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ANTIOXIDANT ACTIVITY TEST AND EFFECT OF ETHANOL EXTRACT OF NUSA INDA PUTIH (*Mussaenda pubescens*) AGAINST CREATININE, URIC ACID AND UREUM LEVELS IN WHITE RATS (*Rattus novergicus*)

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Abstract

The purpose of this study was to examine the antioxidant activity and the effect of the extract of Nusa Indah Putih (*Mussaenda pubescens*) on creatinine, uric acid and urea levels in white rats (*Rattus novergicus*). The study design, the treatment group was divided into 5 groups. The control group (P0), the ethylene glycol group (P1), the kidney drug treatment group (P2), the Nusa Indah Putih ethanol extract group at a dose of 100 mg/kgBW (P3), and the Nusa Indah Putih ethanol extract group at a dose of 200 mg/kgBW (P4). Nusa Indah Putih extract has strong antioxidant activity with IC50 value of 78.27 ppm and AAI value of 1.277. Nusa Indah Putih extract was effective in lowering creatinine, uric acid and urea levels in experimental animals.

Keywords: Antioxidants; *Mussaenda pubescens*; Creatinine; Uric acid; Ureum

INTRODUCTION

The use of synthetic drugs in treating kidney stone disease, besides being expensive, the use of drugs such as diuretics or surgery to remove kidney stones often has a risk and the possibility of recurrence of the disease is very large (Joy *et al*., 2012; Lee *et al*., 1996). Therefore, nowadays many herbal medicines have been developed which are not only easy to obtain but also affordable. One of the herbal medicines that has been used empirically to treat kidney disease is the leaves of the Nusa Indah Putih plant (*Mussaenda pubescens*). Nusa Indah Putih (*Mussaenda pubescens*) can be used as a diuretic, antiphlogistic, antipyretic and to detoxify fungal toxins. Phytochemical analysis of the Nusa Indah Putih plant (*Mussaenda pubescens*) containing triterpenoid saponins (Zhao *et al*., 1997)

In addition, the use of antioxidants is growing rapidly both for food and medicine. Its use as a drug is growing along with the development of knowledge about free radicals against several diseases. For the kidney itself, antioxidants have the benefit of improving the state of the renal tissue and the integrity of the cell membrane and preventing its recurrence. In addition, antioxidants are known to have the ability to inhibit the work of free radicals. Most natural sources of antioxidants are plants and generally are phenolic compounds that are scattered throughout the plant (Sarastani *et al*., 2002). Phenolic or polyphenolic compounds, among others, can be in the form of flavonoids. The ability of flavonoids as antioxidants has

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been widely studied in recent years, where flavonoids have the ability to change or reduce free radicals and also as anti-free radicals.

MATERIALS AND METHODS

Animal model: This study used an animal model of white rats (*Rattus norvegicus*) with a weight of approximately 150-200 g, as many as 20 individuals. Feeding and drinking water is provided ad libitum. The rats were kept in plastic cages measuring 25 X 40 cm with individual wire covers. Before the treatment was carried out, the rats were adapted for 7 days.

Medicinal plants: The medicinal plants used are Nusa Indah Putih (*Mussaenda pubescens*)

Research procedure

The sample of Nusa Indah Putih (*Mussaenda pubescens*) obtained was dried by aerating then blended into powder and stored in a clean and tightly closed container.

Making ethanol extract

The extraction of Nusa Indah Putih was carried out by adding ethanol to the Nusa Indah Putih powder with a ratio of the amount of solvent to the powder 1: 5. The result of the maceration was an ethanol extract of Nusa Indah Putih and then evaporated using a rotary evaporator, to evaporate the solvent so that a thick extract was obtained from Nusa Indah. beautiful white.

Kidney damage activity test

After adaptation, the control mice (P0) were only given feed and drinking water, while the P1 mice were given 0.75% ethylene glycol induction for 14 days. The P2 treatment group was given 0.75% induserethylene glycol for 14 days followed by Batugin. The P3 treatment group was given 0.75% induserethylene glycol for 14 days, followed by the administration of Nusa Indah Putih (*Mussaenda pubescens*) extract 100 mg/kg BW for 14 days. The P4 treatment group was given 0.75% ethylene glycol for 14 days, followed by the administration of 200 mg/kgBW of Nusa Indah Putih (*Mussaenda pubescens*) extract for 14 days.

Test for urea, creatinine and uric acid.

At the end of the experiment, as much as 5 cc of blood was drawn through the heart using a syringe. Blood was centrifuged at 10000 rpm for 10 minutes to obtain blood serum for analysis of urea, creatinine and uric acid levels. Tests for urea, creatinine and uric acid were carried out using the Randox® Kit and read with a Spectrophotometer.

