A study of phytoconstituents, α-glucosidase inhibitory effect and antioxidant activity of Lagerstroemia speciosa L. Leaf and Fruit

Phyu Phyu Myint, May Thu Soe and Htet Htet Hlaing

Abstract
Lagerstroemia speciosa (Banaba) was studied for its bioactive phytoconstituents, α-glucosidase inhibitory and antioxidant activities. The nutritional values and elemental analysis were also conducted. Preliminary phytochemical tests have revealed that the presence of alkaloids, flavonoids, α-amino acid, carbohydrates, starch, organic acids, phenolic compounds, saponin, glycosides, reducing sugar, tannin, steroids and terpenoids in the samples. The samples had relatively high Ca, K, P and S according to EDXRF spectrum. Proximate composition has been found as 10.75 and 14.77 % of dietary fiber, 51.20 and 42.48 % of moisture, 3.36 and 0.26 % of crude fat, 11.24 and 2.88 % of protein, 13.76 and 38.39 % of dietary fiber, 51.20 and 42.48 % of carbohydrate, 9.69 and 2.42 % ash, and 275 and 183 kcal /100 g based on dried leaf and fruit sample. β-sitosterol, gallic acid and corosolic acid were mainly isolated from samples. In the enzymatic α-glucosidase inhibitory assay, the result suggested the order of the extracts of EtOH (leaf) > aqueous (fruit) > EtOH (fruit) > EtOH (leaf) > aqueous (fruit). In vitro antioxidant activity was also assessed by DPPH radical scavenging activity assay. IC50 values were found to be 4.29 and 9.16 µg/mL for H2O extracts and 2.64 and 6.17 µg/mL for 95% EtOH extracts of L. speciosa leaf and fruit. From these results, it is suggested that these plants should be used as a remedy for the treatment of disease related to α-glucosidase inhibitory and antioxidant activities.

Keywords: Lagerstroemia speciosa L. (Banaba), leaf and fruit, nutritional value, phytoconstituents, α-glucosidase inhibitory effect, antioxidant activity

1. Introduction
The study of traditional medicinal plants and their therapeutic properties play a very important role in the health care system of the country. According to Myanmar traditional medical belief there are 96 diseases which afflict humankind using fresh or dried roots, stems, barks, leaves, buds and flowers of medicinal plant and the hair, fat, bones and organs of certain insects, reptiles and mammals. Myanmar indigenous medicine is able to heal and cure all 96 maladies. Indigenous medicines are administered as powder, mixtures, decoctions, infusions, percolates, pastes, extracts, preserves, pills or tablets. Therefore, investigation on such agents from traditional medicinal plants has become more important and researches are competing to find the new effective and safe therapeutic agent for treatment of diseases. Lagerstroemia speciosa L. (Banaba) is a medicinal herb which is a shrub to large tree with multiple trunks or stems diverging from just above the ground level and used as traditional remedies of various ailments in Asia and Africa. Banaba is a deciduous or semi-deciduous, small to medium sized or rarely large tree up to 40-45 m. It is found at low to medium altitudes in comparatively open habitats, in disturbed or secondary forests, grasslands, and along rivers. The native is Cambodia, China, Indonesia, Laos, Malaysia, Myanmar, Philippines, Thailand, and Vietnam. The exotic is Singapore (Ulbricht et al., 2007) [2]. Traditional uses include brewing tea from the Banaba leaves and fruits as a treatment for diabetes. The hypoglycemic (blood sugar lowering) effect is similar to that of insulin (which hydrolyzing enzyme) in the digestive organs (Toelka, 1994) [3]. Plants are potential sources of drugs and many of the currently available drugs have been derived from plants. Therefore, α-glucosidase inhibitors screened from plants have attracted increasing attention in recent years.

On the other hand, free radicals may also cause diabetes mellitus. Although free radicals typically come from the surrounding environment, some physiological and biochemical...
processes in the human body also produce reactive oxygen species, such as the superoxide radical, hydroxyl radicals, and peroxyl radicals, as by-products (Duracková, 2010) [4]. Therefore, antioxidants are considered important because of their many health benefits. Plants such as vegetables, fruits, herbs, and spices contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, and terpenoids, which have high antioxidant activities (Tusevski, et al., 2014; Lara, et al. 2013) [5, 6]. In view of these potential health benefits, intensive research has been conducted on natural antioxidants derived from plants.

