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# The Inhibitory Activity of *Picria fel-terrae* Lour Herbs Extract on Nitric Oxide Production toward RAW 264.7 Cells Induced by Lipopolysaccharide

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**Keywords:** *Picria fel-terrae* Lour Herbs Extract; Nitric Oxide production; Immunosuppressive effects

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**Abstract**

**AIM:** The objective of this study was to evaluate the inhibitory activity of *Picria fel-terrae* Lour on Nitric Oxide production toward RAW 264.7 cells.

**METHODS:** The extraction was obtained by maceration method using *n*-hexane, ethyl acetate and ethanol solvents and then nitric oxide (NO) production was obtained using Griess reagent.

**RESULTS:** Extract of *Picria fel-terrae* Lour herbs can reduce the NO production toward RAW 264.7 cells with induced by lipopolysaccharide has obtained nitric concentrations 12.5 and 25 µg/mL from *n*-hexane extract (72.50 ± 4.51 and 10.42 ± 1.82), ethyl acetate extract: (88.33 ± 6.51 and 30.83 ± 6.86), ethanol extract: (75.00 ± 1.91 and 22.08 ± 2.53).

**CONCLUSION:** *n*-hexane extract of *Picria fel-terrae* Lour Herbs has a high potential to reduce the NO production in LPS-stimulated RAW 264.7 cells compared to ethyl acetate and ethanol extracts of *Picria fel-terrae* Lour Herbs.

## Introduction

Nitric oxide (NO) is a necessary molecule to protect against various pathogens such as bacteria, viruses, fungi, and parasites [1], [2]. Under normal physiological conditions, NO plays a notable role in the regulation of various pathophysiological processes such as neuronal communication, vasodilatation, and neurotoxicity. However, overproduction of NO induces tissue damage associated with acute and chronic inflammations. Therefore, many researchers developed new drug as a potential inhibition on NO production related to the treatment of chronic inflammatory diseases [2]. Macrophages are significant components of the mammalian immune system, and they play a key role by providing an immediate defence against foreign agents before leukocyte migration and production of various pro-inflammatory mediators including the short-lived free radical NO. Lipopolysaccharide (LPS), a component from the cell walls of gram-negative bacteria is one of the most efficacious activators of macrophages and involves the production of pro-inflammatory cytokines. Therefore, inhibition of NO production in LPS-stimulated RAW 264.7 cells is one of the possible ways to screen various anti-inflammatory drugs [2].

Poguntano (*Picria fel-terrae* Lour.) have been various modern pharmacological investigations indicated that the extract of *Picria fel-terrae* Lour exerts diuretic, antioxidant, antipyretic, anti-diabetic, anthelmintic, anti-inflammatory, hepatoprotective, cardioprotective, analgesic activities and have inhibits activity of hepatitis B virus [3], [4], [5], [6], [7], [8], [9], [10], [11]. It can be developed a co-chemotherapeutic regimen for breast cancer, and it has antioxidant and antiproliferative activities of ethyl acetate fraction [12], [13]. Therefore, the present study was aimed to

evaluate reduced of Nitric oxide production in LPS-induced on *Picria fel-terrae* Lour herbs extract toward RAW 264.7 murine macrophage cell line.

## Material and Methods

Fresh *Picria fel-terrae* Lour herbs were collected from Tiga Lingga village, Dairi regency, Sumatera Utara province, Indonesia. Lipopolysaccharide is obtained *Escherichia coli* bacteria O111.B4 (Sigma), Dexamethasone (Harsen), Griess reagent and Nitrite Standard Solution (Biotium), *n*-hexane, ethyl acetate, ethanol 96% were procured from Smart lab.

RAW 264.7 cells were obtained from Parasitology Laboratory, Faculty of Medicine, Gadjah Mada University. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal bovine serum and 100 units/mL each of penicillin and streptomycin was grown at 37°C and 5% $CO_2$ in humidified air [14].

The extracts were prepared by Yuandani et al., 2017. The dried material was sequently macerated, briefly an amount of 500 g of *P. fel-terrae* Lour herbs. The following extracts were obtained after removal of solvents under reduced pressure [14], [15].

The NO production assay was conducted according to a previous paper by Yuandani et al. [14]. Briefly, RAW 264.7 cells ($3 \times 10^3$ cells/mL) were seeded in 96-*well plates* for 24 h. Then, cells were incubated with test samples (12.5 and 25 µg/mL) and dexamethasone (1.25 and 2.5 µg/mL) for another 24h, then stimulated with LPS (1 µg/mL). After incubation for 24h at 37°C, 5% $CO_2$, the production of nitric oxide was determined by measuring the quantity of nitrite in the medium using Griess reagent (0.1% naphthyl ethylenediamine dihydrochloride in 2.5% phosphoric acid and 1% sulfanilamide). One hundred µL of Griess reagent was added to culture supernatant, then incubated for 10 min in a dark room. A microplate reader was used to measure absorbance at 595 nm, and a standard solution of sodium nitrite was used to calculate nitrite concentrations. The concentration of nitrite in the samples was determined concerning a sodium nitrite standard curve (Biotium catalogue #30100).

Data were expressed as means ± standard error minimum (SEM) of the mean from three independent experiments. Statistical analysis was conducted using SPSS software (version 22.0). Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by a Tukey HSD multiple comparison test. Differences were considered statistically significant at $P < 0.05$.

