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Antioxidant and Anti-inflammatory Activity of Pagoda Leaves (Clerodendrum paniculatum L.) Ethanolic Extract in White Male Rats (Rattus novergicus)

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Abstract : Plants produce a broad range of bioactive compounds of secondary metabolites which can be classified into three groups of phenolics, terpenoids, and alkaloids as bioactive components. Pagoda leaves (*Clerodendrum paniculatum* L.) had potential antioxidant and antiinflammation. The *Clerodendrum paniculatum* L. leaves were obtained from Berastagi, North Sumatera Province, Indonesia. The leaves of *C. paniculatum* were dried at 50-60^{o}C and milled into powder. The dried leaves powder was extracted with ethanol by maceration method. Extract from solvent were concentrated by a rotary evaporator. The aim of the study was to evaluate *Clerodendrum paniculatum* L. ethanolic extract as antioxidant and antiinflammatory activity. The antioxidant activity was evaluated by scavenging effect of DPPH (*2,2-diphenyl-1-picrylhidrazyl*) method. The antiinflammatory activity was evaluated by paw edema and granuloma cotton pellet method. The result showed that the extract of *C. paniculatum* leaves had antioxidant activity with IC_{50} value of 27,73376 µg/ml and antiinflammatory activity at dose of 50 mg/kg.
Key word : Clerodendrum paniculatum, antioxidant, antiiflammation.

Introduction

Inflammatory and immune responses are evoked as a host defense against various environmental stimuli. These include external stresses such as infection by pathogens, inhalation of foreign materials like asbestos, exposure to heavy metals, ultraviolet and ionizing radiation as well as internal stresses such as excessive accumulation of metabolites like urate crystals and oxidized lipids, superoxide and nitric oxide, autoimmune responses and cancer.[1]

In recent years, studies of antioxidant and antiinflammatory activity increased because the combination has the potential to prevent various chronic diseases. Many studies showed a relation between increasing of oxidative cell damage due to an imbalance between free radicals and natural antioxidants in the body, so that it becomes a major factor of many diseases such as cardiovascular, cancer, aging, and others.[2]

Pagoda flower (*Clerodendum pamiculatum* L.) is a plant belonging to the genus Clerodendrum among 580 different species and spread widely in Asia, Africa, America, and Australia. Some species of this genus have been used in traditional medicine in Asia and Africa. India, China, Korea, Thailand, and Japan are the countries that have used several species of this genus in medical practice.[3] The ability of some species in this genus such as *C. serratum, C. infortunatum, C. inerme, C. viscosum*, and *C. phlomidis*, have a good antoxidant in scavenging of free radicals DPPH.[4]

The treatment of inflammatory disease such as rheumatoid arthritis and asthma, extracts of leaves and roots of some species of the genus Clerodendrum have been used such as *C. Phlomidis*,[1] *C. Serratum*,[6] *C. Trichotomum*,[1] *C. Chinense*,[7] *C. Petasites*,[1] *C. inerme*,[1,5] and *C. viscosum*.[8]

The aim of this study was to evaluate the antioxidant[29-30] and anti-inflammatory activity of Clerodendrum paniculatum L. ethanolum extract. Antioxidant activity measured by scavenging of DPPH and antiinflammatory activity measured by paw edema and granuloma cotton pellet methods.

Experimental

The *Clerodendrum paniculatum* L. leaves were obtained from Berastagi, North Sumatera Province, Indonesia. The leaves of *C. paniculatum* were dried at 50-60°C and milled into powder. The dried leaves powder was extracted with ethanol by maceration method. Extract from solvent were concentrated by a rotary evaporator.

