Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Antioxidant and anti-inflammatory activities of *Lonicera japonica* Thunb. var. *sempervillosa* Hayata flower bud extracts prepared by water, ethanol and supercritical fluid extraction techniques

Hsia-Fen Hsu\(^a,b\), Pei-Chi Hsiao\(^b\), Tzu-Chen Kuo\(^b\), Shu-Tuan Chiang\(^c\), Shin-Lung Chen\(^d\), Shu-Jiau Chiu\(^e\), Xue-Hua Ling\(^a,f\), Ming-Tsai Liang\(^f\), Wei-Yi Cheng\(^a\), Jer-Yiing Houng\(^a,f,*\)

\(^a\) Department of Nutrition, I-Shou University, Kaohsiung, Taiwan
\(^b\) Metal Industries Research & Development Centre, Kaohsiung, Taiwan
\(^c\) Chuang Song Zong Pharmaceutical Co., Pingtung, Taiwan
\(^d\) Sen Tai Pharmaceutical Industrial Co., Tainan, Taiwan
\(^e\) Biomedical Technology and Device Research Laboratories, Industrial Technology Research Institute, Hsinchu, Taiwan
\(^f\) Department of Chemical Engineering, I-Shou University, Kaohsiung, Taiwan

**A R T I C L E   I N F O**

Article history:
Received 8 September 2015
Received in revised form 18 April 2016
Accepted 5 May 2016
Available online 16 June 2016

Keywords:
Anti-inflammatory activity
Antioxidant activity
Chemical composition analysis
*Lonicera japonica*
Supercritical fluid extraction

**A B S T R A C T**

*Lonicera japonica* Thunberg (LJ) has long been used as an antipyretic, anti-inflammatory and anti-infectious agent in East Asia. The subspecies *L. japonica* Thunb. var. *sempervillosa* Hayata (LJv) is a variant that mainly grows in Taiwan. This study examined the antioxidant and anti-inflammatory activities of the extracts from the flower buds of these two species. The extracts were obtained by three extraction methods: water extraction, ethanol extraction, and supercritical-CO\(_2\) fluid extraction (SFE). The antioxidant activities of dry LJ (dLJ) extracts were superior to those of LJv extracts. Water extracts possessed higher activities than that prepared by ethanol or SFE. The total polyphenols content, total flavonoids content, and the amount of chlorogenic acid and luteolin-7-O-glucoside were all higher in the water extracts compared to the other two. The SFE extracts of these two species all exhibited excellent anti-inflammatory activities. Although the water and ethanol extracts of dLJ extracts had higher anti-inflammatory activity than that of LJv extracts, the SFE extracts prepared from fresh LJv flower buds (fLJv) exhibited the highest activity among all extracts. The SFE effectively isolates the bioactive components of *L. japonica* and can obtain the *L. japonica* extracts with high anti-inflammatory activity.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

*Lonicera japonica* Thunb. (LJ), belonging to the Caprifoliaceae family, is known as Japanese honeysuckle, Jin Yin Hua and Ren Dong. It is a traditional Chinese medicine endowing with antidote, diuretic, tonic, antipyretic, anti-inflammatory and anti-infectious activities. It has been widely used in treating exopatogenic wind, heat, furnaces, epidemic febrile diseases, carbuncles, sores and some infectious diseases. It is also applied to treat chronic enteritis, pneumonia, acute tonsillitis, nephritis, acute mastitis, leptospirosis in some folk prescriptions. Recent uses include the prevention and treatment of human and animal viruses, such as SARS coronavirus and swine H1N1 flu virus (Shang et al., 2011). Several pharmacological studies have shown that LJ and its active ingredients possess wide bioactivities, such as antioxidant, anti-inflammatory, antibacterial, antiviral, blood fat reducing, antipyretic and antiendotoxin (Shang et al., 2011). Therefore, in addition to its folk medicinal uses, LJ is also used in health food and cosmetics.

More than 150 chemical compounds have been isolated from LJ. The major compositions are essential oils, flavones, saponins, iridoids, and organic acids. Chlorogenic acid, luteolin, luteolin-7-O-glucoside and essential oils have good pharmacological effects and are believed to be the active ingredients of LJ (Ikeda et al., 1994;...
Wang, 2010). Different plant parts, including flowers, flower buds, leaves and whole plant, contain different ingredients and have different activities. Among them, flowers and flower buds of LJ are the most frequently used plant parts in Taiwan. However, differences in habitat, harvest time, extraction methods, and flower preparation (fresh versus dry) also cause variations in chemical compositions and the quality of LJ extract (Shang et al., 2011).

