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Research Article

Effect of Different Extraction Methods on Major Bioactive Constituents at Different Flowering Stages of Japanese Honeysuckle (*Lonicera japonica* Thunb.)

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Abstract

Japanese honeysuckle (*Lonicera japonica* Thunb.) is a traditional Chinese medicinal herb usually used to treat inflammatory disorders. The contents of two major active constituents of Japanese honeysuckle, chlorogenic acid and luteolin, vary with the plant's development stage. This study aims to analyze the antioxidants and major active constituents of Japanese honeysuckle using different extraction methods at various flowering stages. To achieve the highest efficacy of major active constituents, methanol extraction was better than water extraction. After treatment with microwaves for 3 Min (M3), the mean contents of chlorogenic acid and luteolin obtained using methanol were 42.96 and 1.22 mg/g DW, respectively, which were highest of all treated groups. Of the different flower bud stages, total phenolic content was highest during the Silver Flower (SF) stage, with mean values of 26.91 and 25.82 mg/g DW for water and methanol extraction, respectively. The highest trolox content was also observed at this stage, with mean values of 27.02 and 28.62 mg/g DW for methanol and water extraction, respectively. The level of chlorogenic acid increased with the development of flower buds from the Juvenile Bud (JB) stage to the Gold Flower (GF) stage. The highest value obtained using methanol extraction was 24.45 mg/g DW at the SF stage and the highest values obtained using water extraction were 11.28 and 11.25 mg/g DW at the White Bud (WB) and SF stages, respectively. On the other hand, the content of luteolin obtained using methanol extraction decreased with flower bud development. The highest luteolin levels (0.60 mg/g DW) were found at the JB and Green Bud (GB) stages. For water extraction, however, luteolin content increased with flower bud development, reaching its highest level (0.142 mg/g DW) at the GF stage. The present results can be applied to determine the optimal harvest stage and postharvest treatment method for Japanese honeysuckle.

Keywords: Chlorogenic acid; Extraction method; Flower bud stages; *Lonicera japonica* thunb.; Luteolin

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Introduction

In Asia, Chinese Traditional Medicine (CTM) has been used to treat various diseases for thousands of years. Functional phytochemicals are acquired from many natural herbal sources with potent bioactivity. *Lonicera* (Caprifoliaceae) is a traditional medicinal herb genus comprised of more than 150 species that is widely distributed in Eastern Asia, including China, Japan, Korea and Taiwan [1]. They are perennial climbing plants with white-to-golden flowers and a rich, sweet smell. The Japanese honeysuckle (*L. japonica* Thunb., also known as Jin Yin Hua in Chinese) is a main species of *Lonicera* widely used in CTM [2].

The dried flower buds of *L. japonica* Thunb. have been used not only for tea but also for treating wind-heat, fever and *respiratory* tract *infection [3]*. Furthermore, *L. japonica* Thunb. is often combined with other medicinal herbs to treat many diseases [4]. For example, it is often combined with Gingyo-San and used as a traditional antipyretic treatment for the common cold and influenza [5]. When combined with Shuang-Huang-Lian, it is used to treat respiratory diseases [6,7] and when combined with Jiangtang tablets, it is an anti-diabetic treatment [8]. Several pharmacological studies have demonstrated that the constituents of *L. japonica* Thunb. Possess much bioactivity and many functions, such as antibacterial [9], anti-inflammatory [10], antioxidant [11] antiviral [12] and hepatoprotective functions [13, 14]. Recently, due to the outbreak of COVID-19 worldwide, *L. japonica* Thunb. has received increasing attention because it is reported to be capable for the prevention of COVID-19.

