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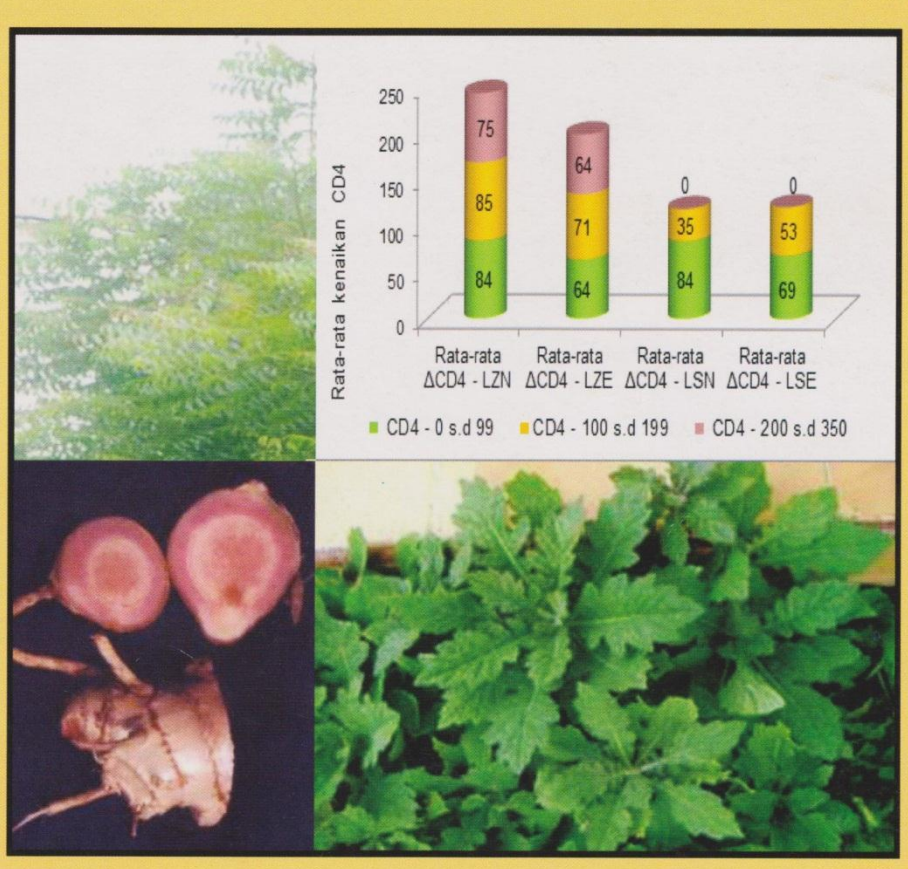

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Free Radical Scavenging and α-Glucosidase Inhibitor Activity of Ethanolic Extract of *Mucuna pruriens* L.

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ABSTRAK

Biji kacang "koro benguk" atau *velvet bean* sudah sangat dikenal tetapi dasar ilmiah penggunaannya sebagai obat tradisional terutama sebagai antioksidan dan antidiabetik masih belum diketahui. Oleh sebab itu dalam penelitian ini dilakukan percobaan untuk mengevaluasi aktivitas antioksidan dan antidiabetik dari ekstrak biji koro benguk, serta fraksi heksana, etil asetat, butanol dan air menggunakan uji *in vitro* menggunakan DPPH (1,1-difenil-2-pikril-hidrazil) dengan pembanding positif kuersetin dan BHA. Hasil penelitian menunjukkan bahwa IC_{50} DPPH untuk ekstrak biji koro benguk, fraksi heksana, etil asetat, butanol dan air berturut-turut sebesar 22,17; 152,60; 113,03; 4,93; dan 9,14 µg/mL, sedangkan untuk kuersetin dan BHA masing masing sebesar 4,28 dan 22,587 µg/mL. Aktivitas antidiabetik ditentukan menggunakan metode penghambatan α-glukosidase dengan glucobay sebagai kontrol positif. Hasil penelitian menunjukkan bahwa IC_{50} antidiabetik untuk ekstrak biji koro benguk, fraksi heksana, etil asetat, butanol, air, dan glucobay berturut-turut sebesar 24,44; 19,55; 8,40; 80,98; 48.97; dan 16,23 µg/mL.

