

Pharmacognostic Specification, Chlorogenic Acid Content, and *In vitro* Antioxidant Activities of *Lonicera japonica* Flowering Bud

Chayanon Chaowuttikul¹, Chanida Palanuvej¹, Nijisiri Ruangrungsi^{1,2}

¹Public Health Sciences Program, College of Public Health Sciences, Chulalongkorn University, Bangkok 10330, ²Department of Pharmacognosy, Faculty of Pharmacy, Rangsit University, Pathum Thani 12000, Thailand

ABSTRACT

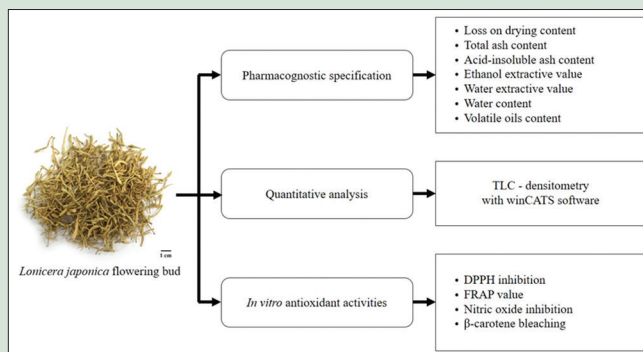
Background: *Lonicera japonica* Thunb. or Japanese Honeysuckle has been widely used in traditional medicine for antipyretic. **Objective:** To establish the pharmacognostic specification of *L. japonica* flowering bud in Thailand and to determine its chlorogenic acid content and *in vitro* antioxidant activities. **Materials and Methods:** Dried *L. japonica* flowering bud from 15 various herbal drugstores throughout Thailand were investigated for pharmacognostic specification. Their chlorogenic acid contents were quantitatively analyzed by thin layer chromatography (TLC) densitometry with winCATS software. The mobile phase for TLC development consisted of ethyl acetate: formic acid: acetic acid: water (10:1.1:1.1:2.6). Antioxidant activities were investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric ion reducing antioxidant power assay, nitric oxide scavenging assay, and β -carotene bleaching assays. **Results:** Qualified *L. japonica* flowering bud in Thailand was presented that the contents of loss on drying, total ash, acid-insoluble ash, and water should not be >10.11%, 6.59%, 1.14%, and 10.82% by weight, respectively. The ethanol and water soluble extractive values should not be <16.46% and 28.88% by weight, respectively. Chlorogenic acid content in *L. japonica* flowering bud was found to be 2.24 ± 0.50 g/100 g of crude drug. *L. japonica* flowering bud showed DPPH and nitric oxide scavenging activities as well as reducing power property. **Conclusion:** This pharmacognostic specification with special reference to the chlorogenic acid content can be used for quality control of *L. japonica* flowering bud in Thailand. The potential antioxidant of this crude drug was demonstrated *in vitro*.

Key words: Antioxidant activity, chlorogenic acid, *Lonicera japonica* Thunb., pharmacognostic specification, quantitative analysis, thin layer chromatography-densitometry

SUMMARY

- Pharmacognostic specification of *Lonicera japonica* flowering bud in Thailand has been established
- The chlorogenic acid content has been quantified by thin layer chromatography-densitometry

- The ethanolic extract of *L. japonica* flowering bud showed antioxidation potential, especially on reducing power property.



Abbreviations Used: TLC: Thin layer chromatography, DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: Ferric ion Reducing Antioxidant Power, WHO: World Health Organization, ICH: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, LOD: Limit of detection, LOQ: Limit of quantitation, BHT: Butylated hydroxytoluene, FeSO_4 : Iron(II) sulfate; DMSO: Dimethyl sulfoxide, TPTZ: 2,4,6-tripyridyl-s-triazine.