*L. japonica* Thunb. var. *semervillosa* Hayata (LJ, Mao Rong Tong) is a variant subspecies of LJ that mainly grows in Pingtung County and Taitung County, Taiwan (Hayata, 1919). The plant LJ resembles LJ except for its leaves, which are thinly hairy, densely villose beneath, and its flowers spread a special fresh fragrance. To the best of our knowledge, this study is the first to examine the bioactivities of LJ.

The *L. japonica* is usually extracted with water or organic solvents, such as methanol or ethanol. One alternative to traditional extraction by organic solvents is supercritical fluid extraction (SFE). The most commonly used supercritical fluid is CO₂ because of its favorable operating temperature and pressure for extracting thermolabile ingredients. Supercritical CO₂ is also non-toxic, non-flammable, widely available, chemically inert, and has low viscosity, low surface tension, high diffusivity and favorable density (Wang, 2011; Quitain et al., 2013). Compared to conventional organic solvent extraction methods, the advantages of SFE are its non-toxicity and high separation selectivity. Especially, the CO₂ gas used in SFE operation can be easily recycled to avoid the concern of greenhouse effects. Therefore, the use of SFE for extracting natural products is rapidly increasing (King, 2014; Uddin et al., 2015; Yen et al., 2015).

This work compared extracts from dry and fresh flower buds of LJ and the dry flower buds of LJ. The fresh flower buds of LJ were not included because the fresh samples produced at the same area and the same season as the dry flower buds of LJ were difficult to purchase throughout the experiment duration. These extracts were prepared with water, ethanol, and supercritical-CO₂ fluid. Their antioxidant, anti-inflammatory activities and their chemical compositions were then analyzed.

### 2. Materials and methods

#### 2.1. Chemicals and plant materials

(+)-Catechin, chlorogenic acid, dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), *Escherichia coli* lipopolysaccharide (LPS), Folin-Ciocalteu reagent, gallic acid, hypoxanthine (HPX), luteolin-7-O-glucoside, N⁵-monomethyl-L-arginine acetate (L-NMMA), nitroblue tetrazolium (NBT), penicillin, streptomycin, xanthine oxidase (XOD) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Dulbecco’s modified Eagle’s medium (DMEM) and fetal bovine serum were from Gibco (Grand Island, New York, USA). All chemicals used in this study were of reagent or higher grade.

The reference dry flower buds of LJ (dLJ) were purchased from Henan, China. Flower buds of LJ (fLJ) freshly harvested from Jhutian Township, Pingtung (Taiwan) were obtained from a local farmer, Mr. Hong Ying. Some of these fresh flower buds were air-dried at 40 °C for 24 h, and then stored at −20 °C (designated as “dLJ”). All samples were harvested in April. The morphological characteristics of LJ were compared with those of a sample authenticated by Prof. Den-En Shieh (Department of Food Science and Technology, Tajen University of Technology, Pingtung, Taiwan). A DNA analysis of the plant materials was performed as described in our previous study (Chiu et al., 2007). The similarity of ITS2 sequences between LJ and LJ exceeded 99%, indicating that these two materials are indistinguishable at the species level.

#### 2.2. Preparation of LJ and LJ extracts

The ethanol and water extracts of dLJ or fLJ were prepared by crushing or drenching 300 g of flower buds in 1800 ml ethanol (50, 75 or 95%) or water for 1 day. After repeating the extraction procedure three times, medicinal gauze was used to filter out insoluble debris. The filtrates were collected and concentrated with a vacuum evaporator. The extract samples were then dried in a freeze-dryer.

The ethanol and water extracts of fLJ were prepared by crushing 800 g of fresh flower buds and drenching them in 4800 ml ethanol (50, 75 or 95%) or water for one day, and repeated this operation for three times. The other operations were the same as above.

The supercritical-CO₂ fluid extracts were prepared by processing the crushed flower buds (1 kg each) in the supercritical fluid extractor (51/1000 bar R&D unit, Natex, Ternitz, Austria). The dynamical extractions were performed at 150, 250 and 350 bar at 45 °C for 2 h. The extract samples were dried in a freeze-dryer.

#### 2.3. Determination of antioxidant activity

##### 2.3.1. Determination of scavenging activity on DPPH radicals

The scavenging activity against DPPH free radical was measured using the method of Hsu et al. (2005). In brief, 0.25 ml of 0.5 M DPPH ethanolic solution was mixed with 1.0 ml extract solution in an eppendorf tube. The absorbance at 517 nm was measured after keeping the solution in the dark for 30 min. The control was the measurement using ethanol to replace the extract sample in the reaction solution. The blank was measured by using ethanol to replace DPPH in the solution. After conducting the scavenging activity measurements under different concentrations of samples, the IC₅₀ value, i.e. the concentration of sample that causes 50% inhibition, was estimated from the plot of scavenging activity against the sample concentration.