Keywords: koro benguk, antioksidan, antidiabetik, radikal bebas, glucobay

ABSTRACT

Velvet bean seeds are ubiquitous but the scientific basis for its medicinal use especially as antioxidant and antidiabetic remains unknown. Therefore, a study was designed to evaluate antioxidant and antidiabetic activities of the extract, hexane, ethyl acetate, butanol and water fractions of velvet bean seeds. In order to evaluate antioxidant activity of extract and fractions using quercetin, butylated hydroxyanisole (BHA) as positive control and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity was determined. The results showed that IC_{50} of DPPH were 22.17; 152.60; 113.03; 4.93; 9.14 µg/mL respectevily; quercetin 4.28 and BHA 22.587 µg/mL. Antidiabetic activity was determined by using of α-glucosidase inhibition assay of extract, four fractions and glucobay as positive control. The results showed that antidiabetic IC_{50} were24.44; 19.55; 8.40; 80.98; 48.97µg/mL respectively and glucobay as positive control 16.23 µg/mL.

Keywords : velvet bean seed, antioxidant, antidiabetic, free radical, glucobay

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemic, glucoseurea and negative nitrogen balance and it is mainly due to lack of insulin secretion in beta cells of pancreas and desensitization of insulin receptors for insulin. It is the most prevalent disease in the world affecting 25% of population. The disease is found in all parts of the world and is rapidly increasing worldwide. People suffering from diabetes cannot produce or properly use insulin, so they have high blood glucose. Most prevalent form of diabetes is non-insulin dependent diabetes mellitus (NIDDM/type 2). Type 2 diabetes, non–insulin-dependent diabetes mellitus, in which the body does not produce enough insulin or properly use it, is the most common form of the disease, accounting for 90%–95% of cases. According WHO projections, the diabetic population is likely to increase to 300 million or more by the year 2025, to reach 366 million by the year 2030 (1,2). Oxidative stress occurs at an early stage in diabetes, preceding the appearance of complications, oxidative stress plays an important role in diabetic complications (3). Another risk for diabetes are the reduced plasma antioxidant level (4), because antioxidants present in plants and herbs could prevent the development of the disease (5). Type 2 diabetes is complicated by several factors inherent to the disease process, typically, insulin resistance, hyperinsulinemia, impaired insulin secretion, reduced insulin-mediated glucose uptake and utilization (3). That has been observed that in diabetes, as a result of the increase in oxidative stress, the production of free radicals increases, but the production of antioxidants decreases. Thus, increased free radical concentration is considered as one of the important complications of diabetes (6). High concentration of blood glucose as well as high glucose fluctuation during postprandial period correlates with the increase in reactive oxygen species (ROS) or oxidative stress. ROS mediates the activation of the imbalance in vasoregulating factors (vasodilators and vasoconstrictors) then affects endothelial homeostasis and triggers atherogenic changes, including increases in low-density lipoprotein oxidation, sympathetic tone, vasoconstriction, and thrombogenicity (7, 8).

The primary therapy for type-2 diabetes used oral hypoglycemic agents currently available also have some side effects, such as hypoglycaemia, lactic acidosis, weight gain and hepatotoxicity (9). Therefore, there still remains a great need for more effective oral anti-diabetic agents. The major advantages of herbal medicine seem to be their efficacy, low incidence of side effects, and low cost (10). α-glucosidase is intestinal enzyme which catalyzes the degradation of diet polysaccharides to absorbable monosaccharide. Natural or synthetic glucosidase inhibitors are of therapeutic interest to delay postprandial hyperglycemia in type 2 diabetes (8, 11).

This present study was motivated by considerations. First, diabetes complications have been linked to oxidant stress, in particular the formation of superoxide (12). Diabetics could benefit from extra antioxidant protection, as diabetes increases free radical production, and may result in damage to the body including increasing risk of heart attack, nerve damage (especially to the eyes—diabetic retinopathies), cataracts and blindness (13).

