Alpha-glucosidase inhibitory activities of bungur (Lagerstroemia loudonii Teijsm. & Binn.) leaves and fruits

S Riyanti*, P S Dewi, A S Windyaswari, and S A N Azizah
Pharmaceutical Biology Research Group, Faculty of Pharmacy, Universitas Jenderal Achmad Yani
Jl. Terusan Jenderal Sudirman PO.BOX 148 Cimahi, 40533, Indonesia

*Corresponding author’s email : anti.piper81@gmail.com

Abstract. Bungur (Lagerstroemia loudonii Teijsm. & Binn.) belongs to the Lythraceae that traditionally was used as antidiabetic, antiinflammatory, antihypertensive and diuretic. Other bungur plants such as Lagerstroemia speciosa have been known for the activity as alpha-glucosidase inhibitor. Based on chemotaxonomy, the distribution of compounds in one family or genus and its activity has similarities, then tested for the activity of alpha-glucosidase of Lagerstroemia loudonii Teijsm. & Binn. The leaves and fruits was extracted by reflux using 96% ethanol. The process of fractionation by liquid-liquid extraction. Testing of alpha-glucosidase inhibitor activity was performed in vitro using colorimetric method at 400.4 nm, acarbose used as standard inhibitor. The results of alpha-glucosidase inhibitory activity of leaves with IC50 showed the ethanol extracts, n-hexane, ethyl acetate and water fractions respectively 262.20 pg/ml; 97.16 pg/ml; 62.73 pg/ml; 145.30 pg/ml; and fruits with IC50 showed the ethanol extracts, n-hexane, ethyl acetate and water fractions respectively 1.50 pg/ml; 3.69 pg/ml; 37.38 pg/ml; 22.59 pg/ml. IC50 standard acarbose was 10.95 pg/ml. The results of this research from Lagerstroemia loudonii Teijsm. & Binn. leaves and fruits can be developed into herbal preparations to lower blood glucose levels.

1. Introduction
Type 2 diabetes mellitus has the highest prevalence in the world with the percentage of 1.9% and it has made it the seventh leading cause of death in the world. Type 2 diabetes mellitus treatments can be done by managing the diet, exercising, and taking oral hypoglycemic drugs. One of the commonly used oral hypoglycemic drugs is a-glucosidase enzyme activity inhibitor which is popularly known as Acarbose. This compound works by inhibiting the a-glucosidase enzyme activity on the walls of the small intestine so that it will reduce the digestion of carbohydrates and its absorption process which will result in a postprandial decrease of blood sugar levels in people suffering from diabetes mellitus [1].

The antidiabetics drugs have many side effect, another alternative treatment for diabetics is the use of herbal medicines. Plants containing alkaloids, steroids, polyphenols, saponins, tannins, triterpenes and flavonoid can serve as alpha-glucosidase inhibitors in type 2 diabetes mellitus. Polyphenol content in plants has long been known to inhibit the activity of digestive enzymes through its ability to bind to proteins. The inhibitory activities of secondary metabolites in plants, including polyphenols, steroids, and alkaloids component against carbohydrates hydrolyzing enzymes can decrease postprandial hyperglycaemia.
**Lagerstroemia loudonii** T.&B (Lythraceae) in Indonesia is well-known as *bungur*. This Lagerstroemia genus grows in tropical areas and has been used in traditional medicine to overcome diabetes mellitus, and it can also be used as antiobesity [2]. Corrosolic acid contained in *Lagerstroemia speciosa* *bungur* leaves is an active component which has a responsibility as antidiabetic [3], besides, the acid also has an effect as an antioxidant, antiinflammatory, and antihypertensive [4]. The corrosolic acid compound contained in the leaves can possibly be distributed in other parts of the plant such as fruits, stems, and barks. Plants which belong in the same family or genus has a possibility of sharing almost the same chemical compounds. Triterpenoids, ellagic acid, coumarin, and neolignan have been identified in *Lagerstroemia specios* L and this chemical compound is similar to other Lagerstroemia species [5]. The purpose of this study is to scientifically prove the effect of leaves and fruits of *L. loudonii* Teijsm. & Binn. as antidiabetic through the mechanism of alpha-glucosidase inhibitors. The alpha-glucosidase inhibitory activity of the leaves and fruits of *L. loudonii* Teijsm. & Binn. was reported for the first time in this study.

2. Materials and methods

Plant Material. Bungur (*Lagerstroemia loudonii* T.&B.) leaves and fruits were gathered from the School of Pharmacy of ITB. Bungur fruits used in this study were the ripe ones with blackish green color. Each levaes and fruits were washed and dried using drying cabinet with temperature below 40°C, then mashed and stored in a closed container.

