Reducing environmental effect of bark waste of Sengon (Paraserianthes falcataria L.) by applying as a source of green ingredients to lower glucose-related diseases

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Abstract. Sengon is a fast growing plant and recently paid many attentions for timber industry sector. New plantations are established in many province of Indonesia. Including in East Java. Plantation is managed by private, public company, and even by individual farmer. The timber logs are produced to support some industries such as pulp, plywood and constructions. However, the processing still remains a huge waste of wood bark and wood scraps. Some companies disposed the waste into environment and some burnt for firewood. And thus, come up to the environmental issue. This paper reports recent chemicals investigation contained in the Sengon bark and also evaluate the potency to lower of the glucose-related diseases. It is found that bark’s crude extract is composed of some group of chemicals, such as alkaloid, flavonoid and triterpenoid. Further separation of the extract into simpler chemicals found the triterpenoid group dominated the composition. UV-VIS and FTIR spectra also confirmed the substituent of the chemicals structures, such as alkyl, hydroxyl, carbonyl, and carboxyl-groups construct the triterpenoid structures. In addition, all these triterpenoids groups can decrease a glucose level in vivo, and the value is comparable to that reference reported.

1. Introduction

Forestation of dry and un-used lands in several region or province in Indonesia has important impact for keeping global and earth atmosphere cool. Also, it prevents negative impact from carbon dioxide warming up the globe, and conversely restore the carbon and nitrogen sources fertilize the earth sustainably. Plantation strategy using Sengon (Paraserianthes falcataria L) has paid government of Indonesia policy, since this plant easily grow in lack-water field and a fast-growing plant that suit for timber industrial sector. This plant is an indigenous of Indonesia, especially from East Timor and Java [1]. It is classified into family of Fabaceae, and known also as Albizia falcataria (L) Fosber, Albizia falcata Back, and Albizia moluccana Miq. Locally, this plant is commonly known as Sengon Laut (East and Central Java), Jeunjing (West Java), Jing Laut (Madura), Tadehu Pute (Sulawesi), Tawasela (Ternate) [2-3]. Industrial sectors have put Sengon as commodity [4-6] in order to support a local timber sources for pulp [7], plywood and home construction [8]. Total area for Sengon plantation in East Java is about 20 kHa, and mostly located in Lumajang, Malang, Probolinggo, and Banyuwangi [9-10]. From this region in average can be produced about 7.5 million of timber logs. However, the process also produces thousands ton of wood scrap and barks waste (Figure 1A). The wood scrap is used directly by
local community for daily firewood, meanwhile wasted bark is generally burnt and disposed in the environment. This disposal can pollute the environment and affect the global atmospheres.

Figure 1. Processing of Sengon to produce timber log and waste bark (A). Processing of waste bark to produce green chemicals (B) in this paper.

Kaida et al. previously reported enzymatic sacharification of wood from Sengon for ethanol production [11]. The bark was just disposed. It is predicted, the bark can not easily to be decomposed by enzyme or some chemicals inhibit the fermentation. From this points, Kaida et al. [11] finding interest to be investigated further for chemicals valorization. Inhibiting enzymatic reaction means disrupt or distract the reaction, and also open potency for disrupting of the other enzymatic reaction such as carbohydrate or glucose metabolism in the body. Disruption glucose metabolism can lead for application in glucose-related diseases such as hypoglycemic, hyperglycemic disorder, and diabetes mellitus. This paper reports a recent investigation of green chemicals composed the waste bark and purifed-triterpenoid group (Figure 1B) as ingredient for declining the glucose concentration \textit{in vivo}. This finding will lead for further application of the waste bark of Sengon as source of green chemicals “ingredients” to treat glucose-related diseases in human or animal [14-17].