The evaluation of antioxidant and α-glucosidase inhibitory activities may be used for preliminary observations on pharmacological activities because natural compounds from plants that are considered to be safe have therapeutic effects and fewer health side effects than synthetic medicines (Deng, et al., 2011) [7]. In the present study, phytochemical screening and the antioxidant and α-glucosidase inhibitor activities of ethanol and water extract from the leaves and fruits of Lagerstroemia speciosa were evaluated. An in vitro assay of α-glucosidase inhibitory activity was conducted using α-glucosidase enzyme from Saccharomyces cerevisiae (S. cerevisiae) yeast while an in vitro antioxidant activity assay was conducted using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. These assays may be used for preliminary observations on the evaluation of pharmacological activities. The results of these assays may then be used to verify the medicinal effects of these samples from plants.

2. Materials and Methods

2.1 Collection and Preparation of the Sample

The leaf and fruit sample of Lagerstroemia speciosa Linn. (Banaba) was collected from Hinthada Township, Ayeyawady Region, Myanmar in December, 2015. After being collected, the scientific name of the sample was identified by authorized botanists at Botany Department, Hinthada University. The fresh samples were cleaned by washing with water and air-dried. The dried sample was grounded using grinding machine. And then this powdered sample was kept in the sealed air-tight container to prevent moisture changes and other contamination.

2.2 Determination of nutritional values, phytoconstituents and elementary analysis

Preliminary phytochemical investigation was carried out according to the standard procedures (Marini, et al. 1981; Shriner et al. 1980) [8, 9]. Heavy toxic metals and macronutrient mineral elements present in dry powder sample for the standardization and quality control were also determined by EDXRF spectrometer. In addition, the amount of nutrients such as moisture, ash, fat, carbohydrate, fiber, protein, vitamins, and calorie contents in the sample were determined by recommended analytical methods (AOAC, 1990) [10].

2.3 Preparation of Different Extracts

Aqueous extracts of each sample were prepared by boiling 50g of sample with 500 mL of distilled water for six hours and filtered. It was repeated three times and the filtrates were combined and followed by removal of the water. The same amount of each sample was packed in the cotton bag and placed in the Soxhlet extractor, equipped with a round bottomed flask (500 mL) containing 250 mL of 95% ethanol. Extractions were done by heating the flask in the water bath. After 6 h of such extraction, it was cooled to room temperature and filtered. The filtrate was then evaporated to dryness and stored under refrigerator. Petroleum (PE) extract was also prepared using soxhlet extractor as similar manner.

2.4 Extraction of Phytochemical Constituents from Lagerstroemia speciosa Leaves and fruits

The PE crude extract (2g) of L. speciosa fruits was placed on the 40g of silica gel in a chromatographic column. The column was eluted consecutively with PE:EtOAc in various ratio. Crystallization of purest fraction was carried out by using ethyl acetate provided colourless needle shaped crystal as an isolated compound A (0.0021%). The defatted marc of L. speciosa leaves was again boiled in the same way with 300 mL of EtOH for another 72 hours. The ethanol extract was dissolved in 50 mL of ethyl acetate. Then the EtOAc extract was fractionated by column chromatography on silica gel, successively eluting with PE:EtOAc in various ratio of 15:1, 9:1, 3:1, 1:1 v/v solvent systems and purest fraction (F-II) was recrystallized from PE to produce white needle shaped crystals as isolated compound B (0.024%). The solid compound obtained from fraction F-III were washed with pet-ether and crystallized from ethyl acetate to yield 0.04% of compound “C” as a yellow needle shaped crystal.

2.5 Structural elucidation and identification

The structures of isolated compounds were elucidated and identified by the modern spectroscopic techniques such as UV, FT IR and 1H NMR. Some physicochemical properties of these compounds such as melting point, solubility test, phytochemical test and thin layer chromatography with various visualizing reagents were also investigated.