**Extraction.** Each crude drug of *L. loudonii* Teijsm. & Binn. leaves and fruits (250 g) was extracted by using reflux method with 96% ethanol for one hour starting from when the solvent mixture and crude drug started to boil. The extraction process was done triplicate. Then, the liquid extract was concentrated using a rotary vacuum evaporator until thick extract was obtained. Fractionation was done by liquid-liquid extraction. Phytochemical screening was done in order to identify the existence of flavonoid, tannin, polyphenol, monoterpenoid, sesquiterpenoid, quinone, steroid, triterpenoid, and alkaloid compounds.

**Alpha-glucosidase Inhibitor Testing.** Alpha-glucosidase enzyme derives from *Saccharomyces cerevisiae* (Sigma Aldrich, G5003-100UN), p-Nitrophenyl a-D-Glucopyranoside substrate (Sigma Aldrich, N1377-1G), dimethyl sulfoxide (DMSO), ovine serum albumin (Sigma Aldrich, A7906-10G), acarbose (PT. Dexa Medica).

The method of the alpha-glucosidase inhibitor testing referred to Watanabe’s with minor modifications using chromogenic substrate [6]. Alpha-glucosidase enzyme hydrolyzed p-nitrophenyl a-D-glucopyranoside (PNPG) into yellow p-nitrophenol, and p-nitrophenol’s uptake was measured at 400.4 nm. 10 pL of sample/extract solution with various concentrations, 500 pL phosphate buffer pH 7.0 and 250 pL PNPG with a concentration of 2.5 mM and 250 pL a-glucosidase concentrate of 0.2 U/mL was incubated for 30 minutes at 37°C. After the incubation period was over, 1000 pL sodium carbonate 200 mM was added. The absorbance of the sample was measured using spectrophotometer at 400.4 nm. The same method was also applied to acarbose as the standard of alpha-glucosidase inhibitor. The control solution which consisted of 10 pL DMSO plus 500 pL phosphate buffer pH 7.0 and 250 pL PNPG with a concentration of 2.5 mM, and 250 pL a-glucosidase enzyme with a concentration of 0.2 U/mL was incubated for 30 minutes at 37°C and then 1000 pL natrium carbonate 200 mM was added. % Inhibition is calculated by this formula:

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\% \text{ inhibition} = \frac{\text{Absorbance Control} - \text{Absorbance Sample}}{\text{Absorbance Control}} \times 100\%
\]

3. Results and discussions

The result of phytochemical screening of crude drug and ethanol extracts of bungur (*Lagerstroemia loudonii* Teijsm.&Binn.) leaves and fruits shows a positive existence of flavonoid, tannin, saponin, polyphenol, quinon, steroid and triterpenoid, and leaves were detected of alkaloids compounds. The result of the phytochemical screening of crude drug, and ethanol extracts of bungur leaves and fruits can be seen in table 1.
Table 1. The results of phytochemical screening of crude drug and extracts

| Class of compounds | Crude drug Leaves | Fruits | Ethanol Extract Leaves | Fruits |
|--------------------|------------------|--------|------------------------|--------|
| Alkaloid           | +                | -      | +                      | -      |
| Flavonoid          | +                | +      | +                      | +      |
| Tannin             | +                | +      | +                      | +      |
| Polyphenol         | +                | +      | +                      | +      |
| Saponin            | +                | +      | +                      | +      |
| Quinone            | +                | +      | +                      | +      |

Notes: (+) shows the existence of the class compound, (-) no class compound.

Alpha-glucosidase inhibitors are the first-line oral antidiabetic drugs used for type 2 diabetes mellitus that work by inhibiting the breakdown of complex carbohydrates, less glucose is absorbed because carbohydrates are not broken down into glucose molecules. One of the oral antidiabetic drugs which is alpha-glucosidase inhibitor is the acarbose which has the function of controlling the glycemic effects associated with postprandial hyperglycemia. The bungur plant is belonging to Lythraceae, is widespread in the Asian region such as Thailand, Vietnam, Laos, Kamboja, Malaysia and Indonesia, which are Lagerstroemia speciosa L., Lagerstroemia floribunda L., Lagerstroemia indica L., and Lagerstroemia loudonii Teijsm. & Binn.. The genus of Lagerstroemia has been reported have antidiabetic activity. The compounds that are responsible for antidiabetic are corosolic acid and ellagitannin. Antidiabetic activity has been proven through several studies including L. speciosa leaves which have been known to contain corosolic acid compounds as activators in glucose transport which generally reduce blood glucose levels and a standardized extract of L. speciosa leaves containing 1% corosolic acid was proven to reduce blood glucose levels at a daily dose of 32 mg and 48 mg with a duration of administration for two weeks [7]. Research on the activity of alpha-glucosidase inhibitors from bungur type Lagerstroemia speciosa has been carried out first, as well as the isolation of the compounds contained in its leaves. One of the compounds that provide alpha-glucosidase inhibitors activity from bungur leaves is corosolic acid which is a triterpene group. The distribution of corosolic acids in these bungur plants is spread to the fruit. Corosolic acid from Lagerstroemia speciosa leaves showed uncompetitive inhibition of alpha-glucosidase activity with IC50 values of 3.53 pg / ml [8]. Corosolic acid content in the leaves are 100.9 mg / g [9]. This study aims to explore antidiabetic activity in vitro through the alpha-glucosidase inhibiting mechanism of ethanol extracts of Lagerstroemia loudonii Teijsm. & Binn. fruits and leaves.