Correspondence:

Dr. Chanida Palanuvej,
College of Public Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand.
E-mail: chanida.p@chula.ac.th
DOI: 10.4103/pr.pr_117_16

Access this article online

Website: www.phcogres.com

Quick Response Code:



INTRODUCTION

Lonicera japonica Thunb. (*Caprifoliaceae*), commonly known in English as “Japanese Honeysuckle” and called “Sai Nam Phueng” in Thai, is native to the eastern Asia and become naturalized in Argentina, Brazil, Mexico, Australia, New Zealand, and the United States.^[1,2] The pharmacological studies of *L. japonica* flowering bud have shown a wide biological activity such as antibacterial,^[3,4] α -glucosidase inhibitory,^[5] antiviral,^[6] anti-inflammatory,^[7,8] antinociceptive,^[9] antiangiogenic,^[9] antioxidant,^[10,11] hepatoprotective,^[12] antifibrotic,^[13] and neuroprotective activities.^[14] In Thailand, the plant is widely used for antipyretic effect.^[2] The chemical constituents have been widely researched. The main compositions such as organic acids, flavones, saponins, iridoids, essential oil, and inorganic elements were isolated and identified. In Chinese Pharmacopoeia, the indicator compound of *L. japonica* is chlorogenic acid, which has been used as characteristics for the quality of this plant.^[1]

Chlorogenic acid [Figure 1] is a kind of polyphenol derivative widely found in plants, fruits, and vegetables. Structurally, it is an ester form

of caffeic acid and quinic acid.^[15] Chlorogenic acid has been shown its biological and physiological activities such as antihyperalgesic,^[16] radioprotective,^[17] anti-inflammatory,^[18] antiulcerogenic,^[19] antihepatotoxic,^[20] neuroprotective effects,^[21] and antioxidant activity of chlorogenic acid-rich extract from *Etlingera elatior* leaves.^[22]

The standardization is important for quality, purity, and authentication of plant crude drug.^[23] Therefore, the aims of this study were to determine the pharmacognostic specification of *L. japonica* flowering bud in

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Chaowuttikul C, Palanuvej C, Ruangrungsi N. Pharmacognostic specification, chlorogenic acid content, and *In vitro* antioxidant activities of *Lonicera japonica* flowering bud. *Phcog Res* 2017;9:128-32.

Thailand, to investigate the content of chlorogenic acid using thin layer chromatography (TLC)–densitometry, and to examine its antioxidant activities by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric ion reducing antioxidant power (FRAP) assay, nitric oxide scavenging assay, and β -carotene bleaching assay for evidence-based efficacy of this crude drug.

MATERIALS AND METHODS

Plant material and chemicals

Dried *L. japonica* flowering bud was purchased from 15 various herbal drugstores throughout Thailand during July–September 2014 and authenticated by one of the authors, Ruangrunsi N. Voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University. Crude drugs were grounded into coarse powder before use. The standard chlorogenic acid (CAS no. 327-97-9) was purchased from Sigma-Aldrich, USA. The chemicals used were of analytical grade.

Morphological identification

The macroscopic study of crude drugs was observed based on the shape, size, color, texture, odor, and taste. The flowering bud powder and cross-section of the corolla were observed under microscope (Carl Zeiss, Germany) to identify tissue and cell structures for microscopic characteristics. Pictures were illustrated by hand drawing in proportional scale related to the original size.

Physicochemical characteristics

Determination of loss on drying, total ash, acid-insoluble ash, ethanol and water extractive values, water content, and volatile oil content were evaluated to establish the pharmacognostic specification of *L. japonica* flowering bud.^[23,24] All samples were analyzed in triplicate. Grand mean and pooled standard deviation were calculated.

Quantitative analysis of chlorogenic acid by thin layer chromatography–densitometry

The powders of flowering bud were extracted with 95% ethanol by Soxhlet apparatus till exhaustion. The extract was filtered, evaporated to dryness, and calculated the percent yield of dried crude drugs. Ten milligrams of the extract was dissolved with 1 ml of 95% ethanol for TLC analysis.

Three microliters of various standard concentrations and 15 ethanolic extracts were spotted on the silica gel 60 GF₂₅₄ TLC plate (20 cm × 10 cm) and developed in a TLC chamber containing ethyl acetate:formic acid:acetic acid:water (10:1.1:1.1:2.6) as mobile phase. The developed TLC plate was scanned by TLC scanner 4 (Camag, Switzerland) at a wavelength of 325 nm and expressed as chromatographic peak by winCATS software. The test was done in triplicate.

