Antioxidant and antidiabetes activity from the fruit shell of *Calophyllum inophyllum*

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ARTICLE INFO	ABSTRACT
<p><i>Article History:</i> Received date: 19 June 2021 Revised date: 22 October 2021 Accepted date: 02 November 2021 Available online at: November 2021</p> <p><i>Keywords:</i> <i>Calophyllum inophyllum</i>, fruit shell, antioxidant, antidiabetes</p>	<p>The purpose of this study was to explore the antioxidant and antidiabetes activity of the <i>Calophyllum inophyllum</i> fruit shell. Hexane fraction from fruit shell of <i>C. inophyllum</i> was prepared and separated by column chromatography methods. The hexane sub fractions (SF1 and SF2) was analysed using DPPH (2,2-diphenyl-1-picrylhydrazyl radical scavenging) for antioxidant activity and inhibition of α-glucosidase enzyme for antidiabetes activity. The sub fractions from fruit shell of <i>C. inophyllum</i> showed moderate antioxidant and antidiabetes activity, although the activities were low. The SF1 had only antidiabetes activity with 16.26% inhibition of α-glucosidase enzyme at 100μg/ml. The SF2 showed antioxidant and antidiabetes activity with 6.31 % inhibition of DPPH and 16.99% inhibition of α-glucosidase enzyme at 100μg/ml, respectively.</p> <p>© 2016 Indonesian Journal of Applied Chemistry. This is an open access article under the CC BY-NC-SA license (https://creativecommons.org/licenses/by-nc-sa/4.0/).</p>

1. INTRODUCTION

C. inophyllum is a large genus in Gutteferae family with about 180-200 species, which is distributed mainly in tropical forests such as China South and Southeast Asia, Eastern Africa, Madagascar, the Pacific island, America, Australia and Brazil. *Calophyllum* species are large hardwood and are generally grown in a mountain forest to coastal swamps. The genus *Calophyllum* has potential for timber and as folk medicines for various diseases such as vaginal discharge, rheumatism, scabies, ulcers, and hair grower [1,2].

In 2016, Li et al. have successfully isolated four coumarins compounds i.e. (-)-12-methoxy inophyllum A, (+)-12-methoxy inophyllum H-1, (-)-12-methoxylinophyllum H-2 and, inophyllum H-J from *C.inophyllum* leaves [2]. Susanto et al.

have analyzed proximate content : i.e. moisture, ash, protein, crude fiber, carbohydrate, mineral and also identified 3 compounds i.e. trans-2[2-(trifluoromethyl) phenyl]-10b, 10c-dimethyl-10b and 10c-dihydropyren from *C.inophyllum* leaves [3]. Dipyrancoumarin has been isolated from callus of *C.inophyllum* Lin, that pyranocoumarin have anti-HIV activity [4]. Ze, Qi Xu [5] proved that Calanolide A is active against both drug-susceptible and drug-resistant strains of *Mycobacterium tuberculosis*.

Many researchers have also observed the activity of some species of *Calophyllum* as anti cytotoxic [6], antimalaria [7], antimicrobial [8], antibacterial [9] toxicology effect [10] and antiulcer [11] and, anti-inflammatory activity [12]. She et al. have isolated inocalophyllins

A, B compound and their methyl ester from the seed of *C.inophyllum* [13]. Unfortunately research on the use of fruit shell of *C.inophyllum* (FSCI) has never been conducted. Therefore this research was importantly conducted to evaluate the antioxidant and antidiabetic activities of fruit shells from *C. inophyllum* (FSCI). *C. inophyllum* fruit shells were considered as waste material from the process of extracting oil from the fruit of *C .inophyllum*. The production of oil from *C. inophyllum* fruit was available in Indonesia. Therefore we used waste material from *C. inophyllum* fruit shells to explore the antioxidant and antidiabetes activity for the possibility of being used as a source for medicines.

2. EXPERIMENTAL

2.1 Materials

The fruits of *C. inophyllum* were obtained from Puspitpek Garden, Serpong, West Java, Indonesia. Indonesia. *C.inophyllum* plants in Puspitpek area were planted by Research Center for Biology – LIPI and the plant name was confirmed by the botanist of Research Center for Biology – LIPI. Solvents used for extraction and column chromatography (n-hexane, ethyl acetate, acetone, and methanol) were purchased from local market and distilled prior to use. Folin-Ciocalteu Reagent, FeCl₃, DPPH (2,2-diphenyl-1-picrylhydrazyl), gallic acid, and quercetin were purchased from Sigma-Aldrich (St. Louis, USA). TLC plates (silica gel 60 F₂₅₄ thin 0.25 mm, 20x20 cm) and silica gel G 60. (70-230 mesh) for column chromatographic were purchased from Merck.

