

Volume 1718

 **Conference collection**

# 5th International Conference and Workshop on Basic and Applied Sciences (ICOWOBAS 2015)



**Surabaya, Indonesia**  
16-17 October 2015

**Editors**  
Moh. Yasin and Professor Dr. Sulaiman W. Harun

**AIP** | Conference Proceedings

[proceedings.aip.org](http://proceedings.aip.org)

## AIP Proceedings: A name your community will know and respect

40 years' experience • 100,000+ papers • 1,600+ volumes

**A world-class proceedings service for all events: From workshops to the largest international conference**

- Online-only proceedings
- Optional printed copies or CDs for participants
- Rapid online and print publication

Our wealth of experience and expertise will ensure an outstanding publication experience.

### Publication fees which work with your budget

- **Simple online publication fees:** Completely independent of page counts, publish substantial papers at no extra cost.
- **Options for online access:** 1-year conference access or select perpetual open access for the entire community.

### Flexibility in the printed medium

Choose from these options to print all papers or just a selection of articles from the conference:

#### **Conference collection**

- Printed copies containing all papers published in the online proceedings.
- For editors who want to reproduce all online papers for their participants.

#### **Selected papers**

- Printed copies containing a selection of papers chosen by the editors.
- Choose to print just the best work, avoid the cost of printing everything.

#### **Workshops and summer schools**

- Printed copies designed especially for summer schools and workshops.
- Visibility and identity for events publishing tutorials and reviews.

### Get a proposal for your proceedings in 3 simple steps

#### **Step 1.**

Obtain a proceedings questionnaire by writing to us at [confproc@aip.org](mailto:confproc@aip.org) or download from [proceedings.aip.org](http://proceedings.aip.org)

#### **Step 2.**

Fill in the questionnaire with details of your conference and return it to [confproc@aip.org](mailto:confproc@aip.org)

#### **Step 3.**

We'll review the questionnaire and your requirements and write to confirm if we can offer a proposal.



ISBN 978-0-7354-1364-1  
ISSN 0094-243X

[proceedings.aip.org](http://proceedings.aip.org)



5th International Conference and Workshop on Basic and Applied Sciences (ICOWOBAS 2015)

Vol. 1718

# 5th International Conference and Workshop on Basic and Applied Sciences (ICOWOBAS 2015)

---

**Surabaya, Indonesia**

16-17 October 2015

## **Editors**

**Moh. Yasin**

Airlangga University, Surabaya, Indonesia

**Professor Dr. Sulaiman W. Harun**

University of Malaya, Kuala Lumpur, Malaysia

## **Sponsoring Organizations**

Universitas Airlangga

Universiti Teknologi Malaysia

Malaysia and Salahaddin-Hawler University

All papers have been peer reviewed.



Melville, New York, 2016  
AIP Conference Proceedings

Volume 1718

---

To learn more about AIP Conference Proceedings visit <http://proceedings.aip.org>

## Editors

### **Moh. Yasin**

Kampus C Universitas Airlangga  
Physics Department  
Jalan Mulyorejo  
Surabaya (60115)  
Indonesia

Email: [yasin@fst.unair.ac.id](mailto:yasin@fst.unair.ac.id)

### **Professor Dr. Sulaiman W. Harun**

Department of Electrical Engineering  
Faculty of Engineering  
University of Malaya  
50603 Kuala Lumpur  
Malaysia

Email: [swharun@um.edu.my](mailto:swharun@um.edu.my)

Authorization to photocopy items for internal or personal use, beyond the free copying permitted under the 1978 U.S. Copyright Law (see statement below), is granted by the AIP Publishing LLC for users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that the base fee of \$30.00 per copy is paid directly to CCC, 222 Rosewood Drive, Danvers, MA 01923, USA: <http://www.copyright.com>. For those organizations that have been granted a photocopy license by CCC, a separate system of payment has been arranged. The fee code for users of the Transactional Reporting Services is: 978-0-7354-1364-1/16/\$30.00



© 2016 AIP Publishing LLC

No claim is made to original U.S. Government works.

Permission is granted to quote from the AIP Conference Proceedings with the customary acknowledgment of the source. Republication of an article or portions thereof (e.g., extensive excerpts, figures, tables, etc.) in original form or in translation, as well as other types of reuse (e.g., in course packs) require formal permission from AIP Publishing and may be subject to fees. As a courtesy, the author of the original proceedings article should be informed of any request for republication/reuse. Permission may be obtained online using RightsLink. Locate the article online at <http://proceedings.aip.org>, then simply click on the RightsLink icon/"Permissions/Reprints" link found in the article abstract. You may also address requests to: AIP Publishing Office of Rights and Permissions, 1305 Walt Whitman Road, Suite 300, Melville, NY 11747-4300, USA; Fax: 516-576-2450; Tel.: 516-576-2268; E-mail: [rights@aip.org](mailto:rights@aip.org).

ISBN 978-0-7354-1364-1

ISSN 0094-243X

Printed in the United States of America

**AIP Conference Proceedings, Volume 1718**  
**5th International Conference and Workshop on Basic**  
**and Applied Sciences (ICOWOBAS 2015)**