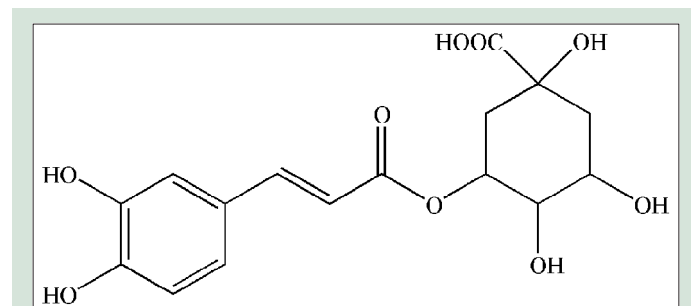


Figure 1: The structure of chlorogenic acid

Method validation

According to the International Conference on Harmonisation guideline,^[25] calibration range, specificity, accuracy, repeatability, intermediate precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness of chlorogenic acid quantitative analysis in flowering bud were validated.

Antioxidant activities

2, 2-diphenyl-1-picrylhydrazyl radical scavenging assay

One hundred microliters of various ethanolic extract concentrations, standard chlorogenic acid, and positive controls (quercetin and butylated hydroxytoluene [BHT]) dissolved in methanol were added to 100 μ l of 120 μ M DPPH methanolic solution in 96-well microplate.^[26] The plate was incubated for 30 min in the dark at room temperature. The absorbance at 517 nm was measured. Each sample was done in triplicate. Percent scavenging activity was calculated:

$$\% \text{Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

Ferric ion reducing antioxidant power assay

Twenty-five microliters of ethanolic extract and standard chlorogenic acid (1 mg/ml) were mixed with 175 μ l of FRAP reagent in 96-well microplate and incubated for 30 min in the dark at room temperature. The absorbance at 593 nm was measured using microplate reader.^[27] Quercetin and BHT was used as a positive control. The FRAP value of samples was calculated using the linear relationship from the calibration curve of FeSO₄ methanolic solutions in the range of 0.5–1.5 mM. Each sample was done in triplicate. The samples and positive controls were expressed as mM of ferrous iron (Fe(II)/mg crude extract).

Nitric oxide scavenging assay

The mixture containing 50 μ l of ethanolic extract, standard chlorogenic acid, or positive controls (quercetin) at concentration 800 μ g/ml dissolved in dimethyl sulfoxide and 50 μ l of sodium nitroprusside (10 mM) in phosphate-buffered saline was incubated at 25°C for 150 min. Then, the mixture reacted with 50 μ l of Griess reagent (0.33% sulphanilamide in 20% glacial acetic acid) and incubated for 10 min. After incubation, 50 μ l of 0.1% naphthyl ethylenediamine dihydrochloride was added and allowed to stand for 30 min.^[28,29] The absorbance at 540 nm was measured. Each sample was done in triplicate. Percent scavenging activity was calculated as aforementioned.

β -carotene bleaching assay

One milliliter of β -carotene solution (2 mg/ml in chloroform) was mixed with 40 μ l of linoleic acid (10 mg/ml) and 400 μ l of Tween 20. The chloroform was removed at 40°C under vacuum. The mixture was diluted with 50 ml of ultrapure water and shaken to form an emulsion. Two hundred microliters of the emulsion was transferred into the 96-well microplate which contained 10 μ l of the various concentrations of ethanolic extract, standard chlorogenic acid, or positive controls (quercetin and BHT) and heated at 50°C.^[30,31] The absorbance at 470 nm was recorded at 30 min intervals for 120 min. Each sample was done in triplicate. The antioxidant activity was calculated:

$$\% \text{Antioxidant activity} = 1 - \left(\frac{A_0 - A_{120}}{C_0 - C_{120}} \right) \times 100$$

Where A_0 , A_{120} : The absorbance values measured at zero time and end time of incubation for sample, C_0 , C_{120} : The absorbance values measured at zero time and end time of incubation for control.

RESULTS AND DISCUSSION

Pharmacognostic specification

Macroscopic and microscopic characteristics are the first step to establish the identity of materials.^[23] A dried flowering bud was yellowish-green color, clavate shape, and 2–3 cm in length with velvet surface [Figure 2a]. Histological and anatomical characters including corolla, glandular and nonglandular trichomes, pollen grain, spiral, parenchyma, and calcium oxalate were illustrated in Figure 2b and c.