The plant *Mucuna pruriens* L.(widely known as velvet beans) is widespread

throughout the tropic countries. The empirical data velvet bean seeds, are used for the management of several free radical-mediated diseases, such as rheumatoid arthritis, diabetes, atherosclerosis, male infertility and nervous disorders (14). In this study, we have examined the antioxidant and antidiabetic activities of ethanolic extract and fractions of velvet bean seeds (*Mucuna pruriens* L.).

MATERIALS AND METHODS

Preparation of extract

Velvet bean seeds (*M. pruriens* L.) was collected from Sukoharjo district, center Java (May, 2007). The velvet bean seeds were kept under dry tunnel (40-45^{0}C) and chopped finely using a proccesor.

Five kilogram of velvet bean seeds were extracted with distilled ethanol by maserasi extraction, filtered and evaporated using rotatory evaporator, yielded ethanolic extract of *M. pruriens* 517.20 g (10.34 %). The ethanolic extract were stored at 4°C. The ethanolic extract (150.356 g) was partitioned between hexane and water (7:3). The aqueous layer was fractioned respectively with ethyl acetate (1:1) and butanol (1:1). The hexane, ethyl acetate, butanol, water fraction were collected and concentrated with vacuum rotary evaporator at 40°C giving the yields 35.334 g (23.50%), 0.586 g (0.39%) , 4.189 g (2.79%), and 4.811 g (3.20%) respectively.

DPPH Free Radical Scavenging Activity Assay

The free radical scavenging activity of the ethanolic extract and its fractions of velvet bean seeds (hexane, ethyl acetate, butanol, and water) were evaluated with DPPH (1,1-diphenyl-2-picryl-hydrazyl). The DPPH assay was carried out as described by Unlu *et al* (2003) and Frum and Viljoen (2006). Pipette 50 µL of sample (extract, fractions, quercetin, BHA) of various concentrations of the samples enter at the microtitre plate and then were added 200 µL of 0.077 mmol/L methanol solution of DPPH and the reaction mixture was shaken vigorously and kept in the dark for 30 min at room temperature, the at room temperature of DPPH was determined by microplate reader at 517 nm. The radical scavenging activity of each sample was expressed by the ratio the of lowering of the absorption of DPPH (%), relative to the absorption (100%) of DPPH solution in the absence of test sample (negative control).

$$\text{scavenging \%} = \frac{A_c - A_s}{A_c} \times 100$$

where A_s and A_c are absorbance at 517 nm of the reaction mixture with samples and without sample respectively.

α-glucosidase Inhibitor Assay

The α-glucosidase inhibitory activities were assayed by the method of (17,18) by partial modification. 500 µL of *Saccharomyces* sp yeast α-glucosidase (contained 200 mM p-nitrophenyl-a-glucopyranoside), 990 µL 100 mM PBS pH 7.0 and 10 µL of the inhibitor (extract, fractions, glucobay : 125; 250; 500 µg/mL) were pre-incubated at 37 C for 5 min. After the reaction had been stopped by adding 200 µL of a 200 mM Na_2CO_3, the absorbance at 400 nm was measured with spectrophotometer. Controls without inhibitors were checked also, as a reference. The α-glucosidase inhibitory activity can be calculated as follows:

$$\text{Inhibitory ratio \%} = \frac{Ac - As}{Ac} \times 100\%$$

where Ac, As represent the absorbance at 400 nm of the control, sample respectively.

RESULTS AND DISCUSSION

The comparison of IC_{50} of DPPH scavenging activity among the ethanol extract, hexane fraction, ethyl acetate, butanol, water fraction, quercetin and BHA as standard is described in Table 1, and the complete data or histogram of DPPH scavenging activity can be seen in Figure 1. It needed 4.28 µg/mL quercetin to inhibit 50% DPPH, while it would need 4.93 µg/mL of butanol fraction and 9.14 µg/mL of water fraction and 22.17 µg/mL of ethanolic extract to scavenge 50% DPPH.

Table 1. IC_{50} of DPPH scavenging activity of velvet bean extract and fractions.

