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December 2018, 18(4)

A review of the phytochemistry and medicinal activities of the popular African food additive:*Parkia biglobosa*seed

Wasiu Gbolahan Balogun, Ismail Abiola Adebayo, Usman Yusuf, Azman Seeni

December 2018, 18(4), p.271 - 279

Food additives have been used for many decades, mainly to enhance food aesthetics and taste. However, considerable research attention has been placed on identifying food additives with additional medicinal benefits. Despite on-going efforts to document food additives with medicinal benefits, including the Food and Drug Administration ...

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Focalization of thrombosis and therapeutic perspectives: a memoir

S. Merlyn Keziah, C. Subathra Devi

December 2018, 18(4), p.281 - 298

The main aim of the review is to recapitulate the effects of thrombotic diseases, clotting disorders, and the methodology of prospecting a range of thrombolytics from various sources and thrombolytic therapies. Thrombolytic medications are administered to infringe dangerous clots inside blood vessels when there is deprivation in this ...

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Antioxidant activity of*Moringa oleifera*seed extracts

Ismet Ara Jahan, M. Hemayet Hossain, Khondoker Shahin Ahmed, Zakia Sultana, Pizush Kanti Biswas, Katrun Nada

December 2018, 18(4), p.299 - 307

The present research evaluates the phytochemical and antioxidant activity ofMoringa oleiferaLam. seed kernel grown in Bangladesh.M. oleiferaseed kernel was extracted with methanol, acetone and water individually. The phytochemical content was evaluated through the determination of total phenolic, total flavonoid and total tannin contents. In vitro antioxidant...

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Phytochemical analysis and antioxidant potential of the crude extract of*Allium*

***oschaniii*scape**

Seenivasaperumal Alagarsamy, Pandiselvam Chellappan, Michel Thomas Jesuraj, Manoj Saravana Guru Mohan, Rengesh Balakrishnan

December 2018, 18(4), p.309 - 316

The present study focuses on the antioxidant potential of aqueous and ethanolic extracts of the scape ofAllium oschaninii. Phytochemical analysis and total phenolic content for both the extracts and Total flavonoid content for ethanolic extract were determined. Anti-microbial activity of different concentrations of both the extracts againstPseudomonas aeruginosa...

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Influence of Moringa (*Moringa oleifera*) leaf extracts on the antioxidant and angiotensin-1 converting enzyme inhibitory properties of lisinopril

Ganiyu Oboh, Ayokunle Olubode Ademosun, Oyewumi John Oyetomi, Taiwo Mary Adewuni

December 2018, 18(4), p.317 - 324

This study investigated the in vitro effect of various combinations of aqueous extracts of moringa (Moringa oleifera) leaves on the ACE inhibitory and antioxidant properties of lisinopril, which is a popular synthetic ACE inhibitor. Moringa leaves were air-dried and blended into powdery form. Thereafter, the aqueous...

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The comparison of two neolignans isolated from red betel leaf and its extract against macrophage phagocytic activity, the level of AST, and histopathological features of the liver in mice

Yustina Sri Hartini, Sitarina Widyarini, Laurentius Hartanto Nugroho

December 2018, 18(4), p.325 - 333

A single active compound isolated from a plant extract may show a lower, equal, or greater activity compared to its extract. The two neolignans (Pc-1 and Pc-2) has been isolated from red betel (Piper crocatumRuiz & Pav.) leaf. The aim of this study ...

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Acute toxicity and in vivo anti-inflammatory effects and in vitro antioxidant and anti-arthritic potential of*Scabiosa stellata*

Chaima Mouffouk, Leila Hambaba, Hamada Haba, Soumia Mouffouk, Chawki Bensouici, Sara mouffouk, Messaoud Hachemi, Hachani Khadraoui

December 2018, 18(4), p.335 - 348

This study is devoted to the estimation of total bioactive contents and the evaluation of acute toxicity and in vivo anti-inflammatory effects and the assessment of in vitro antioxidant and anti-arthritic potential of the speciesScabiosa stellata.The anti-arthritic activity was performed by bovine serum albumin denaturation method...

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Maternal olive oil intake enhances lipid metabolism and lipase activities in offspring of cafeteria diet-induced obese rats

Salima Derouiche, Fatima Zohra Baba Ahmed, Hafida Merzouk, Samira Bouanane, Sid Ahmed Merzouk

December 2018, 18(4), p.349 - 356

It has long been established that maternal nutritional condition and fatty acid intake during pregnancy and/or lactation may influence the modifications in the individual risk for developing metabolic diseases throughout life, but little is known about the possible role of maternal nutrition before conception. The aim of this...

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Evaluation of genotoxicity and mutagenicity of aqueous extracts of*Rhazya stricta*Decne. and*Thymus vulgaris*L.

Shatha Salem Hameed, M-Zaki Mustafa ElAssouli, Ahmed M. Alhejin, Mohammad Zubair Alam, Sufian Mohamad ElAssouli, Faten Zubair Filimban

December 2018, 18(4), p.357 - 363

Traditional herbal medicines have been part of human healthcare systems since ancient times. The studies on herbal medicines have mainly focused on their beneficial aspects and hence their harmful effects have been usually overlooked. Throughout Middle East and South Asia,Rhazya strictaDecne. andThymus vulgarisL. are used as traditional...

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Overexpressed CYP450 mediated apoptosis evaluates cytotoxicity and teratotoxicity of*Cucumis callosus*

Siva Prasad Panda, Bikash Ranjan Jena, Gade Kalyani, Uttam Prasad Panigrahy

December 2018, 18(4), p.365 - 375

Fruits ofCucumis callosus(Rottl.) Cogn. (Family: Cucurbitaceae) plant, are commonly known as “bitter cucumber” (English) and “Kachri” (Hindi) in India and have been traditionally used for antioxidant, rich source of vitamin C, antidiabetic and anticancer actions. Tribal peoples of Odissa and...

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Body constitution and dysmenorrhea: a study on university students in Malaysia

Teek Foh Chong, Xin Ee Ser, Lay Khuan Ooi, Ling Shing Wong

December 2018, 18(4), p.377 - 380

Dysmenorrhea is a common gynecologic condition among women. The relation between the menstrual pain and the Chinese medicine body constitution had never being studied in Malaysia, especially among students in higher education institute. In this study, a survey was carried out to analyze the relationship between the occurrence...