**Table of Contents**

<b>Preface: 5th International Conference and Workshop on Basic and Applied Sciences (5th ICOWOBAS) 2015</b>	010001
<b>Committees: 5th International Conference and Workshop on Basic and Applied Sciences (5th ICOWOBAS) 2015</b>	010002
<b>INVITED SPEAKER</b>	
<b>Microstructure and mechanical changes induced by Q-Switched pulse laser on human enamel with aim of caries prevention</b>	
R. Apsari, D. A. Pratomo, D. Hikmawati, and N. Bidin	020001
<b>BIODIVERSITY</b>	
<b>Sea cucumber species identification of family Caudinidae from Surabaya based on morphological and mitochondrial DNA evidence</b>	
Muhammad Hilman Fu'adil Amin, Ida Bagus Rai Pidada, Sugiharto, Johan Nuari Widyatmoko, and Bambang Irawan	030001
<b>Oil removal from petroleum sludge using bacterial culture with molasses substrate at temperature variation</b>	
Ni'matuzahroh, Alvin Oktaviana Puspitasari, Intan Ayu Pratiwi, Fatimah, Sri Sumarsih, Tini Surtiningsih, and Salamun	030002
<b>MICROBIAL BIOCHEMISTRY AND MOLECULAR BIOLOGY</b>	
<b>Immunofluorescence assay method to detect dengue virus in Paniai-Papua</b>	
Teguh Hari Sucipto, Nur Laila Fitriati Ahwanah, Siti Churrotin, Norifumi Mataka, Tomohiro Kotaki, and Soengeng Soegijanto	040001
<b>Inhibitor candidates's identification of HCV's RNA polymerase NS5B using virtual screening against iPPI-library</b>	
Indah Sulistyawati, Sulisty Dwi K. P., and Mochammad Ichsan	040002
<b>ENVIRONMENTAL AND GREEN CHEMISTRY</b>	
<b>Seasonal radon measurements in Darbandikhan Lake water resources at Kurdistan region-northeastern of Iraq</b>	
Adeeb Omer Jafir, Ali Hassan Ahmad, and Wan Muhamad Saridan	050001
<b>Effect of digestion time on anaerobic digestion with high ammonia concentration</b>	
Nur Indradewi Oktavitri, Hery Purnobasuki, Eko Prasetyo Kuncoro, Indah Purnamasari, and Semma Hadinnata P.	050002
<b>The influence of dicarboxylic acids: Oxalic acid and tartaric acid on the compressive strength of glass ionomer cements</b>	
Ahmadi Jaya Permana, Harsasi Setyawati, Hamami, and Irmina Kris Murwani	050003

<b>The effect of glycerol and sorbitol plasticizers toward disintegration time of phyto-capsules</b> Pratiwi Pudjiastuti, Esti Hendradi, Siti Wafiroh, Muji Harsini, and Handoko Darmokoesoemo	050004
<b>Speciation and bioavailability of some heavy metals in agricultural soils used for cultivating various vegetables in Bedugul, Bali</b> I. Made Siaka, I. Made Supartha Utama, I. B. Putra Manuaba, I. Made Adnyana, and Emmy Sahara	050005
<b>Potential contribution of low cost materials in clean technology</b> Heman A. Smail, Kafia M. Shareef, and Zainab Ramli	050006
<b>Monitoring of coastline change using remote sensing data at South Pamekasan</b> Thin Soedarti, Onny Z. Rinanda, and Agoes Soegianto	050007
<b>The production of sulfonated chitosan-sodium alginate find in brown algae (<i>Sargassum sp.</i>) composite membrane as proton exchange membrane fuel cell (PEMFC)</b> Siti Wafiroh, Pratiwi Pudjiastuti, and Ilma Indana Sari	050008
<b>NATURAL PRODUCTS AND MEDICINAL CHEMISTRY</b>	
<b>Virtual screening using MTiOpenScreen and PyRx 0,8 revealed ZINC95486216 as a human acetylcholinesterase inhibitor candidate</b> Sulistyo Dwi K. P., Arindra Trisna W., Vindri Catur P. W., Erna Wijayanti, and Mochammad Ichsan	060001
<b>Three-step crystallization in synthesis of ZSM-5 without organic template</b> Hartati, Alfa Akustia, Indra Permana, and Didik Prasetyoko	060002
<b>Spermatogenic structure and fertility of <i>Mus musculus</i> after exposure of mangosteen (<i>Garcinia mangostana L</i>) pericarp extract</b> Alfiah Hayati, Melia Eka Agustin, Farida Ayu Rokhimaningrum, Hasan Adro'i, and Win Darmanto	060003
<b>Double layer structure-based virtual screening reveals 3'-Hydroxy-A-Naphthoflavone as novel inhibitor candidate of human acetylcholinesterase</b> Mochammad Ichsan, Ardini Pangastuti, Mohammad Wildan Habibi, and Kartika Juliana	060004
<b>Total flavonoid and phenolic contents of n-butanol extract of <i>Samanea saman</i> leaf and the antibacterial activity towards <i>Escherichia coli</i> and <i>Staphylococcus aureus</i></b> Wiwik Susannah Rita, I. Made Dira Swantara, I. A. Raka Astiti Asih, Ni Ketut Sinarsih, and I. Kadek Pater Suteja	060005
<b>Properties of kojic acid and curcumin: Assay on cell B16-F1</b> Sugiharto, Arbakariya Ariff, Syahida Ahmad, and Muhajir Hamid	060006
<b>Phenolic compounds from the stem bark <i>Erythrina Orientalis</i> and detection of antimalaria activity by ELISA</b> Tjijtjik Srie Tjahjadarie, Ratih Dewi Saputri, and Mulyadi Tanjung	060007
<b>Morphology characterization and biocompatibility study of PLLA (Poly-L-Llactid-Acid) coating chitosan as stent for coronary heart disease</b> Prihartini Widiyanti, Adanti W. Paramadini, Hajria Jabbar, Inas Fatimah, Fadila N. K. Nisak, and Rahma A. Puspitasari	060008

## ANALYTIC AND FORENSIC CHEMISTRY

- Preparation and characterization Al<sup>3+</sup>-bentonite Turen Malang for esterification fatty acid (palmitic acid, oleic acid and linoleic acid)**  
Abdulloh Abdulloh, Nanik Siti Aminah, Triyono, Mudasir, and Wega Trisunaryanti 070001
- Electrochemical degradation of malachite green using nanoporous carbon paste electrode**  
Muji Harsini, Faizatul Fitria, and Pratiwi Pudjiastuti 070002
- Imprinted zeolite modified carbon paste electrode as a potentiometric sensor for uric acid**  
Miratul Khasanah, Alfa Akustia Widati, and Sarita Aulia Fitri 070003
- Potential complex of rhodamine B and copper (II) for dye sensitizer on solar cell**  
Harsasi Setyawati, Aning Purwaningsih, Handoko Darmokoesoemo, Hamami, Faidur Rochman, and Ahmadi Jaya Permana 070004
- Gas chromatography-mass spectrometry of ethyl palmitate calibration and resolution with ethyl oleate as biomarker ethanol sub acute in urine application study**  
Ni Made Suaniti and Manuntun Manurung 070005

