



Wenny Irawaty &lt;wenny\_i\_s@ukwms.ac.id&gt;

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**BISSTECH 2015 submission 56**

1 message

**BISSTECH 2015** <bisstech2015@easychair.org>

Thu, Jun 25, 2015 at 3:59 PM

To: Wenny Irawaty &lt;wenny\_i\_s@ukwms.ac.id&gt;

Dear authors,

We received your paper:

Authors : Wenny Irawaty and Aning Ayucitra

Title : Effect of solvent polarity on antioxidant activity during fractionation of ethanolic extract of Citrus hystrix peel

Number : 56

The paper was submitted by Wenny Irawaty <[wenny\\_i\\_s@ukwms.ac.id](mailto:wenny_i_s@ukwms.ac.id)>.

Thank you for submitting to BISSTECH 2015.

Best regards,

EasyChair for BISSTECH 2015.



Wenny Irawaty &lt;wenny\_i\_s@ukwms.ac.id&gt;

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**Notification of Acceptance**

1 message

**BISSTECH 2015** <bisstech2015@easychair.org>

Mon, Jul 13, 2015 at 1:12 PM

To: Wenny Irawaty &lt;wenny\_i\_s@ukwms.ac.id&gt;

Dear Mr/Mrs Wenny,

Firstly, we would like to express our sincere appreciation for your interest in joining our Bali International Seminar on Science and Technology Seminar (BISSTECH 2015). As of 13/07/2015, we have received more than 125 submissions.

Secondly, we are glad to inform you that your abstract entitled "Effect of solvent polarity on antioxidant activity during fractionation of ethanolic extract of Citrus hystrix peel" has been accepted to be presented in the seminar. Please, kindly prepare your full paper and have it submitted no later than August 14, 2015 through Easy Chair. If you do not have an account at Easy Chair, we kindly advise you to create one, however, if you prefer email correspondence you can still submit your paper to [bisstech2015@gmail.com](mailto:bisstech2015@gmail.com) and we will upload it to Easy Chair for you. For full paper format, please download and use template from <http://2015.bisstech.org/wp-content/uploads/2015/05/Proceeding.rar> (ARPN Journal style) or <http://2015.bisstech.org/wp-content/uploads/2015/05/Journal.rar> (MAS Journal style).

Please be advised, your full paper file should be prepared in PDF format and named using the following format "Submission#Number-1stAuthorfirstName-TemplateName.PDF".

For example, "Submission#112-MohamadIrwan-ARPN.PDF" or "Submission#112-MohamadIrwan-MAS.PDF".

All full papers will be peer-reviewed carefully and thoroughly for novelty, paper organization and clarity, and appropriate use of English. Feedbacks and suggestions will be given individually to each paper submitted for revision and resubmission. Those final papers which have been revised and meet the requirements will be selected for publication to Scopus indexed journal. The rest of the manuscripts will be published in our Bali International Seminar on Science and Technology 2015 Proceedings.

Thank you.

Kind Regards,

BISSTECH 2015 Committee



Wenny Irawaty &lt;wenny\_i\_s@ukwms.ac.id&gt;

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## Guideline and Sample Paper

1 message

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**BISSTECH 2015** <bisstech2015@easychair.org>

Tue, Sep 22, 2015 at 10:28 AM

To: Wenny Irawaty <wenny\_i\_s@ukwms.ac.id>

Dear Wenny,

As we have mentioned in the previous email, attached is zip file of conference proceedings paper guideline and sample.

Please submit your revision (.docx version) to [bisstech2015@gmail.com](mailto:bisstech2015@gmail.com) by September 30, 2015.

Thank you.

Kind Regards,

BISSTECH 2015 Committee



**ConferenceProceedingsGuidelineAndSample.zip**

80K



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**review paper**

3 messages

**bisstech bali** <bisstech2015@gmail.com>

Sun, Nov 15, 2015 at 3:22 PM

To: "Wenny Irawaty , ST., MT., Ph.D." &lt;wenny\_i\_s@ukwms.ac.id&gt;

Dear Authors,

Please find in the attachment, review process results of your paper(s).