The physicochemical characteristics are an essential for qualification of crude drug. The pharmacognostic specification of *L. japonica* flowering bud was demonstrated in Table 1. The ethanol and water soluble extractive values should not be <16.46% and 28.88% by weight, respectively, while the loss on drying, total ash, acid-insoluble ash, and water content should not be >10.11%, 6.59%, 1.14%, and 10.82% by dry weight, respectively. The total ash, acid-insoluble ash, and water content were less than previously reported (10.6%, 1.2%, and 17.5%, respectively).^[32] In Chinese Pharmacopoeia, the total ash, acid-insoluble ash, and water content were not >10.0%, 3.0%, and 12.0%, respectively.^[33] The volatile oil was undetected in this study because the samples were dried crude drug, and the previous study showed the lowest volatile oil content at flowering bud stage.^[34]

Quantitative analysis of chlorogenic acid by thin layer chromatography–densitometry

The yield of ethanolic extract of *L. japonica* flowering bud was 39.44 ± 5.83 g/100 g crude drug. Chlorogenic acid content in the ethanolic

extract was found to be 0.06 ± 0.01 g/g and in the crude drug was found to be 2.24 ± 0.50 g/100 g which was less than previously reported as 2.62%–3.66%.^[35] However, according to Chinese Pharmacopoeia, the content of chlorogenic acid in *L. japonica* flowering bud should not be <1.5%.^[33]

Method validation

The calibration range of chlorogenic acid was polynomial ranged from 0.6 to 3.0 µg/spot [Figure 3]. The specificity was shown by comparing ultraviolet (UV) spectrum of the peak apex among all samples and standard chlorogenic acid for peak identity, and the comparison of UV spectrum recorded at up-slope, apex, and down-slope of the peak for peak purity. The spectra showed the maximum absorbance at the wavelength of 325 nm [Figure 4]. The validity of chlorogenic acid quantification in *L. japonica* flower buds was displayed in Table 2. The recovery was determined to evaluate the accuracy of the method by spiking known three concentrations of standard chlorogenic acid in a sample extract. The recovery value was $94.10\% \pm 4.09\%$. The repeatability and the intermediate precision were determined on the same day and in three different days. The repeatability and the intermediate precision were 1.17%RSD and 3.73%RSD, respectively. The LOD and LOQ of TLC–densitometry were calculated by the residual standard deviation of a regression line and was found to be 0.07 and 0.21 µg/spot, respectively.

Table 1: Physicochemical characteristics of *Lonicera japonica* flowering bud

Parameter	Content (% by weight)
Loss on drying content	10.11±0.06
Total ash content	6.59±0.05
Acid-insoluble ash content	1.14±0.06
Ethanol extractive value	16.46±0.25
Water extractive value	28.88±0.59
Water content	10.82±0.49
Volatile oils content	0

Table 2: The validity of chlorogenic acid in *Lonicera japonica* flower buds

Parameter	Validity
Accuracy: % recovery	94.10±4.09
Precision: Repeatability	1.17%RSD
Precision: Intermediate precision	3.73%RSD
LOD	0.07 µg/spot
LOQ	0.21 µg/spot
Robustness	8.59%RSD

LOD: Limit of detection; LOQ: Limit of quantitation; RSD: Relative standard deviation

