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Chemical characterization and anti-inflammatory activity of Piladang Leaf (*Coleus Atropurpureus*) Extract

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ABSTRACT

Secondary metabolites in medicinal plants were responsible for pharmacological activities of plants. In this research, chemical characterization of piladang (*Coleus atropurpureus*) leaf extract and evaluation of its anti-inflammatory activity had been done. Chemical characterization was done by phytochemical screening as well as the total phenolics and flavonoids content determination in piladang leaf extract. The anti-inflammatory activity was evaluated by using method of carrageenan-induced oedema on the dorsal area of mice. Experimental animals were grouped into 5 groups, consist of group I which was administered with 0.5% Na CMC, group II which was administered with aspirin 130 mg/ kg, group III, IV, and V which were administered with preparation of extract test by doses 200, 400, and 600 mg/kg respectively. Parameters observed were oedema volume and inhibiting percentage of oedema. As results, phytochemical examination showed positive results for alkaloids, phenolics, steroids, saponins and flavonoids, while total phenolic content (TPC) of the extract was 173.69±1.81 mg of gallic acid equivalent/g extract and total flavonoid content (TFC) was 39.73 ± 0.27 mg of quercetin equivalent/g extract. Whereas, the result of anti-inflammatory test showed that ethanolic extracts of piladang (*Coleus atropurpureus*) leaves had anti-inflammatory effect that had been evidenced by decreasing of the exudate volume ($P > 0.05$). From all of doses tested, it can be concluded that the best dose for decreasing the oedema was 600 mg /kg.

KEY WORDS: Piladang, anti-inflammatory, flavonoids, phenolics, *Coleus atropurpureus*.

1. INTRODUCTION

The use of herbs for traditional medicine have been started since human culture exist around the world. Herbal medicine recipes from our ancestor were informed by hereditary system. Some historical evidence also indicated the system of traditional medicine in Indonesia, such as carvings at Borobudur temple which showed the king was drinking herbal medicine in the cup. Ancient books contained recipe potions had written on palm leaves and the presence of the tradition in drinking Jamu to maintain a healthy body. However, most of the use of plants to maintain health still do not have sufficient scientific evidence. Therefore, recently has been growing research on testing of pharmacological activity and search chemical components of medicinal plants that are responsible for the activity.

One of the traditional medicinal plant in West Sumatera, Indonesia is Piladang (*Coleus atropurpureus*). Traditionally, Piladang leaves were used to cure various inflammatory disorders such as ulcers, hemorrhoid, eye inflammation and fever (Zulfahmi, 2010). Several previous studies mentioned that piladang leaf extracts have antimicrobial activity against Salmonella enteridis (Aryati, 2007), tuberculosis (Ahmad, 2014), and some other bacteria gram (+) and (-) (Kumala 2009). The piladang leaves contain phenolic, flavonoids and volatile components which were very effective as an anti-inflammatory agent in dealing with oxidative stress conditions (Sony, 2012).

Based on the above information, there was a need to do research on piladang leaf extract which aimed at chemical characterization of extract and anti-inflammatory activity evaluation. Chemical characterization of extract consists of phytochemical test, determination of total flavonoids and phenolic content. While anti-inflammatory activity evaluation was done by a method of making oedema on the dorsal area of mice induced with karageenan.

2. RESEARCH PROCEDURES

Materials: A set of laboratory glassware, rotary evaporator, Piladang leaves, carrageenan, Na CMC, ethanol, analytical balance, pipettes, drop plate, reagents, phytochemicals, Follin Ciocalteu, Gallic acid, Quercetin, $AlCl_3$, Spectrophotometer UV- Vis.

Plant Collection and Identification: Piladang leaves were collected wildly on March 2014 in the area of Bukittinggi, West Sumatra. The leaves were washed and air dried for a week. Plant specimen was identified and deposited in the Herbarium of Biology Department of Andalas University with collection number of 275/K-ID/ANDA/XI/2014.

Extraction of Piladang Leaves: 2100 g of fresh leaves were dried for 1 week or until the leaves can be crushed and broken by finger. Dry leaves were powdered and had weight of 330 g. Piladang leaf powder was extracted by using maceration method with ethanol as solvent extraction. Maceration was done for 4x 3 days. Each macerate was filtered and the filtrate was concentrated using vacuum distillation and continued with a rotary evaporator to obtain a thick extract.

Chemical Characterizations:

Phytochemical screening: Phytochemical screening was done by Culvenor & Fitzgerald and Simes method. 0.5 g piladang extract was fractionated with aquadest and chloroform (1:1) about 5 ml for each solvent and then shaked vigorously and allowed to stand and formed two layers; organic (chloroform layer and aqua layer). Chloroform layer was used to examine the presence of alkaloids, terpenoids and steroids otherwise aqua layer was used to examine the presence of fenolics, flavonoids and saponins.

Flavonoid test (Cyanidine test): Put 1-2 drops of the water layer on the plate drops, add a magnesium metal powder and a few drops of HCl (p), the incidence of yellow-orange to red indicate the presence of flavonoid compounds.

