Phytochemical, Antioxidant Studies of *Ruellia tuberosa* Linn, Heart, and Lung organs

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Abstract:

Pletekan (*Ruellia tuberosa* Linn.) from Acanthaceae family is widely used traditionally to treat diabetic, diuretic, lowering blood pressure and fever. Pletekan contains phenol and flavonoid compounds which have biological effects as antioxidants. Therefore, in recent work, the content of phenol and flavonoid compounds was analysed and the antioxidant activity of *Ruellia tuberosa* Linn was evaluated with in vivo and in vitro methods. The antioxidant was determined in vitro with Ferric Thiocyanate (FTC) and Phosphomolybdate methods then determined in vitro by measuring lipid peroxidation products, the levels of MDA and SOD enzymes in experimental animals treated with the extract. *R. tuberosa* leaves ethanol extract had a total phenolic and flavonoid content of 86.95 mg GAE/g sample ±s 0,81 and 65.24 mg QE/g, respectively. Extracts provide antioxidant activity with both in vitro methods used, and the activity was increased based on concentration. Furthermore, the extract provides inhibition on MDA and increases the SOD levels characterized by an increase in inhibition value with the highest activity at a concentration of 400 mg/kg BW. Current studies have presented that there are potential activities that can be explored from pletekan leaves as a source of medicinal compounds.

Keywords: *Ruellia tuberosa* Linn.; antioxidant; FTC; Phosphomolybdate; MDA; SOD.

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1. Introduction

Ruellia tuberosa or commonly known in Indonesia as Pletekan, belongs to the family Acanthaceae is a tropical plant and commonly found in the Southeast Asian region and has different ethnobotanical uses in different countries. Traditionally used to treat diuretic, antipyretic, analgesic, antihypertensive, worm medicine, bladder disease, kidney disorders, bronchitis, gonorrhea, and syphilis (Daya et al., 2010). Study about its pharmacological activity has been done and revealed that pletekan has antioxidant activity (Ahmad et al., 2012), antinociceptive and anti-inflammation and antidiabetic, hyperlipid and hepatoprotector activities (Alam et al., 2009; Rajan et al., 2012).

Pletekan (*Ruellia tuberosa* Linn.) known contained phenolic and flavonoid compounds. Cirsimaritin, Cirsimarin, cirsiliol-4-glucoside, sorbifolin and pedalitin were phenolic and flavonoid compounds that has been isolated from this plant (Lin et al., 2006). Many researchers reported that phenols and flavonoids have the potential to prevent free radicals (Ahmad, R.A., Ahmad, M., Berna, 2012). Furthermore, the presence of antioxidant compounds such as phenolics, flavonoids, tannins and proanthocyanidins in plants may provide protection against a number of degenerative disorders (Gülçin, 2012). Previous research conducted by Ahmad et al. (2012) with DPPH method showed that extract and fraction of *R. tuberosa* leaves crude extract able to counteract the negative effects of free radicals with IC_{50} of DCM and methanol fraction were 14.57 and 11.55µg/mL and the IC_{50} of ethyl acetate, *n*-buthanol and water fraction respectively were 8.79, 7.42, and 21.69 µg/mL (Ahmad et al., 2012).

Oxidative stress in different tissues due to environmental oxidants can damage molecular signaling pathways and enzyme activity leading to tissue damage (Sies et al., 2017). Lipids, especially polyunsaturated fatty acids are the biomolecules most affected by oxidative stress causing lipid peroxidation and MDA is biomarker that are produces in this reaction which is known to have mutagenic and toxic effects. Meanwhile, enzymes and antioxidants that are involved in the line of defence against oxidative stress including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), carotenoids, flavonoids, ascorbic acid, and alpha-tocopherol (Valko et al., 2007). Therefore, aim of this study was to determine the phytochemical content which includes phenol and flavonoid content and antioxidant activity of *R. tuberosa* Linn. leaf extracts was study

in-vitro using ferric thiocyanate and phosphomolybdate methods. In addition, the activity of the extract was also identified in vivo in counteracting tissue oxidative stress by analyzing MDA levels in plasma and the increase in SOD activity. Utilization of *R. tuberosa* in Indonesia as a medicinal plant has not yet been maximally explored but tends to be considered as a weed plant. Hence, It is expected that this research will give directions for the optimum use of this plant as a medicinal herbs.