![Fig 1: Photograph of Lagerstroemia speciosa Linn. (Banaba) tree and its parts](Image)
2.6 Determination of α-glucosidase inhibition activity

The alpha-glucosidase inhibitory effects of the aqueous and alcoholic extracts of the L. speciosa were assayed according to the procedure described previously by Dewi et al. (2007) [11] with modifications. Briefly, the enzyme reaction was performed using p-Nitrophenylalpaha- D-glucopyranoside (PNP-glycoside) as a substrate in phosphate buffer, pH 6.8. The PNP-glycoside (2.0 mM) was premixed with samples at various concentrations. The required concentration of extract solution was prepared by dilution with DMSO. The sample assay mixture consist of 122.5 µL of phosphate buffer solution (20 mM, pH 6.8), 62.5 µL of p-nitrophenyl-α-D-glucopyranoside (5 mM) and 2.5 µL of sample solution with various concentration. The assay mixture was incubated for 5 min at 37°C. Then, 62.5 µL of enzyme solution (0.025 U/mL) was added and incubated for 15 min at 37°C. Enzymatic reaction was terminated by adding 250 µL of Na2CO3 solution. Enzymatic activity was quantified by measuring the p-nitrophenol released from PNP-glycoside at 405 nm wave length. All reactions were carried out with three replications. Acarbose was used as a positive control. One set of mixtures prepared with an equivalent volume of buffer instead of tested samples was used as control. The concentration of the extracts required to inhibit 50% of α-glucosidase activity under the assay conditions was defined as the IC50 value.

2.7 Determining antioxidant activity by DPPH radical scavenging ability

The effects of crude extracts and positive controls (ascorbic acid) on DPPH radicals were estimated according to the spectrosocopic method. Aliquots of crude extracts (1.5 mL) at various concentrations were each mixed with 1.5 mL of 60 mM DPPH solution. All the mixtures were shaken vigorously and left to stand at room temperature for 20 min in the dark. The absorbance of the reaction solutions were measured spectrophotometrically at 517 nm. The DPPH decolorizations of the samples were calculated in percentage according to the equation: % decolorization = [1- (ABSsample /ABScontrol)] × 100. IC50 value was the effective concentration in which DPPH 50% of radicals were scavenged and was obtained by interpolation with linear regression analysis. A lower IC50 value indicated a greater antioxidant activity.

3. Results and Discussion

3.1 Phytochemical constituents and Some Elements present in L. speciosa leaf and Fruit

The preliminary phytochemical investigation was carried out for the samples with a view to determine the presence or absence the types of phytochemical constituents. From these results, it was observed that steroids, terpenoids, glycosides, phenolic compounds, α-amino acids, saponins, starch, alkaloids, carbohydrates, organic acids, flavonoids, reducing sugars and tannins are present in the samples but cyanogenic glycosides are absent.

X-ray spectrometer permits simultaneous analysis of light elements to heavy elements. Shimadzu EDX-700 spectrometer can analyze the elements from Na to U under vacuum condition. From the result, it can be seen that K, Ca and S are observed as predominant mineral elements in Banaba leaf. The relative abundance was found as 0.456% for Ca, 0.443% for K, 0.206% for S and 0.017% for Mn. Moreover, K, Ca, P, Fe, Zn, Rb, Cu and Sr are observed as predominant mineral elements in Banaba fruits. The relative abundance were found as 60.848% for K, 25.717% for Ca, 5.561% for P, 3.477% for S, 2.733% for Fe, 0.517% for Zn, 0.491% for Rb, 0.418% for Cu and 0.238% for Sr.

3.2 Nutrient values of L. speciosa leaf and Fruit

The nutritional values such as protein, fiber, fat and carbohydrates were also determined for L. speciosa leaf and fruit samples. The moisture content of leaf and fruit sample was determined by AOAC method and was found 10.75 and 14.77%. The total ash in the sample is the inorganic residue remaining after the organic matter has been burnt away and it was found 9.69% for leaf and 4.42% for the fruit samples. The fat content for leaf and fruit samples was determined by the soxhlet extraction method and 3.36 and 0.26% was obtained. In addition, the sample was also studied for fiber content by acid alkali treatment method, protein content by AOAC method and ash content by using muffle furnace. The fiber and protein contents for L. speciosa leaf were found to be 13.76% and 11.24%, respectively. And 51.20% of carbohydrate was observed to be present in the leaf sample. The fiber, protein and carbohydrate contents for L. speciosa fruit were found to be 35.39%, 2.88% and 42.28%, respectively.

