Antidiabetic potential of *Caesalpinia sumatrana*, a medicinal herbs traditionally used by local tribe in East Kalimantan

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Abstract. The aims of the research was to analyze the content of phytochemicals, to examine the antioxidant and antidiabeticpotentials of *n*-hexane, chloroform, ethyl acetate, and ethanol extracts of *Caesalpinia sumatrana*. Method to measure antioxidant capacity of sample involves the use of the free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical. Analysis the potential of antidiabeticactivity of the extracts was determined by α-glucosidase and α-amylase inhibitory assay. Of all extracts obtained by successive maceration, ethanol maceration gave the highest extract by 2.63% of extract on the dry weigh basis. The result of phytochemicals showed that all extracts contain alkaloid and flavonoid. The highest antioxidant activity was 82.32% with IC_{50} value of 5.00 µg/ml obtained by ethanol extract. The results of enzyme inhibitory assay of α-glucosidase showed that ethanol extract of *C. sumatrana*had IC_{50} value 17.16 µg/mLto inhibit α-glucosidase activity and IC_{50} value 16.78 µg/mLfor α-amylase. The present result displayed potential of the plant to be developed as natural antidiabetic and antioxidant agents.

1. Introduction

Indonesia has approximately 30,000 variety of plants, and 7,000 of them are herbs. This needs to be proved, to be developed and to be used to increase the level of healthiness [1].*Tetracera* sp., *Tristaniopsis whiteana*, *Bauhinia purpurea*, *Syzygium* sp., *Parkia roxburghii*, *Cananga odorata*, *Alpinia galangal*, *Passiflora foetida*, *Cinnamomum burmanii*, and *Ligodium circinatum*are several types of plants used by some local tribes in Kalimantan as natural antidiabetic [2]. Bahau ethnic in Matalibaq village in East Kalimantan uses *Caesalpinia sumatrana*, as herbs for diabetic. These herbs need to be validated scientifically. Diabetes is one of the largest global health emergencies of the 21st century. In 2015 there are 153.2 million adults estimated to have diabetes in the Western Pacific and are expected to continue rising in 2040 to 214,8 million people with diabetes.Indonesia is in position 7 of 10 countries with the largest diabetics in the world with an estimated 10 million people with diabetes[3]. The aims of the research are to analyze the content of phytochemicals, to examine the activity of antioxidant, and to analyze antidiabetic potential of *n*-hexane, chloroform, ethyl acetate, and ethanol extracts.

2. Materials and Methods

2.1. Plant material

Fresh leaves of *C. Sumatrana* were collected from Matalibaq village, Mahakam Ulu district in East Kalimantanand were authenticated by laboratory of Forest Dendrology and Ecology, Faculty of Forestry, Mulawarman University. A voucher specimen is kept in our laboratory for future reference.

2.2. Preparation of Extracts

Plant material was extracted successively with *n*-hexane, chloroform, ethyl acetate, and ethanol in the increasing order of their polarity using rotary evaporator to obtain the extracts. The yield of the extract in each solvent was recorded.

2.3. Phytochemical analysis

The *n*-hexane, chloroform, ethyl acetate, and ethanol extracts of following plants was subjected tophytochemical tests for the detection presence of alkaloid, flavonoid, coumarin, carotenoid, tannin, saponin, steroid as secondary metabolites using standard procedures [4].

2.4. DPPH radical scavenging capacity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method was used to evaluate the antioxidant property. Ascorbic acid is used as a comparator of antioxidant activity. The antioxidant activity of each sample was expressed in terms of IC_{50} and was calculated from the graph after plotting inhibition percentage against extract concentration DPPH assay was carried out of the standard protocol [5]. 1000 µL of 0.1 mM DPPH solution was mixed with 1.5 mg of various concentrations (6.25 to 50 µg/mL) of leaf extract. The mixture was shaken vigorously and incubated at room temperature for 20 min in the dark. DPPH free radical scavenging was measured by a spectrophotometer at 517 nm . DPPH solution with no extract and ethanol is used as a control. The experiment was done in three repetitions. Percentage of DPPH free radical inhibition was calculated by the formula:

$$Inhibition\ (\%) = \frac{Acontrol\ -Asample}{Acontrol} \times 100 \quad (1)$$

Where $A_{control}$ is the absorbance of the control (Ascorbic acid) and A_{sample} is the absorbance of reaction mixture samples (in the presence of sample).