Antioxidant activity

A value of antioxidant activity was evaluated using DPPH free radical scavenging method. 1 ml of extract (0.25 to 2 mg/ml in ethanol) was added to 3 ml of DPPH solution in ethanol (40 mg/l) in a test tube. Control used was DPPH solution 40 mg/l. Extract and DPPH solution were mixed for 1 minute and incubated at room temperature (25°C) for 30 minutes. Absorbance was measured at wavelength 517,5 nm.[9] Antioxidant activity was calculated by the equation:

$$\%\ DPPH\ inhibition = \frac{A^0 - A^1}{A^0} \times 100$$

Explanation : A^0 = control absorbance

A^1 = sample absorbance

The scavenging of DPPH radical ability was indicated by a decrease at the absorbance measurement. IC_{50} value is obtained from the calculation if the percent (%) inhibition is 50%.[10,11.21-25,27]

Anti-inflammatory activity

Paw edema

The different concentrations of ethanolic extract of *C.paniculatum* leaves were suspended with CMC Na 1%. The concentration of extract were 100, 200 and 400 mg/kgbw. Positive control used was acetosa 33 mg/kg dose. Negative control used was suspended of CMC Na 1%. White male rats as experimental animals were divided into five groups and each consisted of six rats.

Leg was marked as the boundary measurements on plethysmometer, then the volume of a normal leg was measured. At fourty (40) minute after administration of the extracts, each animal was given a 0.05 ml carrageenan solution with a concentration of 1% on the soles of the leg. Furthermore, the volume of leg were measured on 30, 60, 120, and 180 minutes after the carrageenan induction. Based on the measurement results obtained edema value data from reducing the volume of edema which were formed at each time of measurement compared to the volume of a normal leg, and the means of percent inhibition of edema is calculating the percentage ratio of edema formed at each measurement time.[12,13,26,28] Percent inhibition of inflammation calculated by the formula:

Edema volume (ml) = $V_0 - V_t$

% inhibition = $\frac{V_{c0} - V_S}{V_{c0}}$ x

Explanation V_0 = Normal leg volume
Vt = Inflammation leg volume (t)
V_{C0} = Negative control leg volume
V_S = Sample leg volume

Granuloma cotton pellet

The test was performed on the rats using the cotton pellet induced granuloma method. The rats were anesthetized under light ether and an incision was made on the lumbar region by blunted forceps, a subcutaneous tunnel was made and cotton pellet (20±1 mg) was inserted in the groin area. All the animals received ethanol extract of *C.paniculatum* leaves (25, 50, and 100 mg/kgbw), Acetosal 33 mg/kgbw as positive control and CMC Na 1% as negative control given orally depending upon their respective grouping for seven consecutive days from the day of cotton pellet insertion. On the day 8, animals were anesthetized again and cotton pellets were removed and dried to constant mass.[14]

Statistical analysis

For statistical analysis, data were analyzed by the one-way ANOVA followed by a Tukey post-hoc for pair-wise comparisons between groups. A $p<0.05$ indicated a significant difference between the groups.[15]

Result and Discussion

Antioxidant Activities

Several concentrations, ranging from 10-50 µg/ml of the ethanolic extract of *C. paniculatum* leaves were tested for their antioxidant antivity in vitro moldels. It was observed that the tested compound scavenged DPPH free radicals. The antioxidant activity of ethanol extract of *C. paniculatum* can be seen in the Table 1.

Table 1. The antioxidant ativity of ethanolic extract of *C. paniculatum*

Concentration	Absorbance	Inhibition Concentration (%)
0 (blanko)	0.93515	0
10 µg/ml	0.69426	25.7792
20 µg/ml	0.59134	36.7675
30 µg/ml	0.37263	60.1595
40 µg/ml	0.30765	67.1068
50 µg/ml	0.15253	83.6737

The simple approach of testing data interpretation antioxidant activity using DPPH is by plotting absorbance value data and concentration, followed by finding the IC_{50} value as an endpoint in the study.[16,17] IC_{50} value is obtained by calculating the linear regression equation obtained by plotting the concentration of the test solution and the percent DPPH antioxidant activity as a parameter. IC_{50} values obtained by measuring the antioxidant activity of ethanol extract of *C. paniculatum* leaves is 27.73326 µg/ml, is included in the category of very strong.[18]

Antiinflammatory Activity

The test results of anti-inflammatory activity indicated by the edema volume and the percent inhibition of edema is presented in Table 2.