2. Materials and Methods

2.1 General methods

Quercetin, gallic acid, and linoleic acid were purchased from Sigma Aldrich. Folin Ciocalteu was purchased from Merck. Ketamine, K_2HPO_4, KH_2PO_4, NaCl 0,1N, KCl, Tricloro acetat acid (TCA) 20%, Thiobarbiturat acid 0,67%, Tetra ethoxy propane (TEP), NaCl 0.9%, CCl_4, NaOH, H_2SO_4, pyrogalol, Tris-Hydrochloride (Tris HCl) Buffer purchased from Merck (Darmstadt, Germany). All chemical reagents used in this research were analytical grade.

2.2 Plant Material

This research material was pletekan fresh leaves collected from Bogor Agricultural Institute (IPB), Bogor, West Java, and authenticated by the Research Center for Biology, Indonesian Institute of Sciences, Cibinong, Bogor, Indonesia.

2.3 Plant Extraction

The extraction procedure was performed according to method by pletekan fresh leaves dried in the oven at 50^{o}C then powdered and sifted through sieve number 40. The powder was extracted with maceration methods with ethanol 70% ratio of 1:10, the process was repeated to achieve optimal extraction (Hanani et al., 2020).

2.4 Phytochemical Screening of the Extract

Pletekan leaf extract was qualitatively identified to detect the presence of secondary metabolites: Phenolic compounds, flavonoids, alkaloids, terpenes, steroids, and saponins (Srisawat et al., 2013).

2.5 Determination of Total Phenolic Contents

The total phenolic content of the extract was identified using Folin-Ciocalteu reagent and gallic acid as a reference according to the methods performed by Baba and Malik (2011) with a few modifications. 0.5 ml (1000 ppm) extract 0.5 ml and added with 2 ml Folin Ciocalteu reagent then added with 4 ml Na_2CO_3 1 M mixed homogeneously. The solution was incubated for 1 h and the absorbance was measured at 650 nm. All determinations were carried out in triplicate. The standard calibration curve was determined from the linear regression equation between the range of gallic acid concentration (x) and the absorbance of the solution from the reaction of gallic acid with the Folin-Ciocalteu reagent (y) (Baba & Malik, 2015).

2.6 Determination of Total Flavonoid Contents

The total flavonoid of pletekan leaves extract was identified with $AlCl_3$ and Quercetin as a standard according to the methods performed by Chang et al. (2002). 100 mg ethanol extract of pletekan leaves was dissolved in a volumetric flask with 10 ml ethanol. 1 ml taken from the main solution (10 mg extract/ml) was added with 3 ml ethanol, 0.2 ml $AlCl_3$ 10%, and 0.2 ml sodium acetate 1M. The solution was added with distilled water up to 10 ml. The mixture was incubated 30 minutes and the absorbance was measured at 427 nm. All determinations were carried out in triplicate. The equation of the standard calibration curve was obtained from a linear regression between several concentrations of quercetin (x) and absorbance (y) (Chang et al. 2002).

2.7 In vitro Antioxidant Assays

2.7.1 Ferric Thiocyanate Methods (FTC)

The ability of pletekan leaves extract to prevent the formation of peroxides from linoleic acid was confirmed with the FTC methods as described by Zahin et al. (2009). 1 ml linoleic acid 50 mM added with 1 ml phosphate buffer solution pH 7 0.1 M and 0.5 ml of extract with various concentration (25, 50, 75, 100 and 125 ppm), then incubated at 37-40^oC in a dark room for 24 hours. 100 µl incubated solution was added with 2.35 ml ethanol and 50 ml ammonium thiocyanate 30%, then 50 µl $FeCl_2$ 0,02 M in 3.5% HCl solution. The mixture was incubated according to the

operating time range and absorbances of solutions were measured at the maximum wavelength (Zahin et al., 2009).

