

# Anti-inflammatory Effect of *Phyllanthus niruri* L. from Indonesia (Pre-clinical Study)

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## ABSTRACT

Indonesian people often use *Phyllanthus niruri* L. (*P. niruri* L.) to treat pain. Pain is the one of symptoms of inflammation. Some people take traditional medicine to treat this pain, because some analgesic drugs have several adverse effects. The aim of this research is to explore the anti-inflammatory effect of ethyl acetate fraction of ethanolic extract of *P. niruri* L. (EAPN) from Indonesia in rats induced by carrageenan. A total of 25 rats were divided into 5 groups. Group 1 (diclofenac sodium 50 mg/kgbw), group 2 (aqueous extract), groups 3,4 and 5 were ethyl acetate fraction at dosage of 250; 500 and 1000 mg/kgbw respectively. Group 1 was treated by diclofenac sodium at dose of 50 mg/kgbw, group II was treated by aqueous 2mL/rats, groups III, IV and V were treated by EAPN at dosage of 250; 500 and 1000mg/kgbw respectively. Thirty minutes later, all rats were injected by carrageenan 0,1 mL 1% w/v sub cutaneous into back rat's left paw. Paw volumes were measured by Plethysmometer on minutes 0, 60, 90, 150, 210, 330. Area under the curve (AUC) were calculated from these results (AUC 0-30; AUC 0-90; 0-150; AUC 0-210 and AUC 0-330). The AUC of EAPN at dosage of 250; 500 and 1000mg/kgbw smaller than negative control significantly ( $P < 0.05$ ). EAPN contains alkaloids, flavonoids, terpenoids, saponins and triterpens. EAPN has anti-inflammatory effect in rats induced by carrageenan.

**Key words:** Anti-inflammatory, *Phyllanthus niruri* L., Carrageenan.

## INTRODUCTION

Indonesia is the largest biodiversity country in the world after Brazilia. Ironically, the development of phytopharmaca in Indonesia is very slow. Traditional Indonesian society often use traditional medicinal plants to cure the disease. One plant that is often used to treat diseases is *P. niruri* L. This plant was used to treat pain.<sup>1</sup> *P. niruri* L. is a species of the genus *Phyllanthus*. This genus consists of more than 600 species of plants.<sup>2</sup> Some other communities in the world, such as in Africa, *P. fraternus* (a species of the genus *Phyllanthus*) is used to treatment of diabetes, flu, colic, dyspepsia, fever, jaundice.<sup>3</sup>

Pain is one sign of inflammation. Inflammation is part of the body's immune system response to various dangerous stimuli, such as bacteria, viruses, damaged cells, toxic compounds, radiation and chemical exposure.<sup>4</sup> Inflammation can caused by infection and non-infection.<sup>5</sup> Due to this stimulation, the body will try to eliminate it and begin the healing process.<sup>6</sup> Cardinal sign of inflammation are swelling/edema, pain, fever, redness, and loss of function.<sup>5</sup>

At the present, the treatment of inflammation using NSAIDs (non-steroidal anti-inflammatory drugs). Research by Teslim *et al*, that published in 2014, found there are many side effect of this drugs, among others: high blood pressure, low blood pressure, indigestion, gastro-intestinal bleeding, ulcer, vomiting, diarrhea, abdominal pain, heart attack, kidney disorders, dizziness, comma/unconsciousness, nausea, constipation, gastric

pain and liver problems.<sup>7</sup> Due to many side effects of NSAIDs, many people used medicinal traditional plant to treat pain and inflammation. Currently, there is no study which is reporting on anti-inflammatory effect of effect ethyl acetate fraction of ethanol extract *P. niruri* L.

The aim of this research is to explore the effect ethyl acetate fraction of ethanol extract *P. niruri* as an anti-inflammatory.

## METHOD

This research has been approved by Health Research Ethics Committee of Medical Faculty of Universitas Muhammadiyah Surakarta with number 2064/A.2/KEPK-FKUMS/III/2019.

## Plant materials

Dry matters of simplisia was found from Gede market, Surakarta, Central of Java in March 2019. The rats wistar strain were found from Pharmacology laboratory of UMS.

Drug and chemical: Diclofenac sodium (Kimia Pharma) and Carrageenan (Sigma Aldrich) were found in April 2019.

## Preparation of extracts

A total of 300 g of plant samples were macerated in 4 liters of ethanol. This immersion is done for 3 days, and re-maceration is carried out. The results of maceration were then fractionated with ethyl acetate and then placed in the rotary evaporator until an ethyl acetate fraction was obtained.

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## Anti-inflammatory activity

A total of 25 rats were divided into 5 groups. Group 1 was treated by diclofenac sodium at dose of 50 mg/kgbw, group II was treated by aqueous 2 ml/rat, groups III, IV dan V were treated by EAPN at dosage of 250; 500 and 1000 mg/kgbw respectively. Thirty minutes later, all rats were injected by carrageenan 0,1 mL 1% w/v sub cutaneous into back rat's left paw.<sup>8</sup> Paw volume were measured on minutes 0; 60; 90; 150; 210; 330 by Plethysmometer. Area under the curve (AUC) were calculated on 0-30; AUC 0-90; 0-150; AUC 0-210 and AUC 0-330.