##### 2.3.2. Determination of scavenging activity on superoxide anions

Superoxide anion radicals were generated in a HPX-XOD system by HPX oxidation and assayed by NBT reduction (Hsu et al., 2005). The solutions of NBT (300 μM), HPX (1.1 mM) and XOD (1.67 IU/ml) were prepared separately in a 0.1 M sodium phosphate buffer (pH 7.4). The HPX (760 μl) was mixed with 100 μl NBT and 100 μl extract solution in a 1-ml cuvette. Next, 40 μl XOD was added. The decrease in absorbance at 560 nm was measured every 15 s for 6 min. The rate of decreasing, designated as R₁, was estimated by enzyme kinetic function of the spectrophotometer (Ultrascop 2100 pro, GE Healthcare, Amersham Place, UK). The control was the measurement using phosphate buffer to replace the extract sample in the reaction solution, and the decreasing rate of the absorbance is designated as R₀. The O₂⁻ scavenging activity was determined by the following equation:

\[
O_2^{-}\text{scavenging activity} (%) = \left( \frac{R_0 - R_1}{R_0} \right) \times 100\%.
\]

#### 2.4. Determination of total polyphenols content

The total amount of phenolic compounds of each extract was determined according to the method of Zielinski and Kozlowska (2000). A 0.15 ml aliquot of the diluted extract solution was mixed with 0.75 ml of 0.2 N Folin-Ciocalteu reagent and 0.6 ml of 7.5% sodium carbonate solution. The mixture was allowed to stand at room temperature for 30 min and then measured at 765 nm with a spectrophotometer. A calibration curve was constructed using gal-
acid as a standard. The total polyphenols content is expressed as percentage of gallic acid equivalents of dry extract.

2.5. Determination of total flavonoids content

The total flavonoids content was determined according to Ismail et al. (2010) with some modifications. An aliquot of 0.3 ml sample, 1.2 ml of deionized water, and 0.075 ml of 15% Na₂CO₃ were added to a test tube. Five minutes later, 0.15 ml of 10% AlCl₃ was added. After 6 min at room temperature, 0.5 ml of 1 M NaOH and 0.275 ml deionized water were added. The absorbance of the solution was measured against a blank at 510 nm. A calibration curve was constructed using catechin as a standard. The total flavonoids content is expressed as percentage of catechin equivalents of dry extract.

2.6. Determination of anti-inflammatory activity

The anti-inflammatory activity was determined by the suppressing effect on nitric oxide (NO) production in LPS-stimulated RAW264.7 macrophage cells (Bioresource Collection and Research Center; Hsinchu, Taiwan). The cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10%(v/v) fetal bovine serum, 1% penicillin/streptomycin and 1.5 g/l sodium bicarbonate. The cells were cultivated in a humidified incubator at 37 °C with 5% CO₂ and 95% air. The inhibitory effect of extract samples on NO production was measured using the method of Hong et al. (2014). RAW264.7 cells (4 × 10⁵) were seeded into 100 μl DMEM medium in 96-well culture plates and incubated at 37 °C with 5% CO₂ for 24 h. The cells were then incubated with 1 μl LPS (1 μg/ml) and 0.5 μl sample solution, which was the freeze-dried extract dissolved in DMSO. Following incubation for 24 h, 80 μl of the supernatant were collected for nitrite assay. The residual medium was removed, and the cell viability was evaluated using the MTT method (Hong et al., 2014). The nitrite concentration in the medium was measured as an index of NO production by using the Griess reaction. Griess reagent was freshly prepared from reagents A (1% sulfanilamide in 2.5% phosphoric acid) and B (0.1% N-1-naphthylethlenediamide dihydrochloride in water) at a ratio of 1:1. Equal volume of Griess reagent was added to the supernatants from the cells treated with the extract samples. Absorbance was measured on an ELISA reader (Model 550, Bio-Rad Laboratories, Hercules, CA, USA) at 540 nm. The NO-suppressing effect was expressed as the IC₅₀ which denotes the concentration of extract sample causing 50% inhibition of NO production by LPS-activated RAW 264.7 cells. The 1-NMMA, a clinical use iNOS inhibitor, was used as the positive control.

