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Introduction

Red betel (*Piper crocatum* Ruiz & Pav) leaves have been used traditionally in Indonesia to maintain people health. Our previous studies, two principal immunostimulant substances were isolated from the red betel MeOH extract. The substances were identified as neolignan (Pc-1) and its deacetyl derivates (Pc-2). There was no histopathological effect on kidneys observed caused by both compounds. However histopathological effects on liver is observed due to Pc-2 treatment, not for Pc-1. The plant extracts may have a higher phagocytic activity compared to isolated compound. This recent study, *in vivo* phagocytic activity of red betel leaf MeOH extract and those of isolated compounds were compared.

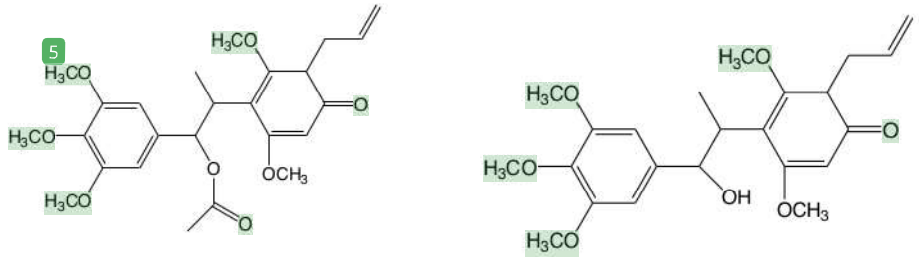
Experimental

Red betle leaves MeOH extract and isolated Pc-1 and Pc-2 (Hartini *et al*., 2014) were used as materials. The phagocytic activity assessment were carried out by macrophage phagocytosis and nitric oxide assay. After 14 days of Red betel leaves MeOH extract (150,300, and 450 mg/kgBW) and the Pc-1 and Pc-2 isolated compounds (5 mg/kgBW each) treatment, the BALB/c mice were infected with *Listeria monocytogenes*. Macrophage phagocytosis activity and nitric oxide production were measured twice, before and 21th day after *L. monocytogenes* infection.

RESULT AND DISCUSSION

The Compounds

The two compound (Pc-1 and Pc-2) isolated from methanolic extract of red betel leaves are the same as Kustiawan[2]'s isolates.



Pc-1 **Pc-2**

Fig 1. Two cpmpounds isolated from Red betel (*Piper crocatum* Ruiz & Pav.), Pc-1 is 2-allyl-4-(1'-acetyl-1'-(3 ", 4", 5 "-trimethoxyphenyl) propan-2'-yl) -3,5-dimethoxycyclohexa- 3,5-dienone,Pc-2 is 2-allyl-4-(1'-hydroxy-1'-(3",4", 5 "-trimethoxyphenyl) propan-2'-yl) -3,5-dimethoxycyclohexa-3, 5-dienone [2] (Kustiawan[2])

They are neolignans, almost have the same structure which were Pc-2 differs from Pc-1 on their C1 binding group. The Pc-1 bind the acetyl while Pc-2 bind the hidroksil. The melting point of Pc-1 was 165-167°C. The GC-MS spektrogram showed that Pc-1 have molecular weigh 460 with retention time at 29.985 minutes (100% of total peak); while Pc-2 have molecular weigh 418 with retention time at 29.495 minutes (96.7% of total peak).

Phagocytic Activity

A day after 14th days treatment there was no significant different in macrophage phagocytosis activity (Phagocytosis Percentage, Phagocytosis Index, and Phagocytosis Efficiency) and nitric oxide production among the groups of treatment. The same results were also found when it was compared to negative control. The following experiment, the treated mice were infected with *L. monocytogenes*. After 21th day of infection, the

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Phagocytosis Percentage (PP) and Phagocytosis Index (PI) showed significantly different between those of treated group and negative control (figure 2 and 3). The PP and PI of groups treated with the isolates (5 mg/kgBW Pc-1 and Pc-2) were equal to 450 mg/kgBW Red betel leaves MeOH extract. The phagocytic activity on 450 mg/kgBW Red betel leaves MeOH extract seemed more efficient than others, but statistical analysis showed the dofferences not significantly (Figure 4). Nitric oxide assay also showed the same results (figure 5). In our previous research reported that at the treatment of 5 mg/kgBW Pc-1 on BALB/c mice showed normal histopathologic effects on the kidneys and liver, whereas at the same dose of Pc-2 treatment showed normal histopathologic effects on the kidneys, but caused liver damages. The structure difference between Pc-1 and Pc-1 is one of their functional groups. Pc-1 had acetyl group which replaces the hydroxyl group on the Pc-2 (Hartini *et al*., 2014). The on going research, the histophatological effect of the extract and isolated compounds treatment on infected BALB/c mice liver and kidneys will be carried out. The damaged on liver will also be analysed by measuring SGOT/SGPT.

