

Full Length Research Paper

Pharmacognostic evaluations of *Lagerstroemia speciosa* leaves

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The current study was conducted to develop the pharmacognostic standards for *Lagerstroemia speciosa* leaves. These evaluations were performed according to the World Health Organization (WHO) guidelines and the Thai Herbal Pharmacopoeia (THP) for herbal standardisation. No other reports are available on the pharmacognostic evaluation of the leaves of *L. speciosa* (L.) Pers. Organoleptic, and we thus reported the anatomical and microscopic characteristics, physico-chemical properties, preliminary phytochemical screening, and thin layer chromatography (TLC) fingerprinting profiles for this plant. Corosolic acid, an active compound of *L. speciosa* leaves, was also analyzed. Anatomical and histological analyses revealed the presence of anomocytic stomata and parenchyma containing rosette aggregate calcium oxalate crystals. Triterpene, sterol and tannin tests were positive. The loss of these compounds after drying, the moisture content, the total ash content and the acid-insoluble ash content were determined to be 8.2141 ± 0.9300 , 7.8593 ± 0.8141 , 7.4725 ± 0.7277 and $1.2176 \pm 0.6223\%$, respectively. These pharmacognostic parameters were useful for detecting low-grade products and for determining extractive values. Ethanol-, dichloromethane-, and water-extractive values were found to be 9.0280 ± 2.2937 , 2.9442 ± 0.8827 and $13.1895 \pm 1.9934\%$, respectively. This study provides important information for the correct identification and herbal standardisation of *L. speciosa* leaves.

Key words: *Lagerstroemia speciosa*, corosolic acid, herbal standardisation, Lthraceae, pharmacognostic evaluation.

INTRODUCTION

Banaba leaves (*Lagerstroemia speciosa* L. Pers, Lythraceae) have been used in traditional medicine to treat diabetes mellitus in Southeast Asia for a many years. Banaba extracts are also known to have anti-obesity (Suzuki et al., 1999), anti-oxidant (Unno et al., 1997) and anti-gout (Unno et al., 2004) effects. Corosolic acid, an active ingredient in these extracts, displays a potential anti-diabetic activity (Murakami et al., 1993; Kakuda et al., 1996; Lui et al., 2001; Judy et al., 2003; Miura et al., 2004, 2006; Fukushima et al., 2006; Shi et al., 2008), as well as anti-oxidant, anti-inflammation, and antihypertension properties (Yamaguchi et al., 2006). However, no scientific standards or pharmacognostic

parameters are available for the standardization of this herb. This study aims to use pharmacognostic evaluation to authenticate the leaves of *L. speciosa* from several sources and drugstores in Thailand.

MATERIALS AND METHODS

Plant materials

Seventeen leaf specimens were collected from both natural sources and the crude drug market in Thailand. The specimens obtained from natural sources were authenticated by Associate Professor Thatree Phadungcharoen. The remaining specimens were deposited

at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

Pharmacognostic evaluations

Macroscopic, microscopic and qualitative evaluations were performed according to the THP (Thai Pharmacopoeia Committee, 2000) and WHO guidelines (Anonymous, 2002).

Macroscopic and microscopic investigation

Macroscopic characteristics such as the size, colour and other visible properties were observed. Transverse sections and ground powders were observed under a microscope (Zeiss model Axiostar, Germany) to determine the anatomical and histological characteristics.

Physicochemical standards

The physicochemical parameters of the powdered drug were determined according to the procedures described in the Thai Herbal Pharmacopoeia. Briefly, to determine loss on drying, samples were dried at 105°C. To determine the moisture content of the leaves, azeotropic distillation was performed. Total ash was obtained from the combustion of powdered banaba leaves at 500°C. The carbonless ash was then weighed. The remaining ash was boiled in 2 N HCl, neutralized in hot water, dried and burned at 500°C until a constant weight was reached, leaving the acid insoluble ash. The acid-insoluble ash is an indicator about the amount of silica present, especially as sand and siliceous earth. Extractions were performed in 95% ethanol, dichloromethane and water to determine the amount of active constituents. Powdered *L. speciosa* leaves were macerated in each solvent for 24 h. These samples were placed in a gentle shaking bath for 6 h and then allowed to stand for 18 h. After incubation, the samples were rapidly filtered, and the volume was adjusted to 100 ml with solvent. A 20 ml aliquot was transferred into an evaporating dish. This aliquot was weighed, evaporated to dryness, and the further dried in an oven at 105°C until a constant weight was obtained.