2.7. Analysis of chemical compositions

The sample analysis was conducted by an Acquity UPLC system coupled to a TQD mass spectrometer equipped with a Z-Spray ESI source (Waters, Manchester, UK). The UPLC system was equipped with an Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm) and maintained at 50 °C with a column oven. The mobile phase consisted of 0.1% acetic acid in water in channel A and 100% acetonitrile in channel B. An isotropic elution was used with a flow rate of 0.4 ml/min at a ratio of 95% A and 5% B for a total of 18 min and the injection volume was 5 μl. The mass spectrometer was operated in electrospray positive mode with the capillary voltage set at 3.2 kV. The TQD mass spectrometer was operated with the follow-
ing optimized source-dependent parameters (ESI source): capillary voltage 3.2 kV, cone voltage 40 V, desolvation temperature 300 °C, desolvation gas flow 500 l/h, cone gas flow 50 l/h, source temperature 120 °C and scanning from 100 to 800 m/z. The MassLynx 4.1 with QuanLynx software (Waters) was used for data processing.

Gas chromatography (GC) analysis was performed using Thermo Trace GC Ultra system (Thermo Fisher Scientific, Waltham, MA, USA). The GC column was DB-WAX capillary column (30 m × 0.32 mm, film thickness 0.25 μm, Agilent Technologies, Santa Clara, CA, USA). Injector and detector temperatures were set at 250 °C and 280 °C, respectively. Oven temperature was kept at 90 °C for 1 min, raised to 180 °C by a rate of 15 °C/min, kept at 180 °C for 3 min, and then raised to 240 °C by a rate of 5 °C/min, kept at 240 °C for 18 min. The carrier gas was helium at a flow rate of 1.8 ml/min. Diluted samples of 2.0 μl was injected manually and in the splitless mode.

2.8. Determination of the amounts of chlorogenic acid and luteolin-7-O-glucoside

The amount of chlorogenic acid and luteolin-7-O-glucoside were determined by HPLC (L-7100, Hitachi, Tokyo, Japan). The extracts were dissolved in 50% methanol and filtered with a 0.22 μm filter. The diluted samples were analyzed by a Cosmosil 5C18-MS-II column (250 mm × 4.6 mm, 5 μm; Nacalai Tesque, Kyoto, Japan) at column oven of 35 °C. The sample injection size was 20 μl. The flow rate was 1.0 ml/min, and the detection was carried out at 330 nm. For chlorogenic acid analysis, the solution of 0.4% phosphoric acid/acetonitrile (90:10, v/v) was used as the mobile phase. For luteolin-7-O-glucoside analysis, the mobile phase consisted of 0.4% phosphoric acid (channel A) and acetonitrile (channel B) using the gradient program as follows: 0–25 min, 90%–85% A; 25–40 min, 85%–80% A; 40–45 min, 80%–70% A; 45–50 min, 70%–60% A.

2.9. Statistical analysis

All experiments were conducted for three to five independent replicates. The data are expressed in terms of mean and standard deviation. The experimental data were analyzed using Microsoft Excel software (Microsoft Software Inc., USA).

3. Results and discussion

3.1. Extraction yield

In this study, 300 g of dry flower buds or 800 g of fresh flower buds (dry weight = 135.2 g) were extracted by ethanol and water, respectively. Additionally, 1 kg each of the dry or fresh flower buds were extracted by supercritical-CO₂ fluid. Fig. 1 shows the extraction yields from different materials and different extraction methods. Because the fresh flower buds contained 83.1% water, for comparison under the same standard, their yields were calculated on the basis of dry material.

The yields of dLJv, fLJv and dLJ extracted by water were all around 38%. In ethanol extraction of dLJv, the yield decreased as the ethanol concentration increased. For fLJv and dLJ, the extraction with 75% ethanol obtained the highest yield. In SFE extraction,
increasing the extraction pressure increased the yield of dLJv extracts but decreased the yield of dLJ extracts. The yields of the fLJv extracts from different extraction pressures were all similar (around 10%).

### 3.2. Antioxidant activity

As reported in the literature, the ethyl acetate extract and the methanol extract of LJ flower buds had good ROS scavenging activities (Cai et al., 2004; Choi et al., 2007). Myung et al. (2004) demonstrated that the LJ extracts prepared by 70% methanol or 70% acetone extraction had good tyrosinase inhibition, xanthine oxidase inhibition, and nitrite scavenging activities. Choi et al. (2007) reported that the ingredients of ethyl acetate extract, such as luteolin, caffeic acid, protocatechuic acid,isorhamnetin-3-O-d-glucopyranoside, quercetin 3-O-d-glucopyranoside, and luteolin 7-O-d-glucopyranoside, had high antioxidant activity. Most of these bioactive components are flavonoids.