Antioxidant test by DPPH method

A total of 10 mg of ethanol extract of pakoba stem bark was weighed and then dissolved in 10 mL of methanol p.a to obtain a concentration of 1000 ppm. Pipette 0.75, 1, 1.5, 2, and 4 mL of mother liquor into a 10 mL volumetric flask and add methanol p.a to obtain concentrations of 75, 100, 150, 200, and

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400 ppm. Pipette 1 mL of each into a test tube and add 1 mL of DPPH then add 2 mL of methanol p.a and vortex until homogeneous, then incubated at 37ºC for 30 minutes. Furthermore, the absorbance of the test solution was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm (Alhabsyi *et al*., 2014; El-Maati *et al*., 2016) and 515 (Hidayati *et al*., 2017).

Determination of inhibition percent, IC50 (Inhibition Concentration) value and AAI (Antioxidant Activity Index) value. The percentage of inhibition of DPPH radicals from each concentration of sample solution can be calculated by the formula:

$$\% \text{ Inhibition} = \frac{A_{blangko} - A_{sample}}{A_{blangko}} \times 100\ \%$$

Where A blangko is the absorbance value of the DPPH solution without extract, A sampel is the absorbance value of the sample being tested. After obtaining the percentage of inhibition from each concentration, the sample concentration and the percentage of inhibition obtained were plotted on the x and y axes respectively in the linear regression equation y = a ± bx. The equation is used to determine the IC50 value of each sample expressed by a y value of 50 and the x value to be obtained as IC50. The IC50 value is the sample concentration that can reduce DPPH radicals as much as 50% of the initial concentration (Alhabsyi *et al*., 2014; El-Maati *et al*., 2016). Calculation of the value of AAI (Antioxidant Activity Index) is used to determine the index of antioxidant activity with the formula:

$$\text{Index AAI} = \frac{Concentration\ DPPH\ (ppm)}{IC_{50}\ Sample\ (ppm)}$$

The concentration of DPPH used in the test (ppm) is divided by the IC50 value obtained (ppm) (Alfira, 2014). The AAI value can be seen in table 1

Table 1. Value of AAI (Antioxidant Activity Index)

Antioxidant Activity	**AAI Value**
Weak	< 0,5
Medium	0,5 – 1
Strong	1 – 2
Very strong	> 2

RESULTS AND DISCUSSION

Antioxidant Activity

Antioxidant activity is the ability of the extract to capture free radicals. To determine the percentage of free radical scavenging by the extract, an antioxidant activity test was carried out using the DPPH radical reduction method with the parameter IC50 value. IC50 is the half maximal antioxidant inhibitory concentration on free radicals. A compound is said to be an antioxidant if the IC50 value is less than 200 ppm and if the obtained ranges from 200 to 1000 ppm, then the substance is less active but still has potential as an antioxidant. The lower IC50 value indicates high activity. IC50 less than 50 ppm is very active or strong, whereas if the IC50 value ranges from 50 ppm to 100 ppm, it is quite active or moderate,

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100 ppm to 200 ppm is slightly active or weak. If the IC50 value is more than 200 ppm it is not active (Herman, 2013). Based on the analysis, the IC50 value of the Nusa Indah Putih extract was 78.27 ppm and the AAI was 1.277. This shows that the extract of Nusa Indah Putih has strong antioxidant activity.

Analysis of urea, creatinine and uric acid levels

The results of the analysis of urea, creatinine and uric acid levels can be seen in table 1. The results showed that the ethylene glycol group experienced an increase in creatinine, uric acid and urea when compared to the control group. The results of the analysis showed that the levels of creatinine, urea and uric acid decreased in the treatment of kidney drugs and the treatment of extracts of Nusa Indah Putih both at doses of 100mg/kgBW and 200 mg/kgBW.

Table 2. Analysis of Creatinine, Uric Acid and Urea

Treatment	Creatinine (mg/dL)	Uric Acid (mg/dL)	Urea (mg/dL)
P0 (Control)	0.60±0.03	2.54±0.11	28.74±1.26
P1 (Ethylene Glycol)	1.11±0.02	4.32±0.44	52.38±2.05
P2 (Kidney Drug)	0.48±0.02	2.56±0.32	34.73±3.92
P3 (Dose100 mg/kgBB)	0.46±0.01	1.66±0.21	35.44±4.57
P4 (Dose 200 mg/kgBB)	0.47±0.01	1.71±0.18	37.57±4.42

The administration of ethylene glycol in this study was to stimulate the formation of urolithiasis, because ethylene glycol is a nephrotoxic agent that is often used in experiments with animal models of mice to stimulate the formation of calcium oxalate in the kidneys (Palmar, 2012).