2.5. Inhibition assay for a-amylase activity

α-Amylase inhibitory activity was assayed according to standard protocol with a slight modification [6]. Amylase activity was determined using soluble starch (1%) as a substrate in 0.05 M sodium phosphate buffer (pH 6.8) containing 1 mM $CaCl_2$. Briefly, 100 µL of a-amylase solution (20 U/mL) was mixed with 250µL of sodium phosphate buffer (pH 6.8) and 50 µL of the extracts in 75% aqueous ethanol (6.25 to 50µg/mL). After incubation at 37^{o}C for 10 min, 75µL of the starch solution (1%) was added and the mixture re-incubated at 37^{o}C for 20 min. The reaction was terminated by adding 50 µL of dinitrosalicylic acid solution and boiled for 10 min in a boiling water bath. The total volume was made up to 2.0 mL of distilled water. The absorbance was measured at 540 nm against a control containing a buffer solution in place of the enzyme solution. Quercetin was used as a positive control. The assay was performed in triplicate. Inhibition of α-amylase was calculated by the formula:

$$Inhibition\ (\%) = \frac{Acontrol\ -Asample}{Acontrol} \times 100 \quad (2)$$

Where Acontrol is the absorbance of the control (quercetin) and A sample is the absorbance of reaction mixture samples (in the presence of sample).

2.6. Inhibition assay for a-glucosidase activity

α-Glucosidase inhibitory activity was assayed according to standard protocol with a slight modification [7]. α-Glucosidase (0.075 unit) was premixed with the extract at various concentrations (12.5 to 100 µg/mL), and 3 mM p-nitrophenyl α-D-glucopyranoside as a substrate in phosphate buffer was added to the mixture to start theiraction. The reaction was incubated at 37°C for 30 min and stopped by adding 2 ml of 0.1 M Na_2CO_3. α-Glucosidase activity was determined by measuring release of *p*-nitrophenol from p-nitrophenyl α-D-glucopyranoside at 400 nm. Quercetin was used as a positive control. The assay was performed in triplicate. Inhibition of α-α-glucosidase was calculated by the formula:

$$Inhibition\ (\%) = \frac{Acontrol\ -Asample}{Acontrol} \times 100 \quad (3)$$

Where Acontrol is the absorbance of the control (quercetin) and Asample is the absorbance of reaction mixture samples (in the presence of sample).

3. Result

The results for phytochemical screening of the plant extracts are presented in Table 1. Phytochemical screening revealed that the all extracts contained alkaloid and flavonoid, while coumarin was only absence in *n*-hexane extracts, carotenoid were presence in *n*-hexane and ethyl acetate extracts. Tannin, saponin, and steroid were absence in all extracts. Flavonoids have been reported to possess anticancer, antiviral, antioxidant, antibacterial, and anti-inflammatory agents. Recent studies have determined that flavonoid compounds can be very effective in inhibiting α-glucosidase activity [8].

Table 1. Phytochemical analysis of*Caesalpinia sumatrana*extracts

Phytochemicals	Plant extracts			
	n-Hexane	Chloroform	Ethyl acetate	Ethanol
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Coumarin	-	+	+	+
Carotenoid	+	-	+	-
Tannin	-	-	-	-
Saponin	-	-	-	-
Steroid	-	-	-	-

+ sign indicates presence and - sign indicates absence.

The results of antioxidant activity showed the radical-scavenging activity of *C. sumatrana* extracts was estimated by comparing the percentage inhibition of formation of DPPH radicals with that of ascorbic acid. Ethyl and ethanol extracts showed moderate antioxidant activity when compared with ascorbic acid. The results of antioxidant activity are presented in Figure 1 and Table 2.

Table 2. Antioxidant analysis of*Caesalpinia sumatrana*extracts

Samples	Extracts	IC_{50} (µg/mL)
Caesalpinia sumatrana	*n*-Hexane	75.28
	Chloroform	66.79
	Ethyl Acetate	12.08
	Ethanol	75.28
Ascorbic acid*		3.12

*Ascorbic acid was used as a positive control

The DPPH radical scavenging activity of *C. sumatrana* extracts increased with increasing the concentration. The ethyl and ethanol extracts appeared to be more active than other extracts. The activities of these plant extracts were 82−89% at 50 µg/mL, which was comparable to that of the positive control ascorbic acid (95% activity). The IC_{50} value obtained was as 5.00 and 3.12 µg/mL for ethanol and ascorbic acid extract respectively. It means ethanol extract of plant at higher concentration captured more free radicals formed by DPPH resulting into decrease in absorbance and increase in IC_{50} value.