2. Experiment section

2.1. Sample, chemicals and instrument analysis

Sample of Sengon (\textit{Paraserianthes falcataria} L) bark is given by local farmer in Malang, East Java. The taxonomy of the specimen previously was identified by Dr. Djati Batoro from Laboratory of Plants Taxonomy in Faculty of Mathematics and Natural Sciences of Brawijaya University, Malang. Meanwhile the animal model for \textit{in vivo} evaluation using \textit{Rattus norvegicus}. It is bought and prepared by a trained laboratory technician from Pharmacology Laboratory in Faculty of Medicine of Brawijaya University. A seven groups of rats and each group consisted of 5 are induced by alloxan 100 mg/Kg of body weight. This procedure is certified by board of standard and ethics treatment using animal as model for research in Brawijaya University.

Chemicals are used directly as bought from manufacturer, i.e. distillated water, methanol (Smart Lab), n-hexane (Smart Lab), ethyl acetate (Smart Lab), iron(III) chloride (Merck), hydrochloric acid (Merck), sulfuric acid (SAP), acetyl chloride (Merck), pre-coated TLC plate (Merck), silica gel for column chromatography (Merck), glibenclamide, sodium chloride (Merck), and alloxan monohydrate (Merck).

Instrument for analysis includes infrared spectrophotometer (FTIR Shimadzu 8400), ultra violet-visible spectrophotometer (Shimadzu 1601), and glucometer.
2.2. Extraction procedure
A dried powder of Sengon bark (3.5 Kg) is extracted n-hexane (5 L). This process is repeated 5 times or until clear solution of n-hexane extract is afforded. Then, the combined of n-hexane extract is concentrated in vacuum with rotary evaporator to afford a crude n-hexane extract as a brown-yellow solid 19.89 g. This extract is further separated under column chromatography (silica gel) with increasing of gradient solvent n-hexane/ethyl acetate (95%, 90%, 70%, 60%, 40%, 0%) and flushing with ethanol. Fractions are grouped based on similarity pattern of retardation factor (Rf) in TLC, and further analysis by UV-VIS and FTIR spectrophotometry.

2.3. Chemicals analysis
Procedure for chemicals analysis contained in the waste bark extract follows the reference previously reported [12-13]. Alkaloid test undergoes following Mayer techniques. Tannin test uses iron(III) chloride reduction and flavonoid extracted with methanol 30% is reacted with sulfuric acid. Meanwhile triterpenoid is reacted to Liebermann-Burchard reagent (acetyl chloride and sulfuric acid).

2.4. Glucose-lowering activity test
Procedure to test glucose-lowering activity follows the reference previously reported [18]. Seven group of model-rat induced with alloxan are treated with fractions (I-VI) 100 mg/Kg of rat body weight. The other is treated with glibenclamide as positive control, and the rest is grouped as normal rat (healthy rat). Fraction VII-VIII are not evaluated due to fraction quantity consideration. Glucose level of each rat in each groups are checked each day for a month.

3. Result and discussion
A dried-bark waste of Sengon (total 3.5 Kg) is immersed in n-hexane for 24 h. The mixture is separated by filtration and further concentrated in vacuum to afford 0.57% yield of crude extract as a brown-yellow concentrate liquid. With a ton of waste bark disposed in each week from one timber company in Lumajang region only, then, approximately can be produced chemicals in 5.7 Kg. This are important quantity of a green chemicals source for chemical industry or medicine, that normally uses in micro- and milli-gram scale. Further chemicals analysis following reference [12-13] affords some secondary metabolite groups composed the extract, such as alkaloid, flavonoid and triterpenoid (Table 1). Alkaloid is secondary metabolites with nitrogen-atom made off the structure. Meanwhile, flavonoid is also a phenolic class of secondary metabolite that its structure consists of benzenoid ring interconnected with 3 carbon chain (C6-C3-C6). Each benzenoid ring generally contain hydroxyl (HO-) and methoxy (CH3O-) group. The hydroxyl-group can be more than two in each ring. Moreover, the triterpenoid compound is constructed by 30 carbon atom and have 6-cyclic chain. Triterpenoid has two different structures, i.e. oleanane and steroid (Figure 2). Oleanane is built from 5 frameworks of hexa-cyclic carbon chain, while steroid is constitute of 3 backbone of hexa-cyclic carbon chain and a penta-cyclic carbon chain. Both structures can have hydroxyl, alkene, carbonyl, carboxyl and alkyl-group. The chemicals profile composed of the extract is displayed as TLC chromatogram (Figure 3). Eight spot is detected, and indicate number of chemicals composed the bark. Further separation by column chromatography under increasing solvent polarity afford 8 fraction (I-VIII).