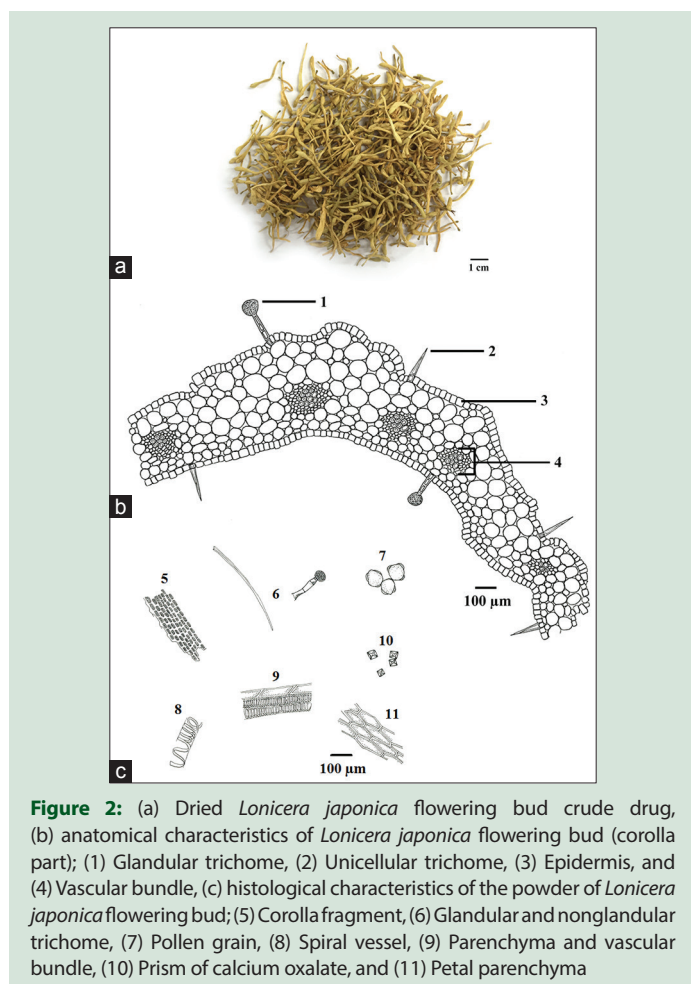


Figure 2: (a) Dried *Lonicera japonica* flowering bud crude drug, (b) anatomical characteristics of *Lonicera japonica* flowering bud (corolla part); (1) Glandular trichome, (2) Unicellular trichome, (3) Epidermis, and (4) Vascular bundle, (c) histological characteristics of the powder of *Lonicera japonica* flowering bud; (5) Corolla fragment, (6) Glandular and nonglandular trichome, (7) Pollen grain, (8) Spiral vessel, (9) Parenchyma and vascular bundle, (10) Prism of calcium oxalate, and (11) Petal parenchyma

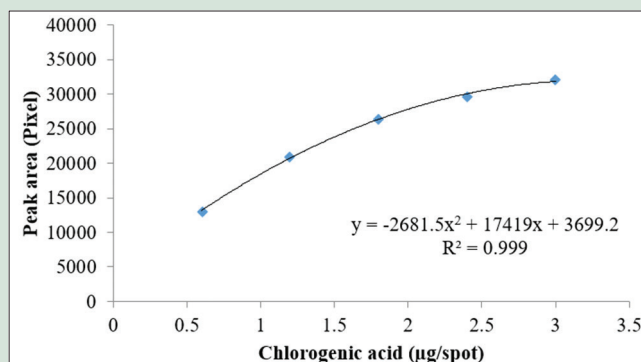


Figure 3: The calibration curve of chlorogenic acid by thin layer chromatography–densitometry

The robustness performed by varying the mobile phase ratio showed the values of 8.59%RSD. The results were close to the previous study reported that the accuracy, repeatability, intermediate precision, LOD, and LOQ were 99.66%–101.59% recovery, 1.01–1.32%RSD, 3.21%RSD, 0.12 µg/spot, and 0.20 µg/spot.^[35] Thus, this method was suitable and reliable to evaluate the quantitative analysis.

Antioxidant activities

The antioxidant activities of *L. japonica* flowering bud ethanolic extract and standard chlorogenic acid were summarized in Table 3.

The character of DPPH is a stable free radical for evaluate the ability of substances that can donate a hydrogen atom or free radical scavengers, caused by the delocalization of DPPH, and to estimate the antioxidant activity.^[36] The result of DPPH radical scavenging activity demonstrated that *L. japonica* flowering bud extract showed IC₅₀ as 54.78 µg/ml. The scavenging activity of *L. japonica* flowering bud was less potent than chlorogenic acid, quercetin, and BHT with IC₅₀ of 7.83, 4.84, and 24.82 µg/ml, respectively. The previous study also presented higher potential DPPH radical scavenging activity of dried *L. japonica* flowering bud as 19.45 µg/ml.^[37]