Phytochemicals are natural chemicals originally extracted from alga, vegetables, fruits and herbs. Dietary phytochemicals may have

beneficial health effects, such as protection against disease and regulatory gene expression [15,16]. Several studies have indicated that *L. japonica* Thunb. is rich with phytochemicals, including volatiles (linalool, epoxylinalool, geraniol, α-trepineol, etc.) [17,18], phenolic acids (chlorogenic acid, isochlorogenic acid, caffeic acid, etc.) [19] and flavonoids (luteolin, rutin, quercetin, etc.) [20]. *According to the Pharmacopoeia of the People's Republic of China (2005 version), the indicator constituents of L. japonica Thunb. (i.e., chlorogenic acid and luteolin) should keep above 1.5 % and 0.05 %, respectively. Medicinally, chlorogenic acid functions primarily as an* anti-tumor [21], anti-inflammatory [22] and hypoglycemic agent [23]. *Luteolin has been reported to have prominent* antioxidant [24], anti-inflammatory [25,26] and anti-cancer effects [27].

The quality and quantity of phytochemicals in plant tissue or organs may be affected by many factors, such as fertilization, growing season and harvest time. Figueiredo *et al. indicated that several factors* affect the volatile components and essential oil of plant extracts [28]. Ramakrishna and Ravishankar demonstrated that the secondary metabolites of plants are influenced by abiotic stress [29]. Jaime *et al.,* found that temperature changes the volatile accumulation of *Salvia lavandulifolia* [30]. Novak *et al.* indicated that thymol increases with decreasing temperatures, while carvacrol increases with increasing temperatures in *Origanum* spp [31]. The accumulation of different compounds in *Eleutherococcus senticosu* is affected by light *quality* [32], whereas light intensity affects anthocyanin accumulation in *Melastoma malabathricum* [33]. In addition, the phenolic content of blueberries is influenced by maturation status; all phenolic compounds are degraded in fully ripe berries [34]. For strawberries, two active compounds comprised of anthocyanin and cinnamic acid derivatives are increased when the fruits develop from white to red [35].

Previous research has demonstrated that the content of phytochemical compounds and constituents of floral organs can be influenced by maturation status or development stage. For example, *Acacia cyclops* is most volatile at the yellow bud stage, which increases aliphatic compounds and irregular terpene content and decreases monoterpenoid levels [36]. After stage 3 (when the petals start to split), when *Camellia sinensis* has more phytochemical compounds than at other stages, the plant's phytochemical compounds decreased until the full bloom stage [37]. The maximum contents of chlorogenic acid, cichoric acid, echinacoside and isobutylamide in *Echinacea purpurea* occur at stages 1, 1, 2 and 3, *respectively*, but total content is highest at stage 3 [38]. The maximum contents of gentiopicroside, sweroside, swertiamarin and longanic acid in *Gentiana macrophylla* occur at stages 2, 3, 3 and 4, *respectively* [39]. The most flavonols and anthocyanin in *Helleborus niger* are present at stages B and POF, *respectively* [40]. Ascorbic acid, β-Carotene, flavone and rutin in *Hemerocallis fulva* increase with the stage of development, whereas flavan-3-ol, quercetin and wogonin decrease with the stage of development [41,42]. The contents of anthocyanin and flavonol in *Lilium* increase during the late stages of flower bud development [43]. The anthocyanin content of *Paeonia suffruticosa* increases with development stage after the half-opening or full-opening stage [44]. *Sandersonia aurantiaca* has the highest flavonol content at stage 1, but the content does not have a monotonic relation with development stage [45].

This study aims to determine the antioxidant and major phytochemical compounds (chlorogenic acid and luteolin) of *L. japonica* Thunb. at different flowering stages. Furthermore, the effects of different extraction methods on major active constituents' content at different flowering stages are also investigated. The results of the present study can provide strong evidence regarding the optimal harvest period and postharvest treatment strategy to enhance the value of CTM.

Materials and Methods

Chemical compounds

Chlorogenic acid (Sigma, USA), DPPH (1, 1-diphenyl-2-picrylhydrazyl) (Sigma, USA), folin-ciocalteu reagent (Merck, Germany), gallic acid (Sigma, USA), l*uteolin* (Sigma, USA), HPLC-grade methanol (Burdick & Jackson, USA), phosphoric acid (Merck, Germany), sodium carbonate (Merck, Germany) and trolox (6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carbonsaure, 97 %) (Sigma, USA) were used in this study.