## ENVIRONMENTAL BIOCHEMISTRY AND BIOTECHNOLOGY

- Tailoring folic acid and methotrexate-attributed quantum dots for integrated cancer cell imaging and therapy**  
Mochamad Zakki Fahmi and Jia-Yaw Chang 080001
- The effect of aqueous extract of *Kalanchoe Folium* on methylprednisolone pharmacokinetic profile**  
Niken Indriyanti, Afrillia Nuryanti Garmana, Finna Setiawan, Elin Yulinah Sukandar, and I. Ketut Adnyana 080002
- Microbial consortium role in processing liquid waste of vegetables in Keputran Market Surabaya as organic liquid fertilizer ferti-plus**  
Fauziah Rizqi, Agus Supriyanto, Intan Lestari, Lita Indri D. L., Elmi Irmayanti A., and Fadilatur Rahmaniayah 080003
- Isolation, transformation, anticancer, and apoptosis activity of lupeyl acetate from *Artocarpus integra***  
Hery Suwito, Wan Lelly Heffen, Herry Cahyana, and Wahyudi Priyono Suwarso 080004

## COMPUTATIONAL PHYSICS, CHEMISTRY & MATHEMATICS

- Contrastive studies of potential energy functions of some diatomic molecules**  
Hassan H. Abdallah and Hewa Y. Abdallah 090001
- Determination the total neutron yields of several semiconductor compounds using various alpha emitters**  
Ramadhan Hayder Abdallah and Barzan Nehmat Sabr 090002
- Forward problem solution as operator of filter and back projection matrix to reconstruct the various of data collection in electrical impedance tomography**  
Khusnul Ain, Deddy Kurniadi, Suprijanto, Oerip Santoso, and R. Arif Wibowo 090003



**Influence of geometrical factor on binding energy of Cooper pairs in  $YBa_2Cu_3O_{7-\delta}$  compound**  
Saeed O. Ibrahim and Bassam M. Mustafa 090004

**Size dependence lattice thermal conductivity for Si nanofilm**  
Hawkar T. Taha and Abdulrahman Kh. Alassafee 090005

#### PHYSICS AND RENEWABLE ENERGY

**The effect of nitrogen on biogas flame propagation characteristic in premix combustion**  
Willyanto Anggono, Fandi D. Suprianto, Tan Ivan Hartanto, Kenny Purnomo, and Tubagus P. Wijaya 100001

**Porous carbon materials synthesized using IRMOF-3 and furfuryl alcohol as precursor**  
Pemta Tia Deka and Ratna Ediati 100002

**Fiber optic displacement sensor for medal detection using fiber bundled probe**  
M. Yasin, Samian, Supadi, Pujiyanto, and Y. G. Yhun Yhuwana 100003

#### STATISTICS, PURE AND APPLIED MATHEMATICS

**Estimation of median growth curves for children up two years old based on biresponse local linear estimator**  
Nur Chamidah and Marisa Rifada 110001

**Segmentation of breast cancer cells positive 1+ and 3+ immunohistochemistry**  
Ause Labellapansa, Izzati Muhimmah, and Indrayanti 110002

**Search and selection hotel system in Surabaya based on geographic information system (GIS) with fuzzy logic**  
Purbandini and Taufik 110003

**Fuzzy multinomial control chart and its application**  
Wibawati, Muhammad Mashuri, Purhadi, and Irhamah 110004

**An implementation of continuous genetic algorithm in parameter estimation of predator-prey model**  
Windarto 110005

#### BIOMEDICAL ENGINEERING

**Chlorophyll mediated photodynamic inactivation of blue laser on *Streptococcus mutans***  
Suryani Dyah Astuti, A. Zaidan, Ernie Maduratna Setiawati, and Suhariningsih 120001

**Nearest patch matching for color image segmentation supporting neural network classification in pulmonary tuberculosis identification**  
Riries Rulaningtyas, Andriyan B. Suksmono, Tati L. R. Mengko, and Putri Saptawati 120002

**Infant breathing rate counter based on variable resistor for pneumonia**  
Novi Angga Sakti, Ardy Dwi Hardiyanto, La Febry Andira R. C., Kesa Camelya, and Prihartini Widiyanti 120003

## **Preface: 5th International Conference and Workshop on Basic and Applied Sciences.**

Foreword from Chairman of 5<sup>th</sup> ICOWOBAS 2015

Ladies and Gentleman,

The 5<sup>th</sup> International Conference and Workshops on the Basic and Applied Sciences (ICOWOBAS) is organized of existing collaborations between Airlangga University, Universiti Teknologi Malaysia and Salahaddin-Hawler University (Erbil, Iraqi Kurdistan) in order to promote the development of sciences and their prospect of application in industry and medical devices. The program of this activity are the scientific program involves the presentation of the paper and poster in the area of chemistry, biology, physics, mathematics and their applications. It also conducts the workshop program was presented the current issues in optical instrumentation. Thus invited many participants as academic researchers, scientists, industrial professionals, government officers, students and other participants. The meeting intends to bring together researcher, scientists and scholars to exchange and share their experiences, new ideas, research novelties in related fields and discuss the practical challenges and the solutions adopted.

The AIP proceedings hold the full papers presented at the 5<sup>th</sup> ICOWOBAS. The conference took place in Surabaya (Indonesia) at the Garuda Mukti Room, Kampus C Universitas Airlangga, October 16<sup>th</sup> - 17<sup>th</sup>, 2015, and the workshop was conducted at the Faculty of Science and Technology, Airlangga University, October 15<sup>th</sup>, 2015.

The conference included: Prof. Dr. Retna Apsari (Universitas Airlangga, Indonesia) as invited speaker. In total, we received 152 abstracts for oral and 29 posters, and 46 full paper selected in AIP proceeding. As the acceptance rates illustrates the competition is stiff, and the accepted submission reflected high rates of reviewer enthusiasm. By design, these papers have been through peer-review process and they are almost accepted.

ICOWOBAS is a lot of work. We could not have done it without help from many people. We would especially like to thank: Scientific board of ICOWOBAS, for inviting us to chair the meeting; The Rector of Airlangga University, for supporting us to conduct the conference; our colleagues in the Faculty of Science and Technology, Airlangga University, for their support in the conference; the local committee, for organizing and handling the conference; the many reviewers, for providing professional reviews; our sponsor: Vitalong C and DGHE through Airlangga University funding.