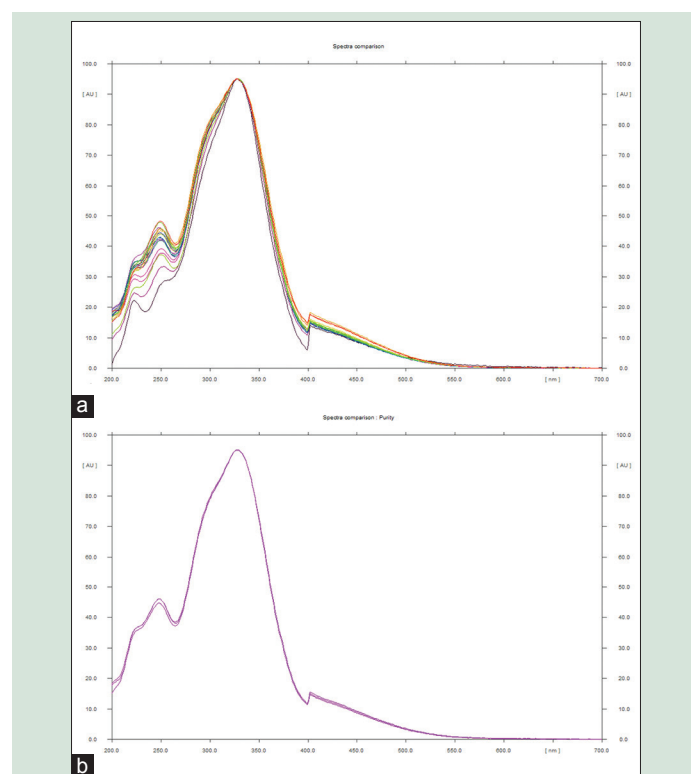


Figure 4: (a) The ultraviolet absorbance spectra of chlorogenic acid in samples and standard chlorogenic acid bands, (b) peak purity determination using up-slope, apex, and down-slope of the peak

The FRAP assay is antioxidant method to measure reducing the power of ferric iron and 2,4,6-tripyridyl-s-triazine to a colored product in plasma and botanicals.^[38] In this study, *L. japonica* flowering bud extract showed the FRAP value as 1.24 ± 0.02 mM FeSO₄/mg crude extract which similarly to chlorogenic acid as 1.17 ± 0.02 mM FeSO₄/mg substance. Quercetin and BHT showed FRAP value of 1.48 ± 0.06 and 1.51 ± 0.01 mM FeSO₄/mg substance, respectively.

Nitric oxide scavenging assay evaluates the ability of the substance to scavenge nitric oxide. The nitric oxide (NO[•]) reacts with oxygen, under aerobic conditions, to produce stable products (nitrate and nitrite), Griess reagent was applied to determine the quantity of nitric oxide scavenging assay.^[39] The result showed percent nitric oxide inhibition of *L. japonica* flowering bud as 49.86% compared to chlorogenic acid and quercetin which showed percent inhibition of 75.97% and 72.27%, respectively.

β-carotene bleaching assay measures the ability of an antioxidant to inhibit lipid peroxidation which produced by linoleic acid. The discoloration of yellow color of β-carotene is due to peroxide free radicals. The activity of β-carotene bleaching can be decrease with the presence of antioxidants.^[39] In this study, *L. japonica* flowering bud at a concentration of 1 mg/ml showed antioxidant activity of 2.71% compared to 1 mg/ml of quercetin and BHT which showed the antioxidant activity of 78.12% and 91.81%, respectively. The antioxidant activities of these extracts, chlorogenic acid, and positive controls demonstrated the dose-response relationship [Figure 5].

CONCLUSION

This study established the scientific information for authentication and quality control of *L. japonica* flowering bud in Thailand including the pharmacognostic specification as well as the chlorogenic acid content by TLC–densitometry. In addition, the ethanolic extract of *L. japonica* flowering bud showed antioxidation potential, especially on reducing power property.

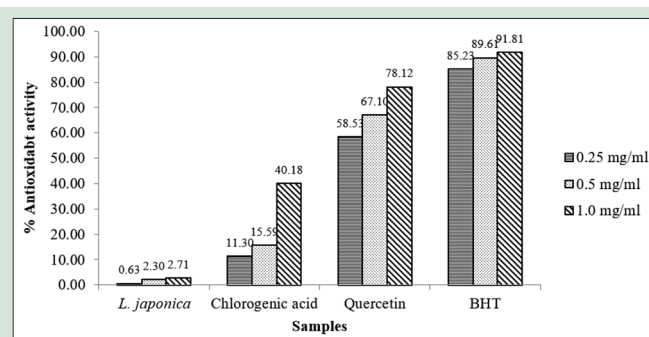


Figure 5: The antioxidant activity of *Lonicera japonica* flowering bud, standard chlorogenic acid, quercetin, and butylated hydroxytoluene by β-carotene bleaching assay

Table 3: The antioxidant activities of *Lonicera japonica* flower buds extract, standard chlorogenic acid, and positive control