Plant material preparation

Plant management and harvest: *Lonicera japonica* Thunb. was planted organically in the screen house of the Department of Post-Modern Agriculture at MingDao University (Changhua, Taiwan). The development of flower buds from visible to wilted can be separated into six stages (Figure 1):

i. Juvenile Bud (JB): Small green buds of 0.8-1.5 cm;
ii. Green Bud (GB): Green buds of 2.1-3.0 cm;
iii. Green-White Bud (GWB): Shallow green buds of 3.1-4.0 cm;
iv. White Bud (WB): White buds getting ready to burst sized 3.8-4.9 cm;
v. Silver Flower (SF): White flower blooms of 4.4-5.2 cm; and
vi. Gold Flower (GF): Yellow petals of 4.2-5.2 cm.

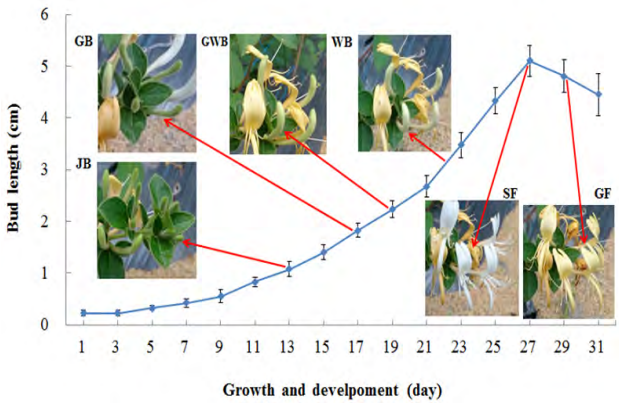

Figure 1: Different growth and developemt stages of *L. japonica*.

JB: Juvenile Bud, GB: Green Bud, GWB: Green-White Bud, WB: White Bud, SF: Silver Flower and GF: Gold Lower.

Flower buds at different stages were harvested in October 2014. Five hundred flowers were collected for each group. The fresh weight was recorded and the dry weight was investigated after the flowers were oven-dried at 40°C for 96 hours until fully dehydrated. The water content was calculated using the following equation:

Water content (%) = [(fresh weight-dry weight/fresh weight)]×100. (1)

The flower bud was then ground into fine powder and stored in a cabinet drier for extraction experiments.

Extraction

Different extraction methods: Water (W) or Methanol (MeOH, M) were used as solvents for the extraction of phytochemical compounds. The powder of *L. japonica* flowers was fully mixed with the solvent at a ratio of 1:10 (m/v). Five treatments, including water bath treatment at 55°C (H55) and 95°C (H95) for 1 hour, ultrasonic treatment (40 KHz) for 1 hour (US) and microwave treatment (500 w) for 1 Minute (M1) and 3 Minutes (M3), were performed. The treated samples were centrifuged at 6,000 rpm for 10 minutes (Hermle Z 323 K, Hermle, USA) and then filtrated (ADVANTEC No.1, TOYO) into tubes. The samples extracted using water and methanol were stored at 4 and -20°C, respectively.

Different flower bud stages: Flower buds at six development stages (i.e. JB, GB, GWB, WB, SF and GF) were treated using Water (W) or Methanol (M) as described in section 2.2.2.1.

Antioxidant activity analysis

Determination of total phenolic content: The total phenolic content of the extract was determined according to the method proposed by Sato et al. [46]. Fresh gallic acid solution (0-500 mg L^{-1}) was prepared with 80 % methanol and 20 % sodium carbonate. Twenty-µL extracted samples of *L. japonica* were mixed with 1 mL Folin-Ciocalteu reagent and left to stand for 5 min. The mixture was added to 0.9 mL of 20 % sodium carbonate and left to stand for 10 min. Then, it was centrifuged at 3,000 rpm for 10 min. The absorbance was measured to be 760 nm. The total phenolic content was calculated from the calibration curve and expressed as mg of gallic acid equivalent to g in dry weight.