Sample	IC_{50} (µg/mL)
Ethanol extract	22.17
Hexane fraction	152.60
Ethyl acetate fraction	113.03
Butanol fraction	4.93
Water fraction	9.14
Quercetin	4.28
BHA	22.587

The comparison of IC_{50} of α-glucosidase inhibition or antidiabetic activity among the ethanol extract, hexane fraction, ethyl acetate fraction, butanol fraction, water fraction, and glucobay as a standard is described in table 2. The complete data or histogram of α-glucosidase inhibition of extract and fractions of velvet bean seeds can be seen in Figure 2. The highest antidiabetic activity among ethanolic extract, fractions and glucobay was hexane fraction, it needed 8.40 µg/ml hexane fraction to inhibit 50% α-glucosidase more active than glucobay. Ethanolic extract, ethyl acetate fraction were more active than glucobay.

The DPPH scavenging activity test if antioxidant or sample which contain antioxidant will be occurred hydrogen (H) captured by DPPH free radical or antioxidant donate hydrogen (H) was indicated purple color to become 1,1-diphenyl-2-picrylhydrazyn yellow color (19). When DPPH$^{\cdot}$ reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep—violet to light—yellow) were measured at 517nm (20). The DPPH assay, showed that sample had highest antioxidant activity will occures the fastest colour changing compared to the others sample or progressive decrease in absorbance. The sample had lowest antioxidant activity may not be reached for several hours, even the sample is still purple (20).

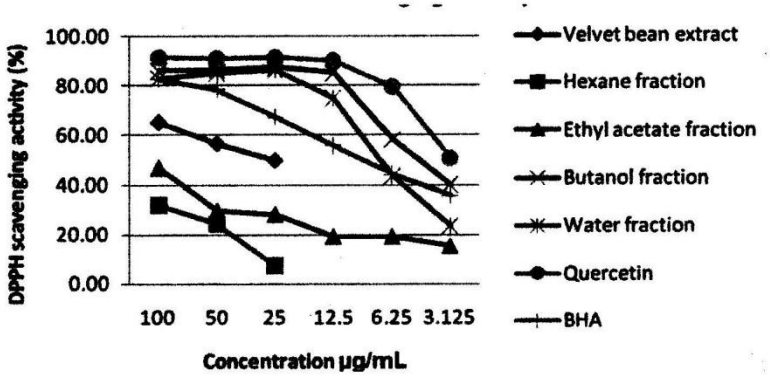

Figure 1. DPPH scavenging activity of extract and fractions of velvet bean seeds

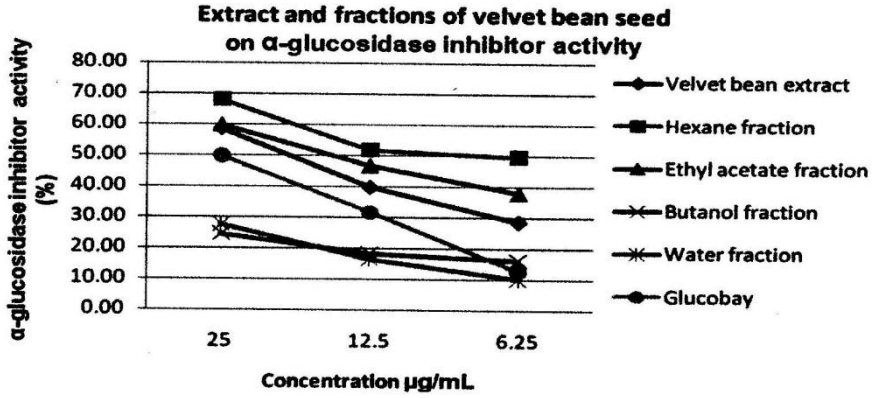

Figure 2. α-glucosidase activity of extract and fractions of velvet bean seeds

Table 2. IC_{50} of α-glucosidase inhibitor activity of velvet bean extracts

Sample	IC_{50} (µg/ml)
Ethanolic extract	19.55
Hexane fraction	8.40
Ethyl acetate fraction	16.23
Butanol fraction	80.98
Water fraction	48.97
Glucobay	24.44