The review was mainly about the paper format (the way you organize your paper according to the template) and novelty (gap between previous research findings and your study). You must show that at least 10 related papers from reputable international journals are referenced in your paper.

Due to limited time given by the journal publisher, we apologize that you will only be given a maximum of 7 days to revise your paper and submit back to us for final decision.

Please be advised that should your paper is selected to be published in the journal, you may be charged an additional fee. We will inform you once final decision is made. If your paper is not selected then it will be published in our BISSTECH 2015 Proceedings.

Thank you for your participation in the seminar.

Good luck to you all.

Regards,

BISSTECH 2015  
Committee

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**2 attachments** **weny irawati.uwm\_review.docx**  
64K **Keterangan Revisi.docx**  
14K

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**Wenny Irawaty , ST., MT., Ph.D.** <wenny\_i\_s@ukwms.ac.id>  
To: bisstech bali <bisstech2015@gmail.com>

Sun, Nov 22, 2015 at 8:39 PM

Dear committee,

I'd like to raise a question regarding the journal indexed by scopus that you have been contacted to publish papers of bisstech 2015, i.e. what are journals that have been contacted to publish our paper so that the cost is US\$ 600? Is that ARPN because we have to follow ARPN format?

Thank you very much in advance for your kind information.

All the best,

Wenny

[Quoted text hidden]

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**2 attachments****weny irawati.uwm\_review.docx**

ISBN 978-602-0856-36-0



# PROCEEDINGS

THE 3rd BALI INTERNATIONAL SEMINAR ON SCIENCE AND TECHNOLOGY (BISSTECH) 2015

Theme

"TOWARDS FUNDAMENTAL AND APPLIED RESEARCH FOR SOCIO-ENVIRONMENTALLY  
SAFE INDUSTRIAL DEVELOPMENT"

October 15-17, 2015 - Bali, Indonesia

Organized By :

UNIVERSITAS PEMBANGUNAN NASIONAL "VETERAN" JAWA TIMUR

Sponsored By: *Patra Logistik*

# PROCEEDINGS



Telah diperiksa kebenarannya dan sesuai dengan aslinya  
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Surabaya, 05-08-2020

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## The 3<sup>rd</sup> Bali International Seminar on Science and Technology

Bali, October 15-16<sup>th</sup>, 2015

**"TOWARDS FUNDAMENTAL AND APPLIED RESEARCH FOR  
SOCIO-ENVIRONMENTALLY SAFE INDUSTRIAL DEVELOPMENT"**

Organized By:

Faculty of Industrial Technology, University of Pembangunan Nasional "Veteran" Jawa Timur,

Bali State Polytechnic, and

College Of Engineering of National Cheng-kung University

# PROCEEDINGS



## The 3<sup>rd</sup> Bali International Seminar on Science and Technology

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Ketut Vini Elfarosa, S.E, M.M