## Results

The inhibitory activity of *n*-hexane, ethyl acetate and ethanol extracts from P. *fel-terrae* Lour on NO production toward RAW 264.7 cells.

All the samples tested revealed significant inhibition with inhibition value at concentration 12.5 and 25 µg/mL had decreased compared to LPS. As shown in Table 1, *n*-hexane extracts of *P. fel-terrae* Lour depicted the strongest NO inhibitory activity with a concentration value of 25 µg/mL (10.42 ± 1.82). However, its value was higher than that of dexamethasone as a positive control (5.83 ± 3.33). The negative control (LPS) shows the highest value of nitrite inhibitory too because the normal cell (CC) was used the normal cell did not release much nitrite like LPS-stimulated cells, so the inhibitory activity becomes high.

**Table 1: Mean of nitric oxide production and Tukey HSD post hoc test of nitrite concentration over the various concentration extract and dexamethasone measured in triplicate**

| Samples | Mean of nitric oxide production ± SEM |
|---|---|
| CC | $2.67 \pm 1.10^{b}$ |
| LPS | $100.83 \pm 2.20^{ac}$ |
| NEPFH 12.5 | $72.50 \pm 4.51^{abc}$ |
| NEPFH 25 | $10.42 \pm 1.82^{b}$ |
| EAEPFH 12.5 | $88.33 \pm 6.51^{ac}$ |
| EAEPFH 25 | $30.83 \pm 6.86^{abc}$ |
| EEPFH 12.5 | $75.00 \pm 1.91^{abc}$ |
| EEPFH 25 | $22.08 \pm 2.53^{abc}$ |
| Dexamethasone 1.25 | $7.42 \pm 4.53^{b}$ |
| Dexamethasone 2.5 | $5.83 \pm 3.33^{b}$ |

Values are mean of three replicated determinations (n = 3) ± Standard error of the mean. [a] $P < 0.05$ vs Cells Control, [b] $P < 0.05$ vs LPS, [c] $P < 0.05$ vs Dexamethasone. NEPFH: *n*-Hexane Extract of *Picria fel-terrae* Lour Herbs, EAEPFH: Ethylacetate Extract of *Picria fel-terrae* Lour Herbs, EEPFH: Ethanol Extract of *Picria fel-terrae* Lour Herbs. CC: Cells Control, LPS: Lipopolysaccharides.

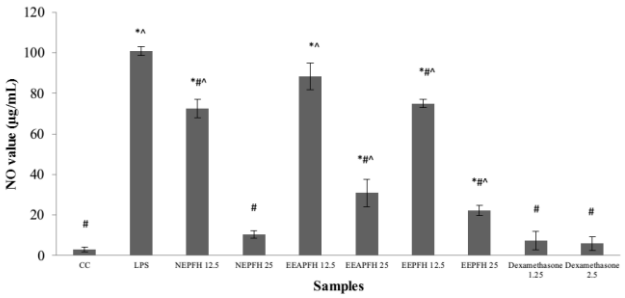


*Figure 1: Inhibitory activity extracts of P.fel-terrae herbs on NO production by LPS-stimulated RAW 264.7 cells; NEPFH: n-Hexane Extract of Picria fel-terrae Lour Herbs; EAEPFH: Ethylacetate Extract of Picria fel-terrae Lour Herbs; EEPFH: Ethanol Extract of Picria fel-terrae Lour Herbs; CC: Cells Control; LPS: Lipopolysaccharides; Values are mean of three replicated determinations (n = 3) ± Standard error of the mean; [*] P < 0.05 vs Cells Control; [#] P < 0.05 vs LPS; [^] P < 0.05 vs Dexamethasone.*

In this study, *n*-hexane, ethyl acetate and ethanol extracts of *Picria fel-terrae* Lour in reduced

the NO production in RAW 264.7 cells with induced by LPS. NO production was measured as nitrite concentration in culture media and compared with normal cell (control) release lower NO production than compared with negative control (LPS) as shown in Figure 1.

## Discussion

NO is a multifunctional signalling molecule. Thus the impact of the extract or compound on NO production likely has further effects on signalling pathways in many cell types [2], [16]. RAW 264.7 cell a murine macrophage cell line had been often used for the screening of anti-inflammatory drugs and immunomodulatory [2]. The extracts showed the reduced of NO production in cells indicating that the presence of antioxidant molecules would be responsible for the inhibitory action [13]. The results study demonstrated that the *n*-hexane extract significantly decreased the nitrite accumulation in LPS-stimulated RAW 264.7 cells. This is caused the secondary metabolite. The *n*-hexane extract of *P. fel-terrae* Lour herb contained steroids [13] likely dexamethasone. Dexamethasone is a steroid agent which can reduce NO production. It used as positive control. While ethyl acetate and ethanol extracts contained flavonoids, saponins, tannin, glycoside reduce NO production too [13], [18], [19], [20], [21], [22], [23].

The results of this study indicate that the *n*-hexane extract from *Picria fel-terrae* Lour Herbs has a high potential to reduce the production of NO in LPS-stimulated RAW 264.7 cells compared ethyl acetate and ethanol extract *Picria fel-terrae* Lour Herbs. The findings of this study provided evidence that supports the traditional use of *Picria fel-terrae* Lour Herbs in the treatment of inflammatory diseases and immunomodulatory agents.

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