Songklanakarin J. Sci. Technol.
42 (2), 314-320, Mar. - Apr. 2020

http://www.sjst.psu.ac.th

Original Article

Development of a high-performance liquid chromatography for analysis of corosolic acid in *Lagerstroemia* species and their hypoglycemic potentials

Gorawit Yusakul[1, 2], Paritad Saensom[3], Nattapol Mitsantia[3], Chattarika Pengdee[3], and Waraporn Putalun[3, 4*]

[1] *School of Pharmacy, Walailak University, Tha Sala, Nakhon Si Thammarat, 80160 Thailand*

[2] *Drug and Cosmetics Excellence Center, Walailak University, Tha Sala, Nakhon Si Thammarat, 80160 Thailand*

[3] *Faculty of Pharmaceutical Sciences, Khon Kaen University, Mueang, Khon Kaen, 40002 Thailand*

[4] *Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology, Khon Kaen University, Mueang, Khon Kaen, 40002 Thailand*

Received: 10 November 2018; Revised: 11 December 2018; Accepted: 18 December 2018

Abstract

Worldwide people suffer from metabolic syndrome and its complications. For these conditions, functional foods are becoming more important. We aimed to determine the amounts of corosolic acid in *Lagerstroemia* species and their α-glucosidase inhibitory potency. In addition, we developed a new source of corosolic acid using the plant tissue culture technique. The HPLC-UV method was reliable and applicable for corosolic acid determination of the *Lagerstroemia* species. Although the corosolic acid standardized extract is usually prepared using *L. speciosa*, our results revealed that *L. macrocarpa* and *L. loudonii* contained much higher amounts of corosolic acid. In addition, the established callus culture of *L. speciosa* produced corosolic acid with higher content than their parental *L. speciosa* mature leaves. The corosolic contents in these samples were also in agreement with α-glucosidase inhibitory activity. Therefore, this method is worthy of antidiabetic standardization of *Lagerstroemia* derived materials.

Keywords: *Lagerstroemia*, corosolic acid, HPLC, α-glucosidase inhibition, diabetes

1. Introduction

Metabolic syndrome is a global health problem. Worldwide, in 1980 and 2013, male adults with a body-mass index (BMI) of 25 kg/m^2 or greater increased from 28.8% to 36.9% and from 28.8% to 38.0% in woman (Ng *et al.*, 2014). It is clear that overweight or obesity, an unhealthy diet, physical inactivity, and smoking increase the risk of type 2 diabetes. Complications from diabetes are associated with damage to the heart, blood vessels, eyes, kidneys, and nerves leading to disabilities and premature death if the conditions cannot be controlled appropriately. Currently, antidiabetic drugs are classified into biguanides, dipeptidyl peptidase-4 (DPP4) inhibitors, insulins, sodium glucose cotransporter 2

*Corresponding author
Email address: waraporn@kku.ac.th

inhibitors, glucagon-like peptide-1 receptor agonist, sulfonylureas, and thiazolidinediones. An undesirable effect of some antidiabetic drugs is weight gain after treatment with insulins, sulfonylureas, and thiazolidinediones. Combinations between classes of antihyperglycemic drugs are widely used in clinical practice for appropriate management of type 2 diabetes. For example, the addition of a DPP4 inhibitor for type 2 diabetic patients who are inadequately controlled with an α-glucosidase inhibitor, achieved better glycemic control (Min, Yoon, Hahn, & Cho, 2018). α-Glucosidase inhibitors used in combination with metformin were associated with significantly lower major adverse cardiovascular risk (Chan *et al.,* 2018). In type 2 diabetic patients, co-treatment with α-glucosidase inhibitors and sulfonylureas also prolongs the duration of good glycemic control compared with sulfonylureas alone. Overall, a combination of α-glucosidase inhibitors with other classes of antidiabetic drugs provides better outcomes of treatment.

