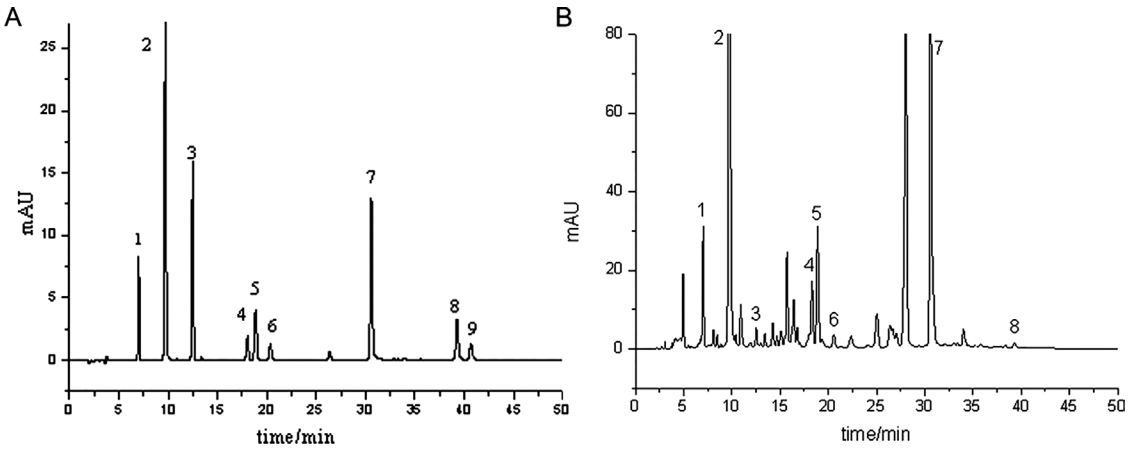

Figure 1. Representative high-performance liquid chromatograms of standard mixture **(A)** and sample **(B)** of honeysuckle leaves. Peaks identification: 1, neochlorogenic acid; 2, chlorogenic acid; 3, caffeic acid; 4, rutin; 5, galuteolin; 6, hyperoside; 7, isochlorogenic acid C; 8, luteolin; and 9, quercetin. Chromatographic conditions are as depicted in the text.

Table 2. The contents of nine components in *L. japonica* Thunb leaves (LE), green buds (GB), and flowers (FL)

Compound	Content/mg/g		
	LE*	GB	FL
Galuteolin	1.80	0.03	0.045
Neochlorogenic acid	0.55	0.51	0.40
Chlorogenic acid	17.75	24.91	16.96
Isochlorogenic acid C	140.47	112.20	120.09
Caffeic acid	0.011	0.03	0.018
Rutin	13.82	0.95	2.50
Hyperoside	–	–	–
Luteolin	0.17	0.066	0.074
Quercetin	–	–	–

*The LE were collected in June 2015 with FL and GB.

was much higher than other components in each part of *L. japonica* Thunb. Furthermore, the results indicated that the contents of galuteolin, luteolin, rutin, and isochlorogenic acid C were obviously higher in LE than those in FL and GB, and neochlorogenic acid content was similar in LE and FL. The contents of caffeic acid and chlorogenic acid detected in GB were the highest. Quercetin was not detected in all the samples even with sample concentration or using different sample disposal method. It can be concluded that quercetin does not exist in this kind of plant that we investigated. Interestingly, hyperoside was not detected in FL, GB, and LE which were collected from April to September. However, it can be found in other 6 months. It may relate to the temperature or the plant metabolism. The specific reason remains unknown at present and needs further research.

Volatile components in LE, GB, and FL

The volatile components in different sections of *L. japonica* Thunb were determined by GC-MS. Their content percentage is expressed by the area normalization method of total ion current (**Figure 2**). As shown in **Table 3**, the contents of 45 volatile components in samples were listed in details. There were 25 types of compounds detected in GB, compared with 21 in LE and 20 in FL. The results showed that there were no significant differences of the main compositions among FL, LE, and GB, and most of the contents were hydrocarbons with 66.07% in LE, 70.75% in FL, and 67.94% in GB, respectively. Apart from hydrocarbons, the content of terpenoids was the highest in LE (15.36%), which was higher than that of GB (10.69%). Interestingly, no terpenoid was found in FL. In addition, the content of (3β)-9,19-cyclolanost-24-en-3-ol was the highest in LE (13.51%), and 17 and 26 methyl carbonate were found only in FL and GB.

