Effect of Sterculia quadrifida R. Br bark to in vitro DPPH Radical and Glutathione Peroxidase on Diabetic Wistar Rats

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Received: 10 June 2020, Revised and Accepted: 15 September 2020

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 IC₅₀ 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.

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-tocopherol, 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 falao, 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 DPPH 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, Sebels 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 January 2017 and authenticated from the Biology Lab, Faculty of Math and Science, Sebels 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.
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 free radical. The result of reduction DPPH radicals causes decoloration from purple color to yellow pale color which indicates the scavenging activity. Percent of absorbance of DPPH 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 IC₅₀ was calculated using regression linear equation. IC₅₀ is the ability of the compounds to inhibit 50% DPPH activity.

The IC₅₀ value of ethanol extract of fakak bark and rutin is 4.86 µg/ml and 4.23 µg/ml, respectively. The previous research by Saragih and Siswadi [14] showed that S. quadrifida bark extracts had DPPH radical scavenging activity by IC₅₀ of 2.51±0.03 µg/ml. These result indicated that the extracts and standard rutin had very strong antioxidant activity. The sample had IC₅₀ <50 ppm, it was very strong antioxidant, 50–100 ppm strong antioxidant, and 101–150 ppm medium antioxidant, while weak antioxidant with IC₅₀ >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 antioxidant activity and varied pharmacological activities [16].

In vivo antioxidant of GPx activity

GPx is an important intracellular enzyme that breakdown hydrogen peroxides (H₂O₂) 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 IC₅₀ value of ethanol extract of Sterculia quadrifida bark and rutin

| Solutions                | Replication | IC₅₀ (µg/ml) | Mean IC₅₀±SD |
|--------------------------|-------------|-------------|--------------|
| Sterculia quadrifida bark | 1           | 4.86        | 4.86±0.01    |
|                         | 2           | 4.86        |              |
|                         | 3           | 4.87        |              |
| bark extract             | 1           | 4.23        | 4.23±0.01    |
|                         | 2           | 4.24        |              |
|                         | 3           | 4.23        |              |
| Standard rutin           | 1           | 4.23        | 4.23±0.01    |
|                         | 2           | 4.24        |              |
|                         | 3           | 4.23        |              |
In the study, S. quadrifida was partially reduce the imbalances between the generation of ROS and the scavenging enzyme activity. According to these results, S. quadrifida could be used as a supplement, as an antioxidant remedy, 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±0.66             |
| Group V: Extract 130 mg/kg bw | 50.87±1.90             |
| Group VI: Extract 260 mg/kg bw | 56.10±0.94             |

*a: significantly different compared to normal control (p<0.05), **: significantly different compared to diabetic control (p<0.05), ***: 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 IC₅₀ value of 4.86 µg/ml. The ethanol extract of *S. quadrifida* bark dose of 260 mg/kg bw showed the highest GPx activity in diabetic rats.

**ACKNOWLEDGMENT**

We sincerely acknowledge to Dr. Rina Herawati for her grammatical editorial.

**AUTHORS’ 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.

**AUTHORS’ FUNDING**

None.

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