Functional foods with α-glucosidase inhibitory properties are complementary choices for diabetic patients. *Lagerstroemia speciosa* has been applied traditionally for antihyperglycemic purposes in traditional medicines. Corosolic acid (Figure 1) and tannins are considered to be bioactive constituents of *L. speciosa* for lowering glucose levels (Miura, Takagi, & Ishida, 2012). In the Philippines, *L. speciosa* has been used in both traditional medicines and food supplements for diabetes and kidney related diseases (Klein, Kim, Himmeldirk, Cao, & Chen, 2007). The standardized extract of *L. speciosa* that contains 1% corosolic acid (Glucosol™) was demonstrated to significantly reduce blood glucose levels in a clinical trial (Judy *et al.,* 2003). Scientific evidence demonstrated that *L. speciosa* and its active ingredient corosolic acid exhibited α-glucosidase inhibition. In human studies, *L. speciosa* standardized extract (1% corosolic acid) in dosages of 32 mg and 48 mg daily for 2 weeks exhibited a 30% decrease in blood glucose levels (Stohs, Miller, & Kaats, 2012). A 10 mg dose of corosolic acid resulted in significant lowering of blood glucose levels compared to control at the 90-min time point when corosolic acid was given 5 min before a 75 g oral glucose tolerance test (Fukushima *et al.,* 2006). Besides the glucose lowering effects, the *L. speciosa* standardized extract and its combination with other medicinal plants showed potential effect on weight loss in humans (Lieberman, Spahrs, Stanton, Martinez, & Grinder, 2005; Tsuchibe, Kataumi, Mori, & Mori, 2006). Corosolic acid was administered with a high fat diet for 9 weeks. The results indicated that corosolic acid significantly deceased fasting plasma glucose, insulin, and triglycerides compared to control (Yamada *et al.,* 2008b), which implied beneficial effects of corosolic acid against metabolic syndrome. Further investigations indicated that corosolic acid suppressed gluconeogenesis and enhanced glycolysis (Yamada *et al.,* 2008a). Among triterpene acids isolated from *L. speciosa*, corosolic acid showed the best bioactivity against α-glucosidase (Hou *et al.,* 2009). Therefore, the compound is considered to be a bioactive marker for the antidiabetic effect of *L. speciosa*.

Although *L. speciosa* is usually studied and reported for antidiabetic activity, there are many *Lagerstroemia* spp. distributed in Thailand. These species may be a good source of corosolic acid. The presence and content of corosolic acid in these species have not been reported. Therefore, we aimed to develop a method to determine the level of corosolic acid and find a good source of corosolic acid. Moreover, we also aimed to determine the correlation between corosolic acid in the extracts and their α-glucosidase inhibition. Finally, we aimed to establish a sustainable source of corosolic acid using the plant tissue culture technique.

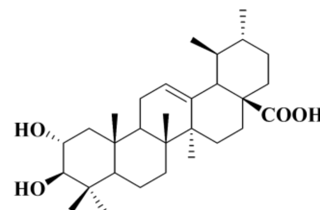

Figure 1. Chemical structure of corosolic acid.

2. Material and Methods

2.1 Chemical reagents

Corosolic acid (≥98%), *p*-nitrophenyl-α-D-glucopyranoside (pNPG), and α-glucosidase from *Saccharomyces cerevisiae* were purchased from the Sigma-Aldrich (MO, USA). Organic solvents including acetonitrile, methanol, and phosphoric acid were analytical-reagent grade supplied by RCI Labscan Limited (Bangkok, Thailand).

2.2 Validation of HPLC method for determination of corosolic acid

The isocratic high-performance liquid chromategraphy (HPLC) method was validated for determination of corosolic acid. The analytical method was performed on an Agilent 1100 series instrument (Agilent Corp., Santa Clara, CA, USA) equipped with a degasser, pump, UV-vis detector, and autosampler. The corosolic acid standard solution (3.12–50.0 µg/mL) or solutions of plant extract were subjected via the autosampler to a reverse phase column (LiChroCart®, 125 ×4 mm, 5 µm particle size; Merck KGaA., Darmstadt, Germany). Then, the column was eluted with an isocratic mobile phase system of acetonitrile and 0.1% (v/v) aqueous phosphoric acid in the ratio of 6:4. The flow rate of the mobile phase was set at l mL/min and the detection wavelength was set at 204 nm. The analytical performance of the HPLC system, that included the sensitivity, precision, and accuracy of corosolic acid determination, was evaluated. The sensitivities in terms of the limit of detection (LOD) and limit of determination (LOQ) of the method were evaluated when serial concentrations of corosolic acid were subjected to the HPLC system. The concentrations of the corosolic acid that yielded a signal-to-noise ratio of 3.3 and 10 were estimated as the LOD and LOQ, respectively. Repeatability of the method was measured by 6 injections (n=6) within one day (intra-day precision) of every concentration (3.13, 6.25, 12.5, 25.0, and 50.0 µg/mL). Inter-day repeatability was analyzed using 3 injections in three consecutive days (n=3). The precisions were expressed as relative standard deviation (%RSD). Accuracy of the analytical methods was determined by a recovery experiment. Corosolic acid in the amounts of 10, 12, and 15 µg were spiked into the extracts of *L. speciosa*. Then, all samples were analyzed using the HPLC method. The percentages of corosolic acid recovery were calculated using the following equation:

$$Recovery\,(\%) = \frac{Css - Cus}{Cs} \times 100$$

where the Css and Cus are the amounts measured in the spiked and unspiked samples, respectively. The Cs is the theoretical spiked amount of corosolic acid.

2.3 Plant samples and their preparation

Plant samples including leaves and branches of *Lagerstroemia speciosa* (L.) Pers., *Lagerstroemia macrocarpa* Wall. ex Kurz, *Lagerstroemia loudonii* Teijsm. & Binn., *Lagerstroemia calyculata* Kurz, and *Lagerstroemia indica* L. were identified by Professor Waraporn Putalun, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. They were collected and dried at 50 ºC for 7 days. All powdered samples were weighted (30 mg) and extracted with 0.5 mL methanol. The extraction was performed using sonication for 15 min. The clear extract was collected after centrifugation for 10 min at 3,000 rpm. The remaining residue of the plant sample was re-extracted with the same process three more times. All extracts were combined and dried at 50 ºC. The obtained dried residuals were dissolved in 1 mL of methanol. In addition, the calluses of *L. speciosa* were also prepared in the same manner. The corosolic acid concentrations in these sample extracts were determined using the HPLC method which was developed and validated.

2.4 Establishment of plant tissue culture system for *L. speciosa*

Plant tissue culture is a high potential technique for a sustainable source of phytochemicals, which are needed worldwide as supplements and cosmeceutical ingredients. Therefore, callus culture of *L. speciosa* was initiated to evaluate the productive capacity of corosolic acid. Initially, young leaves and shoots were washed and sterilized using 1.2% sodium hypochlorite for 20 min. The explant was rinsed with sterilized water to remove the sodium hypochlorite. The explants were sterilized again using 70% (v/v) ethanol for 1 min, and then transferred to Murashige and Skoog (MS) medium supplemented with combinations of plant growth regulators, including thidiazuron (TDZ), 1-naphthaleneacetic acid (NAA), and benzyladenine (BA) (Table 1). When the calluses developed, they were collected and dried. Before the analysis of corosolic acid content in these samples, the dried samples were extracted as described previously.

Table 1. Plant growth regulators for callus induction of *L. speciosa*.

Compositions of plant growth regulators	Concentrations of plant growth regulators (mg/L)		
	Thidiazuron (TDZ)	1-Naphthaleneacetic acid (NAA)	Benzyladenine (BA)
T0.1N0.5	0.1	0.5	-
T0.1N1	0.1	1	-
T0.5N0.5	0.5	0.5	-
T0.5N1	0.5	1	-
N0.5B1	-	0.5	1
N1B0.5	-	1	0.5
N1B1	-	1	1

2.5 α-Glucosidase inhibitory assay

The α-glucosidase activity was evaluated via its capability to release p-nitrophenol from the pNPG substrate. α-Glucosidase inhibitory assay of the plant and callus extracts was performed via the method described previously with minor modifications (Inthongkaew *et al.,* 2017). Sample extracts were prepared using the same method described in the section 2.3 (plant samples and their preparation). Serial concentrations of a test extract (10 µL) were allowed to react with α-glucosidase (100 µL, 0.1 U/mL) at 37 °C for 15 min. The pNPG substrate solution (100 µL, 1 mM) was added to the reaction mixture which was incubated subsequently for 15 min. Finally, Na_2CO_3 solution (50 µL, 1 M) was added to stop the reaction. The absorption (405 nm) of the released p-nitrophenol was recorded using a microplate reader. The percentage of α-glucosidase inhibitory activity was calculated using the following equation:

$$\alpha - \text{Glucosidase inhibitory activity}(\%) = \frac{A\text{control} - A\text{sample}}{A\text{control}} \times 100$$

where *A*control is the absorbance of the control which is absent of test extract and *A*sample is the absorbance where a concentration of test extract was present. A α-glucosidase inhibitory activity (%) curve against the concentrations of the samples was drawn. Finally, the concentration expressed as 50% inhibitory concentration (IC_{50}) that decreased the formation of p-nitrophenol was calculated.

3. Results and Discussion

3.1 Validation of a HPLC method for determination of corosolic acid

HPLC-UV is a universal analytical method applied in pharmaceutical analyses. The isocratic HPLC-UV method was successfully developed for corosolic acid determination. The retention of corosolic acid was 9.2±0.4 min (Figure 2). The peak of corosolic acid was well separated from the other components in the extracts (Figure 2). All of the investigated *Lagerstroemia* spp. could be analyzed using this single isocratic HPLC-UV system. The sensitivities for the determination of LOD and LOQ of corosolic acid were 0.452 and 1.37 µg/mL, respectively. Previously, HPLC-UV methods produced LOD and LOQ of corosolic acid at 0.8 µg/mL and 2.4 µg/mL, respectively (Katta, Murthy, Kannababu, Syamasundar, & Subbaraju, 2006). Similar analytical results were reported for simultaneous determination of corosolic acid, asiatic acid, and β-sitosterol in *L. speciosa* (Joshi, Vaidya, Pawar, & Gadgil, 2013). Although our HPLC-UV system exhibited similar analytical characteristics as these reports, our system was extended to determine corosolic acid in other *Lagerstroemia* spp. The system provided suitable separation of corosolic acid from the other chemical components in the *Lagerstroemia* spp. When the HPLC-UV signals (peak areas) were plotted against concentrations of corosolic acid, the linearity of determination of the HPLC-UV method was between 3.12 to 50 µg/mL (y=9.3200x+1.8422, r=0.9998). The analytical performance in the range of determination was precise and the intra-day (n=6) and inter-day (n=3) variations (%RSD) were in the range of 1.55–3.06% and 1.33–4.56%,

