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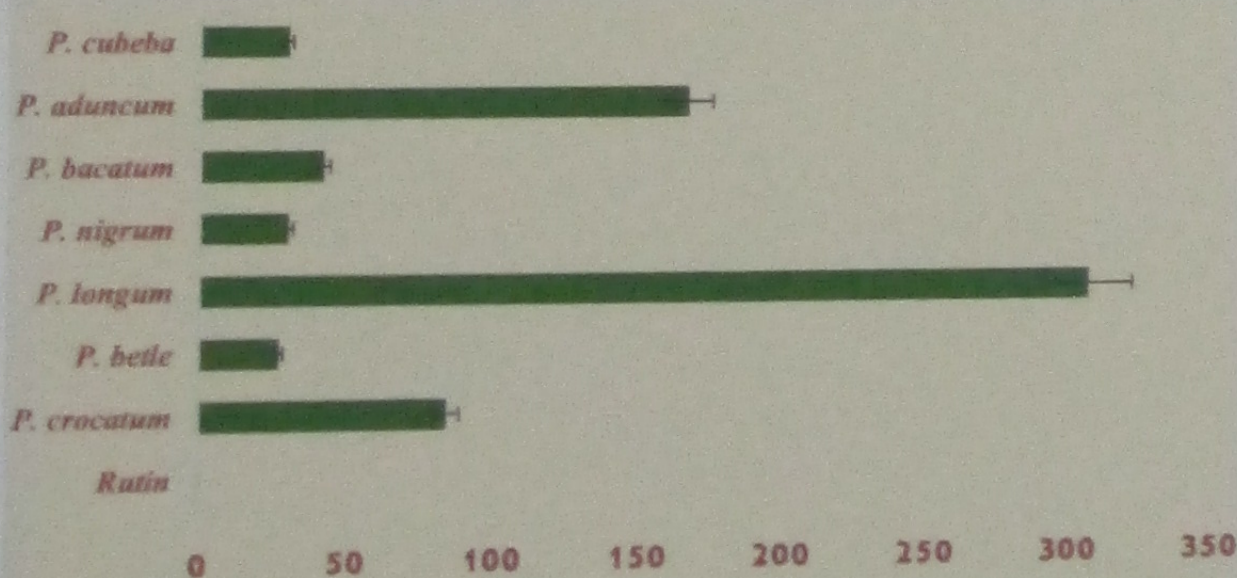


Fig. 1. The IC₅₀ of Piper sp. leaves methanolic extract



Fig. 2. Total Phenolic content of Piper sp. leaves methanolic extract

Abstract

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 Nitric Oxide (NO) productions. In the catalyzed by iNOS enzyme. The methanolic extract of red betel (*Piper crocatum* Ruiz & Pav.) leaf is an immunostimulant, which is able to increase macrophages phagocytic activity known relatively high. However the mentioned activity did not over produce of NO₂. The mention condition may be due to the ability of red betel leaf to keep safe the function of immune cells from the effect of macrophage phagocyte over activity. The aim of the current research was to investigate the red betel leaves methanolic extract immunostimulant and antioxidant while the methanolic extract of *P. betle* is an immunosuppressant and antioxidant. Furthermore, the extracts. The results show that IC₅₀ values of red betel leaf methanol extract, rutin, and *P. betle* leaf methanolic extract were 84.341 ± 6878 µg/ml; 0.135 ± 0.0004 µg/ml, and 26.915 ± 0.455 µg/ml respectively. Statistical test shows that the antioxidant activity of red betel leaf methanol extract is lower than the antioxidant activity of *P. betle* leaf methanolic extract and rutin. *Piper betle* showed the strongest antioxidant activity followed by *P. aduncum*, *P. nigrum*, *P. cubeba*, *P. bacatum*, *P. crocatum*, *P. longum* and *P. nigrum* respectively. The greatest total phenol was *P. betle*, followed by *P. aduncum*, *P. cubeba*, *P. bacatum*, *P. crocatum*, *P. longum* and *P. nigrum* respectively.

Keywords: red betel (*Piper crocatum* Ruiz & Pav.), extract, antioxidant, *Piper* species.

INTRODUCTION

Currently people use traditional medicine because it has a psychological appeal than modern medicine. In certain circumstances, modern drugs tend to be expensive, sometimes painful effect, and have less opportunity to be recovered (Spinedis, 2006). Recently red betel leaves are widely used as a traditional medicine in Indonesia. *In vitro* phagocytic activity test of macrophages showed that the methanol extract of red betel (*Piper crocatum*, Ruiz & Pav.) leaf had immunostimulant activity (Hartini *et al.*, 2013). Unlike the red betel initially used as an ornamental plant, green betel (*P. betle*) is a species of the genus *Piper* was widely used Indonesian people as a traditional medicine. Kanjwani *et al.* (2008) reported that the methanol extract of green betel leaf has immunosuppressive activity. In addition to red and green betel, some species of the genus *Piper* also used as traditional medicine. Rutin is an antioxidant compound and at the levels higher 50 µg/ml showed significant stimulatory effect (Zhao *et al.*, 2007; Yang *et al.*, 2008).