3.3 Characterization and identification of isolated compounds

Compound A was isolated as a colourless crystal from PE crude extract of L. speciosa fruit. Its melting point was observed to be 138-140°C. It was soluble in ethyl acetate, methanol, ethanol and chloroform. Rf value of compound A was found to be 0.54 in solvent system of PE: EA (5:1 v/v). It gave a cherry red spot on TLC while spraying with 5% H2SO4 followed by heating. It also gave purple colour testing with anisaldehyde and blue colour in Libermann-Burchard reagent test that suggested this compound to be a steroidal. It was also observed as a yellow spot on TLC when treated with I2 vapour. Compound A was UV inactive suggesting the absence of conjugated double bond system. The Rf value of A was found to be identical with that of β-sitosterol in any solvent system and they also gave the same behaviours on Co-TLC chromatogram.

In addition, the melting point of A was also identical with that of authentic β-sitosterol. According to FT IR spectrum of isolated compound A, the band at 3377 cm-1 was attributed to O-H stretching vibration of hydroxyl groups. The strong vibration bands at 2935 cm-1 and 2865 cm-1 were due to asymmetric and symmetric CH stretching vibration of aliphatic >CH2 and -CH3 groups. The peak at 1640 cm-1 was appeared due to C=C stretching vibration of alkene group. The band at 1461 cm-1 and 1384 cm-1 was observed to be 138 cm-1 and 1374 cm-1 was appeared due to C-H bending of cyclic (>CH2)n group. The peak at 1050 cm-1 was indicated that compound A possessed CH=OH binding of cyclic alcohol group. All of the above resulting data obtained from physicochemical properties, melting point, Rf value, and FT IR spectrum lead the compound A to be β-sitosterol.

Isolated compound B was obtained as colourless crystal from PE extract of L. speciosa leaf. Its melting point was observed to be 253-255 °C. It was soluble in ethyl acetate, ethanol, chloroform and methanol. Rf value of compound B was 0.5 in PE: EtOAc (1:3 v/v). It was UV inactive, indicating the absence of conjugated double bond system. It gave a pink spot on TLC while spraying with 5% H2SO4 followed by heating. Since it didn’t give blue colouration when treated with Liebermann Burchard test, compound B was not a steroidal compound. However, it was observed yellow colouration when treated with bromothymol blue solution. The melting point of compound B was found to be similar to that of pentacyclic triterpenoid acid, corosolic acid. (melting point 254-256 °C) (Merk Index, 2001).
From the result of FT IR spectrum of isolated compound B, the presence of alcoholic O-H and carboxyl (-COOH) group was confirmed by the broad band appeared at 3377 cm\(^{-1}\) (3700-3300 cm\(^{-1}\)). Asymmetric and symmetric stretching vibration of C-H provided the bands at 2935 cm\(^{-1}\) and 2865 cm\(^{-1}\). The absorption band at 1689 cm\(^{-1}\) was appeared due to C=O stretching vibration of carboxylic acid group. The bands at 1456 cm\(^{-1}\) and 1387 cm\(^{-1}\) suggested the asymmetric and symmetric C-H bending vibration of >CH\(_2\) and CH\(_3\) groups. C-O-C stretching vibration was observed at 1030 cm\(^{-1}\) and the peak due to C-H out of plane bending vibration occurred at 997 cm\(^{-1}\). Therefore, according to the physicochemical properties, melting point, \(R_f\) value, and FT IR spectral data and chemical tests, isolated compound B may be pentacyclic triterpenoid acid compound, corosolic acid.

Compound C was isolated as yellow needle shaped crystal from column chromatographic separation of EtOAc crude extract of L. speciosa leaf using silica gel GF\(_254\) as absorbent and PE:EtOAc (1:1 v/v) as eluent. Its \(R_f\) value was found to be 0.45 in PE:EtOAc (1:5 v/v) solvent system. Compound C gave deep blue coloration with 1% FeCl\(_3\) showing that it contains phenolic –OH group. It did not give pink colour with concentrated HCl and Mg ribbon but it gave brown colouration with KI. Therefore compound F was also assumed as tannin.