Antibacterial activity assays

SA belongs to gram-positive bacteria and is one of the most common pathogens. It can cause systemic infection such as sepsis, pneumonia, and pseudomembranous enteritis, and it is commonly found on the surface of skin and upper respiratory tract mucosa. EC is a gram-negative bacterium, which can cause neonatal meningitis, urinary tract and intestinal tract infection, and other diseases, and it is commonly found in human and animal intestines. Based on these points, SA and EC were selected as the tested strains to study the antibacterial effect of different parts of honeysuckle. The bacteriostatic

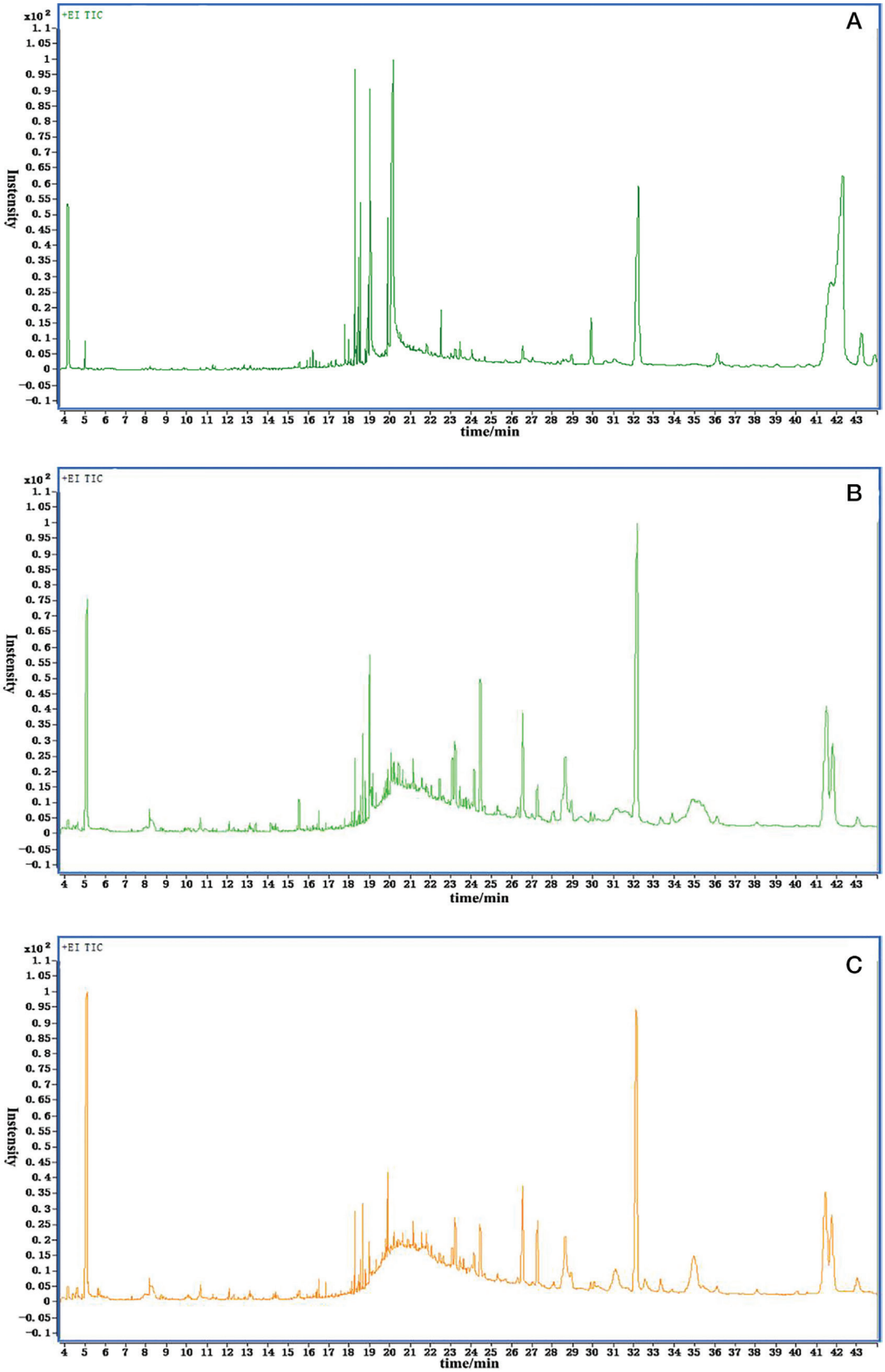

Figure 2. Total ion chromatogram of the volatile components in June in leaves (LE) (**A**), flowers (FL) (**B**), and green buds (GB) (**C**) of *L. japonica* Thunb. The chromatographic and mass spectrometric conditions are as depicted in the text. The LE were collected in June 2015 with FL and GB.

results of inhibition zone are shown in **Figure 3** and **Table 4**. As can be seen, all the samples had antibacterial effect on SA and ESBL-SA. However, there was no obvious effect on EC and ESBL-EC. The cell wall of gram-positive bacteria mainly composed of the peptidoglycan, and flavonoids and organic acids in *L. japonica* Thunb can solubilize peptidoglycan components which can dissolve the cell wall. The cell wall of gram-negative

Table 3. Volatile components in *L. japonica* Thunb leaves (LE), green buds (GB), and flowers (FL)

	Compound	Content/%*		
		LE†	GB	FL
1	1,4-Eicosadiene1	0.94	0.47	0.42
2	Tricosane	–	0.50	0.50
3	(*Z*)-9-Octadecenamide	–	0.37	–
4	3-Ethyl-5-(2-ethylbutyl)-octadecane	–	1.42	–
5	Heptacosane	–	6.70	7.59
6	Tetratriacontane	6.79	4.79	–
7	Nonacosane	10.11	30.71	32.82