Surabaya, February 2016

Dr. Moh. Yasin

## Committees

### Scientific committees:

1. Prof. Dr. Stephen G. Pyne (University of Wollongong, Australia)
2. Prof. Dr. Sugeng Triwahyono (Universiti Teknologi Malaysia, Malaysia)
3. Prof. Dr. Sulaiman W. Harun (University of Malaya, Malaysia)
4. Assoc. Prof. Dr. Yhosiaki Takaya (Meijo University, Japan)
5. Prof. Dr. Retna Apsari (Universitas Airlangga, Indonesia)
6. Dr. Fatmawati, M.Si. (Universitas Airlangga, Indonesia)
7. Prof. Dr. Afaf Baktir, MS. (Universitas Airlangga, Indonesia)
8. Prof. Dr. Suhariningsih (Universitas Airlangga, Indonesia)
9. Prof. Dr. Ir. Agoes Soegianto, DEA. (Universitas Airlangga, Indonesia)
10. Prof. Dr. Bambang Irawan, M.Sc. (Universitas Airlangga, Indonesia)
11. Dr. Y. Sri Wulan Manuhara, M. Si. (Universitas Airlangga, Indonesia)
12. Dr. Pratiwi Pudjiastuti, M.Si (Universitas Airlangga, Indonesia)
13. Dr. Muji Harsini, M.Si (Universitas Airlangga, Indonesia)
14. Dr. Miswanto, M.Si. (Universitas Airlangga, Indonesia)
15. Dr. Nur Chamidah, M.Si. (Universitas Airlangga, Indonesia)
16. Dr. Windarto, M.Si. (Universitas Airlangga, Indonesia)
17. Dr. Mochamad Zakki Fahmi (Universitas Airlangga, Indonesia)

### Organizing Committees:

1. Dr. Moh. Yasin (Universitas Airlangga, Indonesia)
2. Prof. Dr. Hery Purnobasuki (Universitas Airlangga, Indonesia)
3. Dr. Eridani, M.Si. (Universitas Airlangga, Indonesia)
4. Dr. Hewa Y Abdullan (Salahaddin-Hawler University, Erbil, Iraqi Kurdistan)
5. Prof. Dr. Wan Aini Ibrahim (Universiti Teknologi Malaysia, Malaysia)

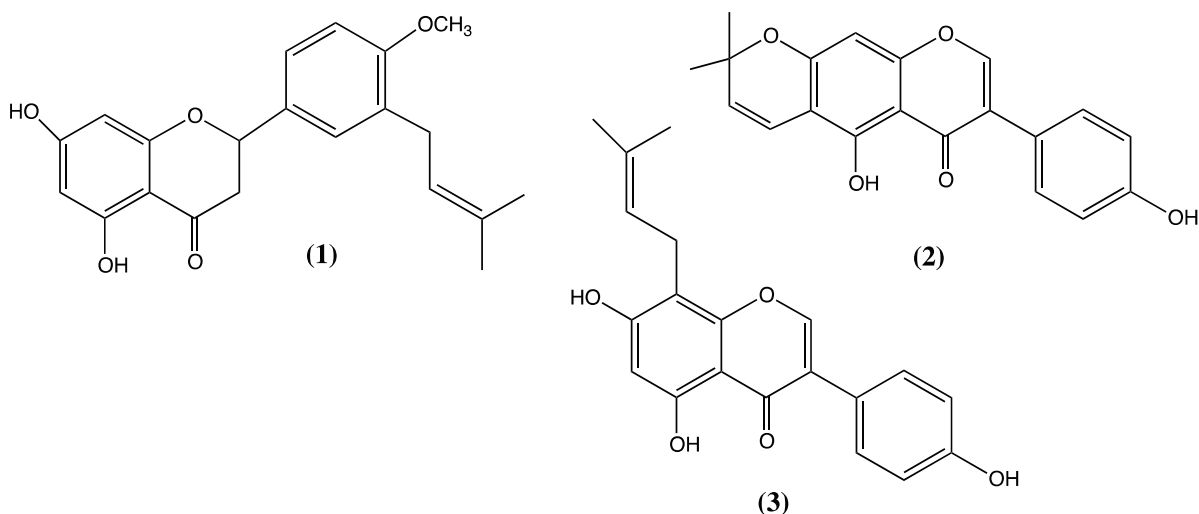
# Phenolic Compounds from The Stem Bark *Erythrina Orientalis* and Detection of Antimalaria Activity by *ELISA*

Tjitjik Srie Tjahjadarie\*, Ratih Dewi Saputri, and Mulyadi Tanjung

Natural Products Chemistry Research Group, Organic Chemistry Division, Department of Chemistry,  
Faculty of Science and Technology, Universitas Airlangga

\*Corresponding author email: tjitjiktjahjandarie@gmail.com

**Abstract.** *Erythrina orientalis* has local name “Dadap”. This plant has known producing alkaloids, flavonoids, pterocarpan, stilbenes, and arylbenzofurans which are active compounds. Three prenylated flavonoids, 8-prenyl-daidzein (1), alpinumisoflavone (2) and 4'-O-methyl licoflavanone (3) had been isolated from the stem bark of *Erythrina Orientalis*. The structures were elucidated on the basis of spectroscopic data, which are IR, UV, MS, and NMR 1D (<sup>1</sup>H-NMR and <sup>13</sup>C-NMR) and 2D (COSY, HMQC, and HMBC).



Compounds 1-3 were evaluated for their antimalaria with *ELISA* methods, which showed percentage inhibition values of 74.46%, 62.06%, and 0% respectively. Based on the inhibition value showed 4'-O-methyl licoflavanone (1) exhibit very high antimalaria activity compared to compounds (2) and (3).

**Keywords:** Pterocarpan, Flavonoid, *Erythrina Orientalis*, Antimalaria

## 1. Introduction

Malaria remains world's one of the most devastating human parasitic infection affecting more than 500 million people and causing about 1–3 million deaths each year. This disease has been found as endemic in all region of Indonesia. Recently, chloroquine and artemisinin have used as antimalaria drug and showed resistance against *Plasmodium* parasites in Indonesia [1]. *Erythrina* is a large genus of Leguminosae family consisting of about 120 species and distributed in the tropical and subtropical region, including Indonesia [1]. The phytochemical studies of *Erythrina* have been known that these plants producing alkaloids [2,3], flavonoids [4,5], pterocarpan [6,7], stilbenes [8], and

arylbenzofurans [9], which are active compounds. *Erythrina orientalis* known local name as "Dadap". The bark or leaves of *Erythrina orientalis* has been used by Indonesian people as a traditional medicine of malaria [10]. In continuation of our research of phenolic compound in this medicinal plant, we reported the isolation of prenylated flavonoids, 4'-O-methyl licoflavanone (**1**), alpinumisoflavone (**2**) and 8-prenyl-daidzein (**3**), from the ethyl acetate extract of the stem bark of *Erythrina orientalis*. The antimalaria properties of compounds **1-3** against *Plasmodium palcifarum* were analysed using *ELISA* method.