TLC analysis

The chemical fingerprint was determined using thin-layer chromatography (TLC). A 10 g pulverized sample was macerated in 100 ml of methanol for 24 h. Extraction and evaporation were then performed. The crude extract was then partitioned twice between dichloromethane (CH₂Cl₂) and water. The CH₂Cl₂ layers were combined, concentrated under vacuum and stored in a well-closed container prior to spotting on a TLC plate. The residue was dissolved in 0.5 ml methanol, and 10 µl of this solution was applied onto a thin-layer plate coated with F254 silica gel (Merck, Germany). The TLC plate was developed in a chamber containing acetone and chloroform (1:4) as the mobile phase. The TLC plate was air-dried and investigated under visible light and ultraviolet light (254 and 365 nm) to visualize the spots produced. The dried TLC was then sprayed with an anisaldehyde-sulphuric acid reagent and heated in an oven for 10 min.

Phytochemical screening

Coarsely powdered leaf samples were subjected to phytochemical screening to assay for the presence of alkaloids, tannins,

flavonoids, triterpenoids, sterol and saponins using standard experimental procedures (Trease and Evans, 1989).

Quantitative estimation of corosolic acid contents by high performance liquid chromatography (HPLC)

Five milligrams of corosolic acid was dissolved in 5 ml of methanol to give a 1 mg/ml stock solution. The stock solution was diluted to various concentrations ranging from 10 to 250 µg/ml. The solutions were then used to construct a calibration curve of corosolic acid using HPLC.

Dry *L. speciosa* leaves were ground into a fine powder. The tissue was sieved, and 200 mg of the ground leaves was weighed into a test tube and macerated in 2 ml of methanol for 24 h. The extract was filtered through a 0.45 µm filter for HPLC analysis. The filtrate was transferred to clean glass vials and used directly for HPLC analysis. The HPLC system was composed of a Shimadzu SIL-20 AHT pump equipped with a ZORBAX Eclipse XDB-C18 column (250 x 0.46 mm, i.d. 5 µm) and a guard column (Agilent Technologies, USA). The column contents were eluted with acetonitrile and 0.1% phosphoric acid in water (75:25) at a flow rate of 1 ml/min. The eluent was monitored at 204 nm using diode array detector (DAD). The amount of corosolic acid in the crude extracts was estimated using the standard curves. All of the measurements were done in triplicate.

RESULTS

Macroscopic evaluation

The morphological evaluation for the identification of *L. speciosa* was described. *L. speciosa* is a tall tree that can grow up to 20 to 25 m in height, but it flowers while it is still a shrub. Its bark is creamy-brown or grey in colour, smooth and peels in thin flakes. The leaves are approximately 11 to 26 cm long and 7 to 12 cm wide and are broadly ovate or oblong in shape. The mature leaves are smooth. There are 10 to 15 pairs of side veins, looped at the margin and quite prominent below. Old leaves are orange-red in colour. The flowers are 5 to 7.5 cm in diameter and bright pink to purple in colour. The fruit is 1.5 to 2.5 cm in size and globose in shape.

In organoleptic evaluation, appropriate parameters like taste, odor, size, shape and color of the leaves and leaf powder were studied. Macroscopically, the colour of their leaves was shown in olive green to yellowish brown. The nearly perfect leaves were 7 to 15 cm wide and 10 to 28 cm long. The petiole was 1 cm long. The shape of leaves was broadly, ovate or oblong. There were some fragments of leaves. Some of crude drugs from traditional drugstore were chopped in small pieces. The odor was slightly characteristic and the taste was slightly bitter. Leaf powder is green and yellowish brown in colour with characteristic odor and slightly bitter in taste.