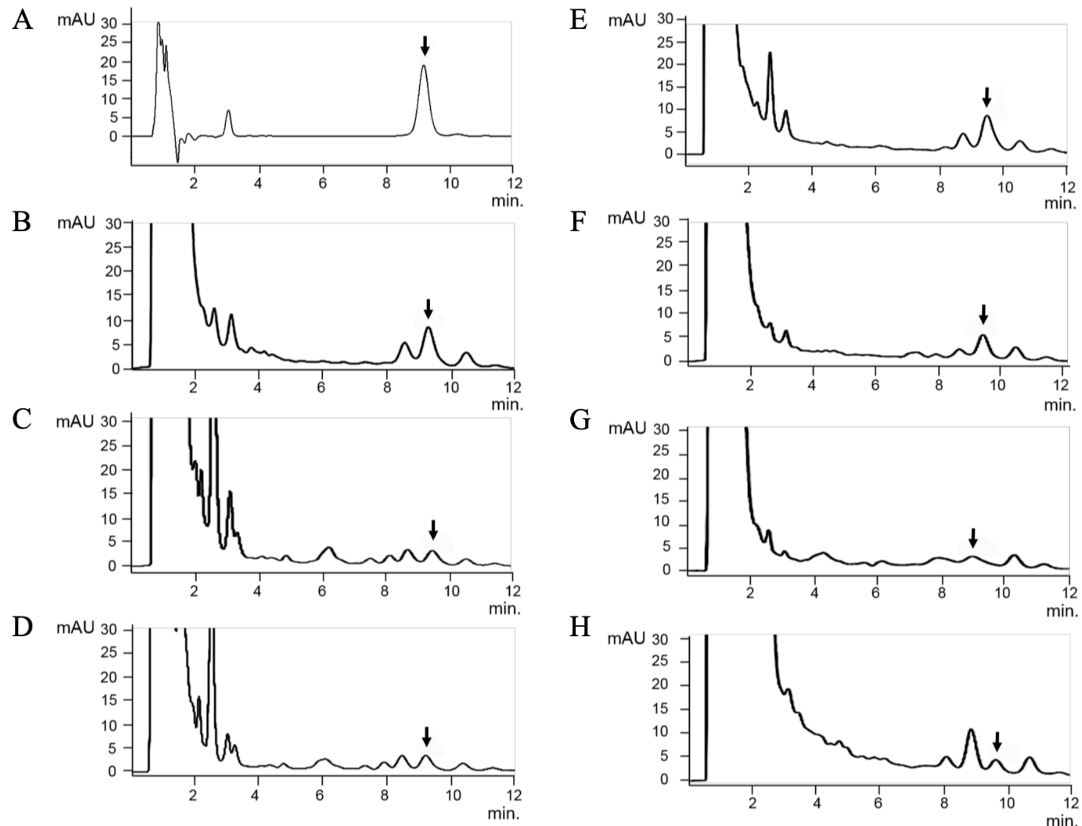

Figure 2. HPLC chromatograms of authentic corosolic acid (A), *L. speciosa* mature leaves (B), *L. speciosa* callus induced MS medium supplemented with T0.1N0.5 (C) and T0.5N0.5 (D), *L. macrocarpa* mature leaves (E), *L. loudonii* mature leaves (F), *L. floribunda* mature leaves (G), and *L. indica* mature leaves (H).

respectively. The accuracy of the determination was evaluated using the corosolic acid recovery experiment. The results indicated 97.9–101% recovery (Table 2) which implied analytical accuracy. When the validated method was applied to determine corosolic acid in the parts of the *Lagerstroemia* spp., the mature leaves of all investigated plants contained corosolic acid and the results correlated well with a previous study (Jayakumar *et al.,* 2014). In comparative analyses of gene expressions in young and mature leaves, the expressed genes that were involved in upstream terpenoid biosynthesis was higher in the young leaves; however, the expression of gene encoding cytochrome P_{450} hydroxylase catalyzing the final step(s) in corosolic acid synthesis was higher in the mature leaves (Vijayan, Padmesh Pillai, Hemanthakumar, & Krishnan, 2015) which underscores the use of the leaf for medical purpose. Interestingly, we found that *L. macrocarpa* contained approximately 15 times more corosolic acid than *L. speciosa* (Table 3). Moreover, we also revealed that other *Lagerstroemia* spp., including *L. loudonii*, *L. floribunda*, and *L. indica*, also contained corosolic acid and the amounts were higher than in *L. speciosa.* Previously, the content of corosolic acid was usually investigated in *L. speciosa*. Therefore, this is the first report of corosolic content in species other than *L. speciosa.* This information provides alternatives and good sources of corosolic acid. Since corosolic acid was present in all of the evaluated *Lagerstroemia* spp, this compound can be selected as an antidiabetic marker for standardization.

3.2 Plant tissue culture condition of *L. speciosa*

To establish a plant tissue culture of *L. speciosa*, the callus was successfully initiated from only the leaf of *L. speciosa*. The callus cannot be initiated from shoot explant. Only MS medium supplemented with combinations of TDZ and NAA can be applied to induce callus of *L. speciosa* but the combinations between NAA and BA were not successful (Figure 3). TDZ was reported to be the most active cytokinin-like substance for woody plant tissue culture (Huetteman & Preece, 1993). In addition, TDZ contributes to secondary metabolism of plant cells which enhances some useful secondary metabolite production (Turkyilmaz Unal, 2018). This proved that TDZ was also effective for *L. speciosa* callus culture. Two-month-old calluses were collected and dried. The productivity of corosolic acid in these tissues was determined. The callus that was induced and maintained in MS medium supplemented with T0.5N0.5 was the best for corosolic acid

Table 2. Recovery of corosolic acid spiked into *L. speciosa* sample.

Spiked amount (µg)	Measured amount (µg)	Recovery (%)
0	10.41	-
10	20.2±0.31	97.9
12	22.5±0.12	101
15	25.5±0.16	101

Table 3. Content of corosolic acid in the different organ of *Lagerstroemia* species.

