Title : The Effect Of Crocatin And Deacetyl Crocatin Isolated From Red Betel (*Piper Crocatum,* Ruiz & Pav.) Leave On Mice Antibody Titer

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Yth.Bu Yuli,

Terlampir adalah revisi atas naskah saya untuk prosiding seminar internasioal yang diselenggarakan oleh POKJANAS TOI bulan Juni 2014 di Tawangmangu, yag berjudul :

**The Effect of Pc-1 and Pc-2 Isolated from Red Betel (*Piper crocatum*, Ruiz & Pav.) Leave on Mice Antibody Titre**

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Trima kasih atas perhatian dan bantuannya Bu.

salam,
yustina

# THE EFFECT OF CROCATIN AND DEACETYL CROCATIN ISOLATED FROM RED BETEL (*Piper crocatum*, Ruiz & Pav.) LEAVE ON MICE ANTIBODY TITER

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**Abstract**

The aim of this research was to investigate antibody titre effect in mice treated with Pc-1 and Pc-2 isolated from red betel (*Piper crocatum* Ruiz & Pav.). The Balb/c mice immune response were induced with *Listeria monocytogenes.* Antibody titre effect was tested using mouse IgG elisa kit. The effect of both Pc-1 and Pc-2 IgG titres, at the dose of 2,5; 5; and 10 mg/kg BW, both compound showed no significantly difference compared to the control group on day 21$^{th}$ after *L. monocytogenes* infection.

Keywords : *Piper crocatum* Ruiz & Pav., Pc-1 and Pc-2, IgG titre

## INTRODUCTION

The activity of the compounds in the extract of red betel leaf (*Piper crocatum* Ruiz & Pav. ) was reported (Wicaksono *et al*., 2009; Rachmawaty *et al*., 2013). Its imunommodulatory activity was also reported (Hartini *et al.*, 2013a). In general, plants that have imunomdulator activity has a stimulating activity of specific and non-specific immunity (Wagner and Proskh, 1985). Some of these plants stimulate the humoral and cellular immunity, while others simply activate the cellular components of the immune system, such as phagocytosis function without effect on humoral and cellular immunity (Bafna and Misrha, 2004*). In vitro* study of two neolignans isolated from red betel leaf etanolic extract showed that both compounds increased macrophage phagocytic activity (Kustiawan, 2012). *In vivo* study of two compound isolated from red betel leaf methanolic extract (Pc-1 and Pc-2) also showed that Pc-1 and Pc-2 increased the macrophage phagocytic activity too (Hartini, *et al*., 2013b). This research aim to know the *in vivo* effect of Pc-1 and Pc-2 on humoral immunity.

## MATERIAL AND METHODS

Preparation of methanol extract of red betel leaves was done by maceration. The extract was further fractionated by the method of Vacuum Liquid Chromatography, successively using n-hexane, chloroform, ethyl acetate, and methanol. Pc-1 and Pc-2 are in the $3^{rd}$ and $4^{th}$ of 5 methanolic extracts fractions. Isolation of the two compounds was conducted by preparative Thin Layer Chromatography.

Male Balb/c mice 8 weeks old weighing about 20-25 g and *Listeria monocytogenes* were used for the experiments. All procedures were approved by The Ethical Clearance Commision for pra-clinically research of Laboratorium Penelitian dan Pengujian Terpadu Gadjah Mada University, Yogyakarta, Indonesia. In the preliminary study, Balb/c mice were divided into treatment group and control group. The treathment group, received 10 mg/kg BW Pc-2 while the control group received 0.7 ml of 1% sodium carboxy methyl cellulose as solvent control, per oral for 14 days. On $15^{th}$ day (= day 0), 0.2 ml *L. monocytogenes* containing $5x10^3$ cfu/ml are injected intraperioneally to all mice. On day 0, day 3, day 10 and the twenty-one days after *L. monocytogenes* infection, 0.5 ml of blood was taken from the *infra-orbital plexus* of mice.

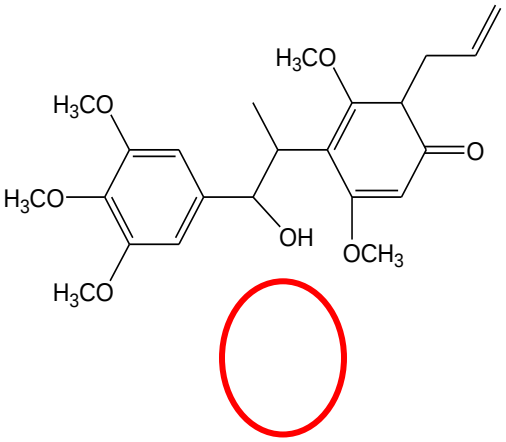
In the main study, Balb/c mice were divided into nine groups. Group A, received 2.5 mg/kg BW Pc-1, Group B, received 5 mg/kg BW Pc-1, Group C, received 10 mg/kg BW Pc-1, Group D, received 2.5 mg/kg BW Pc-2, Group E, received 5 mg/kg BW Pc-2, Group F, received 10 mg/kg BW Pc-2, per oral for 14 days. Group G, didn't received drugs, as normal control, Group H,