Determination of DPPH radical scavenging activity: Analysis of DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity was conducted according to Shimada et al.'s method [47]. Fresh DPPH-methanol solution (26.68 µM) was prepared and stored at 4°C until use. Four mL of the solution was mixed with 20 µL of extracted solutions of *L. japonica*. The mixture was incubated in the dark at 4°C. Methanol solution was used as a blank. The absorbance change at 517 nm was measured 10 min later. All measurements were performed in triplicate. DPPH radical scavenging activity and 50 % scavenging concentration (EC50) were calculated using Equations (2) and (3), respectively. The relative trolox content of samples was calculated from the calibration curve and expressed as trolox equivalents.

$$\text{DPPH radical scavenging effect (\%)} = [(A_{517}\,\text{blank} - A_{517}\,\text{Sample}/A_{517}\,\text{blank})] \times 100 \quad (2)$$

$$\text{EC50 (mg mL}^{-1}\text{)} = (0.05 \times 50/\text{DPPH radical scavenging effect (\%)}) \times 1000 \quad (3)$$

Determination of chlorogenic acid and luteolin contents

In the present study, chlorogenic acid and luteolin contents were determined *by HPLC* according to the method used by Lu et al. with some modification [48]. Briefly, quantification of chlorogenic acid and luteolin in the flower buds of *L. japonica* was performed using the Waters 1525 binary HPLC Pump (Waters Corp.) equipped with a *Waters 2487 UV-Visible Detector* (Waters Corp.). The standard solutions and extracts were separated on a SunFire C18 (5 µm 4.6x250 mm, Waters Corp.). The mobile phase was a methano l-0.4 % phosphoric acid solution (50:50, v/v), the flow rate was 1 mL min^{-1}, the ultraviolet spectrum change was 350 nm and the injection volume was 10 mL. *Next, 100 mM* of prepared c*hlorogenic acid and luteolin was mixed with* Milli-Q® Water (Millipore, Merck, Germany) through a *s*yringe filter with PES of 0.22 µm (Sterlitech Corp.). Then, c*hlorogenic acid* was diluted to 0, 0.25, 0.5, 1, 1.5 and 2 mM and *luteolin was* diluted to 0, 0.5, 1, 2, 5 and 7.5 mM. All samples were through *s*yringe filters with PES of 0.22 µm until use. The chlorogenic acid and luteolin contents of samples were calculated from the calibration curve.

Statistical analysis

All experiments were conducted in triplicate. All results were expressed as means ± Standard Deviation (SD). The significance of differences was determined via LSD (Least Significant Difference) using SAS version 9.4 with a significance level of $p<0.05$.

Results and Discussion

Flower development stages

The basic characteristics of different flower bud stages of *L. japonica* Thunb. found during our investigation are listed in table 1. The bud length and correspondent duration of development are as follows: JB stage (0.8-1.3 cm, 12-14 d), GB stage (2.2-2.7 cm, 17-19 d), GWB stage (3.1-3.7 cm, 21-23 d), WB stage (4.0-4.8 cm, 24-26 d), SF stage (4.5-5.3 cm, 27-28 d) and GF stage (4.2-5.1 cm, 29-30 d) (Figure 1). The bud length increased with development stage until the SF stage. This result was in line with the findings proposed by Wang et al. [18].

Stages	Day of Development	Length (cm)	Fresh Weight (g)z	Dry Weight (g)z	Water Content (%)
JB	12-14	1.08^{e}	2.13^{e}	0.49^{e}	77.14^{d}
GB	17-19	2.68^{d}	6.17^{d}	1.39^{d}	77.54^{d}
GWB	21-23	3.48^{c}	8.01^{c}	1.73^{c}	78.46^{c}
WB	24-26	4.33^{b}	11.65^{b}	2.09^{b}	82.10^{b}
SF	27-28	4.95^{a}	14.00^{a}	2.47^{a}	82.39^{ab}
GF	29-30	4.80^{a}	12.76^{ab}	2.20^{b}	82.74^{a}

Table 1: Basic characteristics of L. japonica Thunb. at different flower development stages.

Means in the same column followed by the same letter are not significantly different at the 5 % level according to the Least Significant Difference (LSD).

[z]Five hundred flowers were collected at every stage.