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*Lantana camara*enhances antibacterial potency of antibiotics and exerts synergistic inhibitory effect against pathogenic bacterial species

Jigisha Anand, Shanu Choudhary, Nishant Rai

December 2018, 18(4), p.381 - 389

The present study aims at evaluating the potentiating ability ofLantana camaraleaves and flower extracts in enhancing the antibacterial efficacy of antibiotics against pathogenic bacterial species. Methanolic extracts ofL. camaraleaves and flowers (pink turn yellow) were prepared by the solvent extraction method. The leaves and flower extracts alone ...

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Gastroprotective and digestive potential of an Ayurvedic asava–arishta preparation

Anjan Adhikari, Amitabha Dey, Satyajyoti Kanjilal, Rajarshi Biswas, Deepa Gandhi, Kamarujjaman Mondal, Bibhuti Nath Bhatt, Tapas Kumar Sur, Satyabrata Mohapatra, Avinash Narwaria, Chandra Kant Katiyar

December 2018, 18(4), p.391 - 401

Asavas and arishtas are considered as important Ayurvedic self-fermented dosage forms which are being used widely to promote health and well-being and for management of digestive and metabolic disorders. The present investigation was carried out to study the potential of an Ayurvedic polyherbal asava–arishta preparation (ZP...

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Comparative potential of hydrocortisone, deoxycorticosterone and dexamethasone in the prevention of cataract: an in silico and in vitro study

Divya Rana, Ruchika Sharma, Anoop Kumar

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Cataract is visual impairment which arises due to disturbance of lens transparency due to aggregation of the protein. Currently, surgery is the only choice for the treatment of cataract. Thus, there is a need for new drugs in the prevention of cataract. In the current investigation, we...

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Issue List

Report a problem

The comparison of two neolignans isolated from red betel leaf and its extract against macrophage phagocytic activity, the level of AST, and histopathological features of the liver in mice

Yustina Sri Hartini, Sitarina Widyarini & Laurentius Hartanto Nugroho

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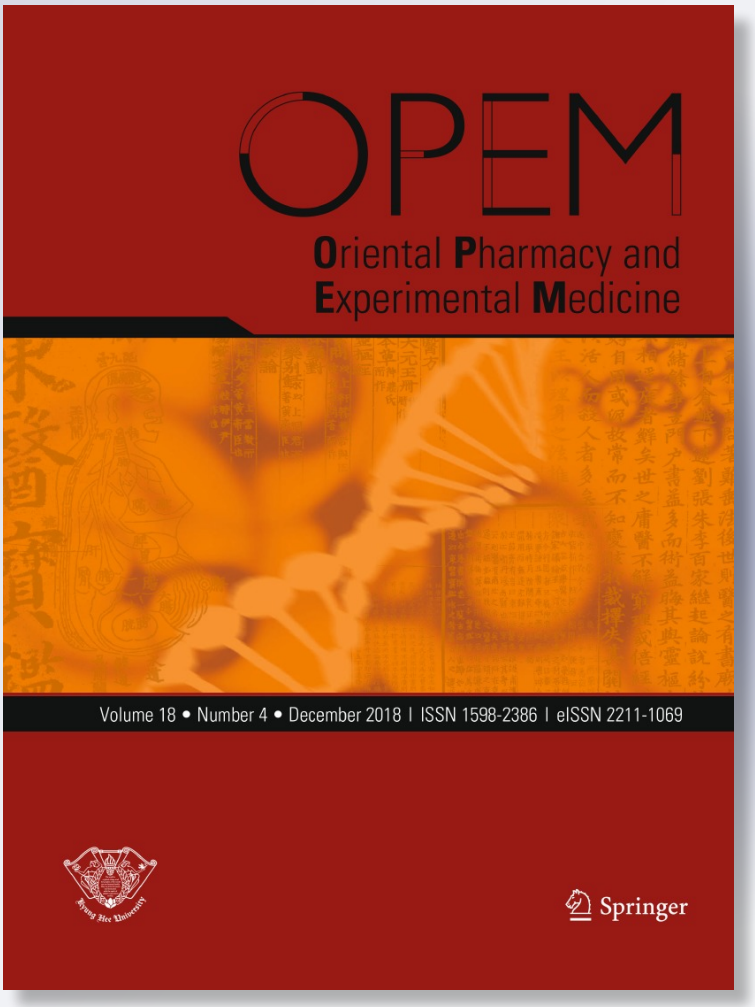

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RESEARCH ARTICLE

The comparison of two neolignans isolated from red betel leaf and its extract against macrophage phagocytic activity, the level of AST, and histopathological features of the liver in mice

Yustina Sri Hartini[1] · **Sitarina Widyarini**[2] · **Laurentius Hartanto Nugroho**[3]

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Abstract

A single active compound isolated from a plant extract may show a lower, equal, or greater activity compared to its extract. The two neolignans (Pc-1 and Pc-2) has been isolated from red betel (*Piper crocatum* Ruiz & Pav.) leaf. The aim of this study was to compare the phagocytic activity of macrophage and histopathological feature in mice treated with Pc-1, Pc-2 and its Pc-extract. *Listeria monocytogenes* was used to induce Balb/c mice immune response. Peroral administration of Pc-1 and Pc-2 and red betel leaf methanolic Pc-extract were carried out. The phagocytic effect was determined by macrophage phagocytosis and nitric oxide (NO) assays. The morphological feature of liver and kidney were observed using light microscope. The level of apartate transaminase (AST serum) and alanine transaminase (ALT serum) were also measured from the blood serum before and after *L. monocytogenes* infection. The results shows that the macrophage phagocytic activity of given 450 mg/kg body weight Pc-extract was equal to 5 mg/kg body weight of Pc-1 and Pc-2. The activity pattern of percentage phagocytosis, index phagocytosis, and efficiency phagocytosis of Pc-extract, Pc-1, and Pc-2 were similar, as well as the NO production. A certain dose of Pc-extract and both isolated compound reduced the level of AST while there were no effect on ALT level. There were no histopathological features differences in the liver after treated with Pc-extract and Pc-1. However, Pc-2 treatment caused hydropic degeneration on liver. Therefore it can be concluded that there were equal activity between Pc-extract and the isolated compound in certain dose. The Pc-extract and isolated compound reduced the level of AST while there were no effect on ALT. The only Pc-2 affected the histopathological features of the liver.