## TABLE OF CONTENTS

### A. Chemical Engineering

#	TITLE	#PAGE
#A1	NOVEL NON RESISTANT ANTIBACTERIAL SULPHATE CHELATING AGENT <i>Haryanto Wardoyo</i>	A.1-A.12
#A2	HYDROPHILICATION OF MESOPOROUS CARBON BY IMPREGNATING-SILICA METHOD AS SOLID-DESICCANT IN GAS DEHYDRATION PROCESS <i>Fadlilatul Taufany, Anita Rahmawati, Dessy Fatmawati, Nurhamidah, Fahmi</i>	A.13-A.16
#A3	APPLICATION OF CHIRALITY ON CHIRAL RECONITION OF ENATIOMERS AND PHOTONIC CRYATALS FABRICATION <i>Chih-Chieh Chien, Jui-Hsiang Liu</i>	A.17-A.20
#A4	SYNTHESIS OF TRIPODAL CONJUGATED PYRRYL- RHODAMINE CHEMOSENSOR AND SELECTIVE SENSING OF Zn <sup>2+</sup> ION <i>Yu-Jen Chen, Wen-I Chang, Jui-Hsiang Liu</i>	A.21-A.23
#A5	FABRICATION AND CHIRAL RECOGNITION OF PREDESIGNED SUPRAMOLECULE VIA CHIRAL DOPED ORGANOGELEATOR <i>Jian-Chin Lin, Bo-Han Huang, Jui-Hsiang Liu</i>	A.25-A.28
#A6	APPLICATION OF BUOYANCY WEIGHING-BAR METHOD TO ESTIMATE THE DROPLET SIZE DISTRIBUTION AND OPTIMAL TIME OF BIODIESEL-GLYCEROL SEPARATION <i>Rondang Tambun, Farida Hanum, Yudhy Sibagariang, Jhoni Manurung</i>	A.29-A.32
#A7	BIODISEL PRODUCTION FROM USED COOKING OIL USING COMPOSITE CATALYST CAO/KI/Γ-AL <sub>2</sub> O <sub>3</sub> <i>Nyoman Puspa Asri, Diah Agustina Puspita Sari, Bambang Poedjojono, Suprpto</i>	A.33-A.37
#A8	ORI BAMBOO POTENTIAL AS BIOETHANOL SOURCE <i>M. T. Safirin, Edahwati Luluk, D. S. Perwitasari</i>	A.39-A.42
#A9	EFFECT OF TEMPERATURE ON MANUFACTURING PROCESS OF POLYETHYLENE GLYCOL DIOLEATE (PDO) <i>Yan Irawan, Ika Juliana</i>	A.43-A.45
#A10	SOLID WASTE MIXTURE AS AN ALTERNATIF SOLID FUEL <i>Sri Rachmania Juliastuti, Nuniek Hendrianie, Imam Naufal, Eka Patriargadani</i>	A.47-A.51

#	TITLE	#PAGE
#C7	MASS TRANSFER ON CONCENTRATION PROCESS OF CANE JUICE USING VACUUM FALLING FILM EVAPORATOR PART II <i>Suhadi, Wiwik Prihartanti, Nyoman Sriwidari</i>	C.29-C.32
#C8	THE CHARACTERISTICS OF EMULSION CREAM CONTAINING GOTUKOLA AND GINGER EXTRACT IN ADDITION OF FRAGRANCE <i>Yenny Meliana*), Witta Kartika Restu, Egi Agustian, Anny Sulaswaty, Yulianti Sampora, Sri Fahmiati, Melati Septiyanti</i>	C.33-C.38
#C9	MODIFICATION OF TARO FLOUR WITH FERMENTATION PROCESS <i>Endang Srihari, Farid Sri Lingganingrum</i>	C.39-C.43
#C10	EFFECT OF SOLVENT POLARITY ON ANTIOXIDANT ACTIVITY DURING FRACTIONATION OF ETHANOLIC EXTRACT OF CITRUS HYSTRIX PEEL <i>Wenny Irawaty, Aning Ayucitra</i>	C.45-C.48
#C11	EFFECT OF CHITOSAN ADDITION AND TEMPERATURE OF HEATING FOR TENSILE STRENGTH AND ELONGATION AT BREAK VELUE OF BIOPLASTICS FROM TARO STARCH (COLOCASIA ESCULANTA) WITH GLYCEROL PLASTICIZER <i>M. Hendra Ginting, Toni Pahri Sirait, Torasman Sidabudar</i>	C.49-C.57
#C12	EXTRACTION OF ESSENTIAL OIL BY ENFLEURATION PROCESS USING VEGETABLE AND ANIMAL FATS <i>Siswati Soe'eib, Nyoman Puspa Asri, A.S Dwi Saptati N.H, Diah Agustina P</i>	C.60-C.62