## 2. Material and Methods

### General

UV and IR spectra were measured with a Shimadzu 1800 and Perkin Elmer Spectrum One FTIR spectrometer, respectively. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded with an Agilent 500 spectrometer operating at 500 (<sup>1</sup>H) and 125 (<sup>13</sup>C) MHz in CDCl<sub>3</sub> or acetone-*d*<sub>6</sub> using TMS as the internal standard. Mass spectra were obtained from a Waters LCT Premier XE. Vacuum liquid chromatography (VLC) and radial chromatography were carried out using Si gel 60 GF254 and Si gel 60 PF254, for TLC analysis, pre-coated silica gel plates (Merck Kiesel gel 60 GF 254, 0,25 mm thickness) were used. Solvents used for extraction and preparative chromatography were of technical grade and distilled before used. Solvent used for purification was pro analysis grade.

### Plant material

The stem bark of *E. orientalis* were collected from Purwodadi, Kabupaten Pasuruan, East Java, Indonesia. The species was identified at the Herbarium Bogorienses, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia and a voucher specimen had been deposited at the Herbarium Bogorienses.

### Extraction and isolation

The stem bark of *E. orientalis* (3.0 kg) was macerated with MeOH two times at room temperature and concentrated to dryness on a rotary evaporator under reduced pressure. The residu was suspended in water and partitioned sequentially with *n*-hexane (56 g) and EtOAc (18 g). The EtOAc extract was fractionated on silica gel by VLC eluting with mixtures of *n*-hexane-EtOAc (9:1, 4:1, 7:3, 1:1, and 3:7) to give four major fractions A-D. Fractions B-C were separated further to isolate flavonoids compounds. Fraction B (1.8 g) was purified using radial chromatography eluted with a mixtures of *n* hexane-CHCl<sub>3</sub> (3:7, 1:1, and 7:3) yielded compound **1** (50 mg). Fraction C (6.8 g) was fractionated on silica gel by VLC eluting with a mixtures of *n*-hexane-acetone (9:1, and 4:1) to give three sub fractions C<sub>1</sub>-C<sub>3</sub>. Sub fractions C<sub>1</sub> purified using radial chromatography eluted with a mixtures of *n*-hexane-acetone (9:1, and 4:1) yielded compound **2** (9 mg). Using the same methodology on fraction C<sub>3</sub> afforded compound **3** (12 mg).

**4'-O-Methyl licoflavanone (1):** Pale yellow solid: UV (MeOH)  $\lambda_{maks}$  (nm) (log  $\epsilon$ ): 203 (4.62), 226 sh (4.43), 288(4.25), 332 sh (3.78); (MeOH+NaOH) 204 (4.67), 218 sh (4.48), 323 (4.41); (MeOH+AlCl<sub>3</sub>) 204 (4.67), 223 (4.54), 310 (4.34), 368 (3.54); (AlCl<sub>3</sub>+HCl) 204 (4.67), 223 (4.54), 309 (4.34), 368 (3.54); (NaOAc): 204 (4.68); 225 sh(4.43); 228 (4.16), 322 (4.10); HR-ESI-MS *m/z* 355.1460 [M+H]<sup>+</sup> (calcd for C<sub>21</sub>H<sub>23</sub>O<sub>5</sub>: 355.1462); <sup>1</sup>H-NMR (500MHz, CDCl<sub>3</sub>)  $\delta_H$  (ppm): 5.33 (1H, dd, *J* = 13,0, 3,0 Hz, H-2), 3.11 (1H, dd, *J* = 17,0, 13,0 Hz, H-3ax), 2.77 (1H, dd, *J* = 17,0, 3,0 Hz, H-3eq), 5.97 (1H, d, *J* = 1.8 Hz, H-6), 5.99 (1H, d, *J* = 1.8 Hz, H-8), 7.19 (1H, d, *J* = 2.0 Hz, H-2'), 6.87 (1H, d, *J* = 8.0 Hz, H-5'), 7.25 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 3.33 (1H, d, *J* = 7.0 Hz, H-1''), 5.29 (1H, tlike, *J* = 7.0 Hz, H-2''), 1.74 (3H, s, H-4''), 1.70 (3H, s, H-5''), 3.85 (3H, s, 4'-OCH<sub>3</sub>), 12.06 (1H, s, 5-OH); <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta_H$  (ppm): 79.3 (C-2), 43.1 (C-3), 196.4 (C-4), 103.1 (C-4a), 164.3 (C-5), 95.5 (C-6), 164.8 (C-7), 96.6 (C-8), 163.4 (C-8a), 129.9 (C-1'), 127.6 (C-2'), 130.8 (C-3'), 157.8 (C-4'), 110.3 (C-5'), 125.1 (C-6'), 28.5 (C-1''), 121.9 (C-2''), 133.0 (C-3''), 25.8

(C-4''), 17.8 (C-5''), 55.5 (4'OCH<sub>3</sub>). Further support for structure **1** was also obtained by HMQC and HMBC spectra. The spectra data of compound **1** was compared with 4'-*O*-methyl licoflavanone from *Macaranga trichocarpa* [11].

**Alpinumisoflavone (2):** Pale yellow solid: HR-ESI-MS  $m/z$  337.1082 [M+H]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>17</sub>O<sub>5</sub>: 337.1076); <sup>1</sup>H-NMR (500 MHz, acetone-*d*<sub>6</sub>) δ<sub>H</sub> (ppm): 8.17 (1H, s, H-2), 6.36 (1H, s, H-8), 7.45 (2H, d,  $J = 8.5$ , H-2'/6'), 6.90 (2H, d,  $J = 8.5$ , H-3'/5'), 5.76 (1H, d,  $J = 10.0$ ), 6.67 (1H, d,  $J = 10.0$ ), 1.46 (6H, s, H-5'/6'), 13.42 (1H, s, 5-OH); <sup>13</sup>C-NMR (125 MHz, acetone-*d*<sub>6</sub>) δ<sub>H</sub> (ppm): 154.4 (C-2), 124.1 (C-3), 181.8 (C-4), 106.0 (C-4a), 157.7 (C-5), 106.7 (C-6), 160.2 (C-7), 95.4 (C-8), 158.1 (C-8a), 122.9 (C-1'), 131.1 (C-2'/6'), 116.0 (C-3'/5'), 158.5 (C-4'), 78.8 (C-2''), 129.4 (C-3''), 115.7 (C-4''), 28.4 (C-5''/6''). Further support for structure **2** was also obtained by HMQC and HMBC spectra. The spectra data of compound **2** was compared with alpinumisoflavone from *Erythrina fusca* [12].

