Title : The Accumulation of Two Neolignans in the Leaves, Stems, and Flowers of Red Betel (Piper crocatum Ruiz & Pav.)

Journal name : Journal of Physics: Conference Series

1/17/23, 3:26 PM

Mail - Yustina Sri Hartini - Outlook

Konfirmasi Ketidak Sesuaian dan Permohonan maaf

ISBCM IPB <isbcm.ipb@gmail.com> Mon 5/29/2017 2:49 AM To: Yustina Sri Hartini <yustinahartini@usd.ac.id>

Yth. Ibu Dr. Yustina Sri Hartini

Di Tempat

Dengan hormat,

Terima kasih sebelumnya kami sampaikan atas partisipasi Ibu dalam kegiatan International Symposium on Bioinformatics, Chemometrics and Metabolomics (ISBCM) 2016 dan mengirimkan artikel ke proceding ISBCM 2016.

Kami konfirmasikan adanya ketidak sesuaian penulisan author kedua dari paper "The accumulation of two neolignan in the leaves, stems, and flower of red betel (*Piper crocatum* Ruiz & Pav.)"yang telah terbit di procceding IOP Journal of Physics: Conference Series Vol 835 (Proc of ISBCM 2016)" dengan penulisan di manuskrip yang dikirimkan ke panitia. Kami sudah mencoba menghubungi pihak penerbit untuk memperbaikinya namun tidak bisa diubah dikarenakan adanya perjanjian bersama IOP- Journal of Physics:conferences series, yang menyatakan bahwa perubahan setelah proceeding diterbitkan tidak dapat dilakukan.

Atas kekurang periksaan dan ketidaknyamanan yang terjadi, kami menyampaikan permohonan maaf yang sebesarnya. Berikutnya, terkait dampak terhadap indeksasi, kami dapat menawarkan bantuan untuk mengusulkan nama author tertulis di proceeding sebagai "name variant" dari apa yang sudah terdaftar di Scopus melalui fasilitas "Author Feedback Wizard" (https://www.scopus.com/feedback/author/home.uri#/).

Demikian kami sampaikan, atas perhatian dan kerjasamanya kami ucapkan terima kasih.

Panitia ISBCM 2016

Ketua,

Dr. Mohamad Rafi, SSi., MSi

Secretariat of ISBCM 2016 Tropical Biopharmaca Research Center IPB Jl. Taman Kencana No. 3, Bogor 16128 West Java, Indonesia Email: <u>isbcm.ipb@gmail.com</u> Website: <u>http://metabolomics.ipb.ac.id/</u>

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ISBCM2016 Review Results-need major revision

ISBCM IPB <isbcm.ipb@gmail.com>

Wed 3/8/2017 2:07 AM

To: Yustina Sri Hartini <yustinahartini@usd.ac.id>;yustinahartini@yahoo.com <yustinahartini@yahoo.com>

Dear Dr. Yustina Sri Hartini

Following the review of your article submitted to Proceeding of the Intl Symposium on Bioinformatics, Chemometrics and Metabolomics 2016 (IOP J of Physics), We recommend your article should be revised taking into account the comments or any changes requested by the reviewer(s) and also follow the IOP J of Physics Conf Series guidelines for preparing the revised manuscript (file attached)

Please submit the revised version of your manuscript (both doc/docx and pdf version) via email to isbcm.ipb@gmail.com no later than March 15, 2017

Please consider the comments of the reviewers carefully and upgrade your paper accordingly.

Best regards,

The Editor

Dr. Wisnu Ananta Kusuma (Bioinformatics)

Prof. Dr. Abdul Rohman M.Si., Apt (Chemometrics)

Dr. Sastia Prama Putri (Metabolomics)

Secretariat of ISBCM 2016 Tropical Biopharmaca Research Center IPB Jl. Taman Kencana No. 3, Bogor 16128 West Java, Indonesia Email: <u>isbcm.ipb@gmail.com</u> Website: <u>http://metabolomics.ipb.ac.id/</u> Mail - Yustina Sri Hartini - Outlook

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Revisi kembali manuskrip ISBMC 2016 no 17