2.7.2 Phosphomolibdate Methods

Determination of antioxidant activity by phosphomolybdate method with the method described by Salamah (2014). 1 ml sample solution with various concentration (30, 45, 60, 75, 90 ppm) added with 1 ml phosphomolibdate reagent then incubated at 95^{o}C in operating time range. Absorbance were measured at the maximum wavelength obtained as much as triplo in each concentration. Data were analyzed with linear regression equations. Calculation of antioxidant activity obtained by equalizing the antioxidant activity of the sample with antioxidant activity of a standard (Quercetin Equivalents) which was expressed as milligrams of Quercetin Equivalents/ gram extract (mgQE/ gram extract) (Salamah & Farahana, 2014).

2.7.3 In vivo Antioxidant Assays with Determination of MDA and SOD Levels

Preparation of Animals

Twenty-five male rats aged 2–3 months, 200–300 g, were obtained from Research Animal Bekasi, Indonesia. The identity of the experimental animal confirmed was white rat (*Rattus norvegicus*) Sprague Dawley strain by the Research Center for Biology, BRIN. Experimental animals used in this study were 24 rats divided into 6 groups. Each group consisted of 4 rats. In the preliminary stage, experimental animals were acclimatized for 7 days and given standard food and drink.

Experimental Design

The experimental design was approved by Faculty of Pharmacy and Sains, Muhammadiyah Prof. Dr. Hamka University with ethical approval number: 02/19.10/0212. MDA and SOD level were determined according to method performed by Ahmed et al.(Ahmed Amar et al., 2019) . The design used includes a randomized design with Curcumin (PT. SOHO) purchased from the market was used a standard. The use of Curcumin is based on an article from Alizadeh (2019) who reviewed several literatures and concluded that Curcumin is effective in reducing MDA levels in tissues and increasing antioxidant levels under oxidative stress conditions (Alizadeh & Kheirouri,

2019). Experimental animals were grouped into normal control (0.5% Na CMC), negative control (0.5% Na CMC), positive control (200 mg/Kg BW curcuma), dose I group (100 mg/kg BW extract), dose II group (200 mg/kg BW extract) and dose III group (400 mg/kg BW extract). All groups were administered a substance once daily for 21 days and on the 22nd day, all groups (except the normal control group) were induced with 1 mL/Kg BW CCl_4 (Merck) intraperitoneally. On day 16, the test animals were administered ketamine, then the collar bone was dislocated, and initiation was performed to view the homogenates of the kidney, heart, and liver. Furthermore, malondialdehyde (MDA) levels and superoxide dismutase (SOD) activities of each organ were determined.

Statistical Analysis

All data were expressed as mean ± standard deviation (mean ± SD). Pearson correlation analysis was used to find correlations among the various parameters of the study. Statistical analysis was performed using the statistical software package SPSS version 17.0.

Rats Organ Preparation

Kidney, heart, and liver organs were cleaned with 0.9% NaCl then stored in plastic pots containing 0.9% NaCl and stored in a freezer. Kidney organs were washed with phosphate pH 7.4 in a 500 mL beaker glass and weighed 0.5 g. Phosphate salt solution of 5 times the volume of the organ weight was prepared, homogenized the organ in a mortar then placed in a container filled with ice, and added phosphate gradually. Pipetted the kidney homogenate into a 10 mL test tube for examination of SOD activities. Then the mixture was photometrically at a wavelength of 540 nm.