Keywords Red betel (*Piper crocatum* Ruiz & Pav.) · Isolated compound · Pc-extract · Phagocytic · Histopathological

Introduction

Genus *Piper* have been used for traditional medicine. One of them is red betel (*Piper crocatum* Ruiz & Pav.). Two neolignans (Pc-1 and Pc-2) were isolated from red betel leaf (Kustiawan 2012). Both compounds were accumulated in the leaves, stems, and flowers of red betel with the highest concentrations in the leaves (Hartini and Nugroho 2017).

The methanol extract of red betel leaf and fractions of the extract is able to increase the activity of macrophage phagocytosis (Hartini et al. 2013). In vitro method showed Pc-1 and Pc-2 from red betel has an effect on macrophage phagocytic activity (Kustiawan 2012). Our previous study showed that Pc-1 and Pc-2 isolated from the red betel leaf increased macrophage phagocytic activity and nitric oxide (NO) production (Hartini et al. 2014). A plant extract produced a higher or smaller response than those of an equivalent dose of the isolated compound considered to be the 'active' one (Mukherjee and Houghton 2009). Therefore, the current research aim to compare the effect of Pc-1, Pc-2 and Pc-extract on macrophage phagocytic activity and histopathological features of the liver in mice.

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Materials and methods

Plant material

The red betel (*Piper crocatum* Ruiz & Pav.) fresh leaves were harvested from Tawangmangu, Central of Java, Indonesia. Plant species identification was done by Wahyono, Department of Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia. The voucer specimen was stored in herbarium unit at The Faculty of Pharmacy, Sanata Dharma University, Indonesia (no. BF/184/Ident/Det/VI/2011).

Extract preparation

The powdered dried leaves were put in macerator, methanol was added to the macerator until all of the powder was submerged. The mixture was then stirred overnight. Liquid immersion results were separated, while the waste were re-macerated two times in the same way. Liquid immersion results of three time macerations were collected, then evaporated using a rotary evaporator to obtain a thick extract (Hartini et al. 2013).

Fractionation preparation

An amount of 10 g silica gel 60 were added to the porcelain cup contained 2 g Pc-extract. The both materials were mix gently. An amount of petroleum ether was added to form free flowing mixture. Column was made by added 15 g silica gel 60 slowly into sintered glass Buchner while it was vacuumed to form solid and compact stationary phase. Free flowing mixture was moved to sintered glass Buchner slowly on the top of stationary phase while it was vacuumed. On the top of free flowing mixture was covered with 2 layers of filter papers. Fractionation was done by pouring solvent slowly on the top of filter papers while it was vacuumed. The solvent used were 50 ml *n*-hexane, 50 ml chloroform, 50 ml chloroform, 50 ml ethyl acetate, 100 ml methanol in sequence resulted in fraction I, II, III and IV. The fractions were collected in porcelain cup. After drying, the fractions were weighed. The compounds in the fraction were monitored using TLC compared to TLC profile of standard compounds (Pc-1 and Pc-2) donated by Kustiawan.

Isolation of compounds

The purposes of the compound isolation was to isolate the Pc-1 and Pc-2 in the red betel extract. The extract was fractionated by vacuum liquid chromatography (VLC) method. After monitoring using TLC and the results were compared to the TLC chromatogram of Pc-1 and Pc-2, it were known that Pc-1 and Pc-2 were found in the third and fourth fractions of VLC. Both fractions were then separated using preparative TLC. The detected spot of the compound was scraped, collected, and then diluted with chloroform:methanol (1:1). The compound was obtained in the form of a crystal after filtration and evaporation. Melting points of both compounds was determined using Mettler Toledo MP70 and the molecular weight stated from gas chromatography–mass spectrometry (GC–MS) analysis using caplary column Agilent 19091S-433.

Animals

Eight weeks old male Balb/c mice with 25–30 g bodyweight were used in this research. Mice were grouped into seven groups. Each group consisted of five mice. Groups A and B were treated with the neolignans Pc-1 and Pc-2 at the dose of 5 mg/kg body weight. Groups C, D, and E were treated the extract with the dose of 150, 300, and 450 mg/kg body weight, respectively. Both neolignans and the Pc-extract were given orally once in a day for 14 days. The day of treatment was done according to Kanjwani et al. (2008). Group F was a normal control and Group H was given orally 1% sodium carboxy methyl cellulose. At 15th day (day 0) and 25th day 0.2 ml *L. monocytogenes* (5×10^3 cfu/ml) injected intraperitoneally to all of the mice. Measurement of macrophage phagocytic activity were done before *L. monocytogenes* injection (day 0) and day 37th (day 21) after *L. monocytogenes* injection. All procedures associated with animal experimentations were approved by the central integrated research (LPPT) Universitas Gadjah Mada Indonesia number: 221/KEC-LPPT/III/2015. European Council Legislation 87/609/EEC protocol for the protection of experimental animals was conducted for handling and sacrificing of the animals (Mitjans et al. 2001).

Macrophage phagocytosis assay

The method of Leijh et al. (1986) using latex beads with a diameter of 13 mm were used for the assay of macrophage phagocytic. Latex beads were suspended in PBS up to the concentration 2.5×10^7/ml. Macrophage cultured a day before was washed twice with RPMI 1640 and to be placed in 24 well plate. The latex beads (200 µL) were added to each well, and then incubated in CO_2 incubator at 37 °C for 60 min. Cells were washed with PBS three times to remove the remaining latex beads. Cover slips containing macrophages were dried at room temperature and fixed with methanol for 30 s. The following process was removing the methanol. The cover slips containing macrophages were dried and stained with 20% Giemsa for 30 min. Coverslips were washed with distilled water

thoroughly (4–5 times), separated from the culture wells and dried at room temperature. The calculation of activated macrophages were done using a light microscope with magnification of 400×. The activity of macrophages was measured by the latex-bead phagocytosis index (PI), the phagocytosis percentage (PP), and the phagocytosis efficiency (PE) (Sanchez et al. 2008).

