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Toxicity, Antioxidant, Analgesic and Anti-inflamantory of Ethanol Extracts of *Laportea aestuans* (Linn.) Chew

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ABSTRACT

Laportea aestuans (Linn.) Chew as original plant from Papua has been using for pain relief as traditional medication in the local community widely. The objective of this study aims to screen active compound chemical, determine LC50, IC50 and analgesic-antiinflamatory activity effect of ethanolic extract from L. aestuans (ELA). Sample that picked up from Biak Indonesia was determined in Herbarium Manokwariensis. Ethanolic extract was tested the toxicity with BSLT and antioxidant effect by DPPH methode. Then ELA was evaluated analgesic with tail flick test and anti-inflammatory effect with carragen-induced paw edema with 0.3 g/kg, 0.6 g/kg and 1.2 g/kg dose on white male rat. This study resulted that ethanolic extract Laportea aestuans (Linn) Chew positively contained alkaloids, flavonoids, saponins and tannins. The extract was toxic with LC50 285 µg/mL and weak antioxidant activity with IC50 of ELA had 554.30 µg/ml. According to tail flick methods, dose maximum of ELA was 1.2 g/kg resulted percent analgesic effect 54.70% and 0.6 g/kg presented 39.62% antiinflamantory activity.

Keywords: *Laportea aestuans* (Linn.) *Chew*; Toxicity; Antioxidant; Analgesic; Anti-inflammatory

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INTRODUCTION

Indonesia *Urticaceae* has been used as a traditional medicine to treat several infectious diseases such as ulcers, carbuncle, dysentery, urinary infectious and itching. Based on data from IPNI, there are 163 species of stinging netlle or genus Laportea spread all over the world [1]. *Urticaceae* on Java island consist of 22 genera and 76 species [2] while on Papua, there was five species has been known for instance *Laportea aestuans*, *Laportea decumana* [3] *Laportea sinuata, Laportea interupta*, *Dendrocnide peltata and Laportea* sp [4-6].

L. aestuans is widely distributed throughout the world from tropical to temperate region especially Indonesia that growing in temperate and tropical wasteland areas around the world. This plant grows 2 to 4 meters high and produces pointed leaves and white to yellowish flowers and belongs to the family of Urticaceaea. The genus name *Urtica* comes from the Latin verb *urere*, meaning 'to burn,' because of these stinging hairs. Stingging nettle or Daun Gatal (the Papuan people call *L. aestuans*) is a plant whose have hairs along the leaf and stem leaves [7].

L. aestuans leaf is original plant from Papua Indonesia has been using for pain relief as traditional medication in the local community widely. This plant is widely used by people local such as Biak, Wamena, Sentani and other places in Papua Indonesia as an analgesic such as pain relief, soreness, stomach pain and fatigue effectively [7-9]. The

plant has found its application in ethnomedicine in Papua [10,11] by picking and applied to painful body. It will give itchy sense as the medicine work according believer people. *L. aestuans* contain of tannins, phlobatannins, flavanoid, glicosides, moderately present in saponins and tracer in alkaloid [12]. Extract ethylacetat has antibacterial activity to *E. coli*, methanol extract againsts *B. cereus* [13].

L. aestuans claimed to have many medical uses. The crushed stem is employed as an inflammatory agent. The leaf is used as an abortifacient, laxative, pain-killer, febrifuge, eye treatment, pulmonary and stomatch troubles. The phytochemical mucilage from the plant is used as ahesives. The leaf and flowers parts of the plant can also be used in medicine to cure diarrhea and dysentery [14,15]. The plant though used in traditional medicine extensively has not enjoyed much pharmacologies research. A recent research however revealed that the phytochemical screening of L. aestuans revealed the presence of saponins, tannins, flavanoids, phlobatanins and ardiac glycosides [12]. The toxicity of crude extract leaf reported hexane faction was very toxic. The essensial oil from the plant is dominated by methyl salicylate and had significant antioxidant and antimicrobial [16-18]. Therefore, there is need to investigate the analgesic, antiinflamtory, antioxidant and toxicity activity of *L. aestuans* leaf extract ethanol.

MATERIALS AND METHODS

Plant Material

The leaves of *L. aestuans* was collected from Biak Papua Indonesia and determined at the Herbarium Manokwariensis the Departement Biology, Universitas Negeri Papua-West Papua. The leaves are sun dried and finally dried in oven at 50°C. The leaves are blended , sieved with 100 um pore and stored in a sealed container.