**8-Prenyldaizein (5):** Pale yellow solid: HR-ESI-MS  $m/z$  321.1120 [M-H]<sup>-</sup> (calcd for C<sub>20</sub>H<sub>17</sub>O<sub>4</sub>: 321.1127); <sup>1</sup>H-NMR (500 MHz, acetone-*d*<sub>6</sub>) δ<sub>H</sub> (ppm): 8.23 (1H, s, H-2), 6.36 (1H, s, H-8), 7.92 (1H, d,  $J = 9.0$ , H-5), 7.03 (1H, d,  $J = 9.0$ , H-6), 7.48 (2H, d,  $J = 9.0$ , H-2'/6'), 6.88 (2H, d,  $J = 9.0$ , H-3'/5'), 3.57 (1H, d,  $J = 7.0$  Hz, H-1''), 5.28 (1H, tm,  $J = 6.8$  Hz, H-2''), 1.83 (3H, s, H-4''), 1.66 (3H, s, H-5''); <sup>13</sup>C-NMR (125 MHz, acetone-*d*<sub>6</sub>) δ<sub>H</sub> (ppm): 153.2 (C-2), 124.7 (C-3), 176.1 (C-4), 118.8 (C-4a), 125.3 (C-5), 114.8 (C-6), 160.1 (C-7), 116.3 (C-8), 156.6 (C-8a), 124.5 (C-1'), 131.0 (C-2'/6'), 115.8 (C-3'/5'), 158.1 (C-4'), 22.6 (C-1''), 122.5 (C-2''), 132.5 (C-3''), 17.9 (C-4''), 25.8 (C-5''). Further support for structure **3** was obtained by HMQC and HMBC spectra. The spectra data of compound **3** was compared with 8-prenyldaizein from *Erythrina fusca* [12].

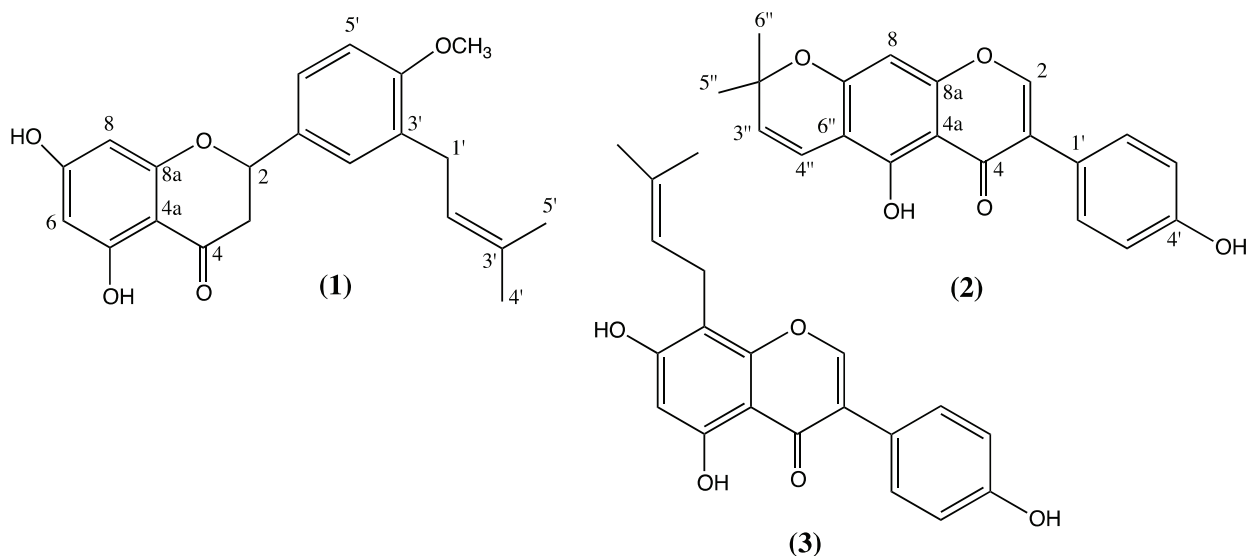


Figure 1. Structures of phenolic compounds

### Antimalaria assay

In vitro antimalarial activity against *Plasmodium falciparum* was carried out according to a modified method of ELISA using PBS liquid (Buffer Phosphat) and 19G7 antibody using *EZ-Link Sulfo-NHS-LC* and 2-(4-hydroxybenzene) (HABA). The isolated compounds **1–3** have been inserted into the well of micro enzyme-linked immune sorbent assay (ELISA) with the addition of 100 μL 19G7 antibodies were incubated for 1 h at 37 ° C. After incubation, to the ELISA well was added 100 μl peroxide



and 100  $\mu$ l TMB (3,3',5,5'-tetramethylbenzidine) and then incubated for 20 min at room temperature. The isolated performed using reagent H<sub>2</sub>SO<sub>4</sub> 2N was measured by UV spectrometer at  $\lambda$  450 nm [17].

### 3. Results and Discussion

Three prenylated flavonoids, 4'-*O*-methyllicoflavanone (**1**), alpinumisoflavone (**2**), and 8-prenyldaidzein (**3**) have been isolated from the stem bark of *E.orientalis*. Their structures were elucidated with extensive by UV, IR, HRESIMS, 1D and 2D NMR spectrum. 4'-*O*-methyl licoflavanone (**1**) was obtained as yellow solid, and its UV spectra exhibited absorption maxima (203, 226, 288 and 323 nm) typical for a flavanone structure [13]. The HRESIMS spectrum showed a quasi-molecular ion [M+H]<sup>+</sup> at  $m/z$  355.1460 consistent to the molecular formula C<sub>21</sub>H<sub>23</sub>O<sub>5</sub>, suggesting that **1** is a prenylated flavanone with contain one methoxyl group. The <sup>1</sup>H-NMR spectra of **1** showed three doublet-doublet proton signals at  $\delta_H$  5.33 (1H, dd,  $J$  = 13.0, 3.0 Hz, H-2), 3.11 (1H, dd,  $J$  = 17.0, 13.0 Hz, H-3ax), and 2.77 (1H, dd,  $J$  = 17.1, 3.0 Hz, H-3eq) confirmed for the flavanone structure. In the <sup>1</sup>H-NMR spectrum of **1** showed ABX system at  $\delta_H$  7.19 (d,  $J$  = 2.0 Hz, H-2'), 6.87 (d,  $J$  = 8.0 Hz, H-5'), 7.25 (dd,  $J$  = 8.0, 2.0 Hz, H-6') characteristic for aromatic in thering B.