Microscopic investigation

Microscopic characteristics were examined both in

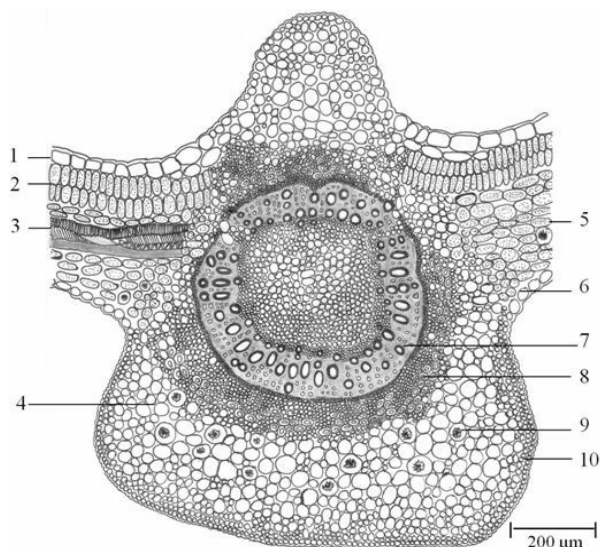


Figure 1. Microscopic characteristics of the transverse section of *L. speciosa* midrib. 1) upper epidermis; 2) palisade parenchyma; 3) group of vascular bundles; 4) parenchyma; 5) spongy parenchyma; 6) lower epidermis; 7) xylem vessel; 8) phloem tissue; 9) rosette aggregate calcium oxalate crystal in a parenchyma cell, and 10) collenchymas.

transverse sections and in the powder. The transverse section of the midrib showed parenchyma, collenchymas, phloem, xylem and parenchyma containing calcium oxalate crystals (Figure 1). The transverse section of the midrib showed that epidermal cells were rectangular to round in shape, with dividing cells occurring regularly. Some epidermal cells contained spherical clusters of rosette aggregate calcium oxalate crystals, and some cells were enlarged and mucilaginous. The mucilage cells tended to protrude into the mesophyll and sometimes appeared to be below the upper epidermis. Cells of the upper epidermis were about twice as large as those of the lower epidermis. The mesophyll was well differentiated and composed of a double palisade layer that made the lamina and spongy layers 4 to 6 cells thick. The lamina in the sectional view of the leaves showed an upper epidermis in which some cells contained mucilage, palisade and spongy parenchyma, and lower epidermis. The upper epidermal cells were polygonal cells. The cell length was approximately equal to the width or twice as long as the straight wall (Figure 2A). The lower epidermal cells were irregularly shaped, and their walls were slightly sinuous. The anomocytic stomata were only found in lower epidermis (Figure 2B). The leaf powders were olive-green colour with a slightly bitter taste. The powdered drugs displayed some of the same microscopic characteristics, such as part of the upper epidermis with the part of palisade mesophyll, stomata, rosette aggregate calcium oxalate crystals, fibres and vessels (Figure 3).