Plant species (Thai name)	Plant organ	Content of corosolic acid (mg/g dried weight)
L. speciosa (อินทนิลน้ำ)	Young leaves	ND
	Mature leaves	0.25±0.01
	Branches	ND
L. macrocarpa (อินทนิลบก)	Young leaves	ND
	Mature leaves	3.77±0.17
	Branches	ND
L. loudonii (เสลา)	Young leaves	0.21±0.01
	Mature leaves	1.41±0.04
	Branches	ND
L. floribunda (ตะแบก)	Young leaves	ND
	Mature leaves	0.65±0.02
	Branches	ND
L. indica (ยี่เข่ง)	Young leaves	ND
	Mature leaves	0.42±0.02
	Branches	ND
L. speciosa calluses	Plant growth regulators	
	T0.1N0.5	0.59±0.04
	T0.1N1	0.45±0.01
	T0.5N0.5	0.86±0.04
	T0.5N1	0.45±0.01

ND = not detected

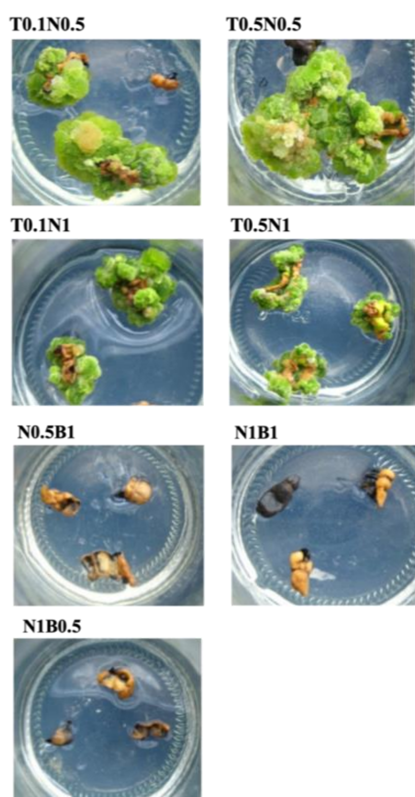

Figure 3. Callus cultures of *L. speciosa* established in MS medium supplemented with various combinations of plant growth regulators.

accumulation which contained 0.86 mg/g dry weight of callus. The amounts of corosolic acid in all calluses were significantly higher than the parental plant leaves (0.25 mg/g dry weight). Therefore, this technique has promise as a useful source of corosolic acid. In addition, this technique is one approach to green chemistry development for phytochemical preparation. It is independent from natural resources and it prevents shortage and extinction of raw materials. Furthermore, the conditions for culturing can be controlled to improve productivity of preferred secondary metabolites.

3.3 α-Glucosidase inhibitory activity

All samples that contained corosolic acid, including mature leaves of *Lagerstroemia* spp. and calluses of *L. speciosa*, were evaluated for α-glucosidase inhibitory effect. The results indicated that the *L. macrocarpa* and *L. loudonii* mature leaves, which contained the highest amounts of corosolic acid, also exhibited the highest α-glucosidase inhibition (IC_{50} =0.09 mg/mL) (Table 4). The α-glucosidase inhibitory activities were significantly higher than *L. speciosa* (IC_{50}=1.68 mg/mL). Calluses of *L. speciosa* also exhibited greater effects than its parental *L. speciosa*, which corresponded to the higher corosolic acid content (Table 4). The IC_{50} values of α-glucosidase inhibition by corosolic acid and acarbose were 0.01 and 0.3 mg/mL, respectively. Therefore, corosolic and its extracts showed high strength as an antidiabetic substance. Overall, the amount of corosolic acid was in the agreement with α-glucosidase inhibition. Among the triterpenoid acids found in *L. speciosa* leaves, corosolic acid showed the best inhibitory activity against α-glucosidase. Its potency was higher than oleanolic acid, arjunolic acid, asiatic acid, maslinic acid, and 23-hydroxyursolic acid (Hou *et al.,* 2009). In addition, the α-amylase inhibitory effect of corosolic acid was also reported (Hou *et al.,* 2009). Therefore, the analysis of corosolic acid content indicated its potency as an antidiabetic agent. This is the first report which described the antidiabetic potentials of *L. macrocarpa*, *L. loudonii*, *L. floribunda*, and *L. indica*. These are new alternative resources for functional food ingredients against metabolic syndrome. Since only *L. speciosa* has been used in traditional medicines, other *Lagerstroemia* spp. must be tested for toxicity prior to the development of products. Although the bioactivity-based standardization directly reflects the bioactivity of plant material, chemical-based quality control is more convenient, especially in industrial