In the results of the study, the creatinine level induced by ethylene glycol was 1.11 mg/dL which was higher than the control group. This indicates that the occurrence of kidney damage due to the administration of ethylene glycol. Administration of 0.75% ethylene glycol in drinking water for 14 days actually caused damage to the glomerulus and renal tubules, which was characterized by the infiltration of inflammatory cells into the tubular lumen. Epithelial cells undergo squamation and even loss of cell nuclei and the presence of microcrystal deposits in the renal tubules. An increase in plasma creatinine levels always indicates a decrease in excretion caused by impaired renal function. Creatinine is an anhydride form of creatine which is mostly synthesized in muscle by non-enzymatic dehydration of creatine phosphate. Creatinine is excreted entirely into the urine by glomerular filtration. Increased levels of creatinine in the blood is an indication of kidney damage. In addition, creatinine levels in the blood and in the urine can be used to estimate the glomerular track (Lu, 1995).

The administration of nusa Indah putih (*Mussaenda pubescens*) leaf extract as much as 100 and 200 mg/KgBW in this study was able to reduce creatinine levels by 0.46 and 0.47 mg/dL, respectively. This indicates that the administration of the ethanol extract can reduce serum creatinine levels. The results of the analysis also showed that ethylene glycol-induced uric acid levels reached 4.32 mg/dL. And decreased when given the treatment of white nusa Indah extract respectively 1.66 mg/dL and 1.71 mg/dL.

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Normal blood uric acid levels in rats are 1.2-5.0 mg/dL (Girindra, 1989). Induction of ethylene glycol causes reduced kidney performance which is characterized by high levels of creatinine and urea in blood serum and less efficient glomerular filtration. This causes increased levels of uric acid in the blood (Syukri, 2007). Uric acid will be carried to the kidneys through the bloodstream to be excreted with urine. The kidneys will regulate the level of uric acid in the blood so that it is always in a normal state. However, excessive uric acid will not be accommodated and completely metabolized by the body, so there will be an increase in uric acid levels in the blood.

Urea levels induced by ethylene glycol increased compared to the normal treatment, which was 52.38 mg/dL. Normal urea levels in white rats are 15.0 – 21.0 mg/dL (Malole & Pramono, 1989). High levels of urea in all treatment groups may be caused by feeding high protein content. Foods with high protein will increase the release of amino acids into the blood, which are then reabsorbed in the proximal tubule, because amino acids and sodium are reabsorbed together by the proximal tubule, the increase in amino acid reabsorption also stimulates sodium reabsorption in the proximal tubule.

In this study, ethylene glycol was used to stimulate the formation of urolithiasis7. This is because ethylene glycol can stimulate the formation of calcium oxalate. Oxalate metabolic compounds can bind to calcium in the blood to form calcium oxalate crystals and precipitate in the kidneys (Palmar, 2012). This causes an increase in creatinine, uric acid and urea.

The use of doses of 100 mg/kgBW and 200 mg/kgBW extract of Nusa Indah Putih can effectively reduce levels of creatinine, uric acid and urea. This is presumably because the content in plant extracts contains flavonoids and phenols. with high antioxidant activity and works as a diuretic thereby increasing the glomerular filtration rate. Nusa Indah Putih (*Mussaenda pubescens*) belongs to the Mussaenda genus which has pharmacologically active natural products, especially iridoids, triterpenes, and flavonoids. The advantages of species of this genus are easy to grow, free of disease and pesticides. Its medicinal activities include cytotoxic, anti-inflammatory, antiviral, antioxidant and antibacterial (Vidyalakshmi *et al*., 2008), diuretic, antipyretic and effective in treating acute laryngoparengitis, gastroenteritis and dysentery and anti-fertility activity (Venkatesh *et al.,* 2013)

Flavonoid compounds can prevent damage to pancreatic beta cells because they have antioxidant activity by capturing or neutralizing free radicals associated with phenolic OH groups so that they can improve the condition of damaged tissues (Sari, 2016). This is also in accordance with the results of antioxidant analysis of the extract of Nusa Indah Putih which has strong antioxidant activity results. Phenolic compounds that act as antioxidants by counteracting free radicals, so it is very important in maintaining the balance between oxidants and antioxidants in the body, neutralize the toxic effects of free radicals by donating hydrogen ions so that the ions become stable. The stable ion state causes a decrease in the state of oxidative stress in the tissue, so that it can cause an increase in the glomerular filtration rate (GFR) (Tandi *et al*., 2017).

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CONCLUSION

Nusa Indah Putih extract has strong antioxidant activity with IC50 value of 78.27 ppm and AAI value of 1.277. The levels of creatinine, uric acid and urea decreased after being given the extract treatment of 100 mg/kgBW and 200 mg/kgBW. This shows that the compound content in Nusa Indah Putih has effectiveness in lowering creatinine, uric acid and urea levels.

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