2.2 Methods

2.2.1 Extraction and fractionation

The fresh fruit samples were chopped into two pieces then were separated into fruit peel, fruit shell and fruit core before finely cut. Image of *C. inophyllum* fruit shell (CIFS) shown in Fig. 1. The CIFS were dried at 45°C then grinded using Laboratory Mill grinder.

The powder of CIFS (0.5 kg) was extracted with 3 L of *n*-hexane, ethyl acetate and methanol for 72 h (n=3x). Then, the solutions were filtered, then evaporated under reduced pressure to give *n*-hexane, ethyl acetate and methanol crude extract are 64.215 g : 66.929 g and 101.387 g respectively.

The *n*-hexane extract (50.0 g) was fractionated on silica gel column. The column was eluted by using *n*-hexane and EtOAc with increasing polarity. Fractions were collected and combined on the basis of TLC analysis. The TLC plate was visualized by spraying with 5% v/v H₂SO₄ in methanol and Folin-Ciocalteu reagent in methanol. Based on the results of the TLC analysis, the fractions that gave the same results were combined, so that two sub-fractions were obtained, namely SF1 and SF2. These two sub fractions were analyzed for the total phenolic content, total flavonoid content, antioxidant activity and also antidiabetic activity.

2.2.2 Total Phenolic Content (TPC)

TPC of the SF1 and SF2 fraction from *C.inophyllum* were identified using the Folin Ciocalteu method. Briefly, the sample solution

Figure 1. *C. inophyllum* tree (a), fruit (b) and fruit shell (c)

(2 µl of SF1 , SF2 extracts or standard solution of gallic acid at different concentrations (5, 10, 15, 20, 25, 30, 40, 50 µg/mL) were combined with 10 µl Folin Ciocalteu, followed by 158 µl of deionized water into a 96-well microplate. After 8 minutes was added 30 µl of 20% sodium carbonate (Na₂CO₃) (total volume 200 µL). Then, the mixture was incubated for 2 hours at room temperature. The formation of the blue color solution was measured at 765 nm against a blank consisting of deionized water and Folin Ciocalteu reagent using a Thermo Scientific Varioskan Spectrophotometer. Total phenolic contents were expressed as mg gallic acid equivalent (GAE) per gram of extract by computing with standard calibration curve. The TPC was performed in duplicate and the results were expressed as average [14,15]

2.2.3 Total Flavonoid Content

Determination of the total flavonoid content (TPC) in SF21, SF2 fraction from *C.inophyllum* was done by using aluminium chloride (AlCl₃) assay. Briefly, a volume of SF1 and SF2 extracts or standard quercetin each 20 µl ranging 5 to 50 µg/mL were mixed with 100 µl of deionized water and 6 µl NaNO₂ solution (5 %) were then vortexed for 10 s and left at room temperature for 5 min. 12 µl AlCl₃ (10 % w/v in water) was added followed by 40 µl NaOH (1 mM) and 22 µl of distilled water in a 96-well microplate (total volume 200 µL) After 15 min incubation at room temperature, the absorbance was measured at 510 nm using a microplate reader. Quercetin 5 to 50 µg/ml were used as standard. The total flavonoid content were expressed as mg quercetin equivqlent (QE) per gram extract [15,16]

2.2.4 Antioxidant activity

2,2-Diphenyl-2-picrylhydrazyl (DPPH) was used to evaluate radical scavenging activity according to the procedure described in previous areferences [17,18,19]. The samples were dissolved in methanol and 20 µL aliquot of different sample concentrations was mixed with 50 µl of DPPH solution (0.2 mM in methanol). Methanol was added up to 200 µl to give different concentrations of the samples in the 96-well microplate, incubated for 30 min.

The absorbance was measured at 517 nm by using a microplate reader (Thermo Scientific Varioskan®).

$$(\%) \text{ Inhibition} = \frac{(\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{blank}}} \times 100\%$$

2.2.5 Antidiabetes activity test.

The antidiabetes activity in SF 1 and SF 2 of fruit shell from *C. inophyllum* was measured as the inhibitory activity for α-glucosidase using the method reported by Da Silva Pinto et al. 2008 (15) with minor modification. A 0.25 mg α- glucocidase was dissolved in 10 ml buffer phosphate (pH 7) containing 20 mg albumin serum bovine. The enzyme was diluted 10 times before used for assay. The assay mixtures were as follow 250 µl p-Nitrophenyl-α-D-glucopyranoside (5 mM) was added to 495 µl 100 mM buffer phosphate (pH 7) and added 5 µl sample in DMSO (1%, 0.5% and 0.25%) and yeast α-glucosidase 250 µl (0.065 units/mL) was used as the enzyme. Incubated at 37°C for 5 minutes, then added 25 µl enzyme, incubated 15 minutes. Reaction was stopped by using 1 mL 200 mM Na₂CO₃. α-glucosidase inhibitory activity was conducted at sample concentrations of 0.25, 0.5 and 1%. The inhibitory effect on α-glucosidase activity was determined by measuring the amount of p-nitrophenol released at 400 nm using UV –Vis Spectrometer [20,21].