This study investigated the antioxidant activities of different extracts by analyzing their DPPH and SOD scavenging activities. The DPPH radicals scavenging activity of antioxidants are attributed to their hydrogen donating abilities. While the SOD anion scavenging activity denotes the ability to remove free radicals, such as peroxy, alkoxyl, hydroxyl, and nitric oxide, which formed from superoxide anions through the Fenton reaction, lipid oxidation or nitric oxidation (Ambrosio and Flaherty, 1992; Kostyuk and Potapovich, 1998).

For all extracts examined in this study, radical scavenging activities were dose-dependent within the concentration range tested. The IC$_{50}$ values of DPPH and SOD scavenging activities of different L. japonica extracts are compared in Fig. 2A and B. In these analyses, the highest concentration tested was 1000 μg/ml. Scavenging activities were high for both DPPH and SOD in all water extracts. The dLJ extracts showed much higher antioxidant activities compared to the fLJv extracts. The dLJ extract prepared by 75% ethanol had the lowest IC$_{50}$ values of DPPH (56.8 ± 0.5 μg/ml) and SOD (134.1 ± 5.7 μg/ml) scavenging activities. For comparison, the IC$_{50}$ values of DPPH and SOD scavenging activities of catechin, which was used as the positive control, were 5.6 ± 1.2 μg/ml and 22.7 ± 0.7 μg/ml, respectively. All SFE extracts had low antioxidant activities.

Phenolic compounds and flavonoids are considered powerful antioxidants because they donate hydrogen or electrons to form stable radical intermediates (Wang et al., 2006; Vijayalaxmi et al., 2015). The antioxidant activities of many fruits, vegetables and edible plant extracts have a strong correlation with their total polyphenols content (TPC) and total flavonoids content (TFC) (Katsube et al., 2004; Kiselova et al., 2006; Klimczak et al., 2007; Jayaprakasha et al., 2008). Fig. 2C and D show the TPC and TFC values of these L. japonica extracts. High TPC and TFC were found for dLJ water and ethanol extracts, but TPC and TFC were all low for all LVj extracts and the SFE extracts.

The extracts of dLJv, fLJv and dLJ prepared by 75% ethanol were further analyzed by UPLC–MS/MS (Fig. 3A–C). Eight components were identified as chlorogenic acid (Compound 1, RT = 2.7 min), loganin (Compound 2, RT = 3.4 min), secologanic acid (Compound 3, RT = 3.8 min), sweroside (Compound 4, RT = 4.9 min), secoxyloganin (Compound 5, RT = 6.0 min), epi-vogeloside (Compound 6, RT = 6.5 min), luteolin-7-O-glucoside (Compound 7, RT = 7.5 min), 3,5-di-O-caffeyloquinic acid (Compound 8, RT = 8.2 min). The dLJ contained higher amounts of chlorogenic acid, secoliganic acid and epi-vogeloside than those of the other two. Chlorogenic acid and luteolin-7-O-glucoside are the bioactive components of LJ and are known to have potent antioxidant and anti-inflammatory activities (Choi et al., 2007; Huo et al., 2003; Guo et al., 2014). Further analyses for determining their concentrations were performed by HPLC. The chlorogenic acid concentration of dLJv, fLJv and dLJ ethanol (75%) extracts were 0.89%, 0.21% and 1.75%, respectively. The luteolin-7-O-glucoside concentration of dLJv, fLJv and dLJ ethanol (75%) extracts were 0.017%, 0.009% and 0.035%, respectively. The dLJ extracts had the highest chlorogenic acid content and the highest luteolin-7-O-glucoside content, which partly explains why the dLJ extracts had the best antioxidant activity.

### 3.3. Anti-inflammatory activity

Many studies have shown that different extracts of LJ can inhibit various inflammatory reactions, and suppress inflammatory cytokines expression. In a mouse model of proteinase activated receptor 2 (PAR2)-mediated edema in the paw, the water extract of LJ significantly inhibited the change in paw thickness, vascular permeability, PAR2 agonists-induced myeloperoxidase (MPO) activity, and TNF-α expression in paw tissue (Tae et al., 2003). Aqueous extract of LJ also attenuated trypsin-induced mast HMC-1 cell activation through inhibition of ERK phosphorylation and the inhibition of trypsin activity (Kang et al., 2004). Water extract of LJ inhibited both COX-1 and COX-2 activity, the expression of IL-1β-induced COX-2 protein and mRNA in A549 cells (Xu et al., 2007). All of these reports suggest that LJ is a good anti-inflammatory agent for treating inflammatory disorders.