Figure 2. Two type of triterpenoid structures. $R_1$ and $R_2$ is substituents may construct of hydroxyl, carbonyl, carboxyl, alkene, and alkyl group.
Chemicals analysis of each fraction give positive result for triterpenoid, except for fraction VIII (Table 2). The other secondary metabolite (alkaloid and flavonoid) is not detected from each fraction. It is predicted the quantity of both flavonoid and alkaloid are very low. Fraction I-IV shows similar pattern in UV-VIS absorption. The maximum wavelength is detected in about 205-219 nm, except for fraction II detected at 450 nm. Meanwhile for fraction V-VIII mostly have maximum wavelength in 217-224 nm (Figure 4). Peaks absorption is detected in UV-VIS spectra correlate to the electronic transition from a highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). For organic molecule, this transition occurs specifically in functional group made of the structure. The similarity in pattern of transitional electronic may predict the similarity of functional group constructs the structure. Fraction I, III-IV and V-VIII have similar UV-VIS pattern, and is predicted these fractions have similar functional group composed the triterpenoid structure.

**Table 1. Tabulation of secondary metabolite composed the bark extract of Sengon**

| No | Secondary metabolite | Reagents test | Result |
|----|----------------------|---------------|--------|
| 1  | Alkaloid             | Mayer reagent | Positive (+) |
|    |                      |               | Indicate yellow precipitate |
| 2  | Tannin               | FeCl₃         | Negative (-) |
|    |                      |               | No indication in color |
| 3  | Flavonoid            | Sulfuric acid/methanol | Positive (+) |
|    |                      |               | Indicates a red in bottom of solution |
| 4  | Triterpenoid         | Liberman-Burchard reagent | Positive (+) |
|    |                      |               | Indicate violet solid as precipitate |

**Table 2. Tabulation of fractions, retardation factor (Rf) and phytochemical analysis**

| Fraction | Number spot in TLC | Rf1 | Rf2 | Phytochemical test |
|----------|-------------------|-----|-----|--------------------|
| I        | -                 | -   | -   | Triterpenoid (+)   |
| II       | 1                 | 0.93| -   | Triterpenoid (+)   |
| III      | 1                 | 0.78| -   | Triterpenoid (+)   |
| IV       | 2                 | 0.78| 0.84| Triterpenoid (+)   |
| V        | 2                 | 0.65| 0.76| Triterpenoid (+)   |
| VI       | -                 | -   | -   | Triterpenoid (+)   |
| VII      | 1                 | 0.38| -   | Triterpenoid (+)   |
| VIII     | -                 | -   | -   | Triterpenoid (-)   |

Note: TLC is performed using ethyl acetate/n-hexane (3/7 v/v).