Preparation of Plant Extracts

A total of 200 g of powder leaves powder was weighed and soaked in 750 ml 95% ethanol then stirred occasionally. Maceration process were repeated 2 times for 3 days. Concentrated extract was obtained by filter paper and evaporated with a rotary evaporator at 40°C then stored.

Toxicity Activities by Brine Shrimp Test

A 100 mg Extract was put into a 100 mL flask and dissolved with ethanol to obtain a stock solution of 1000 ppm. The test sample was made with various concentrations of 25, 50, 75, 100,125 and 150 ppm. Each test solution was poured into a vial of 5 mL then ethanol-free dried. Shrimp eggs weighed as much as 3 g and then bred using sea water from the beach Amai Sentani as much as 2 L with pH 8.64. After two days (2 × 24 hours) the eggs are ready for use. Test sample (150, 125, 100, 75, 50, 25 µg/ml) in vials was made by adding with 1% 50 µl DMSO solution, pouring sea water and putting 10 shrimp larvae. After 24 hours, the number of dead larvae was calculated. The obtained data is calculated LC_{50} by probit method [19].

Antioxidant Activities by DPPH Methods

Amount 3.9432 mg DPPH was added with ethanol in 100 ml in measuring flask so obtained DPPH 100 µM. A total of 50 mg of extract was dissolved with ethanol in a 50 ml measuring flask. Solution was diluted that became 100, 200, 300, 400, 500, 600 and 700 ppm respectively into a 10 ml measuring flask. A mount 3 ml DPPH solution and 3 ml test sample were added in test tube, homogenized and incubated for 30 minutes. Furthermore, solution was measured the absorbance at 518 nm wavelength. The absorbance of the extract obtained was compared with the absorbance of DPPH blanks to obtain a percentage of resistance to DPPH radicals [20]. The same treatment was performed on the positive control of Vitamin C. All test and anylises were determined in triplicate and avaraged witth formula:

$$Percent\ inhibition\ of\ DPPH = \frac{Abs\ Control - Abs\ Sample}{Abs\ Control} \times 100$$

IC_{50} was determined by following linear regression calculations using the equation y=Ax+B, where x is the concentration (ppm) and y is the percent of inhibition (%). Antioxidant activity, expressed by IC_{50} (50% Inhibitor concentration), is concentration of sample which can reduce DPPH radical by 50%. The value of IC_{50} is obtained from the value of x after substituting y with a value of 50.

Analgesic Activity Test by Tail Flick

Each rat was acclimatized for 7 days then administered 18 hours. Initial test with hot test for 3-5 seconds were measured L0 (reaction time) as 0 second. Negative control group was given suspension of CMC Na 0,5%, positive control group was given acetosal suspension and treatment group was given test sample extract stinging nettle. Analgesiometer was set at infrared radiation to observations tail flick. About 2 centimeter of tail rat were irradiated to the light made up to 60 minutes, with a time interval of 15 minutes for each observation. Observations were recorded by measuring the time wagging rat tail when irradiated at 55°C until began to feel the pain/heat (tail response wagging) [21]. The test and anylises were determined in five replication The effect of the extract on the analgesic effect is determined by calculating the tail response time with formula:

$$Vu=Vt-Vo$$

Where, Vu: Tail response time wagging every time t; Vt: Tail response time wails after being treated (t); Vo: Tail response time wags before being treated

Then calculated AUC (Area under curve) was determined by averaging area under the curve which is the average relationship of tail response time wag each unit time, with the formula:

$$AUC\ \frac{n}{n-1} = \frac{Vt(n-1)+Vt(n)}{2}[t(n)-t(n-n)]$$

Where, $V\ t_{n-1}$=tail response time wagging average on $t_{n-1;}$ $V\ t_n$=tail response time wagging average on t_n

Percentage of pain constraint (PPC) was determined with formulation:

$$\%PPC = \frac{AUC\ treatment - AUC\ control}{AUC\ treatment} \times 100\%$$

Where, AUC control = AUC average response time curve to negative control time; AUC treatments = AUC average response time curve to time of treatment group

Dose of Stinging Nettle Extract

The dose conversion for humans with 70 kg in rat with BW 200 g was 0.018. The average Indonesian person weighs 50 kg. The dose of stingging nettle leaf extract commonly used by people is 2.380 g then the dose for the rat is:

= (70/50 × 2.380 g) × 0.018

= 0.06 g/200 g mice

= 0.3 g/kg BW

In the experiment used doses of stemmed leaf extract are:

Group p1=1 × 0.3 g/kg BW=0.3/kg BW

Group p2=2 × 0.3 g/kg BW=0.6 g/kg BW

Group p3=4 × 0.3 g/kgBW=1.2 g/kg BW

Dose of Acetosal

Each tablet contains 500 mg of acetosal. The dose conversion of acetosal dose for humans with BW 70 kg in mice with BW 200 g was 0.018. The average Indonesian person weighs 50 kg. The dose used in the study=0.35 mg/kg BW

Antiinflammatory Activity Test

This research was conducted by evaluation method of udem inhibition on rat foot formed by induction of carrageenan. In this study used white male rats weighing 200-250 g. These rats were acclimatized for one week at room temperature, fed in the form of pellets and drinking water to taste. Rats were grouped into five groups: one group as positive control, one negative control group and three other groups were given the extract stingging nettle with the specified dosage. Each group was consisted of 3 rats in the ethanol extract test group, 5 rats in the positive control group and 5 rats in the negative group. Rats had been fed for 18 hours before the experiment began (drinking water was still given enough). Before rats were given the test material, the rat's leg volume was measured using a pletismometer as the initial volume (V_0). In the negative control group given a 0.5% Na-CMC suspension, a positive control group was given an acetosal suspension at a dose of 200 mg/kg in CMC-Na 0.5%, the test group were each given an extract with a dose of 0.3 g/kg BW, 0.6 g/kg BW and 1.2 g/kg body weight [22].

The effect of the extract on the anti-inflammatory effect is done by calculating the volume of edema

$$Vu=Vt-Vo$$

Where, Vu: volume of mouse foot udem every time t; Vt: the volume of rat foot udem after reconstituted with 1% carrageenan at time (t); Vo: volume of rat foot udem before being given carrageenan 1%

After the data obtained volume of edema, and then were made the ratio of volume vs. edema vs. time. Then the calculated AUC (Area Under Curve) is the average area under the curve which is the relation of the volume of edema on average per unit time, by the formula:

$$AUC\ \frac{n}{n-1} = \frac{Vt(n-1) + Vt(n)}{2}[t(n) - t(n-n)]$$

Where, V t_{n-1} = volume avarage of edema at $t_{n-1;}$ V t_n = volume avarage of edema at
Percent of antiinflamatory effect (% PAE) can be calculated by the following formula:

$$\%PAE = \frac{AUC\ treatment - AUC\ control}{AUS\ treatment} \times 100\%$$

Where, AUC treatment = Average AUC of edema volume curve with time for treatment group ; AUC cotrol = Average AUC of edema volume curve vs. time of negative control

RESULTS AND DISCUSSION

The collection of stingging nettle samples was obtained from Biak Numfor District, Papua (The phytochemical screening showed that stingging nettle (*Laportea aestuans (L)* Chew) contained various secondary metabolite compounds such as alkaloids, flavonoids, saponins, tannins and undetectable quinone compounds (Figure 1).

Figure 1: Stingging nettle leaves and simplicia (L. aestuans (L) Chew)

Brine Shrimp Lethality Test/Cytotoxic Activity Test

This test showed that ethanol extract of *L. aestuans* had LC_{50} 285 µg/mL. The LC_{50} value is smaller than 1000 µg/mL indicated that the extract was toxic. Consequently, stigging nettle has potential cytotoxic activity which can be developed as an anticancer [19, 23]. Based on LC_{50} with BSLT butanol fraction of *L. aestuans* (Gaud) was 117.8520 µg/ml [17] but LC_{50} metyl salicylate dominated essential oil of *L. aestuans* (Gaud) was 367.1805 µg/ml [24]. In this experiment, BSLT used nauplius stage (24 hours) of *A. salina* to determine LC_{50} value because it is more sensitive to toxic compounds [25]. Pathway exposure to toxic compounds ethanol extract of stingging nettle in nauplius began through oral and dermal parts. At the oral, toxic compounds was absorbed into the gastrointestinal tract. While at the dermal, absorption process occurs through the cell membrane. After the absorption process, toxic compounds were distributed into the body A. salina, metabolism reaction process and finally causing death.