Nitric oxide (NO) assay

The overnight incubated macrophage cell culture with the amount of 100 µL were placed in 96 well plate. With the addition of Gries solution (100 µL) to each well and the incubation for 10 min, the optical density was read using Elisa reader at 550 nm. Concentration ranging from 0.078 to 20 µM of NO was used as standard.

Histopathological examination of liver

Peritoneal macrophages were isolated from the spleen, murine peritoneum sheath was opened, and liver were removed. The liver were then immersed in 10% buffered formalin for histopathological examination. The kidney and liver were cut to 4 µm thickness using microtome, and stained using hematoxylin–eosin (HE). Histological slides were observed under a light microscope at a magnification of 400×.

AST/ALT assay

The International Federation of Clinical Chemistry (IFCC) method without pyridoxal phosphate (P-5′-P) were done for analysis of apartate transaminase (AST)/glutamic oxaloacetic transaminase (SGOT) and alanine transaminase (ALT)/Glutamic pyruvic transaminase (SGPT) in serum. The absorbance was measured at 340 nm.

Data analysis

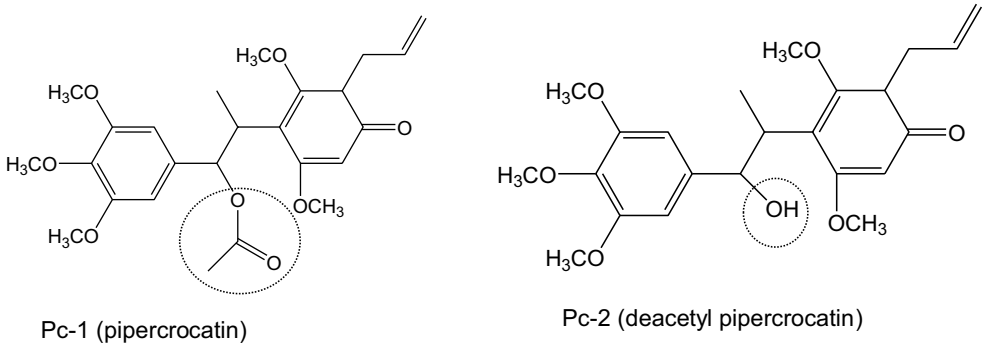
Histopathological features were analysed descriptively, while macrophage phagocytic activity, NO and level of AST/ALT were statistically analysed by one-way analysis of variance (ANOVA) followed by Tukey's test post hoc analysis; p values less than 0.05 were considered statistically significant.

Results

Isolated compounds

Isolation of Pc-1 and Pc-2 from the extract was carried out according to Hartini et al. (2014). The Pc-1 and Pc-2 were elucidated by Kustiawan (2012). The Pc-1 was elucidated as 2-allyl-4-(1′-acetyl-1′-(3″,4″,5″-trimethoxyphenyl) propan-2′-yl)-3,5 dimethoxycyclohexa -3,5-dienone and Pc-2 was elucidated as 2-allyl-4-(1′-hydroxy-1′-(3″,4″,5″-trimethoxyphenyl) propan-2′-yl)-3,5-dimethoxycyclohexa-3,5-dienone. Pc-2 differs from Pc-1 on their C_7 binding group, Pc-1 binds acetyl while Pc-2 binds hydroxyl group (detail structure can be seen in Fig. 1). The trivial name for Pc-1 and Pc-2 are pipercrocatin and deacetyl pipercrocatin, respectively (Table 1).

Both compounds in Fig. 1 are neolignan which have similar structure each other. The melting point of Pc-1 and Pc-2 were 165–167 and 161.5 °C respectively. The GC–MS spectrogram showed that Pc-1 have molecular weight 460 with retention time at 29.985 min (100% of total peak); while Pc-2 have molecular weight 418 with retention time at



Pc-1 (pipercrocatin) Pc-2 (deacetyl pipercrocatin)

Fig. 1 Isolated compounds from red betel (*Piper crocatum*, Ruiz & pav.)

Table 1 The level of SGOT SGPT assay of mice blood serum on 21th day after *L. monocytogenes* infection

Group	Treatment	Dose (mg/kg body weight)	SGOT	SGPT
1	Pc-1	5	85 ± 7*	53 ± 5
2	Pc-2	5	93 ± 4*	56 ± 6
3	Pc-extract	150	84 ± 3*	42 ± 5
4	Pc-extract	300	118 ± 11	68 ± 12
5	Pc-extract	450	126 ± 6	53 ± 7
6	Negative/vehicle control	–	142 ± 4	45 ± 2

Values are presented as mean ± SE (n: 3); *$p < 0.05$ was considered to be significant when compared to negative control weight did not cause the change of liver histopathological features however Pc-2 resulted in hydropic degeneration of the liver

29.495 min (96.7% of total peak). Pc-1 and Pc-2 were identified in the gc–ms chromatograms of extract in the minute of 30.145 and 29.708 respectively (Figs. 2, 3, 4).

Macrophage phagocytic activity

After 14th days treatment (day 0) there was no significant differences in macrophage phagocytic activity (PP, PI, and PE) and NO production among the groups of treatment. The same result were also found when it was compared to negative control. The following experiment, the treated mice were infected with *L. monocytogenes.* At 21th day of infection, the PP and PI showed significantly increased between those of treated group and negative control (Figs. 5, 6).

The PP and PI of groups treated with the isolates (5 mg/kg body weight Pc-1 and Pc-2) were equal to those of 450 mg/kg body weight Pc-extract. The phagocytic activity on 450 mg/kg body weight Pc-extract seemed more efficient than those of the neolignans. However statistical analysis showed there is no significantly differences in the Pc-extract compare to its PP and PI (Fig. 7).