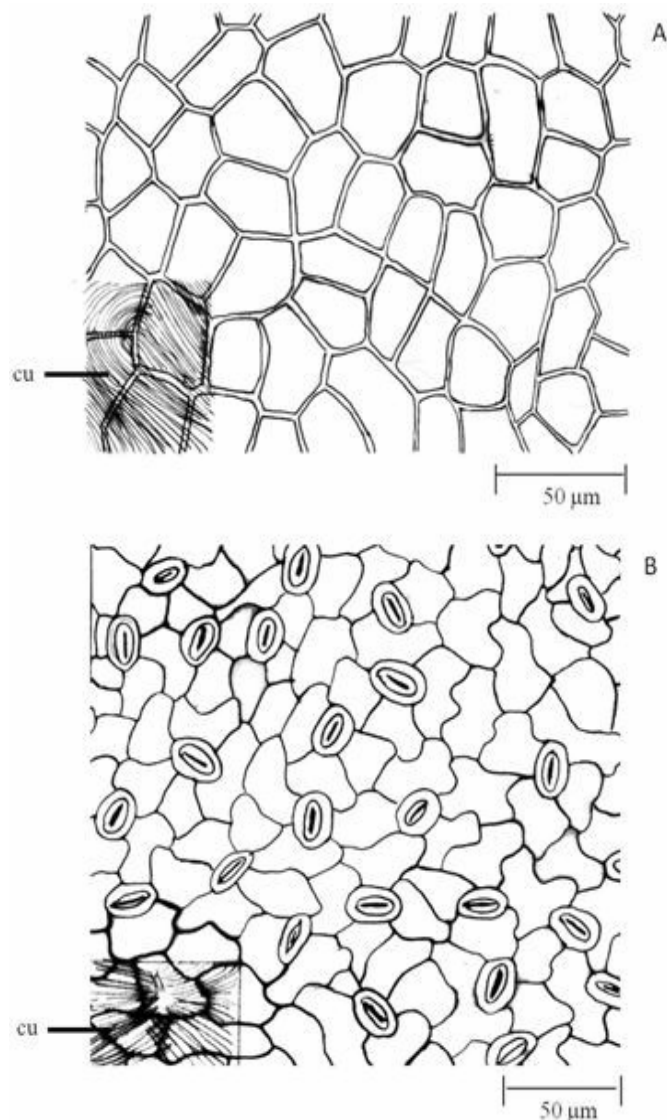


Figure 2. Microscopic characteristics of the surface views of *L. speciosa* lamina. A) Upper epidermis showing striated cuticle (cu), and B) Lower epidermis with anomocytic stomata.

Physicochemical constant

The physicochemical values of *L. speciosa* leaves are displayed in Table 1. Loss on drying, moisture content, total ash content, and acid-insoluble ash content were determined to be 8.2141 ± 0.9300 , 7.8593 ± 0.8141 , 7.4725 ± 0.7277 and $1.2176 \pm 0.6223\%$ of the dry weight, respectively. These parameters were useful for detecting low-grade products as well as for determining the extractive values. Ethanol-, dichloromethane- and water-extractive values were determined to be 9.0280 ± 2.2937 , 2.9442 ± 0.8827 and $13.1895 \pm 1.9934\%$ of the dry weight, respectively.