Table 4. α-Glucosidase inhibitory activities of the *Lagerstroemia* spp. and *L. speciosa* calluses.

Samples	IC_{50} (mg/mL)
L. speciosa (mature leaves)	1.68
L. macrocarpa (mature leaves)	0.09
L. loudonii (mature leaves)	0.09
L. floribunda (mature leaves)	1.31
L. indica (mature leaves)	3.15
L. speciosa (calluses T0.1N1)	0.19
L. speciosa (calluses T0.5 N0.5)	0.52
L. speciosa (calluses T0.1 N0.5)	1.15
L. speciosa (calluses T0.5 N1)	1.03
Corosolic acid	0.01
Acarbose	0.30

scale production. This analytical method of corosolic acid has merit in qualifying and quantifying the contents of *Lagerstroemia* spp. for antidiabetic purposes.

4. Conclusions

According to the analytical performance, that included the precision, sensitivity, and accuracy, the HPLC-UV method used in this study was reliable and applicable for corosolic acid determination in the *Lagerstroemia* spp. The compound exists in various amounts in different *Lagerstroemia* spp. The mature leaves had the highest amount of corosolic acid. Interestingly, *L. macrocarpa* and *L. loudonii* had much higher amounts of corosolic acid than *L. speciosa*. The callus culture of *L. speciosa* also produced a high amount of corosolic acid. The amounts of corosolic acid in the *Lagerstroemia* spp, agreed with the α-glucosidase inhibitory activity. Therefore, this method is worthy of antidiabetic standardization of *Lagerstroemia* derived materials and it is more convenient than the bioassay-based standardization.

Acknowledgements

This research was supported by Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand and The Thailand Research Fund (IRN 61W0005).

References

Chan, C. W., Yu, C. L., Lin, J. C., Hsieh, Y. C., Lin, C. C., Hung, C. Y., . . . Wu, T. J. (2018). Glitazones and alpha-glucosidase inhibitors as the second-line oral anti-diabetic agents added to metformin reduce cardiovascular risk in Type 2 diabetes patients: A nationwide cohort observational study. *Cardiovascular Diabetology, 17*(1), 20. doi:10.1186/s12933-018-0663-6

Fukushima, M., Matsuyama, F., Ueda, N., Egawa, K., Takemoto, J., Kajimoto, Y., . . . Seino, Y. (2006). Effect of corosolic acid on postchallenge plasma glucose levels. *Diabetes Research and Clinical Practice, 73* (2), 174-177.

Hou, W., Li, Y., Zhang, Q., Wei, X., Peng, A., Chen, L., & Wei, Y. (2009). Triterpene acids isolated from *Lagerstroemia speciosa* leaves as alpha-glucosidase inhibitors. *Phytotherapy Research, 23*(5), 614-618.

Huetteman, C. A., & Preece, J. E. (1993). Thidiazuron: A potent cytokinin for woody plant tissue culture. *Plant Cell, Tissue and Organ Culture, 33*(2), 105-119.

Inthongkaew, P., Chatsumpun, N., Supasuteekul, C., Kitisripanya, T., Putalun, W., Likhitwitayawuid, K., & Sritularak, B. (2017). α-Glucosidase and pancreatic lipase inhibitory activities and glucose uptake stimulatory effect of phenolic compounds from *Dendrobium formosum*. *Revista Brasileira de Farmacognosia, 27*(4), 480-487.

Jayakumar, K. S., Sajan, J. S., Aswati Nair, R., Padmesh Pillai, P., Deepu, S., Padmaja, R., & Pandurangan, A. G. (2014). Corosolic acid content and SSR markers in *Lagerstroemia speciosa* (L.) Pers.: A comparative analysis among populations across the Southern Western Ghats of India. *Phytochemistry, 106*, 94-103.