Nitrict oxide (NO) production activity

The results of NO assay showed the similar pattern with macrophage phagocytic activity assay (Fig. 8). At the dose

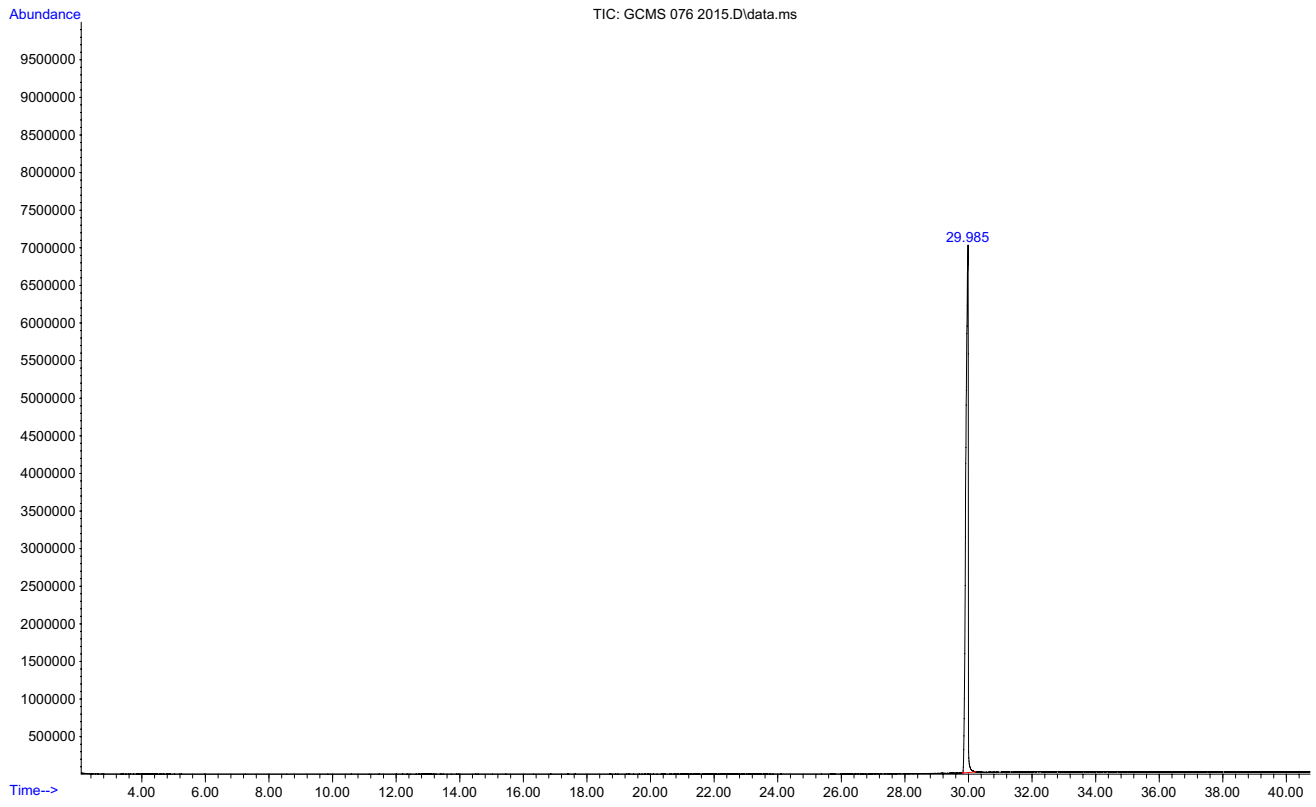


Fig. 2 GC–MS chromatogram of Pc-1

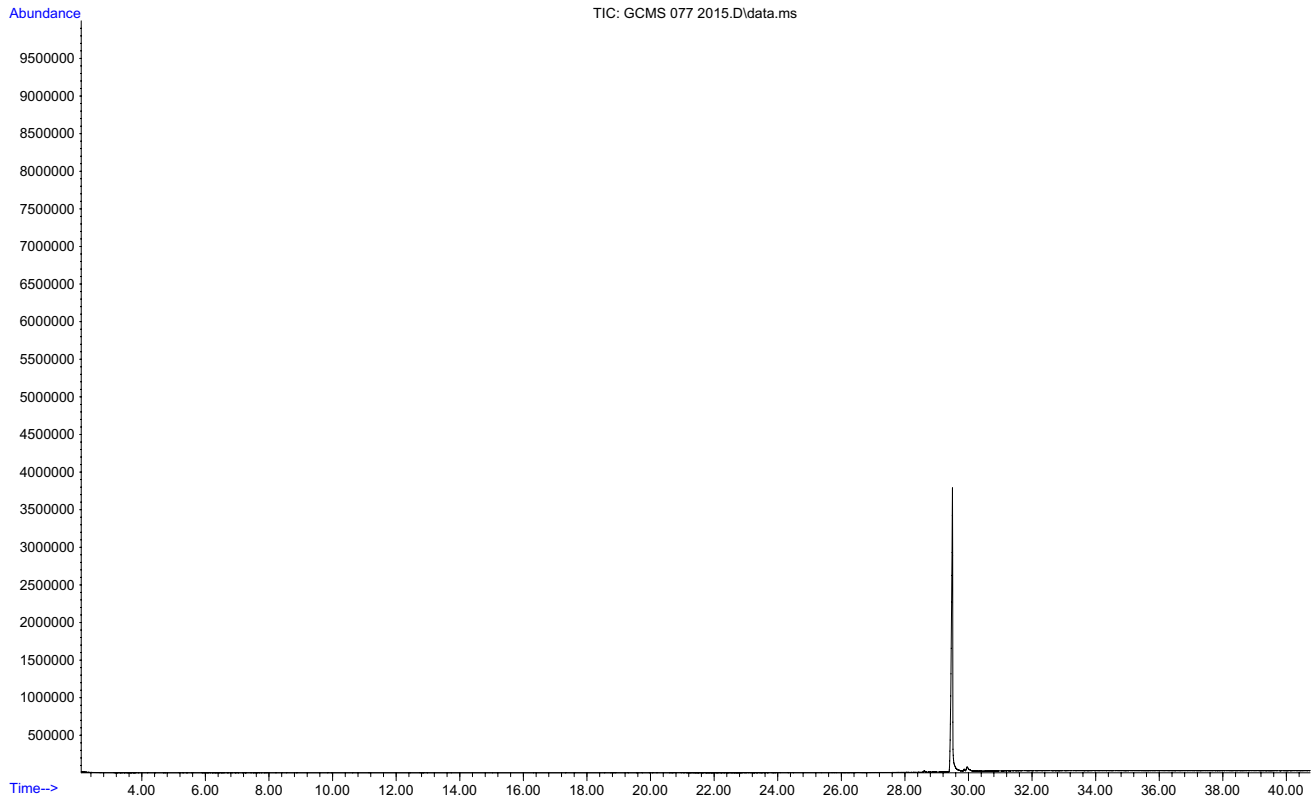


Fig. 3 GC–MS chromatogram of Pc-2

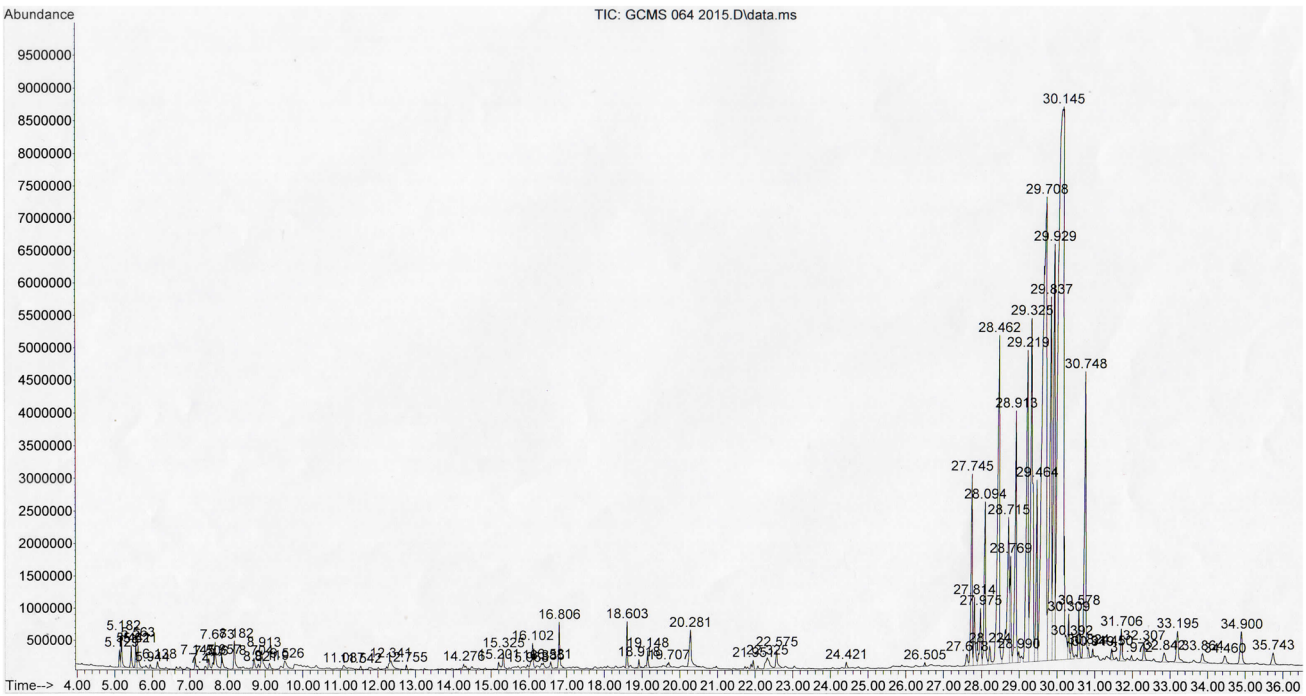


Fig. 4 GC–MS chromatogram of *Piper crocatum* Ruiz & Pav. leaf extract

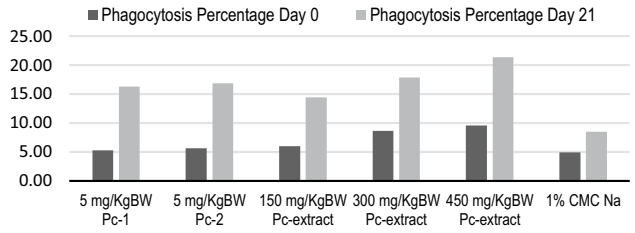


Fig. 5 Macrophage phagocytosis percentage of mice on day 0 and 21th day after *L. monocytogenes* infection

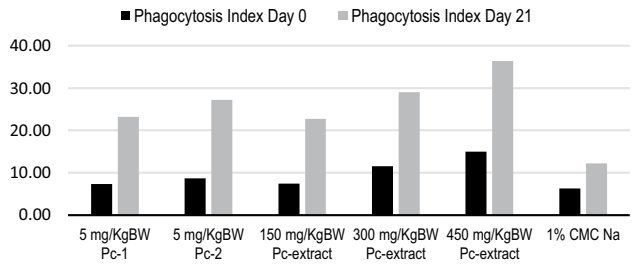