## Identification of alkaloids

A total of 0.5 g extract was inserted in the test tube + 2 mL ethanol 70% then stirred and 5mL Hcl 2N was added and heated it in a bath water. After getting cold, the mixture was filtered and some filtrate was added with Meyer's reagent drops. The emergence of orange red precipitate indicated the presence of alkaloids.<sup>9</sup>

## Identification of flavonoids

A total of 0.5 g of extract is put in a test tube + 2 mL ethanol 70% then stirred and magnesium powder 0.5 g and 3 drops of concentrated Hcl were added. The orange to red solution indicated the presence of flavonoids.<sup>8</sup>

## Identification of saponin

A total of 0.5 g of extract was put in a test tube + 2 mL ethanol 70% then stir and 20 mL of aquadest was added and shaken then left for 15-20 minutes. The magnesium 0.5 g and 3 drops of concentrated Hcl was then added. The formation of foam indicated the presence of saponin.<sup>9</sup>

## Identification of triterpenoids

A total of 0.5 g of extract was put in a test tube + 2 mL ethanol 70% then stirred and added with 1mL of chloroform and 1mL of acetic anhydrous then cooled. After it was cold, a few drops H<sub>2</sub>SO<sub>4</sub> was added. The red color ring indicated the presence of triterpenoids.<sup>9</sup>

## Tanin identification

A total 0.5 g of extract was put in a cup + 2 mL ethanol 70% then stirred and 3 drops of FeCl<sub>3</sub> was added. The occurrence of blue, black blue, green or blue green and sediment indicated the presence of tannins.<sup>9</sup>

## Identification of steroids

0.5 g of extract was put in a test tube + 2 mL ethanol 70% then it was stirred. 2 mL of chloroform and concentrated H<sub>2</sub>SO<sub>4</sub> were added by dropping them slowly through the wall of the test tube. The formation of red rings showed the presence of steroids.<sup>9</sup>

## RESULTS

### Phytochemical screening

The compound content in EAPN is scientifically validated through identification of phytochemicals. This compound is thought to be responsible for the anti-inflammatory effect. The results of the qualitative chemical analysis of EAPN are tabulated in Table 1.

### Anti-inflammatory activity

Carrageenan is used to induce inflammation. The use of carrageenan refers to research conducted by Mostofa, et al, 2017.<sup>8</sup> Diclofenac sodium (50 mg/kgbw p.o) was used as a synthetic drug during the anti-inflammatory evaluation of EAPA. The results of the volumes of rat paw edema in the five groups are presented in Table 2.

The graphic of edema on minutes 30; 60; 90; 150; 210 and 330 were presented in Figure 1. From the rat's paw edema, the AUC was calculated and described in Table 2.

From this table, it can be concluded that EAPN at dosage of 250; 500 and 1000 mg/kgbw can decrease edema volume on minutes 150-330.

## DISCUSSION

The results of this research is in line with previous research. Research by Porto at al., 2013, showed that *Phyllanthus niruri* spray-dried standardized extract has anti-inflammatory effect on mus musculus Swiss male mice (18-30 g) induced by carrageenan (1% w/v, 50 µL).<sup>10</sup> Methanol extract of *Phyllanthus niruri* decreased edema in Swiss albino rats induced by carrageenan. This extract showed a reduction of 46.80%; 55.32% and 69.14% at doses of 100; 200 and 400 mg/kg respectively. In this research, inflammation was induced by injecting 0.1 ml carrageenan (1% w/v) into the left hind paw.<sup>8</sup> The aqueous extract revealed anti-inflammatory activity significantly on carrageenan ( $p < 0.001$ ) and chronic granuloma ( $p < 0.001$ ) rodent models.<sup>11</sup> Ethanolic and aqueous extract of *P. fraternus* whole plant possess anti-inflammatory activity in on carrageenan-induced paw edema in Sprague-Dawley rats.<sup>12</sup>

**Table 1: The phytochemical profile of ethyl asetat fraction of ethanolic extract of *P. niruri* (EAPN).**

Active compounds	Results
Alkaloid	Positive
Flavonoid	Positive
Tannin	Positive
Saponin	Positive
Triterpen	Positive
Steroid	Negative