8	Tetratetracontane	–	0.62	–
9	Triacontane	8.33	14.60	19.67
10	17-Pentatriacontene	17.67	7.24	7.24
11	Squalene	8.72	0.52	0.66
12	Pentacosane	–	–	1.85
13	Hexadecanamide	–	–	–
14	9-Octadecene,1,1 -[1,2-ethanediylbis(oxy)] bis-(*Z*,*Z*)-	–	–	–
	Hydrocarbons/%	66.07	67.94	70.75
15	2-Heptadecanone	–	1.49	1.59
16	Bacteriochlorophyll-*c*-stearyl	0.41	–	–
17	6,10,14-Trimethyl-2-pentadecanone	0.17	–	–
18	2-Nonadecanone	–	0.33	–
19	Phytol	1.85	1.90	0.52
20	(*Z*)-Ethanol, 2-(9-octadecenyloxy)-	–	0.20	–
21	γ-Sitosterol	6.92	6.58	8.35
22	(*Z*,*Z*,*Z*)-9,12,15-Octadecatrien-1-ol	–	–	–
23	Stigmasterol	–	–	–
24	*Trans*-Geranylgeraniol	–	–	0.37
25	14,16-Hentriacontanedione	–	–	6.74
	Alcohol ketones/%	9.35	10.50	17.57
26	*n*-Hexadecanoic acid	–	0.75	3.76
27	Phytol, acetate	3.56	1.36	1.13
28	(*Z*,*Z*,*Z*)-9,12,15-Octadecatrienoic acid	0.74	0.29	–
29	(*Z*)-13-Docosenamide	6.34	–	1.41
	Acids/%	10.64	2.40	4.89
30	15-Isobutyl-(13α*H*)-isocopalane	1.46	–	–
31	8,14-Seco-3,19-epoxyandrostane-8,14-di-one, 17-acetoxy-3β-methoxy-4,4-dimethyl-	0.39	–	–
32	(3β,24*Z*)-Stigmasta-5,24(28)-dien-3-ol	–	–	–
33	(3β)-9,19-Cyclolanost-24-en-3-ol	13.51	10.69	–
	Terpenes/%	15.36	10.69	0
34	Docosanoic acid, methyl ester	–	0.41	–
35	(*Z*,*Z*,*Z*)-9,12,15-Octadecatrienoic acid, methyl ester	–	–	–
36	Tetracosanoic acid, methyl ester	–	3.81	1.72
37	Hexacosanoic acid, methyl ester	–	1.26	0.72
38	Hexadecanoic acid, methyl ester	0.41	–	0.79
39	Heptanoic acid, docosyl ester	–	–	–
40	(*Z*,*Z*)-9-Hexadecenoic acid, 9-octadecenyl ester	–	0.24	–
41	Oleic acid, 3-(octadecyloxy)propyl ester	–	–	–
42	Oleic acid, eicosyl ester	0.37	–	–
	Lipids%	0.78	5.72	3.23
43	2,2 -Methylenebis[6-(1,1-dimethylethyl)-4-methyl-phenol]	1.42	–	–
44	Vitamin E	2.42	2.03	1.51
	Derivatives/%	3.84	2.03	1.51