METHOD

Authentication materials in the form of plants of various species of *Piper* namely sirih merah (*P. crocatum*), sirih lingsu (*P. betle*), sirih lengkung (*P. aduncum*), lada (*P. nigrum*), kemukus (*P. cubeba*), rinu (*P. bacatum*), and cabe jawa (*P. longum*) were conducted at the Faculty of Biology UGM. Maceration method was used to extract the compound of various *Piper* species. Antioxidant activity test used DPPH method. Preparation of plant material refers to the preparation stages of plant extraction according to Kumar *et al.* (2016). Rutin was used as a positive control. Inhibitory Concentration (IC₅₀ values) were calculated from the linear regression equation by plotting the average percent concentration of antioxidants and antioxidant activity (Alam, *et al.*, 2013; Jugdale, *et al.*, 2009; dan Kumar, 2016).

RESULT AND DISCUSSION

The test results showed that antioxidant activity (IC₅₀ values) of *P. crocatum* leaf extract was higher than those of *P. betle* or rutin. The average of IC₅₀ leaf extract *P. crocatum*, *P. betle*, and rutin was 84.341 ± 6878 µg/ml, 26.915 ± 0.455 µg/ml, and 0.135 ± 0.0004 µg/ml respectively. Rutin used as a positive control in this study is a compound isolated from plant known to have an antioxidant activity and an immunostimulatory (Zhao *et al.*, 2007; Yang *et al.*, 2008). Conversely, *P. betle* is known as an antioxidant but an immunosuppressive (Kanjwani *et al.*, 2008). The IC₅₀ value of *P. crocatum* was higher than those of *P. betle* or rutin. In other words, the potency of *P. crocatum* as an antioxidant was lower than those of *P. betle* or rutin. In case of the same methanolic extract, *P. betle* had a higher antioxidant activity compared to those of *P. crocatum*. The phenolic compounds contributed significantly to the antioxidant capacity of the medicinal herbs (Cai *et al.*, 2004). Therefore, it could be that phenolic content of *P. betle* higher than those of *P. crocatum*. Immunostimulant will enhance the immune system's ability to respond to the attendance of antigen. *P. crocatum* which is an immunostimulatory did not show higher antioxidant activity than *P. betle* which is immunosuppressant. Stimulation of the macrophage by *P. crocatum* may activate the enzyme iNOS and ROS, thus causing an adverse reaction to the cell. On the other hand, *P. betle* that do not stimulate macrophage. Therefore, even both species from the same genus, and extracted with the same solvent and method but they have different antioxidant activity. Rutin is an immunostimulatory cell proliferation of mice splenocytes. It is not stimulates phagocytosis of phagocytic cells. Rutin showed significant stimulatory effect and shows the potential for a powerful antioxidant at the levels of higher than 50 µg/ml (Zhao *et al.*, 2007).

The antioxidant activity of various species in the genus *Piper* showed that *P. betle* had a highest antioxidant activity followed by *P. aduncum*, *P. nigrum*, *P. cubeba*, *P. bacatum*, *P. crocatum*, and *P. longum* respectively. The content of total phenolic measured at the extract concentration of 200 µg/ml indicated that the highest total phenol content was *P. betle* followed by *P. aduncum*, *P. cubeba*, *P. bacatum*, *P. crocatum*, *P. longum* and the lowest was *P. nigrum*. According to Cai *et al.* (2004), phenolic compounds contribute significantly to the antioxidant activity of plants. This research results supports previous opinion, total phenolic content of the leaves of *P. betle* was highest compared to those of other species. Concomitant to the current results, the antioxidant activity of *P. betle* was also the highest compared to those of other species. In general, total phenolic content of 7 species of the genus *Piper* were ranging from 133 to 198 µg/ml. It indicates that the species of the genus *Piper* contained relatively high phenolic compounds. The antioxidant activity of *P. longum* was the lowest compared to other species, but the content of total phenols was not the least. It indicates that the compounds responsible for antioxidant activity were not only in the form of phenol compounds. Phenol and other compounds in the extract work together to produce an antioxidant effect. Interactions between compounds occur in the extracts known as endointeraction (Lila and Raskin, 2005) may cause a synergy effect which is more powerful effect compared to those of single compound (Heinrich *et al.*, 2004).

CONCLUSION

1. *Piper betle* was the plant in the genus *Piper* which showed the strongest antioxidant activity followed by *P. aduncum*, *P. nigrum*, *P. cubeba*, *P. bacatum*, *P. crocatum*, and *P. longum* respectively.
2. The greatest total phenol content of 200 µg/ml extract concentration was those of *P. betle*, followed by *P. aduncum*, *P. cubeba*, *P. bacatum*, *P. crocatum*, *P. longum* and *P. nigrum* respectively.

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Certificate of Attendance

This is to certify that

YUSTINA SRI HARTINI

Has successfully attended at

The 26th Asian Congress of Pharmaceutical Sciences

Federation of Asian Pharmaceutical Associations

held in Bangkok, Thailand

on November 9-13, 2016

Assoc.Prof.Dr. Sindhchai Keokitichai
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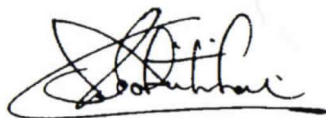
Yustina Sri Hartini

In recognition of the poster presentation entitled

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at the 26th Federation of Asian Pharmaceutical Association Congress (FAPA 2016)

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2. Yang ditugasi memberikan laporan kepada Rektor tentang pelaksanaan tugas tersebut.

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