ISBCM IPB <isbcm.ipb@gmail.com>

Sun 3/19/2017 11:45 PM

To: Yustina Sri Hartini <yustinahartini@usd.ac.id>

Cc: hartantonugroho2005@ugm.ac.id <hartantonugroho2005@ugm.ac.id>

Dear Dr. Yustina Sri Hartini

Terima kasih telah mengirimkan revisi manuskripnya. Ada beberapa hal yang perlu kami sampaikan terhadap paper yang telah anda revisi ini yaitu:

1. Dalam menampilkan gambar di paper belum mengikuti format yang telah ditetapkan oleh petunjuk penulisan IOP J of Physics Conf series (halaman 6 di file ISBCM 2016 - JPCSExampleWordDocument untuk contohnya)

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Terima kasih

The Editor

Dr. Wisnu Ananta Kusuma (Bioinformatics)

Prof. Dr. Abdul Rohman M.Si., Apt (Chemometrics)

Dr. Sastia Prama Putri (Metabolomics)

2017-03-15 12:28 GMT+07:00 Yustina Sri Hartini < yustinahartini@usd.ac.id >:

Yth. Tim Prosiding ISBCM 2016

Trima kasih informasinya.

Terlampir kami sampaikan manuskrip berjudul 'The Accumulation of Two Neolignans in the Leaves, Stems in the Leaves, of Red Betel (Piper crocatum Ruiz & Pav.)' yang sudah direvisi sesuai komentar dari reviewer dan format penulisan IOP J of Physics. File tersebut kami kirim dalam format pdf dan word.

Trima kasih perhatiannya.

salam,

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Dear Colleague:

We request that you review the enclosed article. The article is a manuscript that is submitted to International Symposium on Bioinformatics, Chemometrics and Metabolomics 2016 (ISBCM 2016, metabolomics.ipb.ac.id). It will be considered to be published in Journal of Physics: Conference Series. The review is part of a double-blinded review process in which the identity of the authors and the reviewers are masked. Please make all comments on this form, not on the manuscript. The manuscript should not be shown to anyone without permission from the Editor.

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The second page solicits your comments to the authors. Please be as specific as possible. The review may be returned via e-mail at the address above. Please contact me by e-mail if I may be of assistance.

Thank you, in advance, for your help.

Yours sincerely,

The Editor

Dr. Wisnu Ananta Kusuma (Bioinformatics), w.ananta.kusuma@gmail.com

Prof. Dr. Abdul Rohman M.Si., Apt (Chemometrics), abdulkimfar@gmail.com

Dr. Sastia Prama Putri (Metabolomics), sastia_putri@bio.eng.osaka-u.ac.jp

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Dear authors

- The authors reported about the accumulation of two neolignans in the some organ of red betel. This manuscripts not really sound because some part in the manuscript are missing. For example in the introduction section the authors didn't give any information why they should check the accumulation of the two compound also in the discussion section the authors didn't give any clear statement why in the some organ the two compound found in higher yield. So please improve your manuscript with this kind information or discuss more deeply.
- Grammar need to be improve
- Some figure could be combined, eg figure 2 with 3, 4 with 5 and I think figure 7 is not needed or you can write in table form

The Accumulation of Two Neolignan in the Leaves, Stems, and Flower of Red Betel (*Piper crocatum* Ruiz & Pav.)

Yustina Sri Hartini^{1*} and Laurentius Hartanto Nugroho²

¹Faculty of Pharmacy, Sanata Dharma University, Yogyakarta, Indonesia ²Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia

Email : yustinahartini@usd.ac.id

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. 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 compound. The compounds are made by a series of reaction called metabolism which could be distinguished to be primary and secondary metabolisms. Humans utilize secondary metabolics for various purposes because the compounds show various pharmacological activity. Secondary metabolites synthesized in certain organs of the plant. Plant secondary metabolites accumulate in different ways, tobacco 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* had imunostimulant actitivity [3]. The analysis of the distribution of compounds in *P. crocatum* especially neolignan in the various organ of the plant have not been reported yet. In this study, the accumulation of two compound neolignans (Pc-1 and Pc-2) isolated from the methanolic extract of red betel leaf in the leaves, stems, and flowers was developed.



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

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 of the Department of 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 dried powder of leaves, stems and flowers of red betel were extracted with methanol by maceration method at a room temperature for three days. The extract were filtered, 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 chloroform:methanol (1:1).