*The content was calculated by area normalization method.
†The LE were collected in June 2015 with FL and GB.

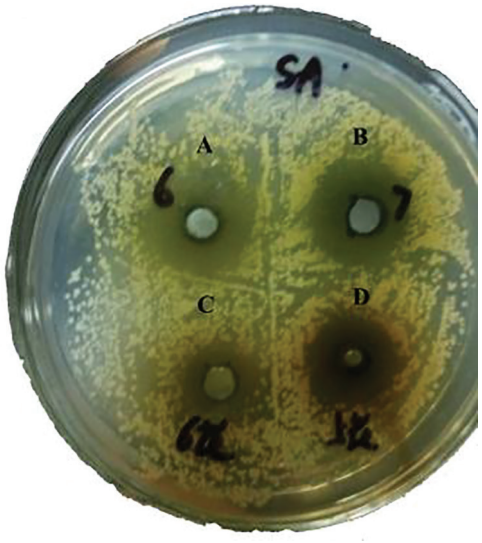

Figure 3. The antibacterial effect on *Staphylococcus aureus*. (**A**) Leaves (LE) were collected in June, (**B**) LE were collected in July, (**C**) green buds (GB), and (**D**) flowers (FL). The FL and GB were collected in June 2015.

Table 4. Bacteriostatic results of *L. japonica* Thunb leaves (LE), green buds (GB), and flowers (FL)

Samples	Concentration (g/mL)	Diameters of the inhibition zone (mm)					
		SA	RSD/%	ESBL-SA	RSD/%	EC	ESBL-EC
LE*	1.0	13	0.78	14	0.74	–	–
GB	1.0	11	1.17	13	0.43	–	–
FL	1.0	12	0.45	11	1.30	–	–

*The LE were collected in June 2015 with FL and GB.
SA, *Staphylococcus aureus*; ESBL-SA, extended spectrum β-lactamases SA; EC, *Escherichia coli*; ESBL-EC, extended spectrum β-lactamases EC.

bacteria is mainly composed of the lipid composition, and the antibacterial ingredient in *L. japonica* Thunb is difficult to dissolve, thus the inhibitory effect on gram-negative-bacteria is relatively poor [32]. Results showed that the LE had better inhibitory effect on SA and ESBL-SA (bacteriostatic ring was 13 and 14 mm, respectively) than GB (bacteriostatic ring was 12 and 11 mm, respectively) and FL (bacteriostatic ring was 11 and 13 mm, respectively) at the same picking period. From **Table 2**, the results indicated that the contents of galuteolin, luteolin, rutin, and isochlorogenic acid C were obviously higher in LE than those in FL and GB. Therefore, the extract of LE more easily dissolves the cell wall of SA and ESBL-SA. As a result, *L. japonica* Thunb LE had the best antibacterial activity that was even better than FL and GB.