This study assayed the anti-inflammatory properties of L. japonica extracts by suppressing the effects of NO production in LPS-activated RAW264.7 cells. Fig. 4A shows the IC$_{50}$ values for the NO-suppressing activities of these extracts. The relative effectiveness of these extracts for suppressing NO formation was SFE extracts > ethanol extracts > water extracts; and dLJ > dLJv > fLJv of ethanol extracts and water extracts. However, for the SFE extracts, the fLJv exhibited the highest activity among the three materials. The IC$_{50}$ values of the fLJv extracts prepared at 150, 250
and 350 bar were $69.3 \pm 6.0$, $71.0 \pm 6.8$, $64.3 \pm 7.5 \mu g/ml$, respectively. The IC$_{50}$ values of the dLJ extracts prepared at 150, 250 and 350 bar were $184.7 \pm 13.1$, $177.6 \pm 8.9$, $218.9 \pm 18.3 \mu g/ml$, respectively. In contrast, the IC$_{50}$ values of the positive control L-NMMA was $22.4 \pm 2.0 \mu g/ml$. Moreover, these extracts did not significantly retard cell growth in a 24-h treatment under a concentration below their respective NO-suppressing IC$_{50}$ value. Fig. 4B shows that the fLJv SFE extract prepared at 350 bar revealed a dose-dependent inhibitory effect on NO formation of RAW264.7 cells, and this extract had no cytotoxic effects on RAW264.7 cells.

The chemical compositions of these extracts were compared by gas chromatography. The analytical chromatograms show that the ethanol extracts of dLJ, dLJv and fLJv (Fig. 5A–C) had comparable chemical composition patterns, but their compositions differed from those of the SFE extracts (Fig. 5D–F). Additionally, the dLJ SFE extract (Fig. 5D) and dLJv SFE extract (Fig. 5E) had comparable compositions, but the fLJv SFE extract (Fig. 5F) had more ingredients than the other two extracts, especially the ingredients at the retention time between 7 and 13 min.

Most of the fresh L. japonica samples had a high water content. Thus, a drying step is usually required before extraction. However, the drying process may result in the loss of some bioactive components. Ji et al. (1990) reported that the content of bioactive ingredient linalool in volatile oil from fresh LJ flowers was more than 26%, and linalool was less than 0.4% in volatile oil from dry LJ flowers. Fragrant composites are lost by heating and lighting in the drying process. Moreover, different extraction methods would result in different contents and compositions of LJ essential oils (Du et al., 2009).

An important feature of supercritical CO$_2$ extraction is its low polarity. Therefore, it can only extract the non-polar components (Quitain et al., 2013). The properties of CO$_2$ in supercritical state resemble those of the solvent ethyl acetate. Thus, more polar impurities are eliminated by SFE than by conventional aqueous and ethanol extractions. This feature leads to the concentration of non-polar bioactive components in the SFE extracts and results in high anti-inflammatory activity. Furthermore, CO$_2$ extraction prevents degradation of extracts because it is performed in a non-oxidizing atmosphere (Jaime et al., 2007).

4. Conclusions

This study examined and compared the antioxidant and anti-inflammatory activities of LJ and LJ extracts and compared the activities of extracts prepared by different extraction methods. To our knowledge, this study is the first to compare the bioactivities of these two species. The antioxidant activities of dLJ extracts were superior to those of dLJv extracts. In terms of anti-inflammatory activity, SFE was a good separation tool that provided excellent activities of L. japonica extracts. Since SFE concentrates non-polar
bioactive components of $I_d$ and $I_{dv}$ in the extraction step, it can obtain the extracts with high anti-inflammatory activity. Additionally, SFE is non-toxic, non-flammable, and easy recycle use of CO$_2$. Therefore, using SFE to recover valuable components from L. japonica is effective and clean, and is a good alternative extraction method.

Although the water and ethanol extracts of dLJ extracts had higher anti-inflammatory activity compared to $I_d$ extracts, the fLJ SFE extracts had superior activity to dLJ extracts. Further studies are needed to compare the active ingredients of fLJ, dLJ, and dLJ SFE extracts; and to investigate the other bioactivities of $I_d$ and $I_{dv}$ extracts, such as anti-microbial, antiviral and antipyretic activities.

