Hypoglycaemic activity of hydroethanolic root extracts of *Ruellia tuberosa* L in diabetic rats

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Abstract. This study aims to determine the antihyperglycemic of the hydroethanolic root extracts of *Ruellia tuberosa* L to blood glucose levels and MDA (malondialdehyde) levels in serum of diabetic rats. Rats were divided into five groups: control, diabetes, and treatment groups with doses of 250, 375, and 500 mg/kg body weights. All treatment groups received hydroethanolic root extracts of *R. tuberosa* L for 21 days by oral administration. The results of the characterization of the extracts showed that the secondary metabolite compounds contained in hydroethanolic root extracts of *R. tuberosa* L were phytosterol compounds (β-sitosterol, stigmasterol, and campesterol). The results showed a decrease in blood glucose levels by 54.56%, 37.70%, and 16.79%, for treatment doses of 250, 375, and 500 mg/kg body weights, respectively, in day 21. The MDA levels in serum were measured by UV-Vis spectrophotometry method. Results show that the most effective therapy doses for decreasing MDA level activities was at 250 mg/kg body weight in the amount of 40%. In summary, hydroethanolic root extracts of *R. tuberosa* L have the potential to be used as a natural cure for diabetes.

1. Introduction
Diabetes mellitus is one of the prevalent lifestyle-related diseases in this century [1]. Diabetes mellitus is typically categorized as either type 1 or insulin-dependent, which is caused by damage of the pancreas that produced insulin, or type 2 non-insulin dependent that is caused by obesity, aging, or other environmental factors [2]. Type 1 diabetic people are dependent to insulin, since they need to take insulin injection continuously for their life endurance [1, 2]. The number of people that have diabetes has been forecasted to increase from 171 million in 2000, to 366 million in 2030, globally [3]. The diabetes epidemic particularly relates to type 2 diabetes, which contributes for over 90-95% of all cases of diabetes worldwide [3]. In Indonesia, Ministry of Health Republic of Indonesia data reveals that there are nearly 8.4 million people in Indonesia diagnosed with diabetes in 2006. By the year of 2030, the quantity of people having diabetes is estimated to increase to 21.3 million [4].

Indonesia has rich diversity of floras that can be used as a source of raw materials for traditional medicines. The advantageous applications of natural products such as plants or flora in many countries are comprehensively acknowledged. Numerous floras have been used as nutritional food and in curing many metabolic disorders even without any information on their accurate functions and their contents. Even though various synthetic drugs were established for the management of diabetes mellitus but the
safety and efficacy of the treatment model have not been completed. Nevertheless, synthetic drugs in the long-term use leads to some drawbacks, including hypoglycaemia, toxic to kidneys and increasing the risk of heart attack [5]. Thus, there are increasing interests to develop and search for medicines for diabetes, particularly in developing countries like Indonesia, where natural resources are plentiful [6, 7].

One of plants that can be utilized and contains bioactive compounds is from the family of Acanthaceae. One member of the Acanthaceae family is genus Ruellia. Ruellia tuberosa L. is a tropical plant that is widely grown in Asia, including Indonesia, Malaysia, and India [8-9]. This plant is locally known as pletekan, pletikan, or ciplukan. Previous phytochemical determination pointed out that ethanolic leaves extract from R. tuberosa L contained flavonoids, glycosides, saponins, phenols, and carotenoids [10]. In addition, those extracts also had nutrients such as tocopherol, ascorbic acid and lycopene [11]. Further research from Manikandan mentioned that the leaf extract of politician has acted as anti-diabetes and anti-oxidant [11].

Based on our previous research, pletekan roots extracted with n-hexane had anti-diabetic activity by conducting an in vivo study [12]. These include reducing blood glucose levels, reduce malondialdehyde (MDA) levels, and repair on the kidney histopathologic profiles [12]. Phytochemical investigation of this study revealed that the pletekan roots extracted with n-hexane contained triterpenoid compounds [12]. However, no further characterizations for the extracts were conducted.

For these reasons, the current work investigates the antidiabetic activity of pletekan roots extract in vivo using rats (Rattus novergicus). Pletekan roots are extracted using maceration technique, using ethanol and water as solvents, followed by identification and characterization of the resulted extracts. Rats are injected with multiple low-dose streptozotocin, to induce diabetic states. The antidiabetic capacity of extracts is determined by its effect on blood glucose and MDA (malondialdehyde) levels in rat serum.