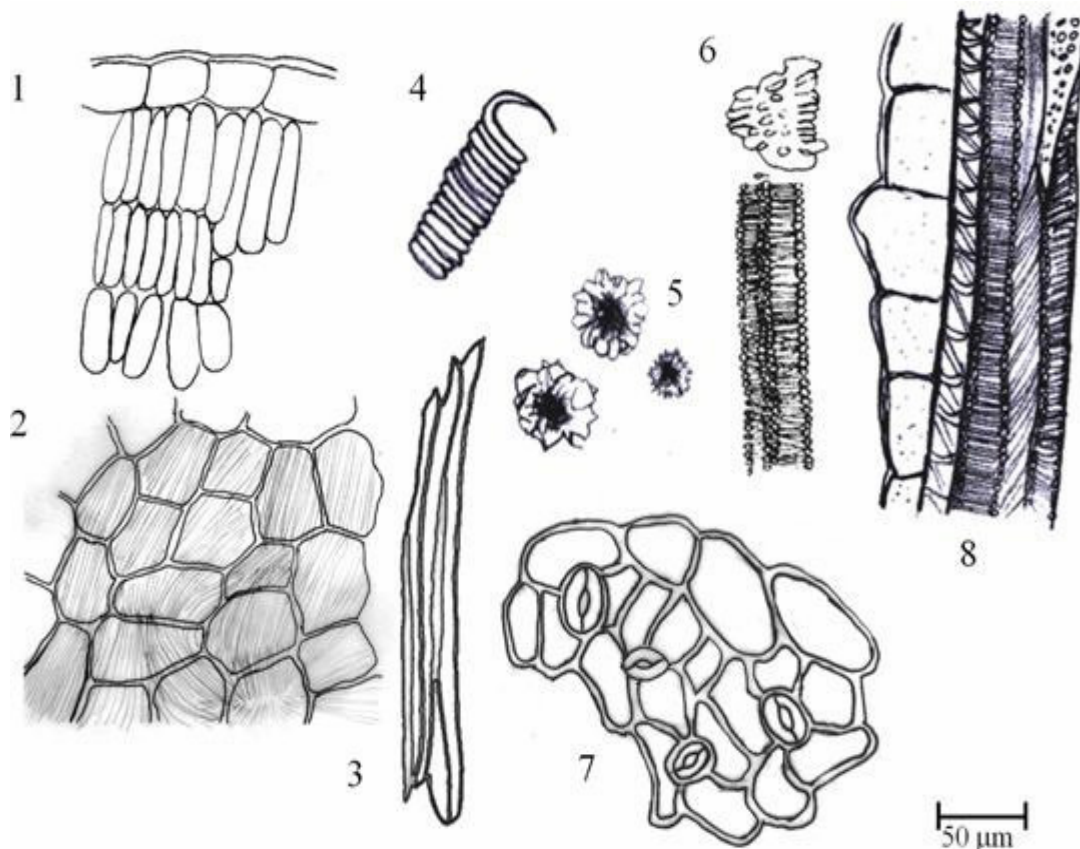


Figure 3. Microscopic characteristics of powdered *L. speciosa*. 1) Part of the lamina in sectional view, showing the upper epidermis, palisade parenchyma and part of the spongy parenchyma; 2) upper epidermis in surface view, showing the striated cuticle; 3) group of lignified fiber; 4) spiral vessel; 5) rosette aggregate crystals of calcium oxalate; 6) reticulate vessel; 7) lower epidermis in surface view showing anomocytic stomata; 8) fibrovascular tissue and parenchyma cell.

Table 1. Physicochemical characteristics of *L. speciosa* leaves.

| Specification | Mean \pm SD | Min - Max |
|----------------------------------|----------------------|------------------|
| Loss on drying | 8.2141 \pm 0.9300 | 6.4844 - 9.9510 |
| Moisture content | 7.8593 \pm 0.8141 | 6.1440 - 9.3782 |
| Total ash content | 7.4725 \pm 0.7277 | 6.3714 - 8.9072 |
| Acid insoluble ash content | 1.2176 \pm 0.6223 | 0.1700 - 2.4995 |
| Ethanol extractive value | 9.0280 \pm 2.2937 | 5.2745 - 13.1487 |
| Dichloromethane extractive value | 2.9442 \pm 0.8827 | 2.2992 - 5.2795 |
| Water extractive value | 13.1895 \pm 1.9934 | 9.9998 - 17.8247 |

Experiments were done in triplicate.

TLC analysis

The TLC pattern is displayed in Figure 4. We used acetone and chloroform (1:4) as the mobile phase and silica gel 60 GF254 as the stationary phase. The R_f value and colour of each spot is tabulated (Table 2).

Phytochemical screening

Phytochemical screening was used to detect therapeutic compounds in the plants. Qualitative chemical examination of *L. speciosa* leaves revealed the presence of tannins, triterpenes and steroids (Table 3), as