The presence of the proton signals of a pair of doublets ( $J$  = 1.8 Hz) in the aromatic region at  $\delta_H$  5.97 and 5.99 ppm, characteristic for H-6 and H-8 in the ring A. Furthermore, in the <sup>1</sup>H-NMR spectra showed one isoprenyl group assignable to a 3-methyl-2-buten-1-yl group at  $\delta_H$  5.29 (1H, t,  $J$  = 7.0 Hz, H-2''), 3.33 (2H, d,  $J$  = 7.0 Hz, H-1''), 1.74 (3H, s, H-4''), 1.70 (3H, s, H-5''), and one methoxyl group at  $\delta_H$  3.85 ppm. The presence of a chelated hydroxyl group ( $\delta_H$  12.06, 5-OH) suggested that methoxyl group ( $\delta_H$  3.85) at C-7 or C-4'. In the <sup>13</sup>C-NMR spectrum, the presence of four oxyaryl ( $\delta_C$  164.8, 164.3, 163.4 and 157.8) and a carbonyl carbon signal at  $\delta_C$  196.4 suggested that the oxygenates functionalities are at C-5, C-7 and C-4' of the flavanone skeleton. The placement of methoxyl group and prenyl group were obtained from the HMQC and HMBC spectra. The one bond and two/three bonds <sup>1</sup>H-<sup>13</sup>C correlations found in the HMQC and HMBC spectra of compound **1** unambiguously placed the methoxyl group at C-4' was observed. In the HMBC spectrum showed correlations between a proton signal of a methoxyl group at  $\delta_H$  3.85 with one oxyaryl signals  $\delta_C$  157.8 and between two proton signals of a aromatic group at  $\delta_H$  7.19 and 7.25 with one oxyaryl signals  $\delta_C$  157.8.

The prenyl group at C-3' showed correlations between them ethylene signal at  $\delta_H$  3.33 with a oxyaryl carbon at  $\delta_C$  157.8 (C-4'). Compound **1**, trivially named 4'-*O*-methyl licoflavanone, was identified as 4;-*O*-methyl-3'-prenyl naringenin. Further support for the structure **1** was also obtained from the comparison of the NMR data with those reported for 4'-*O*-methyl licoflavanone from *Macaranga trichocarpa* [15].

Alpinumisoflavone (**1**) was isolated as pale yellow solid and the HRESIMS spectrum showed a quasimolecular ion [M+H]<sup>+</sup> at  $m/z$  337.1082 consistent to the molecular formula C<sub>20</sub>H<sub>17</sub>O<sub>5</sub>, suggesting that **1** is a prenylated flavonoid. The <sup>1</sup>H-NMR spectra of **1** showed singlet proton deshielding signals at  $\delta_H$  8.17 characteristic for H-2 of their isoflavone structure. The <sup>1</sup>H-NMR spectrum also displayed a pair of doublets ( $J$  = 8.5 Hz), each integrating for two protons, at  $\delta_H$  6.90 and 7.45 assignable to the signals of a *para*-hydroxyphenyl group in the ring B. The presence of a chelated -OH group at  $\delta_H$  13.42 and a singlet signal aromatic proton at  $\delta_H$  6.36 characteristic for 5-OH and H-6 or H-8 in the ring A of isoflavonoid structure. In addition, the <sup>1</sup>H and <sup>13</sup>C-NMR patterns of **1** exhibited the presence of a 2,2-dimethyl pyrano ring due to the presence of a *gem*-dimethyl resonance [ $\delta_H$  1.46 (6H, s, H-5'' and H-6''),  $\delta_C$  28.4 (C-5'' and C-6'')], an olefinic group [ $\delta_H$  5.76 (1H, d,  $J$  = 10.0 Hz, H-3'') and 6.67 (1H, d,  $J$  = 10 Hz, H-4''),  $\delta_C$  129.4 (C-3'') and 115.7 (C-4'')], and an oxygenated quaternary carbon ( $\delta_C$  78.8). Based on <sup>1</sup>H and <sup>13</sup>C-NMR spectra, the placement 2,2-dimethyl pyrano ring were fused at C-6 and C-7 or C-7 and C-8. The location of the 2,2-dimethylpyrano unit at C-6 and C-7 determined on the basis of the HMBC correlations observed. The presence of long-range correlations in the HMBC spectrum of **1** between the proton signal of a chelated 5-OH group at  $\delta_H$  13.42 and three quaternary carbon signals at  $\delta_C$  106.0 (C-4a), 157.7 (C-5), 106.7 (C-6) unambiguously placed the 2,2-dimethyl pyrano fused at C-6 and C-7. Further support for the structure **1** was also obtained from the comparison of the NMR data with those reported for alpinum isoflavone from *Erythrina fusca* [16].

8-Prenylidaizein (**3**) was isolated as yellow solid and the HRESIMS spectrum showed a quasimolecular ion  $[M-H]^-$  at  $m/z$  321.1120 consistent to the molecular formula  $C_{20}H_{17}O_4$ , suggesting that **3** is a prenylated flavonoid. The  $^1H$ -NMR spectra of **3** showed singlet proton signals at  $\delta_H$  8.23 characteristic for H-2 of the isoflavone structure. The presence of the proton signals of a pair of doublets ( $J = 9.0$  Hz) in the aromatic region at  $\delta_H$  6.88 and 7.48 (each 2H), assignable to the signals of a *p*-hydroxy phenyl group in the ring B. The signal of a pair of doublets ( $J = 9.0$  Hz) in the ring A at  $\delta_H$  7.03 (H-6) and 7.92 (H-5), suggested that the prenyl group attached at C-8. Further support for structure **3** was also obtained by HMQC and HMBC spectra. The spectra data of compound **3** was compared with 8-prenylidaizein from *Erythrina fusca* [16]. The result of compounds **1–3** were evaluated for their antimalaria with *ELISA* methods, which showed percentage inhibition values 74.46%, 62.06%, and 0 % respectively. Compound **1** exhibited very high antimalaria activity.