$$\text{Inhibition (\%)} = \frac{[(\text{Ac(o)}-\text{Ac (t)}) - (\text{As}-\text{Ab})]}{(\text{As}-\text{Ab})} \times 100\%$$

3. RESULT AND DISCUSSION

3.1 Extraction and Fractionation

Fractionation of CIFS n-hexane extract using silica column chromatography with hexane and ethyl acetate solvents with increasing polarity resulted in 50 fractions. After being analyzed by TLC, fraction 10-13 were combined as sub fraction 1 (SF1) and fraction 43-50 were combined as SF2 which further analysed.

3.2 Total Phenolic and Total Flavonoid Content

Fig 2. shows total phenolic content and total flavonoid content of in SF 1 and SF 2 of fruit shell from *C. inophyllum* n-hexane extract. Each value in the graph is represented as mean (n = 2). Total flavonoid contents in both sub fractions were higher than total phenolic contents. These results were similar with the reported results from the leaf extract of *C. inophyllum* [15] but none of those studies used fruit shell part and n-hexane as solvent .

Fig 2. Total phenolic and flavonoid content of SF1 and SF2

3.3 Antioxidant and Antidiabetes activities

The DPPH free radical scavenging activity The DPPH free radical scavenging activity measured the ability of the sample to donate hydrogen to the DPPH radical, resulting in bleaching of the stable DPPH free radical from the purple colour of DPPH cation to the yellow-colored diphenyl picryl hydrazine. The greater the bleaching action observed, the higher the antioxidant activity, which is reflected in a lower IC_{50} [17]. The *in vitro* antidiabetes activity were conducted by α -glucosidase inhibitory activity method. Figure 3 show the results scavenger free radical DPPH and as α -glucosidase inhibitor activities of SF 1 and SF 2 from *C.inophyllum* fruit shell n-hexane extract at concentration of 100 µg/ml. At this concentration SF1 did not show any antioxidant activity whereas SF2 showed low antioxidant activity. Both subfractions (SF1

and SF2) showed almost the same α -glucosidase inhibitory activity. Results reported here are relatively lower when compared to antioxidant and antidiabetes results reported in other *C. inophyllum* studies [8,19,20,21]. It is suspected that compounds containing hydroxyl groups such as calanone, coumarin and xanthenes compounds and unsaturated fatty acids such as palmitoleic acid have antidiabetic activity. These suspected compounds might be present in low quantities in SF 1 and SF 2 from *C. inophyllum* fruit shell n-hexane fractions. Whereas in those other *C. inophyllum* studies which used different plant parts and extracted with water (hydrodistillation) and methanol, the suspected compounds might be present in much higher quantities. None of those studies used fruit shell part and n-hexane as solvent, that might explain the difference in the antioxidant and andiabetes activities with our results.

Fig 3. Antioxidant and antidiabetes activities of SF1 and SF2 at 100µg/ml.

4. CONCLUSION

The phytochemical screening from n-hexane fraction of fruit shell *C. inophyllum* namely SF1 and SF2 showed that both contained higher total flavonoid contents compared to total phenolic content. Antioxidant test using DPPH method showed that at 100µg/ml SF1 did not have antioxidant activity and SF 2 have antioxidant activity with inhibition of 6.31 %. The. Antioxidant test using DPPH method showed that at 100µg/ml SF1 did not have antioxidant activity and SF

2 have antioxidant activity with inhibition of 6.31 %. The result of the antidiabetes showed that at 100 µg/ml SF1 and SF2 have 16.26% and 16.99 % inhibition α -glucosidase activity, respectively. Although relatively low, so far that we are aware this is the first report about antioxidant activity and α -glucosidase inhibition of n-hexane fraction of fruit shell of *C. inophyllum*. Therefore SF1 and SF2 might not be too suitable for the source to obtain antioxidant and antidiabetes compounds compared to other reported parts of *C. inophyllum*. Since fruit shells of *C. inophyllum* as waste materials are available in large quantities, studies to explore other bioactivities should be conducted for utilization as a source for medicine development.

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