The bud length of *L. japonica* Thunb. in this study was a little longer than in Wang et al.'s study [18]. This could be the result of different management strategies, such as irrigation and fertilizer, in different cultures or other natural factors, such as soil texture, temperature and daylight length/intensity. With each development stage, the fresh weight (2.13, 6.17, 8.01, 11.65, 14.00 and 12.76 g, respectively), dry weight (0.49, 1.39, 1.73, 2.09, 2.47 and 2.20 g, respectively) and water content (77.14, 77.54, 78.46, 82.10, 82.39 and 82.74 %, respectively) of *L. japonica* Thunb. buds progressively increased (Table 1).

Effects of different extraction methods

The present study showed that methanol's extraction efficiency was superior to that of water for both antioxidants (i.e. scavenging

DPPH radical activity, equivalent trolox content and total phenolic content) and major compounds (i.e. chlorogenic acid and luteolin). For US treatment, methanol (3.81 %) showed significant DPPH radical scavenging activity that was 20.05 times higher than that of water (0.19 %) (Table 2). For H55 treatment, no significant difference was observed between water and methanol extraction of equivalent trolox content (3.48 and 3.48 mg/g DW, respectively). For H95 treatment, water and methanol extraction of equivalent trolox content increased to 19.75 and 28.10 mg/g DW, respectively, indicating that heat can significantly improve extraction efficiency. For microwave treatment, DPPH radical scavenging activity and equivalent trolox content was significantly influenced by both extraction solvent and treatment time (Table 2). Compared with water extraction, methanol extraction with M1 and M3 treatment increased DPPH radical scavenging activity by 1.49 and 1.40 times, respectively. In addition, compared to water extraction, methanol extraction with M3 and M1 treatment increased the equivalent trolox content by 8.42 and 11.11 mg/g DW, respectively (Table 2). Regarding total phenolic content, H95 and M1 and M3 treatment resulted in the most efficient extraction of gallic acid content for both methanol and water (Figure 2). However, the extraction efficiency of methanol extraction with M3 was not as high. Lower extraction efficiency was also observed for H55 and US treatment. For these two treatments, methanol had better extraction efficiency than water.

Extraction Methods	Scavenging Effects (%)		EC_{50} (mg/mL)		Trolox Content (mg/g DW)	
	Water	MeOH[x]	Water	MeOH	Water	MeOH
H55z	1.54^{e}	1.53^{e}	1627.61^{b}	1673.92^{a}	3.48^{c}	3.48^{c}
H95	35.64^{a}	57.32^{a}	70.43^{d}	43.61^{d}	19.75^{a}	28.10^{a}
U	0.19^{d}	3.81^{d}	13316.67^{a}	659.76^{b}	0.52^{c}	4.57^{c}
M1	19.95^{b}	29.73^{c}	125.81^{c}	258.88^{c}	12.21^{b}	16.10^{b}
M3	37.49^{a}	52.66^{b}	67.26^{d}	47.47^{d}	20.63^{a}	27.21^{a}

Table 2: Effects of different extraction methods on the DPPH radical scavenging activity of L. japonica Thunb. Means in the same column followed by the same letter are not significantly different at the 5 % level according to the LSD.

[z]H: heated water bath, U: Ultrasonic, M: Microwave, 55: 55°C, 95: 95°C, 1: 1 minute nd 3: 3 minutes.

[x]: Methanol.

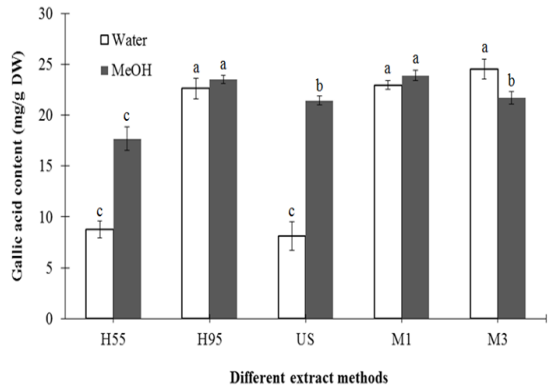

Figure 2: Effects of different extraction methods on the total phenolic content of *L. japonica* Thunb.