Joshi, N. P., Vaidya, V. V., Pawar, S. S., & Gadgil, J. N. (2013). Development and validation of HPLC method for simultaneous determination of bio-active markers corosolic acid, asiatic acid and β-sitosterol from leaves of *Lagerstroemia speciosa* linn. and from marketed formulation. *International Journal of Pharmacy and Pharmaceutical Sciences, 5*, 223-226.

Judy, W. V., Hari, S. P., Stogsdill, W. W., Judy, J. S., Naguib, Y. M. A., & Passwater, R. (2003). Antidiabetic activity of a standardized extract (Glucosol™) from *Lagerstroemia speciosa* leaves in Type II diabetics: a dose-dependence study. *Journal of Ethnopharmacology, 87*(1), 115-117.

Katta, V., Murthy, P. B., Kannababu, S., Syamasundar, B., & Subbaraju, G. V. (2006). Quantitative determination of corosolic acid in *Lagerstroemia speciosa* leaves, extracts and dosage forms. *International Journal of Applied Science and Engineering, 4*, 103-114.

Klein, G., Kim, J., Himmeldirk, K., Cao, Y., & Chen, X. (2007). Antidiabetes and anti-obesity activity of *Lagerstroemia speciosa*. *Evidence-based Complementary and Alternative Medicine : eCAM, 4*(4), 401-407.

Lieberman, S., Spahrs, R., Stanton, A., Martinez, L., & Grinder, M. (2005). Weight loss, body measurements, and compliance: A 12 week total lifestyle intervention pilot study. *Alternative and Complementary Therapies, 11*, 307-313.

Min, S. H., Yoon, J.-H., Hahn, S., & Cho, Y. M. (2018). Efficacy and safety of combination therapy with an α-glucosidase inhibitor and a dipeptidyl peptidase-4 inhibitor in patients with type 2 diabetes mellitus: a systematic review with meta-analysis. *Journal of Diabetes Investigation, 9*(4), 893-902.

Miura, T., Takagi, S., & Ishida, T. (2012). Management of diabetes and its complications with banaba (Lagerstroemia speciosa L.) and corosolic acid. *Evidence-based complementary and alternative medicine : eCAM, 2012*, 871495-871495.

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., . . . Gakidou, E. (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet, 384*(9945), 766-781.

Stohs, S. J., Miller, H., & Kaats, G. R. (2012). A review of the efficacy and safety of banaba (*Lagerstroemia speciosa* L.) and corosolic acid. *Phytotherapy Research, 26*(3), 317-324.

Tsuchibe, S., Kataumi, S., Mori, M., & Mori, H. (2006). An inhibitory effect on the increase in the postprandial blood glucose by Banaba extract capsule enriched corosolic acid. *Journal for the Integrated Study of Dietary Habits, 17*(3), 255-259.

Turkyilmaz Unal, B. (2018). Thidiazuron as an elicitor in the production of secondary metabolite. In N. Ahmad & M. Faisal (Eds.), *Thidiazuron: from urea derivative to plant growth regulator* (pp. 463-469). Singapore: Springer Singapore.

Vijayan, A., Padmesh Pillai, P., Hemanthakumar, A. S., & Krishnan, P. N. (2015). Improved *in vitro* propagation, genetic stability and analysis of corosolic acid synthesis in regenerants of *Lagerstroemia speciosa* (L.) Pers. by HPLC and gene expression profiles. *Plant Cell, Tissue and Organ Culture, 120*(3), 1209-1214.

Yamada, K., Hosokawa, M., Fujimoto, S., Fujiwara, H., Fujita, Y., Harada, N., . . . Inagaki, N. (2008b). Effect of corosolic acid on gluconeogenesis in rat liver. *Diabetes Research and Clinical Practice, 80*(1), 48-55.

Yamada, K., Hosokawa, M., Yamada, C., Watanabe, R., Fujimoto, S., Fujiwara, H., . . . Inagaki, N. (2008b). Dietary corosolic acid ameliorates obesity and hepatic steatosis in KK-Ay mice. *Biological and Pharmaceutical Bulletin, 31*(4), 651-655.