The present of alkaloids and flavanoids can cause death to the larvae of *A. Salina* [26]. Alkaloid (vincristine and vinblastin) compounds inhibited the growth of cancer cells. This compounds act as an antimitotic agent by binding tubulin dimers that can interfere microtubules at metaphase. Because cell mitosis process were disrupted so the proliferation of cancer cells is inhibited [27]. Flavanoids are polyphenol subclass (6000 phenolic coumpounds) which founded in plant with two or more rings aromatics [28]**.** Flavanoids also prevented inflammation associated skin cancer and cardiovascular disease with induce cell death in maloma [29,30].

Antioxidant Activities by DPPH Methods

The value of IC_{50} of ELA had 554.30 µg/ml that was indicated weak antoxidant activity [31]. Because ELA had only tannins and flavanoid was not detected, their antioxidant activity is low. Tannins nd flavonoid or polyphenol group compounds have hydroxyl groups that can donate hydrogen atoms to neutralize free radicals DPPH [30] .

In other side, etanolic extract of L. aestuas using DPPH 1,1-diphenyl-2-pircryl hydraziln obtained IC_{50} value of 15.0 ug/ml [16]. Percent inhibition of L aestuans essential oils showed antioxidant activity with valued at 0.1 mg/ml and 0.2 mg/ml were 84.46% and 86.87% [24]. Methanol fraction showed an inhibition activity with concentration of

0.0625-0.100 mg/ml was 80-92%. Different value of these IC_{50} can be caused by differences in plant habitat *L. aestuans*. This indicates that the L aestuans from Papua has a lower antioxidant compared with itchy leaves from Nigeria

Tail Flick

This study used male rats because biological conditions are more stable than female rats. The animals are acclimated for 8 hours to made the same animal condition and reduce the influence of the food consumed. The stimulus given to test animal by heat stimulation with temperature 55 °C. Pain was started at 45 °C and hot receptors respond to temperatures of 30-45°C. Temperatures above 45°C caused tissue damage and made pain. Pain for the rat response was assessed as a wagging tail movement. Rats began to feel pain when licking the back foot and or jump [32] (Figure 2).

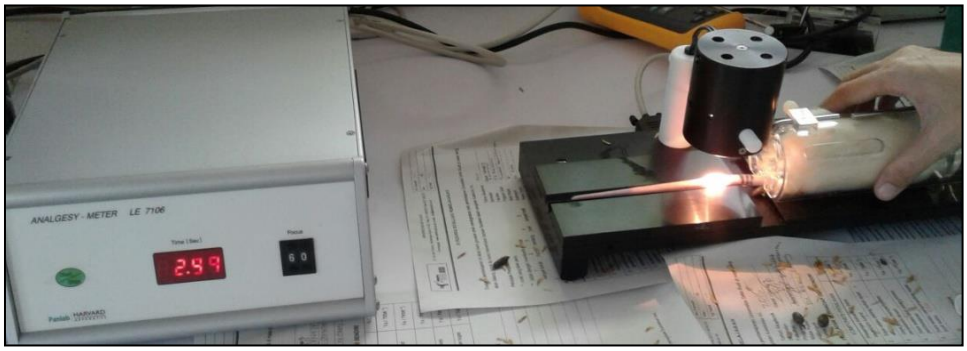

Figure 2: Analgesic activity test used tail flick to male rat

In negative control group, the average number of animal test responses increased and finally decreased. It was caused of negative control is not contained active substances that can reduce pain. In the positive control showed an increase average response of animal testing to pain stimulation. In humans, the analgesic effect of acetosal was at 2-4 hours while in rats at 120 minutes. It was caused by difference species made different metabolism (Table 1).

Table 1: Time of fail flick rat test

Group	Dose	Average Time (second)				
		0	15	30	45	60
Treated 1 (T1)	0.3 g/kg	4.93	6.15	6.71	7.86	9.05
Treated 2 (T2)	0.6 g/kg	5.61	6.50	8.10	9.47	10.91
Treated 3 (T3)	1.2 g/kg	4.89	6.76	9.16	10.82	12.77
Positive control (Acetosa)	0.35 g/kg	4.00	4.73	9.20	11.29	12.43
Negative control (CMC-Na)	0.5%	3.99	4.24	3.98	3.98	3.85

From the treatment group ELA showed that T1 (dose 0.3 g/kgBM) and T2 (dose 0.6 g/kgBM) had analgesic effect smaller than aspirin. But group T3 (dose 1.2 g/kgBM) and acetosal were almost the same. T3 (dose 1.2 g/kgBM) is the maximum dose had analgesic potential is similar with aspirin to reduce pain in rats. T3 had analgetiknya effect in fifteenth minutes until sixteenth minutes and stronger than positive control. This suggested that the higher the dose of ELA the greater the effect of pain reduction. Several similar plants (*L. interupta* and *Dendocidne sinuata)* that have been tested for analgesic activity have lower analgesic power than L. aestuans [33,34] (Table 2).