![Figure 3](image-url). Profile of extract in TLC (Solvent in n-hexane/ethyl acetate 3/7 v/v) (left) and photograph of column chromatography (right)
Identical result is provided by FTIR analysis. The result is summarized in Table 3. In general, the alkyl group is detected in all fractions, and hydroxyl group detected in fraction III-VIII. Combining data for double bond vibration (contained in fraction II-VI), shows that =C-H vibration for aromatics is detected for fraction IV ($3170 \text{ cm}^{-1}$) and V ($3163 \text{ cm}^{-1}$). While other is recorded at $3004 \text{ cm}^{-1}$ that is a general vibration for non-conjugated =C-H alkene. In addition, only fraction V gives a carbonyl absorption at $1708 \text{ cm}^{-1}$ that possibly correlates to flavonoid structure. The rest of fractions are predicted to be triterpenoid compound contains double bond, hydroxyl, carbonyl (VII) and carboxylate (III and VI) groups. While fraction VIII is not triterpenoid nor flavonoid but an alkaloid. These fractions are further analysis for their capability to affect glucose metabolism in vivo. The test only performed for fraction I-VI, and fraction VII-VIII is not evaluated due to a limited quantity.

| Group vibration      | Wavelength value (cm$^{-1}$) of fractions |
|----------------------|------------------------------------------|
| Hydroxyl group (O-H) | I: 3500-2500, II: 3394-2500, III: 3408-2500, IV: 3436-2300, V: 3421 (s), VI: 3421 (s) |
| Alkene group (C=C-H) | I: 3004, II: 3006, III: 3170, IV: 3163, V: 3004, VI: 3004 |
| Alkyl group (C-H)    | I: 2954, 2923, 2852, II: 2923, 2952, 2852, III: 2925, 2854, 2852, IV: 2923, 2852, 2852, V: 2921, 2852, 2852 |
| Carbonyl (C=O)       | I: 1739, 1710, II: 1710, III: 1708, IV: 1708, V: 1710, VI: 1630, VII: 1660 |

The in vivo evaluation of triterpenoid (fraction I-VII) prepared from the waste bark is undertaken using rat model for hyperglycemic diseases [18]. Hyperglycemic correlate to the increasing of glucose concentration in the blood. If the concentration persistent for a long time can effect a diabetes mellitus type 2 diseases. This disorder due to insulin as a hormone or enzyme to degrade the glycogen can not work as it in normal or healthy condition. Thus treatment of the triterpenoid as a green chemical which is as an active ingredient to stimulate insulin work above the condition can reduce glucose concentration in the blood. And that will avoid any diseases correlated to the higher glucose concentration.

The evaluation result is displayed in Table 4. In normal condition, glucose contains in blood between 50-120 mg/dL. Alloxan treatment of the normal rat increase the glucose level to above 150 mg/dL. This level is similar to diabetic mellitus type 2 conditions. Treatment with fraction II-VI indicate the glucose level reduction (59.00-105.00 mg/dL) but not for fraction I (165 mg/dL). Similar result also recorded after treatment with glibenclamide, and especially fraction II and V give comparable activity.
Table 4. Tabulation of anti-diabetic activity of fractions

| Group of rats                          | Glucose level in blood of rats (mg/dL) |
|---------------------------------------|----------------------------------------|
|                                       | I       | II      | III     | IV      | V       | VI      |
| Normal control group                  | 93.33   | 88.67   | 107.33  | 84.33   | 90.67   | 90.00   |
| Alloxan induction group (DB)          | 170.00  | 170.00  | 175.00  | 175.00  | 165.00  | 166.00  |
| Treatment group rat with fractions    | 165.00  | 80.00   | 105.00  | 101.00  | 59.00   | 100.0   |
| Treatment group rat with glibenclamide| 80.00   | 75.00   | 90.00   | 90.00   | 60.00   | 80.00   |

Note: treatment is taken by injection of 100 mg/Kg body weight of rat. Blood test is taken every day until 4 weeks.

4. Conclusion
The waste bark of Sengon (Paraserianthes falcataria L) contain chemicals in 0.57% yield. In approximate, each ton of waste bark can produce about 5.70 kg chemicals. This is a huge source of the class chemicals of alkaloid, flavonoid, and triterpenoid. Purified the triterpenoid group are able to be chemical ingredient to lower glucose concentration in vivo. The capability is comparable to commercial synthetic medicine, glibenclamide. This result lead for further detailed molecular purification and structural elucidation.

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