Fig. 6 Macrophage Phagocytosis Index of mice on day 0 and 21th day after *L. monocytogenes* infection

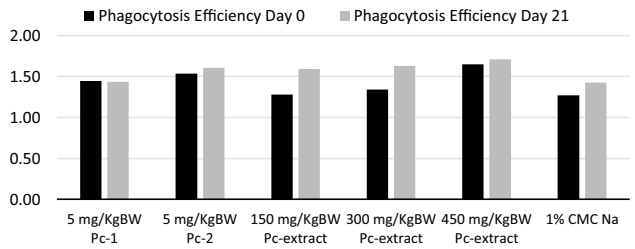


Fig. 7 Macrophage phagocytosis efficiency of mice on day 0 and 21th day after *L. monocytogenes* infection

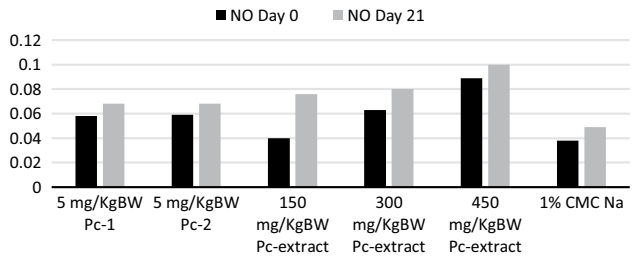


Fig. 8 Nitric oxide production of mice on day 0 and 21th day after *L. monocytogenes* infection

of 150 mg/kg body weight Pc-extract, the NO production was increased significantly. At the dose of 300 and 450 mg/kg body weight Pc-extract did not increase NO production significantly.