Sample	DPPH inhibition (IC ₅₀ µg/ml)	FRAP value (mM FeSO ₄ /mg crude extract)	Nitric oxide inhibition (%)*	β-carotene bleaching (%)**
<i>Lonicera japonica</i> flowering bud ethanolic extract	54.78	1.24±0.02	49.86	2.71
Chlorogenic acid	7.83	1.17±0.02	75.97	40.18
Quercetin	4.84	1.48±0.06	72.27	78.12
BHT	24.82	1.51±0.01	-	91.81

*Concentration of all samples at 800 µg/ml; **Concentration of all samples at 1 mg/ml. DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric ion reducing antioxidant power; BHT: Butylated hydroxyl toluene

Acknowledgement

The authors are supported the scholarship from “The 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship.” The authors are also thankful to College of Public Health Sciences, Chulalongkorn University, and all the staff members for necessary assistance and instrumental support.

Financial support and sponsorship

This study was financially supported by The 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Shang X, Pan H, Li M, Miao X, Ding H. *Lonicera japonica* Thunb.: Ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine. *J Ethnopharmacol* 2011;138:1-21.
- Thanabhorn S, Jaijoy K, Thamaree S, Ingkaninan K, Panthong A. Acute and subacute toxicity study of the ethanol extract from *Lonicera japonica* Thunb. *J Ethnopharmacol* 2006;107:370-3.
- Fu L, Li ZQ, Xie ZC, Tan ZX, Zhang QL. The *in vitro* antibacterial activity of dried flowers used in Guangdong traditional soup. *Int J Food Nutr Saf* 2015;6:106-16.
- Shan B, Cai YZ, Brooks JD, Corke H. The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. *Int J Food Microbiol* 2007;117:112-9.
- Zhang Z, Luo A, Zhong K, Huang Y, Gao Y, Zhang J, *et al.* α -glucosidase inhibitory activity by the flower buds of *Lonicera japonica* Thunb. *J Funct Foods* 2013;5:1253-9.
- Ma SC, Du J, But PP, Deng XL, Zhang YW, Ooi VE, *et al.* Antiviral Chinese medicinal herbs against respiratory syncytial virus. *J Ethnopharmacol* 2002;79:205-11.
- Lee JH, Ko WS, Kim YH, Kang HS, Kim HD, Choi BT. Anti-inflammatory effect of the aqueous extract from *Lonicera japonica* flower is related to inhibition of NF- κ B activation through reducing I- κ B α degradation in rat liver. *Int J Mol Med* 2001;7:79-83.
- Chan BC, Hon KL, Leung PC, Sam SW, Fung KP, Lee MY, *et al.* Traditional Chinese medicine for atopic eczema: PentaHerbs formula suppresses inflammatory mediators release from mast cells. *J Ethnopharmacol* 2008;120:85-91.
- Yoo HJ, Kang HJ, Song YS, Park EH, Lim CJ. Anti-angiogenic, antinociceptive and anti-inflammatory activities of *Lonicera japonica* extract. *J Pharm Pharmacol* 2008;60:779-86.
- Choi CW, Jung HA, Kang SS, Choi JS. Antioxidant constituents and a new triterpenoid glycoside from *Flos Lonicerae*. *Arch Pharm Res* 2007;30:1-7.
- Tang D, Li HJ, Chen J, Guo CW, Li P. Rapid and simple method for screening of natural antioxidants from Chinese herb *Flos Lonicerae Japonicae* by DPPH-HPLC-DAD-TOF/MS. *J Sep Sci* 2008;31:3519-26.
- Yip EC, Chan AS, Pang H, Tam YK, Wong YH. Protocatechuic acid induces cell death in HepG2 hepatocellular carcinoma cells through a c-Jun N-terminal kinase-dependent mechanism. *Cell Biol Toxicol* 2006;22:293-302.
- Teng Y, Sun CH, Li G, Sun G, Nomachi Y, Yokota J, *et al.* Protective effects of *Flos Lonicera* extract on acute liver injury by dimethylnitrosamine-induced in rats. *J Nat Med* 2010;64:288-94.
- Weon JB, Yang HJ, Lee B, Yun BR, Ahn JH, Lee HY, *et al.* Neuroprotective activity of the methanolic extract of *Lonicera japonica* in glutamate-injured primary rat cortical cells. *Pharmacogn Mag* 2011;7:284-8.
- Sato Y, Itagaki S, Kurokawa T, Ogura J, Kobayashi M, Hirano T, *et al.* *In vitro* and *in vivo* antioxidant properties of chlorogenic acid and caffeic acid. *Int J Pharm* 2011;403:136-8.