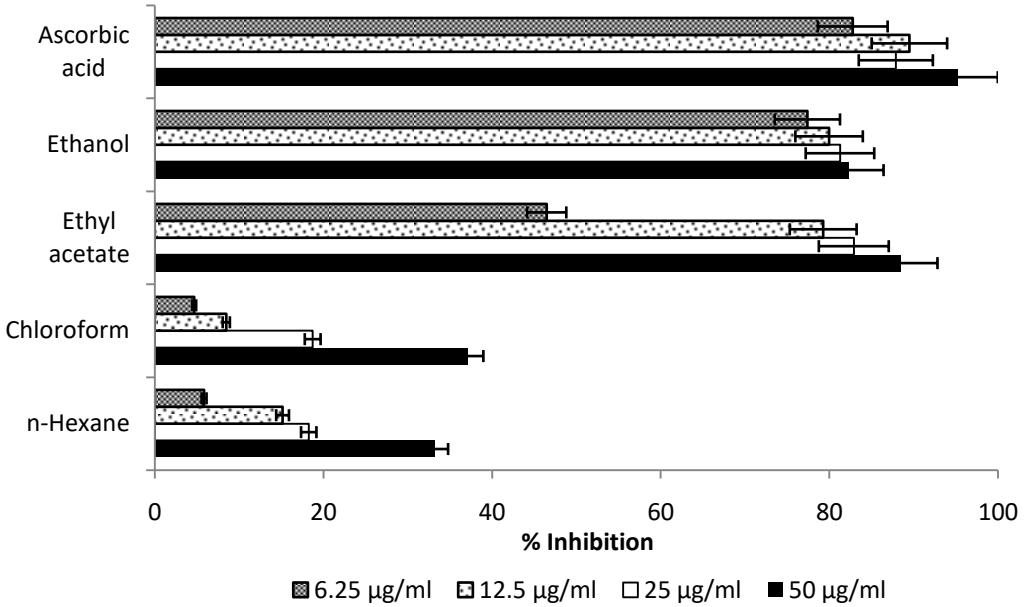

Figure 1. DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity of plant extracts

The inhibitory activity of the *n*-hexane, chloroform, ethyl acetate, and ethanol extract of *C. sumatrana* against yeast α-glucosidase is shown in table 3. The ethanol extracts inhibited yeast α-glucosidase activity by 95.58, 88.76, 75.10 and 64.42% at concentrations of 100, 50, 25 and 12.5 µg/mL, respectively. Quercetin, an α-glucosidase inhibitor used as positive control, inhibited enzyme activity by 98.85, 86.26, 82.06 and 62.90% at concentrations of 100, 50, 25 and 12.5 µg/L respectively. The ethanol extract had an IC_{50} value of 17.16 µg/mL.

Table 3. Inhibitory activity of the extract against α-glucosidase

Samples	Extracts	IC_{50} (µg/mL)
Caesalpinia sumatrana	*n*-Hexane	45.88
	Chloroform	44.36
	Ethyl Acetate	31.74
	Ethanol	17.16
Quercetin*		2.00

* Quercetin was used as a positive control

For α-amylase inhibition, ethanol extracts inhibited α-amylase activity by 95.58, 88.76, 75.10 and 64.42 % at concentrations of 50, 25, 12.5 and 6.25 µg/mL, respectively. Ethanol extracts had the highest inhibition activity with IC_{50} values 16.78µg/mL moderate than quercetin was used as a positive control 4.04 µg/ml. Some flavonoids, such as rutin, myricetin, kaempferol, and quercetin, have been previously reported to inhibit α-glucosidase and α-amylase. These flavonoids exhibit both hypoglycemic and antioxidant effects in diabetic animals [9]. The results of our experiments seemed to agree with this finding, probably due to the presence of flavonoids in phytochemicals analysis.

Table 4. Inhibitory activity of the extract against α-amylase

Samples	Extracts	IC_{50} (µg/mL)
Caesalpinia sumatrana	*n*-Hexane	18.22
	Chloroform	28.50
	Ethyl Acetate	27.31
	Ethanol	16.78
Quercetin*		4.04

* Quercetin was used as a positive control

4. Conclusions

In the light of the results, phytochemicals revealed that alkaloid and flavonoid were present in the extracts and our study indicates that ethanol extracts of*C. sumatrana* leaves have highest α-glucosidase and α-amylase inhibitory and scavenging activities against DPPH. The present result displayed potential of the plant to be developed as natural antidiabetic and antioxidant agents.

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