**Table 2: The AUC of edema volume on minutes 0-60; AUC 0-90; AUC 0-150; AUC 0-210 and AUC 0-330.**

		Groups				
		Diclofenac sodium at dose of 50 mg/kgbw	Aquaous 2 mL/rats	EAPN at dose 250 mg/kgbw	EAPN at dose 500 mg/kgbw	EAPN at dose 1000 mg/kgbw
AUC(ml ± SD)	AUC 0-60	1.53 ± 0.63	1.59 ± 0.71	1.59 ± 0.79	0.84 ± 0.44	1.46 ± 0.94
	AUC 0-90	3.09 ± 1.05	3.60 ± 1.06	3.00 ± 1.41	2.25 ± 0.74	2.92 ± 1.43
	AUC0-150	7.14 ± 0.71	7.11 ± 0.68	4.74 ± 1.14*	4.08 ± 0.47*	4.65 ± 0.81*
	AUC0-210	8.04 ± 1.49*	9.93 ± 0.97	5.76 ± 1.47*	4.56 ± 1.04*	5.66 ± 1.13*
	AUC0-330	10.92 ± 2.73	14.01 ± 2.12	7.20 ± 1.73*	5.04 ± 0.69*	6.84 ± 1.68*

Note: \*: Significantly different from negative control on Anova (p.0.05)

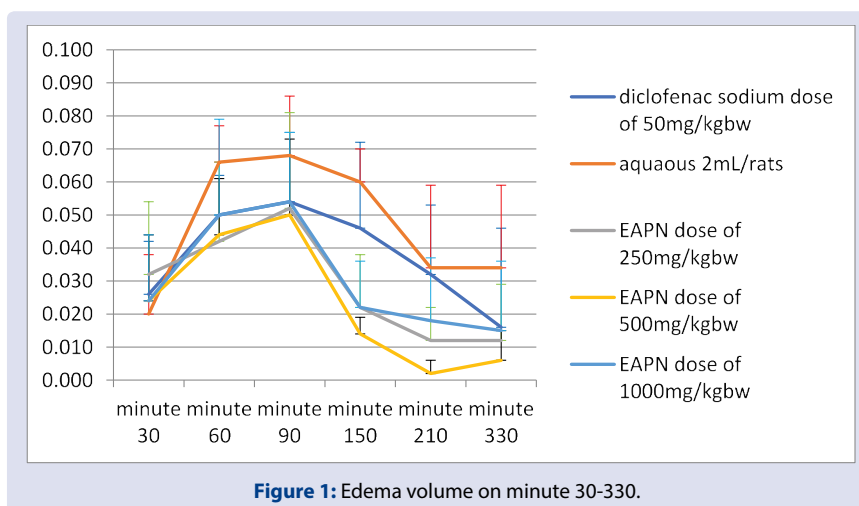


Figure 1: Edema volume on minute 30-330.

This extract contains some phytochemical, among others: alkaloids, flavonoid, tannin, saponin and triterpen. Mechanism of anti-inflammatory effect of this extract is not clear. Some alkaloids have been reported to have antihypertensive, analgesic, antidepressant, muscle relaxant, antimicrobial, anti-inflammatory, antitumor, diuretic, sympatho-mimetic and antiviral activities.<sup>13,14</sup> Flavonoid allegedly has anti-inflammatory effect by several mechanism, among others: inhibit enzymes xanthine oxidase, aldose reductase, cyclooxygenase, phosphodiesterase, Ca(+2)-ATP-ase and lipoxygenase etc.<sup>15</sup> The molecular activity of flavonoids is thought to inhibit NF-kappaB and activate protein-1 (AP-1) and erythroid 2-related factor 2 (Nrf2) factors.<sup>16</sup> One form of tannin is Corilagin ( $\beta$ -1-O-galloyl-3,6- (R)-hexahydroxydiphenoyl-d-glucose). Corilagin is a tannin isolated from several plants, including *Phyllanthus niruri* and has the effect of inhibiting the release of cytokines such as TNF- $\alpha$ , IL-1 $\beta$  and IL-6 and the production of nitric oxide, both of which are inflammatory mediators.<sup>17,18</sup> Research by Bogi *et al*, found that saponin extracted from *Zizyphus lotus*(L.) Lam inhibited paw edema dan production of nitrite.<sup>19</sup> Research by Patricia *et al.*, 2015 found natural triterpenes  $\alpha$ , $\beta$ -amyryn isolated from *Protium paniculatum* Oil-Resins inhibited the expression of COX-2 and also inhibited the formation of paw or ear edema in rats and mice.<sup>20</sup>

## CONCLUSION

Ethyl asetat fraction of ethanolic extract of *P. niruri* has anti-inflammatory effect on rat's paw induced by carrageenan.

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3. Hypolipidemic effect of ethanolic extract of Salam bark (*Syzygium polyanthum* (Wight) Walp.) from Indonesia (Preclinical study)
4. Antidiabetic activity of ethanolic extract of *Eugenia polyantha* Wight leaf from Indonesia in diabetic rat wistar strain induced by alloxan
5. Hypolipidemic effect of *Tamarindus indica* L fruit on Triton X-100-induced hyperlipidemia in Wistar rats

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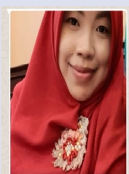
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