#### D. Information and Communication Technologies

#	TITLE	#PAGE
#D1	CONCEPTUAL MODEL TO INCREASE COCOA PRODUCTIVITY WITH SYSTEM DYNAMICS APPROACH <i>Syurfah Ayu Ithriah, Erma Suryani</i>	D.1-D.4
#D2	TEMPLATE MATCHING BASED PATTERN IDENTIFICATION WITH GEOMETRIC MOTIF OF BATIK <i>Eva Yulia Puspaningrum, Wahyu S.J. Saputra, Yisti Vita Via, Suryohadi</i>	D.5-D.7
#D3	PERFORMANCE OF ROBUST REGRESSION METHOD FOR SIDE VIEW FACE RECOGNITION <i>Budi Nugroho, Intan Yuniar Purbasari</i>	D.9-D.12



The 3<sup>rd</sup> Bali International Seminar on Science and Technology (BISSTECH)  
"Towards Fundamental and Applied Research for Socio-Environmentally Safe Industrial  
Development", October 15-17<sup>th</sup>, 2015 - Denpasar Bali, Indonesia

1 <sup>st</sup> Day: Thursday, October 15 <sup>th</sup> , 2015	
07.30-08.30	Registration
08.30-09.00	Opening (MC) + Bali Dance
09.00-09.45	<p>SPEECHES :</p> <ol style="list-style-type: none"><li>1. Chair Of BISSTECH 2015</li><li>2. Rector Of UPN</li><li>3. Director Of State Polytechnic Of Bali</li><li>4. Governor Of Bali</li><li>5. Ministry Of Research, Technology And Higher Education</li></ol> <p>MOU SIGNING :</p> <ol style="list-style-type: none"><li>1. Rector Of University Of Pembangunan Nasional "Veteran" East Java AND Director Of State Polytechnic Of Bali</li><li>2. Rector Of University Of Pembangunan Nasional "Veteran" East Java AND Rector Of University Of Trunojoyo Madura</li><li>3. Rector Of University Of Pembangunan Nasional "Veteran" East Java AND Rector Of University Of WR. Supratman</li><li>4. Dean Of College Of Engineering, NCKU AND Dean Of Faculty Of Industrial Technology, UPN "Veteran" East Java</li><li>5. Dean Of Faculty Of Industrial Technology, UPN "Veteran" East Java AND UPT DISPERINDAG, EAST JAVA, JEMBER</li><li>6. Director Of State Polytechnic Of Bali AND Keiyo University, JAPAN</li></ol>
09.45-10.00	Coffee Break
10.00-12.00	PROF. PAO-SHAN YU PROF. DR. WOLFGANG W. SCHMALL
12.00-13.00	Lunch & Prayer Break
13.00-15.00	Parallel Session
15.00-15.15	Coffee Break
15.15-16.00	Parallel Session
19.00-21.00	Gala Dinner at Governor Office - Courtesy of Governor of Bali (Tentatively)

(Ludwig Maximilians Universität München) xvii

bio minerals: lesson learned



The 3<sup>rd</sup> Bali International Seminar on Science and Technology (BISSTECH)  
"Towards Fundamental and Applied Research for Socio-Environmentally Safe Industrial  
Development", October 15-17<sup>th</sup>, 2015 - Denpasar Bali, Indonesia

2 <sup>nd</sup> Day: Friday, October 16 <sup>th</sup> , 2015	
07.30-08.30	Registration
08.30-09.00	Opening (MC)
09.00-10.30	PROF. YASUSHI KIYOKI PROF. Inocencio E. Buot, JR, PhD *
10.30-10.45	Coffee Break
10.45-12.00	Parallel Session
12.00-13.30	Lunch & Prayer Break
13.30-15.30	Parallel Session
15.30	Closing
15.30-16.30	Break (Preparation To Tampak Siring Palace)
16.30-21.00	Gala Dinner at Courtesy of Bali State Polytechnic (Tentatively)

3 <sup>rd</sup> Day: Saturday, October 17 <sup>th</sup> , 2015	
08.30-Till end	Tour (Registration required)

\* open university, Philippine (organic agriculture, biotechnology, ...)