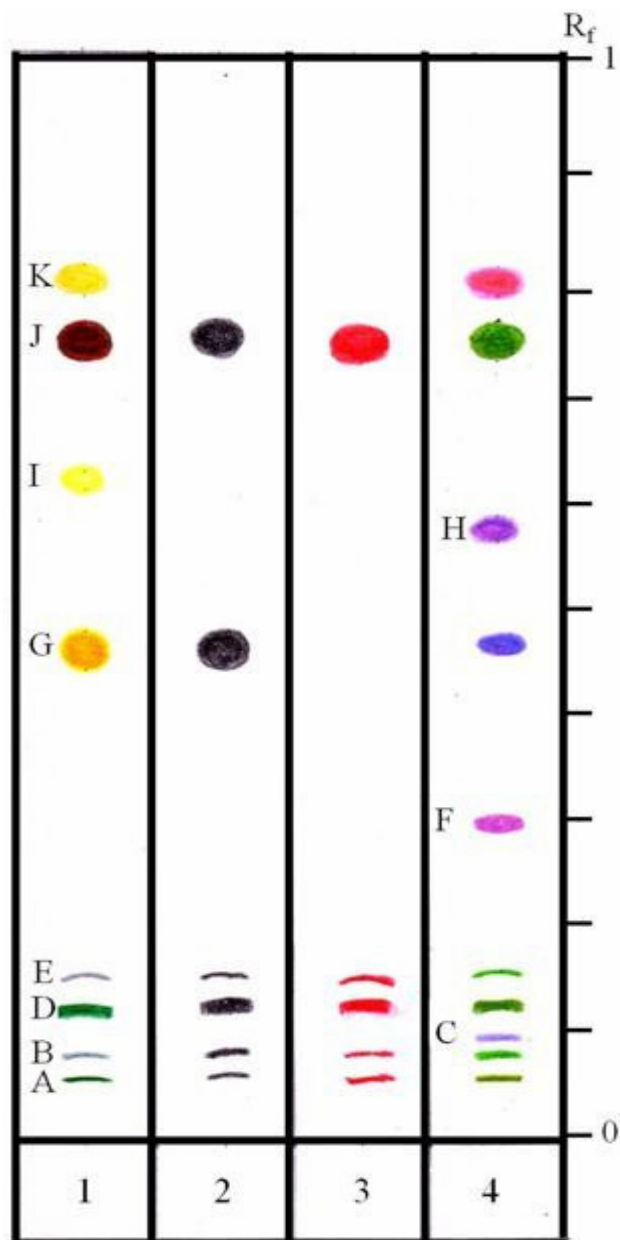


Figure 4. Thin layer chromatography fingerprinting of a methanolic extract of dried *L. speciosa* leaves. 1) Appearance under visible light; 2) under 254-nm UV light; 3) under 365-nm UV light, and 4) Detection with anisaldehyde-sulphuric acid and heat.

previously reported (Murakami et al., 1993; Suzuki et al., 1999; Ragasa et al., 2005; Unno et al., 2004; Yamaguchi et al., 2006).

Corosolic acid content in *L. speciosa* leaves

In the present study, corosolic acid was quantified from the leaves of *L. speciosa* using HPLC. Corosolic acid

could be used as a chemical marker for the standardization of *L. speciosa*. The chromatograms of corosolic acid and of a crude extract of *L. speciosa* leaves are shown in Figures 5A and 5B, respectively. Spiking the crude extract with corosolic acid was used to confirm its peak in the chromatogram (Figure 5C). Under the present chromatographic conditions, the run time for each sample was 15 min. The retention time of corosolic acid was 8.681 min. HPLC analyses of all samples were similar in pattern, but the quantity of corosolic acid ranged from 0.0100 to 0.7496% w/w.

DISCUSSION

Currently, there is an emphasis on the standardization of medicinal plant materials for their therapeutic potentials. The modern techniques available make the identification and evaluation of crude drugs by pharmacognostic studies reliable, accurate and inexpensive. According to the WHO, determining the macroscopic and microscopic characteristics are the first steps towards establishing the identity and the purity of such materials, and these steps should be carried out before any further tests are undertaken.

L. speciosa has been confused with other species due to their relative similarities. The results of these investigations could be serving as a basis for proper identification, collection and investigation of the plant. The macro – and micro – morphological features of the leaf were described, distinguishes it from other members of the genera. Polygonal cells with striated cuticle, rosette aggregate crystals of calcium oxalate and anomocytic stomata are remarkable microscopic features of the drugs. These characteristics would be useful in discrimination *L. speciosa* from its substitutes and adulterants.