Phenolic test: Put 1-2 drops of the water layer on the plate drops, then add 1-2 drops of reagent $FeCl_3$, the formation of a blue color signifies their phenolic content.

Saponin test: Put a layer of water in a test tube and shake, when the foam formed a permanent (± 15 minutes) showed a saponin.

Terpenoid and steroid test (Simes method): Chloroform layer was filtered with norit and put 1-2 drops clear filtrate on the plate drops and allowed to dry, after dried add 2 drops of acetic acid anhydrous and 1 drop of concentrated sulfuric acid (reagent Lieberman-Bouchard) if formed red is positive terpenoids and if formed blue or green color means positive steroid.

Alkaloid test (method Culvenore-First gerald): 2 ml chloroform layer was added with 10 drops of sulfuric acid 2 N, then shake vigorously and let stand until two layers formed, took acid layer and add 1-2 drops of reagent mayer, positive reaction alkaloids characterized by a creamy precipitate.

Determination of Total Phenolic compound (Donald, 2001): Piladang leaf extract solution (250 ppm) and gallic acid standard solution (20, 40, 60, 80 and 100 ppm, 0.5 ml of each solution was pippeted into a separate test tube and mixed with 5 ml Folin – Ciocalteu reagent (diluted 1: 10 distilled water). Then add 4 ml of 1M sodium carbonate solution, shaked homogeneous. Leave at room temperature for 15 minutes and measure the absorbance of each solution at a wavelength of 743 nm using a UV-VIS spectrophotometer. The concentration and the absorbance of standard solution of gallic acid was used to create a calibration curve in order to obtain a regression equation. The absorbance value of the piladang extract solution incorporated into the regression equation in order to obtain the total phenolic compound.

Determination of the total content flavonoids (Chang, 2002): Piladang leaf extract solution (1000 ppm) and standard solution quercetin (25, 50, 75, 100 and 125 ppm 0.5 ml of each solution was pippeted into a separate test tube and mixed with 1.5 ml of ethanol, and added 0.1 ml of 10% aluminum chloride solution, then added 0.1 mL of 1 M sodium acetate and 2.8 ml of distilled water. Let stand for 30 minutes, then measured by UV-Vis spectrophotometer at wavelength of 428nm. The concentration and the absorbance of standard solution of quecetin was used to create a calibration curve in order to obtain a regression equation. The absorbance value of the piladang extract solution incorporated into the regression equation in order to obtain the total phenolic compound.

Evaluation of anti-inflammatory activity: In the carrageenan-induced model, inflammation was produced according to the method of Winter (1962), as described previously in Owoyele (2004). Male Swiss albino mice used were mice with approximately weight of 30 g. The animals received food and water ad libitum and were maintained in a room with light and temperature regulation. Inflammation was induced by injection of carrageenan (200 µL of 1% freshly prepared solution of carrageenan in saline water) into air pouch at dorsal area of mice.

Animals were divided into 5 groups. Group I (carrageenan control) was given only the suspension of Na. CMC 10%; Group II (positive control) was administered with acetosal 130 mg/kg BW; Group III, IV and V received 200, 400 and 600 mg/kg piladang extract respectively suspended in Na CMC 10%. All of test preparations were given orally at a volume of 1% of body weight. Furthermore, the drug is administered every day for 4 days. Parameters of anti-inflammatory activity determined were exudate volume and percentage of oedema inhibition. Blood was drawn from the tail vein of mice.

3. RESULTS AND DISCUSSION

The phytochemical screening of the plant studied showed the presence of phenolic, flavonoids, phytosterol, alkaloids and saponins. (Table 1) .

The content of total phenolic compounds were determined by Folin Ciocalteu methods, using gallic acid as standard compound and measured at a wavelength of 743 nm. While total flavonoid content was determined by a complexion of $AlCl_3$ method, using quercetin as standard compound and measured at a wavelength of 428 nm. Based on the calibration curve that formed from a series of specific standard compound concentration data and linear regression equation was obtained as follows in Table 2. Levels of total phenolic obtained was 173.69 ± 1.81 mg of gallic acid equivalent/ g extract and total flavonoid content was 39.73 ± 0.27 mg of quercetin equivalent/ g extract.

Oedema volume measurement was done on fifth day after administration of suspension of the ethanolic extract of piladang leaves. Average exudate volume measured in the control group (0.5% Na CMC suspension), dose groups of 200 mg / kg, 400 mg / kg, 600 mg / kg and standard group (acetosal 130 mg / kg) in a were 0.1975 mL,

0.0375 mL, 0.0175 mL, 0.0125 and 0.0425 mL mL respectively with percentation of inhibition of inflammatory exudates volume measured in the dorsal area of mice in the control group (0.5% Na CMC suspension), dose groups of 200 mg / kg, 400 mg / kg, 600mg / kg and standard group (aspirin 130 mg / kg) were 81.01%, 91.13%, 93.67%, 78.48 % respectively. From the result of the measurements, it could be obtained a correlation that showed a relationship between exudate volume (ml) with extract dose (mg/kg). Where the average volume of exudate decreased as equal with increased doses of the extract given, the higher the dose the less measurable exudate volume compared to the controls provided by NaCMC suspension.