**Table-1. Antimalaria activities of flavonoids compounds**

Compound	% inhibition antimalaria ( <i>ELISA</i> method) dosage 100 $\mu$ g/ml
4'- <i>O</i> -methyl licoflavanone ( <b>1</b> )	74.46%
Alpinumisoflavone ( <b>2</b> )	62.06%
8-Prenylidaizein ( <b>3</b> )	na %

#### 4. Conclusion

Maceration of dried and powdered stem bark of *E. orientalis* in methanol at room temperature yielded a brown extract. Methanol extract was suspended in water and partitioned sequentially with *n*-hexane, and then ethyl acetate. Fractionation of the ethyl acetate extract by VLC on silica gel gave four major fractions, A-D and then fraction B-C purified with radial chromatography yielded flavonoids compounds 4'-*O*-methyl licoflavanone (**1**), alpinum isoflavone (**2**), and 8-prenylidaizein (**3**). Their structures were elucidated based on UV, IR, HR-ESI-MS, 1D and 2D NMR data by comparing spectroscopic data with reported value. All of isolated compounds **1–3** were assessed for their malaria activity against *Plasmodium falciparum* as shown in Table-1. Compounds **1–3** were evaluated for their antimalarial activity against *Plasmodium falciparum* was carried out according to a modified method of *ELISA*, showing their percentage inhibition values 74.46%, 62.06%, and 0 % respectively. The results of antimalaria activity against *Plasmodium falciparum* showed that compound **1** is more active than two other flavonoid compounds. Compound **1** exhibited very high antimalaria activity, while compound **3** was inactive.

#### Acknowledgment

We would like to thank to Prof. Dr. Yana M Syah from Department of Chemistry, ITB Bandung, Indonesia for HRESIMS spectra measurements and Institute Tropical Disease Center Universitas Airlangga for NMR spectra measurements. We would like to thank to Mr. Ismail Rachman from the Herbarium Bogoriense, Botanical Garden, Bogor, Indonesia for identifying the species.

#### References

- [1] B.B. Oliver., *Medicinal Plants in Tropical West Africa*; Cambridge University Press: New York, **1981**, 5-24.
- [2] S.A. Faggion., A.O.S. Fachim., A.S. Gavin., W.F. Santos., A.M.S. Pereira., and R.O. Beleboni., *Epilepsy and Behavior.*, **2011**, 20, 441-446.
- [3] M. Ozawa., S. Kawamata., T. Etoh., M. Hayashi., K. Komiyama., A. Kishida., C. Kuroda., and A. Ohsaki., *Chem. Pharm. Bull.*, **2010**, 58(8), 1119-1122.
- [4] L. Cui., P.T. Thuong., H.S. Lee., D.T. Ndinthe., J.T. Mbafor., Z.T. Fomum, Z.T., and W.K. Oh., *Bioorg. Med. Chem. Lett.*, **2008**, 18, 10256–10262.



- [5] SA W. Watjen., A.K. Suckow-Ashnitker., R. Rohrig., A. Kulawik., C.W. Wright., and C.M. Passreiter., *J.Nat. Prod.*, **2008**, 71, 735-738.
- [6] P. Innok., T. Rukachaisirikul., S. Phongpaichit., and A. Suksamrarn, *Bioorg. Med. Chem. Lett.*, **2009**, 19, 6745–6749 .
- [7] P.H. Nguyen., T.V.T. Thuong., T.T. Dao., D.T. Nditeh., J.T. Mbafor., J. Park., H. Cheong., and W.K. Oh, *J.Nat. Prod. Comm.*, **2010**, 5(8), 1209-1211.
- [8] P.H. Nguyen., M.K. Na., T.T. Dao., D.T. Nditeh., J.T. Mbafor., K.W. Kang., and W.K. Oh, *Bioorg. Med. Chem.Lett.*, **2010**, 20, 6430–6434.
- [9] M.K. Na., D.M. Hoang., D. Njamen., J.T. Mbafor., Z.T., Fomum., P.T. Thuong., J.T. Ahn., and W.K. Oh., *Bioorg. Med. Chem. Lett.*, **2007**, 17, 3868–3871.
- [10] K. Heyne., *The Useful Indonesian Plants*. Research and Development Agency, Ministry of Forestry, **1987**, Jakarta, Indonesia.
- [11] M.C. Alley., D.A. Scudiero., A. Monks, M.L. Hursey., M.J. Czerwinski., B.J.. Abbot., J.G. Mayo., R.H. Shoemaker., and M.R. Boyd., *Cancer Res.*, **1998**, 48, 589-601.
- [12] W. Li., Y.N. Sun., X.T. Yan., S.Y. Yang., S. Kim, D. Chae., J.W. Hyun., H.K. Kang., Y.S. Koh., and Y.H. Kim., *Arch. Pharm. Res.*, **2014**, 37, 721-727.
- [13] M. Tanjung., T.S. Tjahjandarie., and M.H. Sentosa., *Asian Pac. J. Trop. Dis.*, **2013**, 3(5), 401-404.
- [16] T. Rukachaisirikul., P. Innok., N. Aroonrerk., W. Boonamnuaylap., S. Limrangsun, C. Boonyon., U. Woonjina., and A. Suksamrarn, *J. Ethnopharmacol.*, **2007**, 110, 171-175.
- [14] A. Yenesew., J.O. Midiwo., M. Heydenreich., D. Schanzenbach., and M.G. Peter., *Phytochemistry*, **2000**, 55, 457-459.
- [15] Y.M. Syah., E.H. Hakim., S.A. Achmad, M. Hanafi., and E.L. Ghisalberty., *J. Nat. Prod Comm.*, **2009**, 4, 1137-1140.
- [16] P. Khaomek., C. Ichino., A. Ishiyama., H. Sekiguchi., M. Namatame., N. Ruangrunsi., E. Saifah., H. Kiyohara., K. Otoguro, S. Omura., and H. Yamada., *J. Nat. Med.*, **2008** 62, 217-220.
- [17] Sanchez, Pamela, O., Duggan, E., Nolan, J., Frangos, John A., and Leonardo, JM., *Malaria Journal*, 2012, 11, 366.