## ROOM B1

Friday, October 16<sup>th</sup>, 2015

#	Authors	Title
1	Stefanus Muryanto, Sri Sutanti and Mega Kasmiyatun	Inhibition of struvite crystal growth in the presence of herbal extract <i>Orthosiphon aristatus</i> Bl.Miq
2	Rondang Tambun, Farida Hanum, Yudhy Sibagariang and Jhoni Manurung	Application of buoyancy weighing-bar method to estimate the droplet size distribution and optimal time of biodiesel-glycerol separation
3	Wenny Irawaty and Aning Ayucitra	Effect of solvent polarity on antioxidant activity during fractionation of ethanolic extract of <i>Citrus hystrix</i> peel
4	M. Hendra Ginting and Toni Pahri Sirait	The Effect of Gelatinization Profile For Mechanical Properties Of Bioplastics From Taro Starch ( <i>Colocasia esculenta</i> ) - Chitosan With Glycerol Plasticizer
5	Rini Rahma, Indrawati Yuhertiana and Siti Sundari	The Evaluation Of E-Audit Implementation On Indonesia Government Financial Audit Process
6	Yan Irawan and Ika Juliana	Effect Of Temperature On Manufacturing Process Of Poly (Ethylene Glycol) Diolate (Pdo)
7	Tavio Tavio and Parmo Parmo	A Proposed Clamp System For Mechanical Connection Of Reinforcing Steel Bars
8	Sugeng Winardi, Kusdianto, Tantular Nurtono, Widiyastuti And Siti Machmudah	The Flow Pattern And Mixing Time In Biogas Digester With Side Entering Mixer
9	Kusdianto, Siti Machmudah,	Extraction Of Residual Palm Oil From Palm Mesocarp Pressed- Fiber ( <i>Elaeis Guineensis</i> ) Using

## Effect of Solvent Polarity on Antioxidant Activity during Fractionation of Ethanolic Extract of *Citrus Hystrix* peel

Wenny Irawaty<sup>1</sup> and Aning Ayucitra<sup>2</sup>

Chemical Engineering Department, Faculty of Engineering, Widya Mandala Catholic University Surabaya, East Java, Indonesia  
e-mail: [wenny\\_i\\_s@ukwms.ac.id](mailto:wenny_i_s@ukwms.ac.id)

### ABSTRACT

*Citrus hystrix* has been claimed to exhibit tremendous performance to repair organ damage caused by free radicals developed during metabolism. The present study was undertaken to evaluate the antioxidant power of *Citrus hystrix* peel. Antioxidant activity of several fractions (water, ethyl acetate, and hexane) obtained from the fractionation of ethanolic extract of *Citrus hystrix* peel was investigated. The effect of the solvents on phytochemical content was also evaluated. The research methods involve the *Citrus hystrix* peel preparation (sizing, drying), extraction of the peel by using ethanol 41% for 8 hours, fractionation of the ethanolic extract by different solvents, and then followed by antioxidant activity measurement of the fractions obtained. The results revealed that all fractions of the ethanolic extract of *Citrus hystrix* peel exhibited variable antioxidant activity. Specially, the ethyl acetate fraction showed the highest values of antioxidant capacity (% DPPH scavenging activity). The different activity of the fractions was correlated with phytochemical content in each fraction.