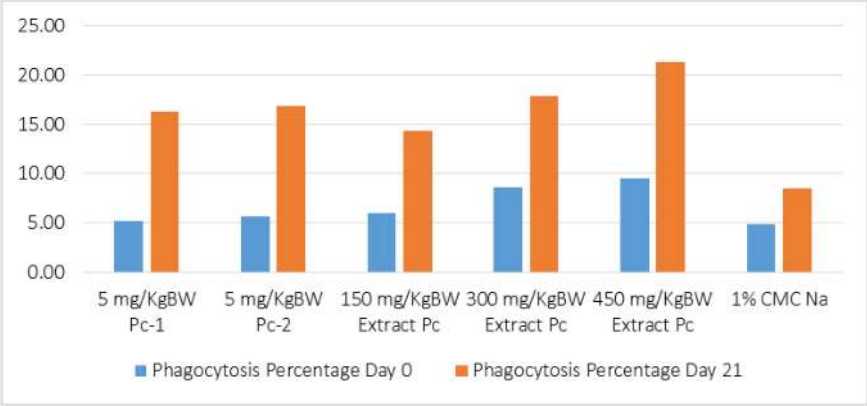

Figure 2. The Phagocytosis Percentage (PP) of treated groups on the day 0 and day 21 after *Listeria monocytogenes* infection

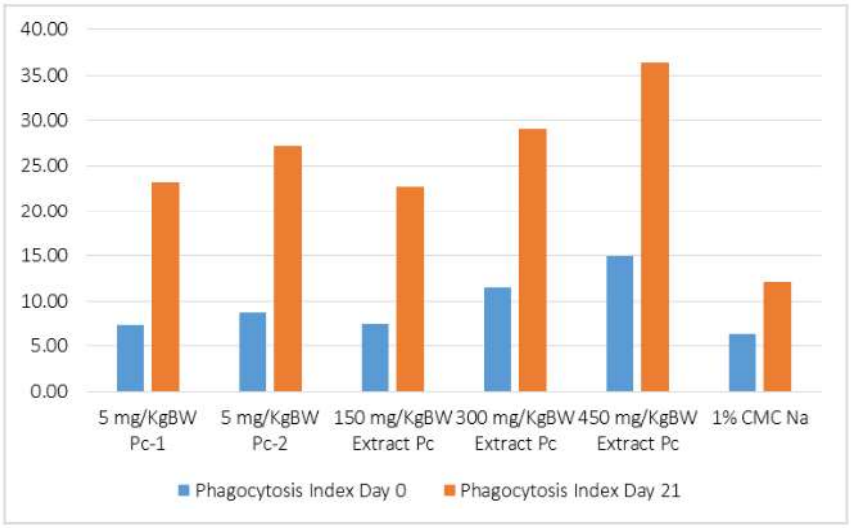

Figure 3. The Phagocytosis Index (PI) of treated groups on the day 0 and day 21 after *Listeria monocytogenes* infection

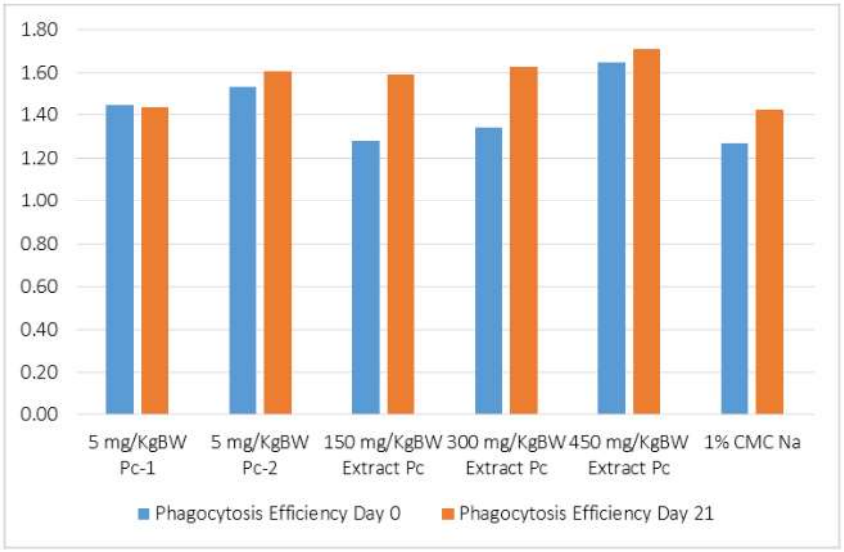

Figure 4. The Phagocytosis Efficiency (PE) of treated groups on the day 0 and day 21 after *Listeria monocytogenes* infection

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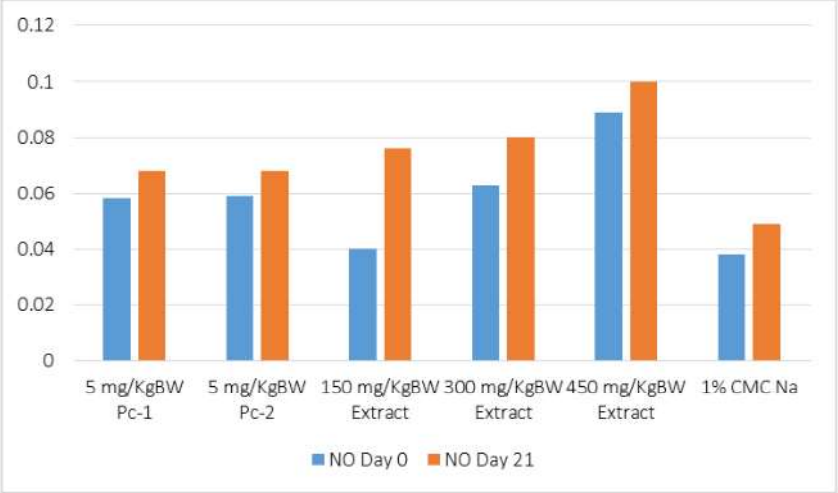

Figure 5. The Nitic Oxide (NO) production of treated groups on the day 0 and day 21 after *Listeria monocytogenes* infection

Conclusion:

The 450 mg/kgBW Red betel leaves MeOH extract had equal phagocytic activity compared to 5 mg/kgBW two compounds (Pc-1 and Pc-2) isolated from red betel (*Piper crocatum* Ruiz & Pav.) leaves

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