2.4. Instrumentation and chromatographic conditions

The analysis were carried out using an Agilent GC 6890N 59765 B MSD with capillary column model number Agilent 19091S-433 HP-5ms 5% phenyl methyl siloxane. Maximal temperature 325°C, nominal length 30,0 m, diameter 250 μ m, film thickness 0.25 μ m, initial flow 1.0 mL/min, nominal initial pressure 8.65 psi, average velocity 37 cm/sec.

3. Result and Discussion

Pc-1 compound in the form of a white crystalline glazed, while the Pc-2 form of greenish-white coloured powder. The Figure 2-5 shows the GC-MS chromatograms of Pc-1 and Pc-2.



Figure 2. The GC-MS chromatogram of Pc-1, Pc-2 and Pc-extract; GC of

The maximum absorption of Pc-1 and Pc-2 was found to be 234,5 nm and 264,5 nm 280 nm, respectively. 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.





Figure 6. The GC cromatogram of red betel leaves extract

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

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

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. 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.

5. 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.

6. Acknowledgment

The author is grateful to Ministry of Research, Technology and Higher Education, Republik of Indonesia for the financial support by Fundamental Research Grant with contract References Grant with contract number: 010/HB-LIT/III/2015.

References

- [1] Shoji T, Kajikawa M, and Hashimoto T 2010 The Plant Cell 22 3390-409
- Felipe DF, Filho BPD, Nakamura CV, Franco SL, and Cortez DAG 2006 J. of Pharm Biomed Anal 41 1371-75

- [3] Hartini YS, Wahyuono S, Widyarini S, Yuswanto A 2014 Trop J. Pharm Res 13 10 1609-14
- [4] Kustiawan PM 2012 Isolasi dan Identifikasi Senyawa Imunostimulan Non Spesifik In Vitro dari Daun Sirih Merah (Piper crocatum Ruiz & Pav.) Thesis (Yogyakarta: Fakultas Farmasi Universitas Gadjah Mada) p 65
- [5] Lewis NG and Davin LB 1994 ACS Symposium series 562 10 202-45, American Chemical Society

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methanol extract of red betel leat (<i>typer crocatum Kum</i> Chromatographic methods used was Gas Chromatograph M5 showed that the Pc-1 with purity of 100%, m/z 446. Pc-2 with purity of 96 681%, m/z 418.3 was detected at to investigate the existence and accumulation of both co GGAMS chromatograms shows that Pc-1 could be for	2.8 PAV) in the leaves, stems, and howers of red betel. >y-Mass Spectromatry (GC-MS). Command prum of GC- 3.3 could be detected at the minute of 20 986, while the the minute of 20 945. Sector Awar Shear Continued impounds in leaves, stem of the forwars of red betel. The end in the leaves in the united of 30 130 with the	- U	Add a reply	
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1. INTRODUCTION T 0 live, grow, and reproduce, plant transform and interconvert a vast number of organic compound.	two neolignams isolated from methanolic extract of P crocentum had immostimulant activity (Harthi <i>et al.</i> , 2014). The analysis of the distribution of compounds on P . crocentum sepacially accelgran in the various			
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International Symposium on Bioinformatics, Chemometr Bogor, Indonesia, October 18, 2016	ics, and Metabolomics 2016			

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International Symposium on Bioinformatics, Chemometrics, and Metabolomics 2016 Bogor, Indonesia, October 18, 2016

different plant. The extracts demonstrate unitary of the state of the source of the source

4 CONCUMENT

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.

ACKNOWLEDGMENT

ACINOWLEDGARENT The author is grateful to Ministry of Research, Technology and Higher Education, Republik of Indonesia for the financial support by Fundamental Research Grant with contract number. 010 HB-LIT/IIL/015.

REFERENCES

- EFFERDED: 1) Feljes DT, Files DD, Nakeman CV, Franco SL, and Corte EAG. Analysis of essignmes composites of the sensitive of the sensitive of the sensitive Biomedical Analysis of the sensitive of the sensitive transmission of the sensitive of the sensitive of the two human sensitive of the sensitive of the sensitive biological solutions for Add Hall Sensitive Biological Sensitive Sensitive of the sensitive biological solutions for the sensitive of the sensitive of the sensitive biological solutions for the sensitive of the sensitive of the sensitive biological solutions for the sensitive of the

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