Determination MDA Level

MDA levels were measured with a thiobarbituric acid reactive substance (TBARS) assay. 1 mL of sample was put into a test tube added with 0.5 mL of 20% TCA, then centrifuged at 3000 rpm for 10 minutes, supernatant was collected. One ml supernatant mixed with 1 ml of 0.67% TBA in the tube, then put the tube into a water bath at 95-100ºc for 10 minutes and cooled with running water. The reaction of MDA and TBA forms a complex that produces a red color to reddish then measured the intensity using a spectrophotometer at 532 nm (Aguilar & Borges, 2020).

Determination of SOD Levels

Measurement of SOD activity using this principle of autoxidation of pyrogallol by SOD. 200 µL of 10 mM pyrogallol was mixed with 2765 µL of 50 mM Tris-HCl buffer (pH 8.5) containing 1 mM EDTA, then 35 µL of organ supernatant was added. The solution was measured for absorbance at a wavelength of 420 nm every 10 seconds for 3 minutes. Determination of the autooxidation inhibition of pyrogallol by SOD was based on the reduction of the area under the pyrogallol autooxidation curve (Potapovitch, 1990).

3. Results

The characteristics of the extract used in the test are presented in Table 1 and showed that the ethanol pletekan leaves extract meets the requirement according to the standard (Table 1) (Indonesian Health Ministry, 2008).

Phytochemical screening aims to examine qualitatively a group of compounds that have biological activity in an extract. The studies showed that pletekan leaf extract gave positive results on phenols, flavonoids, saponins, tannins, steroids, and terpenoids.

Table 1. Extraction Result of Pletekan Leaves Extract

Sample	Powdered Simplicia	Extract weight	Yields (%)	Moisture content (%)	Ash Value (%)
Pletekan leaves powder	1 kg	173.6 g	17.36	8.62	16.05

3.1 Total Phenolic Compounds

The determination of total phenolic content with Folin-Ciocalteu reagent was characterized by the formation of a blue complex compound measured at 765 nm. The hydroxyl groups in phenolic compounds reacted with Folin-Ciocalteu reagents to form a blue molybdenum-tungsten which can be detected with a spectrophotometer. The results showed in Table 2 that the total phenolic content

of Pletekan leaves extract was 86.953 mg GAE/g of the sample which indicates that 1 g of extract contained a total phenolic content of 86.953 mg of phenol which was equivalent to gallic acid.

Table 2. Total Phenolic Content of Pletekan Leaves Extract

Conc.	λ max.	Abs	Phenolic content (mgGAE/gram)
1000 ppm	756 nm	0.574	87.77
		0.566	86.14
		0.570	86.96

3.2 Determination of Flavonoid Content

Determination of flavonoid content is essential to determine the amount of flavonoid compound in the extract as one of the compounds that affect the antioxidant activity. In the measurement of total flavonoids, $AlCl_3$ is added which can form a complex that causes a wavelength shift towards visible light. Characterized by a solution that produces a yellow color (Zahin et al., 2009). The maximum wavelength of quercetin with $AlCl_3$ was obtained at 427 nm which is in the range under the requirements (415-440 nm) (Zahin et al., 2009). The results showed on Table 3 that the total flavonoid content of pletekan leaves extract was 70.897 mg QE/g which indicates that 1 g of leaves extract contains a total flavonoid content of 70.897 mg of flavonoids which were equivalent to quercetin.

Table 3. Total Flavonoid Content of Pletekan Leaves Extract

Conc.	λ max.	Abs	Flavonoid content (mgGAE/gram)
1000 ppm	427 nm	0.487	71.15
		0.487	71.15
		0.482	70.39

3.3 In vitro Antioxidant Assays

3.3.1 Ferric Thiocyanate (FTC) Methods

The study measured the maximum wavelength obtained at 490 nm. Based on the percentage inhibition of linoleic acid oxidation, the antioxidant activity increased according to the

concentration of the extract. The best activity obtained at 125 ppm extract concentration with inhibition was 51.79%, whereas quercetin gave 56.57% inhibition at 16 ppm concentration (Table. 4).