2. Material and Methods

2.1 Materials and Instruments
The animal models for this study were white male rats, Wistar strain, obtained from Institute of Biosains, Brawijaya University, Malang. The experimental in vivo study has obtained a certificate of ethics from the Research Committee of Universitas Brawijaya No: 873-KEP-UB-2018. Materials used in this research included pletekan roots powder obtained from Materia Medica (Batu, East Java) enclosed with the determination letter of the species, Ruellia tuberosa L. Other materials were purchased from Sigma-Aldrich: ethyl alcohol (pure, $d = 0.789$ g/mL), glacial acetic acid (pharmaceutical secondary standard), HCl (37%, analytical grade), H$_2$SO$_4$ (99.999%, analytical grade), NaCl (powder, ≥99.5%, analytical grade), KMnO$_4$ (≥99.0%, reagent grade), magnesium (turnings powder, 98%, reagent grade), sodium citrate solution, citrate buffer solution pH 4.5. Other materials used were streptozotocin (Bioworld), PBS solution (Phosphate Buffer Saline), 1% PBS-acid solution, 10% trichloroacetic acid (TCA), ND MDA reagent. Instruments used was UV-Vis spectrophotometer (1601, Shimadzu).

2.2 Preparation of hydroethanolic extracts of pletekan roots
A 900 g of pletekan root powder (90 mesh) was macerated with water: ethyl alcohol in 1:1 ratio of 3 (24 h. The resulted extracts were filtered, and then concentrated using a rotary evaporator vacuum at 50 °C, 120 RPM. The extracts were stored at 4 °C for further characterization.

2.3 Phytochemical Screening Tests of hydroethanolic extracts of pletekan roots
Phytochemical qualitative tests were conducted based on standard phytochemical tests [13-15]. The extracts were tested for flavonoids, triterpenoids, steroids, phenolic compounds, saponins, tannins, and ascorbic acids.
2.4 Preparation of Diabetic Rats
Two weeks before the assay, rats were adapted in the animal house. Rats were distributed into five groups: (I) control group; (II) diabetic group; (III) treatment group 1; (IV) treatment group 2; and (V) treatment group 3, 5 rats in each group. Rats were maintained in the animal house, in Biosains Laboratory, Brawijaya University. In order to prepare streptozotocin induction, 100 mg of streptozotocin was dissolved in a 3-mL of citrate buffer at pH 4.5. A dose of 20 mg/kg of body weight was injected intraperitoneally to rats in groups II, III, IV, and V for 5 subsequent days, while the rats in group I (negative control) did not receive an injection of streptozotocin.

2.5 Treatment with Root Extracts of Pletekan for Groups III, IV, and V
Rats in groups III, IV, and V were received with pletekan root hydroethanolic extracts at various doses of 250; 375; and 500 mg/kg body weights per day, for 21 days. Changes in blood glucose levels were monitored per week during the treatment. At the end of the experiment (day 21), rats were sacrificed, and the blood serum was collected and stored at 4°C for further analysis.

2.6 Measurement of MDA Levels
Determination of MDA levels was conducted using spectrophotometry UV-Vis technique, with TBA reagents. The rat serum from all groups (I-V) were prepared based on the earlier published method [16]. The color changes on rat serum after adding of TBA reagent were measured using a UV-Vis spectrophotometer at the 530 NM wavelength. The absorbance values were proportional to the concentration of MDA in the samples.

3. Results and Discussion
Table 1 shows phytochemical screening results from hydroethanolic extracts of pletekan roots. The test was a qualitative test, based on the color changes after extracts were reacted with standard reagents for secondary metabolite detection. The secondary metabolites contained in the hydroethanolic extracts of pletekan roots were steroids, flavonoids, phenolics, and ascorbic acids. These results are in agreement with previous studies conducted on hydroethanolic leaves extracts of this species, which reported the presence of ascorbic acid, phenolic, tannin, lycopene, carotenoid, and tocopherol [10].

Table 1. Phytochemical screening results of hydroethanolic extracts of pletekan roots

| No | Parameter   | Result* |
|----|-------------|---------|
| 1  | Flavonoid   | +       |
| 2  | Steroid     | +       |
| 3  | Phenolic    | +       |
| 4  | Ascorbic acid | +    |
| 5  | Terpenoid   | -       |
| 6  | Saponin     | -       |
| 7  | Tannin      | -       |