- Bagdas D, Cinkilic N, Ozboluk HY, Ozyigit MO, Gurun MS. Antihyperalgesic activity of chlorogenic acid in experimental neuropathic pain. *J Nat Med* 2013;67:698-704.
- Cinkilic N, Cetintas SK, Zorlu T, Vatan O, Yilmaz D, Cavas T, *et al.* Radioprotection by two phenolic compounds: Chlorogenic and quinic acid, on X-ray induced DNA damage in human blood lymphocytes *in vitro*. *Food Chem Toxicol* 2013;53:359-63.
- dos Santos MD, Almeida MC, Lopes NP, de Souza GE. Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. *Biol Pharm Bull* 2006;29:2236-40.
- Shimoyama AT, Santin JR, Machado ID, de Oliveira e Silva AM, de Melo IL, Mancini-Filho J, *et al.* Antiulcerogenic activity of chlorogenic acid in different models of gastric ulcer. *Naunyn Schmiedeberg Arch Pharmacol* 2013;386:5-14.
- Kapil A, Koul IB, Suri OP. Antihepatotoxic effects of chlorogenic acid from *Anthocephalus cadamba*. *Phytother Res* 1995;9:189-93.
- Li Y, Shi W, Li Y, Zhou Y, Hu X, Song C, *et al.* Neuroprotective effects of chlorogenic acid against apoptosis of PC12 cells induced by methylmercury. *Environ Toxicol Pharmacol* 2008;26:13-21.
- Chan EW, Lim YY, Tan SP. Standardised herbal extract of chlorogenic acid from leaves of *Etilingera elatior* (Zingiberaceae). *Pharmacognosy Res* 2011;3:178-84.
- World Health Organization. Quality Control Methods for Herbal Materials. Geneva: WHO; 1998.
- Duangyod T, Palanuvej C, Ruangrunsi N. Pharmacognostic specifications and quantification of (+)-catechin and (–)-epicatechin in *Pentace burmanica* stem bark. *Pharmacognosy Res* 2014;6:251-6.
- The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1). Geneva: ICH; 2005.
- Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *Lebensm Wiss Technol* 1995;28:25-30.
- Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Anal Biochem* 1996;239:70-6.
- Rai S, Wahile A, Mukherjee K, Saha BP, Mukherjee PK. Antioxidant activity of *Nelumbo nucifera* (sacred lotus) seeds. *J Ethnopharmacol* 2006;104:322-7.
- Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B. *In vitro* antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. *Pharmacogn Mag* 2009;5:122-6.
- Jayaprakasha GK, Jena BS, Negi PS, Sakariah KK. Evaluation of antioxidant activities and antimutagenicity of turmeric oil: A byproduct from curcumin production. *Z Naturforsch C* 2002;57:828-35.
- Amran N, Rani AN, Mahmud R, Yin KB. Antioxidant and cytotoxic effect of *Barringtonia racemosa* and *Hibiscus sabdariffa* fruit extracts in MCF-7 human breast cancer cell line. *Pharmacognosy Res* 2016;8:66-70.
- Mo Z, Huang N. Identification and determination of adulterant of honeysuckle. *Food Drug* 2010;9:341-3.
- Pharmacopoeia Committee of PR China. Pharmacopoeia of People's Republic of China. Beijing: Chemical Industrial; 2005.
- Wang LM, Li MT, Yan YY, Ao MZ, Wu G, Yu LJ. Influence of flowering stage of *Lonicera japonica* Thunb. on variation in volatiles and chlorogenic acid. *J Sci Food Agric* 2009;89:953-7.
- Rumalla CS, Avula B, Zhao J, Smillie TJ, Khan IA. Quantitative determination of phenolic acids in *Lonicera japonica* Thunb. using high performance thin layer chromatography. *J Liq Chromatogr Relat Technol* 2011;34:38-47.
- Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. *J Food Sci Technol* 2011;48:412-22.
- Dung NT, Bajpai VK, Rahman A, Yoon JI, Kang SC. Phenolic contents, antioxidant and tyrosinase inhibitory activities of *Lonicera japonica* Thunb. *J Food Biochem* 2011;35:148-60.
- Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem* 2005;53:4290-302.
- Alam MN, Bristi NJ, Rafiqzaman M. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharm J* 2013;21:143-52.