**Keywords:** *Citrus hystrix* peel, antioxidant, fractionation

### INTRODUCTION

Plants and plant products are being used as a source of medicine since long time. The medicinal properties of plants have been widely investigated due to their potent antioxidant activities, no side effect, and economic feasibility (Hui et al., 2009). The natural antioxidants or phytochemicals are the secondary metabolites of plants that are widely distributed in foods of plants and count as phenolic compounds. As antioxidant, phenolic compounds comprising flavonoids and phenolic acids play an important role in the prevention of human pathologies by acting as radical scavenger against degenerative diseases such as cardiovascular diseases, neurodegenerative diseases, blood disorder diseases, diabetes mellitus (Zhao et al., 2012), and cancers. Therefore, there is growing interest toward natural antioxidant from herbal sources (Ebrahimzadeh et al., 2008, Sarepoua et al., 2015, Gorinstein et al., 2001). Phenolic compounds are frequently found in fruits (Nizam Uddin et al., 2014, Ebrahimzadeh et al., 2008, Gorinstein et al., 2001), vegetables (Hui et al., 2009, Widyawati et al., 2014, Harbaum et al., 2008), and grains (Singh et al., 2012, Chiremba et al., 2012). The chemical composition of fruits, including leaves and peels have been widely investigated and it was found that the peel possesses higher antioxidant activity compared to other parts of the fruit (Gorinstein et al., 2001, Li et al., 2006). For example, Gorinstein et al. (2001) found that the amount of phenolic compounds in the peels of orange, lemon, and grapefruit were higher than the peeled fruits. Similar result was

reported by Li et al. (2006) when pomegranate was selected in their study (Li et al., 2006).

Citrus fruits contain high content of flavonoids compared to other type of fruits. Citrus fruits contain a wide range of flavonoid compounds which are sub-classed in flavanones, flavones, flavonol, and dihydrochalcone C- and/or O-glycosides (Roowi and Crozier, 2011, Gattuso et al., 2007). Therefore, since in the early nineties the presence of flavonoids in citrus fruits began to attract a number of researchers. This study focused on *Citrus hystrix*. The leaves of this citrus have commercial importance and its byproduct, i.e. the fruit itself has not been utilized yet.

This work evaluates the effect of solvent polarity used during fractionation of *Citrus hystrix* peel extract on the antioxidant capacity. The aim of this work was to propose a suitable solvent for the separation of phenolic compounds in crude *Citrus hystrix* peel extract.

### LITERATURE REVIEW

*Citrus hystrix*, commonly known as Kaffir lime or wild lime, is originated from South East Asia and cultivated throughout the tropical regions. *Citrus hystrix* is greenish yellow, acidic flavor, bumpy, and pear-shaped. This citrus is reported rich in phenolic compounds including flavonoids, limonoids, glycerolglycolipids, furanocoumarins, benzenoid derivative and quinolinone alkaloids with potential health-promoting properties.

Several methods such as maceration (Cha et al., 2010), heat treatment (Xu et al., 2007), microwave (Chiremba et al., 2012), ultrasonic (Ma et al., 2008), far-

infrared radiation (Lee et al., 2006), subcritical water (Plaza et al., 2010), high pressure – pulsed electric field (Sánchez-Moreno et al., 2005), fermentation (Harbaum et al., 2008), and cellulases treatment (Kim et al., 2005) have been studied to extract phenolic compounds from plant materials. Among the methods mentioned earlier, maceration is the most economical feasible and this led to commercial application in the future.

Solvent plays an important role in the extraction of plant natural antioxidant compounds. The amount of compounds extracted is influenced by the polarity of both compounds and solvent used. Hegazy and Ibrahim (2012) investigated the effect of solvent (methanol, ethanol, dichloromethane, acetone, hexane, and ethyl acetate) polarity on the antioxidant capacities of orange peel extracts (Hegazy and Ibrahim, 2012). It was found that the antioxidant capacities vary with solvent polarity and the high polar solvent of ethanol exhibited the highest antioxidant activities. Patel et al. (2011) reported the antioxidant activities of *Hybanthus enneaspermus* (Linn.) F. Muell. (Violaceae) was greatly influenced by solvent polarity that influence phenolic compounds being extracted by certain solvent which in turn its activity (Patel et al., 2011). Similar finding was also reported by other work (Widyawati et al., 2014).