The UV spectra of isolated compound C were recorded in MeOH as well as in the presence of NaOH. Compound C showed \(\lambda_{\text{max}}\) at 221 nm (\(\pi \rightarrow \pi^*\)) and 271 nm (\(\pi \rightarrow \pi^*\)) in MeOH, indicating the presence of unsaturation and non-bonding electron pairs. In the presence of NaOH, the absorption band at shorter wavelength of 209 nm occurred not to be shifted but that at longer wavelength of 271 nm was formed to be shifted to longer wavelength of 287 nm, ascribable to bathochromic shift, red shift. This indicated the presence of phenolic OH group.

The functional groups present in compound C were also studied by FT-IR spectroscopy. The broad band ranging between 3550 cm\(^{-1}\) ~ 2500 cm\(^{-1}\) indicating that compound C born a carboxylic acid –COOH group and it was attributed to –OH stretching vibration. The absorption band appeared at 3371 cm\(^{-1}\) and 3278 cm\(^{-1}\) stretching vibration was confirmed the presence of phenolic OH group. The absorption band at 1701 cm\(^{-1}\) referred to \(\alpha\), \(\beta\)-unsaturated carboxyl group and that at 1620 cm\(^{-1}\) and 1543 cm\(^{-1}\) indicated the presence of C = C group. In addition, the fingerprint region occurred in the range 1454-1311 cm\(^{-1}\) represented the presence of aromatic benzene ring. The absorption bands at 1454, 1338 and 1311 cm\(^{-1}\) were appeared due to C-H in-plane-bending vibration of aromatic ring.

In \(^1\)H NMR spectrum of compound C, only one single signal occurred to appear at the chemical shift of \(\delta\) 7.07 ppm related to two equivalent protons (H-2 and H-6) of tetra-substituted benzene ring. From the UV, FT-IR and \(^1\)H NMR spectra data, compound C may be assigned as gallic acid. Its melting point (253-255°C) was found to be identical with that of gallic acid (258-265°C) (Mercr Index, 2001) and its \(R_f\) value was also coincident with that of authentic gallic acid, checked by Co. TLC chromatography.

Therefore, on the basis of colour reaction, melting point, UV, FT-IR and \(^1\)H NMR spectral data, compound C was identified as gallic acid.

3.4 \(\alpha\) – Glucosidase Inhibitory Effect of Crude Extracts from L. speciosa Leaf and Fruit

The \(\alpha\)-glucosidase inhibitory method is based on the breakdown of p-nitrophenyl-\(\alpha\)-D-glucopyranoside substrate by \(\alpha\)-glucosidase to produce p-nitrophenol, followed by measuring the absorbance of the yellow pigment converted from the glucose over a period of time.

In this experiment, acarbose, glucose – lowering agent, was used as a standard drug. Despite the wide clinical use of Acarbose, the mechanism of its action is not fully understood. The glucose lowering effect of Acarbose is apparently composed of a combination of several distinct activities in various organs and tissue, including decreased hepatic glucose output due to decrease the gluconeogenesis, and increase glycosogenesis and lipogenesis reduced the rate of intestinal glucose absorption and increase glucose uptake by muscle cells and adipocytes. The determination of absorbance was carried out at wavelength 405 nm using UV-visible spectrophotometer.

The absorbance and percent inhibition values of crude extracts were measured at different concentrations and the results are summarized in Table 1. The 50% inhibitory concentrations (IC\(_{50}\)) of 95% ethanol extract and aqueous extract from L. speciosa leaf were found to be 12.2 \(\mu\)g/mL and 18.3 \(\mu\)g/mL, respectively. On the basis of these IC\(_{50}\) values, the inhibitory effects of 95% ethanol extract from L. speciosa leaf on \(\alpha\)-glucosidase activity were found was higher than and aqueous extract. However, in the fruit sample, aqueous extract (3.3\(\mu\)g/mL) on \(\alpha\)-glucosidase activity were found was higher than and 95% ethanol extract (4.3\(\mu\)g/mL). Since the lower the IC\(_{50}\) value, the higher is the \(\alpha\)-glucosidase inhibitory effect. All the extracts tested of L. speciosa possess considerably high \(\alpha\) -glucosidase inhibitory activity. All aqueous ethanol extracts of L. speciosa leaf and fruit sample showed stronger inhibition against \(\alpha\) -glucosidase compared to acarbose (118.4 \(\mu\)g/mL). It is probably that the ability to bind to wide regions of enzyme other than the active site enables these extracts as noncompetitive or mixed inhibitors a broader specificity of inhibition, compared with acarbose, a competitive inhibitor. In addition, phenolic compounds and
terpenoidal compounds present in the samples are able to inhibit the activities of carbohydrate-hydrolyzing enzymes due to their ability to bind with proteins.