The quantitative determination of physicochemical parameters is useful for setting standards for crude drugs. Evaluation of these parameters in crude drugs is important in detecting adulteration or improper handling of the drugs. Excessive water content in crude drugs and temperature are important factors affecting fungal and bacterial growth, which cause spoilage. So, the loss on drying and moisture content for controlling the quality of medicinal plant materials should not be more than 10 and 9% w/w, respectively. Ash content analyses indicate the degree of admixture of foreign inorganic matter either from the storage container or by intentional addition to disguise the appearance of the crude drug. The ash of any organic material was composed of non-volatile inorganic components. The controlled incineration resulted in ash residue consisting of an inorganic material (metallic salt and silica). Ash content were accountable for controlling the admixture of foreign inorganic matter due to their storage, container or intentional add to disguise the appearance of crude drug. We could detect

Table 2. R_f values of components in the methanolic extract of *L. speciosa* leaves using chloroform and acetone (4:1) as a solvent system.

| Spot | R _f | Detecting agents | | | |
|------|----------------|------------------|------------|------------|----------------------------------|
| | | Visible light | UV, 254 nm | UV, 365 nm | Anisaldehyde – sulphuric acid TS |
| A | 0.06 | Green | Quenching | Red | Olive Green |
| B | 0.08 | Pale Grey | Quenching | Red | Green |
| C | 0.09 | - | - | - | Light Purple |
| D | 0.13 | Green | Quenching | Red | Green |
| E | 0.15 | Grey | Quenching | Red | Green |
| F | 0.29 | - | - | - | Purple |
| G | 0.44 – 0.47 | Yellow | Quenching | - | Blue Purple |
| H | 0.55 – 0.58 | - | - | - | Pink Purple |
| I | 0.60 – 0.61 | Yellow | - | - | - |
| J | 0.72 – 0.75 | Brownish Green | Quenching | Red | Emerald Green |
| K | 0.78 – 0.8 | Yellow | - | - | Reddish Pink |

Table 3. Phytochemical screening of *L. speciosa* leaves by preliminary qualitative chemical analysis.

| Phytochemical | Results obtained |
|---|------------------|
| Alkaloids (Dragendorff's, Mayer's test) | - |
| Steroids/Triterpenoids (Liebermann-Burchard test) | + |
| Tannin (Ferric chloride TS; Gelatine precipitation) | + |
| Saponins (Foam test) | - |
| Flavonoids (Shinoda's test) | - |
| Anthraquinone (Borntrager's test) | - |

(+) presence, (-) absence.

the extent of adulterations as well as set up the quality and purity of crude drug by using this method. Here, the value obtained for the *L. speciosa* leaves is around 7% as total ash. The acid insoluble ash determines the acid insoluble material present in the drug materials. The acid insoluble ash values for the *L. speciosa* leaves should not be more than 2% w/w for qualitative specification. The extraction of any crude drug with particular solvent yields a solution containing different phyto-constituents. The composition of these phyto-constituents in that particular solvent depends on the nature of the drugs and solvent used. The extractive values of the crude drugs determined the quality as well as purity of the drug materials. The ethanol-, water-, and dichloromethane-extractive values were determined to be not less than 8, 10 and 2%, respectively. The phytochemicals quantified in this investigation have a great deal of medicinal importance. Mixture of such chemicals shows a spectrum of biological effects and pharmacological properties. The presence of tannins suggests the ability of this plant to play a major role in the treatment infectious diseases (Asquith and Butler, 1986), as tannins have shown anti-oxidant and protein-precipitating properties (Ruch et al., 1989). Triterpenoids and sterols possess anti-inflammatory

and anti-tumour activities (Lui, 1995).