## METHODOLOGY

**Material:** *Citrus hystrix* obtained around East Java was collected in January-March. Chemicals used were ethanol, methanol, hexane, ethyl acetate, ascorbic acid, DPPH, in this work were purchased from Sigma Chemical Co.

**Methods:** Citrus fruits were manually peeled and the edible portions were carefully separated. The peels were further cut into 0.5 x 0.5 cm and air dried for 2 days. The dry peel was then soaked with ethanol solution 41% for 8 h at room temperature. The solid part was then separated by a Whatman filter paper. The filtrate was concentrated under a vacuum by evaporating the ethanol. The water residue was then fractionated under solvents of varying polarity. The phytochemical analysis of the various fractions was carried out independently by using methods described in (Harbone, 1973, Adewole et al., 2014). Antioxidant capacity was quantified by the DPPH radical method. For this assay, a solution of DPPH in methanol (0.2 mM) was prepared freshly. 1.25 mL aliquot of this solution was added to 1 mL sample at different concentrations in the range of 0.03-4 mg/mL. The sample solution was shaken and left to react in the dark for 30 min. Then the solution was transferred into a cuvette, and the absorbance was determined at 520 nm using a spectrophotometer (Shimadzu, UVmini-1240). A decrease in absorbance was recorded and the antioxidant capacity was expressed as IC<sub>50</sub>. The IC<sub>50</sub> is the concentration where 50% inhibition occurs. The control contained all reagents except the fraction was prepared under the same treatment. The antioxidant capacity was compared to standard compound of ascorbic acid which is already known for its

al., 2008), and cellulases treatment (Kim et al., 2005) have been

good antioxidant activity. The percentage inhibition of the radicals was calculated using the formula:

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100 \dots \dots \dots (1)$$

where A<sub>0</sub> is the absorbance of the control, and A<sub>1</sub> is the absorbance of the fraction/standard.

## RESULTS

Free radical scavenging activity expressed as IC<sub>50</sub> ranged from 0.03 to 2.37 mg crude extract/mL as shown in Table 1.

Table 1. DPPH free radical scavenging activity of different fractions of ethanolic *Citrus hystrix* peel extract

Fraction	IC <sub>50</sub> (mg/mL)
Hexane	2.37 ± 0.16
Ethyl acetate	0.03 ± 0.00
Water residue	1.09 ± 0.05
Ascorbic acid (standard)	0.04 ± 0.00

As seen, the three fractions exhibit DPPH radical scavenging capacity and the ethyl acetate shown the best activity among the others with the IC<sub>50</sub> reached a value of 0.03±0 mg/mL. The water residue exhibited the second highest value in the DPPH free radical test after the ethyl acetate fraction where the IC<sub>50</sub> was recorded at 1.09±0.05 mg/mL. With the IC<sub>50</sub> value of 2.37±0.16 mg/mL, hexane fraction occupied the third highest rank in the same test method. Ascorbic acid as the control compound has also exhibited the scavenging activity toward DPPH free radical compound with the IC<sub>50</sub> value of 0.04 mg/mL.

When the concentration of the fractions was increased, it was observed that the antioxidant capacity was improved as shown in Figure 1 for the fraction of ethyl acetate (r<sup>2</sup> = 0.9581). Other fractions showed the similar trend (data not shown).