Acknowledgement
The authors gratefully acknowledge the financial support of Ministry of Economic Affairs, Taiwan (SBIR 2Z1010479) and I-Shou University, Taiwan (ISU101-INT-002). The authors thank Prof. Den-En Shieh, from Department of Food Science and Technology, Tajen University of Technology, Pingtung, Taiwan, for his assistance to identify Lonicera japonica Thunb. var. sempervillosa Hayata.

References
Ambrosio, G., Flaherty, J.T., 1992. Effects of the superoxide radical scavenger superoxide dismutase, and of the hydroxyl radical scavenger mannitol, on reperfusion injury in isolated rabbit hearts. Cardiovasc. Drugs Ther. 6, 623–632.
Cai, Y.Z., Luo, Q., Sun, M., Corke, H., 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 74, 2157–2184.
Chiou, S.J., Yen, J.H., Fang, C.L., Chen, H.L., Lin, T.Y., 2007. Authentication of medicinal herbs using PCR-amplified ITS2 with specific primers. Planta Med. 73, 1421–1426.
Choi, C.W., Jung, H.A., Kang, S.S., Choi, J.S., 2007. Antioxidant constituents and a new triterpenoid glycoside from Flos lonicerae. Arch. Pharm. Res. 30, 1–7.
Du, H.F., Zhang, Y., Wen, D.Q., Yang, D.J., 2009. Identify the contents of fresh flower of L. japonica using GC–MS with the different extraction methods. TCM Res. Chongqing 60, 13–15 (in Chinese).
Guo, A.L., Chen, L.M., Wang, Y.M., Liu, X.Q., Zhang, Q.W., Gao, H.M., Wang, Z.M., Xiao, W., Wang, Z.Z., 2014. Influence of sulfur fumigation on the chemical constituents and antioxidant activity of buds of Lonicera japonica. Molecules 19 (10), 16640–16655.
Hayata, B., 1919. Lonicera japonica thunb. var. sempervillosa hayata. Icones Plantarum Formosanarum, vol. D. Bureau of Forestry, Government of Formosa, Taipei, Taiwan, pp. 47.
Hong, Y.H., Weng, L.W., Chang, C.C., Hsu, H.F., Wang, C.P., Wang, S.W., Houng, J.Y., 2014. Anti-inflammatory effects of Siegesbeckia orientalis ethanol extract in vivo and in vivo models. BioMed Res. Int., 329712.
Hsu, H.F., Houng, J.Y., Chang, C.L., Wu, C.C., Chang, F.R., Wu, Y.C., 2005. Antioxidant activity, cytotoxicity and DNA information of Glissocarpis teminifolia. J. Agric. Food Chem. 53, 6117–6125.
Huo, X.F., Tang, Y.P., Zhang, Q.L., Luo, S.H., 2003. Effect of chlorogenic acid on the macrophages induced by lipopolysaccharide. Acta Acad. Med. Zunyi 26, 507–508 (in Chinese).
Ileda, N., Ishihara, M., Tsuney, T., Kawakita, M., Yoshihara, M., Suzuki, Y., Komaki, P., Inui, M., 1994. Volatile components of honeysuckle (Lonicera japonica Thunb.) flowers. Flavour Frag. J. 9, 325–331.
Ismail, H.I., Chan, K.W., Mariod, A.A., Ismail, M., 2010. Phenolic content and antioxidant activity of cantaloupe (Cucumis melo) edible manchelian. Food Chem. 119, 643–647.
Jaime, L., Mendiola, J.A., Ibáñez, E., Martin-Álvarez, P.J., Cifuentes, A., Reglero, G., Seirócas, F.J., 2007. Carotenoid isomer composition of sub- and supercritical carbon dioxide extracts. Antioxidant activity measurement. J. Agric. Food Chem. 55, 10585–10590.
Jayaprakash, G.K., Girennavar, B., Patil, B.S., 2008. Radical scavenging activities of ripe red grapefruits and sour orange fruit extracts in different in vitro model systems. Biosens. Resour. Technol. 99, 4484–4494.
Ji, L., Pan, J.G., Xu, Z.L., 1990. The GC–MS analysis of volatile oil from Lonicera japonica thunb. J. Chin. Mater. Med. 15, 680 (in Chinese).
Kang, O.H., Choi, Y.A., Park, H.J., Lee, J.Y., Kim, D.K., Choi, S.C., Kim, T.H., Nah, Y.H., Yun, K.J., Choi, S.J., Kim, Y.H., Bae, K.H., Lee, Y.M., 2004. Inhibition of trypsin-induced mast cell activation by water fraction of Lonicera japonica. Arch. Pharm. Res. 27, 1141–1146.