The effect of neolignans and Pc-extract on the liver

The result of histopathological analysis showed that treatment with Pc-extract dose of 150, 300, and 450 mg/kg body weight did not cause the change of liver histopathological features. However Pc-2 resulted in hydropic degeneration of the liver (Fig. 9).

Discussion

Melting point (mp) of Pc-1 and Pc-2 was different from the melting point of neolignan from other Piper species. Neolignan mp from *P. argyrophylum* namely futoquinol and kadsurin B were 97°–98° and 101°–102° respectively. Compounds with a mp similar to the Pc-1 was an alkaloid isolated from *P. argyrophylum* namely (E)-N-feruloyltyramine diacetate and and N-p-coumaroyltyramine diacetate which had a mp 160°–161° and 160° respectively (Singh et al. 1996). Other compounds from Piper has a mp varying between 58° and 305° (Singh et al. 1996; Kuo et al. 2002; Banerji et al. 2002; Dharmaratne et al. 2002).

The ethanolic extract of *Piper guineense* at the dose of 20 mg/kg body weight and higher is a risk factor for hepatic function impairment and the associated disorder (Umoh et al. 2013). Our previous research found that 2:12 g of Pc-extract contained 12.0 mg Pc-1 and 12.1 mg Pc-2 (Hartini et al. 2014). The current study measured the activity of Pc-extract and isolates/pure compounds from its extract to determine the quantitative contribution of pure compounds on the activity of the extract. Mathematically, PP and PI of isolates with a dose of 5 mg/kg body weight would be equivalent to PP and PI generated by Pc-1 contained in 876 mg/kg body weight Pc-extract and Pc-2 contained in 883 mg/kg body weight Pc-extract. However, our current results showed that at a dose of 450 mg/kg body weight Pc-extract, or almost half of mathematical calculation, already indicates PP and PI which is equivalent to 5 mg/kg isolates. This indicates that there is a synergistic or additive effect that is responsible for higher PP and PI Pc-extract than isolates. The same condition was also found in compounds isolated from *P. methysticum* which showed anti-anxiolytic activity. The root Pc-extract showed higher anti-anxiolytic activity than each individual major compound (Umoh et al. 2013). Occasionally, extract the potential to produce active compounds be wasted because the activity was not detected during the fractionation. Failure to isolate active compounds from the extract may also be caused by instability of the active compound during the extraction (Deharo and Ginsburg 2011). Extracts, fractions, as well as isolated compounds from red betel leaves can increase the phagocytic activity of macrophages (Hartini et al. 2013; Kustiawan 2012; Hartini et al. 2014), this indicated that the Pc-1 and PC-2 was relatively

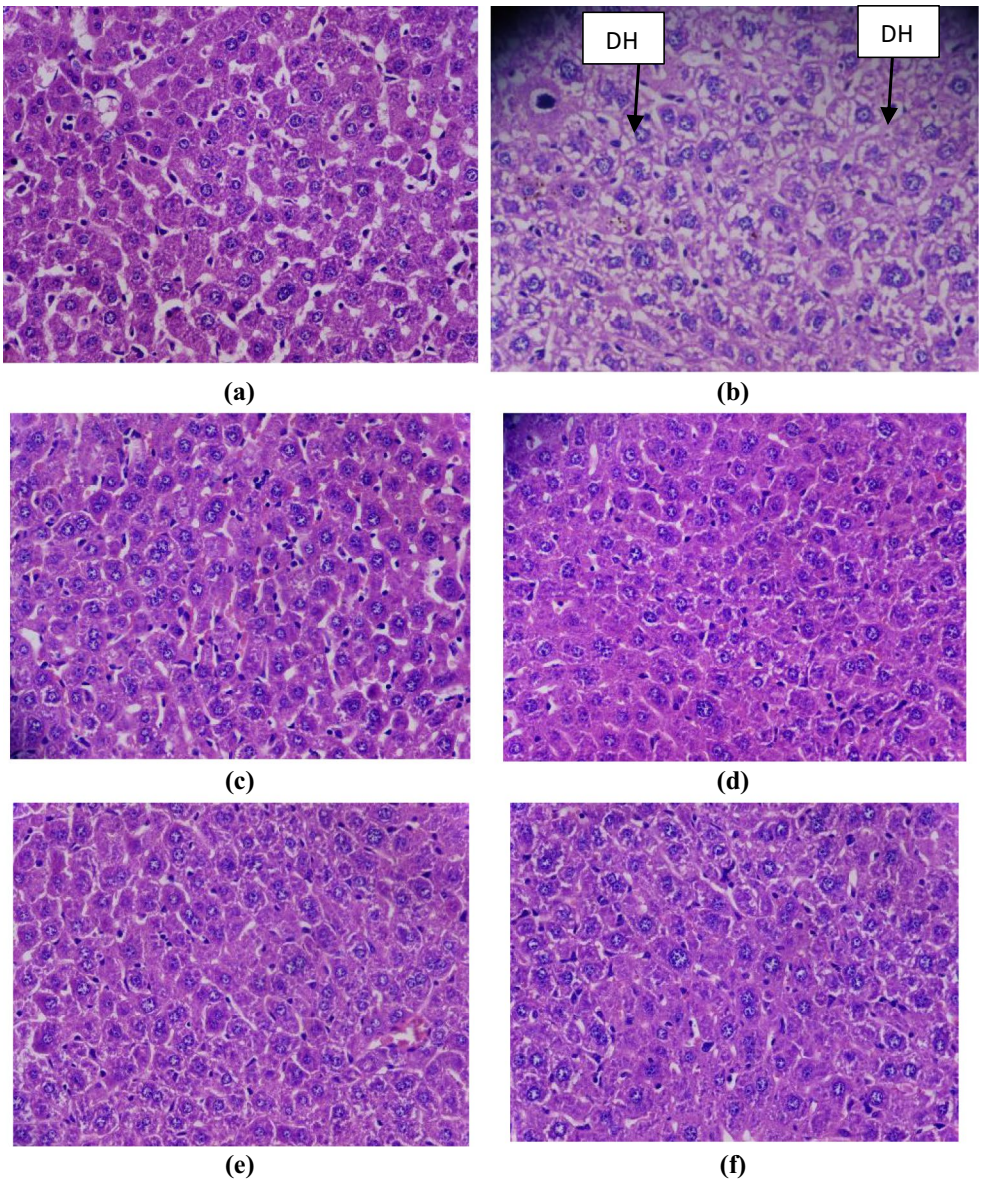


Fig. 9 Photomicrography of liver mice treated with **a** 5 mg/kg body weight Pc-1; **b** 5 mg/kg body weight Pc-2; **c** 150 mg/kg body weight Pc-extract; **d** 300 mg/kg body weight Pc-extract; **e** 450 mg/kg body weight Pc-extract; **f** CMC Na 1%