Means for the same solvent followed by the same letter are not significantly different at the 5 % level according to the LSD.

The effects of different treatment methods and extraction solvents on the major biochemical compounds, chlorogenic acid and luteolin, are displayed in figures 3 and 4, respectively. For all treatments, methanol had higher extraction efficiency than water. Similar to the results for antioxidants, the extraction efficiency of major compounds increased with temperature and time of extraction. As a result, H95 and M3 treatment with methanol extraction led to the highest content of major compounds. Compared to H55 treatment, H95 significantly increased chlorogenic acid and luteolin extraction using water (12.51 and 0.06 mg/g DW, respectively) and methanol (13.93 and 0.38 mg/g DW, respectively). In addition, compared to M1 treatment, M3 increased chlorogenic acid and luteolin extraction by 4.89 and 0.03 mg/g DW, respectively, using water and 5.58 and 0.27 mg/g DW, respectively, using methanol.

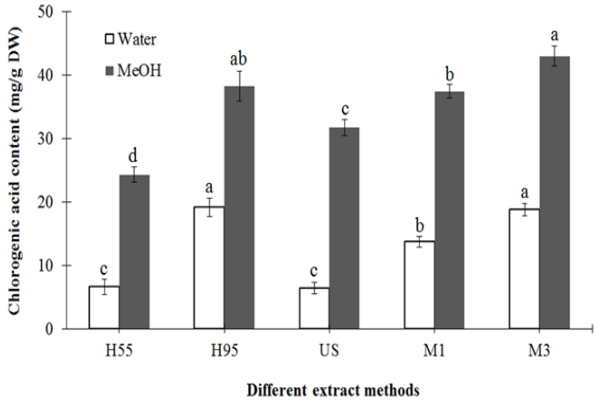

Figure 3: Effects of different extraction methods on the chlorogenic acid content of *L. japonica* Thunb.

Means for the same solvent followed by the same letter are not significantly different at the 5 % level according to the LSD.

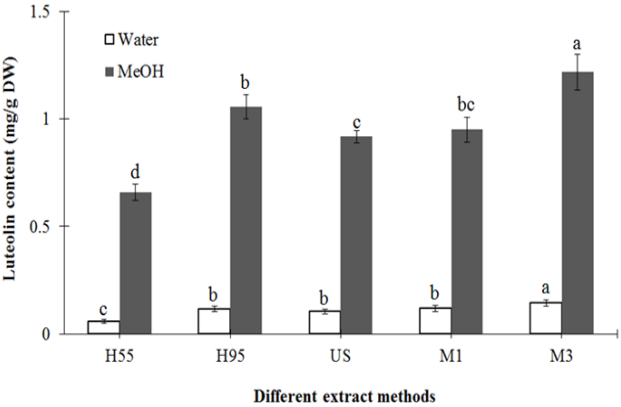

Figure 4: Effects of different extraction methods on the luteolin content of *L. japonica* Thunb.

Means for the same solvent followed by the same letter are not significantly different at the 5 % level according to the LSD.

As mentioned above, antioxidants and chlorogenic acid and luteolin content were substantially increased with temperature and time of extraction (Table 2; Figures 2-4). H95 and M3 treatment with methanol extraction led to the highest equivalent trolox, chlorogenic acid and luteolin contents. In comparison to heating, microwave treatment

saves more time and energy. Therefore, M3 treatment was chosen for further experiments in the present study.

DPPH radical scavenging activity at different flower development stages

The results of the present study showed that DPPH scavenging radical activity and trolox content increased from the JB stage to the SF stage and then slightly decreased at the GF stage (Table 3). In other words, the highest antioxidant content was found at the SF stage (85.01 % DPPH scavenging effect and 27.02 mg/mL DW trolox content for water extraction; 90.04 % DPPH scavenging effect and 28.62 mg/mL DW trolox content for methanol extraction) and the lowest antioxidant content was observed at the JB stage (7.07 % DPPH scavenging effect and 2.29 mg/g DW trolox content for water extraction; 19.30 % DPPH scavenging effect and 6.14 mg/g DW trolox content for methanol extraction). Table 3 again shows that methanol had better overall extraction efficiency than water.