Katsube, T., Tabata, H., Ohba, Y., Yamasaki, Y., Anamurd, E., Shiwaku, K., Yamane, Y., 2004. Screening for antioxidant activity in edible plant products: comparison of low-density lipoprotein oxidation assay, DPPH radical scavenging assay, and Fe(II)-chelating assay. J. Agric. Food Chem. 52, 2391–2396.
King, J.W., 2014. Modern supercritical fluid technology for food applications. Annu. Rev. Food Sci. Technol. 5, 215–238.
Kiselova, Y., Ivanova, D., Chervenkov, T., Gerova, D., Calunuska, B., Yankova, T., 2006. Correlation between the flavonoid content and polyphenol content of aqueous extracts from Bulgarian herbs. Phytother. Res. 20, 961–965.
Klimczak, I., Malecka, M., Szlachta, M., Gliszczynska-Swiglo, A., 2007. Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. J. Food Compos. Anal. 20, 313–322.
Kostyuk, V.A., Potapovich, A.J., 1998. Antiradical and chelating effects in flavonoid protection against silica-induced cell injury. Arch. Biochem. Biophys 355, 44–48.
Myung, W.B., Cheourun, J., Tae, W.J., Cheul, H.H., 2004. Effects of gamma irradiation on color characteristics and biological activities of extracts of Lonicera japonica (Japanese honeysuckle) with methanol and acetone. Lebensm.-Wiss. Technol. 37, 29–33.
Quaitin, A.T., Kai, T., Sasaki, M., Goto, M., 2013. Supercritical carbon dioxide extraction of fucosanthin from Undaria pinnatifida. J. Agric. Food Chem. 61, 5792–5797.
Shang, X., Pan, H., Li, M., Xiao, X., Ding, H., 2011. Lonicera japonica Thunb.: ethnomycnacology, phytochemistry and pharmacology of an important traditional Chinese medicine. J. Ethnomedic. 138, 1–21.
Tao, J., Han, S.W., Yen, J.Y., Kim, J.A., Kang, O.H., Baek, O.S., Lim, J.P., Kim, D.K., Kim, Y.H., Bae, K.H., Lee, Y.M., 2003. Anti-inflammatory effect of Lonicera japonica in proteasome-activated receptor 2-mediated paw edema. Clin. Chim. Acta 330, 165–171.
Tsao, K.D., Hsu, H.F., Chen, Z.H., Wang, Y.T., Huang, S.H., Chen, H.J., Wang, C.P., Wang, S.W., Chang, C.C., Houng, J.Y., 2014. Antioxidant anti-inflammatory, and anti-proliferative activities of extracts from different parts of farmed and wild Glissocarpus teminifolia. Ind. Crop. Prod. 57, 98–105.
Uddin, M.S., Sarker, M.Z., Ferdos, S., Akanda, M.J., Fasim, M.S., Bt Shamsudin, S.H., Bin Yunus, K., 2015. Phytoestrogens and their extraction from various plant matrices using supercritical carbon dioxide: a review. J. Sci. Food Agric. 95 (7), 1385–1394.
Vijayalaxmi, S., Jayalakshmi, S.K., Sreeamulu, K., 2015. Polyphenols from different agricultural residues: extraction, identification and their antioxidant properties. J. Food Sci. Technol. 52 (5), 2761–2769.
Wang, L., Tu, Y.C., Lian, T.W., Hung, J.T., Yen, J.H., Wu, M.J., 2006. Distinctive antioxidant and anti-inflammatory effects of flavonoids. J. Agric. Food Chem. 54, 9798–9804.
Wang, L.J., 2010. The study progress of Lonicera japonica. Med. Inf. 8, 2293–2296 (in Chinese).
Wang, L., 2011. Advances in extraction of plant products in nutraceutical processing. In: Pathak, Y. (Ed.), Handbook of Nutraceuticals. Volume II. Scale-Up, Processing and Automation. CRC Press, Boca Raton, FL, USA, pp. 15–52.
Xu, Y.B., Olverson, B.G., Simmons, D.L., 2007. Trifunctional inhibition of COX-2 by extracts of Lonicera japonica: direct inhibition, transcriptional and post-transcriptional down regulation. J. Ethnopharmacol. 111, 667–670.
Yen, H.W., Yang, S.C., Chen, H.C., Jesica Chang, J.S., 2015. Supercritical fluid extraction of valuable compounds from microalgae biomass. Biosens. Technol. 184, 291–296.
Zielinski, H., Kozlowska, H., 2000. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. J. Agric. Food Chem. 48, 2008–2016.