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JOURNAL LINKS

The accumulation of two neolignan in the leaves, stems, and flower of red betel (*Piper crocatum* Ruiz & Pav.)

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The accumulation of two neolignan in the leaves, stems, and flower of red betel (*Piper crocatum* Ruiz & Pav.)

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Abstract. Organ for the biosynthesis of secondary metabolite is not always a place for its biosynthesis, even the same compound was synthesized in the different organs in different plants. Neolignan is a secondary metabolite, known as an imunostimulant, synthesized through shikimic acid pathway with the important precursor is chorismic acid. The compound was known to be accumulated in the roots, stems and leaves of *Piper regnellii* with the concentration varies depending on the type of neolignan. It has been investigated the accumulation of two compound neolignans (Pc-1 and Pc-2) isolated from the methanol extract of red betel leaf (*Piper crocatum* Ruiz & Pav.) in the leaves, stems, and flowers of red betel. Chromatographic methods used was Gas Chromatography-Mass Spectrometry (GC-MS). Chromatogram of GC-MS showed that the Pc-1 with purity of 100%, m/z 460.3 could be detected at the minute of 29.986, while the Pc-2 with purity of 96.681%, m/z 418.3 was detected at the minute of 29.495. The research was then continued to investigate the existence and accumulation of both compounds in leaves, stems, and flowers of red betel. The GC-MS chromatogram shows that Pc-1 and Pc-2 could be detected in the leaves, stem and flower with various concentration among plant organs. Moreover, leaves contained the highest concentration of Pc-1 and Pc-2 compared to other plant organs.

1. Introduction

To live, grow, and reproduce, plant transform and interconvert a vast number of organic compounds. The compounds are made by a series of reaction called metabolism which could be distinguished to be primary and secondary metabolisms. Humans utilize secondary metabolites for various purposes because the compounds show various pharmacological activities. Secondary metabolites were synthesized in certain organs of the plant. Plant secondary metabolites were accumulated in different ways. Tobacco, for instance, synthesize its alkaloid nicotine in the root but accumulate it in the leaves [1], while *Piper regnellii* accumulate its neolignan in the roots, stems, and leaves [2]. *Piper crocatum* known as red betel is a species of the family Piperaceae. Decoction of the leaves of the plant family Piperaceae used traditionally for the treatment of various diseases. Our previous study reported that two neolignans isolated from methanolic extract of *P. crocatum* leaves had imunostimulant actitivity [3]. Regarding many previous studies, the accumulation of both compounds could be in the organs other than leaves. In order to broaden the provided material for traditional healing, the distribution of both compounds in organs other than leaves should be known. After knowing the distribution of both

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compounds, hopefully the provided material for traditional healing could also be supplied by organs other than leaves. Therefore, the accumulation of two compound neolignans (Pc-1/pipercrocatin and Pc-2/deacetyl pipercrocatin) isolated from the methanolic extract of red betel leaf in the leaves, stems, and flowers were detected in this study.

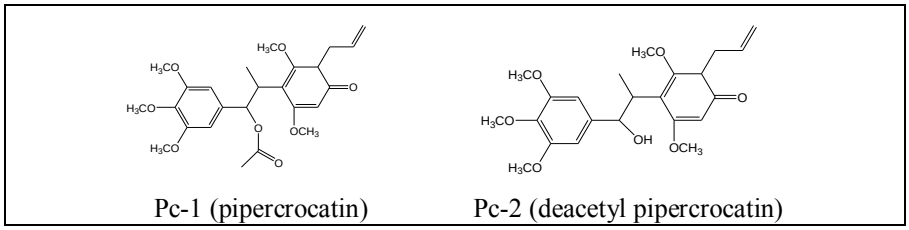

Figure 1. Structures of the neolignans isolated from *P. crocatum* [4,3]

2. Experimental

2.1. Plant material

The leaves, steam, and flowers of red betel were collected in April 2015 at Sleman, Yogyakarta Indonesia. The plant material was identified by Wahyono at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia and a voucer specimen (no.BF/284/Ident/Det/VIII/2011) was deposited in herbarium unit at The Faculty of Pharmacy, Sanata Dharma University, Yogyakarta, Indonesia.

2.2. Extract preparation

The leaves, stems and flowers of red betel were dried and powdered using mortar and pestle. The powder of three organs were then extracted with methanol by maceration method at a room temperature for three days. The extract were filtered and evaporated under vacuum.

2.3. Sample preparation

Stock solution of Pc-1, Pc-2, and the extracts of the leaves, stems and flowers from red betel were prepared in the mixture of chloroform:methanol (1:1).

2.4. Instrumentation and chromatographic conditions

The analysis were carried out using an Agilent GC 6890N 59765 B MSD connected with MS. The capillary column was Agilent 19091S-433 HP-5ms 5% phenyl methyl siloxane. Maximal temperature was 325^{o}C with the nominal length of 30,0 m, the diameter was 250 µm, the film thickness was 0.25 µm, the initial flow was 1.0 mL/min, the nominal initial pressure was 8.65 psi, and the average velocity was 37 cm/sec.

3. Result and Discussion

Pipercrocatin (Pc-1) compound was identified in the form of a white crystalline glazed, while the form of deacetyl pipercrocatin (Pc-2) was greenish-white coloured powder. The Figure 2-4 shows the GC-MS chromatograms of Pc-1 and Pc-2, the MS spectrograms of Pc-1 and Pc-2, and the GC-MS chromatogram of red betel leaves methanolic extract.