Statistical test of variance analysis showed that the suspension of ethanolic extract affected the volume of oedema significantly ($P < 0.05$). The Duncan test result showed that a dose of 200 could decrease the volume of oedema, which was not significantly different with aspirin group. Volume of oedema in the group of dose of 400 mg/kg and 600 mg / kg were not significantly different, and has a better effect in lowering the volume of oedema compared to group of 200 mg/kg and acetosal 130 mg/kg. A material could be having anti-inflammatory property if it experienced a reduction in swelling (% inhibition) of up to 50% or more in animal trials that induced by carrageenan 1%. From the results of research conducted, ethanolic extract of piladang leaf has potential as an anti-inflammatory, as shown by the degree of inhibition of inflammation higher than the rate of inhibition in the control group and the standard group (aspirin 130 mg/kg).

From the whole sample, it could be seen that dose of 400 mg/kg and 600 mg/kg gave better result as ant inflammatory agent. This might be happen because dose of 400 mg/kg and 600 mg / kg increased the activity and capacity of leukocyte cells that act as phagocytic cells which suppress and eliminate damaged cell and decrease the oedema volume, and it is correlated with a decreasing in the volume of oedema. Acetosal in the form of a suspension which was used as a standard drug which is one of anti-inflammatory agent that are widely used anti-inflammatory drugs to treat inflammatory reaction showed the effect that was almost comparable to the suspension of ethanolic extract of piladang leaves at dose of 200 mg/kg. Both could reduce and suppress the degree of inflammation that occurs in animal experiments.

In the chronic inflammation in normal people which were not treated, this inflammation could heal itself which is characterized by diminished or loss of volume in the area of inflammatory oedema. Usually it occurs when there is an increasing in the number of inflammatory cells in blood leukocytes, especially phagocytic cells (neutrophils segments, monocytes / macrophages). This increasing was aimed to improve the digestive process or phagocytic cells that have been damaged and agents that can damage cells attacker subsequently (Robbins, 1995). From the results of research that has been conducted and the data which were statistically analyzed it seems that ethanolic extract of piladang leaf may provide anti-inflammatory effects through its ability to inhibit and reduce the volume of oedema in the inflamed area.

Table.1. Phytochemical constituent of Piladang extract (*Coleus atropurpureus*)

Test	Result
Flavonoids	+ orange colour
Phenolics	+ bluish violet
Saponins	+ foam (20 minutes)
Phytosterols	+/- (violet)
Alkaloids	+ curdy cream)

Table.2. Data from the linear regression calibration curve of total phenolic and flavonoid

Parameter	Value	
	Total Phenolics	Total Flavonoids
Regression equation	y= 0.0096 + 0.00548x	y = - 0.03575+0.00685x
Dynamic Range	20-100 µg/ml	25-125 µg/ml
Limit of Detection	3 µg/ml	10.623 µg/ml
Limit of Quantification	20.731 µg/ml	35.409 µg/ml
Correlation coefficient	0.999	0.997
Relative Standard Deviation	1.759	0.024

Table.3.The Average of exudates volume and inhibition percentages (%) of inflammatory on dorsal area of albino mice males after administration of piladang suspension *C. atropurpureus* extract orally.

Group	Dose (mg/kg)	Exudates volume	Inhibition (%)
Control (Na CMC 0.5%)	-	0.1975 ± 0.0125^{a}	-
C. atropurpureus	200	0.0375 ± 0.005^{b}	81.01
C. atropurpureus	400	0.0175 ± 0.0095^{c}	91.13
C. atropurpureus	600	0.0125 ± 0.005^{c}	93.67
Acetosal	130	0.0425 ± 0.0095^{b}	78.45

Each exudates volume value is the mean ± SEM of 5 mices.

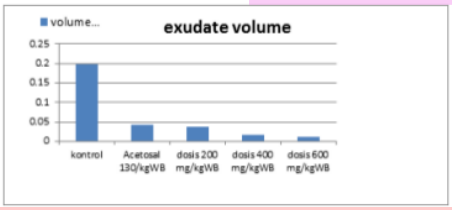

Figure.1.Correlation of exudate volume vs dose

4. CONCLUSION

Piladang leaf extract (*Coleus atropurpureus*) contained total phenolics of 173.69±1.81 mg of gallic acid equivalent/g extract and total flavonoids of 39.73±0.27 mg of quercetin equivalent/g extract. A Dose of 600 mg/kg was an effective dose of piladang leaf extract with inhibiting percentace of oedema by 93.67%. Increasing doses piladang leaf extract would improve its ability to reduce inflammatory exudate volume in the dorsal area of male albino mice ($P < 0.05$).

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