stable and the isolation methods applied to separate Pc-1 and pc-2 from the extract did not cause damage to the two compounds. The effect of increasing phagocytic activity of macrophages probably due to other compounds (other than the PC-1 and PC-2) in Pc-extract which has similar activity, resulting in an additive effect, or there are other compounds that are able to optimize the effects of Pc-1 and Pc-2 resulting in a synergy effect.

It was not recommended to use Pc-extract at the dose of 150 mg/kg body weight. At the dose of 150 mg/kg body weight Pc-extract, the NO production was increased significantly but there was no significant increasing occur on the other groups. Phagocytic cells play an important role in the immune system mechanism. However, when the phagocytic cells were over activated, the cells will be damaged through their reactive oxygen species (ROS) and NO productions. In the phagolysosome process, inducible nitric oxide synthase (iNOS) and ROS were activated. Nitric oxide is a product of arginine reaction catalyzed by iNOS enzyme. NO regulates inflammatory erythema and edema, and regulate the synthesis of inflammatory mediators and some inflammatory cell function. NO is synthesized in large quantities which are activated by macrophages as a vasodilator modulate vascular responses in acute inflammatory reaction. Pc-extract is an immunostimulant, which is able to increase macrophages phagocytic activity known relatively high. As

already mentioned, the dose of 300 and 450 mg/kg body weight did not increase NO production significantly. Similar with our previous results that the treatment of Pc-1 and Pc-2 produced low level of NO although the phagocytic activities were relatively high (Hartini et al. 2014). The condition may be due to the ability of the isolated compound and the extract to maintain the function of immune cells from the effect of macrophage phagocytic over activity. This indicates the activity of antioxidant and anti-inflammatory of the Pc-extract and its two compounds.

The isolated compound is neolignan, a dimer of lignans one subgroup of polyphenols (non-flavonoid). One of the polyphenols as anti-inflammatory mechanism is to inhibit pro-inflammatory enzymes, such as cyclooxygenase (COX), lipooksigenase (LOX), and iNOS; through activation of the peroxisome proliferators activated receptor gamma (PPARγ). Polyphenols inhibit eicosanoid which form the enzyme phospholipase A2, cyclooxygenase, and lipooksigenase causes a decrease in the concentration of prostanoids and leukotriene (Santangelo et al. 2007). Anti-inflammatory activity of Piper species have also been reported. *P. longum* Linn fruit extract is reported to have anti-inflammatory activity (Kumar et al. 2009), as well neolignan derived from extracts of *P. nigrum* L. (Tasleem et al. 2014). The antioxidant and anti-inflammatory activity of both compounds and Pc-extracts need to be further studied.

Treatment with Pc-extract with the dose of 150, 300, and 450 mg/kg body weight, there is no difference in the histopathological features of mice liver in the treatment group compared to its control group. There is no change in histopathological features of the liver after treatment with Pc-1(showed with an arrow in Fig. 9b). Degeneration Hydrophobic is a reversible processes. If the use of the substance is stopped, the condition of DH can be normal again (Dancygier and Schimacer 2010). Pc-2 at a dose of 5 mg/kg body weight was recommended to be used in the short-term treatment.

Apartate transaminase (AST)/glutamic oxaloacetic transaminase (SGOT) and alanine transaminase (ALT)/glutamic pyruvic transaminase (SGPT) serum are the enzymes produced by liver. Although many studies showed that the increase of hepatic enzymes serum was not a directly linked for liver injury, increase levels were responsible to cause inflammation, cellular leakage and damage of cell membrane to cells in the liver (Kausar et al. 2010). The results of the current study showed that mice AST treated with extract and isolated compounds were significantly lower than the negative control mice group, but the ALT value was not significantly different compared to the negative control group mice. In general, 5 mg/kg body weight Pc-1, 5 mg/kg body weight Pc-2, and Pc-extract of 150, 300, and 450 mg/kg body weight reduced the levels of AST. Further research is needed to investigate the hepatoprotective effect of extracts and isolated compounds. Acute toxicity testing is one of the parameter for the measurements of drug safety. Acute toxicity tests on mice showed that treatment with 15 mg/kg body weight piperine (isolated from *P. nigrum* L. extract) or 15 mg/kg body weight extract of *P. nigrum* L. mice resulted zero persen (%) mortality. A 100% of mice mortality occurred in mice treated with the isolated compound and extract at a dose of 25 mg/kg body weight (Kumar et al. 2009). At doses of 450 mg/kg body weight Pc-extract did not cause the death of mice even there was no change in the liver and kidneys histopathological features, this indicates that Pc-extract safer than *P. nigrum* L. To compare the level of isolates safety of both species Piper, it is necessary to conduct further research to establish the LD_{50} of Pc-1 and Pc-2.

Conclusion

The 450 mg/kg body weight of Pc-extract had equal macrophage phagocytic activity compared to 5 mg/kg body weight of two neolignans (Pc-1 and Pc-2) isolated from Pc-extract. The 150 mg/kg body weight of Pc-extract had high production of NO than 300 and 450 mg/kg body weight and 5 mg/kg body weight Pc-1 and Pc-2. The only treatment of 5 mg/kg body weight Pc-2 caused liver hydropic degeneration.

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Compliance with ethical standards

Ethical statement All procedures associated with animal experimentations were approved by the central integrated research (LPPT) Universitas Gadjah Mada Indonesia number: 221/KEC-LPPT/III/2015.

Conflict of interest This manuscript described has not been published before; not under consideration for publication anywhere else; and has been approved by all co-authors.

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