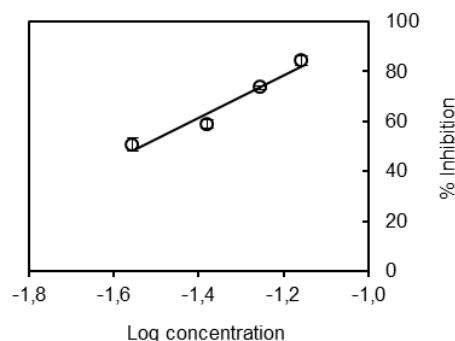


Figure 1. DPPH scavenging activity for different amount of ethyl acetate fraction

In order to get insight into the scavenging activity of each fractions observed in this study, phytochemical

analysis of the three fractions were performed and the results are tabulated in Table 2. As seen, each fractions exhibited different compounds that may contributed to the activity of each fractions. Alkaloids, phenolics, and flavonoids were detected in all fractions. While compounds of saponins, tannins, sugars, and carbohydrates were not identified in the fraction of hexane, however, all the compounds were revealed in the fractions of ethyl acetate and water residue.

## DISCUSSIONS

As shown in Table 1, the IC<sub>50</sub> values varied in the following order: ethyl acetate fraction < water residue < hexane fraction. Similar result was found by other work (Anagnostopoulou et al., 2006) that the fraction of ethyl acetate exhibited the highest antioxidant capacity compared to other fractions (ether, dichloromethane, and water fraction) when sweet orange peel was selected in their study. The IC<sub>50</sub> values were then compared to the standard of ascorbic acid. The standard suggests that its activity was 25% lower than the ethyl acetate fraction, while compared to the other two fractions, the standard was 27 and 59% higher for the fractions of water residue and hexane, respectively. The results show that ethyl acetate is regarded as the most effective solvent to extract compounds from the ethanolic extract of *Citrus hystrix* peel. This can be explained by the possible complex formation of phenolic compounds with other components which are more extractable in ethyl acetate than those of other fractions (hexane and water) (Zhao and Hall, 2008, Zhu et al., 2011).

The antioxidant capacity of plants is contributed by the presence of phenolic compounds or phytochemicals. The fractions of *Citrus hystrix* peel extract contain some potent phytochemical constituents such as phenolics, flavonoids, saponins, alkaloids, carbohydrates, and tannins which may be responsible for this activity (Table 2). Phenolic compounds such as phenolic acid, flavonoids, and tannins

have been well known contribute the antioxidant activity/capacity (Gorinstein et al., 2001). Flavonoids found in citrus juice were reported flavanone aglycones, flavone aglycones, polymethoxyflavones, flavanone-*O*-glycosides, flavone-*C*-glycosides, and flavone-*O*-glycosides. Earlier investigations on saponins found that the compound reduced the risk factor of atherosclerosis (Rodrigues et al., 2005). Tannins was also reported to possess antioxidant activity (Beninger and Hosfield, 2003). Since the fraction of hexane contains least phytochemical compounds (Table 2), thus it is not surprising if the fraction exhibited the lowest activity to quench DPPH free radical compound. The higher antioxidant capacity exhibited by ethyl acetate fraction cannot only be explained by phytochemical analysis performed. However, the mechanism behind this could be due to the presence of high content of phenolic compounds.

## CONCLUSIONS

The fraction of ethyl acetate has shown impressive antioxidant capacity toward DPPH free radical scavenging activity. The comparable antioxidant capacity of ethyl acetate fraction compared to the standard of ascorbic acid indicates that the fraction may have beneficial implication for human health. The mechanism behind this could be due to the presence of high content of phenolic compounds. Accordingly, further investigation on analysis of compounds in the ethyl acetate fraction is required to confirm this.

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Table 2. Phytochemical analysis of various fractions of *Citrus hystrix* peel extract

Fraction	Component						
	Alkaloids	Saponins	Tannins	Sugars	Carbohydrates	Phenolics	Flavonoids
Hexane	+	—	—	—	—	+	+
Ethyl acetate	+	+	+	+	+	+	+
Water residue	+	+	+	+	+	+	+

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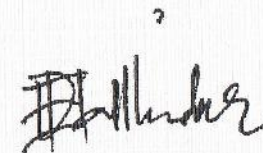
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