Conclusions

In summary, this study investigated the natural biologically active ingredients of the different parts of *L. japonica* Thunb. The content of total flavonoids in LE and GB in June was 6.68 and 7.38%, respectively, higher than that in FL (6.35%). There were no significant differences in the main volatile components among FL, LE, and GB, and dominant constituents are hydrocarbons with 66.07% in LE, 70.75% in FL, and 67.94% in GB. In addition, the contents of galuteolin, luteolin, rutin, and isochlorogenic acid C were higher in LE than those in FL and GB, and neochlorogenic acid content was similar in LE and FL. All samples had only antibacterial effect on SA and ESBL-SA. LE had the best inhibitory effect on SA and ESBL-SA, which was even better than FL and GB.

The results are helpful in understanding the rational use of resources of *L. japonica* Thunb, and they provide additional basic evidence for the quality evaluation of *L. japonica* Thunb. This study also suggested that *L. japonica* Thunb is a sustainably used resource, especially the LE and it deserves further exploiting.

Author contributions. JZ and XL contributed substantially to the conception and design of this study. LY, YW, ML, and DZ contributed substantially to the acquisition of data. LY and YX analyzed and interpreted the data. JZ drafted the manuscript. LY, YX, YW, ML, XL, and DZ contributed substantially to its critical revision. All the authors approved the final version submitted for publication and take responsibility for the statements made in the published article.

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Conflict of interest statement. The authors have completed and submitted the International Committee of Medical Journal Editors Uniform Disclosure Form for Potential Conflicts of Interest. None of the authors disclose any conflict of interest.

References

[1] Shang XF, Pan H, Wang XZ, He H, Li MX. *Leonurus japonicus houtt*: ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine. J Ethnopharmacol. 2014; 152:14–32.

[2] Jiang M, Han YQ, Zhou MG, Zhao HZ, Xiao X, Hou YY, et al. The screening research of anti-inflammatory bioactive markers from different flowering phases of Flos *Lonicerae japonicae*. PLoS ONE. 2014; 9:e96214.

[3] Park KI, Kang SR, Park HS, Lee DH, Nagappan A, Kim JA, et al. Regulation of proinflammatory mediators via nf-κb and p38 mapk-dependent mechanisms in raw 264.7 macrophages by polyphenol components isolated from Korea *Lonicera japonica* Thunb. Evid Based Complement Alternat Med. 2012; 2012:828521.

[4] Rhee KH, Lee KH. Antimicrobial effects of *Lonicera japonica* against gram positive and gram negative anaerobic bacteria. Nat Prod Sci. 2011; 17:23–5.

[5] Chen JJ, Fang JG, Wan J, Feng L, Zhang YW, Zhao JH, et al. An in vitro study of the anti-cytomegalovirus effect of chlorogenic acid. Her Med. 2009; 28:1138–41.

[6] Zhang RL, Hua YP, Liu D. Study on the antiviral effects of Flos *Lonicerae* against Newcastle disease in chick embryo. J Tradit Chin Vet Med. 2010; 4:33–6.