Table 4. Inhibition Result in Antioxidant Assayed of Pletekan Leaves Extract with FTC Methods

Conc.	Abs	% Inhibition
25 ppm	0.7063	11.16 %
50 ppm	0.6420	19.25 %
75 ppm	0.5290	33.46 %
100 ppm	0.4600	42.14 %
125 ppm	0.3833	51.79 %

3.3.2 Phosphomolybdate Methods

In this method, antioxidant activity can be seen from the ability of the sample to reduce Mo (VI) to Mo (V) and form a green Phosphomolybdate complex at the acidic solution and high temperature so that the amount of Mo (V) formed can be measured using a spectrophotometer(Khatoon et al., 2013). Antioxidant activity was measured at the maximum wavelength of a mixed solution of quercetin and Phosphomolibdate reagent at 695.50 nm.

The antioxidant activity of extract with the phosphomolybdate method is shown in Table 5. The best activity obtained at 90 ppm extract concentration with activity was 151.10 mgQE/ gram extract indicated that every 1 gram of pletekan leaves extract is equivalent to 151.10 mg antioxidant activity of quercetin.

The results of the antioxidant studies showed that there was an increase in activity with increasing extract concentration. The FTC method illustrates that pletekan leaf extract requires a concentration of 7.8 times to inhibit fat peroxidation equivalent to quercetin. While in the phosphomolybdate method, pletekan extract requires 6.6 times the concentration to perform antioxidant activity equivalent to quercetin.

Table 5. Antioxidant Activity Equality of Pletekan Leaves Extract with quercetin with Phosphomolybdate method

Concentration (ppm)	mgQE/ gram of extract				
	I	II	III	$\bar{x}$	SD
30	71.91	70.73	71.32	71.32	0.59
45	90.87	91.46	90.87	91.06	0.34
60	110.42	111.01	111.61	111.01	0.59
75	130.56	130.56	131.16	130.76	0.34
90	151.30	150.71	151.30	151.10	0.34

3.4 In vivo Antioxidant Assays with MDA and SOD Determination

SOD activity was determined by calculating the percentage inhibition of pyrogallol autooxidation by comparing the concentration of purpurogallin formed in a solution containing pyrogallol and SOD enzyme with a solution containing only pyrogallol. One unit of SOD is defined as the amount of enzyme required to inhibit the autooxidation reaction by 50%(Marklund & Marklund, 1974). Determination of MDA levels is performed with thiobarbituric acid reactive substance (TBARS) assay. The principle is that malondialdehyde can react with thiobarbituric acid (TBA) at hot temperatures in an acidic atmosphere to produce a pink product that will absorb UV light at a wavelength of 532 nm (Leon & Borges, 2020). The Determination of MDA and SOD levels from various organs are shown in table 6.

Table 6. Determination of MDA Levels and SOD Inhibitory Activities

No	Organs	Groups	MDA Levels (nmol/gram) ± SD	SOD Activities (% inhibition) ± SD
1	Heart	Normal	10.26 ± 0.48	94.89 ± 0.91
		Negative	15.88 ± 0.17	64.43 ± 2.54
		Positive	11.06 ± 0.35	92.95 ± 1.35
		100 mg/Kg BW	14.16 ± 0.32	76.13 ± 0.76
		200 mg/Kg BW	13.38 ± 0.35	80.48 ± 1.19
		400 mg/Kg BW	11.69 ± 0.33	89.17 ± 1.67
2	Kidney	Normal	10.44 ± 0.39	83.17 ± 1.15
		Negative	16.02 ± 0.41	63.67 ± 2.01
		Positive	11.27 ± 0.23	75.98 ± 1.09
		100 mg/Kg BW	13.55 ± 0.33	71.33 ± 2.09
		200 mg/Kg BW	12.42 ± 0.32	73.36 ± 0.53
		400 mg/Kg BW	11.48 ± 0.32	79.41 ± 1.76
3	Lever	Normal	12.75 ± 0.27	86.31 ± 3.87
		Negative	19.06 ± 0.24	64.21 ± 2.73
		Positive	13.37 ± 0.27	78.73 ± 1.89
		100 mg/Kg BW	15.85 ± 0.42	69.97 ± 1.28
		200 mg/Kg BW	14.60 ± 0.30	72.41 ± 0.81
		400 mg/Kg BW	13.59 ± 0.38	74.99 ± 0.89

