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Research Article

EFFECT OF *STERCULIA QUADRIFIDA* R. BR BARK TO *IN VITRO* DPPH RADICAL AND GLUTATHIONE PEROXIDASE ON DIABETIC WISTAR RATS

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ABSTRACT

Objective: The aims of the research were to evaluate the antioxidant effect of *Sterculia quadrifida* R.Br bark by *in vitro* 1,1-biphenyl-2-picryl-hydrazyl (DPPH) radical scavenging method and glutathione peroxidase (GPx) activity on diabetic Wistar rats model.

Methods: *S. quadrifida* bark powder was extracted in ethanol 70% by maceration method. The antioxidant activity of ethanol extract was tested by DPPH radical scavenging method. The activity of GPx was evaluated from the liver of alloxan-induced diabetic rats. The antioxidant activity was determined by measuring the absorbance using ultraviolet-visible spectrophotometry method.

Results: The ethanol extract of *S. quadrifida* bark had a strong DPPH radical scavenging activity, with the IC_{50} value was 4.86±0.01 ug/ml, besides that the extract also showed significantly activity of GPx ($p<0.05$) at a dose of 65, 130, and 260 mg/kg bw.

Conclusion: The ethanol extract of *S. quadrifida* bark had a potent antioxidant activity by DPPH radical scavenging and GPx activity.

Keywords: *Sterculia quadrifida* R.Br, 1,1-biphenyl-2-picryl-hydrazyl, Glutathione peroxidase, Antioxidant.

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INTRODUCTION

The reactive oxygen species (ROS) are produced as by products during the metabolic processes. Normally, these ROS are produced in small amounts in the body for various physiological functions, but if they produced in excessive amounts, they could cause oxidative stress [1]. Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases. These also effect various enzyme systems and cause damage which may further contribute to conditions such as cancer, ischemia, atherosclerosis, aging, rheumatoid arthritis, and neurological disorders [2].

Antioxidants can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. The main characteristic of an antioxidant is its ability to neutralize free radical [3]. The antioxidant components of natural origin have attracted special interest because they can protect human body from free radicals. Antioxidants such as polyphenols, ascorbic acid, Vitamin A, alpha-lipoic acid, thioredoxin, glutathione, melatonin, coenzyme Q, beta-carotenoids, alpha-tocopherols, as well as antioxidant enzymes [4]. Enzymatic antioxidants include primary enzymes such as superoxide dismutase, catalase, glutathione peroxidase (GPx), and secondary enzymes include glutathione reductase [5]. These enzymes have been widely investigated for the prevention and treatment of diseases resulting from oxidative damage.

Sterculia quadrifida R. BR. which is known as faloak, belonging to the family Sterculiaceae, widely distributed in Indonesia, especially in Timor, Sumba, Alor, and Flores Island [6]. Conventionally, in Indonesia, especially East Nusa Tenggara, the bark of this plant is used to treat liver diseases, gastroenteritis and as stamina booster [7], rheumatism, and malaria [8]. The ethanolic extract of *S. quadrifida* bark had been reported to have cytotoxicity activity on hepatocyte cell line Huh7 and inhibition activity to HCV JFH1 replication [9], antidiabetic [10], and immunomodulatory [11]. This study aimed to study antioxidant activity of *S. quadrifida* bark by *in vitro* DPPD radical scavenging method and glutathione reductase activity in diabetic Wistar rat model.

METHODS

Chemicals

Alloxan, rutin, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and phosphate buffer were purchased from Sigma-Aldrich. GPx assay kit was purchased from BioVision. Glibenclamide was obtained from PT Indofarma. Other chemicals and reagents were of analytical grade which was used in the present evaluation.

Animal

Male Wistar rats weighed 180–200 g (age 2–3 months) were obtained from the animal laboratory of Gadjah Mada University, Yogyakarta. The rats were adapted to their environment at constant temperature of 25°C for a week before being used. They were given free access to feed standard pellets and water during the study. All of procedures were approved by Health Research Ethics Committee, Sebelas Maret University, with a certificate no 100/II/HREC/2017.