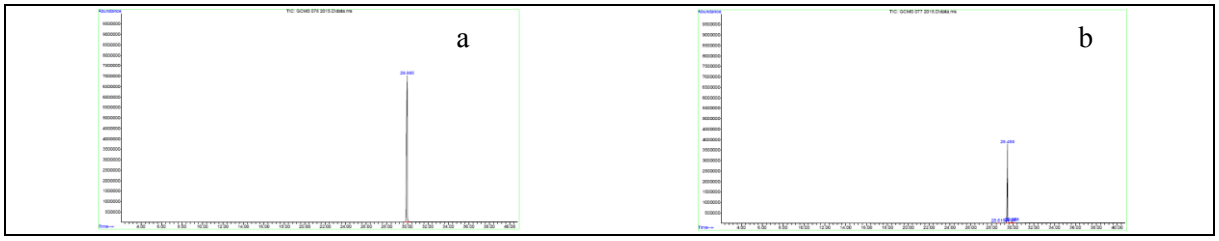

Figure 2. The GC-MS chromatogram of Pc-1 (a) and Pc-2 (b)

The maximum absorption of Pc-1 and Pc-2 were found at 234,5 nm and 264,5 nm 280 nm, respectively. Chromatogram of GC-MS showed that the Pc-1 with the purity of 100%, m/z 460.3 could be detected at the minute of 29.986, while the Pc-2 with the purity of 96. 681%, m/z 418.3 was detected at the minute of 29. 495.

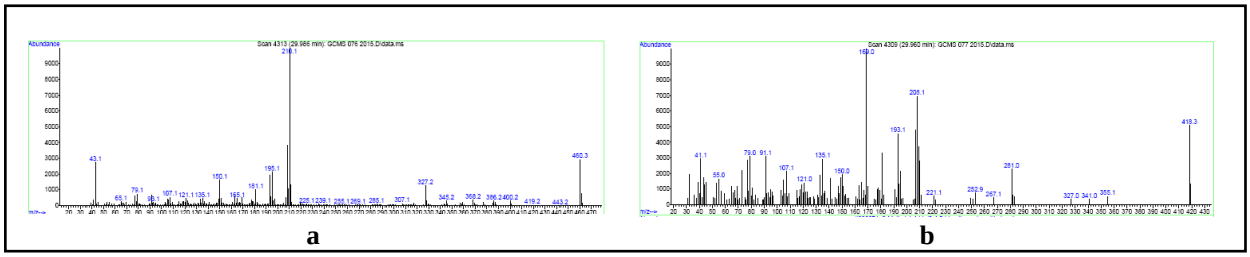

Figure 3. The MS spectrogram of Pc-1 (a) and Pc-2 (b

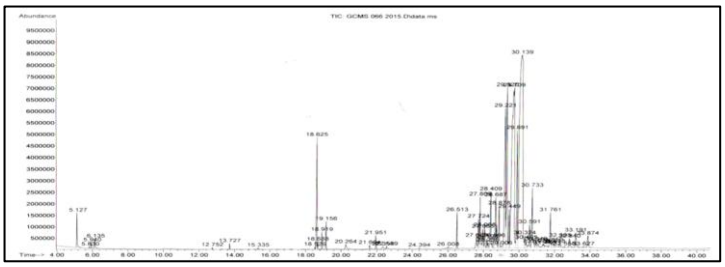

Figure 4. The GC chromatogram of red betel leaves extract

Table 1. Area percent of Pc-1 and Pc-2 in the leavess, stem, and flowers of red betel.

Red betle extract of:	Pc-1	Pc-2
Leaves	31.726%	19.798 %
Stems	29.531 %	13.533 %
Flowers	5.259 %	16.209 %

Organ for the biosynthesis of secondary metabolite is not always a place for its biosynthesis, even the same compound was synthesized in the different organs in different plants. The extracts demonstrate similar chromatographic profile, but there were differences in the concentrations of neolignans. Neolignan is a secondary metabolite, synthesized through shikimic acid pathway. Some neolignans participate in lignin synthesis and hence have important roles in determining physicochemical and mechanical properties of cell walls, whereas others act as antioxidants, biocides (fungicides, bactericides and antiviral agents) and perhaps even as cytokinins [5]. Our previous research found that Pc-1 and Pc-2 showed imunostimulant activity [3]. Table 1 shows the content of neolignans in the different parts of the plant. Leaves presented a highest concentration of Pc-1 and Pc-2. The lowest

concentration of Pc-1 was found in flowers, while stems contain the lowest concentration of Pc-2. The reason why leaves contain the highest concentration of Pc1 and Pc2 may due to the important precursor for various compounds in the shikimic acid pathway, namely chorismic acid was located in the plastids [6]. Therefore, the accumulation of most chorismic acid derivates including lignan were abundance in the organ with higher content of plastid, its means was leaves. Differences in the distribution of neolignan compounds are also shown in the other Piper species namely *P. regnellii*. The compound was known to be accumulated in the roots, stems and leaves of *P. regnellii* with the concentration varies depending on the type of neolignan [2**]**. The results of this study recommend the use of organ leaves to get neolignan compound especially Pc-1 and Pc-2.

4. Conclusion

The neolignans (Pc-1 and Pc-2) isolated from red betel (*Piper crocatum* Ruiz & Pav.) accumulated in the leaves, stems, and flowers of red betel with the highest concentrations in the leaves.

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