Table 2. Antiinflammatory activity by paw edema method

No	Group of Test	Edema Volume (ml)			
		30 min	60 min	120 min	180 min
1	CMC Na 1%	2.06±0.06#	2.89±0.08#	3.67±0.12#	4.59±0.26#
2	Acetosal 33 mg/kg	1.69±0.13*	1.96±0.18*	2.45±0.17*	2.61±0.18*
3	EECL 25 mg/kg	1.89±0.18	2.31±0.28*#	3.15±0.25*#	3.67±0.25*#
4	EECL50 mg/kg	1.81±0.11*	2.18±0.19*	2.72±0.09*	2.82±0.12*
5	EECL 100 mg/kg	1.71±0.11*	2.12±0.16*	2.48±0.23*	2.56±0.31*

Explanation: * (Significantly different to the CMC Na), # (Significantly different to the Acetosal), Significantly different= p>0.05, EECL (ethanolic extract of Clerodendrum paniculatum leaves)

Results of statistical analysis showed that the group of 25 mg/kg showed activity in reducing edema, but has not been able to reach the value of acetosal 33 mg/kg on each measurement. A group of 50 mg/kg and 100 mg/kg showed excellent results, where both groups are able to approach the value of acetosal 33 mg/kg based on the statistical test which states that the results of these two groups did not differ significantly to the positive control.

Carrageenan as the induction of inflammation leads to the release of nitric oxide (NO) in tissue injury. Compensation body on the release of NO as a mediator of inflammation is to release the non-selective NOS inhibitors (Aminoguanidine hemisulfat), NG-monomethyl-L-arginine acetate (L-NMMA) that serves to suppress the release of NO. This compensation process occurs in 2.5 to 8 hours.[19,20]

The result of anti-inflammatory activity by cotton pellet granuloma tissue method is presented in Table 3.

Table 3. Antiinflammatory activities by cotton pellet granuloma tissue method

No	Groupof test	Granuloma tissue (mg)	Inhibition (%)
1.	CMC Na 1%	51.16±5.19#	0#
2.	Acetosal 33 mg/kg	35.00±5.25*	31.60±10.27*
3.	EECL 25 mg/kg	45.50±4.41#	11.07±8.63#
4.	EECL 50 mg/kg	32.50±6.62*	36.48±12.94*
5	EECL 100 mg/kg	24.33±5.16*#	52.44±10.09*#

Explanation: * (Significantly different to the CMC Na), # (Significantly different to the Acetosal), Significantly different= p>0.05, EECL (ethanolic extract of Clerodendrum paniculatum leaves)

Based on the results of measurements of cotton pellet granuluma tissue, dose groups of 50 mg/kg and 100 mg/kg provide activity in suppressing the formation of granuloma tissue indicated by statistical test results that showed a significantly difference to the negative control. Dose group of 50 mg/kg showed the same activity with the positive control. Dose group of 100 mg/kg had higher activity in suppressing the granuloma tissue formation compared to acetosal 33 mg/kg, shown by the percent inhibition values were higher and significantly different statistical test to the positive control.

The test group dose of 25 mg/kg did not inhibit the formation of granuoma tissue as an inflammatory response, indicated by the results of statistical tests did not show any significantly difference to the negative control.

The mechanism of activity of ethanolic extract of *C. paniculatum* leaves as an anti-inflammatory approach to look at the mechanism of phytochemical compounds in suppressing inflammation. Phytochemical compounds that have anti-inflammatory activity are phenolic (flavonoids), alkaloids and terpenoids. Based on the literature, phytochemical compounds as anti-inflammatory activity of the most widely discussed is the flavonoid compound. [21]

Anti-inflammatory activities of flavonoid based on the antioxidant properties of its phenolic groups. Tissue damage will trigger a process of homeostasis in the form acute phase response (APR) initiated by

macrophages or monocytes. Macrophages release cytokines. Locally, stromal cells also release cytokines such as IL-1, IL-6 and TNF. Cytokines and TNF induces gene expression. In this process the released mediators that will promote chemotaxis of inflammatory mediators, dilate blood vessels by NO and affect the fluidity of the blood to form edema. This process can be inhibited by flavonoid antioxidants activity and inhibit the chain reaction that aggrvate inflammatory.

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