Collection and authentication of plant material

The bark of *S quadrifida* was collected from Kupang, East Nusa Tenggara, Indonesia, in Januari 2017 and authenticated from the Biology Lab, Faculty of Math and Science, Sebelas Maret University, Surakarta. Four kilograms bark of *S. quadrifida* was taken and washed properly, and then the materials were sorted while wet, dried, and grinded into powder.

Extraction of plant material and phytochemical screening

The 500 g dried bark powder of *S. quadrifida* was extracted by maceration using 5 l ethanol 70% for 5 days at room temperature and protected from sunlight. After 5 days, the mixture was filtered, and the residue was washed out with ethanol and treated for 5 days as the same as treatment before. The extract was evaporated by rotary evaporator yielding 67.68 g of *S. quadrifida* ethanol extract. Preliminary phytochemical screening of the plant extract was performed for determining the presence of alkaloids, tannins, flavonoids, saponins, and steroid/triterpenoid.

DPPH radical scavenging activity

The antioxidant activity of extracts was determined by DPPH assay using Blois's method [12] with minor modifications. Extract of *S. quadrifida* was prepared in various concentrations. 2 ml of extract were added to 2 ml DPPH 80 µg/ml to initiate the reaction for obtaining a calibration curve. The mixture of 2 ml DPPH solution and 2 ml methanol was used as the control solution. The methanol was used as the blank solution and rutin as reference standard. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min then; the absorbance was measured at 517 nm by ultraviolet-visible spectrophotometer. The analysis was conducted in triplicate for standard and extract. The absorbance value was assessed as the inhibitory percentage (%). The antioxidant activity was revealed as IC_{50} of DPPH scavenging activity by determining the 50% inhibitory concentration for extract using the calibration curve. The percent of DPPH scavenging activity was calculated by following equation:

Percent inhibition = $[A_0\text{-}A_1/A_0]\times100$.

Where A_0 was the absorbance of control reaction and A_1 was the absorbance in presence of test or standard sample.

Determination of GPx activity

The experiment was performed for 14 days using Wistar rats. All of rats were divided into six groups comprising six rats in each group and fed the normal diet throughout the experimental period. Group I was the normal control group and received vehicle (0.5% carboxymethyl cellulose [CMC]-Na) orally. Groups II-VI were induced with alloxan monohydrate intraperitoneally to obtain diabetic with stress oxidative condition. Group II as diabetic control was orally administered vehicle. Glibenclamide was orally administered at a dose of 0.45 mg/kg bw to Group III as drug control group. Groups IV, V, and VI were orally treated with *S. quadrifida* extract in CMC-Na suspension at dose of 65, 130, and 260 mg/kg bw, respectively. Before inducing by alloxan monohydrate, the blood sample was collected for glucose level assay on 0^{th} day (T_0). Briefly, the rats were injected intraperitoneally with 180 mg/kg bw alloxan monohydrate (suspended in NaCl 0.9%) to increase the glucose level, except for normal control group. After 5^{th} days of alloxan induction, the blood glucose level was measured. The rats with glucose level above 200 mg/dl were assigned for 14 days treatment. At the end of the experimental period (15^{th} days), the rats were starved overnight and then sacrificed, the liver was used for the measurement of GPx activity. The GPx activity assay of the extract was performed by the method of Lawrence and Burk [13] with minor modification. Briefly, 200 µl of liver supernatants were added to the mixture consisting of 200 µl phosphate buffers 0.1 mM pH 7.4 (contains EDTA 0.1 M), 200 µl glutathione (GSH) 10 mM, 200 µl glutathione reductase 2.4 U/ml, and 200 µl NADPH 1.5 mM. The mixture was incubated at 37°C 10 min and then 200 µl H_2O_2 1.5 mM was added to above mixture. The change in absorbance of the sample was measured at λ 340 nm using spectrophotometer for 3 min. The GPx enzymatic activity was calculated as µmol NADPH that oxidized to $NADP^+$ $minute^{-1}$ mg^{-1} protein with extrinsic coefficient NADPH 6.22 mM^{-1} cm^{-1}, the results were expressed as units of GPx activity/mg cell protein and were calculated using equation:

$$\text{M unit GPx} = \frac{\text{Abs}\times\text{Vt}\times2\times1000\times1/\text{mg protein}}{6.22\times\text{Vs}}$$

Where Abs was the change of the absorbance; Vt was the total volume (ml); Vs was the sample volume (ml); 2 was the equivalent of 2 mol GSH with 1 mol NADPH; 6.22 was the NADPH molar extinction coefficient (in $Mm.cm^{-1}$); and 1000 was the conversion of ml unit.