Stages	Scavenging Effects (%)		EC_{50} (mg/mL)		Trolox content (mg/mL)	
	Water	MeOH	Water	MeOH	Water	MeOH
JB	7.07^{e}	19.30^{e}	353.42^{a}	130.06^{a}	2.29^{e}	6.14^{e}
GB	26.37^{d}	27.91^{d}	94.82^{b}	89.62^{b}	8.38^{d}	8.87^{d}
GWB	53.90^{c}	64.28^{c}	46.40^{c}	38.89^{c}	17.14^{c}	20.43^{c}
WB	80.70^{b}	89.77^{a}	30.99^{d}	27.85^{d}	25.65^{b}	28.53^{a}
SF	85.01^{a}	90.04^{a}	29.41^{d}	27.76^{d}	27.02^{a}	28.62^{a}
GF	81.47^{b}	87.62^{b}	30.69^{d}	28.53^{d}	25.90^{b}	27.85^{b}

Table 3: DPPH radical scavenging activity and trolox content of *L. japonica* Thunb. at different flower development stages

Means in the same column followed by the same letter are not significantly different at the 5 % level according to the LSD.

Total phenolic content at different flower development stages

Total phenolic content at different flower development stages followed the same trend as DPPH radical scavenging activity; however, no significant difference was found between water and methanol extraction (Figure 5). Total phenolic content was highest at the SF stage (26.91 and 25.82 mg/g DW for water and methanol extraction, respectively) and then decreased dramatically at the GF stage (13.55 and 14.46 mg/g DW for water and methanol extraction, respectively).

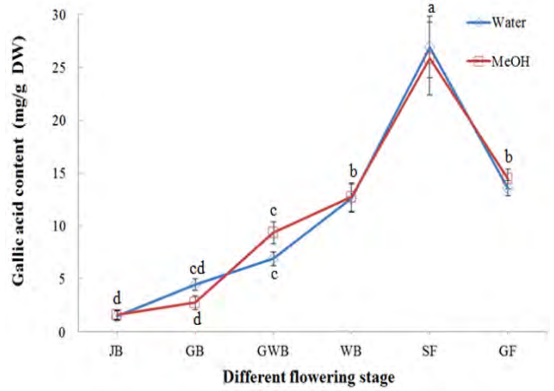

Figure 5: Total phenolic content of *L. japonica* Thunb. at different flower development stages Means for the same solvent followed by the same letter are not significantly different at the 5 % level according to the LSD.

Chlorogenic acid content at different flower development stages

Chlorogenic acid is a major biochemical compound that accounts for approximately 1-2 % of the composition of dried *L. japonica* buds. In the present study, chlorogenic acid content substantially increased with flower development stage until the SF stage, similar to antioxidant content (Figure 6). The highest chlorogenic acid content obtained using methanol extraction (24.45 mg/g DW) was found at the SF stage and the highest obtained using water extraction (11.28 mg/g DW) was found at the WB stage. The maximum extraction efficiency of methanol was 2.17 times higher than that of water. At the GF stage, the chlorogenic acid content was decreased to 15.94 and 8.20 mg/g DW for methanol and water extraction, respectively.

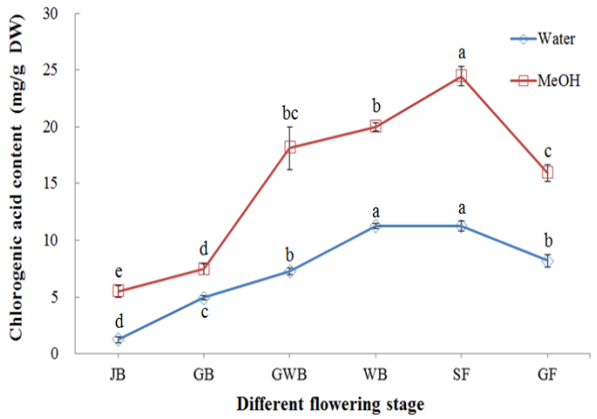

Figure 6: Chlorogenic acid content of *L. japonica* Thunb. at different flower development stages.