4. Discussion

Oxidants provide a range of detrimental effects to the human body and therefore are essential to prevent. Free radical-induced cell damage affects many organs and physiological systems in the body that can lead to the pathogenesis of chronic diseases (Ghosh & Sarkar, 2016). Ultimately, oxidative stress affected the pathology of several diseases including diabetic, vascular, and nervous disorders (Pasupuleti et al., 2020; Pham-Huy et al., 2008). Antioxidants are compounds that can help counteract oxidative stress. Numerous studies have proven that natural products particularly polyphenol and flavonoid are the main source of active compounds as antioxidants (Dimitrios, 2006; Pasupuleti et al., 2020; Mziouid et al., 2022).

Ruellia tuberosa is one of the traditional plants that has been widely used in folk medicine and Ayurvedic medicine (Hosseinimehr et al., 2005). Different authors proved that extracts of the aerial parts of *R. tuberosa* showed potent antioxidant activity with DPPH method (Chen et al., 2006; Khachitpongpanit et al., 2016). Some studies showed that leaves exhibit free radical scavenging activity (Khachitpongpanit et al., 2016). Phenol and flavonoid are widespread compounds in nature and have been proven by many researchers to have important biological activities in humans. Current studies report that the phenolic and flavonoid content of *R. tuberosa* leaves extract were 86.953 mg GAE/g and 70.897 mg QE/g respectively. The analysis of flavonoid and phenolic compound levels in the extracts showed significant amounts compared to other plants in the genus Acanthaceae where the benefits of polyphenolic compounds are very diverse, one of which supports antioxidant activity (Sawadogo et al., 2006). The measurement of phenol and flavonoid compounds is also included as part of the standardization process of the test sample (Mutha et al., 2021). Several studies reveal that polyphenols present in foods and beverages exhibit enhanced plasma antioxidant activity as the accumulation of these reducing polyphenols, along with endogenous antioxidants in plasma, aids iron absorption like pro-oxidative dietary components (Scalbert et al., 2005). Based on free radical scavenging test of leaf extract with the FTC method, it is known that there is a significant inhibition of 125 ppm extract with an inhibition value was 51.79%, compared to quercetin with 56.57% inhibition at 16 ppm concentration. Besides that, with phosphomolybdate observed that 151.10 mgQE/ gram extract indicated that every 1 gram of pletekan (*R. tuberosa*) leaves extract is equivalent to 151.10 mg antioxidant activity of quercetin.

Guo et al. (2020) observed the effect of polyphenol extract of *Apocynum venetum* on serum and tissues of oxidized mice and found that SOD activities in mice were significantly increased compared with the model group, whereas NO and MDA levels were significantly decreased. The result of this study indicated that polyphenol extract can prevent oxidative stress in mice (Guo et al., 2019). Another study proved the effect of polyphenols compound from *Coix lachryma-jobi* L. var. ma-yuen Stapf significantly effective in decreasing the serum levels of MDA. Moreover, to study the activity of *R. tuberosa* extract to protect from pathology mechanisms, SOD and MDA levels were tested in the current study from three vital organs including lung, heart, and liver. MDA is one of the lipid oxidation products in the body that is used as an indicator of the lipid oxidation process due to free radicals. MDA has been used widely in biomedical research as a biomarker of lipid peroxidation due to its easy reaction with thiobarbituric acid (TBA) to produce colored MDA-TBA2 conjugates (Leon & Borges, 2020). In the lipid peroxidation reaction, the addition of oxygen radicals occurs which results in oxidative damage to polyunsaturated fatty acids (PUFA). This reaction attacks the methylene C-H bond resulting in the loss of hydrogen and the generation of an unpaired electron on the carbon. These unpaired electrons are stabilized by molecular rearrangements of double bonds forming conjugated dienes that combine with oxygen to form peroxyl radicals. Those peroxyl radicals can attack the hydrogen atoms of other polyunsaturated fatty acids resulting in the initiation of a chain reaction (Chang et al., 1992). Superoxide dismutase (SOD) is an endogenous antioxidant that can catalyze superoxide anion into hydrogen peroxide. The presence of SOD through the fenton reaction will help capture free radicals in tissues, thereby inhibiting oxidative damage by lowering free radical levels in various organs of the body (Wang et al., 2018).