Data analysis

The results were represented as the mean±standard deviation. The statistical significance of difference to DPPH radical scavenging activity was calculated by t-test, and for GPx activity was calculated by Mann–Whitney test. Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Identification of the compound of *S. quadrifida* bark extract

Identification of the compounds using chemical reaction method showed that the ethanol extract of *S. quadrifida* bark contains flavonoid, triterpenoid, saponin, tannin, and alkaloid.

***In vitro* DPPH radical scavenging activity**

The relatively stable DPPH radical had been used widely to test the ability of compounds to act as free-radical scavengers or hydrogen donors. This capability was used to evaluate antioxidant activity. Compounds with radical scavenger capacity are able to reduce DPPH radical using donor hydrogen atom to DPPH free-radical based on the type and concentration of sample. Interaction of antioxidant compound with DPPH is based on transfer electron or hydrogen atom to DPPH radical and converts it to 1 DPPH. The result of reduction DPPH radicals causes discoloration from purple color to yellow pale color which indicates the scavenging activity. The decrease of absorbance of DPPH radicals was measured by spectrophotometer. The absorbance was measured at 517 nm for each concentration of extract and standard. The percentage inhibition of all concentration series of extract and rutin was calculated. The value of IC_{50} was calculated using regression linear equation. IC_{50} is the ability of the compounds to inhibit 50% DPPH activity.

The IC_{50} value of ethanol extract of faloak bark and rutin is 4.86 µg/ml and 4.23 µg/ml, respectively. The previous research by Saragih and Siswadi [14] exposed that *S. quadrifida* bark extracts had DPPH radical scavenging activity by IC_{50} of 2.51+0.03 µg/ml. These result indicated that the extracts and standard rutin had very strong antioxidant activity. The sample had IC_{50} <50 ppm, it was very strong antioxidant, 50–100 ppm strong antioxidant, and 101–150 ppm medium antioxidant, while weak antioxidant with IC_{50} >150 ppm [15].

A potential source of antioxidant agents may be derived from the natural products. Flavonoids are groups of plant polyphenols, which display antioxidant activity and varied pharmacological activities [16]. The study of ten flavonoids catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, myricetin, quercetin, apigenin, kaempferol, and luteolin showed that these compounds had the radical scavenging capacity toward DPPH radical [17].

The result of statistical analysis using independent samples t-test to compare the average of IC_{50} rutin and extract showed that the values were significantly different ($p<0.05$). The result of antioxidant activity of *S. quadrifida* bark extract and standard rutin is shown in Table 1.

***In vivo* antioxidant of GPx activity**

GPx is an important intracellular enzyme that breakdown hydrogen peroxides (H_2O_2) to water and lipid peroxides to their corresponding alcohols, mainly in the mitochondria and sometimes in the cytosol. GPx plays a key role in protecting the cell from free-radical-induced damage, particularly lipid peroxidation. The enzyme also catalyzes the reduction of hydrogen peroxide and organic peroxides (ROOH) to water and corresponding stable alcohols (ROH), respectively, using

Table 1: The IC_{50} value of ethanolic extract of *Sterculia quadrifida* bark and rutin

Solutions	Replication	IC_{50} (ug/ml)	Mean IC_{50}±SD
Sterculia quadrifida bark extract	1	4.86	4.86±0.01
	2	4.86	
	3	4.87	
Standard rutin	1	4.23	4.23±0.01
	2	4.24	
	3	4.23	

reduced glutathione as a source of reducing equivalents. When oxidized glutathione is produced upon reduction of organic peroxide by GPx, it was recycled to its reduced state by glutathione reductase with oxidation of NADPH to NADP+. The process of NADPH oxidation was accompanied by a decrease in absorbance at 340 nm for monitoring the activity of GPx.