Butanol fraction of velvet bean seeds showed the highest antioxidant activity compared to among ethanolic extract, fractions and BHA and was comparable with quercetin. Butanol fraction of *M. pruriens* L. had high antioxidant activity, its validated with previous research using phytochemical assay by (21) butanol fraction of velvet bean seeds contain very high flavonoids (+++), high tannins (+++) and moderate phenols (++). The most widely recognized properties of polyphenols are their antioxidant activities, arising from their ability to scavenge reactive oxygen species (22). The antioxidant activity of phenolics is mainly because of their redox properties which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (23, 24). Flavonoids, a group of compounds in plant foods with antioxidant effects (25). Flavonoids are phenolic substances isolated from a wide range of vascular plants, with over 8,000 individual compounds known, they act in plants as antioxidants (26). Flavonoids are most commonly known for their antioxidant activity. These effects are due to the physiological activity of flavonoids in the reduction of oxidative stress (27). IC_{50} value of DPPH scavenging activity of ethanolic extract was 22.17 µg/mL was similar with BHA. This result was validated with previous research by R(14) that methanolic extract of velvet bean seeds have high antioxidant activity including DPPH scavenging activity, nitric oxide (NO) and super oxide (SO) anions and hydrogen peroxide (H_2O_2) radical inhibition.

Hexane fraction of velvet bean seeds showed the highest antidiabetic activity compared to among ethanolic extract, fractions and glucobay. Glucobay is the α-glucosidase inhibitor acarbose is now marketed worldwide and is used in the therapy of diabetes type II (non-insulin-dependent), in order to enable patients to better control blood sugar contents while living with starch-containing diets (28). The α-

glucosidase enzyme (AGIs) is located in the brush border of the small intestine and is required for the breakdown of carbohydrates to absorbable monosaccharides. The AGIs delay, but do not prevent, the absorption of ingested carbohydrates, reducing the postprandial glucose and insulin peaks. Table 2. showed that glucobay had IC_{50} α-glucosidase 24.44 µg/mL, its was lower than hexane fraction (8.40 µg/mL). Hexane fraction contain low terpenoids (+) (21), ethanolic extract and the other fractions didn't contain terpenoid. Terpenoid-type quinones are obtained from the leaves and stems of *Pycnanthus angolensis* as agents for the treatment of diabetes, the novel terpenoid quinones are useful for treating insulin-dependent (type I) and/or non-insulin-dependent (type II) diabetes (29). Butanol, ethyl acetate water fractions which contain high flavonoids (+++) and moderate phenols (++) (21), base on the data (Table 2.) butanol and water fractions had very low α-glucosidase inhibition, meanwhile ethyl acetate fraction had α-glucosidase inhibition, was more active than glucobay. This results (butanol and water fractions) were not validated but ethyl acetate fraction was validated with previous research that flavonoids and polyphenols, as well as their sugar derivates, are found to be effective inhibitors of α-glucosidase (2). According to the glucosidase inhibitory activity of *Ezoishige*, the active compound of *E. stolonifera* was expected to be polyphenols in *E. stolonifera* are phlorotannins (30). The polymeric polyphenols were observed to contribute to this strong α-glucosidase inhibition (31). Antidiabetic activity of ethyl acetate fraction was consistent with the empirical data that velvet bean seeds had hypoglycemic activity (14).

α-glucosidase inhibitory activity of hexane fraction of velvet beans (IC_{50} = 8.40 µg/mL) was lowest compared with plants extract of *Terminalia* species with IC_{50} 0.27 – 7.02 µg/mL (Anam *et al.*, 2009). The previous research of α-glucosidase inhibitory activity showed that extracts of *Viscum album*, *Urtica dioica*, *Myrutus communis*, *Taraxacum officinale* with baker's yeast as α-glucosidase respectively IC_{50} were 11.7; 3.7; 0.038; 2.3; mg/mL and glucobay drug had IC_{50} 0.5 mg/mL (Onal *et al.*, 2005). This results showed that IC_{50} of α-glucosidase inhibition of glucobay was more active than previous research with our result (24.44 µg/mL).

CONCLUSION

Butanol and water fractions of velvet bean seeds (*M. pruriens* L) had a potential antioxidant activity. Hexane and ethyl acetate fractions had a potential α-glucosidase inhibitor activity.

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