received 0.7 ml of 1% sodium carboxy methyl cellulose per oral as solvent control, and Group I, received 100 mg/kgBW product-X® (contain echinacea extract) per oral as positive control. On $15^{th}$ day (= day 0) and $25^{th}$ day 0.2 ml *L. monocytogenes* containing $5x10^3$ cfu/ml are injected intraperioneally to all mice. On day 0, day 10 and the twenty-one days after *L. monocytogenes* infection, 0.5 ml of blood was taken from the *infra-orbital plexus* of mice.

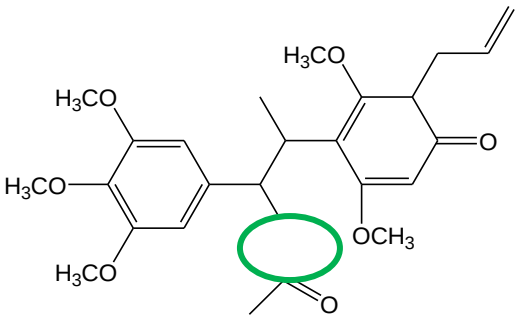
The humoral immune response determined by measuring the titre of immunoglobulin G (IgG). Measurement of IgG titers using mouse IgG elisa kit. The data were analyzed by one-way ANOVA followed by Tukey test.

## RESULT AND DISCUSSION

The compounds isolated from red betel are neolignan. The existence of an acetyl group ($OC_2H_3$) at $C_{1'}$ to distinguish Pc-1 of Pc-2 having hydroxyl groups (OH). The chemical structure differences Pc-1 and Pc-2 are shown in Figure 1. Pc-1 is *2-allyl-4-(1'-hydroxy-1'-(3 ", 4", 5 "-trimethoxyphenyl) propan-2'-yl) -3,5-dimethoxycyclohexa-3, 5-dienone and Pc-2 is 2-allyl-4-(1'-acetyl-1'-(3 ", 4", 5 "-trimethoxyphenyl) propan-2'-yl) -3,5-dimethoxycyclohexa- 3,5-dienone* (Kustiawan, 2012). Aside from the relatively high rendemen, size Pc-1 and Pc-2 spotting on TLC chromatogram is relatively large and the color intensity of damping patches on UV detection at 254 nm is very strong. Processes, equipment, and means of detection croctin and Pc-2 fairly simple, allowing the two compounds used as chemical markers for leaves of *P. crocatum*. Pc-1 and Pc-2 can be used as a marker compound, which is a therapeutic components for *P. crocatum*.




**Pc-1**




**Pc-2**

Figure 1. The chemical structure differences between Pc-1 and Pc-2 (Kustiawan,, 2012).