Means for the same solvent followed by the same letter are not significantly different at the 5 % level according to the LSD.

Luteolin content at different flower development stages

The efficiency of methanol extraction of luteolin was 2.08 to 4.23 times higher than that of water (Figure 7). Luteolin content slightly increased with flower development stage and reached its highest value (0.192 mg/g DW) at the GF stage after water extraction. However, it decreased from 0.60 mg/g DW at the JB stage to 0.4 mg/g DW at the GF stage.

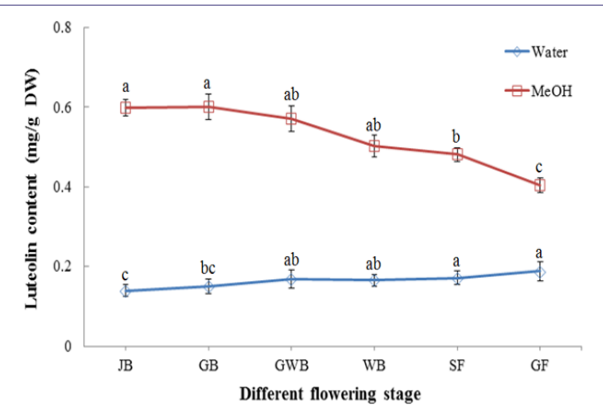

Figure 7: Luteolin content of L. japonica Thunb. at different flower development stages.

Means for the same solvent followed by the same letter are not significantly different at the 5 % level according to the LSD.

Microwave extraction is an approach that heats the solvent by microwave energy, disruption of hydrogen bonds, and enforcement of ion migration [49]. This approach is widely applied to rapidly extract biochemical compounds from many plants, such as the green tea [50], pistachio [51] and tomato plants [52]. In the present study, M3 treatment with methanol extraction led to the highest chlorogenic acid content (40 mg/g DW) *in L. japonica* buds (Figure 3). Zhang et al. used microwaves to extract chlorogenic acid from *L. japonica* and proposed that the maximum extraction content could reach 61.4 mg/g [53]. With regards to the extracting solvent, the results of the present study revealed that methanol was better than water for increasing antioxidant activity and major active constituents' content. Similar results were also obtained for the antioxidant activity of olive leaves [54] and the total phenolic content of cactus flowers [55].

It is generally recognized that the biochemical compounds of plants change depending on the flower development stage [36,39,40]. In this study, antioxidant activity and major biochemical compounds (except *l*uteolin) varied with the *flower development stages of L. japonica* and reached their highest value at the SF stage. According to Joshi et al., antioxidant activity in *Camellia sinensis* flowers increased with *development* stage and reached its maximum value when *p*etals started to split, whereas chlorogenic acid content was highest at the stage of flower *o*pening [37]. This result was in line with the present study. In a study investigating *Echinacea purpurea*, the maximum chlorogenic acid content was observed at the initial stage of flower bud development [38]. However, Zhu and Xiong found the highest chlorogenic acid content during the GWB stage of *L. japonica* [56]. Wang *et al.* also investigated the chlorogenic acid content at different development stages of *L. japonica* flowers and found that the highest content was present at the GB stage and decreased thereafter [18]. The present study found the highest chlorogenic acid content at the WB and SF stages. This controversy may be due to several factors, such as plant species, growing environment, growing season and extraction methods. Therefore, further studies are necessary to study the effects of these factors on the extraction of bioactive components when determining optimal harvest time for commercial demand.

Conclusion

Analysis of various treatment methods showed that *the* M3 treatment was the most efficient for extraction of antioxidant and bioactive components of Japanese honeysuckle (*Lonicera japonica* Thunb.). Except for luteolin content, methanol had better extraction efficiency than water. *We believe that the SF stage is the optimal harvest stage because* DPPH scavenging radical activity, equivalent trolox content and chlorogenic acid content were at their highest. The present results can be applied to determine the best harvest time and treatment method for enhancing the value of Chinese Traditional Medicine (CTM). However, further investigations are still needed to study the influence of environmental and biogeochemical factors on the bioactive components of *L. japonica* Thunb.

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