The determination of MDA levels by the thiobarbituric acid reactive substance (TBARS) assay method, studies indicated the effect of giving test extracts on reducing MDA levels with increasing doses. The statistical analysis of MDA levels showed that there was a significant difference between the negative control group and the positive control group given curcumin which has been tested as an antioxidant that can counteract free radicals and inhibit the formation of lipid peroxidation so that prevent the formation of MDA ($P>0.05$) (Alizadeh & Kheirouri, 2019). In the group of rats given 70% ethanol extract of pletekan leaves, the MDA levels were different according to the dose given, the higher the dose given, the significant decrease in MDA levels occurred ($P>0.05$). These results indicated that the group of rats given 70% ethanol extract of

pletekan leaves has a significant difference with the negative control group. The administration of 70% ethanol extract of pletekan leaves at a dose of 400 mg/kgBB has the best antioxidant activity with average MDA levels comparable to the positive control group but not comparable to normal control.

In addition, SOD of each organ was also analyzed further to study the effect of the extract in protecting the organs. The analysis of SOD in the current study used a method based on the pyrogallol autooxidation reaction. Pyrogallol or benzene-1,2,3-triol or benzenetriol is a strong reductant compound that is obtained from the heating of gallic acid and the mixture of parachlorophenoldisulfonic acid with KOH. In an alkaline solution, pyrogallol compounds will react with oxygen in the air to produce purpurogallin compounds and change color from colorless to yellow. The principle of this method is that SOD will inhibit the autooxidation process of pyrogallol compounds into purpurogallin compounds by capturing oxygen. SOD activity was determined by calculating the percentage of pyrogalol autooxidation inhibition by comparing the concentration of purpurogalin formed in the solution containing pyrogalol and SOD enzyme with the solution containing pyrogalol only. One unit of SOD is defined as the amount of enzyme required to inhibit the autooxidation reaction by 50% (Marklund & Marklund, 1974). This research proved that in the 100 mg/kgBB dose group, 200 mg/kgBB dose, and 400 mg/kgBB dose of 70% ethanol extract of pletekan leaves showed significant oxidative inhibition from the negative control group in each organ. Administration of 70% ethanol extract of pletekan leaves at a dose of 400 mg/kgBB dose showed oxidative inhibition that was comparable to the control but not significant with the positive control group given curcumin.

The present study showed a protective effect of pletekan (*R. tuberosa*) leaf extract which can inhibit pathogenic activity that occurs in each test organ. The results of statistical analysis showed that the higher the dose given, the more it can prevent a significant decrease in SOD activity of the three doses given and the extract provides antioxidant activity that is on average comparable to the positive control group.

5. Conclusion

It can concluded that the pletekan leaves extract (*Ruellia tuberosa* Linn.) showed a significant content of phenol and flavonoid compounds that many studies proved its capacity in reducing

oxidative stress. In addition, the antioxidant activity obtained in the in vitro was linear with those obtained in the in vivo assay which indicates an opportunity to further develop and research of pletekan plant as a source of medicinal plants. However, the use of these herbs as traditional medicine still needs to be studied further regarding toxicity and effective doses to provide appropriate pharmacological activity for the community.

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