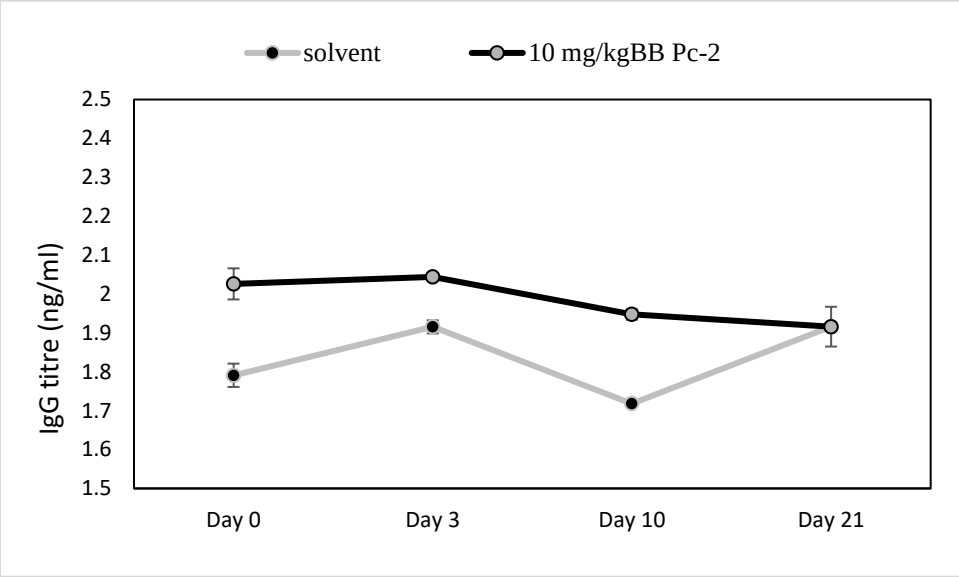



Figure 2. IgG titer levels after the mice were infected by *L. monocytogenes*

Figure 1 shows the result of preliminary study. In this study, IgG titers of mice treated with 10 mg/kgBW Pc-2 showed increase on day 3$^{th}$, then decreased on day 10$^{th}$ and it was as same as the control group on day 21$^{th}$. Although any differences IgG titres on day 3$^{th}$ and 10$^{th}$, but statistically analysis showed no significant difference between treatment group and control group. It indicates that treatment with 10 mg/kgBW Pc-2 have no IgG titres differences compare to control group. Probably due to on the day-10, it need to boost the mice immune responses, so that in the main study we use twice *L. monocytogenes* infection. In the preliminary study the dose of 10 mg/kgBW Pc-2 showed increasing IgG titre, in order to know the potential level of the compound, we use 2 lower doses in the main study. The main study tested 3 range doses of Pc-1 and Pc-2 ie: 2,5; 5; and 10 mg/kgBW. The result of main study can see on figure 3.

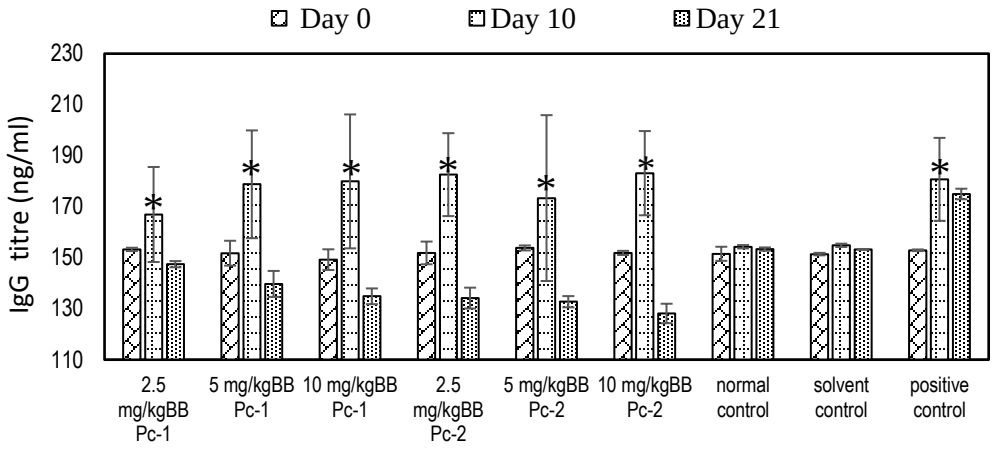



Figure 3. The effect of Pc-1 and Pc-2 against IgG titers in mice after twice infection with *L. monocytogenes*. Values are mean ± SD of 3 replicate, * denotes significant difference ($P < 0.05$) to the normal control and the solvent control

The normal control and solvent control showed the same level of IgG titres, the solvent did not give unexpected effect, 1% sodium carboxy methyl cellulose is an appropriate solvent for this study. In the day 0 (before infection with *L. monocytogenes*) there are no differences effect on all of groups. There are no differences IgG titres of mice before *L. monocytogenes* infection, It indicates that treatment with Pc-1, Pc-2 (at dose of 2.5; 5; 10 mg/kgBW) and product-X® (contain echinacea extract, at dose of 100 mg/kgBW) per oral for 14 days, didn't effect on IgG titres. At day 10 after infection of *L. monocytogenes*, the treatment group showed significantly different IgG titers, but on day 21$^{th}$ IgG titres decline, in contrast to the control group but the difference was not significant. The SD value on day 10 data was high, therefore from the day 0, day 10, and day 21, it can be concluded that Pc-1 and Pc-2 have no effect on antibody titre.

Echinacea was reported to have no effect on the stimulation of IgG immune response, one week following the secondary sheep RBC's subcutaneously infection (Dennis, 1999). In this study, Product-X® (contain echinacea and zinc picolinate) was used for positive control.

The IgG titre of mice treated with Pc-1 and Pc-2 at the day of 21$^{st}$ after the infection with *L. monocytogenes* (day 11 after re-infection) showed no significantly difference compared to the control group. *Listeria monocytogenes* which is an intracellular bacterium maybe induce cellular immunity, but the humoral immune response, so that the IgG titre production did not increase. As it has reported, the differences of both neolignan are Pc-1 not

cause toxic effects on the kidneys and liver either, but Pc-2 that have OH at $C_{1'}$ cause liver damage even though no effect on the kidneys (Hartini *et al*., 2013).

## CONCLUSION

There are no differences patern effect of Pc-1 and Pc-2 on the IgG titres of mice infected with *L. monocytogenes*. Both of the neolignans didn't show significantly effect on the mice IgG titre, compare to control group.

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