[7] Wang DY, Zhao XM, Liu YL. Hypoglycemic and hypolipidemic effects of a polysaccharide from flower buds of *Lonicera japonica* in streptozotocin-induced diabetic rats. Int J Biol Macromol. 2017; 102:396–404.
[8] Chen HL. Flos *Lonicerae* health-promoting tobacco shred and preparation method. 2016; Patent: C N. CN 105795504 A.
[9] Meng H. External use skin care composition and lotion having radiation-proof and skin barrier repair effect, and preparation method. 2015; Patent: C N. CN 105193649 A.
[10] Tu H. *Lonicerae* flos blueberry wine production process. 2017; Patent: CN. CN 106434155 A.
[11] Xiang L, Yang ZJ, Kang HP, Chen ZB, Li N. A kind of *Lonicerae* Flos instant solid beverage and its preparation method. 2017; Patent: C N. CN 106387597 A.
[12] Ryu KH, Rhee HI, Kim JH, Yoo H, Lee BY, Um K, et al. Anti-inflammatory and analgesic activities of SKLJI, a highly purified and injectable herbal extract of *Lonicera japonica*. Biosci Biotechnol Biochem. 2010; 74:2022–8.
[13] Wang LN, Jiang Q, Hu JH, Zhang Y, Li J. Research progress on chemical constituents of *Lonicerae japonicae* flos. Bio Med Res Int. 2016; 2016:1–18.
[14] Kumar N, Singh B, Bhandari P, Gupta AP, Uniyal SK, Kaul VK. Biflavonoids from *Lonicera japonica*. Phytochem. 2005; 66:2740–4.
[15] Zhang DY, Yao XH, Duan MH, Wei FY, Wu GH, Li L. Variation of essential oil content and antioxidant activity of *Lonicera* species in different sites of China. Ind Crop Prod. 2015; 77:772–9.
[16] Yu Y, Song W, Zhu C, Lin S, Zhao F, Wu X. Two homosecoiridoids from the flower buds of *Lonicera japonica*. J Nat Prod. 2011; 74:2151–60.
[17] Atiqur R, Kang SC. In vitro control of food-borne and food spoilage bacteria by essential oil and ethanol extracts of *Lonicera japonica* Thunb. Food Chem. 2009; 116:670–5.
[18] Oku H, Ogawa Y, Iwaoka E, Ishiguro K. Allergy-preventive effects of chlorogenic acid and iridoid derivatives from flower buds of *Lonicera japonica*. Biol Pharm Bull. 2011; 34:1330–3.
[19] Ma JL, Shang KY, Zhou YH. Protective effects of total flavonoids from leaves of *Lonicera japonica* on CCl_4-induced acute liver injury in mice. Chin J Exp Tradit Med Form. 2012; 18:304–7.
[20] Rendeiro C, Rhodes JS, Spencer JP. The mechanisms of action of flavonoids in the brain: direct versus indirect effects. Neurochem Int. 2015; 89:126–39.
[21] Wang YP, Xue XY, Zhang FF, Liang XM. Separation and analysis of volatile oil from *Lonicera japonica* thumb. World Sci Technol. 2008; 10:45–55.
[22] Wang LJ. The study progress of *Lonicera japonica*. Med Inform. 2010; 8:2293–6.
[23] Yang ML, Zhao YG. A comparative study of composition of volatile oil of Flos *Lonicerae* of Yuanzhou in Ningxia in different months. J Ningxia Univ (Nat Sci Edit). 2007; 28:140–2.
[24] Wang XQ, Wei FY, Wei ZF, Zhang L, Luo M, Zhang YH, et al. Homogenate-assisted negative-pressure cavitation extraction for determination of organic acids and flavonoids in honeysuckle (*Lonicera japonica* thunb.) by LC-MS/MS. Sep Purif Technol. 2014; 135:807.
[25] National Pharmacopoeia Committee. Pharmacopoeia of the People's Republic of China. 1st ed. Beijing: China Medical Science Press; 2010. p. 250.
[26] Williamson EM, Lorenc A, Booker A, Robinson N. The rise of traditional Chinese medicine and its materia medica: a comparison of the frequency and safety of materials and species used in Europe and China. J Ethnopharmacol. 2013; 149:453–62.
[27] Liu P. Profile on the research of hydrosoluble antioxidant in China. China Food Addit. 2003; 6:70–2.
[28] Wang K, Wang YY, Zhao DB, Liu XH, Li MJ, Xu PH. Determination of the contents of luteolin and luteoloside in different parts of *Lonicera japonica* by HPLC. J Henan Univ. 2011; 41:39–43.
[29] Wang K, Wang Y, Zhao DB, Liu XH, Li MJ, Xu PH. Determination of the contents of total flavonoids in *Lonicera japonica* and its leaves by three wavelength spectrophotometry. Chin J Anal Lab. 2011; 30:28–41.
[30] Qing WX, Wang YY, Wang YY, Liu XH, Zhao DB. Preparative separation of luteolin-7-O-β-D-glucoside and lonicerin from leaves of *Lonicera japonica* by high speed countercurrent chromatography. Chin J Pharm. 2015; 46:145–8.
[31] Zhu JH, Wang YY, Qing WX, Liu XH, Zhao DB. Determination of nine active constituents in the leaves of *Lonicera Japonica* Thunb by HPLC. Chem Res. 2017; 28:70–4.
[32] Gu HM, Wang HX. The bacteriostasis of chrysanthemum and honeysuckle. J Zhoukou Norm Univ. 2010; 27:85–7.