The result of alpha-glucosidase inhibitor testing towards ethanol extract, n-hexane fraction, ethyl acetate, and water fractions can be seen on table 2. Acarbose is used as the standard of alpha-glucosidase inhibitor.

Table 2. Alpha-glucosidase inhibitory activity of bungur leaves and fruits

| Sample               | IC50 (μg/ml) |
|----------------------|--------------|
|                      | Leaves       | Fruits      |
| Ethanol extract      | 262.20       | 1.50        |
| n-hexane fraction    | 97.16        | 3.69        |
| Ethyl acetate fraction | 62.73    | 37.38        |
| Water fraction       | 145.30       | 22.59        |
| Acarbose             |              | 10.95        |
The results showed that the alpha-glucosidase inhibitory activity of ethanol extract of *L. loudonii* Teijsm. & Binn. fruits gave a very strong activity compared to the leaves. IC<sub>50</sub> ethanol extract of *L. loudonii* Teijsm. & Binn. fruit is 1.5 pg/mL while the leaves are 262.20 pg/mL. Likewise, the fractions of *L. loudonii* Teijsm. & Binn. fruit provide alpha-glucosidase inhibitory activity smaller than 100 pg/mL, whereas on the leaves only fractions of n-hexane and ethyl acetate. Acarbose as a comparison of alpha-glucosidase inhibitors gave IC<sub>50</sub> values of 10.95 pg/mL. Alpha-glucosidase inhibitor activity of ethanol extract of *L. loudonii* Teijsm. & Binn. fruit is 7 times stronger than that of acarbose, whereas ethanol extract of *L. loudonii* Teijsm. & Binn. leaf is 24 times weaker than acarbose. This activity of enzymatic inhibition is stated with the IC<sub>50</sub> value by seeing the lowest value of IC<sub>50</sub> which shows a strong activity in inhibiting enzymatic activity. *L. loudonii* Teijsm. & Binn. fruits used in this study were the ripe ones. Generally, the result of alpha-glucosidase inhibition from the *L. loudonii* Teijsm. & Binn. fruit extract and fraction shows a strong activity. The extract gives a quite decent inhibitory activity rather than the standard inhibitor. The reported chemical compound of *Lagerstroemia loudonii* T.&B. bungur fruits is corosolic acid, ursolic acid and oleanolic acid [10]. The biological activity for ursolic and oleanolic acids is as an anticancer, antioxidant, antiinflammatory, hepatoprotector, anti-TB, and antimalarial [10]. Corosolic acid belongs to the pentacyclic triterpenoid class contained in bungur leaves which have activities as antidiabetes and antiobesity [11], and there is a possibility that this corosolic acid is also distributed in bungur fruits so that it gives a good activity in the alpha-glucosidase inhibition. The highest and the lowest levels of corosolic acid content based on the research which is conducted consecutively are found in old leaves with reddish yellow and fallen leaves, flower petals, and green leaves [12].

This is an introductory study for alpha-glucosidase inhibitory of fruits and leaves of *Lagerstroemia loudonii* Teijsm. & Binn. More thorough research on the study of marker and active compound as alpha-glucosidase inhibitor are indispensable. Thus, further research to complete the data on the components of marker compound and active compound in *Lagerstroemia loudonii* Teijsm. & Binn. fruits is currently conducted. The activity of alpha-glucosidase inhibitor by bungur leaves and fruits can be developed for safety testing and traditional medicine preparations which can help lower the blood glucose level.

4. Conclusion
Ethanol extract of fruits and leaves have potential alpha-glucosidase inhibitory activity. alpha-glucosidase inhibitory activity in *L. loudonii* Teijsm. & Binn. fruit was 175 times stronger than the leaf extract. The activity of the ethanol extract in fruits along with its fractions gives a significant result in inhibiting alpha-glucosidase. Furthermore, the identified bioactive on *L. loudonii* Teijsm. & Binn. fruits may be responsible for its antidiabetic (alpha-glucosidase inhibitor) and further studies may investigated its antidiabetic potential.

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