* + sign = detected; - sign = undetected

Administration of multiple-low-dose streptozotocin with a dose of 20 mg/kg body weights for 5 days caused rats suffered from diabetes type 1. The treatments to the rats were administered for 21 days, after the rats in group III-V had blood glucose levels more than 200 mg/dL. Table 2 presents the blood glucose levels in rats in all groups in the last day of the assay (day 21). Based on the data in Table 2, it can be seen that blood glucose levels in rats at the group I was at a range of normal rats (non-diabetic), 134.25 ± 20.77 mg/dL, whereas blood glucose levels in rats at group II (untreated) were very high at 542.04 ± 18.02 mg/dL.
Table 2. Blood Glucose Levels in Five Groups of Rats

| No | Group | Blood glucose level (mg/dL) |
|----|-------|----------------------------|
| 1  | I     | 134.25 ± 20.77a            |
| 2  | II    | 542.04 ± 18.02e            |
| 3  | III   | 246.25 ± 15.38b            |
| 4  | IV    | 337.50 ± 14.20c            |
| 5  | V     | 451.11 ± 13.88d            |

*different letters (a-e) show significant statistical different effects in each group (p <0.05)

The treatments of diabetic rats to groups III, with pletekan root extracts with the dose of 250 mg/kg body weight decreased blood glucose level to 246.25 ± 15.38 mg/dL, with a percentage of decrease was 54.56%. However, at higher doses, 375 and 500 mg/kg body weights, did not lead to an increasing reduction in blood glucose levels. At doses of 375 and 500 kg/body weights, blood glucose levels of rats decreased to 37.70% and 16.79%, respectively. Therefore, the effective dose to decrease blood glucose level of diabetic rats in this study is 250 m/kg body weight.

Table 3 shows the MDA levels in rat serum in all groups. Malondialdehyde is one of the end products of cell membrane lipid peroxidation by excess ROS. Hence, MDA levels are an indicator of the oxidative stress levels in the body [18]. Measurement of MDA levels (Table 3) shows that MDA levels in rats at group I were 0.81 ± 0.013 µg/mL, diabetic rat group (group II) were 2.62 ± 0.029 µg/ml. There were decreases in MDA levels in all treatment groups (groups III-V). Again, the effective dose to decrease MDA levels of diabetic rats was the lowest dose, 250 mg/kg body weight, resulting in a 39.99 % decrease. The 375 and 500 mg/body weight doses had 25.74% and 10.94% decreases, respectively.

Decreases in blood glucose and in MDA levels of diabetic rats treated with pletekan root extract can be caused by the content of flavonoids and ascorbic acids in the composition of pletekan root extracts (Table 1). In the diabetic states, hyperglycemia condition leads to increasing levels of oxidative stress and free radicals [17, 18]. In this study, the presence of flavonoids, ascorbic acids, and phenolic compounds in the extracts, as shown from phytochemical test, lead to anti-oxidant activity of the extracts. In addition, we have studied the anti-oxidant activity of the extracts using DPPH free radical scavenging activity [19]. In that study, hydroethanolic pletekan root extracts had high anti-oxidant activity with the IC_{50} value of 2.48 µg/mL [19].

Table 3. MDA Levels in Five Groups of Rats’s Serum

| No | Group | MDA levels (µg/dL) |
|----|-------|-------------------|
| 1  | I     | 0.81 ± 0.013b     |
| 2  | II    | 2.62 ± 0.029c     |
| 3  | III   | 1.57 ± 0.017b     |
| 4  | IV    | 1.94 ± 0.042c     |
| 5  | V     | 2.33 ± 0.055d     |

*different letters (a-e) show significant statistical different effects in each group (p <0.05)
Figure 1. Proposed mechanism of action of flavonoid in hydroethanolic root extracts of pletekan acting as free radical scavengers (adapted from Ref. 20).

One of the proposed mechanism of actions of secondary metabolites compound contained in the extracts acting as anti-oxidants are shown in Figure 1. Figure 1 shows the flavonoid compounds act as free radical scavengers. The presence of -OH groups in ortho position; and conjugated C=C double bonds contribute to antioxidant properties of flavonoids. Flavonoid compounds donate H atom(s) from the -OH groups, and bind to free radicals (ONOO•). This will produce phenoxy flavonoid radicals, and the phenoxy radicals will be stabilized by conjugated C=C double bonds in the flavonoid rings [20].

4. Conclusion
In summary, the current work has investigated hypoglycaemic activity of R. tuberosa L root extracts in diabetic rats as animal models. The hypoglycemic activity of the extracts has been determined from decreases in blood glucose concentration, and decreases in the MDA levels of rats’ serum. The effective dose was at 250 mg/kg body weight. The hypoglycaemic activity of pletekan root extracts can be caused by the content of flavonoids, phenolic compounds and ascorbic acids within the extracts.

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