The results of GPx activity assay from extract of *S. quadrifida* bark and standard rutin are shown in Table 2. The extracts treatment groups at a dose of 65, 130, and 260 mg/kg bw were significantly ($p<0.05$) increased the GPx activity in liver tissue (36.7±0.66; 50.87±1.90; and 56.10±0.94 U/mg tissue, respectively) compared to the diabetic control group (25.87±1.07 U/mg tissue). Activity of faloak extract at dose of 300 mg was higher than two other doses. Furthermore, the GPx activity of treatment groups rat, extract, and standard glibenclamide, was significantly different compared to normal control group ($p<0.05$), which was only received vehicle (CMC-Na). These data indicated that all of the extracts had potential activity. However, GPx increasing activity of *S. quadrifida* extract and standard glibenclamide has not reached the normal level.

The diabetic control group showed the lowest GPx activity value (25.87±1.07 U/mg) and significantly different compared to the normal group ($p<0.05$). The diabetic control group was only given vehicle (0.5% Na CMC) for 14 days after have hyperglycemia conditions due to alloxan induction. Normal control and glibenclamide showed GPx activity value 77.79±3.28 U/mg and 62.83±1.44 U/mg, respectively. These values were close to the value of GPx activity that has been reported [18].

Alloxan as a reactive compound, quickly to the pancreas and accelerate the formation of ROS that can cause molecular modification. This is the beginning of oxidative damage due to an imbalance between protective antioxidants and increased free-radical production. The production of free radical cannot be totally neutralized by endogenous antioxidant (GPx). The administration of *S. quadrifida* extract to the diabetic rats increased the activities of these enzymes almost achieved the normal level, especially at dose 260 mg/kg bw.

Phytochemical screening of *S. quadrifida* extract showed the presence of alkaloid, terpenoid/steroid, saponin, tannin, and flavonoid compounds. Flavonoids, such as quercetin and rutin, showed antioxidant effects against damage caused by excess ROS of sickle cell anemia [19]. Moreover, many studies also indicated that flavonoid compounds induced the GPx activity. Flavonoid rutin could induce glutathione and GPx activities in the testis of adult rats that were induced using cadmium [20]. The treatment with silymarin significantly lowered the level of lipid peroxidation and enhanced the antioxidant status and resulted a reduction of the necrotic damage caused by nickel and thereby restored the previous biochemical parameters [21].

The study suggested that *S. quadrifida* had been partially reduce the imbalances between the generation of ROS and the scavenging enzyme activity. According to these results, *S. quadrifida* could be used a supplement, as an antioxidant therapy, and preventing diabetic complications due to lipid peroxidation and free radicals.

Table 2: Activity of glutathione peroxidase

Groups	GPx activity (U/mg)±SD
Group I: Normal control	77.79±3.28
Group II: Diabetic control	25.87±1.07
Group III: Glibenclamide 0.45 mg/kg bw	62.83±1.44
Group IV : Extract 65 mg/kg bw	36.73^{abc}±0.66
Group V: Extract 130 mg/kg bw	50.87^{abc}±1.90
Group VI: Extract 260 mg/kg bw	56.10^{abc}±0.94

[a]significantly different compared to normal control ($p<0.05$), [b]significantly different compared to diabetic control ($p<0.05$), [c]significantly different compared to glibenclamide ($p<0.05$)

CONCLUSION

The ethanol extract of *S. quadrifida* bark had very strong antioxidant activity by DPPH radical scavenging with IC_{50} value of 4.86 ug/ml. The ethanol extract of *S. quadrifida* bark dose of 260 mg/kg bw showed the highest GPx activity in diabetic rats.

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AUTHOURS' CONTRIBUTIONS

The author declares that all the named authors have contributed equally to this article.

CONFLICTS OF INTEREST

All authors report no conflicts of interest regarding this manuscript.

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