Table 2: Percentage of analgesic activity ELA with acetosal

Group	Dosis	AUC	% PPC
T1	0.3 g/BW	173.98	41.86
T2	0.6 g/BW	243.35	50.18
T3	1.2 g/BW	291.85	54.70
Postive control (acetosal)	0.35 g/BW	558.4	51.82

Antiinflamatory Activity

After the injection of karagen, the rat showed swelling and redness in the legs and could not walk as well. Measurement of anti-inflammatory activity was shown by ability of ELA in reducing the swelling of the legs after injected with carragen (Table 3).

Tabel 3: Volume oedema of rat

Group	Dose	Value	Time							AUC	%PAE
			0	30	60	120	180	240	300		
T1	0.3 g/kg BW	Vol oedema rat	0.41	0.54	0.62	0.58	0.59	0.62	0.55	52.6	17.93
		Vu		0.13	0.21	0.18	0.19	0.22	0.14		
		AUC		1.95	5.15	11.7	10.9	12.1	10.8		
T2	0.6 g/kg BW	Vol oedema rat	0.34	0.5	0.58	0.58	0.61	0.63	0.56	71.5	39.62
		Vu		0.16	0.25	0.25	0.28	0.29	0.23		
		AUC		2.4	6.1	14.8	15.7	17	15.5		
T3	1.2 g/kg BW	Vol oedema rat	0.29	0.44	0.52	0.52	0.57	0.6	0.56	70.85	39.07
		Vu		0.14	0.22	0.22	0.28	0.31	0.26		
		AUC		2.15	5.5	13.4	15.1	17.6	17.1		
+ control	360 mg/kg BW	Vol oedema rat	0.218	0.31	0.388	0.31	0.36	0.408	0.36	46.65	7.46
		Vu		0.092	0.17	0.092	0.142	0.19	0.36		
		AUC		1.38	3.93	7.86	7.02	9.96	16.5		
- control	0.005	Vol oedema rat	0.222	0.3	0.396	0.326	0.4	0.416	0.37	43.17	
		Vu		0.078	0.174	0.104	0.178	0.194	0.148		
		AUC		1.17	3.78	8.34	8.46	11.16	10.26		

Based on % PAE in table 4, the maximum anti-inflammatory effect of ELA was dose 0.6 g/KgBM. So it can be seen that the greatest anti-inflammatory effect is ELA dose 0.6 g/KgBW, then dose 1.2 g/KgBW, followed dose 0.3 g/KgBW eventhough positive control (acetosal) 360 mg/KgBW. It explained that ELA have great potential in reducing inflammation when compared with acetosal.

The ability of an active coumpond in ELA reduced inflammation in the legs of rat due to carragen injection which expressed as anti-inflammatory. The value of anti-inflammatory activity was obtained by comparing the area under the volume curve of inflammation of the ELA and positive control with the area under the negative control curve. The area under the curve provides information about the potential of ELA to reduce inflammation when compared with negative control (CMC Na 0.5%). The greater the area under the curve means the greater the volume of inflammation caused.

Saponin antiinflammatory activity of various plants has been widely reported but their mechanisms were not explained. Saponins are composed of steroids or triterpine groups that have action such as detergents. The most likely anti-inflammatory mechanism is that saponins are thought to interact with lipid membranes such as phospholipids that are the precursors of prostaglandins and other inflammatory mediators [35].

As usual, antiinflammatory mechanisms was performed by flavonoids through several pathways are inhibition of COX enzyme activity (cyclooxygenase) and or lipooxigenase [36,37] due to COX inhibition or lipooxigenase. Inhibition of these pathways also directly leads to inhibition of eicosanoid biosynthesis and leukotrien, which is the end product of COX and lipooxigenase pathways [38,39]**.**

CONSLUSION

Ethanolic extract *Laportea aestuans* (Linn) Chew positively contained alkaloids, flavonoids, saponins and tannins. The study showed The extract was toxic with LC_{50} 285 µg/mL and weak antioxidant activity with IC_{50} of ELA had 554.30 µg/ml. According to tail flick methods, dose maximum of ELA was 1.2 g/kg resulted percent analgesic effect 54.70%) and 0.6 g/kg presented 39.62% antiinflamantory activity.

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