According to results, the tested extracts are acknowledged as sources of natural antioxidants that can protect from oxidative stress and thus play an important role in the chemoprevention of diseases that have their etiology and pathophysiology in reactive oxygen species. The medicinal properties of these samples are mainly attributed to the presence of organic and inorganic compounds such as steroids, terpenoids, phenolic acids and antioxidant micronutrients.

### 4. Conclusion

From overall assessment of the present work concerning with investigation of phytochemical constituents, α-glucosidase inhibition and antioxidant activities from *Lagerstroemia speciosa* L. (Banaba) leaf and fruit, the plant is a good remedy for some disease due to the presence of important phytoconstituents, such as phenolic compounds, tannins, alkaloids, terpenoids and steroids and so on. Moreover, K, Ca, P and S were found as a major constituents in these samples. And higher content of nutrients was observed to be present in the sample and assumed to be good source of carbohydrate, fiber and protein. Steroids, terpenoid and phenolic compounds such as β-sitosterol, corosolic acid and gallic acid could be observed as the predominant constituents and played an important role in the therapeutic action of *L. speciosa* leaf and fruit. The order of α-glucosidase inhibitory effect was observed to be aqueous extract (fruit) (IC\textsubscript{50} = 3.3 μg/mL) > ethanol extract (fruit) (IC\textsubscript{50} =4.3 μg/mL) > ethanol extract (leaf) (IC\textsubscript{50} =12.2 μg/mL) > aqueous extract (leaf)(IC\textsubscript{50} =18.3 μg/mL). The antioxidant activity screening of two crude extracts such as ethanol and aqueous extracts, using DPPH assay according to the spectrophotometric methods. In this experiment, the order of antioxidant activity was as ethanol extract (IC\textsubscript{50} = 2.6 μg/mL) > aqueous extract (IC\textsubscript{50} = 4.3 μg/mL) > ethanol extract (IC\textsubscript{50} = 6.2 μg/mL) > aqueous extract (IC\textsubscript{50} = 9.2 μg/mL). From these observations, the radical scavenging activity of *L. speciosa* (Pyin-ma) leaf ethanol extract was found to be more effective than aqueous extract in antioxidant activity. Consequently, from these results, it can be inferred that this leaf and fruit possesses nutrients, α-glucosidase inhibitory and antioxidant properties and applied as nutraceuticals potential for the treatment of malnutrition and diabetes mellitus.

### 5. References


### Table 1: Inhibition of Crude Extracts from *L. speciosa* and Standard Acarbose against α- Glucosidase Enzyme Activity

<table>
<thead>
<tr>
<th>Sample</th>
<th>Extract</th>
<th>IC\textsubscript{50} (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. speciosa</em> (leaf)</td>
<td>aqueous</td>
<td>18.3</td>
</tr>
<tr>
<td><em>L. speciosa</em> (leaf)</td>
<td>EtOH</td>
<td>12.2</td>
</tr>
<tr>
<td><em>L. speciosa</em> (fruit)</td>
<td>aqueous</td>
<td>3.3</td>
</tr>
<tr>
<td><em>L. speciosa</em> (fruit)</td>
<td>EtOH</td>
<td>4.3</td>
</tr>
<tr>
<td>acarbose</td>
<td></td>
<td>118.4</td>
</tr>
</tbody>
</table>

### Fig 3: Inhibition activity of aqueous and ethanol crude extracts of *L. speciosa* leaf and fruit in compare with standard acarbose against α-glucosidase enzyme