The TLC fingerprint showed characteristic fingerprint profiles that could be used as markers for quality evaluation and standardization of the crude drug. The R_f values indicate the position at which the substance was located on the chromatogram. The R_f value is widely recognised as a guide for the identification of medicinal plants. The amount of corosolic acid from the sample which collected in Bangkok showed a higher content than those from Saraburi and Chiang Mai. The crude drug sample from Lampang was greenish in colour, representing a high concentration of corosolic acid, while the lowest content was found in dry leaves with a brownish colour. The difference in corosolic acid content in the crude drugs may be due to the age of plants, the geographic conditions where the leaves were cultivated, the duration of storage, differences in the drying process, or genetic variations. Moreover, the season of collection and the storage conditions may also lead to fluctuations in the corosolic acid content (He et al., 2009).

The results obtained from this study will play a significant role in setting standards for this medicinal plant. This study provides useful information for the identification of *L. speciosa* leaves and will help those

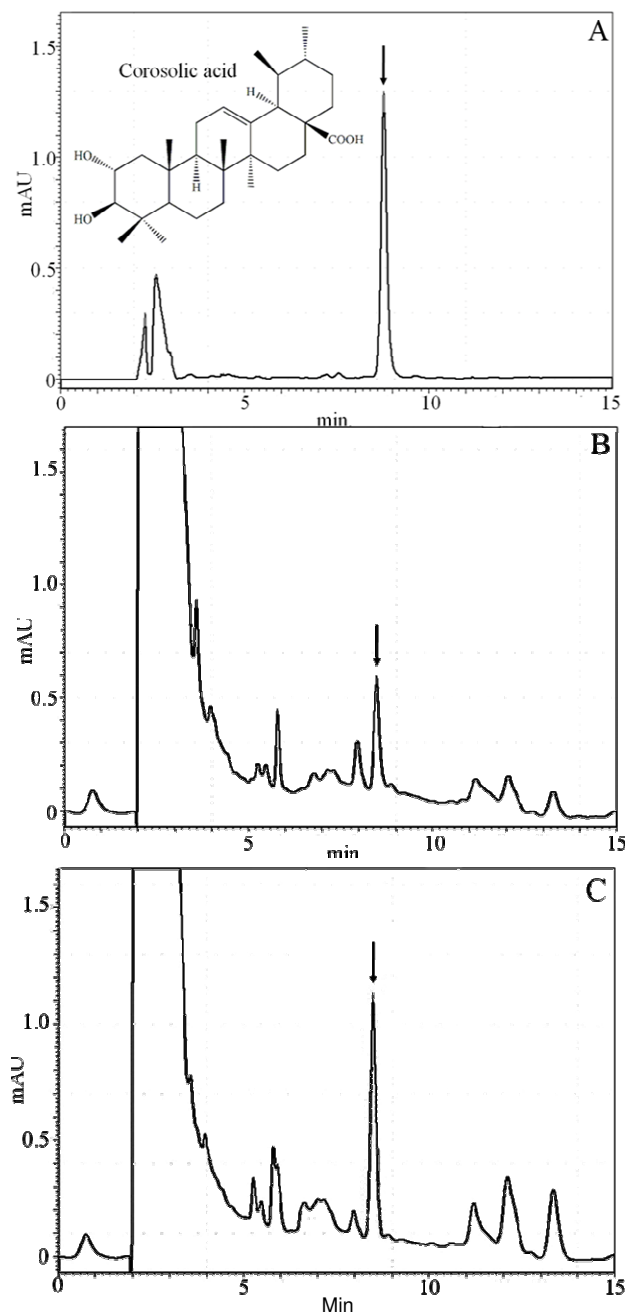


Figure 5. High performance liquid chromatography (HPLC) chromatograms. A) Standard corosolic acid; B) crude methanolic extract of *L. speciosa* leaves, and C) crude methanolic extract spiked with standard corosolic acid detected using photo diode array detector at 204 nm. The mobile phase was acetonitrile and 0.1% phosphoric acid in water (75:25, v/v) at the flow rate of 1 ml/min.

who handle this plant to maintain its quality. Thus, the standards presented in this study will help minimize the adulteration of *L. speciosa* samples and will be of great use for future researchers in selecting correct herbal

specimens. In addition, the results of this investigation may be useful in the preparation of a Thai material medical monograph for this plant.

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